EFFECT OF PROCESSING ON THE STARCH AND GLYCEMIC PROPERTIES OF DIGITARIA SPP.

MICHELLE BEHNERTA JORDAAN
Effect of Processing on the Starch and Glycemic Properties of *Digitaria spp.*

by

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Thesis presented in partial fulfilment for the degree of:

**Master of Technology (Food Technology)**

Department of Food Technology
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DECLARATION

I, Michelle Jordaan, hereby declare that the work contained in this proposal is my own original work and that it has not previously, in its entirety or in part been submitted at any other university for a degree.

Michelle Bernitta Jordaan

Date

13. 06. 2013
Abstract

Acha starch was isolated and purified from clean and milled acha grain. Functional, thermal and physicochemical properties of acha starch were analysed using appropriate methods. Wheat starch was used as the reference standard. Acha bread from acha grain was baked and the consumer sensory acceptability was evaluated and white wheat bread was used as the reference standard. The effect of baking, boiling, steaming and microwaving on the starch and glycemic properties of the acha starch was evaluated. With regard to thermal properties, gelatinisation temperature of acha and iburu starches typifies that of waxy starch. Acha starch has similar retrogradation temperature profiles as that of wheat. There were however significant differences in some of the functional properties (pasting and turbidity) and physico-chemical properties (in vitro starch digestibility), but no significant difference in the texture profile analysis (TPA) and water binding capacity (WBC). WBC of both acha varieties was higher than that for wheat starch. Due to its high break down viscosity, white acha starch can be included in foods that are subjected to high temperature processing. This indicates that both acha starch varieties can be used for hot and cold desserts as well as for soft jelly like sweets and confectionery toppings. A pre-screening exercise using carboxymethyl cellulose (CMC), Xanthan gum, yeast and acha starch as the variables was successful in concluding a recipe which rendered acha bread with the optimum specific loaf volume for both white and black acha bread. The optimum recipe consisted 8.0 % acha starch, 2.0 % xanthan gum, 2.0 % CMC and 1.0 % yeast. The majority of the consumer panellists found the crust colour, taste and aroma to be moderately desirable. This implies that most consumers find acha bread to have the potential to be marketed as wheat free bread. The different processing methods baking, boiling, microwaving and steaming, affected the black and white acha starch hydrolysis. The amount of starch hydrolysed for the different processing methods was in the following order: baking > boiling > microwaving > steaming. It can thus be concluded that different processing methods affects the micro structure and physical properties of the acha and wheat samples.
which thus influence their starch hydrolysis. The equilibrium percentage of starch hydrolysed after 180 min incubation was affected differently for the various starches, black acha, white acha and wheat starch by the different processing methods and times. In the case of baking black acha starch and wheat bread were affected similarly. However, this was not the case for microwaving, steaming and boiling, where both acha starch varieties and wheat starch were affected in the same way. The rate of starch hydrolysis for both acha varieties and wheat grain for the different processing methods, steaming, boiling, microwaving and baking was affected to the same degree respectively.

Language and style used in this thesis are in accordance with the requirements of the International Journal of Food Science and Technology. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.
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Dedication

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CHAPTER 1
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

*Digitaria* *spp.* belongs to the family Graminae and the tribe Poaceae (Dalziel, 1937; Jideani, 1999) and includes 230 species that are widely distributed in the tropics and the subtropics (Adoukonou-Sagbadja *et al.*, 2006). Two types exist namely *Digitaria exilis* (acha, fonio or hungry rice) and *Digitaria iburua* (iburu, black fonio or petit mil) [Jideani, 1999; Philip and Itodo, 2006]. In this study the name Acha will be used to indicate the *Digitaria* sp. Acha is by large the oldest African cereal, it has been cultivated for thousands of years in West Africa (Mali, Burkina Faso, Guinea and Nigeria) and Dominican Republic (Morales-Payán *et al.*, 2003; Jideani *et al.*, 2008) where it is used as a staple food (National Research Council, 1996). *Digitaria exilis* grows under varying conditions from poor dry upland soils to hydro-morphic valleys. Acha was once classified as one of the “lost crops of Africa”, but is now “rediscovered” and considered for improvement as cultivated species (Ibrahim, 2001; Morales-Payán *et al.*, 2003). It is valued because of its unique taste and nutritional value (Philip and Itodo, 2006).

Acha grains contain about 7-9% crude protein with high leucine (9.8%), methionine (5.6%) and valine (5.8%). It has been reported that its methionine content is twice as high as those of egg proteins (Temple and Bassa, 1991; National Research Council, 1996; Ghana Grains Development Project, 1993). Hence, it is the most nutritious grain, containing high essential amino acids that are deficient in today’s major cereals such as maize, sorghum, barley, wheat and rye. Consequently, acha has important potential as a complement for standard diets (National Research Council, 1996).

Food products where acha grains have been used include porridge, couscous and it is also mixed with other flours to make bread, pastries and is even oven popped (Jideani, 1999; Philip and Itodo, 2006) as well as traditional beverages such as beer (Philip and Itodo, 2006). Jideani *et al.* (2007, 2008) demonstrated the possibility of producing gluten free loaf from acha flour. In Togo acha is a staple food crop that is cultivated for home consumption and
used during religious and cultural festivals (Adoukonou-Sagbadja et al., 2006). Acha grains prepared similar to a bean type meal, were reported as the most popular acha-based diet in Togo—mostly prepared at special occasions for chiefs, dignitaries and guests. The more common acha meals were acha couscous, rice, porridge and paste. In some regions acha is used solely or mixed with sorghum, pearl millet and maize to prepare local beer named Tchoukouto. Togolese farmers also consider acha as having medicinal value in being useful for those suffering from diabetes among others (Adoukonou-Sagbadja et al., 2006). Similar medicinal use has also been associated with acha in Nigeria (Jideani, 1999).

Starch is the main storage carbohydrate of plants. It is deposited as insoluble, semi-crystalline granules in storage tissues (grains, tubers, roots) and to lesser extent in most vegetative tissues of plants (Copeland et al., 2009). Energy (50-70%) in the human diet is obtained from starch, providing a direct source of glucose—an essential substrate in brain and red blood cells for generating metabolic energy (Copeland et al., 2009). Starch is a food ingredient with a wide applications—thickener, gelling agent, bulking agent and water retention agent (Pomeranz, 1991; Singh et al., 2003), making them an excellent ingredient for the manufacture of foods such as custards, porridges, puddings, cookies and sausages. There is a growing demand for starches for the modern food industry creating interest for new sources of this polysaccharide. Perry et al. (2003) reported that the availability of a reliable source of starch from agriculture is considered to have an important factor in human development. This is important especially as the glycemic response to excessive consumption of rapidly digesting starch may be a factor in some diet-related illnesses.

Starchy foods like acha usually are not eaten raw and must undergo one type of heat processing or the other for palatability and bioavailability. Different processing methods include boiling, microwave, baking, drying, extrusion, steaming, drum-drying, popping, pressure-cooking and others. Therefore, processing methods may modify starch in various ways consequently affecting digestion and nutritional value. It is thus of interest to investigate the physicochemical, functional and thermal properties of acha grain as well as the effect of processing on the acha starch.
1.2 Statement of the Research Problem
The properties of acha have received some attention. De Lumen et al. (1993) reported acha as a promising underutilised African cereal because of its high content of sulphur-amino acids. Jideani et al. (1994) studied the proteins of acha. Lasekan (1994) studied the effect of germination on alpha-amylase activities of acha. The carbohydrates of acha have been investigated (Jideani et al., 1999). Jideani (2007, 2008) studied the potential of acha flour in non-gluten bread. However, the effect of processing on the starch component and glycemic properties has not received attention from scientists.

1.3 Broad Objectives
The aim of the research was to determine the effect of different processing methods on the starch and glycemic properties of acha with a view to establishing relationship between form and functional properties of starch constituent of acha.

1.3.1 Specific objectives
The objectives of the research were to:
1. Establish some functional and thermal properties of acha starch.
2. Establish the physicochemical properties of acha starch.
3. Evaluate the changes in glycemic properties of acha due to different processing methods.

1.4 Research Hypothesis
The hypotheses in this study comprised of
1. The starches from the two acha cultivars (D. exilis and D. iburu) differ in their functional and thermal properties.
2. Different processing methods differ in their effect on the starch and glycemic properties of acha.
3. The effect of processing on the starch and glycemic properties of acha differs among the cultivars.
1.5 Research Delineation
Two cultivars (*D. exilis* and *D. iburua*) were used in the study.

1.6 Significance of the Research
This study on the effects of different processing techniques on the starch and glycemic properties of acha will benefit the following sectors; research and development, patients suffering from diet dependent illnesses, health food manufacturers, fast moving consumer goods manufacturers, industrial food manufacturers and the economy.

1.6.1 Research and development
1. It will put focus on acha as a crop that can be utilized for other uses apart from being a staple crop i.e. low GI snack foods especially in this era of global obesity, or as a partial substitute for wheat flour based products i.e. bread
2. Research and development departments of manufacturing companies can assist in optimizing procurement by making use of locally procured acha grain instead of opting for imported foreign grains.

1.6.2 Health Foods
It will draw attention to the benefits of acha as a dietary supplement for diabetic patients, the development of products aimed at weaning of toddlers, the old and frail as well as immune-compromised individuals. It will aid in controlling and preventing many metabolic diseases such as cardiovascular diseases and patients suffering from hyperlipidaemia. It will be beneficial for use by patients with celiac disease as well as for promoting and maintaining large bowel health.

1.6.3 Manufacturing
It will create a spin off in the technological development specifically with the aim of improving mechanised processing technique for acha grain.

1.6.4 Economy
It will draw closer attention to this indigenous underutilized crop and its
usefulness, especially in this time of increasing food prices and world-wide food shortages.

1.7 **Expected outcomes, results and contributions of the research**

1. Identification of the processing techniques that slows down the rate of acha starch digestion.

2. New research data on the effect of processing on acha starch properties.

3. New starch source made available.

4. Novel use of this indigenous African cereal as a starch based material.

5. Non wheat bread from acha with nutritional and health benefits.

6. At least one scientific article will be published from the work and the information will be presented at a scientific conference.

**REFERENCES**


CHAPTER 2
LITERATURE REVIEW

2.1 Overview of Digitaria spp.

*Digitaria exilis* (white) and *Digitaria iburua* (black) are two species of acha grains (Figure 2.1). Other common names are fonio or hungry rice. It is indigenous to Africa particularly regions of Mali, Burkina Faso, Guinea and Nigeria (Ayo, 2003) and the Dominican Republic (Morales-Payán et al., 2003; Jideani et al., 2008). Acha falls under the grass family Gramineae (Poaceae) and belongs to the same sub-family as maize, sorghum, pearl millet and barley. This is illustrated in Figure 2.2.

Acha has a unique small size of 0.4 to 0.5 mm (Adoukonou-Saghbadja et al., 2006). Gomez & Gupta (1993) reported acha grains to be very small with an individual grain weight of 0.5 to 0.6 mg. Ayo (2004) recorded this tiny grain weight to be about 0.5 mg with a diameter of between 500 to 700 nm. Acha's kernel is much smaller than the average pearl millet, sorghum and maize kernel (Phillip & Itodo, 2006). Irving & Jideani (1997) reported that acha can be used for the same technological processes as cereals similar to its anatomical structure. It is thus of interest for this research to determine the exact physical characteristics of these two *Digitaria* spp.

2.2 Uses of Acha

Acha is essentially cultivated as a staple food crop for most ethnic groups of West Africa (Phillip & Itodo 2006). Acha can be used in a variety of meals like breakfast and supper. It can be made into a porridge and couscous, or it can be ground and mixed with other flours to make bread, pastries, oven popped and even brewed into a beer.

In Togo acha is known for its medicinal values hence it is used to treat blood clots, chronic diarrhoea, loss of appetite, stomach aches and as a useful diet for individuals suffering from diabetes (Adoukouno-Saghbadja et al., 2006). Although classified as a lost crop of Africa it is one of the world's best palatable cereal. Assessing the effects of processing on acha starch properties and glycemic index can lead to increased focus on the potential of
Figure 2.1  *Digitaria iburua* (A), *Digitaria exilis* (B), *Digitaria iburua* grain size (C).
Figure 2.2 Taxonomic relationship of grasses (Clayton and Renvoize, 1986)
this crop in terms of developing food products that will be beneficial to those suffering from diabetes and related illnesses.

2.3 Nutritional Composition of Acha

Phillip & Itodo (2006) reported that acha contains about 8-11% proteins and its digestibility is better than that of sorghum or millet. Moisture content of acha grain is 7.2% compared to millet of 6.5% (Ayo, 2004). This moisture content is lower than that reported by Chukwu (2008). The total lipid content (Table 2.1) for acha is higher than that of brown rice, wheat and barley but lower than that for maize and millet (CIRAD, 2004). The protein content of hulled acha has been reported to be lower than that of brown rice, wheat and barley, slightly higher than that of maize and millet (CIRAD, 2004).

With a high essential amino acid, leucine 9.7%, methionine 5.6% and valine 5.8% acha is considered as one of the world’s most nutritious grain (Phillip & Itodo, 2006). Essential amino acids content of acha compared to Obatanpa (quality protein maize) and Okomasa (full season maize) as depicted in Table 2.2, indicates that acha contain significantly higher methionine, valine and leucine values than the all season maize and the high quality protein maize (National Research Council, 1996 & Ghana Grains Development Project, 1993).

According to Ayo (2003), the high water absorption capacity of acha is a characteristic that is linked to high amount of pentosans; acha contains 33 g/kg pentosans. The high amount of pentosans in acha will be beneficial during baking due the consequent increase in water absorption capacity during the baking process. Ayo & Nkama (2004) reported an increase in bread moisture content with an increase in acha flour to the recipe.

Acha residue protein has been shown to contain high levels of glycine, glutamate/glutamine and leucine (Jideani, 1999). The structural role of glycine in cereal proteins depends on the amino acid sequence of the glycine-containing peptides, which is responsible for the elastic properties of gluten necessary in bread making and which only occurs in wheat gluten (Jideani, 1999).
Table 2.1 Nutritional composition of acha compared with other cereals

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Hullled acha</th>
<th>Brown rice</th>
<th>Sorghum</th>
<th>Millet</th>
<th>Maize</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar (%)</td>
<td>85.0</td>
<td>86</td>
<td>82.5</td>
<td>80.0</td>
<td>82.0</td>
<td>82.0</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>10.0</td>
<td>9.0</td>
<td>12.0</td>
<td>13.0</td>
<td>11.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>4.0</td>
<td>2.5</td>
<td>4.0</td>
<td>5.0</td>
<td>5.5.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
<td>1.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>


Table 2.2 Essential amino acid content of acha compared to Obatanpa and Okomasa maize on percentage dry matter

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Fonio</th>
<th>Okomasa (Full season maize)</th>
<th>Obatanpa (Quality protein maize)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>0.37</td>
<td>0.24</td>
<td>0.34</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.25</td>
<td>0.19</td>
<td>0.26</td>
</tr>
<tr>
<td>Valine</td>
<td>0.55</td>
<td>0.33</td>
<td>0.48</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.45</td>
<td>0.17</td>
<td>0.15</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.4</td>
<td>0.23</td>
<td>0.30</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.05</td>
<td>0.77</td>
<td>0.88</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.57</td>
<td>0.31</td>
<td>0.39</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.25</td>
<td>0.23</td>
<td>0.36</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.60</td>
<td>0.06</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Source: NRC (1996), GHANA GRAINS DEVELOPMENT PROJECT (1993)

1 Amino acids expressed as % of protein
2.4 Physical Properties of Acha Starch

Starch is laid down in the tissue of many plants and according to Kearnsley & Dsiedzia (1995) the commercial reality of starch recovery is limited to mainly wheat, maize, potato, tapioca and rice. Starch is found in the granules and its shape and size is relative to its source. The size of starch granules varies from 2-150 micron in size; from round to polygonal and truncated in shape. Rice starch is the smallest of the cereal starches, and also generally the most uniform in size.

Jideani (1999) reported that white and black acha have not been used to produce starch for industrial applications, but that the starch from these two grains are similar in structure and physiochemical properties to starch from conventional cereal grains. Electron photomicrographs of dry acha starch granules from two varieties (Hothia and Koulli) of Digitaria exilis grown in Ethiopia, at different magnifications, are presented in Figures 2.3 (Carcea & Acquistucci, 1997). Microscopic examination conducted by Carcea & Acquistucci (1997) revealed that starch granules from these two varieties are morphologically very similar, being in both cases, small in size and mostly polygonal. In both varieties the average diameter of the granules was 8 µm but Koulli appeared to be more uniform. Carcea & Acquistucci (1997) also observed that acha starch has a morphology (small size, angular, irregular shape) which is common to other millet starches. Starch granule dimension is a characteristic peculiar to each plant species. Due to the potential of small-granule starches to be used in many ways such as fat substitutes, as stabilizers in baking powder, as laundry stiffening as well as in the manufacture of degradable plastic films, it has received some investigations by Daniel & Whistler (1990) as well as by Muir & O'Dea (1992). Polygonal starch granule contains deep indentations on their surfaces (Figure 2.3). These indentations could be due to the dense packing of the endosperm and especially the dense packing of the adjacent protein bodies on the developing starch granules (Carcea & Acquistucci, 1997).

2.5 Chemical Properties of Cereal Starch

Kearnsley & Dsiedzia (1995) reported that starch granules consist of two glucose polymer structures, namely amylose and amylopectin (Figure 2.4), as
Figure 2.3  Scanning electron micrograph of fonio (Digitaria exilis Staph) starch. (a) Koulli variety (1310x); (b) Hothia variety (2100x). (Carcea & Acquistucci, 1997)
Figure 2.4  Amylose and Amylopectin structure (Ophardt, 2003)
well as moisture, lipids, proteins and mineral ions. The backbone of this molecule is the $\alpha$ 1-4 linked anhydro-glucose unit but with branching points occurring at the carbon 6 primary alcohol groups, every 10 to 12 glucose units. The side chain of the main backbone is 20 to 30 glucose units in length. Amylose has traditionally been considered to be a linear polymer composed of glucopyranose units linked by $\alpha$-D-(1-4) glycosidic linkages, while amylopectin is a branched polymer (Karim et al., 2000).

The molecular structures and physicochemical properties of starches from waxy barley and two low-amylose cultivars of barley were examined by Yoshimoto et al. (2002) and compared with those of a normal barley cultivar. The waxy barley starch had no detectable amylose. The amylose content was 22% and 11.4% in the two low-amylose barley starches, respectively, being much lower than that in the normal barley cultivar starch 25.4%. The two low-amylose barley starches contained the branched amylose with a lower number of chains (6.1 to 10.4) than normal barley starch cultivar (Yoshimoto et al., 2002).

Acha starches were reported to be of the non-waxy type, and there were significant differences in amylose between the two acha varieties Hothio (22.6%) and Koulli (26.1%) (Carcea & Acquistucci, 1997). The amylose in the Koulli starch was higher than that of Hothio and wheat (25%) starch, but was much higher than that of pearl millet starch reported by Badi et al. (1976). It is unknown at this stage if the same differences exist between Digitaria exilis and Digitaria iburua. Cereal starches normally have a moisture content of 13 to 14% while other starches have a moisture content of 18 to 20%. The moisture content of Hothio variety has been reported to be higher than that of Koulli and wheat starch (Carcea & Acquistucci, 1997).

### 2.6 Functional Properties of Acha Starch

Starch is an important ingredient for the food industry and is extensively used as a binding agent, thickener, gelling agent, bulking agent and water retaining agent (Sandhu & Singh, 2007). A brief review of the functionality of starch in general and acha will follow.
Starch swelling characteristics

Starch granules swell when heated in excess water and their volume fraction and morphology plays an important role in the rheological behaviour of starch dispersions (Sandhu & Singh, 2007). Pyler (1988) reported that when an aqueous starch suspension is heated, the starch granules imbibe water at 50% of its weight, without showing any change in its appearance. At a critical temperature of 60°C the weaker bonds are dissociated and the granules begin to swell progressively and start to lose some of the higher opacity and birefringence. With continued increase in temperature to the gelatinization range, there is a more extensive disruption of the hydrogen bonds and continues swelling of the granules. According to Pyler (1988) this process continues until the swollen granules have taken up all the surrounding free water, to form a starch paste or gel of various clarity and viscosity.

Various starches consist of a birefringence pattern; the centre of this pattern can either be eccentric or concentric and will correspond to the hilum of the starch granule. The different birefringence patterns of different starches indicate different degrees of crystallinity in individual's starches (Kearnsley & Dsiedzig, 1995). The intra- and inter- molecular hydrogen bonding, responsible for the structural integrity of the starch granule, accounts for its insolubility in water. When a starch suspension is heated in water, heat overcomes the hydrogen bonding forces which holds the granules together, allowing swelling or gelatinization of the starch granule to occur. Acid and enzymes have little hydrolyzing effect on the un-gelatinized starch (Kearnsley & Dsiedzig, 1995). Thermal gelatinization of starch in the presence of alkali, using different temperature regimes, is therefore, likely to result in wide variations in the properties of gelatinized starch such as colour, paste viscosity, gel properties and retrogradation tendency (Karim et al., 2000).

González-Reyes et al. (2002) reported the swelling pattern of Okenia (Okenia hypogaea) and corn starches with 5% (w/v) total solids. Okenia starch presented a slight increase in swelling when heated from 30 to 60°C. However, the swelling of corn starch was constant in a similar temperature range. When increasing the temperature, a gradual increase in swelling of both starches was observed with increase in temperature up to 95°C. This
was due to the breaking of the molecular bonds when increasing the
temperature of the starch granules and the subsequent incorporation of water
molecules within their structure (Gonzales & Perez, 2002).

Cereal starches exhibit a two stage swelling and solubility pattern that
is indicative of two sets of internal bonding forces. Whereas waxy starches
swells more readily than do the normal starches, presumably because of the
lack of reinforcement of the internal network provided by the linear fractions.
Potato starch expands at a markedly lower temperature of 75°C and expands
more rapidly and to a greater extent, so that the outline of the granules
become vague. The swelling power of acha and wheat starch over a
temperature range of 50-90°C was investigated by Carcea & Acquistuicci
(1997). Swelling in the early stages indicated disintegration of the granular
structure and significant difference (p ≤ 0.05) were observed in the swelling
power. Acha starch swelled from 60°C and up to 65°C and had lower swelling
power than wheat starch. This is indicative of the degree of bonding forces
within the acha starch granules.

Acha solubility characteristics
Although a significant difference (p ≤ 0.05) was observed between fonio
Hothio and fonio Koulli during exposure to the entire temperature range, the
two fonio varieties behaved similarly for both swelling and solubility power
compared to wheat starch. These stronger bonding forces within the fonio
granules can be observed in the solubility of the two fonio types compared to
wheat starch. Both fonio types showed lower solubility values than wheat for
temperature of 50°C - 65°C, thus less solute were released from fonio than
wheat starch.

In the case of solubility for Okenia and corn starch at 5% solids, over a
range of 30°C - 60°C, the solubility increased slightly, increasing significantly
from 70°C (Gonzales et al., 2003). At this temperature, the granular structure
is lost and solubilisation of the constituent polymers was promoted.

Starch paste viscosity characteristics
Holm et al. (1988), reported that hot paste viscosity at 95°C and final cooked
paste viscosity (after cooling to 25°C or 50°C) of extruded flour and starches are closely related in terms of their molecular size distribution. Carcea & Acquistucci (1997) investigated the pasting properties of 5% wheat and fonio starch by means of a viscoamylograph and observed that wheat starch showed the typical two step pasting curve whereas both fonio varieties showed a single step pasting curve. These studies indicated that in both fonio slurries, the onset of gelatinization, indicated by an increase in viscosity is apparent for fonio at higher temperatures (> 50°C) than for wheat starch. The Hothia variety showed the highest peak viscosity followed by wheat starch. Koulli starch gave the lowest viscosity values and this can be attributed to the higher power of Koulli starch at temperatures higher than 70°C. As reported by Carcea & Acquistucci (1997), there might be negative correlation between amylose content and peak viscosity. Hothia gave the lowest amylose content (22.6%) and showed the highest peak viscosity, whereas Koulli gave the highest amylose (26.1%) and the lowest peak viscosity.

Starch textural characteristics
Understanding gel textural properties of starch is imperative for selecting the appropriate starch for end use suitability. Textural properties of corn starch varieties differ significantly from each other (Sandhu & Singh, 2007). The gel firmness of corn starch is mainly caused by retrogradation of starch which in turn is associated to syneresis of water and crystallization of amylopectin, leading to harder gels. Starches that exhibit harder gels tend to have higher amylose content and longer amylopectin chains. The mechanical properties of starch gels depends on various factors including the rheological characteristics of the amylose matrix, the volume fraction and the rigidity of the gelatinized starch granules as well as the interaction between the dispersed and continuous phases of the gel. These factors in turn depend on the amylose content and the structure of the amylopectin (Sandhu & Singh, 2007).

2.7 Overview of Starch in relation to Digestion
Starches contribute as much as 70-80% of the total carbohydrate in normal
diet and can thus be classified on the basis of the rate at which digestive enzymes hydrolyse starch into three categories (Figure 2.5) namely, rapidly digestible starch (RDS), slowly digestible starch, (SDS) and resistant starch (RS) [Chung et al., 2008a]. RDS and SDS is measure of the glucose released after 20 and 100 min of incubation, respectively. RS is the starch not hydrolysed after 120 min incubation (Englyst et al., 1992). However, RS is commonly defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy humans. RS escapes digestion and is fermented in the colon (Asp, 1992). Resistant starch is starch that escapes digestion in the small intestine of healthy individuals and passes through to the large intestine where it acts as dietary fibre. There are four types of resistant starch (Figure 2.5). RS1 is physically inaccessible or digestible e.g. unprocessed whole grain and those found in legumes. RS2 occur in its natural form such as in uncooked potato and green banana flour. RS3 are formed when starch containing foods are cooked and cooled as in bread, cornflakes and cooked and chilled potatoes. RS4 are formed in starches that have been chemically modified to resist digestion (Asp et al., 1996). Different processing techniques and type of raw materials thus affects the resistant starch content and type within any given food stuff (Hyun-Jung et al., 2008). Chewing can also affect the amount of starch escaping digestion, that is, decrease in RS is relative with increase in chewing (Muir & O’Dea, 1992).

Resistant starch has major health benefits in areas such as individuals colonic health, their energy and glycemic management. Research on dietary fibre has demonstrated the importance of fermentation of indigestible carbohydrates in the human colon. It provides a substrate for microbial fermentation in the large intestine, and therefore as a dietary fibre is beneficial in areas such as diabetes, cardiovascular disease and bowel cancer (Buttriss, 2002). Jideani, (1999) reported that acha is usually consumed as whole grain. It is known that whole grain consumption reduces the risk of certain cancers, stroke, diabetes and cardiovascular disease. Jideani et al. (2008) developed non wheat acha bread where a deliberate attempt was made to remove the sugar from this product and as such is believed to serve as a good alternative to wheat bread for diabetic patients and those allergic to
Figure 2.5 Categories of starch on the basis of enzyme digestion

- Starch
  - Digestible
    - Rapidly digestible starch (RD)
  - Resistant
    - Slowly digestible starch (SDS)
      - RS1
      - RS2
      - RS3
      - RS4
gluten. Acha has been identified as a major food for diabetic patients in Nigeria (Jideani, 1999).

2.8 Glycemic Properties of Starch

The glycemic index is the rate of increase in blood sugar level relative to the glucose taken as 100. The rate of increase in blood sugar level is the area under the curve for two hours following ingestion of 50 g of available carbohydrates (Bird et al., 2000). Glycemic index (GI) of food is the degree to which the food specifically carbohydrates increases blood glucose in proportion to glucose itself. GI is used as a measure of the effects of carbohydrates on blood sugar levels. High GI indicates that the carbohydrate will be broken down rapidly releasing glucose rapidly into the bloodstream. Low GI on the other hand is indicative of a slow break down of the carbohydrates thus releasing glucose slowly and gradually into the bloodstream (Buttriss, 2002).

The GI plays a major role in blood sugar control of diabetics since a lower GI is relative to a lower insulin demand thus a better long term blood glucose control and a reduction in blood lipids (Buttriss, 2002). Following a low GI diet thus reduces the risk of individual developing type-2 diabetes. However, a high GI food which releases glucose rapidly in the bloodstream is suitable for athletes who need quick energy release during a fast event where speed and spurge of energy is required as well as diabetic individuals undergoing hypoglycaemia (Buttriss, 2002). On the other hand, endurance athletes also consume low GI food before and during an endurance event to ensure a slow release of energy during long distance running (Buttriss, 2002).

Pawlak et al. (2002) reported a 71% increase in body fat in male rats fed with high GI diet compared to those fed with a low GI diet. GI values can be interpreted as percentages and are commonly categorised as: Low GI 55% or less, Medium GI 56-79%, high GI 70-99% and with straight glucose as 100% (Pawlak et al., 2002). The GI of foods depends on a number of variables e.g. the type of starch i.e. amylase vs. amylpectin, the chemical and physical nature of the food as well as the combination of the meal e.g. protein and fat content (Buttriss, 2002). The presence of protein and fat in food may lower the GI of the food; this however is not advisable due to risk
<table>
<thead>
<tr>
<th>Factor</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size</td>
<td>Intact grains such as whole wheat, barley, whole corn and whole rye have much lower GI values than flours (tiny particles) made from grains.</td>
</tr>
<tr>
<td>Processing</td>
<td>Milling, beating, grinding, mixing, mashing and refining foods raise the GI index of that foods</td>
</tr>
<tr>
<td>Protein and Fat</td>
<td>The presence of protein and fat in food may lower the GI.</td>
</tr>
<tr>
<td>Cooking</td>
<td>Cooking usually decreases the digestibility of the food and would have an effect of raising the GI of that food.</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>When starches are cooked and then cooled, the crystalline structure within the food changes to resistant starch which is more difficult to digest. Therefore cold cooked starch has a lower GI. This is especially true for mealy meal. The cooked cold maize has a lower GI than the hot freshly prepared porridge.</td>
</tr>
</tbody>
</table>

Source: (Steenkamp, 2008)
associated with high fat content meals (Steenkamp, 2008). Other factors affecting the glycemic properties of foods are outlined in Table 2.3 and include particle size, processing, protein and fat, cooking, resistant starch (Steenkamp, 2008).

The GI and resistant starch content are two important indicators of starch digestibility (Hu et al., 2004). RS and SDS are highly desirable forms of dietary starch as they are known to have low glycemic index (GI) (Eyaru et al., 2008). The amount of resistant starch and digestibility rate of acha starch is not known. As stated by Hu et al. (2004) resistant starch is slowly digestible and may be used as a means of slow release of glucose.

2.9 Measurement Techniques for Resistant Starch
Englyst et al. (1992) devised a scheme for the classification and measurement of nutritionally important starch fractions including the three main forms of RS. The method is based upon analysis of starch fractions in foods as eaten, which is based on the recovery of even the physically enclosed RS1. There are however various in vitro assay systems and in vivo methods to measure the amount of resistant starch escaping digestion in the small intestine.

An in vitro assay system was developed that mimics the physiological conditions for starch digestion. This essay is useful in predicting which foods and processing techniques results in high amounts of starch escaping digestion in the small intestine (Muir & O' DEA, 1992). Another in vitro method that includes the measurement and classification of starch into rapidly available glucose (RAG), slowly available glucose (SAG) and starch fraction, involves the measurement by means of HPLC of the glucose released from a test food during incubation with digestive enzymes under controlled conditions (Englyst, 1999). In this work, extracted acha starch was analyzed for rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) utilizing the modified Goñi et al. (1997) methods.

2.10 Effect of Processing on Functional and Glycemic Properties of Starch
Starch can be modified by various chemical and physical processing techniques (Gonzales & Perez, 2002). For this reason different processing
techniques are currently in use for the production of pre-cooked, modified and instant starches, which has led to an increase in ready-to-eat cereals, ready-to-eat meals, infant formulae and snack foods (Gonzales & Perez, 2002). Examples of different processing techniques are baking, soaking, microwaving and cooking.

Effect of milling on cereal starch
Various grain or dry bran fractionation processes have been developed to produce safe and healthy whole wheat products (Hemery et al., 2007). Some of the biochemical markers were starch, aleurone cell walls, aleurone content and endosperm. Three different grain or bran dry fractionation processes, namely: conventional milling, de-branning process and the production of aleurone-rich fractions from coarse bran were tested. This lead to the development of new processes in order to exploit all the nutritional benefits of whole grain as well as to produce new wheat foods and wheat-based ingredients with improved nutritional quality (Hemery et al., 2007). Various whole grain products with improved nutritional benefits in terms of low GI foods can be found on the market, namely whole grain low GI bread and whole grain low GI porridge/cereal (Eyaru et al., 2008). No investigation could be found where the nutritional benefit of whole acha grain flour has been compared to whole wheat grain flour.

Effect of baking on cereal starch
Collado-Fernandez (2003) reported three stages during the baking process as oven spring; gelatinization of starches (70 to 90°C) and browning and aroma formation above 100°C. During the oven spring stage, yeast activity continues until the dough reached about 50°C and enzymatic activity continues at about 60°C where conversion of starch to sugar continues. Enzymatic activity increases to until about 80°C. The enzymatic activity plays a major role in the quality of the bread with regard to structure, loaf volume and crumb structure (Collado-Fernandez, 2003). Inadequate enzyme activity leads to a small loaf volume due to the starch that becomes too rigid too soon, whereas too much activity on the other hand leads to weak structure, which
collapses completely. Gelatinization of starch is promoted at temperatures of about 50 to 60°C and therefore denaturation of proteins with the coagulation of gluten (Collado-Fernandez, 2003). During baking, dehydration of gluten occurs and water is moved from the gluten to the starch, swelling occurs as the starch gelatinizes. When gluten collapses, the starch not only supports the structure of the dough but also contributes to the firmness of the bread when the starch crystallizes upon cooling of the bread.

Investigating the microstructure of starch in dough, fresh bread and aged bread by means of light microscopy, Hu et al. (2004) observed a partial segregation of starch from the protein phase. On baking, the starch gelatinization yielded a continuous starch network which itself was homogenous and consisted of swollen and connected starch granules. The same studies also revealed that two polymers, namely amylose and amylopectin were phased separately and the amylose was accumulated in the centre of the starch granule. The baking quality rendered by various wheat and non-wheat flour is attributable to factors such as starch structure, namely the crystalline organisation of the starch granules as well as the chemical structure of amylopectin and amylose (Cauvain, 2003).

**Effect of soaking on cereal starch**

Native starch does not absorb water at room temperature and its viscosity is nearly zero, whereas extruded starches absorbs water rapidly to form a paste at room temperature. This paste is formed by solubilised macromolecules but also includes particles swollen with water (gel) (Colonna et al., 1981). These two properties are affected by the particle size, with smaller particles showing increased rates and levels of solubilisation. Water activity index correlates well with cold-paste viscosity because only damaged starch granules absorb water at room temperature and swell, creating increased viscosity. Miao et al. (2009) reported that swelling waxy maize starch were characterised by an initial phase of slight swelling, a second phase of rapid swelling and a final stage of maximum swelling. A similar trend has been observed for waxy rice starch (Miao et al., 2009). No evidence could be found of investigations into a trend of this nature for acha starch granules.
Soaking red kidney beans in water significantly reduced the RDS, TS, RS and SDS as compared to raw beans. Similarly, both varieties of peas (yellow and green) showed a high degree of loss in RDS. The loss in RDS was accompanied by a gain in SDS. Eyaru et al. (2008) further reported that the soaking of seeds in plain water produces swelling of the tissues and water uptake without cell separation. This could have been the cause of increase in solubility of the starch granules during soaking which might have subjected the RS to enzymatic hydrolysis, therefore, yielding more SDS (Eyaru et al., 2008). The effect of soaking on the glycemic properties of acha starch has not been investigated.

Effect of microwaves cooking on cereal starch
Microwaves are non-ionising energy that can generate heat deep inside the penetrated medium by the molecular friction in an alternating electromagnetic field. Microwave irradiation appears to be applicable to starch processing, but has not been used on a commercial scale. It causes several changes in the functional, rheological and morphological characteristics of lentil starches (Gonzales & Perez, 2002). Microwave irradiation also reduced the retrogradation tendency of lentil starch. The effect of microwave irradiation on fonio starch properties have not been an object of research.

Effect of boiling on cereal starch
According to Frame (1994), this is the simplest type of cooking process and one with the least mechanical action and least starch damage. The glycemic responses of boiled products are less than the corresponding extruded products (Mercier & Conterrali, 1989).

Eyaru et al. (2008) reported that boiling of previously soaked legumes brought changes in starch fractions that were different to that of unsoaked-boiled legumes. Boiling soaked red kidney beans caused a lesser increase in RDS (232%) compared to the unsoaked beans (339%) but the SDS contents were almost the same for both cooked beans. There was a higher decrease in RS content during boiling of soaked samples which meant that soaking of beans prior to cooking made them more digestible than cooking raw beans (Eyaru et al., 2008).
Processed rice seeds must be boiled or steamed before consumption. Studies conducted by Frei et al. (2003) to investigate the in vitro starch digestibility and the GI of six different indigenous rice cultivars from Philippines indicated that substantial differences exist in the estimated GI between rice cultivars. Values ranged between 68 and 109 for cooked rice and between 64 and 87 for stored rice containing retrograded starch (Frei et al., 2003). Waxy rice starches exhibit a similar trend as starch granules when starch (100 mg) was soaked in water (5 ml) and heated in a water bath at the required temperature (60 to 80°C) for 30 minutes with constant shaking (Miao et al., 2009). Acha grain is also consumed as rice or couscous (Phillip & Itodo, 2006). The effect on the glycemic properties of processing acha grain into rice or couscous has not been investigated.

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Frame, N.D. (1994). Extrusion cooking In: *The technology of extrusion cooking*, Published by Chapman and Hall, USA.


CHAPTER 3
Physicochemical, Functional and Thermal Properties of Acha Starch

Abstract
The thermal, functional and physicochemical properties of two acha cultivars (*Digitaria exilis*, white and *Digitaria iburua*, black) were investigated. Wheat was used as reference. The acha starch was extracted from cleaned and dried acha grains. The thermal, functional and physicochemical properties of both acha starches and wheat starch as reference were investigated. There was no significant difference \( p > 0.05 \) between white acha, black acha and wheat starches. Wheat starch had the highest gelatinization temperature 34.75°C and 74.56°C for \( T_o \) and \( T_p \). White and black acha recorded 25.05, 26.7°C and 68.20, 70, 54°C for \( T_o \) and \( T_p \) respectively. The gelatinization temperature range \( (T_c - T_o) \) for white and black acha were between 62 and 66°C whereas that of wheat starch was 61°C. No significant \( p > 0.05 \) difference existed between, white and black acha gels in hardness, resilience and springiness. There were significant differences \( p < 0.05 \) in peak viscosity and final viscosity between the black and white acha and wheat starch. There was a significant difference \( p < 0.05 \) in the water binding capacity between wheat starch (0.083 g/100g) and both acha cultivars, black acha starch (0.83 g/100g) and white acha starch (1.33 g/100g). However there was no significant \( p > 0.05 \) difference between black acha and white acha starch. There was similarity in the nutritional composition and amylose content of white acha starch and black acha starch. There was however a significant difference \( p < 0.05 \) in the nutritional composition with regard to the protein, fat and carbohydrate content between black, white acha and wheat starch. Black and white acha starch had a higher fat and protein content than wheat starch, whereas wheat starch had higher carbohydrate content.

3.1 Introduction
*Digitaria* *spp.* belongs to the family Graminaceae and the tribe Poaceae (Dalziel, 1937; Jideani, 1999) and includes 230 species that are widely distributed in
the tropics and the subtropics (Adoukonou-Sagbadja et al., 2004). Two types exist namely *Digitaria exilis* (acha, fonio or hungry rice) and *Digitaria iburua* (iburu, black fonio or petit millet) (Jideani, 1999; Philip & Itodo, 2006). Acha is by large the oldest African cereal, it has been cultivated for hundreds of years in West Africa (Mali, Burkina Faso, Guinea and Nigeria) and the Dominican Republic (Morales-Payán et al., 2003; Jideani et al., 2008). Acha grows under varying conditions from poor dry upland soils to hydro-morphic valleys (National Research Council, 1996). Acha was once classified as one of the “lost crops of Africa”, but is now “rediscovered” and considered for improvement as cultivated species (Ibrahim, 2001; Morales-Payán et al., 2003). It is valued because of its unique taste and nutritional value (Philip & Itodo, 2006).

Acha grains contain about 7 to 9% crude protein with high leucine (9.8%), methionine (5.6%) and valine (5.8%). It has been reported that its methionine content is twice as high as those of egg proteins (Ghana Grains Development Project, 1993; National Research Council, 1996; Temple & Bassa, 1991). Hence, it is the most nutritious grain, containing high levels of essential amino acids that are deficient in today’s major cereals such as maize, sorghum, barley, wheat and rye. Consequently, acha has potential as a complement for standard diets (National Research Council, 1996).

Food products where acha grains have been used include porridge, couscous and it is also mixed with other flours to make bread, pastries and is even oven-popped (Jideani, 1999; Philip & Itodo, 2006). It is also used in traditional beverages such as beer (Philip & Itodo, 2006). Jideani et al. (2007, 2008) demonstrated the possibility of producing gluten free loaf from acha flour.

Starch is the main storage carbohydrate of plants. It is deposited as insoluble, semi-crystalline granules in storage tissues (grains, tubers, roots) and to a lesser extent in most vegetative tissues of plants (Copeland & Blazek, 2009). Energy (50 to 70%) in the human diet is obtained from starch, providing a direct source of glucose, an essential substrate in brain and red blood cells for generating metabolic energy (Copeland & Blazek, 2009). Starch is a food ingredient with wide applications such as thickener, gelling agent, bulking agent and water retention agent (Pomeranz, 1991; Singh et al.,
2003), making it an excellent ingredient for the manufacture of foods such as: custards, porridges, puddings, cookies, and sausages. There is a growing demand for starches for the modern food industry creating interest for new sources of this polysaccharide. Perry & Colman (2003) reported that the availability of a reliable source of starch from agriculture is considered to have an important factor in human development. This is important; especially as the glycemic response to excessive consumption of rapidly digested starch may be a factor in some diet-related illnesses. Togolese farmers also consider acha as having medicinal value in being useful for those suffering from diabetes among others (Adoukonou-Sagbadja et al., 2006). Similar medicinal use has also been associated with acha in Nigeria (Jideani, 1999). Currently there is however a lack of information on the physicochemical, thermal and functional properties of acha starch. The objective of this chapter was to investigate the physicochemical, thermal properties as well as the functional properties of acha starch.

3.2 Materials and Methods

The experimental outline for this chapter is detailed in Figure 3.1. Description of each step in the study will follow.

3.2.1 Source of acha grain and materials

Two acha cultivars namely, Digitaria exilis (white) and Digitaria iburua (black) were purchased from Grace Africa, Salt River, Cape Town, South Africa.

All equipment and materials were either obtained from the Department of Food Technology, Cape Peninsula University of Technology (CPUT) or Pioneer Foods Laboratories (Bokomo Foods pilot plant and SASKO technical laboratory), Cape Town, South Africa.

All chemicals were obtained from Laboratory and Scientific Ltd., Maitland, Cape Town, Republic of South Africa and the Department of Food Technology CPUT.

3.2.2 Cleaning of acha grain

The cleaning procedure entailed screening the grains for 3 minutes through a 1000, 750, 500 and 125 micron screens to remove all foreign matter such as
Acha grain

Cleaning and de-stoning

Drying (40°C for 48h)

Cleaned and dried grain

Starch extraction

Acha Starch

<table>
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<tr>
<th>Physicochemical Properties</th>
<th>Functional</th>
<th>Thermal</th>
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</thead>
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<td>-Gel texture</td>
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<td></td>
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<tr>
<td></td>
<td>-Turbidity</td>
<td></td>
</tr>
</tbody>
</table>

*RDS = rapidly digestible starch; SDS = slowly digestible starch

Figure 3.1 Experimental outlines for first research chapter
stones, foreign grain and small sticks. The process of dry cleaning was then followed by rinsing the grains three times with water and drying at 40°C for 48 h (Jideani & Podgorski, 2010). The cleaned and dried samples were then placed in sealed polyethylene bags and stored in a refrigerator at a temperature of 3 ± 2°C until required.

3.2.3 Isolation and purification of starch from acha grain
The method described by Betancur & Torruco-Uco (2001) as outlined in Figure 3.2 was used to extract starch from milled acha grain. Cleaned, washed and dried acha grain were milled and sieved through a 20 mesh screen. The acha flour was then dispersed in distilled water at a ratio of 1:8 w/v. The pH was then adjusted to 11 with 1 N NaOH and the solution was stirred for 1 h. The suspension was then passed through an 80 and 100 mesh screens to separate the fibre solids from the liquid containing the protein and the starch. The suspension was allowed to sediment for 30 min to recover the starch, and then the solubilised protein was separated. The starch was washed 3 times with distilled water, and centrifuged at 4250 rpm for 10 min during the last wash to recover the starch. The product was then dried at 60°C in a drying oven (Defy, Gemini Ltd., Germiston) for 1 h then milled and sieved through a 20 mesh screen. The isolated starch was then analysed for physicochemical, functional and thermal properties.

3.3 Physicochemical Properties of Acha Starch
3.3.1 Starch characteristics of acha starch
Extracted acha starch was analyzed for rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), amylose and total starch utilizing the modified Goñi et al. (1997) methods.

3.3.2 Total starch analysis for acha and wheat starch
Total starch was determined enzymatically according to the modified Goñi et al. (1997) method. Triplicate samples of both acha starches and wheat starch were analyzed separately. Starch (50 mg) was dispersed in 50 ml centrifuge tubes with 6 ml of KOH. The samples were shaken for 30 min at room
Cleaned and washed acha grain

Milling (hammer mill)

Sieving (20 mesh)

Acha flour (1:8)

Adjust pH (pH = 11)

Stirring (400 rpm for 1 h)

Sieving (100 mesh)

Sedimentation (30 min)

Residue (starch)

Drying (60°C)

Acha starch

Figure 3.2 Extraction of starch from Acha grain (Betancur & Torruco-Uco, 2001)
temperature. The solubilized starch was then hydrolyzed by the addition of 3 ml 0.4 M sodium acetate buffer (pH 4.8) and 60 µl of amylglucosidase (A7420 Sigma Aldrich) from Aspergillus niger (67.4 U/mg 50 mg AMG in 50 ml buffer solution). The samples were transferred into Erlenmeyer flasks and then incubated at 60°C for 45 min in a shaking water bath. The samples were then centrifuged for 10 min at 3000 rpm. After centrifugation, the glucose concentration was determined in the supernatant using a glucose oxidase-peroxidase (GAGO-20 Sigma Aldrich) kit. Sample aliquots (1 ml) of the supernatant were measured into test tubes and to that 2 ml of the assay reagent was added. The sample were then incubated at 37°C for 30 min, 2 ml H₂SO₄, was carefully added to the test tubes after incubation and were then thoroughly mixed. Colour absorption was measured against a reagent blank at a wavelength of 540 nm. The glucose concentration was converted into starch by applying the factor 0.9.

3.3.3 Resistant starch analysis of acha and wheat starch
Resistant starch (RS) content in acha starch and wheat starch were determined according to the Goñi et al. (1997) method. Dry starch (100 mg) was weighed into a 50 ml centrifuge tube. An aliquot (10 ml) of KCl-HCl buffer (pH 1.5) and 0.2 ml of pepsin (P7000 Sigma-Aldricht) solution (1 g pepsin /10 ml buffer KCl-HCl) were added. The solution was mixed well and was placed in a shaking water bath for 60 min at 40°C. After cooling samples to room temperature, 9 ml of 0.1M Tris - maleate buffer (pH 6.9) was added and was followed by adding 1 ml of α-amylase (A3176 Sigma-Aldrich) solution (40 mg α-amylase/ml Tris-maleate buffer). The samples were then mixed well and incubated for 16 h in a water bath at 37°C with constant shaking. The samples were centrifuged for 15 min at 3000 rpm, and the supernatants were discarded. The residues were moistened with 3 ml distilled water and 3 ml of 4 M KOH then mixed well for 30 min at room temperature with constant shaking. Thereafter, 5.5 ml of 2 M HCL and 3 ml of 0.4 M sodium acetate buffer (pH 4.75) were added, followed by the addition of 80 µl of amylglulucosidase. The solution was then mixed well and was placed in a water bath at 60°C for 45 min with constant shaking. The solution was then centrifuged at 3000 rpm for 15 min and the supernatants were collected and
saved in a 50 ml volumetric flask. To 1 ml of the sample in a test tube was added 2 ml of the assay reagent. The samples were then incubated at 37°C for 30 min. Sulphuric acid (2 ml) was carefully added to the test tubes after incubation and were then thoroughly mixed. Colour absorption was measured against the reagent blank at a wavelength of 540 nm and the glucose concentration was converted into starch content by multiplying by the factor 0.9.

3.3.4 Digestible starch
DS was calculated as the difference between TS and RS (Goñi et al., 1997).

3.3.5 Determination of rapidly and slowly digestible starch by in vitro rate of starch digestion
The procedure and model established by Goñi et al. (1997) was used to measure the in vitro starch hydrolysis. Triplicate samples of 50 mg of starch were dispersed in 10 ml of KCI-HCl buffer. Pepsin solution (0.2 ml) containing 1 g of pepsin in 10 ml HCl-KCl buffer (pH 1.5) was added to the samples. The samples were incubated at 40°C for 60 min in a shaking water bath. The volume was raised to 25 ml by adding 15 ml Tris-maleate buffer (pH 6.9) and adjusting the pH carefully. Starch hydrolysis was started by adding another 5 ml of Tris-maleate buffer containing 2.6 UI α-amylase to each sample.

The flasks were placed in a shaking water bath at 37°C with moderate agitation. Sample (1 ml) was taken from each flask every 30 min from 0-3 h. These aliquots were placed in a test tube at 100°C and were energetically shaken for 5 min to inactivate the α-amylase. The 2 ml of 0.4 M sodium acetate buffer (pH 4.75) was added to each aliquot and 60 μl of amyloglucosidase was used to hydrolyze the digested starch into glucose after 45 min at 60°C in a shaking water bath. The volume was adjusted to 5 ml with distilled water and 0.5 ml was incubated with the glucose oxidase-peroxidase kit. The samples were analyzed in triplicate. The glucose was finally converted into starch by multiplying by 0.9. The rate of starch digestion was expressed as percentage of starch hydrolyzed at different times (30, 60, 90, 120, 150 and 180 min). RDS and SDS were determined according to Englyst et al. (1992). The RDS was defined as the percentage of starch
digested at 30 min and the SDS as the percentage of starch digested at 120 min. The digestion curve was modeled with the non-linear equation (Eq 1) established by Goñi et al. (1997) to describe the kinetics of starch hydrolysis.

\[ C = C_\infty (1 - e^{-kt}) \quad \ldots \ldots (1) \]

Where \( C \) is the percentage of starch hydrolyzed at time \( t \) (min); \( C_\infty \) is the equilibrium percentage of starch hydrolyzed after 180 min, and \( k \) is the kinetic constant. The parameters \( C_\infty \) and \( k \) were estimated for each sample using SPSS for Windows 19.0 non-linear regression.

3.3.6 Determination of total amylose content of acha starch

The method by Hoover & Ratnayake, (2001) was used to determine the amylose content of starch. Defatting of starch samples were conducted by weighing 5 g of starch accurately into a cellulose extraction thimble and covering the mouth with cotton wool. Lipids were then extracted with 120 ml of 75% n-Propanol at 85°C with a heating mantle for 7 h in a Soxhlet extractor. The lipid free starch was then air dried for 12 h and removed from the thimble and oven dried for 24 h at 30°C. Lipid-free starch (20 mg) was weighed into a round bottom screw-cap tube fitted with a Teflon-faced rubber liner in the cap. A series of mixtures of pure potato amylose and amyllopectin (0%, 10%, 20%, 40%, 50% amylose) were prepared after which 20 mg of each was weighed into round-bottom tubes with caps. Eight millilitres of 90% DMSO was added to each round-bottom tube and vigorously mixed for 2 min using a vortex mixer. The tubes were then heated in a water bath at 85°C for 15 min with intermittent mixing. The tubes were then allowed to cool to room temperature (45 min). The samples were then diluted to 25 ml with water in a volumetric flask.

Determination of absorbance of the dispersed starch solution was then conducted after completion of the following steps. Diluted solution (1 ml) and 40 ml of distilled water were added into a 50 ml volumetric flask. Iodine solution (5 ml) was added and mixed vigorously. The volume was adjusted to 50 ml with distilled water and mixed vigorously. The colour was then allowed to develop for 15 min. The contents of the flask were then vigorously mixed by hand. The absorbance of the samples and each of the standard mixtures
were then measured at 600 nm against a reagent blank as the reference. The reagent blank contained all reagents in the same amounts without the sample containing starch.

Following the above procedure a standard curve was then plotted. The regression equation for the standard curve was then measured and used to calculate the total amylose content of the sample.

3.3.7 Proximate composition of acha starch
The moisture, fat, protein, fiber and ash content of the acha and wheat starch were determined in accordance with the standard methods of AOAC (1990). Carbohydrates were calculated by difference.

3.4. Functional properties of the Starches
3.4.1 Textural properties of starch gels
Texture profile analysis was conducted as described by Sandhu & Narpinder (2007) on an Instron apparatus (model 2519, UK, 3300 series, 2000 N capacity) to evaluate the textural properties of acha and wheat starch gels. A suspension of 45 g of starch in 455 ml of cold water was prepared. The suspension was then boiled on a hot plate (Model Z341 Ohaus Instr. US) with stirring until a gel was formed. The hot starch gel formed was then poured into small aluminium dishes (10 cm in diameter) and stored at 4°C. The gels were evaluated for their textural properties by means of texture profile analysis.

Each dish was placed on the food support plate (200 x 145 mm) of the Instron, the gel was compressed at a speed of 0.5 mm/s to a distance of 10 mm (30% of the original sample height) with a cylindrical plunger (5 mm diameter). The compressive force was then removed and the sample was re-compressed. Such a compressive sequence represents two bites (Karim et al., 2000). During the test, compressive force was recorded as a function of the amount of compression (distance). Thus two, forces against distance, TPA curves could be derived from which hardness (height of first peak) and springiness (ratio between recovered height after the first compression and the original gel height) were determined. Chewiness was obtained by multiplying gumminess and springiness (Sandhu & Narpinder, 2007). These
were calculated automatically with the Blue-Hill 2 software.

3.4.2 Turbidity of starch suspensions
The turbidity of the acha starch suspension was measured as described by Perera & Hoover (1999). A 1% aqueous suspension of acha starch as well as the reference wheat starch was heated separately, in a water batch at 90°C for 1 h with constant stirring. The samples were then stored for 5 days at 4°C and turbidity was determined after 5 days by measuring the absorbance at 640 nm (UV-VIS Split Beam 8 Auto Cell Model UVS-2800 spectrophotometer Shanghai Jingke Scientific Instruments Co. Ltd. China) against a water blank. The turbidity was measured in Nephelometric Turbidity Units (NTU). NTU measures how much light is scattered in the suspension. The greater the scattering, the higher the NTU; thus the lower the clarity of the suspension.

3.4.3 Water binding capacity
Water binding capacity (WBC) of both the acha starch as well as the reference starch was measured as described by Medcalf & Gilles (1965). A suspension of 5 g starch in 75 ml distilled water was agitated for 1 h and centrifuged at 3000 rpm for 10 min. The free water was drained for 10 min and the drained wet starch was then weighed. The water binding capacity was then expressed as grams per hundred grams.

3.4.4 Pasting properties
The method of Holm et al. (1988) was used to determine the pasting properties of acha starch as well as the reference sample using a rapid visco analyzer (RVA 4 from Newport Scientific, Warriewood, Australia). The viscosity profiles of white acha, black acha and wheat starch were recorded using starch suspensions of 9% (w/w, dry basis) and 500 g total sample weight. The starch suspensions were prepared and then transferred to a rapid visco analyzer (RVA4 from Newport Scientific, Warriewood, Australia). The capacity of the RVA was 50 – 50 000 cP at 80 rpm. A programmed heating and cooling cycle were used where the samples were held at 25°C for 1 min and heated to 95°C at 1.5°C/min, a holding at 95°C for 30 min before
cooling from 95° to 50°C, 1.5°C/min and holding at 50°C for 2 min. Parameters recorded were peak viscosity, trough viscosity, final viscosity, breakdown viscosity and setback viscosity. Analysis was conducted in triplicate.

3.5. Thermal properties of the starches

Differential scanning calorimeter (DSC, 20 to 120°C, Perkin-Elmer instrument) was used to measure the thermal characteristics of the isolated starch. Both the acha starches and wheat starch (3.5 mg, dry weight) was separately loaded into a 40 µl capacity aluminium pan (Mettler ME-27331) and distilled water added using a Hamilton micro syringe to achieve a 70% starch-water suspension. Samples were sealed and allowed to stand for 5 h at room temperature before it was heated in the DSC. The DSC analyzer was calibrated using indium, and an empty aluminium pan was used as a reference (Sandhu and Narpinder, 2007). Sample pans were heated at a rate of 10°C/min from 20°C to 100°C. Thermal transition of starch samples was defined as T_o (onset temperature), T_p (peak of gelatinization), T_c (conclusion temperature) and ΔH_{gel} the enthalpy of gelatinization. Enthalpies were calculated on a starch dry weight basis automatically. The gelatinization temperature range (R) and peak height index (PHI) were calculated as described in equations 1 and 2.

\[
R = 2(T_p - T_o) \quad \text{(1)}
\]

\[
\text{PHI} = \Delta H / (T_p - T_o) \quad \text{(2)}
\]

After conducting thermal analysis, the samples were stored at 4°C for 7 days, for retrogradation studies. The aluminium sample pans containing the starches were reheated at the rate of 10°C/min from 25°C to 100°C to measure retrogradation. The enthalpies of retrogradation (ΔH_{gel}) T_o, onset temperature; T_p, peak temperature; H_1, peak height; H, height; ΔH, enthalpy of gelatinization; T_c, end (conclusion) temperature, ΔT_r gelatinization temperature range (T_c - T_o), were calculated automatically by the Pyris
3.6 Results and Discussion
3.6.1 Proximate composition of acha starch

Table 3.1 shows the proximate content of two acha starch varieties and wheat starch. The moisture content of wheat, black and white acha starches was 11.0, 11.9 and 10.7% respectively. There was no significant difference between the white acha starch and wheat starch. The moisture content recorded for white acha starch and black acha starch were slightly lower than that reported for two acha cultivars (Fonio Hothia and F. Koilli), 15 and 13% respectively. The moisture value for white acha starch (10.7%) was in the same range of that reported for corn starch 10.1% (Gonzales-Reyes et al., 2003).

The protein content of white (1.7%) and black (1.2%) acha differed significantly from that of wheat starch (0.5%). There was also a significant difference in the protein content between white (1.7%) and black (1.2%) acha starch. Protein content of acha starch reported in literature ranged between 0.3 and 0.7% for Hothia and Koulli respectively (Carcea and Acquistucci, 1997). Classical studies at the University of Illinois demonstrated that the variability of various chemical compounds such as the protein content in maize is of genetic, environment and cultural origin and that chemical composition can thus be changed through appropriate manipulation (FAO, 2012). The protein content of starch is one of the critical characteristics when starch is used in the manufacture of glucose syrup. The protein content of corn starch from Quinoa Corporation investigated by Lindeboom et al. (2005) was reported to be 1.2%; this is similar to the protein of black acha starch (1.2%) in this study. The lower the protein contents the better the chances of avoiding Maillard reaction (Gonzales-Reyes et al., 2003). Although the protein content of acha starch (1.2% for black and 1.7% for white acha starch) being significantly higher than that of wheat starch, the protein content is within the acceptable range in starches that would possibly avoid Maillard reaction.

There was no significant difference (p > 0.05) between wheat and black acha starch in terms of dietary fibre, 2.2% and 1.5% respectively. There was
<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture</th>
<th>Fat</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Amylose content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>11.0 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.0 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.8 ± 16.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Black acha</td>
<td>11.9 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.0 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.23 ± 1.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.1 ± 16.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>White acha</td>
<td>10.7 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.0 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.73 ± 1.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61.3 ± 15.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are mean ± standard deviation.

<sup>2</sup>Any two means with different superscript in each column differ significantly (p < 0.05).
however a significant difference between black acha (1.5%) and white acha starch (0.45%). The difference in dietary fibre could be attributed to genetic difference between white acha and black acha starch. Dietary fibre recorded for white acha starch (0.45%) is higher than that reported in literature for lentil starches (0.21%) (Gonzales et al., 2002).

The fat content of white acha starch and black acha differed significantly from that of wheat starch. This difference could be due to their genetic differences. The fat content of black acha (2.9%), white acha (2.0%) and wheat starch (1.8%) are much higher than that reported for corn and Okenia starch, 0.17% and 0.1% respectively (Gonzales-Reyes et al., 2003). Higher values could affect gelatinization due to the formation of amylose-lipid complexes (Gonzales-Reyes et al., 2003).

The carbohydrate content of white acha, black acha and wheat starch were 86.0, 86.0 and 88.0 g/100 g respectively. The carbohydrate content for wheat starch and the two acha varieties did not differ significantly (p > 0.05).

There were no significant differences between either the white or the black acha starches and wheat starch with regard to moisture content, dietary fibre and carbohydrate content. There was thus no significant difference in the nutritional composition of white acha starch and black acha starch with regard to carbohydrate, moisture and fat content. There was however significant differences in the nutritional composition with regard to the protein content and dietary fibre of black and white acha starches. This supports the hypothesis that there is significant difference between the two acha starch varieties.

3.6.2 Amylose content of acha starch

The amylose content of acha and wheat starches is detailed in Table 3.1. There was no significant difference in amylose content between black acha (42.1%), white acha (61.4%) and wheat (45.8%) starches. Lower amylose values were reported in literature for two other varieties of acha (Hothia and Koulli) 22.6 and 26.1% (Carcea and Acquistucci, 1997). In this instance the amylose content was measured in the presence of lipids which may complex with amylose and reduce
its iodine binding capacity (Morrison, 1988). Taylor et al. (1997) however reported amylose content of starch, extracted from sorghum cultivars, to be in the region of 35.7 to 36.9% for high pasting peak viscosity sorghum starch and 27.1 to 47.3% for low peak viscosity sorghum starch that received supplementary irrigation. Literature revealed that environmental effects may exert more influence on amylose content than genetic differences (Taylor et al., 1997). There was a negative correlation between glycemic index, resistance starch and amylose content of starch (Chung et al., 2008a). Higher amylose content may contribute to a lower hydrolysis index (HI) and estimated glycemic index (eGI). The results do not support the hypothesis that there is significant difference in the amylose content between black and white acha starches. Hence, amylose contents of the two acha starches are similar.

3.6.3 In vitro starch digestibility of acha and wheat starches
The starch hydrolysis of starch isolated from black and white acha and wheat grain with respect to incubation time are presented in Table 3.2. The analysis of variance (ANOVA) for the effect of time and type of starch on starch hydrolysis for the two acha variants and wheat grain are presented in Table 3.3. The amount of hydrolyzed starch was significantly ($p < 0.05$) affected by both time and starch type in the in-vitro starch digestibility. There was no significant ($p > 0.05$) difference in the amount of starch hydrolysed within 30, 60, 120 and 150 min for all types of starches. There was no significant difference in the total amount of starch hydrolysed after 180 min for wheat (56.79%) and white acha starch (57.13%). However, there was a significant difference ($p < 0.05$) between white acha starch (56.79%) and black acha starch (39.02%) in the amount of starch hydrolysed within 180 min. The values for white acha starch and wheat starch although slightly higher, still in the same range as those reported by Zhang et al. (2006), for waxy maize (46.6%), maize (53.0%) and wheat (50.0). The amount of starch hydrolysed in black acha starch was however much lower than the value reported by Zhang et al. (2006).

The two acha starch cultivars had fairly low RDS contents, white acha
Table 3.2  The percentage of starch hydrolyzed within 0 - 180 min of starch from acha and wheat grain<sup>1,2,3</sup>

<table>
<thead>
<tr>
<th>Grain</th>
<th>Time (Min)</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>87.4 ± 1.1</td>
<td>83.2 ± 1.4</td>
<td>82.9 ± 1.1</td>
<td>54.5 ± 1.6</td>
<td>66.9 ± 16.0</td>
<td>22.7 ± 5.3</td>
<td>56.8 ± 32.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>White acha</td>
<td></td>
<td>75.0 ± 4.3</td>
<td>72.8 ± 17.7</td>
<td>69.9 ± 27.2</td>
<td>67.7 ± 50.7</td>
<td>66.7 ± 28.6</td>
<td>47.8 ± 2.87</td>
<td>57.1 ± 33.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Black acha</td>
<td></td>
<td>63.8 ± 1.9</td>
<td>62.1 ± 0.8</td>
<td>40.1 ± 0.9</td>
<td>38.8 ± 4.8</td>
<td>36.7 ± 2.9</td>
<td>31.7 ± 0.2</td>
<td>39.0 ± 20.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>75.4 ± 10.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.7 ± 12.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.3 ± 23.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.7 ± 22.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.7 ± 28.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.1 ± 11.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are mean ± standard deviation.
<sup>2</sup>Any two means with different superscript in the total column differ significantly (p < 0.05).
<sup>3</sup>Any two means with different superscript in the total row differ significantly (p < 0.05).

Table 3.3  Analysis of variance (ANOVA) for the effect of time and type of starch grain on hydrolysed starch

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>45977.356&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td>2298.868</td>
<td>10.057</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>163747.985</td>
<td>1</td>
<td>163747.985</td>
<td>716.325</td>
<td>.000</td>
</tr>
<tr>
<td>Starch type</td>
<td>4509.892</td>
<td>2</td>
<td>2254.946</td>
<td>9.864</td>
<td>.000</td>
</tr>
<tr>
<td>time</td>
<td>37542.264</td>
<td>6</td>
<td>6257.044</td>
<td>27.372</td>
<td>.000</td>
</tr>
<tr>
<td>Starch type * time</td>
<td>3925.199</td>
<td>12</td>
<td>327.100</td>
<td>1.431</td>
<td>.190</td>
</tr>
<tr>
<td>Error</td>
<td>9600.966</td>
<td>42</td>
<td>228.594</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>219326.307</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>55578.322</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>R Squared = 0.827 (Adjusted R Squared = 0.745)
(75.0%) and black acha starch (63.8%) compared to wheat starch (87.42%) whereas the SDS for wheat (54.47%) and white acha starch (67.67%) was higher than that of black acha starch (38.79%). No literature values for RDS and SDS for both acha starch cultivars could be found. Chung et al., (2008b), reported that isolated bean starch (63-65%) contained a substantially larger amount of SDS than those reported for cereal starches, maize (53.0%), waxy maize (47.6%), wheat (50.0%). The SDS for white acha starch (67.7%) is higher than the SDS reported by Chung et al., (2008b) for maize and wheat. RDS content of acha starch was substantially higher than that reported for corn (24.4%), wheat (40.1%) and rice (32.4%) starches (Zhang et al., 2006). On the contrary, the amounts of starch hydrolysed for white acha and wheat starch is much lower than that reported for bean starch which ranged from 63.1 – 65.8% (Chung et al., 2008a). In another study by Chung et al. (2008b), unmodified starches showed a higher level of hydrolysis than did the modified starches after 180 min. After 180 min of digestion the amount of starch hydrolysed for white acha starch was much higher that the amount of starch hydrolysed from black acha starch. This difference supports the hypothesis that a significant difference exist in the in-vitro starch digestibility between black and white acha starches. White acha starch appears to have a faster rate of digestibility than black acha starch.

3.6.4 Functional Properties of Acha Starch

Textural characteristics of the starch gels

The textural characteristics of the acha and wheat starches are indicated in Table 3.4. The hardness for white acha starch, black acha starch and wheat starch were 0.9367 N, 0.9467 N and 0.9500 N, respectively. No significant difference in gel hardness was observed between black acha starch gel and white acha starch gel or between the wheat starch and the two acha starch varieties. These low hardness values indicate that both acha and wheat starches produced soft gels. Acha starch would thus be suitable for manufacturing of foodstuff where soft gels are required. Sandhu & Singh (2007) reported that starches which exhibit harder gels tend to have higher amylose content. They also reported that the gel
Table 3.4  Textural characteristics of acha and wheat starch gels$^{1,2}$

<table>
<thead>
<tr>
<th>Cereal starch</th>
<th>Hardness (N)</th>
<th>Resilience (ratio)</th>
<th>Springiness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White acha</td>
<td>0.936 ± 0.041$^a$</td>
<td>1.199 ± 0.090$^a$</td>
<td>0.709 ± 0.112$^a$</td>
</tr>
<tr>
<td>Black acha</td>
<td>0.946 ± 0.015$^a$</td>
<td>1.239 ± 0.087$^a$</td>
<td>0.709 ± 0.132$^a$</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.950 ± 0.010$^a$</td>
<td>1.240 ± 0.033$^a$</td>
<td>0.374 ± 0.273$^a$</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard deviation.

$^2$Any two means with different superscript in each column differ significantly (p < 0.05).
firmness is mainly caused by retrogradation which is associated with crystallization of amylopectin, leading to harder gels.

The resilience value is the first force before the maximum force (F) which is the capability of the gel to return to an original shape or position after having been compressed. The resilience for black and white acha and wheat starch gels were 1.1994, 1.2399 and 1.2402, respectively. There was no significant difference (p > 0.05) in resilience between the gels. Springiness for black acha gel was 0.7090 mm, white acha gel was 0.7090 mm and wheat gel was 0.3746 mm. There was no significant difference (p > 0.05) in the springiness of both acha starch gels and wheat gels. Springiness refer to the degree of gel rubberiness in the mouth (Lau et al., 2000). A high degree of springiness is depicted by gel structure that breaks into large pieces during the initial compression; whereas a low degree of springiness is represented by a gel that breaks into smaller pieces during the initial compression test (Huang et al., 2007). The starch gels broke into large pieces during compression. Hence, both acha and wheat gels are highly springy. Highly springy gels would not break down easily during mastication (Lau et al., 2000). Acha starch gels were springy and resilient. This indicates that both acha starch varieties can be used for hot and cold desserts as well as for soft jelly like sweets and confectionery toppings.

Since there was no significant differences in the hardness, resilience and springiness between the two acha starch gels, the hypothesis that the starch from the two acha cultivars (D. exilis and D. iburu) differ in textural properties is rejected.

Pasting properties of acha starch
The pasting properties of wheat starch, black acha starch as well as white acha starch are summarized in Table 3.5. Peak viscosity is the ability of starch to swell before their physical breakdown, an indication of the water binding capacity of starch (Ikegwu et al., 2010). The peak viscosity was 3506 cP for wheat starch, 3994 cP for black acha starch and 4936 cP for white acha gel. There was a
<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak1 (cp)</th>
<th>Trough1 (cp)</th>
<th>Breakdown viscosity (cp)</th>
<th>Final viscosity (cp)</th>
<th>Setback viscosity(cp)</th>
<th>Peak Time (min)</th>
<th>Peak Temp. (°C)</th>
<th>Pasting Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>3506 ± 16a</td>
<td>1960 ± 41a</td>
<td>369 ± 21a</td>
<td>4436 ± 82a</td>
<td>2004 ± 62a</td>
<td>4.18± 0.04a</td>
<td>78.03 ± 0.46a</td>
<td></td>
</tr>
<tr>
<td>Black acha</td>
<td>3994 ± 36b</td>
<td>3125 ± 15b</td>
<td>869 ± 34b</td>
<td>5129 ± 60b</td>
<td>2332 ± 54b</td>
<td>5.78± 0.10b</td>
<td>80.15 ± 0.44b</td>
<td></td>
</tr>
<tr>
<td>White acha</td>
<td>4936 ± 58c</td>
<td>3137 ± 6b</td>
<td>2976 ± 95c</td>
<td>5470 ± 50c</td>
<td>2476 ± 190b</td>
<td>6.69± 0.03c</td>
<td>85.88 ± 0.49c</td>
<td></td>
</tr>
</tbody>
</table>

1Values are mean ± standard deviation.
2Any two means with different superscript in each column differ significantly (p < 0.05)
significant difference ($p < 0.05$) in the peak viscosity between white acha starch and black acha starch. Acha starch has a higher peak viscosity than that reported in literature for corn starch (Sandhu & Singh, 2007). The peak viscosity of various corn starches ranged between 804 and 1552 cP. The higher peak viscosity of acha starch is indicative of their higher water binding capacity and thus higher degree of starch swelling, an indication that acha starch may be good for products requiring high gel strength and elasticity. Trough viscosity is the measurement of the holding strength of the starch paste before it breaks down and viscosity decreases. This depends on the temperature and degree of mixing or shear stress. The trough viscosity for two acha starch varieties were similar, 3125 cP and 3137 cP for black and white acha starch. There was however a significant difference between the two acha starch varieties and wheat starch trough viscosity, 1960, 3125, 3137 cP for wheat starch, black acha and white acha, respectively. This significant difference ($p < 0.05$) in trough viscosity implies a difference in the paste holding strength of wheat starch and the two acha starch varieties. The holding strength of black acha and white acha starch is similar while the holding strength of wheat starch paste is much lower than the two acha starch paste varieties. This signifies that acha starch gels may be the better option for products requiring high holding strength without breaking down and thus lead to a decrease in viscosity. The trough viscosity are much higher than the values recorded for African Tall (662 cP) and Ageti (652 cP) corn starch (Sandhu & Singh, 2007).

The break down viscosity for wheat, black acha and white acha starches were 369, 869, 2976 cP respectively. The break down viscosity for white acha starch was significantly higher ($p < 0.05$) than those of wheat and black acha starches. Breakdown viscosity is the measure of disintegration of cooked starch. It is the difference between the peak viscosity and the trough viscosity. The higher the breakdown viscosity; the lower the ability of the sample to withstand heating and shear stress during cooking (Adebowale, 2004). This indicates that white acha starch being higher in break down viscosity, will have a lower ability to withstand heating and shear stress during cooking.
The final viscosity for black acha starch was 5129 cP, white acha starch 5470 cP and wheat starch 4436 cP. The final viscosity, which is the measure of the ability of starch to form a viscous paste for white starch was significantly higher ($p < 0.05$) than those of black acha starch and wheat starch. This difference could be due to the difference detected in the peak time and pasting temperatures of all three starches, white acha starch 6.69 min at 85.88°C, black acha starch 5.7 min at 80.15°C and wheat starch 4.18 min at 78.03°C. The higher pasting temperatures for acha starches indicated the higher resistance of their starches to swelling. The increase in viscosity with temperature may be contributed by the removal of water from the exuded amylose as the starch granules swell (Sandhu & Singh, 2007).

The setback viscosity is the measure of the degree of syneresis of starch upon cooling of the cooked starch paste, for white acha, black acha and wheat starch were 2476 cP, 2332 cP and 2004 cP respectively. There was no significant difference between the acha starches in setback viscosity. However, the wheat starch was significantly lower ($p < 0.05$) in setback viscosity compared to the acha starches. The high setback values for acha starches make them unsuitable for use where low syneresis rate is required, such as in frozen or refrigerated foods. The setback values are also indicative of the retrogradation tendency of starch gels. The higher the setback viscosity the lower the retrogradation during cooling of the products made from the flour (Ikegwu et al, 2010). This implies a significant difference in retrogradation tendency between wheat starch and the two acha starch varieties. Acha starches have lower retrogradation tendencies during cooling compared with wheat starch, suggesting that the degree of re-association of the wheat starch molecules was higher than that of acha starch molecules, upon cooling. Sandhu & Singh (2007) reported that the pasting properties of starch depend upon various factors such as the rigidity of starch granules, which in turn affects the degree of swelling of the starch granules. The significant differences in peak, break down, and final viscosity support the hypotheses that there are differences in the pasting properties between white and black acha starches.
Turbidity of acha starch

The turbidity for wheat, black acha and white acha starches are reported in Table 3.6 the values were 0.2097 NTU, 0.0003 NTU and 0.0193 NTU respectively for wheat, black and white acha starches. There was significant difference (p < 0.05) in the turbidity of the starches, with wheat starch being the highest followed by white acha starch and lastly black acha starch. The turbidity of starch in foods is important when used as thickener in sauces, as a carrier of flavours in beverages or as a suspending agent in liquid foods. The higher the NTU value the lower the clarity of the suspension. This signifies that both acha starch varieties are clearer than wheat starch, and would be the better option to use in products where starch clarity is required. Sandhu & Singh (2007) reported that turbidity development of starches is affected by inter-related factors such as starch granule swelling, leached amylose and amylopectin, amylose and amylopectin chain length. The substantial difference in turbidity between white acha starch and black acha starch supports the hypotheses that the white acha starch and black acha starch will differ in their functional properties.

Water binding capacity of acha starch

The water binding capacity (WBC) for acha and wheat starches are detailed in Table 3.6. The water binding for wheat, black and white acha starches were 0.83, 1.33 and 1.36 g/100g respectively. The WBC for both acha starches were significantly higher (p < 0.05) than for wheat starch. This difference in terms of WBC can be due to structural differences in starch and proteins of the starches (Celik et al., 2005). Different proportions of crystalline and amorphous regions within the granules may be the result of the variations in WBC. Thus weakly bonded amorphous starch granules will imbibe less water (Carcea & Acquistucci, 1997). It appears that acha starch granules possess stronger bonded amorphous granules compared to wheat starch. There was however no
Table 3.6 Turbidity and WBC of acha starch\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Starch</th>
<th>Turbidity (NTU)\textsuperscript{3}</th>
<th>Water binding capacity (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black acha</td>
<td>0.0003 ± 0.001\textsuperscript{a}</td>
<td>1.333 ± 0.06\textsuperscript{b}</td>
</tr>
<tr>
<td>White acha</td>
<td>0.0193 ± 0.002\textsuperscript{b}</td>
<td>1.367 ± 0.06\textsuperscript{b}</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.2097 ± 0.009\textsuperscript{c}</td>
<td>0.833 ± 0.23\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Values are mean ± standard deviation.

\textsuperscript{2}Any two means with different superscript in each column differ significantly (p < 0.05).

\textsuperscript{3}NTU, nephelometric turbidity units
significant difference ($p > 0.05$) in WBC between black acha starch 1.33 g/100g and white acha starch 1.36 g/100g. This similarity does not support the hypotheses that the white acha starch and black acha starch will differ in their functional properties. Water binding capacity (WBC) is considered very important in foods such as sauces, batters, dough(s) and baked products.

The WBC of the starch affects important physical attributes such as the viscosity of sauces and batters and the texture of baked products. Sandhu & Singh (2007) reported WBC for starches from different corn varieties in the range of 0.82 to 0.97 g/100 g. The water binding capacity for acha starch is higher than the values reported by Sandhu & Singh (2007). This is also true in practice. Acha is known to swell far more than other cereals (Carcea & Acquistucci, 1997). Acha starch can thus be included as part of the ingredients in the manufacturing of sauces, batters, and dough(s) and baked products. It can be used as thickener, bulking agent and most importantly due to its high WBC as water retention agent.

3.6.5 Thermal properties of starches

**Gelatinization of acha starch**

The onset, peak and conclusion temperatures ($T_o$, $T_p$, and $T_c$) and enthalpy ($\Delta H$) of wheat and acha starches are shown in Table 3.7. There was no significant difference ($p > 0.05$) in peak and onset temperature between the acha and wheat starches. Wheat starch had the highest onset ($T_o$) and peak ($T_p$) temperatures 34.8°C and 74.6°C, although it was not significant. White and black acha starches recorded (25.1° & 26.8°C) and (68.2° & 70.8°C) for ($T_o$) and ($T_p$), respectively.

Acha starches and the wheat starch differed significantly ($p < 0.05$) in conclusion temperature $T_c$ and peak $T_p$ temperatures. For wheat starch it was 96.0°C, black acha starch was 87.8°C and for white acha starch was 93.7°C. Miao et al. (2009) reported gelatinization temperatures for waxy maize starch of 59.9° ($T_o$), 69.1° ($T_p$) & 78.1°C ($T_c$). The onset temperature range of acha starch is much lower than that of waxy maize starch. This means that less energy is
<table>
<thead>
<tr>
<th>Starch</th>
<th>$T_o$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$\Delta H_{gel}$ J/kg</th>
<th>$\Delta T_f = (T_c - T_o)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>34.7 ± 25.20$^a$</td>
<td>74.56 ± 0.97$^a$</td>
<td>96.03 ± 0.49$^b$</td>
<td>-213.21 ± 169$^a$</td>
<td>58.33 ± 24.71$^a$</td>
</tr>
<tr>
<td>White acha</td>
<td>25.05 ± 12.12$^a$</td>
<td>68.20 ± 1.64$^a$</td>
<td>93.67 ± 0.57$^b$</td>
<td>188.42 ± 250$^b$</td>
<td>66.92 ± 11.55$^{ab}$</td>
</tr>
<tr>
<td>Black acha</td>
<td>26.75 ± 7.56$^a$</td>
<td>70.54 ± 6.07$^a$</td>
<td>87.85 ± 2.15$^a$</td>
<td>691.04 ± 106$^c$</td>
<td>62.8 ± 5.41$^a$</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard deviation

$^2$Any two means with different superscript in each column differ significantly ($p < 0.05$). $T_o$, onset temperature; $T_p$, peak temperature; $H_1$, peak height; $\Delta H$, enthalpy of gelatinization; $T_c$, end (conclusion) temperature, $\Delta T_f$, gelatinization temperature range ($T_c - T_o$)
needed to induce gelatinization of acha starches. The peak gelatinization temperature of acha starch is the same as that reported in literature (Miao et al., 2009). Tester & Morrison (1990) reported that due to the structural differences in amylopectin, starches with the low gelatinization temperature has less crystallinity than the high gelatinization temperature starches, this was attributed to structural differences in their amylopectins. Hence, acha starches may have lower degree of crystallinity compared to wheat starch. Ezekiel et al. (2007) reported that low transition temperatures of between 50°-67°C for Kufri and Chandramukhi potato starch was due to the lower crystallinity, meaning that the higher the transition temperatures the higher the degree of crystallinity. This would lead to structural stability causing the starch granules to have a higher degree of resistance to gelatinization. Acha starches have low transition temperature which implies a low crystallinity and high amorphous regions (Miao et al., 2009). The gelatinization temperature range (T<sub>c</sub> - T<sub>d</sub>) for white and black acha was 66.9° and 62.8°C respectively, whereas that of wheat starch was 58.3°C. The similarity of the gelatinization temperature range amongst the starches could be attributed to possible similarity in protein content and starch structure.

The melting enthalpy (∆H) of gelatinization of black acha was 188.41 J/kg and white acha was 691.6 J/kg whereas that of wheat starch was 213.21 J/kg. The melting enthalpy values for white acha starch and black acha starch differed significantly (p < 0.05). The melting enthalpy for both acha starch varieties as well as wheat starch was higher than that reported for waxy maize starch (Chung et al., 2008b). The higher melting enthalpy could be due to the difference in the alignments of the hydrogen bonds in the starch molecules. This is caused by the difference in the bonding forces between the double helices that form the amylopectin crystallites (Sandhu & Singh, 2007). Sandhu & Singh (2007) reported melting enthalpy values between 11200 and 12700 J/kg for various corn starches. This is much higher than the melting enthalpy of acha starch.

The similarity of the gelatinization and gelatinization temperature range of white and black acha starches do not support the hypotheses that there is a
significant difference between white acha and black acha starch in thermal properties. However, the significant difference in peak height and melting enthalpy substantiate the hypotheses.

**Retrogradation properties of acha starch**

The retrogradation of acha starch compared to that of wheat starch is summarised in Table 3.8. Retrogradation is the hydrogen bonding between starch chains that occurs after cooling of gelatinized starch paste (Hoover & Zhou, 2003). The transition temperatures of retrogradation for both acha starches and wheat starch are much lower than the transition temperatures of gelatinization. The onset temperature for wheat, white acha and black acha starches were 19.97°, 17.58° & 19.05°C respectively. Wheat starch had the highest onset temperature followed by black and white acha starch. However, the differences were not significant ($p > 0.05$). Sandhu & Singh (2007) reported onset temperatures for retrogradation in the range between 41.50° & 43.10°C for two corn starch varieties. These values are much higher than that recorded for white acha and black acha starches. According to Sandhu & Singh (2007) this could be due to a less organized manner of recrystallization of the amylopectin branched chains of the gels. The lower onset temperature for the acha starches could be due to weaker molecular bonding of the starch chains.

Values for peak temperature of retrogradation were 64.13, 67.05° & 64.84°C for wheat, white acha and black acha starches, respectively. There was no significant difference ($p > 0.05$) between the starches in peak temperatures. Literature values for different corn varieties varied between 52.40° & 54.50°C for African Tall corn starch and Partap corn starch respectively. These values were much lower than that recorded for white acha and black acha starch. Higher peak temperatures of acha starches could be indicative of the need for higher thermal energy for retrogradation.

Retrogradation enthalpy is an indication of the disentanglement and melting of the double helices formed during cooling (Adebowale et al., 2004). The retrogradation enthalpy ($\Delta H$) for white acha starch was 37.61 J/kg and for
<table>
<thead>
<tr>
<th>Starch</th>
<th>$T_o$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$\Delta H_{\text{retro}}$ J/kg</th>
<th>$\Delta T_r = (T_c - T_o)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>17.97 ± 0.02$^a$</td>
<td>64.13 ± 5.35$^a$</td>
<td>94.73 ± 1.78$^a$</td>
<td>56.87 ± 32.62$^{ab}$</td>
<td>76.76 ± 1.76$^a$</td>
</tr>
<tr>
<td>White acha</td>
<td>17.58 ± 0.48$^a$</td>
<td>67.06 ± 4.58$^a$</td>
<td>94.83 ± 1.48$^a$</td>
<td>37.61 ± 12.88$^a$</td>
<td>77.25 ± 1.00$^a$</td>
</tr>
<tr>
<td>Black acha</td>
<td>19.05 ± 1.30$^a$</td>
<td>64.84 ± 0.53$^a$</td>
<td>95.95 ± 0.52$^a$</td>
<td>118.00 ± 40.58$^b$</td>
<td>76.90 ± 0.78$^a$</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard deviation.

$^2$Any two means with different superscript in each column differ significantly ($p<0.05$). $T_o$, onset temperature; $T_p$, peak temperature; $H_1$, peak height; $H$, $\Delta H$, enthalpy of gelatinization; $\Delta H_f$, $T_c$, end (conclusion) temperature.
black acha starch was 118.00 J/kg. There was a significant difference between black and white acha starches, with black acha starch being higher than that of white acha starch. No significant difference was observed for retrogradation enthalpy between wheat starch and both acha starch varieties. Wheat starch had a recorded value of 56.87 J/kg, white acha 37.61 J/Kg and black acha starch 118.00 J/Kg. The differences in the retrogradation enthalpy could be due to the difference in the degree of starch crystallinity of the retrograded starches (Sasaki et al., 2000).

The conclusion melting temperature values for wheat, white acha and black acha starches were 94.73°C, 94.82°C and 95.95°C respectively. There was no significant difference in conclusion temperature of retrogradation between white acha and black acha starches. Whenever a material undergoes a change in physical state for example melting, or transforms from one crystalline form to another, or when it reacts chemically, heat is either absorbed (endothermic) or liberated (exothermic) (Karim et al., 2000). Wheat and both acha starch cultivars presented an endothermic transition (retrogradation). Retrogradation has been identified as the dissociation of the amylopectin crystallites (Gonzales et al., 2003). The retrogradation phenomenon is a process of re-crystallization of the starch polymers (Gonzales-Reyes et al., 2003). In the case of retrograded starch, ΔH (J/kg) provides a quantitative measure of the energy transformation that occurs during the melting of recrystallized amylopectin as well as precise measurement of the transition temperatures (onset, T_o, peak, T_p and conclusion (end), T_c) (Karim et al., 2000). There is a significant difference in the ΔH and peak height values between white and black acha starches, supporting the hypotheses that there is a significant difference in the thermal properties between white and black acha starch. There were however no significant difference amongst the retrogradation temperature profile (T_o, T_p and T_c) for both white and black acha starch, this opposes the hypotheses.

### 3.7 Conclusion

The physicochemical, functional and thermal properties of acha starches
compared to wheat starch are reported in this chapter. The following conclusion can be made:

1. The two acha starch cultivars were low in RDS compared to wheat starch. SDS for wheat and white acha starch was higher than that of black acha starch.

2. Although there was no significant difference in the nutritional composition of white acha starch and black acha starch with regard to carbohydrate, moisture and fat content, there was however significant differences with regard to the protein content and dietary fibre between black and white acha starches.

3. There was no significant difference in amylose content between black acha (42.1%), white acha (61.4%) and wheat (45.8%) starches.

4. The peak time and pasting temperatures for acha starches, white acha starch 6.69 min at 85.88°C, black acha starch 5.7 min at 80.15°C is higher than that of wheat starch 4.18 min at 78.03°C. The higher pasting temperatures for acha starches indicated the higher resistance of their starches to swelling. Consequently, the water absorption capacity (WBC) of both acha varieties was higher than that for wheat starch. This indicates that both acha starch varieties can be used for hot and cold desserts as well as for soft jelly like sweets and confectionery toppings.

5. Acha starch cultivars and wheat starch are similar in thermal properties. The observed gelatinisation temperature of acha and iburua starches typifies that of non-waxy starch. Acha starch has similar retrogradation temperature profiles as that of wheat starch.

6. Acha starches may not be suitable for use where low syneresis rate is required, such as in frozen or refrigerated foods.

7. White acha has a lower ability to withstand heating and shear stress during cooking.
References


CHAPTER 4
PRODUCTION OF ACHA BREAD AND ITS SENSORY CHARACTERISTICS

Abstract

Bread was baked with two acha cultivars (*Digitaria exilis*, white and *Digitaria iburua*, black) and their specific loaf volume as well as consumer acceptability were investigated and compared against that of white wheat bread. The sensory properties investigated were appearance, crust colour, crumb colour, aroma, taste, firmness, mouthfeel and overall acceptability on a 5-point hedonic scale (1 = undesirable, 5 = desirable). Results indicated that the majority of panel members found both varieties of acha bread, white acha and black acha to be moderately desirable 83.3 % and 81.7% respectively in terms of crust colour, taste and aroma.

4.1 Introduction

Acha is by large the oldest African cereal, it has been cultivated for years in West Africa (Mali, Burkina Faso, Guinea and Nigeria) and the Dominican Republic (Morales-Payán et al., 2003; Jideani et al., 2008) where it is used as a staple food (National Research Council, 1996). Food products where acha grains have been used are porridge, couscous mixed with other flours to make bread, pastries and oven-popped (Jideani, 1999; Philip & Itodo, 2006). It is valued because of its unique taste and nutritional value (Philip & Itodo, 2006). Togolese farmers also consider acha as having medicinal value in being useful for those suffering from diabetes among others (Adoukonou-Sagbadja et al., 2004). Similar medicinal use has also been associated with acha in Nigeria (Jideani, 1999). Being gluten free, food products from acha, like acha bread has the potential to be consumed by individuals suffering from wheat intolerance and gluten allergies namely celiac disease. Jideani et al. (2007, 2008) demonstrated
the possibility of producing gluten free loaf from acha flour. However, nothing is known about the effect of processing on the starch and glycemic property of such bread. The objective of this study was thus to produce bread from whole white and black acha flour, assess its consumer acceptability and study the rate of starch hydrolysis of the loaf. The latter objective will be discussed in Chapter 5.

4.2 Materials and Methods

4.2.1 Source of acha grain and materials
Two acha cultivars namely: *Digitaria exilis* (white) and *Digitaria exilis* (black) were purchased from Grace Africa, Salt River, Cape Town, South Africa. All equipment and materials were obtained from the Department of Food Technology Cape Peninsula University of Technology (CPUT) or Pioneer foods laboratories (Bokomo Foods pilot plant and SASKO technical laboratory), Cape Town, and South Africa. All ingredients were obtained from Pick and Pay retail store in Brackenfell, Cape Town.

4.2.2 Cleaning of acha grain and production of acha flour
The cleaning procedure entailed screening the grains through a 1000, 750, 500 and 125 micron screens to remove all foreign matter such as stones, foreign grain and small sticks. The process of dry cleaning was then followed by washing three times by rinsing with water and drying at 40°C for 48 h (Jideani & Podgorski, 2010). The cleaned and dried grain samples were then placed in sealed polyethylene bags and stored in a refrigerator at a temperature of 3 to 5°C until it was required. Cleaned and dried acha grain was milled through a Hammer mill (9FQ-500 series, ZMA manufacturing Co.Ltd. China) and screened through a 20 mesh sieve to produce acha flour.

4.2.3 Experimental design for acha bread
Screening experiment ($2^{4-1}$) with design resolution IV was used to identify the ingredients (acha starch, xanthan gum, CMC and yeast) with significant effect on the loaf volume. The variables and their proportions as well as the runs are
detailed in Tables 4.1 and 4.2. There was ten design points in total with each design point conducted in triplicate. The ingredients that gave the optimal loaf volume were used to bake the optimal loaf following the procedure described in section 4.2.4. The baked bread was assessed for consumer acceptability as described in section 4.2.6.

4.2.4 Production of acha bread
The method of Jideani et al. (2007) was modified and used to produce acha bread. The standard recipe consisted of 100% flour, 1% salt, vegetable fat 2.5% and water (52%). Lite apple juice (7%) was used as a source of sugar for yeast fermentation. The quantities for acha starch, xanthan gum, CMC and yeast were added according to the factorial design (Tables 4.1 and 4.2).

The method used for production of acha flour and acha bread is explained in Figure 4.1. The batter was prepared by pre-mixing the dry ingredients together for 5 minutes at low speed in a Hobart mixer (model 1/6-H.P.). The wet ingredients were added and mixed at high speed for 10 minutes. The batter was divided into equal weights and poured into greased baking pans (10 x 20 cm). The batter in the pans was placed in the proof oven (Macadam’s, Johannesburg) for 10 minutes at 40°C. The proofed batter was then baked at 180°C for 55 minutes in a rotary air oven (Macadam’s, Johannesburg), in triplicate. After baking, the bread was de-panned and allowed to cool for 20 minutes at room temperature.

4.2.5 Physical properties of acha bread
The physical quality attributes that were analysed on the baked bread were loaf height, loaf weight and loaf volume. Loaf weight was weighed on a Mettler Toledo scale (RSA) with an accuracy of 0.01 g and maximum capacity of 2 kg. The loaf volume was measured by means of seed displacement method using a rectangular plastic container. The container was filled with acha grain, levelled and poured out and noted as the volume that filled the container. The acha bread was then placed in the same container and filled with the same acha grain
Table 4.1 Ingredients and levels used for acha bread production\(^1,2\)

<table>
<thead>
<tr>
<th>Variable (%)</th>
<th>Symbol</th>
<th>(-1)</th>
<th>0</th>
<th>(+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acha starch</td>
<td>(X_1)</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Xanthan gum</td>
<td>(X_2)</td>
<td>1.2</td>
<td>1.6</td>
<td>2.0</td>
</tr>
<tr>
<td>CMC</td>
<td>(X_3)</td>
<td>1.2</td>
<td>1.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Yeast</td>
<td>(X_4)</td>
<td>1.2</td>
<td>2.0</td>
<td>2.8</td>
</tr>
</tbody>
</table>

\(^1\)Transformation of coded variable \(x_i\) to un-coded variable \(X_1\) levels could be obtained from \(X_1 = 2x_1 + 6, X_2 = 0.4x_2 + 1.6, X_3 = 0.4x_3 + 1.6, X_4 = 0.4x_4 + 2\)

\(^2\)CMC: carboxyl methyl cellulose

Table 4.2 Fractional \((2^4-1)\) factorial design with resolution IV for acha bread production

<table>
<thead>
<tr>
<th>Design point</th>
<th>Acha starch</th>
<th>xanthan</th>
<th>CMC</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>+1</td>
<td>-1</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>4</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>5</td>
<td>-1</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>6</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>7</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>8</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Production of acha bread

Acha flour

Mixing of dry ingredients for 5 min

Premixing of dry ingredients

Mixing for 10 min

Batter weighing and panning

Proofing for 10 min at 40°C

Baking at 180°C for 55 min

Bread loaf de-panning and cooling

Figure 4.1

Ingredients:
- Xanthan gum, CMC, salt, yeast
- Water 30°C, Lite Apple juice
and levelled. The volume of the spilled acha grains was taken as the volume of the loaf (Ayo and Nkama, 2004; Jideani and Onwubali, 2009). The specific loaf volume was estimated as loaf volume divided by the loaf weight and expressed in ml/g.

4.2.6 Sensory properties of acha bread

The sensory evaluation method as described by Jideani et al. (2008) was used to conduct the consumer acceptability test on the optimal acha bread loaf. An evaluation panel of 30 members were used to evaluate the acha bread samples. A slice (3 mm in thickness) of white acha, black acha and wheat bread were served on separate white paper plates (15 cm). Random numbers were used for sample identification. Panellists were required to taste the sample and rate their preference for appearance, taste, aroma, crust colour, crumb colour, firmness, mouthfeel and overall acceptability using a 5-point hedonic scale (1, undesirable; 2, undesirable; 3, neither desirable nor undesirable; 4, moderately desirable; 5, desirable).

4.2.7 Data analysis

Analysis of variance (ANOVA) was used to establish significant differences among treatments. Duncan’s multiple range tests was used to separate means where significant difference existed. Sensory attributes as judged by the consumers were subjected to principal component analysis (PCA) in order to obtain three factors that will explain at least 80% of the variability in the panellist preference for acha bread. Hierarchical cluster analysis was used to determine the clusters inherent in the data. K-means cluster analysis was performed to classify the bread samples into the inherent cluster on the basis of these attributes or parameters (IBM SPSS, 2010).

4.3 Results and Discussion

4.3.1 Effects of ingredients on acha bread loaf volume

Tables 4.3 indicates the analysis of variance for the effect of ingredients on
Table 4.3  Analysis of variance for black acha specific loaf volume

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2.01</td>
<td>3</td>
<td>0.67</td>
<td>6.96</td>
<td>0.0222</td>
</tr>
<tr>
<td>Acha starch</td>
<td>0.034</td>
<td>1</td>
<td>0.034</td>
<td>0.36</td>
<td>0.5728</td>
</tr>
<tr>
<td>CMC</td>
<td>0.96</td>
<td>1</td>
<td>0.96</td>
<td>9.93</td>
<td>0.0198</td>
</tr>
<tr>
<td>Acha starch * CMC</td>
<td>1.02</td>
<td>1</td>
<td>1.02</td>
<td>10.59</td>
<td>0.0174</td>
</tr>
<tr>
<td>Residual</td>
<td>0.58</td>
<td>6</td>
<td>0.096</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>0.56</td>
<td>5</td>
<td>0.11</td>
<td>8.02</td>
<td>0.2615</td>
</tr>
<tr>
<td>Pure Error</td>
<td>0.014</td>
<td>1</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor Total</td>
<td>2.59</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 CMC = carboxymethyl cellulose

Table 4.4  Analysis of variance for white acha specific loaf volume

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>0.56</td>
<td>2</td>
<td>0.28</td>
<td>7.75</td>
<td>0.0217</td>
</tr>
<tr>
<td>Acha starch</td>
<td>6.355E-003</td>
<td>1</td>
<td>6.355E-003</td>
<td>0.18</td>
<td>0.6884</td>
</tr>
<tr>
<td>CMC</td>
<td>0.52</td>
<td>1</td>
<td>0.52</td>
<td>14.40</td>
<td>0.0090</td>
</tr>
<tr>
<td>Residual</td>
<td>0.22</td>
<td>6</td>
<td>0.036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>0.20</td>
<td>5</td>
<td>0.040</td>
<td>2.41</td>
<td>0.4519</td>
</tr>
<tr>
<td>Pure Error</td>
<td>0.016</td>
<td>1</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor Total</td>
<td>0.77</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
specific loaf volume (SLV) of black acha bread. Carboxyl methyl cellulose (CMC) and the interaction between it and the acha starch significantly affected the specific loaf volume. A significantly greater effect (39.4%) was from the interaction between CMC and acha starch and 36.9% from CMC. Hence, the effectiveness of the CMC depends on the level of acha starch. A 6.8% of the effect came from the yeast and 0.23% from xanthan. Table 4.4 details the analysis of variance for the effect of ingredients on white acha SLV. CMC significantly (p < 0.05) affected the specific loaf volume contributing to 66.9% of the overall effect. The effects from acha starch, xanthan and yeast were 0.8%, 0.01% and 4.0% respectively. Figure 4.2 details the mean of SLV for the black and white acha bread. The bread produced with 8% acha starch, 1.2% xanthan gum, 2.0% CMC and 1.0% yeast had the lowest SLV (1.4 ml/g) for both acha varieties (run 6). The bread produced with 8.0% acha starch, 2.0% xanthan gum, 2.0% CMC and 1.0% yeast had the highest specific loaf volume for both black (3.1 ml/g) and white acha (2.2 ml/g) bread (run 4). This recipe was taken as the optimum loaf recipe for acha bread. The SLV differed significantly (p < 0.05) among the varieties, with black acha higher in SLV.

4.3.2 Sensory properties of acha bread

Table 4.5 details the demography of the respondents. A total of 30 consumers participated in the sensory evaluation of the acha breads. Of the 30 panel members 63.3% were female and 36.7% males. In terms of occupation 13.3% was students and 86.7% was employed. Black panel members were 10%, white 13.3% and coloured 76.7%. The age group younger than 20 was 3.3%, 16.7% between 20 and 24, 13.3% between 25 and 29 years, 20% between 30 and 34 and 46.7% between 35 and 39 years of age.

The mean scores of the consumer panellist rating are shown in Figure 4.3. Multivariate analysis of variance indicated that the panellist differed significantly (p < 0.05) in their preference for the acha bread and wheat bread. However, the panellists could not detect any difference between the black acha bread and that from the white acha bread. There was a significant interaction between the
Figure 4.2 Specific loaf volume of acha bread
1 = acha starch (4%), xanthan (1.2%), CMC (1.2%), yeast (1.2%);
2 = acha starch (8%), xanthan (1.2%), CMC (1.2%), yeast (2.8%);
3 = acha starch (4%), xanthan (2%), CMC (1.2%), yeast (2.8%);
4 = acha starch (8%), xanthan (2%), CMC (2.0%), yeast (1.2%);
5 = acha starch (4%), xanthan (1.2%), CMC (2%), yeast (2.8%);
6 = acha starch (8%), xanthan (1.2%), CMC (2%), yeast (2.8%);
7 = acha starch (8%), xanthan (1.2%), CMC (2.0%), yeast (2.8%);
8 = acha starch (8%), xanthan (2%), CMC (2%), yeast (2.8%);
9 = acha starch (6%), xanthan (1.6%), CMC (1.6%), yeast (2.0%);
10 = acha starch (6%), xanthan (1.6%), CMC (1.6%), yeast (2.0%).
Table 4.5  Demography of respondents (N = 30) for acha and wheat bread consumer acceptability.

<table>
<thead>
<tr>
<th>Item</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>19 (63.3)</td>
</tr>
<tr>
<td>Male</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Employed</td>
<td>26 (86.7)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>3 (10.0)</td>
</tr>
<tr>
<td>White</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Coloured</td>
<td>23 (76.7)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>Less than 20</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>20-24</td>
<td>5 (16.7)</td>
</tr>
<tr>
<td>25-29</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>30-34</td>
<td>6 (20)</td>
</tr>
<tr>
<td>35-39</td>
<td>14 (46.7)</td>
</tr>
</tbody>
</table>
Figure 4.3 Mean sensory scores for acha bread on a 5-point hedonic scale
1 = very undesirable; 2 = moderately undesirable; 3 = neither desirable nor undesirable; 4 = moderately desirable; 5 = very desirable
Figure 4.4 Bread from different grains (A) wheat bread; (B) white acha and (C) black acha bread
panellists and type of bread. This implies that the significant difference noted earlier among the panellists was in bread type as panellist score differed according to the type of bread. For appearance black acha scored higher than white acha bread while wheat bread scored higher than black and white acha bread. There was no difference in preference between the bread samples in crust and crumb colour. White acha bread was rated 4.2 and 4.2 respectively, for crust and crumb colour. Black acha bread 4.0 and 4.2 and wheat bread 4.3 and 4.3 respectively, for crust and crumb colour. For aroma wheat bread (4.1) was more preferred followed by black acha bread (3.7) which was more preferred than white acha bread (3.6). The acha bread and wheat bread were similar in firmness, however, they differed significantly \( p < 0.05 \) in taste and mouthfeel with high preference for wheat bread. The bread samples differed significantly \( p < 0.05 \) in overall acceptability, 4.4 for wheat bread and 3.9 for both acha breads. The high preference for wheat bread in some of the sensory attributes is expected as consumers are generally familiar with wheat bread. However, the high rating for acha bread indicating moderating acceptance in most of the sensory attributes as well as the similarity of acha bread to wheat bread in crust and crumb colour indicates the potential of acha bread to make it into the food basket of Africa.

Lack of differences \( p > 0.05 \) between white and black acha loaves for aroma, taste, mouthfeel and overall acceptability does not support the hypotheses that there will be a significant difference in sensory parameters between black and white acha loaves.

### 4.3.3 Sensory attributes of interest to the consumer panellists

The suitability of the sensory data for factor analysis was assessed prior to performing principal component analysis (PCA). Inspection of the correlation matrix revealed the presence of many coefficients of 0.4 and above. The Kaiser-Meyer-Oklin value (0.898) exceeded the recommended value of 0.6. The Barlett's test of sphericity was significant \( P < 0.05 \). Hence, factorability of the correlation matrix was supported. PCA revealed that the variation in the sensory
attributes of acha bread could be explained by three components with eigen values exceeding 1. Much of the variation (35.7%) in the data is explained by component 1, component 2 (32.2%) and component 3 (17.0%), with a cumulative variation of 84.9% (Table 4.6).

Varimax rotation was performed to aid in the interpretation of these three components. Table 4.6 indicates pattern or structure for coefficients obtained using varimax rotation of three factor solution for sensory attributes of acha bread. Crust colour and appearance loaded strongly on component 1, taste and mouthfeel on component 2 while aroma loaded strongly on component 3. The implication is that acha bread can be categorised into groups on the basis of crust colour, taste and aroma. K-means cluster analysis was performed to classify the bread samples into two groups (inherent in the data as indicated by a hierarchical cluster analysis) on the basis of these attributes or parameters.

Cluster 1 was the case for consumers who neither desired nor undesired (3) acha bread in crust colour and the samples were moderately undesirable in taste and aroma (17.5%, 21 out of 120 cases). Cluster 2 was the case where the bread samples were moderately desirable in crust colour, taste and aroma (82.5%, 99 out of 120 cases). This implies that most consumers find acha bread to have the potential to be marketed amongst other types of bread. It also implies that with small improvements in the taste and aroma the remaining 17.5% of consumers will switch to desirable.

Majority of the consumers in all age group moderately desired acha bread in crust colour, taste and aroma (Figure 4.5). The potential of acceptance for acha bread is high across all ages. This implies that acha bread has the potential to be consumed as an alternative to other wheat or non-wheat bread across all age groups. Majority of the cases for males (92.3%) and females (76.3%) were in cluster 2- moderately desirable in crust colour, taste and aroma (82.5% cases) (Table 4.7). Majority (53.7%) of the black panel members were in cluster 1, implying that they neither desired nor undesired (3) acha bread in crust colour (Table 4.8). Majority of the cases for white (81.3%) and coloured (88%)
Table 4.6  Pattern/structure for coefficients obtained using varimax rotation of three factor solution for sensory attributes of acha bread

<table>
<thead>
<tr>
<th>Component*</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>.415</td>
<td>-.122</td>
<td>-.115</td>
</tr>
<tr>
<td>Crust colour</td>
<td>.512</td>
<td>-.198</td>
<td>-.179</td>
</tr>
<tr>
<td>Crumb colour</td>
<td>.308</td>
<td>-.044</td>
<td>-.056</td>
</tr>
<tr>
<td>Aroma</td>
<td>-.222</td>
<td>-.272</td>
<td>1.111</td>
</tr>
<tr>
<td>Firmness</td>
<td>.135</td>
<td>-.134</td>
<td>.387</td>
</tr>
<tr>
<td>Taste</td>
<td>-.235</td>
<td>.572</td>
<td>-.132</td>
</tr>
<tr>
<td>Mouth feel</td>
<td>-.133</td>
<td>.523</td>
<td>-.192</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>.047</td>
<td>.364</td>
<td>-.219</td>
</tr>
<tr>
<td>% of variance explained</td>
<td>35.7</td>
<td>32.182</td>
<td>17.0</td>
</tr>
</tbody>
</table>

*Extracted method: Principle Component Analysis. Rotation Method: Varimax with Kaiser Normalization
Figure 4.5. Cluster count per age group
### Table 4.7 Gender * Cluster Number of Case Cross tabulation

<table>
<thead>
<tr>
<th>Gender</th>
<th>Cluster Number of Case*</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (23.7%)</td>
<td>2 (76.3%)</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>58</td>
<td>76 (100%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3 (6.8%)</td>
<td>41</td>
<td>44 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21 (17.5%)</td>
<td>99</td>
<td>120 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

*Cluster 1 = consumers who neither desired nor undesired (3) acha bread in crust colour and who moderately undesired acha bread in taste and aroma; Cluster 2 = consumers who moderately desired the acha bread in crust colour, taste and aroma (82.5%, 99 out of 120 cases).

### Table 4.8 Race * Cluster Number of Case Cross tabulation

<table>
<thead>
<tr>
<th>Race</th>
<th>Cluster Number of Case*</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (58.3%)</td>
<td>2 (41.7%)</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>7</td>
<td>5</td>
<td>12 (100%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>3 (18.8%)</td>
<td>13</td>
<td>16 (100%)</td>
<td></td>
</tr>
<tr>
<td>Coloured</td>
<td>11 (12%)</td>
<td>81</td>
<td>92 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21 (17.5%)</td>
<td>99</td>
<td>120 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

*Cluster 1 = consumers who neither desired nor undesired (3) acha bread in crust colour and who moderately undesired acha bread in taste and aroma; Cluster 2 = is the case where the consumers who bread samples were moderately desired the acha bread in crust colour, taste and aroma (82.5%, 99 out of 120 cases).
panel members were in cluster 2- the case where the bread samples were moderately desirable in crust colour, taste and aroma. Acha bread has the potential to serve a niche market amongst white and coloured consumers.

Majority of the cases with regards to variety [black acha (83.3%), white acha (81.7%)] belong to cluster 2 indicating moderately desirable in crust colour, taste and aroma (Table 4.09). Hence, either of the acha varieties could be used in the production of acha bread. Acha bread thus has the potential to be consumed as alternative wheat free bread. The overall acceptability ratings for all consumer quality characteristics were the same for both white and black acha bread. The objective to produce black and white acha bread that is acceptable to the consumer and thus be used as a wheat free bread for individuals suffering from diabetes and celiac disease have been met.

Table 4.09 Acha varieties * Cluster Number of Case Cross tabulation

<table>
<thead>
<tr>
<th>Acha variety</th>
<th>Cluster Number of Case*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>White</td>
<td>10(16.7%)</td>
</tr>
<tr>
<td>Black</td>
<td>11(18.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>21(17.5%)</td>
</tr>
</tbody>
</table>

*Cluster 1 = consumers who neither desired nor undesired (3) acha bread in crust colour and who moderately undesired acha bread in taste and aroma; Cluster 2 = is the case where the consumers who moderately desired the acha bread in crust colour, taste and aroma (82.5%, 99 out of 120 cases).

**4.4 Conclusion**

Optimum acha bread was baked using 8.0 % acha starch, 2% xanthan gum, 2.0% CMC and 1% yeast. Majority of the panellist found the crust colour, taste and aroma to be moderately desirable. This implies that acha bread have the potential to be marketed as wheat free bread. Male and female as well as consumers of all ages found acha bread to be moderately desirable in crust colour, taste and aroma.
References


CHAPTER 5
EFFECT OF PROCESSING ON THE STARCH AND GLYCEMIC PROPERTIES OF ACHA

Abstract
The effect of baking, steaming, boiling and microwaving on the starch and glycemic properties of two acha cultivars (*Digitaria exilis*, white and *Digitaria iburua*, black) is reported. Processing method, sample and incubation time significantly (*p* < 0.05) affected starch hydrolysis. However, the processing times did not affect the starch hydrolysis significantly. Significantly (*p* < 0.05) more starch was released from baked products (49.4%) compared to steaming (7.2%), microwaving (10.7%) and boiling (19.4%). Significantly more starch was released from black acha (19.8%) compared to white acha (16.3%) and wheat (12.4%). The amount of starch hydrolysed for the different processing methods was in the following order: baking > boiling > microwaving > steaming. For baking, the wheat and both acha bread samples did not differ significantly in their response to hydrolysis. On the effect of microwaving on starch hydrolysis samples, incubation time, interaction between sample and incubation time affected starch hydrolysis significantly (*p* < 0.05) of wheat and black and white acha. During boiling, processing time, cereals, incubation time, interaction between processing time * incubation time and sample * incubation time significantly influenced the hydrolysed starch. The cereals differed significantly in the amount of starch released with high starch (28.1%) for white acha compared to black acha (23.8%) and wheat (6.3%). During steaming the processing time, cereals, incubation time as well as the two-way and three-way interaction all affected starch hydrolysis significantly (*p* > 0.05). Slowly digestible starch (SDS) and rapidly digestible starch (RDS) analysis results indicate that black acha grain (*Digitaria iburua*) cultivar is the better cultivar to use than white acha grain (*Digitaria exilis*) since it consisted of higher slowly digestible starch (SDS) and lower rapidly digestible starch content after baking, microwaving, boiling and steaming. Whereas white wheat bread, microwaved, boiled and steamed wheat
grain had a higher RDS and SDS compared to black acha. Acha white bread and processed (boiled, steamed and microwaved) acha and wheat grain consisted of low GI values and is thus suitable as a food supplement or meal replacer for those individuals suffering from diabetes and celiac disease.

5.1 Introduction

Digitaria exilis (white) and Digitaria iburua (black) are two species of acha grains. Other common names are fonio or hungry rice. It is indigenous to most African countries particularly regions of Mali, Burkina Faso, Guinea and Nigeria (Ayo, 2003) and the Dominican Republic (Morales-Payán et al., 2003; Jideani et al., 2008). Starchy foods like acha are not usually eaten raw and must undergo one type of heat processing or the other for palatability and bioavailability. Different processing methods include boiling, microwave, baking, drying, extrusion, steaming, drum-drying, popping, pressure-cooking and others. Therefore, processing methods may modify starch in various ways, consequently affecting digestion and nutritional value. De Lumen et al. (1993) reported acha as a promising underutilised African cereal because of its high content of sulphur-amino acids. Acha is recommended as a cereal suitable for the management of diabetes mellitus in West Africa (Jideani et al., 1994). However, not much is known about the starch and glycemic properties of this grain. It is thus of interest to investigate the effect of processing specifically baking, boiling, steaming and microwaving on the starch and glycemic properties of acha in order to ascertain its potential in low glycemic food products. The outline of the investigation can be seen in Figure 5.1.

5.2 Materials and Methods

5.2.1 Source of acha grain and materials

Two acha cultivars Digitaria exilis (white) and Digitaria exilis (black) were purchased from Grace Africa, Salt River, Cape Town, South Africa.

All equipment and materials were either obtained from the Department of Food Technology Cape Peninsula University of Technology (CPUT) or Pioneer
foods laboratories (Bokomo Foods pilot plant and SASKO technical laboratory), Cape Town, South Africa.

All chemicals were obtained from the Department of Food Technology CPUT and Laboratory and Scientific Ltd. in Maitland, Cape Town, Republic of South Africa.

5.2.2 Cleaning of acha grain
The cleaning procedure entailed screening the grains through a 1000, 750, 500 and 125 micron screens to remove all foreign matter such as stones, foreign grain and small sticks. The process of dry cleaning was then followed by washing three times by rinsing with water and drying at 40°C for 48 h (Jideani & Podgorski, 2010). The cleaned and dried samples were then placed in sealed polyethylene bags and stored in a refrigerator at a temperature of 3 to 5°C until required.

5.2.3 Steaming of acha
Cleaned and dried acha grains (20 g) were rinsed in 500 ml distilled water. The water was drained through a sieve for 5 min. The drained grain was steamed using a steamer and three samples were drawn at 10 min interval for 15 to 35 minutes. The samples were cooled immediately on crushed ice for 10 min and thereafter were assayed for SDS, RDS, RS, TS and glycemic index as outlined in section 5.2.6 to 5.2.9

5.2.4 Microwaving of acha
Cleaned and dried acha grains (20 g) in 250 ml distilled water were processed by microwaving at medium for 3, 6 and 9 minutes, respectively. The samples were cooled immediately on crushed ice for 10 min and thereafter were assayed for SDS, RDS, RS, TS and glycemic index. See section 5.2.6 to 5.2.9.

5.2.5. Production of acha bread
The method of Jideani et al. (2007) was modified and used to produce acha
Acha grain

Cleaning & de-stoning

Drying
(40°C for 48 h)

Cleaned and dried grain

Processed Acha
- Boiling
- Steaming
- Microwaving
- Baking

IN-vitro starch digestibility
- Rapidly Digestible Starch (RDS)
- Resistant Starch (RS)
- Slowly Digestible Starch (SDS)
- Total Starch (TS)
- Glycemic properties

Figure 5.1 Experimental outlines for the effect of processing on acha
bread as described in Chapter 4. Bread samples (50 mg) were assayed for SDS, RDS, RS, TS and glycemic index following the method described in section 5.2.6 – 5.2.9 of this chapter.

5.2.6 Analysis of total starch of processed acha and wheat products

Total starch was determined enzymatically according to the modified method of Goñi et al. (1997). Triplicate samples of both processed acha and wheat products were analyzed for total starch. 50 mg of processed sample was dispersed in 50 ml centrifuge tubes with 6 ml of KOH. The samples were shaken for 30 min at room temperature. The samples were then hydrolyzed by the addition of 3 ml 0.4M Sodium acetate buffer (pH 4.8) and 60 µl of amyloliglucosidase (A7420 Sigma Aldrich) from Aspergillus niger (67.4 U/mg 50 mg AMG in 50 ml buffer solution). The samples were transferred into Erlenmeyer flasks and then incubated at 60°C for 45 min in a shaking water bath. The samples were then centrifuged for 10 min at 3000 rpm. After centrifugation, the glucose concentration was determined in the supernatant using a glucose oxidase-peroxidase (GAGO-20 Sigma Aldrich) kit. 1 ml sample aliquots of the supernatant were measured out into test tubes and to that 2 ml of the assay reagent was added. The samples were then incubated at 37°C for 30 min, 2 ml H2SO4, were carefully added to the test tubes after incubation and were then thoroughly mixed. Colour absorption was measured against a reagent black at a wavelength of 540 nm and the glucose concentration was converted into starch by applying the factor 0.9.

5.2.7 Resistant starch analysis of processed acha and wheat products

RS content in acha grain bread and wheat grain and bread were determined according to the method of Goñi et al. (1997). Dry grain and processed grain samples (100 mg) was weighed into a 50 ml centrifuge tube. An aliquot (10 ml) of KCL-HCL buffer (pH 1.5) and 0.2 ml of pepsin (P7000 Sigma-Aldrich) solution (1 g pepsin /10 ml buffer KCL-HCL) were added. The solution was mixed well and was placed in a shaking water bath for 60 min at 40°C. After cooling
samples to room temperature, 9 ml of 0.1M Tris-maleate buffer (pH 6.9) was added and was followed by adding 1 ml of α-amylase (A3176 Sigma-Aldrich) solution (40 mg α-amylase / ml Tris-maleate buffer). The samples were then mixed well and incubated for 16 h in a water bath at 37°C with constant shaking. The samples were centrifuged for 15 min at 3000 rpm, and the supernatants were discarded. The residues were moistened with 3 ml distilled water and 3 ml of 4 M KOH then mixed well for 30 min at room temperature with constant shaking. Thereafter, 5.5 ml of 2 M HCL and 3 ml of 0.4 M sodium acetate buffer (pH 4.75) were added, followed by the addition of 80 µl of amyglolucosidase. The solution was then mixed well and was placed in a water bath at 60°C for 45 min with constant shaking. Thereafter the solution was centrifuged at 3000 rpm for 15 min and the supernatants were collected and saved in a 50 ml volumetric flask. 1 ml of the sample was measured into test tubes and to that 2 ml of the assay reagent added. The samples were then incubated at 37°C for 30 min. Sulphuric acid (2 ml) was carefully added to the test tubes after incubation and were then thoroughly mixed. Colour absorption was measured against the reagent blank at a wavelength of 540 nm and the glucose concentration was converted into starch content by multiplying by the factor 0.9.

5.2.8 Digestible starch of processed acha and wheat products
Digestible starch (DS) was calculated as the difference between TS and RS (Goñi et al., 1997).

5.2.9 Determination of rapidly and slowly digestible starch by in vitro rate of starch digestion
The procedure and model established by Goñi et al. (1997) was used to measure the in vitro starch hydrolysis. Triplicate samples of 50 mg of unprocessed grain, processed grain and acha and wheat bread were dispersed in 10 ml of KCL-HCL buffer. 0.2 ml pepsin solution containing 1 g of pepsin in 10 ml HCL-KCL buffer (pH 1.5) was added to the samples. The samples were incubated at 40°C for 60
min in a shaking water bath. The volume was raised to 25 ml by adding 15 ml Tris-maleate buffer (pH 6.9) and adjusting the pH carefully. Starch hydrolysis was started by adding to each sample another 5 ml of Tris-maleate buffer containing 2.6 UI α-amylase.

The flasks were placed in a shaking water bath at 37°C with moderate agitation. Aliquot samples (1 ml) were taken from each flask every 30 min for 3 h. These aliquots were placed in a test tube at 100°C and were energetically shaken for 5 min to inactivate the α-amylase. The 2 ml of 0.4 M sodium acetate buffer (pH 4.75) was added to each aliquot and 60 μl of amylglucosidase was used to hydrolyze the digested starch into glucose after 45 min at 60°C in a shaking water bath. The volume was adjusted to 5 ml with distilled water and 0.5 ml was incubated with the glucose oxidase-peroxidase kit. The samples were analyzed in triplicate. The glucose was finally converted into starch by multiplying with 0.9. The rate of starch digestion was expressed as percentage of starch hydrolyzed at different times (30, 60, 90, 120, 150 and 180 min). RDS and SDS were determined according to Englyst et al. (1992). The RDS was defined as the percentage of starch digested at 30 min and the SDS as the percentage of starch digested at 180 min. The digestion curve was modeled with the non-linear equation (Eq1) established by Goñi et al. (1997) to describe the kinetics of starch hydrolysis.

\[ C = C_\infty (1 - e^{-kt}) \]

where \( C \) is the percentage of starch hydrolyzed at time t (min); \( C_\infty \) is the equilibrium percentage of starch hydrolyzed after 180 min, and \( k \) is the kinetic constant. The parameters \( C_\infty \) and \( k \) were estimated for each sample using IBM SPSS for Windows 19.0 non-linear regression.

5.3. **Statistical analysis**

Analysis of variance (ANOVA) was used to determine differences among treatments. Duncan's multiple range tests was used to separate means where significant difference existed (IBM SPSS, 2010).
5.4 Results and Discussion

5.4.1 Effect of processing on starch hydrolysis

Table 5.1 indicates the effect of incubation time on the hydrolysed starch from baking, microwave, boiling and steaming of acha and wheat. Processing method, sample and incubation time significantly \((p < 0.05)\) affected starch hydrolysis. However, the processing times did not affect the starch hydrolysis significantly. Significant interaction effect exists between processing * processing time, processing time * incubation time, processing * sample, processing * incubation time, sample * incubation time, processing * processing time * sample, processing * sample * incubation time, and processing * processing time * sample * incubation time.

There was a significant difference \((p < 0.05)\) in the amount of starch released during baking, steaming, microwaving and boiling. More starch was released from baked products (49.4%) compared to steaming (7.2%), microwaving (10.7%) and boiling (19.4%) from wheat and both acha variants. Steaming resulted in significantly \((p < 0.05)\) less hydrolysed starch compared to the others. Significantly more starch was released from black acha (19.8%) compared to white acha (16.3%) and wheat (12.4%). This variation is in line with other investigations. Eyaru et al. (2009) reported that starch digestibility of three legumes varied with the different processing techniques, pressure cooking raw grain, boiling raw grain, pressure cooking soaked grain, and boiling soaked grain. Increase in incubation time of acha and wheat grain resulted to increase in hydrolysed starch. Lee et al. (2005) reported that the hydrolysis rate of corn samples treated with microwaves were marginally lower than other samples treated with other cooking methods. There was a correlation between changes in starch hydrolysis rates and the differences in disruption of the crystalline regions due to the various processing methods. Different processing methods affects the microstructure of the samples which thus influences their physical properties and starch hydrolysis (Lee et al., 2005). Effect of baking on starch hydrolysis is depicted in Figure 5.2 and Table 5.2. The bread samples did not differ
Table 5.1  Analysis of variance for the effect of processing, processing time, incubation time and sample on starch hydrolysis of acha and wheat

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
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<td>208</td>
<td>1199.3</td>
<td>13.4</td>
<td>0.000</td>
</tr>
<tr>
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<td>1</td>
<td>205816.2</td>
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<td>0.000</td>
</tr>
<tr>
<td>Processing</td>
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<td>1</td>
<td>13033.3</td>
<td>145.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time</td>
<td>561.5</td>
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<td>140.4</td>
<td>1.6</td>
<td>0.183</td>
</tr>
<tr>
<td>Sample</td>
<td>4160.6</td>
<td>2</td>
<td>2080.3</td>
<td>23.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Incubation time</td>
<td>57118.5</td>
<td>6</td>
<td>9519.6</td>
<td>106.1</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing * Processing time</td>
<td>804.4</td>
<td>2</td>
<td>402.1</td>
<td>4.5</td>
<td>0.012</td>
</tr>
<tr>
<td>Processing time * sample</td>
<td>1180.7</td>
<td>8</td>
<td>147.6</td>
<td>1.6</td>
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</tr>
<tr>
<td>Processing time * Incubation time</td>
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<td>24</td>
<td>137.7</td>
<td>1.5</td>
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</tr>
<tr>
<td>Processing * sample</td>
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<td>2</td>
<td>3428.0</td>
<td>38.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing * Incubation time</td>
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<td>6</td>
<td>2084.2</td>
<td>23.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Sample * Incubation time</td>
<td>4263.9</td>
<td>12</td>
<td>355.3</td>
<td>3.9</td>
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<td>1.0</td>
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<tr>
<td>Processing * Processing time * Incubation time</td>
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<td>12</td>
<td>192.3</td>
<td>2.1</td>
<td>0.014</td>
</tr>
<tr>
<td>Processing time * sample * Incubation time</td>
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<td>48</td>
<td>117.3</td>
<td>1.3</td>
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</tr>
<tr>
<td>Processing * sample * Incubation time</td>
<td>4879.5</td>
<td>12</td>
<td>406.6</td>
<td>4.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing * Processing time * sample * Incubation time</td>
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<td>23</td>
<td>233.3</td>
<td>2.6</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>37432.3</td>
<td>417</td>
<td>89.8</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>450359.9</td>
<td>626</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>286881.1</td>
<td>625</td>
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</tr>
</tbody>
</table>

*R Squared = 0.870 (Adjusted R Squared = 0.804)
Figure 5.2  Effect of incubation time on starch hydrolysis from acha and wheat bread

Table 5.2  Tests of between-subjects effects of baking on starch hydrolysis from acha and wheat bread

<table>
<thead>
<tr>
<th>Source</th>
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<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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<td>Corrected Model</td>
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<td>2536.2</td>
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</tr>
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<td>153926.5</td>
<td>517.8</td>
<td>0.000</td>
</tr>
<tr>
<td>sample</td>
<td>131.5</td>
<td>2</td>
<td>65.8</td>
<td>0.221</td>
<td>0.803</td>
</tr>
<tr>
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<td>32709.6</td>
<td>6</td>
<td>5451.6</td>
<td>18.4</td>
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</tr>
<tr>
<td>sample * time</td>
<td>17883.6</td>
<td>12</td>
<td>1490.3</td>
<td>5.0</td>
<td>0.000</td>
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<tr>
<td>Incubation Error</td>
<td>12486.1</td>
<td>42</td>
<td>297.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>217137.3</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>63210.8</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

{superscript}R{superscript}² = .802 (Adjusted R Squared = 0.708)
significantly in their response to hydrolysis. This is in agreement with the report of Sayed et al. (2008) indicating that the differences in starch hydrolysis of spelt and wheat grain disappeared after baking. However, incubation time and interaction between the samples and the incubation time had significant effect on the starch hydrolysis. Hydrolysis of starch increased with increase in incubation time peaking at 120 min. Bread normally has an open cell structure where the enzyme solution is exposed to a greater protein-starch complex surface area (Van der Merwe et al., 2001). This means that there is a higher accessibility of starch to enzymatic action and would thus lead to a higher starch digestibility rate in the bread than within the rest of the processed (steaming, microwaved and boiled) samples. Goni et al. (1996) found 76.1% starch digestion for wheat bread after 180 minutes, this however is still higher than the results in this study 49.4%.

Effect of microwave on starch hydrolysis is detailed in Figure 5.3 and Table 5.3. Samples, incubation time and interaction between sample and incubation time significantly \((p < 0.05)\) affected starch hydrolysis. Significantly more starch was released from black acha (14.8%) and wheat (13.9%) compared to white acha (3.4%). There was no significant difference between the starch released from wheat and that of black acha. Significantly more starch was released after 90, 120 and 150 min of incubation. Processing time did not have significant effect on starch hydrolysis.

Effect of boiling on starch hydrolysis is detailed in Figure 5.4 and Table 5.4. Processing time, cereals, incubation time, interaction between processing time * incubation time and sample * incubation time significantly influenced the hydrolysed starch. Boiling for 15 min resulted in significant amount (22.7%) of hydrolysed starch. A significant decrease in starch hydrolysis resulted when boiled for 25 min (16.7%) and 35 min (18.9%). The cereals differed significantly in the amount of starch released with higher starch (28.1%) for white acha compared to black acha (23.8%) and wheat (6.3%). Significantly more starch was release after 90, 120 and 150 min of incubation.

Effect of steaming on starch hydrolysis is detailed in Figure 5.5 and Table 5.5. Processing time, cereals, incubation time as well as the two-way and three-
Figure 5.3. Effect of incubation time on starch hydrolysis from microwaved acha and wheat
Table 5.3. Analysis of variance for effect of microwave on starch hydrolysis

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>20248.1(^a)</td>
<td>62</td>
<td>326.6</td>
<td>4.7</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>21616.5</td>
<td>1</td>
<td>21616.5</td>
<td>312.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time</td>
<td>230.1</td>
<td>2</td>
<td>115.0</td>
<td>1.7</td>
<td>0.193</td>
</tr>
<tr>
<td>Sample</td>
<td>5092.5</td>
<td>2</td>
<td>2546.2</td>
<td>36.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Incubation time</td>
<td>8664.3</td>
<td>6</td>
<td>1444.0</td>
<td>20.9</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time * sample</td>
<td>488.3</td>
<td>4</td>
<td>122.1</td>
<td>1.8</td>
<td>0.140</td>
</tr>
<tr>
<td>Processing time * Incubation</td>
<td>504.0</td>
<td>12</td>
<td>42.0</td>
<td>0.6</td>
<td>0.832</td>
</tr>
<tr>
<td>sample * Incubation</td>
<td>3140.3</td>
<td>12</td>
<td>261.7</td>
<td>3.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time * sample * Incubation</td>
<td>2128.5</td>
<td>24</td>
<td>88.7</td>
<td>1.3</td>
<td>0.189</td>
</tr>
<tr>
<td>time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>8707.4</td>
<td>126</td>
<td>69.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50572.0</td>
<td>189</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>28955.5</td>
<td>188</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a^{R^2} = 0.699\) (Adjusted \(R^2 = 0.551\))
Figure 5.4. Effect of incubation time on starch hydrolysis from boiled acha and wheat
Table 5.4. Analysis of variance for the effect of boiling on starch hydrolysis of acha and wheat

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>70069.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61</td>
<td>1148.7</td>
<td>12.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>69233.8</td>
<td>1</td>
<td>69233.8</td>
<td>724.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time</td>
<td>925.0</td>
<td>2</td>
<td>462.5</td>
<td>4.8</td>
<td>0.009</td>
</tr>
<tr>
<td>Sample</td>
<td>16134.7</td>
<td>2</td>
<td>8067.4</td>
<td>84.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Incubation time</td>
<td>34410.4</td>
<td>6</td>
<td>5735.1</td>
<td>60.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time * sample</td>
<td>603.9</td>
<td>4</td>
<td>151.0</td>
<td>1.6</td>
<td>0.184</td>
</tr>
<tr>
<td>Processing time * Incubation time</td>
<td>3100.9</td>
<td>12</td>
<td>258.4</td>
<td>2.7</td>
<td>0.003</td>
</tr>
<tr>
<td>Sample * Incubation time</td>
<td>11155.9</td>
<td>12</td>
<td>929.6</td>
<td>9.7</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time * sample * Incubation time</td>
<td>2543.8</td>
<td>23</td>
<td>110.6</td>
<td>1.2</td>
<td>0.296</td>
</tr>
<tr>
<td>Error</td>
<td>12041.8</td>
<td>126</td>
<td>95.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>152743.0</td>
<td>188</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>82111.3</td>
<td>187</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>R<sup>2</sup> = 0.853 (Adjusted R<sup>2</sup> = 0.782)
Figure 5.5. Effect of incubation time on starch hydrolysis from steamed acha and wheat
<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>16101.8(^a)</td>
<td>62</td>
<td>259.7</td>
<td>7.6</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>9972.7</td>
<td>1</td>
<td>9972.7</td>
<td>292.3</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time</td>
<td>222.3</td>
<td>2</td>
<td>111.2</td>
<td>3.3</td>
<td>0.042</td>
</tr>
<tr>
<td>Sample</td>
<td>820.3</td>
<td>2</td>
<td>410.1</td>
<td>12.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Incubation time</td>
<td>4585.8</td>
<td>6</td>
<td>764.3</td>
<td>22.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time * sample</td>
<td>431.5</td>
<td>4</td>
<td>107.9</td>
<td>3.2</td>
<td>0.016</td>
</tr>
<tr>
<td>Processing time * Incubation time</td>
<td>2002.2</td>
<td>12</td>
<td>166.9</td>
<td>4.9</td>
<td>0.000</td>
</tr>
<tr>
<td>Sample * Incubation time</td>
<td>2471.4</td>
<td>12</td>
<td>206.0</td>
<td>6.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Processing time * sample * Incubation time</td>
<td>6039.4</td>
<td>24</td>
<td>251.6</td>
<td>7.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>4197.0</td>
<td>123</td>
<td>34.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29907.6</td>
<td>186</td>
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<tr>
<td>Corrected Total</td>
<td>20298.8</td>
<td>185</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)R^2 = 0.793 (Adjusted R^2 = 0.689)
way interaction all affected starch hydrolysis significantly \((p > 0.05)\). There was no significant difference in the amount of starch released after 15 min (6.2%) and 25 min (6.4%) of steaming. However, steaming for 35 min (8.9%) significantly increased the amount of hydrolysed starch. The increase in hydrolysed starch with increase in steaming time could be explained by the disruption of complexes such as starch-lipid complexes, which renders the weaker bonds to hydrolysis \((\text{Jideani} \& \text{Scott, 2011})\). Wheat and white acha did not differ significantly in the amount of starch released during steaming. However, black acha released higher amount of starch (10.4%) compared to white acha and wheat. Grain sizes may have also contributed to the different amount of starch hydrolysed. Given that the acha grains are very small compared to wheat, they may be subjected to enzyme digestion faster thereby resulting in comparatively more starch hydrolysed. Incubation time increased significantly the amount of starch released with more starch released from 90 to 180 min of steaming.

The different processing methods, microwaving, boiling and steaming as well as the different processing times, affects the structure of the cereals and thus influenced the amount of starch hydrolysed in the different cereal samples. Van der Merwe et al. (2001) reported that different cooking methods of bread and cereal porridge and the way that cooking affects the structure of the food could greatly contribute to the differences in starch hydrolysis. Shorter processing time will lead to less disrupted starch granules and therefore less prone to enzyme digestion, whereas longer processing time would lead to increased starch granule disruption and increase in the formation of retrograded amylose during the cooling period.

5.4.2 In vitro starch hydrolysis rate

**Modelling the effect of baking on in vitro acha starch hydrolysis**

Table 5.6 and Figure 5.6 detail the model parameters for bread prepared from black acha, white acha and wheat. The equilibrium percentage of starch hydrolysed after 180 min did not differ significantly \((p > 0.05)\) among the cereals. However, the rate of starch hydrolysis \((k)\) differed significantly \((p < 0.05)\) among
Table 5.6 Model parameters for bread from black acha, white acha and wheat bread\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Bread</th>
<th>C_\infty</th>
<th>k</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black acha</td>
<td>77.5 ± 5.7\textsuperscript{a}</td>
<td>0.039 ± 0.015\textsuperscript{a}</td>
<td>0.713</td>
</tr>
<tr>
<td>White acha</td>
<td>59.1 ± 5.5\textsuperscript{a}</td>
<td>1.394 ± 0.015\textsuperscript{b}</td>
<td>0.461</td>
</tr>
<tr>
<td>Wheat</td>
<td>91.4 ± 21.7\textsuperscript{a}</td>
<td>0.011 ± 0.005\textsuperscript{a}</td>
<td>0.763</td>
</tr>
</tbody>
</table>

\textsuperscript{1}C_\infty = \text{equilibrium percentage of starch hydrolyzed after 180 min}; k = \text{kinetic constant}; R^2 = 1 - (\text{Residual Sum of Squares}) / (\text{Corrected Sum of Squares})

\textsuperscript{2}Values with the different superscript in a column are significantly different (p < 0.05).

Figure 5.6. Predicted hydrolysed starch from black acha, white acha and wheat bread
the cereals. White acha showed a higher rate of hydrolysis compared to black acha and wheat. Wheat and black acha did not differ significantly in their rate of hydrolysis. White acha showed a higher rate of hydrolysis compared to black acha and wheat. Wheat and black acha did not differ significantly in their rate of hydrolysis. Sayed et al. (2008) reported a similar difference in starch hydrolysis of spelt whole grain bread and common wheat bread. The same results were reported by Skrabanja et al. (2001); spelt white bread had a significantly higher starch hydrolysis index than common wheat white bread. These differences in starch hydrolysis rate and extent could be attributed to difference in the chemical and physicochemical properties due to the genetic differences. Although there was no significant difference, the equilibrium percentage of starch hydrolyzed from black acha and wheat bread were higher compared to white acha bread. This does not support the hypothesis that white and black acha bread will differ in the equilibrium percentage of starch hydrolyzed after 180 min.

5.4.3 Modelling the effect of boiling on acha starch hydrolysis
The model parameters for black acha, white acha and wheat grain subjected to different boiling times are detailed in Table 5.7 and Figure 5.7. The equilibrium percentage of starch hydrolysed after 180 min did not differ significantly among the cereals for the same boiling time but differed significantly \((p < 0.05)\) amongst the different boiling times (15, 25, 35 min). This difference in the equilibrium starch hydrolysis can be attributed to the disruption of the crystalline regions due to the various boiling times. Lee et al. (2005) reported similar results for different corn samples subjected to various processing methods and times. A positive correlation could be drawn for the changes in the starch hydrolysis and the different cooking times (Lee et al., 2005). The equilibrium percentage of starch hydrolyzed for cereals boiled for 15 min was the highest for white acha 62.2% followed by black acha 48.1% and the lowest for wheat at 7.7%. The difference in the equilibrium percentage of starch hydrolyzed after 180 min for the different cereal samples could possibly be due to genetic and grain sizes differences.
Table 5.7  Model parameters for black acha, white acha and wheat grain as affected by boiling\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Boiling time (min)</th>
<th>$C_\infty$</th>
<th>$k$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black acha</td>
<td>15</td>
<td>$48.1 \pm 10.5^a$</td>
<td>$0.015 \pm 0.009^a$</td>
<td>0.603</td>
</tr>
<tr>
<td>White acha</td>
<td>15</td>
<td>$62.2 \pm 31.9^a$</td>
<td>$0.011 \pm 0.011^a$</td>
<td>0.448</td>
</tr>
<tr>
<td>Wheat</td>
<td>15</td>
<td>$27.1 \pm 40.1^a$</td>
<td>$0.004 \pm 0.007^a$</td>
<td>0.535</td>
</tr>
<tr>
<td>Black acha</td>
<td>25</td>
<td>$25.9 \pm 4.0^b$</td>
<td>$0.029 \pm 0.019^b$</td>
<td>0.431</td>
</tr>
<tr>
<td>White acha</td>
<td>25</td>
<td>$46.4 \pm 17.3^b$</td>
<td>$0.014 \pm 0.013^b$</td>
<td>0.420</td>
</tr>
<tr>
<td>Wheat</td>
<td>25</td>
<td>$7.7 \pm 1.7^b$</td>
<td>$0.019 \pm 0.013^b$</td>
<td>0.454</td>
</tr>
<tr>
<td>Black acha</td>
<td>35</td>
<td>$34.6 \pm 9.1^c$</td>
<td>$0.018 \pm 0.014^c$</td>
<td>0.423</td>
</tr>
<tr>
<td>White acha</td>
<td>35</td>
<td>$38.7 \pm 10.7^c$</td>
<td>$0.017 \pm 0.013^c$</td>
<td>0.267</td>
</tr>
<tr>
<td>Wheat</td>
<td>35</td>
<td>$8.8 \pm 1.5^c$</td>
<td>$0.036 \pm 0.029^c$</td>
<td>0.373</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Values are mean ± standard error. $C_\infty$ = equilibrium percentage of starch hydrolyzed after 180 min; $k$ = kinetic constant; $R^2 = 1 - \frac{\text{Residual Sum of Squares}}{\text{Corrected Sum of Squares}}$.

\textsuperscript{2}Values with the different superscript in a column (within boiling time) are significantly different ($p < 0.05$).
Figure 5.7. Predicted hydrolysed starch from black acha, white acha and wheat after boiling for (A) 15 min; (B) 25 min; (C) 35 min
amongst the cereals. The equilibrium percentage of starch hydrolyzed after 180 min for samples boiled for 25 min was 25.9% for white acha and 27.7% for wheat and black acha, 46.1%. Chung et al. (2008) reported similar differences in starch hydrolysis and physicochemical properties among pulse from the same species.

The rate of starch hydrolysis \( (k) \) did not differ significantly \( (p > 0.05) \) among the cereals for the same processing times. However the rate of starch hydrolysis increased with increase in boiling time of the different cereal grains. The highest rate of starch hydrolysis \( (k) \) was for cereal samples boiled for 35 min, with wheat 0.036 followed by black acha 0.018 and white acha 0.017. The increase in hydrolysis rate \( (k) \) was influenced by the difference in their microstructures caused by longer boiling times. Lee et al. (2005) demonstrated that the highest kinetic constant was experienced Table 5.8 and Figure 5.8 detail with the longest method (autoclaving) followed by stone pot cooking, electric pot cooking and lastly microwaving. Englyst et al. (1992) reported that the variation on rate and extent of starch digestion in foods depends on several intrinsic and extrinsic factors such as physical form of the food and its composition. There are significant differences \( (p < 0.05) \) in the rate of starch hydrolysis \( (k) \) and the equilibrium percentage of starch hydrolysed after 180 min of incubation for white acha, black acha and wheat grain for the different boiling times (15, 25 and 35 min). This supports the hypothesis that white acha and black acha will differ in their hydrolysis rate and kinetic constant.

### 5.4.4 Modelling the effect of steaming on the acha starch hydrolysis

The model parameters for samples from black acha, white acha and wheat grain subjected to different steaming times is detailed in Table 5.8 and Figure 5.8. Apart from wheat and white acha steamed for 25 min, the equilibrium percentage of starch hydrolysed after 180 min did not differ significantly \( (p > 0.05) \) among the cereals for the different steaming times (15, 25, 35 min). This could be due to minimal disruption of the starch crystalline regions at the various steaming times. The amount of moisture to which the cereals were subjected to, played a role in the degree of disruption of the cereals starch crystallinity during steaming. Less
Table 5.8  Model parameters for black acha, white acha and wheat grain as affected by steaming$^1, 2$

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Steaming time (min)</th>
<th>C$^\infty$</th>
<th>k</th>
<th>R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black acha</td>
<td>15</td>
<td>12.3 ± 2.3$^a$</td>
<td>0.558 ± 0.017$^a$</td>
<td>0.201</td>
</tr>
<tr>
<td>White acha</td>
<td>15</td>
<td>15.4 ± 13.2$^a$</td>
<td>0.012 ± 0.024$^b$</td>
<td>0.167</td>
</tr>
<tr>
<td>Wheat</td>
<td>15</td>
<td>3.2 ± 1.3$^a$</td>
<td>0.025 ± 0.038$^b$</td>
<td>0.145</td>
</tr>
<tr>
<td>Black acha</td>
<td>25</td>
<td>11.7 ± 2.4$^a$</td>
<td>0.024 ± 0.017$^a$</td>
<td>0.413</td>
</tr>
<tr>
<td>White acha</td>
<td>25</td>
<td>4.9 ± 1.0$^b$</td>
<td>0.055 ± 0.078$^a$</td>
<td>0.168</td>
</tr>
<tr>
<td>Wheat</td>
<td>25</td>
<td>2.5 ± 0.6$^b$</td>
<td>0.043 ± 0.054$^a$</td>
<td>0.210</td>
</tr>
<tr>
<td>Black acha</td>
<td>35</td>
<td>8.6 ± 0.7$^a$</td>
<td>0.024 ± 0.007$^a$</td>
<td>0.817</td>
</tr>
<tr>
<td>White acha</td>
<td>35</td>
<td>10.6 ± 3.5$^a$</td>
<td>0.024 ± 0.028$^a$</td>
<td>0.220</td>
</tr>
<tr>
<td>Wheat</td>
<td>35</td>
<td>12.9 ± 6.5$^a$</td>
<td>0.010 ± 0.010$^a$</td>
<td>0.444</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard error. C$^\infty$ = equilibrium percentage of starch hydrolyzed after 180 min; $k$ = kinetic constant; $R^2 = 1 - (\text{Residual Sum of Squares}) / (\text{Corrected Sum of Squares})$

$^2$Values with the different superscript in a column (within steaming time) are significantly different (p < 0.05).
Figure 5.8. Predicted hydrolysed starch from black acha, white acha and wheat after steaming for (A) 15 min; (B) 25 min; (C) 35 min
disruption of the crystalline regions leads to less water infiltration and hydration of the starch granules (Sagum & Argot, 2000). A slight increase in the equilibrium percentage starch hydrolysis from the shortest steaming time of 15 min to the longest steaming time of 35 min was observed. This is in agreement with reports by Sargum & Argot (2000) who reported an increase in the starch hydrolysis with increase in gelatinization and thus an increase in the breakdown of the crystallinity of the starch molecules. However, the equilibrium percentage of starch hydrolyzed for wheat (2.5%) and white acha (4.9%) did not differ significantly (p < 0.05) after 25 min steaming, but differed significantly (p < 0.05) from black acha (11.7%) at the same steaming time. Van der Merwe et al. (2001) reported that the way the cooking affects the structure of the different samples would greatly affect the starch hydrolysis. Chung et al. (2008) reported similar differences in extent of starch hydrolysis for pulse species of the same cultivar and specie. They attributed these differences in extent of starch hydrolysis to differences in protein content, amylose content and melting temperatures.

The rate of starch hydrolysis (k) differed significantly (p < 0.05) for wheat and white acha after 15 min of steaming and black acha at the same steaming time. The rate of starch hydrolysis was the highest for black acha after 15 min, followed by wheat and white acha. There was however no significant differences (p < 0.05) in the rate of starch hydrolysis between black acha, white acha and wheat at the rest of the different processing times (25, 35 min). This does not support the hypothesis that white acha grain and black acha grain will differ in the hydrolysis rates when subjected to different processing times.

5.4.5 Modelling the effect of microwaving
Table 5.9 and figure 5.9 depicts the model parameters for samples from black acha, white acha and wheat grain subjected to different microwaving times. There were no significant difference (p > 0.05) in the equilibrium amount of starch hydrolyzed after 180 min for black acha microwaved for 3 min and black acha, white acha and wheat microwaved for different microwaving times (6, 9 min),
Table 5.9  Model parameters for black acha, white acha and wheat grain as affected by microwave\(^1, 2, 3\)

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Microwave time (min)</th>
<th>$C^\infty$</th>
<th>$k$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black acha</td>
<td>3</td>
<td>22.9 ± 5.5(^a)</td>
<td>0.027 ± 0.026(^a)</td>
<td>0.260</td>
</tr>
<tr>
<td>White acha</td>
<td>3</td>
<td>4.7 ± 0.8(^b)</td>
<td>0.027 ± 0.017(^a)</td>
<td>0.403</td>
</tr>
<tr>
<td>Wheat</td>
<td>3</td>
<td>16.0 ± 3.5(^a)</td>
<td>0.028 ± 0.025(^a)</td>
<td>0.304</td>
</tr>
<tr>
<td>Black acha</td>
<td>6</td>
<td>19.2 ± 5.6(^a)</td>
<td>0.022 ± 0.022(^a)</td>
<td>0.296</td>
</tr>
<tr>
<td>White acha</td>
<td>6</td>
<td>3.8 ± 0.5(^a)</td>
<td>0.031 ± 0.017(^a)</td>
<td>0.504</td>
</tr>
<tr>
<td>Wheat</td>
<td>6</td>
<td>14.5 ± 2.5(^a)</td>
<td>0.049 ± 0.053(^a)</td>
<td>0.268</td>
</tr>
<tr>
<td>Black acha</td>
<td>9</td>
<td>18.1 ± 3.8(^a)</td>
<td>0.038 ± 0.041(^a)</td>
<td>0.237</td>
</tr>
<tr>
<td>White acha</td>
<td>9</td>
<td>5.4 ± 0.9(^a)</td>
<td>0.019 ± 0.010(^a)</td>
<td>0.561</td>
</tr>
<tr>
<td>Wheat</td>
<td>9</td>
<td>37.2 ± 26.8(^a)</td>
<td>0.009 ± 0.012(^a)</td>
<td>0.320</td>
</tr>
</tbody>
</table>

\(^1\)Values are mean ± standard error. $C_\infty$ = equilibrium percentage of starch hydrolyzed after 180 min; $k$ = kinetic constant; $R^2 = 1 - (\text{Residual Sum of Squares}) / (\text{Corrected Sum of Squares})$

\(^2\)Values with the different superscript in a column (within microwave time) are significantly different (p < 0.05).
Figure 5.9. Predicted hydrolysed starch from black acha, white acha and wheat after microwave for (A) 3 min; (B) 6 min; (C) 9 min
respectively. This similarity indicates a similar degree of disruption of the cellular structure and therefore degree of susceptibility of starch to amylolytic enzymes after 3, 6, and 9 min of microwaving. There was however significant difference in the equilibrium percentage amount of starch hydrolyzed for white acha (4.7%) and the equilibrium amount of starch hydrolyzed for black acha (22.9%) and wheat (16.0%) after 3 min of microwaving. This difference in the equilibrium percentage hydrolysed starch could be due to genetic differences and therefore difference in the degree of disruption of the crystalline region of the starch molecules of the different cereals after 3 min of microwaving. Lee et al. (2005) reported that the overall starch hydrolysis of cooked rice was influenced by the differences in their microstructure, which were caused by the different processing methods. Similar results were reported by Eyaru et al. (2009). They highlighted that cooking increases starch digestibility and decreases enzyme resistant starch. There were no significant differences in the rate (k) of starch hydrolysis amongst the different samples or the different processing times. There was very little literature available on the effect of microwave processing on the rate of starch hydrolysis. However this similarity in the rate (k) of starch hydrolysis amongst the different samples or the different processing times does not support the hypothesis that white and black acha grain will differ in their rate of starch hydrolysis at different microwaving times.

5.5 Total, Resistant and Digestible starch in Acha and Wheat bread
The amount of total, resistant and digestible starch in white acha, black acha and wheat bread is shown in Table 5.10. There are significant difference (p < 0.05) in the total percentage starch between wheat bread (WB), white acha bread (WAB) and black acha bread (BAB). Total starch in WB, WAB and BAB were 6.6%, 42.0% and 52.3%, respectively. Hallstrom et al. (2011) reported that the starch content of white wheat bread to be 40.7%. Hence, the result is in agreement with the value of total WB reported in this work. The significant difference in the total starch between the acha bread varieties and the wheat bread could be due to the fact that the acha bread recipe consisted of added
Table 5.10 Total starch, resistant starch and digestible starch in bread samples\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Bread</th>
<th>Total</th>
<th>Resistant</th>
<th>Digestible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>6.6 ± 4.2\textsuperscript{a}</td>
<td>0.94 ± 0.09\textsuperscript{a}</td>
<td>5.7 ± 4.2\textsuperscript{a}</td>
</tr>
<tr>
<td>White acha</td>
<td>42.0 ± 2.5\textsuperscript{b}</td>
<td>0.74 ± 0.03\textsuperscript{b}</td>
<td>41.2 ± 2.5\textsuperscript{b}</td>
</tr>
<tr>
<td>Black acha</td>
<td>52.3 ± 4.1\textsuperscript{c}</td>
<td>0.93 ± 0.01\textsuperscript{a}</td>
<td>51.3 ± 4.1\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Values are mean ± standard error.
\textsuperscript{2}Values with the different superscript in a column are significantly different (p < 0.05).
acha starch, whereas the wheat bread did contain added starch in the recipe. There was no significant difference (p > 0.05) in the resistant starch between BAB (0.93%) and WB (0.94%). There was however significant difference (p < 0.05) in resistant starch between WAB (0.74%) and WB as well as between the two acha cultivars, WAB (0.74) and BAB (0.93). The results in this study were higher than that reported by Elmstahl (2002) for Hallakaka wheat bread (0.60%). Elmstahl (2002) however also reported wholemeal bread to contain 1.5% resistant starch. This contrast in quantity of resistant starch could be due to part of the wholemeal to consist of intact cereal grain which keeps the starch encapsulated in the grain structure. The difference in the resistant starch content between WAB and BAB could be due to genetic difference of the two acha grain varieties.

In terms of digestible starch, a significant difference (p < 0.05) was detected between the two acha bread variants and WB. The digestible starch was 5.7%, 41.2% and 51.3%, respectively for WB, WAB and BAB. The higher percentage of digestible starch in WAB (41.2%) and BAB (51.3%) could be attributed to the added acha starch as well as the lite apple juice in the bread recipe. Hence, addition of acha starch and lite apple juice to acha bread recipe is discouraged as it may add to the glycemic load. This suggests effect of ingredients on GI of a food product is additive. Consequently, combining low GI ingredients in a recipe could result in high glycemic load.

The significant difference in the total starch, resistant starch and digestible starch supports the hypothesis that significant differences exists in the starch of the two acha varieties.

5.6 Total, Resistant and Digestible Starch in Acha and Wheat Grain

The percentage of total, resistant and digestible starch of acha and wheat grain is show in Table 5.11. There are no significant difference (p > 0.05) in the percentage total starch between wheat and white acha grain. There was however significant difference (p < 0.05) between black acha grain and wheat grain in terms of total starch content as well as between white and black acha.
<table>
<thead>
<tr>
<th>Grain</th>
<th>Total</th>
<th>Resistant</th>
<th>Digestible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>15.02±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.89±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.14±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>White acha</td>
<td>16.23±0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.07±0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.17±1.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Black acha</td>
<td>12.16±1.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.14±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.03±2.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are mean ± standard error.

<sup>2</sup>Values with the different superscript in a column are significantly different (p < 0.05).
The difference in the percentage total starch between white acha and black acha grain could be due to differences in their genetic structure. A resistant starch value of 3.1% was however reported by Hallstrom et al. (2011) for elevated amylose wheat grain. Resistant starch has many health benefits such as provision of dietary fibre, prebiotic benefits to the gut as well as overall health benefit. The higher resistant starch reported in the study could be due to the grains being whole and intact. Whole and intact grains are good sources of resistant starch, since the starch is encapsulated in the intact grain.

The resistant starch for wheat grain is 5.9%, for white acha starch grain, 6.07% and for black acha starch grain is 6.1%. There were no significant differences \((p > 0.05)\) in resistant starch amongst the different grains. Hallstrom et al. (2011) reported a resistant starch value of 2.9% for whole grain wheat. This is much lower than the values reported in this study, wheat grain was 5.9%, white acha grain, 6.1% and black acha grain 6.1%. The digestible starch for white acha grain was highest at 10.2%, followed by wheat grain at 9.1% and black acha grain at 6.0%. There was no significant difference in the percentage digestible starch between white acha grain and wheat grain. There was however significant difference \((p < 0.05)\) in digestible starch between white and black acha grain. The difference in total starch and digestible starch between white and black acha grain supports the hypothesis that a difference exist in the two acha grain varieties.

### 5.7 Total, resistant and digestible starch in processed acha and wheat grain.

Rapidly digestible starch (RDS) and slowly digestible starch (SDS) contents of acha and wheat bread are shown in Figures 5.10, microwaved acha and wheat grain in Figure 5.11 and boiled acha and wheat grain in Figure 5.12, steamed acha and wheat grain in Figure 5.13. Table 5.13 shows the starch hydrolysis of acha and wheat grain with respect to incubation time.

The RDS for baked bread ranged from 12% for wheat bread (WB), 51% for black acha bread (BAB) and 60% for white acha bread (WAB) (Figure 5.10).
Figure 5.10 Rapidly digestible starch (RDS), and slowly digestible starch (SDS) of milled acha and wheat bread.

Figure 5.11 Rapidly digestible (RDS), and slowly digestible starch (SDS) of microwaved acha and wheat grain. 1: acha and wheat grain microwaved for 3 minutes, 2: acha and wheat grain microwaved for 6 minutes, 3: acha and wheat grain microwaved for 9 minutes.
Figure 5.12  Rapidly digestible (RDS), and slowly digestible starch (SDS) of boiled acha and wheat grain. 1: acha and wheat grain boiled for 15 minutes, 2: acha and wheat grain boiled for 25 minutes, 3: acha and wheat grain boiled for 35 minutes.

Figure 5.13  Rapidly digestible (RDS), and slowly digestible starch (SDS) of steamed acha and wheat grain. 1: acha and wheat grain steamed for 15 minutes, 2: acha and wheat grain microwaved for 25 minutes, 3: acha and wheat grain microwaved for 35 minutes.
The SOS content for BAB was the highest at 89% followed by WB, 62% and WAB at 56%. Goñi *et al.* (1997) reported a RDS in the region of 20% and SDS of about 25% for wheat bread. This SDS for wheat bread is much lower than that reported in this study (62%) and the RDS reported by Goñi *et al.* (1997) is much higher than the RDS results in this study (12%). Whereas Sayed *et al.* (2008) reported RDS of 41.9% and SDS of 5.8% for common wheat bread and a RDS of 42.3% and SDS of 6.5% for spelt bread. This difference might be due to differences in genetics and composition of the wheat cultivars, baking formulas and baking conditions. The higher RDS of acha breads compared to the wheat bread could be due to the difference in the recipe, since the acha bread recipe consisted of additional acha starch whereas the white bread recipe did not contain added starch.

The SDS for microwaved black acha grain (MBA) was higher than that of microwaved wheat grain (MWG) at 3 and 6 minutes of microwaving (Figure 5.11). For MBA the SDS was 34.0% for 3 minutes and 20.9% for 6 minutes of microwaving. Whereas for MWG, it was 18.5% at 3 minutes of microwaving and 15.5% at 6 minutes of microwaving. The SDS for microwaved white acha (MWA) was much lower than that of MBA as well as MW. The SDS for MWA was 6.69% for 3 minutes, 5.07% for 6 minutes and 6.72% for 9 minutes of microwaving. The SDS after 9 minutes of microwaving for MBA was 19.0% and for MW was 36.10%. The RDS for all microwaved grain samples were much lower than the SDS microwaved grain samples for the different processing times.

The SDS content of 15, 25 and 35 minutes boiled black acha (BBA), boiled white acha (BWA) and boiled wheat grain (BW) grain were significantly higher than the RDS for BBA, BWA and BW grain.

The SDS for steamed black acha (SBA), steamed white acha (SWA) and steamed wheat grain (SW) after 15 minutes of steaming was 13.94% (SBA) and 26.15% (SWA) and 1.32% (SW). The RDS for steamed acha and wheat grain was significantly higher than SDS for 15, 25 and 35 minutes of steaming. The SDS for SBA after 15, 25 and 35 minutes of steaming was higher than the SDS
for SWA and SW at the same steaming times.

A study RDS and SDS of wholes grain acha porridge were conducted by Podgorski (2009) showed a RDS of 13.73% for white acha and 16.26% for black acha porridge. The RDS reported for white and black acha porridge was in the same range as the results for RDS in this study for boiling after 15 and 25 minutes, but much higher than the results for boiling of 35 minutes. The SDS content for white acha porridge was 18.47% and black acha porridge was 39.97%. This is lower than that reported in this study for BWA, 71.4% after 15 minutes and 43.8% after 35 minutes, but higher than the 9.45% after 25 minutes of boiling. The SDS for black acha porridge reported by Jideani & Podgorski (2010) is much lower than that reported in this study for 15 minutes of boiling (57.98%), 25 minutes (58.62%) and 35 minutes (42.62%). This difference in SDS and RDS can be attributed to the difference in boiling processing method with regard to sample size and boiling times. No evidence could be found of investigation of the effect of steam and microwave processing on acha and wheat grains.

According to Englyst et al. (1999), the nutrients in minimally processed cereal grains are encapsulated within the cell walls which retard the release and thus digestion of starch which thus lead to low RDS. High RDS foods are harmful for health, whereas high SDS foods are beneficial for reducing chronic diseases (FAO/WHO, 1997).

Results indicate that black acha bread as well as microwaved, boiled, steamed black acha grain is the better alternative in the dietary management of metabolic disorders such as diabetes and hyperlipidemia due to its lower RDS and higher SDS compared to the higher RDS and SDS for white wheat bread, microwaved, boiled and steamed wheat grain.

5.8 Estimated Glycemic index (EGI) of processed Acha and Wheat grain and bread.

Figure 5.14 shows the GI of acha and wheat breads. Black acha and wheat bread the GI differed significantly. For white wheat bread and white acha bread it
Figure 5.14 Estimated Glycemic Index (EGI) of black acha bread, white acha bread and wheat bread.
was 146 and 110 respectively whereas for black acha bread it was 44. Englyst (1999) reported that white wheat bread, corn flakes, and spaghetti are all examples of highly processed foods. The starch in white wheat bread is fully gelatinized and thus likely to be rapidly digested and absorbed and white wheat breads thus have high GI values. Glycemic index categories are as follows, low GI (0-55), intermediate (56-69) and high GI (>70). Based on this, the black acha can be categorised as low GI whereas white wheat bread and white acha bread as high GI. Shangumam et al. (2007) however reported that the average GI values, calculated, taking white bread as a control (GI =100), for wheat-based, millet-based, expanded rice-based and popped rice based foods were 55.4, 93.4, 105 and 109 respectively. White acha bread in this study was thus in line with GI results reported for millet and popped rice based breads reported by Shanmugam et al. 2007. The difference in the GI between white acha, black acha and wheat bread could be attributed to the nature of available as well as non-available carbohydrates since both acha bread recipes consisted of added starch whereas the white wheat bread did not consist of added starch. The difference in GI values between white and black acha bread could be due to genetic differences. Englyst et al. (2003) reported that white wheat bread, corn flakes, and spaghetti is all examples of highly processed foods. The starches in white wheat breads are fully gelatinized and thus likely to be rapidly digested and absorbed and thus have high GI values.

After boiling for 15, 25 and 35 minutes, both acha varieties and wheat grain were categorised as low GI according to their GI index results (Figure 5.15). The GI ranged between 40 and 46 for both acha varieties and wheat grain, with wheat grain (46) the highest followed by white acha grain (45) after 35 minutes of boiling. Black acha grain had the lowest GI at all processing times, 40 after 15 minutes boiling and 41 after 25 and 35 minutes of boiling respectively. For steaming the highest GI was for black acha grain (58) after 15 minutes of steaming and wheat grain with a GI of 56 after 35 minutes of steaming (Figure 5.16). This would categorise black acha grain steamed for 15 minutes and
Figure 5.15 EGI of acha and wheat grain boiled for 15, 25 and 35 minutes.

Figure 5.16 EGI of acha and wheat grain steamed for 15, 25 and 35 minutes.

Figure 5.17 EGI of acha and wheat grain microwaved for 3, 6 and 9 minutes.
wheat grain steamed for 35 minutes as intermediary GI products. Steenkamp (2008) reported that the longer the processing, time the higher the GI value. White acha grain however consisted of GI value of 43 after 15 minutes steaming and 40 after 25 and 35 minutes steaming respectively and would thus be categorised as low GI. The average GI for both acha varieties and wheat grain were the lowest after 9 minutes of microwaving (Figure 5.17) and did not differ significantly, ranging between 40 for both wheat and white acha and 44 for black acha grain. Black acha and wheat grain had the highest GI value after 3 and 6 minutes of microwaving, with black acha consisting of 46 and wheat grain 48 after 3 minutes of microwaving and 42 and 52 after 6 minutes of microwaving respectively. White acha grain remained a constant 40 after 3, 6 and 9 minutes of microwaving. Both acha varieties and wheat grain have been categorised as low GI in this study and thus offer the possibility of being used for diabetic dietary management.

The physical form of food and the degree of processing imparted to the foods also influences the glycemic response of the food (Shanmugam et al., 2007). Since the majority of the foods under this study was intact grain and has undergone minimum disruption, the low GI values for boiled, steamed and microwaved acha and wheat grain were to be expected. In many plant foods, such as legumes and minimally processed cereal grains (e.g., pearled barley), nutrients are encapsulated within cell walls (dietary fibre), which retard the release and hence digestion and absorption of starch and sugars, and these foods thus have low GI values (Englyst et al., 1999).

5.8 Conclusion and Recommendation

The effect of processing on the starch and glycemic properties of acha is reported in this chapter. The following conclusion can be drawn:

1. The different processing methods baking, boiling, microwaving and steaming, affected the black and white acha starch hydrolysis. The amount of starch hydrolysed for the different processing methods was in the following order: baking > boiling > microwaving > steaming.
2. The equilibrium percentage of starch hydrolysed was affected differently for the various starches, black acha, white acha and wheat starch by the different processing methods and times. In the case of baking black acha and wheat bread was affected similarly. However, this was not the case for microwaving, steaming and boiling, where both acha starch varieties and wheat starch were affected in the same way.

3. The rate of starch hydrolysis for both acha varieties and wheat grain for the different processing methods, steaming, boiling, microwaving and baking was affected to the same degree.

4. SDS and RDS analysis results indicate that black acha grain (*Digitaria iburua*) cultivar is the better cultivar to use than white acha grain (*Digitaria exilis*) for the treatment and prevention of chronic disease due to its higher slowly digestible starch (SDS) and lower rapidly digestible starch content after baking, microwaving, boiling and steaming. White wheat bread, microwaved, boiled and steamed wheat grain had a higher RDS and SDS compared to black acha.

5. This study indicated that the acha white bread and processed (boiled, steamed and microwaved) acha and wheat grain consisted of low GI values and is thus suitable as a food supplement or meal replacer for those individuals suffering from diabetes and celiac disease.

References


De Lumen, O.B., Odegard, W.J. & Thompson, S. (1993). Sulphur amino acid-rich...


CHAPTER 6
Summary, Conclusion and Recommendation

The physicochemical, thermal and functional properties of two acha cultivars (*Digitaria exilis*, white and *Digitaria iburua*, black) were investigated. Wheat was used as reference. The acha starch was extracted from cleaned and dried acha grain. The physicochemical properties investigated were, proximate nutritional content, amylose content, SDS and RDS. The thermal properties investigated were gelatinization and retrogradation. The functional properties investigated were texture profile analysis, turbidity, pasting properties and water binding capacity. Bread was baked with two acha cultivars (*Digitaria exilis*, white and *Digitaria iburua*, black) and the sensory and physical properties investigated and compared against that of white wheat bread. The physical properties that were investigated were specific loaf volume as well as consumer acceptability. The sensory properties investigated were appearance, crust colour, crumb colour, aroma, taste, firmness, mouthfeel and overall acceptability. The effect of baking, steaming, boiling and microwaving on the starch and glycemic properties of two acha cultivars (*Digitaria exilis*, white and *Digitaria iburua*, black) was investigated. The aim of this research to determine the effect of different processing methods; baking, boiling, steaming and microwaving on the starch and glycemic properties of acha were met. The specific objective to evaluate the changes in glycemic properties of acha due to different processing methods (baking, boiling, steaming and microwaving) was also met.

The following conclusions were made.

1. Although there was no significant difference in the nutritional composition of white acha starch and black acha starch with regard to carbohydrate, moisture and fat content, there was however significant differences with regard to the protein content and dietary fibre between black and white acha starches. Wheat and white acha starch had no significant difference in the nutritional composition with regard to Moisture, fat and carbohydrates. There was no significant difference in amylose content between black acha, white acha and wheat starches.
2. Significant differences in some of the functional properties with regard to pasting and turbidity were observed, but no significant difference in the TPA and WBC. WBC of both acha varieties was higher than that for wheat starch. This indicates that both acha starch varieties can be used for hot and cold desserts as well as for soft jelly like sweets and confectionery toppings.

3. No significant differences with regard to the thermal properties of the two acha starch cultivars and wheat starch were observed. The observed gelatinisation temperature of acha and iburua starches typifies that of non-waxy starch. Acha starch has similar retrogradation temperature profiles as that of wheat starch.

4. Optimum acha bread could be baked using 8% acha starch, 2% xanthan gum, 2% CMC and 1.2% yeast.

5. Majority of the panellists found the crust colour, taste and aroma to be moderately desirable. Hence, acha bread has the potential to be marketed as wheat free bread.

6. Few differences in \textit{in vitro} starch digestibility and physicochemical properties were observed among two acha cultivars and wheat starch. Starch hydrolysis was significantly (\(P < 0.05\)) affected by both time and starch grain. There was no significant difference in the amount of hydrolyzed starch between wheat and white acha starch after 180 min. The two acha starch cultivars had fairly low RDS contents compared to wheat starch whereas the SDS for wheat and white acha starch was higher than that of black acha starch.

7. The different processing methods baking, boiling, microwaving and steaming, affected the black and white acha starch hydrolysis. The amount of starch hydrolysed for the different processing methods was in the following order: baking \(>\) boiling \(>\) microwaving \(>\) steaming.

8. The rate of starch hydrolysis for both acha varieties and wheat grain for the different processing methods, steaming, boiling, microwaving and baking was affected to the same degree.

9. On the basis of slowly digestible starch (SDS) and rapidly digestible starch (RDS), black acha grain (\textit{Digitaria iburua}) cultivar is the better cultivar to use than white acha grain (\textit{Digitaria exilis}) for the treatment
and prevention of chronic disease due to its higher SDS and lower RDS after baking, microwaving, boiling and steaming.

10. This study indicated that the acha white bread and processed (boiled, steamed and microwaved) acha and wheat grain consisted of low GI values and is thus suitable as a food supplement or meal replacer for those individuals suffering from diabetes and celiac disease.

Some of the hypotheses stated below were supported while others were not supported by the test results in this study:

1. For the hypothesis that starches from the two acha cultivars (*D. exilis* and *D. iburu*) differed in their functional, physicochemical and thermal properties, test results indicated that only some functional and physicochemical properties supported this hypotheses while results from thermal analysis did not support the hypothesis.

2. The hypothesis that different processing methods differed in their effect on the starch and glycemic properties of acha was not supported. The rate of starch hydrolysis for both acha varieties and wheat grain for the different processing methods, steaming, boiling, microwaving and baking was affected to the same degree.

3. The effect of processing on the starch and glycemic properties of acha differed among the cultivars. Results of the baked samples supported this hypothesis whereas processing such as boiling, steaming and microwaving did not support this hypothesis. All processed acha grain could be categorised as low GI products.
Dear Prof. Jideani:

A manuscript entitled Physicochemical, Thermal and Functional Properties of Acha (Digitaria spp.) Starch (CCHEM-03-13-0042-R) has been submitted by Mrs. Michelle Jordaan to Cereal Chemistry. You are listed as a co-author for this manuscript. The online peer-review system, Manuscript Central, automatically creates a user account for you. The USER ID and PASSWORD for your account is as follows:

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Physicochemical, Thermal and Functional Properties of Acha (Digitaria spp.) Starch

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<td>Jordaan, Michelle; Cape Peninsula University of Technology, Food Technology Jideani, Victoria; Cape Peninsula University of Technology, Food Technology</td>
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Physicochemical, Thermal and Functional Properties of Acha (Digitaria spp.) Starch

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Abstract
Acha [Digitaria exilis, white (WTAS) and Digitaria iburua, black (BLAS)], consumed as whole grain, has been reported to be high in resistant and slowly digestible starches but low in rapidly digestible starch. It is a grain of choice in the management of diabetes in Africa. Our objective was to investigate the physicochemical, thermal and functional properties of starches from the two acha cultivars compared to wheat starch (WHTS). There were no significant differences in amylase between BLAS (42.1%), WTAS (61.4%) and WHTS (45.8%). WHTS had highest onset (T_o) and gelatinization (T_p) temperatures 34.7°C and 74.5°C, respectively, although not significant. BLAS and WTAS were 25.0, 26.7°C and 68.2, 70.5°C, respectively. The gelatinization temperature ranges did not differ significantly between the varieties. Acha starches with lower transition temperature imply a low crystallinity with high amorphous regions. Temperature of retrogradation was 64.1, 67.0 and 64.8°C for WHTS, WTAS and BKAS, respectively. The starches were similar (p > 0.05) in gelatinization and retrogradation temperatures. There were significant differences (p < 0.05) in peak viscosity and final viscosity between wheat and acha starches. However, no significant difference (p > 0.05) existed between WTAS (3125 cP) and BLAS (3137 cP) for trough viscosity and setback viscosity 2332 cP and 2476 cP respectively. Gels produced from the three starches did not differ significantly in hardness, resilience and springiness. All the starches produced highly springy soft gels. Water binding capacity differed significantly between WHTS (0.83 g/100g) and both acha cultivars 1.33 g/100g for WTAS and 1.36 g/100g for BLAS. However, the acha cultivars did not differ significantly in WBC. Acha starches can thus be utilized as an alternative to wheat starch when required in application where thermal properties such as gelatinization are important.

Introduction
Digitaria spp. belongs to the family Gramineae and the tribe Poaceae (Jideani 1999) and includes 230 species that are widely distributed in the tropics and the subtropics. Acha is by large the oldest African cereal, it has been cultivated for thousands of years in West Africa (Mali, Burkina Faso, Guinea and Nigeria) and Dominican Republic (Jideani et al. 2008). Two types exist namely Digitaria exilis (acha, fonio or hungry rice) and Digitaria iburua (iburua, black fonio or petit millet), (Philip and Itodo 2006). It consists of a unique small size of 0.4 to 0.5 mm seeds. Acha is high in essential amino acids, leucine 9.7%, methionine 5.6% and valine 5.8%. It is considered as one of the world’s most nutritious grains (Philip and Itodo 2006). It is also used for the management of diabetes in West Africa. Not much is
known about the thermal and functional properties of acha starch. Starch is a food ingredient with wide applications such as thickener, gelling agent, bulking agent and water retention agent, making them an excellent ingredient for the manufacture of foods such as: custards, porridges, puddings, cookies, and sausages. There is a growing demand for starches for the modern food industry creating interest for new sources of this polysaccharide. The availability of a reliable source of starch from agriculture is considered to have an important factor in human development (Asp et al. 1996). Our objective was to investigate the physicochemical, thermal and functional properties of acha starch.

MATERIALS AND METHODS

Materials
Two acha cultivars namely, *Digitaria exilis* (white) and *Digitaria iburua* (black) were purchased from Grace Africa, Salt River, Cape Town, South Africa.

All equipment and materials were either obtained from the Department of Food Technology, Cape Peninsula University of Technology (CPUT) or Pioneer Foods Laboratories (Bokomo Foods pilot plant and SASKO technical laboratory), Cape Town, South Africa.

All chemicals were obtained from Laboratory and Scientific Ltd., Maitland, Cape Town, Republic of South Africa and the Department of Food Technology CPUT.

Cleaning of acha grain
The cleaning procedure entailed screening the grains for 3 minutes through a 1000, 750, 500 and 125 micron screens to remove all foreign matter such as stones, foreign grain and small sticks. The grains were washed three times with water, drained and dried at 40°C for 48 h. The cleaned and dried samples were then placed in sealed polyethylene bags and stored in a refrigerator at a temperature of 5°C until required.

Isolation and purification of starch from acha grain
The method described by Betancur and Torruco-Uco (2001) was used to extract starch from milled acha grain. Wheat starch (S2760) bought at Sigma Aldrich was used as the reference. Cleaned, washed and dried acha grain were milled and sieved through a 20 mesh screen. The acha flour was then dispersed in distilled water at a ratio of 1:8 w/v. The pH was then
adjusted to 11 with 1 N NaOH and the solution was stirred for 1 h. The suspension was then passed through an 80 and 100 mesh screens to separate the fibre solids from the liquid containing the protein and the starch. The suspension was allowed to sediment for 30 min to recover the starch, and then the solubilised protein was separated. The starch was washed 3 times with distilled water, and centrifuged at 4250 rpm for 10 min during the last wash to recover the starch. The product was then dried at 60°C in a drying oven (Defy, Gemini Ltd., Germiston) for 1 h then milled and sieved through a 20 mesh screen. Starch from white acha is denoted as WTAS and that from black acha as BLAS. The isolated acha starches and wheat starch (WHTS) were then analysed for physicochemical, functional and thermal properties.

**Physicochemical Properties of Acha Starch**

**Proximate composition and total amylose content of the starches**

The moisture, fat, protein, fiber and ash content of the acha and wheat starch were determined in accordance with the standard methods of AOAC (1990). Carbohydrates were calculated by difference.

The method by Hoover and Ratnayake (2001) was used to determine the amylose content of starches. Defatting of starch samples were conducted by weighing 5 g of starch accurately into a cellulose extraction thimble and covering the mouth with cotton wool. Lipids were then extracted with 120 ml of 75% n-Propanol at 85°C with a heating mantle for 7 h in a Soxhlet extractor. The lipid free starch was then air dried for 12 h and removed from the thimble and oven dried for 24 h at 30°C. Lipid-free starch (20 mg) was weighed into a round bottom screw-cap tube fitted with a Teflon-faced rubber liner in the cap. A series of mixtures of pure potato amylose (0%, 10%, 20%, 40%, 50% amylose) were prepared after which 20 mg of each was weighed into round-bottom tubes with caps. Eight millilitres of 90% DMSO was added to each round-bottom tube and vigorously mixed for 2 min using a vortex mixer. The tubes were then heated in a water bath at 85°C for 15 min with intermittent mixing. The tubes were then allowed to cool to room temperature (45 min). The samples were then diluted to 25 ml with water in a volumetric flask.

Determination of absorbance of the dispersed starch solution was then conducted after completion of the following steps. Diluted solution (1 ml) and 40 ml of distilled water were added into a 50 ml volumetric flask. Iodine solution (5 ml) was added and mixed vigorously. The volume was adjusted to 50 ml with distilled water and mixed vigorously. The colour
was then allowed to develop for 15 min. The contents of the flask were then vigorously mixed by hand. The absorbance of the samples and each of the standard mixtures were then measured at 600 nm against a reagent blank as the reference. The reagent blank contained all reagents in the same amounts without the sample containing starch.

Following the above procedure a standard curve was then plotted. The regression equation for the standard curve was then measured and used to calculate the total amylose content of the sample.

**Thermal properties of the starches**

Differential scanning calorimeter (DSC, 20 to 120°C, Perkin-Elmer instrument) was used to measure the thermal characteristics of the isolated starch. Both the acha starches and wheat starch (3.5 mg, dry weight) were separately loaded into a 40 μl capacity aluminium pan (Mettler ME-27331) and distilled water added using a Hamilton micro syringe to achieve a 70% starch–water suspension. Samples were sealed and allowed to stand for 5 h at room temperature before it was heated in the DSC. The DSC analyzer was calibrated using indium, and an empty aluminium pan was used as a reference (Sandhu and Narpinder, 2007). Sample pans were heated at a rate of 10°C/min from 20°C to 100°C. Thermal transition of starch samples was defined as $T_o$ (onset temperature), $T_p$ (peak of gelatinization), $T_c$ (conclusion temperature) and the enthalpy of gelatinization ($\Delta H_{gel}$). Enthalpies were calculated on a starch dry weight basis automatically. The gelatinization temperature range ($R$) and peak height index (PHI) were calculated as described in equations 1 and 2.

\[
R = 2(T_p - T_o)
\]

\[
\text{PHI} = \frac{\Delta H}{(T_p - T_o)}
\]

After conducting thermal analysis, the samples were stored at 4°C for 7 days, for retrogradation studies. The aluminium sample pans containing the starches were reheated at the rate of 10°C/min from 25°C to 100°C to measure retrogradation. The enthalpies of retrogradation ($\Delta H_{gel}$) $T_o$, onset temperature; $T_p$, peak temperature; $H_I$, peak height; $H$, height; $\Delta H$, enthalpy of gelatinization; $T_c$, end (conclusion) temperature, $\Delta T_r$ gelatinization temperature range ($T_c - T_o$). were calculated automatically by the Pyris R9.1.software.

**Functional properties of the Starches**

**Textural properties of starch gels**

Texture profile analysis was conducted as described by Sandhu and Narpinder (2007) on an
Instron apparatus (model 2519, UK, 3300 series, 2000 N capacity) to evaluate the textural properties of acha and wheat starch gels. A suspension of 45 g of starch in 455 ml of cold water was prepared. The suspension was then boiled on a hot plate (Model Z341 Ohaus Instr. US) with stirring until a gel was formed. The hot starch gel formed was then poured into small aluminium dishes (10 cm in diameter) and stored at 4°C. The gels were evaluated for their textural properties by means of texture profile analysis. These were calculated automatically with the Blue-Hill 2 software.

Turbidity of starch suspensions
The turbidity of the acha starch suspension was measured as described by Perera and Hoover (1999). A 1% aqueous suspension of acha starch as well as the reference wheat starch was heated separately, in a water bath at 90°C for 1 h with constant stirring. The samples were then stored for 5 days at 4°C and turbidity was determined after 5 days by measuring the absorbance at 640 nm (UV-VIS Split Beam 8 Auto Cell Model UVS-2800 spectrophotometer Shanghai Jingke Scientific Instruments Co. Ltd. China) against a water blank.

Water binding capacity
Water binding capacity (WBC) of both the acha starch as well as the reference starch was measured as described by Medcalf and Gilles (1965). A suspension of 5 g starch in 75 ml distilled water was agitated for 1 h and centrifuged at 3000 rpm for 10 min. The free water was drained for 10 min and the drained wet starch was then weighed. The water binding capacity was then expressed as grams per hundred grams.

Pasting properties
The method of Holm et al. (1988) was used to determine the pasting properties of acha starch as well as the reference sample using a rapid visco analyzer (RVA 4 from Newport Scientific, Warriewood, Australia). The viscosity profiles of WTAS, BLAS and WHTS were recorded using starch suspensions of 9% (w/w, dry basis) and 500 g total sample weight. The starch suspensions were prepared and then transferred to a rapid visco analyzer (RVA4 from Newport Scientific, Warriewood, Australia). The capacity of the RVA was 50 – 50 000 cP at 80 rpm. A programmed heating and cooling cycle were used where the samples were held at 25°C for 1 min and heated to 95°C at 1.5°C/min, a holding at 95°C for 30 min before cooling from 95° to 50°C, 1.5°C/min and holding at 50°C for 2 min. Parameters recorded were peak
viscosity, trough viscosity, final viscosity, breakdown viscosity and setback viscosity. Analysis was conducted in triplicate.

Data analysis

Analysis of variance (ANOVA) was used to establish significant differences among treatments. Duncan's multiple range tests was used to separate means where significant difference existed (IBM SPSS 2010).

Results and Discussion

Physicochemical properties

Proximate composition of the starches

Table 1 details the proximate and amylose composition of the starches. The moisture content of WHTS, BLAS and WTAS was 11.0, 11.9 and 10.7% respectively. There was no significant difference between the WTAS and WHTS. The moisture content recorded for acha starches was slightly lower than that reported for two acha cultivars (Fonio Hothia and F. Koulli), 15 and 13% respectively. The moisture content for WTAS (10.7%) was in the same range as that reported for corn starch 10.1% (Gonzales-Reyes et al., 2003).

The protein content of WTAS (1.7%) and BLAS (1.2%) differed significantly (Table 1) from that of WHTS (0.5%). There was also significant difference in the protein content between WTAS (1.7%) and BLAS (1.2%). Protein content of acha starch reported in literature ranged between 0.3 and 0.7% for Hothia and Koulli, respectively (Carcea and Acquistucci 1997). Classical studies at the University of Illinois demonstrated that the variability of various chemical compounds such as the protein content in maize is of genetic, environment and cultural origin and that chemical composition can thus be changed through appropriate manipulation (FAO 2012). The protein content of starch is one of the critical characteristics when starch is used in the manufacture of glucose syrup. The lower the protein contents the better the chances of avoiding Maillard reaction (Gonzales-Reyes et al., 2003). Although the protein content of acha starch (1.2% for BLAS and 1.7% for WTAS) being significantly higher than that of wheat starch, the protein content is within the acceptable range in staches that would possibly avoid Maillard reaction.

There was no significant difference (p > 0.05) between WHTS and BLAS in terms of dietary fibre, 2.2% and 1.5% respectively (Table 1). There was however a significant difference between BLAS (1.5%) and WTAS (0.45%). The difference in dietary fibre could be attributed to genetic difference between acha cultivars. Dietary fibre recorded for WTAS
(0.45%) is higher than that reported in literature for lentil starches (0.21%), (Gonzales and Perez 2002).

The fat content of WTAS and BLAS (Table 1) differed significantly from that of WHTS. This difference could be due to their genetic differences. The fat content of BLAS (2.9%), WTAS (2.0%) and WHTS (1.8%) were higher than that reported for corn and Okenia starch, 0.17% and 0.1% respectively (Gonzales-Reyes et al 2003). Higher fat content could affect gelatinization due to the formation of amylose-lipid complexes (Gonzales-Reyes et al, 2003). The carbohydrate content of WTAS, BLAS and WHTS was 86.0, 86.0 and 88.0 g/100 g respectively. The carbohydrate content for WHTS and the two acha varieties did not differ significantly (p > 0.05).

There are no significant differences in amylose content between BLAS (42.1%), WTAS (61.4%) and WHTS (45.8%) [Table 1]. Lower amylose content was reported in literature for two other varieties of acha [Hothia (22.6%) and Koulli (26.1%)] (Carcea and Acquistucci 1997). In this instance the amylose content was measured in the presence of lipids which may complex with amylose and reduce its iodine binding capacity (Morrison, 1988). Taylor et al (1997) however reported amylose content of high pasting peak viscosity sorghum starch (35.7 to 36.9%) and 27.1 to 47.3% for low peak viscosity sorghum starch. Environmental effects may exert more influence on amylose content than genetic differences (Taylor et al., 1997). A negative correlation between glycemic index, resistance starch and amylose content of starch (Chung et al., 2008a) was noted. Higher amylose content may contribute to a lower hydrolysis index (HI) and estimated glycemic index (eGI).

**Functional properties of the starches**

**Viscosity**

Pasting properties of acha starch is indicated in Table 2. There were significant differences (p < 0.05) in peak viscosity and final viscosity between the two acha gels. Peak viscosity is the ability of starch to swell before their physical breakdown, an indication of the water binding capacity of starch (Ikegwu et al 2010). The peak viscosity was 3506 cP for WHTS, 3994 cP for BLAS and 4936 cP for WTAS. There was a significant difference (p < 0.05) in the peak viscosity between WTAS and BLAS. Acha starch had a higher peak viscosity than that reported in literature for corn starch (Sandhu and Singh 2007). The peak viscosity of various corn starches ranged between 804 and 1552 cP. The higher peak viscosity of acha starch is indicative of their higher water binding capacity and thus higher degree of starch swelling, an indication that acha starch may be good for products requiring high gel strength.
and elasticity. Trough viscosity is the measurement of the holding strength of the starch paste before it breaks down and viscosity decreases. This depends on the temperature and degree of mixing or shear stress. The trough viscosity for two acha starch varieties was similar, 3125 cP and 3137 cP for BLAS and WTAS. There was however a significant difference between the two acha starch varieties and wheat starch trough viscosity, 1960, 3125, 3137 cP for WHTS, BLAS and WTAS, respectively. This significant difference (p < 0.05) in trough viscosity implies a difference in the paste holding strength of wheat starch and the two acha starch varieties. The holding strength of BLAS and WTAS were similar while the holding strength of WHTS paste is much lower than the two acha starch paste varieties. This signifies that acha starch gels may be the better option for products requiring high holding strength without breaking down. The trough viscosity are much higher than the values recorded for African Tall (662 cP) and Ageti (652 cP) corn starch (Sandhu and Singh 2007).

The break down viscosity for WHTS, BLAS and WTAS were 369, 869, 2976 cP respectively. The break down viscosity for WTAS was significantly higher (p < 0.05) than those of WHTS and BLAS. Breakdown viscosity is the measure of disintegration of cooked starch. It is the difference between the peak viscosity and the trough viscosity. The higher the breakdown viscosity; the lower the ability of the sample is to withstand heating and shear stress during cooking (Adebowale et al. 2004). This indicates that WTAS being higher in break down viscosity will have a lower ability to withstand heating and shear stress during cooking.

The final viscosity for BLAS was 5129 cP, WTAS 5470 cP and WHTS 4436 cP. The final viscosity, which is the measure of the ability of starch to form a viscous paste for WTAS, was significantly higher (p < 0.05) than those of BLAS and WHTS. This difference could be due to the difference detected in the peak time and pasting temperatures of all three starches, WTAS (6.7 min, 85.9°C), BLAS (5.7 min, 80.2°C) and WHTS (4.2 min, 78.0°C). The higher pasting temperatures for acha starches indicated the higher resistance of their starches to swelling. The increase in viscosity with temperature may be contributed by the removal of water from the exuded amylose as the starch granules swell (Sandhu & Singh, 2007).

The setback viscosity is the measure of the degree of syneresis of starch upon cooling of the cooked starch paste, for WTAS, BLAS and WHTS were 2476 cP, 2332 cP and 2004 cP respectively. There was no significant difference between the acha starches in setback viscosity. However, the WHTS was significantly lower (p < 0.05) in setback viscosity compared to the acha starches. The high setback values for acha starches make them
unsuitable for use where low syneresis rate is required, such as in frozen or refrigerated
foods. The setback values are also indicative of the retrogradation tendency of starch gels.
The higher the setback viscosity the lower the retrogradation during cooling of the products
made from the flour (Ikegwu et al. 2010). This implies a significant difference in
retrogradation tendency between wheat starch and the two acha starch varieties. Acha
starches have lower retrogradation tendencies during cooling compared with wheat starch,
suggesting that the degree of re-association of the wheat starch molecules was higher than
that of acha starch molecules, upon cooling. Sandhu and Singh (2007) reported that the
pasting properties of starch depend upon various factors such as the rigidity of starch
granules, which in turn affects the degree of swelling of the starch granules.

**Turbidity and water binding capacity (WBC) of the starches**

The turbidity for WHTS, BLAS and WTAS are reported in Table 3. The values were 0.2097
NTU, 0.0003 NTU and 0.0193 NTU, respectively for WHTS, BLAS and WTAS. There was
significant difference ($p < 0.05$) in the turbidity of the starches, with WHTS being the highest
followed by WTAS and lastly BLAS. The turbidity of starch in foods is important when used
as thickener in sauces, as a carrier of flavours in beverages or as a suspending agent in liquid
foods. The higher the NTU value the lower the clarity of the suspension. This signifies that
both acha starch varieties are clearer than wheat starch, and would be the better option to use
in products where starch clarity is required. Sandhu and Singh (2007) reported that turbidity
development of starches is affected by inter-related factors such as starch granule swelling,
leached amylose and amylopectin, amylose and amylopectin chain length.

There were significant differences in the WBC (Table 3) between WHTS, BLAS and
WTAS (0.83, 1.33 and 1.36 g/100g, respectively), whereas there were no significant ($p >
0.05$) differences between BLAS and WTAS. This difference in WBC can be due to
structural differences in starch and proteins of the starches (Celik et al. 2005). Different
proportions of crystalline and amorphous regions within the granules may be the result of the
variations in WBC. Thus weakly bonded amorphous starch granules will imbibe less water
(Carcea and Acquistucci, 1997). It appears that acha starch granules possess stronger bonded
amorphous granules compared to wheat starch. The WBC of the starch affects important
physical attributes such as the viscosity of sauces and batters and the texture of baked
products. Sandhu and Singh (2007) reported WBC for starches from different corn varieties
in the range of 0.82 to 0.97 g/100 g. The water binding capacity for acha starch is higher than
the values reported by Sandhu and Singh (2007). Acha is known to swell far more than other
cereals (Carcea and Acquistucci, 1997). Acha starch can thus be included as part of the ingredients in the manufacturing of sauces, batters, dough’s and baked products. It can be used as thickener, bulking agent and most importantly due to its high WBC as water retention agent.

Textural properties of the starches

The textural characteristics of the acha and wheat starches are indicated in Table 4. The hardness for WTAS, BLAS and WHTS were 0.9367 N, 0.9467 N and 0.9500 N, respectively. No significant difference in gel hardness was observed between BLAS gel and WTAS gel or between the WHTS and the two acha starch gels. These low hardness values indicate that both acha and wheat starches produced soft gels. Acha starch would thus be suitable for manufacturing of foodstuff where soft gels are required. Sandhu and Singh (2007) reported that the gel firmness is mainly caused by retrogradation which is associated with crystallization of amylopectin, leading to harder gels. The resilience value is the first force before the maximum force (F) which is the capability of the gel to return to an original shape or position after having been compressed. The resilience for BLAS and WTAS and WHTS gels were 1.1994, 1.2399 and 1.2402, respectively. There was no significant difference (p > 0.05) in resilience between the gels. Springiness for BLAS gel was 0.7090 mm, WTAS gel was 0.7090 mm and WHTS was 0.3746 mm. There was no significant difference (p > 0.05) in the springiness of both acha starch gels and WHTS gels. Springiness refers to the degree of gel rubberiness in the mouth (Lau et al. 2000). A high degree of springiness is depicted by gel structure that breaks into large pieces during the initial compression; whereas a low degree of springiness is represented by a gel that breaks into smaller pieces during the initial compression test (Huang et al. 2007). The starch gels broke into large pieces during compression. Hence, both acha and wheat gels are highly springy. Highly springy gels would not break down easily during mastication (Lau et al., 2000). Acha starch gels were springy and resilient. This indicates that both acha starch varieties can be used for hot and cold desserts as well as for soft jelly like sweets and confectionery toppings.

Thermal properties of acha starch

Gelatinization of acha starch

The gelatinization temperatures (To, Tp, and Tc) and enthalpy (ΔH) of wheat and acha starches are shown in Table 5. There was no significant difference (p > 0.05) in peak and onset temperature between the acha and wheat starches. WHTS had the highest onset (Tc)
and peak (Tp) temperatures 34.8°C and 74.6°C, although not significant. WTAS and BLAS were 25.1, 26.8°C and 68.2, 70.8°C for To and Tp respectively. Acha starches and the wheat starch differed significantly (p < 0.05) in conclusion Tc and Tp temperatures. For WHTS it was 96.0°C, BLAS was 87.8°C and for WTAS was 93.7°C. The gelatinization temperatures of waxy maize starch were 59.9, 69.1 and 78.1°C for To, Tp and, Tc respectively (Miao et al 2009). The onset gelatinization temperature of acha starch is much lower than that of waxy maize starch. This means that less energy is needed to induce gelatinization of acha starches. The peak gelatinization temperature of acha starch is the same as that reported in literature (Miao et al 2009). Tester and Morrison (1990) reported that due to the structural differences in amilopectin, starches with the low gelatinization temperature has less crystallinity than the high gelatinization temperature starches, this was attributed to structural differences in their amilopectins. Hence, acha starches may have lower degree of crystallinity compared to wheat starch. Ezekiel et al (2007) reported that low transition temperatures of between 50-67°C was due to the lower crystallinity, meaning that the higher the transition temperatures the higher the degree of crystallinity. This would lead to structural stability causing the starch granules to have a higher degree of resistance to gelatinization. Acha starches have low transition temperature which implies a low crystallinity and high amorphous regions (Miao et al 2009). The gelatinization temperature range (Tc – To) for WTAS and BLAS was 66.9 and 62.8°C respectively, whereas that of WHTS was 58.3°C. The similarity of the gelatinization temperature range amongst the starches could be attributed to possible similarity in protein content and starch structure. The melting enthalpy (ΔH) of gelatinization of BLAS was 188.41 J/kg and WTAS was 691.6 J/kg whereas that of WHTS was 213.21 J/kg. The melting enthalpy values for WTAS and BLAS differed significantly (p < 0.05). The melting enthalpy for both acha starch varieties as well as WHTS was higher than that reported for waxy maize starch (Chung et al 2008b). The higher melting enthalpy could be due to the difference in the alignments of the hydrogen bonds in the starch molecules. This is caused by the difference in the bonding forces between the double helices that form the amilopectin crystallites (Sandhu and Singh 2007). Sandhu and Singh (2007) reported melting enthalpy values between 11200 and 12700 J/kg for various corn starches. This is much higher than the melting enthalpy of acha starch.

Retrogradation properties of acha starch

The retrogradation of acha starch compared to that of wheat starch is summarised in Table 6.
Retrogradation is the hydrogen bonding between starch chains that occurs after cooling of gelatinized starch paste (Hoover and Zhou 2003). The transition temperatures of retrogradation for both acha starches and WHTS are much lower than the transition temperatures of gelatinization. The onset temperature for WHTS, WHTS and BLAS were 19.97, 17.58 and 19.05°C respectively. WHTS had the highest onset temperature followed by BLAS and WTAS. However, the differences were not significant (p > 0.05). Sandhu and Singh (2007) reported onset temperatures for retrogradation in the range between 41.50 and 43.10°C for two corn starch varieties. These values are much higher than that recorded for white acha and black acha starches. According to Sandhu and Singh (2007) this could be due to a less organized manner of recrystallization of the amylopectin branched chains of the gels. The lower onset temperature for the acha starches could be due to weaker molecular bonding of the starch chains.

Values for peak temperature of retrogradation were 64.13, 67.05 and 64.84°C for WHTS, WTAS and BLAS, respectively. There was no significant difference (p > 0.05) between the starches in peak temperatures. Literature values for different corn varieties varied between 52.40 and 54.50°C for African Tall corn starch and Partap corn starch respectively. These values were much lower than that recorded for white acha and black acha starch. Higher peak temperatures of acha starches could be indicative of the need for higher thermal energy for retrogradation.

Retrogradation enthalpy is an indication of the disentanglement and melting of the double helices formed during cooling (Adebowale et al 2004). The retrogradation enthalpy (ΔH) for WTAS was 37.61 J/kg and for BLAS was 118.00 J/kg. There was a significant difference between BLAS and WTAS, with BLAS being higher than that of WTAS. No significant difference was observed for retrogradation enthalpy between WHTS and WTAS. The differences in the retrogradation enthalpy could be due to the difference in the degree of starch crystallinity of the retrograded starches (Sasaki et al 2000).

The conclusion melting temperature values for WHTS, WTAS and BLAS were 94.73°C, 94.82°C and 95.95°C respectively. There was no significant difference in conclusion temperature of retrogradation between the acha starches. Whenever a material undergoes a change in physical state for example melting, or transforms from one crystalline form to another, or when it reacts chemically, heat is either absorbed (endothermic) or liberated (exothermic) (Karim et al 2000). Wheat and both acha starch cultivars presented an endothermic transition (retrogradation). Retrogradation has been identified as the dissociation of the amylopectin crystallites (Gonzales et al 2002). The retrogradation
phenomenon is a process of re-crystallization of the starch polymers (Gonzales-Reyes et al., 2003). In the case of retrograded starch, $\Delta H$ (J/kg) provides a quantitative measure of the energy transformation that occurs during the melting of recrystallized amylopectin as well as precise measurement of the transition temperatures (onset, $T_o$; peak, $T_p$ and conclusion (end), $T_c$) (Karim et al 2000). There is a significant difference in the $\Delta H$ and peak height values between white and black acha starches. There were however no significant difference amongst the retrogradation temperature profile ($T_o$, $T_p$ and $T_c$) for both white and black acha starch.

**Conclusion**

Acha starches differ in moisture, dietary fibre and carbohydrate content and are similar to wheat starch in amylose content. Water absorption capacity of acha starches were higher than that of wheat starch and hence acha starch can be included as part of the ingredients in the manufacturing of sauces, batters, and dough’s and baked products. It can be used as thickener, bulking agent and most importantly due to its high WBC as water retention agent. Acha starch gels were springy and resilient indicating its potential use in hot and cold desserts as well as for soft jelly like sweets and confectionery toppings. Acha starch cultivars and wheat starch are similar in thermal properties. Acha starch has similar retrogradation temperature profiles as that of wheat starch. Wheat and both acha starch cultivars presented an endothermic transition (retrogradation).

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Table 1. Proximate and amylose composition of acha starch\(^1, 2\)

<table>
<thead>
<tr>
<th>Starch</th>
<th>Moisture</th>
<th>Fat</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Amylose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>11.0 ± 0.00(^a)</td>
<td>0.5 ± 0.00(^a)</td>
<td>88.0 ± 0.00(^a)</td>
<td>0.47 ± 0.47(^a)</td>
<td>45.8 ± 16.56(^a)</td>
</tr>
<tr>
<td>Black acha</td>
<td>11.9 ± 0.25(^b)</td>
<td>0.8 ± 0.00(^a)</td>
<td>86.0 ± 0.00(^a)</td>
<td>1.23 ± 1.23(^b)</td>
<td>42.1 ± 16.35(^a)</td>
</tr>
<tr>
<td>White acha</td>
<td>10.7 ± 0.26(^a)</td>
<td>1.3 ± 0.00(^a)</td>
<td>86.0 ± 0.00(^a)</td>
<td>1.73 ± 1.73(^c)</td>
<td>61.3 ± 15.46(^a)</td>
</tr>
</tbody>
</table>

\(^1\)Values are mean ± standard deviation.

\(^2\)Any two means with different superscript in each column differ significantly (p < 0.05).
Table 2. Pasting properties of Acha starch$^1,2$

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak1 (cp)</th>
<th>Trough1 (cp)</th>
<th>Breakdown Viscosity (cp)</th>
<th>Final Viscosity (cp)</th>
<th>Setback Viscosity (cp)</th>
<th>Peak Time (min)</th>
<th>Pasting Temp. ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>3506 ± 16$^a$</td>
<td>1960 ± 41$^a$</td>
<td>369 ± 21$^a$</td>
<td>4436 ± 82$^a$</td>
<td>2004 ± 62$^a$</td>
<td>4.18 ± 0.04$^a$</td>
<td>78.03 ± 0.46$^a$</td>
</tr>
<tr>
<td>Black acha</td>
<td>3994 ± 36$^b$</td>
<td>3125 ± 15$^b$</td>
<td>869 ± 34$^b$</td>
<td>5129 ± 60$^b$</td>
<td>2332 ± 54$^b$</td>
<td>5.78 ± 0.10$^b$</td>
<td>80.15 ± 0.44$^b$</td>
</tr>
<tr>
<td>White acha</td>
<td>4936 ± 58$^c$</td>
<td>3137 ± 6$^c$</td>
<td>2976 ± 95$^c$</td>
<td>5470 ± 50$^c$</td>
<td>2476 ± 190$^b$</td>
<td>6.69 ± 0.03$^c$</td>
<td>85.88 ± 0.49$^c$</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard deviation.

$^2$Any two means with different superscript in each column differ significantly (p < 0.05)
Table 3  Turbidity and water binding capacity of acha starch\(^1,2\)

<table>
<thead>
<tr>
<th>Starch</th>
<th>Turbidity (NTU)(^3)</th>
<th>Water binding capacity (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black acha</td>
<td>0.0003 ± 0.001(^a)</td>
<td>1.333 ± 0.06(^b)</td>
</tr>
<tr>
<td>White acha</td>
<td>0.0193 ± 0.002(^b)</td>
<td>1.367 ± 0.06(^b)</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.2097 ± 0.009(^c)</td>
<td>0.833 ± 0.23(^a)</td>
</tr>
</tbody>
</table>

\(^1\)Values are mean ± standard deviation.

\(^2\)Any two means with different superscript in each column differ significantly (p < 0.05).

\(^3\)NTU = Nephelometric turbidity units
Table 4. Textural characteristics of acha and wheat starch gels $^{1,2}$

<table>
<thead>
<tr>
<th>Cereal starch</th>
<th>Hardness (N)</th>
<th>Resilience (ratio)</th>
<th>Springiness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White acha</td>
<td>$0.936 \pm 0.041^a$</td>
<td>$1.199 \pm 0.090^a$</td>
<td>$0.709 \pm 0.112^a$</td>
</tr>
<tr>
<td>Black acha</td>
<td>$0.946 \pm 0.015^a$</td>
<td>$1.239 \pm 0.087^a$</td>
<td>$0.709 \pm 0.132^a$</td>
</tr>
<tr>
<td>Wheat</td>
<td>$0.950 \pm 0.010^a$</td>
<td>$1.240 \pm 0.033^a$</td>
<td>$0.374 \pm 0.273^a$</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard deviation.

$^2$Any two means with different superscript in each column differ significantly ($p < 0.05$).
Table 5 Gelatinization of acha starch\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Starch</th>
<th>To (°C)</th>
<th>Tp (°C)</th>
<th>Tc (°C)</th>
<th>$\Delta H_{gel}$ J/kg</th>
<th>$\Delta T_T$ (Tc - To)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>34.7 ± 25.20\textsuperscript{a}</td>
<td>74.56 ± 0.97\textsuperscript{a}</td>
<td>96.03 ± 0.49\textsuperscript{b}</td>
<td>213.21 ± 169\textsuperscript{a}</td>
<td>58.33 ± 24.71\textsuperscript{a}</td>
</tr>
<tr>
<td>White acha</td>
<td>25.05 ± 12.12\textsuperscript{a}</td>
<td>68.20 ± 1.64\textsuperscript{a}</td>
<td>93.67 ± 0.57\textsuperscript{b}</td>
<td>188.42 ± 250\textsuperscript{b}</td>
<td>66.92 ± 11.55\textsuperscript{ab}</td>
</tr>
<tr>
<td>Black acha</td>
<td>26.75 ± 7.56\textsuperscript{a}</td>
<td>70.54 ± 6.07\textsuperscript{a}</td>
<td>87.85 ± 2.15\textsuperscript{a}</td>
<td>691.04 ± 106\textsuperscript{c}</td>
<td>62.8 ± 5.41\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Values are mean ± standard deviation
\textsuperscript{2}Any two means with different superscript in each column differ significantly (p < 0.05). To, onset temperature; Tp, peak temperature; H1, peak height; $\Delta H$, enthalpy of gelatinization; Tc, end (conclusion) temperature, $\Delta T_T$ gelatinization temperature range (Tc - To)
Table 6 Retrogradation of cereal starch

<table>
<thead>
<tr>
<th>Starch</th>
<th>To (°C)</th>
<th>Tp (°C)</th>
<th>Tc (°C)</th>
<th>$\Delta H_{retro}$ J/kg</th>
<th>$\Delta T = (T_c - T_o)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>17.97 ± 0.02</td>
<td>64.13 ± 5.35</td>
<td>94.73 ± 1.78</td>
<td>56.87 ± 32.62</td>
<td>76.76 ± 1.76</td>
</tr>
<tr>
<td>White acha</td>
<td>17.58 ± 0.48</td>
<td>67.06 ± 4.58</td>
<td>94.83 ± 1.48</td>
<td>37.61 ± 12.88</td>
<td>77.25 ± 1.00</td>
</tr>
<tr>
<td>Black acha</td>
<td>19.05 ± 1.30</td>
<td>64.84 ± 0.53</td>
<td>95.95 ± 0.52</td>
<td>118.00 ± 40.58</td>
<td>76.90 ± 0.78</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.

Any two means with different superscript in each column differ significantly ($p < 0.05$). $T_o$, onset temperature; $T_p$, peak temperature; $H_1$, peak height; $H$, $\Delta H$, enthalpy of gelatinization; $\Delta H_f$, $T_c$, end (conclusion) temperature.