CHARACTERIZATION OF FLOUR AND STARCH FROM ZAMBIAN CASSAVA CULTIVARS AND APPLICATION IN FROZEN WHEAT BREAD DOUGH

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PREFACE

The research contained in this dissertation was completed by the candidate while based in the Discipline of Bioresources Engineering (Biosystems), School of Engineering, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

ABSTRACT

The utilization of cassava flour is increasing in Africa. However, increased diversity of cassava genetic factors accounts for differences in the end-user properties of cassava roots. This would require characterization of cassava varieties to determine the suitability for processing and culinary use. Six different cassava varieties: one local landrace (*Katobamputa*), five improved and officially released varieties (*Bangweulu, Mweru, Kariba, Kampolombo* and *Chila*) cultivated in Zambia were characterized for physicochemical, functional and rheological properties of starch and flour, and application of cassava flour in frozen wheat breads dough. The chemical composition and cyanide contents were determined using standard methods of AOAC, respectively, while particle size was determined using standard sieving method. The microscopic morphologies and granule size of starches were examined using scanning electron microscopy and image analysis methods, while crystallinity was determined using powder X-ray diffractometry method. The amylose and resistant starch content were determined using a Megazyme starch assay procedure. The swelling power was investigated using dispersion methods, while gelatinization and pasting were determined using differential scanning calorimetry and rapid visco analyser, respectively. The gel freeze-thaw stability was determined using water syneresis analysis. Dough rheology and bread baking were determined using the Brabender Farinograph and straight-dough method, respectively. Dough stickiness was determined using a Texture Analyzer (TA-XT2)**.** The percentage moisture, protein, lipid, ash and fiber contents of the cassava flour varieties ranged from 10.43-11.18, 1.21–1.87, 0.15–0.63, 1.21-1.78, and 0.03-0.60%, respectively. The flour particle size distribution at 90% (D90) and 10% (D10) cumulative of particles of finer particles than sieve were in the range 250.44-334.34 and 35.56-48.52 μm, respectively, and varied ($p<0.05$) among varieties. The cassava root dry matter contents and starch extraction yields ranged from 40.04–47.25% and 20.76-28.31%, respectively. The cassava cyanide contents for roots and flours were in the range 23.60-238.12 and 8.62-15.48 mg $HCN.kg^{-1}$, respectively. On processing of cassava roots to cassava flours, the cyanide reduction in the range 60.76-93.86% was observed. The cassava starch granules were rounded, truncated and oval granule in shape. The granule size was in the range 7.91-10.19 μm. The cassava starch crystallinity ranged from 31.06-33.40% and showed type A X-ray diffraction patterns. One factor experiment was designed to determine the variety effect on the composition of starch. The results indicated that amylose content (16.04-26.95%) varied ($p<0.05$) among cassava varieties, and negatively correlated ($p<0.01$) with

crystallinity. The resistant starch (RS) content in the starch (1.12-4.14%) and flour (1.36-5.21) varied ($p<0.05$) among varieties. The non-resistant starch (digestible starch) contents were 66.14-78.33% and 64.35-70.20% for starches and flours, respectively. A two-factor experiment was designed to investigate variety and temperature effect on swelling properties of starch and flour. The peak swelling powers were ranged 2.22-15.63 $g.g^{-1}$ and 2.77-13.00 $g.g^{-1}$ for starches and flours, respectively. The onset gelatinization temperature (T_o) for starches (56.33-63 ^oC) and flours (63.2-68.13 ^oC) varied among varieties ($p<0.05$). The peak gelatinization temperatures were in the range 62-71.29 ^oC and 70.53-74.20 ^oC for starches and flours, respectively. The conclusion gelatinization temperature ranged 69.1-77.12 ^oC and 75.47-81.17 ^oC for starches and flours, respectively. The onset, peak and conclusion gelatinization temperatures positively correlated with amylose contents ($r = 0.530$, $p<0.05$; $r = 0.360$, $p<0.01$; and $r = 0.292$, $p<0.001$, respectively). The pasting temperatures were in the range 67.19-74.35 ^oC and 69.33-71.33 ^oC, for starches and flour, respectively. The peak viscosity of starch (782.3-983.5 cP) and flour (651.9-910 cP) varied among varieties ($p<0.05$). The syneresis for freeze-thaw storage ranged from 0.00-29.11% while for the five freeze-thaw cycles were in the range 0.00-42.40%, and significantly varied ($p<0.05$) among varieties. A two-factorial experiment consisting of cassava variety (CV) and cassava flour substitution level (CFSL) (10-30%) was designed. The effect of CV and CFSL on wheat dough development were investigated in regular and frozen dough conditions, and subsequently on baked bread quality characteristics and amylolytic digestibility. The results indicated that CV and CFSL had a significant effect ($p<0.05$) on gluten contents (6.88–13.00%) of flour blends, whereas only CFSL influenced water absorption capacity (WAC) (59.57–61.70%) significantly ($p<0.05$). Development time and stability time of dough were in the range 1.53–10.60 min and 6.27–12.27 min. Bread volume (91.67–148.17 cm^3) and specific volume (1.49–2.46 $g.cm^{-3}$) varied significantly ($p<0.05$) with CV and CFSL, and correlated positively ($r = 0.78$, $p<0.05$) and ($r = 0.76$, $p<0.05$) with gluten, respectively. The particle size of cassava flour was negatively correlated with WAC ($r = -0.26$), dough stability time ($r = -0.51$, $p<0.05$), weight loss ($r = -0.50$, $p<0.05$), bread density ($r = 0.67$, $p<0.05$), bread specific volume ($r = -0.72$, $p<0.05$) and bread volume ($r = -0.68$, $p<0.05$). The CV and CFSL had a significant ($p<0.05$) influence on the stickiness of unleavened (34.14-122.17 g), leavened (13.53-83.94 g) and leavened frozen (126.88-146.82 g) dough. Irrespective of CV and CFSL, the frozen dough had the highest stickiness. Gluten content and WAC had a significant ($p<0.01$) negative influence on stickiness ($r=-0.44$ and -0.44, respectively). The bread loaf volume from flour blends of frozen doughs: frozen-before-proofing (68.67-105.00

cm^3) and frozen-after-proofing (67.67-101.67 cm^3) were significantly lower than the volume of the bread (91.67-140 cm^3) from fresh dough. The bread volume loss for wheat bread from frozen-before-proofing and frozen-after-proofing were 29.94±6.70% and 46.76±2.99%, respectively. The bread volume loss for composite bread made from frozen-before-proofing and frozen-after-proofing were in the range 0.81-40.47% and 4.52-27.04%, respectively. The resistant starch (RS) in wheat-cassava blends were ranged from 1.98-6.90% and 4.24-5.63%, for bread baked from fresh and frozen doughs, respectively. The RS in wheat bread was 4.29% and 6.84%, for fresh and frozen condition, respectively. The digestible starch for frozen dough bread were in the range 81.50-92.64, 76.77-93.86, and 77.85-93.98 %, at 10, 20 and 30% CFSL, respectively. The study showed that the variations in physicochemical properties of cassava varieties were due to differences in amylose, protein, and lipid contents, and starch granule and flour particle size distribution. The high percentage cyanide reduction on processing cassava varieties into flours suggests suitability for human consumption and application in food processing. All cassava varieties exhibited maximum swelling power in the temperature range 60-70 ^oC, indicative of slightly low starch gelatinization temperature as compared to tropical cereal grain starches but are slightly higher than temperate cereal grain starches. The variety *Katobamputa* and *Mweru* were distinguished by smaller flour particle size distribution. Moreover, *Katobamputa* showed low dry matter content, low cyanide content and showed high resistant starches content. The flour particle size, WAC, and gluten content are significant flour properties influencing dough rheology and bread quality. As CFSL increased in the blend the visco-elastic properties of the dough decreased due to wheat gluten dilution. The study showed that wheat can be substituted with cassava flour from cassava varieties *Mweru*, *Kariba* and *Katobamputa* in bread making up to a level of 10%, without affecting bread quality negatively. Nevertheless, the frozen doughs of frozen-after-proofing for *Mweru*, *Kariba*, and *Katobamputa* showed the highest stickiness and yielded the lowest bread volume. The composite frozen doughs for frozen-before-proofing at 20 and 30% CFSL, across varieties yielded intermediary bread quality between that of fresh and frozen-after-proofing doughs.

Keywords: Amylose, bread digestibility, bread quality, cassava, composite flours, dough rheology, farinograph, flour, freeze-thaw stability, frozen dough, gelatinization, particle size, pasting, proximate composition, resistant starches, starch, stickiness, swelling, wheat

DECLARATION AND PLAGIARISM

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DECLARATION OF PUBLICATIONS

Published papers

Chisenga, SM, Workneh, TS, Bultosa, G and Alimi, BA. 2019. Progress in research and applications of cassava flour and starch: a review. *Journal of Food Science and Technology*, 56(6): 2799-2813. DOI: https://doi.org/10.1007/s13197-019-03814-6

Chisenga, SM, Workneh, TS, Bultosa, G. and Alimi, BA. 2019. Effects of cassava flour on the stickiness properties of wheat bread dough: unleavened, leavened and frozen dough. *Acta agriculturae Slovenica*, 114(1): 33-46. DOI: http://dx.doi.org/10.14720/aas.2019.114.1.4

Chisenga, SM, Workneh, TS, Bultosa, G and Laing, M. 2019. Proximate composition, cyanide contents, and particle size distribution of cassava flour from cassava varieties in Zambia. AIMS Agriculture and Food, 4(4), p.869-891. DOI: 10.3934/agrfood.2019.4.869.

Chisenga, SM, Workneh, TS, Bultosa, G and Laing, M. 2019. Characterization of physicochemical properties of starches from improved cassava varieties grown in Zambia. AIMS Agriculture and Food, 4(4): 939–966. DOI: 10.3934/agrfood.2019.4.939.

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Chisenga, SM, Workneh, TS, Bultosa, G, Alimi, BA and Siwela M. 2019. Dataset for cassava flour particle size distribution and bread quality characteristics. *Food Bioscience* (FBIO_2018_1050_R2) – Elsevier.

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Chisenga, SM, Workneh, TS, Bultosa, G and Alimi, BA. 2019. Morphologies, Crystallinity, color and proximate analysis of cassava starches from improved cassava (Manihot esculenta Crantz) varieties. *Acta Agriculture Slovenica.* Under review with manuscript number 1059-6499-2-SM.

Manuscript prepared and ready for submission to Journals

Chisenga, SM, Workneh, TS, Bultosa, G and Alimi, BA. 2019. Effect of frozen storage on wheat-cassava dough and bread quality.

Chisenga, SM, Workneh, TS, Bultosa, G and Laing M. 2019. Composition of starch: amylose and resistant starch contents of improved cassava varieties grown in Zambia.

Paper presented at international conference

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In all the above papers and manuscripts, the conceptualization of the idea was done by SM Chisenga (student), and TS Workneh and G Bultosa (supervisors). SM Chisenga conducted the research and the write up while the supervisors corrected and proof-read the manuscripts.

Prof Mark Laing, Prof Siwela and Dr Alimi contributed to the manuscripts by proof-reading the text materials on genetic factors/traits, bread quality and rheological properties, respectively. The supervisors gave the final concurrence to the manuscript.

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To my wife Nadine, my daughter Muweme, my sons Southall and Northall

SUPERVISORS' APPROVAL

Subject to the regulation of the School of Engineering, we the supervisors of the candidate consent to the submission of this dissertation for examination.

Supervisor: _____ Date:……/……../2019

Prof Tilahun Seyoum Workneh

Co-Supervisor:_____ Date:……/……./2019

Prof Geremew Bultosa

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1. INTRODUCTION

1.1 Introduction

Cassava (*Manihot esculenta* Crantz) is a staple food for over 800 million people in the tropics (Howeler *et al.*, 2013). Cassava root flour is typically a carbohydrate material predominantly comprising of starch (Chiwona-Karltun *et al.*, 2015). Among the starches, including from main cereal crops, cassava is the highest producer of carbohydrates per hectare and can be grown at a considerably lower cost (Emmanuel *et al.*, 2016; Tadesse *et al.*, 2016). Cassava derived raw material of domestic and commercial importance includes flours and starches. Application of cassava flour in product development and food formulations is guided by end-use properties such as composition, physicochemical and functional properties of cassava starch (Omodamiro *et al.*, 2007). However, much research attention on physicochemical and functional properties has been given to rice, wheat, barley, maize, and Irish potato starches. Thus, cereals and potato continue to dominate world markets for starches in food and non-food industries. There is an increasing industrial demand for starches with a global demand of 182 million $t.yr^{-1}$ (Jin *et al.*, 2018). Considering the increased applicability of starch in food systems applications (Abass *et al.*, 2016), alternative sources of cheap starches should be investigated for physicochemical and structural properties. Proximate contents (moisture, protein, lipids, fiber and ash), color, dry matter, starch yield, and cyanide contents are some of the primary quality indicators for selection of cassava root raw materials in the food industry.

Starch granules consist of two main types of glucans, amylose, and amylopectin (Pérez *et al.*, 2010). The proportion and amylose and amylopectin glucans structures fundamentally influence the physicochemical properties of starches and starch based foods. The physicochemical properties of flour starches include swelling, solubility, gelatinization, freeze-thaw stability, pasting, gelation, retrogradation and resistant starches. The physicochemical properties are affected by chemical composition and structural characteristics of starch granules (Mtunguja *et al.*, 2016). The composition include amylose/amylopectin ratio, lipids, proteins, ash, fiber, phosphorus and other trace elements that may be associated with the starch granules (Eleazu and Eleazu, 2012; Mbougueng *et al.*, 2012; Somendrika *et al.*, 2016). Whereas the structural properties include starch granule shape, granule size distribution, degree of polymerization, degree of crystallinity and

molecular weight, chain length of amylopectin and amylose (Rolland-Sabaté *et al.*, 2013; Zhu, 2015). It is worth noting that the behavior of cassava flour in water and food system is the function of the physicochemical properties of cassava starches. The starch granule is a primary unit for physicochemical changes in the whole flour system. The variation in starch composition is the reason that relates to diversity in starch properties of different genotypic sources of cassava.

Native cassava flours and starches have limited applications. Thus, cassava flours are blended with other starches/flours to widen and improve their utilization (Zhu, 2015; Abass et al., 2016). The composite flours of cassava-wheat flour for bread making have been investigated on baking characteristics (Abass et al., 2016). Nevertheless, the increased number of new improved cassava varieties would require that their suitability for processing are evaluated on a variety basis. Moreover, investigations on cassava flour and starches rheological properties are limited, particularly for the cassava varieties grown in Zambia. When cassava flour is used for bread making, the blended dough mixing properties and stickiness would require characterization on the management of machinability and handling of the dough. Some cassava starches were reported to exhibit low syneresis after frozen storage and thus possessed the potential property for formulating frozen or refrigerated foods (Sánchez *et al.*, 2010). One such food formulation is the possibility to incorporate cassava flour in the frozen wheat dough for bread making.

In Zambia, cassava crop play an important role in contributing to food security and the most important staple crop after maize (Haggblade *et al.*, 2012). The current national cassava strategy is focused on developing a viable cassava industry to contribute toward wealth creation and food security for improved livelihoods. Improvement of cassava through breeding is the Zambian Government's agricultural priority and the breeding objective is to produce high yielding, early bulking, pests and disease resistant varieties (Chikoti *et al.*, 2016), including breeding for reduced cyanide content in cassava (Dixon *et al.*, 1994) to produce sweet varieties (Sarkiyayi and Agar, 2010). Thus, there are nine cassava varieties that have been bred and released in Zambia. However, information on the composition, structural, functional and physicochemical properties of flours and starches derived from cassava varieties grown in Zambia are limited. There is a need to characterize the common cassava varieties for end-use properties and suitability for food product development and formulations. Documentation and cataloguing on properties of technological importance will

form a baseline of information to enhance the selection of the most appropriate cassava flours and starches to meet the needs of cassava end-users (Chiwona-Karltun et al., 2015). The characterization work will feed back into breeding programs to generate suitable varieties for increased farmer-adoption. The inclusion of cassava flour into wheat in bread making is an important area towards the sustainable utilization of cassava. Thus, the present work was undertaken to evaluate variety- factor effects on physicochemical properties of starches, and application of flours from local landraces and officially released improved cassava varieties cultivated in Zambia.

1.2 Problem statement

The consumption of cassava flour and starch is increasing in Zambia, eliciting interest in brewing and composite flour (cassava-wheat) bakery industry. The breeding objectives on cassava have generated several varieties for increased yields, disease resistant, early bulking, and improved nutrition. However, there is limited information on composition, structural, functional and physicochemical properties of flour and starch derived from cassava varieties grown in Zambia. The inclusion of cassava flour into wheat in bread making is an important area towards the sustainable utilization of cassava. Wheat-based bread is widely consumed in Zambia and ranks third after maize and cassava in terms of supplying daily caloric intake. Nevertheless, the behavior and interactions of cassava flour and starches in blends with wheat flour composition in freezing and frozen conditions have not been investigated on the cassava-derived products of the Zambian cultivars. While cassava flour has been used in the formulation of cassava-wheat composite flour, the impact of freezing and frozen storage on cassava-wheat based bread dough are limited.

1.3 Research questions

The research work on the characterization of cassava flours and starches from Zambian cultivars and application in the frozen wheat-based dough was based on the following research questions:

1. What is the variety effect on the chemical composition, cyanide contents and particle sizes of flours from Zambian cassava cultivars?
2. What is the variety effect on composition, structural, and physicochemical properties of extracted starches from Zambian cassava cultivars?

3. What is the variety effect on composition, structural, physicochemical and end-use properties of cassava starches?
4. What is the variety effect on the performance of cassava flour inclusions in the frozen wheat dough and its baked products?

1.4 Research hypothesis

There is no significant difference in the chemical composition and α-amylase susceptibility of starches extracted from common cassava varieties consumed in Zambia. There is no significant varietal variation on composition, structural, physicochemical properties and starches end-use properties on cassava starches. The addition of cassava flours for processing of frozen wheat dough has no significant impact on bread qualities.

1.5 Main objective

The main objective of this research work was to conduct a study on characterization of flours and starches of common Zambian cassava cultivars for swelling, solubility, gelatinization, enzymatic digestibility, freeze-thaw stability and pasting properties. The application focuses on the behavior of cassava flour inclusion in the wheat-based frozen doughs for bread making.

1.6 Specific objectives and study methodology approach

The investigations in the current study were based on six common cassava varieties cultivated in Zambia (*Bangweulu, Katobamputa, Mweru, Kariba, Kampolombo* and *Chila*). The cassava roots were planted and harvested from root and root research station located in Mansa District, Luapula Province of Zambia. At harvest, four to five marketable roots were purposefully collected from 9 healthy plants of each variety and were brought to the laboratory for analysis, extraction of starch and production of flour. The parameters such as dry matter content and starch yield were analyzed immediately after harvest to avoid root deterioration. The dried cassava flours and starches were packed and transported to the laboratory of the University of KwaZulu-Natal, South Africa. The dried samples were stored at -18 ^{o}C until the analysis was conducted. The investigations were based on native cassava flours and starches. In objective 1, dry matter, starch yields, chemical composition (proteins, lipids, ash, and fiber), total cyanides were evaluated. The morphology, granule size, color and

crystallinity of starches were evaluated in objective 2. The biopolymer of native starches was investigated for their amylose contents including lipids, ash, proteins, and fiber, and resistant starches in objective 3. The physicochemical properties (swelling, gelatinization, pasting, gel retrogradation-freeze thaw stabilities) were investigated in objective 4. The property optimization of different starches was conducted in objective 4. In objective 5 investigations were made on blends of cassava flour with wheat flour in formulations of frozen dough for bread making. Correlation coefficients were used to develop structure-functional property relationships. All measurements were replicated three times.

1.7 Thesis organization

The thesis comprises of ten chapters:

1. Chapter 1: Introduction
2. Chapter 2: Review of progress in cassava flour and starch research: findings and applications and gaps.
3. Chapter 3: Proximate, cyanide contents, and particle size distribution of cassava flour from improved varieties in Zambia
4. Chapter 4: Morphology, starch granule size, color and crystallinity starches from cassava (*Manihot esculenta* Crantz) varieties grown in Zambia
5. Chapter 5: Composition of starch: amylose contents, proximate analysis and resistant starch contents from cassava varieties grown in Zambia
6. Chapter 6: Swelling, solubility, gelatinization, pasting, gel freeze-thaw stabilities of starches extracted from cassava varieties grown in Zambia
7. Chapter 7: Dough rheology and loaf quality of wheat-cassava bread made using different cassava varieties and wheat substitution levels
8. Chapter 8: Effects of cassava flour on the rheological properties of wheat bread dough: stickiness in unleavened, leavened and frozen dough
9. Chapter 9: Effect of frozen storage on wheat-cassava dough and bread and their resistant starch contents
10. Chapter 10: Conclusion

1.8 References

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2. REVIEW OF PROGRESS IN CASSAVA FLOUR AND STARCH RESEARCH: FINDINGS, APPLICATIONS AND GAPS

This chapter is based on the following publication

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2.1 Overview on cassava

Cassava is an important tropical root crop widely cultivated and produced for its edible roots and leaves. It is a staple food crop to approximately 800 million inhabitants in tropics and subtropics (Howeler *et al.*, 2013; Anyanwu *et al.*, 2015). The total cassava production in 2012 was estimated to be around 263 million tons (Howeler et al., 2013). Sub-Saharan Africa ranks as the number one (Figure 2.1), with 50% of the world's cassava production with an average yield of 12.8 $t.ha^{-1}$ (Chipeta *et al.*, 2016). However, given optimal conditions, cassava yields are estimated at 80 $t.ha^{-1}$ (Howeler *et al.*, 2013). In Zambia, cassava crop play an important role in contributing to food security and the most important staple crop after maize (Haggblade *et al.*, 2012). It is cultivated as a perennial crop for its starchy roots that can be harvested at 16 to 24 months after planting (El-Sharkawy, 2003; Chipeta et al., 2016), which also varies according to genotype and environments (Howeler et al., 2013a). The consumption of storage roots comes in a variety of different forms for human food (Falade and Akingbala, 2010; Karri and Nalluri, 2016), livestock feed (Bokanga, 1995; Okike *et al.*, 2015; Sudarman *et al.*, 2016) and starch extraction and its various industrial uses (Bokanga, 1995; Anyanwu et al., 2015). There is also great potential for bread making using wheat-cassava blend flours (Abass *et al.*, 2016). Cassava is a low input crop that is able to grow in low marginal soil fertility, tolerate drought, and grow without application of fertilizer and other agrochemicals. This makes cassava easy to produce by low-income households and small-scale farmers with limited resources.

2.2 Origin, distribution, botany and cultivation

2.2.1 Origin

The cassava crop has been reported to have originated from subspecies *flabellifolia* of the Amazon basin (Olsen and Schaal, 1999). The cassava crop was reported to have been domesticated at about 10,000 years ago (Uchechukwu-Agua *et al.*, 2015). The diverse results obtained concerning the phylogeny of cassava (Olsen and Schaal, 1999) appears to support the view that *Manihot flabellifolia* were the progenitor of the crop (Olsen and Schaal, 1999). It is believed that early European sailors carried the cassava crop from Brazil to West Africa, and through sailing and trading, cassava crop spread into East Africa and Asia (Ortiz *et al.*, 2016). The worldwide cassava distribution is as shown in Figure 2.1. The figure was drawn based on worldwide cassava production data of FAOSTAT (2013).

2.2.2 Botany

Cassava crop is procumbent, semi-herbaceous and sub-shrubs (Nassar *et al.*, 2008). Cassava crop grows between 30^{o} N and 30^{o} S (El-Sharkawy, 2003; Falade and Akingbala, 2008; Iyer *et al.*, 2010; Ortiz et al., 2016). The highest production can be achieved in tropical lowland with optimal temperatures of 25-35^{o}C (El-Sharkawy, 2003). Cassava can grow in low nutrient and acidic soils (El-Sharkawy, 2003). Depending on botanical source and type of cultivar, the roots can be harvested after planting, over the range of time from 8 to 24 months (El-Sharkawy, 2003; Mtunguja *et al.*, 2016a; Nduwumuremyi *et al.*, 2016b). For most common varieties grown by people in rural Zambia, harvesting duration can go beyond 24 months. The prolonged harvesting period serves as the means of storing fresh roots under the ground (Nduwumuremyi et al., 2016b).

2.3 Cassava varieties in Zambia

There are a number of local cassava varieties cultivated in Zambia. The cassava crop is grown for its bulking roots, and the leaves serve as the main vegetable in both rural and urban areas. The breeding of cassava in Zambia is the Zambian Government's agricultural priority. The breeding objective is to produce high yielding, early bulking, pests and disease resistant varieties (Chikoti *et al.*, 2016), for reduced cyanide content in cassava (Dixon *et al.*, 1994) to produce sweet varieties (Sarkiyayi and Agar, 2010). The research institute with a mandate for crop breeding is the Zambian Agriculture Research Institute (ZARI). The root and tuber

research station is based in Mansa District, Luapula province in the northern parts of Zambia. The cassava varieties including local landraces are shown in Table 2.1. The bio-fortified cassava is yellow fleshed and is the most recent genotype which has been bred for improved nutrition to supply pro-vitamin A carotenoids (La Frano *et al.*, 2013). The micronutrients, mainly of provitamin A, and dry matter content traits are among the primary selection objectives in cassava breeding (Rabbi *et al.*, 2017). The yellow-fleshed cassava has other advantages such as delayed postharvest deterioration due to anti-oxidant carotenoids contained in the root (Sánchez *et al.*, 2006; Nduwumuremyi *et al.*, 2016a; Nduwumuremyi et al., 2016b). The physicochemical traits such as water binding capacity, swelling power, solubility, gelatinization, and pasting properties of cassava-derived flour and starch remain under-researched in Zambia. Bechoff *et al.* (2016), reported that the research on technological properties such as swelling, gelatinization, and pasting including end-user acceptance traits of cassava starches have been ignored. Studies on characterization of end-use properties were based on common cassava flour and starches collected from markets, farms and varieties in breeding sites. Muñoz *et al.* (2015), purchased cassava starch from the local markets in the study of microstructural and thermal properties of native starch. Some studies were based on commercial native cassava starch collected from industry and markets (Bahnassey and Breene, 1994; Abera and Rakshit, 2003; Farahnaky *et al.*, 2009; Aichayawanich *et al.*, 2011; Dhillon and Seetharaman, 2011; Beninca *et al.*, 2013a; Colman *et al.*, 2014; Bie *et al.*, 2016; Chandanasree *et al.*, 2016; Dries *et al.*, 2016). Other studies were based on cassava from farms (Moorthy *et al.*, 2006; Chantaro *et al.*, 2013; Babajide *et al.*, 2014; Figueroa *et al.*, 2016) and research breeding sites (Abera and Rakshit, 2003; Ceballos *et al.*, 2007; Beninca et al., 2013a; Eriksson, 2013). In the current study, characterization of flours and starches were conducted based on cassava varieties from research breeding site.

2.4 Economic importance of cassava

Cassava has been reported to supply the second highest dietary calories in sub-Saharan Africa and is mainly produced for human consumption (Uchechukwu-Agua et al., 2015). Asia is the major exporter of cassava products while in the Americas, 40% of produced cassava is used as human food, and 30% for livestock feed. Cassava provides income, employment, and support food security (Iyer et al., 2010; Howeler et al., 2013). Though the crop is grown widely in Africa, average yield varies from one country to another for example in Ghana 12 $t.ha^{-1}$ (Angelucci, 2013; Peprah *et al.*, 2016), and in Zambia dry matter yields of cassava

amounts to 9-12 $t.ha^{-1}$ (Barratt *et al.*, 2006). Given suitable conditions for growth, the potential yield for cassava is estimated to be 70-90 $t.ha^{-1}$ fresh cassava (Howeler *et al.*, 2013).

Cassava starch is a multi-billion-dollar business worldwide with several industrial applications in the food and non-food industry (Tonukari, 2004). In Brazil, cassava waste which includes thick stalks, thin stalks, and seed stems have been characterized for use as an energy source and fuel (Veiga *et al.*, 2016). Cassava has gained tremendous use in food and beverages. It is added to wheat flour to form composite flour for making bread and other bakery products (Komlaga *et al.*, 2012). The percent inclusion of cassava to wheat is product dependent but ranges from 10 to 25% have been reported (Komlaga et al., 2012; Abass *et al*., 2016; Serventi *et al.*, 2016b). In Nigeria, where the policy of cassava flour inclusion is mandatory, flour mills are required to partially substitute wheat flour with minimum of 10% cassava flour for bread making and other bakery products (Amannah *et al.*, 2017).

Table 2.1 Cassava varieties at Mansa Root and Tuber Research Station Zambia

Entry	Cultivar	Root /color	Source	Entry	Cultivar	Root/color	Source
1	Mweru	Improved	Mansa Research Station	16	MM96/1757	Yellow	Introduced by IITA
2	Chila	Improved	Mansa Research Station	17	MM96/1759	Yellow	Introduced by IITA
3	Tanganyika	Improved	Mansa Research Station	18	99/0395	Yellow	Introduced by IITA
4	Bangweulu	Improved	Mansa Research Station	19	00/0093	Yellow	Introduced by IITA
5	Kampolombo	Improved	Mansa Research Station	20	00/1093	Yellow	Introduced by IITA
6	Nalumino	Improved	Mansa Research Station	21	01/1172	Yellow	Introduced by IITA
7	Kapumba	Improved	Mansa Research Station	22	99/3575	Yellow	Introduced by IITA
8	MM06/0130		Mansa Research Station	23	00/0779	Yellow	Introduced by IITA
9	MM06/0013		Mansa Research Station	24	Kasweshi	Local	Mansa Research Station
10	L9-304/147/81		Mansa Research Station	25	Kariba	Local	Mansa Research Station
11	MM06/0074		Mansa Research Station	26	Nakapai	Local	Mansa Research Station
12	L9-304/147/87		Mansa Research Station	27	Namumba	Local	Mansa Research Station
13	L9-304/147/96		Mansa Research Station	28	Katobamputa	Local	Mansa Research Station
14	L9-304/147/83		Mansa Research Station	29	Namunyongo	Local	Mansa Research Station
15	01/1646	Yellow	Introduced by IITA	30	Mukondezi 5B4		Mansa Research Station

IITA, International Institute of tropical Agriculture

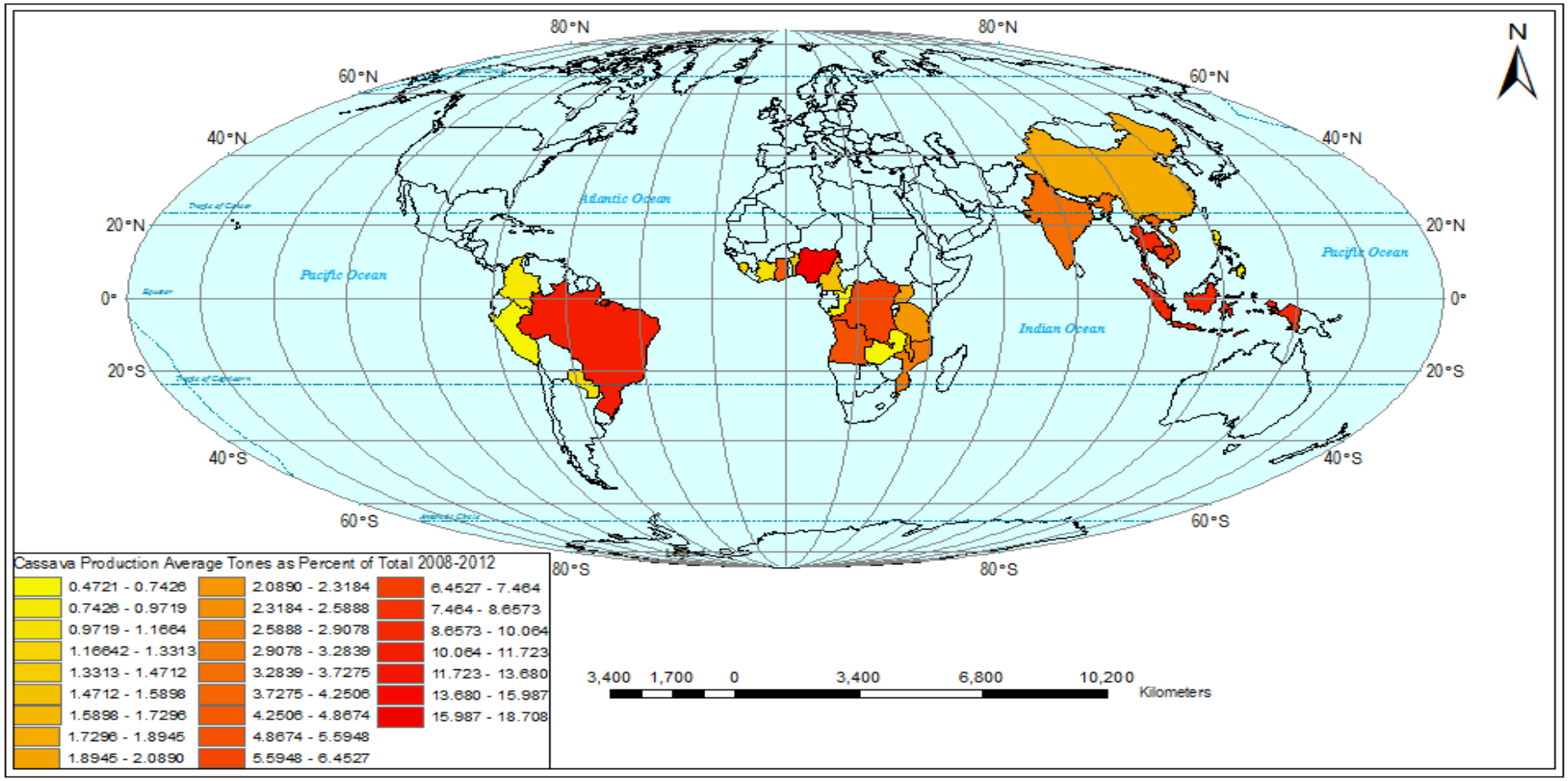

Figure 2.1 Cassava producing countries throughout the world (produced as part of PhD study on ‘cassava flours and starches at Department of Bioresources Engineering, University of KwaZulu-Natal)

2.5 Postharvest quality and products

The shelf life stability of fresh cassava is limited due to rapid postharvest quality deterioration. Thus fresh cassava is processed into shelf-stable products immediately after harvest.

2.5.1 Postharvest deterioration

Fresh cassava roots undergo rapid physiological postharvest deterioration (PPD) after harvest. This is the major constraint to the cassava value chain, production, and utilization (Ma *et al.*, 2016; Uarrota *et al.*, 2016). The PPD brings about rapid quality deterioration within 24-72 hours after harvest making the roots unpalatable (Uchechukwu-Agua et al., 2015). The deterioration reactions are induced on the detachment of the root from the mother plant (Nduwumuremyi et al., 2016a). Other factors include storage and transportation conditions (Venturini *et al.*, 2015). The deterioration is characterized by discoloration which appears as dark streaks on xylem tissue (Iyer et al., 2010). The PPD reduces starch content resulting in poor functional properties (Sánchez *et al.*, 2013). To delay deterioration and extend shelf life, several methods have been suggested such as pruning before harvest, wax coating, storage of root underground, and cold storage (Ravi *et al.*, 1996; Reilly *et al.*, 2004; Sánchez et al., 2013). However, these techniques have been reported to be neither effective nor practical (Sánchez et al., 2013). Thus it is essential to immediately transform fresh cassava roots into shelf-stable dried products such as flour, chips, and starches.

2.5.2 Cassava primary products

The factors limiting utilization of fresh cassava include poor shelf life and high amounts of cyanides (Mtunguja et al., 2016a). The cassava varieties with low cyanide contents such as sweet fresh cassava root variety (Oluwole *et al.*, 2007) can be consumed raw with minimal processing. Processing of cassava roots leads to decreased cyanide content and improved shelf life stability. Traditionally, various methods of processing cassava roots into various products vary depending on local customs and preferences and differ in between and within countries (Falade and Akingbala, 2010). The cassava products are either fermented or unfermented (Uchechukwu-Agua et al., 2015; Zhu, 2015) and contribute to the growing industrial application of cassava. Cassava is an industrial crop in Thailand, Brazil, and Indonesia, where it is processed into biofuel and livestock feed (Zhang *et al.*, 2016).

2.5.2.1 Unfermented cassava products

Unfermented cassava products include chips, starch, flour, and pellets. Chips are transformed into pellets through the process of pelletizing to obtain hardened cylindrical or cube materials (Nguyen *et al.*, 2007; Falade and Akingbala, 2010). Cassava starches are produced from fresh cassava roots. The cassava starches are applied as a thickening and gelling agents in the food industry (Banerjee and Bhattacharya, 2012; Uchechukwu-Agua et al., 2015). High-quality cassava flour (HQCF) is unfermented flour useful in bakery products and in the production of starch and glucose syrup (Ofori *et al.*, 2016). Cassava flours are added to wheat to make composite flour for bread making as a sustainable alternative to wheat bread (Abass et al., 2016; Serventi *et al.*, 2016), and alcohol beverage production (Taiwo, 2006; Freire *et al.*, 2015). The introduction of HQCF has promoted the use of cassava in different products such as bread (Uchechukwu-Agua et al., 2015).

2.5.2.2 Fermented cassava products

In Zambia fermentation is the most common traditional technique of processing cassava into shelf-stable raw and dried cassava products with a longer storage period of two years as the practice in rural households (personal observation). Some of the documented fermented products are *fufu, lafun, agbelima and akyeke* (Falade and Akingbala, 2010; Uchechukwu-Agua et al., 2015), are similar in production but carry different native names depending on the country of origin. *Fufu* is produced in Nigeria and other West African countries (Bamidele *et al.*, 2015; Thomas and Philips, 2015). *Agbelima* is a traditional product of Ghana, Benin and Togo (Panda and Ray, 2016; Salvador *et al.*, 2016) produced by using starter culture obtained from previously fermented cassava (Amoa-Awua and Jakobsen, 1995; Sefa Dedeh, 1995; Rosales-Soto *et al.*, 2016).

2.5.3 Cassava based foods in Zambia

The common uses of cassava in Zambia include flour, which is used for domestic purposes. The Zambian fermented cassava flour is produced through various operational stages involving peeling of fresh cassava and soaking in stagnant water for 3-5 days. During this period peeled cassava roots undergo spontaneous fermentation and become softened. The softened fermented roots are collected and cleaned, followed by open air-drying in the sun. The dried roots are milled into flour. The dried roots can be stored in sacks for a period of up to two years. However, household storages can result in fungal and mold growth. The

fermented cassava flour is used for the preparation of cassava *nshima*, a thickened gelatinized porridge serving as main meal and source of dietary calories. The cassava *nshima* is consumed daily by the households in the cassava belt of northern, central, and western provinces of Zambia. Furthermore, the flour is used in the preparation of porridges for children and serve as weaning food. The other products are roasted fermented cassava roots consumed as a snack and are commonly sold in the urban streets and markets. The roasted cassava is derived from fermented cassava roots. The dried fermented cassava roots are soaked and sliced into longitudinal pieces of chips which are salted and roasted on firewood or charcoal. The low cyanide cassava, usually found as selections from local indigenous cultivars are consumed raw after peeling or can be boiled. The raw and boiled peeled cassava is commonly sold in large cities. Literature regarding the chemical composition of Zambian cassava products is limited.

2.6 Chemical composition of cassava

The chemical composition is dependent on a number of factors such as cultivar, the geographical location, maturity stage of the plant, and environmental conditions (Burns *et al.*, 2012; Agiriga and Iwe, 2016). On the wet basis, cassava roots are composed of 56-60% moisture, 0.3-0.6% protein, 30-35% carbohydrate, and 0.1-0.3% fat (Charles *et al.*, 2004; Montagnac *et al.*, 2009). Other constituents include calcium in the range of 0.16-0.45 mg g^{-1} (Charles *et al.*, 2005b). Cassava root contains a small amount of sugars in the form of sucrose, fructose, glucose, and maltose (Afoakwa *et al.*, 2012a; Li *et al.*, 2016). It is difficult to compare chemical constituents based on data derived from literature because analyses were based on different cassava varieties and variation in harvest time, and lack of complete description of sample materials in terms of genotypic traits. Table 2.2 shows the chemical composition of cassava. Cassava is rich in carbohydrates and deficient in proteins and fats. On a dry matter basis, cassava root has carbohydrate content of 70-82%, which is made up of starch containing amylopectin and amylose (Charles *et al.*, 2005b; Chiwona-Karltun *et al.*, 2015).

Table 2.2 Proximate composition of different cassava varieties

Cassava source, Country	Number of variety	White/yellow fleshed	Dry/wet basis	Harvest time (MAP)	Moisture (%)	Ash (%)	Protein (%)	Lipids (%)	Fiber (%)	Author
Gannoruwa, SriLanka	1	-	-	-	62.92	-	0.72	0.41	0.92	Somendrika *et al.* (2016)
Umudike, Nigeria	3	-	-	10	61.05-69.95	-	-	-	-	-
Umudike, Nigeria	3	-	-	13	62.85-70.21	-	-	-	-	Agiriga and Iwe (2016)
Umudike, Nigeria	3	-	-	16	49.96-62.02	-	-	-	-	Agiriga and Iwe (2016)
Pokuase, Ghana	6	-	-	12	33.14-45.86	-	1.76-3.48	0.74-1.49	1.38-3.20	Emmanuel *et al.* (2012)
Nassau, Bahamas	6	-	-	9	56.50-68.80	2.27-3.24	1.20-2.10	0.20-041	-	-
Chapare, Bolivia	6	-	-	-	-	1.46-2.71	1.46-2.49	0.58-1.4	7.40-8.50	Rojas *et al.* (2007)
IITA Ibadan, Nigeria	2	-	-	-	55.44-58.79	1.90-2.84	0.90-1.43	-	3.62-5.45	Charles et al. (2005b)
Nokornratchasrima, Thailand	1	-	dry	10	-	2.41	1.83	0.14	1.79	Chotineeranat *et al.* (2006)
Nokornratchasrima, Thailand	1	white	dry	12	-	2.52	1.41	0.08	2.59	Chotineeranat et al. (2006)
Umudike, Nigeria	1	white	dry	-	12.28	1.92	-	0.95	1.78	Eleazu and Eleazu (2012)
Umudike, Nigeria	5	yellow	dry	-	8.40-9.85	1.44-2.35	-	0.80-2.75	1.65-2.32	Eleazu and Eleazu (2012)

MAP=months after planting, hyphen (-) implies value or information not found. Dry matter basis, analysis based on dried cassava samples. Wet basis, analysis based on fresh cassava samples.

2.7 Starch extraction

The common practice and primary method of extracting starch from fresh cassava is wet milling. Starch is extracted after wet milling through filtration, sedimentation, and decantation. The wet starch is dried through sun drying and oven drying at 65 ^{o}C for 12 h (Benesi *et al.*, 2004). A similar method has been reported by Nand *et al.* (2008). Peeling is a critical step. Cassava starch prepared from unpeeled or not properly peeled roots develops a grey color during wet storage (Jyothi *et al.*, 2007) and develops a purple color during drying (personal observations). The retained color lowers the quality and thus affecting its value. The starch extraction methods are not standardized. The search in the literature shows several researchers applied a different amount of water for extraction. For example a ratio of water to cassava slurry 2:1 was used where 700 mL of water was mixed with 333 g of grated cassava (Abera and Rakshit, 2003), the ratio of water to cassava slurry 10:1 (Nand et al., 2008). Information is limited regarding the effect of extraction ratio on the end product. In some methods, the grating is conducted with sulfur-containing water and also storing of fresh starch in sodium meta-bisulfite solution to inhibit the microbial growth (Jyothi et al., 2007; Zhu, 2015). Where centrifugation is used (Moorthy *et al.*, 1996), a step of sedimentation is eliminated.

2.7.1 Starch yield

Starch is the main constituent of cassava and a wide range of starch yields have been reported based on wet weight, 24% (Osunsami *et al.*, 1989), and 20.7-27.8% (Abera and Rakshit, 2003). On dry weight, the starch yield from cassava root was estimated at 80% (Olomo and Ajibola, 2003). Various factors affecting starch yield have been reported. Genotype was reported to have a huge influence on starch yield (Benesi et al., 2004; Zhu, 2015). Pérez *et al.* (2011), reported that starch yield is dry matter dependent as high starch yields were associated with high dry matter content of the cultivars root.

2.7.2 Composition of starch

The cassava starch is composed of amylose which is essentially a linear molecule (Morante *et al.*, 2016), and amylopectin a highly branched molecule (Zhu, 2015). Zhu (2015) compiled a wide ranges of minor chemical composition data for starch as: ash 0.03-0.29% (Eke *et al.*, 2007; Nwokocha *et al.*, 2009); protein 0.05-0.75% (Asaoka *et al.*, 1991; Charles et al., 2004); lipid 0.01-1.2% (Sriroth *et al.*, 1999; Freitas *et al.*, 2004); phosphorus 0.0029-0.0095%

(Raemakers *et al.*, 2005; Mbougueng *et al.*, 2009) and fiber 0.11-1.90% (Moorthy et al., 1996; Olomo and Ajibola, 2003). Minor components such as phosphorus may affect functional properties of starch (Biliaderis, 1991). Phosphate monoester attached to cassava starch macromolecules promotes hydrophilic nature and thus increases starches paste transmittance and swelling power (Swinkels, 1985). The formation of amylose-lipid complexes are reported to increase the viscosities during starches pasting and to retard the starches gelling properties (Peroni *et al.*, 2006; Nwokocha et al., 2009). The effect of other minor components such as lipids and phosphorus on functional properties has not been examined on cassava starch of the Zambian cultivars.

The cassava starch functionalities as stabilizers are associated with polysaccharide fractions amylose (17 - 24%) and amylopectin (76 - 83%) (Bahnassey and Breene, 1994; Charles *et al.*, 2005a; Suriyakul Na Ayudhaya *et al.*, 2016). Amylose consists of α-(1-4) linked D-glucose units which are characteristic of the degree of polymerization (DP) in the range of between 500 and 6000 glucose units. Amylopectin is a highly branched molecule structure with a DP ranging between $3x10^5$ and $3x10^6$ glucose units (Zobel, 1988; Jacobs and Delcour, 1998; Gu *et al.*, 2013), and consist of α-(1-4) glucan chains linked with α-(1-6) at branch points (Jacobs and Delcour, 1998; Laohaphatanaleart *et al.*, 2010). Several authors have reported amylose content for cassava starches in the range of 16-20% (Asaoka et al., 1991), 17.9-23.6% (Defloor *et al.*, 1998), 15.9-25.31% (Sriroth et al., 1999; Charles et al., 2004) and 15.2-26.5% (Sánchez *et al.*,2009).

2.7.3 Quantifying amylose

Amylose content in starches can be determined by iodine-binding (Kaufman *et al.*, 2015; Morante et al., 2016), differential scanning calorimetry (Biliaderis *et al.*, 1980; Mestres *et al.*, 1996; Moorthy et al., 2006; Ceballos et al., 2007; Beninca *et al.*, 2013b), enzymatic method using Megazyme amylose-amylopectin assay kit (Zhu, 2015) and size exclusion chromatography (Ovando-Martínez et al. 2013; Kobayashi et al. 1985). The iodine binding method is widely used. However, the iodine binding method with amylose has been reported to be not consistent due to some minor complex formation of iodine with amylopectin polymers (about 1 mg iodine per 100 mg amylopectin) that has maximum absorbance wavelength of 540 nm (Zhu *et al.*, 2008; Zhu, 2015). Such small binding of iodine with amylopectin even though the wavelength absorbance maxima are different from amylose

iodine binding (about 620) were indicated to overestimate the amylose percentage. Other methods include potentiometric (Soto *et al.*, 2014; Castaño *et al.*, 2016), amperometric (Jansen *et al.*, 2012), and paper-based microfluidic chip (Hu *et al.*, 2015). Megazyme International's amylose/amylopectin assay kit is reported as an efficient and rapid measurement of amylose content (Hu *et al.*, 2010). This kit is applicable to all pure starch sample analyses (Zhu et al., 2008). The various amylose content determination methods, amylose % ranges and proximate composition of cassava flour starches from different cassava varieties reported by different past works are summarized in Table 2.3.

Table 2.3 Amylose content determination methods and composition of starch granules from different cassava varieties

Source of cassava starch	Country/region	Method	Amylose (%)	Ash (%)	Protein (%)	Lipid (%)	Phosphorus (%)	Fiber (%)	Author
IITA varieties	Nigeria	Starch assay procedure	21.0-22.5	0.36-0.37	0.13-0.17	0.37	-	0.20-0.23	Abioye *et al.* (2017)
Varieties	Tanzania	K-AMYL	11.9-19.4	-	-	-	-	-	Mtunguja *et al.* (2016b)
Varieties	India	Iodine-S	18.66	-	-	-	-	-	Remya *et al.* (2017)
Variety	Malaysia	Enzymatic	16.6	0.31	0.55	0.24	-	-	Edhirej *et al.* (2017)
Variety	Cameroon	Iodine-S	23.81	-	-	-	-		Mbougueng *et al.* (2012a)
Producer	Brazil	-	32.5	-	-	-	-	-	Santana *et al.* (2017)
Producer	India	-	20.7	-	0.34	0.23	-	-	Nair *et al.* (2017)
Varieties	Mexico	Iodine-S	19.25-32.12	-	-	-	-	-	Hernández-Fernández *et al.* (2016b)
Varieties	Nigeria	Iodine-S	26.73	0.26	0.10	0.79	-	1.50	Eke-Ejiofor (2015)
Varieties	Uganda	K-AMYL	17.9-19.7	0.12-0.23	0.27	0.22	-		Nuwamanya *et al.* (2010)
Producer	Nigeria	Iodine-S	22	0.2	0.1	0.1	0.007	-	Abiola (2014)
Producer	China	Iodine-S	28.6	0.13	0.08	-	-	-	Ren *et al.* (2015)
Producer	Thailand	Iodine-S	26.85	-	-	-	-	-	Suriyakul Na Ayudhaya et al. (2016)
Varieties	Brazil	K-AMYL	14.8-24.38	-	-	-	0.0034-0.0093	-	Justamante Händel Schmitz *et al.* (2017)
IITA varieties	Nigeria	K-AMYL	19.2	0.1	0.8	1.0	-	-	Oladunmoye *et al.* (2014)
-	-	DSC and Iodine-A	0-30.3	-	-	-	-	-	Rolland-Sabaté *et al.* (2012)
Farm roots	Brazil	Potentiometric	22.81-25.52	-	-	-	-	-	Moraes *et al.* (2013)
Producer	Brazil	Iodine-S	21.0	0.10	0.26	0.12	0.014	-	Gutiérrez *et al.* (2014)
Varieties	Côte d'Ivoire	Iodine-S	14.20-25.31	-	-	-	-	-	Doué *et al.* (2014)
CIAT varieties	Colombia	Iodine	19.5-20.3	-	-	-	-	-	Morante et al. (2016)
Greenhouse varieties	Netherland	Con A	6.0-18.4	-	-	-	-	-	Gomand *et al.* (2010)

Where: DSC = differential scanning calorimetry based method for amylose measurement; K-AMYL= starch assay procedure of Megazyme; Iodine-S = iodine-spectrophotometry/colorimetry based method; Iodine-A = iodine-amperometry based method; Con A = concanavalin A based precipitation method. Producer's starch (native starch produced by commercial companies).

2.7.4 Cassava starch granular shape and size

Cassava starches granule size ranges between 9 and 20 µm (Niba *et al.*, 2002; Hoover *et al.*, 2010). Several other studies have reported a wide variation of granular sizes; 2.4-31.1 µm (Asaoka et al., 1991); 12.5-23.8 µm (Onitilo *et al.*, 2007); and 9-20 µm (Niba et al., 2002). Granule sizes influence water absorption (Hedayati *et al.*, 2016). Small granules have the high surface area and hence enhanced water absorption capacity (Lindeboom *et al.*, 2004). Cassava starch granules are mostly rounded, truncated or oval (Niba et al., 2002). Enzymatic susceptibility of starches were associated with granule shape (Oates, 1997). Techniques for studying starch granular size and shape are scanning electron microscopy (Colivet and Carvalho, 2017; Pineros-Hernandez *et al.*, 2017) and light microscopy (Fan *et al.*, 2017). The starches granular surfaces were investigated using atomic force microscopy (Lindeboom et al., 2004; Zhu, 2015). Information on granule size and shape of cassava starches of the Zambian cultivars are limited.

2.7.5 Crystallinity of cassava starches

As observed using small-angle X-ray scattering analysis (SAXS), the starch granule consists of amorphous and semi-crystalline shells with a thickness in the range of between 100 and 400 nm (Jenkins and Donald, 1995; Oates, 1997; Pérez and Bertoft, 2010). The starch granule crystallinity is strongly associated with the orderly arrangement of amylopectin molecules (Jenkins and Donald, 1995; Gallant *et al.*, 1997). Amylose molecules were indicated to be at large in the amorphous lamellae (Jenkins and Donald, 1995; Waterschoot *et al.*, 2015b). Amylopectin forms crystalline lamellae (Jenkins *et al.*, 1993; Tester *et al.*, 2004; Bie et al., 2016). The structural crystallinity was identified as type A, B, and C using X-ray diffraction analysis (Pérez and Bertoft, 2010). The A-types crystallinity are short chains, and B-type crystallinity associated with long chains, while C-type crystallinity exhibited intermediate chains (Jenkins and Donald, 1995; Zhu, 2015). Cassava starches exhibited either A- or C-type (Zhu, 2015; Garcia *et al.*, 2016). Other studies reported type B or C cassava starch crystallinity (Vamadevan and Bertoft, 2015; Dries et al., 2016). Conflicting results on cassava starch polymorph type require careful examination. The effect of the polymorphic composition of cassava starch on functional properties remains under-researched on cassava starches of the Zambian cultivars.

2.8 Physical properties

The behavior of starch in water includes swelling, solubilization, gelatinization, pasting, gel formation, and retrogradation. Before starches get gelatinized when heated in water, it absorbs water in the amorphous region and the starch granules swelled. On further heating, the crystalline structure in the granules is disrupted and some starch molecules will leach out from the granule and then starch forms paste. The paste clarity of gels can be characterized by light transmittance, while the cold storage behavior is characterized by freeze-thaw properties. On cooling the pasted starch forms gel and then over prolonged storage, the gel will be converted to retrograded starches.

2.8.1 Swelling and gelatinization

Most past works reported swelling and solubility properties of cassava flours and starches in the temperatures ranges of 50–95 ^{o}C (Gomand *et al.*, 2010; Mtunguja et al., 2016b) (Table 2.4). The degree of granular swelling can be quantified as swelling power (SP) and solubilization of starch molecules (Hermansson and Svegmark, 1996; Waterschoot *et al.*, 2015a). Hydration and swelling capacity of flours/starches are influenced by flour particle size and granulation (Mutungi *et al.*, 2009). However, works conducted based on particle/granule size are limited. There is limited information on solubility and swelling properties of cassava flours. The swelling power of cassava flour was reported to be 13.80 g.g^{-1} (Gomes *et al.*, 2005). Van Hung *et al.* (2017), generalized that swelling power, solubility volume, and water-binding capacity of cassava flours were higher than that of wheat flour.

Gelatinization is an irreversible change manifested by swelling, disruption of hydrogen bonds, crystallite melting, the disappearance of Maltese cross, viscosity development, and starch molecules solubilization when heated in water (Zhu, 2015; Charles *et al.*, 2016; Garcia et al., 2016). Starch granule gelatinization can also happen on food extrusion. The transformation results in changes in viscosities and formation of a paste are influenced by starch granule shape, swelling power, and amylopectin/amylose ratio (Tabilo-Munizaga and Barbosa-Cánovas, 2005; Ai and Jane, 2015). Gelatinization processes are characterized by the temperatures and enthalpies of gelatinization. The gelatinization temperatures of cassava starches reported in the past are summarised in Table 2.5. The onset, peak, and conclusion gelatinization temperatures were reported in the range of 54–72 ^{o}C, 62.0–77.6 ^{o}C), and 65.0–85.5 ^{o}C, respectively (Ren *et al.*, 2010; Charoenkul *et al.*, 2011; Mbougueng *et al.*, 2012b;

Beninca et al., 2013a; Rolland-Sabaté *et al.*, 2013). The gelatinization enthalpies of cassava starches are reported in the range of 5.0–50.2 $J.g^{-1}$ (Ren et al., 2010; Herceg *et al.*, 2013; Mweta *et al.*, 2015). Water acts as a plasticizer (Perdomo *et al.*, 2009). Other reported plasticizers used were glycerol, ethylene glycol, and 1, 4-butanediol (Perry and Donald, 2000; Nashed *et al.*, 2003; Ai and Jane, 2015). Gelatinization properties are determined using several techniques, differential scanning calorimetry (Nakayoshi *et al.*, 2015; Xia *et al.*, 2015; Morante et al., 2016), polarised light microscopy equipped with a hot stage (Muñoz et al., 2015; Ortolan *et al.*, 2015), amylograph (Chantaro et al., 2013; Wongsagonsup *et al.*, 2014), rapid visco analyser (Wongsagonsup et al., 2014) and nuclear magnetic resonance spectroscopy (Warren *et al.*, 2016; Zhu, 2016).

Table 2.4 Swelling power (SP, $g.g^{-1}$) and solubility (S, %) of cassava flours and starches

			Temperature (°C)								
Source of starch	**Country**	**SP/S**	50	60	70	75	80	85	90	95	References
Varieties	Tanzania	SP	-	-	8.9 - 12.3	-	-	-	13.5 - 16.3	-	Mtunguja et al. (2016b)
Purchased on local market	Indonesia	SP	-	13.8	-	-	-	-	-	-	Kusumayanti *et al.* (2015)
Purchased on local market	Indonesia	S	-	3.02	-	-	-	-	-	-	Kusumayanti et al. (2015)
Varieties	Ugandan	SP	5.62 - 7.97	7.53 - 10.77	10.18-13.61	-	18.05 - 20.79	-	-	-	Nuwamanya et al. (2010)
Variety	Cameroon	SP	-	15	18	-	20	-	27	-	Mbougueng et al. (2012a)
Purchased on local market	Brazil	SP	-	-	-	-	-	-	29.11	-	Klein *et al.* (2013b)
Purchased on local market	Brazil	S	-	-	-	-	-	-	25.66	-	Klein *et al.* (2013a)
CIAT variety	Colombia	SP	-	-	-	49.7	-	-	51	-	Sánchez *et al.* (2010)
Variety	Nigeria	SP	-	-	-	-	-	-	-	1.23 - 7.41	Akpa and Dagde (2012)
-		SP	-	30 - 35	37 -40	-	41 - 41	-	50 - 59	-	Gomand et al. (2010)
-		SP	0.4 - 13.9	0.4 - 21.4	0.5 - 31.4	-	0.3 - 40.3	-	0.4 - 47.6	-	Demiate and Kotovicz (2011)
Food grade starches (32)	Brazil	S	1.1 - 90.9	1.2 - 90.7	2.1 - 90.7	-	2.4 - 96.4	-	3.4 - 95.9	-	
Variety	Nigeria	SP	-	3.3	7.2	-	10.8	-	18.0	-	Chinma *et al.* (2013)
Varierty	Nigeria	SP	17.01	13.27	-	-	-	-	-	-	Aviara *et al.* (2010)
Variety	Nigeria	SP	-	-	-	-	-	29.6	-	-	Osundahunsi and Mueller (2011)
-	Nigeria	S	-	-	-	-	-	22.09	-	-	Osundahunsi and Mueller (2011)
Variety	India	SP	-	-	16.11	-	-	-	-	-	Bala *et al.* (2015)
-	India	S	-	-	14.0	-	-	-	-	-	Bala et al. (2015)
Varieties	Ghana	SP	-	-	-	-	-	10.5 – 12.0	-	-	Eriksson (2013)
Varieties	Ghana	S	-	-	-	-	-	11.0 - 20.8	-	-	Eriksson (2013)

Hyphen (-) implies value or information not found

Table 2.5 Gelatinization temperatures and enthalpies measured by differential scanning calorimetry (DSC)

Source of cassava starch	Country	Method	Starch: water ratio	Heating rate (^oC/min)	Scanning temp range (^oC)	T_o (^oC)	T_p (^oC)	T_c (^oC)	ΔT_R (^oC)	ΔH (J/kg)	References
Variety	Thailand	DSC	1:2.3	10	-	42.64	54.88	65.65	23.01	7.41	(Suriyakul Na Ayudhaya et al., 2016)
Producer	Brazil	DSC	-	10	-	63.79	77.57	70	-	8.37	Beninca et al. (2013a)
Producer	China	DSC	-	10	-	57.59	65.34	72.91	-	5.53	Ren et al. (2010)
Producer	Brazil	DSC	-	-	-	55.8	62.8	70	-	11.5	Cavallini and Franco (2010)
Varieties	Thailand	DSC	1:2	5	-	63.49 - 71.33	66.78 - 77.22	78.40 - 85.49	-	15.08 - 16.36	Charoenkul et al. (2011)
Variety	-	DSC	-	5	25 - 110	59	64.7	71.2	12.2	12.8	Campanha and Franco (2011)
Producer	China	DSC	-	10	20 - 130	57.8	67.08	77.29	-	10.53	Mei *et al.* (2015)
Producer	Brazil	DSC	-	5	20 - 90	55.95	63.11	68.96	-	10.73	Leite *et al.* (2012)
Variety	Cameroon	DSC	-	20	-	55.22	62.54	65.01	-	11.49	Mbougueng et al. (2012b)
-	-	DSC	-	10	25 - 100	64.3	68.3	74.4	10.1	14.7	Ai and Jane (2015)
-	-	DSC	1:3		-	55 - 64	61 - 68	71 - 74	10 - 16	15 - 19	Waterschoot et al. (2015a)
Producer	Thailand	DSC	1:3	10	25 - 100	64.1	69.9	79.5	-	14.9	Wongsagonsup et al. (2014)
Producer	Thailand	DSC	1:2.3	10	-40 - 120	64.1	-	-	16.79	16.97	Chatakanonda *et al.* (2011)
Producer	Argentina	DSC	-	5	25 - 125	57.47	-	-	9.71	9.34	Colombo *et al.* (2011)
Producer	Venezuela	DSC	1:3	10	30 - 130	63.65	68.5	79.19	-	14.93	Yussof *et al.* (2013)
Producer	China	DSC	1:3	10	20 - 100	65.8	71.9	82.5	-	18.1	Hong *et al.* (2016b)
CIAT varieties	Colombia	DSC	-	10	20 - 130	54.0 - 61.2	63.3 - 69.4	72.7 - 78.3	18.7	12.9	Rolland-Sabaté et al. (2013)
Local variety	Malawi	DSC	1:3	10	20 - 95	58.2 - 62.1	65.4 - 68.6	76.2 - 77.5	14.1- 19.3	13.1-15.1	Mweta et al. (2015)
Producer	-	DSC	-	10	25 - 95	65.93	70.48	81.55	15.62	50.62	Herceg et al. (2013)

T_o = Onset temperature, T_p =Peak temperature, Tc = Conclusion temperature and ΔH is change in enthalpy of gelatinization $\Delta T_{R=}$ ($T_c - T_{c)}$. Producer's starch (native starch produced by commercial companies)

2.8.2 Pasting

Pasting occurs after gelatinization leading to the formation of amylose-amylopectin paste and gel network (Ai and Jane, 2015). At higher gelatinization temperatures, cassava starches formed a clear paste with high viscosity (Chantaro et al., 2013; Wongsagonsup et al., 2014; Waterschoot et al., 2015a). Pasting properties are characterized by rapid visco-analyser (Oladunmoye *et al.*, 2014b; Wongsagonsup et al., 2014) or Brabender visco-amylograph (Afoakwa *et al.*, 2012b; Cappa *et al.*, 2016). Factors affecting pasting behavior are composition of starch granules and other ingredients present in the food system (Ketjarut and Pongsawatmanit, 2015; Zhu, 2015b). The pasting properties are investigated in terms of pasting temperatures, and viscosities, which are characterized as peak, breakdown, and setback viscosities. Cassava starches have lower pasting temperatures than cereal starches due to high levels of negatively charged phosphate groups in cassava starch, and viscosity development starts at lower temperatures (Srichuwong *et al.*, 2005; Ketjarut and Pongsawatmanit, 2015). During cooling, the paste transforms into a gel. The cold paste of cassava has low peak viscosities due to significant starch granule breakdown on shearing (Jane *et al.*, 1999; Gomand et al., 2010a). The pasting temperatures for cassava starches were varied in the range 64.5–78.7 ^{o}C (Adeniji *et al.*, 2010; Klein et al., 2013b) (Table 2.6). Cassava starches with low peak viscosities ($1.6x10^3$–$2.3x10^3$ cP) exhibited better culinary properties than starches with higher peak viscosity ($2.3x10^3$-$2.8x10^3$ cP) (Nuwamanya et al., 2010). Waxy cassava starches exhibited a narrow range of viscosities (544–746 cP) (Morante et al., 2016). Paste clarity was negatively related to amylose content (Craig *et al.*, 1989; Hernández-Fernández *et al.*, 2016a). Amylose content has a strong influence on the pasting characteristics of cassava starches. There is limited information on the effect of the amylose/amylopectin ratio on pasting properties of the Zambian cassava starches.

2.8.3 Paste clarity of starch

At higher gelatinization temperatures, cassava starches form a clear paste with high starch paste viscosity (Chantaro et al., 2013; Wongsagonsup et al., 2014; Waterschoot et al., 2015a). The chemical components such as phosphorus may affect functional properties of starch (Biliaderis, 1991). Phosphate monoester attached to starch macromolecules promote its hydrophilic nature and thus increases paste transmittance and swelling power of starches (Swinkels, 1985). Hernández-Fernández et al. (2016a) reported cassava paste transmittance (%T) values in the 27.74-49.52% making cassava starch a potential ingredient in the processing of confectionaries, jams, and jellies because of clarity. Paste clarity was

negatively related to amylose content (Craig et al., 1989; Hernández-Fernández et al., 2016a). Transparent gels can be used as carriers of active ingredients composed of oils, surfactants, vitamins, sunscreen agent and antioxidants in the formulation of multifunctional cosmetic gels (Comelles *et al.*, 1992). There is limited information on the use of cassava starch gels with high paste clarity in the solubilization of active ingredients of lipophilic and hydrophilic in nature.

2.8.4 Freeze-thaw properties

On cooling, the gelatinized starch undergoes re-association of starch chains resulting in re-ordering of the system and a partial recrystallization of starch molecules (Ai and Jane, 2015; Charles et al., 2016; Pornsuksomboon *et al.*, 2016; Wang *et al.*, 2016). Amylose re-association is largely responsible for initial hardening of gel. The long term gelling and retrogradation are mostly determined by amylopectin re-crystallization (Waterschoot et al., 2015a; Lara and Salcedo, 2016). Syneresis is the physical phenomenon in which the water is expelled and released from the starch gel (Karim *et al.*, 2000). Syneresis is the method to study retrogradation and has been used in the understanding of the freeze-thaw stability of food systems (Karim et al., 2000; Zhu, 2015; Hernández-Fernández et al., 2016). Low syneresis during storage or freeze-thaw cycle is required for stability and shelf life of some frozen food. Cassava starch paste was unstable and had poor gelling properties compared to maize and wheat starches (Zhu, 2015). A comparative study showed that waxy cassava starch gel had no syneresis after five weeks of storage at -20^{o}C and thus possessed the superior potential for formulating frozen or refrigerated foods (Sánchez et al., 2010). The low syneresis of waxy starch is attributed to low or zero amylose content and amylopectin structure (Dhillon and Seetharaman, 2011). Low viscosities attributed to low amylose content in cassava starches and significant loss of starch granule structure during gelatinization (Karam *et al.*, 2005). Freeze-thaw stability is an important quality parameter of starch gels. When a starch gel undergoes repeated freezing and thawing cycles, it releases water. The extent of syneresis is a measure of its freeze-thaw stability (Waterschoot et al., 2015a). Starch extracted from fresh roots had lower syneresis than from the dried roots (Abera and Rakshit, 2003). A comparison of reported data on pasting properties is deficient as genotypic traits were not reported. There is no information on pasting properties of starches from Zambia cassava varieties.

Table 2.6 Pasting properties of cassava starches

Starch source	Country	Method	Starch (%) Starch: water (w/v)	T range	Rate of heating (°C/min)	PV (RVU)	BD (RVU)	SB (RVU)	PT(°C)	Reference
Varieties	Nigeria	RVA	3g/25mL	50 - 95	12	308.50 - 466.63	194.83 - 320.25	34.71 - 42.71	63.10 - 64.13	Adegunwa *et al.* (2011)
Producers	Austria	BVA	10	30 - 95	7.5	1363	1083	831	64.7	Saeleaw and Schleining (2010)
Varieties	Brazil	RVA	-	30 - 95	6	172-286	-	26.8-81.7	62-68	(Justamante Händel Schmitz et al., 2017)
Varieties	Nigeria	RVA	10	-	-	308.4 - 324.5	120.3 - 161.8	50.9 - 78.6	3.7 - 4.7	Adeniji et al. (2010)
Varieties	Nigeria	RVA	10	50 - 95	12	160	74.8	55	71	Chinma et al. (2013)
Variety	Thailand	RVA	8	50 - 95	12.2	134.9	59	46.6	72.7	Zaidul *et al.* (2007)
Producers	India	RVA	-	-	-	2880.5 mPa s	-	981 mPa s	69.1	Jyothi *et al.* (2006)
Varieties	Uganda	RVA	8.9	50 - 95	5.7	170.79 - 344.96	76.26 - 264.42	57.99 - 89.17	64.75 - 70.4	Nuwamanya et al. (2010)
Producers	Brazil	RVA	9	50 - 95	-	364.3	215.8	69.6	64.5	Klein et al. (2013b)
Producers	Thailand	RVA	5	50 - 95	6	397.55	249.14	101.65	72.8	Aviara et al. (2010)
Producers	China	RVA	6	50 - 95	7.5	1252 mPa s	487 mPa s	326 mPa s	73.3	Zhang *et al.* (2013a)
Producers	Brazil	RVA	3g/25mL	50 - 95	-	301.7	164.2	63.3	67.1	Klein *et al.* (2014)
Producers	China	BVA	6	45 - 95	2	735	483	240	65.6	Zhang *et al.* (2013b)
Variety		RVA	5	50 - 95	6	1119 cP	631 cP	595 cP	67.4	Sánchez et al. (2010)
Varieties	Nigeria	RVA	3g/25mL	50 - 95	-	243.75 - 289.08	126.25 - 162.83	39,92 - 44.42	71.9 - 73.6	Uzomah and Ibe (2011)
	Brazil	RVA	2.5g/28mL	50- 95	6	2118 cP	1335 cP	696 cP	67.2	Beninca et al. (2013a)
Producers	Thailand	RVA	5.7	-	-	165.5	80.7	64.5	68.8	Wongsagonsup et al. (2014)
Producers	Brazil	RVA	-	50 - 95	6	247.7	165.3	70.7	65	Leite et al. (2012)
Variety	Nigeria	RVA	-	-	-	364.3	210.8	62.5	50.3	Oladunmoye et al. (2014a)
Variety	Nigeria	RVA	-	-	-	7015.5	3558	668	70.2	Eke-Ejiofor (2015)
Producers	Thailand	RVA		50 - 95	6	793 mPa s	329 mPa s	419 mPa s	68.9	Chantaro et al. (2013)

Brabender viscograph=BVA, Rapid Visco-Analyzer=RVA, PV=Peak viscosity, BD=Breakdown viscosity, SB=Setback viscosity, Tr=Temperature range of the scanning program, RVU as viscosity unit, 1 RVU = 12 centipoise. Producer's starch (native starch produced by commercial companies)

2.9 Resistant starches

The *in vitro* enzyme susceptibility of cassava starch to α-amylases have been an area of several investigations (Pereira and Leonel, 2014; Ogbo and Okafor, 2015; Mtunguja et al., 2016b). Amylose content was inversely related to cassava starch digestibility (Mtunguja et al., 2016b). The resistance of a starch material to digestion is associated with the extent of starch availability to enzymatic hydrolysis in the human digestive system (Alimi *et al.*, 2017). The resistant starch (RS) and inclusion in human diets have elicited interest because it restricts calorie load for individuals such as diabetic patients (Sajilata *et al.*, 2006; Raigond *et al.*, 2015). RS is a dietary fiber that does not get digested in the small intestine (Saura-Calixto *et al.*, 1993) and has the potential for human health benefits (Sajilata et al., 2006; Raigond et al., 2015). RS is influenced by the degree of gelatinization (Goñi *et al.*, 1996), and amylopectin branch chain length distribution (Jane and Chen, 1992). Other factors affecting food RS content are amylose/amylopectin ratio, the degree of milling, heat applied under moist conditions, cooling, α-amylase inhibitors, and non-starch polysaccharides (Sajilata et al., 2006; Pereira and Leonel, 2014). There is limited information on RS in cassava flours. However, high levels of RS in the range 50–196 g/kg recorded in cassava flour samples, were characteristic of C-type X-ray diffraction pattern, which are highly associated with slow or incomplete digestion *in vitro* and *in vivo* (Mejía-Agüero *et al.*, 2012). Enzymatic susceptibility of cassava starches was due to the amylose/amylopectin ratio, crystalline structure, and granular structure (Van Hung et al., 2017). Alcázar-Alay and Meireles (2015) described five types of RS. In the first type (RS I) starch is physically not accessible to enzymes and the breakdown of the granular structure is limited. The second type, RS II, is gelatinized starch and is the common type in most starchy foods. The RS III type is produced following starch retrogradation. The other types, RS IV is due to chemical modifications of starch, and type RS V is starch consisting of amylose-lipid complexes and characterized with high gelatinization temperatures and insolubility in water. Mejía-Agüero et al. (2012) reported that the inverse relationship between RS and α-amylolysis index were due to limited accessibility of amylase enzymes to RS zones in the starch granule. The RS concept could be utilized as the basis of describing nutrition quality and potentially as a criterion parameter for classification of cassava varieties. However, cassava of the Zambian cultivars remains under-researched in the area of resistant starches. There is a need to characterize and profile RS in flour and starch derived from cassava varieties.

2.10 Application of cassava flours/starches

2.10.1 Blending with other starches

Inclusions of cassava flour and starch with other starches in food formulation can give rise to a wide range of properties to the finished product such as bread (Shittu *et al.*, 2007; Oladunmoye et al., 2014; Abass et al., 2016) and noodles (Charles *et al.*, 2007; Qazi *et al.*, 2014). Starches and flours in the blend can influence each other's gelatinization and pasting properties (Tharise *et al.*, 2014; Waterschoot et al., 2015b; Waterschoot *et al.*, 2016). Commercial starches are derived from cereals, legumes, roots, and tubers. The derived starches are usually limited in certain desired properties and exhibit variation in pasting properties. For example, wheat starch has higher phospholipids and produces pastes with lower transmittance than potato starches with low content of phospholipids (Das *et al.*, 2010; Hong *et al.*, 2016a). The potato starch exhibits the highest swelling power, produces the highest viscosities of pasting and break down properties compared to cereal starches (Zaidul et al., 2007). The phosphate content is higher in potato starches than in cassava starches (Vamadevan and Bertoft, 2015). The cereal starches exhibit higher final viscosities than cassava starch (Ai and Jane, 2015). The water-binding and absorption capacities increased with increased proportion of cassava starch inclusion into wheat starches (Oladunmoye et al., 2014). There is a search for alternative natural ways to alter starch properties. The alternative procedure is the mixing of different starches to produce desired physical properties. Commercial starches and their properties are summarised in Table 2.7. Microscopic investigations concluded that swelling of the starch with the largest granules decreased when is in a mixture of smallest granules (Puncha-arnon *et al.*, 2008; Lin *et al.*, 2013). Amylose content and granule size of starches are important parameters in the behavior of starch and flour blends (Wu *et al.*, 2016). Native cassava flour and starches have limited use in chilled and frozen food system due to high retrogradation and syneresis (Seetapan *et al.*, 2015).

2.10.2 Cassava-wheat composite flour for bread making

The inclusion of cassava flour into wheat in bread making is an important area towards the sustainable utilization of cassava. Wheat based bread is widely consumed in Zambia and ranks third after maize and cassava in terms of supplying daily caloric intake (Chapoto, 2010). With increased wheat prices there are challenges on the economic concern about vast importation of wheat grains. Thus, there is growing interest to promote the use of local sources of flour for partial substitution of wheat flour bakery applications (Shittu *et al.*, 2009; Abass et al., 2016; Oladunmoye *et al.*, 2017). The unfermented cassava flour has been

identified as an alternative to replace a portion of wheat flour in composite flours, and substitution levels of 10-30% cassava flour into wheat flour were investigated (Shittu *et al.*, 2008; Eriksson, 2013). The cassava inclusion into wheat-based dough for bread making has been the subject of recent but limited investigations in various areas pertaining to rheological properties and quality issues (Gunaratne and Hoover, 2002; Hoover, 2010; Klein et al., 2013a; Oladunmoye et al., 2014; Abass et al., 2016). The previous research efforts have concluded significant genotypic influence on physical, chemical and functional characteristics of cassava-wheat composite flour and bread quality (Shittu *et al.*, 2007; Shittu *et al.*, 2008). The bread loaf quality varies according to cassava variety and percentage cassava flour inclusion into wheat flour (Almazan, 1990; Shittu et al., 2007). The leavening ability (Aboaba and Obakpolor, 2010), and cassava flour concentration (Eduardo *et al.*, 2013) were some of the quality parameters of investigating the dough made from cassava-wheat composite flours. However, optimization of functional characteristics such as pasting and rheological properties of composite flours from various cultivars was not investigated. There is limited information on associating physical parameters such as bulk density, water absorption capacity, and swelling power to rheological and pasting properties and consequently to dough development and baking characteristics.

2.10.3 Frozen dough for bread making

Common fresh bread has a short shelf life due to staling (Demiate and Kotovicz, 2011). To reduce bread staling and associated problems such as loss of freshness and taste, Ribotta *et al.* (2001) suggested the use of technologies to produce dough with long shelf life. Freezing is a suitable technology for preserving dough quality (Adams *et al.*, 2017; Wang *et al.*, 2017). The use of frozen doughs has been the area of huge interest in the bakery industry and traditional chain stores (Buddhi and Sahoo, 1997; Demiate and Kotovicz, 2011; Klein et al., 2013a). Relative to freshly baked bread, frozen doughs have been reported to produce bread characterized with long proof time, hard texture, and low specific volume (Kusumayanti et al., 2015). However, the addition of glycerol as a plasticizer improved the leavening capacity and reduced proof time (Sharma *et al.*, 2005; Yadav *et al.*, 2008). Waxy cassava starch gel had no syneresis after five weeks of storage at -20^{o}C and thus possessed the superior potential for formulating frozen or refrigerated foods (Sánchez et al., 2010). While cassava flour has been used in the formulation of cassava-wheat composite flours, the impact of freezing and frozen storage on cassava-wheat based bread dough was not studied.

2.11 Summary

The breeding objectives of cassava have led to increased cassava varieties with the focus on increased yields and disease tolerance (Uchechukwu-Agua et al., 2015; Chikoti et al., 2016; Chipeta et al., 2016). Variety has a huge role in the production of diversified food products due to inherent properties which vary from one cassava to the other, and such properties include amylose and starch contents. For example, the level of amylose content affect physicochemical properties and amylose content can vary with the variety. However, information on cassava varieties toward end-use properties in Zambian is limited. The introduction and the official release of improved varieties in Zambia would require screening for suitability of processing and culinary usage. Since the development of such starch properties is mainly determined by genetic factors, it would be beneficial to identify cassava roots suitable for processing on a variety basis. The physicochemical and structural properties of cassava flour and starch are major quality criteria for selection of raw cassava flours and starches for use in food formulations and product development. These physicochemical properties are swelling, solubility, gelatinization, retrogradation, enzymatic susceptibility, and pasting. The physicochemical and functional properties are affected by chemical composition and structural characteristics of granular starch (Mtunguja et al., 2016b). The composition include amylose, lipids, protein, ash, fiber, phosphorus (Eleazu and Eleazu, 2012; Mbougueng et al., 2012b; Somendrika et al., 2016) while structural properties include starch granule shape, granule size distribution, degree of polymerization, degree of crystallinity and molecular weight, chain length of amylopectin and amylose (Rolland-Sabaté et al., 2013; Zhu, 2015). Several authors have reported wide variation in starch granule size (Niba et al., 2002; Onitilo et al., 2007; Hoover et al., 2010; Rolland-Sabaté et al., 2013) and their physical properties such as swelling varied accordingly. However, information on the variety effect on starch granule size of cassava starches of the Zambian cultivars is not available.

The amylose and amylopectin ratio effect on swelling, solubility, gelatinization, retrogradation, enzymatic susceptibility and pasting (Mtunguja et al., 2016b; Suriyakul Na Ayudhaya et al., 2016) were not well investigated on cassava starch as the study approach was limited to native starch. There is a need to analyze the amylose and amylopectin ratio of the cassava varieties and consequently investigating the effect of amylose and amylopectin ratio on the physicochemical properties of starches and cassava utilization end products. The search in the literature shows that limited studies have been reported on non-starchy contents

such as phosphorus, lipids and proteins (Asaoka et al., 1991; Mishra and Rai, 2006; Mbougueng et al., 2012a). The non-starchy components such as proteins, ash, and phosphorus even though their quantity is small they were reported to influence starch properties (Nwokocha et al., 2009; Kawai *et al.*, 2012; Maphalla and Emmambux, 2016). The gap is that the non-starchy components were not analyzed in most of the reported cassava cultivars. Consequently, the influence of these components on swelling, solubility, gelatinization, retrogradation, enzymatic susceptibility, and pasting properties cannot be properly ascertained. There is no work done on non-starchy contents of the Zambian cultivars.

The composite flours of cassava-wheat for bread making have been investigated on rheological properties and baking characteristics (Abass et al., 2016), and were cultivar dependent (Shittu et al., 2008a). However, information is limited on the cassava flour effect on rheological properties of the blends and subsequently on the baking characteristics. It was reported that the waxy cassava starch gel had no syneresis after five weeks of storage at -20 ^{o}C (Sánchez et al., 2010) and thus possessed the superior potential for formulating frozen or refrigerated foods. There is a need to investigate the performance of selected cassava flours from different cultivars in the frozen wheat dough for bread making.

In conclusion, the physicochemical and structural properties are principal selection criteria of cassava flour and starch for use in the food industry. The properties such as swelling, solubility, gelatinization, pasting, retrogradation, enzymatic susceptibility are genetic factor dependent. The cassava flour and starches of the Zambian cultivars remain under-researched on value-added products. Besides genetic factors, the amylose and amylopectin ratio, granular size and shape are some of the factors for investigation of swelling, solubility, gelatinization, pasting, enzymatic susceptibility and retrogradation properties of cassava flours and starches. The information, particularly on cassava varieties grown in Zambia is limited on the genetic factor effect on the performance of cassava flours and starches in freezing conditions and frozen doughs for bread making.

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3. PROXIMATE, CYANIDE CONTENTS, AND PARTICLE SIZE DISTRIBUTION OF FLOUR FROM IMPROVED CASSAVA VARIETIES IN ZAMBIA

This chapter is based on the following publication

Chisenga, SM, Workneh, TS, Bultosa, G and Laing, M. 2019. Proximate composition, cyanide contents, and particle size distribution of cassava flour from cassava varieties in Zambia. AIMS Agriculture and Food, 4(4), p.869-891. DOI: 10.3934/agrfood.2019.4.869.

Abstract

Cassava flours were processed from six cassava varieties (*Bangweulu, Katobamputa, Mweru, Kariba, Kampolombo* and *Chila*) cultivated in Zambia using completely randomized block design. The cassava flours obtained were assessed for particle size distribution, dry matter, starch yields; moisture, protein, lipid, fiber and cyanide contents, and whiteness index. The variety effect was significant ($p<0.05$) on all the analyzed quality traits except for protein content. The proximate and cyanide contents were determined using standard methods of AOAC and spectrophotometric procedure, respectively, while particle size was determined by standard sieve methods. The cassava root dry matter contents and starch yields ranged from 40.04–47.25% and 20.76–28.31%, respectively. *Kariba* variety had the lowest starch yield and significantly ($p<0.05$) varied from other varieties. The moisture, protein, lipid, ash and fiber contents of the cassava flours were in the range 10.43-11.18, 1.21–1.87, 0.15–0.63, 1.21-1.78, and 0.03-0.60%, respectively. The average particle size distribution at D90 (250.44-334.34 μm) and D10 (35.56-48.52 μm) varied ($p<0.05$) among varieties. The bulk and packed density ranged 0.40-0.47 and 0.62-0.67 $g.cm^{-3}$, respectively. Bulk density correlated positively with moisture content ($r = 0.56$, $p<0.05$). Carbohydrate content (84.32-86.57%) correlated negatively with lipid ($r = -0.39$, $p<0.01$), ash ($r = -0.73$, $p<0.05$) and positively with fiber ($r = 0.40$, $p<0.01$) contents. The cassava cyanide contents were in the range 23.60-238.12 and 8.62-15.48 mg HCN/kg for roots and flours and were reduced on processing to flours by 60.76-93.86%. The cassava flours attained high degrees of lightness (L*) (93.65 and 94.55) because the flour is dominantly starch. The yellowness (b*) and greenness ranged between 6.52–8.15 and -0.03 to 0.44, respectively. The yellowness most probably imparted by residual carotenoid contents. The whiteness index of flours ranged

from 89.90 to 91.46 and correlated negatively with fiber ($r = -0.44$, $p<0.01$) and dry matter ($r = -0.51$, $p<0.05$) contents. The high root dry matter and starch contents in the cassava varieties show that they are a valuable starchy raw material for the cassava products utilization in the industry.

Keywords: Cassava, flour, particle size, dry matter, starch yield, proximate, cyanides, color

3.1 Introduction

Proximate, dry matter, starch yield and cyanide contents are some of the primary quality indicators for the selection of raw materials in the food industry. Particle size measurement is a quality technique for characterizing the distribution of particles in flours and can be the basis for investigating the physical properties of flours and behavior in water and food systems.

The chemical composition of cassava roots is influenced by factors that include genetics of the cultivar, the geographical location, maturity stage of the plant, and environmental conditions (Burns *et al.*, 2012; Agiriga and Iwe, 2016). The dry matter, lipids, proteins, and fiber contents in cassava roots have been reported. Cassava is rich in carbohydrates and deficient in proteins and fats. On a dry matter basis, cassava root has carbohydrate content of 70-82%, which is predominantly starch containing amylopectin and amylose polymers (Charles *et al.*, 2005; Chiwona-Karltun *et al.*, 2015). The functionality of flours may vary due to the chemical composition of flours. Protein and lipid may affect the rate of hydration (Lu and Lu, 2012). Fresh cassava root is highly perishable due to high moisture content, 33-72% moisture (Emmanuel *et al.*, 2012; Agiriga and Iwe, 2016) and has short postharvest life of less than 72 hours. Thus, immediately after harvest, cassava is required to be transformed into shelf-stable primary products such as flour, chip, and pellets. Processing fresh cassava roots into flour reduces cyanide contents (Kasankala *et al.*, 2019).

Cyanide glucosides content is a limiting quality trait for both human and animal consumption of cassava roots and their derived products (Mtunguja *et al.*, 2016). Consumption of high dietary cyanogens causes a disease known as Konzo, a permanent and clinically upper motor neuron disease (Kashala-Abotnes *et al.*, 2018). The groups at risk include children and women of child-bearing age. Furthermore, cassava dietary toxicity causes tropical ataxic neuropathy in the elderly, a progressive myeloneuropathy that was first described in Nigeria and is characterized by a progressive onset of ataxia (Kashala-Abotnes *et al.*, 2018). Primary processing techniques have been developed with a common goal of reducing cyanides to safe levels in shelf-stable products such as cassava flour, chips, and starches (Montagnac *et al.*, 2009b). High cyanide contents (1090-1550 mg HCN/kg) in cassava roots have been reported (Mlingi and Bainbridge, 1994; Montagnac et al., 2009b). Cyanide content is used as criteria for selecting cassava varieties for use in the breeding programs and food industry (Dixon *et al.*, 1994; Sarkiyayi and Agar, 2010).

Particle size characterization of powder materials is a requirement for food product development and product specification applications. The particle size distribution of flours may have a significant effect on the final product performance on content uniformity of hydration, dissolution, and stability. Foods are commonly used in the form of fine particles during processing and marketing (Barbosa-Cánovas *et al.*, 2012). The physical properties such as bulk density, compressibility, and flowability of a food powder are highly dependent on particle size and size distribution. In the product manufacturing, particle size distribution can influence processes such as conveying, mixing, granulation, drying, milling, and blending, which ultimately impact the quality of the final product (Barbosa-Cánovas et al., 2012). For quality control and system property specifications, particle diameters of flours are described using appropriate procedures such as sieving and laser diffraction (Roa *et al.*, 2015), and are often used in the classification of powder material to obtain cumulative particle size distribution (Scientific, 2012). For example, the use of cassava flours in various food industry applications would require that particle size distribution is specified to ascertain sifting size aperture. The inclusion of cassava flour into wheat in bread making is an important area towards the sustainable utilization of cassava. The quality of wheat bread depends on the particle size of wheat flour, and the finer fractions produced high-quality bread than coarser (Sakhare *et al.*, 2014). The reduced particle size of cassava flours was reported to decrease peak, trough and final viscosities (Hossen *et al.*, 2011).

There is limited information on chemical composition and particle size of cassava flours of the Zambian varieties. In view of this, the aim of this study was to evaluate the proximate composition, cyanides and particle size distribution of six different cassava varieties, one local variety (*Katobamputa*) and five released improved varieties (*Bangweulu, Mweru, Kariba, Kampolombo* and *Chila*) (Table 3.1).

Table 3.1 Selected agronomic characteristics of some released improved cassava varieties in Zambia

	Variety					
Trait	*Kariba*	*Bangweulu*	*Katobamputa*	*Chila*	*Mweru*	*Kampolombo*
Tip colour	purplish green	Purple	Light purple	Light green	Light green	Light purple
Petiole colour	Green	Purple	red	Light green	Green purple	Light green
Old Leaf colour	Green	Dark green	Green	Light green	Light green	Green
Leaf shape	Lanceolate	Spear shape	Lanceolate	Broad	Lanceolate	Lanceolate
Number of lobes per leaf		5	7	5	7	5
Stem colour	Grey	Grey	Silvery green	Light green	Grey	Reddish brown
Outer root skin colour	Light Brownn	Greyish	Cream	Cream	Brown	Brown
Inner skin colour	White	White	White	White	Cream	Pink
Flesh colour	White	White	White	White	White	White
Root shape	Cylindrical	Oblong	Conical/Cylindrical	Long	Fusiform/Long	Cynical-cylindrical
Plant architecture	Semi-branching	Semi-branching	Branching	Semi-branched	Un-branched	Highly branched
Taste	Sweet	Bitter	Flat	Bitter	Sweet	Sweet
Flowering ability	Good	Poor	flowering	Flowering	Non flowering	Flowering
Plant height at maturity	3-4 m	2-3 m	1-2 m	2-3 m	1.6 m	2-3 m
Maturity	16 months	12-16 months	16-24 months	16 months	16 months	16 months
Yield (t/ha)	34 at 16 months	31 at 16 months	29 at 24 months	35 at 24 months	41 at 24 months	38 at 24 months
Reaction to pests/diseases	Tolerant	Moderately resistant	Moderately susceptible	Moderately tolerant	Tolerant	Moderately tolerant
Root Dry matter (%)	41	39	41	41	42	40
Year Released	2003	1993	Indigenous (local)	2003	2003	2003

Source: Mansa Root and Tuber Research Station, Zambia Agriculture Research Institute (ZARI).

3.2 Materials and methods

3.2.1 Source of materials

Six cassava varieties were planted at Mansa Root and Tuber Research Station, a branch of Zambian Agriculture Research Station (ZARI), Mansa District, Luapula Province, Zambia. The station is located 29^{o} 00' E, 11^{o} 30'E, and elevation of about 1200 m. The region receives rainfall (1000 and 1500 mm per year) and has mean annual minimum temperature of 10˚C and maximum temperature 31˚C. The six cassava varieties (*Bangweulu*, *Katobamputa, Mweru, Kariba, Kampolombo* and *Chila)* were planted in a completely randomized block design in triplicates on a plot of 5m x 5m with a plant spacing of 1m x 1m in January 2016 and were harvested at 18 months after planting (June 2017). The roots were collected from five cassava plants randomly selected from each block

3.2.2 Dry matter content

The dry matter content was determined as described in Benesi *et al.* (2004). A 200 ± 05 g fresh peeled cassava roots from undamaged roots selected randomly from 3 plants after medial sections were chipped into strips, mixed thoroughly and dried at 65 ^{o}C until constant weight (about for 72 h) in triplicates. The dry matter content was estimated as the difference between the mass before drying and the mass loss on drying.

$$\text{Dry matter content}, \% = \frac{W_f - W_d}{W_f} \times 100 \qquad (3.2)$$

where

W_f = weight of fresh cassava strips

W_d = weight of dried cassava strips

3.2.3 Native starch isolation and starch yield

The cassava roots were brought to the laboratory for analysis immediately after harvest and starch extraction was conducted as described in Numfor and Walter Jr (1996). The fresh cassava roots were washed, peeled, chopped into small pieces and then pulverized in a blender (Marlex, Ecella model, Kanchan International Limited, Daman, India*)*. The pulp was suspended in 10x its volume of potable water, and the well-stirred mixture was filtered using double cheesecloth. The collected filtrate was allowed to sediment, and after decanting of the supernatant, the sediment was washed six times. The resultant starch was washed using

distilled water, and after decanting, the starch was oven-dried at less than 40 ^{o}C for 12 h. Starch yield was determined based on 400 g of peeled and blended cassava.

$$\text{Starch yield}, \% = \frac{S_f - S_d}{400\ \text{g}} \times 100 \quad (3.1)$$

where:

S_f = weight of fresh starch

S_d = weight of dried starch

3.2.4 Production of cassava flour

The cassava roots were processed into flour using the method of Eriksson *et al.* (2014b). The fresh cassava roots were peeled, washed, grated, dewatered and then sun-dried followed by oven drying at about 45 ^{o}C for 12 h. The dried grits were milled using a centrifugal mill (Retsch ZM200, Haan, Germany).

3.2.5 Fractionation of cassava flours based on particle size

Flour particle size distribution was determined as described in Sonaye and Baxi (2012) by sieving 250 g of sample for 5 min using seven sieves with opening dimensions of 425, 300, 180, 150, 106, 90 and 38μm. The sieves were serially stacked in descending order with the receiver pan at the base. The sample was loaded on the largest sieve on top and covered. The column was placed on the vibratory mechanical shaker (DuraTap, Model DT168, Advantech Mfg. Co., New Berlin). After shaking was completed the sample weight on each sieve was measured. The weight of the materials on each sieve was then divided by sample weight to obtain the percentage retained on each sieve. The next step was then to find the cumulative percent of the retained in each sieve. The cumulative percent passing was calculated by subtracting the percent cumulative retained from 100%.

$$\text{Retained}\ (\%) = \frac{W_{\text{sieve}}}{W_{\text{sample}}} \quad (3.3)$$

$$\text{Cumulative}\ (\%) = 100 - \%\text{Cumulative Retained} \quad (3.4)$$

where:

W_{sieve} = weight of fraction retained on the sieve

W_{sample} = weight of the sample

The percent passing (finer than size) was plotted as the function of sieve sizes. The limits of D10, D30, D50, D60, and D90 were selected as they are commonly used in the classification of powder materials. These parameters refer to the percentages cumulative size distribution of passing particles finer than the particular sieve size. D10, D30, D50, D60 and D90 is defined as the size value corresponding to cumulative size distribution at 10%, 30%, 50%, 60% of 90% by weight, which represents the size of particles below which 10%, 30%, 50%, 60% of 90% of the sample lies. The D10, D30, D50, D60 and D90 were obtained from the plot by performing particle size trend analysis using Excel. For example, particle size of *Bangweulu* flour (312 μm) at D90 was obtained by performing trend formula: TREND(B3:B4,C3:C4,90), where B3=425 μm mesh, B3=300 μm mesh, C3=95.03% finer particles passing through 425 μm mesh, C4=89.46% finer particles passing through 300 μm mesh.

3.2.6 Bulk and packed density

The bulk density was determined as described in Eleazu *et al.* (2014) by adding 50 g of flour sample to a graduated cylinder, and the volume recorded.

$$\text{Bulk density, g/cm}^3 = \frac{V_b}{\text{Weight of sample}} \quad (3.5)$$

where:

V_b = volume of flour

Packed density

The tapped density was determined by mechanically tapping (100x) a graduated cylinder containing flour sample (V_b) until no further volume change was observed. The final volume (V_f) was recorded.

$$\text{Packed density g/cm}^3 = \frac{V_b - V_f}{\text{Weight of sample}} \quad (3.6)$$

where:

V_f = final volume

3.2.7 Moisture content

The moisture content of the dried flour sample was determined in a triplicate according to AOAC (2012) method 925.10 by drying of about 3.0 g sample at 105 ^oC overnight.

$$\text{Moisture content, \%} = \frac{W_b - W_d}{W_b} \times 100 \quad (3.7)$$

Where:

W_b = weight of cassava flour before drying

W_d = weight of dried cassava flour

3.2.8 Ash content

The starch ash content was determined according to AOAC (2012) method 923.03 by taking about 3.0 g sample after carbonization and ignition at 500 ^oC for 6 h in the muffle furnace (J M Ney furnace, model 2-525).

$$\text{Ash content}, \% = \frac{W_b - W_a}{W_b} \times 100 \quad (3.8)$$

where:

W_b = weight of cassava flour before ashing

W_a = weight of ash

3.2.9 Determination of nitrogen content and crude protein

The crude protein content was determined as described in Nuwamanya *et al.* (2010) using Dumas combustion method of nitrogen content analysis (Leco Truspec Model FP-528, St Joseph Mi, USA) by taking about 0.3 g of sample. Percentage protein was calculated as %N x 6.25. The control sample (nutritious flour) contained 16% of proteins.

3.2.10 Determination of crude lipid

The crude lipid content was determined using standard AOAC (2012) method No of 920.39 by taking about 5 g of sample in a Soxhlet extraction unit (Soxhlet, BÜCHI 810, Switzerland) using petroleum ether as a solvent.

$$\text{Crude lipid}, \% = \frac{W_3 - W_2}{W_1} \times 100 \quad (3.9)$$

where:

W_1 = Mass of a sample (g)

W_2 = Mass of the Buchi fat beaker (g)

W_3 = Mass of the Buchi fat beaker with extracted residue (g**)**

3.2.11 Determination of crude fiber

The crude fiber content was determined using AOAC (2012) method No 962.09 after sequential digestion with 0.3M H_2SO_4 and 0.25M of sodium hydroxide, sieving, drying and ignition in a muffle furnace to subtract ash from fiber content. Weighed 5 g sample was

boiled in 50 mL of 0.3M H_2SO_4 under reflux for 30 min, followed by filtering under suction pressure. The residue was washed with hot distilled water to remove the acid. The residue was then boiled in 100 mL, 0.25M of sodium hydroxide under reflux for 30 min and filtered under suction. The insoluble was washed with hot distilled water to free the alkaline. The insoluble was dried to the constant weight in the oven at 100 ^{o}C, 2 h, then cooled in the desiccator. The sample was then carbonized in a blue Bunsen burner and then ashed in a muffle furnace to subtract ash from the fiber.

$$\text{Crude fiber}, \% = \frac{(W_2 - W_1) - (W_3 - W_1)}{\text{Weight of sample}} \quad (3.10)$$

where:

W_1 = weights of residue before drying

W_2 = weight of residue after drying for 2hrs at 100 °C

W_3 = weight of residue after ignite

3.2.12 Total carbohydrate content

The carbohydrate content was determined by difference as described in Emmanuel *et al.* (2012).

$$\text{Carbohydrate content}, \% = 100 - (\text{moisture} + \text{ash} + \text{protein} + \text{lipid} + \text{fiber}) \quad (3.11)$$

3.2.13 Determination of cyanides

The cyanide contents of cassava root and flour samples were determined as described in Orjiekwe *et al.* (2013) with modification. The standard curve for cyanide assay was drawn using a standard stock of potassium cyanide solution (0.2%). From the stock solution, varying concentrations (1 to 10 mg/kg) was prepared. The potassium cyanide solutions in glass bottles were subsequently acidified with 20% hydrochloric acid solution in ratio 1:1 to release the free cyanide and immediately sealed with 3 picrate impregnated filter paper strips. The resulting solution was incubated in a water bath at 95 ºC for 5 min and was subsequently removed from the bath and kept in the laboratory at room temperature for 24 h. The red colored picrate paper strips from each glass bottle were removed and rinsed in 5 mL of 50% ethanol solution and kept for 30 min. The absorbance of the solution was measured using a spectrophotometer at 490 nm wavelength against a similarly prepared blank developed without potassium cyanide solution. The standard curve, $y = 0.0098x + 0.00303$ (R^2 =

0.849) where y = absorbance and x = concentration of sample, was subsequently used for evaluation of cyanide concentration in the test samples.

3.2.14 Whiteness of flours

The whiteness of flours was analyzed using a HunterLab ColorFlex instrument (Hunter Associate Laboratories Inc., Reston, CA, USA). The color of flours regarding L^*, a^*, and b^* were measured after being standardized using Hunter Lab Color standards and their Hunter 'L' (degree of lightness), 'a' (redness to greenness) and 'b' (yellowness to blueness). The whiteness index was calculated as described by Zhu *et al.* (2016) using the equation:

$$\text{Whiteness index} = 100 - [(100 - L)^2 + a^2 + b^2]^{\frac{1}{2}} \quad (3.12)$$

$$\text{Chroma} = (a^2 + b^2]^{\frac{1}{2}} \quad (3.13)$$

where:

L* = lightness

a* = redness to greenness

b* = yellowness to blueness

3.2.15 Experimental design and analysis

The experiment was conducted in a completely randomized design of one factor (variety). A triplicate data were analyzed using one-way ANOVA by using GenStat 18th Edition software. The mean differences were determined using Fisher's Least Significance Difference (LSD) test at the 5% significant level. Correlation coefficients were analyzed by using Pearson's correlation test.

3.3 Results and discussion

3.3.1 Root dry matter contents

The dry matter contents of the cassava root varieties ranged between 40.04 and 47.25% (Table 3.2), and varied ($p<0.05$) among varieties with the highest recorded in *Bangweulu* and lowest in *Kariba*. The indigenous variety *Katobamputa* did not differ significantly ($p>0.05$) from the improved varieties of *Mweru, Kariba,* and *Chila.* The dry matter content is a basis of accepting raw materials in the food industry such as for cassava processing. Dry matter contents in cassava roots were reported to be varied among the accessions and were in the

average range of between 20 and 47% (Teye *et al.*, 2011). The dry matter contents above 30% are considered to be high. Teye et al. (2011) reported the dry matter in the 31.45-40.74% for cassava root harvested at 13 months after planting, which are lower than the values reported in the current study for cassava roots harvested at 18 months after planting. The differences could be attributed to the roots composition differences which are influenced by the genetics of the varieties, harvest age, seasons and growing locations (Buddhakulsomsiri *et al.*, 2018). In this work, *Bangweulu* exhibited the highest dry matter content also has the highest fiber content. Beyene *et al.* (2018) reported that bio-fortification of nutrients in cassava reduced dry matter contents.

3.3.2 Starch yield expressed as fresh weight of cassava

The starch extraction yield expressed as a percentage of isolated starch to the fresh weight of peeled cassava was in the range of 20.76-28.31% (Table 3.2). *Kariba* had lowest starch yield and was significantly different ($p<0.05$) from the other five varieties. The highest starch yield was recovered from *Chila*. Starch is the main constituent of cassava, and a similarly wide range of starch yields have been reported based on wet weight, 20.7-27.8% (Abera and Rakshit, 2003). The starch yield was found to be influenced by various factors. And among others genotype was reported to have a huge influence on starch yield (Benesi et al., 2004). The weak positive correlation between starch yield and dry matter content ($r = 0.07$, $p\leq0.0001$) could be ascribed to loss of materials (non-starchy) during filtration, decantation, and washing. Nevertheless, lowest starch yields (20.76±0.59) were recovered from *Kariba* which had the lowest dry matter contents (40.04±1.62). This is in agreement with Pérez *et al.* (2011) who reported that starch content is dry matter dependent since the high dry matter in the cassava roots is correlated with high starch contents. The industry is focused on high starch yielding cultivars, and thus dry matter content is the basis of selecting cassava on a variety basis.

Table 3.2 Percentage (%) dry matter content and starch yield of six varieties grown in Zambia

Variety	Dry matter	Starch yield
Bangweulu	47.25(2.80) [a]	25.98(1.44)[a]
Katobamputa	40.27(4.31) [b]	26.80(1.83)[a]
Mweru	40.31(1.34) [b]	28.31(4.12)[a]
Kariba	40.04(1.62) [b]	20.76(0.59)[b]
Kampolombo	46.59(1.61) [a]	26.92(3.37)[a]
Chila	43.01(1.27) [ab]	28.11(1.01)[a]
Level of significance		
Variety	$p<0.05$	$p<0.05$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test

3.3.3 Particle size distribution

Table 3.3 shows results for percentage cumulative particle passing (finer than size). Table 3.4 shows results for particle size distribution at selected percentage cumulative of particles passing finer than sieve size.

The percentage of flour particles passing through 38, 90, 106, 150, 180, 300, and 425 μm standard sieves were in the ranges 6.47-11.77, 17.13-49.53, 20.51-61.69, 68.21-78.96, 77.03-82.05, 88.22-93.15, and 94.64-95.85%, respectively, and significantly varied ($p<0.05$) among varieties across all sieve sizes. This suggests that flours were a mixture of various particle sizes. The flour particle size distribution between 90 and 10% cumulative of particles passing finer than sieve were estimated from the particle distribution curve (Figure 2.1). The average particle sizes of flours at D90, D60, D50, D30, and D10 were in the ranges 250.44-334.34, 103.76-142.42, 90.59-133.19, 63.09-114.75 and 35.56-48.52 μm, respectively, and varied ($p<0.05$) among the varieties. *Kampolombo* recorded the largest particle size across the distribution levels except at D10 and exhibited a smaller amount of flour passing at all sieve sizes except at lowest aperture sieve (38 μm). The particle size can affect the pasting and functional characteristics of flours and starches. In a related study on quinoa flours, Ahmed *et al.* (2018) reported that the onset gelatinization temperature decreased from 72.9 to 60.8 °C with decreasing particle size, which suggested that the smallest particle fraction had a lower initiation temperature of gelatinization because of high water absorption for smaller particle size. Oladunmoye *et al.*, (2014) reported that the particle size of flours affect the rate of water absorption during processing as fine particles resulted in faster absorption of water. Lazaridou *et al.* (2019) reported that coarse flour doughs exhibited increased stiffness and resistance to deformation and flow. A related study on rice reported that coarse particles had lower solubility compared with fine and medium particles, and large particle size retarded digestion (Farooq *et al.*, 2018). The selection of sieve size would depend on the end-use of

flours and food system nature. The reduced digestibility in large particle could suggest application in the formulation of resistant starch products. When wheat flour was fractioned by sieving into finer fractions (<75 and 75–118 μm) and coarser fractions (118–150 and >150 μm), the finer fractions were reported to produce high-quality bread (Sakhare et al., 2014). Wang *et al.* (2017) reported that reducing the particle size strengthened the gluten network of dough, and resulted in shorter development time and longer mixing stability of dough because of fast and high water absorption. The reduced particle size of cassava flours from 16.30 to 5.60 μm were reported to result in a decreased peak, trough and final viscosities (Hossen et al., 2011).

Table 3.3 Percentage cumulative particle passing (finer than size) of cassava flours from six cassava varieties grown in Zambia

	Cumulative particle passing finer than sieve size (µm)						
Variety	38	90	106	150	180	300	425
Bangweulu	9.17(0.10)^{d}	34.76(0.06)^{n}	45.58(0.07)^{o}	67.64(0.15)^{r}	74.87(0.15)^{u}	89.19(0.24)^{E}	95.24(0.39)J
Katobamputa	11.77(0.00)^{f}	49.53(0.03)^{p}	61.69(0.01)^{q}	77.63(0.05)^{w}	81.81(0.01)^{z}	91.30(010)^{F}	95.67(0.15)^{K}
Mweru	9.51(0.01)^{e}	33.74(0.04)^{l}	49.46(0.05)^{p}	78.96(0.05)^{y}	85.53(0.02)^{B}	93.15(0.04)^{H}	96.79(0.01)^{M}
Kariba	6.47(0.01)^{a}	28.43(0.00)^{k}	34.46(0.05)^{m}	72.96(0.005)^{t}	77.03(0.01)^{v}	88.41(0.01)^{D}	94.51(0.01)^{l}
Kampolombo	8.46(0.00)^{c}	18.66(0.00)^{h}	20.51(0.00)^{i}	68.21(0.00)^{s}	74.76(0.00)^{u}	88.22(0.00)^{C}	94.64(0.03)^{l}
Chila	8.19(0.00)^{b}	17.13(0.00)^{g}	22.42(0.01)^{j}	78.46(0.04)^{x}	82.05(0.00)^{A}	91.73(0.00)^{G}	95.85(0.00)^{L}
Level of significance							
Variety	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

All values are means of three replications. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test

Table 3.4 Flour particle size at selected percentage cumulative of particles passing finer than sieve size

	Percentage cumulative				
Variety	D90	D60	D50	D30	D10
Bangweulu	312.00(0.00) ^{A}	134.80(0.01)^{o}	114.71(0.01) ^{n}	80.29(0.01)^{h}	39.78(0.01)^{c}
Katobamputa	282.53(0.03) ^{z}	103.76(0.01)^{l}	90.59(0.01) ^{j}	63.09(0.01)^{g}	35.56(0.01)^{a}
Mweru	250.43(0.03) ^{x}	121.69(0.01)^{p}	123.71(0.01)^{q}	81.92(0.02)^{i}	39.02(0.00)^{b}
Kariba	332.52(0.02) ^{B}	135.17(0.03)^{u}	123.72(0.03) ^{q}	94.12(0.01)^{k}	46.35(0.00)^{e}
Kampolombo	334.43(0.01)^{C}	142.42(0.01)^{w}	133.19(0.01) ^{s}	114.75(0.01)^{o}	45.82(0.00)^{d}
Chila	278.49(0.00) ^{y}	135.48(0.00)^{v}	127.64(0.00) ^{r}	111.94(0.03)^{m}	48.52(0.00)^{f}
Level of significance					
Variety	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

All values are means of three replications. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test.

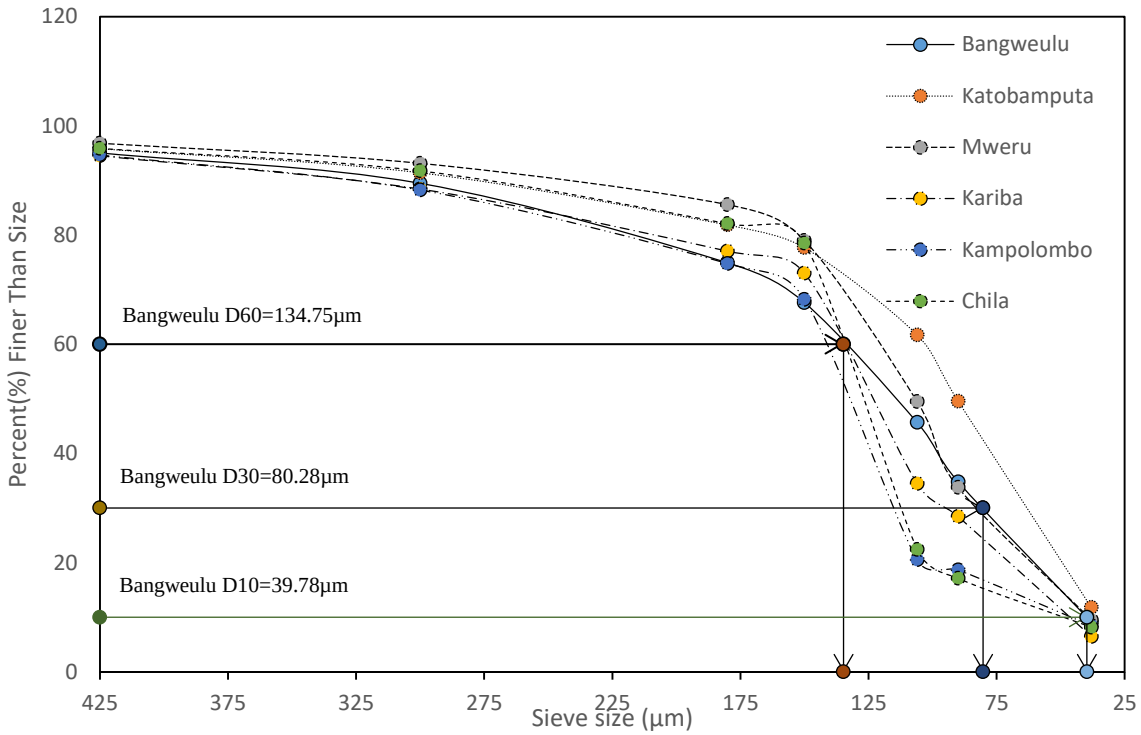

Figure 3.1 Particle size distribution curves for cassava flours from six different varieties at selected percentage cumulative of particles passing finer than standard sieve size at 5% significance level LSD test (Variety=0.055, Sieve size=0.059). Classification and uniformity criteria of D10, D30, and D60 for the particle size distribution of flours derived from Bangweulu variety using TREND in Excel.

3.3.4 Bulk density

Table 3.5 shows the results for the bulky and packed density of cassava flours from six different varieties.

The bulk density ranged between 0.40 and 0.47 g/cm^3 and differed significantly ($p<0.05$) among the varieties. The highest bulk density was recorded in *Mweru* and lowest in *Chila*. The bulk density showed weak positive correlation with protein ($r = 0.31$, $p<0.01$), and lipid ($r = 0.42$, $p<0.01$), and negative with fiber ($r = -0.17$, $p<0.001$). This suggests that flour with high bulk density had high protein and lipid contents. This is in agreement with Oladunmoye *et al.* (2010), who attributed low bulk density in cassava flour to low protein and fat content of cassava flour. Eleazu *et al.* (2014) reported bulk density of cassava flour in the range 0.59-0.68 g.cm^{-3} lower than 0.77 g.cm^{-3} in wheat flour. Wheat flour contained higher protein than cassava flour. Flours with high bulk density exhibited low fiber content, implying that decreased fiber content resulted in finer flour particle size. The positive correlation between bulk density and moisture content ($r = 0.56$, $p<0.05$) suggests that bulk density increases with increase in moisture content. In a related study on roasted Bengal gram flour, an increase in moisture content resulted in an increase in bulk density (Raigar and Mishra, 2015). The negative correlation between bulk density and particle size (D90) ($r = -0.71$, $p<0.05$) suggests that varieties with lower particle size at D90 had higher bulk density. *Mweru* recorded the lowest particle size (250.43 μm) and highest bulk density. The bulk density values can find use in packaging, handling, and processing requirements. Reduced bulk density is a requirement for instant products (Sharma *et al.*, 2012).

3.3.5 Packed density

The packed density was in the range 0.62–0.67 g.cm^{-3}, and varied ($p<0.05$) among the varieties. Packing of powder is the indication of the maximum packing density of flours attained under the influence of defined externally applied forces. The packed densities were higher than bulk densities. This variation could be due to factors such as geometry, size, solid density and surface properties of the flour materials and could be improved when the particles are small, compactable, properly tapped/vibrated and with suitable packaging material (Iwe *et al.*, 2016). Bulk density influences flowability of flours, package design and can be used in determining the requirements of packaging material (Abdullah and Geldart, 1999). It follows that the higher the bulk density, the denser the packaging material required. *Mweru* exhibited both the highest bulk and packed densities. The increase in packed density is desirable as it

offers greater packaging advantage as a greater quantity may be packed within a constant unit volume (Van Toan, 2018).

Table 3.5 Bulky and packed density of cassava flours from six different varieties

	Bulky Density ($g.cm^{-3}$)	Packed Density ($g.cm^{-3}$)
Bangweulu	$0.42(0.004)^{b}$	$0.66(0.007)^{bc}$
Katobamputa	$0.47(0.003)^{d}$	$0.64(0.003)^{b}$
Mweru	$0.56(0.003)^{e}$	$0.67(0.009)^{d}$
Kariba	$0.41(0.004)^{a}$	$0.62(0.009)^{a}$
Kampolombo	$0.43(0.002)^{c}$	$0.67(0.009)^{d}$
Chila	$0.40(0.002)^{a}$	$0.66(0.007)^{c}$
Level of significance		
Variety	$p<0.05$	$p<0.05$

All values are means of three replications. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

3.3.6 Proximate composition

Table 3.6 shows moisture, protein, lipid, fiber, carbohydrates and ash contents for the cassava varieties evaluated.

3.3.6.1 Moisture content

The moisture content of the cassava flour varieties ranged between 10.43 and 11.76%, and varied ($p<0.05$) among the varieties. Manano *et al.* (2017) reported moisture contents of cassava flour varieties in Uganda in the average range of 5.43-10.87%. The differences could be attributed to differences in chemical constituents. Moisture correlated positively with protein ($r = 0.36$, $p<0.01$), lipid ($r = 0.45$, $p<0.05$), and negatively with fiber contents ($r = -0.32$, $p<0.01$). This shows that in high lipid and possibly protein containing materials on drying of flour, moisture loss can be high since water binding capacity is low particularly for lipids. Whereas on drying of cellulosic fiber and starches there can be high water binding by glucose molecules and on drying water loss can be resisted. Moisture content is one of the most common tests in foods since the water content in foods has an important relationship between preservation and the chemical, physical and microbiological changes during the storage (Passos *et al.*, 2013).

3.3.6.2 Protein content

The cassava flour protein contents ranged from 1.21–1.87% and were not significantly different among the varieties ($p>0.05$). The highest protein contents were recorded in

Bangweulu, and the lowest was in *Chila*. Almost similar protein content in cassava flours were reported by Manano et al. (2017) in the range 0.74-1.52% and Emmanuel et al. (2012) in the range 1.76-3.46%. Other authors have reported lower protein values in the range 0.3-0.6% (Charles *et al.*, 2004; Montagnac *et al.*, 2009a), and 0.72% (Somendrika *et al.*, 2016). The differences can be accounted for in terms of environmental conditions such as soil fertility and environmental conditions (Burns et al., 2012; Agiriga and Iwe, 2016). Nitrogen-rich fertilizer was reported to contribute to increase in protein contents in cassava varieties from the range of 4.3-19.30% in unfertilized cassava varieties to the range 9.6-20.9% in fertilized varieties (Shittu *et al.*, 2008). However, these results are alarmingly too high levels for protein in cassava flours. The high levels can be ascribed to additional nitrogen from cyanides during alkaline distillation of acid-digested samples. While it is not certainly clearly understood, the nitrogen in cyanide compounds can contribute to the crude content of nitrogen levels attributed to proteins. In the current study, the proteins content showed a weak positive correlation ($r = 0.12$, $p<0.001$) with cyanide contents in the roots. Protein in cassava flours may possibly influence the four starch pasting properties as a positive correlation with moisture content ($r = 0.36$, $p<0.01$) was observed since moisture is required for flour starch gelatinization and pasting. The entanglement of protein and starch can contribute to viscosity changes during gelation and the resulting matrix could restrict swelling of starch granules during gelatinization. Proteins and starch compete for binding (interaction) with water which could limit starch granule swelling (Lu and Lu, 2012) at low heating temperatures. Protein correlated negatively with carbohydrates ($r = -0.70$, $p<0.05$). This follows the 'dilution hypothesis' which explains the reduction of molecular interactions between protein molecules (aggregation) by increased saccharide contents (Costantino *et al.*, 1998). During drying, saccharides may replace water molecules bonded to proteins. The elimination of water may alter the binding sites of proteins which affect their activities, and presumably decreasing the protein contents.

3.3.6.3 Lipid content

The crude lipid contents for the cassava flour varieties ranged from 0.15–0.63%, and varied ($p<0.05$) among the varieties. The lipid contents in previous studies reported in the range 0.1-0.3% (Charles et al., 2004; Montagnac et al., 2009a), 0.74-1.49 (Emmanuel et al., 2012), and 0.41 (Somendrika et al., 2016). Lipids exhibited negative correlation with carbohydrates ($r = -0.56$, $p<0.05$) and dry matter contents ($r = -0.39$). Lipid such as monoglycerides and

phospholipids can interact with water through hydrophilic (polar heads) or hydrophobic (methyl) groups. The polar lipid, due to their surface-active nature, accumulates at the interface (Larsson, 1982), and have the tendency to interact with water, which justifies their positive correlation with moisture contents in flours. It follows that higher contents of lipids can delay or inhibit hydration of starchy food systems. The formation of amylose-lipid complexes was reported to influence the viscosities of starch pasting and to retard the starch gelation process (Peroni *et al.*, 2006; Nwokocha *et al.*, 2009).

3.3.6.4 Ash content

The ash contents for the cassava flour varieties ranged from 1.21–1.78%, and varied ($p<0.05$) among the varieties. The cassava ash contents in previous studies were ranged from 1.46-2.71 (Rojas *et al.*, 2007), 1.90-2.84 (Emmanuel et al., 2012) and 1.44-2.35 (Eleazu and Eleazu, 2012). The differences in cassava flour ash contents could be attributed to differences in dry matter contents and flour processing methods. Ash contents correlated negatively with dry matter ($r = -0.73$, $p<0.05$), while dry matter and fiber contents were positively correlated, which suggests fiber contents as a significant contributor to ash contents in varieties. In a related study, wheat flour varieties with higher fiber content had higher ash contents (Pavlovich-Abril *et al.*, 2015). Ash content is an indicator of mineral contents and is used as a measurement of quality of flours in the food industry. The redness-greenness (a^*) correlated positively with ash content in wheat flours (Katyal *et al.*, 2016), which suggests higher ash contents can impact the whiteness of flours and bread. Increased mineral content may promote metal chelating activities to form metal ion-pigment complexes (Yu and Nanguet, 2013) which can confer greenness/redness or yellowness color on the final flour product. Nevertheless, in the current study ash negatively correlated with a* and b*, which is justified as starchy vascular ground tissue of cassava do not contain pigments, and formation metal ion-pigment complexes are prominent in the cassava peels (Schwantes *et al.*, 2016).

3.3.6.5 Fiber contents

The fiber contents for the cassava flour varieties ranged from 0.03 to 0.60%. Bangweulu differed significantly ($p<0.05$) from other varieties. The highest and lowest fiber contents were recorded in Bangweulu and Kampolombo, respectively. Fiber content showed a very weak positive correlation with smaller particle size D90 ($r = 0.05$, $p<0.0001$). This may suggest high fibrous cassava would be characteristically coarse while less fibrous is likely to be finer. This was further reflected in the positive correlation of fiber with dry matter ($r = 0.40$, $p<0.05$), which suggests that higher dry matter contents are likely to be associated with

high amounts of fiber and larger flour particle size. The negative correlation between fiber and ash contents ($r = -0.35$, $p<0.05$) could be an indicator of loss of mineral content in high fibrous cassava roots during dewatering (pressing). An increased rate of nutrients release (loss) in highly permeable fibers during processing was reported (Grundy et al., 2016). Furthermore, the negative correlation between fiber and moisture contents ($r = -0.35$, $p<0.01$) could suggest that the high fiber cassava flour has high tendency to lose moisture during dying due to weak water-fiber interaction (bonding). Edible fibers are mainly composed of polysaccharides such as cellulose, hemicellulose, lignin, pectin, and gums. The combination of cellulose microfibrils and cross-linking hemicelluloses with an inter-penetrating pectin network provides strength and rigidity to the cell wall. In cellulose, a network of microfibrils formed by close packing of unbranched β-1,4-glucan chains, which are stabilized by intra- and inter-molecular hydrogen bonds, makes this polymer impermeable and water-insoluble (Grundy et al., 2016). When fiber is present along with starch, it competes for the limited amount of water available in the food system. The partial solubilization of fiber present in mixtures can affect the initial viscosity. Fiber correlated negatively with protein ($r = -0.19$, $p<0.001$) and lipid ($r = -0.18$, $p<0.001$). Fiber contents in cassava flours were observed to increase with the increase in the age of the plant while protein and lipids were found to decrease (Oluwaniyi and Oladipo, 2017). Since age was not the factor in the current study, the variation in fiber can be ascribed to differential genetic responses of varieties.

3.3.6.6 Total carbohydrates

The total carbohydrates for the cassava flour varieties ranged from 84.32–86.57%. The total carbohydrate contents were high in *Chila* and *Kampolombo* ($p>0.05$) with significant difference ($p<0.05$) from other varieties. Similar total carbohydrate contents (80.1–86.3%) were reported by Charles *et al*. (2005). Carbohydrate contents exhibited negative correlation with protein ($r= -0.70$, $p<0.05$), lipid ($r = -0.56$, $p<0.05$), ash ($r = -0.2$, $p<0.01$) and moisture ($r = -0.83$, $p<0.05$) contents. Carbohydrates can interact with proteins through hydrogen bonding via hydroxyl group on saccharides and amine group on proteins (del Carmen Fernández-Alonso *et al.*, 2012), which may result in carbonyl substituted with amide, and subsequently, such bonding will limit the protein activity and availability. Carbohydrate interacts with lipids to form glycolipids through a glycosidic bond (Pomeranz and Chung, 1978) which reduces free lipids. The carbohydrates bind water molecules through hydrogen bonding (Li *et al.*, 2018), hence limiting water mobility which justifies the inverse

relationship between moisture and carbohydrates. There was a weak positive correlation between carbohydrates and dry matter contents ($r = 0.39$, $p<0.01$), suggesting that higher dry matter were associated with higher carbohydrate contents in the cassava flours. The negative correlation between carbohydrate content and bulk density ($r = -0.58$, $p<0.05$) suggests that reduced bulk density had higher carbohydrates contents. The carbohydrates content correlated positively with flour particle sizes, D10 ($r = 0.60$, $p<0.05$), D30 ($r = 0.62$, $p<0.05$), D50 ($r = 0.31$, $p<0.05$), D60 ($r = 0.39$, $p<0.01$), and D90 ($r = 0.28$, $p<0.01$). The strong correlation with D10 and D30 suggests that the smaller flour particle size were associated with reduced fiber contents and increased carbohydrate contents.

Table 3.6 Percentage (%) moisture, protein, lipid, ash, fiber and total carbohydrate contents of cassava flours from six varieties grown in Zambia

Variety	Moisture	Protein	Lipid	Ash	Fiber	Total Carbohydrates
Bangweulu	11.02(1.00)^{ab}	1.87(0.78)^{a}	0.39(0.04)^{a}	1.16(0.05)^{a}	0.60(0.49)^{b}	84.95(1.04)^{ab}
Katobamputa	11.05(1.46)^{ab}	1.45(0.03)^{a}	0.41(0.05)^{ab}	1.78(0.32)^{d}	0.15(0.15)^{a}	85.16(0.18)^{ab}
Mweru	11.76(1.61)^{b}	1.78(0.28)^{a}	0.59(0.18)^{bc}	1.51(0.08)^{c}	0.05(0.06)^{a}	84.32(0.32)^{a}
Kariba	11.18(0.72)^{ab}	1.43(0.41)^{a}	0.63(0.06)^{bc}	1.48(0.06)^{c}	0.04(0.02)^{a}	85.24(1.08)^{ab}
Kampolombo	10.69(0.62)^{a}	1.58(0.15)^{a}	0.32(0.19)^{cd}	1.21(0.09)^{ab}	0.03(0.02)^{a}	86.17(0.58)^{bc}
Chila	10.43(0.37)^{a}	1.21(0.09)^{a}	0.15(0.04)^{d}	1.48(0.07)^{bc}	0.15(0.05)^{a}	86.57(0.41)^{c}
Level of significance						
Variety	p>0.05	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test

Table 3.7 Correlation coefficients among bulk density, proximate composition, and particle size distribution and cyanides contents

Parameters	Bulk Density	Protein	Lipid	Fiber	Ash	Moisture	Carbs	Dry matter	D10	D30	D50	D60	D90	Cyanides Roots	Cyanides Flours
Bulk Density	1														
Protein	0.31**	1													
Lipid	0.42**	0.20	1												
Fiber	-0.17***	0.29	-0.13	1											
Ash	0.26	-0.22	0.14	-0.35	1										
Moisture	0.56*	0.36**	0.45**	-0.32**	0.19	1									
Carbohydrates	-0.58*	-0.70*	-0.56*	-0.08	-0.21	-0.83*	1								
Dry matter	-0.34	-0.02*	-0.39**	0.40**	-0.73*	-0.36**	0.39*	1							
D10	-0.62*	-0.32	-0.31	-0.24**	-0.33	-0.40	0.60	0.17*	1						
D30	-0.46**	-0.23	-0.41	-0.25**	-0.44	-0.41	0.62	0.33*	0.93	1					
D50	-0.16	-0.02	-0.10	-0.22**	-0.55	-0.13	0.31	0.28*	0.80	0.88	1				
D60	-0.53*	0.00	-0.20	0.04	-0.72	-0.29	0.39	0.52*	0.82	0.85	0.88	1			
D90	-0.71*	-0.02	0.01	0.05***	-0.45	-0.28	0.28	0.39	0.44	0.38	0.26	0.59	1		
Cyanides Roots	-0.30	0.12***	0.12	0.35	-0.50	0.03	-0.07	0.18	0.48	0.32	0.56	0.67	0.21	1	
Cyanides Flours	0.21	-0.01	0.12	-0.23	-0.22	0.29	-0.08	0.22	-0.23	-0.19	0.02	0.03	-0.01	0.07	1

D10, D30, D50, D60 and D90 = 10%, 30%, 50%, 60% and 90% cumulative size distribution, respectively. Significance: p<0.05*, p<0.01**, p<0.001***, p<0.0001****

Table 3.8 Correlation coefficients of color parameters, and proximate composition and cyanide contents

Parameter	L*	a*	b*	Whiteness	Chroma	Dry matter	Protein	Lipid	Fiber	Cyanides flours	Cyanides varieties
L*	1										
a*	0.16	1									
b*	-0.06	0.74	1								
Whiteness	0.51*	-0.56*	-0.89	1							
Chroma	-0.06	0.75*	1.00	-0.89	1						
Dry matter	-0.21	0.33**	0.49	-0.51*	0.49	1					
Protein	-0.20	-0.27	-0.29	0.16	-0.29	-0.02	1				
Lipid	0.23	-0.34	-0.77	0.77*	-0.77*	-0.39	0.21	1			
Fiber	-0.47	0.29	0.26	-0.44**	0.26	0.58	-0.19	-0.18	1		
Cyanides flours	0.24	-0.09	-0.12	0.21	-0.11	0.08	0.82	0.16	-0.16	1	
Cyanides varieties	0.44	0.39	0.19	0.04	0.19	0.23	0.02	0.13	0.35	0.47	1

Significance: p<0.01**, p<0.001***, p<0.05*

3.3.7 Cyanide contents in cassava roots

Table 3.9 shows the results for cyanide contents in cassava roots and flours.

The cyanide contents ranged from 23.60 to 238.12 mg/kg and varied significantly ($p<0.05$) among varieties. The lowest and highest cyanide content were recorded in *Katobamputa* and *Bangweulu*, respectively. Mtunguja *et al*. (2016) reported average cyanide contents in the range 133.30-346.70 mg/kg in six cassava varieties of the Tanzanian cultivars, and these cyanide contents varied with genotype and environment. The author observed that the variety *Kiroba* recorded 800, 200, and 40 mg mg/kg from three separate regional sites (Chambezi, Amani and Magadu, respectively) at 15 months after planting. In the current study, the varieties were cultivated on the same site and were rain fed. Thus, the differences in cyanides among varieties could be due to variations in genotype. The dry spell experienced during the rainy season of the cassava plant growth may have affected cyanide contents. Ndubuisi and Chidiebere (2018) reported that the cyanide content of cassava increased during a period of drought due to water stress on the plant. The root cyanide contents exhibited positive weak correlations with genetic traits, protein ($r= 0.12$, $p<0.001$), lipid ($r = 0.12$, $p<0.001$), and fiber ($r = 0.35$, $p<0.01$). The xylem and phloem are fibrous nature (Figueiredo *et al.*, 2015), and retain higher cyanides after harvest. This suggests that higher fiber contents would produce higher cyanides. Cassava roots contain cyanides in different forms. The major glycosides linamarin and lotaustraline are essentially chemically bound (Zidenga *et al.*, 2017). The non-glycosides which are hydrogen cyanide (HCN) and cyanohydride are considered free (Samson *et al.*, 2017). This cyanide can lead to human toxicity problems and would require that cassava for food is processed to remove cyanide-containing substances to safe levels (Montagnac *et al*., 2009b)

3.3.8 Cyanide contents in cassava flours

The cyanide content in cassava flours variety ranged between 8.62 and 15.48 mg/kg, and differed insignificantly ($p<0.05$) among the varieties. The lowest cyanide contents were recorded in *Katobamputa*, and differed significantly ($p<0.05$) from the improved varieties. Lower cyanides were recorded in flours than roots. Percentage cyanide reductions in flours were 93.27, 60.76, 90.94, 93.86, 88.03, and 93.71, for *Bangweulu, Katobamputa, Mweru, Kariba, Kampolombo,* and *Chila*, respectively. This is attributed to the method of processing. Cyanide is largely removed by the traditional processing methods of grating, dewatering (pressing), fermenting, and drying (Montagnac *et al*., 2009b). The highest cyanide retention

was in local variety *Katobamputa*. The improved varieties had lower cyanide retention levels which could indicate the presence of free cyanides such as hydrogen cyanide and cyanohydride and probably also a low level of cyanide-bearing compounds. Cyanide is soluble in water and volatile (25 ^{o}C boiling point) and can be removed on soaking and air drying temperatures (28-40 ^{o}C) (Akande *et al.*, 2017). Cassava varieties are classified as sweet variety when cyanide contents are in the range 15-50 mg/kg, and bitter variety when the contents are from 15-400 mg/kg of fresh cassava (Ndubuisi and Chidiebere, 2018). The recommended safe cyanide level in a final food product is ≤10 ppm (FAO/WHO, 1991). Since cassava flour is a raw material, the cyanides are expected to reduce further in the processing streams. The temperatures for bread dough proofing (30-32 ^{o}C) and baking (178-193 ^{o}C) can significantly reduce cyanides in the final product.

Table 3.9 Cyanide content (mg/kg) and percent cyanide reduction of six cassava varieties at 18 months after planting

Variety	Cassava root	Cassava flour	Cyanide reduction (%)
Bangweulu	238.12(31.11)^{d}	15.48(4.78)^{b}	93.27(2.93)^{b}
Katobamputa	23.60(6.87)^{a}	8.62(0.58)^{a}	60.76(13.82)^{a}
Mweru	167.33(8.92)^{c}	15.16(1.98)^{b}	90.94(1.00)^{b}
Kariba	229.00(24.25)^{d}	13.69(3.55)^{b}	93.86(2.29)^{b}
Kampolombo	113.50(7.15)^{b}	13.59(0.84)^{b}	88.03(0.03)^{b}
Chila	190.61(7.18)^{c}	11.97(0.72)^{ab}	93.71(0.60)^{b}
Level of significance			
Variety	p<0.05	p<0.05	p<0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test. Units: mg/kg = mg HCN/kg

3.3.9 Color of cassava flours

Table 3.10 shows the results for the color of cassava flours.

3.3.9.1 Lightness (L*)

The lightness (L^{*}) of cassava flours varied significantly between 93.65 and 94.69 (p<0.05). Omolola *et al.* (2017) reported the lightness value (88.30-93.57) of cassava flours dried at temperatures 60-72 ^{o}C for 15-20 h. The differences in lightness could be attributed to drying temperature differences. In the current study cassava flours were sun-dried followed by oven drying at 40±5 ^{o}C for 12 h. Drying of flours at elevated oven temperatures can cause scorching and discolorations (personal observation) leading to reduced lightness. Depending on the moisture content of flours, high temperatures reported in Omolola *et al*. (2017) can

gelatinize the flours leading to loss of birefringence properties which can affect the pasting quality of cassava flours.

3.3.9.2 Redness-greenness (a^*)

The redness-greenness (a^*) of the cassava flours ranged between -0.03 (green) and 0.44 (red), and significant differences were observed between *Mweru* and *Chila*. The source of greenness possibly could be due to the residue of cassava peels in the flours. Nevertheless, in the current study, the a* values were lower compared to -0.22 to -0.31 reported by Eriksson *et al.* (2014a).

3.3.9.3 Yellowness (b^*)

The yellowness (b^*) of the cassava flours varied from 6.52 to 8.14 significantly ($p<0.05$) among the varieties. Similar cassava flour yellowness values were reported in the range 5.00-5.15 (Eriksson et al., 2014a). The differences could be attributed to the varied yellowness imparting compounds. In the present study, variation in yellowness could be due to inadequate dewatering of grated cassava. The water in the fresh cassava is the medium of reactive oxygen species (oxidants) (Hu *et al.*, 2018), and can taint the flours yellowish during drying. Also, the yellowness may be due to residual pro-carotenoids compounds or minor Maillard and/or caramelization reaction products formed on drying. During processing toward flours, it requires that the water is expressed out from the grated cassava followed by granulating of mass before drying. Granulation with use of pulverizer or hands is critical to crumble the mass into smaller particles for an increased surface area during drying.

3.3.9.4 Whiteness index

The whiteness index value (89.90-91.46) of the cassava flours varied significantly among the varieties ($p<0.05$) (Table 8). In a similar study, reported whiteness in the range 82.88-89.42 (Omolola et al., 2017). The differences could be attributed to differences in drying temperature and time. Higher temperatures and longer times can impact scorching effect on flours resulting in increased a* (redness) and b* (yellowish) values, which contributes to decreased whiteness. The whiteness correlated positively with L* ($r = 0.51$, $p<0.05$), negatively with a* ($r = -0.56$, $p<0.05$) and b* ($r = -0.89$, $p<0.05$). This implies that the high whiteness index values could be attributed to low a* and b* values, and high L* values. The

fiber contents impacted negatively on whiteness ($r = -0.44$, $p<0.01$), suggesting that high fiber contents decreased whiteness of the flour. The negative correlation of whiteness with dry matter ($r = -0.51$, $p<0.05$) implies that a decrease in fiber contents increased whiteness. Varieties *Mweru* and *Kariba* had low dry matter and fiber contents and yielded high whiteness index.

3.3.9.5 Chroma

The Chroma of the cassava flours were in the range of 6.52-8.16, and varied ($p<0.05$) among varieties. Chroma correlated positively with dry matter ($r = 0.49$, $p<0.01$) and fiber contents ($r = 0.26$, $p<0.01$). This suggests that increased dry matter and fiber content increased chroma. *Bangweulu* had the highest chroma, and exhibited the highest dry matter and fiber content.

Table 3.10 Lightness, redness-greenness, yellowness, whiteness and chroma of flour from six cassava varieties grown in Zambia

	Color parameters				
Variety	L*	a*	b*	Whiteness	Chroma
Bangweulu	94.05(0.02) [b]	0.27(0.02) [ab]	7.48(0.28) [c]	90.46(0.09) [b]	7.48(0.09) [c]
Katobamputa	93.65(0.27) [a]	0.19(0.04) [ab]	7.29(0.42) [c]	90.33(0.69) [b]	7.29(0.59) [c]
Mweru	94.49(0.16) [c]	-0.03(0.05) [a]	6.52(0.20) [a]	91.46(0.14) [c]	6.52(0.16) [a]
Kariba	94.69(0.08) [d]	0.01(0.01) [a]	6.74(0.05) [ab]	91.38(0.21) [c]	6.79(0.21) [ab]
Kampolombo	94.55(0.09) [cd]	0.15(0.03) [ab]	6.97(0.09) [b]	91.15(0.19) [c]	6.97(0.19) [b]
Chila	94.05(0.36) [b]	0.44(0.12) [b]	8.15(0.58) [d]	89.90(0.49) [a]	8.16(0.49) [d]

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. L*=lightness, a*=redness-greenness, b*=yellowness

Color is the consumer preference quality attribute, and it is perceived as a measure of quality. The desired color quality criteria for selection of cassava flours for industrial applications are high value for lightness (L^*) and low value for chroma (Sankhon *et al.*, 2014; Vasconcelos *et al.*, 2017). The small degree shifts in yellowness and greenness impacted on the whiteness of the cassava flours. The low whiteness value of *Chila* was due to high Chroma value. The high

whiteness value of *Kariba* exhibited low Chroma value. In general, factors affecting the color of flours include variety, maturity stage (McClements *et al.*, 2017), and processing procedure (Rodriguez-Sandoval *et al.*, 2017). Cassava flour prepared from unpeeled or not properly peeled roots develops a grey color during wet storage (Jyothi *et al.*, 2007) and develops a purple color during drying (personal observations). The retained color lowers the quality and thus affecting its flour value. The production of flour with increased lightness (L^*) would require processes that include controlled sorting, peeling, enhanced washing through the use of potable water, grating and high press dewatering.

3.4 Multivariate analysis

The principal component analysis was used to determine clusters of similar cassava varieties to ascertain the variety effect on chemical composition and flour particle size. Based on the plot (Figure. 3.2), the varieties *Katobamputa*, *Mweru* and *Chila* were distinguished from other varieties, suggesting that these varieties were significantly different ($p<0.05$). The variety *Kampolombo* was disparate but close to the coordinates of *Kariba*, an indication of insignificant differences ($p>0.05$) between these two varieties. The varieties *Bangweulu* and *Kariba* clustered together, an indication of strong similarities and hence insignificant differences ($p>0.05$). Figure 3.3 shows the quality traits which were the source of variations among cassava varieties. The variety *Katobamputa* negatively associated with the axis of flour particle size, (D90, D60, and D50) and dry matter content, is an indication that *Katobamputa* were strongly influenced by smaller particle sizes (at D90, D60 and D50) and lower dry matter contents than those of other cassava varieties. The variety *Kampolombo* positively associated with the axis of D50, D30, and D10, suggests that was strongly distinguished by larger particle sizes (at D50, D30, and D10) than other varieties. On the contrary, *Mweru* was strongly distinguished by smaller particle size at D30 and D10. The variety Chila was strongly distinguished by higher carbohydrate content and smaller particle size at D30 and D10 than other varieties. The strong similarities between *Kariba* and *Bangweulu* were due to low amount of starch yields. The proximate contents (protein, lipid, and fiber) were not the source of variations among the varieties, except for proteins in *Bangweulu*, and fiber content in *Kariba*. Furthermore, *Bangweulu* was strongly impacted by high cyanide contents.

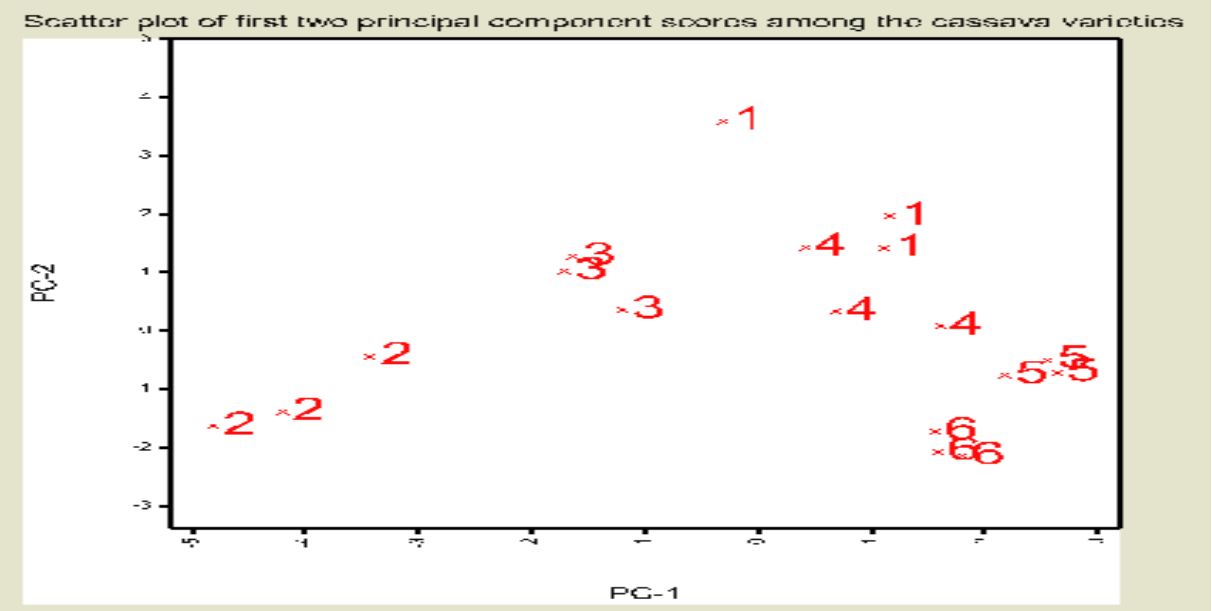

Figure 3.2 Principal component analysis of cassava varieties. Variety 1=Bangweulu,2=Katobamputa, 3=Mweru, 4=Kariba, 5=Kampolombo, 6=Chila

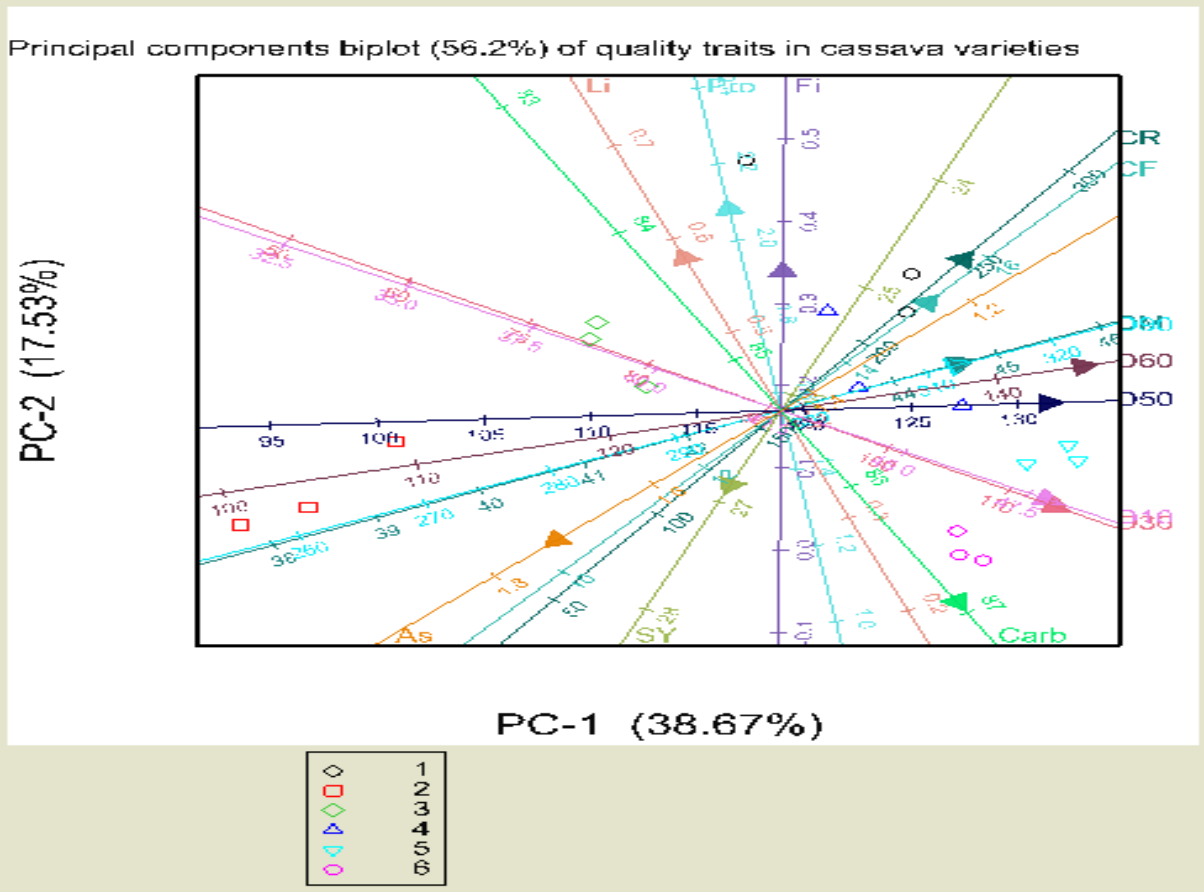

Figure 3.3 Principal component biplot of quality traits in cassava flour varieties. Percentage cumulative of flour particles (D90, D60, D50, D30, and D10), Pr=Protein, Li=Lipid, Fi=fiber, DM=dry matter, SY=Starch yield, Carb=Carbohydrates, CR=cyanides in roots, CF=Cyanide flour.

3.5 Conclusion

The variety effect on the quality traits was significant which suggests that variety can be targeted as the basis of selecting cassava with potential to produce required quality characteristics. The particle size distribution at 90 percent, were within the acceptable size limits for application in the baking industry. The varieties were characterized with high dry matter contents and starch extraction yields. *Kariba* exhibited the lowest dry matter and starch extraction yields. The protein, lipids, fiber and ash contents in cassava flours were generally low which indicates that cassava flour is rich in starches and poor in terms of other nutrients. The cyanide content of cassava roots varied among the cassava varieties. The local variety *Katobamputa* exhibited lowest cyanide content in cassava roots and flours within the recommended safe levels. However, this variety had the highest cyanide retention when processed into flours. The higher reduction of cyanides in flours can be attributed to grating and dewatering processing. The two operation steps can be considered as critical control points in the primary processing of transforming cassava roots into safe shelf-stable product. The high whiteness index and low ash content are some of the primary desirable quality traits for application of cassava flours in the food and non-food industry where the whiteness of the food products are demanded. Nevertheless, to ascertain the suitability for cassava flours applications, it would require that the extracted starches are investigated for physicochemical and structural properties such as morphology, starch granule size, crystallinity, gelatinization, pasting, gel formation, and retrogradation properties.

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4. MORPHOLOGY, STARCH GRANULE SIZE, COLOR AND CRYSTALLINITY OF STARCHES FROM CASSAVA VARIETIES GROWN IN ZAMBIA

This chapter is based on the following paper

Chisenga, SM, Workneh, TS, Bultosa, G and Alimi, BA. 2019. Morphologies, Crystallinity, color and proximate analysis of cassava starches from improved cassava (Manihot esculenta Crantz) varieties. *Acta Agriculture Slovenica.* Under review with manuscript number 1059-6499-2-SM.

Abstract

The extracted starches from six cassava varieties were evaluated for granule- shapes, size, crystallinity, whiteness; and paste clarity. The cassava starches exhibited rounded, truncated and oval granule shapes. The microscopic morphologies and granule size of starches were examined using scanning electron microscopy and image analysis methods, while crystallinity was determined using X-ray diffractometry powder method. The color parameters were examined using Hunter Lab method. The starches granule size were in the range 7.91-10.19 μm and varied significantly ($p<0.05$) among varieties. The cassava starch crystallinity (31.06-33.40%) exhibited insignificant variations ($p>0.05$) and showed prominent features of type A X-ray diffraction patterns. The starches showed high degrees of lightness (L*) obtained were in the range between 93.02 and 94.67, yellowness (b*) 6.86–7.72 with redness in the range -2.32 to -2.01. The calculated whiteness indexes of the starches were ranged from 89.53 to 91.11. The starches paste clarity were ranged from 75.20–78.87% light transmittance with no significant differences ($p>0.05$) and decreased to the range between 44.77 and 63.97% light transmittance during cold storage. The purity of starches was high as evidenced by a high degree of whiteness, and high paste clarity.

Keywords: cassava, morphologies, crystallinity, proximate, paste clarity, starch whiteness index

4.1 Introduction

Starch is an ingredient for various applications in the food industries. Specifying starch regarding end-user properties is required to ascertain desirable functional attributes such as starches gelatinization, paste viscosity, resistance to shear breakdown and starches paste clarity in the food system (Zhu, 2015). The clarity of starch is a quality attribute of starch paste and characterized by measuring light transmittance. High transparent starch pastes are preferred in thickening juicy pie filling, and opaque starch pastes can be used in salad creams (Pereira *et al.*, 2016). Very small size starch granules are used as fat mimetics (Bartz *et al.*, 2017). Starch serves as an ingredient in bread making, meat binder, in confectionary and additive in food and beverages. Other applications of starch granule size include the production of biodegradable plastic and carbonless copy paper. The global demand for starch was estimated at 182 million $t.yr^{-1}$ (Jin *et al.*, 2018). The increased industrial use of starch in food systems and non-food applications would require alternative sources of starches that are cheap but satisfy the end-user product properties. The quality of starch is the main determinant in the selection of starch for the industry. The characteristics of cassava starches could potentially provide useful information on end-use properties for product formulation and development and in the development of national standards as requirements for trade.

Some of the quality traits for starches include color, granule shape and size, starch crystallinity gelatinization and pasting properties, gel clarity retrogradation tendency. The cassava starches granule shapes are oval and truncated, and their granule sizes varied in the range between 4 and 45 μm diameter (Bertoft *et al.*, 2010). Lindeboom *et al.* (2004) categorized starch granule sizes into four classes: large – above 25 μm, medium – from 10 to 25 μm, small – from 5 to 10 μm, and very small – below 5 μm. Hernández-Fernández *et al.* (2016) reported cassava starch paste transmittance (%T) values in the range of 27.74-49.52%.

The starches characteristics are influenced by the genetics of the plant and to some degree also by environmental conditions. The genotype, soil condition, and environmental factors have been reported as important source of variations on starch content and physicochemical traits in cassava roots (Mtunguja *et al.*, 2016a). Several authors (Cadavid *et al.*, 1998; Benesi *et al.*, 2004;

Mtunguja et al., 2016a) reported that genotype had a more significant influence on the cassava root dry matter than the environment. Hence, variety plays a major role in the production of varied food products due to inherent attributes which vary from one cassava to the other, and such properties include morphology, granule size, and crystallinity, gelatinization, pasting and paste clarity. However, these properties were not studied on cassava starches of the Zambian varieties. In this chapter variety effect on starch granule shapes and sizes, color, crystallinity and paste clarity for starches extracted from six cassava root varieties are reported.

4.2 Materials and methods

4.2.1 Source of materials

Starches were collected and extracted as described in Chapter 3 method 3.2.1 and 3.2.2, respectively.

4.2.2 Starch granule and size characteristics

The morphology of starch granules was studied using a scanning electron microscope (SEM) as described in Fannon *et al.* (1992). The double adhesive tape cut into a small piece was attached to a circular (10 mm diameter) specimen stub. The starch sample was then splashed on the adhesive tape to form a film of finely distributed starch particles which were then sputter coated with gold using a sputter coater (Quorum, Q150 ЄS, West Sussex, UK). The prepared gold-coated samples were examined for granule size and shape using SEM (ZEISS, EVO, LS15, Jena, Germany), set at a magnification of 1.00kX with signal A at SEI, I Probe = 30 pA, and EHT = 5.00kV. The obtained microscopic granules from SEM were then subjected to image analysis for starch granule size (diameter) estimation as shown in Figure 4.1 using Soft Imaging System GmbH (Johann-Krane-Weg, Munster, Germany) at the Laboratory of Microscopic Analysis (University of KwaZulu Natal, Pietermaritzburg, South Africa).

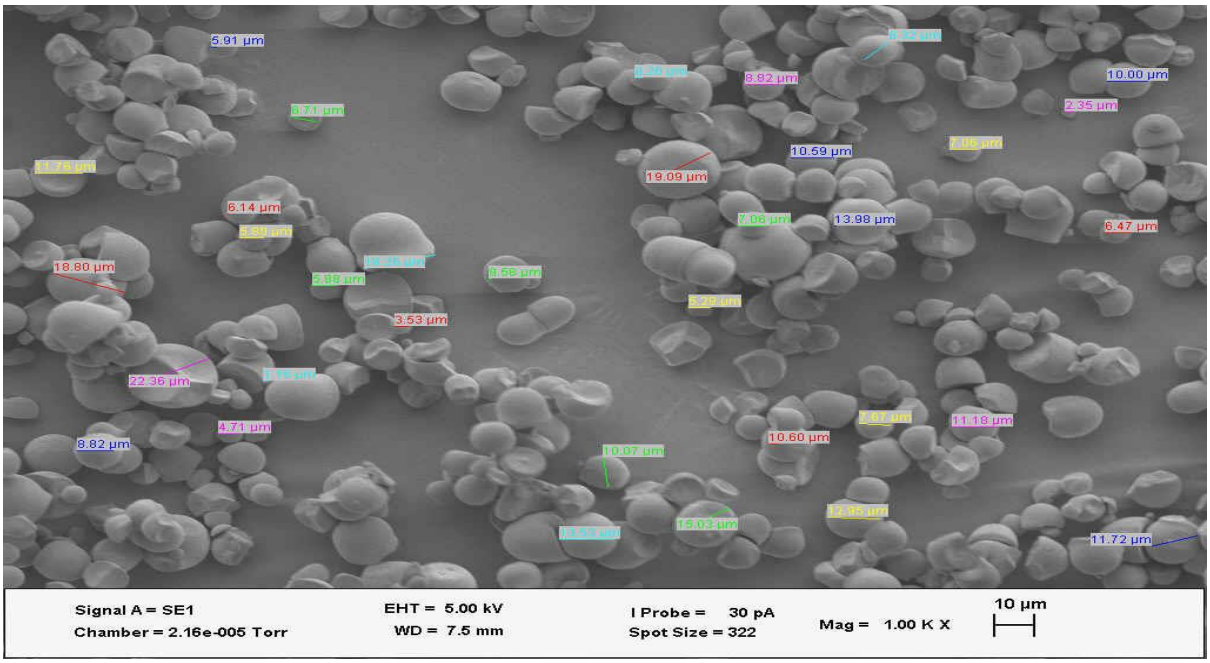

Figure 4.1 Cassava starch granule size evaluated using Soft Imaging System GmbH (Johann-Krane-Weg, Munster, Germany)

4.2.3 Crystallinity

The X-ray diffraction pattern of starch powder samples were studied using analytical X-ray diffractometer equipped with photon counter as described in Huang *et al.* (2007). The X-ray diffractometer (D8 Advance, BRUKER AXS Germany) was equipped with a copper anode X-ray tube (Cu-Kα radiation, $\lambda K\alpha_1$=1.5406Å) and position sensitive detector (LynxEye). Starch samples (1 g) were equilibrated in a 100% relative humidity chamber at room temperature for 24 h prior to the analysis. The diffractometer was operated at 40 mA and 45 kV, and the spectra were scanned over a diffraction angle (2θ) range of 5-40 ° at a step size of 0.1° and a count time of 2 s. The crystalline peak and total area of the diffractogram were measured. The percent crystallinity was calculated as the percentage of peak area to the total diffraction area.

4.2.4 Whiteness of starch granules

The whiteness of starch granules were analyzed as described in Chapter 3 method 3.2.14.

4.2.5 Starches paste clarity

The past clarity of the starch was determined as described in Craig *et al.* (1989) with modification. A 100 mg starch sample was suspended in 10 mL water in screw cap test tubes was

placed in a boiling water bath and heated above cassava starches gelatinization temperature for 30 min by shaking thoroughly every 5 min. After cooling to room temperature, the percent transmittance at 650 nm was determined against water blank using a UV-Visible spectrophotometer.

4.2.6 Experimental design and data analysis

The experimental design comprises a completely randomized design of one-factor cassava variety. A triplicate data were analyzed using one-way ANOVA by using GenStat 18th Edition software. The mean differences were determined using Fisher's Least Significance Difference (LSD) test at the 5% significant level. Correlation coefficients results on dry matter contents and starch yield (Table 3.1, Chapter 3) with starch granule size and color characteristics were performed using Pearson's correlation.

4.3 Results and Discussion

4.3.1 Starch microscopic characteristics

4.3.1.1 Starch morphologies

The cassava starch granules observed through SEM were rounded, oval and truncated in their morphological shapes (Figure 4.2). The truncated granules were observed in all varieties supporting the findings other authors who have reported that cassava starch granules are mostly rounded, truncated or oval (Vasconcelos *et al.*, 2017). Common starches from different plants exhibited distinct morphologies ranging from round and lenticular (wheat), polygonal (rice), round and polygonal (corn) or oval and polygonal (potato) (Bertoft, 2017). The differences in morphology of starch granules could be due to variations in the biochemistry of the chloroplast or amyloplast, and the physiology of the plant (Vandromme *et al.*, 2019). The starch granules contain minor non-starch compounds (protein, lipid, fiber, and phosphorus) which ultimately influences the swelling, gelatinization, pasting and enzymatic digestibility of flours and starches. Enzymatic and acid susceptibility of starches were associated with granule shape (Oates, 1997; Srichuwong *et al.*, 2017).

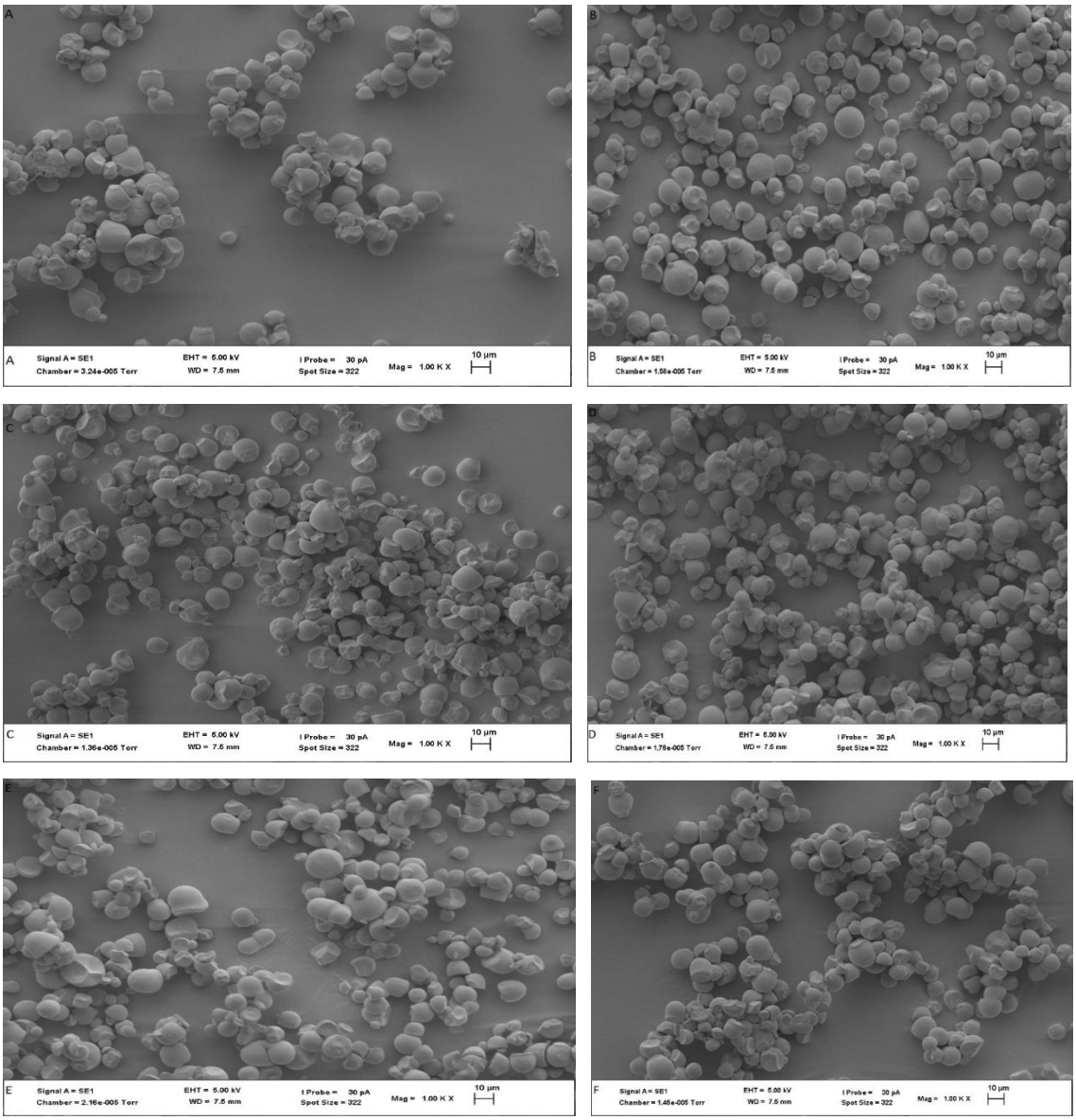

Figure 4.2 Scanning electronic micrographs of starch from cassava varieties: Bangweulu (A), Chila (B), Kariba (C), Kampolombo (D), Katobamputa (E) and Mweru (F).

4.3.1.2 Starch granule size

Table 4.1 shows the results for starch granule sizes and percentage crystallinity.

The granule sizes were distributed in the range between 1.17 and 22.22 µm with an inter-cultivar average of 9.16±3.32 µm among the cassava varieties. Similar granule sizes (12.5 to 13.8 µm) of cassava starch were reported by Mtunguja *et al.* (2016b). According to Lindeboom et al. (2004), the granules size distribution obtained in this study can be classified as very small to medium size. The starch granule size positively correlated with starch yields ($r = 0.518$, $p<0.05$),

implying that smaller granule size had lower starch extraction yield. The largest and smallest starch granule sizes were observed in *Mweru* and *Kariba*, respectively. The granule sizes of *Kariba* were significantly different from the other varieties. There were no significant differences ($p<0.058$) among the granule sizes of starches from *Bangweulu, Kampolombo, Katobamputa,* and *Chila*. The starch granule size between *Mweru* and *Chila* were not significantly different ($p>0.05$). Rice starches were reported to exhibit small granule sizes of sub-microns (0.8-8.7 μm), while large granules sizes were found in potato starches (45-100 μm). The variations in wheat starch granule sizes were ascribed to differences in genotype (Singh *et al.*, 2010). Starch granule sizes influence water absorption, solubility, and swelling (Hedayati *et al.*, 2016). Small granules have a high surface area that can lead to high water absorption capacity (Lindeboom et al., 2004). The small starch granule sizes exhibited higher solubility and increased water absorption capacity (Agnes *et al.*, 2017). In the current study, granule sizes less than 2 μm were recorded, and very small cassava starch granules with a diameter similar to lipid micelles (approximately 2 μm) can be applied as fat mimetics (McClements *et al.*, 2017).

Table 4.1 Starch granule size and percent crystallinty of six cassava varieties grown in Zambia

Variety	Granule size (μm)	Crystallinity (%)
Bangweulu	9.11(4.14)^{b}	32.77(0.88)^{a}
Katobamputa	9.23(3.82)^{b}	32.84(0.71)^{a}
Mweru	10.19(3.52)^{c}	33.34(0.55)^{a}
Kariba	7.91(4.23)^{a}	31.50(1.87)^{a}
Kampolombo	9.09(3.69)^{b}	31.06(1.26)^{a}
Chila	9.44(4.67)^{bc}	32.59(1.85)^{a}
Level of significance		
Variety	$p<0.05$	$p<0.05$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

4.3.2 Crystallinity

The crystallinity of starches were in the range 31.06-33.40% (Table 4.1) and exhibited insignificant ($p<0.05$) differences among the cassava varieties. The crystallinity values are in agreement with previously reported research works, 28.9 - 37.4% (Nuwamanya *et al.* 2010) and 35-40% (Morante *et al.*, 2016). However, the differences in crystallinity could be due to variation in amylose content. Amylose-free cassava starch (waxy) had crystallinity 49% (Gomand *et al.*, 2010; Morante et al., 2016). In a related study, Cheetham and Tao (1998) reported that the degree of crystallinity of free-amylose corn starch was 41.8%, while high amylose content (84%) exhibited 17.2%. Also, crystallinity was reported to be influenced by water content, as crystallinity increased with an increase in hydration (Cheetham and Tao, 1998; Morante et al., 2016).

Starch granule is a semi-crystalline material. The amorphous region consists of amylopectin branching chains and amylose. The crystalline region consists of mainly amylopectin. The starch granules structural crystallinity patterns classified into three types of crystallinity patterns: Type A (Bragg angle 2θ at about 15.3; 17.1; 18.2; and 23.5), Type B (Bragg angle 2θ at about 5.6; 14.4; 17.2; 22.2; and 24) and Type C (Bragg angle 2θ at approximately 5.6; 15.3; 17.3; and 23.5). In the current study, all the starches exhibited prominent crystalline peaks (2θ-scale) at around 15^{o} and 23^{o}, and unresolved double peak at 17^{o}, 18^{o} 2θ (Figure 4.3 and Figure 4.4) This suggests that all the cassava starches fell in the range of Type A crystallinity, a characteristic feature of waxy starches. A similar range of XRD on cassava starch was obtained by Lemos *et al.* (2018).

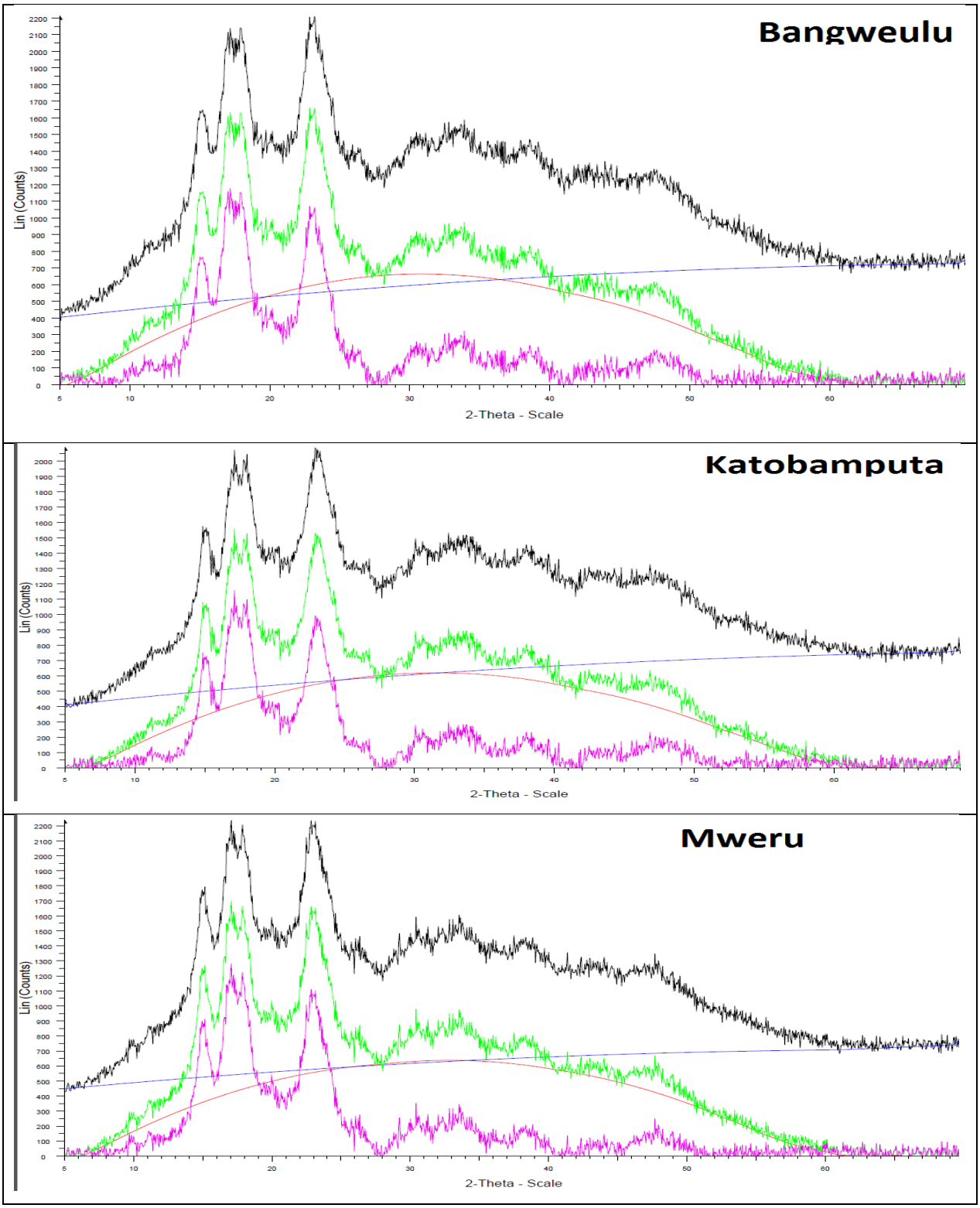

Figure 4.3 X-ray crystalline diffractogram (XRD) (replicated three times) for cassava starches (Bangweulu, Katobamputa and Mweru)

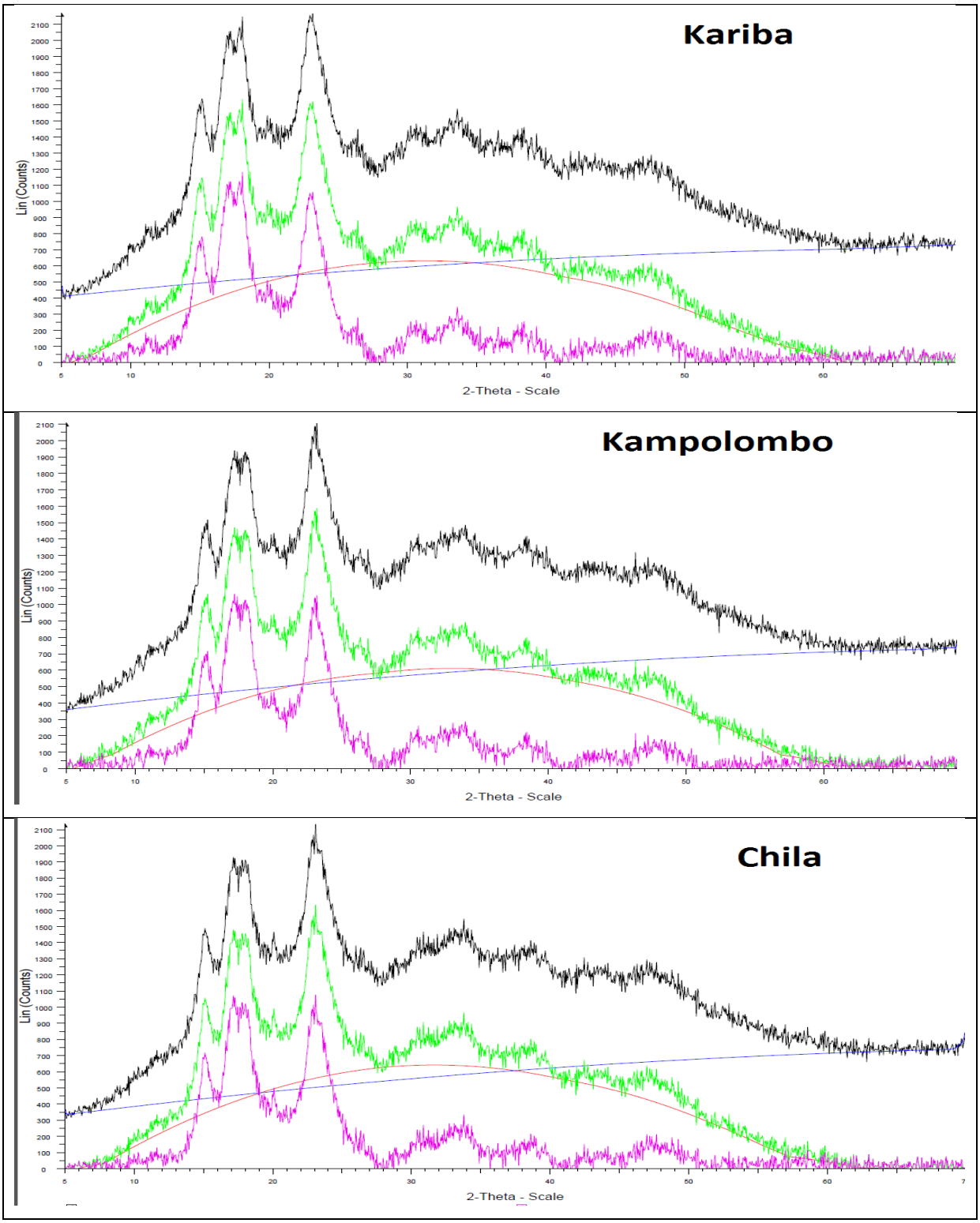

Figure 4.4 X-ray crystalline diffractogram (XRD) (replicated three times) for cassava starches (Kariba, Kampolombo and Chila)

4.3.3 Color characteristics of cassava starches

The results of the color parameters are shown in Table 4.2.

4.3.3.1 Lightness of starch

The lightness (L^*) ranged between 92.02 and 95.47, and varied among cassava varieties ($p<0.05$). The highest and lowest L* were recorded in *Kariba* and *Mweru*, respectively. The L* showed weak negative correlation with granule size ($r = -0.135$, $p<0.001$) (Table 3). This suggests that starches with smaller granule sizes gave higher lightness.

4.3.3.2 Redness-greenness

The greenness (a^*) ranged between -2.48 and -1.69, and, varied among the cassava varieties. The redness was not observed.

4.3.3.3 Yellowness

The yellowness (b^*) ranged from 6.56 to 8.59 and varied among cassava varieties. The L* exhibited negative correlation with a* ($r = -0.85$, $p<0.05$), and b* ($r = -0.728$, $p<0.05$). This suggests that lightness is impacted negatively by traces of redness and yellowness. Color is the consumer preference quality attribute, and it is perceived as a measure of quality. The desired color quality criteria for selection of starches for industrial applications is a high value for lightness (L^*) (Sankhon et al., 2014; Vasconcelos et al., 2017). The results on L^*, a^*, and b^* shows that cassava starches were typically white in color, and were similar to the findings of Benesi et al. (2004) and Bartz et al. (2017).

4.3.3.4 Whiteness index of starch

The whiteness index were in the range 89.53-91.11, and varied ($p<0.05$) among varieties. The highest and lowest whiteness indexes were recorded in *Kariba* and *Mweru*, respectively. Whiteness showed a weak negative correlation with granule size ($r = -0.122$, $p<0.0001$), suggesting that smaller granule size starches showed higher whiteness index. In general, factors affecting the color of starch include variety, maturity stage, and degree of non-starch component removal on starches extraction (McClements et al., 2017), and processing procedure (Rodriguez-Sandoval *et al.*, 2017). Cassava starch prepared from unpeeled or not properly peeled roots develops a grey color during wet storage (Jyothi *et al.*, 2007) and develops a purple color during

drying (personal observations). The retained color lowers the quality and thus affecting its value. The isolation of starch with increased lightness (L^*) and hence whiteness would require extraction processes characterized with controlled sorting, thorough peeling and grating, and use of potable extraction water. Sedimentation of starch is time-dependent, and starch can sediment within approximately 2-3 h, as observed in this study. The suspension (waste water) contains inorganic and organic materials (Pereira et al., 2016), which can settle and form an unwanted layer on top of starch and can taint starch when sedimentation is prolonged, which would require thorough washing after decantation. It is recommendable that decantation follows immediately after sedimentation to prevent tainting of wet starch which would consequently affect the whiteness of the final product. The whiteness of starch influences the starch paste clarity.

Table 4.2 Lightness, redness-greenness, yellowness and whiteness of starches isolated from six cassava varieties grown in Zambia

	Color parameters			
Variety	L*	a*	b*	Whiteness
Bangweulu	94.64(0.88)^{c}	-2.32(0.17)^{a}	6.86(0.24)^{ab}	90.97(0.66)^{bc}
Katobamputa	94.31(0.14)^{c}	-2.23(0.05)^{a}	7.08(0.12)^{b}	90.65(0.14)^{b}
Mweru	93.02(0.74)^{a}	-2.06(0.26)^{b}	7.51(0.70)^{c}	89.53(0.88)^{a}
Kariba	94.67(0.24)^{c}	-2.32(0.06)^{a}	7.72(0.24)^{a}	91.11(0.73)^{c}
Kampolombo	94.43(1.11)^{c}	-2.27(0.19)^{a}	8.85(0.17)^{ab}	90.85(0.14)^{bc}
Chila	93.64(0.27)^{b}	- 2.01(0.03)^{b}	7.52(0.11)^{c}	89.94(0.73)^{a}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. L*=lightness, a*=redness-greenness, b*=yellowness

Table 4.3 Correlation coefficients of color parameters and granule size

Parameter	L	a	b	Whiteness	Granule size	Paste	Dry matter	Starch yield
L	1							
a	-0.795*	1						
b	-0.588*	0.945	1					
Whiteness	0.968*	-0.915	-0.772	1				
Granule size	-0.135**	0.092	0.050	-0.122***	1			
Paste	-0.001	-0.082	-0.104	0.033***	0.020***	1		
Dry matter	0.568*	-0.327	-0.151	0.480	-0.010	-0.466*	1	
Starch yield	-0.357*	0.465	0.529	-0.458	0.518*	-0.236	0.074	1

P<0.05*, p<0.001**, p<0.0001***

4.3.4 Starch paste clarity

The values of transmittance (T) of the 1% starch pastes of the native cassava starches are shown in Table 4.4. The high transmittance values of paste were recorded on the first day in the range

from 75.20 to 78.87% and were not significantly different (P>0.05). Craig et al. (1989) recorded similar results 73% T at 650 nm on 1% cassava starch paste. Paste clarity exhibited negative correlation with starch granule size ($r = -0.020$, $p<0.0001$) and whiteness ($r = 0.033$, $p<0.0001$) implying that smaller granules and a higher degree of whiteness showed higher paste clarity. Paste clarity significantly ($p<0.05$) decreased during cold storage. The paste clarity was observed to rise on the third day to levels ranging between 62.53 and 63.97% and decreased to almost constant levels of 50 to 53 % T (Figure 4.5) for the remaining period of cold storage. The interaction of light with starch granules in the presence of water is expressed as paste clarity. The assumption is that starch in water transmits, reflect and refract light. The native starch in raw granular form appears white and opaque and is characterized with a birefringence with the presence of Maltese cross when examined under the polarized light microscopy (Bartz et al., 2017). However, during gelatinization, degree of starch granule to refract and reflect light diminishes with increased swelling (Craig et al., 1989) leading to the increased transmittance of light. On cooling, the gelatinized starches undergo re-association of starch chains resulting in re-ordering of the system and a partial recrystallization of molecules (Ai and Jane, 2015). These physicochemical changes expel and releases water from the pasted gel (Karim *et al.,* 2000) and thus creating clear spaces in gel filled with water which could have led to increased light transmittance on the third day. Hernández-Fernández et al. (2016) reported cassava paste clarity in the range 27.74-49.52% and suggested cassava starch as a potential ingredient in the processing of confectionaries, jams, and jellies. Transparent gels can be used as carriers of active ingredients composed of oils, surfactants, vitamins, sunscreen agent and antioxidants in the formulation of multifunctional cosmetic gels (Comelles *et al.*, 1992). However, there is limited information on the use of cassava starch gels with high paste clarity for solubilization of active ingredients of lipophilic and hydrophilic in nature.

Table 4.4 Evolution of paste clarity with storage time of cassava starch gels from six cassava varieties

	%Light Transmittance per Day					
Variety	1	2	3	4	5	6
Bangweulu	75.20(3.21)^{a}	55.00(1.53)$^{b-h}$	63.73(1.91)^{bc}	52.93(2.00)$^{c-h}$	51.53(2.05)^{fgh}	50.77(1.80)^{gh}
Katobamputa	76.53(4.55)^{a}	54.13(1.81)$^{b-h}$	63.00(2.26)$^{b-e}$	52.57(1.48)$^{e-h}$	52.00(1.25)^{fgh}	50.20(1.20)^{gh}
Mweru	77.57(2.13)^{a}	56.27(2.30)$^{b-h}$	63.97(4.38)^{b}	53.17(3.32)$^{c-h}$	52.37(2.70)^{fgh}	53.10(0.87)$^{c-h}$
Kariba	78.87(7.82)^{a}	60.77(12.58)$^{b-g}$	63.73(2.57)^{bc}	52.50(1.47)$^{e-h}$	51.93(1.16)^{efgh}	52.43(3.35)^{efgh}
Kampolombo	76.43(2.62)^{a}	54.93(0.72)$^{b-h}$	63.53(0.58)^{bcd}	52.77(0.89)$^{d-h}$	51.93(0.66)^{fgh}	50.97(0.80)^{gh}
Chila	75.30(2.78)^{a}	54.07(1.88)$^{b-h}$	62.53(2.82)$^{b-f}$	53.33(3.02)gh	49.77(3.86)h	50.60(3.74)gh
Level of significance						
Variety	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. The numbers 1,2,3,4,5 and 6 refer to storage days.

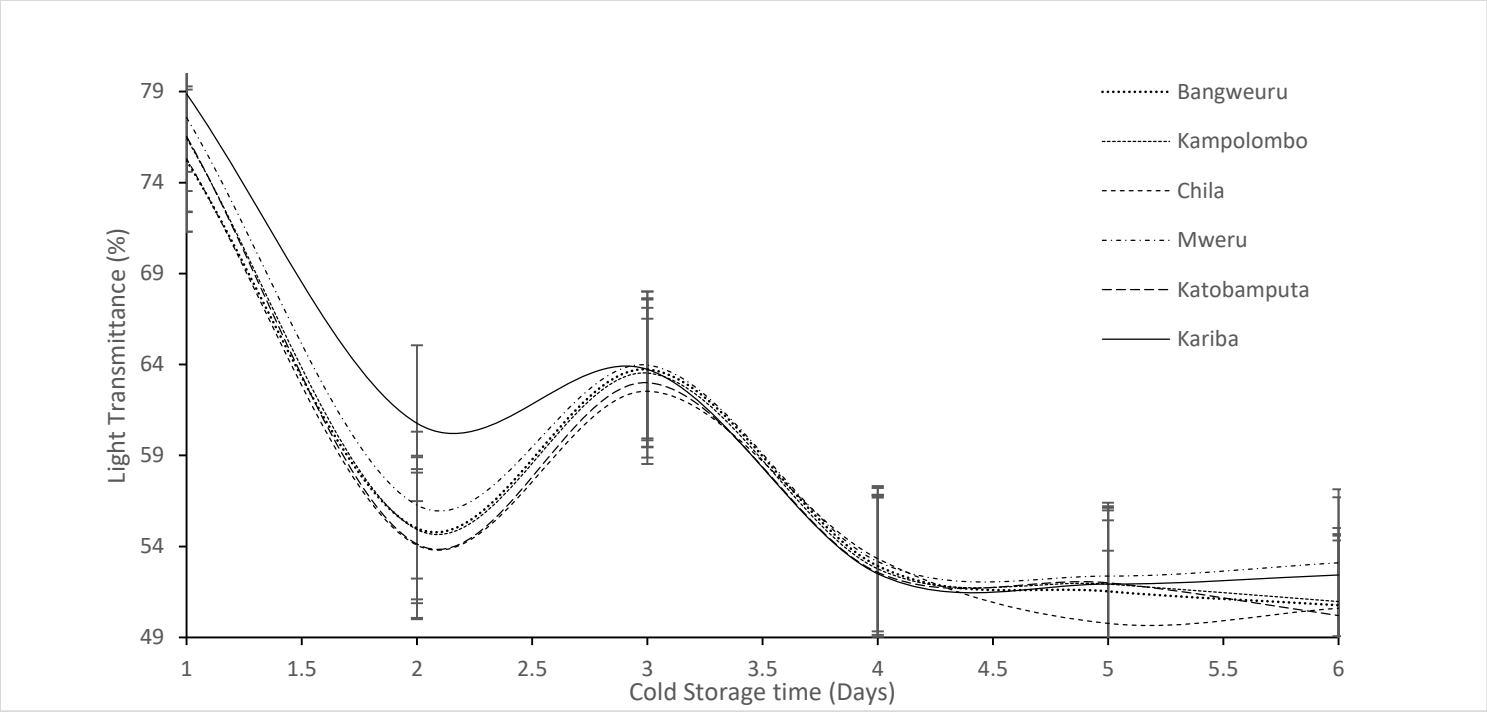

Figure 4.5 The graph of light transmittance (%) as the function of cold storage time (days). All values are means of three replications.
Variety-cold storage time interaction insignificantly different, $p>0.05$ ($p=0.999$), LSD=5.42)

4.4 Conclusion

The microscopic morphologies of cassava starch granules were rounded, oval and truncated. The starches exhibited small to medium granules sizes. The starches showed Type A crystallinity characteristic of high amylopectin content. The high levels of starch paste clarity were recorded in fresh gel and decreased with cold storage time. The values of whiteness were characterized with high levels of lightness and traces of greenness and yellowness. The variety of *Kariba* exhibited the highest values of whiteness and paste clarity. Furthermore, *Kariba* was observed with the smallest granule sizes, potentially an indication for high swelling power starches, and significant starch granule breakdown during gel formation as evidenced in highest paste clarity. The high degree of whiteness is some of the primary desirable quality traits for application of starch in the food and non-food industry. The granule shape is an indicator of the botanical source of starch can be used as a parameter for characterizing product adulteration in powdery products of food and non-food. The variations in starch granule sizes were due to differences in cassava genotype. To ascertain these differences would require determination of amylose content and minor components (phosphorus, protein, lipids, and fiber). Therefore, the next chapter is focussed on the composition of starches.

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5. COMPOSITION OF STARCH: AMYLOSE CONTENTS, PROXIMATE ANALYSIS AND RESISTANT STARCH

This chapter is based on the following paper prepared for submission

Chisenga, SM, Workneh, TS, Bultosa, G and Laing M. 2019. Composition of starch: amylose and resistant starch contents of improved cassava varieties grown in Zambia

Abstract

The composition (moisture, protein, lipid, fiber, ash, amylose contents) and resistant starch (RS) were investigated in raw native starch extracted from six cassava varieties (*Bangweulu, Katobamputa, Mweru, Kariba, Kampolombo* and *Chila*). Variety effect was tested. The proximate contents were determined using standard AOAC methods, while amylose and resistant starch content were investigated using Megazyme starch assay procedure, respectively. The protein content (0.37-0.61%) and fiber contents (0.33-0.46%) insignificantly varied ($p>0.05$) among varieties, while lipids varied ($p<0.05$) among varieties. The ash contents correlated negatively with whiteness index ($p<0.05$), and positively with and starch crystallinity ($p<0.01$). The amylose content (16.04-26.95%) varied ($p<0.05$) among cassava varieties. The amylose contents correlated negatively with crystallinity ($p<0.01$). The RS content in starch (1.12-4.14%) and flour (1.36-5.21) varied ($p<0.05$) among varieties. The RS positively correlated with amylose content ($p<0.001$), ash ($p<0.001$), protein ($p<0.001$), fiber ($p<0.0001$), lipid ($p<0.05$), and granule size ($p<0.001$). The non-RS (digested starch) contents were 66.14-78.33% and 64.35-70.20% for starches and flours, respectively. The total starch contents were 67.36-81.13% and 69.56-72.60% for starches and flours, respectively. The sources of variations in varieties were due to amylose and non-starch contents. The study showed that amylose content is the main genetic trait causing inter-cultivar variations for resistant starches.

Keywords: cassava starch, whiteness index, amylose, resistant starch, non-starch contents

5.1 Introduction

The cassava starch functionalities are associated with polysaccharide fractions of amylose and amylopectin molecules (Zhu, 2015). The differences in amylose contents account for variations in end-user properties. The high amylose contents were reported to exhibit higher resistant starches and lower α-amylase digestibility (Mejía-Agüero *et al.*, 2012).

The *in vitro* enzyme susceptibility of cassava starch by α-amylases have been an area of investigations (Mtunguja *et al.*, 2016b). The resistance of a starch material to digestion is associated with the extent of starch availability to enzymatic hydrolysis in the human digestive system. The resistant starch (RS) is a dietary fiber that does not get digested in the small intestine, and its inclusion in human diets have elicited interest (Raigond *et al.*, 2015) for potential human health benefits. However, enzymatic susceptibility of cassava starches can be influenced by amylose contents, crystalline and granular structure (Van Hung *et al.*, 2017). Digestibility tests were mainly conducted on gelatinized starch based on the rationale that starchy foods are cooked before consumption. Nevertheless, in Zambia, low cyanide fresh cassava (sweet varieties) are consumed raw after peeling and soaking in fresh waters. Noda *et al.* (2008) suggested that estimating the digestibility of raw starch is important when making value-added food products. In the current study, the RS concept is suggested to be utilized as the basis of describing nutrition quality and potentially as criterion parameter for classification and selection of cassava varieties for breeding, product formulations, and development. The diversity in genetic traits accounts for differences in cassava genotypes.

The breeding of cassava in Zambia has led to increased varieties for high yielding, early bulking, pests and disease resistant varieties, reduced cyanide content in cassava to produce sweet varieties. Nonetheless, there is limited information on amylose, resistant starch and non-starch components, and their interrelations with structural properties (crystallinity and granule sizes) for newly released cassava varieties. Variety plays a very important role in the production of diversified food products due to inherent characteristics which vary from one cassava to the other. Thus, the aim of the study was to determine the composition of starch and analyze the variety effect on structural properties (granule size and crystallinity) and resistant starch.

5.2 Materials and methods

5.2.1 Source of materials

Starches extracted according to Chapter 3, method 3.2.3 were used in the study.

5.2.2 Proximate analysis

Chemical constituents were determined according to Chapter 3 methods: Moisture (3.2.7), ash (3.2.8), protein (3.2.9), lipid (3.2.10) and fiber (3.2.11).

5.2.3 Total phosphorus contents

The total phosphorus contents of starch were determined according to AOAC (2012) method 931.01 with some modification. Starch sample about 2.0g was mixed with 1mL $Mg(NO_3)_2$ solution in a porcelain crucible and placed on a water bath with the gradual addition of few drops of hydrochloric acid (HCl) until the material sample approaches complete dryness. The sample was then transferred to the cold furnace and ignited at 500 ^oC for 6 h until uniform grey ash was obtained. After cooling, ash was transferred to 100 mL beaker followed by addition of 5 mL HCl, 50 mL distilled water with heating in a water bath for few minutes followed by cooling and dilution to the volume. To the 5 mL filtrate collected 1 mL ammonium molybdate, 1 mL hydroquinone solution and 1 mL sodium sulfite (Na_2SO_3) solution were mixed and diluted to volume. The absorbance of the phosphomolybdate blue color developed were measured at 650 nm with UV-Vis Spectrophotometer (Evolution 60S, Thermo Scientific, Loughborough, UK). The phosphorus level was estimated from a series of a standard calibration curve prepared from the 25 mg/kg stock solution of dipotassium hydrogen phosphate (K_2HPO_4). The concentrations standard solutions were prepared in the range of 0 to 10 mg/kg. The absorbance against concentration of standard solutions produced calibration curve: $y = 0.0912x + 0.0163$ (y=absorbance, x=concentration of standard solutions) and R^2=0.9982.

5.2.4 Amylose contents

The amylose content in cassava and wheat flour samples was determined using a Megazyme amylose/amylopectin assay kit (K-AMYL 12/16 Megazyme International, Ireland). A 20 mg sample was dispersed by heating in dimethyl sulfoxide (DMSO) solution, lipids were removed by precipitating dispersed starch in ethanol. After the dissolution of the precipitated starch sample in an acetate/salt solution, amylopectin was selectively precipitated by the

addition of lectin concanavalin A (Con A) and removed by centrifugation. The amylose, in the supernatant, was enzymatically hydrolyzed by 0.1 mL amyloglucosidase/α-amylase enzyme system to glucose which was then treated with glucose oxidase/peroxidase (GOPOD) reagent and absorbance of color developed was measured by UV-Vis spectrophotometer at 510 nm. Total starch in a separate acetate/salt solution was hydrolyzed to D-glucose and was reacted with GOPOD reagent and its absorbance was measured similarly. The concentration of amylose in the starch sample was then estimated as the ratio of GOPOD absorbance of the supernatant at 510 nm of the Con A precipitated sample to that of the total starch sample.

$$\text{Amylose, \%(w/w)} = \frac{\text{Absorbance (CoA supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \text{ x } 66.8 \qquad (5.1)$$

5.2.5 Resistant starch assay procedure

The resistant starch (RS) contents in cassava flours and starches were determined using starch assay kit (K-RSTAR 2/17, Megazyme International, Ireland) as starch components that resisted digestion by pancreatic α-amylase and amyloglucosidase (AMG). About 0.15 g sample was used. The non-resistant starch in the sample were hydrolyzed and solubilized by adding 4.0 mL of pancreatic α-amylase containing amyloglucosidase (AMG). Then, the mixture was incubated in water bath at 37 ^{o}C for 16 h. After removing the tubes from water bath, 4 mL ethanol (99% v/v) was added, stirred (vortex) and centrifuged (1500 g, 10 min). The supernatants were decanted into separate collecting tubes, and the pellet (sediment) re-suspended in 8 ml ethanol (50% v/v) and mixed, followed by centrifugation. The supernatant decanted were collected into the respective collecting tube. Suspension and centrifugation was repeated once more by carefully decanting the supernatant into the same collecting tubes. The tube with pellet were inverted to drain off excess water. Then, 2 mL of 2 M KOH was added to dissolve RS under water bath with stirring. Sodium acetate buffer (8 mL of 12 M, pH 3.8) was added followed by 0.1 mL of AMG with mixing placing sample in water bath at 50 ^{o}C for 30 min. The contents were transferred to 100 mL volumetric flask (washing down contents using water) with mixing, and adjusted to 100 mL with distilled water followed by centrifugation. The supernatant of 0.1 mL aliquots were transferred into glass test tubes, to which 3 mL GOPOD reagent was added, and incubated at 50 ^{o}C for 20 min. Absorbance of the diesgted RS solution was measured at 510 nm against the blank. To measure non-RS, the combined supernatants were mixed, and 0.1 mL of diluted AMG solution in 100 mM sodium maleate buffer (pH 6.0) at 20 min at 50 ^{o}C water bath. To non-RS sample tube solutions, 3.0

mL GOPOD reagent was added, incubated (20 min at 50 ^{o}C) and then the absorbance was measured at 510 nm similar as that for digested RS solution.

The percent resistant starch (RS), Non-RS and total starch were calculated on dry weight basis as follows:

$$\mathrm{RS}(\%) = \Delta E \times \frac{F}{W} \times 90 \quad (5.2)$$

$$\mathrm{Non-RS}\ (\%) = \Delta E \times \frac{F}{W} \times 90 \quad (5.3)$$

$$\mathrm{Total\ starch}\ (\%) = \mathrm{RS} + \mathrm{Non-RS} \quad (5.4)$$

where:

ΔE = absorbance (reaction) read against the reagent blank

F = conversion from absorbance to micrograms

W = dry weight of sample analyzed.

5.2.6 Experimental design and analysis

A completely randomized design of one factor was used. Variety is a factor. Triplicate data were analyzed using one-way ANOVA by using GenStat 18th Edition software. The mean differences were determined using Fisher's Least Significance Difference (LSD) test at the 5% significant level. The results from Chapter 4 on starch granule size, crystallinity (Table 4.1) and color characteristics (Table 4.2) were correlated with amylose, proximate and starch resistant contents using Pearson's correlation.

5.3 Results and Discussion

5.3.1 Proximate analysis of starches

Table 5.1 shows the summary of data on moisture, protein, lipids, fiber ash, and phosphorus contents of native starch derived from six cassava varieties.

5.3.1.1 Moisture content

The moisture contents ranged between 5.50 and 6.91 % and varied ($p<0.05$) among the cassava varieties. The lowest and highest moisture content were recorded in *Kariba* and *Mweru*, respectively. The moisture content negatively correlated with lightness (L*) ($r = -0.555$, $p<0.05$) and whiteness index ($r = -0.612$, $p<0.05$) of starches. This suggests that high moisture contents in starches decreased L* and whiteness index. The recommended moisture level for storing commercial starches is 10-12%. The moisture contents greater than 12% encourages microbial contamination and induces degradative biochemical reactions leading

to spoilage of starches during storage (Abdullah *et al.*, 2000). This may possibly increase the redness-greenness (a*) and yellowness (b*) in starches as evidenced in the positive correlation coefficients of moisture content with a*($r = 0.569$, $p<0.05$) and b*($r = 0.535$, $p<0.05$). The moisture content exhibited positive correlation with crystallinity ($r = 0.380$, $p<0.01$). This implies that high moisture content starches had a high degree of crystallinity. The moisture content meets the requirements 5.5 to 7.0% moisture levels for processing starch molded confections. Nevertheless, low moisture contents (<5.5%) can be dusty and may expose to potential explosive hazards, and furthermore, lower moisture contents have reduced flowability (Cooke, 1997). However, starch conditioning is recommended for processing specifications requiring high moisture contents (Delgado and Bañón, 2015). Moisture contents lower than 10% is specified for incorporation into low-density polyethylene matrix in the production of biodegradable products (Kormin *et al.*, 2017). Moisture content showed positive correlation with starch granule size ($r = 0.535$, $p<0.05$). This suggests an increase in moisture content can result in swelling (increase in size) of starch granules due to the plasticizing effect of water.

5.3.1.2 Protein content

The protein content were in the range 0.37-0.61%, and showed insignificant ($p>0.05$) variations, except in *Chila*. The lowest and highest protein contents were recorded in *Mweru* and *Chila*. The protein contents in cassava starches were lower than those recorded in cassava flours (Table 3.5. Chapter 3). The protein value for Nigerian cassava starches were in the range 0.13-0.17% (Abioye *et al.*, 2017). The Malaysian cassava starch had a protein value of 0.55% (Edhirej *et al.*, 2017). Elsewhere, the protein values were 0.34% (Nair *et al.*, 2017), 0.27% (Nuwamanya *et al.*, 2010) and 0.26% (Gutiérrez *et al.*, 2014).

5.3.1.3 Lipid content

The lipid content ranged between 0.03 and 0.17%, and significantly varied ($p<0.05$) among cassava starch varieties. The lowest and highest lipid contents were recorded in *Kampolombo* and *Katobamputa*, respectively. The lipid values from previous studies were reported 0.37% (Abioye et al., 2017), 0.79% (Eke-Ejiofor, 2015), and 1.00% (Oladunmoye *et al.*, 2014a).

5.3.1.4 Fiber content

The fiber content ranged 0.33–0.46% and varied insignificantly ($p>0.05$) among varieties. The fiber contents from previous studies were reported, 0.20-0.23% (Abioye et al., 2017), and 1.50% (Eke-Ejiofor, 2015).

5.3.1.5 Ash content

The ash content were in the range 0.14 to 0.23% and varied among the cassava varieties. The lowest and highest were recorded in *Bangweulu* and *Mweru*, respectively. Previous studies reported ash contents in the range of 0.36-0.37% (Abioye et al., 2017) and 0.12-0.23% (Nuwamanya et al., 2010). The granular surface features such as surface pores (Lindeboom *et al.*, 2004), and these pores including crevices can accumulate non-starch components such as inorganic matter and thus contributing to variations in the ash content. Ash contents correlated negatively with L*($r = -0.640$, $p<0.05$) and whiteness index ($r = -0.555$, $p<0.05$), and positively with a*($r = 0.438$, $p<0.05$) and b*($r = 0.215$, $p<0.01$). This implies that higher ash content had a reducing effect on lightness and whiteness index, and an increasing effect on impurities (a* and b*). The ash content correlated positively with crystallinity ($r = 0.239$, $p<0.01$). This suggests that the increase in ash content (impurities) can result in high degree of crystallinity.

5.3.2 Phosphorus contents

The phosphorus contents of cassava starches were in the range 0.00444 to 0.00754%, and there were no significant differences among the varieties ($p>0.05$). Similar phosphorus content in cassava starches were reported in the range 0.00342-0.00932% (Justamante Händel Schmitz *et al.*, 2017), 0.007% (Abiola, 2014) and 0.014% (Gutiérrez et al., 2014). The phosphorus contents were not detected in six varieties reported by Mtunguja et al. (2016b). The low phosphorus content in cassava varieties is probably attributed to low level of starch molecules phosphorylation and weak bondage of phosphorus to amylopectin, which might be easily washed away during extraction processes. Phosphorus is a minor non-starchy component of the starch granules but can affect functional properties of starch. Swinkels (1985) reported that phosphate monoester in root and roots promote its hydrophilic nature and thus increases paste transmittance.

Table 5.1 Moisture, protein, lipid, fiber, ash and phosphorus contents (dry basis) of cassava starches from different varieties

Variety	Moisture (%)	Protein (%)	Lipid (%)	Fiber (%)	Ash (%)	Phosphorus (%)
Bangweulu	5.98(0.26)^{bc}	0.47(0.13)^{a}	0.10(0.01)^{a}	0.41(0.35)^{a}	0.14(0.02)^{b}	0.007($1.5x10^{-3}$)^{a}
Katobamputa	6.56(0.61)ab	0.48(0.06)^{a}	0.17(0.06)^{ab}	0.33(0.28)^{a}	0.23(0.01)^{a}	0.006($1.5x10^{-3}$)^{a}
Mweru	6.91(0.27)^{a}	0.37(0.02)^{a}	0.15(0.02)^{bc}	0.15(0.15)^{a}	0.23(0.04)^{a}	0.005($6.4x10^{-4}$)^{a}
Kariba	5.51(0.13)^{c}	0.40(0.02)^{ab}	0.09(0.05)^{c}	0.11(0.19)^{a}	0.22(0.01)^{a}	0.004($6.2x10^{-4}$)^{a}
Kampolombo	6.39(0.19)^{ab}	0.49(0.08)^{ab}	0.02(0.01)^{cd}	0.46(0.32)^{a}	0.14(0.02)^{b}	0.005($1.6x10^{-3}$)^{a}
Chila	6.54(0.49)^{ab}	0.61(0.08)^{b}	0.03(0.02)^{d}	0.38(0.52)^{a}	0.19(0.02)^{ab}	0.008($2.2x10^{-3}$)^{a}
Level of significance						
Variety	p<0.05	p>0.05	p<0.05	p>0.05	p<0.05	p>0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test.

5.3.3 Amylose content

The amylose contents in cassava varieties were in the range 16.04–26.95% (Table 5.2). The amylose contents in *Katobamputa* was significantly different (p<0.05) from other cassava varieties. Similar cassava amylose contents have been reported, 19.50-20.30% (Morante et al., 2016), 22.60±1.30 (dos Santos *et al.*, 2018), and 17.06-25.72% (Liu *et al.*, 2019). The amylose content for Tanzanian cassava starches were within the range 11.9-19.4% (Mtunguja et al., 2016b). The reported values of amylose contents for Nigerian cassava starches were 21.0-22.5% (Abioye et al., 2017), 26.73% (Eke-Ejiofor, 2015), 22% (Abiola, 2014), and 19.2 % (Oladunmoye *et al.*, 2014). The amylose content for Thailand cassava starch was reported 26.85% (Suriyakul Na Ayudhaya et al., 2016). The differences in amylose contents could be due to differences in genotype (Mejía-Agüero et al., 2012; Mtunguja *et al.*, 2016a) and differences in methods of analysis. The iodine binding method is widely used, however, it has been reported to be not consistent because of variations in the starch dispersion procedures, starch lipids that potentially complex with amylose and residual starch proteins (Vilaplana *et al.*, 2012). Amylopectin molecules also weakly form complexes (about 1 mg iodine/100 mg amylopectin) with absorption maxima at 540 nm with iodine as compared to amylose (20 mg iodine/100 mg amylose) which has absorption maxima at 620 nm (Vilaplana et al., 2012). Such weak iodine binding by amylopectin could lead to a slight overestimation of amylose content even though the interference in the absorption measurement is insignificant (Zhu, 2015). Other methods include potentiometric (Soto *et al.*, 2014; Castaño *et al.*, 2016), amperometric (Jansen *et al.*, 2012), and paper-based microfluidic chip (Hu *et al.*, 2015). Megazyme International's amylose/amylopectin assay kit is reported as an efficient and rapid measurement of amylose content. This kit is applicable to all pure starch sample analyses.

Table 5.2 Amylose contents of cassava starches from different varieties

Variety	Amylose (%)
Bangweulu	22.22(2.78)[ab]
Katobamputa	26.95(2.30)[b]
Mweru	17.95(8.02)[a]
Kariba	18.47(7.30)[a]
Kampolombo	16.15(3.88)[a]
Chila	20.83(0.45)[ab]
Level of significance	
Variety	$p<0.05$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

The amylose content is the basis of classifying starches into waxy, semi-waxy, normal/regular and high-amylose types when amylose content is 0-2%, 3-15% 20-35%, and higher than 40% of the total starch, respectively (Tester et al., 2004; Morante et al., 2016; Botticella *et al.*, 2018). The result shows all the cassava flour varieties were generally classified as normal/regular starches. Amylose contents exhibited a negative correlation with crystallinity ($r = -0.442$, $p<0.01$), implying that high amylose starches had low crystallinity. This result is in agreement with the finding of Cheetham and Tao (1998), who demonstrated that high maize amylose gave low starch crystallinity. Similar was observed by Morante et al. (2016) who reported that free-amylose cassava starch showed a high degree of crystallinity (49%). Nevertheless, Zobel (1988) reported that it is not always obvious that X-ray diffraction of starches is a function of amylose contents, but the type of crystal (A, B, C) transition are likely to result from the presence of amylose extender (ae) gene expression which causes amylopectin to have longer size chain. Furthermore, crystal types were reported to depend on the weight of average chain length (Zobel, 1988). A-type crystallinity are short chains, and B-type crystallinity associated with long chains, while C-type crystallinity exhibited intermediate chains (Jenkins and Donald, 1995; Zhu, 2015). Amylose content showed a negative correlation with starch granule size ($r = -0.024$, $p<0.0001$), suggesting that high amylose starches had small granule sizes. Similar was observed by Charles et al. (2005), who reported that cassava starch varieties with smaller granule dimensions were characteristic of higher amylose contents. This could be due to reduced interference from fiber contents. Smaller granule size had lower fiber contents ($r = 0.164$, $p<0.0001$). The high amylose contents can increase resistant starches (Hallström et al., 2011).

5.3.4 Resistant starch

The resistant starch (RS) for extracted cassava starches were in the range 1.12-4.14% RS (starch basis) (Table 5.3), while cassava flours gave 1.36-5-5.21% RS, and varied ($p<0.05$) among varieties. The lowest and highest RS both starches and flours were recorded in *Mweru* and *Katobamputa*, respectively. The insignificant difference between extracted starch and flour is owing to RS analytical protocol based on the precipitated purified starch of the sample. RS in cassava starch were reported in the range 5.99-6.01% RS (Abioye et al., 2017), 2.2-4.5% RS (Vatanasuchart *et al.*, 2009) and 9.69% RS (Moongngarm, 2013), and 0.19-2.21% RS (Pereira and Leonel, 2014b). The RS values in cassava starch (10.4±1.21%) and flours (19.3±3.80%) have been reported (Aprianita *et al.*, 2014). The differences could be due to variations in non-starch components (protein, lipid, fibe, and ash). The non-starch contents of cassava flour (Table 3.3, Chapter 3) were higher than in extracted starch. In the current study, RS positively correlated with ash ($r = 0.104$, $p<0.0001$), protein ($r = 0.171$, $p<0.001$), fiber ($r = 0.195$, $p<0.0001$) and lipid ($r = 0.555$, $p<0.05$) (Table 5.4). This suggests that higher non-starch contents increase RS. The non-starch components might have competed for available water (Moorthy *et al.*, 1996; Defloor et al., 1998) which may possibly inhibit enzymatic hydrolysis of starch. High levels of RS values (5.00–19.6%) recorded in cassava flour samples (Mejía-Agüero et al., 2012) were characteristic of C-type X-ray diffraction pattern, which is highly associated with slow or incomplete digestion *in vitro* and *in vivo* (Mejía-Agüero et al., 2012). The RS positively correlated with amylose content ($r = 0.214$, $p<0.001$) (Table 5.4). This suggests that high amylose starches had high RS content. Similar was observed by Mtunguja et al. (2016b) who reported that amylose was inversely proportional to starch digestibility. RS positively correlated with granule size ($r = 0.166$, $p<0.001$). This suggests that larger size particles could result in increased RS. Noda *et al.* (2008) reported that starch granule size influences the digestibility of raw starch by amylase. Large starch granules are characteristic of smaller surface area which decreases the α-amylase digestion rate. The RS content of common white bread is naturally low (<2%) (Sullivan and Small, 2019), however, bread has been suggested as medium for the fortification of dietary RS (Sullivan and Small, 2019). Nevertheless, the specific volume of bread was reported to be inversely related with RS content (Tien *et al.*, 2019). This suggests that increasing RS in bread decreases the bread volume.

5.3.5 Non-resistant starch (Non-RS)

The digested (non-RS) for the starches and flour were in the range 66.14-78.33% and 64.35-70.20%, respectively (Table 5.3). The non-RS was reported in cassava starch (44.4±2.90%) and flours (72.1±2.90%) (Aprianita *et al*., 2014). The percentage of digested cassava starch were reported in the range 70-80% (Mtunguja et al., 2016b), 75.00-94.80% (Pereira and Leonel, 2014b). The differences could be attributed to variations in digestibility time, and form of starch (raw or gelatinized). A related study by Park *et al.* (2018) on maize starch, reported that swelling and rupture of the starch granule and melting of crystalline structures during gelatinization accelerated the digestion of native starch. Mtunguja *et al*. (2016b) demonstrated that differences in α-amylase hydrolysis rate between raw and gelatinized starch were more prominent for digestibility time range 1-6 h, and digestibility time (16-24 h) did not exhibit significant differences. In the current study, the α-amylase digestibility incubation were based on 16 h.

5.3.6 Starch contents

The total starch content (dry weight) of cassava starches and flours were in the range 67.36-81.13% and 69.56-72.60%, respectively. The variety *Bangweulu* was significantly ($p<0.05$) different from other cassava starch varieties. There was no significant ($p>0.05$) variations in total starch content among the cassava flours. Generally, total starch contents in starches and flours were similar ($p>0.05$) except for high value in *Bangweulu*. Starch content (dry matter) in cassava were reported in the range 87.8-89.2% (Eriksson *et al.*, 2014), 77.4% (Aprianita et al., 2014), 74.3-80.3% (Mtunguja et al., 2016b) and 70.4-89.9% (Nuwamanya et al., 2010). The sources of differences in starch contents were reported to be more likely due to variations in genotype than environmental factors. The genotype had huge influence on the variability of starch contents and yields, although the effects due to variation in environmental factors were insignificant (Mtunguja et al., 2016a). Similarly, Mejía-Agüero et al. (2012) screened and compared starch content among twenty-five cassava cultivars planted and harvested simultaneously in a single plantation, and observed significant differences in starch contents due to inter-cultivar variability with insignificant influence from environmental factors.

Table 5.3 Resistant starch (RS) content, non-resistant starch (Non-RS) and total starch contents of six different cassava varieties

	RS (%)		Non-RS (%)		Total starch (%)	
Variety	Starch	Flour	Starch	Flour	Starch	Flour
Bangweulu	2.81(0.62)^{cd}	2.32(0.52)^{bcd}	78.33(6.84)^{b}	68.22(0.58)^{a}	81.13(7.03)^{b}	70.55(1.06)^{a}
Katobamputa	4.14(1.04)^{e}	5.21(0.67)^{f}	66.47(2.05)^{a}	64.35(2.69)^{a}	70.6(1.76)^{a}	69.56(3.05)^{a}
Mweru	1.12(0.42)^{a}	1.35(0.22)^{a}	68.11(5.82)^{a}	69.08(1.84)^{a}	69.23(5.84)^{a}	70.43(2.02)^{a}
Kariba	1.22(0.64)^{a}	2.06(0.81)$^{a-d}$	66.14(10.22)^{a}	69.36(1.84)^{a}	67.36(10.28)^{a}	71.43(1.48)^{a}
Kampolombo	1.40(0.46)^{ab}	1.48(1.12)^{abc}	69.51(2.13)^{a}	69.42(2.82)^{a}	70.91(2.42)^{a}	70.9(2.27)^{a}
Chila	1.68(0.05)^{abc}	2.40(0.95)^{cd}	69.07(5.39)^{a}	70.2(4.41)^{a}	70.74(5.44)^{a}	72.6(4.20)^{a}
Wheat flour (control)		2.06(0.16)$^{a-d}$		68.02(3.17)^{a}		70.08(3.10)^{a}
Level of significance						
Cassava variety	P<0.05	P<0.05	P<0.05	P>0.05	P<0.05	P>0.05

Table 5.4 Correlation coefficients of cassava starch quality parameters

Parameter	L*	a*	b*	Whiteness	Granule size	Crystallinity	Ash	Moisture	Amylose	Protein	Fiber	Lipid	RS Starch
L*	1												
a*	-0.795	1											
b*	-0.588	0.945	1										
Whiteness	0.968	-0.915	-0.772	1									
Granule size	-0.135	0.092	0.050	-0.122	1								
Crystallinity	-0.381***	0.308	0.233	-0.376	-0.096	1							
Ash	-0.640*	0.438**	0.215***	-0.555	-0.093	0.239	1						
Moisture	-0.555*	0.569	0.535	-0.612	0.535	0.380***	0.106	1					
Amylose	0.505*	-0.285	-0.149	0.444	-0.024	-0.442**	-0.306	-0.029	1				
Protein	0.056*	0.363	0.483	-0.100	0.243	-0.033	-0.263	0.159	0.050	1			
Fiber	0.259	0.000	0.144	0.151	0.164	-0.192	-0.434	0.219	0.151	0.498	1		
Lipid	-0.262	0.024	-0.142	-0.156	0.019	0.540	0.516	0.180	-0.101	-0.394	-0.277	1	
RS Starch	0.244	0.038	0.097	0.170	0.166	0.292	0.104	0.154	0.214***	0.171	0.195	0.555	1

Significance: p<0.05*, p<0.01**, p<0.001***, p<0.0001****, L*=lightness, a*=redness-greenness, b*=yellowness

5.4 Multivariate analysis

Figure 5.1 shows principal component analysis of quality traits of cassava varieties. The variety *Katobamputa* was near the axis of ash, lipid, amylose contents, and RS. The axes of amylose and RS clustered together and were in the same direction but opposite to that of the axes for granule size, and protein and fiber contents. This suggests that RS was significantly ($p<0.05$) influenced positively by lipid and amylose contents, and negatively by granule sizes, protein, and fiber contents. The varieties *Chila* and *Kariba* associated with the axis of granule size but in the opposite direction, which suggests that Chila was significantly ($p<0.05$) distinguished by large granule sizes, while Kariba was distinct by small granule size. The variety *Bangweulu* was distinguished by a higher axis of whiteness, non-RS, and total starch contents. Moreover, the whiteness, non-RS and total starch closely associated in the same direction. This suggests that highly digested starches have a high content of total starches. Furthermore, high whiteness color of starches could potentially indicate highly digested starches. In addition, whiteness axis was in the opposite direction of moisture content, and impurities (redness-greenness), an indication that b* and a* and moisture content have significant ($p<0.05$) negating effect on the whiteness color of the starches.

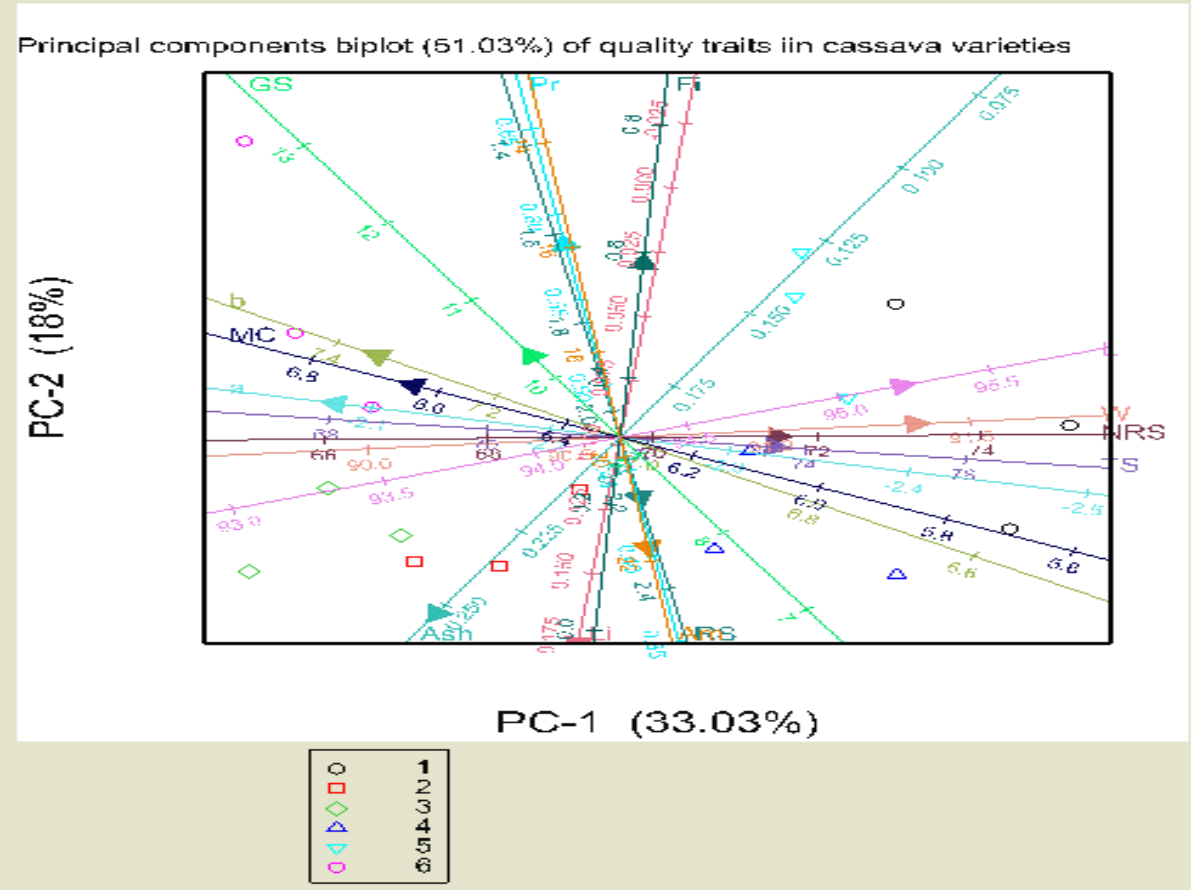

Figure 5.1 Principal component biplot of quality parameters in six different cassava varieties. Variety 1=*Bangweulu*, 2=*Katobamputa*, 3=*Mweru*, 4=*Kariba*, 5=*Kampolombo*, 6=*Chila*. L=Lightness, W=Whiteness, NRS=Non-RS, TS=Total starch content, Amy=Amylose, RS=Resistant starch, GS=Granule size, Am=Amylose, Pr=Protein, Fi=Fiber, Li=Lipid, MC=Moisture content, a* and b* (redness-greenness).

5.5 Conclusion

The moisture and ash contents showed increasing effect on redness (a*) and yellowness (b*), and reducing effect on whiteness of starches. The moisture and ash contents positively affected starch crystallinity, which indicated that crystallinity is not only dependent on amylose contents. The cassava varieties were characteristic of normal regular starches felling in the range 15-30% amylose contents. The results showed that differences in amylose contents among the cassava varieties were insignificant ($p>0.05$), except in *Katobamputa* which recorded high amylose content. The amylose contents exhibited negative correlations ($p<0.01$) with starch crystallinity and granule size. Smaller starch granule sizes showed high amylose contents and high crystallinity. The results revealed that resistant starch was significantly ($p<0.01$) affected by amylose content. *Katobamputa* had high amylose content and recorded high resistant starch, which justifies the previous reports on the inverse relationship between amylose and starch digestibility. The non-starch components (protein,

lipid, fiber, and ash) increased resistant starch and can be associated with decrease in α-amylase hydrolysis. The sources of variation in varieties were due to differences in amylose contents, lipids and fiber contents, and starch granule size. *Katobamputa* can be suggested for the formulation and development of dietary resistant starch food products. The effect of amylose content on the behavior of cassava starches and flours in water under heating conditions would require characterization to ascertain suitability of use of varieties. The next chapter is focused on swelling, solubility, gelatinization, pasting and freeze-thaw stability of starches.

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6. SWELLING, SOLUBILITY, GELATINIZATION, PASTING, GEL FREEZE-THAW STABILITIES OF STARCHES EXTRACTED FROM CASSAVA VARIETIES GROWN IN ZAMBIA

This chapter is based on the following publication

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Abstract

Cassava starches and flours processed from six cassava varieties (*Bangweulu, Katobamputa, Mweru, Kariba, Kampolombo and Chila*) were assessed for variety effect on swelling, solubility, gelatinization, pasting and gel freeze-thaw stability properties. The swelling power was investigated using dispersion methods, while gelatinization and pasting were determined using differential scanning calorimetry and rapid visco analyser, respectively. The gel freeze-thaw stability was determined using syneresis methods. The peak swelling power ranged between 2.22-15.63 and 2.77-13.00 $g.g^{-1}$ for starches and flours, respectively. The solubility index was in the range 1.62-71.15 and 4.58-54.56% for starches and flours, respectively. The swelling power exhibited a weak negative correlation with amylose ($p<0.0001$). Solubility index showed a weak positive correlation with amylose ($p<0.0001$). There was a weak negative correlation between the swelling power of starches and resistant starch content ($p<0.0001$). The onset gelatinization temperature (T_o) for starches (56.33-63 ^oC) and flours (63.2-68.13 ^oC) varied among varieties ($p<0.05$). The peak gelatinization temperature (T_p) was in the range 62-71.29 ^oC and 70.53-74.20 ^oC for starches and flours, respectively. The conclusion gelatinization temperature (T_c) ranged 69.1-77.12 ^oC and 75.47-81.17 ^oC for starches and flours, respectively. The T_o, T_p and T_c positively correlated with amylose content ($r = 0.530$, $p<0.05$; $r = 0.360$, $p<0.01$; and $r = 0.292$, $p<0.001$, respectively). The pasting temperatures were in the range 67.19-74.35 ^oC and 69.33-71.33 ^oC, for starches and flours, respectively. The peak viscosity starch (782.3-983.5 cP) and flour (651.9-910 cP) varied among varieties ($p<0.05$). The breakdown viscosity ranged 383.8-506.8 cP and 268.1-480.9 cP for starches and flours, respectively, and varied among the varieties ($p<0.05$). The

final viscosity for starches (462.0-569.7 cP) and flours (438.7-571.0 cP) varied significantly among the varieties. The pasting temperature positively correlated with amylose ($r = 0.231$, $p<0.001$). The peak, breakdown and final viscosity correlated negatively with amylose ($r = -0.561$, $p<0.05$; $r = -0.418$, $p<0.01$; and $r = -0.383$, $p<0.01$, respectively). The syneresis for freeze-thaw storage were ranged from 0.00-29.11% while for the five freeze-thaw cycles were in the range 0.00-42.40%, and significantly varied ($p<0.05$) among varieties. The sources of variations in physicochemical properties among the cassava varieties were due to differences in amylose, protein, lipids and starch granule and flour particle size.

Keywords: swelling power, solubility, gelatinization, pasting, freeze-thaw stability

6.1 Introduction

The swelling of cassava starches in water is one of the most important structural characteristics towards ascertaining the suitability toward processing and culinary applications of cassava starches and flours. Starches can undergo different stages of swelling from water absorption initially through amorphous regions of starch granules to the disintegration of the granules. Water absorption and heating can result into swelling of starch granules, disruption of hydrogen bonds, increase in granule sizes, and crystallite melting leading to amylose-amylopectin separation, exudation of amylose and small amylopectin molecules (Zhu, 2015; Charles *et al.*, 2016). Starches in excess water form dispersions, when dispersions are heated, swelling, starch granule gelatinization and starch solubilization occur which influence the properties of both continuous and dispersed phase and paste development. The properties of the paste and gels resulting from heating, freezing and thawing processes can be the basis of selecting cassava varieties for suitability in industrial application. The factors influencing the dispersed phase are genetically inherent in cassava varieties and these include starch granule shape, size, and molecular composition which include amylose, lipids and protein contents (Wang *et al.*, 2015). The concept of resistant starch in the food system can be related to swelling powers of starch granules. Park *et al.* (2018) reported that swelling and rupture of the starch granular and melting of crystalline structures during gelatinization accelerated the digestion of native maize starch. Nevertheless, information on correlations between swelling and digestibility are limited. Proteins, lipid, and phosphorus are present in small amounts within the starch granules. The presence of these minor components associated with starch granules can affect functional properties of starches. Other factors affecting swelling include flour particle size and starch granule size distribution (Lindeboom *et al.*, 2004).

In most part of literature, swelling power of cassava starches and flours were conducted at 1 % concentrations in distilled water, however, using different heating temperature of 90 ^{o}C (Mtunguja *et al.*, 2016b), from 60 to 90 ^{o}C (Mbougueng *et al.*, 2012), 60 ^{o}C (Kusumayanti *et al.*, 2015) and 70 ^{o}C (Eriksson, 2013). Peak swelling values for cassava starch granules were reported to be in the range between 70 and 95 ^{o}C (Chinma *et al.*, 2013). However, at high temperatures, swelling power decrease as more solutes leaches out. High swelling powers of starches at lower temperature 60 ^{o}C were characterized with high viscosities, however, an

increase in shear rate will decrease the viscosities of starch paste due to shear thinning. Food quality can be associated with swelling and gelatinization of starch granules, and susceptibility of starches to enzymatic hydrolysis (Rocha *et al.*, 2010). High soluble starches find applications in the preparation of gum candies and as carriers of active ingredients in cakes, noodles, pie fillings, soups and sauces (Saha and Bhattacharya, 2010). There is no information on the physicochemical properties of flour and starches from cassava of the Zambian cultivars.

The work was undertaken to evaluate variety- and temperature factor effects on swelling, solubility, gelatinization, and pasting, and freeze-thaw properties of cassava starches from local landraces and officially released improved cassava varieties cultivated in Zambia.

6.2 Materials and methods

6.2.1 Source of materials

Cassava varieties were as described in method 3.2.1, Chapter 3. The starches were extracted as described in method 3.2.3. The cassava flour production was conducted as described in method 3.2.4, Chapter 3.

6.2.2 Swelling power and solubility

The swelling power and solubility patterns of cassava flour and starch were determined as described in Kusumayanti et al. (2015) at different temperature (50, 60, 70, 80 and 90 °C). Sample (0.5 g dry weight) was suspended in 20 mL water in centrifuge tube (50 mL) of known weight, heated for 30 min at specified temperature, swirling at every 5 min, centrifuged (8000 rpm for 20 min) using Beckman Coulter Centrifuge (Avant J-26 XPI, High Performance Centrifuge, USA), supernatant discarded and the sediment mass was measured. The swelling power ($g.g^{-1}$) was calculated as the ratio of sediment mass to the original sample weight. The supernatant separated was collected on pre-weighed evaporating crucible dish, oven dried (105 °C for 12 h) and the dried residue was weighed. The solubility index was then expressed as a percentage of dried supernatant weight to the original sample weight (Ws).

$$\text{Swelling power g.g}^{-1} = \frac{W_{sd}}{W_{s\ \text{dry basis}}} \quad (6.1)$$

$$\text{Solubility, \%} = \frac{W_d}{W_{s\ \text{dry basis}}} \times 100 \quad (6.2)$$

where:

W_{sd} = dried sediment mass

W_s = original sample weight

W_d = dried residue

6.2.3 Starch gelatinization properties

The starch gelatinization properties (onset, peak and conclusion gelatinization temperature and enthalpy of gelatinization) were determined using differential scanning calorimetry (Perkin Elmer system (Model DSC7; Norwalk, CT, USA) as described in Huang *et al.* (2007). A starch sample (4 mg) was placed in aluminium pan and deionized water was added to obtain a starch to water ratio of 1:4 (w/w). The sample was sealed hermetically and equilibrated for 4 h. The prepared sample was then scanned in the heating program of 30 °C to 150 °C at the scanning rate of 10 °C.min^{-1} using nitrogen as a purging gas at the rate of 30 mL.min^{-1}. Parameters analyzed from the thermogram were onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{gel}).

6.2.4 Starch pasting properties

The starch pasting properties were determined using Rapid Visco Analyser (Model: RVA-4, Newport Scientific, Warriewood, Australia) as described in Colman *et al.* (2014). The starch samples (5 g dry basis) was suspended in 25 mL of distilled water. A heating and cooling cycle program was utilized. The samples were held at 50 °C for 1 min, followed by heating at 95°C for 7.5 min at the heating rate of 6 °C.min^{-1}, holding at 95 °C for 5 min followed by cooling to 50°C in 7.5 min and holding at 50 °C for 1 min. Parameters measured were pasting temperature (PT), peak viscosity (PV), hot paste viscosity at the end of the plateau (HPV), cooled paste viscosity (CPV) at 50 ^{o}C, and final viscosity (FV), breakdown viscosity (BD) to be estimated as PV-HPV, setback viscosity (SB) to be estimated as CPV-HPV.

6.2.5 Syneresis (freeze-thaw stability)

6.2.5.1 Syneresis after freezing

The starch paste was prepared by suspending starch samples in distilled water (5% w/v) and heated in a boiling water bath for 30 min with constant stirring. After cooling to room temperature, 15 centrifuge tubes per starch gel variety were filled with approximately 6 g of gel (WG) and stored at -20 ^{o}C for five weeks. Every week (after every 7 days), three tubes were drawn out of the freezer and thawed for 90 min in a water bath at 30 ^{o}C, followed by centrifugation of samples at 5000 rpm/10 min. After centrifugation, the mass of the supernatant separated and weighted (WS). Syneresis was calculated using the following formula:

$$\text{Syneresis}, \% = \frac{\text{WG}}{\text{WS}} \text{ x } 100 \qquad (6.3)$$

where:
WG = weight of gel
WS = weight of supernatant.

6.2.5.2 Syneresis after consecutive freeze-thaw cycles

The syneresis after consecutive freeze-thaw cycles was studied as described above in Morante *et al.* (2016), and sample preparation and calculations were done as described above. Fifteen (15) centrifuge tubes per starch sample of each variety were filled with approximately 6 g of gel and stored at -20^{o} C. Every seven days, the whole set of tubes were removed from the freezer and held at room temperature for 90 min in a water bath (30 ^{o}C). Three random tubes were taken out and centrifuged (8000 rpm for 10 min) and supernatant separated and weighed. The remaining tubes were frozen again for another freeze-thaw cycle.

6.2.6 Experimental design and analysis

Swelling power and solubility: a two factor (variety, temperature) Completely Randomized Design comprising was used in the experiment. Gelatinization and pasting properties: one factor (variety) Completely Randomized Design was used. Freeze-thaw stability: a two factor (variety, storage period) Completely Randomized Design was used. Triplicate data

were analyzed using ANOVA using GenStat 18th edition software. The mean differences were determined using Fisher's Least Significance Difference (LSD) test at the 5% significant level. The starch granules size and crystallinity (Table 4.1, Chapter 4), amylose content (Table 5.2, Chapter 5), proximate content for flour (Table 3.5, Chapter 3) and starch (Table 5.1, Chapter 5), and resistant starch (Table 5.3, Chapter 5) contents were correlated with swelling, solubility, gelatinization, pasting and freeze-thaw properties using Pearson's correlation.

6.3 Results and discussion

6.3.1 Swelling power and solubility of starches

Table 6.1 shows the swelling capacities of starches. At 50 ^{o}C, the average swelling power of starches were in the range 2.22-2.49 g.g^{-1}. At 60 ^{o}C, the average swelling power across all the varieties was in the range 9.04-12.53 g.g^{-1}, while the swelling powers at 70 ^{o}C ranged between 11.24 and 15.63 g/g. The peak swelling powers observed at 60 ^{o}C for *Kampolombo* and *Chila* indicates that these varieties have the capacity to swell at relatively low temperature. The highest and lowest peak swelling powers at 70 ^{o}C were recorded in *Bangweulu* and *Katobamputa*, respectively. In the previous studies swelling power of normal cassava starches were reported, 10.80 g.g^{-1} at 1% starch suspension (Gbadamosi and Oladeji, 2013), 8.9–16.3 g.g^{-1} at 1% starch suspension (Mtunguja et al., 2016b), 5.62–20.79 g.g^{-1} (Nuwamanya *et al.*, 2010a) and 3.3–18 g.g^{-1} (Chinma et al., 2013). The differences in swelling powers could be ascribed to variations in amylose contents and protein content. The swelling power showed weak negative correlation with amylose ($r = -0.038$, $p<0.0001$) and protein ($r = -0.080$, $p<0.001$) (Table 6.5). This suggests that high swelling starches had low amylose and protein contents. Similarly, Mtunguja *et al*. (2016b) reported that low swelling powers of cassava starches were due to high amylose contents. This is in agreement with Sánchez *et al.* (2010) who reported the highest swelling power (49.7–51 g.g^{-1} at 1% starch suspension) for waxy cassava starches. Furthermore, protein are known to restrict swelling of the starch granules (Uthumporn *et al.*, 2017) because of increased hydrophobicity leading to reduced uptake of water of decreased starches granule swelling (Muoki *et al.*, 2015). The swelling power had a weak positive correlation with starch granule size ($r = 0.066$, $p<0.0001$), implying that large granule sizes had higher swelling powers. The large surface

area in large starch granules may be due to loss of non-starch components during starch extraction.

Except in the cassava starches gelatinization region (>60 ^{o}C), the source of variation due to variety x temperature interaction on swelling capacities was insignificant ($p>0.05$). The swelling powers of cassava starch varieties had varied significantly ($p>0.05$) with temperature and increased from 60 to 70 ^{o}C and thereafter decreased in temperature ranges of 80-90 ^{o}C. The swelling power of starches exhibited a two-stage process, an initial slow swelling in the heating temperature range 50–60 ^{o}C with insignificant variations among cassava varieties ($p>0.05$) followed by a significant increase in the range 60–70 ^{o}C ($p<0.05$). Similar was reported by Akinwale *et al.* (2017), and Demiate and Kotovicz (2011). During the initial heating stages, hydrogen bonding of amylose molecules form complex with lipids, entanglement with amylopectin branches and proteins might restrict swelling. However, with an increase in temperature above 60 ^{o}C, water penetrates into the crystalline region of starch granules leading to disruption of hydrogen bonding, crystalline melting and an increased swelling (Nuwamanya *et al.*, 2010b). The decrease in the swelling is indicative of increased exudation of amylose and solubilization of starch molecules. Starch granules swell to the peak value, after which the swollen granules disintegrate to release the soluble materials including amylose molecules (Demiate and Kotovicz, 2011).

Table 6.1 Swelling power ($g.g^{-1}$) of cassava starches from different varieties at different heating temperatures

Variety	50 °C	60 °C	70 °C	80 °C	90 °C
Bangweulu	2.22(0.20)^{a}	10.36(0.33)^{hijk}	15.63(3.30)^{m}	8.30(0.50)^{fgh}	3.58(0.43)^{abc}
Katobamputa	2.49(0.16)^{ab}	9.56(0.21)^{hij}	12.69(2.96)^{kl}	6.67(0.68)^{defg}	3.70(0.55)^{abc}
Mweru	2.23(0.07)^{a}	9.04(1.28)^{ghi}	14.66(2.69)^{lm}	6.79(1.48)^{efg}	6.73(2.96)^{efg}
Kariba	2.31(0.09)^{a}	11.50(0.96)^{ijk}	12.48(1.72)^{kl}	5.52(0.76)^{cde}	6.28(3.19)^{def}
Kampolombo	2.44(0.09)^{a}	12.53(0.74)^{kl}	11.24(0.50)^{ijk}	4.93(0.18)^{bcde}	4.23(0.14)^{abcd}
Chila	2.32(0.08)^{a}	11.73(0.51)^{jk}	11.26(0.41)^{ijk}	6.28(2.00)^{def}	5.30(2.59)^{cde}

Level of significance

Variety	$p>0.05$
Temperature	$p<0.05$
Variety x Temperature	$p<0.05$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test

Table 6.2 Solubility (%) of cassava starches from different varieties at different heating temperatures

Variety	50 °C	60 °C	70 °C	80 °C	90 °C
Bangweulu	2.61(1.13) [a]	9.15(1.15) [abc]	13.53(1.71) [bc]	32.11(0.87) [e]	71.15(10.05) [kl]
Katobamputa	1.62(0.55) [a]	4.61(1.19) [ab]	24.08(2.89) [de]	42.06(6.39) [fg]	75.79(6.61) [l]
Mweru	2.59(1.08) [a]	5.31(2.33) [ab]	18.19(8.85) [cd]	33.84(5.18) [ef]	57.36(16.28) [hi]
Kariba	3.27(1.13) [a]	10.54(1.26) [abc]	10.41(3.06) [abc]	68.13(6.67) [jkl]	56.92(9.26) [hi]
Kampolombo	1.97(0.02) [a]	10.56(2.48) [abc]	7.78(3.06) [ab]	63.41(2.57) [ijk]	59.95(4.88) [hij]
Chila	5.91(3.37) [ab]	7.85(0.15) [ab]	8.55(1.08) [abc]	53.83(13.78) [hi]	51.25(10.61) [gh]

Level of significance

Variety	$p>0.05$
Temperature	$p<0.05$
Variety x Temperature	$p<0.05$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test

A significant difference in the solubility of cassava starches was observed in the gelatinization region (> 60 ^{o}C) (Table 6.2). At 50 ^{o}C, the solubility of starches ranged between 1.62 and 5.91 %, with the lowest and highest in *Katobamputa* and *Chila*, respectively. The solubility at 60 ^{o}C and 70 ^{o}C ranged from 4.61-10.56 % and 7.78-24.08 %, respectively. The solubility index values for starches were reported, 10.0 - 46.7 % (Gbadamosi and Oladeji, 2013). There was a weak negative correlation between swelling power and solubility of cassava starches ($r = -0.302$, $p<0.01$). This implies that a decrease in swelling particularly after peak values led to increased solubility. The solubility of starches showed a very weak positive correlation with amylose content ($r = 0.051$, $p<0.001$). This may suggest that high solubility starches had high amylose contents. Starch granules swell to the peak value, after which the swollen granules disintegrate to release the soluble materials including amylose molecules (Demiate and Kotovicz, 2011; Akpa and Dagde, 2012). The decrease in solubility index after peak values could be attributed to the pasting phenomenon of amylose molecules and some amylopectin molecules entanglement through increased gel junction zones and partial aggregation of double-helices formations in the continuous phase that occurs on cooling resulting in the formation of starches gel (Tako *et al.*, 2014).

6.3.2 Swelling power and solubility of cassava flours

The swelling power of cassava flours were not significantly different in the temperature range 50-70 ^{o}C ($p<0.05$) (Table 6.3). The peak swelling power (13±1.01 g.g^{-1}) ^{of} cassava flours were recorded at 70 ^{o}C, after which a significant decrease between 80 and 90 ^{o}C was observed. The cassava flours peak swelling values were reported, 16.11 g.g^{-1} at 1% flour suspension (Bala *et al.*, 2015) and 13.8 g.g^{-1} at 1% flour suspension (Kusumayanti et al., 2015). The differences could be due to variations in amylose contents. The swelling power of flours had a weak negative correlation with fiber content ($r = -0.185$, $p<0.001$). This suggests that high swelling cassava flours had low fiber contents.

Table 6.3 Swelling power ($g.g^{-1}$) of cassava flours from different varieties at different temperature

Variety	50 ^{o}C	60 ^{o}C	70 ^{o}C	80 ^{o}C	90 ^{o}C
Bangweulu	3.14(0.17) ^{a}	7.27(0.43) ^{bcd}	12.21(0.74) ^{h}	8.52(0.51) ^{cdef}	7.91(0.56) ^{bcde}
Katobamputa	3.22(0.11) ^{a}	7.50(0.43) ^{bcde}	12.79(0.31) ^{h}	8.11(1.66) ^{cde}	8.08(1.16) ^{cde}
Mweru	2.92(0.19) ^{a}	6.43(0.69) ^{b}	13.00(1.01) ^{h}	9.98(2.23) ^{fg}	8.50(0.18) ^{cdef}
Kariba	2.96(0.33) ^{a}	7.69(0.81) ^{bcde}	12.50(0.91) ^{h}	7.22(0.48) ^{bcd}	8.66(0.38) ^{def}
Kampolombo	2.81(0.21) ^{a}	7.74(0.25) ^{bcde}	11.94(0.17) ^{h}	7.78(0.47) ^{bcde}	10.35(2.66) ^{g}
Chila	2.77(0.20) ^{a}	8.910.48) ^{efg}	12.75(0.26) ^{h}	7.03(1.15) ^{bc}	8.21(1.38) ^{cde}
Level of Significance					
Variety		p>0.05			
Temperature		p<0.05			
Variety x Temperature		p<0.05			

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test

The solubility of cassava flours ranged between 4.58 and 7.19 % at 50 ^{o}C, and no significant (p>0.05) differences were observed among the varieties except between *Kariba* and *Kampolombo* at 70 ^{o}C (Table 6.4). The highest peak solubility values were observed for *Chila* (56.72±8.22 %) and *Bangweulu* (51.64±4.14 %) at 80 and 90 ^{o}C, respectively. The cassava flour solubility index values were reported, 3.02 % of cassava flour at 60 ^{o}C by Kusumayanti et al. (2015) and 14.00 % by Bala et al. (2015). The solubility index showed weak negative correlation with amylose content (r = -0.047, p<0.001) (Table 5). This correlation is contrary to what was observed in starch solubility. However, this may suggest that regular to waxy starches are likely to exhibit higher solubility values. The high amount of protein, fiber, and lipid in flours may compete with amylose for water molecules, and thus inhibiting solubilization of amylose molecules.

Table 6.4 Solubility (%) of cassava flours from different varieties at different temperatures

Variety	50 °C	60 °C	70 °C	80 °C	90 °C
Bangweulu	5.86(1.86) ^{ab}	8.37(2.13) ^{abc}	9.81(0.19) ^{abc}	47.39(2.46) ^{d}	51.62(4.14) ^{def}
Katobamputa	5.29(1.12) ^{a}	4.58(1.13) ^{a}	7.23(1.07) ^{abc}	49.33(7.21) ^{de}	46.43(3.43) ^{d}
Mweru	4.58(1.13) ^{a}	6.45(1.07) ^{ab}	9.68(1.92) ^{abc}	49.02(3.39) ^{de}	48.67(4.59) ^{de}
Kariba	5.92(1.96) ^{ab}	6.45(1.07) ^{ab}	11.49(5.14) ^{ab}	54.56(1.24) ^{ef}	46.78(4.34) ^{d}
Kampolombo	7.19(1.13) ^{abc}	5.11(1.15) ^{a}	12.84(4.04) ^{c}	47.90(5.15) ^{d}	47.39(2.46) ^{d}
Chila	6.78(0.86) ^{ab}	5.22(1.03) ^{a}	9.75(5.22) ^{abc}	56.72(8.22) ^{f}	46.81(7.48) ^{d}
Level of significance					
Variety		$p>0.05$			
Temperature		$p<0.05$			
Variety x Temperature		$p<0.05$			

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

The peak swelling and solubility values were higher in starches than flour samples. The differences could be due to the interference of lipid, protein and fiber contents which were higher in flours than starches. The solubility of flours exhibited weak negative correlation with protein ($r = -0.042$, $p<0.001$) and lipid ($r = -0.025$, $p<0.001$). Solubility index values increased as the temperature increased resulting in increased mobility of the starch molecules in the granules which led to exudation of amylose and other soluble compounds, and thus, enhanced dispersion of starch molecules in water. However, non-starchy components such as protein and lipid contents in flours can restrict swelling leading to reduced solubility (Lu and Lu, 2012; Uthumporn et al., 2017).

6.3.3 Swelling and resistant starch

Cassava starches swelling power exhibited very weak negative correlation with resistant starch ($r = -0.072$, $p<0.0001$) and positively with digested starch ($r = 0.026$, $p<0.0001$). Swelling powers of cassava flours showed negative correlation with resistant starch ($r = -0.276$, $p<0.01$), and weak positive correlation with digested starch ($r = 0.059$, $p<0.0001$) (Table 6.5). This suggests that high swelling starches had low resistant starches and high susceptibility to amylolytic digestion. The intact molecular structure in raw starches restricts the accessibility of amylases. The resistant nature of raw starch granules has been observed (Nissar *et al.*, 2017). Swelling disrupts the double helical structure of starch granules and increased interaction of hydroxyl and water molecules renders the swollen granules susceptible to digestive enzymes.

Table 6.5 Correlation coefficients of swelling, resistant starch, and digested starch

Parameter	Swelling	Solubility	Amylose	Protein	Lipid	Fiber	RS	DS
Cassava starches								
Swelling	1							
Solubility	-0.302**	1						
Amylose	-0.038****	0.051***	1					
Protein	-0.080****	0.028	-0.075	1				
Lipid	0.031	0.010	0.435	-0.394	1			
Fiber	0.015	0.019	0.049	0.498	-0.277	1		
RS	-0.072****	0.037	0.656	0.149	0.481	0.156	1	
DS	0.026****	-0.042	0.121	0.103	-0.136	0.232	0.173	1
Cassava flours								
Swelling	1							
Solubility	-0.040	1						
Amylose	0.010	-0.047***	1					
Protein	0.207	-0.042***	0.299	1				
Lipid	0.162	-0.025***	0.058	0.207	1			
Fiber	-0.185***	0.004	0.162	0.285	-0.132	1		
RS flour	-0.276***	-0.026	0.566	-0.180	-0.127	0.108	1	
DS	0.059****	0.047	-0.618	-0.034	-0.154	-0.183	-0.908	1

RS=Resistant starches, DS=Digestible starch. Significance: $p<0.05$*, $p<0.01$**, $p<0.001$***, $p<0.0001$****

6.3.4 Gelatinization properties

Table 6.6 shows the onset, peak and conclusion gelatinization temperatures for cassava and flour starches from six different cassava varieties. Table 6.7 shows correlation coefficients of swelling, solubility, amylose, gelatinization and pasting properties of starch. Table 6.8 shows correlation coefficients of swelling, solubility, amylose, gelatinization and pasting properties of starch. Table 6.9 shows correlation coefficients swelling, solubility, gelatinization and pasting properties of flours.

6.3.4.1 Onset gelatinization temperature

The onset gelatinization temperature for starches (56.33-63 ^{o}C) and flours (63.2-68.13 ^{o}C) varied ($p<0.05$) among cassava variety. The lowest and highest onset gelatinization temperature for cassava starches were recorded in *Kampolombo* and *Kariba*. The lowest and highest onset gelatinization temperatures for cassava flour were recorded in *Chila* and

Kariba. Similar was reported for cassava starches, 64.3 ^{o}C (Ai and Jane 2015), 63.70 ^{o}C (Muñoz *et al.*, 2015). The variation in gelatinization temperatures of starches are dependent on starch types, water availability on heating of starches granules, heating conditions and is also influenced by the differences in amylose and non-starch granule component (lipid, proteins and phosphate contents) (Joye, 2019). The gelatinization temperature can also be slightly different within a species among given botanical origin of the starch. The onset gelatinization temperature for cassava starches positively correlated with amylose content ($r = 0.530$, $p<0.05$), and showed a weak negative correlation with swelling power of starches ($r = -0.090$, $p<0.0001$). This suggests that high amylose inhibited swelling resulting in low swelling powers and thus making starch granule more resistant to gelatinization. The high amylose starches would require high onset gelatinization temperatures to overcome resistance against swelling. This is not in agreement with Morante *et al*. (2016) who reported that waxy cassava starch had higher gelatinization temperatures because the gelatinization is fundamentally related to the degree of crystalline lamella variations in the starch granules. Nevertheless, it should be noted that all the cassava starches in the present study were classified as regular/normal starches (16-26% amylose contents), and exhibited Type A crystallinity (polymorph) of the X-ray diffraction pattern. The A-polymorph double helical structure densely packed with about 8 water molecules in a unit cell exhibit more molecular compact than B-polymorph with 36 water molecules in a unit cell (Bertoft, 2017; Chisenga *et al*., 2019). In the current study, A-polymorph in all six cassava starches may have contributed to the significant relationship between amylose and gelatinization temperatures. Competing action between starch granule and amylose for water molecules increases gelatinization temperatures (Chisenga *et al*., 2019). The onset gelatinization temperature exhibited weak positive correlation with protein ($r = 0.390$, $p<0.01$), lipid ($r = 0.100$, $p<0.001$) and fiber contents ($r = 0.386$, $p<0.01$). This suggests that an increase in non-starch components increased onset gelatinization temperature. The presence of amylose-lipid complex inhibits gelatinization of starch granules (Charles *et al.*, 2005). High levels of lipids decrease starch granule susceptibility to gelatinization. Lipids may affect the diffusion of water into the starch granules, and their presences on starch granules can retard gelatinization. Li *et al.* (2016b) reported that defatted starch resulted in decreased gelatinization temperatures. The protein and starch granules compete for water molecules (Uthumporn et al., 2017) which probably results in inhibited swelling and increased gelatinization temperature. There was a

weak positive correlation between the solubility index for starches and onset gelatinization temperature ($r = 0.047$, $p<0.001$), implying that solubilization of solutes increased linearly with gelatinization temperature. The cassava flours onset gelatinization showed similar correlations with starches regarding amylose contents ($r = 0.403$, $p<0.01$), lipids ($r = 0.443$, $p<0.01$) and swelling power ($r = -0.065$, $p<0.001$). The higher onset gelatinization temperatures in cassava flours than starches may, in part, be influenced by high protein and lipid contents in flours.

Table 6.6 Gelatinization properties of starches and flours from six cassava varieties

	T_o		T_p		T_c		Enthalpy (J/g)	
Variety	Starch	Flour	Starch	Flour	Starch	Flour	Starch	Flour
Bangweulu	56.56(1.89)^{a}	63.57(2.40)^{cd}	63.23(1.66)^{a}	71.47(3.36)^{bcd}	70.8(4.23)^{ab}	76.7(0.52)^{cd}	13.57(0.45)^{c}	7.97(1.25)^{a}
Katobamputa	58.33(2.51)^{ab}	64(1.00)^{cd}	70.93(0.81)^{b}	74.2(0.82)^{d}	77(2.00)^{cd}	81.17(1.77)^{d}	14.1(1.01)^{c}	8.5(0.53)^{a}
Mweru	61(2.64)^{bc}	66.1(1.01)^{bc}	70.67(2.08)^{b}	74(1.00)^{cd}	76.67(1.52)^{cd}	79.07(1.10)^{cd}	10.67(1.25)^{b}	8.67(0.80)^{a}
Kariba	63(1.00)^{cd}	68.13(0.91)^{e}	71.29(1.58)^{bc}	74(1.00)^{cd}	77.12(2.10)^{cd}	81.00(1.00)^{d}	14.7(0.26)^{c}	8.53(0.63)^{a}
Kampolombo	56.33(1.52)^{a}	65.43(2.38)^{de}	62(1.00)^{a}	73.03(0.95)^{bcd}	71.1(4.74)^{ab}	77.27(2.53)^{cd}	13.73(2.19)^{c}	9.43(0.63)^{ab}
Chila	57.31(2.84)^{cd}	63.2(1.73)^{cd}	64.73(1.27)^{a}	70.53(2.28)^{b}	69.1(4.00)^{a}	75.47(4.10)^{bc}	11.13(0.90)^{b}	7.93(0.98)^{a}
Significance level								
Variety	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p>0.05$

To=onset gelatinization, Tp=peak gelatinization, Tc=Conclusion gelatinization temperature. All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

Table 6.7 Correlation coefficient swelling, solubility, amylose, gelatinization and pasting properties of starch

Parameter	Swelling	Solubility	Amylose	To	Tp	Tc	PV	FV	PT	GS
Swelling	1									
Solubility	-0.302	1								
Amylose	-0.038	0.051	1							
To	-0.090****	0.047***	0.530*	1						
Tp	-0.127****	0.074	0.360**	0.686	1					
Tc	-0.061****	0.040	0.292***	0.653	0.846	1				
Enthalpy	-0.079	0.068	0.261***	0.321**	0.586*	0.508*				
PV	0.018	-0.027****	-0.561*	-0.677	-0.425	-0.418	1			
FV	-0.003	-0.023	-0.383**	-0.330	-0.409	-0.425	0.640	1		
PT	0.025	-0.019	0.231***	-0.304	0.028	-0.052	0.178	0.0742	1	
GS	0.066	-0.052	-0.155	0.275	0.132	0.247	-0.227	-0.3693	-0.366***	1

Swe=Swelling, Sol=Solubility, AM=Amylose, Pr=Protein, Lip=Lipids, Fib=Fiber, To=onset gelatinization, Tp=peak gelatinization, Tc=Conclusion gelatinization temperature, Ent=Enthalpy, PV=Pasting viscosity, BV=Breakdown viscosity, FV=Final viscosity, SV=Setback viscosity, PT=Pasting temperature, GS=Starch granule size

Significance: $p<0.05$*, $p<0.01$**, $p<0.001$***, $p<0.0001$****

Table 6.8 Correlation coefficient protein, lipid, fiber, gelatinization and pasting properties of starch

Parameter	Protein	Lipid	Fiber	To	Tp	Tc	PV	FV	PT	GS
Protein	1									
Lipid	-0.394	1								
Fiber	0.498	-0.277	1							
To	0.390**	0.100***	0.386**	1						
Tp	0.360**	0.084****	0.220	0.686	1					
Tc	0.146***	0.268***	0.264	0.653	0.846	1				
Enthalpy	-0.194	0.008	0.097	0.321	0.586	0.508				
PV	0.005	-0.453**	-0.172	-0.677	-0.425	-0.418	1			
FV	0.162	-0.559*	0.130	-0.330	-0.409	-0.425	0.640*	1		
PT	-0.109	0.063	-0.188	-0.304	0.028	-0.052	0.178	0.0742	1	
GS	0.243	0.019	0.164	0.275	0.132	0.247	-0.227***	-0.3693	-0.366	1

Swe=Swelling, Sol=Solubility, AM=Amylose, Pr=Protein, Lip=Lipids, Fib=Fiber, To=onset gelatinization, Tp=peak gelatinization, Tc=Conclusion gelatinization temperature, Ent=Enthalpy, PV=Pasting viscosity, BV=Breakdown viscosity, FV=Final viscosity, SV=Setback viscosity, PT=Pasting temperature, GS=Starch granule size

Significance: p<0.05*, p<0.01**, p<0.001***, p<0.0001****

Table 6.9 Correlation coefficients swelling, solubility, gelatinization and pasting properties of flours

Parameter	Swe	Sol	AM	To	Tp	Tc	Ent	PV	BV	FV	SV	PT	FS
Swe	1												
Sol	-0.040	1											
AM	0.010	-0.047	1										
To	-0.065***	-0.022	0.403**	1									
Tp	-0.319**	-0.003	0.314**	0.412	1								
Tc	-0.228	-0.027	0.343**	0.626	0.813	1							
Ent	-0.084	0.004	0.040	-0.127	-0.006	0.032	1						
PV	0.163	0.031	-0.483	-0.166	-0.286	-0.296	0.316	1					
BV	0.183	0.027	-0.374	0.006	-0.173	-0.055	0.479	0.924	1				
FV	-0.016	0.013	-0.312	-0.445	-0.239	-0.471	-0.103	0.503	0.216	1			
SV	-0.205	-0.031	0.393	-0.078	0.196	0.069	-0.418	-0.856	-0.939	0.014	1		
PT	-0.244	0.109	-0.407	-0.103	0.068	0.075	0.132	-0.028	0.031	-0.044	-0.014	1	
FS	-0.477	0.061	-0.093	-0.009	0.352	0.020	0.197	-0.038	-0.052	-0.163	-0.054	0.257***	1

Swe=Swelling, Sol=Solubility, AM=Amylose, Pr=Protein, Lip=Lipids, Fib=Fiber, To=onset gelatinization, Tp=peak gelatinization, Tc=Conclusion gelatinization temperature, Ent=Enthalpy, PV=Pasting viscosity, TV=Trough viscosity, BV=Breakdown viscosity, FV=Final viscosity, SV=Setback viscosity, PT=Pasting temperature, FS=Flour particle size

Significance: p<0.05*, p<0.01**, p<0.001***, p<0.0001****

Table 6.10 Correlation coefficients protein, lipids, fiber, gelatinization and pasting properties of flours

	Pr	Lip	Fib	To	Tp	Tc	Ent	PV	BV	FV	SV	PT	FS
Pr	1												
Lip	0.207	1											
Fib	0.285	-0.132	1										
To	-0.166	0.443**	-0.424	1									
Tp	-0.345	-0.425	-0.176	0.412	1								
Tc	-0.361	-0.249	-0.124	0.626	0.813	1							
Ent	0.088	0.186	0.053	-0.127	-0.006	0.032	1						
PV	-0.359	0.058	-0.620	-0.166	-0.286	-0.296	0.316	1					
BV	-0.360	0.125	-0.591	0.006	-0.173	-0.055	0.479	0.924	1				
FV	-0.140	-0.320	-0.228	-0.445	-0.239	-0.471	-0.103	0.503	0.216	1			
SV	0.326	-0.271	0.577	-0.078	0.196	0.069	-0.418	-0.856	-0.939	0.014	1		
PT	-0.138	-0.185	0.244	-0.103	0.068	0.075	0.132	-0.028	0.031	-0.044	-0.014	1	
FS	-0.021	0.013	0.053	-0.009	0.352	0.020	0.197	-0.038	-0.052	-0.163	-0.054	0.257	1

Swe=Swelling, Sol=Solubility, AM=Amylose, Pr=Protein, Lip=Lipids, Fib=Fiber, To=onset gelatinization, Tp=peak gelatinization, Tc=Conclusion gelatinization temperature, Ent=Enthalpy, PV=Pasting viscosity, TV=Trough viscosity, BV=Breakdown viscosity, FV=Final viscosity, SV=Setback viscosity, PT=Pasting temperature, FS=Flour particle size

Significance: $p<0.05$*, $p<0.01$**, $p<0.001$***, $p<0.0001$****

6.3.4.2 Peak gelatinization temperature

The peak gelatinization temperature for starches and flours ranged from 62-71.29 ^{o}C, and 70.53-74.20 ^{o}C, respectively, and varied ($p<0.05$) among cassava variety. The lowest and highest peak gelatinization temperatures for starches were registered in *Kampolombo* and *Kariba* while in cassava flours were recorded in *Chila* and *Mweru*. The peak gelatinization temperatures were reported, 65.5 ^{o}C (Mweta *et al.*, 2015), 68.69 ^{o}C (Muñoz et al., 2015), 61.2-69.9 ^{o}C (Morante et al., 2016) and 68.3 ^{o}C (Ai and Jane, 2015). The differences among cassava starch varieties could be due to variations in amylose, protein and lipid contents. The peak and onset gelatinization temperature were positively correlated and yielded similar behavior against amylose and non-starchy contents. The peak gelatinization temperature showed weak positive correlation with amylose content ($r = 0.360$, $p<0.01$), protein ($r = 0.360$, $p<0.01$) and lipid contents ($r = 0.084$, $p<0.0001$). This implies that high amylose content had high peak gelatinization temperatures. Nevertheless, high peak gelatinization temperatures were recorded in waxy cassava starches due to high degree of amylopectin crystallite formation in waxy starches (Morante et al., 2016). Amylopectin chain length degree of polymerization has been reported as the major factor for discriminating gelatinization transition temperatures. In a related study on wheat starch, amylopectin chain lengths were reported to influence gelatinization temperature as starches with high short amylopectin chain length (degree of polymerization less than 12) exhibited lower gelatinization temperature than long chain (Kaur *et al.*, 2016) justifying suggestions that it is not always that gelatinization depends on amylose content. Jane *et al.* (1999) cautioned that starch gelatinization could be influenced by many external factors such as growing and processing conditions, and asserted that chemically extracted starches exhibited higher gelatinization temperatures than starch extracted using mild-chemical and enzymatic methods. There was negative correlation between peak gelatinization temperature and swelling power for starches ($r = -0.127$, $p<0.001$) and flours ($r = -0.319$, $p<0.01$). Similar correlations were observed by Mtunguja et al. (2016b), who reported negative coefficients between peak gelatinization temperature and swelling powers of cassava starches. This suggests that high peak gelatinization temperatures could occur in low swelling starch granules. The cassava flours peak gelatinization temperature correlated positively with amylose content ($r = 0.314$, $p<0.01$).

6.3.4.3 Conclusion gelatinization temperature (T_c)

The conclusion gelatinization temperatures for starches (69.1-77.12 ^{o}C) and flours (75.47-81.17 ^{o}C) varied ($p<0.05$) among cassava variety. The lowest and highest conclusion gelatinization temperatures for starches were recorded in *Chila* and *Kariba* while in flours were registered in *Chila* and *Katobamputa*. Similar conclusion gelatinization temperatures for cassava starches were reported to 78.9 ^{o}C (Mweta et al., 2015) 66.7-75.1 ^{o}C (Morante et al., 2016) and 74.8 ^{o}C (Ai and Jane, 2015). The variation among the cassava starch varieties could be due to differences in amylose, protein and lipid contents. The conclusion gelatinization temperature positively correlated with the onset and peak gelatinization temperatures. The conclusion gelatinization temperatures for cassava starches positively correlated with amylose ($r = 0.292$, $p<0.001$), protein ($r = 0.146$, $p<0.001$) and lipid ($r = 0.268$, $p<0.001$). The conclusion gelatinization temperatures for cassava flours positively correlated with amylose content ($r = 0.343$, $p<0.01$) and negatively correlated with swelling powers ($r = -0.061$, $p<0.001$).

6.3.4.4 Enthalpy of gelatinization

The enthalpy of gelatinization of cassava starches ranged from 10.67-14.10 J/g, and varied among the cassava varieties ($p<0.05$), while for cassava flours (7.93-9.43 J/g) were insignificant ($p>0.05$) among the varieties. The lowest and highest enthalpies for starches were recorded in *Mweru* and *Kariba*. The enthalpy values for cassava starch varieties were reported, 13.1-15.1 J/g (Mweta et al., 2015), 14.70 J/g (Ai and Jane, 2015) and 9.8-14.2 J/g (Morante et al., 2016). The differences in enthalpies could be due to variations in amylose contents. The enthalpy of gelatinization positively correlated with amylose contents ($r = 0.268$, $p<0.001$). This suggests that the amylose structures were more organized and would require high energy to break hydrogen bonding. Furthermore, amylopectin structures could be suspected to comprise of shorter chain lengths than longer chains. The enthalpy of gelatinization for cassava starches positively correlated with onset ($r = 0.321$, $p<0.01$), peak ($r = 0.586$, $p<0.05$) and conclusion ($r = 0.508$, $p<0.05$) gelatinization temperatures. This implies that an increase in gelatinization transition temperatures would result in higher enthalpy of gelatinization.

6.3.5 Pasting properties

Table 6.11 shows the results on pasting properties of six cassava starches and flours.

6.3.5.1 Pasting temperature

The pasting temperature for starches ranged from 67.19-74.35 ^{o}C, and variations among the five cassava varieties (*Bangweulu*, *Mweru*, *Kariba*, *Kampolombo* and *Chila*) were not significant ($p>0.05$). The lowest and highest pasting temperatures were recorded in *Kariba* and *Katobamputa*. The pasting temperatures for cassava flours (69.33-71.33 ^{o}C) were not significantly different ($p>0.05$) among the varieties. Similar starch pasting temperatures were reported, Tanzanian varieties (66.4-69.6 ^{o}C) (Mtunguja et al., 2016b), CIAT varieties (63.7-71.7 ^{o}C) (Morante et al., 2016), Colombian varieties (starches: 67.85-74.35 ^{o}C and flours: 71.25-75.95 ^{o}C) (Aldana and Quintero, 2013) and Brazilian varieties (62-68 ^{o}C) (Justamante Händel Schmitz *et al.*, 2017). The differences in pasting temperatures among the starches could be due to variations in amylose contents in the varieties. The pasting temperature positively correlated with amylose content ($r = 0.231$, $p<0.001$) suggesting that high amylose starches had high pasting temperatures. *Katobamputa* had high amylose content and displayed higher pasting temperatures. High pasting temperature could indicate resistance against swelling of starch granules. The other source of variations could be due to differences in starch granule sizes. The pasting temperature negatively correlated with granule size ($r = -0.369$, $p<0.001$), implying that higher pasting temperature starches had lower granule sizes. Similar was reported in a study on potato starches, larger granule size fraction recorded lower pasting temperature than smaller granule sizes (Kaur et al., 2007). The positive correlation between pasting temperature and flour particle size ($r = 0.257$, $p<0.001$) could be due to heterogeneous nature of whole flours containing a wider particle size distribution and more lipid, fiber, and protein than in extracted starches.

6.3.5.2 Peak viscosity (PV)

The peak viscosity for starch (782.3-983.5 cP) and flour (651.9-910 cP) varied ($p<0.05$) among the cassava varieties. The lowest and highest peak viscosities for starches were recorded in *Katobamputa* and *Chila,* while for flours were recorded in *Bangweulu* and *Kariba,* respectively. High peak viscosity in starches could indicate the high water-holding capacity of swollen granule and their resistance against shear and swelling. Peak viscosity showed a weak negative correlation with solubility ($r = -0.027$, $p<0.0001$) an indication of

restricted solubility resulting from starch granules resistance to swelling. The peak viscosity exhibited negative correlation with amylose content ($r = -0.561$, $p<0.05$). This suggests that starches with higher amylose content had lower peak viscosity. *Katobamputa* recorded the highest amylose content and lowest peak viscosity. This is in agreement with Morante et al. (2016) who reported that waxy cassava starches recorded higher peak viscosity than normal starches. In a study on wheat starch, peak viscosity exhibited a negative correlation with amylose content (Zeng *et al.*, 1997). In other studies, native starches from waxy maize and waxy rice showed higher peak viscosities than normal starches (Ai and Jane, 2015). There was a negative correlation between peak viscosity and lipid content ($r = -0.453$, $p<0.01$). The amylose-lipid complexes form entanglements with amylopectin structure restricting swelling of starch granule and subsequently lowering the peak viscosity. The negative correlation between peak viscosity and starch granule size ($r = -0.227$, $p<0.001$) is an indication that smaller granule sizes contribute to high peak viscosity. Smaller granules have a large surface area (Lindeboom et al., 2004). The small starch granule sizes were reported to display higher solubility and increased water absorption capacity (Agnes *et al.*, 2017).

6.3.5.3 Hot and cold paste viscosity

The hot paste viscosity (HPV) for starches (385.2-481.7 cP) and flours (383.8-448.3 cP) were two times lower than peak viscosity. The cold paste viscosity (CPV) for starches (445.0-551.7 cP) and flours (418.8-535.7 cP) were slightly higher than HPV.

6.3.5.4 Breakdown viscosity

The breakdown viscosity for starches (383.8-506.8 cP), and flours (268.1-480.9 cP) varied among varieties ($p<0.05$). The lowest and highest breakdown viscosity were recorded in *Bangweulu* and *Chila*, while in flours were recorded in *Bangweulu* and *Kampolombo* respectively. Breakdown viscosity negatively correlated with amylose content in starches ($r = -0.418$, $p<0.01$) and in flours ($r = -0.374$, $p<0.01$). This suggests that high breakdown viscosity occurred in starches with low amylose content. The breakdown viscosity exhibited positive correlation with peak viscosity for both starches ($r = 0.924$, $p<0.05$) and flours ($r = 0.895$, $p<0.05$). Similar correlations were observed in Charles et al. (2005) who reported that high peak viscosities and huge breakdown values were due to low levels of amylose and their

failure to re-associate with amylopectin and reinforce the molecular network within the granule. The lowest breakdown viscosity value in *Bangweulu* could suggest resistance against disruption/dissolution of starch granules to shearing at high temperatures.

6.3.5.5 Final viscosity

The final viscosity for starches (462.0-569.7 cP) and flours (438.7-571.0 cP) varied among the varieties ($p<0.05$). The lowest and highest final viscosity for both starches and flours were recorded in *Katobamputa* and *Chila*. The differences among the varieties were ascribed to variations in amylose and lipid contents. The final viscosity negatively correlated with amylose ($r = -0.383$, $p<0.001$) and lipid ($r = -0.559$, $p<0.05$) contents. This suggests that starches with lower amylose content were associated with higher final viscosities. Similarly, low lipid starches yielded high final viscosity. The presence of lipid-amylose complexes tends to restrict swelling, and thus affecting the viscosity. In a related study on wheat, the defatted wheat starch exhibited high viscosities (Li *et al.*, 2016a). The final viscosity positively correlated with peak viscosity ($r = 0.640$, $p<0.05$), and final viscosity values were significantly lower ($p<0.05$) than peak values. The cold paste of cassava starches is low in viscosities due to significant starch granules breakdown during cooling (Jane et al., 1999; Gomand *et al.*, 2010).

6.3.5.6 Setback viscosity

The setback viscosity for starches was in the range 278.1-487.0 cP and varied among the varieties ($p<0.05$). The lowest and highest viscosity was recorded in *Bangweulu* and *Mweru*. The differences in setback viscosity among varieties could be attributed to variations in amylose contents. There was a negative correlation between setback viscosity and amylose content ($r = -0.432$, $p<0.01$). This suggests that starches with high amylose content recorded lower setback viscosity values. This is in agreement with Morante et al. (2016) who reported higher setback viscosity values in cassava waxy starches than normal starches. This could be attributed to the level of short chains unable to form double helices, and therefore accountable for less organized granular structure (Rolland-Sabaté *et al.*, 2012), and such structural defects are likely to exhibit a high rate of retrogradation during cooling and hence higher setback values. The paste (viscoelastic gel) is an interaction of water with the biphasic system characteristic of both amylopectin enriched swollen granules (dispersed) and amylose

network (continuous) phases (Biliaderis, 1991). The tendency of amylose molecules to crystallize causes phase separation between polymer and water, and this is likely to result in increased setback values.

Table 6.11 Pasting properties of starch and flour from six cassava varieties

Variety	Material	PV	HPV	CPV	BV	FV	SV
Bangweulu	Starch	790.4(94.99)^{bc}	406.7(37.86)^{abc}	512.3(93.54)^{bc}	383.8(66.21)^{b}	514.3(113.47)^{abc}	278.1(33.1)^{bc}
	Flour	651.9(45.25)^{a}	383.8(37.12)^{ab}	442.3(38.55)^{ab}	268.1(72.14)^{a}	463(38.16)^{ab}	209.6(74.24)^{c}
Katobamputa	Starch	782.3(17.09)^{b}	385.2(31.32)^{ab}	445(30.05)^{ab}	397.1(23.35)^{b}	462(29.14)^{ab}	337.3(21.77)^{b}
	Flour	733(28.56)^{ab}	346.8(12.44)^{a}	418.8(6.35)^{a}	386.2(29.37)^{b}	438.7(5.13)^{a}	314.2(23.05)^{b}
Mweru	Starch	963.5(37.57)^{def}	476.7(64.29)^{d}	476.5(21.84)^{ab}	486.8(66.52)^{c}	498.7(20.01)^{abc}	487(35.09)^{a}
	Flour	892.5(6.61)^{de}	410.3(10.6)^{bc}	468.9(16.75)^{ab}	482.2(10.54)^{c}	492.3(18.72)^{abc}	423.6(19.05)^{a}
Kariba	Starch	960.2(52.63)^{def}	484.3(29.77)^{d}	504.7(46.31)^{bc}	475.8(24.77)^{c}	523.3(52.29)^{bc}	455.5(49.11)^{a}
	Flour	910(20.00)^{def}	431.1(41.33)^{bcd}	470.3(11.08)^{ab}	478.9(23.41)^{c}	495.3(14.15)^{abc}	439.7(29.10)^{a}
Kampolombo	Starch	966.2(57.39)^{ef}	481.7(51.03)^{d}	502.7(26.69)^{bc}	484.5(12.31)^{c}	521.5(25.34)^{bc}	463.5(39.14)^{a}
	Flour	876.9(68.17)^{cd}	396(13.00)^{abc}	456.3(19.22)^{ab}	480.9(64.94)^{c}	475.7(19.14)^{ab}	420.5(58.06)^{a}
Chila	Starch	983.5(76.10)^{f}	476.7(31.64)^{d}	551.7(19.63)^{c}	506.8(44.88)^{c}	569.7(19.63)^{c}	431.8(57.92)^{a}
	Flour	893.5(40.47)^{de}	448.3(42.25)^{cd}	535.7(89.51)^{c}	445.2(24.68)^{bc}	571.7(89.54)	341.9(62.42)^{b}
Level of significance							
Variety		$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	

PV=Pasting viscosity, HPV=Hot paste viscosity, CPV=Cold paste viscosity, BV=Breakdown viscosity, FV=Final viscosity, SV=Setback viscosity, PT=Pasting temperature,
All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

6.3.6 Freeze-thaw stability and syneresis

Table 6.12 shows syneresis during freezing storage at -20 ^{o}C for six different cassava starch varieties.

6.3.6.1 Syneresis at -20 ^{o}C freezing storage

The syneresis of starch gels in Week 1, were in the range 0.00-12.55% and varied insignificantly ($p<0.05$) among the cassava varieties. In week 2, the syneresis (3.58-20.01%) significantly varied ($p<0.05$) among the varieties. The lowest and highest syneresis was recorded in *Chila* and *Katobamputa*, respectively. In week 3, the syneresis (0.00-29.11%) varied significantly ($p<0.05$) among the varieties, and the lowest and highest syneresis was recorded in *Katobamputa* and *Kampolombo*, respectively. In week 4, the syneresis (1.26-18.32) varied significantly ($p<0.05$) among varieties, and the lowest and highest syneresis recorded in *Bangweulu* and *Katobamputa*, respectively. In week 5, the syneresis (0.00-19.69%) varied significantly ($p<0.05$) among varieties, and the lowest and highest syneresis recorded in *Chila* and *Katobamputa*, respectively. The source of variations in syneresis among varieties could be ascribed to differences in amylose contents. The percentage syneresis correlated positively with amylose contents ($r = 0.119$, $p<0.0001$; $r = 0.417$, $p<0.01$; $r = 0.554$, $p<0.05$; $r = 0.363$, $p<0.001$ and $r = 0.380$, $p<0.001$; for weeks 1, 2, 3, 4 and 5, respectively) (Table 6.14). This suggests that syneresis in starch gels increases with amylose contents. The syneresis is attributed to amylose content and amylopectin structure (Dhillon and Seetharaman, 2011). The waxy cassava starch (zero amylose starch) had zero syneresis throughout five weeks of freeze storage at -20 ^{o}C (Morante et al., 2016). Waxy cassava starch gel had no syneresis after 5 weeks of storage at -20^{o}C (Sánchez et al., 2010). Mtunguja *et al.* (2016a) reported syneresis of cassava starches in the range 31.7-57.7 and attributed the lowest syneresis value to low amylose content (17.1%). During cooling of starch gels, amylose tendency to crystallize and self-associated causes phase separation which results in loss of gel structure leading to the formation of water zones. The water zones transform into ice crystals during freezing, and upon thawing the ice crystals transforms into water. The other sources of variations of syneresis could be due to differences in lipid contents in varieties. The syneresis positively correlated with lipid contents ($r = 0.464$, $p<0.01$; $r = 0.793$, $p<0.05$; $r = 0.743$, $p<0.05$; $r = 0.504$, $p<0.05$; $r = 0.794$, $p<0.05$; for weeks 1, 2, 3, 4 and 5, respectively. This implies that the presence of lipids accelerated syneresis by stabilizing the water molecules in an ice-like arrangement. The interaction of negatively

charged phosphates in lipid molecules with hydrogen atoms is likely to promote heterogeneous ice nucleation, and their growth into ice crystals could be enhanced by available water during phase separation due to the crystallization of amylose molecules. There was a negative correlation between proteins and syneresis. This suggests that proteins retarded the Syneresis possibly by binding of water to protein. In the current study, proteins inhibited granular swelling and leaching of amylose thereby making starch molecules to remain intact in their swollen state. This may possibly facilitate the reduction of phase separation.

6.3.6.2 Freeze-thaw cycles freezing storage at -20 ^{o}C

Table 6.13 shows syneresis in storage freezing at -20 ^{o}C for five weeks freeze-thaw cycles for six different cassava starch varieties.

The syneresis in the freeze-thaw cycle week 1 was ranged from 0.40-42.50%. The lowest and highest syneresis was recorded in *Bangweulu* and *Katobamputa,* respectively. The syneresis in freeze-thaw cycle week 2 were in the range 12.80-41.60% and varied significantly ($p<0.05$) among the varieties with the lowest and highest syneresis recorded in *Chila* and *Mweru,* respectively. The freeze-thaw cycle week 3 had syneresis in the range 2.60-37.40% and varied ($p<0.05$) among the varieties with the lowest and highest recorded in *Kampolombo* and *Katobamputa*, respectively. The syneresis in the freeze-thaw cycle week 4 ranged from 0.00-41.70% and varied ($p<0.05$) among the varieties with the lowest and highest recorded in *Bangweulu* and *Katobamputa*, respectively. The syneresis in freeze-thaw cycle week 5 (2.00-30.30%) varied ($p<0.05$) among the varieties, and the lowest and highest recorded in *Kariba* and *Katobamputa,* respectively. The variety *Katobamputa* recorded the highest syneresis throughout freeze-thaw cycles. The high level of amylose contents in this variety could be the reason for high syneresis. The decrease in syneresis values in cycle 3, 4 and 5 could possibly suggest that repeated freeze-thaw cycles increased the entanglement of amylopectin with water molecules through hydrogen bonding. The syneresis for five freeze-thaw cycles in cassava starches were reported, 0.00-0.40% (Morante et al., 2016) and 50-67% (Waterschoot *et al.*, 2015). These results are higher than syneresis values in the current study.

Table 6.12 Syneresis in freezing storage at -20 ^{o}C for six different cassava starch varieties

Variety	% Syneresis				
	Week 1	Week 2	Week 3	Week 4	Week 5
Bangweulu	10.76(13.66)^{abc}	18.07(8.76)^{cd}	2.05(2.89)^{ab}	1.26(1.74)^{ab}	1.38(1.73)^{ab}
Katobamputa	2.45(2.45)^{ab}	18.07(5.51)^{cd}	29.11(6.83)^{d}	18.32(9.72)^{cd}	19.69(12.95)^{cd}
Mweru	12.55(12.91)^{bc}	20.01(5.25)^{cd}	11.45(14.00)^{abc}	4.62(1.30)^{ab}	17.15(10.17)^{c}
Kariba	0.06(0.09)^{a}	9.37(6.11)^{abc}	0.85(0.14)^{ab}	11.71(16.93)^{abc}	4.02(4.35)^{ab}
Kampolombo	0.03(0.05)^{a}	4.47(2.23)^{ab}	0.00(0.00)^{a}	5.23(4.51)^{ab}	0.00(0.00)^{a}
Chila	0.00(0.00)^{a}	3.58(2.31)^{ab}	0.26(0.45)^{a}	2.27(2.54)^{ab}	0.00(0.00)^{a}
Level of significance					
Variety	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test.

Table 6.13 Syneresis in storage freezing at -20 ^{o}C for five weeks freeze-thaw cycles for six different cassava starch varieties

Variety	Syneresis (%)				
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Bangweulu	0.4(0.72)^{a}	27.3(11.76)$^{d-g}$	4.2(4.27)^{abc}	0.00(0.00)^{a}	2.00(1.74)^{ab}
Katobamputa	42.5(7.04)^{g}	29.4(24.84)^{efg}	37.4(13.08)^{fg}	41.7(6.67)^{g}	30.3(25.19)^{efg}
Mweru	15.1(15.06)$^{a-e}$	12.8(7.28)$^{a-e}$	6.1(3.27)^{abc}	4.7(4.44)^{abc}	21.7(30.86)$^{c-f}$
Kariba	4.3(3.15)^{abc}	14.4(4.08)$^{a-e}$	7.8(8.93)^{abc}	10.5(15.86)$^{a-d}$	1.4(1.23)^{ab}
Kampolombo	0.8(0.98)^{a}	18.9(2.05)$^{b-e}$	2.6(2.20)^{ab}	2.2(1.23)^{ab}	2.00(1.19)^{ab}
Chila	3.3(1.72)^{ab}	41.6(18.09)^{g}	2.8(2.93)^{ab}	2.2(2.93)^{ab}	14.7(11.27)$^{a-e}$
Level of significance					
Variety	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test.

Table 6.14 Correlation coefficients of syneresis for freeze-thaw storage at -20 ^{o}C

	AM	Pr	Lip	Fib	Cr	GS	Swk1	Swk2	Swk3	Swk4	Swk5
AM	1										
Pr	-0.075	1									
Lip	0.435	-0.394	1								
Fib	0.049	0.498	-0.277	1							
Cr	0.194	-0.033	0.540	-0.192	1						
GS	-0.155	0.243	0.019	0.164	-0.096	1					
Swk1	0.119****	-0.409	0.464**	-0.087	0.438	0.172	1				
Swk2	0.417**	-0.476	0.793*	-0.144	0.440	0.159	0.814	1			
Swk3	0.554*	-0.157	0.734*	-0.062	0.305	0.131	0.146	0.617	1		
Swk4	0.364***	-0.171	0.504*	-0.143	-0.041	-0.063	-0.384	0.204	0.750	1	
Swk5	0.380***	-0.380	0.794*	-0.202	0.365	0.121	0.373	0.726	0.905	0.618	1

AM=Amylose, Pr=protein, Lip=Lipids, Fib=Fiber, Cr=Crystallinity, GS=Starch granule size, Swk1=Syneresis week 1
Significance: p<0.05*, p<0.01**, p<0.001***, p<0.0001****

6.4 Multivariate analysis

The principal component analysis (Figure 6.1) was conducted on gelatinization and pasting properties of starches to determine the differences among the cassava varieties. The axes of T_o, T_p and T_c associated closely in the same direction, and there was no variety which was significantly differentiated by high gelatinization temperatures. The varieties, *Kariba* and *Mweru* were located on the lower axes of granule size, and T_o, T_p and T_c. This suggests that *Kariba* and *Mweru* were significantly distinguished ($p<0.05$) by small granule sizes and low gelatinization temperatures. Furthermore, *Mweru* was distinguished by high pasting temperatures. Amylose and final viscosity clustered on the same axis but in the inverse direction. Also, amylose associated with peak and breakdown viscosities in the opposite direction. This indicates that amylose had a significant negating effect on the final viscosity, and also negatively impacted peak viscosity and breakdown viscosities. The variety *Bangweulu* was disparate by low amylose content and high viscosities. *Katobamputa* clustered towards the axis of setback viscosity and was the only variety located close to the coordinates of the axis of amylose. *Katobamputa* was significantly distinct by high setback viscosity. The swelling and solubility at peak values closely associated together and did not cause differences among the cassava varieties. This suggests that all varieties showed similar ($p>0.05$) response to swelling and solubility.

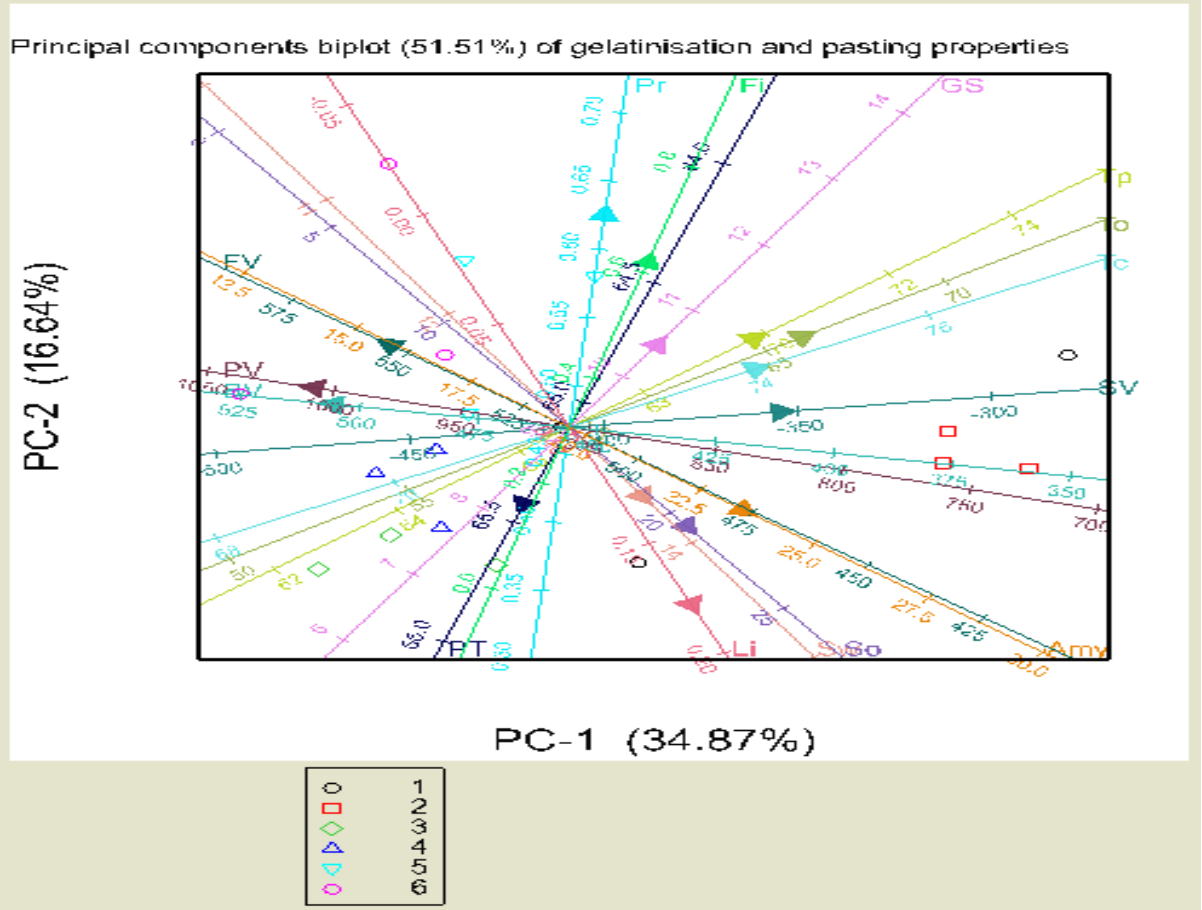

Figure 6.1 Principal component biplot of gelatinization and pasting properties of starches from different six cassava starches. Variety 1=*Bangweulu*, 2=*Katobamputa*, 3=*Mweru*, 4=*Kariba*, 5=*Kampolombo*, 6=*Chila*. To= Onset, Tp=Peak, and Tc=Conclusion gelatinization temper temperatures, GS=Granule size, PT=Pasting temperature, PV=Peak viscosity, BV=Breakdown viscosity, SV=Setback viscosity, FV=Final viscosity. Pr=Protein, Li=Lipid, Fi=Fiber, Amy=Amylose

6.5 Potential application of cassava starches

Quality of food products is influenced by swelling powers of starches (Kusumayanti et al., 2015; Olanrewaju and Idowu, 2017). The starches and flours of *Chila* and *Kampolombo* exhibited the capacity to swell and gelatinize at low temperature ranges (60-74 ^{o}C) and pasted in the ranges 70-74 ^{o}C. Some starches molecules leached and solubilized at 80 ^{o}C. These properties are indicative of the formation of viscosities with low energy requirements during cooking (8-14 J/g), and therefore demonstrate the potential use of cassava flours as an inclusion ingredient into wheat flour for bread making, and in the formulation and development of near-instant porridge products. Efforts to combat protein-energy malnutrition in Zambia identifies dietary protein-energy rich porridge products for children. However,

given the high cost of energy requirements for cooking, such efforts must seek energy serving food materials which can justify the role of starches and flours derived from *Chila* and *Kampolombo* for their capacity to get gelatinized at low temperature. This characteristic is consistent with the requirements for instant porridges (Srikaeo and Sopade, 2010)

The starches of *Bangweulu* and *Mweru* exhibited high solubilization of their molecules and probably starches in these varieties are more susceptible towards amylolytic enzymes (Oates, 1997). High swelling powers were associated with the high digestibility of starches (Abioye *et al.*, 2017). This characteristic is desirable in the brewing and starches liquefaction industry. Some of the most important factors for efficient conversion of starch into fermentable sugars is temperature program of the mashing and solubilization processes. The mashing temperatures were in the range 48 – 72 ^{o}C, and effective enzymatic hydrolysis was reported to occur after the starch has been solubilized (MacGregor *et al.*, 2002; Rübsam *et al.*, 2013). Therefore the higher solubility values obtained in this study suggest flours and starches can find relevance for use as adjunct materials in the brewing industry, local liquefied beverages such as Maheu and Munkoyo drink, and other local traditional sweet beers in Zambia. The swelling properties of starches in this study have potential application in soup, cream, salad, and sauce products since the starches were able to absorb water and swell almost 18 times the original volume (Saha and Bhattacharya, 2010).

6.6 Conclusions

The amylose, protein and lipid contents were the sources of variations evidenced in different peak swelling, solubility and gelatinization, and viscosity values. Higher amylose variety (*Katobamputa*) had high resistant starch content and showed restricted swelling. At low heating temperatures, the swelling was inhibited due to the hydrophobic nature of proteins and lipids. Swelling and solubility values were higher in starches than flours because of lower protein and lipid contents in starches than flours, and this is consistent with the removal of some of exterior proteins and lipids on the surface of starch granules during wet extraction of starches from cassava. The peak swelling of starches and flours of *Chila* and *Kampolombo* at 60 and 70 ^{o}C in is indicative of early gelatinization, rapid solubilization, and high amylolytic susceptibility which suggest potential application of cassava starches in food such as instant pudding, pie filling, cake frosting and soups, and in the manufacture of syrups such as

glucose and fructose. The amylose and protein contents significantly influenced gelatinization and pasting temperatures, and viscosities of starches and flours. The cold paste viscosity were generally two times lower than peak viscosity, an indication of significant ruptures of swollen starch granules. The breakdown and final viscosities were negatively influenced by amylose content. Syneresis values showed high amylose content starches can be susceptible to retrogradation under freezing storage conditions. Nevertheless, the syneresis values were within acceptable ranges to suggest the application of cassava starches and flours in frozen food systems. The significant breakdown viscosities could be suggested for stabilization potentially through blending with other commercial starches and flours. The subsequent chapters are focused on the application of cassava flours in the frozen wheat dough for bread making.

6.7 References

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7. DOUGH RHEOLOGY AND LOAF QUALITY OF WHEAT-CASSAVA BREAD MADE USING DIFFERENT CASSAVA VARIETIES AND WHEAT SUBSTITUTION LEVELS

This chapter is based on the following paper accepted for publication

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Abstract

Flours obtained from six cassava varieties grown in Zambia were incorporated into wheat flour for bread making. The effect of cassava variety (CV) and cassava flour substitution level (CFSL) (wheat: cassava, 90:10, 80:20, 70:30) on dough rheology and bread quality were investigated. Dough rheology and bread baking were determined by the Brabender Farinograph and straight-dough method, respectively, while chemical composition analysis was done using AOAC and AACC standard methods**.** There was a positive correlation ($r = 0.60$, $p<0.05$) between gluten content (6.88-13.00%) and water absorption capacity (WAC) (59.57-61.70%). Development time and stability time of the composite doughs ranged from 1.53-10.60 min and 6.27-12.27 min, respectively. Dough consistency (475.67-512.00 FU) positively correlated ($r = 0.54$) with gluten content. Bread volume (91.67-148.17 cm^3) and specific volume (1.49-2.46 g/cm^3) varied significantly ($p<0.05$) with CV and CFSL, and correlated positively ($r = 0.78$, $p<0.05$) and ($r = 0.76$, $p<0.05$) with gluten content, respectively. The average pore area of the breadcrumb was in the range 0.39-0.85 mm^2. Flour particle size negatively correlated with WAC ($r = -0.26$, 0.001), dough stability time ($r = -0.51$, $p<0.05$), weight loss ($r = -0.50$, $p<0.05$), bread density ($r = 0.67$, $p<0.05$), bread specific volume ($r = -0.72$, $p<0.05$), bread volume ($r = -0.68$, $p<0.05$) and crumb porosity ($r = -0.57$, $p<0.05$). The flour particle size, WAC, and gluten content are significant flour properties influencing dough rheology and bread quality. The results show that wheat can be substituted with cassava flour from cassava varieties *Mweru*, *Kariba* and *Katobamputa* in bread making up to a level of 10%, without affecting bread quality negatively. Cassava inclusion generally

led to reduced bread weight loss. Further work, however, needs to be done to explore the use of higher levels of cassava in composite bread.

Keywords: bread quality, cassava, composite flours, farinograph, gluten, wheat substitutes

7.1 Introduction

Wheat is widely consumed in many African countries and ranks third after maize and cassava for daily caloric supply (Chapoto, 2010). However, continuous increase in the price of wheat in international markets, due to inflation and changes in the exchange rate, is raising serious concern about the economic sustainability of huge importation of wheat grain by some African countries, including Zambia. Thus, there is a growing interest to promote the use of locally produced staples for partial substitution of wheat flour in baking (Abass *et al.*, 2016). Cassava flour has been identified as a candidate for partial replacement of wheat flour in baked goods (Eriksson *et al.*, 2014).

The gluten proteins (glutenins and gliadins) in wheat are responsible for the unique viscoelastic dough that is suitable for leavened baked products (Ribeiro *et al.*, 2018). During dough making, the hydration of glutenins and gliadins results in the development of the gluten structure, a viscoelastic network held together by covalent bonds, and to some extent non-covalent bonds (Jekle and Becker, 2015; Chen *et al.*, 2018a).

Gluten is not present in cassava flours. However, cassava flour has some attractive properties such as low tendency of starch retrogradation, good stability, high water binding capacity and good adhesive strength (Sriroth *et al.*, 1999; Jyothi *et al.*, 2005; Shittu *et al.*, 2016), which could complement dough mixing properties and subsequent bread quality. Thus, it would be worthwhile to determine the influence of partial substitution of wheat flour with cassava flour on dough rheology and consequently bread quality. Currently, there are limited published data on the effect of partially substituting wheat flour with cassava flour on dough rheology and bread quality, particularly on cassava varieties grown in Zambia.

Previous studies have found that genotypes of both cassava and wheat significantly influenced the physical, chemical and functional characteristics of cassava-wheat composite flour and that bread quality varied with cassava genotype and substitution levels (Eriksson *et al.*, 2014). Further, in related studies, product physical characteristics were not only due to processing conditions but also varied with genotype (Ngobese and Workneh, 2018). The amylose content was reported to vary with genotype (Mejía-Agüero *et al.*, 2012), and variations in starch types, proteins, lipids, and fiber were ascribed to differences in genotype

with subtle influence by growing conditions (Halford *et al.*, 2014; Zhu, 2015). However, the previous studies did not establish the chemical components of cassava flour that influenced dough rheology and bread quality. Differences in particle size distribution can affect water absorption capacity of flours, which subsequently influences dough rheological properties, and bread quality (Liu *et al.*, 2015b). Sakhare *et al.* (2014) reported that when wheat flour was fractioned by sieving, the finer (<75 and 75–118 μm) fractions produced higher bread quality than the coarser (118–150 and >150 μm).

The cassava root is perishable due to physiological deterioration of the root immediately after harvest (Zainuddin *et al.*, 2018). Thus, processing of cassava into flour for bread making is one strategy to reduce postharvest loss and end-use diversification for cassava root. In Zambia, cassava is the most important staple crop after maize (Haggblade *et al.*, 2012) and as such, the Zambian Government has prioritized improvement of cassava through breeding. One of the national agricultural strategies is to develop a viable cassava industry. As a result, a number of cassava varieties have been developed and released into the market in Zambia. Nonetheless, there is no cassava variety that was developed for a specific culinary and/or food processing purpose, e.g. bread making. Therefore, there is a need to evaluate the developed cassava varieties for their potential and/or suitability for partial replacement of wheat flour in bread making. Positive evaluation of some of the cassava varieties for bread making would enhance their market value thereby encouraging their cultivation. These would have the attendant effect of enhancing the income of the stakeholders along the value chains (Alimi and Workneh, 2018). Thus, in this work, the effects of cassava variety and substitution level on dough rheology and bread quality were evaluated.

7.2 Materials and methods

7.2.1 Source of materials

White bread wheat flour was procured from the local market in the city of Pietermaritzburg, South Africa. Cassava varieties were sourced as described in method 3.2.1, Chapter 3. The cassava flour production was conducted as described in method 3.2.4, Chapter 3. Flour particle size analysis was conducted as described in method 3.2.5, Chapter 3.

7.2.2 Proximate analysis of wheat flour

Chemical constituents were determined according to methods in Chapter 3: Moisture (3.2.7), Protein (3.2.9), Lipid (3.2.10) and Fiber (3.2.11).

7.2.3 Amylose contents of wheat flour

The amylose content in wheat flour sample was determined using a Megazyme amylose/amylopectin assay kit (K-AMYL 12/16 Megazyme International, Ireland) as described in method 5.2.4 of Chapter 5.

7.2.4 Blending of wheat and cassava flour

Three levels of wheat: cassava (90:10, 80:20, 70:30) composite flours were prepared as described in Aboaba and Obakpolor (2010). Wheat flour (100 %) was used as a control in the analysis. The blends were packed in polyethylene- paper bags and stored at -18 ^{o}C until use.

7.2.5 Rheological properties

The rheological properties of doughs prepared from wheat flour alone (control) and composite wheat-cassava flours were determined with Brabender Farinograph (Model 820603, Brabender OHG, Duisberg, Germany) at 30±0.2 ^{o}C using a 300 g mixing bowl operated at 63 revmin^{-1} according to AACC (2011) Method 54-21 of constant dough weight method in triplicate. From the farinogram developed, water absorption (14% mb), dough development time (min.), dough stability (min.), mixing tolerance index (FU), and consistency (FU) were evaluated using Farinogram software (Farinograph®-E).

7.2.6 Gluten content

The gluten content was determined by hand washing method using 2% sodium chloride solution by taking about 10g flour sample as described in AACC (2011) Method 38-10. The dough was developed by working with hand after gradual addition of small amount of water, placed on the muslin cloth held over sieve, kneaded gently in the stream of washing solution until the dough was free of starch and water-soluble pentosans. The viscoelastic mass obtained was press dried between hand palms, rolled in to ball and determined as wet gluten content. The wet gluten was dried at 100 ^{o}C for 24 h and the mass was determined as dry gluten content.

7.2.7 Preparation of bread

Bread was baked by optimized Straight-Dough Bread-Making Method (AACCI Method 10-09) (AACCI, 2000) using 250 g wheat flour, 25 g sugar, 3 g salt, 5 g baking fat and 2.5 g baker's yeast. The dough was made in a mixing bowl using 150 mL water kneaded by hand until smooth and continuous dough surface obtained, proofed for 45 min in the SelfCookingCentre® (Rational AG, Landsberg am Lech, Germany) at 30 ^{o}C, 100% humidity where upon loading of dough, the cooker automatically adjusted to 32 ^{o}C, 65% humidity for about 3 min before remaining constant at 30 ^{o}C, 100%. After the 1st proof, the dough was re-kneaded and divided into 70 g equal portions (three portions for each blend) per variety, molded and placed in separate oil greased baking pans of equal size. The baking was conducted in the SelfCookingCentre®, preheated at 178 ^{o}C which remain constant upon loading of dough in baking pans for about 3 min, followed by rise to a constant temperature of 193 ^{o}C for about 9 min, with steam conditioning dropping the temperature to 182 ^{o}C with further steaming interval down to 178 ^{o}C at 60% humidity for about 5 min (total baking time of 16 min). The probe sensor was dipped into one of the dough at the beginning of baking. The baked bread quality was evaluated after cooling at room temperature overnight.

7.2.8 Crumb and crust color

The bread crumb and crust color were evaluated using a HunterLab ColorFlex instrument (Hunter Associate Laboratories Inc, Reston, CA, USA) at three bread locations. The Hunter L* (degree of lightness), a* (redness to greenness) and b* (yellowness to blueness) were measured after being standardized using Hunter lab color standards. The whiteness value of crumb and brownness of crust was calculated as described by Zhu *et al.* (2016) using the equation:

$$\text{Whiteness index} = 100 - [(100 - L)^2 + a^2 + b^2]^{\frac{1}{2}} \quad (7.1)$$

$$\text{Brownness Index (BI)} = \frac{100 \text{ x } (\chi - 0.31)}{0.17} \quad (7.2)$$

where

$$\chi = \frac{a + 1.75L}{5.645L + a - 3.01b} \quad (7.3)$$

where:

L=Lightness,

a=redness-greenness

b=yellowness

7.2.9 Bread specific volume and density

The loaves bread mass (W) was measured using digital balance. The bread loaf volume (BV) was determined by a modification of the AACC Method 10-05 rapeseed replacement method using maize grit instead of rapeseeds (Eriksson et al. 2014). The bread was put into a round container of known volume (VC) and the basin filled to the brim with grits, bread was removed and the volume of the grits (VG) was measured with a measuring cylinder.

$$\text{Loaf volume, BV } (\text{cm}^3) = \text{VC} - \text{VG} \quad (7.4)$$

$$\text{Density } (\text{g/cm}^3) = \frac{\text{W}}{\text{BV}} \quad (7.5)$$

$$\text{Specific volume } (\text{cm}^3/\text{g}) = \frac{\text{BV}}{\text{W}} \quad (7.6)$$

where:

BV = bread volume

VG = known volume of container

W = bread mass

7.2.10 Weight loss

The weight loss of the bread in percent was determined as described in Bakare *et al.* (2016) by measuring mass of dough before baking and mass of baked bread

$$\text{Weight loss } (\%) = \frac{\text{A}-\text{B}}{\text{A}} \text{ x } 100 \quad (7.7)$$

where:

A = weight of dough

B = weight of baked bread.

7.2.11 Bread crumb pore size characteristics

The bread morphology and pore size of crumb was studied using scanning electron microscope (SEM) as described in Hayta and Ertop (2018) with modifications. The breadcrumb samples of approximately 5x5x3 mm^3 were prepared and mounted on the SEM sample stubs with double adhesive tape. In order to obtain the actual crumb pores (gas cells), sample treatments such freeze-drying and gold sputtering were skipped. The prepared samples were examined for microscopic pore morphologies using SEM (ZEISS, EVO, LS15, Jena, Germany), set at a magnification of 100 X signal A = VPSE G3, EHT = 20.00 kV, chamber = 2.07e-001 Torr, I Probe = 253 pA, Spot size = 442 and scale of 200µm. The level of magnification was chosen in such a way that the images could encompass a number of pores so that a more realistic estimation of the void fraction could be determined. The SEM images were submitted to image analysis for pore size (cross section area mm^2) estimation (Figure 1) using Soft Imaging System GmbH (Johann-Krane-Weg, Munster, Germany) at the Laboratory of Microscopic Analysis (University of KwaZulu Natal, Pietermaritzburg, South Africa). The porosity was expressed as total pore area to a total surface area of the image.

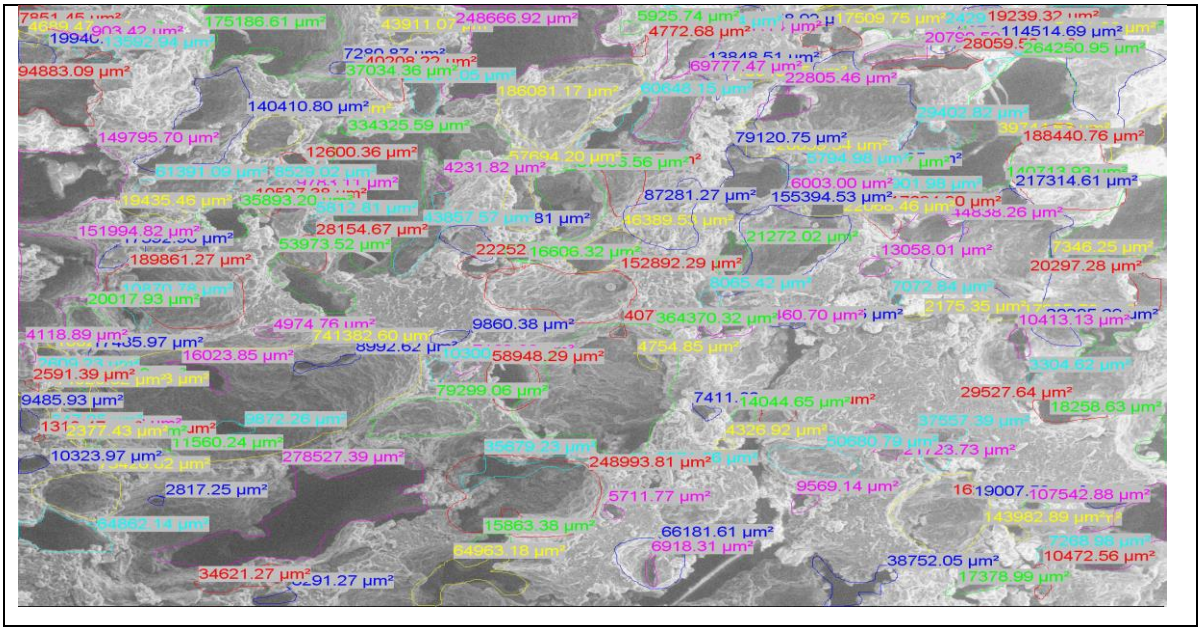

Figure 7.1 Bread crumb pore size (cross section area) characterization using Soft Imaging System.

7.2.12 Experimental design and data analysis

A completely randomized design comprising of two factors cassava variety and blend ratio (cassava concentration) was used. Triplicate data were analyzed using two-way ANOVA of GenStat 18th Edition software and mean differences were determined using Fisher's Least Significance Difference (LSD) test at 5% significant level. The correlation coefficients of flour proximate content (Table 3.5, Chapter 3), particle size distribution at 90% cumulative of finer particles (Table 3.3, Chapter 3) and amylose content (Table 5.2, Chapter 5) with Farinogram properties and bread quality characteristics were analyzed using Pearson's correlation.

7.3 Results and Discussion

7.3.1 Proximate composition of cassava and wheat flours

Table 7.1 shows the amylose contents and proximate composition of cassava and wheat flours.

7.3.1.1 Moisture contents

The moisture content of the cassava flours ranged from 10.43 to 11.76%. The moisture content of wheat flour was 14.05±0.93%.

7.3.1.2 Protein contents

The cassava flours protein were in the range 1.21–1.87%. A significant difference was observed only between *Bangweulu* and *Chila* which recorded the highest and lowest protein contents, respectively. The protein content of the cassava flours was in the range 1.21–1.87% (Table 1). The protein content of the cassava flours was very low compared to that of wheat flour (11.03±0.27%). Wheat flour proteins contain about 85% gluten proteins (glutenins and gliadins) (Avramenko *et al.*, 2018; Ribeiro *et al.*, 2018), while cassava flour protein is gluten-free (Chakrabarti *et al.*, 2017).

7.3.1.3 Lipid contents

The cassava flour lipid content ranged between 0.15 and 0.63%. The lipid contents in all cassava flour varieties were significantly ($p<0.05$) lower than 1.72±0.16% in wheat flour. Significant variations ($p<0.05$) in lipid contents were observed between *Bangweulu* and

Chila. The lipid reinforces gluten structure through lipid-protein interactions (Avramenko et al., 2018).

7.3.1.4 Fiber content

The fiber contents ranged 0.03–0.60% and were significantly ($p<0.05$) lower than wheat flour fiber contents (2.90±0.10%). The fiber content (0.03–0.60%) of the cassava flour was significantly ($p<0.05$) lower than that of the wheat flour (2.90±0.10%). Leavened aerated bread cannot be made without wheat flour because of viscoelastic dough making properties of wheat gluten proteins (Ceresino et al. 2018). Blending of cassava flours in wheat flour influences the blended dough rheological properties and bread nature by diluting wheat protein content and gluten proteins functionality. Also, variation in fiber and lipid contents has an additional effect on baked bread quality.

Table 7.1 Percentage (%) moisture, protein, lipid, fiber and amylose contents of wheat and cassava flours

Variety	Moisture	Protein	Lipid	Fiber	Amylose
Bangweulu	11.02(1.00)^{ab}	1.87(0.78)^{b}	0.40(0.04)^{bc}	0.60(0.49)^{b}	22.22(2.78)^{ab}
Katobamputa	11.05(1.46)^{ab}	1.45(0.03)^{ab}	0.41(0.05)^{bc}	0.15(0.15)^{a}	26.95(2.30)^{b}
Mweru	11.76(1.61)^{b}	1.78(0.28)^{ab}	0.59(0.18)^{cd}	0.05(0.06)^{a}	17.95(8.02)^{a}
Kariba	11.18(0.72)^{ab}	1.43(0.41)^{ab}	0.63(0.06)^{d}	0.04(0.02)^{a}	16.04(1.16)^{a}
Kampolombo	10.69(0.62)^{a}	1.58(0.15)^{ab}	0.32(0.20)^{ab}	0.03(0.02)^{a}	18.47(7.30)^{a}
Chila	10.43(0.37)^{a}	1.21(0.09)^{a}	0.15(0.04)^{a}	0.15(0.05)^{a}	16.15(3.88)^{a}
Wheat (control)	14.05(0.93)^{c}	11.03(0.27)^{c}	1.72(0.16)^{c}	2.90(0.10)^{c}	20.83(0.45)^{ab}
Level of significance					
Variety	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

7.3.2 Amylose content

The amylose content in cassava varieties was in the range 16.04–26.95% and 20.83±0.45% for wheat flour. The amylose content of cassava was reported previously, 19.50-20.30% (Morante *et al.*, 2016), 22.60±1.30 (dos Santos *et al.*, 2018), and 17.06-25.72% (Liu *et al.*, 2019). The amylose content is the basis of classifying starches into waxy, semi-waxy, normal/regular and high-amylose types when amylose content is 0-2%, 3-15% 20-35%, and

higher than 40% of the total starch, respectively (Tester *et al.*, 2004; Morante *et al.*, 2016; Botticella *et al.*, 2018). The result shows all the cassava flour varieties including wheat flour were generally classified as normal regular starches. The amylose content in *Katobamputa* was significantly different ($p<0.05$) from other cassava varieties. There was no significant difference ($p>0.05$) between the amylose content of the wheat flour and that of any of the cassava varieties. High amylose content can reduce starch granules swelling and significantly increase the level of resistant starches (Hallström *et al.*, 2011).

7.3.3 Particle size of cassava varieties and wheat flours

Average particle size of flours from the cassava varieties ranged from 250.43–333.43 µm (Table 7.2) and varied among varieties. The highest and lowest particle size of cassava flour were recorded in *Bangweulu* and *Mweru*, respectively. The average particle size of the wheat flour was low (206.67±6.81 µm) compared to the particle size of cassava flours. The particle size of flour is an important factor that can affect the baking properties and end product quality (Vouris *et al.*, 2018). Particle size is influenced by the milling technique applied and inherent hardness differences of wheat grain and cassava flour varieties (Liu *et al.*, 2015). Reduction of flour particle size during milling can result in a high proportion of damaged starch granules leading to high water absorption capacity of the flour and high susceptibility of starches to enzymatic hydrolysis, both of which can affect bread quality (Wang *et al.*, 2017).

Table 7.2 Distribution of particle size of cassava flour at 90% cumulative of finer particles passing through sieve

Variety	Size (µm)
Bangweulu	312.01(0.001) [a]
Katobamputa	282.53(0.02)[c]
Mweru	250.43(0.03)[b]
Kariba	332.52(0.02)[e]
Kampolombo	334.43(0.01)[e]
Chila	278.49(0.001)[c]
Control (Wheat)	206.67(6.81)[f]

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

7.3.4 Water absorption capacity (WAC)

The moisture content of the composite flour blends ranged from 13.13 to 13.83% (Table 7.3) and increased with increase in CFSL ($r = 0.37$, $p<0.001$). The WAC results for the flour blends at 10%, 20%, and 30% were in the range 60.43–62.10%, 61.03–61.50%, and 59.57–60.33%, respectively, and negatively correlated with CFSL ($r = -0.65$, $p<0.05$) suggesting that higher CFSL resulted in decreasing WAC, in part, due to the large particle size of cassava flour with low water absorption capacity. There was a weak positive correlation between WAC and protein ($r = 0.34$, $p<0.01$), lipid ($r = 0.36$, $p<0.01$) and fiber ($r = 0.36$, $p<0.01$) contents. The high protein and fiber levels in wheat flours are significant contributors toward water absorption. The protein contents were generally very low in cassava with no significant difference among the cassava varieties ($p>0.05$) (Table 1). The fiber contents of the cassava varieties (≤ 0.6%) were low compared to wheat flours (2.9%) ($p<0.05$) and hence the contribution of cassava fiber to WAC was likely to be low. Nevertheless, the difference in fiber content can bring a difference in water absorption of wheat flours. A study by Struck *et al.* (2018) found that addition of almond fiber significantly reduced WAC of wheat flour.

In a similar study on potato-wheat flour, higher protein contents increased WAC of wheat flour (Sarker *et al.*, 2008). According to Liniņa *et al.* (2014), water absorption of weak flour is below 55%, of medium flour 54–60%, and strong above 58%. The WAC of the flour blends in the present study was characteristic of strong flours and showed significant correlations with gluten content ($r = 0.60$, $p>0.05$), an indication that high gluten content resulted in a high WAC. There was a weak negative correlation between WAC and flour particle size ($r = -0.26$, $p<0.01$), which indicates that smaller particle size flours had higher water hydration capacity.

Table 7.3 Moisture content, water absorption capacity and gluten content of cassava-wheat flour blends

Variety	CFSL (%)	Moisture (%)	Water absorption capacity (%)	Gluten (%)
Bangweulu	10	13.23(0.31)^{ab}	60.43(1.62)^{abcd}	10.41(0.04)^{de}
Katobamputa	10	13.47(0.06)^{bcd}	62.10(0.10)^{e}	11.25(0.01)^{ef}
Mweru	10	13.27(0.15)^{ab}	61.53(0.20)^{e}	10.41(0.02)^{de}
Kariba	10	13.37(0.06)^{abc}	61.37(1.19)^{cde}	11.26(0.01)^{ef}
Kampolombo	10	13.27(0.05)^{ab}	61.63(0.46)^{e}	11.28(0.01)^{ef}
Chila	10	13.27(0.06)^{ab}	61.57(0.30)^{e}	11.28(0.01)^{ef}
Bangweulu	20	13.13(0.15)^{a}	61.10(0.87)^{bcde}	9.56(0.02)^{cd}
Katobamputa	20	13.83(0.06)^{ef}	61.40(0.20)^{cde}	8.62(0.87)^{bc}
Mweru	20	13.27(0.21)^{ab}	61.03(1.00)^{bcde}	8.67(0.85)^{bc}
Kariba	20	13.67(0.06)^{cde}	61.53(0.50)^{e}	7.77(0.87)^{ab}
Kampolombo	20	13.23(0.15)^{av}	61.50(0.20)^{de}	12.15(0.03)^{fg}
Chila	20	13.37(0.15)^{abc}	61.37(0.55)^{cde}	10.40(0.02)^{de}
Bangweulu	30	13.40(0.20)^{abcd}	59.90(0.53)^{a}	8.66(0.02)^{bc}
Katobamputa	30	13.67(0.59)^{cde}	59.57(0.06)^{a}	8.06(1.01)^{bc}
Mweru	30	13.47(0.15)^{ab}	59.67(0.35)^{a}	6.92(0.87)^{a}
Kariba	30	14.00(0.10)^{f}	60.17(0.15)^{ab}	6.88(0.86)^{a}
Kampolombo	30	13.43(0.16)^{abcd}	60.33(0.58)^{abc}	9.23(0.51)^{c}
Chila	30	13.70(0.10)^{def}	60.07(0.90)^{ab}	10.36(0.01)^{de}
Wheat	100	13.37(0.15)^{abc}	61.70(0.61)^{e}	13.00(0.87)^{g}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. CFSL = Cassava flour substitution level

7.3.5 Gluten content

The dry gluten content of wheat flour was 13.00±0.87% (Table 7.3) and decreased with increase in CFSL ($r = -0.84$, $p<0.05$). The mixing of wheat flour with water transforms gluten proteins into viscoelastic gluten structures that ultimately determine the quality of the final bread product (Sissons and Smit, 2018). The negative correlation between protein content and CFSL ($r = -0.77$, $p<0.05$) suggests that the inclusion of cassava flour resulted in decreasing gluten proteins of wheat flour. Cassava flour does not contain proteins found in wheat and hence has a diluent effect during wheat gluten development, an effect also observed by Collar and Armero (2018). Thus the partial replacement of wheat flour with cassava flour could reduce bread volume because of dilution in wheat gluten functionalities (Šárka *et al.*, 2017). Flour particle size had a significant negative correlation ($r = -0.53$, $p<0.05$) with gluten development implying that smaller particles hydrate faster and thereby promote migration of excess water to the gluten network.

7.3.6 Dough rheological properties

The dough development time, consistency, stability time and mixing tolerance index are shown in Table 7.4.

7.3.6.1 Dough development time (DDT)

The DDT of composite flours was in the range 1.53-10.60 min and increased with increase in CFSL ($r = 0.62$, $p<0.05$). The DDT of the control (wheat) flour (2.13±0.11 min) was not significantly different from that of composite flours of *Katobamputa*, *Mweru,* and *Kampolombo* at 10%, Kariba at 20%, and *Mweru* at 30%. The DDT showed weak negative correlation with WAC ($r = -0.44$, $p<0.01$), protein ($r = -0.28$, $p<0.001$) and gluten content ($r = -0.28$, $p>0.001$). Reduced WAC inhibits gluten development. Additionally, excess water beyond that required for gluten development can cause weakening of gluten matrix leading to delayed dough development. Higher WAC increases hydration of gluten and hence contributes to quicker dough development. Jafari *et al.* (2018) reported that decreased gluten hydration is the main reason for high DDT. The high DDT observed in this study could be attributed to decreasing gluten content with increasing cassava flour content, which might have disrupted the formation of the gluten network (Zhang *et al.,* 2018), thus increasing dough development time (Eduardo *et al.,* 2013). DDT is influenced by protein content (Huang *et al.,* 2016). The positive correlation between DDT and flour particle size ($r = 0.45$, $p<0.05$) suggest that large particle size is a significant contributor to the low hydration capacity that might have delayed gluten development resulting in an extended period of dough development.

7.3.6.2 Consistency

Cassava variety and main interaction (CV x CFSL) had significant ($p<0.05$) influence on the consistency of dough. The peak consistency value of the wheat dough (512 FU) was higher than those of the composite doughs, which varied among flour blends, showing a decreasing trend with increasing CFSL ($r = -0.65$, $p<0.05$). This indicates that the inclusion of cassava flour resulted in a decrease in dough consistency. Dough consistency positively correlated with gluten content ($r=0.54$, $p<0.05$), which is expected because gluten is largely responsible for dough structure and strength. Dough consistency was negatively correlated with dough development time ($r = -0.45$, $p<0.01$), and positively correlated with water absorption

capacity (r = 0.45, p<0.01) and protein content (r = 0.60, p<0.05) indicating that strong, high gluten content dough would be of high dough consistency. The dough consistency correlated negatively with flour particle size (r = -0.48, p<0.01) implying that doughs with smaller particles had higher consistency than doughs made with flour of large particle size.

Table 7.4 Mixing properties (development time, consistency, stability time, tolerance index) of wheat flour and cassava-wheat composite flours

Variety	Blending level	Development time (min)	Consistency (FU)	Stability (min)	Tolerance index (FU)
Bangweulu	10	1.87(0.28)^{bcde}	478.00(1.73)^{cd}	7.20(0.10)^{b}	30.33(1.52)^{hi}
Katobamputa	10	2.10(0.10)^{de}	478.00(1.73)^{cd}	9.87(0.05)^{ef}	25.33(0.58)^{efg}
Mweru	10	2.03(0.06)^{cde}	501.00(0.00)^{h}	10.57(0.06)^{gh}	29.00(3.60)^{hi}
Kariba	10	1.70(0.10)^{ab}	516.33(1.16)^{i}	7.23(0.25)^{b}	34.67(0.57)^{jk}
Kampolombo	10	2.07(0.11)^{de}	475.67(0.50)^{c}	9.37(0.47)^{de}	35.33(0.57)^{jk}
Chila	10	2.17(0.06)^{e}	490.00(0.00)^{fg}	7.10(0.10)^{b}	35.33(0.58)^{jk}
Bangweulu	20	1.83(0.15)^{abcd}	510.33(0.57)^{i}	8.23(0.11)^{c}	18.33(0.57)^{bc}
Katobamputa	20	1.83(0.06)^{abcd}	511.33(0.57)^{i}	6.27(0.06)^{a}	32.33(0.57)^{ij}
Mweru	20	1.53(0.06)^{a}	501.33(0.60)^{h}	6.70(1.13)^{ab}	36.67(5.77)^{k}
Kariba	20	2.07(0.11)^{de}	483.67(21.38)^{def}	10.17(0.28)^{fg}	21.67(1.15)^{k}
Kampolombo	20	7.80(0.20)^{h}	492.00(1.73)^{g}	6.60(0.17)^{ab}	28.00(5.29)^{a}
Chila	20	1.73(0.06)^{abc}	481.67(0.57)^{cde}	6.90(0.10)^{ab}	28.33(0.57)^{gh}
Bangweulu	30	7.23(0.06)^{g}	493.00(0.00)^{g}	9.10(0.10)^{d}	40.33(0.57)^{l}
Katobamputa	30	4.00(0.00)^{f}	488.00(1.00)^{i}	9.47(0.06)^{de}	11.33(0.57)^{a}
Mweru	30	2.13(0.11)^{de}	452.67(0.50)^{h}	10.83(1.27)^{hi}	15.33(3.77)^{b}
Kariba	30	9.37(0.55)^{j}	480.67(0.60)^{def}	12.27(0.11)^{k}	23.67(1.15)^{de}
Kampolombo	30	10.60(0.53)^{k}	462.00(0.00)^{g}	11.23(0.32)^{ij}	24.33(1.15)^{def}
Chila	30	8.73(0.06)^{i}	467.33(0.58)^{cde}	11.70(0.10)^{jk}	27.33(0.58)^{fgh}
Wheat	100	2.13(0.11)^{de}	512.33(0.50)^{i}	12.20(0.17)^{k}	23.67(1.15)^{fgh}
Level of significance					
Variety		<0.001*	<0.001*	<0.001*	<0.001*
Blend ratio		<0.001*	<0.001*	<0.001*	<0.001*
Variety x Blend		<0.001*	<0.001*	<0.001*	<0.001*

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test. *significance differences

Table 7.5 Correlation coefficients of dough mixing properties, bread quality, amylose and proximate contents

	CFSL	M	WAC	DDT	DST	MTI	DC	Amy	Prt	Lip	Fib	BV	WB	BD	SP	WL	PA	P	D90	G
CFSL	1																			
M	0.37*	1																		
WAC	-0.65***	-0.30*	1																	
DDT	0.62***	0.32*	-0.44**	1																
DST	-0.27*	0.19	0.00	0.19	1															
MTI	-0.06	-0.19	0.16	0.00	-0.48	1														
DC	-0.65***	-0.19	0.45**	-0.45	0.03	0.12	1													
Amy	-0.10	0.12	-0.12	-0.16	0.00	-0.21	0.18	1												
Prot	-0.77***	-0.14	0.34**	-0.28	0.64***	-0.25	0.60***	0.15	1											
Lip	-0.74***	-0.08	0.36**	-0.31	0.65***	-0.27	0.61***	0.14	0.96	1										
Fib	-0.76***	-0.15	0.36**	-0.29	0.61***	-0.23	0.60***	0.16	0.98	0.93	1									
BV	-0.87***	-0.21	0.66***	-0.51	0.36	-0.06	0.60***	0.16	0.77***	0.78	0.74	1								
WB	0.04	0.13	0.08	0.21	-0.27	0.25	-0.02	0.06	-0.23	-0.32	-0.20	-0.13	1							
BD	0.85***	0.24	-0.65***	0.58	-0.30	0.05	-0.57***	-0.13	-0.71***	-0.73	-0.66	-0.98	0.24	1						
SV	-0.86***	-0.22	0.63***	-0.52*	0.39	-0.09	0.59***	0.14	0.79***	0.81	0.75	0.99	-0.25	-0.98	1					
WL	-0.04	-0.13	-0.08	-0.21	0.27	-0.25	0.02	-0.06	0.23	-0.32	-0.20	0.13	-1.0***	0.24	-0.25	1				
PA	0.19	0.00	-0.05	0.12	-0.23	0.40	-0.20	0.26	-0.29	0.36	0.24	-0.16	0.38	-0.20	0.20	-0.38	1			
P	-0.71***	-0.23	0.35	-0.30	0.43	-0.09	0.41	0.40	0.75***	-0.68	-0.78	0.66***	-0.0	0.58	-0.64	0.01	0.18	1		
D90	0.64***	0.13	-0.26	-0.45*	-0.51***	0.24	-0.48**	-0.16	-0.83***	0.79	0.81	-0.68***	0.50	-0.67	0.72	-0.50	-0.29	-0.57	1	
G	-0.84***	-0.44	0.60	-0.28*	0.25	0.10	0.54***	0.08	0.70***	0.62	0.70	0.78***	0.07	-0.72	0.76	-0.07	-0.08	0.64	-0.53	1

WAC=Water Absorption Capacity, DDT=Dough Development Time, DST=Dough Stability Time, MTI =Mixing Tolerance Index, DC=Dough Consistency, VB= Volume of Bread, WB= Weight of Bread, DB= Density of Bread, SP= Specific Volume, WL= Weight loss, P= Porosity, D90=Particle Size Distribution at 90% finer particles pass, G= Gluten Significance differences at p<0.001* p<0.01**, p<0.05***

7.3.6.3 Dough stability time (DST)

The DST at 10% cassava flour substitution level was in the range 7.10–10.57 min and decreased when cassava flour substitution level was increased to 20% except for varieties *Bangweulu* and *Kariba*. DST showed a weak negative correlation with CFSL ($r = -0.27$, $p<0.001$) suggesting that high DST were associated with low CFSL. The DST of the composite doughs increased when CFSL was increased to 30%, DSTs were similar ($p>0.05$) to the DST of the control (12.20±0.17). Dough stability time (DST) indicates the tolerance of the dough to mixing stress. Flour with a DST greater than 10 min is resistant to mechanical stress (Edun *et al.*, 2018), and is classified as flour of excellent quality, and flour of poor quality has stability time of about less than 3 min (Liniņa *et al.*, 2014). DST had a negative correlation ($r = -0.51$, $p<0.05$) with particle size implying that DST increased with reduced particle size, presumably because smaller particle size favor uniform and high water absorption that in turn enhances gluten development. Wang *et al.* (2017) reported that reducing the particle size strengthened the gluten network, and resulted in shorter development time and longer mixing stability of the dough. DST showed significant correlation with protein ($r = 0.64$, $p<0.05$), lipid ($r = 0.65$, $p<0.05$) and fiber content ($r = 0.61$, $p<0.05$). The DSTs of the composite flours were generally poor at 10% and 20% CFSL but improved at 30% substitution level. Increased fiber along with starch content might have contributed to an increase in the water absorption required for the development of gluten structure (Hrušková and Švec, 2018). The increased starch-protein interaction may have contributed to increased hydrogen bonding in the starch-gluten interaction and thus contributed to the stability of the gluten network. A similar, observation was made by Zhang *et al.* (2018) on tapioca starch-wheat composite flours.

7.3.6.4 Mixing tolerance index (MTI)

The MTI ranged from 11.33–40.33 FU across varieties for all blend ratios and did not vary with CFSL ($p>0.05$). The MTI for the control was 23.67±1.15 FU and was not significantly different from *Katobamputa* (25.33±0.58 FU) at 10%, *Kariba* (21.67±1.20) at 20%, *Kariba* and *Kampolombo* at 30%. Mixing tolerance index (MTI) indicates the degree of dough softening over a period of mixing (Srikanlaya *et al.*, 2018). The lower the MTI value the better quality. According to Liniņa *et al.* (2014), dough mixing quality is considered satisfactory if mixing tolerance is below 70 FU. Doughs with mixing tolerance values higher

than 110 FU are considered weak and are characterized by difficulties in mechanical handling during dough making. Depending on its quantity and composition (Gómez *et al.*, 2003), dietary fiber can have a dilution effect on gluten proteins (Ho and Aziah, 2013). All the composite flour samples in this study, irrespective of variety and CFSL, had satisfactory mixing tolerance, which indicates good dough mixing quality. Values obtained in this study were in the range considered satisfactory for good dough mixing quality. However, the mixing tolerance showed poor correlation with other mixing properties. Similar results were reported by Isah (2017) in the study of African locust bean pulp flour incorporated into wheat flour at different ratios. It seems likely that high levels of cassava flour contributed a large amount of starches which may have weakened gluten structure.

7.3.7 Bread quality

Table 7.6 shows Volume, specific volume and density of bread baked from cassava-wheat flours blends.

7.3.7.1 Bread volume (BV)

The BV of the control (0% cassava flour) was 148.17±10.16 cm^3. The BV decreased with increase in CFSL ($r = -0.87$, $p<0.05$). The volume of bread made from flour blends at 10%, 20%, and 30% CFSL ranged 103.00–140 cm^3, 103.00–120.00 cm^3, and 91.67–105.00 cm^3, respectively. The BV correlated strongly and positively with gluten content ($r = 0.78$, $p<0.05$), protein ($r = 0.77$, $p<0.05$), WAC ($r = 0.66$, $p<0.05$), dough consistency ($r = 0.60$, $p<0.05$) and negatively with DDT ($r = -0.51$, $p<0.05$). The correlations indicate that flour samples with relatively high gluten content had good mixing properties, including good consistency, short mixing time and resistance to stress and yielded quality bread with respect to BV. Further, the BV showed a negative correlation ($r = -0.68$, $p<0.05$) with particle size, indicating flours of small particle size produced bread of large volume. Similarly, Jacobs *et al.* (2018) reported that flours of smaller particle size produce large volume bread. This observation can be attributed mainly to the observation described earlier, that flours of small particle size have high water absorption capacity (WAC), which promotes good gluten development.

7.3.7.2 Bread specific volume (SV)

The SV for bread from 100% wheat was the highest (2.46±0.19 g/ cm) and the value decreased as cassava flour level increased. The SV correlated positively with WAC ($r = 0.63$, $p<0.05$), gluten content ($r = 0.76$, $p<0.05$), DST ($r = 0.39$, $p<0.01$), and negatively to DDT ($r = -0.52$, $p<0.05$). The highest SV was obtained at 10% cassava flour substitution of *Katobamputa, Mweru* and *Kariba*, and was not significantly different ($p>0.05$) from that of control wheat bread. These findings are similar to those of Eriksson *et al.* (2014), who observed insignificant variation in cassava variety effect on SV of bread. The decrease in bread SV with an increasing amount of cassava flours has been reported by several authors (Eggleston *et al.*, 1993; Aboaba and Obakpolor, 2010; Eriksson *et al.*, 2014). The SV values obtained in the previous studies were higher than the SV values of the present study, which was probably influenced by additional ingredients. Ingredients such as concentrated milk have an enhanced emulsifying effect (Julianti *et al.*, 2017), which promotes emulsification of the shortening, which improves bread quality. The negative correlation between flour particle size and SV ($r = -0.72$, $p<0.05$) again indicating that smaller flour particle size is associated with large bread volume likely due to high WAC of the flour.

7.3.7.3 Bread density (BD)

The density of wheat bread (control) was the lowest (0.41±0.03 g/cm^3) and BD values increased with increase in CFSL ($r = 0.85$, $p<0.05$). Increased CFSL favored starch-starch/starch-protein system more than protein-protein interactions, which can weaken gluten structure. During baking, starch granules lose birefringence properties through swelling and leaching of amylose and this results in an increase in viscosity and migration of plasticizing water from gluten to starch (Verbauwhede *et al.*, 2018). Higher absorption capacities due to damaged starch of cassava flour (Nindjin *et al.*, 2011) can deplete water to a level lower than is required for gluten structure, which can lead to inhibition of expansion of gas cells and hence a dense (less foam) crumb structure at the end of the oven spring. The bread density positively correlated with flour particle size ($r = 0.67$, $p<0.05$) indicating that flours of larger particle size had low expansion capacity when processed into the dough and then bread. Bread density was significantly negatively correlated with gluten content ($r= -0.72$, $p<0.05$), dough consistency ($r = -0.57$, $p<0.05$), and WAC ($r = -0.65$, $p<0.05$) but positively correlated with DDT ($r = 0.58$, $p<0.05$), indicating that high BD values were associated with flours of low gluten content, low WAC and high dough development time.

7.3.7.4 Weight loss (WL)

The WL of wheat bread (control) was 13.86±0.87% and was similar to the WL of the bread containing the cassava varieties *Bangweulu* at 20%, *Chila* at 20% and *Bangweulu* at 30% CFSL. Weight loss ranged from 10.43-18.19% across all CFSLs, and there was no clear pattern of change in WL. Vouris *et al.* (2018) reported weight loss of wheat bread in the range of 16.11-18.06%, values somewhat higher than those obtained in this study. Weight loss occurring during the baking stage of bread processing may be due to both fermentation processes, evaporation of water as well as volatilization of low molecular weight compounds produced during fermentation, including ethanol (Bakare *et al.*, 2016; Verbauwhede *et al.*, 2018) during baking (Shittu *et al.*, 2007b).

Table 7.6 Volume, specific volume and density of bread baked from cassava-wheat flours blends

Variety	Cassava flour blending level	Volume (cm^3)	Specific volume ($cm^3.g^{-1}$)	Density ($g.cm^{-3}$)	Weight loss (%)
Bangweulu	10	103.00(6.03)^{abcd}	1.67(0.09)^{abc}	0.60(0.03)^{fghi}	12.00(0.25)^{bcde}
Katobamputa	10	140.00(5.00)^{gh}	2.27(0.07)^{ij}	0.44(0.01)^{ab}	12.05(0.58)^{bcde}
Mweru	10	123.33(7.64)^{ef}	2.13(0.12) ^{hi}	0.47(0.03)^{bc}	17.14(1.43)^{gh}
Kariba	10	128.33(7.64)^{fg}	2.08(0.10)^{ghi}	0.48(0.02)^{bc}	11.67(0.95)^{abcd}
Kampolombo	10	121.67(2.89)^{ef}	1.94(0.06)^{efgh}	0.52(0.01)^{cd}	10.43(1.38)^{a}
Chila	10	118.33(7.63)^{ef}	1.90(0.11)^{defg}	0.53(0.03)^{cde}	10.91(0.36)^{ab}
Bangweulu	20	103.00(6.08)^{abcd}	1.69(1.69)^{abcd}	0.59(0.03)^{fgh}	12.95(0.41)^{def}
Katobamputa	20	120.00((8.66)^{ef}	1.93(1.92)^{efgh}	0.52(0.03)^{def}	11.10(1.03)^{abc}
Mweru	20	115.00(5.00)^{de}	1.96(1.96)^{fgh}	0.51(0.03)^{def}	16.33(1.79)^{g}
Kariba	20	111.67(5.77)^{cde}	1.82(1.81)^{cdef}	0.55(0.03)^{def}	12.29(0.49)^{cde}
Kampolombo	20	111.67(5.77)^{cde}	1.81(1.81)^{cdef}	0.55(0.02)^{def}	11.95(1.03)^{bcde}
Chila	20	111.67(2.88)^{cde}	1.83(1.83)^{cdef}	0.55(0.01)^{def}	12.95(0.72)^{def}
Bangweulu	30	92.67(6.24)^{ab}	1.52(0.10)^{ab}	0.66(0.04)^{ij}	13.14(0.25)^{ef}
Katobamputa	30	100.00(13.22)^{abc}	1.63(0.23)^{abc}	0.62(0.08)ghij	12.48(0.95)^{de}
Mweru	30	103.33(11.55)^{abcd}	1.80(0.19)^{cdef}	0.56(0.06)^{def}	18.19(0.91)^{h}
Kariba	30	95.00(8.66)^{ab}	1.54(0.13)^{ab}	0.65(0.06)^{hij}	12.00(0.43)^{bcde}
Kampolombo	30	105.00(0.00)^{bcd}	1.72(0.13)^{bcde}	0.58(0.00)^{efg}	12.81(0.46)^{def}
Chila	30	91.67(2.88)^{a}	1.49(0.01)^{a}	0.67(0.02)^{j}	11.91(0.52)^{bcde}
Control Wheat flour	0	148.17(10.61)^{h}	2.46(0.19)^{j}	0.41(0.03)^{a}	13.86(0.87)^{f}
Level of Significance					
Variety		0.001*	<0.001*	<0.001*	
Blend ratio		<0.001*	0.020*	<0.001*	
Interactions: Variety x Blend ratio		0.114	0.607	0.023*	

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. *significance differences

7.3.8 Bread crumb color characteristics

Table 7.7 color parameters of the crumb of the bread baked with six cassava varieties-wheat flour blends.

7.3.8.1 Crumb Lightness (L^*)

The bread crumb lightness values (L* values) for flour blends at 10%, 20, and 30% ranged from 72.62–75.42, 71.27–72.83, and 71.17–75.55, respectively, and did not significantly vary with CFSL ($p>0.05$). The L value (74.45±0.02) of the control was similar ($p>0.05$) to the L values of bread samples containing cassava flour at 20% CFSL for *Mweru*, 30% CFSL for

Kampolombo and 30% for *Chila*. These results indicate that cassava flour of different cassava varieties can be incorporated at different substitution levels to obtain bread with lightness similar to that of the control.

7.3.8.2 Crumb redness-greenness (a^*)

The bread crumb redness-greenness (a^*) ranged between -0.35 (green) and 0.67 (red), and varied significantly ($p<0.05$) across blend ratios. However, their values were too low to significantly reduce lightness. These traces of weak red and green could be attributed to carotenoid pigments in wheat flour (Zhai *et al.*, 2018) and residual pigment due to reddish peels of cassava.

7.3.8.3 Crumb yellowness (b^*)

The crumb yellowness (b^*) ranged between 19.62 and 20.89 across the blend ratios, and varied significantly ($p<0.05$). Crumb yellowness could be attributed, in part, to the non-enzymatic reaction between reducing sugar and proteins to develop a yellow-brown color. Flour yellowness was most probably as a result of the accumulation of carotenoids in the wheat grain flour used (Zhai *et al.*, 2018). A combination of lower a* and higher b* values increased the whiteness index.

7.3.8.4 Crumb whiteness index

The whiteness index ranged between 65.38 and 67.54 and did not vary with CFSL ($p>0.05$). The whiteness of the control was 66.60±0.01 and was similar ($p>0.05$) to the whiteness of *Bangweulu, Kariba, Katobamputa,* and *Chila* at 30% CFSL. The whiteness of the crumb significantly correlated positively ($r = 0.85$, $p<0.05$) with lightness, implying that increased lightness produced a high level of whiteness. Nevertheless, whiteness is affected by the yellowness of the crumb. The increased crumb yellowness reduced lightness resulting in decreased whiteness. Crumb color affects consumer preference of bread because it is perceived as a measure of quality. Crumb color is influenced by the color of flours and other ingredients (Shittu *et al.,* 2007), as well as non-enzymatic reactions, which can contribute to yellowness or brownness. The desired color of wheat flours for industrial applications is a high value for lightness (L^*) and low value for Chroma (Sankhon *et al.,* 2014; Vasconcelos *et al.,* 2017).

7.3.8.5 Crumb chroma

The Chroma was in the range 19.42-22.34 and varied ($p<0.05$) with CFSL. However, there was no clear trend in the variations across the flour blend ratios. Chroma is influenced by redness-greenness and yellowness. Chroma positively correlated with a* ($r = 0.74$, $p<0.05$) and b* ($r = 1.00$, $p<0.05$), suggesting that higher levels of crumb a* and b* increased the chroma.

Table 7.7 Color parameters of the crumb of the bread baked with six cassava varieties-wheat flour blends

Variety	Blend ratio	L*	a*	b*	Whiteness	Chroma
Bangweulu	10	73.57(0.13)^{fg}	0.00(0.05)^{c}	20.56(0.08)^{cd}	66.51(0.16)^{efg}	20.56(0.08)^{cd}
Katobamputa	10	72.62(0.26)^{cd}	0.13(0.06)^{ef}	21.20(0.14)^{fgh}	65.38(0.12)^{bc}	21.20(0.14)^{fgh}
Mweru	10	73.20(0.14)^{def}	0.58(0.04)^{l}	22.33(0.06)^{j}	65.11(0.08)^{ab}	22.34(0.05)^{j}
Kariba	10	73.49(0.33)^{fg}	0.04(0.03)^{cd}	20.75(0.26)^{cde}	66.33(0.42)^{ef}	20.75(0.25)^{cde}
Kampolombo	10	73.28(0.31)^{ef}	-0.35(0.09)^{a}	19.62(0.37)^{a}	66.85(0.47)^{ghi}	19.62(0.37)^{a}
Chila	10	75.42(0.11)^{j}	0.26(0.07)^{gh}	21.39(0.19)^{gh}	67.42(0.39)^{j}	21.40(0.19)^{gh}
Bangweulu	20	72.38(0.56)^{cd}	-0.31(0.07)^{ab}	20.47(0.10)^{c}	65.62(0.39)^{cd}	20.47(0.11)^{c}
Katobamputa	20	72.59(0.31)^{cd}	-0.25(0.09)^{b}	19.42(0.49)^{a}	66.40(0.04)^{bc}	19.42(0.49)^{a}
Mweru	20	74.52(0.52)^{hi}	0.65(0.03)^{jk}	22.49(0.13)^{j}	66.01(0.31)^{de}	22.50(0.12)^{j}
Kariba	20	71.27(1.23)^{a}	0.21(0.03)^{fg}	20.63(0.17)^{cd}	64.63(0.89)^{a}	20.63(0.17)^{cd}
Kampolombo	20	72.81(0.22)^{cde}	0.39(0.01)^{ijk}	20.99(0.14)^{ef}	65.65(0.25)^{cd}	20.99(0.13)^{ef}
Chila	20	72.83(0.29)^{cde}	0.10(0.02)^{de}	21.14(0.13)^{fg}	65.57(0.29)^{bcd}	21.14(0.13)^{fg}
Bangweulu	30	71.79(0.57)^{ab}	0.05(0.11)^{cde}	20.03(0.63)^{b}	65.40(0.39)^{fgh}	20.03(0.63)^{b}
Katobamputa	30	75.17(0.01)^{ij}	0.46(0.02)^{k}	20.89(0.04)^{def}	67.54(0.03)^{j}	20.90(0.04)^{def}
Mweru	30	75.55(0.52)^{j}	0.41(0.05)^{jk}	21.81(0.09)^{i}	67.23(0.40)^{ij}	21.81(0.09)^{i}
Kariba	30	73.57(0.01)^{fg}	0.31(0.05)^{hi}	20.53(0.06)^{c}	66.53(0.03)^{fg}	20.53(0.07)^{c}
Kampolombo	30	74.29(0.48)^{h}	0.62(0.05)^{lm}	20.60(0.07)^{cd}	67.05(0.36)^{hij}	20.61(0.07)^{cd}
Chila	30	73.93(0.71)^{gh}	0.67(0.11)^{m}	21.15(0.25)^{fg}	66.42(0.39)^{efg}	21.16(0.25)^{fgh}
Wheat	0	74.45(0.02)^{h}	0.36(0.02)^{ij}	21.50(0.03)^{hi}	66.60(0.01)^{fgh}	21.51(0.03)^{hi}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

7.3.9 Bread crust color characteristics

Table 7.8 Color parameters of the crust of the bread baked with six cassava-wheat flour blends.

7.3.9.1 Crust lightness (L*)

The bread crust lightness values (L*) at 10%, 20% and 30% CFSL ranged from 51.01–63.50, 51.17–61.71, and 54.40–63.80, respectively (Table 6). The bread crust color of the control

(59.46±0.20) was similar to the bread processed with the inclusion of cassava flour at 30%, CFSL for *Mweru* and at both 10% and 20% CFSL for *Kampolombo* ($p>0.05$). Crumb color was lighter than crust color. The acceptable range of lightness (L^* value) for bread crust is 54 to 62 (Fu *et al.*, 2018). It is promising to note that most of the bread samples of this study had L values within this range.

7.3.9.2 Crust redness-greenness (a*)

Crust redness values (a*) ranged from 12.00 to 16.88, and varied ($p<0.05$) with CFSL without a clear trend.

7.3.9.3 Crust yellowness (b*)

Crust yellowness (b*) values were in the average range of 35.16-38.84, and varied ($p<0.05$) across CFSL. The color shift from crumb to crust was characterized by increased a* and b* values. It is generally acceptable to have darker crust than crumb, therefore the relative color of the crust and crumb of the bread samples of the current study are acceptable.

7.3.9.4 Brownness index (BI)

The brownness index of the wheat bread (control) was 108.57±1.85, and the value was similar ($p>0.05$) with bread samples containing flours of the cassava varieties *Bangweulu* and *Katobamputa* at 20% CFSL, and *Chila* at 30% CFSL($p>0.05$). The reduced lightness and increased chroma resulted in an increased brownness index. The BI correlated negatively with WL ($r = -0.59$, $p<0.05$) and BV ($r = -0.35$, $p>0.01$) and positively ($r = 0.59$, $p<0.05$) with the weight of the bread (Table 7). In addition, the weight of the bread was strongly correlated negatively ($r = -1.00$, $p<0.05$) with weight loss. The high BI of the bread was typical of reduced BV and WL with increased bread weight. Browning can be ascribed to the products of the Maillard and caramelization reactions that occur during dry heating as in baking (Shen *et al.*, 2018). The BI showed weak negative correlation with gluten ($r = -0.20$, $p<0.001$) and protein content ($r = -0.24$, $p<0.001$). The flour blends with higher CFSL had lower protein content and higher BI. Increased starch contents in the flour blends may possibly lead to higher levels of reducing sugars available for the Maillard reaction that results in high BI of the bread (Buckman *et al.*, 2018).

7.3.9.5 Crust chroma

Crust chroma of the control was 39.44±0.35, and for crust chroma of the bread samples containing cassava flour was in the average range of 36.92-41.80, and varied across CFSL, without a clear pattern. Compared to the crumb, the crust color had higher a* and b* values.

Table 7.8 Color parameters of the crust of the bread baked with six cassava-wheat flour blends

Variety		L*	a*	b*	BI	Chroma
Bangweulu	10	52.59(0.40)^{b}	16.88(0.17)^{k}	37.66(0.06)^{fgh}	137.34(1.47)^{j}	41.27(0.01)^{ij}
Katobamputa	10	58.00(1.66)^{d}	14.45(0.65)^{def}	37.20(0.24)^{def}	114.51(6.59)^{e}	39.91(0.47)^{cde}
Mweru	10	51.01(0.77)^{a}	14.98(0.14)^{gh}	33.75(0.61)^{a}	122.19(0.38)^{f}	36.92(0.62)^{a}
Kariba	10	57.52(0.01)^{d}	14.57(0.04)^{efg}	37.00(0.09)^{de}	115.06(0.31)^{e}	39.77(0.08)^{cd}
Kampolombo	10	59.77(0.01)^{efg}	14.68(0.03)^{fg}	38.84(0.04)^{i}	115.98(0.13)^{e}	41.52(0.03)^{j}
Chila	10	63.50(0.01)^{j}	12.07(0.02)^{a}	37.87(0.14)^{gh}	100.15(0.44)^{b}	39.75(0.12)^{cd}
Bangweulu	20	61.71(0.52)^{h}	12.91(0.05)^{b}	37.96(0.04)^{h}	105.58(1.20)^{cd}	40.09(0.03)^{def}
Katobamputa	20	60.53(0.43)^{g}	13.95(0.36)^{cd}	37.34(0.60)^{def}	107.57(3.66)^{cd}	39.86(0.69)^{cd}
Mweru	20	51.17(0.10)^{a}	15.04(0.17)^{gh}	35.16(0.36)^{b}	128.26(1.81)^{cd}	38.24(0.40)^{b}
Kariba	20	53.44(0.04)^{b}	16.34(0.07)^{j}	37.80(0.60)^{gh}	134.07(0.44)^{ij}	41.18(0.07)^{hij}
Kampolombo	20	60.06(0.42)^{fg}	13.56(0.20)^{c}	37.49(0.14)^{efgh}	108.79(0.71)^{d}	39.87(0.09)^{cd}
Chila	20	53.04(1.08)^{b}	16.42(0.18)^{jk}	37.05(0.36)^{de}	132.19(2.85)^{hi}	40.52(0.28)^{efg}
Bangweulu	30	54.40(0.34)^{c}	15.38(0.60)^{hi}	37.66(0.47)^{fgh}	128.72(3.18)^{gh}	40.68(0.67)^{fghi}
Katobamputa	30	63.80(0.58)^{j}	12.00(0.47)^{a}	36.37(0.53)^{c}	94.59(3.40)^{a}	38.30(0.65)^{b}
Mweru	30	59.01(0.07)^{e}	14.12(0.08)^{de}	37.75(0.12)^{gh}	113.22(0.47)^{e}	40.30(0.15)^{defg}
Kariba	30	63.07(0.82)^{ij}	12.83(0.45)^{b}	38.60(0.37)^{i}	104.51(3.68)^{c}	40.68(0.49)^{fghi}
Kampolombo	30	55.29(0.92)^{c}	15.77(0.65)^{i}	37.73(0.29)^{fgh}	126.58(4.85)^{g}	40.89(0.47)^{ghij}
Chila	30	62.49(0.68)^{hi}	12.60(0.21)^{b}	38.54(0.42)^{i}	105.35(1.01)^{cd}	40.54(0.45)^{efgh}
Wheat	100	59.46(0.20)^{ef}	14.29(0.24)^{def}	36.76(0.28)^{cd}	108.57(1.848)^{cd}	39.44(0.35)^{c}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test.

7.3.10 Bread crumb pore area distribution

Figure 7.2 shows bread crumb pore characteristics as determined by image analysis. Table 7.9 shows correlation coefficients of color parameters of crumb and crust of bread baked from flour blends of wheat and cassava flour.

7.3.10.1 Crumb pore area

The pore area of the control was 0.47 mm^2, whilst the average crumb pore area of experimental bread increased with cassava flour substitution level (CFSL) from 0.39-0.76

mm^2, 0.38-0.69 mm^2, and 0.27-0.85 mm^2 at10%, 20%, and 30%, respectively, across the varieties (Figure 7.2).

7.3.10.2 Crumb porosity

The porosity of the control 71.48±0.52% (Figure 7.3) differed significantly ($p<0.05$) from the porosities of experimental bread, which decreased with increasing CFSL. Crumb porosity was negatively correlated ($r = -0.57$, $p<0.05$) with cassava flour particle size. This indicates that flours with smaller particles produced bread of higher porosity. Crumb porosity correlated positively with SV ($r = 0.66$, $p<0.05$), gluten ($r = 0.64$, $p<0.05$), and negatively with BD ($r = -0.58$, $p<0.05$). The bread of large volume was associated with higher gluten content, which promotes appreciable pore formation and better gas retention during proofing. Figure 7.4 shows photos of typical crust and crumb structures. Espinosa-Ramírez *et al.* (2018) reported that large bread volume is related to better retention of carbon dioxide during proofing. Increasing CFSL led to increased bread density and a decrease in crumb porosity. Analysis of the crumb structure of the experimental bread, which had low porosity, by scanning electron microscopy (SEM) showed that the microstructures of the bread crumbs were characterized by a continuous dense mass (Figure 7.5). The increased levels of cassava flour seem to have weakened the gluten network by disrupting the intermolecular disulfide bonds in the glutenin and gliadins molecules, thereby limiting protein-protein interaction. The fat and protein contents at higher cassava flour substitution possibly increased hydrophobicity (Muoki *et al.*, 2015; Uthumporn *et al.*, 2017), thereby limiting available water for gluten development. Hydration is primarily responsible for the development of the gluten network (Chen *et al.*, 2018), hence any other flour component with strong water absorption capacity is likely to limit gluten development by reducing the hydration of the gluten proteins and consequently affect bread quality negatively.

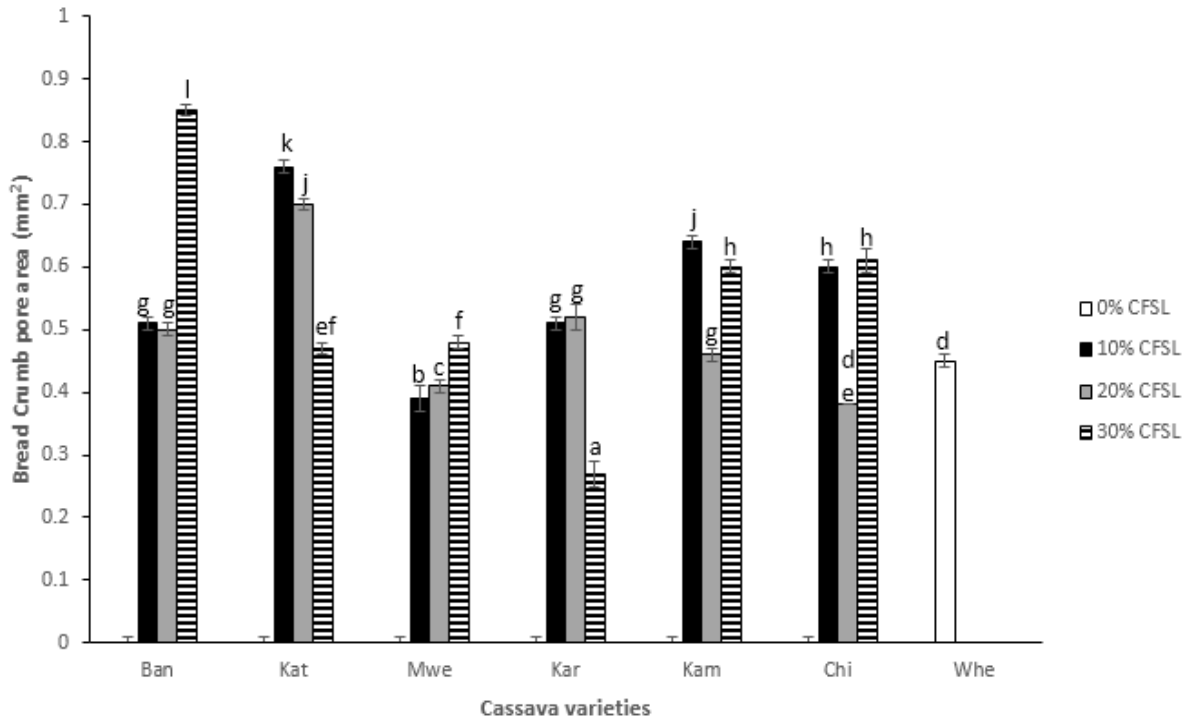

Figure 7.2 Bread Crumb pore cross surface area (mm^2) per cassava flour substitution level (CFSL). Ban=Bangweulu, Kat=Katobamputa, Mwe=Mweru, Kar=Kariba, Kam=Kampolombo, Chi=Chila, Whe=Wheat (Control). Values are presented as mean ± standard deviation (n = 3). Error bars represent standard deviations based on 3 replicates. Bars marked with different letters within the same CFSL indicated significant difference ($p < 0.05$) among cassava varieties.

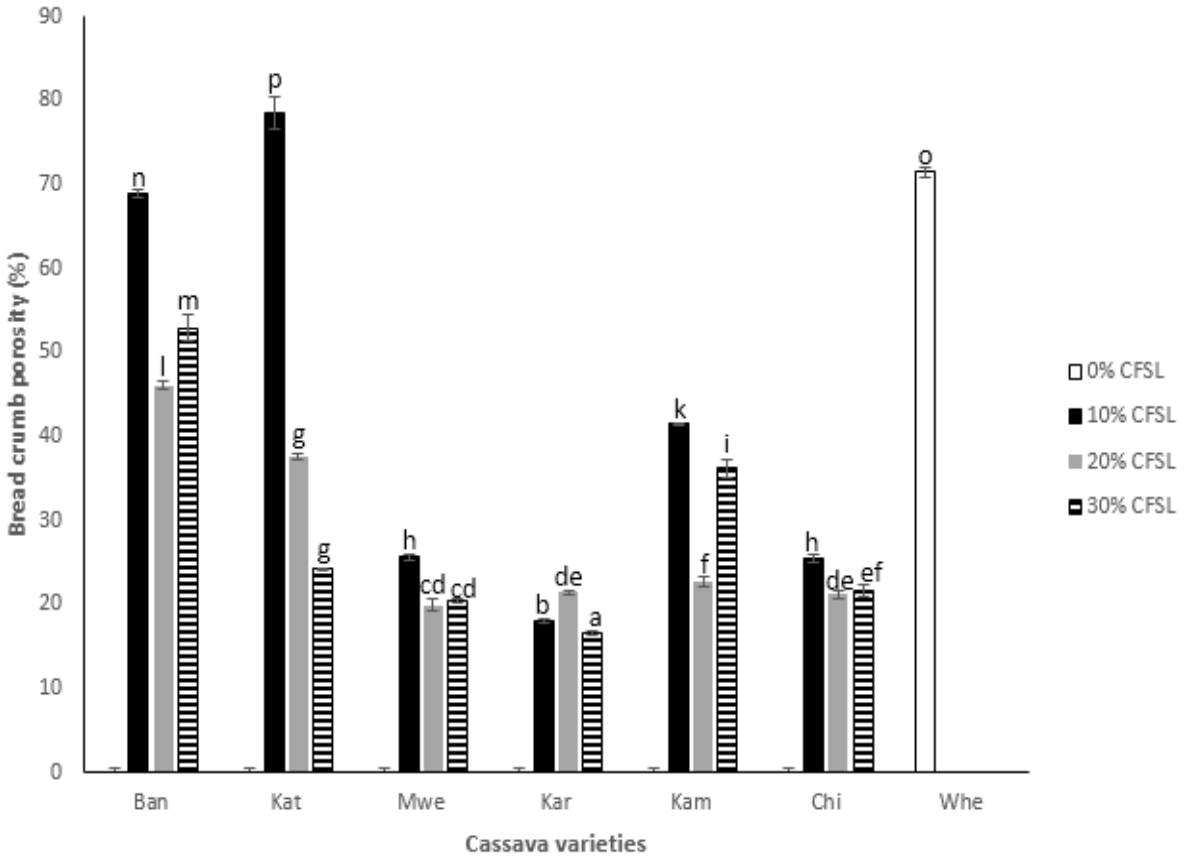

Figure 7.3 Bread crumb porosity (%) per cassava flour substitution level (CFSL). Ban=Bangweulu, Kat=Katobamputa, Mwe=Mweru, Kar=Kariba, Kam=Kampolombo, Chi=Chila, Whe=Wheat (Control). Values are presented as mean ± standard deviation (n = 3). Error bars represent standard deviations based on 3 replicates. Bars marked with different letters within the same CFSL indicated significant difference ($p < 0.05$) among cassava varieties.

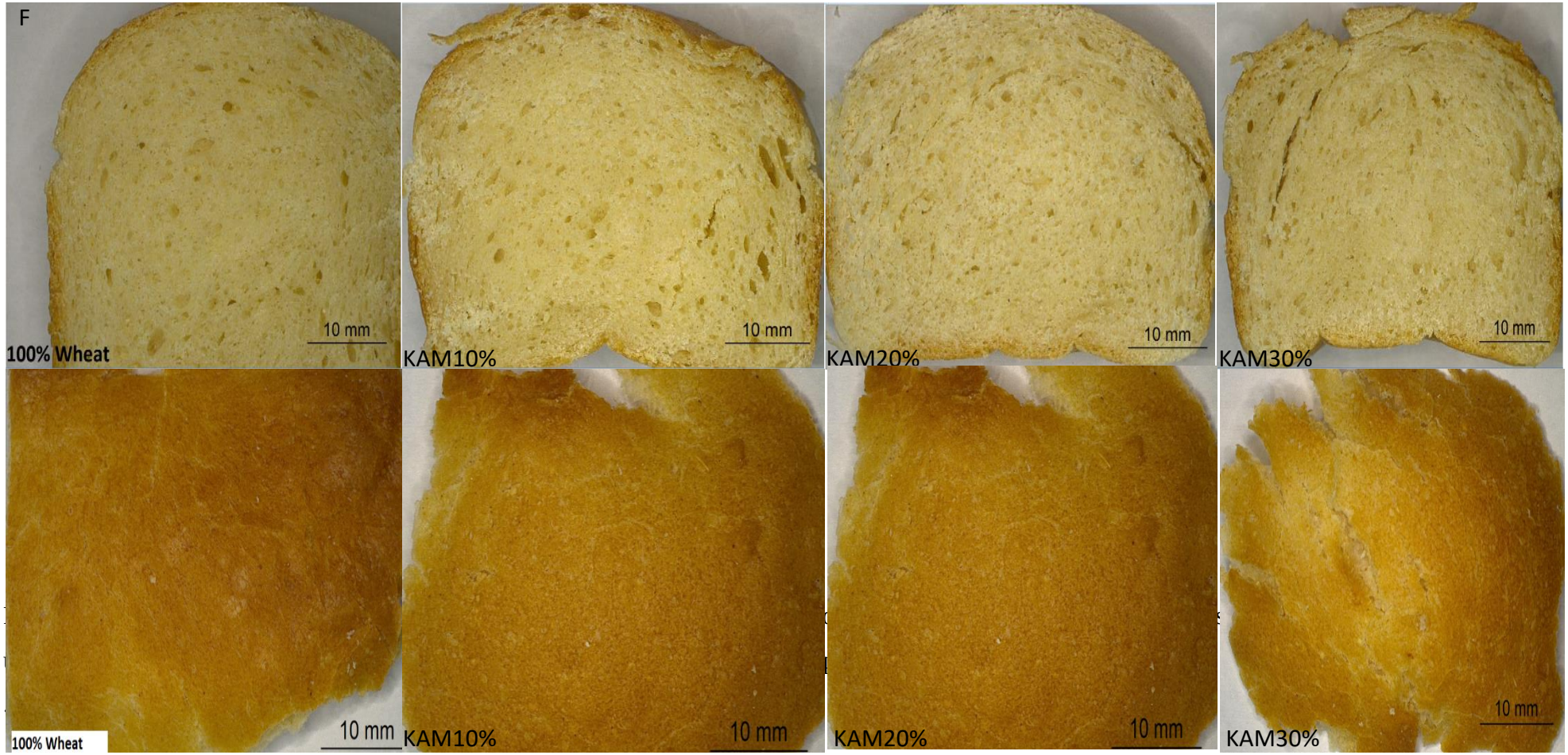

Figure 7.4 Bread crumb and crust from flour blend of cassava/wheat in Kampolombo at 10, 20 and 30% CFSL. Photos at scale 10 mm obtained using microscope (Leica MZ16) with Leica camera (Leica DFC450C), Leica Application Suite (LAS).

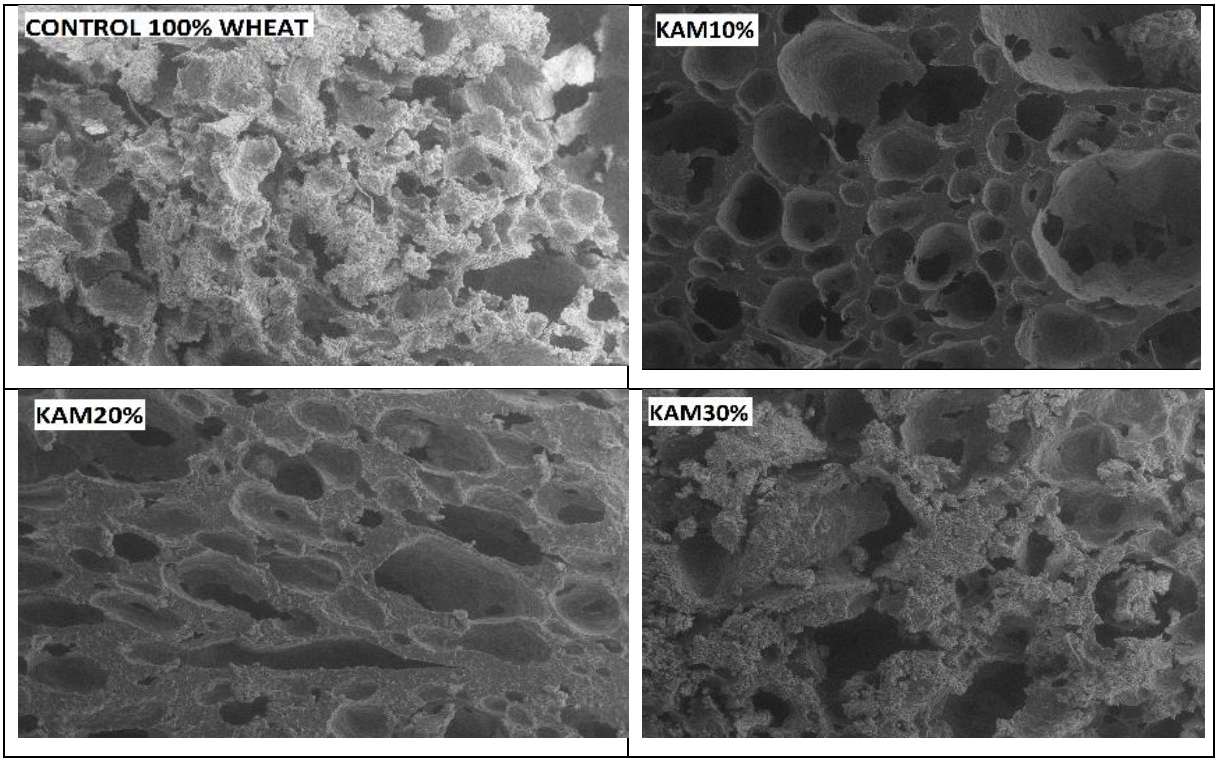

Figure 7.5 Scanning microscopic images of bread made from cassava-wheat flour blends at 10%, 20% and 30% CFSL. Variety KAM: Kampolombo Control: 100% Wheat flour. Image properties: Signal A = VPSE G3, EHT = 20.00 kV, Chamber = 2.07e-001 Torr, I Probe = 253 pA, Spot Size = 442, Mag = 100X, Scale: 200µm

Table 7.9 Correlation coefficients of color parameters of crumb and crust of bread baked from flour blends of wheat and cassava flour

	Crumb						Crust				
Parameter	CFSL	L	a	b	Whiteness	Chroma	CFSL	L	a	b	BI
CFSL	1						1				
L	-0.20	1					0.01	1			
a	0.08	0.51**	1				-0.12	-0.87	1		
b	-0.32*	0.52**	0.74	1			0.32	0.46	-0.12	1	
Whiteness	-0.05	0.85**	0.15	-0.01	1		0.32	0.46	-0.12	1.00	1
Chroma	-0.31	0.52	0.74**	1.00	0.00	1					
Bread volume	-0.87	0.23	0.04	0.35	0.06	0.35	-0.87	0.05	0.06	-0.35	-0.35
Weight of Bread	0.04	-0.31*	-0.48	-0.67	0.06	-0.67**	0.04	0.37	-0.15	0.59**	0.59**
Bread density	0.85	-0.24	-0.07	-0.42	-0.03	-0.42*	0.85	0.05	-0.12	0.43*	0.43*
Specific volume	-0.86	0.27	0.11	0.43	0.05	0.43*	-0.86	0.00	0.08	-0.42*	-0.42*
Weight loss	-0.04	0.31	0.48	0.67	-0.06	0.67**	-0.04	-0.37	0.15	-0.59**	-0.59**
Aver Pore Area	0.19	-0.33	-0.37	-0.50	-0.09	-0.50**	0.19	0.02	0.06	0.33	0.33

Significance differences at p<0.01*, p<0.05**

7.4 Multivariate analysis

Principal component analysis (PCA) was used to provide an in-depth analysis of the differences among the cassava varieties. The scree plot (Fig. 5A) showed that the cassava flour properties had low percentage variations resulting in no significant differences among the cassava varieties ($p<0.05$). This implies that the cassava flour properties did not exhibit distinct separation among the cassava varieties. The scree plot (Fig. 5B) showed that partial replacement of wheat with cassava flour yielded high percentage variations resulting in distinct separation among cassava varieties. This suggests that wheat flour properties were significant contributors to differences among cassava varieties across the blend ratios. The dough mixing properties (Fig. 5C) showed wheat clustered separately from cassava varieties. It is worth noting that based on experimental design and the plot, all cassava varieties at zero (0%) CFSL overlapped with the actual coordinates of 100% wheat flour resulting in over clouding (hence black shade). Based on the coordinates of the plot *Mweru* formed distinct separation from other varieties and closely associated with *Katobamputa* and *Kariba*. These varieties (*Mweru, Katobamputa* and *Kariba*) were near the coordinates of wheat and did not cluster together with other varieties. The variety *Kampolombo* was disparate but strongly overlapped with *Bangweulu* and *Chila*. For the bread quality characteristics (Fig. 5D), bread baked from composite flour of *Mweru* could be distinguished from other varieties and was close to the coordinates of wheat bread without interference from other varieties. Similarly, *Katobamputa* and *Kariba* clustered separately on the same coordinates as those for wheat bread, however with little overlaps with the other varieties. The PCA plot (Fig. 5E) described the effect of flour properties on dough mixing characteristics. According to Mtunguja *et al.* (2016), based on coordinates of the plot, values close to the origin have a smaller impact on the plot pattern, while those further away are significant contributors. The plot showed that the impact of flour properties on dough characteristics varied with CFSL. The control (wheat) dough was distinguished from cassava varieties. The flour proximate properties (protein, fiber, and lipids) closely associated (clustered together) on the PCA plot and strongly correlated with D90-axis but in the opposite direction. This indicates that proximate contents had a similar effect on dough mixing properties (Equations 7.8 and 7.9), and are impacted negatively by D90. Gluten content, flour particle size, and water absorption capacity were distinctly separated on the plot, suggesting that these properties impacted dough mixing differently. The DDT is closely associated with WAC but in the opposite direction, indicative

of the negative effect of water absorption capacity on dough development time. The Mixing dough properties of cassava varieties *Mweru*, *Katobamputa*, *Kampolombo* and *Kariba* (clustered in the bottom left of the plot) were strongly influenced by WAC and gluten content. However, all cassava varieties clustered strongly along D90-axis, indicative of variations due to flour particle size. The PCA plot (Fig. 5F) explained the effect of flour properties on bread quality characteristics. Flour particle size, water absorption capacity and gluten contents showed different correlation coefficients (Equations 7.10 and 7.11) and were distinct on the plot suggesting that they were significant source of variations on the bread quality. The proximate contents (protein, fiber and lipid) were clustered together in the plot suggesting similar correlations and contributions to the bread quality characteristics. The coordinates of bread volume and bread specific volume were close together, indicative of similar response to flour properties. The bread made from *Mweru* and *Katobamputa* clustered separately along the axis of WAC and gluten, respectively, indicative of strong influence from water absorption and gluten content. The bread from all cassava composite varieties showed strong presence along and towards D90-axis. This suggests that flour particle size distribution were the source of variations among the cassava varieties.

$$PC1 = 0.09X_1 - 0.37X_2 + 0.40X_3 + 0.33X_4 + 0.40X_5 + 0.40X_6 + 0.22X_7 + 0.30X_8 - 0.20X_9 + 0.27X_{10} - 0.12X_{11} \quad (7.8)$$

$$PC2 = 0.10X_1 - 0.07X_2 + 0.08X_3 - 0.21X_4 + 0.09X_5 + 0.09X_6 - 0.43X_7 - 0.30X_8 + 0.38X_9 + 0.47X_{10} - 0.52X_{11} \quad (7.9)$$

Where X_1=Amylose, X_2=D90, X_3=Fiber, X_4=Gluten, X_5=Lipids, X_6=Protein, X_7=Water absorption capacity, X_8=Dough consistency, X_9=Dough development time, X_{10}=Dough stability time, X_{11}=Mixing tolerance index

$$PC1 = 0.06X_1 - 0.32X_2 + 0.34X_3 + 0.31X_4 + 0.35X_5 + 0.35X_6 + 0.23X_7 + 0.35X_8 - 0.34X_9 + 0.35X_{10} + 0.11X_{11} \quad (7.10)$$

$$PC2 = 0.19X_1 - 0.32X_2 + 0.13X_3 - 0.27X_4 + 0.17X_5 + 0.16X_6 - 0.53X_7 - 0.16X_8 + 0.12X_9 - 0.09X_{10} - 0.60X_{11} \quad (7.11)$$

Where X_1=Amylose, X_2=D90, X_3=Fiber, X_4=Gluten, X_5=Lipids, X_6=Protein, X_7=Water absorption capacity, X_8=Bread volume, X_9=Bread density, X_{10}=Bread specific volume, X_{11}=Weight loss

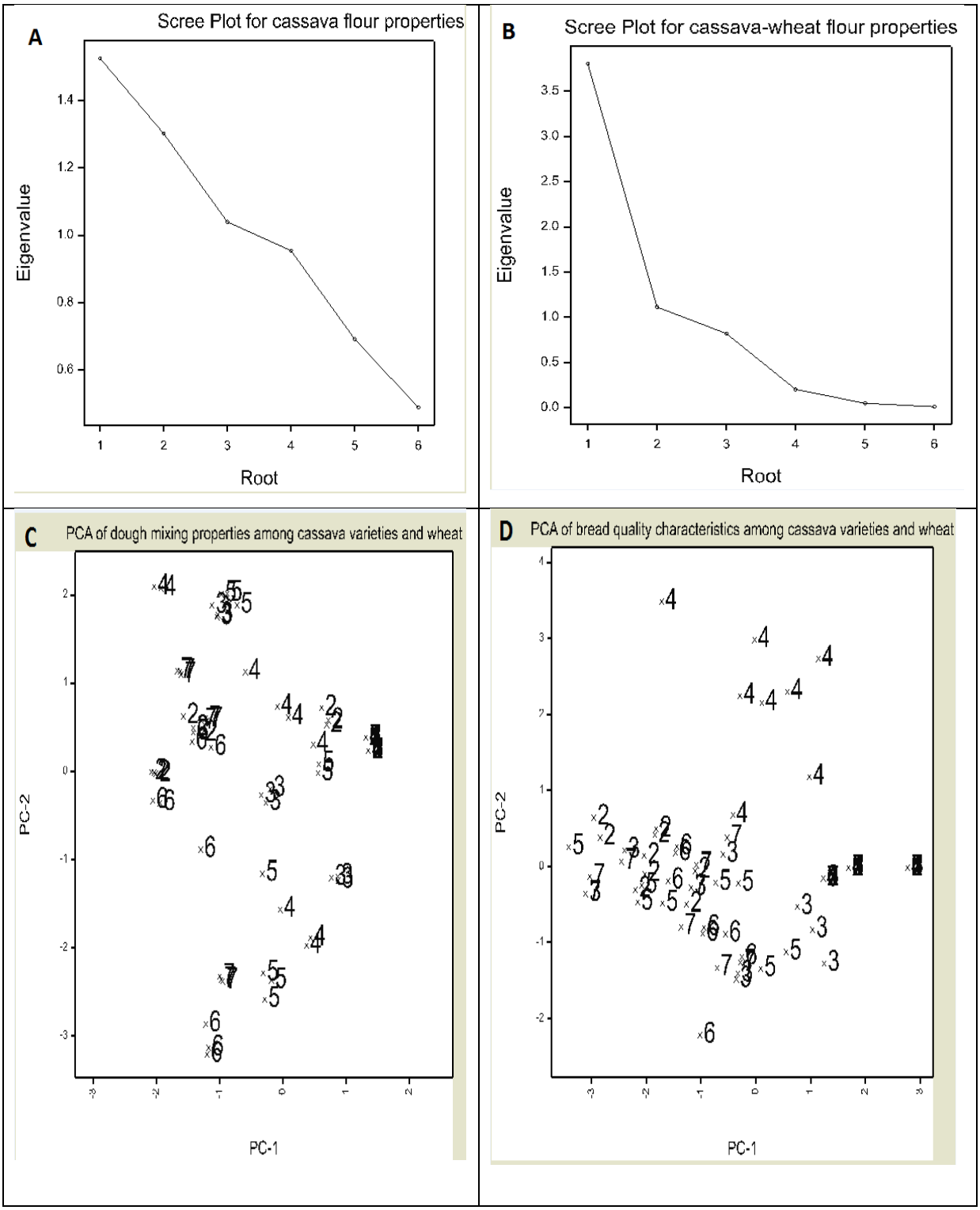

Figure 7.6 Principal component analysis (PCA). (A) Scree plot for cassava flour properties, Latent roots PC 1 = 1.52, PC2 = 1.30. Percentage variation PC1 = 25.41, PC2 = 21.69. (B) Scree plot for cassava flour with wheat flour, Latent roots PC1 = 3.709, PC2 = 1.121. Percentage variation PC1 = 61.82, PC2 = 18.89. (C) PCA of dough mixing properties and (D) PCA of bread quality characteristic among cassava varieties and wheat. Varieties: 1=Control (Wheat shaded black), 2=Bangweulu, 3=Katobamputa, 4=Mweru, 5=Kariba, 6=Kampolombo and 7=Chila.

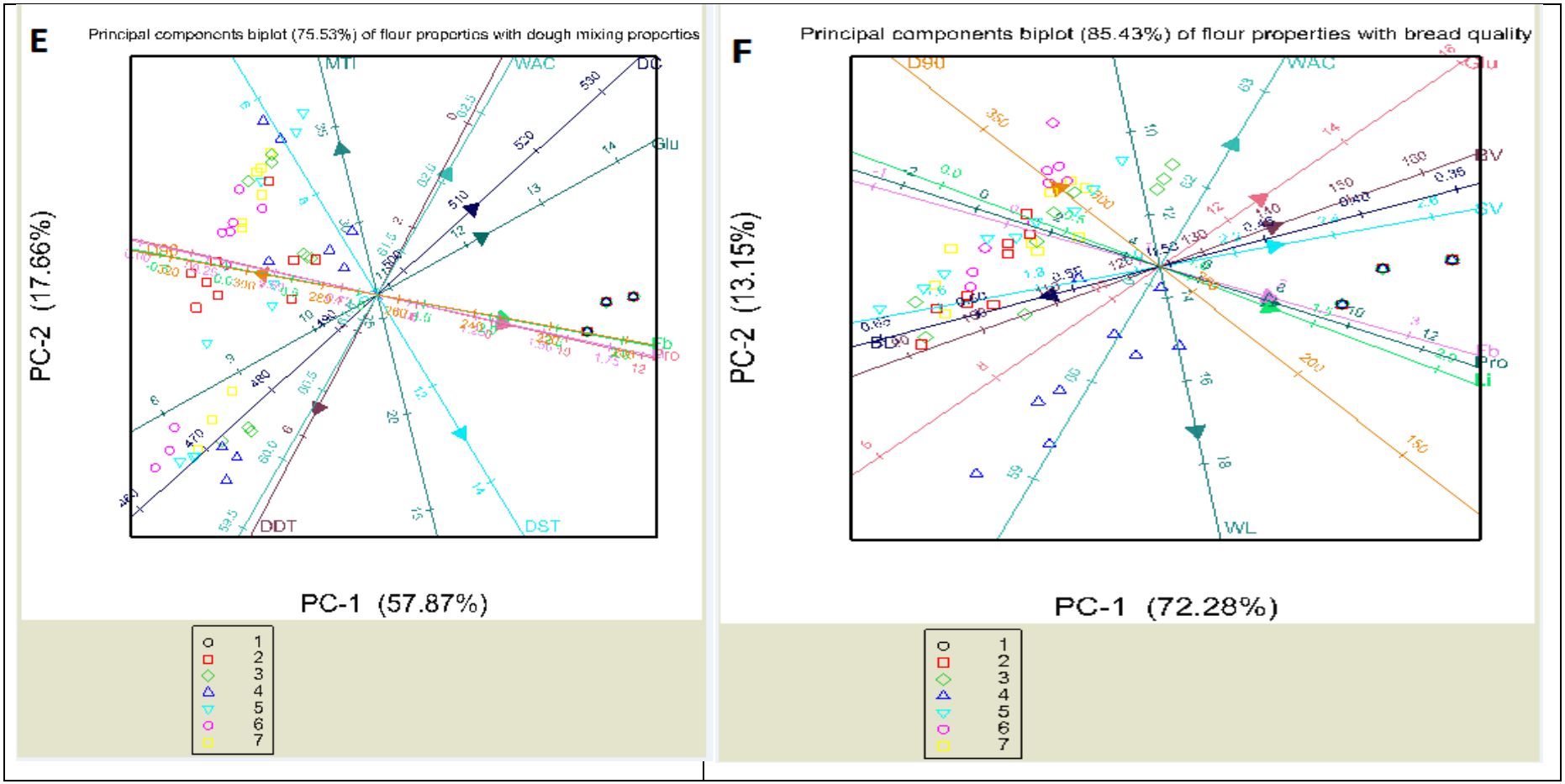

Figure 7.7 Principal components biplot of flour properties with dough mixing properties (E). Latent roots: PC1 = 5.82, PC2 = 1.77. Percentage variations: PC1 = 52.93, PC2 = 16.13 (F) Principal components of biplot of flour properties with bread quality characteristics. Latent roots: PC1 = 7.25, PC2 = 1.32. Percentage variations: PC1 = 65.95, PC2 = 12.06. Flour properties WAC=water absorption capacity, D90=flour particle size, Pro=protein, Li=Lipid, Fb=Fiber, Glu=Gluten. Dough properties: DDT=dough development time, DC=dough consistency, DST=dough stability time, MTI=mixing tolerance index. Bread characteristics: BV=bread volume, SV=bread specific volume, BD=bread density, and WL=weight loss. Varieties: 1=Wheat, 2=*Bangweulu*, 3=*Katobamputa*, 4=*Mweru*, 5=*Kariba*, 6=*Kampolombo* and 7=*Chila*.

7.5 Conclusion

This study showed that cassava variety (CV) and cassava flour substitution level (CFSL) had varying effects on dough rheology and ultimately bread quality. Although gluten content was the major determinant of dough rheology and bread quality attributes, the different responses among the cassava varieties could be attributed to variation in cassava flour particle size distribution, a parameter that significantly affected the water absorption capacity and specific bread volume. Irrespective of CV, low cassava inclusion ratio (10%) did not significantly limit flour hydration. Generally, all the composite flour samples had satisfactory mixing tolerance, which indicates good dough mixing quality. It is noteworthy to mention that cassava inclusion reduced weight loss. Bread specific volume was high (2.13, 2.08, and 2.27 cm^3/g), specifically at 10% CFSL of flour for the cassava varieties of *Mweru*, *Kariba,* and *Katobamputa,* respectively. The specific bread volume for the bread sample containing 10% *Katobamputa* cassava flour was not statistically different from the wheat bread (control) (2.46 cm^3/g). The results show that bread with acceptable quality can be processed from these varieties at about 10% cassava flour substitution level. Further research works could target the effect of the particle size distribution (fractionated flour) on rheology and bread quality characteristics to ascertain effective flour size for substitution into wheat flour.

The bread specific volume is a response function of flour particle size and water absorption capacity. Particle size can be a significant differentiating genetic trait among cassava varieties. In the current study, cassava genotypes were cultivated simultaneously in a single plantation and harvested at the same time. In addition, the milling conditions were the same across varieties. The variations observed in flour particle size were attributed to genetic differences among the cassava varieties. The particle size distribution influences the hydration properties of flours, which in turn, is responsible for gluten development in a dough system and ultimately bread quality. Optimizing the flour particle size between the blends of wheat and cassava flours in response to water absorption capacity can be the basis for formulating composite flours of improved properties for dough performance and processing of bread quality.

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8. EFFECTS OF CASSAVA FLOUR ON THE RHEOLOGICAL PROPERTIES OF WHEAT BREAD DOUGH: STICKINESS IN UNLEAVENED, LEAVENED AND FROZEN DOUGH

This chapter is based on the following publication

Chisenga, SM, Workneh, TS, Bultosa, G. and Alimi, BA. 2019. Effects of cassava flour on the stickiness properties of wheat bread dough: unleavened, leavened and frozen dough. *Acta agriculturae Slovenica*, 114(1): 33-46. DOI: http://dx.doi.org/10.14720/aas.2019.114.1.4

Abstract

Cassava utilization in the form of cassava-wheat bread is on the increase in Africa. However, information on stickiness properties of dough handling under normal and frozen conditions is limited. In view of this the gluten contents and water absorption of doughs, and stickiness of unleavened, leavened and leavened-frozen doughs processed from 0 to 30% cassava flour substitution level (CFSL) as compared to wheat flour were determined. The gluten contents of flour blends (6.88–13.00%) decreased significantly ($p<0.05$) with increasing CFSL. Water absorption capacity (WAC) was ranged from 59.57-61.70% and showed positive correlation with gluten contents ($r=0.595$, $p<0.05$). Cassava variety (CV) and CFSL had significant $p<0.05$) influence on stickiness of unleavened (34.14-122.17 g), leavened (13.53-83.94 g) and leavened frozen (126.88-146.82 g) dough. Irrespective of CV and CFSL, leavened frozen dough had highest stickiness. Gluten content and WAC had significant ($p<0.01$) negative influence on stickiness in unleavened ($r=-0.445$ and -0.437, respectively) and leavened ($r=-0.457$ and -0.434, respectively) dough. The variation in stickiness was influenced by gluten contents and CFSL. The unfrozen doughs and frozen dough exhibited higher stickiness in lower and higher gluten content flour blends, respectively.

Key words: cassava, composite flours, gluten, stickiness, wheat

8.1 Introduction

Stickiness, a surface related property, is a tendency of dough to adhere to contact surface of equipment and hands during mixing and kneading. This tendency affects dough handling (Villanueva et al., 2018). Moreover, sticky dough is considered a problem to high speed mixing and can cause disruption to production schedule, and subsequent loss of quality. Stickiness is associated with physical factors such as adhesive force, combined effects of adhesive and cohesive forces (Hoseney and Smewing, 1999; Král *et al.*, 2018) and viscoelasticity.

Stickiness properties of dough are influenced by several factors. The most important is the extent of mixing and water quantity (Ahmed and Thomas, 2018). However, studies have shown that excessive water plays the most significant role in dough stickiness. Water acts as a plasticizer in a dough system due to its influence on molecular mobility (Liu *et al.*, 2018a). Some properties of dough such as surface tension and solvation are dependent on the plasticizing effect of water (Fonseca-Florido *et al.*, 2018). Constituents of food systems such as proteins, in particular, glutenin and gliadins (Stone *et al.*, 2018), alpha-amylase activity (Zadeike *et al.*, 2018), and proteolytic enzyme activity (Zadeike *et al.*, 2018) are also reported to affect the stickiness of the dough. Therefore, information on the water absorption capability and intrinsic composition of the base material are necessary to estimate the stickiness of resulting dough.

The compressive force is applied during the mixing of ingredients to form dough and kneading of resulting dough. Force of adhesion between the contact surface and dough may result in stickiness depending on the strength and cohesion forces of the dough. Stickiness has been reported to be dependent on the rheological properties of the dough. Grausgruber *et al.* (2003) proposed that if the dough is strong and elastic, the adhesive force is overcome, and the dough will separate from the surface (i.e., the dough is not sticky). On the other hand, if the dough is viscous, it will flow and not overcome the adhesive force (i.e. the dough is sticky). Therefore, understanding the stickiness properties of dough from a formulation is important for its handling and machination. In this regard, scientific report on the stickiness properties of composite wheat-cassava flour dough is virtually absent.

Rheological tests on doughs are used as quality indicators of the gluten and starch polymers molecular structure in ascertaining the dough's functional behavior. The viscoelastic network of the dough is dependent on gluten development properties during mixing of wheat flour and can influence the handling characteristics of dough during processing. The inclusion of cassava flour

into wheat flour in bread making is an important issue in the sustainable utilization of cassava. Increasing acceptance of bread form composite cassava-wheat flour would stir interest in the storage of the composite flour dough through freezing. However, frozen storage of the dough could have an additional effect on its subsequent handling and machination during bakery process. Cassava flour consists mainly of starches and some minor amounts of fiber. In a dough system, starches impart high water binding capacity (Kaushik *et al.*, 2015) but favors more starch-starch interaction than wheat gluten protein-protein. Thus, incorporation of cassava flours in frozen wheat dough system may lead to reduced gluten network deteriorations and ameliorate the rate of ice crystal formation during freezing which is detrimental and contribute to gluten network disruptions. However, there is limited information on dough stickiness characteristics in the cassava-wheat frozen dough system. Also, baking ingredients such as yeast, sugar, salt, and fat can influence the stickiness of the dough. Differences in chemical constituents and flour particle size (Sakhare *et al.*, 2014) can affect the stickiness of the dough. Ascertainable stickiness based purely on raw material and water, and subsequently on developed dough is a reflection of industrial quality acceptance criteria based on the raw material. In the present article, stickiness measurements were conducted on three doughs: (1) dough made from a mixture of flour and water, (2) developed dough with ingredients, and (3) frozen developed dough. The hypothesis: (1) stickiness of the wheat dough decreases with increasing percentage of cassava flour concentration and (2) there is a variation in the stickiness of the three different doughs.

8.2 Materials and Methods

8.2.1 Source of materials

The wheat flour for white bread was procured from the local market in Pietermaritzburg, South Africa. Cassava flours from six varieties used in this study were prepared as described in Chapter 3, method 3.2.3.

8.2.2 Blending of wheat-cassava flour

Three levels of wheat: cassava (90:10, 80:20, 70:30) were prepared as described in method 7.2.3 of Chapter 7.

8.2.3 Dough preparation

The unleavened dough was prepared as described in Grausgruber et al. (2003) with modification. A 20 g flour was mixed with distilled water at the rate of water absorption rates from Farinogram. The mixture was kneaded until the dough was formed. The leavened dough was prepared as described in method 7.2.7 of Chapter 7. The dough was divided into 20 g portion, of which three portions were wrapped in polyethylene plastic bags and stored at a frozen temperature of -18 ^{o}C for three weeks to produce frozen dough.

8.2.4 Stickiness of doughs

Dough stickiness was determined according to the procedure of Sangnark and Noomhorm (2004) using a Texture Analyzer (TA-XT2, Stable Micro Systems Ltd., England). The dough was molded and divided into about 5 g portions for each test. The dough portion was placed into the chamber of Stable Micro system/Chen–Hoseney Dough Stickiness Cell and then closed with a die by screwing. After which, the dough was extruded through the holes on the die by rotating the internal screw. The first extrusion was discarded from the die surface using a spatula. The screw was then rotated once again until a 1mm height of dough sample was extruded through the die. The stickiness of the dough was determined using an adhesive test at test speed 0.5 mm s^{-1}, and post-test speed 10 min s^{-1} with a 25mm perspex cylinder probe at the applied force of 80 g (0.785 N) with trigger type: button. The maximum force reading from the highest positive peak is an indicator of the stickiness of the dough.

8.2.5 Experimental design and data analysis

A completely randomized design comprising of two factors cassava variety and blend ratio (cassava concentration) was used. Triplicate data were analyzed using two-way ANOVA of GenStat 18th Edition software and mean differences were determined using Fisher's Least Significance Difference (LSD) test at 5% significant level. The correlation coefficients of flours particle size distribution (D90) (Table 7.2, Chapter 7), dough mixing properties (Table 7.3, Chapter 7) and stickiness characteristics were analyzed using Pearson's' correlation.

8.3 Results and Discussion

8.3.1 Stickiness description

Figure 8.1 shows the typical stickiness curve drawn from the TA.XT2i texture analyzer. Adhesiveness is the work necessary to overcome the attractive force between the surface of the food and the surface of other materials with which the food comes in contact. The work of adhesion is generated during compression. The contact point where the curves are observed to shift away from the x-axis occurs when the surface of the probe contacts the surface of the dough. The *interfacial tension* and work of adhesion between two materials such as two polymers or a polymer and a solid surface are of great importance to the production of dough and application of flours. Energy due to the surface tension on the dough is released when probe solid surface comes in contact with the surface of the dough. The energy released will equal the work of adhesion, and the work of adhesion is negative because the force due to the dough is acting in the opposite direction of the displacement. The bonding between adhesive (dough) and adhered (probe surface) is essential for stickiness, however, the mechanism of failure of this bond is equally important (Figure 8.2). The clear failure of adhesive and the adhered surface is termed adhesive failure while the failure within the adhesive with residue on the adhered surface is known as a cohesive failure (Kilcast and Roberts, 1998; Adhikari *et al.*, 2001). The strength of the dough is influenced by covalent or ionic bonding developing (Dobraszczyk, 1997) upon hydration and kneading.

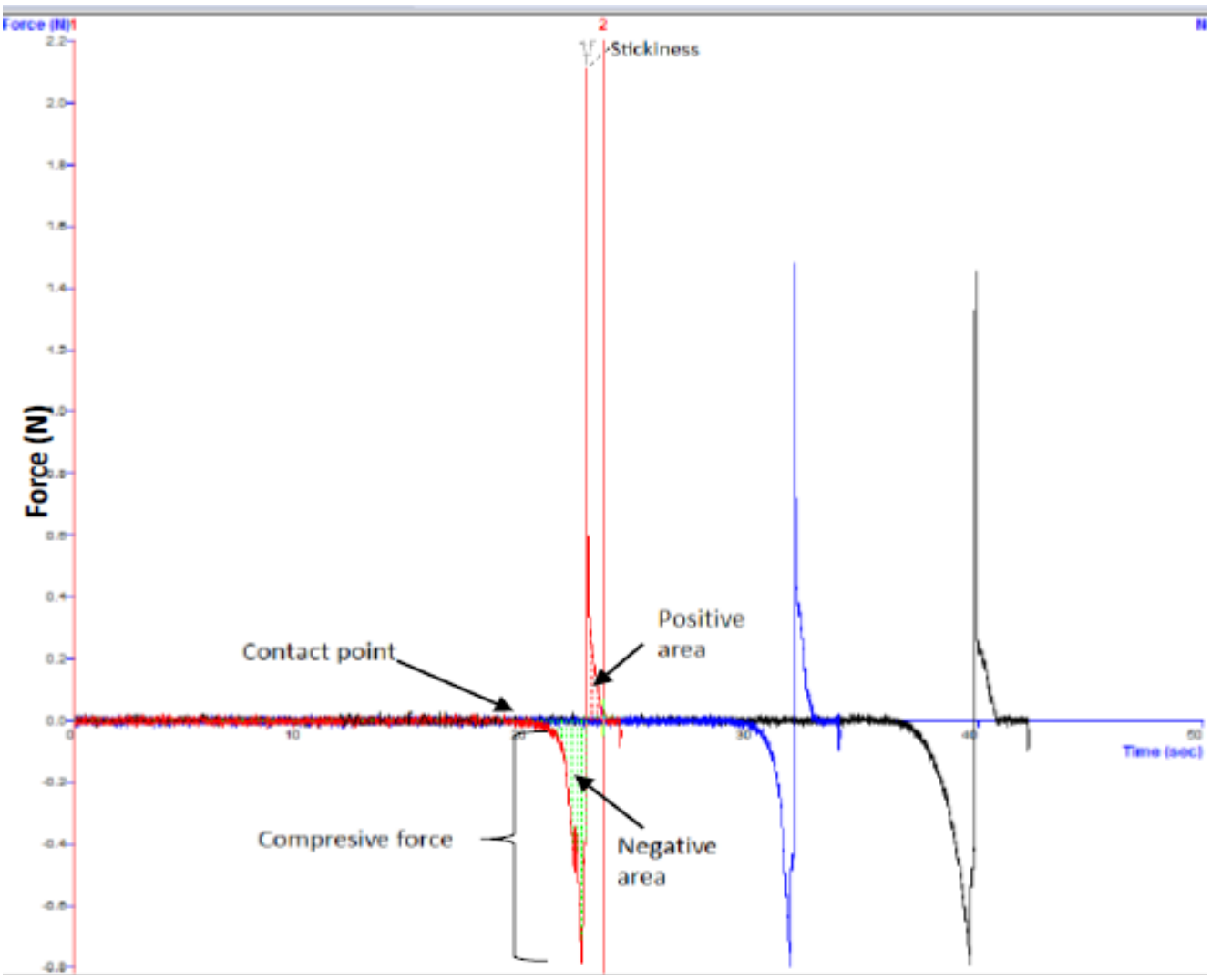

Figure 8.1 Typical curves of force and stickiness test of frozen wheat dough (n = 3) using TA.XT2i texture analyzer

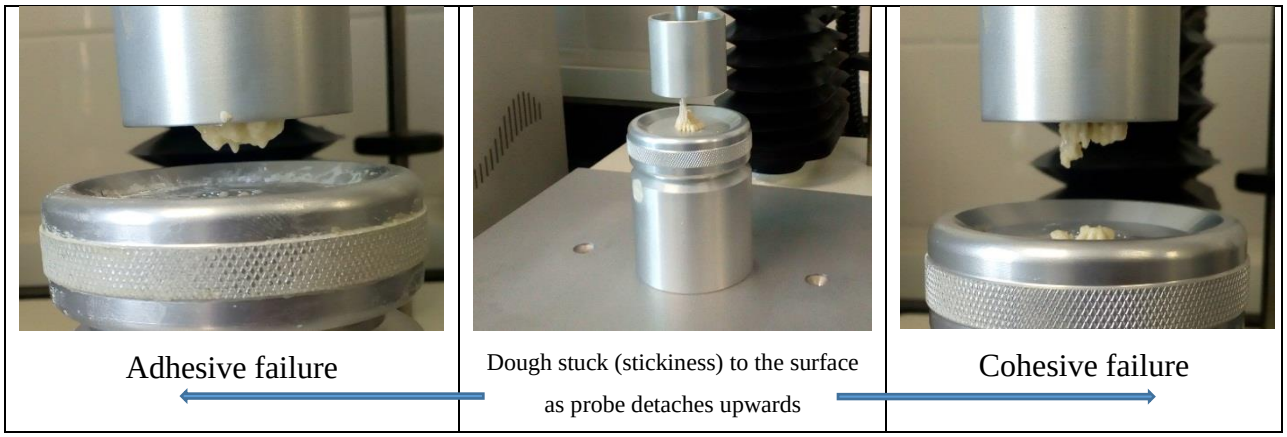

Figure 8.2 Mechanism of failure between the dough (adhesive) and probe surface (adhered)

8.3.2 Stickiness of unleavened dough

8.3.2.1 Peak positive force

The peak positive force of unleavened dough were in the range 0.44–0.77 N, 0.61–1.26 N, and 0.57–1.20 N, at 10, 20 and 30% cassava flour level, respectively (Table 8.1), and increased significantly ($p<0.05$) with increase in CFSL ($r = 0.678$, $p<0.05$). The peak positive force is the maximum force required to pull a compression surface (probe) from a sample after the compression. Stickiness above the 1N value was characteristic of dough handling difficulties.

8.3.2.2 Work of adhesion

The work of adhesion of the unleavened dough was from -35.40 to -90.70 g.s, -68.40 to -90.50 g.s, and -33.10 to -90.30 g.s, at 10, 20 and 30% CFSL, respectively. The control (wheat) sample (-72.50±10.31 g.s) significantly ($p<0.05$) increased at high cassava flour levels. The work of adhesion correlated positively with force ($r = 0.515$, $p<0.05$) and weak positive with gluten ($r = 0.137$, $p<0.0001$). The adhesion is influenced by cohesion forces which are governed by chemical bonds due to crosslinking of the polymers, glutenin, and gliadins within the dough resulting in cohesive and viscoelastic gluten (Guo et al., 2018).

8.3.2.3 Peak positive area

The positive area for wheat sample in unleavened dough was 0.03±0.01 N.s and increased with increase in CFSL ($r = 0.321$, $p<0.05$). Positive area exhibited strong positive correlation with

both stickiness and peak positive force ($r = 0.779$, $p<0.05$) and work of adhesion ($r = 0.710$, $p<0.05$). This means that area of displacement is larger in sticky doughs and may result in adhesive and cohesive failure. The peak positive area is the maximum area of displacement in the dough as the probe (contact surface) detaches upwards from the surface of the dough (Figure 8.2)

8.3.2.4 Stickiness

The unleavened dough of flour blends had stickiness in the range 34.14–74.10 g, 49.58–77.20 g, and 57.91–122.17 g, at 10, 20 and 30% CFSL, respectively, and increased significantly with CFSL ($r = 0.678$, $p<0.05$). The wheat sample exhibited stickiness of 42.63 g and was observed to increase with increase in CFSL. Stickiness correlated positively with work of adhesion ($r = 0.515$, $p<0.05$), peak force ($r = 1.000$, $p<0.05$) and positive area ($r = 0.779$, $p<0.05$). This implies that unleavened sticky doughs were associated with high forces and work of adhesion. The stickiness of unleavened dough correlated negatively with gluten ($r = -0.445$, $p<0.01$), protein ($r = -0.592$, $p<0.05$), WAC ($r = -0.437$, $p<0.01$), and positively with flour particle size ($r = 0.412$, $p<0.05$) suggesting that unleavened doughs with high gluten and protein content, and high hydration capacity yielded low stickiness values. The large flour particle size hydrate slowly and thus limiting the development of gluten structure resulting in high stickiness. High positive area values were characteristic of sticky doughs. Similar was observed in a related study by Amonsou et al. (2013) on the adhesiveness of marama bean protein. The reduction in work of adhesion and peak area decreased stickiness. The differences in varieties could be attributed to variations in amylose contents. The unleavened dough stickiness exhibited a negative correlation with amylose contents ($r = -0.340$, $p<0.01$). This suggests that higher amylose varieties were less sticky.

The stickiness of unleavened dough exhibited weak to good correlations with dough mixing properties, development time ($r = 0.41$, $p<0.01$) and consistency ($r = -0.67$, $p<0.05$). High dough development time increased stickiness. Stickiness is an important textural property of dough as sticky dough adheres to machine surface, giving difficulties during bread preparation. Dough stickiness may result in chewy bread that adheres to the mouth, and often the dough would seem unbaked and thus decreasing consumer acceptance (Caramanico et al., 2018). Grausgruber et al.

(2003) classified sticky and nonsticky dough as stickiness greater than 90 g results in sticky dough, and less than 80 g produces nonsticky dough.

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Table 8.1 Stickiness and peak positive force of doughs from wheat (control) and flour blends

Variety	Dough type	Stickiness (g)				Peak positive force (N)			
		Wheat (100%)	10% CFSL	20% CFSL	30% CFSL	Wheat (100%)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	ULD	42.68(0.61)^{fghij}	47.44(7.60)^{ghijk}	61.95(12.17)^{klm}	57.91(3.66)^{jkl}	0.42(0.01)^{defgh}	0.47(0.07)^{efghi}	0.61(0.11)^{ijk}	0.57(0.03)^{hij}
	LD	19.26(6.68)^{ab}	26.29(3.02)^{abcde}	21.69(2.12)^{abc}	32.16(1.78)^{bcdefh}	0.19(0.07)^{a}	0.26(0.03)^{abc}	0.21(0.02)^{ab}	0.32(0.17)^{abcde}
	FLD	150.60(1.66)^{wxyz}	137.30(9.89)^{tuvw}	128.98(6.98)^{stu}	93.78(11.36)^{qr}	1.48(0.02)^{uvwx}	1.35(0.09)^{rstu}	1.26(0.07)^{qrs}	0.92(0.11)^{op}
Katobamputa	ULD	42.68(0.61)^{fghij}	69.43(3.05)^{lmno}	49.58(6.48)^{hijk}	67.85(5.67)^{lmn}	0.42(0.01)^{defgh}	0.68(0.03)^{jklm}	0.49(0.06)^{fghi}	0.67(0.05)^{kjl}
	LD	19.26(6.68)^{ab}	25.49(0.98)^{abcde}	38.83(10.28)^{efghi}	83.94(9.77)^{opq}	0.19(0.07)^{a}	0.25(0.01)^{abc}	0.38(0.10)^{cdefg}	0.82(0.09)^{mno}
	FLD	150.60(1.66) wxyz	153.12(13.42)^{xyzA}	179.52(42.29)^{B}	120.91(6.79)^{s}	1.48(0.02)^{uvwx}	1.50(0.13)^{vwx}	1.76(0.41)^{z}	1.19(0.07)^{q}
Mweru	ULD	42.68(0.61)^{fghij}	45.32(9.56)^{ghij}	74.12(5.31)^{mnop}	103.88(9.02)^{r}	0.42(0.01)^{defgh}	0.44(0.09)^{efgh}	0.73(0.05)^{klmn}	1.02(0.08)^{p}
	LD	19.26(6.68)^{ab}	13.53(10.69)^{a}	23.11(2.75)^{abcd}	25.07(8.26)^{abcde}	0.19(0.07)^{a}	0.22(0.03)^{ab}	0.23(0.03)^{ab}	0.25(0.08)^{abc}
	FLD	150.60(1.66) wxyz	162.89(6.89)^{zA}	133.66(13.53)^{stuv}	146.82(6.88)^{vwxy}	1.48(0.02)^{uvwx}	1.60((0.07)^{xy}	1.31(0.13)^{qrst}	1.44(0.07)^{tuvw}
Kariba	ULD	42.68(0.61) ^{fghij}	74.10(14.47)^{mnop}	75.61(2.76)^{mnop}	62.09(1.14)^{klm}	0.42(0.01)^{defgh}	0.73(0.14)^{klmn}	0.74(0.03)^{klmn}	0.61(0.01)^{ijk}
	LD	19.26(6.68)^{ab}	28.76(1.08)^{abcdef}	35.36(5.44)^{cdefghi}	36.87(0.12)^{cdefghi}	0.19(0.07)^{a}	0.28(0.01)^{abcd}	0.35(0.05)^{bcdefg}	0.36(0.00)^{bcdefg}
	FLD	150.60(1.66) wxyz	126.88(12.70)^{stu}	136.79(10.59)	93.67(8.77)^{qr}	1.48(0.02)^{uvwx}	1.24((0.12)	1.34(0.10)^{rstu}	0.92(0.09)^{op}
Kampolombo	ULD	42.68(0.61) ^{fghij}	57.00(19.30)^{jkl}	76.34(5.58)^{mnop}	85.55(5.94)^{pq}	0.42(0.01)^{defgh}	0.56(0.18)^{hij}	0.75(0.05)^{klmn}	0.84(0.06)^{no}
	LD	19.26(6.68) ^{wxyz}	35.15(2.15)^{cdefghi}	38.67(4.96)^{stuvw}	42.45(2.39)^{fghij}	0.19(0.07)^{a}	0.34(0.02)^{bcdef}	0.38(0.05)^{cdefg}	0.42(0.025)^{defgh}
	FLD	150.60(1.66)	154.14(5.54)^{yzA}	135.02(7.53)^{defghi}	138.21(17.31)^{uvwx}	1.48(0.02)^{uvwx}	1.51(0.05)^{wxy}	1.32(0.07)^{qrstu}	1.36(0.17)^{stuv}
Chila	ULD	42.68(0.61) ^{fghij}	78.48(2.21)^{nopq}	77.20(3.43)^{mnop}	122.17(8.41)st	0.42(0.01)^{defgh}	0.77(0.02)^{klmn}	0.76(0.03)^{klmn}	1.20(0.08)^{qr}
	LD	19.26(6.68)^{ab}	34.14(1.34)^{bcdefgh}	50.68(7.45)^{ijk}	36.1(1.34)^{cdefghi}	0.19(0.07)^{a}	0.33(0.02)^{abcdef}	0.50(0.07)^{ghi}	0.35(0.01)^{bcdefg}
	FLD	150.60(1.66) wxyz	205.66(19.98)^{C}	168.63(30.88)^{AB}	140.15(4.69)^{uvwxy}	1.48(0.02)^{uvwx}	2.02(0.19)^{A}	1.65(0.30)^{yz}	1.37(0.05)^{stuvw}
Level of significance									
Variety			<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Blend ratio			<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
variety x blend ratio			<0.05	<0.01	<0.05	<0.05	<0.05	<0.05	<0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test. ULD=Unleavened dough. LD=Leavened dough, FLD=Frozen Leavened dough, CFSL=cassava flour substitution level.

8.3.3 Stickiness of leavened dough

8.3.3.1 Peak positive force

The positive force ranged 0.25–0.34 N, 0.21–0.50 N, and 0.25–0.82 N, at 10, 20 and 30% cassava flour level, respectively. The positive force for the control sample was 0.19 N, and was observed to increase with increase in CFSL, and was lower than the positive force of the unleavened dough.

8.3.3.2 Work of adhesion

The work of adhesion increased in the leavened dough ranged from -130.20 to -194.40 g.s, -123.90 to -210.40 g.s, and -72.80 to -234.80 g.s, at 10, 20, and 30% CFSL, respectively. The WA for the wheat was -167.2 g.s, and increased with increase in CFSL. The work of adhesion exhibited poor correlations with dough mixing properties and flour particle size distribution. This is as the result of strong cohesive dough due to the addition of ingredients.

8.3.3.3 Peak positive area

The peak positive area of the wheat sample had lower value (0.01±0.01 N.s) than that of the wheat sample from unleavened dough. The differences in peak positive area upon inclusion of cassava flour were not significant ($p>0.05$) except in *Mweru* at 10 and 20% CFSL.

8.3.3.4 Stickiness

The stickiness of the leavened dough were in the range 13.53–35.15 g, 21.69–50.68 g, and 25.07–83.94 g, at 10, 20 and 30% CFSL, respectively, and varied significantly ($p<0.05$) according to CFSL ($r = 0.578$, $p<0.05$). The wheat sample had the stickiness of 19.26±6.68 g which increased with increasing CFSL. The stickiness of leavened dough negatively correlated with protein ($r = -0.465$, $p<0.05$). In a related study, Gujral et al. (2018) reported that blending gluten-free flours with wheat resulted in protein weakening due to increased starch-starch, and starch-protein interaction resulting in low level of gluten formation. The negative correlation of gluten content with stickiness in leavened dough ($r = -0.457$, $p<0.01$) was similar with unleavened ($r = -0.445$, $p<0.01$). The Stickiness increased with reduced gluten contents in flour blends in both unleavened and leavened doughs. High CFSL were associated with low gluten contents and thus they had lower stickiness. The negative correlation of stickiness with WAC in leavened ($r = -0.434$, $p<0.01$) was similar with WAC in unleavened ($r = -0.437$, $p<0.01$) doughs.

This implies that high WAC produced less sticky doughs. Similar was observed in the work of Amonsou et al. (2013) in which pure gluten isolates were characterized with lower forces of adhesion (low stickiness) as moisture content increased. The amylose contents in the leavened dough did not influence stickiness ($r = 0.078$, $p>0.01$). This may suggest that addition of leavening ingredients reduced the influence of amylose contents on stickiness. In the current study, the amylose contents of the flours were classified as normal or regular starches, and have been reported to be highly susceptible to enzymatic hydrolysis (Adefegha et al., 2018). The positive correlation of stickiness with flour particle size in leavened ($r = 0.423$, $p<0.01$) was similar with flour particle size in unleavened ($r = 0.412$, $p<0.01$) doughs. There was reduction in stickiness upon inclusion of ingredients (yeast, salt, fat and sugar). The stickiness trend was unleavened>leavened dough. The development of dough is key in baking since it combines the ingredients and develops a unique viscoelastic gluten network. Chen et al. (2018) reported that salt increases dough mixing resistance, and decreases dough stickiness during processing as higher levels of salt induced stronger gluten interactions via sulfhydryl-disulfide cross-linking. Salt has been identified as an ingredient in the dough that influences the level of protein–protein interactions and strength of the gluten network by changing the level of gluten hydration. Salt shields around the protein surface and thus induce charge on amino acids on the protein's surface, thus reducing the thickness of the electric double layer, and strengthening gluten interactions, which would subsequently yield a stronger network (Avramenko et al., 2018). Fat enhances dough plasticity (Mert and Demirkesen, 2016), softens and improves smoothness of the dough (Öztürk and Ova, 2018) which would probably contribute to reduced surface tension between probe surface and dough. Adhesion is negligibly small in smooth surfaces (McFarlane and Tabor, 1950; Liu et al., 2018b). Dough stickiness may result in chewy bread that adheres to the mouth. Often the dough would seem unbaked, and would thus contribute to decreased consumer acceptance (Caramanico et al., 2018).

Table 8.2 Work of adhesion and peak positive area of doughs from wheat (control) and flour blends

Variety	Dough type	work of adhesion (g.s)				Peak positive area (N.s)			
		Wheat (100%)	10%CFSL	20% CFSL	30% CFSL	Wheat (100%)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	ULD	-72.5(10.31)$^{h-m}$	-65.5(11.40)$^{i-m}$	-83.7(24.27)$^{g-l}$	-90.3(12.57)$^{g-k}$	0.03(0.01)$^{a-e}$	0.04(0.02)$^{a-f}$	0.06(0.03)$^{a-i}$	0.06(0.00)$^{a-h}$
	LD	-167.2(91.22)$^{b-e}$	-194.4(69.98)$^{a-c}$	-123.9(41.89)$^{e-h}$	-209.8(32.69)$^{a-c}$	0.01(0.01)^{ab}	0.01(0.00)^{ab}	0.00(0.00)^{a}	0.01(0.00)^{a}
	FLD	-31.7(16.75)^{lm}	-60.4(11.30)i-m	-90.8(11.74)$^{g-k}$	-26.4(30.52)^{m}	0.21(0.00)$^{l-o}$	0.17(0.003)$^{i-o}$	0.14(0.02)$^{f-n}$	0.11(0.02)$^{a-l}$
Katobamputa	ULD	-72.5(10.31)$^{h-m}$	-65.1(4.22)$^{i-m}$	-91.50(2.09)$^{g-j}$	-78.3(8.40)$^{g-m}$	0.03(0.01)$^{a-e}$	0.19(0.04)$^{k-o}$	0.03(0.01)$^{a-e}$	0.07(0.01)$^{a-j}$
	LD	-167.2(91.22)$^{b-e}$	-178.9(23.10)$^{b-d}$	-186.0(35.27)$^{a-c}$	-156.7(27.55)$^{c-f}$	0.01(0.01)^{ab}	0.01(0.00)^{ab}	0.03(0.02)$^{a-d}$	0.11(0.04)$^{b-l}$
	FLD	-31.7(16.75)^{lm}	-40.5(5.79)$^{i-m}$	-73.2(37.31)$^{h-m}$	-30(2.49)^{lm}	0.21(0.00)$^{l-o}$	0.13(0.03)$^{d-m}$	0.21(0.07)$^{l-o}$	0.13(0.002)$^{d-m}$
Mweru	ULD	-72.5(10.31)$^{h-m}$	-90.7(9.49)$^{g-k}$	-91.4(9.63)$^{g-j}$	-33.1(7.86)^{lm}	0.03(0.01)$^{a-e}$	0.06(0.05)$^{a-h}$	0.10(0.04)$^{a-k}$	0.42(0.19)^{qr}
	LD	-167.2(91.22)$^{b-e}$	-180.7(40.44)$^{a-d}$	-191.4(27.43)$^{a-c}$	-207.4(13.03)$^{a-c}$	0.01(0.01)^{ab}	0.32(0.43)^{pq}	0.23(0.02)$^{m-p}$	0.01(0.00)^{a}
	FLD	-31.7(16.75)^{lm}	-36.3(7.21)$^{k-m}$	-60.2(40.09)$^{i-m}$	-63.9(10.75)$^{i-l}$	0.21(0.00)$^{l-o}$	0.14(0.01)$^{e-n}$	0.10(0.01)$^{a-k}$	0.16(0.01)$^{i-o}$
Kariba	ULD	-72.5(10.31)$^{h-m}$	-65.2(14.11)$^{i-m}$	-75.1(8.10)$^{h-m}$	-102.6(7.17)$^{f-i}$	0.03(0.01)$^{a-e}$	0.26(0.12)^{op}	0.14(0.07)$^{f-n}$	0.05(0.02)$^{a-g}$
	LD	-167.2(91.22)$^{b-e}$	-173.9(8.83)$^{b-e}$	-178.9(31.16)$^{b-d}$	-234.8(31.45)^{a}	0.01(0.01)^{ab}	0.01(0.00)^{a}	0.01(0.01)^{ab}	0.02(0.00)^{ab}
	FLD	-31.7(16.75)^{lm}	-70.7(13.27)$^{h-m}$	-40.3(4.42)$^{j-m}$	-49.8(8.02)$^{i-m}$	0.21(0.00)$^{l-o}$	0.16(0.03)$^{h-o}$	0.10(0.01)$^{a-k}$	0.07(0.01)$^{a-j}$
Kampolombo	ULD	-72.5(10.31)$^{h-m}$	-67.8(20.51)$^{i-m}$	-68.4(10.33)$^{i-m}$	-77.3(3.21)$^{g-m}$	0.03(0.01)$^{a-e}$	0.09(0.09)$^{a-k}$	0.15(0.02)$^{h-o}$	0.16(0.05)$^{h-o}$
	LD	-167.2(91.22)$^{b-e}$	-130.2(38.75)$^{d-g}$	-207.1(19.67)$^{a-c}$	-219.9(17.05)^{ab}	0.01(0.01)^{ab}	0.02(0.00)^{ab}	0.02(0.00)^{abc}	0.02(0.01)^{abc}
	FLD	-31.7(16.75)^{lm}	-62.6(14,93)$^{i-m}$	-67.4(31.43)$^{i-m}$	-56.9(23.97)$^{i-m}$	0.21(0.00)$^{l-o}$	0.18(0.01)$^{k-o}$	0.16(0.02)$^{h-o}$	0.13(0.01)$^{c-m}$
Chila	ULD	-72.5(10.31)$^{h-m}$	-35.4(14.57)^{lm}	-77.7(7.53)$^{g-m}$	-39.4(9.61)$^{j-m}$	0.03(0.01)$^{a-e}$	0.50(0.14)^{r}	0.15(0.01)$^{g-n}$	0.46(0.05)^{r}
	LD	-167.2(91.22)$^{b-e}$	-181.8(7.12)$^{a-d}$	-210.4(26.40)$^{a-c}$	-72.8(49.36)$^{b-e}$	0.01(0.01)^{ab}	0.02(0.02)^{ab}	0.03(0.01)$^{a-e}$	0.01(0.01)^{ab}
	FLD	-31.7(16.75)^{lm}	-60.2(5.00)$^{i-m}$	-30.3(2.28)^{lm}	-55.2(5.85)$^{i-m}$	0.21(0.00)$^{l-o}$	0.24(0.03)^{nop}	0.17(0.02)$^{j-o}$	0.16(0.01)$^{h-o}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. ULD=Unleavened dough. LD=Leavened dough, FLD=Frozen Leavened dough, CFSL=cassava flour substitution level

Table 8.3 Correlation coefficient of stickiness with water absorption, amylose and gluten contents in unleavened and leavened dough

Parameter	CFSL	ST	WA	PPF	PA	WAC	Amy	Pro	Lip	Fib	D90	Glu
Unleavened dough												
CFSL	1											
ST	0.678*	1										
WA	-0.055	0.515*	1									
PPF	0.678*	1.000*	0.515*	1								
PA	0.321	0.779*	0.710*	0.779*	1							
WAC	-0.652*	-0.437*	-0.082	-0.437*	-0.186	1						
Amy	-0.099	-0.340**	-0.199	-0.340**	-0.388	-0.119	1					
Pro	-0.772*	-0.592*	-0.014	-0.592*	-0.401	0.337**	0.152	1				
Lip	-0.741*	-0.620*	-0.128	-0.620*	-0.443	0.359**	0.138	0.957	1			
Fib	-0.760*	-0.613*	-0.027	-0.613*	-0.411	0.356**	0.157	0.982	0.935	1		
D90	0.644*	0.412*	-0.099	0.412**	0.188	-0.264	-0.159	-0.830	-0.787	-0.809	1	
Glu	-0.839*	-0.445*	0.137	-0.445*	-0.159	0.595*	0.082	0.703	0.620	0.704	-0.531*	1
Leavened dough												
CFSL	1											
ST	0.578*	1										
WA	-0.239	-0.192	1									
PPF	-0.051	-0.091	-0.057	1								
PA	0.278	-0.123	-0.107	-0.101	1							
WAC	-0.652*	-0.434**	0.270	0.042	-0.268	1						
Amy	-0.099	0.078	0.087	-0.184	-0.112	-0.119	1					
Pro	-0.772*	-0.465*	0.154	-0.117	-0.134	0.337**	0.152	1				
Lip	-0.741*	-0.520*	0.207	-0.084	-0.059	0.359**	0.138	0.957	1			
Fib	-0.760*	-0.466*	0.174	-0.139	-0.177	0.356**	0.157	0.982	0.935	1		
D90	0.644*	0.423**	-0.104	-0.072	-0.148	-0.264	-0.159	-0.830	-0.787	-0.809	1	
Glu	-0.839*	-0.457*	0.287	-0.025	-0.403	0.595*	0.082	0.703	0.620	0.704	-0.531*	1

ST=Stickiness, WA=Work adhesion, PPF=Peak Positive Force, WAC=Water absorption capacity, PA=Positive area, Amy=Amylose, Pro=Protein, Lip=Lipid, Fib=Fiber, Glu=Gluten. CFSL=cassava flour substitution level. $p<0.05$*, $p<0.01$**, $p<0.001$***

Table 8.4 Correlation coefficient of stickiness with water absorption, amylose and gluten contents in frozen leavened dough

Parameter	CFSL	ST	WA	PPF	PA	WAC	Amy	Pro	Lip	Fib	D90	Glu
CFSL	1											
ST	-0.409*	1										
WA	-0.243	0.034	1									
PPF	-0.409*	1.000*	0.034	1								
PA	-0.629*	0.566*	-0.009	0566*.	1							
WAC	-0.652*	0.344**	0.134	0.344**	0.365	1						
Amy	-0.099	0.012	0.013	0.012	0.085	-0.119	1					
Pro	-0.772*	0.130	0.397**	0.130	0.545	0.337	0.152	1				
Lip	-0.741*	0.034	0.365**	0.034	0.427	0.359	0.138	0.957	1			
Fib	-0.760*	0.102	0.381**	0.102	0.559	0.356	0.157	0.982	0.935	1		
D90	0.644*	-0.330	-0.420*	-0.330**	-0.540	-0.264	-0.159	-0.830	-0.787	-0.809	1	
Glu	-0.839*	0.325**	0.164	0.325**	0.645	0.595	0.082	0.703	0.620	0.704	-0.531	1

ST=Stickiness, WA=Work adhesion, PPF=Peak Positive Force, WAC=Water absorption capacity, PA=Positive area, Amy=Amylose, Pro=Protein, Lip=Lipid, Fib=Fiber, Glu=Gluten, CFSL=cassava flour substitution level. $p<0.05$*, $p<0.01$**, $p<0.001$***

8.3.4 Interrelations of mixing properties, stickiness, and gluten

The correlation of stickiness parameters of unleavened and leavened doughs with mixing properties produced poor to good coefficients. The stickiness increased with decrease in gluten content in flour blends in both unleavened and leavened doughs. Water absorption capacity negatively correlated with stickiness in both unleavened and leavened doughs. This implies that high water absorption capacity produced less sticky doughs. Similar was observed in the work of Amonsou et al. (2013) in which pure gluten isolates were characterized by lower forces of adhesion (low stickiness) as moisture content increased. Dough development time positively correlated with stickiness in both unleavened and leavened doughs implies sticky doughs were characteristic of higher development times. In a related study, Gujral *et al.* (2018) reported that blending gluten-free flours with wheat resulted in protein weakening due to the low level of gluten formation, thus produced longer dough development time, which justifies the present results. The consistency (FU) negatively correlated with stickiness in both unleavened and leavened doughs. Sticky doughs were characterized with lower consistency as cassava flour level increased. A series of rigorous processes during the production of cassava flour such as grating, dewatering, drying, and fine milling could damage the starch. The damaged starch granule lowers paste viscosity and was observed to decrease maximum dough consistency (Pasqualone *et al.,* 2010). The damaged starch has more hydrating sites prominent of producing a molecular force which may weaken the stability of the gluten structure. In a related study, Ma *et al.* (2016) observed an increase in flour weakness (FU) with damaged starch content and ascribed this observation to the inverse relationship between damaged starch and protein in gluten matrix.

8.3.5 Stickiness of frozen leavened dough

8.3.5.1 Peak positive force

The peak positive force (PPF) for frozen dough showed higher levels of peak force in the range 1.24–2.02 N, 1.26–1.65 N, 0.92–1.44 N at 10, 20 and 30% CFSL, respectively, and significantly decreased with increase in CFSL ($r = -0.409$, $p<0.01$). The PPF for the wheat sample was 1.48±0.02 N. The forces were generally higher at 10 and 20% CFSL than those at 30% CFSL. These variations were due to differences in gluten content. The PPF showed a weak positive correlation with gluten ($r = 0.325$, $p<0.01$), implying that high gluten content doughs needed high forces to detach from the adhering surfaces.

8.3.5.2 Work of adhesion

The work of adhesion (WA) for frozen dough ranged from -36.30 to -70.70, -30.30 to -90.80 and -26.40 to -63.90 g.s, at 10, 20 and 30% CFSL, respectively, and decreased significantly ($p<0.001$) with increase in CFSL. The WA value (31.7±16.75 g.s) for the wheat sample was lower than those of unleavened and leavened doughs. This implies that the probe surface would require a small amount of work to adhere to sticky doughs (sticky doughs easily adhere to surfaces).

8.3.5.3 Peak positive area

There was significant ($p<0.05$) increase of PPA in frozen dough compared to the unfrozen leavened dough. This implies adhesive material (dough) displaced from the dough increased. The wheat sample for frozen dough exhibited high PPA value (0.21±00) and showed insignificant differences ($p>0.05$) with PPA in flour blends.

8.3.5.4 Stickiness

There was a huge increase in the stickiness of the frozen dough compared to unfrozen doughs. The stickiness of frozen leavened were in the range 126.88–205.66 g, 128.98–179.52 g and 93.67–146.82 g, at 10, 20 and 30% CFSL, respectively. The stickiness of wheat sample (150±60 g) decreased significantly ($r = -0.409$, $p<0.05$) with increase in CFSL. The stickiness of frozen doughs was high at 10% CFSL, and generally low at subsequent blend ratios. This trend is opposite to that of unfrozen doughs, in which higher CFSL doughs yielded low stickiness. This variation could suggest that depolymerization of gluten during frozen storage generates low molecular weight compounds which are hydrophobic nature. During frozen storage, the water solidifies into ice through crystallization, and subsequent expansion of solid water can cause physical rupture of protein (disulfide) films, thus limiting protein-protein interactions leading to weakening of gluten. During thawing the ice melts and separates irreversibly away from the starch–gluten matrix which reduces interaction of water with hydration sites of gluten–starch system. Hence, the resulting water phase is in weak interaction with the gluten structure (Zhao et al., 2013; Ma et al., 2016). The unbound water probably might have contributed to increased adhesion. Similar was observed by Amonsou et al. (2013) as high moisture content doughs

exhibited a higher force of adhesion. Moreover, the development of gluten structure is the function of disulfide bonds in glutenin and gliadins, and thus depleting disulfide bonds weaken the gluten matrix. The developed gluten matrix undergoes deterioration exhibited through molecular changes during frozen storage (Wang et al., 2018). Zhao et al. (2013) reported free sulfhydryl groups increased in the wheat dough during frozen storage time, which indicated a decrease of a number of disulfide bonds. The higher peak stickiness (Figure 8.3) in low levels of cassava flour could be as the result of the increased number of low molecular weight oligomers due to depolymerization of glutenin which occurs via the breakage of interchain disulfide bonds and thus weakening the viscoelasticity resulting in high stickiness. Also, the decrease in stickiness at higher CFSL could be attributed to the high contents of starches in cassava flour with higher water binding capacity than wheat gluten.

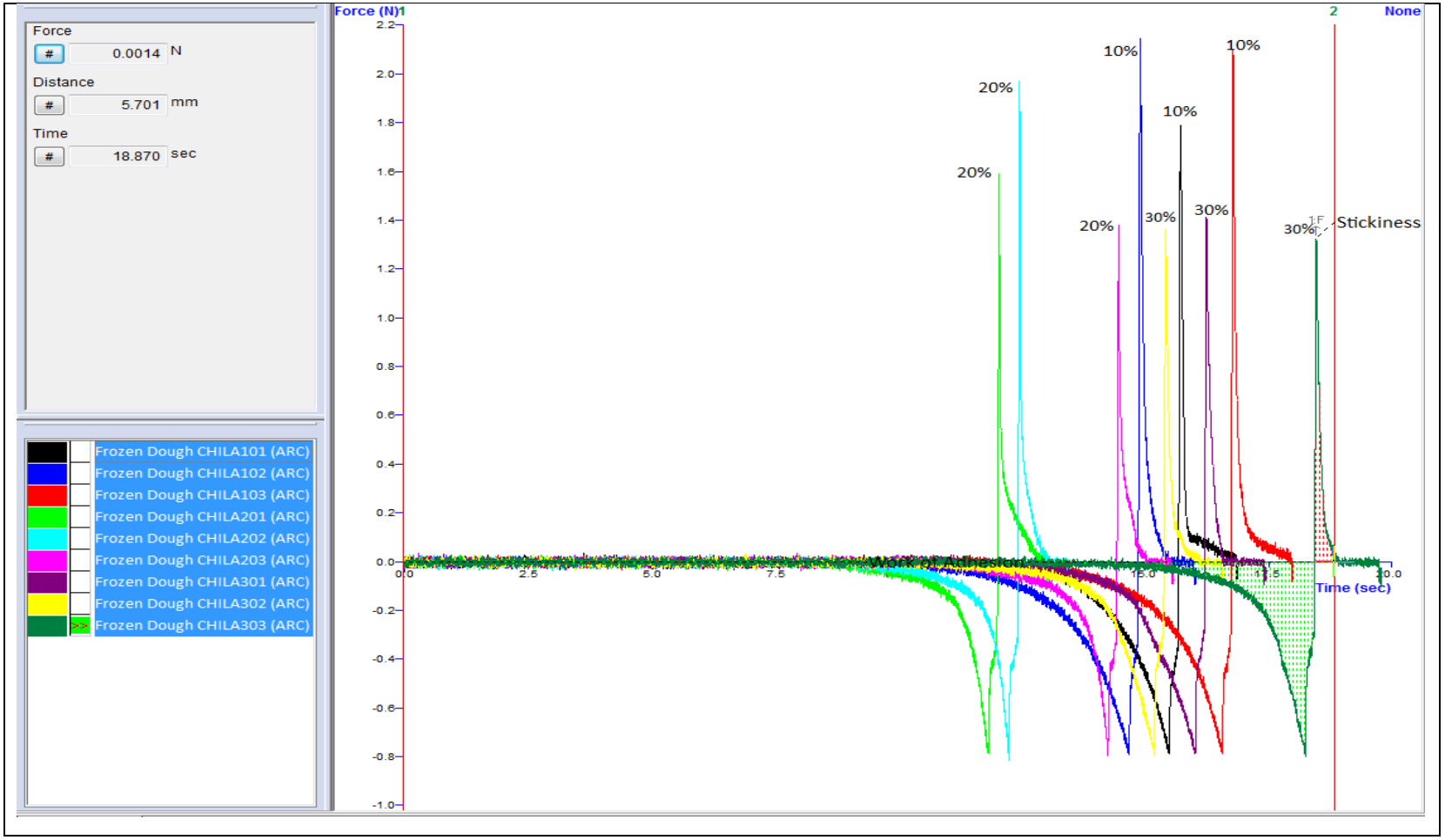

Figure 8.3 Stickiness curves for frozen dough of wheat-cassava blend flour at 10, 20 and 30% cassava flour concentration levels. Cassava variety: Chila

8.4 Conclusion

The stickiness of wheat related blends is dependent on water absorption and gluten development. The stickiness in the unleavened and leavened doughs increased with increasing CFSL. The opposite was observed in the frozen dough in which the stickiness decreased with increasing cassava flour level. Cassava flour acted as diluent against gluten content in wheat and caused excess water in the aqueous phase at higher CFSL. In the frozen dough, the deterioration of gluten may possibly lead to increased unbound water resulting in high stickiness values at low CFSL. The stickiness of unleavened dough decreased upon inclusion of ingredients. Therefore, leavened exhibited lower stickiness values than those of unleavened dough. The frozen dough would be recommended for re-kneading to re-develop the desired consistency with reduced stickiness. Future investigation should focus on the effect of dough stabilizers (ingredients) on the stickiness of frozen dough system.

There is limited information on the effect of stickiness on the quality of bread processed from frozen dough system of wheat-cassava composite flours. Therefore, the effect of frozen storage and stickiness on bread quality is the focus of the next chapter.

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9. EFFECT OF FROZEN STORAGE ON WHEAT-CASSAVA DOUGH AND BREAD AND THEIR RESISTANT STARCH CONTENTS

This chapter is based on the following paper prepared for submission

Chisenga, SM, Workneh, TS, Bultosa, G and Alimi, BA. 2019. Effect of frozen storage on wheat-cassava dough and bread quality.

Abstract

The volume of wheat bread baked from fresh, frozen-before-proofing (FBP) and frozen-after-proofing (FAP) doughs were 148.17±10.61, 103.33±2.89, and 78.67±1.15 cm^3, respectively. The bread volume of composite flours baked from fresh, FBP and FAP ranged from 91.67-140 cm^3, 68.67-105.00 cm^3 and 67.67-11.67 cm^3, respectively. The bread volume loss for wheat bread baked from FBP and FAP were 29.94±6.70% and 46.76±2.99%, and their composite flours recorded bread volume loss in the range 0.81-40.47% and 4.52-27.04%, respectively. Specific volume of fresh wheat bread (2.46±0.19 cm^3/g) was higher than FBP (1.71±0.05 cm^3/g) and FAP (1.29±0.02 cm^3/g). Stickiness showed negative ($r = -0.76$, $p<0.05$), ($r = -0.75$, $p<0.05$) and positive ($r = 0.76$, $p<0.05$) correlation with bread volume, specific volume, and bread density, respectively. The variations among the cassava varieties were due to differences in particle size distribution. The trend for whiteness index of bread crumb was fresh>FBP> FAP. The trend for brownness index was FBP> FAP>fresh bread. The resistant starch (RS) content were in the range 1.98-6.90% and 4.24-5.63%, for bread from fresh and frozen dough, respectively. The RS for wheat bread was 4.29% and 6.84%, for fresh and frozen dough, respectively. High stickiness doughs yielded low bread volume and high RS. The varieties *Katobamputa*, *Mweru*, and *Kariba* at 10% CFSL exhibited highest and lowest specific bread volume in fresh and FAP, respectively.

Key word: frozen dough, wheat-cassava flour, stickiness, bread volume

9.1 Introduction

The fresh dough for bread making has a short shelf life. The wheat dough is characteristically high in water content, making it susceptible to deterioration of microbial, enzymatic and biochemical nature (Giannou *et al*., 2003), and if not controlled can cause dough weakening and degradation of quality. Bread making processes requires that fresh dough is baked immediately after proofing. Increased demand for fresh bread products would require continuous availability of fresh dough. Dependence on daily preparation of dough to meet production requirements and customer need is likely to result in lagged processes. Freezing is a suitable technology for preserving dough quality (Adams *et al*., 2017; Wang *et al*., 2017), and a means of continuous supply of dough in the production. The use of frozen doughs has been an area of huge interest in the bakery industry and traditional chain stores (Buddhi and Sahoo, 1997; Demiate and Kotovicz, 2011; Klein *et al*., 2013). To overcome short shelf life and freshness associated with the dough, Ribotta et al. (2001) suggested the use of technologies to produce long-enduring dough. Nevertheless, freezing and frozen storage resulted in dough deterioration and undesirable bread quality characteristics.

Frozen doughs have been reported to be associated with problems such as reduced yeast activity (Halagarda, 2017; Luo *et al*., 2017b) and thinning of gluten structure (Xuan et al., 2017; Wang et al., 2018). Consequently, the bread baked from frozen doughs has been characterized with low specific volume, decrease in the retention capacity of CO_2 and deterioration in the texture of the final product (Adams *et al*., 2017; Jia *et al*., 2017). During frozen storage, temperature fluctuations can cause separation of water from the gluten and subsequent crystallization (Lu and Grant, 1999). The volume expansion of ice crystals causes serious damage to the gluten-starch structure (Wang *et al*., 2017) that can account for the poor baking performance of frozen dough. The frozen storage of wheat dough was reported to increase *in vitro* slowly digestible starch in their baked products (Ronda *et al.*, 2010). However, information is limited on the enzymatic digestibility and resistant starch of bread products baked from frozen dough of wheat-cassava flour blends.

Cassava flour contains a large amount of starch and minor fiber contents. In a dough system, starches impart higher water binding capacity than wheat gluten proteins. Thus, the inclusion of

cassava flours in frozen wheat dough system may lead to reduced gluten network deteriorations and ameliorate the rate of ice crystal formation during frozen storages which are detrimental to gluten network disruptions. Increasing acceptance of bread from composite cassava-wheat flour would stir interest in the storage of the composite flour dough through freezing. The frozen storage of the dough could have additional effect on its subsequent handling and machination during bakery process. However, dough stickiness characteristics in the cassava-wheat frozen dough system and subsequently bread quality have not been researched. Thus in this study, the effect of stickiness on bread quality baked with cassava-wheat frozen dough were investigated.

9.2 Materials and Methods

9.2.1 Source of materials

The wheat flour for white bread was procured from the local market in the city of Pietermaritzburg, South African. Cassava flours from six varieties were prepared as described in method 3.2.4, Chapter 3.

9.2.2 Blending of wheat-cassava flour

Three levels of wheat: cassava (90:10, 80:20, and 70:30) used in the study were as described in method 7.2.4, Chapter 7.

9.2.3 Dough preparation

The regular dough was processed as described in Chapter 7, method 7.2.7 and frozen dough was processed as described in method 8.2.3, Chapter 8.

9.2.4 Preparation of bread

Bread was baked as described in method 7.2.7, Chapter 7.

9.2.5 Crumb and crust color

The bread crumb and crust color were measured as described in Chapter 7, method 7.2.8.

9.2.6 Bread specific volume, density and weight loss

The bread volume, specific volume, and density were measured as described in Chapter 7, method 7.2.8 and 7.2.9

$$\text{Bread volume loss (\%)} = \frac{V_{fresh} - V_{frozen}}{V_{fresh}} \times 100 \quad (1)$$

where,

V_{fresh} = volume of fresh bread

V_{frozen} = volume of bread

9.2.7 Bread crumb pore size characteristics

The bread pore size characteristics were evaluated as described in Chapter 7, method 7.2.11.

9.2.8 Resistant starch assay procedure

Resistant starch (RS) contents in bread samples of fresh and frozen (frozen before proofing) were determined as described in method 5.2.5, Chapter 5.

9.2.9 Experimental design and analysis

A Completely Randomized Design comprising of two factors (cassava variety and CFSL) was used. Triplicate data were analyzed using two-way ANOVA of GenStat 18th Edition software and mean differences were determined using Fisher's Least Significance Difference (LSD) test at 5% significant level. The correlation coefficients of flour proximate and amylose content (Table 7.2, Chapter 7), gluten (table 7.4, Chapter 7), particle size distribution at 90% cumulative of finer particles (Table 7.3, Chapter 7), stickiness (table 8.1, Chapter 8) and bread quality characteristics were performed using Pearson's correlation.

9.3 Results and Discussion

9.3.1 Bread quality characteristics

9.3.1.1 Bread volume

Table 9.1 shows results for bread volume of bread processed from doughs frozen before and after proofing

The average bread volume of the control (wheat) for the fresh dough was 148.17±10.61 and was observed to decrease significantly ($p<0.05$) with increase in cassava flour substitution levels. The bread volume from flour blends at 10%, 20%, and 30% were ranged 103.00–140 cm^3, 103.00–120.00 cm^3, and 91.67–105.00 cm^3, respectively. The lowest and highest bread volumes were recorded in *Chila* and *Katobamputa* at 30 and 10% cassava flour blend, respectively. *Katobamputa* showed insignificant differences ($p>0.05$) with *Bangweulu* at 30% cassava flour level. The variations in varieties were due to differences in flour particles size. The bread volume was negatively correlated with particle size ($r = -0.68$, $p<0.05$). The effective size (D10) of *Katobamputa* (35.56 μm) was significantly lower ($p<0.05$) than *Chila* (48.52 μm). In addition, the highest percentage cumulative of finest particles were recorded in *Katobamputa* (11.77%), and was significantly higher ($p<0.05$) than *Chila* (8.19%). Smaller flour particle sizes have larger contact surface area permitting huge interactions of hydroxyl groups with water through hydrogen bonding (Bressiani *et al.*, 2017) thus enhancing optimal dough development resulting in higher bread volumes.

The average bread volume of the control (wheat) for frozen before proofing (FBP) was 103.33±2.89, representing a 29.94±6.7% reduction in bread volume from fresh dough. Frauenlob *et al.* (2017a) reported that bread volume decreased with increasing frozen storage, and ascribed the changes to yeast damage. The reduction in bread volume can be attributed to decrease in the ability of gluten network to retain CO_2 during proofing (Adams et al., 2017). Jia et al. (2017) demonstrated depolymerization of glutenin macropolymer via breakage of inter-chain disulphide bonds resulted in decreased high molecular weight and increase in low molecular weight glutenin subunits. The low molecular weight compounds are hydrophobic in nature (Jia et al., 2017), and might cause formation of excess water during thawing. The FBP bread volume of the composite flours were in the range 76.33-105.00, 71.67-94.67 and 68.67-92.67 cm^3 at 10, 20 and 30% cassava flour, respectively, and were significantly different among the varieties. The lowest

and highest FBP bread volume was recorded in *Chila* and *Mweru* at 30 and 10% cassava flour, respectively. The variation in varieties could be due to differences in particle size. The frozen bread volume negatively correlated with flour particle size ($r = -0.24$, $p<0.01$). Smaller flour particle size in *Mweru* (250.43±0.03 μm) had higher bread volume compared to *Chila* (278.49±0.00 μm), and their finest particles were cumulatively higher in *Mweru* (9.51±0.01%) than *Chila* (8.19±0.00%). The frozen bread volume positively correlated ($r = 0.37$, $p<0.01$) with water absorption capacity. The doughs with high water absorption capacity yielded high bread volume. *Mweru* at 10% cassava flour had higher ($p<0.05$) water absorption than *Chila*.

The frozen-after-proofing (FAP) bread volume were in the range 88.33-111.67, 75.00-93.33, 67.67-90.00 cm^3 at 10, 20 and 30% CFSL, respectively, and varied among the varieties. The lowest and highest were recorded in *Bangweulu* and *Katobamputa* at 30 and 10% cassava flour level, respectively. The variation in varieties might have been due to differences in particle size. The FAP bread volume negatively correlated ($r = -0.56$, $p<0.05$) with flour particle size. This suggests that the finer flour gave better bread volume. *Katobamputa* exhibited finer particle size (35.56±0.01 μm) than *Bangweulu* (39.78±0.01 μm), and cumulative finer particles in *Katobamputa* (11.77%) were higher than *Bangweulu* (9.17%). Furthermore, the higher crude fiber content in *Bangweulu* might have contributed to decreased bread volume. Edible fibers are mainly composed of polysaccharides such as cellulose, hemicellulose, and pectin. The combination of cellulose microfibrils and cross-linking hemicelluloses with an inter-penetrating pectin network provides strength and rigidity to the cell wall. In cellulose, a network of microfibrils formed by close packing of unbranched β-1,4-glucan chains, which are stabilized by intra- and inter-molecular hydrogen bonds, makes this polymer impermeable and water-insoluble (Grundy *et al.*, 2016), such characteristics are likely to contribute to weakening of gluten network and hinder gas cell (pore) formation. Lauková *et al.* (2017) concluded that cellulose fiber lowered bread rolls volume. The average bread volume of the control (wheat) for (FAP) was 78.67±1.15 cm^3 and was significantly lower ($p<0.05$) than FBP and fresh bread volume. The trending pattern of bread volume for wheat bread was fresh>FBP>FAP. The dough stickiness negatively correlated ($r = -0.76$, $p<0.05$) with bread volume. This suggests that high stickiness values in frozen doughs resulted in reduced bread volume. Luo et al. (2017) reported that bread made with frozen dough showed lower bread volume than that baked from fresh dough.

Table 9.1 Bread volume of bread made from flour blends of wheat and cassava flours

Variety	Dough type	Volume (cm^3)			
		Control (Wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	Fresh	148.17(10.61)^{x}	103.00(6.08)$^{q-t}$	103.00(6.08)$^{p-s}$	92.67(6.43)$^{j-p}$
	FBP	103.33(2.89)^{rst}	102.00(2.00)$^{p-t}$	94.67(4.62)$^{l-r}$	86.67(2.88)$^{f-l}$
	FAP	78.67(78.67)$^{c-g}$	88.33(2.89)$^{g-m}$	75.00(5.00)$^{f-l}$	67.67(2.52)^{a}
Katobamputa	Fresh	148.17(10.61)^{x}	140.00(5.00)^{x}	120.00(8.66)^{uvw}	100.00(13.22)$^{o-s}$
	FBP	103.33(2.89)^{rst}	83.33(2.89)$^{e-j}$	86.33(11.84)$^{f-l}$	90.00(0.00)$^{h-n}$
	FAP	78.67(78.67)$^{c-g}$	111.67(2.89)^{tu}	93.33(14.43)$^{k-q}$	90.00(5.00)$^{h-n}$
Mweru	Fresh	148.17(10.61)^{x}	123.33(7.64)^{vw}	115.00(5.00)^{uv}	103.33(11.55)^{rst}
	FBP	103.33(2.89)^{rst}	105.00(5.00)st	83.33(2.88)$^{e-j}$	70.00(0.00)^{abc}
	FAP	78.67(78.67)$^{c-g}$	96.67(2.88)$^{m-s}$	91.67(2.88)$^{i-o}$	78.33(14.43)$^{b-f}$
Kariba	Fresh	148.17(10.61)^{x}	128.33(7.64)^{w}	111.67(5.77)^{tu}	95.00(8.66)$^{l-r}$
	FBP	103.33(2.89)^{rst}	76.33(15.88)$^{a-e}$	71.67(2.88)$^{a-d}$	70.00(0.00)^{abc}
	FAP	78.67(78.67)$^{c-g}$	98.33(2.88)$^{n-s}$	84.00(3.60)$^{e-l}$	80.00(0.00)$^{d-g}$
Kampolombo	Fresh	148.17(10.61)^{x}	121.67(2.88)^{vw}	111.67(5.77)^{tu}	105.00(0.00)st
	FBP	103.33(2.89)^{rst}	101.67(2.88)$^{p-s}$	80.67(1.16)$^{d-h}$	71.33(1.16)$^{a-d}$
	FAP	78.67(78.67)$^{c-g}$	95.27(0.46)$^{l-s}$	90.33(0.57)$^{h-o}$	82.67(4.04)$^{d-h}$
Chila	Fresh	148.17(10.61)^{x}	118.33(7.46)^{uv}	111.67(2.88)^{tu}	91.67(2.88)$^{i-o}$
	FBP	103.33(2.89)^{rst}	101.67(2.88)$^{p-s}$	82.33(0.58)$^{e-i}$	68.67(1.16)^{ab}
	FAP	78.67(78.67)$^{c-g}$	101.67(2.88)$^{p-s}$	93.33(2.88)$^{k-q}$	87.33(6.43)$^{f-m}$
Significance level					
Variety		p<0.05	p<0.05	p<0.05	p<0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test. FBP=frozen before proofing, FAP=frozen after proofing, CFSL=cassava flour substitution level. FBP = Frozen-before-proofing, FAP = Frozen-after-proofing, CFSL=cassava flour substitution level.

9.3.1.2 Bread volume loss

The volume loss for bread from FBP were in the range 0.81-40.47, 7.78-35.61 and 6.14-32.06% at 10, 20 and 30% CFSL, respectively. The lowest volume loss was recorded in *Bangweulu* across all blend ratios. The highest volume loss was recorded in *Katobamputa* and was significantly similar (p>0.05) to *Kariba* at 10% cassava flour. The volume loss in FBP wheat bread was 29.94±6.70%, and was not different (p>0.05) from *Katobamputa* and *Kariba* at 10%, but was significantly different (p<0.05) from only *Bangweulu* at 20%, and was found to be

similar ($p>0.05$) with *Mweru*, *Kariba*, *Kampolombo* and *Chila* at 30% cassava flour. This observation suggests that many varieties and wheat bread from FBP exhibited similar volume losses. The volume loss in the FAP bread were in the range 13.86-21.67, 16.40-27.04, 4.52-26.79% at 10, 20 and 30% CFSL, respectively. The lowest and highest volume losses for FAP bread were recorded in *Chila* and *Bangweulu* at 30%. The volume loss values in FAP bread were generally lower than those of FBP bread. The wheat bread volume for FAP exhibited the highest volume loss (46.76±2.99%). The volume losses in FBP may be attributed to the impact of the freeze-thaw effect on yeast viability and gluten network. During freezing, water crystallizes into ice, and the expansion effect of ice crystals can cause rupture of yeast cells, and this chilling injury can lead to loss of yeast viability. Wang *et al.* (2017) reported that frozen storage of dough decreased three-quarters of the original yeast activity resulting in reduced gassing power and release rate of yeast. Since the FAP doughs were frozen after proofing, the volume losses could be alluded to deterioration of protein polymer during freezing (Silvas-García *et al.*, 2014) and thus weakening gluten network. The other factor could be the scorching effect of freezing due to ice sublimation may possibly cause loss of moisture (Tang *et al.*, 2018) which can limit hydration of gluten network structure and thus hindering gas cell formation. Lu and Grant (1999) reported the wheat bread volume decreased from an average of 912 cm^3 for fresh doughs to 738 cm^3 for frozen doughs. This reduction represented 19.07% volume loss, similar to volume losses of wheat bread from FBP.

Table 9.2 Reduction (%) of bread volume from straight dough to frozen before proofing, and frozen after proofing dough

	FBP			FAP		
Variety	10% CFSL	20% CFSL	30% CFSL	10% CFSL	20% CFSL	30% CFSL
Bangweulu	0.81(4.30)^{a}	7.78(8.68)^{ab}	6.14(7.86)^{ab}	14.09(4.61)^{abc}	27.04(6.22)^{d}	26.79(4.92)^{d}
Katobamputa	40.47(1.07)^{g}	27.30(15.77)^{ef}	9.00(11.34)^{ab}	20.13(4.79)^{bcd}	21.33(18.47)^{cd}	8.7(15.06)^{ab}
Mweru	14.81(1.56)^{bcd}	27.38(5.42)^{ef}	31.65(8.16)^{fg}	21.49(3.57)^{cd}	20.25(1.84)^{cd}	24.07(11.47)^{cd}
Kriba	40.00(15.08)^{g}	35.61(6.09)^{fg}	25.88(7.13)^{def}	23.28(2.55)^{cd}	24.55(6.77)^{cd}	15.29(8.15)^{abc}
Kampolombo	16.44(0.38)^{bcde}	27.65(3.43)^{ef}	32.06(1.10)^{fg}	21.67(2.04)^{cd}	18.94(4.85)^{bcd}	21.27(3.84)^{cd}
Chila	13.92(4.19)^{bc}	26.27(0.92)^{def}	25.07(1.08)^{cdef}	13.86(5.58)^{abc}	16.40(2.42)^{bcd}	4.52(9.82)^{a}
Wheat	29.94(6.70)^{fg}			46.76(2.99)^{e}		

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP = Frozen-before-proofing, FAP = Frozen-after-proofing, CFSL=cassava flour substitution level.

9.3.1.3 Specific volume

The specific volume of the composite bread for fresh dough were ranged 1.67-2.27, 1.69-1.96 and 1.49-1.80 $cm^3.g^{-1}$ at 10, 20 and 30% CFSL, respectively (Table 9.3). The specific volume of wheat bread for the fresh dough was 2.46±0.19 $cm^3.g^{-1}$. The specific volume of FBP for wheat bread was 1.71±0.05 $cm^3.g^{-1}$. The specific volume of the composite bread for FBP 1.37-1.78, 1.27-1.55 and 1.16-1.49 $cm^3.g^{-1}$ at 10, 20 and 30% CFSL, respectively. The specific volume of the composite bread for FAP 1.47-1.86, 1.25-1.60 and 1.12-1.53 $cm^3.g^{-1}$ at 10, 20 and 30% CFSL, respectively, and varied among varieties. The specific volume of wheat bread for FAP was 1.29±0.02 $cm^3.g^{-1}$ and recorded lowest compared to fresh and FBP. The trend for reduction in specific bread volume was fresh>FBP>FAP. Frauenlob *et al.* (2017b) observed a similar trend in which frozen dough yielded low specific volume than fresh dough.

Table 9.3 Specific volume of bread made from flour blends of wheat and cassava flours

Variety	Dough type	Specific volume ($cm^3.g^{-1}$)			
		Control (Wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	Fresh	2.46(0.19)^{A}	1.67(0.10)$^{n-s}$	1.69(0.10)$^{n-t}$	1.52(0.10)$^{g-n}$
	FBP	1.71(0.05)$^{o-t}$	1.69(0.04)$^{n-t}$	1.55(0.07)$^{i-o}$	1.42(0.05)$^{e-l}$
	FAP	1.29(0.02)$^{a-e}$	1.47(0.05)$^{f-m}$	1.25(0.08)$^{a-d}$	1.12(0.04)^{a}
Katobamputa	Fresh	2.46(0.19)^{A}	2.27(0.06)^{z}	1.93(0.11)^{vwx}	1.63(0.23)$^{m-r}$
	FBP	1.71(0.05)$^{o-t}$	1.38(0.05)$^{c-h}$	1.42(0.18)$^{e-l}$	1.49(0.01)$^{f-m}$
	FAP	1.29(0.02)$^{a-e}$	1.86(0.04)$^{t-w}$	1.57(0.26)$^{j-p}$	1.53(0.07)$^{g-n}$
Mweru	Fresh	2.46(0.19)^{A}	2.13(0.12)^{yz}	1.96(0.10)^{wxy}	1.80(0.19)$^{s-w}$
	FBP	1.71(0.05)$^{o-t}$	1.78(0.09)$^{r-v}$	1.38(0.03)$^{c-h}$	1.16(0.00)^{ab}
	FAP	1.29(0.02)$^{a-e}$	1.58(0.05)$^{k-q}$	1.54(0.03)$^{h-n}$	1.30(0.22)$^{b-e}$
Kariba	Fresh	2.46(0.19)^{A}	2.07(0.11)^{xy}	1.82(0.10)$^{s-w}$	1.54(0.13)$^{h-m}$
	FBP	1.71(0.05)$^{o-t}$	1.37(0.29)$^{c-g}$	1.27(0.04)$^{a-e}$	1.24(0.01)^{abc}
	FAP	1.29(0.02)$^{a-e}$	1.63(0.05)$^{m-r}$	1.42(0.05)$^{e-k}$	1.35(0.02)$^{c-f}$
Kampolombo	Fresh	2.46(0.19)^{A}	1.94(0.06)^{vwx}	1.81(0.07)$^{s-w}$	1.72(0.01)$^{p-t}$
	FBP	1.71(0.05)$^{o-t}$	1.77(0.05)$^{r-v}$	1.41(0.03)$^{d-k}$	1.26(0.02)$^{a-e}$
	FAP	1.29(0.02)$^{a-e}$	1.59(0.01)$^{l-q}$	1.50(0.01)$^{f-m}$	1.38(0.07)$^{c-i}$
Chila	Fresh	2.46(0.19)^{A}	1.90(0.12)^{uvw}	1.83(0.06)$^{s-w}$	1.49(0.05)$^{f-m}$
	FBP	1.71(0.05)$^{o-t}$	1.74(0.04)$^{q-u}$	1.41(0.04)$^{c-j}$	1.16(0.02)^{ab}
	FAP	1.29(0.02)$^{a-e}$	1.73(0.05)$^{p-u}$	1.60(0.04)$^{m-q}$	1.49(0.12)$^{f-m}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. CFSL=cassava flour substitution leve

9.3.1.4 Bread density

The density of wheat bread for fresh dough sample (0.41±0.03 $g.cm^{-3}$) (Table 9.4) increased with increase in CFSL and was significantly smaller ($p<0.05$) than densities of frozen doughs. Similar fresh bread density was reported 0.41 $g.cm^{-3}$ (Ayati *et al.*, 2017). The bread density of the bread from flour blends of FBP were in the range 0.56-0.72, 0.65-0.79 and 0.67-0.86 $g.cm^{-3}$ at 10, 20 and 30% CFSL, respectively. The FBP density of wheat bread was 0.58 $g.cm^{-3}$ and was similar ($p>0.05$) to *Bangweulu, Mweru, Kariba* and *Chila* at 10% CFSL. Bread density had significant inverse relationship ($r = -0.61$, $p<0.05$) with gluten content. This suggests low gluten content yielded high bread density. Cassava flours were reported to contain higher levels of damaged starch than wheat (Oladunmoye *et al.*, 2014), and excess damaged starch can limit migration of volatiles and gas cell expansion, with high water absorption capacity can result in high bread density. The bread density for FAP in flour blends were in the range 0.47-0.68, 0.63-0.81 and 0.66-0.89 $g.cm^{-3}$ at 10, 20 and 30% CFSL, respectively. The density of FAP wheat bread was 0.78 $g.cm^{-3}$ and decreased with increase in CFSL except for an increase in *Bangweulu* at 30%. The interaction of fiber and gluten in *Bangweulu* might have increased bread density. Dietary fiber components such as arabinogalactan peptides can interact with gluten molecule to form a complex structure, resulting in reduced water absorption (Sivam *et al.*, 2010), and thus inhibiting gluten development with the subsequent limited formation of crumb gas cells. The bread density trend fresh<FBP<FAP justifies that frozen storage of dough affects bread volume.

The stickiness correlated positively ($r = 0.76$, $p<0.05$) with bread density. This suggests that high stickiness dough yielded high bread density. This can be ascribed to the survival of yeast after frozen storage (Miyazaki *et al.*, 2008). The FAP were frozen at their maximum aeration and gas retention capacity, at which yeast viability diminished. Ice crystallization and recrystallization of fermented doughs (FAP) contracted dough gassing power resulting in increased gelatinization temperatures during baking (Miyazaki et al., 2008). The low bread density in FBP could be attributed to surviving yeast cells, and development of gluten structure during re-kneading.

Table 9.4 Bread density and weight loss bread made from flour blends of wheat and cassava flours

Variety	Dough type	Density ($g.cm^{-3}$)				Weight loss (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL	Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	fresh	0.41(0.03)^{a}	0.60(0.003)$^{i-p}$	0.59(0.03)$^{h-p}$	0.66(0.04)$^{p-t}$	13.86(0.42)$^{k-q}$	12.00(0.24)$^{b-f}$	12.95(0.41)$^{e-l}$	13.14(0.25)$^{g-m}$
	FBP	0.58(0.02)$^{f-o}$	0.59(0.01)$^{h-p}$	0.65(0.03)$^{n-s}$	0.70(0.03)$^{s-w}$	13.71(0.00)$^{j-p}$	13.57(0.42)$^{h-o}$	12.81(0.29)$^{e-k}$	13.10(0.36)$^{f-m}$
	FAP	0.78(0.01)^{xy}	0.68(0.02)$^{r-v}$	0.81(0.06)^{yz}	0.89(0.04)^{A}	12.71(0.87)$^{d-j}$	14.38(0.30)$^{n-s}$	14.00(0.24)$^{l-r}$	14.05(0.50)$^{l-r}$
Katobamputa	fresh	0.41(0.03)^{a}	0.44(0.01)^{ab}	0.52(0.03)$^{c-g}$	0.62(0.08)$^{k-r}$	13.86(0.42)$^{k-q}$	12.05(0.58)$^{c-g}$	11.10(1.03)^{abc}	12.48(0.95)$^{d-h}$
	FBP	0.58(0.02)$^{f-o}$	0.72(0.03)$^{u-x}$	0.71(0.09)$^{s-w}$	0.67(0.01)$^{q-u}$	13.71(0.00)$^{j-p}$	13.81(0.50)$^{j-p}$	13.43(0.75)$^{h-n}$	13.91(0.86)$^{k-q}$
	FAP	0.78(0.01)^{xy}	0.54(0.01)$^{d-i}$	0.65(0.10)$^{o-s}$	0.66(0.03)$^{p-s}$	12.71(0.87)$^{d-j}$	14.10(0.29)$^{m-r}$	14.95(1.03)$^{q-u}$	15.81(0.87)$^{t-w}$
Mweru	fresh	0.41(0.03)^{a}	0.47(0.03)^{abc}	0.51(0.03)^{cde}	0.56(0.06)$^{e-k}$	13.86(0.42)$^{k-q}$	17.14(1.43)^{xy}	16.33(1.79)^{vwx}	18.19(0.91)^{yzA}
	FBP	0.58(0.02)$^{f-o}$	0.56(0.03)$^{e-l}$	0.73(0.02)$^{u-x}$	0.86(0.00)^{zA}	13.71(0.00)$^{j-p}$	15.81(1.15)$^{t-w}$	13.62(0.82)$^{i-p}$	13.67(0.32)$^{i-p}$
	FAP	0.78(0.01)^{xy}	0.63(0.02)$^{m-r}$	0.65(0.01)$^{p-s}$	0.79(0.12)^{xy}	12.71(0.87)$^{d-j}$	12.57(0.29)$^{d-i}$	14.71(0.98)$^{p-t}$	13.67(0.86)$^{i-p}$
Kariba	fresh	0.41(0.03)^{a}	0.48(0.03)^{bcd}	0.55(0.03)$^{e-j}$	0.65(0.06)$^{p-s}$	13.86(0.42)$^{k-q}$	11.67(0.95)^{bcd}	12.29(0.49)$^{d-g}$	12.00(0.43)$^{b-f}$
	FBP	0.58(0.02)$^{f-o}$	0.76(0.18)^{wxy}	0.79(0.02)^{xy}	0.81(0.01)^{yz}	13.71(0.00)$^{j-p}$	20.05(0.67)^{C}	19.29(0.50)^{ABC}	19.43(0.93)^{BC}
	FAP	0.78(0.01)^{xy}	0.61(0.02)$^{j-q}$	0.71(0.03)$^{s-w}$	0.74(0.01)$^{v-y}$	12.71(0.87)$^{d-j}$	14.00(0.29)$^{l-r}$	15.29(0.75)$^{s-v}$	15.10(1.00)$^{r-u}$
Kampolombo	fresh	0.41(0.03)^{a}	0.52(0.02)$^{c-f}$	0.55(0.02)$^{e-j}$	0.58(0.00)$^{f-m}$	13.86(0.42)$^{k-q}$	10.43(1.38)^{a}	11.95(0.79)$^{b-e}$	12.81(0.45)$^{e-k}$
	FBP	0.58(0.02)$^{f-o}$	0.56(0.02)$^{e-l}$	0.71(0.02)$^{s-w}$	0.80(0.02)^{y}	13.71(0.00)$^{j-p}$	18.10(0.50)^{yz}	18.38(1.03)^{zAB}	18.86(1.07)^{zAB}
	FAP	0.78(0.01)^{xy}	0.63(0.00)$^{l-r}$	0.67(0.00)$^{q-u}$	0.72(0.04)$^{t-x}$	12.71(0.87)$^{d-j}$	14.52(0.17)$^{n-o}$	14.10(0.86)$^{m-r}$	14.67(0.35)$^{o-s}$
Chila	fresh	0.41(0.03)^{a}	0.53(0.03)$^{c-h}$	0.55(0.02)$^{d-i}$	0.67(0.02)$^{q-u}$	13.86(0.42)$^{k-q}$	10.91(0.36)^{ab}	12.95(0.71)$^{e-l}$	11.91(0.50)$^{b-e}$
	FBP	0.58(0.02)$^{f-o}$	0.58(0.01)$^{e-m}$	0.71(0.02)$^{s-w}$	0.86(0.02)^{zA}	13.71(0.00)$^{j-p}$	16.48(0.58)^{wx}	16.48(0.35)^{wx}	15.38(0.71)$^{s-w}$
	FAP	0.78(0.01)^{xy}	0.58(0.01)$^{f-m}$	0.63(0.02)$^{l-r}$	0.67(0.05)$^{p-u}$	12.71(0.87)$^{d-j}$	16.00(0.65)^{uvw}	16.43(0.37)^{wx}	16.29(0.42)^{vwx}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen before proofing, FAP=frozen after proofing, CFSL=cassava flour substitution level.

9.3.1.5 Bread weight loss

Weight losses for fresh bread ranged from 10.43–13.14% (Table 9.4) across all levels of cassava flour, and there was no clear pattern of changes in weight loss. The weight loss in the fresh wheat bread was 13.86±0.87% and similar to those of *Bangweulu* and *Chila* at 20% and *Bangweulu* at 30% CFSL. The weight loss for FBP were in the range 13.57-20.05, 12.81-19.29 and 13.10-19.43% at cassava flour substitution levels of 10, 20 and 30%, respectively, and varied among varieties. The lowest and highest weight losses were recorded in *Bangweulu* and *Kariba* at 20% and 10% cassava flour, respectively. The weight loss for FBP wheat sample was 13.71%, and was similar ($p>0.05$) to *Katobamputa* at 10% cassava flour level. The weight loss for FAP were in the range 14.00-14.50, 14.00-16.43 and 14.05-16.29% at 10, 20 and 30% CFSL, respectively, and varied among cassava varieties. The lowest weight was recorded in *Kariba* and *Bangweulu* at 10 and 20% CFSL, respectively, and highest recorded in *Chila* at 30%. The FAP wheat bread had weight loss of 12.71% and was significantly lower ($p<0.05$) than weight loss of wheat bread from FBP and fresh. The trend for weight losses was fresh>FBP>FAP.

The stickiness negatively correlated ($r = -0.33$, $p<0.001$) with weight loss, implying that frozen doughs (FBP and FAP) with higher stickiness exhibited higher bread weight loss compared to fresh bread. This might be due to yeast damage and ice crystallization during frozen storage resulting in thinning of gluten network and exudation of water (Lu and Grant, 1999) which collects into pools, and thus can evaporate during thawing and re-kneading.

Table 9.5 Correlation coefficients of gluten contents, particle size (D90), and rheological and bread characteristics

Parameter	DS	WAC	DDT	DC	D90	BV	BD	SV	WL	PA	P	AMY	Pr	Lip	Fib	Glu
Stickiness	1															
Water Absorption	-0.44**	1														
Development Time	0.41**	-0.44	1													
Consistency	-0.67*	0.45**	-0.45**	1												
D90	0.41**	-0.26***	0.45	-0.48**	1											
Bread Volume	-0.76*	0.37	-0.53	0.57	-0.56	1										
Bread Density	0.76*	-0.40	0.49	-0.55	0.45	-0.98	1									
Specific Volum	-0.75*	0.40	-0.50	0.55	-0.48	0.99	-0.99	1								
Weight loss	0.33**	0.02	0.39	-0.30	0.64	-0.46	0.32	-0.31	1							
Pore Area	-0.03	0.01	-0.06	0.01	-0.12	0.04	-0.04	0.04	0.03	1						
Porosity	-0.64*	0.37	-0.30	0.41	-0.58	0.63	-0.56	0.56	-0.57	-0.01	1					
Amylose	-0.34	-0.12	-0.16	0.18	-0.16	0.23	-0.19	0.16	-0.49	0.00	0.37	1				
Protein	-0.59	0.34**	-0.28	0.60	-0.83	0.60	-0.53	0.55	-0.41	0.12	0.76	0.15	1			
Lipid	-0.62	0.36**	-0.31	0.61	-0.79	0.52	-0.46	0.48	-0.35	0.05	0.70	0.14	0.96	1		
Fiber	-0.61	0.36	-0.29	0.60	-0.81	0.62	-0.55	0.57	-0.47	0.08	0.80	0.16	0.98	0.93	1	
Gluten	-0.44**	0.60*	-0.28***	0.54*	-0.53*	0.62	-0.61	0.63	-0.17	0.06	0.66	0.08	0.70*	0.62	0.70	1

Significance: p<0.05*, P<0.01**, P<0.001***. DS=dough stickiness, WAC=water absorption capacity, DDT=dough development time, DC=dough consistency, D90=particle size at 90% cumulative distribution, SV=specific volume, BD=bread density, BV=bread volume, WL=weight loss, PA=pore area, P=porosity, Amy=amylose, Pr=protein, Lip=lipid, fib=fiber, Glu=gluten

9.3.2 Bread pore size characteristics

9.3.2.1 Bread pore area

The average pore area of bread from *fresh* varied according to cassava flour level, 0.39–0.76 mm^2 at 10%, 0.38–0.69 mm^2 at 20%, and 0.27–0.85 mm^2 at 30% (Table 9.6). The pore size of the wheat (control) bread sample was 0.47 mm^2 and generally increased with an increase in CFSL. The average bread pore size for FBP were in the range 0.06-0.82, 0.50-1.99 and 1.80 mm^2 at 10, 20 and 30% CFSL, respectively. The lowest and highest pore areas were recorded in *Katobamputa* and *Chila* at 10 and 20% CFSL, respectively. The wheat bread had average pore area of 0.91 mm^2, and was observed to decrease with increase in CFSL except in *Kariba*, *Chila* and *Kampolombo* at 10, 20 and 30% cassava flour level, respectively.

The average pore area for FAP were in the range 0.49-1.78, 0.38-1.38 and 0.30-1.63 mm^2, at 10, 20 and 30% CFSL, respectively. The lowest and highest pore sizes were recorded in *Chila* and *Kariba*, at 30 and 10% CFSL, respectively. The pore area of wheat bread for FAP was 1.18 mm^2 and decreased with increase in CFSL except for significant ($p<0.05$) increase in *Mweru* and *Kariba* at 10% CFSL. The wheat bread crumb pore size was higher than those of bread from FBP and FAP. The frozen doughs were characterized with large crumb pore areas, the formation of fissures and depressions, and were not uniformly distributed (Figure 9.1). The large crumb pore areas in frozen doughs were random but few, and not uniformly distributed. Similar was observed by Matuda *et al.* (2008) in wheat frozen doughs, however, demonstrated that frozen dough formulations with shortening and emulsifiers contained large numbers of small gas cells of uniform size.

Table 9.6 Cross sectional area (mm^2) of pores in bread crumb made from flour blends of wheat and cassava flours

Variety	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	fresh	0.45(0.01)^{abcd}	0.51(0.010)^{abcd}	1.01(1.42)^{abcd}	0.74(0.66)^{abcd}
	FBP	0.91(0.90)^{abcd}	0.62(0.91)^{abcd}	0.50(0.01)^{abcd}	0.22(0.19)^{ab}
	FAP	1.18(1.00)^{abcd}	0.49(0.61)^{abcd}	0.74(0.96)^{abcd}	1.09(1.72)^{abcd}
Katobamputa	fresh	0.45(0.01)^{abcd}	0.76(0.01)^{abcd}	0.70(0.01)^{abcd}	0.47(0.00)^{abcd}
	FBP	0.91(0.90)^{abcd}	0.06(0.06)^{a}	0.51(0.31)^{abcd}	0.30(0.32)^{abc}
	FAP	1.18(1.00)^{abcd}	1.01(1.42)^{abcd}	1.00(0.92)^{abcd}	0.44(0.48)^{abcd}
Mweru	fresh	0.45(0.01)^{abcd}	0.39(0.01)^{abc}	0.41(0.01)^{abc}	0.48(0.01)^{abcd}
	FBP	0.91(0.90)^{abcd}	0.82(0.97)^{abcd}	1.04(1.70)^{abcd}	0.67(0.57)^{abcd}
	FAP	1.18(1.00)^{abcd}	1.76(1.18)^{bcd}	1.24(2.05)^{abcd}	0.67(0.57)^{abcd}
Kariba	fresh	0.45(0.01)^{abcd}	0.51(0.02)^{abcd}	0.52(0.02)^{abcd}	0.27(0.02)^{abc}
	FBP	0.91(0.90)^{abcd}	0.51(0.02)^{abcd}	0.78(0.93)^{abcd}	0.12(0.17)^{a}
	FAP	1.18(1.00)^{abcd}	1.78(30.2)^{cd}	0.78(0.93)^{abcd}	1.63(1.51)^{abcd}
Kampolombo	fresh	0.45(0.01)^{abcd}	0.64(0.01)^{abcd}	0.46(0.01)^{abcd}	0.60(0.01)^{abcd}
	FBP	0.91(0.90)^{abcd}	0.74(0.66)^{abcd}	0.74(0.66)^{abcd}	1.80(2.02)^{abcd}
	FAP	1.18(1.00)^{abcd}	0.74(0.66)^{abcd}	1.38(2.34)^{abcd}	0.45(0.65)^{abcd}
Chila	fresh	0.45(0.01)^{abcd}	0.60(0.01)^{abcd}	0.38(0.00)^{abc}	0.61(0.02)^{abcd}
	FBP	0.91(0.90)^{abcd}	0.57(0.59)^{abcd}	1.99(2.29)^{d}	0.29(0.37)^{abc}
	FAP	1.18(1.00)^{abcd}	1.32(1.56)^{abcd}	0.68(0.91)^{abcd}	0.89(1.22)^{abcd}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen-before-proofing, FAP=frozen-after-proofing, CFSL=cassava flour substitution level.

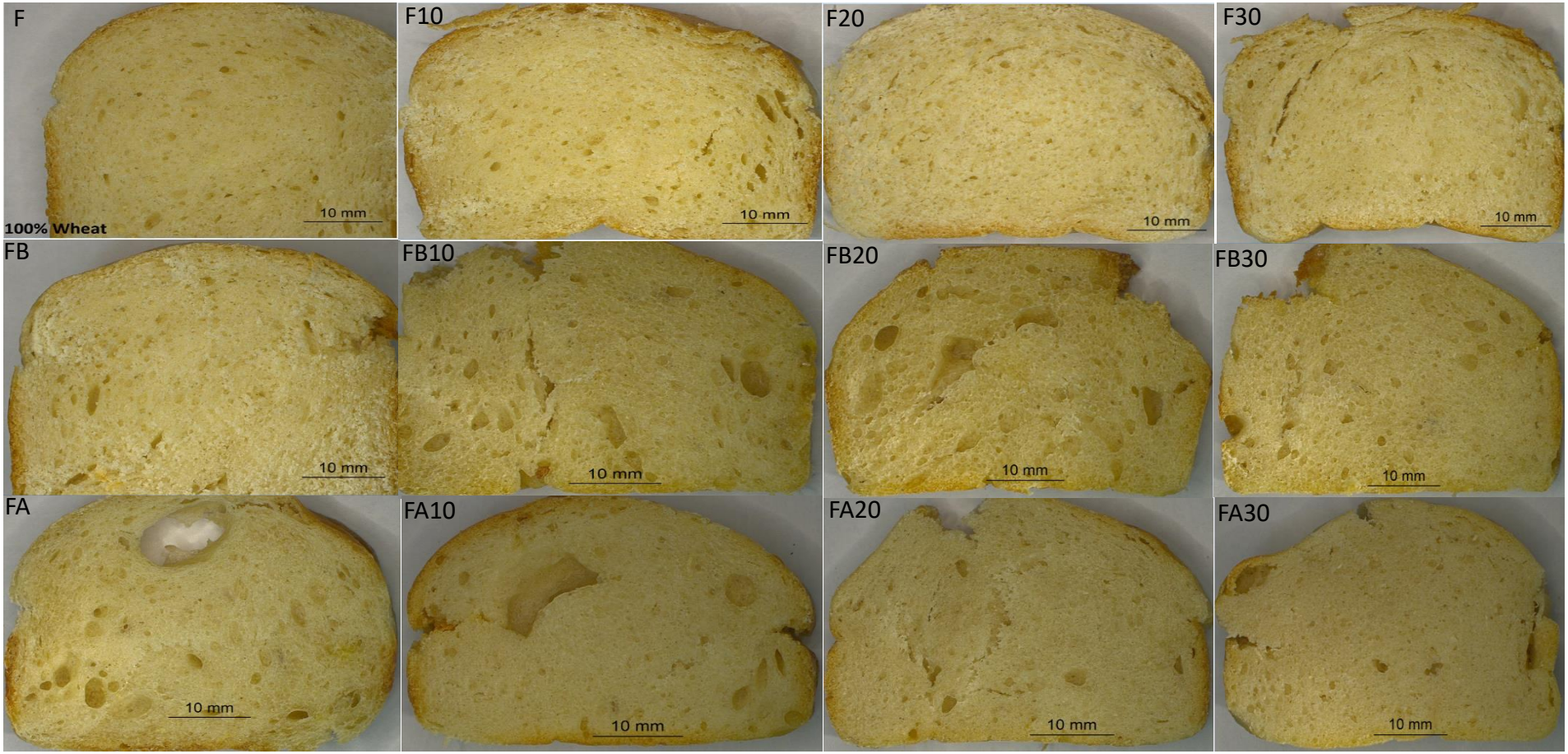

Figure 9.1 Bread crumb from flour blend of cassava/wheat of variety Kampolombo at 10, 20 and 30% CFSL. Photos at scale 10 mm obtained using microscope (Leica MZ16) with Leica camera (Leica DFC450C), Leica Application Suite (LAS). Bread from fresh dough: F=control (100%whaeat), F10=10%CFSL, F20=20%CFSL, F30=30%CFSL; Bread from frozen before proofing: FB=control (100%whaeat), FB10=10%CFSL, FB20=20%CFSL, FB30=30%CFSL; Bread from frozen after proofing: FA=control (100%wheat), FA10=10%CFSL, FA20=20%CFSL, FA30=30%CFSL. CFSL=cassava flour substitution level.

9.3.2.2 Bread porosity

The porosity of wheat bread sample from fresh dough was 71.48±0.52% (Table 9.7) and decreased with increase in CFSL. The bread crumb porosity for FBP were in the range 17.76-69.39, 18.99-40.01 and 15.30-48.73 at 10, 20 and 30% CFSL, respectively. The lowest and highest porosity were recorded in *Kariba* and *Bangweulu*, respectively, at 30% CFSL. The FBP wheat bread had a porosity of 65% and decreased with increase in CFSL. The bread crumb porosity for FAP were in the range 18.63-63.65, 17.55-37.22 and 17.33-43.55 at 10, 20 and 30% CFSL. The lowest and highest porosity were recorded in *Mweru* and *Katobamputa* at 30 and 10% CFSL, respectively. The porosity of the FAP wheat bread was 55.34 and decreased with increase in CFSL. The porosity of fresh wheat bread was significantly higher ($p<0.05$) than those of bread from FBP and FAP.

In frozen dough, stickiness showed a weak positive correlation ($r = 0.10$, $p<0.0001$) with bread crumb porosity. Similarly, gluten positively correlated with stickiness ($r = 0.33$, $p<0.01$) and porosity ($r = 0.66$, $p<0.05$). This suggests that regardless of stickiness, gluten network was the determining factor in the gas cell production which justifies the porosity trend fresh>FBP>FAP. The trend is similar to that reported by Halagarda (2017) in which bread crumb from frozen dough had lower porosity compared to fresh bread. The crumb walls forming the gas cell can be damaged by expanded ice crystals during frozen storage, which may cause collapse and reduction of porosity. Carr *et al.* (2006) reported shrinkage and reduction of crumb porosity of frozen dough bread.

Table 9.7 Porosity (%) in bread crumb made from flour blends of wheat and cassava flours

Variety	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	Fresh	71.48(0.52)^{B}	68.82(0.47)^{B}	46.08(0.51)^{uv}	52.91(1.61)^{w}
	FBP	65.00(2.00)A	60.77(1.33)^{yz}	40.01(1.01)^{rs}	48.73(1.60)^{v}
	FAP	55.34(5.27)^{wx}	57.82(2.80)^{xy}	37.22(3.22)^{qr}	43.55(1.26)^{tu}
Katobamputa	Fresh	71.48(0.52)^{B}	78.54(1.91)^{C}	37.62(0.29)^{qr}	24.19(0.17)^{jk}
	FBP	65.00(2.00)A	69.39(0.78)^{B}	31.89(1.64)^{no}	20.34(0.59)$^{d-i}$
	FAP	55.34(5.27)^{wx}	63.65(2.65)^{zA}	27.67(2.88)^{lm}	18.68(0.55)$^{b-g}$
Mweru	Fresh	71.48(0.52)^{B}	25.72(0.35)^{kl}	19.98(0.80)$^{c-h}$	20.40(0.27)$^{d-i}$
	FBP	65.00(2.00)A	22.55(2.34)^{hijk}	18.99(0.66)$^{c-g}$	18.22(1.34)$^{b-f}$
	FAP	55.34(5.27)^{wx}	19.78(1.51)$^{c-h}$	17.55(0.51)^{bcd}	17.33(1.52)^{bcd}
Kariba	Fresh	71.48(0.52)^{B}	18.09(0.33)$^{b-e}$	21.50(0.23)$^{f-j}$	16.67(0.23)^{abc}
	FBP	65.00(2.00)A	17.76(1.49)^{bcd}	19.44(0.76)$^{c-h}$	15.30(1.17)^{ab}
	FAP	55.34(5.27)^{wx}	18.63(3.73)$^{b-g}$	18.89(1.64)$^{c-g}$	13.63(0.55)^{a}
Kampolombo	Fresh	71.48(0.52)^{B}	41.52(0.09)st	22.79(0.64)$^{h-k}$	36.21(1.02)^{pq}
	FBP	65.00(2.00)A	36.62(2.89)^{qr}	20.29(0.52)$^{d-i}$	32.31(1.14)^{no}
	FAP	55.34(5.27)^{wx}	33.12(3.75)^{op}	18.50(0.88)$^{b-g}$	29.69(0.57)^{mn}
Chila	Fresh	71.48(0.52)^{B}	25.4(0.47)^{kl}	21.22(0.46)$^{e-j}$	21.74(0.74)$^{g-j}$
	FBP	65.00(2.00)A	23.44(2.71)^{ijk}	19.96(0.34)$^{c-h}$	20.11(0.30)$^{d-i}$
	FAP	55.34(5.27)^{wx}	20.33(1.62)$^{d-i}$	19.15(0.51)$^{c-g}$	18.35(0.56)$^{b-f}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP = Frozen-before-proofing, FAP = Frozen-after-proofing, CFSL=cassava flour substitution level.

9.3.3 Bread crumb color characteristics

The results on bread crumb color characteristics are shown in tables; lightness (Table 9.8), redness-greenness (Table 9.9), yellowness (Table 9.10) and whiteness index (Table 9.11).

9.3.3.1 Bread crumb lightness

The bread L* of *fresh* ranged from 72.62–75.42, 71.27–72.83, and 71.17–75.55 (Table 12) at 10%, 20%, and 30% CFSL, respectively, and were not significantly different ($p<0.05$) from wheat bread control (74.45±0.02). The lightness of bread crumb for FBP were in the range 69.03-71.18, 68.19-73.07 and 69.22-73.32 at 10, 20 and 30% CFSL, respectively. The lowest and highest were recorded in *Kariba* and *Katobamputa* at 10 and 20% cassava flour, respectively. The lightness for FBP bread crumb was 71.74 and decreased significantly ($p<0.05$) with an increase in CFSL. Wheat glume color can possibly be associated with gluten color (Leisle *et al.*, 1981). Thus high lightness of cassava was diluent against the color of gluten (brownish). The lightness of bread crumb for FAP ranged from 68.63-72.14, 69.50-71.92 and 65.66-72.32 at 10, 20 and 30% CFSL. The lowest and highest lightness were recorded in *Kariba*

at 10 and 30% CFSL, respectively. The lightness of FAP wheat bread crumb was 70.39. The L* trend was fresh>FBP>FAP. Similarly, Ayati et al. (2017) reported low crumb lightness in frozen doughs. Stickiness negatively correlated ($r = -0.36$, $p<0.001$) with crumb L*, suggesting that sticky doughs had a low lightness. The desiccation effect due to ice sublimation on the dough surface during freezing conditions (Ramírez-Wong and Magaña-Barajas, 2018) may contribute to the decreased lightness of frozen doughs. The variation of lightness among varieties could be due to differences in flour particle size. The L* of crumb negatively correlated ($r = -0.23$, $p<0.001$) with particle size. This implies that bread baked from finer flour yielded high lightness.

Table 9.8 Lightness (L*) color of bread crumb made from flour blends of wheat and cassava flours

Variety	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	fresh	74.45(0.02)^{yzA}	73.57(0.14)$^{v-y}$	72.38(0.56)$^{p-v}$	71.79(0.05)$^{m-t}$
	FBP	71.74(0.29)$^{l-s}$	70.73(1.14)$^{g-o}$	71.44(0.31)$^{k-r}$	69.22(0.12)$^{b-g}$
	FAP	70.39(0.54)$^{e-m}$	72.14(1.86)$^{o-v}$	71.25(2.31)$^{i-q}$	69.87(1.65)$^{c-j}$
Katobamputa	fresh	74.45(0.02)^{yzA}	72.62(0.26)$^{o-w}$	72.59(0.30)$^{p-v}$	75.17(0.01)^{zA}
	FBP	71.74(0.29)$^{l-s}$	71.18(0.67)$^{i-q}$	73.07(0.03)$^{s-y}$	73.32(0.03)$^{u-y}$
	FAP	70.39(0.54)$^{e-m}$	71.84(0.28)$^{m-u}$	71.07(1.49)$^{i-p}$	65.66(0.66)^{a}
Mweru	fresh	74.45(0.02)^{yzA}	73.20(0.15)$^{s-y}$	74.52(0.52)^{yzA}	75.55(0.52)^{A}
	FBP	71.74(0.29)$^{l-s}$	69.12(0.16)$^{b-f}$	72.82(1.06)$^{r-x}$	73.19(0.07)$^{s-y}$
	FAP	70.39(0.54)$^{e-m}$	69.83(0.88)$^{c-j}$	70.26(0.72)$^{d-l}$	72.15(0.44)$^{o-v}$
Kariba	fresh	74.45(0.02)^{yzA}	73.49(0.33)$^{v-y}$	71.27(1.23)$^{j-q}$	73.57(0.01)$^{v-y}$
	FBP	71.74(0.29)$^{l-s}$	69.03(0.22)$^{b-e}$	68.19(0.16)^{b}	70.10(2.25)$^{c-k}$
	FAP	70.39(0.54)$^{e-m}$	68.63(0.16)^{bc}	69.50(1.28)$^{b-h}$	72.31(0.89)$^{p-v}$
Kampolombo	fresh	74.45(0.02)^{yzA}	73.28(0.30)$^{t-y}$	72.81(0.22)$^{r-x}$	74.29(0.48)^{xyzA}
	FBP	71.74(0.29)$^{l-s}$	68.76(3.93)^{bcd}	69.90(0.12)$^{c-j}$	70.57(0.72)$^{f-n}$
	FAP	70.39(0.54)$^{e-m}$	69.75(2.02)$^{c-i}$	71.02(1.22)$^{i-p}$	70.58(0.03)$^{f-n}$
Chila	fresh	74.45(0.02)^{yzA}	75.42(0.11)^{yzA}	72.83(0.29)$^{r-x}$	73.93(0.70)$^{w-z}$
	FBP	71.74(0.29)$^{l-s}$	69.88(0.15)$^{c-j}$	69.51(1.84)$^{b-h}$	69.40(0.19)$^{b-g}$
	FAP	70.39(0.54)$^{e-m}$	70.93(1.63)$^{h-p}$	71.92(0.10)$^{n-u}$	70.09(1.64)$^{c-k}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen before proofing, FAP=frozen after proofing, CFSL=cassava flour substitution level.

9.3.3.2 Crumb redness-greenness

The a^* for fresh ranged between -0.35 (green) and 0.67 (red) and varied significantly ($p<0.05$) across blend ratios. The bread crumb redness for FBP were in the range 0.76-1.20, 0.42-1.10 and 0.86-1.61 at 10, 20 and 30% CFSL, respectively. The lowest and highest redness were recorded

in *Mweru* and *Chila* at 20 and 30% CFSL, respectively. The FBP wheat bread crumb had redness value of 0.66 and increased with increase in CFSL except for *Mweru* at 20% cassava flour. The FAP greenness (-0.17) of bread crumb was recorded in *Bangweulu* at 10%, and redness ranged from 0.44-1.33, 0.81-1.13 and 0.92-1.59 at 10, 20 and 30% CFSL. The redness value (1.02) for FAP wheat bread did not show a clear trend against CFSL. The non-enzymatic and Maillard reactions during baking (Helou *et al.*, 2016), and condensation of volatiles (alcohols, acids) (Pico *et al.*, 2015) in the bread crumb, and may contribute to redness and a reduced lightness of the crumb.

Table 9.9 Redness-greenness (a*) of bread crumb made from flour blends of wheat and cassava flours

Variety	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	fresh	0.36(0.02)$^{f-j}$	0.00(0.05)^{abc}	-0.31(0.07)^{a}	0.05(0.10)$^{b-e}$
	FBP	0.66(0.13)$^{j-p}$	0.96(0.20)$^{p-w}$	0.79(0.03)$^{m-r}$	1.22(0.04)$^{w-z}$
	FAP	1.02(1.02)$^{r-x}$	-0.17(0.36)^{abc}	0.82(0.37)$^{m-t}$	0.92(0.10)$^{n-w}$
Katobamputa	fresh	0.36(0.02)$^{f-j}$	0.13(0.06)$^{c-g}$	-0.25(0.09)^{ab}	0.46(0.02)$^{h-l}$
	FBP	0.66(0.13)$^{j-p}$	0.76(0.01)$^{l-r}$	0.59(0.07)$^{i-m}$	0.93(0.03)$^{o-w}$
	FAP	1.02(1.02)$^{r-x}$	0.44(0.13)$^{h-k}$	0.81(0.58)$^{m-s}$	1.59(0.01)^{A}
Mweru	fresh	0.36(0.02)$^{f-j}$	0.58(0.04)$^{i-m}$	0.65(0.02)$^{j-o}$	0.41(0.05)$^{g-k}$
	FBP	0.66(0.13)$^{j-p}$	0.87(0.06)$^{m-u}$	0.42(0.03)$^{g-k}$	0.86(0.03)$^{m-u}$
	FAP	1.02(1.02)$^{r-x}$	1.12(0.50)$^{t-y}$	1.04(0.37)$^{r-x}$	0.98(0.13)$^{r-w}$
Kariba	fresh	0.36(0.02)$^{f-j}$	0.04(0.03)$^{b-e}$	0.21(0.03)$^{d-h}$	0.31(0.02)$^{e-i}$
	FBP	0.66(0.13)$^{j-p}$	0.91(0.02)$^{n-v}$	1.02(0.02)$^{r-x}$	0.96(0.06)$^{p-w}$
	FAP	1.02(1.02)$^{r-x}$	1.10(0.01)$^{s-y}$	0.66(0.38)$^{j-p}$	1.31(0.62)^{xyzA}
Kampolombo	fresh	0.36(0.02)$^{f-j}$	-0.35(0.08)^{a}	0.39(0.01)$^{f-k}$	0.62(0.05)$^{j-n}$
	FBP	0.66(0.13)$^{j-p}$	0.85(0.21)$^{m-u}$	0.97(0.04)$^{q-w}$	0.98(0.02)$^{r-w}$
	FAP	1.02(1.02)$^{r-x}$	1.23(0.14)$^{w-z}$	1.13(0.35)$^{u-y}$	1.39(0.31)^{yzA}
Chila	fresh	0.36(0.02)$^{f-j}$	0.26(0.07)$^{d-h}$	0.10(0.02)$^{c-f}$	0.67(0.10)$^{k-q}$
	FBP	0.66(0.13)$^{j-p}$	1.20(0.10)$^{v-y}$	1.10(0.06)$^{s-y}$	1.61(0.04)^{A}
	FAP	1.02(1.02)$^{r-x}$	1.33(0.41)^{xyzA}	0.95(0.01)$^{o-w}$	1.52(0.47)^{zA}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen before proofing, FAP=frozen after proofing, CFSL=cassava flour substitution level.

9.3.3.3 Crumb Yellowness (b*)

The b^* for fresh bread ranged between 19.62 and 20.89 (Table 9.10) across the blend ratios. The FBP yellowness of the bread crumb ranged 20.49-21.76, 20.28-21.20 and 20.26-22.26 at 10, 20 and 30% CFSL, respectively. The lowest yellowness was registered in *Mweru* and *Kariba* at 20%, and highest in *Chila* at 10% CFSL. The wheat bread crumb had b* value of 21.30. The b*

of the bread crumb for FAP were in the range 18.61-21.15, 19.03-20.90 and 19.62-21.46 at 10, 20 and 30% CFSL, respectively. The lowest and highest b* were registered in *Bangweulu* and *Chila* at 10 and 20% cassava flour, respectively. The FAP yellowness of wheat bread crumb was 22.28 and decreased with increase in CFSL. The b* of wheat breadcrumb for fresh, FBP and FAP were insignificantly different ($p<0.05$). The yellowness could be attributed to residues of carotenoids (Hidalgo *et al.*, 2010). The variation in varieties could be attributed to differences in flour particle size distribution. The crumb b* negatively correlated ($r = -0.46$, $p<0.01$) with flour particle size, an indication that yellowness was more pronounced in bread baked from finer flours. The migration of wheat pigments and volatiles would be quicker in finer flours. *Chila* (278.49±0.00 μm) had higher crumb yellowness than *Bangweulu* (312±0.00 μm) at 90% cumulative of finer particles.

Table 9.10 Yellowness (b*) of bread crumb made from flour blends of wheat and cassava flours

Variety	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	fresh	21.50(0.03)$^{h-m}$	20.56(0.08)$^{c-k}$	20.47(0.10)$^{b-j}$	20.03(0.63)$^{a-g}$
	FBP	21.30(0.79)$^{g-m}$	21.07(0.08)$^{e-m}$	20.53(0.28)$^{c-j}$	21.17(0.04)$^{f-m}$
	FAP	22.28(0.87)^{lm}	18.61(0.10)^{a}	19.56(1.44)$^{a-d}$	19.62(0.79)$^{a-e}$
Katobamputa	fresh	21.50(0.03)$^{h-m}$	21.20(0.14)$^{f-m}$	19.42(0.49)^{abc}	20.89(0.04)$^{d-l}$
	FBP	21.30(0.79)$^{g-m}$	20.49(0.11)$^{b-j}$	20.63(0.25)$^{c-k}$	20.26(0.10)$^{b-h}$
	FAP	22.28(0.87)^{lm}	20.40(0.61)$^{b-j}$	19.39(0.59)^{abc}	22.01(0.17)^{klm}
Mweru	fresh	21.50(0.03)$^{h-m}$	22.33(0.05)^{lm}	22.49(0.12)^{m}	21.81(0.09)$^{j-m}$
	FBP	21.30(0.79)$^{g-m}$	21.07(0.03)$^{e-m}$	20.28(0.26)$^{b-h}$	21.04(0.30)$^{e-m}$
	FAP	22.28(0.87)^{lm}	20.03(2.75)$^{a-g}$	20.90(2.36)$^{d-l}$	20.71(0.85)$^{c-k}$
Kariba	fresh	21.50(0.03)$^{h-m}$	20.75(0.25)$^{c-k}$	20.63(0.17)$^{c-k}$	20.53(0.06)$^{c-j}$
	FBP	21.30(0.79)$^{g-m}$	20.96(0.07)$^{d-l}$	20.28(0.08)$^{b-h}$	21.36(0.39)$^{g-m}$
	FAP	22.28(0.87)^{lm}	19.75(0.02)$^{a-f}$	19.03(1.28)^{ab}	20.76(2.25)$^{c-k}$
Kampolombo	fresh	21.50(0.03)$^{h-m}$	19.62(0.37)$^{a-e}$	20.99(0.13)$^{d-l}$	20.60(0.07)$^{c-k}$
	FBP	21.30(0.79)$^{g-m}$	21.07(0.79)$^{e-m}$	20.63(0.15)$^{c-k}$	20.32(0.09)$^{b-j}$
	FAP	22.28(0.87)^{lm}	21.15(0.27)$^{f-m}$	20.46(1.78)$^{b-j}$	21.74(1.49)$^{i-m}$
Chila	fresh	21.50(0.03)$^{h-m}$	21.39(0.19)$^{g-m}$	21.14(0.13)$^{f-m}$	21.15(0.25)$^{f-m}$
	FBP	21.30(0.79)$^{g-m}$	21.76(0.30)$^{i-m}$	21.20(0.79)$^{f-m}$	22.26(0.17)^{lm}
	FAP	22.28(0.87)^{lm}	20.31(1.93)$^{b-i}$	20.67(0.12)$^{c-k}$	21.46(2.21)$^{g-m}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen-before-proofing, FAP=frozen-after-proofing, CFSL=cassava flour substitution level.

9.3.3.4 Crumb whiteness index

The whiteness for fresh bread crumb ranged between 65.38 and 67.54 (Table 9.11) and increased with increase in CFSL. The whiteness of the fresh wheat bread was 66.60±0.01 and was similar ($p>0.05$) to fresh bread from *Bangweulu, Kariba, Katobamputa* and *Chila* at 30% CFSL. The whiteness index of bread crumb for FBP ranged from 62.29-64.63, 62.08-66.07 and 62.13-66.49 at 10, 20 and 30% CFSL, respectively. The lowest and highest whiteness for FBP bread were recorded in *Mweru* and *Katobamputa* at 20 and 30% CFSL, respectively. The whiteness of the FBP wheat bread was 64.61 and was similar ($p>0.05$) with *Katobamputa* at 10 and 20% CFSL. The whiteness of bread crumb for FAP were in the range 62.91-65.22, 63.62-65.21 and 59.18-65.35 at 10, 20 and 30% CFSL, respectively. The lowest and highest whiteness were registered in *Katobamputa* and *Kariba* at 30% CFSL. The whiteness of FAP wheat bread crumb was 62.93 and increased with increase in CFSL. The differences in whiteness could be ascribed to degeneration of yeast and depolymerization of gluten. Yeast cell death is accompanied by the release of reactive species (superoxide anion, hydroxyl radical, hydrogen peroxide and minerals) (Carmona-Gutierrez *et al.*, 2018). These compounds including accumulation low molecular weight compounds due to gluten deterioration may trigger oxidative reactions upon thawing with subsequent colorations which may possibly contribute to decreased whiteness.

Table 9.11 Whiteness index of bread crumb made from flour blends of wheat and cassava flours

Variety	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	fresh	66.60(0.00)$^{r-u}$	66.51(0.16)$^{f-o}$	65.62(0.39)$^{m-t}$	65.40(0.39)$^{l-s}$
	FBP	64.61(0.63)$^{g-p}$	63.92(0.88)$^{c-m}$	64.82(0.10)$^{h-q}$	62.62(1.00)$^{b-e}$
	FAP	62.93(0.83)$^{b-g}$	66.46(1.17)$^{q-u}$	65.21(2.73)$^{j-r}$	64.02(1.00)$^{d-m}$
Katobamputa	fresh	66.60(0.00)$^{r-u}$	65.38(0.12)$^{l-s}$	66.40(0.04)$^{q-u}$	67.54(0.03)^{u}
	FBP	64.61(0.63)$^{g-p}$	64.63(0.47)$^{g-p}$	66.07(0.12)$^{o-u}$	66.49(0.03)$^{q-u}$
	FAP	62.93(0.83)$^{b-g}$	65.22(0.58)$^{j-r}$	65.15(2.70)$^{j-r}$	59.18(0.47)^{a}
Mweru	fresh	66.60(0.00)$^{r-u}$	65.11(0.08)$^{j-r}$	66.01(0.31)$^{o-u}$	67.23(0.40)^{tu}
	FBP	64.61(0.63)$^{g-p}$	62.60(0.14)$^{b-e}$	66.08(0.69)$^{o-u}$	65.91(0.12)$^{n-u}$
	FAP	62.93(0.83)$^{b-g}$	63.74(2.25)$^{b-l}$	63.62(1.92)$^{b-k}$	65.28(0.86)$^{k-r}$
Kariba	fresh	66.60(0.00)$^{r-u}$	66.33(0.41)$^{p-u}$	64.63(0.89)$^{g-p}$	66.53(0.03)$^{q-u}$
	FBP	64.61(0.63)$^{g-p}$	62.59(0.14)$^{b-e}$	62.26(0.09)^{bc}	63.22(1.58)$^{b-h}$
	FAP	62.93(0.83)$^{b-g}$	62.91(0.12)$^{b-g}$	64.04(1.77)$^{e-m}$	65.35(2.22)$^{k-s}$
Kampolombo	fresh	66.60(0.00)$^{r-u}$	66.85(0.47)$^{r-u}$	65.65(0.25)$^{m-t}$	67.05(0.36)^{stu}
	FBP	64.61(0.63)$^{g-p}$	62.29(3.69)^{bcd}	63.49(0.08)$^{b-j}$	64.22(0.55)$^{e-n}$
	FAP	62.93(0.83)$^{b-g}$	63.05(1.54)$^{b-g}$	64.50(2.03)$^{f-o}$	63.38(0.19)$^{b-i}$
Chila	fresh	66.60(0.00)$^{r-u}$	67.42(0.07)^{u}	65.57(0.29)$^{m-t}$	66.42(0.39)$^{q-u}$
	FBP	64.61(0.63)$^{g-p}$	62.82(0.05)$^{b-f}$	62.82(1.08)$^{b-f}$	62.13(0.26)^{b}
	FAP	62.93(0.83)$^{b-g}$	64.51(2.45)$^{f-o}$	65.12(0.00)$^{i-r}$	63.15(2.63)$^{b-h}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen before proofing, FAP=frozen after proofing, CFSL=cassava flour substitution level.

9.3.4 Bread crust color characteristics

9.3.4.1 Lightness of bread crust

The L* for fresh bread crust at 10%, 20%, and 30% were ranged from 51.01–63.50, 51.17–61.71 and 54.40–63.80, respectively. The L* of crust for fresh wheat bread (59.46±0.20) was significantly ($p>0.05$) similar to *Mweru* at 30%, and *Kampolombo* at both 10% and 20%. Similar wheat bread crust L* (53.92) was reported by Fu *et al.* (2018). The L* of bread crust for FBP were in the range 54.13-58.27, 53.86-65.03 and 60.60-67.51 at 10, 20 and 30% CFSL, respectively. The lowest and highest FBP crust L* were recorded in *Bangweulu* and *Katobamputa* at 20 and 30% CFSL, respectively. The FBP wheat bread crust had L* value of 50.76 and increased with increase in CFSL. The L* for FAP bread crust ranged from 49.7-60.80, 57.59-67.43 and 60.49-72.94 at 10, 20 and 30% CFSL, respectively. The lowest and highest L* FAP crust were recorded in *Chila* and *Mweru* at 10 and 30% CFSL, respectively. The wheat bread crust had L* value of 56.61 and increased with increase in CFSL. The differences in crust

L* could be attributed to non-enzymatic browning reaction between protein and sugar (Nguyen and van Boekel, 2017) under dry heating of baking conditions. The crust L* correlated negatively ($r = -0.50$, $p<0.05$) with gluten contents, suggesting that high lightness of crust occurred in high CFSL. The variations among cassava varieties could be attributed to differences in reducing sugars (Nguyen and van Boekel, 2017).

Table 9.12 Lightness of bread crust of bread made from flour blends of wheat and cassava flours

Factor	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	fresh	59.46(0.20)$^{l-q}$	52.59(0.40)^{bcd}	61.71(0.52)$^{r-u}$	54.40(0.34)^{def}
	FBP	50.76(0.65)^{ab}	54.17(0.99)^{def}	53.86(0.93)^{de}	63.92(1.29)$^{v-y}$
	FAP	56.61(1.20)$^{i-j}$	60.80(6.17)$^{p-s}$	67.43(1.03)^{A}	67.53(1.72)^{A}
Katobamputa	fresh	59.46(0.20)$^{l-q}$	58.00(1.66)$^{h-m}$	60.53(0.42)$^{o-s}$	63.80(0.58)$^{t-y}$
	FBP	50.76(0.65)^{ab}	58.27(1.43)$^{i-n}$	65.03(0.02)$^{w-z}$	67.51(0.86)^{A}
	FAP	56.61(1.20)$^{g-j}$	50.44(0.80)^{a}	65.16(1.53)$^{x-z}$	68.39(0.62)^{A}
Mweru	fresh	59.46(0.20)$^{l-q}$	51.01(0.77)^{abc}	51.17(0.12)^{abc}	59.01(0.07)$^{k-p}$
	FBP	50.76(0.65)^{ab}	54.13(0.61)^{def}	58.48(0.10)$^{j-o}$	61.30(0.03)$^{q-t}$
	FAP	56.61(1.20)$^{g-j}$	53.65(0.14)^{de}	63.72(0.76)$^{u-y}$	72.94(0.31)^{B}
Kariba	fresh	59.46(0.20)$^{l-q}$	57.52(0.01)$^{h-l}$	53.44(0.03)^{de}	63.07(0.81)$^{t-x}$
	FBP	50.76(0.65)^{ab}	56.22(0.88)$^{f-i}$	63.16(0.28)$^{t-x}$	62.99(0.27)$^{t-w}$
	FAP	56.61(1.20)$^{g-j}$	53.01(2.82)^{cd}	61.36(0.64)$^{q-t}$	63.17(0.30)$^{t-x}$
Kampolombo	fresh	59.46(0.20)$^{l-q}$	59.77(0.01)$^{m-r}$	60.06(0.42)$^{m-r}$	55.29(0.92)
	FBP	50.76(0.65)^{ab}	56.02(0.74)^{fgh}	56.83(1.44)$^{g-j}$	66.60(0.23)^{zA}
	FAP	56.61(1.20)$^{g-j}$	57.08(1.69)$^{g-k}$	57.59(1.26)$^{h-l}$	60.12(0.78)$^{n-r}$
Chila	fresh	59.46(0.20)$^{l-q}$	63.50(0.01)$^{u-y}$	53.04(1.08)^{cd}	62.49(0.68)$^{s-v}$
	FBP	50.76(0.65)^{ab}	56.22(0.25)$^{f-i}$	58.63(0.65)$^{j-o}$	65.30(0.59)^{yz}
	FAP	56.61(1.20)$^{g-j}$	49.57(5.59)^{a}	60.20(0.59)$^{n-r}$	64.97(0.36)$^{w-z}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen before proofing, FAP=frozen after proofing, CFSL=cassava flour substitution level.

9.3.4.2 Crust redness-greenness

The crust a* for *fresh* were in the range 12.00 to 16.88 (Table 9.13), and varied ($p<0.05$) with CFSL. The redness of bread crust for FBP were in the range 12.38-16.72, 7.18-16.73 and 5.00-10.72 at 10, 20 and 30% CFSL, respectively. The lowest and highest redness were recorded in *Kariba* and *Bangweulu* at 30 and 20% CFSL, respectively. The FBP wheat bread had a redness value of 17.21 and decreased with increase in CFSL. The redness for FAP bread crust ranged from 13.86-16.54, 8.39-14.45 and 4.09-13.31 at 10, 20 and 30% CFSL, respectively. The lowest

and highest FAP crust redness values were recorded in *Mweru* and *Katobamputa* at 30 and 20% CFSL. The redness of the wheat bread crust was 14.94 and decreased with increase in CFSL except for *Katobamputa, Kariba* and *Chila* at 10% cassava flour level. The crust a* positively correlated ($r = 0.54$, $p<0.05$) with gluten. This implies that high crust redness was associated with high gluten contents.

Table 9.13 Redness-greenness color of bread crust of bread made from flour blends of wheat and cassava flours

Variety	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	Fresh	14.29(0.24)$^{m-s}$	16.88(0.17)^{AB}	12.91(0.05)$^{h-l}$	15.38(0.60)$^{t-x}$
	FBP	17.21(0.41)^{B}	16.72(0.28)^{zAB}	16.73(0.29)^{zAB}	10.72(0.79)^{g}
	FAP	14.94(0.48)$^{q-v}$	13.86(3.16)$^{l-p}$	8.39(0.84)^{de}	7.55(1.23)^{cd}
Katobamputa	Fresh	14.29(0.24)$^{m-s}$	14.45(0.65)$^{n-t}$	13.95(0.35)$^{m-q}$	12.00(0.47)^{h}
	FBP	17.21(0.41)^{B}	12.38(1.79)^{hi}	10.91(0.09)^{g}	7.43(0.49)^{cd}
	FAP	14.94(0.48)$^{q-v}$	16.08(0.16)$^{v-zA}$	9.24(0.97)^{ef}	7.51(0.41)^{cd}
Mweru	Fresh	14.29(0.24)$^{m-s}$	14.98(0.13)$^{q-v}$	15.04(0.17)$^{r-w}$	14.12(0.08)
	FBP	17.21(0.41)^{B}	16.33(0.28)$^{x-zAB}$	14.57(0.10)$^{o-u}$	10.59(0.45)^{g}
	FAP	14.94(0.48)$^{q-v}$	15.19(0.35)$^{s-w}$	10.54(0.38)^{g}	4.09(0.27)^{ab}
Kariba	Fresh	14.29(0.24)$^{m-s}$	14.57(0.03)$^{o-u}$	16.34(0.07)^{xyzAB}	12.83(0.45)$^{h-l}$
	FBP	17.21(0.41)^{B}	13.71(0.02)$^{l-p}$	7.18(0.28)^{c}	5.00(0.26)^{b}
	FAP	14.94(0.48)$^{q-v}$	16.41(1.53)$^{x-AB}$	12.46(0.73)^{hij}	12.02(0.09)^{h}
Kampolombo	Fresh	14.29(0.24)$^{m-s}$	14.68(0.03)$^{p-u}$	13.56(0.20)$^{k-o}$	15.77(0.65)
	FBP	17.21(0.41)^{B}	15.55(0.41)$^{u-y}$	13.25(0.67)$^{i-m}$	7.44(0.34)^{cd}
	FAP	14.94(0.48)$^{q-v}$	14.98(0.97)$^{q-v}$	14.45(1.01)$^{n-t}$	13.31(0.11)$^{i-m}$
Chila	Fresh	14.29(0.24)$^{m-s}$	12.07(0.02)^{h}	16.42(0.18)^{yzAB}	12.60(0.21)$^{h-k}$
	FBP	17.21(0.41)^{B}	15.24(0.16)$^{s-w}$	12.21(0.26)^{h}	3.96(0.12)^{a}
	FAP	14.94(0.48)$^{q-v}$	16.54(0.64)$^{y-AB}$	13.47(0.48)$^{-n}$	10.24(0.13)^{fg}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen before proofing, FAP=frozen after proofing, CFSL=cassava flour substitution level.

9.3.4.3 Yellowness of bread crust

The fresh crust b* ranged from 35.16-38.84 (Table 9.14), and varied ($p<0.05$) across the CFSL. The FBP bread crust b* ranged from 37.33-40.27, 34.95-39.96 and 29.30-38.26 at 10, 20 and 30% CFSL, respectively. The lowest and highest b* were observed in *Chila* and *Mweru* at 30 and 20% CFSL. The b* of wheat bread crust was 36.69 and increased with increase in CFSL except for *Katobamputa* at 30%, *Kariba* at 20 and 30%, and *Chila* at 30% CFSL. The b* of the bread crust for FAP were in the range 33.55-40.31, 33.60-38.94 and 26.95-38.95 at 10, 20 and

30% CFSL, respectively. The lowest and highest b* for FAP were recorded in *Mweru* and *Kampolombo* at 30 and 10% CFSL. The b* value of wheat bread crust for FAP was 37.95 and was not significantly different ($p>0.05$) from *Kariba* at all blend ratios. The yellowness of the crumb could be ascribed to caramelization of sugars and degradation of carbonyls (Pico et al., 2015) during oven baking.

Table 9.14 Yellowness color of bread crust of bread made from flour blends of wheat and cassava flours

Variety	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	Fresh	$36.76(0.28)^{klm}$	$37.66(0.06)^{m-s}$	$37.96(0.04)^{n-v}$	$37.66(0.47)^{m-s}$
	FBP	$36.69(0.53)^{kl}$	$39.20(0.91)^{yzAB}$	$38.83(0.57)^{u-zA}$	$38.26(0.59)^{q-y}$
	FAP	$37.95(0.34)^{n-v}$	$38.76(0.63)^{t-z}$	$35.46(0.67)^{ghi}$	$34.58(0.96)^{efg}$
Katobamputa	Fresh	$36.76(0.28)^{klm}$	$37.20(0.25)^{k-o}$	$37.34(0.60)^{l-q}$	$36.37(0.53)^{ijk}$
	FBP	$36.69(0.53)^{kl}$	$37.33(0.85)^{k-q}$	$37.98(0.05)^{o-w}$	$35.58(0.93)^{hi}$
	FAP	$37.95(0.34)^{n-v}$	$33.70(0.84)^{de}$	$34.18(0.39)^{def}$	$30.64(0.22)^{c}$
Mweru	Fresh	$36.76(0.28)^{klm}$	$33.75(0.61)^{de}$	$35.16(0.36)^{gh}$	$37.75(0.12)^{n-s}$
	FBP	$36.69(0.53)^{kl}$	$39.33(0.12)^{zABC}$	$39.96(0.08)^{BCD}$	$37.38(0.38)^{l-r}$
	FAP	$37.95(0.34)^{n-v}$	$33.55(0.97)^{d}$	$33.60(0.17)^{d}$	$26.95(0.36)^{a}$
Kariba	Fresh	$36.76(0.28)^{klm}$	$37.00(0.09)^{k-n}$	$37.80(0.06)^{n-t}$	$38.60(0.37)^{s-z}$
	FBP	$36.69(0.53)^{kl}$	39.76(0.27)	$34.95(0.41)^{fgh}$	$31.22(0.54)^{c}$
	FAP	$37.95(0.34)^{n-v}$	$37.48(0.68)^{l-r}$	$38.32(0.73)^{r-y}$	$38.23(0.05)^{p-x}$
Kampolombo	Fresh	$36.76(0.28)^{klm}$	$38.84(0.04)^{v-zA}$	$37.49(0.14)^{l-r}$	$37.73(0.29)^{n-s}$
	FBP	$36.69(0.53)^{kl}$	$40.27(0.18)^{CD}$	$38.91(0.28)^{v-zA}$	$36.57(0.29)^{jkl}$
	FAP	$37.95(0.34)^{n-v}$	$40.31(0.45)^{D}$	$39.06(0.38)^{xyzAB}$	$38.95(0.21)^{xyzA}$
Chila	Fresh	$36.76(0.28)^{klm}$	$37.87(0.14)^{n-u}$	$37.05(0.36)^{k-o}$	$38.54(0.41)^{s-z}$
	FBP	$36.69(0.53)^{kl}$	$39.79(0.15)^{A-D}$	$37.49(0.10)^{l-r}$	$29.30(0.15)^{b}$
	FAP	$37.95(0.34)^{n-v}$	$35.69(3.35)^{hij}$	$38.94(0.42)^{w-zA}$	$37.28(0.28)^{k-p}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen before proofing, FAP=frozen after proofing, CFSL=cassava flour substitution level.

9.3.4.4 Brownness index of bread crust

The brownness index (BI) for wheat (control) from fresh was 108.57±1.85 (Table 9.15 and was significantly similar ($p>0.05$) to *Bangweulu* and *Katobamputa* at 20%, and *Chila* at 30% CFSL. The BI for composite bread from FBP at 10, 20 and 30% CFSL ranged from 111.69-138.65, 85.48-137.88 and 62.22-101.67, respectively. The lowest and highest BI for FBP was recorded in *Chila* and *Bangweulu* at 30 and 10% CFSL, respectively. The FBP wheat bread crust had

140.30±2.69 and decreased with increase in CFSL. The BI of crust from FAP was in the range 113.55-139.75, 82.44-123.15 and 49.16-113.79 at 10, 20 and 30% CFSL. The FAP BI for wheat bread crust was 122.18±3.80 and decreased with increase in CFSL. The BI trend was FBP>FAP>fresh. BI positively correlated with crust redness-greenness ($r = 0.96$, $p<0.05$), and gluten contents ($r = 0.50$, $p<0.05$), suggesting that high gluten doughs yielded high brownness. Maillard reaction between protein and sugars is responsible for brownness of crust (Leiva-Valenzuela *et al.*, 2018).

Table 9.15 Brownness Index of bread crust made from flour blends of wheat and cassava flours

Variety	Dough type	CFSL (%)			
		Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	fresh	108.57(1.84)$^{k-n}$	137.34(1.48)^{uvw}	105.58(1.20)$^{i-l}$	128.72(3.18)$^{q-t}$
	FBP	140.3(2.69)^{w}	138.38(0.93)^{vw}	137.88(3.21)^{uvw}	99.03(5.86)^{g}
	FAP	122.18(3.80)^{oq}	114.10(23.97)^{mn}	81.09(4.46)^{ef}	77.63(6.88)^{de}
Katobamputa	fresh	108.57(1.84)$^{k-n}$	114.51(6.59)^{mn}	107.57(3.67)$^{i-m}$	94.59(3.41)^{gh}
	FBP	140.3(2.69)^{w}	111.69(9.37)^{lmn}	95.86(0.20)^{gh}	80.14(2.96)^{ef}
	FAP	122.18(3.80)^{opq}	125.55(1.97)^{qrs}	82.22(4.76)^{ef}	65.94(1.81)^{bc}
Mweru	fresh	108.57(1.84)$^{k-n}$	122.19(0.38)^{opq}	128.26(1.81)$^{q-t}$	113.22(0.47)^{mn}
	FBP	140.3(2.69)^{w}	138.65(2.15)^{vw}	124.13(0.87)^{qr}	101.67(1.84)$^{h-k}$
	FAP	122.18(3.80)^{opq}	113.31(4.19)^{mn}	84.44(2.33)^{ef}	49.16(1.31)^{a}
Kariba	fresh	108.57(1.84)$^{k-n}$	115.06(0.31)^{no}	134.07(0.45)$^{t-w}$	104.51(3.68)$^{i-l}$
	FBP	140.3(2.69)^{w}	129.59(1.87)$^{q-t}$	85.48(1.89)^{f}	71.89(1.47)^{cd}
	FAP	122.18(3.80)$^{o-q}$	134.81(11.68)$^{t-w}$	107.24(5.12)$^{j-m}$	102.00(0.80)$^{h-k}$
Kampolombo	fresh	108.57(1.84)$^{k-n}$	115.98(0.14)^{nop}	108.79(0.71)$^{k-n}$	126.58(4.85)^{qrs}
	FBP	140.3(2.69)^{w}	134.98(2.66)$^{t-w}$	123.37(5.19)^{pq}	84.51(1.68)^{ef}
	FAP	122.18(3.80)^{opq}	130.91(8.29)$^{r-u}$	123.15(6.84)^{pq}	113.79(1.54)^{mn}
Chila	fresh	108.57(1.84)$^{k-n}$	100.15(0.44)$^{g-j}$	132.19(2.86)$^{s-v}$	105.35(1.01)$^{i-l}$
	FBP	140.3(2.69)^{w}	131.69(0.91)$^{s-v}$	110.88(2.54)^{lmn}	62.22(1.28)^{b}
	FAP	122.18(3.80)^{opq}	139.75(7.43)^{w}	113.74(3.60)^{mn}	92.99(0.24)^{g}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p<0.05$ by LSD test. FBP=frozen before proofing, FAP=frozen after proofing, CFSL=cassava flour substitution level.

Table 9.16 Correlation coefficients of bread quality and color characteristics

	Volume of Bread	Weight of Bread	Density of Bread	Specific Volume	weight loss	Aver Pore Area	Porosity	Bulk Density	D90
Volume of Bread	1								
Weight of Bread	0.28	1							
Density of Bread	-0.96	-0.20	1						
Specific Volume	0.99	0.17	-0.97	1					
weight loss	-0.28	-1.00	0.20	-0.17	1				
Aver Pore Area	-0.13	-0.01	0.12	-0.14	0.01	1			
Porosity	0.43	0.35	-0.35	0.41	-0.35	-0.04	1		
Bulk Density	0.24	0.11	-0.18	0.24	-0.11	0.08	0.405	1	
D90	-0.29	-0.19	0.22	-0.28	0.19	-0.05	-0.548	-0.83	1
L crumb	0.55	0.36	-0.49	0.52	-0.36	-0.16	0.217	0.22	-0.23
a crumb	-0.54	-0.40	0.51	-0.51	0.40	0.16	-0.321	-0.03	0.00
b crumb	0.08	-0.05	-0.05	0.09	0.05	0.05	0.145	0.36	-0.43
Whiteness	0.47	0.35	-0.43	0.44	-0.35	-0.16	0.150	0.07	-0.06
L-Crust	-0.28	-0.06	0.37	-0.29	0.06	-0.11	-0.337	-0.23	0.26
a-crust	0.44	0.30	-0.51	0.42	-0.30	0.07	0.424	0.23	-0.27
b-Crust	0.09	0.13	-0.14	0.08	-0.13	-0.02	0.133	-0.19	0.15
BI	0.26	0.13	-0.36	0.26	-0.13	0.08	0.336	0.13	-0.17
Chroma	0.07	-0.05	-0.04	0.08	0.05	0.05	0.139	0.35	-0.42
Stickiness	-0.41	0.02	0.39	-0.42	-0.02	0.19	-0.217	-0.06	0.02
Gluten	0.38	0.20	-0.34	0.37	-0.20	0.06	0.638	0.31	-0.53

Significance: P<0.05*, P<0.01**, P<0.001***

9.3.5 Resistant starch of fresh and frozen dough bread

The resistant starch (RS) for fresh bread of the flour blends were in the range 1.98-3.50, 1.84-6.40, and 2.30-6.90% (Table 9.17), at 10, 20 and 30% CFSL, respectively. The lowest and highest RS were recorded in *Kariba* and *Mweru* at 20 and 30 % CFSL, respectively. The RS for wheat fresh bread was 4.29% and did not exhibit significant differences with cassava varieties across CFSL. The RS value of ordinary (fresh) bread was reported, 3.0% (starch basis) (Liljeberg *et al.*, 1996) and 1.18% (Amaral *et al.*, 2016). The RS slightly increased in bread made from blends of frozen doughs and were in the range 4.24-7.05, 4.74-5.66 and 4.72-5.63%, at 10, 20 and 30% CFSL, respectively. The lowest and highest RS were recorded in *Bangweulu* at 10% CFSL. The RS for frozen wheat bread was 6.84% starch basis and was higher than the RS value of *fresh* bread. The dislocation and ice-crystallization of water molecules may induce irreversible molecular changes in the structural arrangement of amylose and amylopectin (Ribotta *et al.*, 2003) resulting in retrograded starch which is resistant to digestive enzymes (Gujral *et al.*, 2018). The digested starch in fresh bread were in the range 79.73-95.04, 83.41-96.88 and 89.17-96.16% at 10, 20 and 30% CFSL, respectively. The lowest and highest digested starch were recorded in

Bangweulu and *Kampolombo* at 10 and 20% cassava flour substitution level. The wheat bread recorded 81.63% digested starch and increased with increase in CFSL. The digested starch in white wheat bread were reported, 75% (Björck *et al.*, 1986). The digested starch for frozen dough bread were in the range 81.50-92.64, 76.77-93.86, and 77.85-93.98 % (Table 9.18), at 10, 20 and 30% CFSL, respectively. The lowest and highest digested starch were recorded in *Chila* and *Kampolombo* at 20 and 30% CFSL. The digested starch in frozen wheat bread was 76.31±1.06% and was insignificantly ($p>0.05$) lower than that of fresh bread. There was a weak positive correlation ($r = 0.163$, $p<0.0001$) between resistant starch and gluten content (Table 9.19). This suggests that resistant starch increased with an increase in the gluten content. Thus digested starch increased with increase in cassava flour substitution level. Björck et al. (1986) reported that the presence of protein (gluten) in flour restricted starch digestibility.

Table 9.17 Resistant starch content (%) in bread baked from wheat-cassava flour blends

Variety	Bread type	CFSL (%) Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	Fresh	4.29(0.22)$^{a-g}$	1.98(0.13)^{ab}	2.67(0.03)$^{a-s}$	2.62(0.01)$^{a-e}$
	Frozen	6.84(1.14)^{fg}	4.49(0.25)$^{a-g}$	4.74(0.22)$^{a-g}$	5.63(0.46)$^{b-g}$
Katobamputa	Fresh	4.29(0.22)$^{a-g}$	3.45(0.07)$^{a-g}$	4.62(0.05)$^{a-g}$	2.27(0.05)^{abc}
	Frozen	6.84(1.14)^{fg}	4.24(0.39)$^{a-g}$	5.42(0.09)$^{a-g}$	5.25(0.23)$^{a-g}$
Mweru	Fresh	4.29(0.22)$^{a-g}$	3.50(0.64)$^{a-g}$	2.87(0.16)$^{a-e}$	6.90(0.41)^{fg}
	Frozen	6.84(1.14)^{fg}	4.80(0.12)$^{a-g}$	5.01(0.00)$^{a-g}$	4.72(0.09)$^{a-g}$
Kariba	Fresh	4.29(0.22)$^{a-g}$	1.51(0.12)^{a}	1.84(0.55)^{ab}	3.01(0.48)$^{a-f}$
	Frozen	6.84(1.14)^{fg}	4.89(0.57)$^{a-g}$	5.28(0.98)$^{a-g}$	5.24(0.06)$^{a-g}$
Kampolombo	Fresh	4.29(0.22)$^{a-g}$	2.59(0.18)$^{a-e}$	2.32(0.03)$^{a-d}$	3.59(0.16)$^{a-g}$
	Frozen	6.84(1.14)^{fg}	7.05(0.49)^{g}	6.20(1.24)$^{c-g}$	5.30(0.21)$^{a-g}$
Chila	Fresh	4.29(0.22)$^{a-g}$	2.28(0.62)^{abc}	6.40(6.88)^{efg}	2.30(0.09)^{abc}
	Frozen	6.84(1.14)^{fg}	6.25(0.33)$^{d-g}$	5.66(0.18)$^{b-g}$	4.77(0.23)$^{a-g}$
Significance level					
Variety			p>0.05	p>0.05	p>0.05
Treatment		p>0.05	p<0.05	p<0.05	p<0.05
Variety x Treatment			p<0.05	p<0.05	p<0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test. Frozen = frozen-after-proofing

Table 9.18 Digested starch in wheat-cassava flour blends

Variety	Bread type	CFSL (%) Control (wheat)	10% CFSL	20% CFSL	30% CFSL
Bangweulu	Fresh	81.63(2.50)^{abc}	79.73(2.01)^{ab}	83.41(1.18)^{bcd}	89.17(8.38)$^{d-i}$
	Frozen	76.31(1.06)^{a}	92.64(1.24)$^{e-k}$	90.89(8.76)$^{e-k}$	88.81(1.42)$^{d-h}$
Katobamputa	Fresh	81.63(2.50)^{abc}	87.31(0.07)^{cde}	93.19(0.58)$^{e-k}$	93.53(2.70)$^{f-k}$
	Frozen	76.31(1.06)^{a}	89.52(6.89)$^{e-i}$	90.74(1.48)$^{e-j}$	87.74(0.61)^{def}
Mweru	Fresh	81.63(2.50)^{abc}	93.82(2.80)$^{g-k}$	94.67(1.48)$^{h-k}$	91.12(3.32)$^{e-k}$
	Frozen	76.31(1.06)^{a}	81.5(4.44)^{abc}	80.23(6.43)^{ab}	92.88(2.47)$^{e-k}$
Kariba	Fresh	81.63(2.50)^{abc}	95.04(1.62)^{ijk}	91.32(6.20)$^{e-k}$	94.63(4.92)$^{h-k}$
	Frozen	76.31(1.06)^{a}	88.04(9.78)$^{d-g}$	88.29(4.24)$^{d-g}$	77.85(1.88)^{ab}
Kampolombo	Fresh	81.63(2.50)^{abc}	92.04(2.47)$^{e-k}$	96.88(0.87)^{k}	96.15(1.62)^{jk}
	Frozen	76.31(1.06)^{a}	91.23(4.61)$^{e-k}$	93.86(5.39)$^{g-k}$	93.98(3.18)$^{g-k}$
Chila	Fresh	81.63(2.50)^{abc}	92.96(2.10)$^{e-k}$	92.44(6.01)$^{e-k}$	90.65(0.28)$^{e-j}$
	Frozen	76.31(1.06)^{a}	92.5(4.18)^{ek}	76.77(0.45)^{a}	89.21(1.73)$^{d-i}$
Level of significance					
Variety			p<0.05	p<0.05	p<0.05

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at p<0.05 by LSD test. Frozen = frozen-after-proofing, CFSL=cassava flour substitution level.

Table 9.19 Correlation coefficients of resistant starch and bread quality

parameter	Bread volume	Weight of Bread	Density of Bread	Specific Volume	Resistant starch	Gluten
Bread volume	1					
Weight of Bread	-0.318	1				
Density of Bread	-0.981	0.430	1			
Specific Volume	0.993	-0.424	-0.988	1		
Resistant starch	0.079	-0.256	-0.154	0.108	1	
Gluten	0.031	0.543	0.023	-0.031	0.163****	1

P<0.05*, P<0.01**, P<0.001***, p<0.0001

9.4 Multivariate analysis

The principal component analysis was conducted to determine the effect of freezing on dough and bread quality (Figure 9.2). The bread volume (BV) and specific volume (SP) clustered together on the same axis. This suggests perfect correlation between BV and SP, and exhibited similar response on bread quality. This means that either BV or SP can be targeted as measure of quality for bread quality. The coordinates of BV and SP were on the opposite direction of stickiness (ST) and resistant starch (RS). This implies that stickiness and resistant starch had a negating effect on BV and SP. High resistant starch may possibly yield high stickiness doughs during freezing. Furthermore, resistant starch content was positively influenced by protein (including gluten), lipid and amylose contents. The frozen after proofing (FAP) and fresh were located on coordinates of upper and lower RS-axis, respectively. This suggests highest and lowest RS were recorded in FAP and fresh, respectively, and similar trend was showed in the stickiness axis. All types of doughs/bread, fresh (8), FBP (9) and FAP (10) exhibited a pattern that was strongly influenced by flour particle size (D90-axis) in the order of fresh>FBP>FAP.

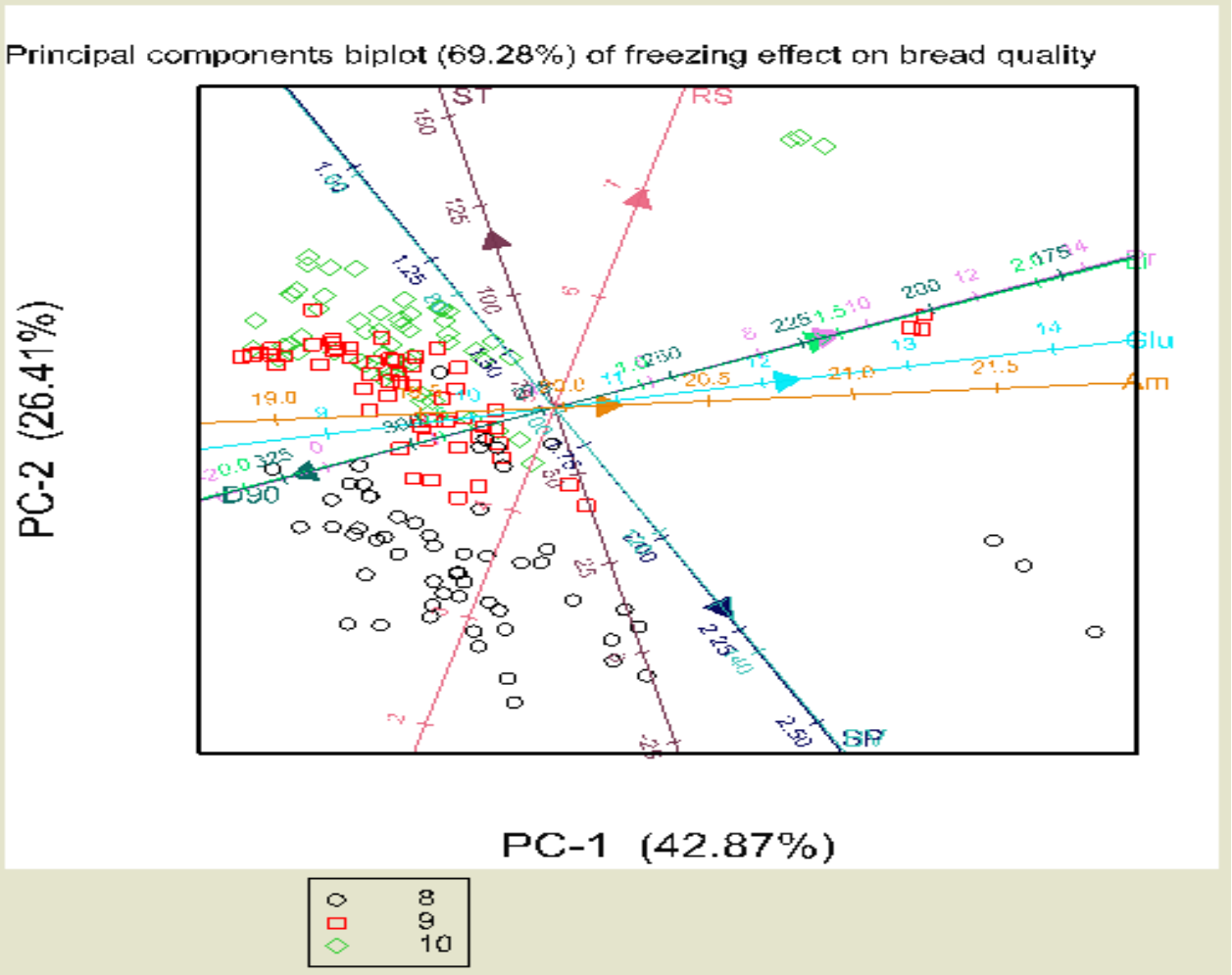

Figure 9.2 Principal component analysis on effect of freezing on dough and bread quality. Types of dough: 8=Fresh, 9=Frozen-before-proofing; 10=Frozen-after-proofing. ST=Stickiness, RS=Resistant starch, Pr=protein, Li=Lipid, Glu=Gluten, Am=Amylose, D90=flour particle size at 90% cumulative of finer particles. SP=specific volume, BV=Bread volume

Figure 9.3 shows the effect of cassava variety on quality of bread processed from frozen dough. Based on the plot, wheat was distinguished with varied coordinates ranging from upper and lower BV-, ST-, and RS-axis. This variation suggests the impact of proofing and freezing on the quality. The sticky frozen dough yielded lower bread volume and higher RS than those of fresh dough. The partial replacement of wheat with cassava varieties produced dough and bread quality with even variation along the ST-and BV-axis. The *Katobamputa*, *Mweru*, and *Kariba* closely associated with both upper and lower BV- and ST-axis, indicative of bread quality from fresh and frozen doughs at 10% CSFL, respectively. This suggests that fresh composite dough with low stickiness yielded high loaf volumes and vice versa in frozen composite doughs. Furthermore, this could mean that composite frozen doughs at 20 and 30% CFSL from FBP

yielded intermediary quality (strongly clustered in the middle of BV and ST axes) across all the cassava varieties.

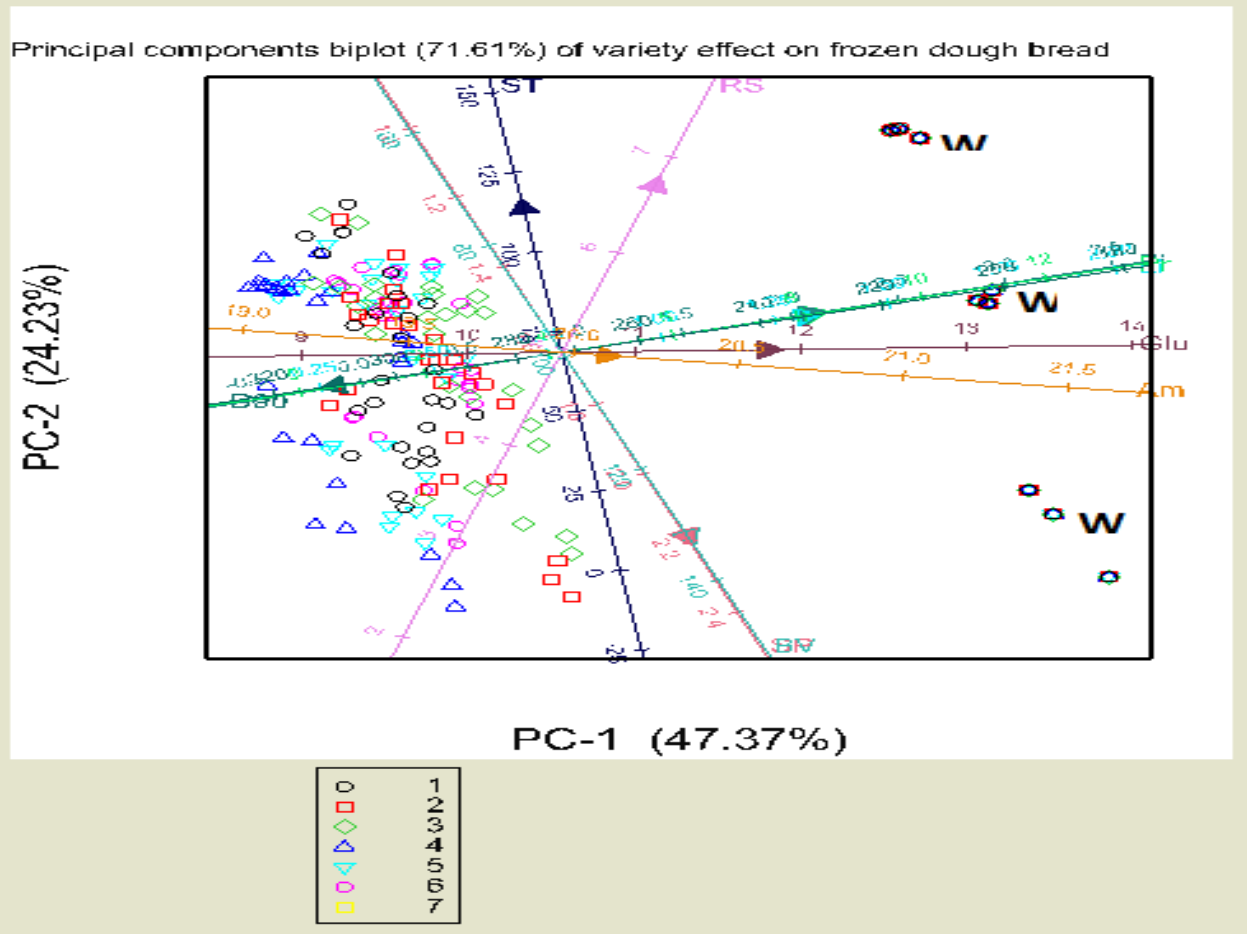

Figure 9.3 Principal component analysis of effect of variety on bread quality baked from frozen dough. Varieties: 1=Wheat, 2=*Bangweulu*, 3=*Katobamputa*, 4=*Mweru*, 5=*Kariba*, 6=*Kampolombo* and 7=*Chila*. ST=Stickiness, RS=Resistant starch, Pr=Protein, Li=Lipid, Glu=Gluten, Am=amylose, D90=Flour particle size at 90% cumulative of finer particles, SP=specific volume, BV=Bread volume.

Figure 9.4 shows the effect of CFSL on dough and bread quality. The CFSL (10-30%) were evenly distributed along the axes of BV and stickiness, whereas the 0% CFSL (wheat) was disparate, and parallel the axes of BV and ST, indicative of variation in doughs (fresh to frozen types) at same percentage CFSL. The fresh doughs clustered in the upper BV-axis and lower ST-axis, while frozen doughs grouped at the lower BV-axis and upper ST-axis.

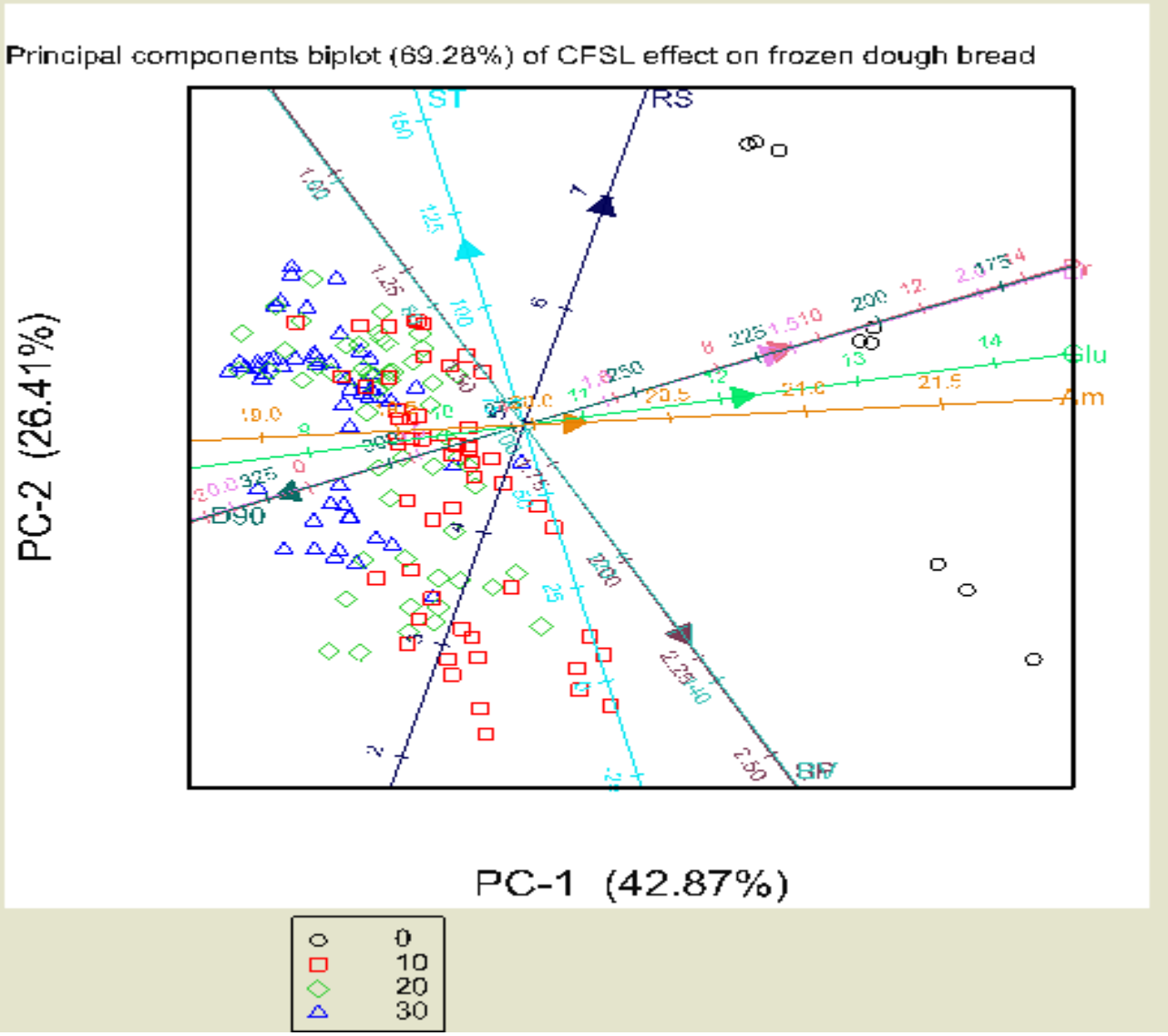

Figure 9.4 Principal component analysis on the effect of CFSL (0, 10, 20, and 30%) on bread quality baked from frozen dough. ST=Stickiness, RS=Resistant starch, Pr=Protein, Li=Lipid, Glu=Gluten, Am=Amylose, D90=Flour particle size at 90% cumulative of finer particles, SP=specific volume, BV=Bread volume

9.5 Conclusion

The bread volume of wheat bread from fresh and frozen doughs decreased with increase in cassava flour substitution level. The variations among the varieties in bread quality were associated with differences in flour particle size. Stickiness influenced the bread quality as sticky doughs exhibited reduced bread volume. The bread volume loss for wheat bread in frozen after proofing (46.76±2.99%) was higher than volume losses (4.52-27.04%) recorded in bread baked from composite flours of frozen before proofing doughs. The bread volume loss for wheat in

frozen before proofing dough (29.94±6.70%) and the lowest bread volume loss values (9.00-40.47%) were recorded in composite flours except for lowest volume losses in *Bangweulu* (0.81-7.78%). This could indicate that cassava flour has a negating effect against weakening of gluten during frozen storage. Resistant starch contents were influenced by cassava substitution levels and gluten content. The frozen storage condition increased resistant starch contents.

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10. CONCLUSION AND RECOMMENDATIONS

This study evaluated six different cassava root varieties: one local landrace (*Katobamputa*), five improved and officially released varieties (*Bangweulu, Mweru, Kariba, Kampolombo and Chila*) from Zambia for physicochemical properties of starch and application of cassava flour in bread from the frozen wheat dough. The chemical composition (moisture, protein, lipid, fiber and cyanide contents), dry matter, starch extraction yields, particle size distribution and whiteness index significantly varied among the cassava flour varieties ($p<0.05$). *Katobamputa* was significantly ($p<0.05$) impacted by containing smallest flour particle size at 90, 60 and 50% cumulative of finer particles, while *Mweru* showed smallest flour particle size at 30 and 10% cumulative of finer particles. There was a strong positive correlation between dry matter content and 90% cumulative of finer particles ($p<0.05$). However, decreasing percentage of cumulative distribution showed a reducing effect on dry matter contents. This suggests that *Katobamputa* and *Mweru* can be targeted for the product formulation of low dry matter content food system. Moreover, the selection of sieve is guided by product nature because decreasing cumulative of particles passing finer than sieve results in reduced proportion amount of flour. In this study, D90 was selected for bread baking since it yielded a large proportion amount of flour in the range 90-96% finer particle at sieve size 300-425 μm, respectively. The insignificant differences among *Kampolombo*, *Kariba*, and *Bangweulu* ($p>0.05$) were due to similarities in large flour particle sizes, high dry matter and high cyanide contents. The higher percentage cyanide reduction (88.03-93.94%) was recorded in *Kampolombo, Kariba*, and *Bangweulu* than *Katobamputa*. The variety *Katobamputa* recorded lowest cyanide values in cassava root (23.60 mg/kg) and flour (8.62 mg/kg) but yielded the lowest cyanide reduction percentage (60.76%). The cyanide reduction highlighted the importance of primary processing of fresh cassava through peeling, grating, and dewatering before drying. The insignificant differences ($p<0.05$) between *Kariba* and *Bangweulu* were due to the low amount of starch yields. *Chila* was significantly ($p<0.05$) distinguished by containing high carbohydrate content and larger particle sizes at 30 and 10% cumulative of finer particles. The variation among the varieties due to proximate contents (protein, lipid, and fiber) were not significant ($p>0.05$) except for proteins in *Bangweulu,* and fiber content in *Kariba*. Moreover, the low protein content recorded in all cassava varieties is indicative of the poor nutritional quality and can be suggested for blending with other starches/flours such as wheat flour for improved proteins and bread baking properties. The

high whiteness color combined with low ash contents is primary desirable quality traits for application of cassava flours in the baking industry. The whiteness color of cassava flour is significantly affected by moisture, ash, and impurities (redness-greenness). The results on particle size distribution, proximate and cyanide contents can find use in the development and improvement of national standards on quality and safety of cassava flours in Zambia. The data on cyanide content can feed into plant breeding programs for the objectives of producing low cyanide (sweet) cassava varieties.

The scanning electron microscope showed that the cassava starch granules were rounded, truncated and oval granule in shape. The cassava starches showed prominent crystalline peaks (2θ-scale) at around 15^{o} and 23^{o}, and unresolved double peak at 17^{o}, 18^{o} 2θ, a characteristic feature of type A X-ray diffraction pattern of waxy starches. This means that the cassava varieties contained high amylopectin by weight (starch matrix nature with more of waxy than high amylose). The amylopectin chains form double-helices responsible for the crystallinity in starch, and thus the levels of crystallinity for cassava starches in the current study could be attributed to high amylopectin content. There were no significant differences in the degree of crystallinity among the cassava varieties ($p>0.05$). Nevertheless, the cassava starches granule size was significantly different ($p<0.05$), which correlated with differences in flour particle size distribution. The cassava varieties were classified as regular starches based on the level of amylose content (16.04-26.95%) indicative of low amylose starches which are highly susceptible to amylase enzymatic digestibility. Amylose content can be suggested as a basis for selecting flours/starches from different botanical sources for blending application. Starches with similar amylose contents can exhibit similar functionalities. *Katobamputa* starch was significantly different from other varieties because it contained highest amylose content and yielded the highest resistant starch content among the cassava varieties after digestion with α-amylase. On the basis of resistant starch content, *Katobamputa* can find use in the product development and formulation of special food with low glycemic index suitable for individuals with a diabetic health condition. Nevertheless, the interrelations among the variables showed that non-starch content (protein, lipid, ash, and fiber) can significantly ($p<0.05$) influence resistant starch content. Furthermore, small starch granule sizes had an increasing effect on resistant starch, suggesting that smaller granules had an intact structure which resisted digestibility and possibly contained non-starch materials. The starch granules of variety *Kariba* showed the smallest granule sizes, lowest dry matter, and lowest starch yields, and exhibited the highest values of whiteness index and paste clarity

among the varieties. The variety *Bangweulu* was distinguished by high levels of whiteness index, non-resistant starch, and total starch contents. This suggests that highly digestible starches (*Bangweulu*) have a high amount of total starch. Furthermore, high whiteness color of starches could potentially indicative of high susceptibility to α-amylase digestibility.

The swelling power of starches showed a weak inverse relationship with amylose and resistant starch contents. The higher peak swelling power and solubility values in starches than flours were due to the fact that flours contained high non-starch contents. The presence of non-starch compounds (lipids and proteins) restricts swelling power. Lipid promotes the formation of the amylose-lipid complex which could limit exudation of amylose resulting in decreased solubility, amylose resulting in decreased solubility, while probably protein hydrophobicity also may limit uptake of water. The gelatinization temperatures (onset, peak, and conclusion) positively associated ($p<0.05$) with amylose content. The cassava varieties were significantly differentiated ($p<0.05$) by low gelatinization temperatures which suggest that cassava starches had low gelatinization temperature. This result supports the peak swelling temperatures (60-70 ^{o}C). The varieties, *Kariba* and *Mweru* with low amylose content were significantly distinguished ($p<0.05$) by small granule sizes and low gelatinization temperatures. The low amylose starches can restrict the formation of the amylose-lipid complex which would otherwise limit gelatinization of starch granules. The low gelatinization temperatures could also possibly mean that the starches are comprised of high short amylopectin chain length (degree of polymerization less than 12). The failure in short chains to form double helical structures results in a defect of crystalline nature, giving a granule a weak structure susceptible to disruption at low temperatures. There was no variety that was distinguished by high pasting temperature. Generally, all the cassava starches/flours recorded low pasting temperatures and narrow range of viscosity probably due to low amylose contents. *Katobamputa* was significantly distinct by high setback viscosity probably due to higher amylose content than other varieties. The swelling and solubility at peak values closely associated together and did not cause differences among the cassava varieties. This suggests that all varieties showed similar ($p>0.05$) response to swelling and solubility.

The low peak swelling values and early gelatinization temperature make cassava flours potential candidate for partial substitution into wheat for bread making. The cassava variety significantly influenced wheat bread quality ($p<0.05$). Nevertheless, the study showed that wheat can be substituted with cassava flour from cassava varieties *Mweru, Kariba* and

Katobamputa in bread making up to a level of 10%, without significantly affecting bread quality negatively. The flour particle size, water absorption capacity, and gluten content are significant flour properties influencing dough rheology and bread quality. The bread from all cassava composite varieties were significantly ($p<0.05$) influenced by flour particle size (D90), suggesting that flour particle size distribution was the major source of variations among the cassava varieties. The bread specific volume is a response function of gluten flour particle size, gluten content and water absorption capacity. The particle size distribution influences the hydration properties of flours, which in turn, is responsible for gluten development in a dough system and ultimately bread quality. Optimizing the flour particle size between the blends of wheat and cassava flours in response to water absorption capacity can be the basis for formulating composite flours of improved properties for dough performance and processing of bread quality.

The cassava variety significantly ($p<0.05$) influenced the stickiness of the fresh and frozen dough and bread quality. The stickiness of fresh wheat dough increased with increase in cassava flour substitution level, whereas the stickiness of frozen doughs decreased with increase in cassava flour substitution level. This stickiness behavior was significantly ($p<0.05$) influenced by water absorption capacity and gluten content. The stickiness in fresh doughs was due to excess water which increased the surface tension between the surface of the probe and dough. Water tends to promote interfacial tension through hydrogen bonding, and Van der Waals forces at the contact between probe and dough surface. Hydration is responsible for dough development, and thus at high cassava flour levels, the water sufficiently hydrated low gluten content by weight, and excess water participated in the interaction of starch-starch bonding which on saturation increased stickiness. The high stickiness in the frozen wheat dough and composited doughs of low cassava flour substitution level could also be attributed to exudate water during the deterioration of gluten molecules. De-polymerization of high molecular weight (gluten) into low molecular weight is probably accompanied by the release of water, and this water is in weak interaction with low molecular weight compounds. The unbound water can contribute to interfacial surface tension. The dough stickiness significantly ($p<0.05$) affected the loaf volume and digestibility of bread baked from frozen dough. The frozen dough yielded decreased bread volume and increased resistant starch contents.

This study has indicated that properties such as swelling, solubility, gelatinization, pasting, freeze-thaw stability, and resistant starch content are genetic factor (variety) dependent. The source of significant variations in cassava varieties was due to chemical constituents (amylose, protein, lipid, fiber) and structural properties (starch granule size, flour particle size). The flour particle size, amylose, and protein content were the main sources of variations among cassava varieties in the performance of cassava flour inclusions in the frozen wheat dough and its bread quality. Flour particle size can be a significant differentiating genetic trait among cassava varieties. In the current study, cassava genotypes were cultivated simultaneously in a single plantation and harvested at the same time. In addition, the milling conditions were the same across cassava varieties. The variations observed in flour particle size were attributed to genetic differences among the cassava varieties.

Recommendation

- The current study can build up for future study focused on selectively targeting the cassava varieties with enhanced genetic traits such as high amylose and waxy varieties for the investigation of end-user properties and their subsequent behavior in frozen wheat bread dough.
- Flour particle size could be targeted as a genetic trait. In processing conditions where main genetic factors were degraded, flour particle size variations remained the differentiating trait among the varieties.
- The cassava flour in frozen wheat dough had a decreasing effect on the stickiness of the dough. Furthermore higher cassava substitution level doughs yielded lower bread volume losses compared to the wheat dough. There is a need for follow up study to investigate the interaction on gluten depolymerization and cassava starch molecules in frozen storage and baking conditions. Also, the future investigation should focus on the effect of dough stabilizers (ingredients) on the stickiness of the frozen dough system.
- Increased utilization of cassava starches would require that future studies investigate the performance of starches in food systems such as soup, cream, salad, and sauce products.
- Future studies should determine the effect of cassava cultivation conditions on properties of cassava flours and starches.