CASSAVA ROOT (*MANIHOT ESCULENTA* CRANTZ) CHARACTERISATION AND EVALUATION OF PROCESS-INDUCED CHANGES ON FUNCTIONALITY OF ITS FLOUR

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is the world's most important root crop, highly perishable, mostly grown and consumed in the tropics and subtropics of Asia, South America and Africa. Despite the popularity and utilisation of cassava in tropical and sub-tropical regions of Africa, it is not well known in South Africa. In this study, morphological, physicochemical, structural, elemental, and metabolic characterisation was conducted on two South African cassava landraces red (RCLR) and white (WCLR) highlighting their potential end-use properties. Response surface methodology (RSM) was employed in determining the linear, interactive and quadratic effect of varying concentrations of pre-treatment (0.6 – 3.4%w/v) and drying temperature (45 – 74ºC) on thermal, functional and physicochemical properties of cassava flour (CF) from both landraces. Calcium chloride and citric acid were separately applied in pre-treatment of the flours. Four experimental groups: CF from red landrace pre-treated with citric acid (CAR); CF from red landrace pre-treated with calcium chloride (CCR); CF from white landrace pre-treated with citric acid (CAW); and CF from white landrace pre-treated with calcium chloride (CCW) were comparatively evaluated. In each experimental group, the experimental samples were compared with control samples by One-way ANOVA and separation of means using Duncan Multiple Range Test in SPSS statistics software Version 25 (IBM Corp., New York, USA). Experimental design, analysis, response plots, one-way analysis of variance (ANOVA) of model parameters and process optimisation was done with Stat-Ease design expert software (Version 12). Visual assessment of the root parenchyma showed no distinct features as both landraces appeared cream in colour. However, colorimetric analysis revealed that L^* (87.42), a^* (0.06), b^* (14.17), whiteness (89.45) and brownness index (4.15) of WCLR were significantly different ($p < 0.05$) from the L^* (83.30), a^* (0.67), b^* (16.07), whiteness (86.50) and brownness index (5.17) of RCLR. Peel thickness of the RCLR (2.27 mm) was significantly higher ($p < 0.05$) than that of WCLR (1.85 mm) while the percentage flour yield of RCLR (36.15) was significantly lower than WCLR (37.03). Flour from the roots showed significant variance ($p < 0.05$) in a^*, b^* and brownness index but the lightness and whiteness index were not significantly different ($p > 0.05$). Cyanide content (RCLR – 3.62 mg/kg; WCLR – 3.51 mg/kg) of the root was not significantly ($p > 0.05$) different, but the flour (RCLR – 2.92 mg/kg; WCLR – 1.83 mg/kg) was significantly ($p < 0.05$) different. Cyanide content of the root and flour were below WHO recommended safe consumption level of 10.00 mg/kg. Scanning electron micrographs of both flours showed spherical and truncated starch granules clustered and dispersed in no regular pattern. Pattern and main peaks (2θ = 43º, 23º, 17º and 15º) of X-ray diffractometry spectra of the flours were the same, exhibiting A-type starch crystallinity. Metabolic profiling, with the aid of gas chromatography-mass

spectrometry, revealed that phenolic acids identified were higher in WCLR than RCLR. A reverse trend was observed with identified FAMEs in the landraces. Of all sugars identified, sucrose had the highest concentration in both landraces. X-ray fluorescence spectrometry of CF revealed that both landraces contained potassium (RCLR - 26.10 mg g^{-1}; WCLR – 30.30 mg g^{-1}), magnesium (RCLR – 23.40 mg g^{-1}; WCLR – 16.80 mg g^{-1}), calcium (RCLR – 11.50 mg g^{-1}; WCLR – 5.60 mg g^{-1}), aluminium (RCLR – 1.50 mg g^{-1}; WCLR – 1.50 mg g^{-1}), phosphorus (RCLR – 0.80 mg g^{-1}; WCLR – 1.50 mg g^{-1}), iron (RCLR – 0.50 mg g^{-1}; WCLR – 0.50 mg g^{-1}), chromium (RCLR – 0.20 mg g^{-1}; WCLR – 0.20 mg g^{-1}), and titanium (RCLR – 0.20 mg g^{-1}; WCLR – 0.20 mg g^{-1}). Differential scanning calorimetry showed that pre-treatment had an increasing effect on the gelatinisation temperatures and enthalpy of CF. Citric acid treatment had a decreasing effect on water holding capacity of CF when compared to calcium chloride. Loose bulk density (LBD) ranged between 0.34 – 0.41 g/cm^3 (CAR), 0.37 - 0.45 g/cm^3 (CCR), 0.35 – 0.43 g/cm^3 (CAW) and 0.37 – 0.44 g/cm^3 for CCW respectively. The LBD increased with an increase in DT. Packed bulk density (PBD) of CF treated with calcium chloride and citric acid were similar with the least and highest PBD of 0.62 and 0.73 g/mL respectively. An increase in drying temperature and concentration increased ash content. Calcium chloride and citric acid pre-treatments improved the lightness and whiteness index of CF. The *L** values of CF were between 91.37 and 93.65 with the control (not pre-treated) samples significantly lower ($p < 0.05$) than the experimental samples in all four groups. The study reveals that the pre-treatments have a mitigating effect against enzymatic browning associated with cassava root processing. An increase in thermal properties indicates that the processing conditions confer on CF more stability in the presence of heat and water. Chemical characterisation shows that both landraces are of the sweet type, with low cyanide content which makes them safe for human consumption. The flours contain minerals that are useful for proper body function and metabolism. The A-type starch crystallinity of flour exhibited, positions the flours as a suitable wheat replacement in flour-based food applications. Therefore, the roots of these landraces can be processed into minimally processed foods such as chips. The flours can be utilised in food applications such as baked products, gels and stabilisers.

Keywords: Cassava; landraces; root; flour; characterisation; cyanide; phenolic acids; fatty acid methyl esters; sugars; scanning electron microscopy; X-ray diffractometry; X-ray fluorescence; pre-treatment; drying temperature; gelatinisation; enthalpy; functional properties; bulk density; colour; optimisation; response surface methodology

DECLARATION

I hereby declare that this submission is my work and to the best of my knowledge it contains no material previously submitted, in whole or in part, to qualify for any other academic award; the intellectual content of the thesis is the result of work that has been carried out since the official commencement date of the approved research program. Any contribution made to the research by others is explicitly acknowledged in the thesis.

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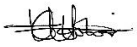
Signature:  Date: 26/02/2021

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This work is dedicated, with a heart of gratitude, to the following persons:

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LIST OF ACRONYMS

CF – Cassava flour

RCLR – Red cassava landrace

WCLR – White cassava landrace

COP – Concentration of pre-treatment

DT – Drying temperature

CA – Citric acid

CC – Calcium chloride

CAR – Flour processed from red cassava landrace pre-treated with CA

CAW – Flour processed from white cassava landrace pre-treated with CA

CCR – Flour processed from red cassava landrace pre-treated with CC

CCW – Flour processed from white cassava landrace pre-treated with CC

FAMEs - Fatty acid methyl esters

SEM - Scanning electron microscopy

XRD - X-ray diffractometry

NIRS – Near-Infrared Spectroscopy

LBD – Loosed bulk density

PBD – Packed bulk density

ΔBD – Difference in PBD and LBD

WHC – Water holding capacity

To – Onset gelatinisation temperature

Tp – Peak gelatinisation temperature

Tc – Conclusion gelatinisation temperature

ΔH – Gelatinisation enthalpy

RSM – Response surface methodology

ER – Experimental run

ANOVA – Analysis of variance

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CHAPTER ONE: INTRODUCTION

1.1 Brief Description and Processing of Cassava

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub that belongs to the family *Euphorbiaceae*. The leaves and roots are the nutritionally valuable parts of the plant and they constitute 6% and 50% of the mature plant respectively (Afoakwa *et al.*, 2012). The starch-rich tuberous roots are the main storage organ in cassava and it is important not just because it forms the bulk of the plant but it is the main part of the plant that is mostly consumed (Aloys and Ming, 2006; Montagnac *et al.*, 2009a; Maieves *et al.*, 2012). Cassava, also called manioc or tapioca, is the most important staple root crop in the world (FAO, 2013) mainly grown in the continents of Asia, South America and Africa (FAO, 2015). Since the introduction of cassava to Africa in the 16th century, cassava (*M. esculenta*) has become one of the most important crops on the continent ranking after rice and maize providing food security and subsistence (Sajeev *et al.*, 2010; FAO, 2013). Cassava is eaten by approximately one billion people every day, predominantly in the tropical and subtropical regions of Asia, Latin America and Africa and is the major staple for 35–50% of people living in different areas of sub-Saharan Africa (Brown *et al.*, 2016). In cassava processing several variables are involved which include type of variety, drying methods, drying temperatures, fermentation method and fermentation time/duration. All these variables are altered towards getting the desired quality of the product. The major processed forms of cassava fall into four general categories: chips, meal, starch and flour (Kimaryo *et al.*, 2000; Taiwo, 2006).

There exist one major setback to the utilisation of cassava, that is the high perishability of the root due to a physiological process known as rapid postharvest physiological deterioration (PPD) (Atieno *et al.*, 2018; Liu *et al.*, 2019). Cassava roots cannot be stored for more than a few days after harvesting therefore the roots are quickly processed to stable products (Udoro *et al.,* 2008) such as cassava flour (Figure 1.1).

1.2 Description, Production, and Application of Cassava Flour

One major processing step for extending the shelf life of cassava root is drying (Agbemafle, 2019). Cassava flour is a dry powdery stable product of the root with simple process technology (Cazumba da Silva *et al.*, 2017). It is directly derived from milling dried cassava chips after which it maybe sieved or it can also be processed from fresh cassava roots by grating and further subjected to other processing steps (Dziedzoave *et al.*, 2006) such as fermentation (Nkoudou and Essia, 2017).

Figure 1.1. Photos of cassava root and flour. **A** - Highly perishable cassava root; **B** - Shelf-stable cassava flour. Source: Photos by author.

Charoenkul *et al.* (2011) describe cassava flour as a suitable representative of the edible portion, of the highly perishable cassava root with reduced moisture and cyanogenic content. Drying cassava is widely practiced in eliminating cyanide and improve the shelf life of the root by reducing the moisture content to a low level. The roots are chipped into smaller sizes for fast drying and help in the process of detoxification (Irinkoyenikan *et al.*, 2008; Pornpraipech *et al.*, 2017). Open sun, solar and oven drying can be used to produce the dry cassava chips out of which the cassava flours are obtained (FIIRO, 2005; Agbemafle, 2019). Cassava flour has been found to elicit various applications in the food and non-food industry such as in baking, edible film, syrup, glucose, alcohol and soups production (Chisenga *et al.*, 2019a). To reduce dependence on wheat flour, especially in countries where cassava is very well cultivated, bakers are encouraged to use cassava flour as a composite in bakery products such as bread, though the baking properties are poor and modification of the flour to better suit this purpose is a research in progress (Jensen *et al.*, 2015; Dudu *et al.,* 2019; Ramirez *et al.*, 2019). The application of cassava flour in product development and food formulations is guided by their end-use properties such as composition, physicochemical and functional properties (Chisenga *et al.*, 2019a).

1.3 Background to the Study

It is common in literature to find studies related to the characterisation of new breeds, clones, accession, varieties and landraces of cassava (Charoenkul *et al.*, 2011; Eleazu and Eleazu, 2012; Chiwona-Karltun *et al.*, 2015a; Mtunguja *et al.*, 2016; Alamu *et al.*, 2017; Ayetigbo *et al.*, 2018; Chisenga *et al.*, 2019b; Falade *et al.*, 2019) because preharvest factors such as variety, environmental conditions during growth of the plant, biofortification, age at harvest, and breeding techniques (Aryee *et al.*, 2006; Onitilo *et al.*, 2007a; Montagnac *et al.*,

2009a) influence the quality of the roots. Afoakwa *et al.* (2012) studied the cassava mosaic disease (CMD) resistant cassava varieties developed by the Crop Research Institute of Ghana and reported wide variations in chemical composition of the improved and traditional cassava cultivars. Screening of their cyanogenic potentials revealed that they were below 10 mg/kg that makes them safe for human consumption. Assessment in variation of the physicochemical, structural and functional properties of Tanzanian cassava landraces was conducted by Mtunguja *et al* (2016). Chisenga *et al* (2019b) studied the proximate composition, particle size distribution and cyanide content of cassava varieties in Zambia. There appears to be a dearth of information on South African cassava landraces.

Pre- and post-harvest factors affect the properties of cassava flour (Tewe and Lutaladio, 2004; Onitilo *et al.*, 2007a; Sajeev *et al.*, 2010). Types and properties of cassava flour vary depending on the process it undergoes (Aryee *et al.*, 2006; Sajeev *et al.*, 2010; Udoro and Anyasi, 2018). Several studies have been conducted on the effect of pre- and post-harvest factors on the quality attributes of cassava flour and intended use. Charoenkul *et al.* (2011) investigated the relationship of textural variety on the physicochemical characteristics of cassava flour and starches and reported that chemical composition, pasting, thermal and structural properties of starch from the 12 varieties studied were not significantly different. But for cassava flours (which consist of starch and non-starch components) showed wider variation in these properties. Chiwona-Karltun *et al.* (2015a) investigated the effect of varietal diversity and processing on the cyanogenic glucoside potential, starch quality and appearance of cassava flours from local and new cassava breeds from Southern-Eastern African region. The authors reported that the cassava varieties contained high cyanide however, the appearance of the flour was an advantageous end-user property. Eduardo *et al.* (2013) reported that differently processed cassava (sun-dried, roasted, and fermented) gave different product quality. The moisture, protein, crude fat, sugar, carbohydrate contents, and functional properties of cassava flour produced from chips dried in the open sun, solar dryer, and oven were affected by the drying methods (Agbemafle, 2019). Fermentation and duration of fermentation of cassava roots influenced the moisture, carbohydrate, water absorption capacity, swelling, solubility index, and resistant starch content of its flour. Pasta processed from these flours exhibited significant variation in their cooking loss, texture and sensory properties which was attributed to the duration of fermentation (Odey and Lee, 2020). Ramirez *et al.* (2019) optimised hot-air drying conditions of cassava flour for its application in gluten-free pasta formulation. The experimental design used by the authors was to produce cassava flour similar to the traditional raw material (wheat). Their results showed that to produce hot-air-dried cassava flour with higher water holding capacity was 57°C at 3m/s and optimal formulation for pasta was 26 g/100 g. The combined effect of drying time and temperature

influenced the thermal and physical properties of cassava flour (Omolola *et al.*, 2017). The drying behaviour of cassava chips using two cutting shapes (rectangular and circular) evaluated under different temperatures (60, 80, 100 and 120°C) was carried out by Pornpraipech *et al.* (2017). The study revealed that the rectangular chips dried at 100°C was optimal because they required less drying time and they had a soft, white colour desirable for cassava flour production.

The susceptibility of cassava roots to enzymatic browning during handling and processing is closely related to PPD. Brito *et al.* (2017) evaluated the phenol metabolism of minimally processed "baby cassava" under different storage temperatures (5 ± 2 and 25 ± 2°C) for 12 h and /or 10 days. They reported that the non-refrigerated product lost overall quality at 12 h while refrigerated pieces had great quality for 10 days which makes them suitable for market. Finding low-cost materials that preserve visual quality and are unharmful to human health is common in food processing. The use of antioxidants, such as citric acid and ascorbic acid, was found to be effective for reducing post-harvest browning for up to 9 days of storage in minimally processed-cassava of the "Cacauzinha" cultivar in a study by Medeiros (2009). In a similar study, Coelho *et al.* (2019) treated minimally-processed cassava with antioxidants (3% citric acid and 3% ascorbic acid) and a starch-based edible coating. The authors applied a 4 x 6 factorial completely randomized design consisting of the storage period (0, 3 6, 9, 12 or 15 days) and treatment (control, coating, antioxidant, or coating and antioxidant). Treatment with antioxidants was effective for reducing browning in minimally-processed cassava, retaining the quality of cassava pieces stored for 15 days at 5 ± 2°C. The combination of antioxidants and the edible coating showed no improvement compared to treatment with antioxidants alone. These chemical pre-treatments are commonly applied in fruit and vegetable processing and minimally processed foods such as cassava chips. There abound studies related to application of various food grade antioxidant pre-treatments on flours from crops such as breadfruit (Arinola and Akingbala, 2018), yam (Wahab *et al.,* 2016; Falade and Ayetigbo, 2015) and potato (Ahmed *et al.,* 2010a,b; Hutasoit *et al.,* 2018; Kuyu *et al.,* 2018) but there are limited studies on the use of these safe antioxidants on the pre-treatment of cassava flour. Therefore, a preliminary study was conducted, in this research, to investigate the effect of calcium chloride and citric acid, separately, on some physical and functional properties of solar-dried cassava flour, from two South African landraces (RCLR and WCLR). It was revealed that pre-treatment had no adverse effect on the physicochemical properties investigated (Nemaungani *et al.,* 2019).

Having this preliminary understanding, further prompted the extensive characterisation of the two identified South African landraces (RCLR and WCLR). Chemical (elemental analysis, metabolic profiling, and cyanide determination), physical (texture, bulk

density, and colour properties) and structural (scanning electron microscopy and XRD) of the root and flour were conducted. Furthermore, in this study, response surface methodology was used to evaluate the interactive effect of two key processing variables, drying temperature and concentration of pre-treatment on the thermal, colour, chemical, and functional properties of cassava flour. Process-induced changes, selected optimal processing conditions and advantageous end-use properties were highlighted. The study aims to fill the knowledge gap to promote utilisation of the crop in this region and globally.

1.4 Problem Statement

The challenge of food insecurity facing South Africa is a complex one. Therefore, there is a need for more crops to be explored and incorporated into the diet of the populace to curtail the menace of hunger and food insecurity. Though the country appears fundamentally food secure at the national level, this changes when the scale of analysis is reduced to the local level, at which many food-insecure households emerge (Altman *et al.*, 2009). The food security situation has been on the decline (WWF-SA, 2010). With a growing population at 2% yearly, the population of South Africa is expected to increase from 49 million in 2009 to 82 million by 2035. Therefore, food production or imports to feed the steadily increasing populace is needed (Stats SA, 2016). Despite cassava's popularity for food security and sustenance in Africa, it is not widely known in South Africa. It is grown as a secondary crop by mostly small-scale farmers and the sweet type is more commonly cultivated (Drimie and McLachlan, 2013). Cassava in South Africa is yet to be fully explored and there appears to be a dearth of information on the characteristics and potential use of landraces from this region for improved food security.

Post-harvest physiological deterioration (PPD) is associated with mechanical damage that occurs during the cassava harvesting process as the root is separated from the plant creating a wound (Coelho *et al.*, 2019). This unavoidable physiological disorder, if not controlled, could progress during processing leading to an increased respiratory rate, and the accumulation of reactive oxygen species (ROS). ROS are produced continuously and removed by various antioxidant mechanisms, including the enzyme superoxide dismutase (SOD), which catalyses the conversion of O_2^- into H_2O_2 and catalase (CAT), which in turn removes H_2O_2 and produces H_2O and O_2 (Sowmyapriya *et al.,* 2017). However, under conditions of stress, there is an oxidative burst and a higher production of ROS induced by damage (Xu *et al.*, 2013). This induces blue/black or brown discolouration of the vascular parenchyma from phenol oxidation via the action of polyphenol oxidase (PPO) and peroxidase (POD), triggering the phenomenon known as enzymatic browning or PPD (Salcedo and

Siritunga, 2011; Soares *et al.*, 2015; Djabou *et al.*, 2017; Coelho *et al.,* 2019). Discolouration during drying is an unpleasant occurrence associated with food processing. Drying is described as the reduction of material moisture to the required dryness values as a definite process and it is of major interest in food processing (Kazeem *et al.*, 2018). Apart from PPD, the drying method influences the quality of cassava flour (Eduardo *et al.,* 2013; Agbemafle, 2019). The use of optimum duration and temperature of drying cassava chips is a key factor in preserving the colour and thermal properties of cassava flour Omolola *et al.* (2017). Calcium chloride and citric acid are two anti-browning reagents, regarded as safe for food treatment. Studies are scarce on the application of these chemicals in pre-treatment of cassava flour. Furthermore, evaluation of the interactive effect of drying temperature and chemical pre-treatment on the physicochemical, thermal and functional properties of cassava flour is yet to be investigated.

1.5 Hypothesis

1.5.1 Null hypothesis

a. Cassava landraces used in this study will not differ in physical, chemical, and structural characteristics.
b. Chemical pre-treatment and drying temperatures will not influence the physical, chemical, thermal, and functional properties of cassava flour.

1.5.2 Alternative hypothesis

a. Cassava landraces used in this study will differ in physical, chemical, and structural characteristics.
b. Chemical pre-treatment and drying temperature will influence the physical, chemical, thermal, and functional properties of cassava flour.

1.6 Justification

Characterisation of cassava starches is well documented however, there are limited studies on the analysis of non-starch components of most cassava cultivars reported (Chisenga *et al.*, 2019a) and very few on the starch and non-starch components of South African cassava landraces. Application of flour from various plant sources in food systems depends greatly on information about the physicochemical properties of such food materials (Nkoudou and Essia, 2017) and screening them is quite imperative to determine their potential and most suitable end use (Shittu *et al.*, 2007). The heterogeneity in cassava genotypes reckons for variation in end-product properties, thus, documentation and catalogue of

properties of technological importance will form a baseline of information to enhance selection of the most appropriate genotypes to meet the needs of cassava end-users such as farmers, breeders, and industry (Chisenga *et al.,* 2019a). These South African cassava landraces, in this study, may offer some variations that could be important to industrial and food applications of their roots in this region and the world at large.

Multifaceted application of cassava both at domestic and industrial front positions it as one of the most important crops in the world's tropical and subtropical regions. It is expected that data generated from this study would project cassava as a valuable crop that can be used for purposes other than starch production, with emphasis on its flour as a possible composite in preparation of various foods. This is with a huge hope that the utilisation base of the under-utilised crop will increase and that value addition to cassava will have an overall positive impact on food security and economy of South Africa.

Processing cassava root to flour is a means to curtail post-harvest loss which entails several vital processing steps such as drying. Discolouration that is associated with PPD and processing can be controlled by using safe antioxidants to control enzymatic browning. The chemicals, citric acid and calcium chloride, selected in this study are expected to mitigate discolouration. However, factoring in the simultaneous effect of the concentration of pre-treatment (1 - 3%w/v) and drying temperature (50 – 70°C) of cassava chips, with the aid of response surface methodology (RSM), on selected properties of cassava flour is worth investigating. Providing data on optimal processing conditions will be useful for processors and consumers alike. The flour quality is important to determine its usefulness in food applications (Olatunde *et al.*, 2015). Cassava flour, with desired end-use properties, could act as a partial substitution for maize and wheat in processing of various food products. This would lower overall production costs and enhance food security. It will also reduce the over-dependence on maize and wheat as staples. The findings from this study may underpin future studies on cassava in South Africa.

1.7 Research Aim

Understanding the properties of the root is an important prerequisite for facilitating its utilisation. Therefore, this research work aims to characterise selected cassava landraces and evaluate process-induced changes in the physical, chemical, and functional properties of its flour.

1.8 Research Objectives

The specific objectives of this work are to:

1. Characterise the physical, chemical, textural, and structural properties of different landraces of cassava root and flour;
2. Determine the potential end-use properties of different landraces of cassava root and flour;
3. Evaluate the effect of different chemical pre-treatment and drying temperature on the physical and functional properties of cassava flour; and
4. Determine optimum processing conditions for production of cassava flour with specific qualities.

1.9 Thesis Outline

This thesis write-up consists of six chapters. The first chapter, being the introductory chapter gives a brief overview of the botanical description, agronomy and diverse utilisation of cassava root. The set back to utilisation of the root and importance of processing the highly perishable root to stable products such as cassava flour was briefly discussed. Background to the current study and problem statement was discussed, bringing to light the gap in knowledge and importance of this study. The research hypothesis, aim and objectives were also stated. Chapter two is an extensive literature review of cassava as an important staple root in the tropics and subtropics. Advances made in cassava breeding and the pending challenge of PPD were discussed. Variations in cassava flour processing and the impact on the quality of cassava flour were reviewed in-depth. The literature review revealed sparseness of studies related to the application of chemical pre-treatment in cassava flour processing, hence this study.

Chapter three specifically addresses the first and second research objectives of this study which focuses on characterisation of the red and white cassava landraces used. It entails comparative morphological, physical, chemical and structural characterisation of the landraces. This chapter provides information on the non-starch components of cassava which is lacking in literature. The similarities and differences in the morphological, physical, metabolic profile, elemental composition and structural properties of the red and white cassava landraces were established. Potential end-use properties of both landraces were mentioned.

Chapters four and five were designed to investigate process-induced changes on the thermal, functional and physical properties of cassava flour with the aid of RSM. Calcium chloride and citric acid were applied separately in the design of experiment and their effect on the quality of cassava flour was compared. The linear, quadratic, and interactive effects of varying pre-treatment concentration and drying temperature on the quality of cassava flour were determined with the aid of RSM. Process optimisation with specific objectives was also conducted for all parameters investigated. ANOVA of experimental and control samples was

conducted using the SPSS statistics software Version 25 (IBM Corp., New York, USA). Both result chapters addressed the third and fourth objectives of this study as well as investigating the validity of the second research hypothesis. Data generated is aimed at providing processors with information on the effect of the processing conditions adopted in this study on the quality of cassava flour. This information may be helpful to consumers as well.

Chapter six gives a general conclusion on the outcome of investigations made in the study. The findings of each result chapter are discussed, addressing the hypothesis and objectives of the research. Recommendation for further research related to cassava flour processing was highlighted. Research outputs such as; journal publication, award received, conference presentations, consortium and workshop attended were listed.

CHAPTER TWO: LITERATURE REVIEW

2.1 Botanical Description of Cassava

Cassava (*M. esculenta*) is a perennial wooden shrub belonging to the family *Euphorbiaceae*. It produces enlarged tuberous roots which are the main storage organs in the plant. The thick root is connected to the plant by a short thick wooden neck. Figure 2.1 shows the plant and roots of cassava. Cassava root has a longish, round form and can grow to between 15 and 100 cm and reach a weight of 0.5 to 2.5 kg. Mature cassava storage roots have three distinct tissues: bark (periderm), peel (or cortex), and parenchyma. The periderm (3% of the total weight) is a thin layer made of a few cells thick.

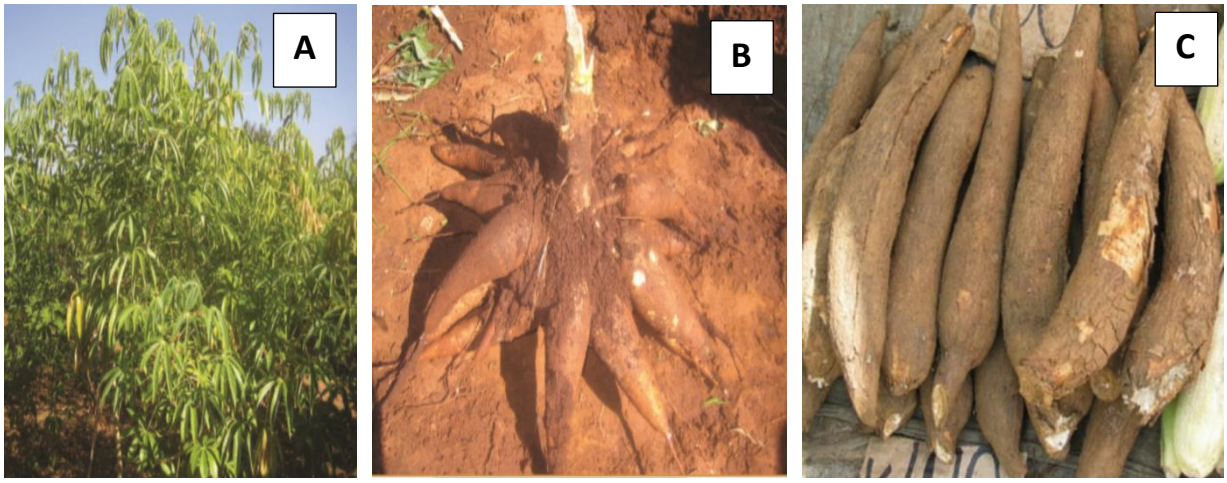

Figure 2.1. Cassava plant and roots. **A** - Cassava plant (DAFF, 2010); **B** - harvested roots attached to stem (DAFF, 2010); **C** - cleaned unpeeled roots displayed for sale (Lim, 2016).

The peel layer, which is of sclerenchyma, cortical parenchyma and phloem constitutes 11-20% of the root weight. The parenchyma, the edible portion of the fresh root, comprises approximately 85% of the total weight consisting of xylem vessels radially distributed in a matrix of starch-containing cells (Alfredo, 2002; Carvalho *et al.*, 2018). The growth cycle of a typical cassava crop is close to one year. The roots start bulging about three months after planting and continue to increase in weight until about 9 to 15 months when the crop is usually harvested (Aloys and Ming, 2006; Carvalho *et al.*, 2018). The crop has growth advantages and production can take place in soil where other crops such as maize, sorghum and sweet potatoes cannot grow (Reynolds *et al.,* 2015).

2.2 Agronomic Description of Cassava

Cassava is a perennial crop that is handled as annual, unlike cereals that have a pre-established development (germinate, grow, flower, fill the grain, mature, and die). Cassava offers the flexibility of harvest when it is needed (Cellabos *et al.,* 2010). Most varieties of cassava grow well in marginal conditions that other crops will not thrive; they are resistant to drought, major pests and diseases (Reynolds *et al.*, 2015). Farmers often abuse this advantageous nature of the crop thereby negating the minimum requirements for a competitive and sustainable production (Cellabos *et al.*, 2010). The trait of drought tolerance and survival in marginal conditions has put cassava expansion high on the agenda of many governments, especially in the context of climate change adaptation strategies (FAO, 2015). Cassava is an important cash crop and has moved beyond merely being a food security crop in Sub–Saharan Africa to becoming a commercial crop (Nweke, 2004; Fermont *et al.*, 2010; Tumuhimbise *et al.*, 2014).

Scientific efforts have been made to improve the relevance of cassava for tropical and subtropical agriculture. With the creation of the International Centre of Tropical Agriculture (CIAT) in Columbia and the International Institute of Tropical Agriculture (IITA) in Nigeria and with cassava related research conducted in national research institutes of several countries like China, Cuba, India, Thailand, Indonesia, Vietnam amongst others have led to successful breeding projects, modernisation of cultural practices, development of new processing methods, and expansion of utilisation (Cellabos *et al.*, 2010). Cassava is indeed gaining in importance after maize as presented in Figure 2.2 (Haggblade *et al.*, 2012) and in climate change debates (Burns *et al.*, 2010; Jarvis *et al.*, 2012; Rosenthal and Donald, 2012). However, there remain challenges, especially when it comes to varietal adoption (Alene *et al.*, 2013; Chiwona-Karltun *et al.*, 2015a; Ayetigbo *et al.*, 2018).

2.3 Production and Advances in Breeding of Cassava

Native to South America, cassava was first introduced from Brazil to West Africa by Portuguese traders in the 1500s. An independent introduction was made to East Africa in the 1700s also by the Portuguese (Jones, 1959). Cassava spread to Central Africa along trade routes from the Congo Basin. Natural cross-pollination and selection by farmers is most probably responsible for the large number of morphologically distinct local varieties found today in Africa (Fregene *et al.*, 2000). Cassava is the favoured root of the tropics and subtropics. Africa accounts for about half the world's production, followed by Asia and then Latin America as shown in Figure 2.3 (FAO, 2015).

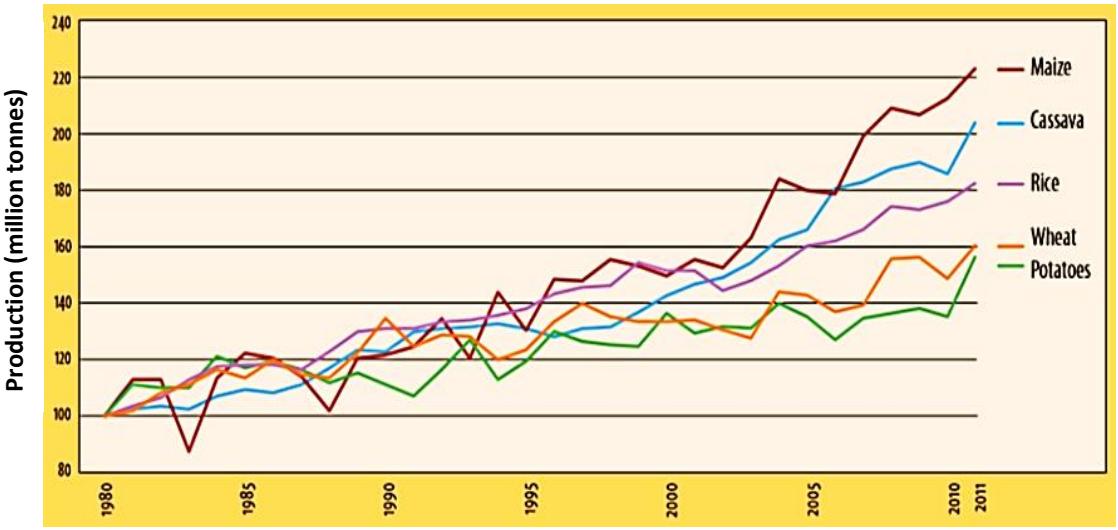

Figure 2.2. Growth in world production of major crops, 1980-2011 (index 1980 = 100). Source: FAO (2013).

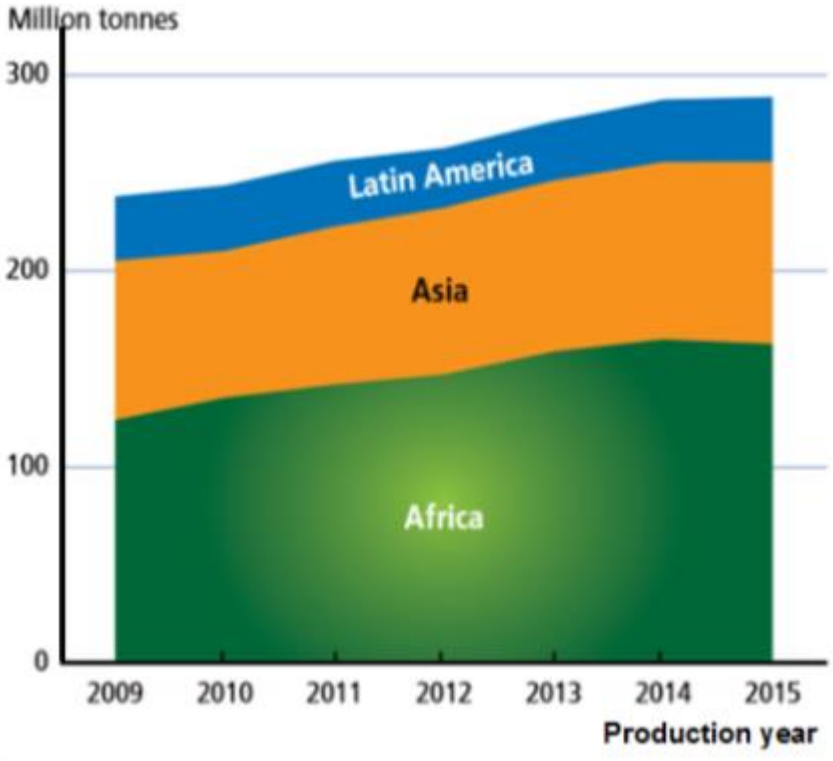

Figure 2.3. World cassava production. Source: FAO (2015).

In recent decades, the expansion of cassava production has been relatively steady owing to the release of improved varieties (Hillocks, 2002; IITA, 2004; Montagnac *et al.,* 2009a; Chisenga *et al.*, 2019a) as well as its relevance with regards to utilisation, economic value and food security. The growing interest in the utilisation of cassava has increased research effort towards application of genetics in cassava breeding and cultivation to improve storage, starch production, nutritional values (Carvalho *et al.*, 2018) and other desirable qualities. New hybrids, clones, cultivars, accessions, varieties and landraces of cassava have been developed across the globe with remarkable impact made in terms of yield, disease tolerance and improvement of quality (Ferguson *et al.*, 2012). For instance in Africa, disease-resistant varieties of cassava were developed to combat a decrease in cassava production caused by an outbreak of cassava mosaic disease (Shittu *et al.*, 2007; Maredza *et al.*, 2016). In other instances, biofortified cassava cultivars with improved vitamin A content (Eleazu and Eleazu, 2012; Ayetigbo *et al.,* 2018) and less cyanogenic glucosides (Montagnac *et al.*, 2009b) have also been developed. Total cassava production in Africa increased by approximately 20% from year 2010 to 2016 with a yield of 132,200,764 tons to 157,271,697 tons (FAOSTAT, 2018). The world's production is estimated to reach 290 million tons per year in 2020 (Zhu, 2015).

2.4 Classification of Cassava Varieties

The classification of cassava into two distinct classes 'sweet' and 'bitter' has been established by both farmers and scientists based on its inherent cyanogenic glucoside potential and intended end use (Mkumbira *et al.*, 2003; Chiwona-Karltun *et al.*, 2004, 2015a). The bitter cassava is easily recognised first by its green leaf-stalk and the whitish outer cortical layer of the root. The sweet cassava is known by a red leafstalk and purplish outer cortical layer. Farmers ethno botanically classify cassava varieties based on their taste. The classification dichotomy based on taste is an important factor in determining potential toxicity (Muhlen *et al.*, 2000; Wilson and Dufour, 2002).

Several studies have shown that the bitter taste is correlated with the cyanogenic glucosides, mainly linamarin and that the cyanogenic glucoside contained in the sweet varieties is much lower than that contained in the bitter varieties. The sweet type has less than 100 mg of total cyanogens per kg of peeled fresh roots while the bitter type contains more than 100 mg cyanogens per kg of peeled fresh roots (Muhlen *et al.*, 2000; Mkumbira *et al.*, 2003; Chiwona-Karltun *et al.*, 2004; Bradbury *et al.*, 2013). The sweet varieties can be eaten raw, boiled, or cooked without prior processing while bitter varieties are processed to

reduce risk of poisoning by residual cyanogens after consumption (Chiwona-Karltun *et al.*, 2000; Ellen and Soselisa, 2012).

The selection and adoption of varieties are dependent on location, human groups, and cultural preferences (Chiwona-Karltun *et al.*, 2015a). An ethno-botanical survey of cassava farmers' preference criteria by Agre *et al.* (2017) showed that the culinary, technological, agronomic, and economic properties of the roots were considered important. These authors recommended that molecular and morphological characterisation and classification be done for better identification of cultivars due to the occurrence of duplication and synonyms during their survey. The diversity in cassava genotypes accounts for differences in end-product properties and it would require characterisation of cassava varieties to determine their most suitable application (Chisenga *et al.*, 2019a). Characterisation of a food crop is a scientific process by which its properties are screened and ascertained. It is fundamental for the scientific understanding of food material. This involves investigating the physical, chemical, structural, functional, microbial and thermal properties of food material. This is necessary for a better understanding of the quality of the material and to optimise processing and utilisation.

2.5 Cassava Utilisation for Food and Non-food Products

Cassava cultivars are generally used as human food in the form of fresh vegetables, after boiling, flour after drying and other food products after fermentation. Traditional cassava processing methods involve several steps including peeling, soaking, grating, drying, milling, roasting, fermenting, sieving, steaming, baking and frying (Aloys and Ming, 2006). Specific combinations of these steps lead to a myriad of different cassava products with acceptable taste to a wide range of consumers **(Table 2.1). In** Figure 2.4 is a flow production of various products from cassava. The high carbohydrate content of cassava makes it a suitable raw material for industrial production of ethanol, starch, adhesives, bio-fuels, glucose syrup among other uses (Tonukari, 2004; Osun *et al.*, 2014). Likely products from fermentation of cassava because of its carbon content as substrate in bioprocessing include bulk chemicals, value-added products, enzymes, organic acids, amino acids, and biologically active metabolites (Sajeev *et al.*, 2010). In recent times, cassava starch and bagasse are used in manufacture and reinforcement of eco-compatible plasticised films for food packaging (Edhirej *et al.*, 2017; Valencia-Sullca *et al.*, 2018). As food, feed and industrial markets are promising (FAO, 2005), there is an increasing focus on cassava by governments, research and development institutes in Africa (Fermont *et al.*, 2010; Adenle *et al.*, 2017; Poku *et al.*, 2018).

Table 2.1. Cassava food products from various world regions

Products	Category	Major processing steps	Region	Reference(s)
Flour	Flour	Dry milling, drying & sieving	Africa, Asia, & Latin America	Charoenkul *et al.* (2011); Agbemafle (2019); Ramirez *et al.* (2019)
Starch/Tapioca	Starch	Wet milling, decanting, drying and sieving	Africa, Asia, & Latin America	Aryee *et al.* (2006); Falade *et al.* (2019)
Dried chips	Chips	Chipping and drying	Asia, Africa, & Latin America	Udoro *et al.* (2008); Kaaya and Eboku (2010)
Minimally processed chips	Chips	Chipping, cooking, chemical pre-treatment	Asia & Latin America	Brito *et al.* (2017); Coelho *et al.* (2019)
Gari	Ready-to-eat gritty meal	Grating, fermenting and roasting	West Africa	Olagunju *et al.* (2012); Udoro *et al.* (2014)
Fufu	Dough meal	Soaking, pounding and cooking	West Africa	Obadina *et al.* (2008); Ogbo &Okafor (2015)
Lafun	Flour meal	Chipping, fermenting, drying and dry milling	West Africa	Padonou *et al.* (2009); Taiwo *et al.* (2016)
Mingao	Juice	Fermenting, boiling and simmering	South America	Balagopalan (2002)
Manicuera	Drink	Boiling	Northwest Amazon	Aloys and Ming (2006)
Farinha	Granular flour	Crushing and dewatering	Latin America	Cardoso *et al.* (2005)
Landang /cassava rice	Meal	Squeezing and pelleting	South East Asia	Elegado *et al.* (2016)
Sago wafers	Meal (snack)	Steaming, gelatinising and drying	Asia	Srinivas and Anantharaman (2000)

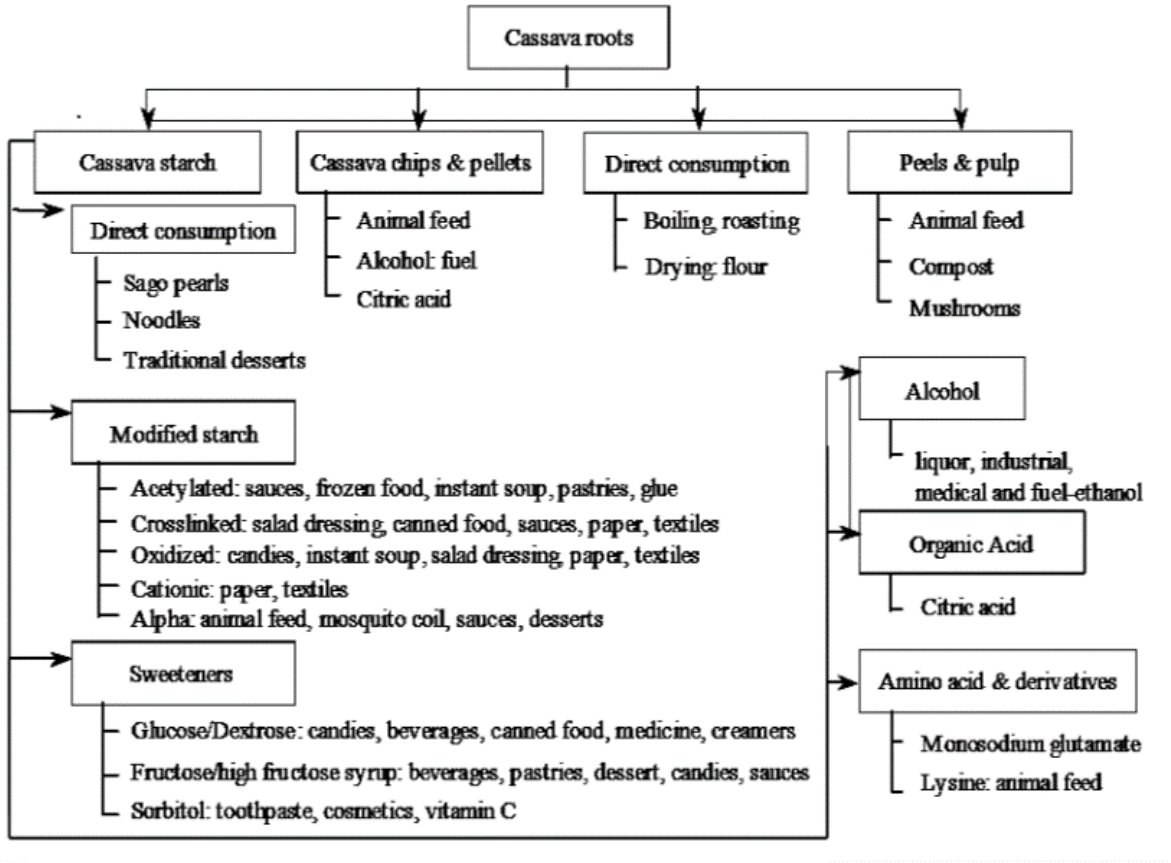

Figure 2.4. Cassava root processing into value-added products. Source: CIAT (2012).

2.5.1 Potential of cassava in the production and volatility of flavour-active compounds

Volatiles are organic compounds with low molecular weight (<2550 g/M) and high vapour pressure at ambient temperature. A wide range of volatiles are produced by plants and these volatiles are grouped into esters, acids, alcohols, ketones, terpenoids, and alcohols (Madrera *et al.*, 2015). Volatiles are one of the three major components of flavour in foods; taste (due to non-volatile compounds), aroma (due to some volatile compounds) and texture (mouthfeel). All three components interact to produce a flavour response (Jansky, 2010). In a specific crop hundreds of volatiles may be identified but only a few may be responsible for the characteristic aroma or flavour of the crop (Kües *et al.*, 2018). Apart from giving the plant its characteristic aroma, the volatile and semi-volatile compounds in plant constituents play major roles in the plant's defense against pest, insects, herbivores, and pathogens (Yang *et al.*, 2013). The volatile profile of the crop cassava is poorly studied. Knowledge of the flavour profile of cassava can facilitate a better understanding of the crop and contribute to the characteristic studies of cassava.

In terms of value, the flavour market accounts for more than 50% of the global market for flavours and fragrances, which is expected to rise (IAL Consultants, 2013). Presently, most flavours are either extracted from plant sources or synthesized by chemical means. Through the use of sophisticated extraction techniques and advanced chromatographic methodologies, flavour-active compounds are identified. Flavour-active compounds contribute significantly to the organoleptic properties of so many food products and flavour control is a crucial element in food production processes. Flavour synthesis via biotechnological process is encouraged by the rising concern of consumers for natural products. Biotechnologically derived products based on microorganisms, cell structures and enzymes are also regarded as natural (Longo and Sanroman, 2006; Dubal *et al.*, 2008; Saerens *et al.*, 2010; Braga *et al.*, 2015). In the production of natural flavour compounds, the major constituents are the substrate used and the enzymatic activities or enzymes used during the process. In most cases the natural process is modified, by introducing microorganisms and altering the substrate, to get the desired flavour. Agro-industrial waste products such as cassava bagasse, sugar cane bagasse, orange peel, bread waste and others have been used to produce volatile compounds as shown in Table 2.2 (Mantozouriduo *et al.*, 2015).

Cassava bagasse is the fibrous residue left of starch extraction from the roots and on a dry weight basis, it contains 50 - 60% residual starch (Edhirej *et al.*, 2017). The rising trend of bio-flavour synthesis by microorganisms is hindered by the high manufacturing costs, partially attributed to the cost of the starting material (Mantozouriduo *et al.*, 2015). Cassava as an energy-dense root, highly rich in carbohydrate, naturally prone to spoilage within a few days after harvesting makes it a suitable substrate for enzymatic activities that may emit flavour-active volatiles. This has not been researched.

2.5.2 Cassava utilisation for food security in South Africa

Altman *et al.* (2009) stated that at the national level South Africa looks food secure but when the scale of analysis is reduced to the local level, many food-insecure households emerge. With a steadily increasing population and no matching increase in food production, food insecurity may increase. A National Development Plan (NDP), an innovative framework, to inform action required across society to deal with pervasive hunger was launched in South Africa. The NDP calls for collaboration between government, the private sector, civil society and citizens to establish “self-sustainable” local food systems that would underpin universal access and utilisation over time. Such an approach is envisaged to reduce hunger and poverty, increase agricultural development and address malnutrition, which will in turn contribute towards skills development and improve inclusive economic growth and job creation (National Planning Commission-SA, 2012).

Table 2.2. Volatiles from cassava bagasse and other agro-industrial waste

Microorganisms	Substrates	Volatile compounds
Ceratocystis fimbriata	Cassava bagasse, wheat bran, sugarcane bagasse	Acetaldehyde, 3-methyl butanol, 3-methylbutyl acetate, ethyl acetate, ethyl propionate
Kluyveromyces marxianu	Cassava bagasse, giant palm bran	Isoamylic alcohol, ethyl acetate, propyl acetate, butylacetate, ethylpropionate, ethyl isobutyrate, isoamyl acetate
Rhizopus oryzae	Cassava bagasse, soybean meal, apple pomace	Acetaldehyde, 3-methyl butanol, 1-propanol, ethyl acetate, ethyl propionate
Ceratocystis fimbriata	Coffee husk	Isopropanol, ethyl acetate, ethyl isobutyrate, isobutyl acetate, isoamyl acetate, ethyl-3-hexanoate
Ceratocystis fimbriata	Mixture of citric pulp and soya bran, sugarcane molasses, soya molasses	Isoamyl acetate

Source: Mantzuoridou *et al.* (2015)

Studies on the feasibility of partial substitution of food raw materials with alternative ingredients, to control costs and maintain the supply of foods, particularly staples will be a step in the right direction. In the light of the above mentioned, cassava is one root crop that offers the advantages of high yield and calorie content and forms a vital part of the energy and nutritional requirements of millions of people in developing countries (Scott *et al.*, 2000; FAO, 2015).

2.6 Genetic Improvement of Cassava

The significant role of cassava as a staple around the world has made it a target for biofortification. Global efforts to develop cassava germplasm enriched with bioavailable nutrients; increased zinc, iron, protein, vitamins A and E content, decreased cyanogen content, delayed post-harvest deterioration, and virus-resistant varieties is a work in progress (Montagnac *et al.*, 2009a). The IITA and CIAT have been at the forefront of executing breeding projects and introducing improved varieties to farmers.

2.6.1 Cassava biofortified with vitamin A

Cassava root contains small amounts of β-carotene, a provitamin A carotenoid, which can be converted as needed into retinal, reduced to retinol, and stored in the liver esterified to fatty acids (Pauleikhoff *et al.,* 2001). Vitamin A is a fat-soluble vitamin playing an important role in vision, bone growth, reproduction, and the maintenance of healthy skin, hair, and mucous membranes (FAO/WHO, 2002). Vitamin A deficiency (VAD) has been identified as a health problem that results in partial or complete blindness mostly in children (UNICEF, 2005; IITA, 2014). VAD is most prevalent in regions, like Africa, Brazil and Asia, where cassava is a staple crop (WHO/FAO, 2003; Montagnac *et al.*, 2009a). Biofortification of staple crops with provitamin A carotenoid is an emerging strategy to address the vitamin A status of the poor (Tanumihardjo, 2008; Tanumihardjo *et al.*, 2008; Saltzman *et al.*, 2013). The bioconversion of β-carotene to vitamin A in the body is naturally regulated and therefore β-carotene has little potential for toxicity compared with high intake of vitamin A-fortified foods (Tanumihardjo, 2008). Utilising biofortified cassava with enhanced β-carotene would be a sustainable strategy to reduce the prevalence of vitamin A deficiency in areas where cassava is a staple food (Saltzman *et al.,* 2013). Other carotenoids that are not provitamin A that are contained in cassava include lycopene, xanthophylls, lutein, and zeaxanthin (Tanumihardjo, 2008). Lycopene appears to be particularly efficient at quenching the destructive potential of singlet oxygen. Lutein and zeaxanthin might act as antioxidants in the macular region of the human retina (Pauleikhoff *et al.*, 2001). The possible role of lutein in preventing age-related eye disease is currently under investigation. Screening, selection, and crossbreeding varieties of cassava with a high content of carotene are currently underway and show potential as a dietary source (Nassar *et al.,* 2005).

A broad distribution of carotene concentrations in cassava leaves and roots has been observed. Carotene content has been reported to be 100 times higher in cassava leaves (12 - 97 mg/100 g FW) than in roots (0.102 - 1.069 mg/100 g FW). The colour intensity of the cassava root and the carotene concentration were positively correlated (Chavez *et al.*, 2003). Boiling the cream-coloured biofortified cassava revealed the β-carotene through the deepening of colour to yellow. This is more clearly observed when the outer brown peel is removed before boiling (Howe *et al.*, 2009). The carotene concentration was 0.13 mg/100 g in white cassava roots, and 0.39 mg/100 g, 0.58 mg/100 g, 0.85 mg/100 g, and 1.26 mg/100 g in cream, yellow, deep yellow, and orange cassava roots, respectively. Moreover, 5 orange cassava genotypes have been found with β-carotene contents ranging from 2.04 to 2.55 mg/100 g FW in the Amazonian region of Brazil and Colombia (Montagnac *et al.,* 2009a). These results are encouraging because using conservative conversion factors of 12 µg β-carotene to 1 µg vitamin A proposed by the Institute of Medicine, Food and Nutrition Board (2001), this level of β-carotene would result in 170 to 210 µg vitamin A. This range of vitamin

A includes the estimated average requirement for a young child 1 to 2 y old (that is, 210 µg vitamin A) and represents about 40% of the estimated average requirement for a woman of childbearing age (that is, 485 µg vitamin A). Yellow and orange cassava roots are a viable alternative for delivering provitamin A carotenoids to vitamin A deficient populations that consume cassava. Considering the high daily intake of cassava in several African countries (FAO, 2006), cassava biofortified with β-carotene could readily impact the prevalence of night blindness due to vitamin A deficiency in women if widely adopted (WHO, 2008).

2.6.2 Biofortified cassava with improved protein value

Cassava roots have a very low protein content (≤ 1% FW). To engineer improved storage proteins with balanced amino acid composition in cassava roots, Zhang *et al.* (2003) used *Agrobacterium* to successfully transfer a 284 bp synthetic gene (ASP1) coding for 11.2 kDa-storage protein rich in essential amino acids (80%) into embryonic suspensions of cassava. They observed stable integration and expression of ASP1 in cassava leaves and primary roots. In a further breeding study by Zhang *et al.* (2004) analysis of 1-y-old cassava plants from the 2nd vegetative generation grown under greenhouse conditions showed an increase in protein content and essential amino acid composition in storage roots of several transgenic lines. They identified two cassava root specific promoters related to vascular expression and secondary growth which represent valuable candidates for targeting the protein ASP1 in storage roots for genetic improvement. Researchers have also tried to improve the nutritional value of cassava by crossbreeding wild-type varieties. The interspecific hybrid of cassava has been identified to possess characteristics such as higher protein content, moderate cyanide content, higher content of manganese and zinc than those of typical cassava cultivars (Nassar *et al.*, 2004). Further research has continued to indicate the feasibility of selecting interspecific hybrids that are rich in both crude protein and amino acids to improve the protein value. This interspecific cassava hybrid has an improved amino acid profile with 10 times more lysine and 3 times more methionine than the common cultivar (Nassar and Souza, 2007).

2.6.3 Improved cassava variety with less cyanogen content

The use of conventional breeding and development of transgenic cassava plants are currently applied in generating cassava varieties with reduced cyanide content. The breeding methods entail selective inhibition of expression of genes which catalyse the synthesis of linamarin in cassava (Montagnac *et al.,* 2009b). The expression of two cytochrome P450

genes (CYP79D1 and CYP79D2) which catalyse the 1st dedicated step in linamarin synthesis in leaves and roots were selectively inhibited (Siritunga and Sayre, 2003, 2004). There was a significant reduction of linamarin content (60-90%) when cyanogenic glucosides were selectively inhibited in the leaves. Although, the expression of CYP79D1 and CYP79D2 in cassava roots had not been modified compared to wild-type cassava roots, a 99% reduction of linamarin content was observed. However, normal root linamarin levels were present when the cyanogenic glucosides were inhibited in the roots. Therefore, it was suggested that linamarin is transported to the roots from the leaves the site of its synthesis. Transgenic approaches to reduce cyanogen in cassava have focused on suppressing cyanogen synthesis or accelerating cyanogen breakdown. One potential benefit of lowering cyanogen content is the facilitation of free cyanide assimilation into amino acids. Thus, reducing toxic cyanogens would have the added benefit of improving the protein value of the roots (Siritunga and Sayre, 2007). The complete removal of cyanogens in transgenic cassava plants would guarantee complete safety for human consumption and could make them palatable for herbivorous animals and insects (Nweke *et al.*, 2002).

The development of transgenic cassava plants entails the increased expression of hydroxynitrile lyase (HNL), an enzyme which catalyses the conversion of acetone cyanohydrin to cyanide accelerated cyanogenesis which in turn increased cyanide volatilisation or extraction during processing (White *et al.*, 1998). Siritunga *et al. (*2004) reported that foods with activated HNL (during frying and boiling) had a lower amount of acetone cyanohydrin. They reported 80% conversion of root linamarin to acetone cyanohydrin, at pH 5 and 25ºC and within 2h following tissue maceration. Acetone cyanohydrin was substantially reduced in transgenic cassava plants expressing HNL in their roots. Transgenic cassava plants have the advantage of retaining the state linamarin level in intact plants over the acyanogen plants therefore deterring herbivores and insects (Siritunga and Sayre, 2003; Siritunga *et al.*, 2004).

2.7 Setbacks to Cassava Utilisation

Despite the increase in cassava production, there are two major setbacks to the utilisation of cassava which are the hydrocyanogenic potential and high perishability of the root (Mtunguja *et al.*, 2016; Nkoudou and Essia, 2017; Atieno *et al.*, 2018; Liu *et al.*, 2019).

2.7.1 Hydrocyanogenic potential of cassava

Cassava is majorly grouped into two (bitter and sweet) based on their inherent cyanogenic glucoside potential and intended end use (Chiwona-Karltun *et al.*, 2015a). The bitter cassava contains more bitter juice in its root. The bitter taste is correlated with the

amount of cyanogenic glucosides, mainly linamarin in the root (Chiwona-Karltun *et al.*, 2004). Cyanogenic content in the sweet varieties is much lower than that contained in the bitter varieties. Cyanide is extremely toxic to humans and animals; therefore, the roots are processed to reduce the cyanide content to safe levels before consumption (Abraham *et al.*, 2016). WHO recommended safe consumption level of cyanide for human consumption is 10.00 mg/kg. The sweet varieties can be eaten raw, boiled, or cooked without prior processing while bitter varieties are subjected to more rigorous processing, in most cases fermented, to reduce the risk of poisoning by residual cyanogens before consumption (Chiwona-Karltun *et al.*, 2000; Irinkoyenikan *et al.*, 2008; Ellen and Soselisa, 2012; Nkoudou and Essia, 2017).

2.7.2 Post-harvest deterioration of cassava

Cassava roots cannot be used for reproductive purposes and the fresh roots cannot be stored because they deteriorate rapidly due to a process known as post-harvest physiological deterioration (PPD). High perishability of cassava root, due to rapid postharvest physiological deterioration (PPD), is a major challenge yet to be surmounted (Atieno *et al.*, 2018; Coelho *et al.*, 2019). Post-harvest physiological deterioration is a complex biochemical and physiological process that starts with vascular streaking which is a blue-black colouration later followed by a microbial activity that causes complete spoilage of the root (Salcedo and Siritunga, 2011). This process is mainly attributed to an increase of oxidative enzyme activity of polyphenol oxidase (PPO) and peroxidase (POD) present in the vascular tissues of cassava resulting in generation of phenols including leucoanthocyanidins and catechins which polymerise to produce condensed tannins (Uarrota and Maraschin, 2015).

The utilisation of cassava root in food and other industrial applications is limited by rapid PPD, which reduces the shelf life and degrades its quality attributes (Sánchez *et al.*, 2006; Sowmyapriya *et al.*, 2017). Post-harvest physiological deterioration (PPD) of cassava reduces the shelf-life and degrades the quality of the root during handling and processing (Sowmyapriya *et al.*, 2017). The PPD happens so fast, that roots spoil 2-3 days after harvest (Coelho *et al.*, 2019) hence the roots are quickly processed to stable products.

Studies have been conducted to understand the complex phenomenon responsible for PPD of cassava storage roots but still the problem persists (Reilly *et al.*, 2001, 2004, 2007; Morante *et al.*, 2010; Atieno *et al.,* 2018; Liu *et al.,* 2019). Phytochemical analysis of cassava fresh roots and roots undergoing PPD by Bayoumi *et al.* (2010) identified and isolated galactosyl diacylglycerides as well as b-carotene, linamarin, and β-sitosterol glucopyranoside from fresh roots while hydroxycoumarin scopoletin and its glucoside scopolin were identified from cassava roots during PPD, as well as trace quantities of esculetin and its glucoside esculin. The early signs leading to PPD though not fully understood has been attributed to

increased cellular respiration and enzymatic activities such as phenylalanine ammonia lyase (PAL) glucanase, proteinase, polyphenol oxidase and peroxidase (Iyer *et al.,* 2010) as well as the accumulation of secondary metabolite (Bayoumi *et al.,* 2008, 2010). Analysis of biochemical changes and starch functional qualities of newly bred cassava genotypes with PPD tolerance roots showed a relation of PPD to scopoletin synthesis. Scopoletin and its glucoside scopolin show antioxidant properties and which may by oxidation and polymerisation give rise to the blue/black discolouration (Bayoumi *et al.*, 2008). There was a loss of starch, a decrease in gel clarity, an increase in gel viscosity and PPD levels significantly differed between clones (Sanchez *et al.,* 2013). To reduce losses due to PPD, the roots are quickly converted to shelf stable products. Cassava chips and flour are products that are easily processed from the roots which can subsequently be used for both industrial and traditional purposes (Amoa-Awua *et al.*, 2005; Udoro *et al.*, 2008).

2.8 Nutritional Composition of Cassava Root

Cassava like other roots and tuber crops is primarily a source of carbohydrate which account for about 85% of its proximate dry matter composition. The tuber consists of the peel and the flesh. The peel comprises 10 - 20% of the tuber. The edible starchy flesh comprises some 80 - 90% of the root and includes about 62% water, 35% carbohydrate, 0.5 - 1.5% protein, 0.3% fat, 1 - 2% fibre and 1% mineral matter (Charles *et al.*, 2005). Most of the carbohydrate fraction is starch, which makes up 20 - 25% of the tuber (Montagnac *et al.*, 2009a). Cassava has been criticised for its low and poor-quality protein content even though the plant advantageously produces more weight of carbohydrate per unit area than other staple food crops under comparable agro-climatic conditions. To address the issue of nutrient deficiency of cassava, efforts are being made to breed biofortified varieties of the crop and at another end enrich ready-to-eat cassava products (Olatunde *et al.,* 2016; Ayetigbo *et al.*, 2018).

2.9 Starch Composition of Cassava

Cassava contains more than 80% starch (dry weight) which makes it an energy-rich food. The starch granules are formed from a heterogeneous mixture of two homopolymers- amylose and amylopectin- the former is linear and the latter is highly branched in structure (Herrero-Martinez *et al.*, 2004). Amylose is formed by units of D-glucose linked in α (1-4) while amylopectin is branched at the D-glycosidic α- (1-6) points as depicted in Figure 2.5. These macromolecules are deposited within the granule in successive layers, forming rings of growth, which are constituted of interspersed crystalline and amorphous regions, forming a

semicrystalline region (Singh *et al.*, 2007). The crystalline layers are formed mainly of amylopectin chains stabilised by hydrogen bonds which are the main components of the crystal layer, while the amorphous layers have branching points of amylopectin and amylose in an unordered conformation (Jane, 2006; Copeland *et al.*, 2009).

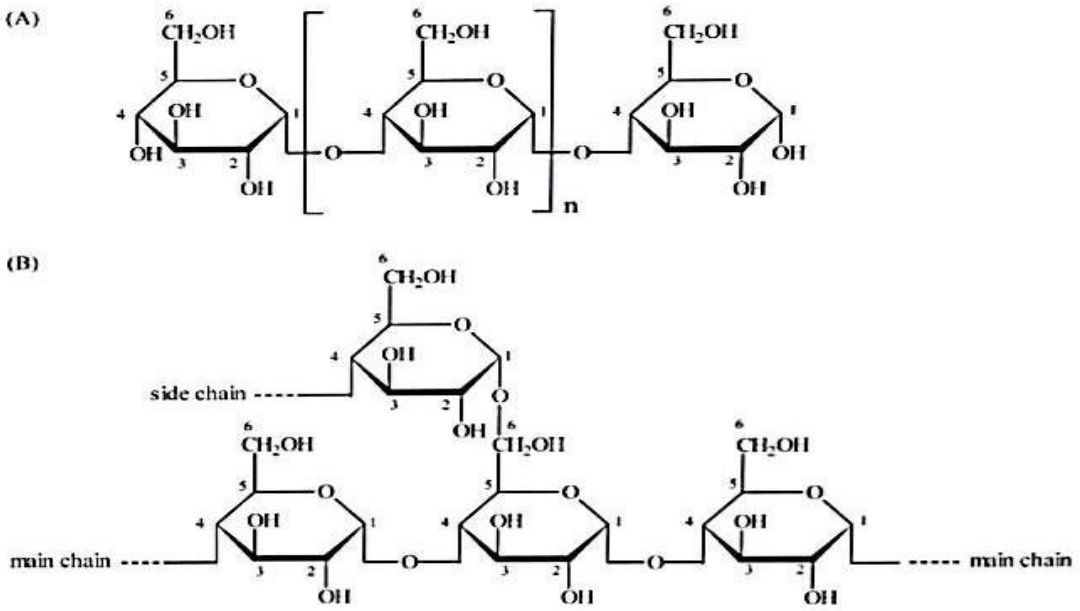

Figure 2.5 Structure of amylose (A) and amylopectin (B) showing the two different types of chain linkages. Source: Herrero-Martinez *et al.* (2004).

Starch is used in food preparations as a gelling and thickening agent, stabiliser and texture modifier. Starch undergoes gelatinisation which involves granule swelling, amylose leaching and amylopectin fusion and upon cooling due to retrogradation, solubilised starch forms a viscous dispersion or paste or gel depending on the temperature of processing and concentration of dispersion, varieties, harvesting age, and growth season (Lagarigue and Alvarez, 2001; Moorthy, 2001; Brouillet-Fourmann *et al.*, 2003). The viscoelasticity of a food material is commonly used as a measure of food consistency and it is influenced by the nature of starch it contains. Starch can be classified nutritionally as rapidly digestible, slowly digestible and resistant starch. Resistant starches have been shown to have similar impacts on human health to that of conventional fibre-enriched foods and positive impacts on some kinds of cancer, cardiovascular diseases, colonic health, obesity, and osteoporosis (Lunn and Buttriss, 2007; Morales-Medina *et al.*, 2014). However, the potential health benefit depends on the source, type, and level of resistant starch consumed (Buttriss and Stokes, 2008). Cassava has been reported to contain resistant starch (RS) and cassava flour alongside other products

of cassava can be considered a good source of RS which is beneficial to the intestinal tract (Pereira and Leonel, 2014).

2.10 Comparison of Cassava Flour and Starch: Physicochemical and Functional Properties

Cassava flour and starch are two different products with some similarities, obtained from the root. They are fine and powdery materials derived from milling and sifting pre-processed cassava root. The processing technology of cassava flour is easier than that of starch. While cassava flour is traditionally obtained by milling the dried root, the starch is extracted as slurry from wet milling of the root (Dziedzoave *et al.* 2006). The flour requires less use of water and a lower amount of byproducts and waste (Abass *et al.* 1998). The components often found in flours include starch, non-starch polysaccharide, sugar, protein, lipid and inorganic materials (Charoenkul *et al.*, 2011). Although starch is the major component of cassava flour, other components may play a significant role in influencing the properties of the flour (Niba *et al.,* 2002; Charoenkul *et al.,* 2011). Due to the very high starch content of cassava flour, it is sometimes referred to as starch. Navia and Villada (2012); Sulistyo *et al.* (2016) used the terms cassava flour and cassava starch interchangeably when characterising the microstructure of cassava flours probably because the most evident component was starch.

A study conducted on 12 cassava varieties of different textural quality reveals the properties of cassava starch and its corresponding flour. It shows that the viscosity and pasting temperatures of the former was substantially higher than the latter and the reverse being the case for the gelatinisation temperatures. Moorthy *et al.* (1996) attributed this trend to the presence of fats and sugars and Niba *et al.* (2002) proposed that the amylase activity and interference of non-starch components maybe responsible for this trend. Strong correlations between firmness and alpha amylase activity, firmness, lipid contents and fiber, paste viscosity and ash, as well as starch content and alpha-amylase activity was reported by Charoenkul *et al.* (2011). The application of these products is similar and both maybe used in textile, paper, pharmaceutical and food industries as a binder, thickener or glazing agent (Tonukari, 2004; Dziedzoave *et al.* 2006). However, cassava flour is mainly consumed by humans, in a reconstituted dough form and suitable as composite flour in the production of baked foods such as biscuits and bread.

2.11 Enrichment of Cassava Food Products

Protein-energy balance is critical for development of children and wellbeing of adults with most studies on cassava food product development aimed at increasing the protein content of the products. Akinwande *et al.* (2008) carried out a study on the evaluation of the quality of high-protein biscuits prepared from ginger flavoured soy-cassava flour. In this study, soy flour augmented the nutrient content of the biscuit by increasing the protein content. The typical beany flavour of soy flour was effectively masked by the flavour of the ginger powder. Sensory evaluation showed a good aroma and positive acceptability. The product was projected as one that can be used in combating protein-energy malnutrition, thereby enhancing protein-energy balance in children particularly in developing countries.

Kolapo and Sanni (2009) carried out a comparative evaluation on the nutritional profile on fortified and unfortified *gari* and tapioca (two important staples from cassava consumed in many African countries). The products, regarded as nutritionally inferior, were fortified with soybean flour at 25% dry weight. Analysis of the samples showed that the fortification of both *gari* and *tapioca* significantly ($p < 0.05$) increased the contents of crude protein, phosphorus, fat and ash, as well as all the, monitored macro- and micronutrients. These values (%) increased from 1.50 - 9.31, 0.06 - 0.11, 1.56 - 4.31 and 1.35 - 1.64 for *gari*-soybean fortified *gari*; and 0.31 - 12.56, 0.03 - 0.12, 0.17 - 5.45 and 0.34 - 1.41 for *tapioca* to soybean fortified *tapioca* respectively. It was deduced that the fortified products could serve as effective diets for meeting the daily requirements of minerals, protein and energy. They suggested that the products could be used to fight macronutrient and micronutrient deficiencies, not only in Nigeria and other African countries where these two cassava products are staples, but also in other cassava-consuming developing nations.

Oboh and Akindahunsi (2003) used *Saccharomyces cerevisae* in the fermentation of cassava pulp to enhance the nutritional quality of flour and *gari* (two popular forms in which cassava is consumed). The cyanide content, proximate and mineral compositions of the products were analysed. They reported a significant increase in the protein (4.4 - 10.9%) and fat (3.6 - 4.5%) content of the flour and a significant decrease in the cyanide (21.3 - 9.0 mg/kg) content. From their findings, it was inferred that *S. cerevisae* could be a cheap and non-pathogenic aerobe for enhancing nutritional content of cassava products.

In a similar study, Oluwamukomi *et al.* (2015) incorporated soy-bean flour and soy-bean concentrate at a 10% level of enrichment in the processing of *gari* to give a fortified product named soy-*gari* and soy-concentrate *gari*. A comparative evaluation of the fortified products with the unfortified conventional *gari* showed that the functional properties were generally lower in soy-*gari* than the other samples (soy-concentrate and unfortified *gari*)

except in bulk density. Water absorption capacity (WAC) for soy-concentrate and unfortified *gari* was the same value of 438.59%. The swelling index value for unfortified *gari* (6.48 v/v) was higher than that of soy-*gari* and soy-concentrate *gari*, 4.72 and 5.52 v/v, respectively. There were no noticeable differences in the moisture content, ash, and crude fibre except crude fat, protein content, and carbohydrate. The protein and carbohydrate content of the samples ranged from 0.90 - 6.13% and 74.69 - 84.33% with unfortified *gari* having the highest carbohydrate and least protein values. The mineral content was higher in the soy-concentrate *gari* than in other samples. They concluded that incorporation of defatted soy-concentrate at a 10% level to produce enriched *gari* improved the nutrient quality of *gari* better than that from soy-flour.

A better understanding of the interaction between soy protein and cassava starch during the fortification of cassava products to ensure that new products have acceptable sensory properties inspired Tivana *et al.* (2013) to study the texture and colour of fortified *gari* as a function of pH and heat treatment of soybean flour used for enrichment. They reported that fortification of *gari* with soy-flour to a protein content of 15% w/w affected the texture of *gari* agglomerates and that the addition of soy-flour suspensions that were not boiled to the shredded cassava roots increased hardness of swollen *gari* particles. However, pre-treatment of the soy suspension in terms of pH adjustment to 4.5 and boiling, restored the hardness to the level of non-fortified *gari*. Colour analysis revealed that the hue turned more yellow and the darkness of fortified *gari* increased because of the soy flours added.

From the reviewed studies, it can be shown that enriched cassava products can be developed with minimal effect on the textural quality if the processing conditions are controlled. However, the apparent detection of the change in appearance of the products is a setback. Therefore, it is advisable that raw materials used in developing enriched products be carefully selected putting into consideration consumers' preference and sensory appeal.

2.12 Production of Cassava Flour

Cassava flour is about the easiest and quickest shelf-stable product to be derived from cassava roots. It is a fine powdery product obtained from drying then grinding after washing, peeling and chipping the roots. It usually contains about same components as the raw root save for the reduced moisture (Charoenkul *et al.*, 2011). The processing steps and time is relatively short compared to other fermented cassava products. Therefore, to produce cassava flour of 10 mg HCN equivalents/kg flour, the WHO safe level, by sun-drying or heap fermentation requires starting with sweet cassava containing 12–32 ppm total cyanide (Cardoso *et al.*, 2005).

Cassava flour is composed of mainly starch and other nutrients. Aryee *et al.* (2006) evaluated the physicochemical and pasting properties of cassava flour processed from 31 different varieties. These varieties were not well adapted because of their poor cooking quality and high cyanogenic potential. Their results showed that starch content ranged from 67.92 - 88.11%. The amylose content of cassava flour varied from 10.9 - 44.3%. The cassava flour had low swelling power values ranging from 5.87 - 13.48. Water binding capacity varied from 113.66 - 201.99%. Gelatinisation temperature was in the range of 66.8 - 70.4 °C with peak temperatures varying between 73.1 - 84.5 °C (94/0107). The cyanogenic potential (CNp) ranged from 0.58 - 20.0 mg HCN per 100 g of dry weight. From the data obtained, they recommended that these varieties could be used for other purposes such as starch production, glucose, adhesives, fuel alcohol, animal feed, and other industrial uses.

The chemical composition and residual cyanogens of cassava flour, processed by wet fermentation, solid-state fermentation, and sun-drying were determined by Muzanila *et al.* (2000). Sun-dried cassava samples (sweet varieties) had 6.8 mg HCN $kg^{-}1$. The total cyanogen content of 5.84 mg HCN $kg^{-}1$ for samples processed by wet fermentation was less than that for samples processed by solid-state fermentation which had a residual cyanogens content of 14.0 mg HCN $kg^{-}1$. Wet fermentation was very effective in reducing cyanogen content in bitter varieties but resulted in lower contents of vitamin C, reducing sugars and protein, and low pH values compared to samples processed by solid-state fermentation and sun-drying. Solid-state fermented samples had higher reducing sugars and protein contents than sun-dried samples.

2.12.1 Assessment of microbial safety

Cassava flour is, in very few instances, produced on an industrial scale, the majority is produced in artisanal units, which do not adhere to the rules of food safety (Cazumba da Silva *et al.,* 2017). The challenge of standardising small scale processing is that the processors have various target flour in mind and one of the constraints in the commercialisation of locally produced cassava products is that the quality of the products varies from one processor to the other and even from one processing batch to the other by the same processor (Oyewole and Sanni, 1995; Padonou *et al.,* 2009). The standard Codex 176-1989; EAS 740:2010 microbiological limits for cassava flour are total viable count of 5.00 log cfu g^{-1}, *S. aureus* limits 2.00 log cfu g^{-1} and zero coliform count (CAC, 2013). But the result of the studies (Padonou *et al.,* 2009; Adebayo-Oyetoro *et al.,* 2013; Gacheru *et al.,* 2016; Cazumba da Silva *et al.,* 2017), motivated from a concern for the safety of the product for consumption, indicates that some of the microbial limits were exceeded. Although cassava

flour is not in its ready-to-eat form, it is worrisome that a very high percentage of the cassava flour samples analyzed were contaminated with very high microbial counts. However, cassava flour sample prepared in the laboratory had a low microbial load compared to samples collected from various processing sites and markets (Adebayo-Oyetoro *et al.* 2013) which goes to show that if more hygienic measures are taken, contamination can be avoided and the safety of the product guaranteed.

2.13 Classification, Nomenclature and Properties of Cassava Flours

Cassava flours are broadly categorised into fermented and unfermented (Dziedzoave *et al.,* 2006). Unfermented cassava flour is white, odourless and bland (Taiwo, 2006) while fermented cassava flour, as the name implies, has fermentation as one of its major processing steps. In most literature (Dziedzoave *et al.*, 2006; Taiwo, 2006; Uchechukwu-Agua *et al.*, 2015a), unfermented cassava flours are referred to as high quality cassava flour (HQCF)**,** it appears white in colour, has low-fat content, is not sour like fermented cassava flour and does not give an off odor or taste to food products. The odourless attribute is an advantageous quality of HQCF which makes it a very suitable composite for various food products (Taiwo, 2006). HQCF is made within a day of harvesting the root, mechanised techniques have been developed to reduce the time and energy involved in the process (Jekayinfa and Olajide, 2007) local farmers are encouraged to adopt these newly developed modern techniques which make the process fast and guarantees better product quality (Olaoye *et al.*, 2011).

The traditional methods may take too long and fermentation sets in mostly during dewatering and drying of grated pulps which adversely affects the functionality of the flour as a composite (Falade and Akingbala, 2010). Most traditional cassava meals are obtained from the fermented flour. These flours are mostly consumed in the reconstituted dough form eaten with soups in most African countries. The fermented flours and their corresponding dough are given various traditional names, such as *Fufu, Lafun, Agbelima, Kivunde, Kokonte, Ugali, Wikau maombo* in different regions (Iwuoha *et al.*, 2003; Okoro, 2007; Tomlins *et al.*, 2007; Jumah *et al.*, 2008a,b; Padonou *et al.,* 2009; Omodamiro *et al.,* 2012; Adebayo-Oyetoro *et al.*, 2013; Bamidele *et al.*, 2015; Lim, 2016; Taiwo *et al.,* 2016; Wahyuni *et al.*, 2016). From a critical point of view, although the term high quality may suggest higher nutritional content, it may only apply to the starch content. It has been shown in the literature that HQCF has a lower nutritional value but high quality and quantity in starch than fermented cassava flour (Sulistyo *et al.,* 2016). Some authors term unfermented cassava flour as raw (Sira *et al.*, 2007; Silva *et al.,* 2012), others native (Sulistyo *et al.,* 2016) and others simply dry (Murayama *et al.*, 2014).

There are large variations in cassava flour due to different conditions the root is subjected to during processing (Pereira and Leonel, 2014) which has given rise to various prefixes for CF such as modified, enriched, fortified, pre-gelatinised, roasted, water group, dry group, wet-milled and dry milled. Terminologies vary across ethnic groups and regions. For instance cassava flour milled directly from dried chips maybe termed dry group cassava flour (Chiste *et al.*, 2015) or dry milled (Murayama *et al.,* 2014). As depicted in Table 2.3 the proximate composition of the unmodified/native cassava flour is different from the modified flour.

2.14 Effect of Variables in Processing Cassava Flour

It has been reported in the literature that variety, maturity, environmental conditions, locations, postharvest practices affect the properties of cassava (Tewe and Lutaladio, 2004; Sajeev *et al.*, 2010; Apea-Bah *et al.,* 2011; Manano *et al.*, 2018) and by way of extension the quality of its flour. The focus of discussion, in this section, will be on the quality of cassava flour as influenced by some of these postharvest procedures and variety.

2.14.1 Variety of root

It is recognised that the quality of flour varies with the variety of cassava from which they are processed (Table 2.4). An extensive study of over 670 cassava varieties grown at the IITA research farm, Nigeria in 2000 and 2001 was evaluated for genotypic variations in cyanogenic potential and pasting properties (Maziya-Dixon *et al.,* 2005). The results showed that there were variations in the cyanide content as well as the genotype x year interactions on the cyanide contents. There were significant ($p < 0.05$) genotypic variations in all the pasting properties except pasting temperature and peak time in 2001. On this basis, the clones were screened and characterised for food, feed and industrial applications.

The evaluation of the physicochemical and pasting properties of cassava flour processed from 31 different varieties was conducted by Aryee *et al.* (2006). These varieties were not well adapted because of their poor cooking quality and high cyanogenic potential. Their results showed that starch content ranged from 67.92 - 88.11%. The amylose content of cassava flour varied from 10.9 - 44.3%. The cassava flour had low swelling power values ranging from 5.87 - 13.48. Water binding capacity varied from 113.66 - 201.99%. Gelatinisation temperature was in the range of 66.8 - 70.4 °C with peak temperatures varying between 73.1 - 84.5 °C. The cyanogenic potential (CNp) ranged from 0.58 - 20.0 mg HCN per 100 g of dry weight.

Table 2.3 Types of cassava flour: physicochemical composition

Types of cassava flour	CHO (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	Moisture (%)	HCN (mg/kg)	Dextrose equivalent	Total sugars	Water activity (a_w)	Reference
Native or unmodified	82.09	1.14	0.93	2.30	2.19	11.49	7.75	2.00			
Modified (enzymatically)	88.80	2.19	0.78	1.44	1.60	5.80	3.88	2.50			Sulistyo *et al.* (2016)
Fortified (fermented with protein hydrosylates)	85.40	11.26	0.75	1.51	1.61	6.44	2.50	3.30			
Pregelatinised	64.10 – 75.31	1.19 – 1.42	0.38 – 0.60	1.89 – 3.28	Nd	8.46 – 9.76	-	-	-	-	Murayama *et al.* (2014)
Dry milled, ungelatinised & unfermented	72.99 – 78.76	1.31 – 1.98	0.48 – 1.03	2.13 – 3.36	-	10.57 – 11.66	-	-	-	-	
Water group	68.32	1.10	1.04	0.75		8.28	-	-	0.42	0.45	Chiste *et al.* (2015)
Dry group	76.57	0.52	0.26	0.83		9.17	-	-	1.10	0.53	

- Not determined; CHO – carbohydrate; HCN – Hydrogen cyanide

Table 2.4 Studies on varietal differences in cassava flour

Number of varieties	Description of varieties	Source/Country	Difference in CF properties associated with varietal	Reference(s)
3	Local varieties	Accra, Ghana	No significant difference in physicochemical properties Difference in functional characteristics	Eriksson (2013)
2	White and yellow	National Root Crops Research Institute (NRCRI), Umudike, Nigeria	Significant difference in proximate composition, colour attributes, exhibited slightly different hygroscopic behaviors during storage	Uchechukwu-Agua *et al.* (2015a); Opara *et al.* (2016)
17	Bitter yellow	State of Bahia, Brazil	Variation in total carotenoid content	Oliveria *et al.* (2010)
6	New elite yellow and white	National Root Crops Research Institute (NRCRI), Umudike, Nigeria	Higher residual cyanide, quantities of reducing sugar and carotenoid in yellow varieties compared to the white.	Eleazu and Eleazu (2012)
31		Crop Research Institute (CRI), Ghana	A wide variance in physicochemical and functional properties	Aryee *et al.* (2006)
12	Low cyanide varieties with different cooked texture: mealy, firm and mealy & firm	Rayong Field Crops Research Center, Thailand	Wide variation in pasting characteristics	Charoenkul *et al.* (2011)
11	Different genotypes	International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria	Variation in total dietary fibre and viscosity profile	Niba *et al.* (2002)
5	-	Philippines	Slight significant difference in physicochemical properties	Murayama *et al.* (2014)

From the data obtained, they recommended that these varieties could be used for other purposes such as starch production, glucose, adhesives, fuel alcohol, animal feed and other industrial uses. Charoenkul *et al.* (2011) studied the physicochemical characteristics of 12 cassava varieties with low cyanide content from Thailand and reported that all the flours showed wide variation in their properties.Five varieties of cassava namely, Lakan 1, Sultan 6, Sultan 7, Rajah 2 and Rajah 4, bred and cultivated in the Philippines were researched by Murayama *et al.* (2014). The dry and pre-gelatinised flours from these varieties displayed different properties however the Lakan 1, Sultan 6, and Sultan 7 varieties were found to be more suitable for pre-gelatinisation most due to the greater retention of chemical components. The attributes of cassava flour during storage can be significantly influenced by cultivars therefore; proper selection of cultivars is recommended (Uchechukwu-Agua *et al.,* 2015b). The study of Eleazu and Eleazu (2012) indicates that some cassava cultivars of the yellow varieties may have dual utility both for human consumption and for industrial purposes while the white variety may be confined to domestic use. Pictures of cross sections of white and yellow varieties are shown in Figure 2.6. It can be deduced from these studies that although cassava may be used for diverse applications, each variety of cassava best suits a particular application and depending on the variety and end-use, the right processing condition should be applied.

Figure 2.6. Pictures of yellow and white-fleshed cassava root. **A** – Cross-section of pro-vitamin A cassava variety UMUCASS 36; **B** – Cross-section of a white cassava variety TME 419. Source: Uchechukwu-Agua *et al.* (2015a)

2.14.2 Pregelatinisation

Pre-gelatinisation of cassava flour may be achieved by cooking or steaming the roots before drying and milling. During application of heat in presence of water, the starch in the

root gelatinises. One alternative to supplying cassava for industrial use is transforming to roots into precooked cassava flour which can be used as a raw material for making high value-added products like cassava dough, croquette, fried chips or snacks. Murayama *et al.* (2014) investigated the effect of pre-gelatinisation on the proximate, mineral and soluble sugar composition, starch, pasting and thermal properties, solubility, swelling power and particle size distribution of cassava flour. The pre-gelatinised flours showed significantly lower values for viscosity, pasting temperature and α-amylase activity than their corresponding ungelatinised flours. The use of a differential scanning calorimeter revealed a complete amorphorisation of the starch contained and it was deduced that pre-gelatinisation causes an increase in the fructose, glucose, amylose content, damaged starch and mean particle size compared to the corresponding flours that were not gelatinised. From the study, it could be inferred that pre-gelatinisation has a great potential for textural and structural improvement by reduction of starch retrogradation in bakery products. Rodriguez-Sandoval *et al.* (2008a) studied the effects of cooking (steaming and boiling) method on the retrogradation of starch in flour and it was reported that cassava flour was pre-gelatinised by steaming showed an increase in starch retrogradation which maybe as a result of higher amylose content.

2.14.3 Fermentation

The positive roles that microorganisms play during fermentation include detoxification, flavor development, biological enrichment, product preservation and a decrease in cooking time (Patel and Shah, 2017). Fermentation, either naturally or with selective inoculation of microorganisms has been extensively used to enhance the nutrient potentials of cassava for human consumption (Aro, 2008). Akindahunsi *et al.* (1999) fermented cassava pulp with *Rhizopus oryzae* which caused a 97% increase in the protein content of flour, a 5% decrease in the carbohydrate content and no considerable increase in the fat, ash and lipid content. The level of anti-nutrients, tannin and cyanide except for phytate which increased, were considerably low. The level of phytate increased. The mechanism of this increase could not be ascertained but it was inferred that it may be because some of the plant metabolite or nutrient content in the solution may have been converted to phytate or phytate-like products. Phytate can chelate divalent cationic minerals like calcium, Iron, Magnesium and Zinc therefore impairing their bioavailability, however, phytate functions as an antioxidant, inhibiting the formation of free radicals, by sequestering iron (Manano *et al.,* 2018). A similar trend was reported by Oboh and Akindahunsi (2003) in the use *S. cerevisae* for fermenting cassava increased in the protein and fat content of the flour. There was no significant change in the tannin, crude fibre or ash content of the flour but there was a significant decrease in the

cyanide content, carbohydrate and mineral content. The chelating activity of phytate may be responsible for the decrease in mineral content.

Fermentation with these microorganisms (*R. oryzae* and *S. cerevisae*) greatly influences the chemical composition of cassava flour positively by increasing the protein level of cassava flour and at the same time reducing the level of some anti-nutrients specifically total cyanide. These microorganisms (*R. oryzae* and *S. cerevisae*) could efficiently improve the nutritional content of cassava flour however the knowledge of secretion of some harmful metabolites associated with microbial activities (Oboh *et al.,* 2003) prompted further research by Oboh and Akindahunsi (2005) on the nutritional and toxicology of cassava flour fermented with *S. cerevisae*. They reported high digestibility and no negative haematological effect. But a significant ($p > 0.05$) rise in pyruvate transaminase and serum glutamate oxaloacetate transaminase activities in the serum was observed which indicates hepatotoxicity and cardiotoxicity. Upon further pathological investigation, the spleen showed some dark red coloration while the liver had some necrotic lesions.

2.14.4 Drying and processing temperatures

Murayama *et al.* (2014) dried chipped cassava roots in a hot air oven at 40ºC however the duration it took to dry was not mentioned. Rodriguez-Sandoval *et al.* (2008a) incorporated resting time after precooking into the stored fresh cassava chips at 5 and -20ºC before drying and milling. The flour stored at -20ºC showed no significant differences in the retrogradation of starch. Rodriguez-Sandoval *et al.* (2008b) reported that the temperature during storage was the most important factor affecting the textural properties of cassava dough. Omolola *et al.* (2017) reported that the use of optimum duration and temperature of drying of cassava chips is a key factor in preserving the color and thermal properties of cassava flour. Three traditional processing methods (sun-drying, roasting and fermentation before sun-drying) were used to produce three types of cassava flour in a study carried out by Eduardo *et al.* (2013). Their findings inferred that the sun-drying method gave a higher yield than roasting. However, upon use as a composite in bread making, the roasted cassava flour had a significantly higher volume compared with sun-dried or fermented cassava flour.

2.14.5 Milling and sieving

In whatever order, the process (dry or wet) flow takes, milling precedes sieving (Eduardo *et al.,* 2013). Sieving is usually the last step in the flow chart of processing cassava flour before packaging for storage. Milling and sieving are both physical and mechanical

processing factors that to a large extent influence the yield and particle size of cassava flour (Adesina and Bolaji, 2013) but these processing steps are not given much research attention as others hence there appears to be a dearth of information on the yield, particle size distribution and average particle size of different types of cassava flours. The fineness of cassava flour is a function of the efficiency and type of milling machine used (Oladunmoye *et al.*, 2010a) and it is also controlled by the attritions on the screen of the mechanical mill. One kg sample of dried cassava chips milled using pin, hammer, attrition and mortar mills gave percentage flour recoveries of 96, 87, 75 and 62 respectively (Adesina and Bolaji, 2013). To some extent, the fibre content of cassava makes it difficult to fine mill thus its average particle size (228 µm) and most frequently occurring particle size (256 µm) was significantly higher than that of wheat flour (Oladunmoye *et al.* 2010a).

Some processors do not sieve after milling but sieving of the flour gives a good quality product (Uchechukwu-Agua *et al.,* 2015b). Sahin and Sumnu (2006) reported that the average particle size of various floury foods depends not only on the cell structure but also the degree of processing that the material undergoes. The use of aperture-sized sieves of 180 µm was reported by Murayama *et al.* (2014) while Eduardo *et al.* (2013) reported a lower size of 125 µm which is within the range (100-150 µm) reported by Lepiz-Aguilar *et al.* (2013). Aperture sizes of 50 and 550 µm were used to sieve cassava flour in the study of Adesina and Bolaji (2013). In the molding of thermoplastic material from cassava flour, the particle size (ranging from 250-600 µm) was included as one of the design factors by Navia and Villada (2012). They established, with the aid of response surface analysis, that the molded material with highest tensile strength was that with 600 µm particle size. Sieving is an important step in processing because it determines the particle size, an important physical property, of the flour which further influences the functional properties of the flour and the subsequent products from them (Oladunmoye *et al.,* 2010a).

2.14.6 Fortification

Due to the high carbohydrate content of cassava flour, fortification is done to improve the nutritional quality. The addition of flours of legume and/ or cereal grains to cassava flour is a means of fortification (Jisha and Padmaja, 2008). Co-processing the root with fermented protein hydrolysates not only resulted in an increase in the protein content but the cyanide content of the fortified flour decreased and that there was a significant increase in the viscosity with increasing level of protein hydrolysates (Sulistyo *et al.*, 2016). Another form of fortification is addition of enzymes such as Termamyl, a thermostable alpha-amylase, to moistened cassava flour to produce malted cassava flour (Jisha *et al.,* 2010).

2.14.7 Packaging materials and storage conditions

Retaining the quality of cassava flour during storage is a very important factor that directly affects the quality of the flour at end-use. During storage flours maybe packed in low-density polyethylene (LDPE) bags, plastic buckets, sacks, jute and paper bags (Ogiehor and Ikenobomeh, 2006). The appropriate packaging material, temperature and relative humidity are critical for the retention of product quality (Opara and Al-Ani, 2010). The use of improper packaging materials could lead to a reduction in the quality and shelf life of flour. Cassava flour is best stored at ambient temperature, storage in refrigeration temperature causes an increase in microbial count (Uchechukwu-Agua *et al.,* 2015a). During storage of cassava flour the whiteness, cyanide, and total carotenoids content reduce, in the course of transportation during sales and storage. Opara *et al.* (2016) investigated the effect of a plastic bucket, LPDE and paper bag on the physicochemical and microbial stability of flour of 2 cassava cultivars under the same temperature and humidity (23 ± 2 ºC and 60% relative humidity). Total color difference (ΔE) increased with storage time with flours packed in plastic buckets giving the least change in color. Total carotenoid decreased but flour packed in plastic had the highest total carotenoid retention. Cassava flour in paper bags had the lowest microbial count for the total aerobic mesophilic bacteria and fungi.

2.15 Pre-treatments in Root Processing

In food processing before storage, the fresh products are most times subjected to various pre-treatments (chemical, thermal and physical) to retain the quality of the products and extend their shelf life. In flour processing from roots before the stage of drying after peeling, the roots maybe dipped in chemical solutions, blanched or both are done on the roots (Campos *et al.,* 2016; Chen *et al.,* 2017). One major unpleasant occurrence during drying is discolouration of the crops. Discolouration of the products may be caused by oxidative browning reactions by enzymes in the sliced roots (Reilly *et al.*, 2004), non-enzymatic browning that results when reducing sugars condense with amino groups at high temperatures (Ulomo *et al.*, 2005) and the drying conditions (FAO, 2006; Oghenechavwuko *et al.*, 2013). It has been reported that the faster the drying process, the brighter the chips of cassava and the rate of drying depends on the method and temperature used (FAO, 2006; Irinkonyenikan *et al.*, 2008). According to Xiao *et al.* (2009), citric acid pre-treatment of potato could significantly improve the drying rate of sweet potato slices. To reduce discolouration during drying, roots are treated with chemicals generally regarded as safe. Ahmed *et al.* (2010b) evaluated the effect of peeling, drying temperature and pre-treatment on the physicochemical properties and

nutritional quality of sweet potato dipped in 0.5% sodium hydrogen sulphite ($NaHSO_3$). They reported that flours pre-treated with sulphite had higher *L**, *a**, and *b** values, swelling capacity, and ascorbic acid than the control. Irrespective of the drying temperatures, the best quality product was obtained when samples were treated with sulphite before drying.

Ahmed *et al.* (2010a) investigated the comparative effect of $NaHSO_3$ and calcium chloride (CC) at drying temperatures 55, 60 and 60ºC on the quality of sweet potato flour. Flour treated with CC had a higher amount of ascorbic acid and β-carotene than that treated with $NaHSO_3$. It was observed that the total phenolic content and water absorption index of flour treated with $NaHSO_3$ was higher than flour treated with CC and dried at 65ºC.

Jyothi *et al.* (2007) subjected freshly extracted cassava starch to wet storage for up to eight weeks with different concentrations of sodium metasulphite and acetic (0.5 to 2 %w/v). They reported that till the sixth week of storage, no change was observed in the viscosity and pasting properties of the starch. Both chemicals were effective in inhibiting microbial contamination during storage, however, 1% sodium metabisulphite was found to be effective for storage of freshly extracted starch in wet conditions up to eight weeks without microbial contamination and no significant alteration in its properties. It was also reported that most of the attacks by enzymes occur in the amorphous regions of the starch granules. It can be deduced that the processing variables which include variety, fermentation, fortification, pregelatinisation, sieving and temperature influence the quality of the cassava flour. It can also be deduced that specific varietal selection and manipulation of processing conditions is important to derive different cassava flours to suit specific purposes. The quest of making cassava flour more suitable for baking maybe achieved by objectively controlling the modification process of cassava flour to become wheat-like. This is a promising means to advance the utilisation of cassava flour globally. The ultimate concern of product safety has to be guaranteed by adhering to safety guidelines during processing as well as proper packaging and storage.

Summarily, a review of literature, in this chapter, gives information on the botany, agronomy and nutritional composition of cassava root. Genetic breeding efforts towards improving the root quality, setback to its utilisation, classification, starch composition, and application were also discussed. The distinction between cassava starch and flour was highlighted. Production, nomenclature, classification, application, and the influence of processing variables on cassava flour quality were extensively discussed. It can be observed that there is a dearth of knowledge on the effect of chemical pretreatment on the quality of cassava flour. Therefore, this study seeks to fill this knowledge gap by investigating the effect of different pre-treatments on the physical, thermal and functional properties of cassava flour.

CHAPTER THREE: CHARACTERISATION OF THE ROOT AND FLOUR OF SOUTH AFRICAN *MANIHOT ESCULENTA* CRANTZ LANDRACES AND THEIR POTENTIAL END-USE PROPERTIES

Abstract

The root and flour of South African *Manihot esculenta* Crantz landraces (red and white) were characterised for their morphological, physicochemical, metabolic, structural and elemental properties. The results of the colourimetric analysis of the root parenchyma revealed that the *L* a* b**, whiteness and brownness index of the white landrace were significantly different ($p < 0.05$) from that of the red landrace. Cassava flour showed significant variance ($p < 0.05$) in the *a**, *b** and brownness index but the lightness and whiteness index were not significantly different ($p > 0.05$). Cyanide content of the root samples (red – 3.62 mg/kg; white – 3.51 mg/kg) were not significantly ($p > 0.05$) different, but the flour samples (red – 2.92 mg/kg; white – 1.83 mg/kg) were significantly ($p < 0.05$) different. Granular morphology of flour samples showed spherical and truncated starch granules, clustered and dispersed in no regular pattern. The X-ray diffractometry pattern and main peaks ($2\theta = 43^{o}$, 23^{o}, 17^{o} and 15^{o}) of the flours showed the A-type pattern for both flour samples. Results of all phenolic acids (benzoic and cinnamic acids groups) identified in root samples were not significantly higher ($p > 0.05$) in the white landrace. However, a reverse trend was observed with identified fatty acid methyl esters in the landraces. The elemental composition of flour samples showed the presence of essential macro and trace elements in flours. The A-type starch crystallinity of flour exhibited in the red and white landraces positions the flours as a suitable wheat replacement in food applications.

Keywords: *Manihot esculenta;* cyanide; elemental composition; starch crystallinity; metabolic profile; scanning electron microscopy

3.1 Introduction

Manihot esculenta Crantz (cassava) is a woody shrub that is of the family *Euphorbiaceae*. The starch-rich tuberous root of the plant is the part that is mostly consumed (Maieves et al., 2012). Cassava, also called manioc or tapioca is mainly grown in the continents of Asia, South America and Africa (FAO, 2016). The multifaceted application of cassava both at domestic and industrial front positions it as the most important staple root crop in the world (FAO, 2013). There is a growing interest in the utilisation of cassava which has increased research effort towards the application of genetics in cassava breeding and cultivation to improve storage, starch production and nutritional values (Carvalho et al., 2018).

A remarkable impact has been made on cassava production in terms of yield, disease tolerance and improvement of quality (Ferguson *et al.,* 2012). Over the years, new hybrids, clones, cultivars, accessions, varieties and landraces of cassava have been developed across the globe.

The term “landrace” is defined as a genetically heterogeneous variety, cultivated and evolved in a certain ecogeographical area and has over time adapted to the edaphic and climatic conditions as well as its traditional management and uses (Casanas *et al.,* 2017). Landraces are mostly cultivated and accepted because of specific desirable traits. Factors such as economic practices, historical relationships and environmental conditions of a region influence the changing diversity of cassava within that region (Lima *et al.,* 2012). It is common in the literature to find studies related to the characterisation of new breeds, clones, accessions, varieties and landraces of cassava because of preharvest factors such as variety, environmental conditions during growth, biofortification, age at harvest and breeding techniques (Aryee *et al.,* 2006; Onitilo *et al.*, 2007a, b; Mtunguja *et al.*, 2016; Chisenga *et al.*, 2019b, c) influence the quality of the roots. Afoakwa *et al.* (2012) studied cassava mosaic disease-resistant cassava varieties developed by the Crop Research Institute of Ghana and reported wide variations in the chemical composition of the improved and traditional cassava cultivars. Screening of their cyanogenic potential makes them safe for human consumption. The effect of genotype and genotype by environment interaction on starch yield and cyanide content in farmer-preferred cassava landraces in Tanzanian was conducted by Mtunguja *et al*. (2016). The study revealed that genotype by environment interaction significantly influenced cyanide content and starch yield of the roots. Cultivars said to be superior in terms of starch yield were identified and maximum starch yield was reported to be at 9 months after planting. Chisenga *et al*. (2019 b, c) who investigated the physical, chemical and functional quality of officially released improved cassava varieties cultivated in Zambia, showed that the effect of variety was significant on the physical and chemical qualities of cassava, while inter-cultivar variation was not highly significant for the functional properties of the flour and starches of the roots. The study further indicated that syneresis values of the starches and flours were within the acceptable range suitable for application in frozen food systems. Eleazu and Eleazu (2012) studied the proximate composition, reducing sugars, total carotenoid, and residual cyanide levels of flours of 6 new white and yellow cassava varieties cultivated in Nigeria. It was reported that residual cyanide was higher in the yellow varieties compared with the white varieties. The authors postulated that the white varieties may be most suitable for domestic use while the yellow varieties for both industrial and domestic applications. Similarly, flours processed from local and improved cassava varieties cultivated within the South-eastern African region were assessed for varietal diversity and processing effects on their biochemical

quality, cyanogenic safety and appearance (Chiwona-Karltun *et al.,* 2015b). The study indicated that colour of the flour is deemed to be the most important factor for the preference and adoption of the varieties.

Despite the popularity of cassava in Africa, it is less known in South Africa (Drimie and McLachlan, 2013). There also appears to be a dearth of information on South African cassava landraces which makes the characterisation of these identified landraces (red and white) important. The characterisation is a scientific process that entails ascertaining the properties of food materials. This is necessary for a better understanding of the quality of the material and to optimise processing and utilisation. Thus, screening these identified South African cassava landraces is quite imperative to determine their potential and most suitable end-use as they may offer variations needed for the industrial and food applications of their roots. Varietal preference based on general utilisation as well as more targeted end-use for preferred local and improved cassava varieties is poorly understood and not well documented. (Chiwona-Karltun *et al.,* 2015b). With the steady release of new clones, varieties, hybrids and landraces it is imperative to update the global database of cassava with information generated from this study. Therefore, the morphological, physical, structural and metabolic characterisation is conducted on two identified South African cassava landraces with emphasis on highlighting their potential end-use properties.

3.2 Materials and Methods

3.2.1 Sourcing and preparation of *Manihot esculenta* root

Cassava root of two landraces (red and white) was obtained from the Institute of Tropical and Subtropical Crops - Agricultural research council (ITSC-ARC) Levubu, Limpopo Province, South Africa (22.946º S 30.485º E). The roots, harvested at 14 months after planting, were sorted before washing with tap water generously to avoid contamination with dirt. Analysis of the raw root and processing of flour was done within 24 h of harvest before the onset of deterioration. This was to ensure that the roots were in a fresh state.

3.2.2 Processing of *Manihot esculenta* flour

Peeling and chipping of the washed roots were done manually with a sharp knife. The fresh cassava chips ranged between 0.5 to 1 cm in thickness. The fresh chips of cassava root were dried in an oven (Ecotherm, 240L digital oven, South Africa) at 60°C for 24 h and milling was done using a dry milling machine (Polymix PX-MFC 90D, Kinematic AG, Switzerland).

The flour was sieved through a 500 µm sized aperture sieve and stored in bags made of paper material at ambient temperature till further analysis.

3.2.3 Instrumental textural characteristics

Textural properties of unpeeled and peeled cassava root for both landraces were measured using a food texture analyser (TA.XT PLUS, Texture Analyzer, Stable Micro Systems, Surrey GU7 1YL, UK) with the Exponent texture analyser software under the following conditions: pre and post-test speed 10 mm/s, mode - measure force in penetration, test speed – 2 mm/s and distance of penetration – 10 mm using a stainless steel cylindrical probe (P/0.5) 5 mm in diameter. Semi-circle shaped slices were obtained by cutting the roots transversely and then through their longitudinal axis. Slices with thickness and maximum transverse height of 5 ± 1 cm were then used for texture determination. The parameters derived from the force-deformation (time) curve included maximum peak force as firmness (N), the gradient of the curve from the origin to the peak force as stiffness (g/sec), and toughness (N.sec) as the area under the positive curve (Sajeev *et al.*, 2010). Diagram of a typical texture profile curve is shown in Appendix A.

3.2.4 Flour yield of *Manihot esculenta* root

The yield of flour processed from red and white cassava landraces was calculated in relation to the weight of unpeeled fresh root. The weight of flour obtained was divided by the weight of the whole root and multiplied by 100 as shown in Equation 3.1 (Oghenechavwuko *et al.,* 2013).

$$\%\ \text{flour yield} = \frac{\text{weight of flour}}{weight\ of\ whole\ root}\ X\ 100 \qquad (3.1)$$

3.2.5 Determination of pH of *Manihot esculenta* root and flour

The pH of samples was determined at room temperature using a Fisher Accumet Model 15, pH Meter (Fisher Scientific, Edmonton, AB, Canada). Three-point calibration was accomplished employing pH 7.0, 4.0 and 2.0 buffers before the analysis. Ten grams of sample was dispersed in 100 mL of distilled water and allowed to stand for 5 min. The pH electrode was completely submerged in the mixture and readings were taken.

3.2.6 Determination of colour properties of *Manihot esculenta* parenchyma and flour

Colour characteristics, *L** (lightness/darkness), *a** (redness/greenness) and *b** (yellowness/blueness), of cassava parenchyma and flour were determined with the aid of a colorimeter (ColorFlex, HunterLab, USA). The colorimeter was calibrated with a standard black and white (*L** = 93.71, *a** = –0.84 and *b** = 1.83) plate before use. The whiteness index (WI) and brownness index (BI) of the samples were calculated with Equations 3.2 and 3.3 respectively (Anyasi *et al.*, 2017).

$$WI = \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (3.2)$$

$$BI = 100\ X\ \frac{x-0.31}{0.71} \quad (3.3)$$

Where $x = \frac{(a^* + 1.75L^*)}{5.645L^* + a^* - 3.012b^*}$

3.2.7 Near-infrared spectroscopy analysis of *Manihot esculenta* flour

The percentage of moisture, crude fiber, ash and starch contents of the cassava flour were determined using NIR analyser (DA 7250 Perten Instruments, Hagersten, Sweden). The analyser's open-faced sample dish filled with cassava flour was placed in the NIR analyser scanned by infrared rays, results obtained were displayed on the computer screen. The NIR analyser was previously standardised by a selection of calibration and validation samples, and reference data obtained by routine laboratory analysis according to the modified method of Alamu *et al.* (2019). The modification was the use of cassava flour samples for standardisation.

3.2.8 Cyanide content of *Manihot esculenta* parenchyma and flour

Cyanide content in cassava root and flour was determined using the acid titration method described by Chiwona-Karltun *et al.* (2015a). Steam distillation was carried out on a 500 mL Kjeldahl flask containing 3 g of the sample and 100 mL distilled water. The distillate was collected in 20 mL 0.02 N $AgNO_3$ acidified with 1 mL HNO_3. The apparatus was set so that the tip of the condenser dipped below surface of the receiver liquid. After 150 mL had passed over, excess $AgNO_3$ was titrated with 0.02 KSCN using Fe alum as an indicator of brown ferric thiocyanate indicated the equivalent point when silver nitrate was used up.

3.2.9 Scanning electron microscopy of *Manihot esculenta* flour

Cassava flour samples were evaluated using a digital scanning electron microscope. Approximately 2 g of the sample, previously dehydrated in ethanol, was sprayed on a metal plate covered with double-sided adhesive tape and a 20-nm gold layer was applied with the aid of a sputter coater according to the method of Garcia *et al.* (2016).

3.2.10 X-ray diffractometry of *Manihot esculenta* flour

The flour samples were equilibrated in a desiccator with 90% relative humidity at 25 °C for 10 days. The X-ray diffraction patterns were determined in a diffractometer (XRD, PW1830, Philips Co., Netherlands) with CuKα (λ = 1.542 Å) radiation. The scanning speed was 2° min^{-1} at 30 kV and 10 mA (Garcia *et al.*, 2016).

3.2.11 Metabolic profiling of *Manihot esculenta* parenchyma

3.2.11.1 Determination of fatty acids methyl esters in Manihot esculenta parenchyma - sample preparation and chromatographic separation

A total of 1.0 mL hexane was added to 250 mg of the sample, followed by adding 1 mL of 20% sulfuric acid in methanol. The sample tubes were placed in a beaker with water and extracted in the oven at 80°C for 1 h. The samples were cooled and 1.5 mL of 20% NaCl solution added, vortexed and centrifuged. Approximately 150 µL of the hexane (top) layer was transferred into an insert (positioned in a 2 mL vial) and analysed on the GC-MS system.

The separation was performed on a gas chromatograph (6890N, Agilent Technologies Network) coupled to an Agilent Technologies inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent Technologies Inc., Palo Alto, CA). The GC-MS system was coupled to a CTC Analytics PAL autosampler. Separation of fatty acids methyl esters (FAMEs) was performed on a polar ZB-WAX (30 m, 0.25 sec ID, 0.25 µm film thickness) Zebron 7HG-G007-11 capillary column. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 250°C. Approximately 1 µL of the sample was injected in a splitless mode. The oven temperature was programmed as follows: 50°C for 2 min; and ramped up to 180°C at a rate of 25°C/min for 5 min; followed by a ramping rate of 3°C/min for 2 min until 250°C and eventually to a maximum temperature of 260°C and held for 2 min. The MSD was operated in a full scan mode and the source and quad temperatures were maintained at 230°C and 150°C, respectively. The transfer line temperature was maintained at 250°C. The mass spectrometer was operated under electron impact mode at ionisation energy of 70eV, scanning from 35 to 500 m/z. A 20 ppm standard FAME mix was used as a reference in identification and quantification of FAMEs in parenchyma of both landraces.

3.2.11.2 Determination of sugars in Manihot esculenta parenchyma - sample preparation, derivitisation and chromatographic separation

Approximately 1 mL 70% methanol was added to 100 mg of the samples. The samples were vortexed and extracted in the oven at 60°C for 3-4 h. Approximately 250 µL of each sample was transferred into a 2 mL tube and completely dried under a gentle stream of nitrogen. The samples were derivitised with 100 µL of 2% methoxyamine in pyridine at 40°C for 2 h, followed by adding 50 µL BSTFA and derivitised again at 60°C for 30 min, vortexed and transferred to an insert (positioned in a vial) and injected into a GC-MS instrument. The separation was performed on a gas chromatograph (6890N, Agilent Technologies Network) coupled to an Agilent Technologies inert XL EI/CI Mass Selective Detector (MSD) (5975, Agilent Technologies Inc., Palo Alto, CA). The GC-MS system was coupled to a CTC Analytics PAL autosampler. Separation of sugars was performed on a ZB-Semi-volatile (30 m, 0.25 sec ID, 0.25 µm film thickness) Zebron 7HG-G027-11-GGA capillary column. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 250°C. Approximately 1µL of the sample was injected in a 10:1 split ratio. The oven temperature was programmed as follows: 80°C for 5 min; and ramped up to 250°C at a rate of 8°C/min for 1 min; followed by a ramping rate of 20°C/min for 5 min until 320°C and eventually to a maximum temperature of 325°C and held for 0.25 min. The MSD was operated in a full scan mode and the source and quad temperatures were maintained at 240°C and 150°C, respectively. The transfer line temperature was maintained at 250°C. The mass spectrometer was operated under electron impact mode at ionisation energy of 70 eV, scanning from 40 to 650 m/z. A 25 ppm standard sugar mix was used as a reference in identification and quantification of sugars in parenchyma of both landraces.

3.2.11.2 Determination of Phenolic acids in Manihot esculenta parenchyma - sample preparation, derivitisation and chromatographic separation

Approximately 1 mL of 70% methanol was added to 100 mg of the samples. The samples were vortexed and extracted in the oven at 60°C for 3-4 h. Approximately 250 µL of each sample was transferred into a 2 mL tube and completely dried under a gentle stream of nitrogen. The samples were derivitised in the oven at 80°C for 1 h by adding 100 µL of acetonitrile and 50 µL BSTFA to the dried samples. Samples were transferred into an insert (positioned in a vial) and injected into a GC-MS instrument. Approximately 1 µl of the sample was injected on a Thermo TSQ 8000 triple quadrupole MS (operated in a selected reaction monitoring (SRM) mode. Separation of the phenolic acids was performed a Rxi ® 1310 gas

chromatography coupled with a non-polar Rxi®-5Sil MS w/integra-Guard (15 m, 0.25 sec ID, 0.25 µm film thickness) capillary column. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 250°C. An amount of 1 µl of the sample was injected in a splitless mode. The oven temperature was programmed as follows: 100°C for 4 min, ramped to 180°C at a rate of 10 °C/min for 2 min; followed by a ramping rate of 20 °C/min for 5 min until 320°C. The ionisation source temperature was set at 250°C and emission current of 50 µA was used with Argon collision. A 100 ppb phenolic standard mix was used as a reference in identification and quantification of phenolic acids in parenchyma of both landraces.

3.2.12 X-ray fluorescence spectroscopy of *Manihot esculenta* flour

The elemental composition of the samples was determined using a PANanalytical Axios X-ray fluorescence (XRF) spectrometer equipped with a 4 kW Rh tube. Approximately two grams of cassava flour was transferred directly to sample cup assembled with a 5-µm thick polypropylene film. The polypropylene film with two small holes (~ 1 mm diameter) was also used for covering the XRF sample cup to avoid sample spreading in the irradiation chamber when the vacuum was released at the end of the analysis. Measurements of the X-rays fluorescence were performed in triplicate per sample (Peruchi *et al.,* 2014).

3.2.13 Statistical analysis

Analysis of the quantitative parameters of both landraces was done to determine the significant difference between the means of both landraces. Independent samples *t*-test was conducted at 95% confidence level and assumption of equal variances. Statistical analysis was conducted using the SPSS statistics software Version 25 (IBM Corp., New York, USA).

3.3 Results and Discussion

3.3.1 Morphological characterisation of red and white *Manihot esculenta* landraces

Plants are easily identified by their morphological features such as colour, shape, size and overall appearance. The red and white cassava landraces at the point of harvest were distinctly identified by the colour of their leaves and stalk (Figure 3.1a, c and d).

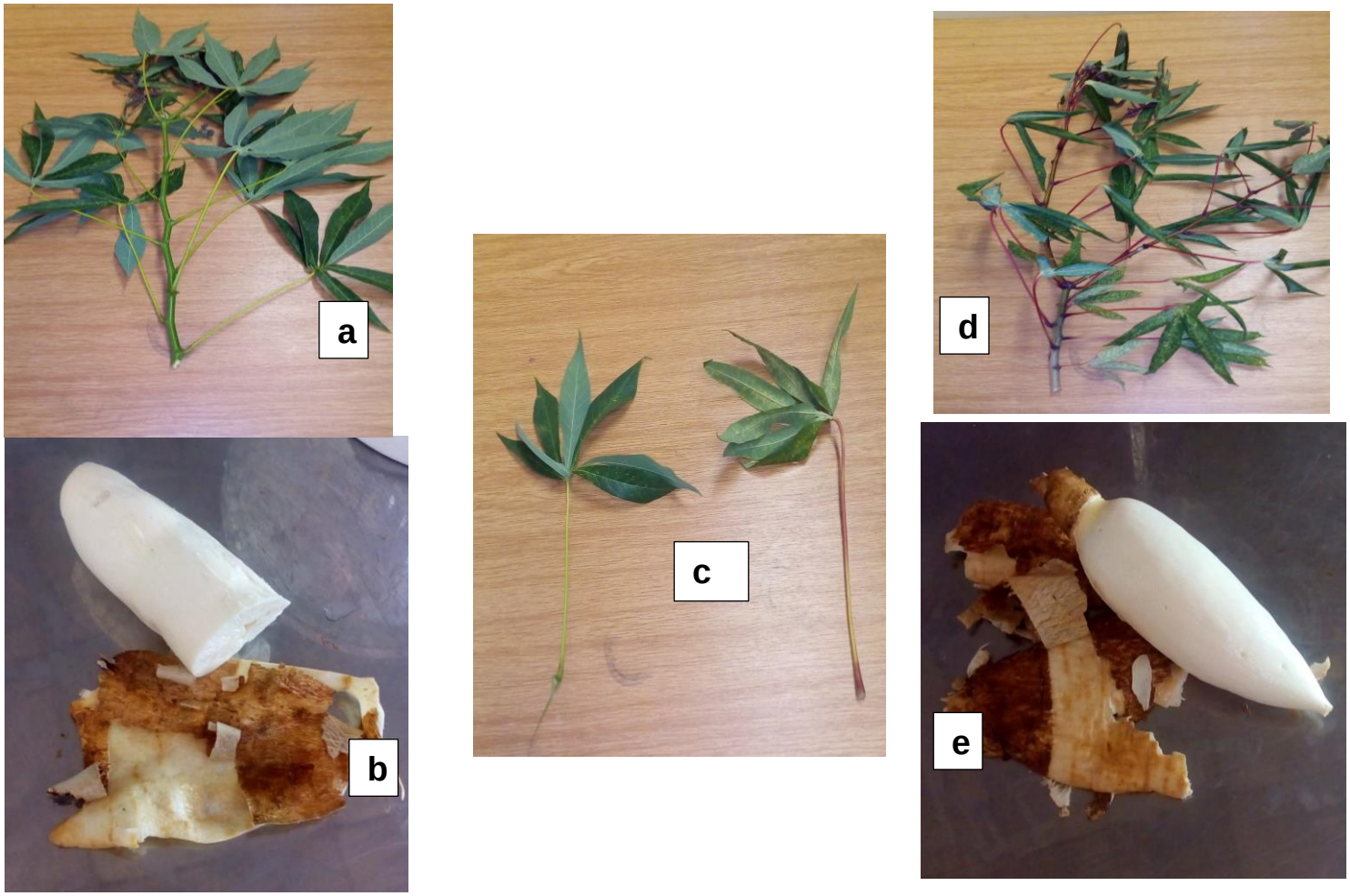

Figure 3.1 Morphological features of red and white *Manihot esculenta* landraces. **a** – stem and leaves of white landrace; **b** – root of white landrace; **c** - Leaves of white (left) and red (right) landrace; **d** – stem and leaves of red landrace; **e** – root of red landrace

The visual assessment clearly shows that the leaves stalk of the red landrace was red and the stem was brown while the stem and leaves stalk of the white landrace was green. In Figure 3.1b and e are the peeled cassava roots of both landraces, showing their parenchyma, cortex and periderm. Upon visual assessment the periderm and cortex of the red landrace appeared more reddish than the white landrace. This was confirmed by the results (Table 3.1) obtained from colorimetric analysis and sample paired t-test of the peels were some of the colour properties, including the degree of lightness, were significantly ($p < 0.05$) different. Cassava periderm colours of light and dark brown; cortex colour of pink, purple, light brown, cream and yellow; parenchyma colours of red, yellow and white have been reported (Anggraini *et al.*, 2009; Fukuda *et al.*, 2010; Gu *et al.*, 2013; Ayetigbo *et al.*, 2018). Yellow fleshed cassava is one of the recent bio-fortified genotype, genetically cultivated via biofortification with β-carotene, to increase nutritional quality of the root to humans, specifically pro-vitamin A but the white-fleshed varieties are still widely cultivated (Uchechukwu-Agua *et al.*, 2015a; Ayetigbo *et al.*, 2018).

3.3.2 Textural characterisation of freshly harvested *Manihot esculenta* root

The textural properties of a material are important parameters that affect its processing and handling. Understanding the textural properties of roots is a requirement for examining the response of roots during application of mechanical forces (Sajeev *et al.*, 2010). It aids in design of fitting and efficient machines to facilitate postharvest processing. Figure 3.2 shows the values of the textural properties of unpeeled cassava root. Toughness (321.81 N.sec), firmness (45.28 N) and stiffness (634.44 g/sec) of the red root were higher than those (301.77 N.sec toughness, 39.06 N firmness and 548.76 g/sec stiffness) of the white landrace. However, the comparison of the means, using the independent *t*-test at an assumption of equal variances, shows that there was no significant difference ($p > 0.05$) in all the texture properties between both landraces. For the peeled cassava roots, a different trend was observed (Figure 3.2). Firmness and stiffness of the red landrace (41.03 N and 794.58 g/sec) were significantly higher ($p < 0.05$) than the corresponding values of the white landrace (38.20 N and 471.24 g/sec), while toughness of the white landrace (303.88 N.sec) was higher but not significantly different ($p > 0.05$) from that (301. 40 N.sec) of the red landrace. The values of firmness obtained in this study are similar to those (24.88 – 42.77 N) reported by Maives *et al.* (2012) but less than the values (90.9 – 127 N) reported by Sajeev *et al.* (2010). The texture of raw plant tissues is determined by the composition and anatomy of the plant (Beleia *et al.*, 2004).

Table 3.1 Instrumental analysis of the colour properties of *Manihot esculenta* roots

Colour Properties	PERIDERM			CORTEX			PARENCHYMA		
	Red	White	p-value	Red	White	p-value	Red	White	p-value
*L**	35.50 ± 1.29	43.39 ± 2.66	0.01**	67.37 ± 1.98	76.05 ± 2.72	0.01**	83.30 ± 2.35	87.42 ± 0.53	0.04**
*a**	13.34 ± 1.03	13.18 ± 0.72	0.84	13.38 ± 1.86	4.26 ± 3.26	0.01**	0.67 ± 0.16	0.06 ± 0.03	<0.01**
*b**	18.17 ± 1.43	20.90 ± 0.58	0.04**	31.00 ± 12.1	24.87 ± 5.50	0.47	16.07 ± 0.65	14.17 ± 0.76	0.03**
Chroma	22.54 ± 1.75	24.71 ± 0.84	0.12	33.77 ± 2.65	25.35 ± 5.72	0.08	16.09 ± 0.65	14.17 ± 0.76	0.03**
WI	77.02 ± 0.06	75.55 ± 0.25	<0.01**	82.18 ± 2.39	83.85 ± 1.65	0.16	86.50 ± 1.72	89.45 ± 0.34	0.04**
BI	23.16 ± 1.20	20.68 ± 0.80	0.07	17.99 ± 2.39	10.42 ± 3.16	0.03**	5.17 ± 0.38	4.15 ± 0.27	0.02**

Values are mean ± standard deviation (n=3); ** - Means across rows for each morphological property are significantly different ($p < 0.05$). *L** - Lightness; *a** - redness/greenness; *b**- Blueness/yellowness; WI -Whiteness index; BI – Brownness index; White – Cassava flour from white landrace; Red – Cassava flour from red landrace.

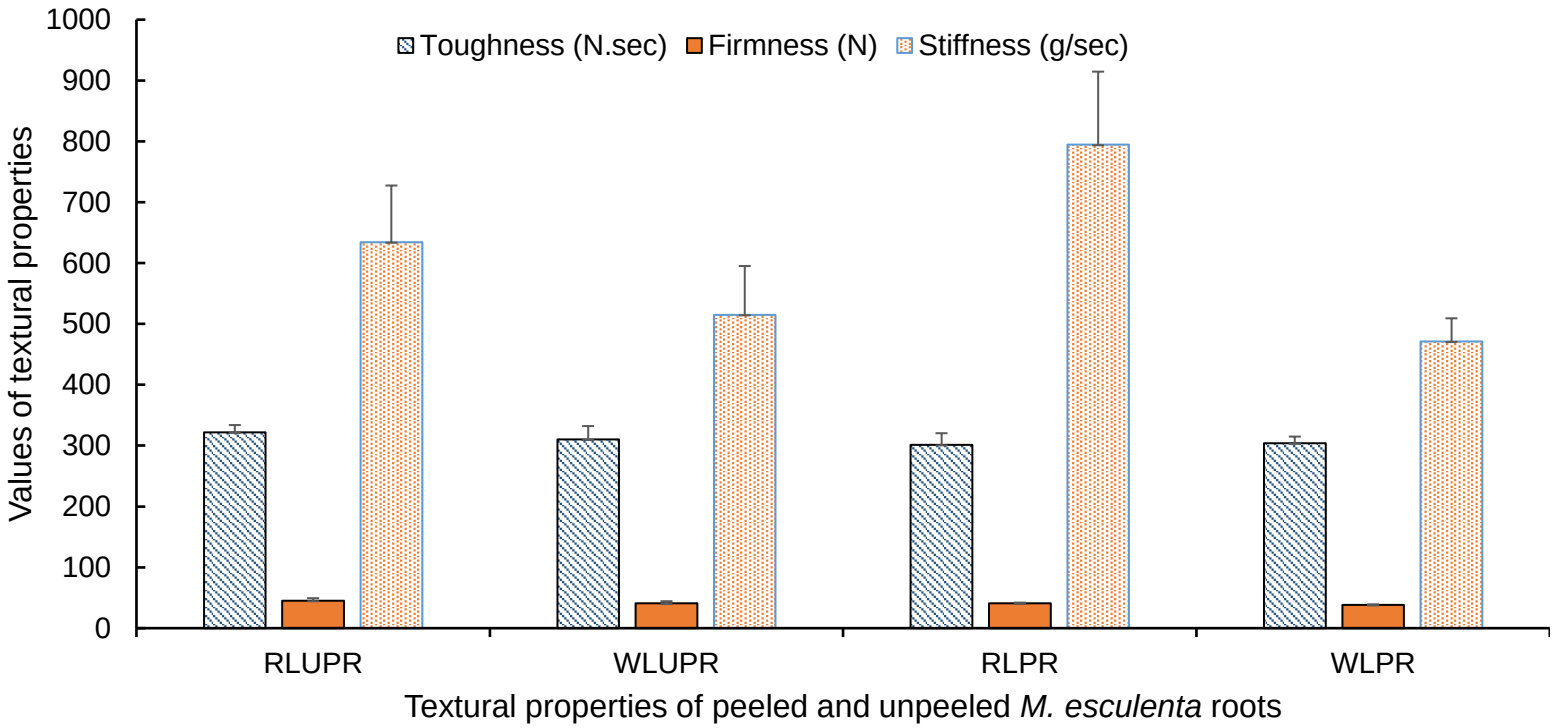

Figure 3.2 Textural properties of peeled and unpeeled *Manihot esculenta* root. RLUPR – Red landrace's unpeeled root; WLUPR - white landrace's unpeeled root; RLPR - Red landrace's peeled root; WLPR - white landrace's peeled root. Values are mean and error bars are standard deviation (n = 3)

Mature cassava root consists of the peel and the flesh also known as the parenchyma. The peel is made up of the periderm, scelerenchyma, cortical parenchyma and tissues while the parenchyma consists of xylem vessels radially dispersed in a matrix of starch-rich cells (Breuninger *et al.,* 2009). The parenchyma is approximately 85 % of the root weight, the most valuable part and eatable portion of the whole cassava plant. From the results obtained it maybe deduced that the anatomy of the root peels of the red and white landrace did not influence the texture properties of the roots. A significant difference ($p < 0.05$) in the toughness between the peeled roots of both landrace indicates that the orientation of the xylem vessels may differ. Carvalho *et al.* (2018) give details of the complex structure of the parenchyma which buttresses the findings of this study for the textural properties of whole root.

3.3.3 Metabolic profile of *Manihot esculenta* red and white parenchyma

Phenolic acids belong to a class of secondary metabolites in plants regarded as the main dietary phenolic compounds. Phenolic acids made up of benzoic and cinnamic acids and their derivatives, are referred to as strong antioxidant substances that can scavenge almost all oxidant molecules such as free radicals using their hydroxyl groups. Due to their highly hydroxylated molecular properties, each compound can scavenge one or two strong oxidant molecules (Sevgi *et al.*, 2015). A strong positive correlation exists between phenolic content and antioxidant potential of some plant foods (Bordoloi *et al.*, 2016**)**. When phenolic rich foods are incorporated in diets it reduces the risk of chronic degenerative diseases such as cancer (Colomer *et al.*, 2017). Identified phenolic acids present in the landraces and belonging to the benzoic acid group include: vanillic, protocatechuic, syringic and gallic acid while those belonging to the cinnamic acids include trans-cinnamic, m-coumaric, p-coumaric, ferulic and caffeic acid (Table 3.2). The chromatograms of phenolic acid identified in cassava root of the red and white landraces are shown in Appendix B.

These phenolic acids have been identified in literature as active phytochemicals with varying sources, degrees and specificity of scavenging abilities (Gultekin-Ozguven *et al.*, 2015; Sevgi *et al.*, 2015; Yashin *et al.*, 2017; Isaikina *et al.*, 2018; Hiraishi *et al.*, 2019) in diverse plants other than cassava. Literature abound on the total phenolic content of cassava root (Uarrota and Maraschin, 2015; Coelho *et al.*, 2019), however, that of phenolic acid profile is rare. Irondi *et al.* (2019) studied the phenolic composition of cassava flour using HPLC and identified five phenolic acids: chlorogenic, caffeic, rutin, gallic and quercetin acids. The variance in phenolic acids composition maybe attributed to variation in cultivar types, method of analysis and the state of the samples. Irondi *et al.* (2019) also reported a dearth of

knowledge of phenolic composition of cassava. Therefore, it is an aspect worth investigating further considering the benefits of these phytochemicals. Data obtained in this study shows that the relative abundance of all the phenolic acids identified was higher in the white landrace but not significantly ($p > 0.05$) except in vanillic acid (Table 3.2).

A reverse trend however was observed in the Fatty acids methyl esters (FAMEs) identified in the landraces. Identified FAMEs in the landraces include: hexadecanoic acid methyl ester, octadecanoic acid methyl ester, 9-octadecanoic acid methyl ester, (Z.Z) 9,12-octadecanoic acid methyl ester and alpha-Linolenic acid methyl ester (Table 3.2). The amount of FAMEs was higher in the red landrace than the white and they were all significantly different ($p < 0.05$) except for (Z.Z) 9,12-Octadecanoic acid methyl ester (Table 3.2). Appendix C shows the chromatograms of FAMEs identified in cassava root of both landraces.

All sugars were higher in the red landrace; Arabinose (red – 7.77 µg/g, white – 5.25 µg/g), d-Ribose (red – 151.85 µg/g, white – 148.77 µg/g), d-Fructose (red – 422.01 µg/g, white – 139.72 µg/g), d-Mannose (red – 174.14 µg/g, white – 127.15 µg/g), Glucose (red – 1064.62 µg/g, white – 217.01 µg/g) and Myo-Inositol (red – 638.60 µg/g, white – 350.50 µg/g) except d-Maltose (white – 284.46 µg/g, red – 207.16 µg/g) and sucrose (white – 5830.67, red – 3300.84) which was significantly higher ($p > 0.05$) in the white landrace (Table 3.2). Sugars such as fructose, maltose, glucose and sucrose have been identified in cassava roots (Onitilo *et al.* 2007b). Sweet cassava varieties have been reported to contain a high concentration (approximately 17% of total sugar) of sucrose in their roots (Ayetigbo *et al.,* 2018). The high concentration of sucrose (Appendix D) recorded for these landraces suggests that the landraces are of the sweet variety.

3.3.4 Scanning electron micrograph of *Manihot esculenta* red and white flour

Instrumentations for studying granular shape and size include light and scanning electron microscopy, while atomic force microscopy is employed in investigation of granular surface features such as surface pores (Zhu, 2015). The study of scanning electron microscopy is significant in elucidating the orientation and distribution of micro-components within a material. Starches, from various plants, possess distinct morphologies ranging from oval, round, truncated, lenticular or polygonal (Chisenga *et al.*, 2019a).

Table 3.2 Quantification of metabolic components of *Manihot esculenta* root

Compounds	Red landrace	White landrace	p-value
Phenolic acids(ng/g)			
trans-cinnamic_acid	21.13 ± 1.56	21.82 ± 0.66	0.52
Vanillic acid	10.39 ± 0.56	14.21 ± 0.94	0.004**
Protocatechuic_acid	30.31 ± 1.81	31.43 ± 1.40	0.44
m-coumaric acid	56.69 ± 3.50	58.16 ± 2.54	0.59
Syringic acid	29.86 ± 1.72	30.66 ± 1.27	0.55
p-coumaric acid	50.27 ± 2.94	51.63 ± 2.24	0.56
Gallic acid	58.98 ± 2.43	60.67 ± 2.60	0.46
Ferulic acid	57.96 ± 3.41	60.30 ± 3.41	0.45
Caffeic acid	67.50 ± 4.01	69.15 ± 3.16	0.61
Fatty acids methyl esters (FAMEs) (µg/g)			
Hexadecanoic acid methyl ester (C16:0)	135.06 ± 16.01	80.14 ± 10.16	0.01**
Octadecanoic acid methyl ester (C18:0)	10.66 ± 0.65	5.68 ± 1.12	0.003**
9-Octadecanoic acid methyl ester (C18:1)	393.55 ± 82.68	128.90 ± 17.86	0.01**
9,12-Octadecanoic acid (Z, Z) methyl ester (C18:2)	361.56 ± 10.51	340.11 ± 16.60	0.13
alpha-Linolenic acid methyl ester (C18:3n3)	257.43 ± 17.49	164.72 ± 5.18	0.001**
Sugars (µg/g)			
L-Arabinose	7.77 ± 2.56	5.25 ± 0.31	0.17
d-Ribose	151.85 ± 3.16	148.77 ± 7.33	0.54
d-Fructose	422.01 ± 75.65	139.72 ± 5.16	0.003**
d-Mannose	174.14 ± 12.00	127.15 ± 4.47	0.003**
Glucose	1064.62 ± 193.85	217.01 ± 3.57	0.002**
Myo-Inositol	638.60 ± 17.92	350.50 ± 42.01	0.00**
Sucrose	3300.84 ± 144.50	5830.67 ± 592.33	0.002**
d-Maltose	207.16 ± 11.53	284.46 ± 5.61	0.00**

Values are mean ± standard deviation (n=3); ** - Means across rows for each metabolic component are significantly different ($p < 0.05$).

The shape of starch granules influences the changes in viscosity and paste formation during gelatinisation (Pérez *et al.*, 2013). In a study by Sulistyo *et al.* (2016), unfermented cassava flour had a smooth surface of starch granules in contrast to fermented and modified cassava flour with broken, rough and eroded surfaces which was similar to what was observed in this study as the surface of the starch granules appeared smooth. It was further observed that within the starch granules are thin parenchymatous materials, an observation also reported by Maieves *et al.* (2012). Navia and Villada (2012) distinctly captured fibre and starch with the application of toluidine blue dye on cassava flour samples. This could suggest that thin parenchymatous materials are fibre. The granules are dispersed yet clustered in no regular pattern, therefore no conspicuous distinctions were observed in the granular morphology of flour from both landraces.

3.3.5 X-ray diffraction pattern of *Manihot esculenta* flour

Starch is the major component of cassava flour and X-ray diffractometry (XRD) is an indispensable technique that reveals the particularities of starch molecular arrangement characterised by the linkage of its two major polysaccharides amylopectin and amylose (Toraya, 2016; Bertoft, 2017). The X-ray diffractograms of cassava flour from the white and red landrace (Figure 3.4) are similar in shape characterised by a broad region between 15º and 45º with conspicuously distinct peaks. The main peaks are observed at 2θ = 43.40º, 23.02º, 17.14º and 15.07º for flour from the white landrace and at 2θ = 43.53º, 23.47º, 17.11º and 15.25º for flour from the red landrace. Similar results (major peaks at 2θ = 23º, 18º, 17º and 15º) were reported by Mei *et al.* (2015) for unmodified cassava starch.

Starch is categorised as semi-crystalline material having both amorphous and crystalline phases (Jane, 2006). The existence of these two phases can be observed in the XRD spectra of flours from red and white cassava landrace (Figure 3.4). Amylose is mostly found in the amorphous lamellae and amylopectin forms crystalline lamellae therefore starch crystallinity is strongly associated with amylopectin molecule (Zhu, 2015). The structural crystallinity of starch can be classified as type A, B and C based on the XRD pattern (Bertoft, 2017). Starches from root and tuber typically exhibit the B-type pattern (Yuan *et al.*, 2007).

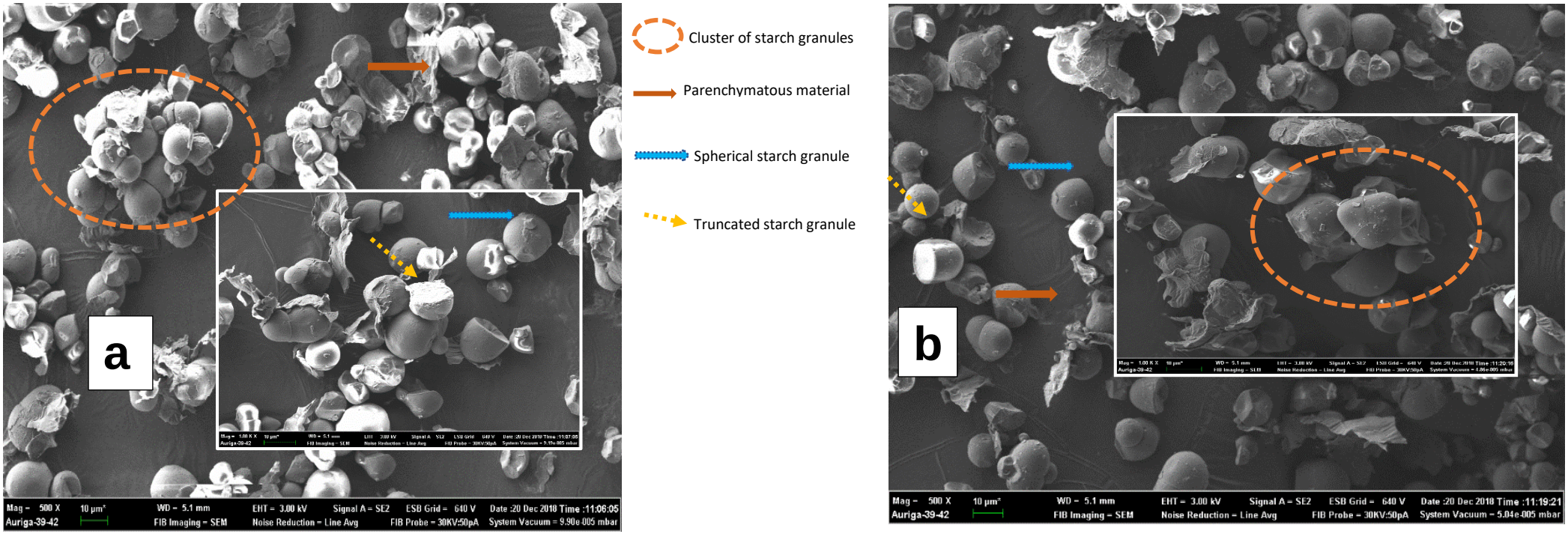

Figure 3.3 Scanning electron micrograph of *Manihot esculenta* flour at 500X and 1.00K resolution. **a** – Red landrace, **b** – White landrace.

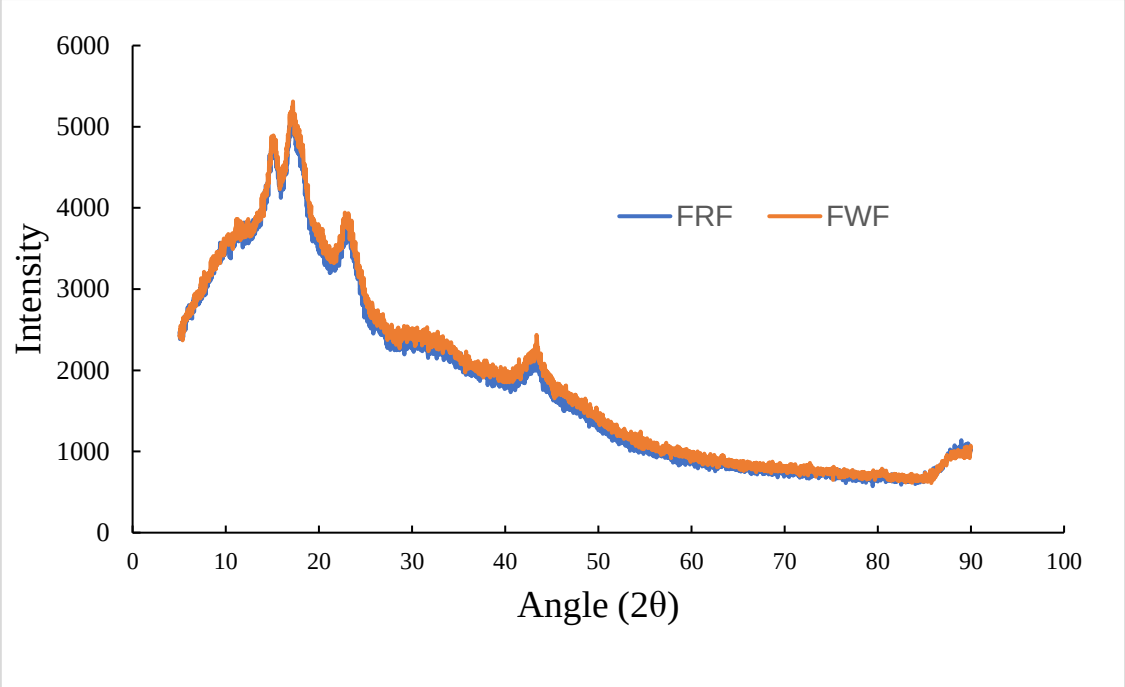

Figure 3.4 X-ray diffraction spectrum of flour from red and white cassava root. FRF – Flour from red landrace; FWF – Flour from white landrace. Values are mean (n = 3)

However, cassava root crop has been reported to exhibit the A-type pattern (Charoenkul *et al.*, 2011; Mei *et al.*, 2015; Zhu, 2015; Oyeyinka *et al.*, 2019), B-type (Chisenga *et al.*, 2019a) and C-type (Zhu, 2015). The A-type crystallinity is crystallites of double-helices of short chains, and are closely packed into a monoclinic unit cell consisting of eight water molecules. The B-type crystallinity is loosely packed crystallites of, double-helices in hexagonal unit cell consisting of thirty-six water molecules. Some starches possess both patterns (A and B) which is classified as the C-type (Bertoft, 2017). A-type XRD pattern all have characteristic peaks crystal around 18° and 23° 2θ while B-type crystal has around 5° 2θ (Guo *et al.*, 2020). The flours in this study showed the characteristic A-type pattern but with a less intense peak at 2θ = 43.40º and 43.53º for the white and red landraces. The presence of another major peak around 2θ = 40º is an indication of an extension of the crystalline domain and this implies a higher percentage of amylopectin in the starch structure of these landraces. The difference between the findings in this study and those earlier referenced maybe due to the composition of the materials investigated. As stated by Chisenga *et al.* (2019a), the difference in structures and composition is associated with diversity in starch properties of various genotypic cassava sources. Variations in crystallinity within plant varieties could be a result of differences in moisture content.

Liu *et al.* (2019) stated that crystallinity was influenced by heating conditions and moisture content. The molecular weight, chain length distribution and ratio of amylose and amylopectin are basic factors that influence the physicochemical properties of starches and starch-based foods. (Chisenga *et al.*, 2019b). Singh *et al.* (2010) stated that difference in double helices and packing pattern affects both morphology and size of starch granules. Understanding starch properties is necessary to provide important information on end-use properties for formulation and development of products. Starches with similar amylose contents can display similar functionalities. However, differences in the structural properties; starch granule size and crystallinity, chain length of amylopectin structures, degree of polymerisation, would likely cause variations in starch functionalities (Chisenga *et al.*, 2019a). Therefore, the flours from the red and white cassava landraces have potentials to function as a substitute for wheat flour which also exhibits A-type of starch crystallinity.

3.3.6 X-ray fluorescence analysis of *Manihot esculenta* flour

X-ray fluorescence spectrometry is a fast, low cost and suitable method for screening the concentration of elements in a given sample. This method is particularly useful when large number of samples are to be screened within a short time (Sosa *et al.*, 2018). The results of elemental analysis of cassava flour using XRF are shown in Table 3.3. Elements identified; potassium (K), magnesium (Mg), calcium (Ca), aluminium (Al), phosphorus (P), iron (Fe), chromium (Cr), and titanium (Ti), in the flours from both cassava landraces are shown in descending order of their amount or composition.

These elements in foods, also referred to as minerals, are classified into macro and micro minerals. Calcium, phosphorus, sodium (Na), potassium and chloride fall into the category of macrominerals. Magnesium, manganese (Mn), zinc (Zn), iron, copper (Cu), molybdenum, selenium, iodine, cobalt and chromium are grouped as microminerals which the body requires in minute quantity for healthy metabolism (Danbaba *et al.*, 2015). The elemental constituents were similar in both landraces except lead, detected in trace amount (0.20 mg g^{-1}) in the red landrace. Independent samples t-test at equal variance shows that there was no significant difference ($p > 0.05$) in the elemental composition of both samples except for their K, Ca and P contents. Afoakwa *et al.* (2012) identified Na and Zn in some traditional and improved varieties of cassava and these were identified in the cassava roots analysed by Oboh and Elusiyan (2007). Charles *et al.* (2005) reported that Na, Zn, Mn and Cu were present in all five genotypes of cassava analysed. These elements were not identified in the landraces used in this study. Similarly, Al identified in the landraces used in this study were not identified in the studies of Charles *et al.* (2005), Oboh and Elusiyan (2007) and Afoakwa *et al.* (2012).

Table 3.3 Elemental analysis of cassava flour as a function of landrace

Elements (mg g^{-1})	Red landrace	White landrace	p-value
Potassium	26.10 ± 0.20	30.30 ± 0.30	<0.01**
Magnesium	23.40 ± 8.70	16.80 ± 8.40	0.34
Calcium	11.50 ± 0.30	5.60 ± 0.10	0.00**
Aluminium	1.50 ± 0.40	1.50 ± 0.50	1.00
Phosphorus	0.80 ± 0.10	1.50 ± 0.10	<0.01**
Iron	0.50 ± 0.04	0.50 ± 0.04	1.00
Chromium	0.20 ± 0.03	0.20 ± 0.03	1.00
Titanium	0.20 ± 0.10	0.20 ± 0.10	1.00
Lead	0.20 ± 0.00	ND	-

Values are mean ± standard deviation (n = 3); **- elements were significantly different ($p < 0.05$) between the landraces; ND - not detected.

Variations that exist in elemental constituents of cassava are attributed to variety, growth environment, breeding condition, age at harvest and postharvest processing (Afoakwa *et al.*, 2012; Djabou *et al.*, 2018).

Potassium was identified in both cassava flour samples as one of the major elements and with the highest compositional value (Red – 26.10 mg g^{-1}; white - 30.30 mg g^{-1}). Dietary potassium intake, in the right amount, has a protective effect of reduction of kidney stones, blood pressure, risk of stroke and age-related bone loss in humans (Weaver, 2013). Potassium in the white landrace (30.30 mg g^{-1}) was significantly higher than that of the red landrace (26.10 mg g^{-1}). The quantity of potassium (221–328 mg g^{-1}) reported by Charles *et al.* (2005) for five cassava genotypes was higher than the values reported in this study. Afoakwa *et al.* (2012) reported that the potassium content (0.25 – 0.36 mg/100 g) of all the varieties of cassava investigated in their study was significantly different ($p < 0.05$) which was lower than the values obtained in this study. Magnesium in the red landrace (23.40 mg g^{-1}) was considerably higher than that of the white landrace (16.80 mg g^{-1}). However, an independent sample t-test shows that there was no significant difference between both landraces. The values obtained in this study are within the range (15.2–32.3 mg g^{-1}) reported by Charles *et al.* (2005) but higher than the values (1.35 mg/100 g to 2.52 mg/100 g) reported by Afoakwa *et al.* (2012).

Calcium was identified as one of the major elements in this study with its content in the white landrace (5.60 mg g^{-1}) significantly lower ($p < 0.05$) than that of the red landrace (11.50 mg g^{-1}). In most studies, calcium is reported as one of the major minerals in cassava. A

comparison of the edible portion of cassava root with other staple crops showed that calcium in cassava was relatively higher (Montagnac *et al.*, 2009b). The range, 0.06 to 1.60 mg/100g, reported by Afoakwa *et al.* (2012) is lower than both values obtained in this study. The calcium content of the white landrace was less than the values (10.9 – 39.9 mg g^{-1}) of Charles *et al.* (2005) but that of the red landrace was within the range. Montagnac *et al.* (2009b) reported that processing 100 g of cassava root to flour using different methods yielded calcium 6 – 8 mg (retting and no peel) and 7-15 mg (retting and peel). It is important to take in adequate calcium to support normal levels of bone and calcium levels (Waheed *et al.*, 2013). Calcium aids in regulation of cardiovascular function and blood pressure levels and in nerve conduction for stabilising the DNA and RNA protein structures (Soetan *et al.*, 2010). Since human bones are made of complex crystalline structure in microcrystalline hydroxyapatite pattern of Ca together with Mg, P and other trace elements such as Zn, Cu and Mn as cofactors of specific enzymes are also vital in bone metabolism, therefore any disease of the bone will decrease all these minerals (Waheed *et al.*, 2013).

The amount of Al (1.50 mg g^{-1}) detected in both flour samples was the same with a p-value of 1.00 which implies that there was no significant variation for the Al content in both landraces. The amount of phosphorus in both samples significantly differed ($p < 0.05$) with the white landrace (1.50 mg g^{-1}) higher than the red landrace (0.80 mg g^{-1}). Afoakwa *et al.* (2012) identified Phosphorus as a major element present in cassava flour reported a range of 1.06 to 2.13 mg/100 g and significant differences among all varieties investigated at $p < 0.05$. Montagnac *et al.* (2009b) reported a range of 9 -21 mg of phosphorus for 100 g of cassava root processed to flour using different methods.

The amount of Fe (0.05 mg g^{-1}), Cr (0.20 mg g^{-1}) and Ti (0.20 mg g^{-1}) in the white and red landrace was the same with a p-value of 1.00. These elements could be classified as the minor elements in the landraces used in this study. The trend in this study is similar to that of Charles *et al.* (2005) where magnesium and calcium were reported as the major elements while Fe, chromium and Titanium as the minor elements. Afoakwa *et al.* (2012) reported a lower amount of Fe (0.16 – 0.24 mg/100 g) in flour from different varieties of cassava root. Fe is a minor mineral present in cassava root which due to its essential characteristic, is a target for biofortification in cassava breeding (Montagnac *et al.*, 2009b). Biofortification is a scientific approach to develop micronutrient-dense crops especially staples that are deficient in these nutrients in a bid to reduce hidden hunger (Sosa *et al.*, 2018). The lack of essential vitamins and minerals in diets causes hidden hunger which is a major public health problem. Retarded mental development, illness, blindness and premature death, associated with hidden hunger, afflicts above two billion people in the world (Umar *et al.*, 2019). Although these elements

generally represent a small portion of food composition, they play major and essential roles in food chemistry and nutrition (Danbaba *et al.*, 2015).

3.3.7 Physicochemical characteristics of white and red *Manihot esculenta* landraces

A primary cassava breeding objective is to produce varieties for increased yields (Chisenga *et al.*, 2019b) and maximised economic benefit. In Table 3.4 are results of peel thickness, percentage yield, colour properties and bulk densities of the flour processed from the red and white cassava landrace. According to Ugwu and Ozioko (2015) the thicker the root peel, the higher the ease of peel. Measured with a vernier callipre, the sample t-test shows that the peel of the red landrace (2.27 mm) was significantly thicker ($p < 0.05$) than the peel of the white landrace (1.85 mm). This result correlates with the percentage flour yield of both landraces with the yield of the white landrace (37.03%) significantly higher than the yield of the red landrace (36.15%). Kaur *et al.* (2016) reported that the yield percentage of cassava starch was 40% and was found to be slightly higher than the yield percentage observed in this study. The yield (13.91 and 20.67% on a wet basis) of cassava flour reported by Falade *et al.* (2019) is below the yield of cassava flour obtained in this study. Dziedzoave *et al.*, (2006) postulated that the yield of cassava flour should fall within the range of 13 to 19%. Eriksson (2013) reported the average flour yield, of three cultivars, as percentage of fresh cassava weight to be 18.50, the peel and water accounts for the remaining weight. The processing method and the basis (dry or wet) of calculation may affect the percentage yield of a product.

Table 3.4 Physical and chemical properties of white and red *Manihot esculenta* landrace

Parameters	Red landrace	White landrace	p-value
Peel thickness (mm)	2.27 ± 0.03	1.85 ± 0.13	<0.01**
Flour yield (%)	36.15 ± 0.01	37.03 ± 0.06	0.00**
LBD (g/ml)	0.39 ± 0.02	0.39 ± 0.01	1.00
PBD (g/ml)	0.63 ± 0.01	0.69 ± 0.01	0.00**
ΔBD (g/ml)	0.30 ± 0.01	0.30 ± 0.02	0.06
Starch (%)	79.13 ± 1.37	78.92 ± 1.48	0.70
Ash (%)	1.30 ± 0.14	1.51 ± 0.10	0.10
Crude fibre (%)	3.27 ± 0.19	3.04 ± 0.18	0.20

Values are mean ± standard deviation (n = 3); ** - means across rows are significantly different ($p< 0.05$). LBD – Loose bulk density; PBD – Packed bulk density; ΔBD – Difference in bulk density.

In some instances, the yield of cassava products maybe calculated based on the whole, peeled or dried root weight (Oghenechavwuko *et al.*, 2013). The maturity and moisture of the root also influence the flour yield (Apea-Bah *et al.*, 2011). The milling and sieving process also influences the recovery of flour. The pin mill gives a higher recovery (approximately 100%) when compared to the hammer, attrition and mortar mills (Adesina and Bolaji, 2013).

The moisture content and pH of the red and white cassava landrace roots and their corresponding flour are shown in Figure 5. There was no significant difference ($p > 0.05$) in the moisture content of fresh cassava root of the red (59.14%) and white (57.67%) landrace. These values are higher than the findings of Afoakwa *et al.* (2012) who reported a significant difference in the moisture content (33.14 – 45.86%) of improved and traditional varieties of cassava obtained from the West African region. Padonou *et al.* (2006) reported a moisture content of 60.3 – 87.1% for cassava root which is higher than the values obtained in this study. It can be observed from results of moisture content that a wide variation occurs which may be attributed to the age at harvest, variety, the season of harvest/planting and climatic conditions. The moisture content of the flour processed from the red landrace (9.31%) was higher than that of the white landrace (9.15%). The moisture content of cassava flour in this study is similar to the reported values of 11 – 16.5% (Shittu *et al.*, 2007), 9.2 – 12.3% (Charles *et al.*, 2005), 7.48 – 9.66% (Afoakwa *et al.*, 2012) and 10.78 – 12.72% (Alamu *et al.*, 2017) for cassava flour. Carvalho *et al.* (2018) stated that the chemical composition of the storage root varies depending on the plant genotype. In some of the studies referenced, there was significant variance in the moisture content of the roots which was not observed in this study. The processing from root to flour allows for sufficient loss of moisture via the drying process. The low moisture content of both flours confers on it high level of resistance to microbial infestation which is an advantage for prolonged shelf life.

The pH (6.49) of the white landrace was significantly higher ($p < 0.05$) than that of the red landrace (6.32) in the root form. In the flour state, the pH of both landraces increased slightly. However, a reverse trend occurred with the pH (6.71) of the red landrace significantly higher than the pH (6.60) of the white landrace. The increase in pH from the root form to the flour form maybe attributed to the loss of moisture. With a decrease in the level of moisture acidity increases. The values of pH in this study are slightly lower than the value (6.82) reported by Odey and Lee (2020) for unfermented cassava flour.

The ash (1.30%), crude fibre (3.27%) and starch (79.13%) contents of the red landrace (Table 3.4) were not significantly different ($p > 0.05$) from the ash (1.51%), crude fibre (3.04%) and starch (78.92%) content of the white landrace. The values of ash and starch reported in this study are within the range of values: ash (0.77-1.43%) and starch (67.08-82.42%) reported by Alamu *et al.* (2017) for high-quality cassava flour processed from β-carotene enriched roots.

The high starch content in these landraces makes them suitable for starch production. The percentage crude fibre content (red, 3.27; white 3.04) for cassava flour in this study is within the range (1.38 – 3.2) reported by Afoakwa *et al.* (2012) and corroborates the recommendations of Gil and Buitrago (2002) that fibre content in cassava flour should not exceed 4%. Consumption of an adequate amount of dietary fibre reduces the risk of diseases such as constipation, coronary heart diseases, obesity, gallstones, diabetes and other related diseases (Dahl and Stewart, 2015).

3.3.8 Colour properties of *Manihot esculenta* parenchyma and flour

Colour is an important organoleptic property that influences the acceptance of crop products. The colour of cassava parenchyma is important in cassava flour production because the flour retains the colour of the root flesh (Ayetigbo *et al.*, 2018). Visual assessment of the root parenchyma showed no distinct features as both landraces appeared cream in colour. However, the instrumental analysis with the aid of a colorimeter, revealed that the *L** (87.42), *a** (0.06), *b** (14.17), whiteness (89.45) and brownness index (4.15) of the white landrace were significantly different ($p < 0.05$) from the *L** (83.30), *a** (0.67), *b** (16.07), whiteness (86.50) and brownness index (5.17) of the red landrace (Table 3.1). This indicates that all colour properties investigated for the edible portion of the root were significantly different at $p < 0.05$. It indicates that visual and instinctive assessment maybe misleading. Values of *L** (72.43 - 81.19), *a** (-0.9 - -1.63) and *b** (14.08-16.29) similar to these findings were reported by Ladeira *et al.* (2013) for cream-fleshed Brazilian cassava roots. The colour parameters *L**, *a**, *b**, whiteness and brownness index of the root and corresponding flour are shown in Figure 3.5. There was no significant difference ($p > 0.05$) in the lightness (91.37) of flours of both landraces. The brownness index (BI) of roots of both landraces was significantly different ($p < 0.05$) and so was the flour. Unlike the whiteness index, the degree of redness/greenness, yellowness/blueness and brownness index of the flour was lower when compared to the root. This shows that the reaction of both landraces under the same processing condition varies. It was also observed from the study that the removal of moisture from the fresh root increased the whiteness of its corresponding flour. A reason for more intense retention of the colour could be due to the lipid-soluble nature of colour-active carotenoids, the complex mucilage and latex as well as starch-lipid, protein-lipid and fibre-lipid interactions (Moorthy, 2002; Ayetigbo *et al.*, 2018). Other factors that may influence the colour of cassava flour are extraction rate, particle size, flour treatment, temperature and drying duration (Oladunmoye *et al.*, 2010b).

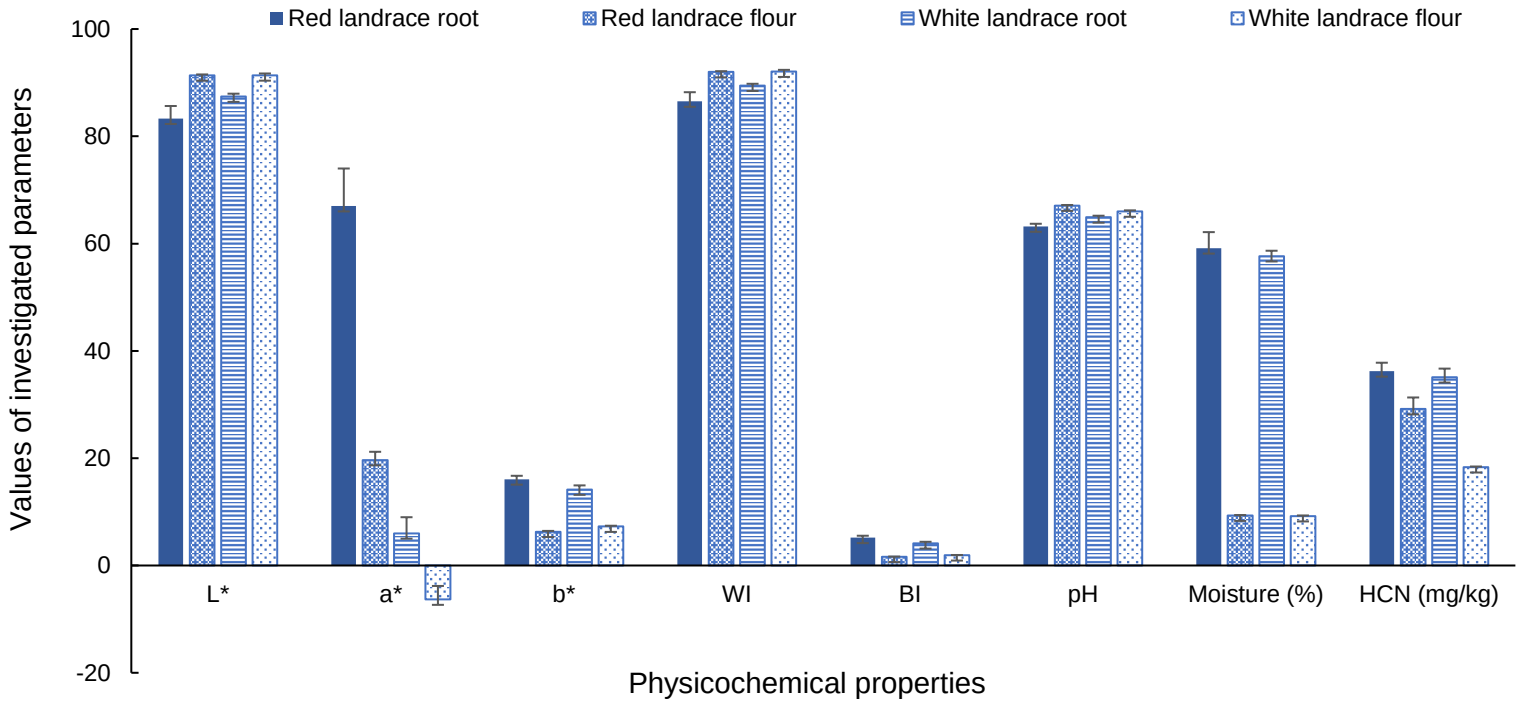

Figure 3.5 Physicochemical properties of *M. esculenta* root and their corresponding flour. HCN – Hydrogen cyanide content; BI - brownness index; WI – whiteness index; Values of pH, HCN and BI were multiplied by 10; a* was multiplied by 100. Values are mean of triplicate runs and error bars are standard deviation of mean.

The characteristic and acceptable colour associated with cassava flour is white (Omolola *et al.*, 2017) and the relatively high whiteness index and *L** values, recorded in this study, implies that the flours are white in colour. This is an advantageous consumer property especially with respect to for substitution of wheat flour.

3.3.9 Processing effect on the cyanide content of *Manihot esculenta* parenchyma and flour

One major setback to the utilisation of cassava is the hydrocyanogenic potential of the root (Mtunguja *et al.*, 2016) and fear of poisoning. Cassava is majorly grouped into two (bitter and sweet) based on their inherent cyanogenic glucoside potential and intended end-use (Chiwona-Karltun *et al.*, 2015a). Cyanide content of the root was not significantly different ($p > 0.05$) in both landraces but the cyanide content of the flours was significantly ($p < 0.05$) different. The process of chipping allows for linamarase in the plant tissue to be in contact with linamarin, the major cyanogenic glucoside in cassava. This enzymatic action is responsible for the decrease in the cyanogenic content of the flour when compared to the root (Uchechukwu-Agua *et al.,* 2015b; Kasankala *et al.,* 2019). Other process steps such as milling and drying also result in loss of cyanide in cassava (Piero *et al.*, 2015; Atlaw, 2018).

The cyanoglucosides content in the root parenchyma normally ranges from 1 - 1550 mg HCN/kg fresh weight. Internationally, the Codex Standard for sweet cassava is ≤ 50 mg/kg fresh weight basis (FAO/WHO, 2005). The values of cyanide obtained in this study are below 10 mg/kg (Figure 3.5), which implies that the landraces can be classified as sweet cassava. This agrees with the report of Drimie and McLachlan (2013) that cassava grown in South Africa is mostly of the sweet variety. Chiwona-Karltun et al. (2015b) referred to cassava flours with HCN ranging from 30.1 – 64.3 mg/kg as having high cyanogenic potentials. Therefore, the root and flour in this study are safe for human consumption with minimal processing. An increase in the pH value which implies a decrease in acidity of the material aligns with the decrease in cyanide content (Figure 3.5). Processing, therefore, results in loss of some cyanogenic acids which are mostly volatile (Sagar, 2018).

3.4 Conclusion

Morphological differences in the colour of leaves, stalk, cortex and periderm aid easy identification of the red and white *M. esculenta* landraces at the point of harvest and during processing. The whiteness of the parenchyma and flour of both landraces is a quality desired by consumers and makes flours of these landraces suitable for food applications. Percentage

flour yield of the red and white landraces was significantly ($p < 0.05$) influenced by root peel thickness. Phenolic acids composition, fatty acid methyl esters and sugar composition of the parenchyma of both landraces were similar but varied in quantity. Elemental components of the flours indicate that both landraces contain vital minerals for proper body metabolism. Structural characterisation revealed that both landraces displayed A-type starch crystallinity, as in wheat, which suggests that the flours may be suitable for wheat replacement in flour-based foods. The recorded high and low concentration of sucrose and cyanide confirms that the landraces are of the sweet variety. This makes the landraces safe for human consumption and suitable for the production of minimally processed foods.

CHAPTER FOUR: INVESTIGATING THE THERMAL AND FUNCTIONAL PROPERTIES OF CHEMICALLY PRE-TREATED CASSAVA FLOUR USING RESPONSE SURFACE METHODOLOGY

Abstract

Cassava flour (CF) is mostly constituted to “dough form” in various food applications which involves the incorporation of water in the presence of heat. This study, using response surface methodology, comparatively investigated the effect of two pre-treatments (calcium chloride and citric acid) interacting with varying drying temperatures on the loose and packed bulk densities, water holding capacity and thermal properties of CF from two South African landraces (red and white). Differential scanning calorimetry was used in determining the gelatinisation temperatures and enthalpy of CF under varying conditions. Gelatinisation temperatures of onset (60.32-120.30ºC), peak (71.85-126.84ºC), conclusion (93.31-140.98ºC) and enthalpy of gelatinisation (0.14 to 54.95 J/g) were recorded for all CF samples. The processing conditions had an increasing effect on the gelatinisation enthalpy of the flour. Citric acid treatment had a decreasing effect on the water holding capacity of the flours when compared to calcium chloride. Drying temperatures significantly influenced bulk densities of CF, while the loosed bulk density increased with drying temperature. An increase in enthalpy of gelatinisation for chemically pre-treated CF infers that more energy will be required for gelatinisation to occur. Therefore, the processing conditions confer on CF more stability in the presence of heat and water.

Keywords: Cassava flour; water holding capacity; pre-treatment; thermal properties; bulk densities

4.1 Introduction

Cassava flour (CF) is a major product of the world’s most important staple root (*Manihot esculenta* Crantz) also known as tapioca or manioc (FAO, 2013). The advantageous agronomic traits of the root guarantee low cost and allyear availability of the flour making it a viable substitute to wheat flour in countries where cassava is a major food staple (El-Sharkawy, 2014; Zhu, 2015; Dudu *et al.*, 2019). Cassava flour, mostly consumed by humans, is currently being explored as a convenient alternative of wheat for producing gluten-free products and developing biofortified and fortified foods (Aristizábal *et al.*, 2017; Ramírez *et al.*, 2019). Cassava flour can be processed into popular flour-based food products such as bread (Agunbiade *et al.*, 2017; Alobo and Arueya, 2017), biscuits (Olatunde *et al.*, 2016; Lu *et al.*,

2020), noodles (Abidin *et al.*, 2013) and other confectionaries (Falade and Akingbala, 2008) both as composite and the base flour.

Bulk density of CF is a measure of its heaviness (Oladele and Aina, 2007) which is not only useful for packaging but also influences its porosity, dispersibility, flowability and fluidity (Falade et al., 2019). A processing step common in CF utilisation is the incorporation of water into the flour structure and application of heat during dough formation for various food products. Water holding ability (WHC) is highly desirable in food systems to improve consistency and yield (Omowaye-Taiwo *et al.*, 2015). Thermal properties are associated with energy required for the occurrence of an irreversible change in starch structure as a result of heat and water application referred to as gelatinisation (Zhu, 2015). Although CF consists mostly of starch, its non-starch components also influence the thermal properties and its WHC is the ability of components of the flour to bind with water at hydrophilic sites (Ayetigbo *et al.*, 2018). High hydration increases the swelling and gelatinisation ability of starch granules (Cornejo-Ramírez *et al.*, 2018). Thus, the ability of flour to take up water and its behaviour during heat application is dependent on the nature of starch in the flour as well as treatment conditions during processing (Jyothi *et al.*, 2010, 2011; Dudu *et al.*, 2019).

Lu *et al.* (2020) investigated the effects of xanthan gum and inulin on the thermal properties of CF and quality of short biscuits from the flour. It was reported that addition of inulin improved starch gelatinisation resulting in decreased hardness and brittleness of biscuit processed from CF. Based on elaborating the utilisation of CF in gluten-free pasta formulation, Ramírez *et al.* (2019) conducted a study to determine the optimal hot-air drying conditions for CF. The authors reported the operational conditions needed to minimise the hot-air drying time (57 min) to produce CF with higher water holding capacity as 57℃ at 3 m/s. Dudu *et al.* (2019) reported that thermal stability and gelatinisation enthalpy of CF increased and decreased respectively at optimal steam-heat-moisture treatments. Falade *et al.* (2019) found out that annealing reduced the WHC while citric acid cross linking increased the packed bulk density (PBD) and loosed bulk density (LBD) of CF. Studies on the effect of chemical pre-treatment on the WHC and thermal properties of CF are scarce in literature, although some (Villanueva *et al.*, 2018; Zambelli *et al.*, 2018) have been reported for cassava starch.

The background knowledge that non-starch components influence the bulk density, WHC and thermal properties of CF prompted this study. Preliminary findings indicated no adverse effect on the bulk density and WHC of pre-treated CF. The study also showed improved colour quality of solar-dried CF as a result of calcium chloride and citric acid pre-treatment (Nemaungani *et al.*, 2019). Therefore, this study, using response surface methodology, comparatively determined the influence of citric acid and calcium chloride pre-

treatment at varying concentrations and drying temperatures on the WHC, thermal properties and bulk densities of CF.

4.2 Materials and Methods

4.2.1 Preparation of cassava root

Two landraces of cassava (white and red) were sourced from the Institute of Tropical and Subtropical Crops - Agricultural Research Council (ITSC-ARC) Levubu, Limpopo Province, South Africa (22.946º S 30.485º E). The roots, fourteen months after planting, were harvested and sorted before washing with tap water to remove adhering dirt and avoid contamination during processing. Within 24 h after harvest processing commenced, to ascertain that the roots were in a fresh state, before the onset of deterioration (Coelho *et al.*, 2019).

4.2.2 Experimental design

Factored into the design of this experiment is the concentration of chemical pre-treatment (citric acid and calcium chloride separately at 1-3% w/v) and drying temperature (50-70ºC) as independent variables in CF processing. While WHC, onset gelatinisation temperature (To), peak gelatinisation temperature (Tp), conclusion gelatinisation temperature (Tc), gelatinisation enthalpy (ΔH), packed bulk density (PBD), loose bulk density (LBD) and difference in bulk density (ΔBD) were the dependent processing variables. Before the conduct of the experiment, the Design Expert (DE) software Version 11 (Stat Ease Inc., Minneapolis, USA) was used in generating the experimental conditions for processing the flour samples. The central composite design was used in this study as shown in Table 4.1. The four experimental groups obtained are; citric acid pre-treated flour from the red landrace (CAR), calcium chloride pre-treated flour from the red landrace (CCR), citric acid pre-treated flour from the white landrace (CAW) and calcium chloride pre-treated flour from the white landrace (CCW).

4.2.3 Processing of pre-treated cassava flour

The method described by the Federal Institute of Industrial Research (2005) was employed, with modifications, in processing the flour. Modifications applied in this study are the application of different concentrations of chemical pre-treatment and drying temperatures for each experimental run as generated by the design expert central composite design (Figure 4.1). The washed roots were peeled and chipped manually with a knife. Fresh cassava chips (1 ± 0.5 cm thick) were pre-treated by steeping them in the chemical (citric acid and calcium

chloride) solution for about 20 min after which they were dried in the oven (Ecotherm, 240L digital oven, South Africa) at different temperatures (50-70ºC) for 24 h. Milling of dried chips was done with the aid of a dry milling machine (Polymix PX-MFC 90D, Switzerland). Flour obtained from the miller was subjected to sieving through a 500 µm sized aperture sieve and stored in sample bags made of paper material at room temperature, till further analysis. Two control samples were processed, one from each landrace, using same procedure as the experimental samples save for pre-treatment and drying temperature was 60ºC. The control samples served as reference samples, within a landrace during statistical analysis.

Table 4.1 Levels of independent variables used for central composite design

Pre-treatment	Code	DT (º C)	COP (g/100 ml)
Citric acid	-1	50	1
	0	60	2
	1	70	3
Calcium chloride	-1	50	1
	0	60	2
	1	70	3

-1 = upper limit; 0 = central point; 1 = lower limit; DT- Drying temperature, COP- Concentration of pre-treatment. NB: Two pre-treatments (citric acid and calcium chloride) were used separately

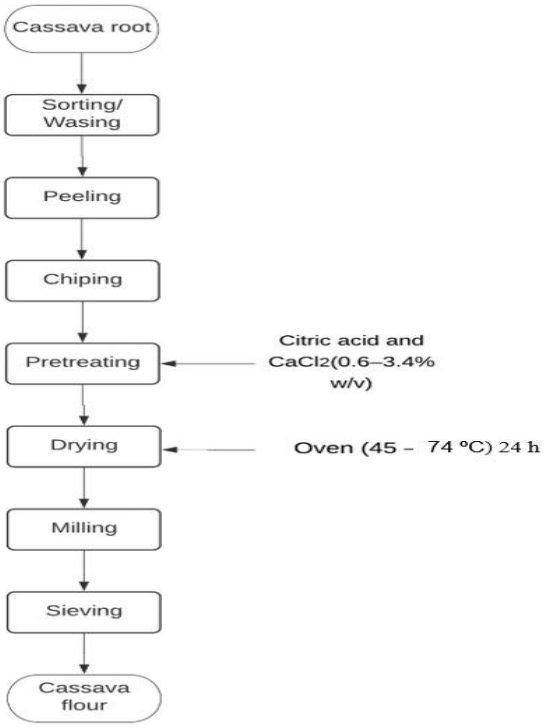

Figure 4.1. Process flow chart for pre-treated cassava flour

4.2.4 Determination of water holding capacity of cassava flour

The method described by Onwuka and Onwuka, (2005) was used. About 1 g of the flour sample was weighed into a 15 mL centrifuge tube and suspended in 10 mL of water. It was shaken on a vortex for 1 min at room temperature. The sample was allowed to stand for 30 min and centrifuged at 1200 x g for 30 min. The volume of free water was read directly from the centrifuge tube. The water absorption capacity was calculated using Equation 4.1.

$$WHC\% = \frac{Amount\ of\ water\ added - free\ water}{weight\ of\ the\ sample\ (dry\ bases)} \times density\ of\ water \times 100 \quad (4.1)$$

4.2.5 Determination of thermal properties of cassava flour

Onset gelatinisation temperature (To), peak gelatinisation temperature (Tp), conclusion gelatinisation temperature (Tc) and gelatinisation enthalpy (ΔH) of CF was determined with a differential scanning calorimeter (DSC 4000, Perkin-Elmer, Shelton, CT, USA) as described by Anyasi *et al.* (2017). To approximately 10 mg of cassava flour 10 µL of distilled water was added in an aluminum pan. The pan was hermetically sealed with a crimper press (Perkin-Elmer, Shelton, CT, USA). The sample was refrigerated (4ºC) overnight to equilibrate it. Afterward, the sample was heated at 10ºC/min from 20-140ºC and thermal properties extrapolated from the curves with the aid of Pyris software (Perkin Elmer Shelton, CT, USA).

4.2.6 Determination of bulk density of cassava flour

Bulk density was determined as described by Maninder *et al.* (2007). The flour samples were gently filled into 10 mL graduated cylinders. The bottom of each cylinder was tapped gently on a laboratory bench several times until diminution of the sample level ceases after filling to the 10 mL mark to determine the packed bulk density (PBD). For the loose bulk density (LBD), the bottom of the cylinder was not tapped. Bulk density was calculated as weight per unit volume of sample (g/mL) as expressed in Equation 4.2. The difference in the bulk densities (ΔBD) was calculated as shown in Equation 4.3.

$$Bulk\ density = \frac{weight\ of\ sample\ (g)}{volume\ of\ sample\ (ml)} \quad (4.2)$$

$$\Delta BD = PBD - LBD \quad (4.3)$$

4.2.7 Statistical analysis and optimisation

Design expert software version 11 was employed in the analysis of variance (ANOVA) of the linear, interactive and quadratic effect of the model parameters on CF properties. Regression models, coefficient of determination (R^2), p-values, F-values, response surface plots, contour plots and optimisation conditions were generated by the software for all four experimental groups. Statistical data analysis was done with the IBM SPSS statistics software Version 25 (IBM Corp., NY, USA) to ascertain the significant difference between means of the experimental and reference/control sample within each experimental group at a 95% confidence level. One-way ANOVA and separation of means were done using Duncan Multiple Range Test.

4.3 Results and discussion

4.3.1 Effect of pre-treatment and drying temperature on water holding capacity of cassava flour

Water holding capacity is a measure of the concentration of water a material can retain and it is dependent on the nature of the material (Aremu *et al.*, 2009). This property is important for reconstitution of flour to dough. It is highly desirable in food systems to improve yield and consistency of the product (Omowaye-Taiwo *et al.*, 2015). Analysis of variance of the means showed significant variance between samples of the experimental runs. However, the trend of variance as a result of the processing conditions was different in all experimental groups (Table 4.2). This is an indication that the landrace and processing conditions have a different effect on the WHC of CF. The WHC of pre-treated CF processed from the red landrace ranged from 41.95% (CAR/60DT/2COP) to 99.37% (CCR/70DT/3COP). Assessment of the influence of processing on WHC of CF from the white landrace shows that the least (45.54%) and highest (111.45%) values were exhibited by CAW/50DT/3COP and CCW/70DT/3COP respectively. Agunbiade *et al.* (2017) reported a value of 62.67% for CF which is within the range reported in this study. This value (101%) obtained by Odey and Lee (2020) for non-fermented CF is also similar to the values (41.95 – 111.45%) reported in this study. It was observed that CF pre-treated with calcium chloride had higher WHC than those pre-treated with citric acid for all corresponding experimental runs. A similar trend was observed in the white landrace for most experimental runs between calcium chloride pre-treated flour (CCW) and their corresponding citric acid pre-treated flour (CAW). Higher WHC is attributed to the loose structure of the starch polymer while a low value indicates the compactness of the molecular structure (Onitilo *et al.*, 2007b).

Table 4.2 Mean ANOVA of water holding capacity of cassava flour

			WHC (%)			
Exp Run	DT (ºC)	COP (%w/v)	CAR	CCR	CAW	CCW
1	50	1	$59.18^{c} \pm 6.54$	$66.71^{de} \pm 8.00$	$61.00^{cde} \pm 6.05$	$68.91^{bcd} \pm 3.43$
2	70	1	$74.50^{ab} \pm 1.46$	$75.52^{c} \pm 4.24$	$73.32^{abc} \pm 1.34$	$84.90^{b} \pm 11.72$
3	50	3	$49.59^{cd} \pm 4.43$	$57.16^{f} \pm 4.51$	$45.54^{e} \pm 4.01$	$62.07^{bcd} \pm 9.38$
4	70	3	$62.46^{bc} \pm 14.88$	$99.37^{a} \pm 5.26$	$76.64^{abc} \pm 6.86$	$111.45^{a} \pm 23.18$
5	45	2	$85.97^{a} \pm 9.48$	$94.04^{a} \pm 4.31$	$76.23^{abc} \pm 16.25$	$59.68^{cd} \pm 8.66$
6	74	2	$60.41^{c} \pm 6.32$	$67.31^{cde} \pm 3.06$	$72.93^{abc} \pm 7.19$	$69.10^{bcd} \pm 15.36$
7	60	0.6	$82.00^{a} \pm 8.96$	$98.06^{a} \pm 5.30$	$86.47^{a} \pm 12.30$	$105.75^{a} \pm 16.93$
8	60	3.4	$52.17^{cd} \pm 5.54$	$59.71^{ef} \pm 2.80$	$52.01^{de} \pm 5.85$	$56.45^{d} \pm 2.15$
9	60	2	$41.95^{d} \pm 2.45$	$72.15^{cd} \pm 3.89$	$83.69^{ab} \pm 20.13$	$80.57^{bc} \pm 5.51$
Control	60	0	$85.89^{a} \pm 2.67$	$85.89^{b} \pm 2.67$	$66.37^{bcd} \pm 6.54$	$66.37^{bcd} \pm 6.54$

Exp Run- Experimental run; DT- Drying temperature, COP- Concentration of pre-treatment; WHC- water holding capacity; CAR- cassava flour from red landrace pre-treated with citric acid; CCR- cassava flour from red landrace pre-treated with calcium chloride; CAW- cassava flour from white landrace pre-treated with citric acid; CCW- cassava flour from white landrace pre-treated with calcium chloride Means in the same column with different superscripts are significantly different ($p < 0.05$).

This implies that, in comparison with calcium chloride, citric acid may have a more compacting effect on the molecular structure of starch in the flour samples under the conditions investigated resulting in a decrease in the WHC of the CAR samples. This observation is buttressed by the ANOVA of model parameters about the WHC of the samples (Table 4.3) generated by RSM. In this study, a factor common to CAR and CAW is that the quadratic effect of B^2 (COP) are significant model terms with p-values less than 0.0500. P-values less than 0.0500 indicate model terms are significant. For CCR and CCW all model parameters had p-values higher than 0.05 which infers that those model parameters did not significantly affect the WHC of the samples. The decreasing effect of citric acid and significance of its quadratic effect on the WHC of flour from both landraces is further buttressed by the coefficient of determination (R^2) values shown in Table 4.4 displayed alongside regression models relating WHC and model parameters. The coefficient of determination (R^2) of citric acid-treated experimental group (CAR – 0.7614, CAW – 0.7251) was higher than calcium chloride treated experimental group (CCR – 0.2640, CCW – 0.5274).

Table 4.3 ANOVA of the effect of model parameters on water holding capacity of cassava flour

Source	CAR		CCR		CAW		CCW	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Model	4.47	0.0379*	0.5021	0.7672	3.69	0.0590	1.56	0.2851
A-DT	0.0768	0.7897	0.0941	0.7680	2.27	0.1752	3.60	0.0994
B-COP	4.94	0.0617	0.8587	0.3849	5.61	0.0497*	1.46	0.2668
AB	0.0146	0.9074	1.20	0.3093	1.07	0.3357	1.30	0.2921
A^2	12.19	0.0101*	0.2679	0.6207	3.56	0.1010	1.10	0.3299
B^2	7.28	0.0307*	0.1315	0.7276	7.09	0.0323*	0.2084	0.6618

*- significant at ($p > 0.05$); DT- Drying temperature; B- concentration of pre-treatment; A^2-quadractic effect of DT; B^2-quadractic effect of COP; AB- interactive effect of A and B. ER-Experimental run; DT- Drying temperature; COP- Concentration of pre-treatment; CAR-cassava flour from red landrace pre-treated with citric acid; CCR- cassava flour from red landrace pre-treated with calcium chloride; CAW- cassava flour from white landrace pre-treated with citric acid; CCW- cassava flour from white landrace pre-treated with calcium chloride.

Table 4.4 Regression models relating WHC and model parameters for cassava flour

Experimental group	Models	R^2
CAR	$41.95 – 0.9947A – 7.98B – 0.6125AB + 13.44A^2 + 10. 39B^2$	0.7614
CCR	$72.15 + 1.65A - 4.99B + 8.35AB + 2.99A^2 + 2.10B^2$	0.2640
CAW	$83.69 + 4.84A - 7.61B + 4.70AB -6.50A^2 - 9.17B^2$	0.7251
CCW	$80.57+ 9.84A- 6.25B + 8.35AB- 5.82A^2+ 2.54B^2$	0.5274

R^2- Coefficient of determination; WHC- Water holding capacity; CAR- Cassava flour from red landrace pre-treated with citric acid; CCR- Cassava flour from red landrace pre-treated with calcium chloride; CAW- Cassava flour from white landrace pre-treated with citric acid; CCW- Cassava flour from white landrace pre-treated with calcium chloride.

Water holding capacity of flour is a useful indicator of whether the protein can be incorporated within aqueous food formulations, especially those involving dough handling (Osungbaro *et al.*, 2010). Relatively high WHC allows for flour to absorb water and swell for improved consistency in food. It is an advantageous baking quality because it allows bakers to add more water to dough, resulting in improved handling characteristics and maintenance of bread freshness (Peluola-Adeyemi *et al.*, 2019). Conversely, lower water absorption capacity would be desirable formulating thinner porridges for infants (Msheliza *et al.*, 2018). Hence, optimisation was conducted to minimise and maximise the WHC within the experimental groups. Optimal processing conditions and WHC of the flours as generated by the Design-Expert software are shown in Table 4.5.

4.3.2 Effect of pre-treatment and drying temperature on bulk densities of cassava flour

Bulk density is an important property that has a direct impact on the packaging requirement of flours (Msheliza *et al.*, 2018). The LBD ranged between 0.34 – 0.41 g/cm^3 (CAR), 0.37 - 0.45 g/cm^3 (CCR), 0.35 – 0.43 g/cm^3 (CAW) and 0.37 – 0.44 g/cm^3 (CCW) respectively. Chisenga *et al.* (2019a) reported a similar range of 0.40 to 0.47 g/cm^3. The flours from the red cassava landrace exhibited a similar trend as in WHC where the LBD of all the CCR samples was higher than the CAR under the same drying temperature and concentration of pre-treatment. This was also observed for most CCW samples in comparison with their corresponding CAW samples. This suggests that the BD and WHC of the flours under this processing condition may positively correlate. It was also observed that the least LBD for all the experimental groups was exhibited at 45DT/2COP) while the highest LBD was exhibited at 70DT/3COP for CAR and 74DT/2COP for CCR, CAW and CCW.

Table 4.5 Processing conditions for optimal water holding capacity of cassava flour

Desired level	Predicted value (%)	COP (%w/v)	DT (ºC)	Desirability
Cassava flour from red landrace pre-treated with citric acid (CAR)				
Minimum	41.411	2.081	59.712	1.000
Maximum	74.144	1.000	50.000	0.731
Cassava flour from red landrace pre-treated with calcium chloride (CCR)				
Minimum	62.241	3.000	50.000	0.880
Maximum	88.925	1.000	50.000	0.753
Cassava flour from white landrace pre-treated with citric acid (CAW)				
Minimum	50.869	3.000	50.000	0.870
Maximum	85.624	1.648	62.457	0.979
Cassava flour from white landrace pre-treated with calcium chloride (CCW)				
Minimum	52.853	3.000	50.000	0.821
Maximum	86.721	1.100	67.500	1.000

COP - Concentration of pre-treatment; DT - Drying temperature; WHC - Water holding capacity

It can be observed that the least LBD was below the lower limit at drying temperature (45°C) while the highest LBDs were at the other extreme, 70 and 74°C. This infers that drying temperature influences the bulk density of the flours. As seen in the response contour plots for LBD of CAR, CCR, CAW and CCW (Figure 4.2), LBD increased along the X1-DT axis for cassava flour from the red landrace irrespective of pre-treatment type. For the white landrace CCW and CAW, an initial increase and decrease were observed along the X1-DT axis. This finding is consistent with Taiwo *et al.* (2016) who reported that fermented CF from cassava chips dried at 70°C in the oven had higher bulk densities than fermented CF from sundried cassava chips. Taiwo *et al.* (2016) also stated that temperatures at which the chips were dried significantly ($p < 0.05$) influenced the BD of their respective flours.

It is expected that the PBD will be higher than the LBD due to the compacting effect of tapping on the flour particles. The PBD of CF pre-treated with calcium chloride and citric acid were similar with the least and highest PBD of 0.62 and 0.73 g/mL (Figure 4.3). The values of LBD (0.51-0.54 g/mL) and PBD (0.58-0.71 g/mL) of CF reported by Falade *et al.* (2019) is similar in range to the obtained results reported in this study.

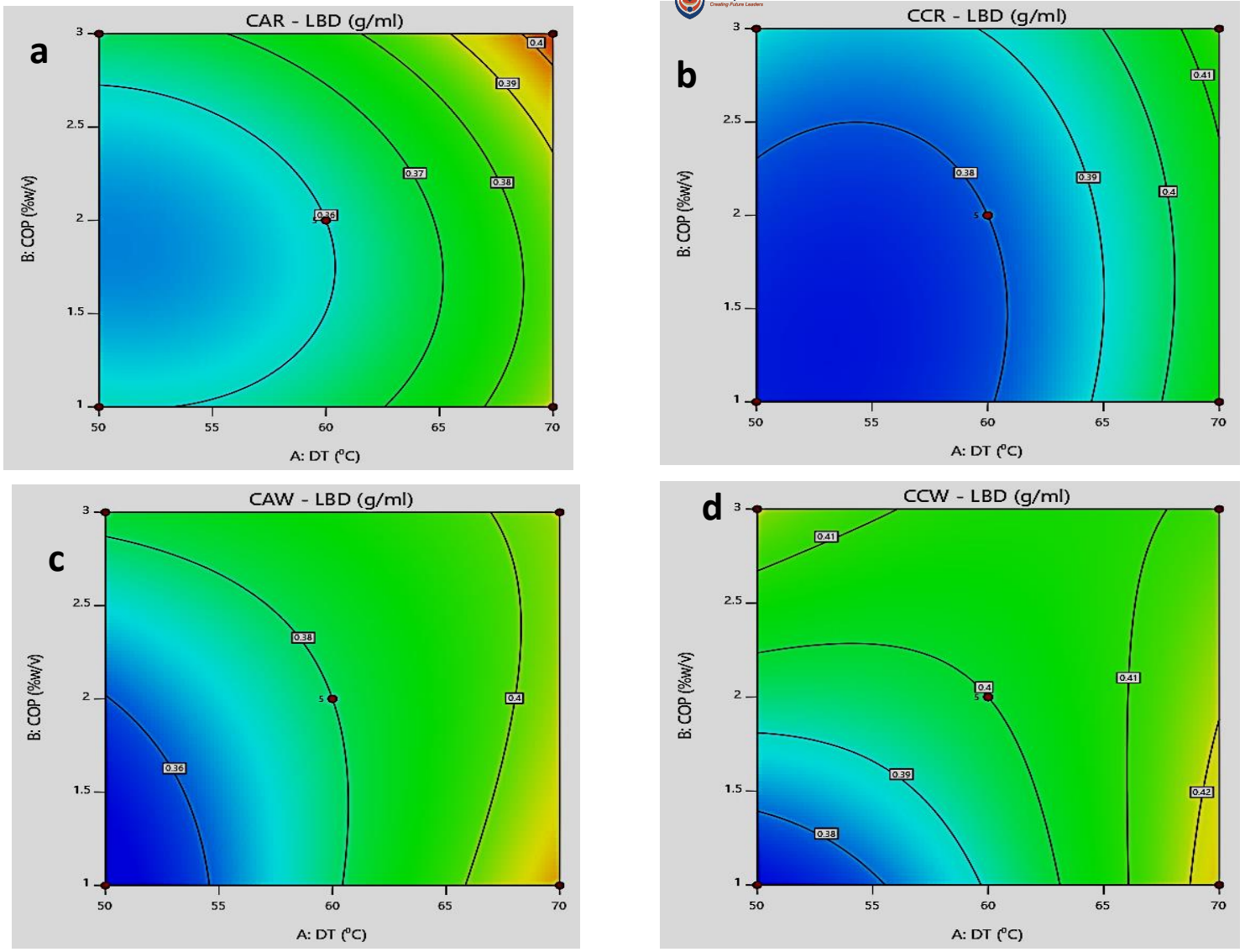

Figure 4.2. Contour response surface plots for effect of process variables on loose bulk densities of cassava flour. **a** = CAR - cassava flour from red landrace pre-treated with citric acid; **b** = CCR - cassava flour from red landrace pre-treated with calcium chloride; **c** = CAW - cassava flour from white landrace pre-treated with citric acid; **d** = CCW - cassava flour from white landrace pre-treated with calcium chloride.

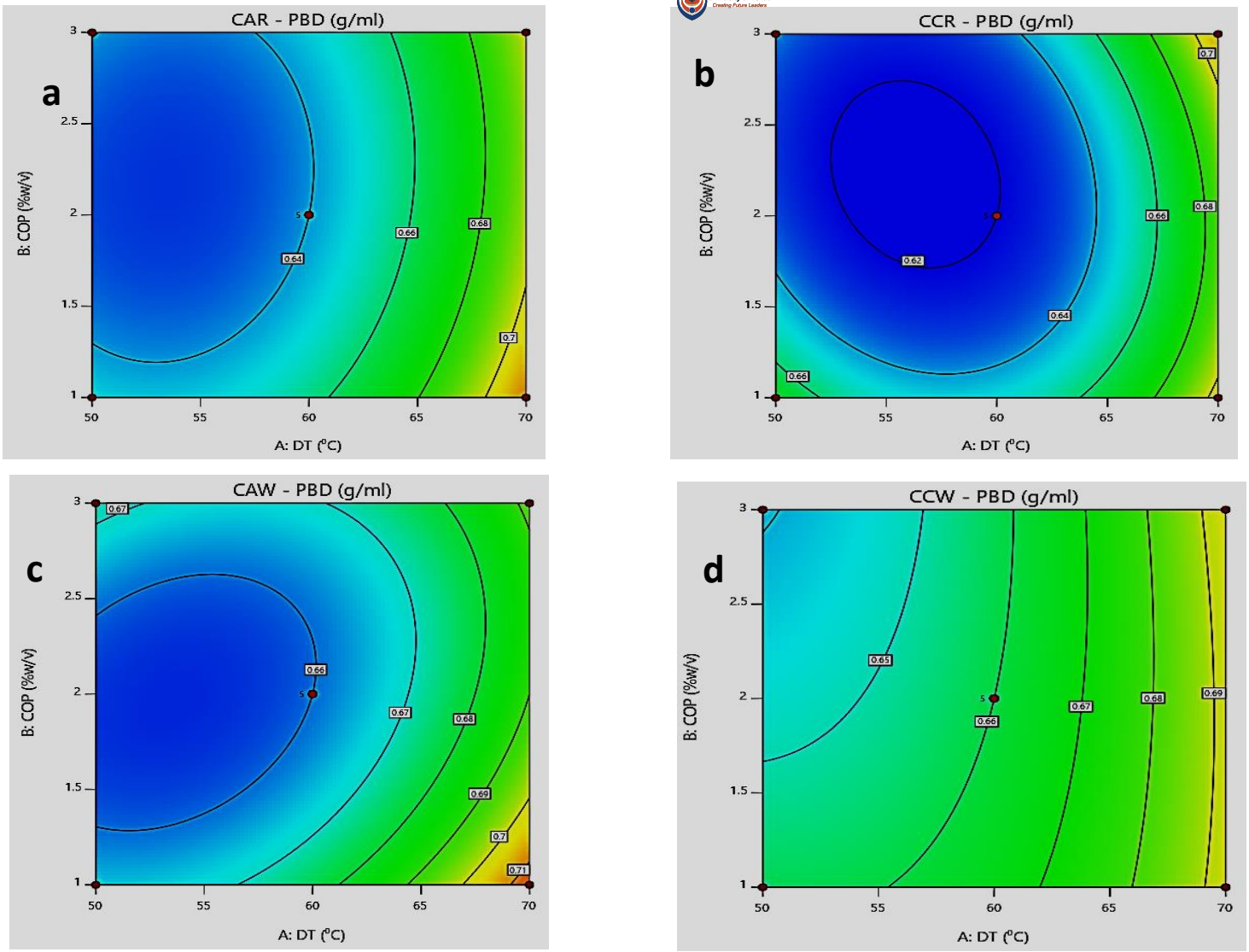

Figure 4.3. Contour response surface plots for effect of process variables on packed bulk densities of cassava flour. **a** = CAR - cassava flour from red landrace pre-treated with citric acid; **b** = CCR - cassava flour from red landrace pre-treated with calcium chloride; **c** = CAW - cassava flour from white landrace pre-treated with citric acid; **d** = CCW- cassava flour from white landrace pre-treated with calcium chloride.

Atlaw (2018) recorded similar values (0.49-0.58 g/ml) for PBD of CF dried using different methods. Figure 4.4 shows the contour response plots for the difference between LBD and PBD (ΔBD), which was determined to give a clue on the average particle size of the flour samples. The difference in the BD for CAR (0.26-0.34 g/mL) was slightly higher than that of CCR (0.24 - 0.30 g/ml) which may suggest that CAR has smaller particle sizes than CCR. The decrease in particle size of flour results in an increase of WHC and swelling power (Rao *et al.*, 2016). Bulk density of flour is important not only for packaging but also for product development. Flowability, compressibility and fluidity of a material are directly influenced by the BD (Falade *et al.*, 2019). High BD indicates higher ease of dispersibility which is a vital parameter in dough formation and infant food formulation (Atlaw, 2018). Contour response surface plots generated by RSM aid visualisation of changes in the response variable of the experimental factors. The patterns of the contour plots were different in all experimental groups. This suggests that the landrace and pre-treatment type influenced the BD of CF. This is buttressed by the variation in the ANOVA for model parameters (Table 4.6) and a relatively high coefficient of determination values (0.6042 – 0.9458) shown in Table 4.7.

4.3.3 Effect of pre-treatment and drying temperature on thermal properties of cassava flour

Gelatinisation is an irreversible change that occurs when starch is heated in water, characterised by gelatinisation temperatures and enthalpy, which can be determined using the differential scanning calorimetry (Zhu, 2015). Onset gelatinisation temperature (To) of 60.32 to 120.30ºC; peak gelatinisation temperature (Tp) of 71.85 to 126.84ºC and conclusion gelatinisation temperature (Tc) of 93.31 to 140.98ºC was recorded for all CF samples. The response plots for thermal properties of CF processed from the red and white cassava landraces are shown in Figures 4.5 and 4.6 respectively. Cassava flour from the red landrace pre-treated with citric acid (CAR) shows that gelatinisation temperatures increased with drying temperature and concentration of pre-treatment. As for CCR, the gelatinisation temperatures decrease and increase along both axis of the independent variables. The peak and conclusion gelatinisation temperatures of CF from the white landrace pre-treated with citric acid increased with an increase in COP and no increase with DT. It could be observed that gelatinisation temperature of CCW increased with concentration of pre-treatment. Variation in the trend displayed by the experimental groups for gelatinisation temperatures implies that the type of landrace and processing variables influenced the thermal properties of the flours.

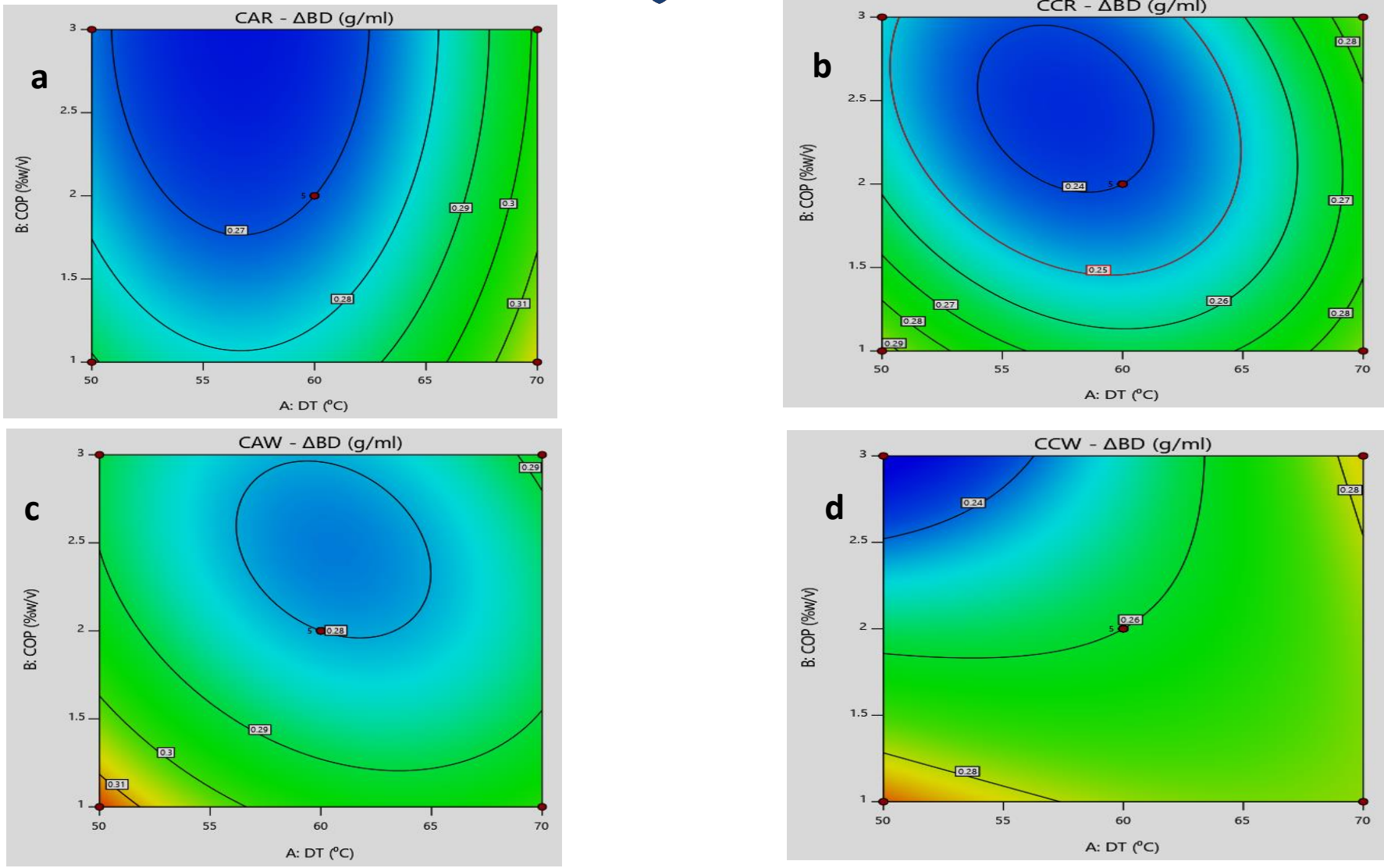

Figure 4.4. Contour response surface plots for effect of process variables on difference in bulk density (ΔBD) of cassava flour; **a** = CAR - cassava flour from red landrace pre-treated with citric acid; **b** = CCR - cassava flour from red landrace pre-treated with calcium chloride; **c** = CAW - cassava flour from white landrace pre-treated with citric acid; **d** = CCW - cassava flour from white landrace pre-treated with calcium chloride.

Table 4.6 ANOVA of the effect of model parameters on bulk densities of cassava flour

Source	CAR		CCR		CAW		CCW	
	F - value	P – value	F - value	P - value	F- value	P - value	F - value	P - value
				Loose bulk density				
Model	8.40	0.01*	2.99	0.09	13.30	<0.01*	2.30	0.15
A-DT	25.13	0.001*	8.50	0.02*	52.03	<0.01*	4.62	0.07
B-COP	2.69	0.14	1.09	0.33	4.26	0.08	2.20	0.18
AB	0.28	0.62	0.11	0.75	7.76	0.03*	3.40	0.11
A^2	5.89	0.05*	4.87	0.06	0.54	0.49	1.24	0.30
B^2	9.73	0.02*	0.78	0.41	2.16	0.19	0.01	0.92
				Packed bulk density				
Model	6.75	0.01*	16.14	<0.01*	11.48	<0.01*	3.66	0.06
A-DT	21.23	<0.01*	23.91	<0.01*	27.58	<0.01*	15.14	0.01*
B-COP	0.65	0.45	1.35	0.28	1.37	0.28	0.54	0.49
AB	0.07	0.80	1.68	0.24	2.73	0.14	0.37	0.56
A^2	10.54	0.01	45.41	<0.01*	14.54	0.01*	2.25	0.18
B^2	2.37	0.17	14.01	0.01*	14.54	0.01*	0.09	0.77
				Difference in bulk density (g/ml)				
Model	4.52	0.04*	4.12	0.04*	2.14	0.18	24.45	<0.01*
A-DT	6.69	0.04*	1.45	0.27	0.59	0.47	28.11	<0.01*
B-COP	2.71	0.14	2.40	0.17	2.90	0.13	47.41	<0.01*
AB	3.42	1.00	1.07	0.34	0.59	0.47	36.76	<0.01*
A^2	13.11	<0.01*	13.42	0.01*	4.68	0.07	8.15	0.02*
B^2	0.52	0.49*	3.84	0.09	2.74	0.14	2.94	0.13

*- significant at ($p > 0.05$); DT - Drying temperature; COP - concentration of pre-treatment; A^2 -quadractic effect of DT; B^2 - quadractic effect of COP; AB - interactive effect of A and B; CAR - Cassava flour from red landrace pre-treated with citric acid; CCR - Cassava flour from red landrace pre-treated with calcium chloride; CAW - Cassava flour from white landrace pre-treated with citric acid; CCW - Cassava flour from white landrace pre-treated with calcium chloride.

Table 4.7 Regression models relating bulk densities and model parameters for cassava flour

Response	Models	R^2
	Cassava flour from red landrace pre-treated with citric acid	
LBD	$+0.3600 + 0.0169A + 0.0055B + 0.0025AB + 0.0087A^2 + 0.0113B^2$	0.8571
PBD	$+0.6400 + 0.0314A - 0.0055B - 0.0025AB + 0.0237A^2 + 0.0113B^2$	0.8283
ΔBD	$+0.2700 + 0.0146A – 0.0093B + 0.0000AB + 0.0219A^2 + 0.0044B^2$	0.7633
	Cassava flour from red landrace pre-treated with calcium chloride	
LBD	$+0.3800 + 0.0154A + 0.0055B – 0.0025AB + 0.0125A^2 + 0.0050B^2$	0.6809
PBD	$+0.6200 + 0.0266A – 0.0063B + 0.0100AB + 0.0394A^2 + 0.0219B^2$	0.9202
ΔBD	$+0.2400 + 0.0082A – 0.0106B + 0.0100AB + 0.0269A^2 + 0.0144B^2$	0.7462
	Cassava flour from white landrace pre-treated with citric acid	
LBD	$+0.3800 + 0.0229A + 0.0066B – 0.0125AB + 0.0025A^2 + 0.0050B^2$	0.9048
PBD	$+0.6600 + 0.0169A – 0.0038B – 0.0075AB + 0.0131A^2 + 0.0131B^2$	0.8913
ΔBD	$+0.2800 – 0.0035A – 0.0078B + 0.0050AB + 0.0106A^2 + 0.0081B^2$	0.6042
	Cassava flour from white landrace pre-treated with calcium chloride	
LBD	$+0.4000 +0.0124A + 0.0085B – 0.0150AB + 0.0069A^2 - 0.0006B^2$	0.6221
PBD	$+0.6600 + 0.0227A – 0.0043B + 0.0050AB + 0.0094A^2 + 0.0019B^2$	0.7233
ΔBD	$+0.2600 + 0.0108A – 0.0141B + 0.0175AB + 0.0063A^2 + 0.0038B^2$	0.9458

R^2 - Coefficient of determination; LBD - loose bulk density; PBD - packed bulk density; ΔBD - difference in bulk density;

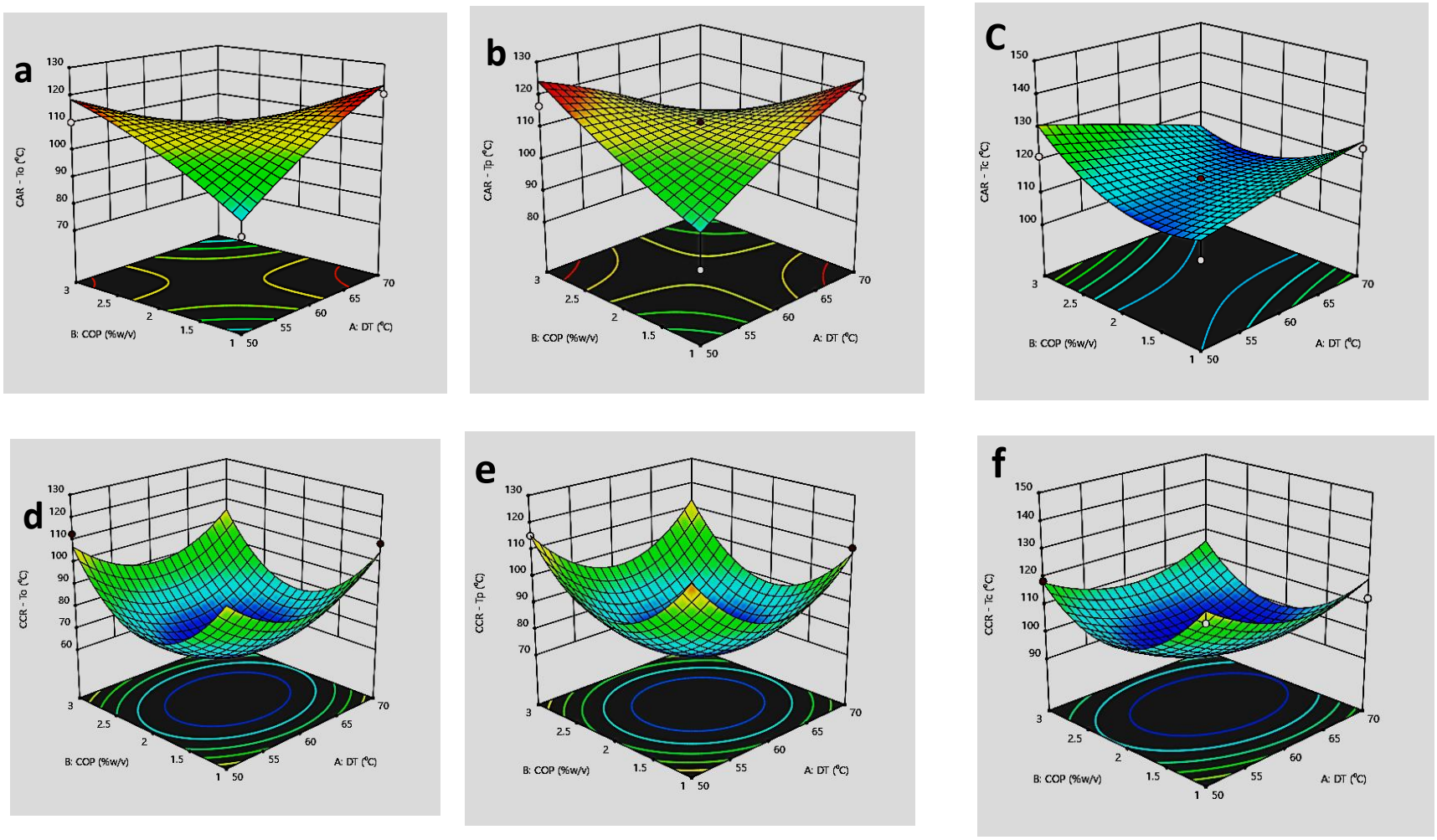

Figure 4.5. Response surface plots for thermal properties of cassava flour from red landrace; DT- drying temperature, COP- Concentration of pre-treatment; CAR – Citric acid pre-treated cassava flour from red landrace; CCR – Calcium chloride pre-treated cassava flour from red landrace; **a** – Onset gelatinisation temperature of CAR; **b** – Peak gelatinisation temperature of CAR; **c** – Conclusion gelatinisation temperature of CAR; **d** - Onset gelatinisation temperature of CCR; **e** – Peak gelatinisation temperature of CCR; **f** – Conclusion gelatinisation temperature of CCR..

The differences in gelatinisation could be ascribed to variations in non-starch content (Chisenga *et al.*, 2019a). Gelatinisation enthalpy (ΔH) is associated with the energy required for breaking double helices in starch granules. It reflects the loss of double-helical order or overall crystallinity of starch (Oyeyinka *et al.*, 2019) in the flour. Gelatinisation enthalpy (ΔH) within the range of 0.14 to 54.95 J/g was recorded for all the samples in this study. Wide variations were observed in the endothermic enthalpies of the samples within each experimental group (CAR, CCR, CAW, CCW). For example, ANOVA using Duncan's Multiple Test to identify the significant difference between the experimental runs in CAW (Table 4.8) produced 10 homogenous subsets with each experimental run significantly different ($p < 0.05$) from the other. It is also interesting to note that in most groups, the control exhibited low values of enthalpy. This indicates that the processing conditions have an increasing effect on the enthalpy of the starch granules in the flour. As reported by Huang *et al.* (2008) high endothermic value, appears around 100ºC, the boiling point of water, and presumably reflects the vaporization of water in the starch sample and the destruction of the crystalline structure between water and starch. Effective moisture diffusivity, thermal conductivity and heat capacity of CF increases with temperature (Sanni *et al.*, 2016). Table 4.9 are regression models relating thermal properties to drying temperature and concentration of pre-treatment. The equation in terms of coded factors (A- DT and B- COP) can be used for predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The coefficient of determination (R^2) obtained was relatively high.

The values of gelatinisation temperatures reported (Zhang *et al.*, 2013; Wongsagonsup *et al.*, 2014; Hong *et al.*, 2016; dos Santos *et al.*, 2019; Zhao and Saldaña, 2019) for cassava starch were below the values of this study. Although CF consists mostly of starch, their gelatinisation temperatures (onset, peak and conclusion) were higher than that for cassava starch reported by Charoenkul *et al.* (2011). Relatively high values of To (93.68-114.21ºC), Tp (100.35-118.49ºC), Tc (108.73-123.51ºC) and ΔH (3.74-11.54 J/g) reported by Omolola *et al.* (2017) for cassava flour not chemically pre-treated are within the range obtained in this study. Omolola *et al.* (2017) studied the influence of drying time (15-20 h) and temperature (60-70ºC), using response surface methodology, on the thermal properties of CF. The authors reported that the drying conditions investigated did not influence the thermal properties of cassava flour. Non-starch components in CF such as fat could be responsible for high gelatinisation temperatures and enthalpy. Lipids may affect diffusion of water into the starch granules, and their presence on starch granules was demonstrated to retard gelatinisation.

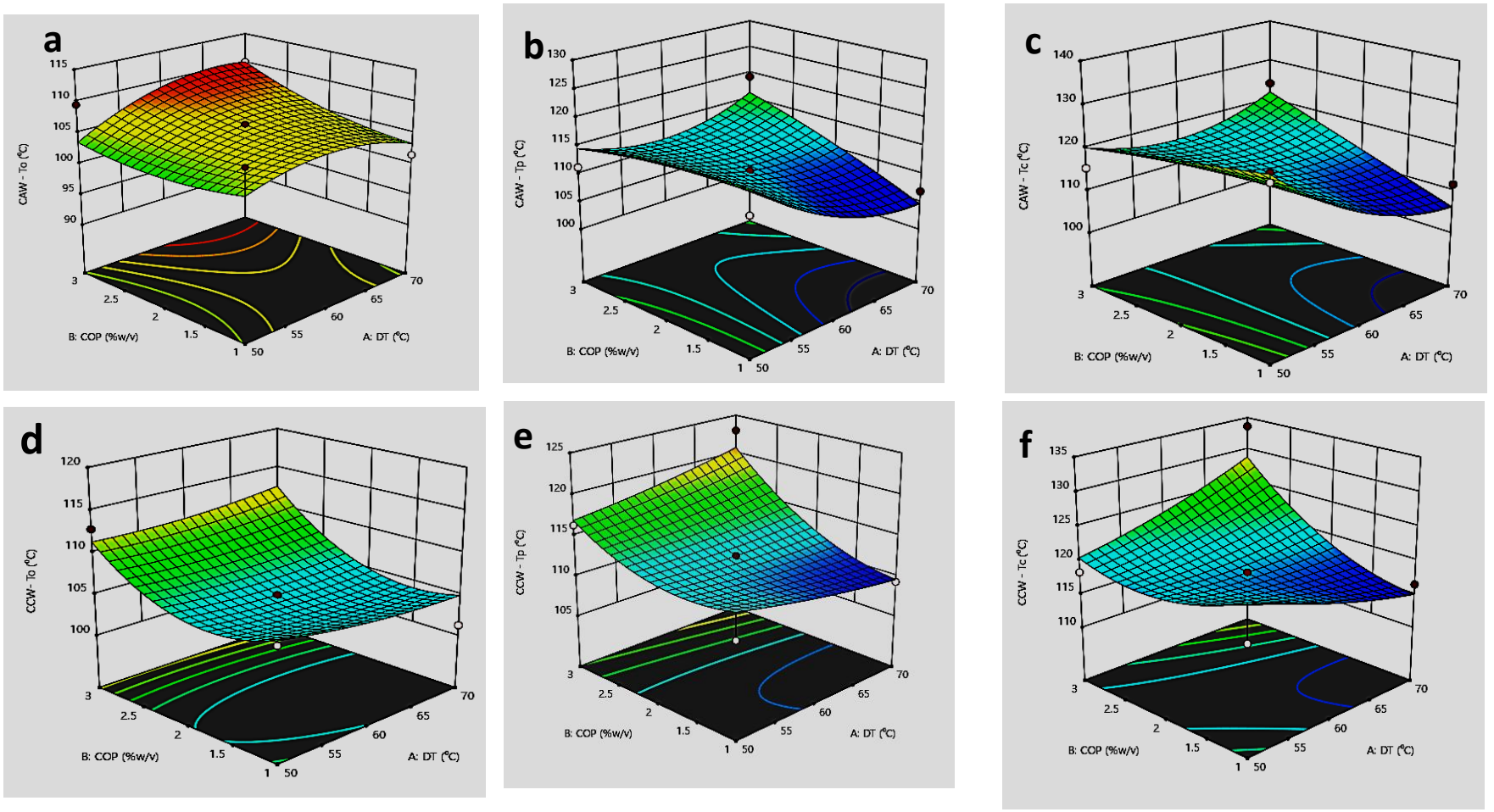

Figure 4.6. Response surface plots for thermal properties of cassava flour from white cassava landrace; **a** – Onset gelatinisation temperature of CAW; **b** – Peak gelatinisation temperature of CAW; **c** – Conclusion gelatinisation temperature of CAW; **d** - Onset gelatinisation temperature of CCW; **e** – Peak gelatinisation temperature of CCW; **f** – Conclusion gelatinisation temperature of CCW;DT- drying temperature, COP- Concentration of pre-treatment.

Table 4.8 Gelatinisation enthalpy of pre-treated flour from red and white cassava landrace

Independent variables			Gelatinisation enthalpy			
ER	DT (ºC)	COP (%w/v)	CAR	CCR	CAW	CCW
1	50	1	$0.44^{h} \pm 0.04$	$0.94^{e} \pm 0.04$	$39.64^{d} \pm 0.55$	$0.89^{g} \pm 0.03$
2	70	1	$1.07^{g} \pm 0.03$	$1.11^{e} \pm 0.01$	$4.51^{i} \pm 0.02$	$8.35^{e} \pm 0.31$
3	50	3	$7.33^{e} \pm 0.03$	$1.95^{e} \pm 0.05$	$17.34^{g} \pm 0.29$	$1.59^{f} \pm 0.08$
4	70	3	$6.16^{f} \pm 0.14$	$1.27^{e} \pm 0.03$	$15.58^{h} \pm 0.07$	$1.18^{fg} \pm 0.02$
5	45	2	$42.12^{a} \pm 0.10$	$33.38^{b} \pm 0.33$	$32.64^{e} \pm 0.04$	$24.54^{d} \pm 0.51$
6	74	2	$17.80^{d} \pm 0.26$	$54.95^{a} \pm 2.62$	$21.14^{f} \pm 0.04$	$43.36^{c} \pm 0.53$
7	60	0.6	$23.49^{c} \pm 0.42$	$17.43^{c} \pm 0.51$	$42.35^{c} \pm 0.30$	$47.42^{b} \pm 0.36$
8	60	3.4	$38.33^{b} \pm 0.16$	$6.35^{d} \pm 0.30$	$46.88^{b} \pm 0.10$	$1.60^{f} \pm 0.10$
9	60	2	$1.01^{g} \pm 0.02$	$1.62^{e} \pm 0.11$	$52.07^{a} \pm 0.06$	$49.79^{a} \pm 0.09$
10	60	0	$1.22^{g} \pm 0.02$	$1.22^{e} \pm 0.02$	$0.14^{j} \pm 0.02$	$0.15^{h} \pm 0.01$

ER – experimental run; DT - Drying temperature; COP - concentration of pre-treatment; CAR - Cassava flour from red landrace pre-treated with citric acid; CCR - Cassava flour from red landrace pre-treated with calcium chloride; CAW - Cassava flour from white landrace pre-treated with citric acid; CCW - Cassava flour from white landrace pre-treated with calcium chloride; WHC – Water holding capacity.

Table 4.9 Regression models relating thermal properties and model parameters of cassava flour

Response variables	Models	R^2
	Cassava flour from red landrace pre-treated with citric acid	
To (ºC)	$+108.95+0.2174A-1.67B-16.76AB-2.40A^2-2.88B^2$	0.8079
Tp (ºC)	$+111.48+0.5867A+0.1812B-13.70AB-1.80A^2+1.24B^2$	0.6513
Tc (ºC)	$+114.36-1.11A+1.34B-6.54AB-0.3350A^2+7.89B^2$	0.6572
ΔH (J/g)	$+1.01-4.37A+4.12B-0.4508AB+7.80A^2+8.28B^2$	0.4077
	Cassava flour from red landrace pre-treated with calcium chloride	
To (ºC)	$+60.32-0.5113A+0.7556B-0.4050AB+16.87A^2+28.40B^2$	0.9166
Tp (ºC)	$+71.85-2.66A+0.7340B+1.63AB+19.44A^2+22.43B^2$	0.9759
Tc (ºC)	$+93.31-2.87A-3.52B+1.72AB+9.16A^2+18.13B^2$	0.9508
ΔH (J/g)	$+1.62+3.75A-1.81B-0.2125AB+14.59A^2-1.54B^2$	0.5186
	Cassava flour from white landrace pre-treated with citric acid	
To (ºC)	$+106.39+1.54A+1.64B+1.76AB-2.49A^2+1.32B^2$	0.5082
Tp (ºC)	$+110.61-2.94A+2.03B+3.74AB+3.48A^2-0.7146B^2$	0.7433
Tc (ºC)	$+114.23-4.87A+2.14B+5.82AB+4.84A^2-0.0408B^2$	0.8158
ΔH (J/g)	$+52.07-6.64A-0.6018B+8.34AB-16.71A^2-7.85B^2$	0.8153
	Cassava flour from white landrace pre-treated with calcium chloride	
To (ºC)	$+104.95-0.2586A+2.95B+0.8725AB+0.5538A^2+3.50B^2$	0.8062
Tp (ºC)	$+112.55+0.0563A+3.73B+1.85AB+0.3300A^2+2.23B^2$	0.8355
Tc (ºC)	$+118.25+0.0776A+2.81B+4.09AB+0.7594A^2+2.80B^2$	0.6043
ΔH (J/g)	$+49.79+4.21A-8.91B-1.97AB-14.48A^2-19.20B^2$	0.7027

To - Onset gelatinisation temperature, Tp - Peak gelatinisation temperature, Tc - Conclusion gelatinisation temperature, ΔH - gelatinisation enthalpy

Li *et al.* (2016) reported that defatting starch results in decreased gelatinisation temperatures which agrees with Charles *et al.* (2005) who postulated that the presence of amylose–lipid complex inhibits gelatinisation of starch granules. Zhao and Saldaña (2019) reported higher onset, peak and conclusion gelatinisation temperatures for cereal and potato starches which contain a high amount of lipids while cassava starches exhibited lower onset gelatinisation temperatures. The samples in this study contained non-starch components and were not defatted. High gelatinisation temperatures were associated with a high degree of crystallinity (Singh *et al.*, 2010), which relates to starch granule structural stability, thus making it resistant to heating. The X-ray diffractometry (XRD) studies for the flours from the red and white cassava landraces (Figure 3.4) indicate that they exhibit an A-type XRD pattern with a high degree of crystallinity which could be responsible for the high gelatinisation temperatures. High crystalline region tends to increase the gelatinization temperature in flour because it interrupts water penetration within starch molecules (Alcázar-Alay and Meireles, 2015). A-type starch has high gelatinisation temperatures when compared to the B and C type of starch (Guo *et al.*, 2020).

Optimum processing conditions generated by the design expert software are displayed graphically as overlay plots (Figure 4.7). The design space is used in determining the optimum variable values for the target goals. The starch of low gelatinisation temperature is cost-effective in industrial food production as it limits energy expenses (Ayetigbo *et al.*, 2018). Conversely, flour with higher gelatinisation temperature is more stable and resistant to heat in the presence of water which may also be desirable in some food formulations. However, the thermal properties of samples used in this study were relatively high, compared to literature, therefore, optimisation goal was to minimise their thermal properties. Multi-response optimisation was thus carried out for all the experimental groups and the optimum values of To, Tp, Tc and ΔH at the various optimised drying conditions are depicted in their respective overlay plots (Figure 4.7). The optimal processing conditions for minimised gelatinisation temperatures and enthalpy are 50DT/1COP with a desirability of 0.805 for CAR, 57.23DT/1.75COP with 0.841 desirability for CCR, 70DT/1COP with 0.754 desirability for CAW and 50DT/1COP with 0.732 desirability for CCW.

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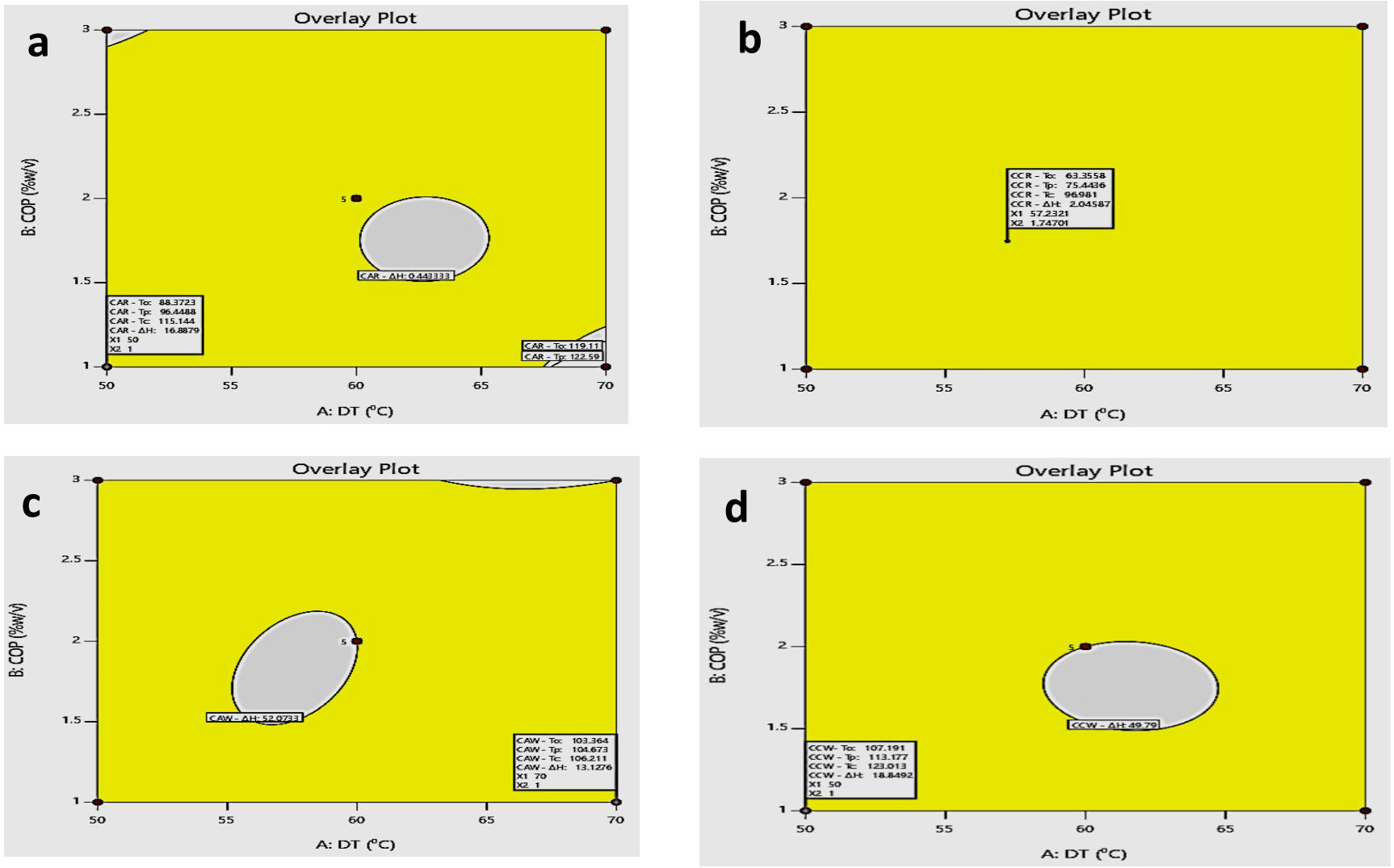

Figure 4.7. Multi-response optimisation overlay plots of processing conditions and thermal properties of cassava flour; DT- drying temperature, COP- Concentration of pre-treatment. **a-** cassava flour from red landrace pre-treated with citric acid;**b** - cassava flour from red landrace pre-treated with calcium chloride; **c** - cassava flour from white landrace pre-treated with citric acid; **d** - cassava flour from white landrace pre-treated with calcium chloride.

4.4 Conclusion

This study shows that pre-treatment of cassava flour with citric acid and calcium chloride influences the bulk density, water holding capacity and thermal properties of the flour. The pre-treatment causes an increase in the amount of energy required for gelatinisation to occur. Higher R^2 values were recorded for water holding capacity of citric acid pre-treatment cassava flour when compared to calcium chloride. This was consistent with the significant influence of the quadratic effect of citric acid pre-treatment on water holding capacity of cassava flour. The drying temperature influences the bulk density of cassava flour with an increase in loose bulk density as temperature increased. Results obtained in this study, using response surface methodology, provide useful information for cassava flour processing and product development.

CHAPTER FIVE: INTERACTIVE EFFECTS OF CHEMICAL PRE-TREATMENT AND DRYING ON THE PHYSICOCHEMICAL PROPERTIES OF CASSAVA FLOUR USING RESPONSE SURFACE METHODOLOGY

Abstract

Cassava flour processing varies and the quality of flour is determined by the process it undergoes. With the aid of response surface methodology (RSM), calcium chloride and citric acid (0.6 – 3.4%w/v) were separately applied in the pre-treatment of two South African cassava landraces (white and red) processed to flour at drying temperatures of 45 – 74ºC. A colorimeter and near-infrared analyser (NIR) were employed in determination of colour properties and proximate components of the flour respectively. Optimisation using RSM showed that ash (0.79 - 4.42%) and crude fibre (2.77 - 5.12%) increased as the drying temperature (DT) and concentration of pre-treatment (COP) increased. The starch content (78.06 - 84.71%) was not influenced by the processing variables. Optimal processing conditions of 70ºC DT and 3%w/v COP were the same for proximate composition of cassava flour from all experimental groups. Both pre-treatments improved the lightness and whiteness index of cassava flour. Enhanced whiteness is an advantageous consumer property especially for substitution of wheat flour in production of baked food products.

Keywords: Cassava; flour; optimisation; pre-treatment; drying; colour properties

5.1 Introduction

The world's most important staple root crop is cassava (*Manihot esculenta* Crantz), also known as tapioca or manioc (FAO, 2013). It is a woody shrub belonging to the family *Euphorbiaceae*. The tuberous roots, rich in starch, are the main storage organ in cassava and the major part of the plant that is mostly consumed (Aloys and Ming, 2006; Montagnac *et al.*, 2009a; Maieves *et al.*, 2012). Approximately one billion people rely on cassava for food daily, predominantly in the tropical and subtropical regions of Latin America, Asia and Africa (Brown *et al.*, 2016). A predominant setback to cassava's utilisation is the high perishability of the root as a result of rapid postharvest physiological deterioration (PPD) (Atieno *et al.*, 2018; Liu *et al.*, 2019). PPD is a complex physiological and biochemical process that begins with vascular streaking characterised by blue-black discoloration ensued by microbial activity that results in total deterioration of the root (Salcedo and Siritunga, 2011). Postharvest physiological deterioration of cassava reduces the shelf-life and degenerates the quality of the root during handling and processing (Sowmyapriya *et al.*, 2017). Due to rapid PPD, the roots cannot be

stored for more than a few days after harvest hence the roots are rapidly processed to stable products (Udoro *et al.*, 2008) such as cassava flour.

Cassava flour is one major product of the root that has found various applications in both domestic and industrial fronts. It is a dry powdery product obtained from the root with simple process technology (Cazumba da Silva *et al.*, 2017). Cassava flour processing varies and the quality of flour is determined by the process it undergoes (Udoro and Anyasi, 2018). Factors such as the variety of the root (Charoenkul *et al.*, 2011; Chiwona-Karltun *et al.*, 2015a; Falade *et al.*, 2019), drying methods (Agbemafle, 2019), drying temperatures (Omolola *et al.*, 2017), fermentation type and duration (Zullaikah *et al.*, 2015; Nkoudou and Essia, 2017; Odey and Lee, 2020), roasting (Eduardo *et al.*, 2013), pre-gelatinisation (Murayama *et al.*, 2014), milling and sieving (Oladunmoye *et al.,* 2010b; Adesina and Bolaji, 2013) and packaging materials (Opara *et al.*, 2016) have been reported to influence the physical and chemical properties of cassava flour. The use of chemicals regarded as safe, such as calcium chloride, citric acid and ascorbic acid in food processing to control enzymatic browning and discoloration is common practice. There is available literature on the use of ascorbic and citric acid in treatment of white yam flour (Akubor, 2013**);** sulphite, calcium chloride and citric acid treatment on potato flour (Ahmed *et al.*, 2010a,b); and calcium chloride treatment for the control of enzymatic browning of minimally processed cassava chips (Medeiros, 2009; Coelho *et al.* 2017, 2019). But there is sparse knowledge in literature on the use of safe chemical pre-treatment in the processing of cassava flour.

Response surface methodology (RSM) is a mathematical and statistical technique used for defining the effect of more than one processing variable on a response (parameter) of interest. Processing cassava flour involves several units which can be varied. RSM was employed to determine the effect of drying temperature and time on thermal and physical properties of cassava flour (Omolola *et al.*, 2017). Drying behaviour of cassava chips using two cutting shapes (rectangular and circular) evaluated under different temperatures (60, 80, 100 and 120°C) was carried out by Pornpraipech *et al.* (2017). Presently, there exists no information on the interactive effects of drying temperature and chemical pre-treatment on cassava flour using RSM. In this study, citric acid and calcium chloride (0.6 - 3.4 %w/v) are separately applied in pre-treatment of flour from two South African cassava landraces (white and red) under drying temperature (45 - 74ºC). RSM is employed to determine the linear, interactive and quadratic effect of these processing variables on the colour properties (*L**, *a**, *b**, chroma, colour difference, whiteness and brownness index) and proximate components (starch, ash, moisture and fibre content) of cassava flour.

5.2 Materials and Methods

5.2.1 Sourcing of cassava root and processing of pre-treated flour

Two landraces of cassava (white and red) were sourced from the Institute of Tropical and Subtropical Crops - Agricultural research council (ITSC-ARC) Levubu, Limpopo Province, South Africa (22.946º S 30.485º E). The roots were harvested fourteen months after planting and sorted before washing with tap water generously to remove adhering dirt and avoid contamination during processing. Within 24 h after harvest, processing commenced ascertaining that the roots were in a fresh state, before the onset of deterioration (Coelho *et al.*, 2019).

The method described by the Federal Institute of Industrial Research (2005) was employed, with modifications, in processing the flour. Modifications involved the application of different concentration of chemical pre-treatment and drying temperatures for each experimental sample as generated by the central composite design. The washed roots were manually peeled and cut into small slices with a knife. Fresh cassava root slices (1 ± 0.5 cm thickness) were pre-treated by steeping them in calcium chloride and citric acid solution (0.6 – 3.4%w/v) separately for about 20 min. The pre-treated chips were dried in the oven (Ecotherm, 240L digital oven, South Africa) at different temperatures (45-74ºC) for 24 h. Milling of dried chips was done with the aid of a dry milling machine (Polymix PX-MFC 90D, Switzerland). Flour obtained from the miller was subjected to sieving through a 500 µm sized aperture sieve and stored, till further analysis, in sample bags made of paper material at room temperature. Two control samples were processed, one from each landrace, using same procedure as the experimental samples save for pre-treatment and DT was 60ºC. The control samples served as reference samples to experimental samples, during statistical analysis within a landrace.

5.2.2 Design of experiment

Before the conduct of the experiment, Design Expert (DE) software Version 11 generated the experimental conditions for processing the flour samples. The conduct of the experiment was done using the central composite design with two independent variables: concentration of pre-treatment (COP) and drying temperature (DT). Two pre-treatments, citric acid (CA) and calcium chloride (CC) were used separately with the same variation in DT giving rise to four experimental groups. The four experimental groups are; citric acid pre-treated flour from the red landrace (CAR), calcium chloride pre-treated flour from the red landrace (CCR), citric acid pre-treated flour from the white landrace (CAW) and calcium chloride pre-treated

flour from the white landrace (CCW). The dependent variables are; proximate components (starch, ash, moisture and fibre content) and colour properties (*L**, *a**, *b**, chroma, colour difference, whiteness and brownness index) of cassava flour.

5.2.3 Determination of colour properties of cassava flour

A colorimeter (ColorFlex, HunterLab, USA) was employed in determination of colour properties; *L** (lightness/darkness), *a** (redness/greenness) and *b** (yellowness/blueness) of the samples. The colorimeter was calibrated with a standard white (*L** = 93.71, *a** = –0.84 and *b** = 1.83) and black plate before use. Chroma, colour difference (ΔE), whiteness index (WI) and brownness index (BI) were calculated using equations 5.1, 5.2, 5.3 and 5.4 (Anyasi *et al.*, 2017).

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad (5.1)$$

$$\Delta E = \sqrt{(L0 - L^{*})^2 + (a0 - a*)2 + (bo - b*)2} \quad (5.2)$$

$$WI = \sqrt{(100 - L^{*})^2 + a^{*2} + b^{*2}} \quad (5.3)$$

$$BI = 100\,X\,\frac{x-0.31}{0.71} \quad (5.4)$$

Where $x = \frac{(a^{*} + 1.75L^{*})}{5.645L^{*} + a^{*} - 3.012b^{*}}$

5.2.4 Near-infrared spectroscopy analysis of cassava flour

The percentage of moisture, crude fiber, ash and starch contents of the cassava flour were determined using NIR analyser (DA 7250 Perten Instruments, Hagersten, Sweden). The analyser's open-faced sample dish filled with cassava flour was placed in the NIR analyser scanned by infrared rays, results obtained were displayed on the computer screen. The NIR analyser was previously standardised by a selection of calibration and validation samples, and reference data obtained by routine laboratory analysis according to the modified method of Alamu *et al.* (2019). The modification was the use of cassava flour samples for standardisation.

5.2.5 Statistical analysis and optimisation

Design expert software version 11 was employed in the analysis of variance (ANOVA) of the linear, interactive and quadratic effect of the model parameters on cassava flour properties. Regression models, coefficient of determination (R^2), p-values, F-values,

response surface plots, contour plots and optimisation conditions were generated by the software for all four experimental groups. Statistical data analysis was done with the IBM SPSS statistics software Version 25 (IBM Corp., NY, USA) to ascertain the significant difference between means of the experimental and reference/control sample within each experimental group at a 95% confidence level. One-way ANOVA and separation of means were done using Duncan multiple range test.

5.3 Results and Discussion

5.3.1 Effect of chemical pre-treatment and drying temperature on ash content of cassava flour

Ash content of cassava is an indication of its non-volatile content and mineral richness (Montagnac *et al.*, 2009a). All four experimental groups, CAR, CCR, CAW and CCW, had similar ash content within the range of 0.79 to 4.42% and they varied significantly within the groups. Significance in variation implies that the processing conditions affected the ash content of flour. Some values are similar while others were above the range (0.33 – 1.04%) obtained by Ukenye *et al.* (2013) for cassava root. The values (0.74 - 1.43%) reported by Aniedu and Omodamiro (2012) for cassava flour are within the range obtained in this study. Response surface plots for ash content of all four experimental groups had a similar shape (Figure 5.1) in which the ash content increased with concentration of pre-treatment and drying temperature. Increase in the ash content observed in this study disagrees with the report of Montagnac *et al.* (2009b) that cassava processing significantly reduces ash content of the roots and Ayetigbo *et al.* (2018) who postulated that severe processing involving application of high temperatures and chemicals may significantly decrease ash in cassava diets. The increase in ash content as COP increased could be attributed to elements from pre-treatment solutions used in soaking fresh cassava chips in water, before drying, which may be retained in the samples after processing. This observation was buttressed by ANOVA of the model parameters F- and p- values in Table 5.1 which indicate that the linear, interactive and quadratic effect of the experimental factors significantly influenced the ash content of cassava flour. Regression models generated by response surface methodology (Table 5.2) and the coefficient of determination (R^2) of the ash content were relatively high between 0.9446 and 0.9710, which shows that the model fits.

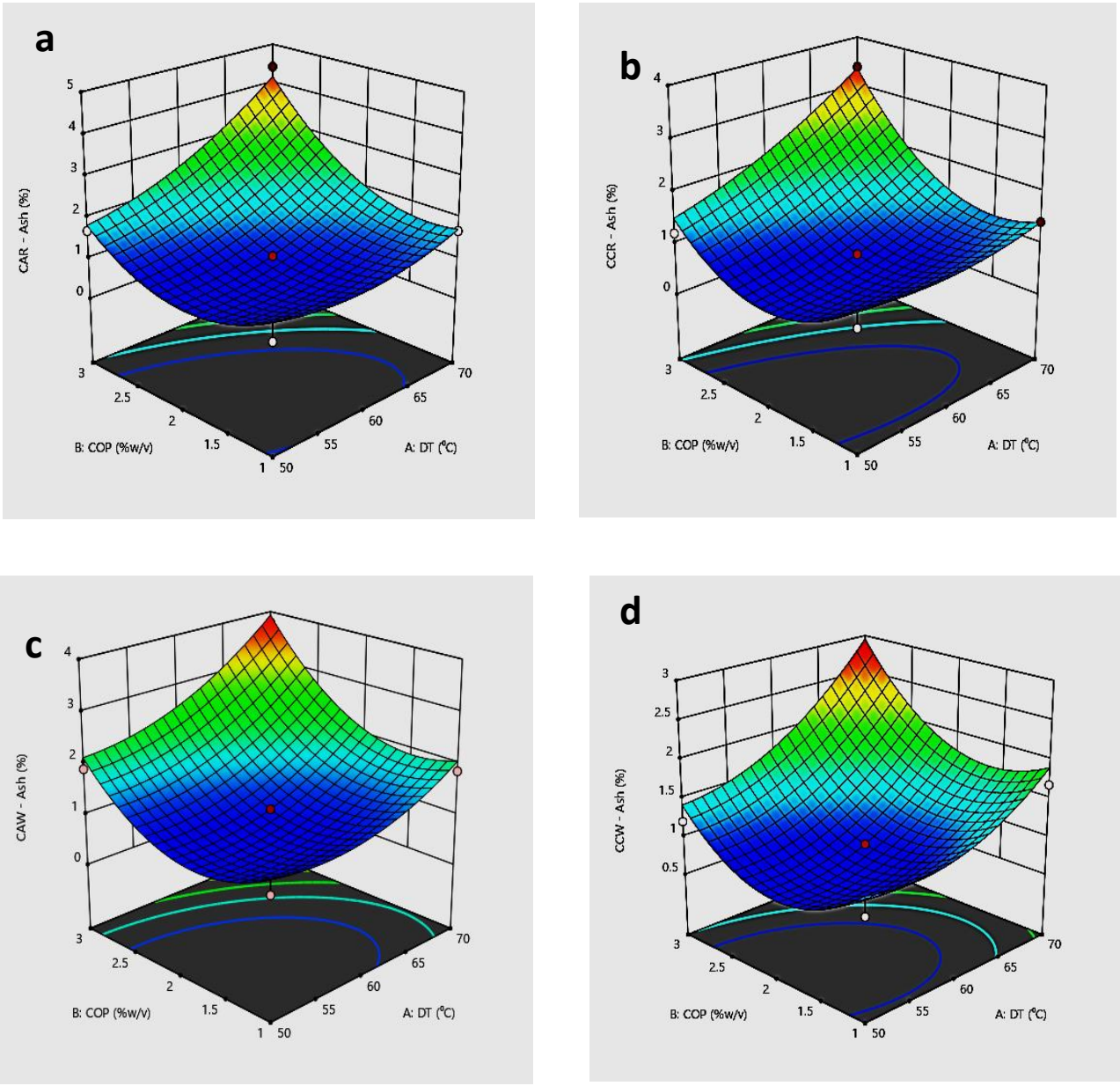

Figure 5.1 Response surface plots for ash content of cassava flour. **a** - Cassava flour from red landrace pre-treated with citric acid; **b** - Cassava flour from red landrace pre-treated with calcium chloride; **c** - Cassava flour from white landrace pre-treated with citric acid; **d** - Cassava flour from white landrace pre-treated with calcium chloride. DT - drying temperature (ºC), COP- Concentration of pre-treatment (%w/v).

Table 5.1 ANOVA of the effect of model parameters on proximate components of cassava flour

Source	Ash		Crude fibre		Moisture		Starch	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Cassava flour from red landrace pre-treated with citric acid (CAR)								
Model	36.47	< 0.0001*	8.91	0.0061*	10.52	0.0037*	1.13	0.4265
A-DT	45.87	0.0003*	16.48	0.0048*	17.73	0.0040*	1.50	0.2605
B-COP	52.69	0.0002*	6.09	0.0430*	2.22	0.1801	1.84	0.2175
AB	13.48	0.0079*	3.41	0.1073	2.34	0.1701	1.31	0.2901
A^2	12.89	0.0089*	1.33	0.2861	0.7218	0.4237	0.8951	0.3756
B^2	63.71	< 0.0001*	18.20	0.0037*	30.28	0.0009*	0.1811	0.6832
Cassava flour from red landrace pre-treated with calcium chloride (CCR)								
Model	41.22	< 0.0001*	7.22	0.0109*	12.47	0.0022*	0.3767	0.8501
A-DT	39.44	0.0004*	6.46	0.0386*	17.17	0.0043*	1.52	0.2570
B-COP	45.69	0.0003*	4.17	0.0805	5.19	0.0568	0.0219	0.8866
AB	14.39	0.0068*	3.04	0.1246	1.39	0.2764	0.0003	0.9870
A^2	5.90	0.0455*	0.1369	0.7223	0.2770	0.6149	0.0900	0.7729
B^2	105.39	< 0.0001*	22.37	0.0021*	38.49	0.0004*	0.2843	0.6104
Cassava flour from white landrace pre-treated with citric acid (CAW)								
Model	46.82	< 0.0001*	7.21	0.0110*	8.82	0.0062*	4.94	0.0296*
A-DT	61.23	0.0001*	15.90	0.0053*	12.47	0.0096*	3.69	0.0960
B-COP	67.66	< 0.0001*	2.18	0.1833	2.78	0.1393	15.15	0.0060*
AB	4.90	0.0624	1.37	0.2807	0.4646	0.5174	0.0187	0.8952
A^2	21.74	0.0023*	1.36	0.2820	1.61	0.2456	0.7732	0.4084
B^2	88.29	< 0.0001*	16.21	0.0050*	28.07	0.0011*	5.51	0.0513
Cassava flour from white landrace pre-treated with calcium chloride (CCW)								
Model	23.86	0.0003*	11.49	0.0029*	9.49	0.0051*	0.6463	0.6740
A-DT	44.24	0.0003*	31.18	0.0008*	17.60	0.0041*	1.94	0.2068
B-COP	14.02	0.0072*	2.95	0.1298	7.36	0.0301*	1.06	0.3381
AB	3.21	0.1163*	2.04	0.1962	1.23	0.3036	0.1602	0.7009
A^2	14.45	0.0067*	3.11	0.1213	1.32	0.2887	0.0789	0.7869
B^2	49.37	0.0002*	19.84	0.0030*	20.93	0.0026*	0.0003	0.9869

DT- Drying temperature; COP- Concentration of pre-treatment; A- Linear effect of DT; B- Linear effect of COP; AB- Interactive effect of DT and COP; A^2- Quadratic effect of DT, B^2- Quadratic effect of COP; *- significant at 95% confidence level.

Table 5.2 Regression models relating proximate components of cassava flour and model parameters

Dependent variables	Models	R^2
	Cassava flour from red landrace pre-treated with citric acid (CAR)	
Ash	$+1.06+0.6651A+0.7128B+0.5100AB+0.3781A^2+0.8406B^2$	0.9630
Crude fibre	$+3.08+0.4897A+0.2976B+0.3150AB+0.1494A^2+0.5519B^2$	0.8642
Moisture	$+10.13-0.6962A-0.2461B-0.3575AB-0.1506A^2-0.9756B^2$	0.8825
Starch	$+80.33+0.4879A+0.5401B+0.6450AB+0.4044\ A^2+0.1819B^2$	0.4459
	Cassava flour from red landrace pre-treated with calcium chloride (CCR)	
Ash	$+0.7900+0.5063A+0.5450B+0.4325AB+0.2100A^2+0.8875B^2$	0.9672
Crude fibre	$+3.05+0.3684A+0.2959B+0.3575AB+0.0575A^2+0.7350B^2$	0.8376
Moisture	$+10.14-0.6516A-0.3582B-0.2625AB-A^2-1.05B^2$	0.8990
Starch	$+79.18+0.5180A-0.0621B-0.0100AB+0.1350A^2+0.2400B^2$	0.2120
	Cassava flour from white landrace pre-treated with citric acid (CAW)	
Ash	$+1.11+0.6435A+0.6765B+0.2575AB+0.4112A^2+0.8288B^2$	0.9710
Crude fibre	$+3.19+0.5005A+0.1853B+0.2075AB+0.1569A^2+0.5419B^2$	0.8375
Moisture	$+10.20-0.6668A-0.3157B-0.1825AB-0.2573A^2-1.08B^2$	0.8631
Starch	$+80.17+0.6714A+1.36B+0.0675AB+0.3294A^2+0.8794B^2$	0.7792
	Cassava flour from white landrace pretreated with calcium chloride (CCW)	
Ash	$+0.9000+0.5578A+0.3140B+0.2125AB+0.3419A^2+0.6319B^2$	0.9446
Crude fibre	$+3.18+0.4836A+0.1487B+0.1750AB+0.1638A^2+0.4138B^2$	0.8914
Moisture	$+9.97-0.5879A-0.3802B-0.2200AB-0.1725A^2-0.6875B^2$	0.8714
Starch	$+79.91+0.4301A+0.3178B+0.1750AB-0.0931A^2-0.0056B^2$	0.3158

R^2 – coefficient of determination; A- Linear effect of drying temperature; B- Linear effect of concentration of pre-treatment; AB- Interactive effect of drying temperature and concentration of pre-treatment; A^2- Quadratic effect of drying temperature, B^2- Quadratic effect of concentration of pre-treatment

5.3.2 Effect of chemical pre-treatment and drying temperature on moisture content of cassava flour

High moisture content in foods aid growth of microbes (Makinde and Ladipo, 2012) and drying is applied to eliminate moisture. Materials, like flour, having above 12.5% moisture possess less storage stability than those with less moisture content hence moisture content of not more than 12.5% is generally specified for flours (Adedeji *et al.*, 2015). The cassava flour samples processed under varying conditions had moisture content between 7.43 and 10.50%. Onitilo *et al.* (2007a) reported percentage moisture within the range of 3.59 to 11.53 for flour from white and yellow-fleshed cassava roots. The moisture content of the yellow-fleshed cassava root was higher than the white-fleshed roots. In Figure 5.2, the response surface plots of the moisture content of all experimental groups displayed same pattern. The moisture content of the samples increased and decreased along the X2 (concentration of pre-treatment) axis. A slight decrease in moisture was observed with an increase along the X1 (drying temperature) axis. ANOVA of model parameters produced p-values less than 0.05 for the linear effect of DT and quadratic effect of COP (Table 5.1). Regression models yielded a coefficient of variation 0.8631 – 0.8990 and all had positive intercepts (Table 5.2).

5.3.3 Effect of chemical pre-treatment and drying temperature on fibre content of cassava flour

Textural behaviour and *invitro* digestibility of cassava flour is influenced by its residual fibre (Ayetigbo *et al.*, 2018). The crude fibre was between 2.77 and 5.12%. The least and highest crude fibre was exhibited by samples of experimental run 1 (50ºC/1%w/v) and 4 (70ºC/3%w/v) in all the experimental groups It gives a clue that the crude fibre content increased with DT and COP. The response surface plots for all experimental groups (Figure 5.3) aligns with this observation. Aniedu and Omodamiro (2012) reported lower values (0.62 – 1.63%) for flour processed from white-flesh and yellow-flesh cassava varieties. The authors reported that fibre in cassava flour from white-flesh variety is generally higher than the yellow-flesh variety. Ukenye *et al.* (2013) also reported relatively higher fibre content (0.62-4.92%) in roots of white-flesh cassava than in yellow-flesh varieties.

Differences in fibre content maybe attributed to differences in varietal composition and age of harvest (Montagnac *et al.*, 2009b). The process flow, in this study, did not involve the removal of fibre from the roots as is done in starch extraction and in some cassava flour processing methods. The omission of this step (removal of fibre) could be responsible for the relatively high fibre content in these samples.

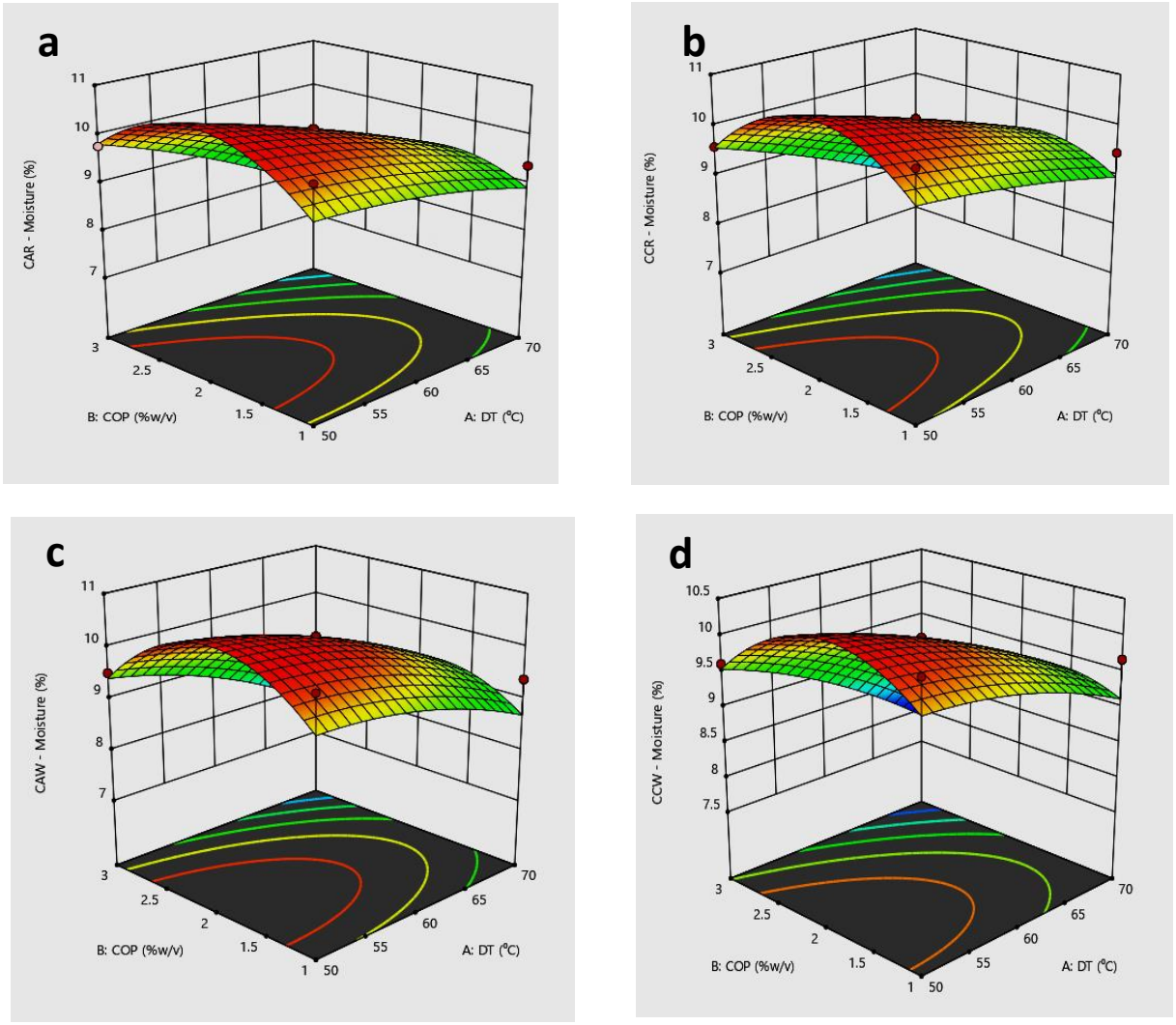

Figure 5.2. Response surface plots for percentage moisture of cassava flour. **a** - Cassava flour from red landrace pre-treated with citric acid; **b** - Cassava flour from red landrace pre-treated with calcium chloride; **c** - Cassava flour from white landrace pre-treated with citric acid; **d** - Cassava flour from white landrace pre-treated with calcium chloride. DT - drying temperature (ºC), COP- Concentration of pre-treatment (%w/v).

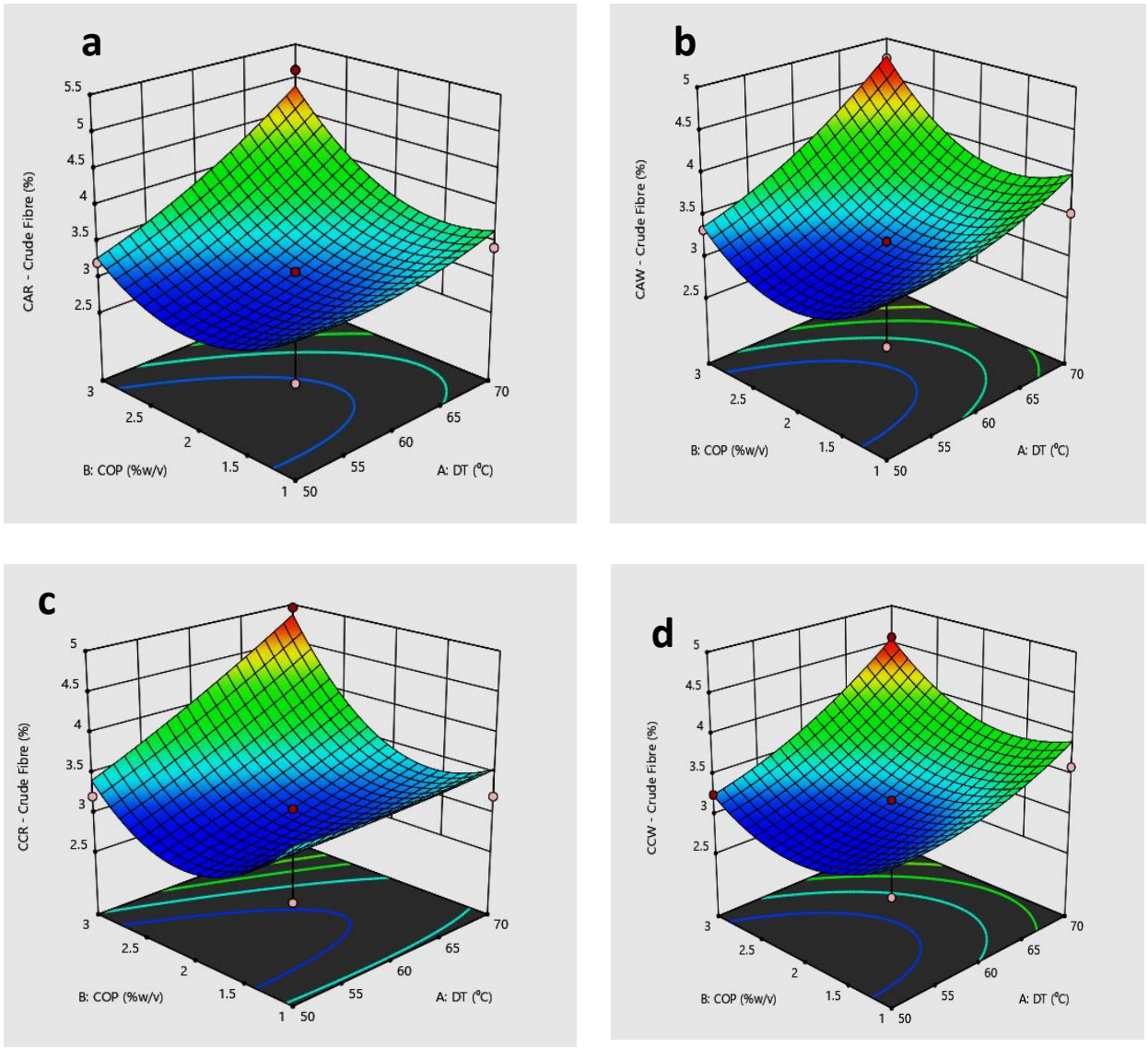

Figure 5.3. Response surface plots for crude fibre content of cassava flour. **a** - Cassava flour from red landrace pre-treated with citric acid; **b** – Cassava flour from red landrace pre-treated with calcium chloride; **c** - Cassava flour from white landrace pre-treated with citric acid; **d** - Cassava flour from white landrace pre-treated with calcium chloride. DT - drying temperature (ºC), COP- Concentration of pre-treatment (%w/v).

ANOVA of model parameters shows that the linear effect of drying temperature and quadratic effect of concentration of pre-treatment significantly influenced ($p < 0.05$) the fibre content of the flours. High fibre content obtained in this study may positively influence dietary fibre available in the flours. Consumption of an ample quantity of dietary fibre decreases the risk of diseases such as obesity, constipation, gallstones, diabetes and coronary heart diseases (Dahl and Stewart, 2015). This is an indication that the landraces and processing conditions are suitable to produce cassava flour advantageous to the health of consumers.

5.3.4 Effect of chemical pre-treatment and drying temperature on starch content of cassava flour

Percentage starch composition of 78.06 – 84.71 was observed with significant variation ($p < 0.05$) across all experimental groups. The coefficient of determination (0.2120 – 0.7792) was relatively low compared to other proximate components of the flour (Table 5.2). The ANOVA of model parameters produced p-values below 0.05 for the model and linear effect of COP for samples pre-treated with citric acid from the white landrace (CAW). This is evident in the response surface plots (Figure 5.4) of CAW and the R^2 of 0.7792 which was higher than all other experimental groups. All other p-value were above 0.05 indicating that the model did not fit, while the linear, interactive and quadratic effect of the processing variables did not have a significant effect on the starch content of the flours.

5.3.5 Effect of chemical pre-treatment and drying temperature on colour properties of cassava flour

Colour is a physical yet vital parameter that determines consumers' preference for a product. Tables 5.3 and 5.4 are results of ANOVA between means of the colour properties of cassava flour pre-treated with calcium chloride. ANOVA reveals that processing cassava flour under varying COP and DT produced samples with significantly variant ($p < 0.05$) L^*, a^*, b^* chroma, whiteness index (WI), brownness index (BI) and colour change (ΔE). Regression models relating the colour properties to actual levels of processing conditions are depicted in Table 5.5. The coefficient of determination was between 0.4241(ΔE - CAR) and 0.9981 (b^* CCW). L^* values of the cassava flour samples were between 91.37 and 93.65 with the control sample significantly lower ($p < 0.05$) than the experimental samples in all four groups. This shows that pre-treatment of cassava flour from both landraces with citric acid or calcium chloride enhances the lightness of the flour.

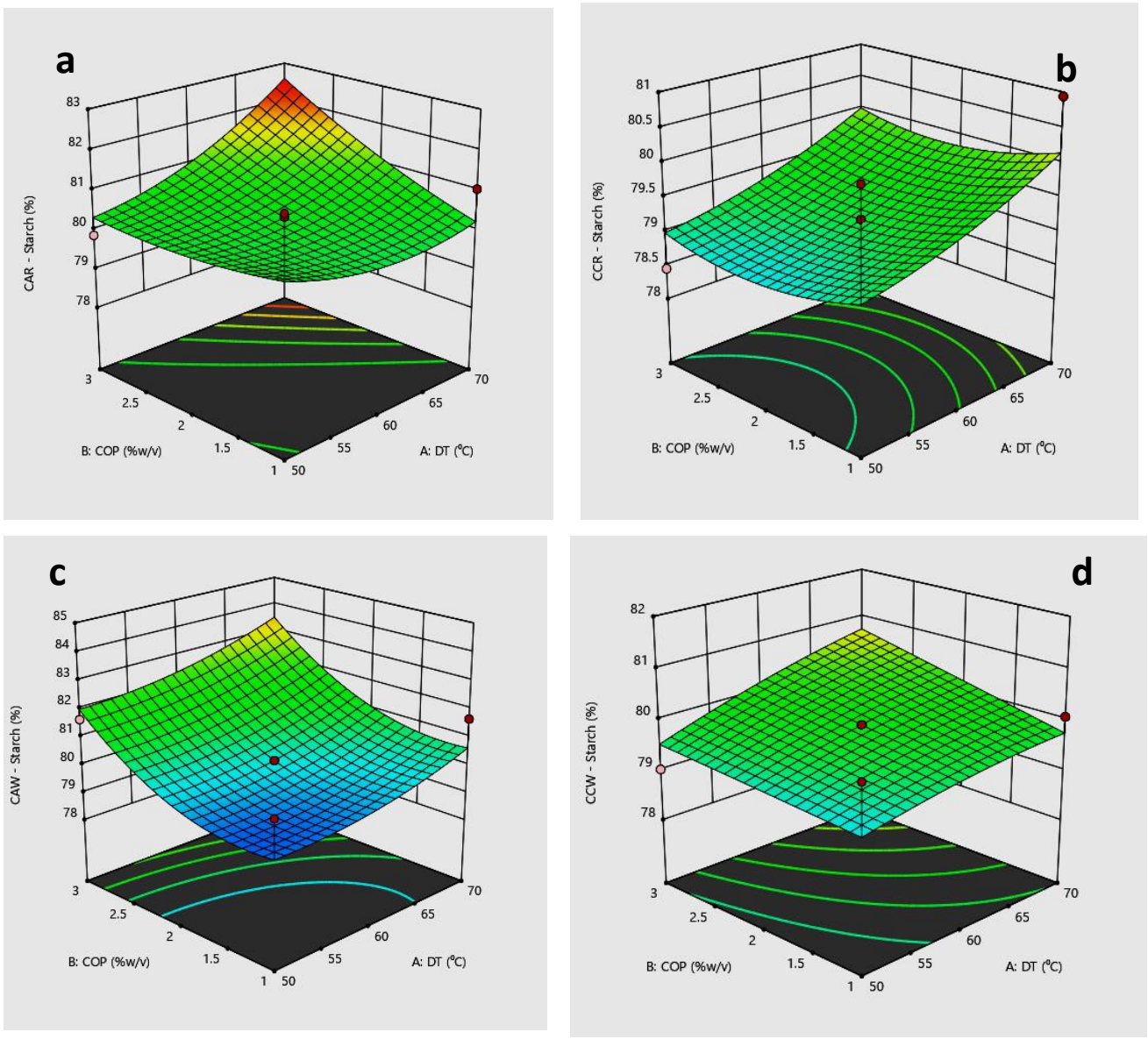

Figure 5.4. Response surface plots for starch content of cassava flour. **a** - Cassava flour from red landrace pre-treated with citric acid; **b** - Cassava flour from red landrace pre-treated with calcium chloride; **c** - Cassava flour from white landrace pre-treated with citric acid; **d** - Cassava flour from white landrace pre-treated with calcium chloride. DT - drying temperature (ºC), COP- Concentration of pre-treatment (%w/v).

Table 5.3. Experimental runs and colour properties of cassava flour under different processing conditions

CCR	Independent variables		Dependent variables						
	DT (ºC)	COP (%w/v)	*L**	*a**	*b**	Chroma	WI	BI	ΔE
1	50	1	$93.43^{c} \pm 0.01$	$0.08^{b} \pm 0.01$	$5.17^{d} \pm 0.05$	$5.17^{d} \pm 0.05$	$93.80^{a} \pm 0.01$	$1.34^{cde} \pm 0.01$	$2.37^{bc} \pm 0.01$
2	70	1	$93.46^{c} \pm 0.09$	$-0.25^{g} \pm 0.03$	$5.84^{b} \pm 0.26$	$5.84^{b} \pm 0.26$	$92.57^{c} \pm 0.00$	$1.46^{b} \pm 0.06$	$2.15^{cd} \pm 0.10$
3	50	3	$93.35^{c} \pm 0.09$	$0.19^{a} \pm 0.02$	$4.55^{f} \pm 0.15$	$4.55^{e} \pm 0.15$	$93.70^{a} \pm 0.09$	$1.20^{g} \pm 0.04$	$2.68^{a} \pm 0.04$
4	70	3	$93.62^{bc} \pm 0.03$	$-0.52^{h} \pm 0.02$	$5.80^{b} \pm 0.08$	$5.82^{b} \pm 0.08$	$92.57^{c} \pm 0.00$	$1.40^{c} \pm 0.02$	$2.33^{c} \pm 0.05$
5	45	2	$92.98^{d} \pm 0.09$	$0.03^{c} \pm 0.03$	$5.11^{de} \pm 0.22$	$5.11^{d} \pm 0.22$	$92.57^{c} \pm 0.00$	$1.32^{de} \pm 0.06$	$2.01^{d} \pm 0.19$
6	74	2	$92.57^{e} \pm 0.41$	$-0.10^{d} \pm 0.01$	$5.41^{c} \pm 0.11$	$5.41^{c} \pm 0.11$	$93.03^{b} \pm 0.37$	$1.39^{cd} \pm 0.03$	$1.51^{e} \pm 0.37$
7	60	0.6	$93.92^{a} \pm 0.02$	$-0.28^{g} \pm 0.02$	$5.50^{c} \pm 0.05$	$5.51^{c} \pm 0.05$	$92.57^{c} \pm 0.00$	$1.36^{cde} \pm 0.01$	$2.67^{a} \pm 0.03$
8	60	3.4	$93.77^{ab} \pm 0.04$	$-0.14^{e} \pm 0.03$	$4.95^{de} \pm 0.08$	$4.96^{d} \pm 0.08$	$92.57^{c} \pm 0.00$	$1.24^{fg} \pm 0.02$	$2.75^{a} \pm 0.07$
9	60	2	$93.54^{bc} \pm 0.05$	$0.22^{a} \pm 0.03$	$4.91^{e} \pm 0.10$	$4.91^{d} \pm 0.10$	$93.89^{a} \pm 0.05$	$1.30^{ef} \pm 0.02$	$2.61^{ab} \pm 0.02$
10	60	0	$91.37^{f} \pm 0.16$	$-0.02^{f} \pm 0.02$	$6.30^{a} \pm 0.14$	$6.31^{a} \pm 0.14$	$91.99^{d} \pm 0.15$	$1.63^{a} \pm 0.03$	-

DT - Drying temperature; COP - Concentration of pre-treatment, CCR - Cassava from red landrace pre-treated with calcium chloride, *L** - Lightness/darkness, *a** - redness/greenness, *b** - yellowness/blueness, WI - whiteness index, BI - brownness index, ΔE - colour difference. Values are mean ± standard deviation (n = 3). Means in the same column with different superscripts are significantly different at 95% confidence level.

Table 5.4 Experimental runs and colour properties of cassava flour (CCW) under different processing conditions

CCW	Independent variables		Dependent variables						
	DT (ºC)	COP (%w/v)	*L**	*a**	*b**	Chroma	WI	BI	ΔE
1	50	1	92.98^{e} ± 0.16	0.11^{d} ± 0.01	5.88^{de} ± 0.12	5.88^{de} ± 0.12	93.43^{a} ± 0.15	1.55^{e} ± 0.03	2.21^{c} ± 0.14
2	70	1	92.75^{c} ± 0.13	0.02^{e} ± 0.04	6.15^{c} ± 0.14	6.15^{c} ± 0.14	92.57^{b} ± 0.00	1.61^{d} ± 0.04	1.87^{de} ± 0.12
3	50	3	93.29^{b} ± 0.15	0.55^{a} ± 0.01	4.78^{h} ± 0.03	4.78^{h} ± 0.03	93.66^{a} ± 0.14	1.33^{g} ± 0.01	3.26^{a} ± 0.09
4	70	3	92.78^{c} ± 0.08	-0.14^{g} ± 0.03	6.58^{b} ± 0.10	6.58^{b} ± 0.10	92.57^{b} ± 0.00	1.69^{c} ± 0.03	1.68^{e} ± 0.11
5	45	2	93.07^{bc} ± 0.06	0.23^{c} ± 0.02	5.03^{g} ± 0.08	5.03^{g} ± 0.08	92.57^{b} ± 0.00	1.34^{fg} ± 0.02	2.89^{b} ± 0.09
6	74	2	91.88^{d} ± 0.34	0.36^{b} ± 0.04	6.55^{b} ± 0.12	6.56^{b} ± 0.12	92.47^{b} ± 0.30	1.79^{b} ± 0.03	1.04^{f} ± 0.31
7	60	0.6	92.96^{bc} ± 0.05	-0.12^{g} ± 0.01	6.05^{cd} ± 0.05	6.05^{cd} ± 0.05	92.57^{b} ± 0.00	1.55^{e} ± 0.01	2.09^{cd} ± 0.04
8	60	3.4	93.65^{a} ± 0.03	-0.25^{h} ± 0.02	5.56^{f} ± 0.06	5.56^{f} ± 0.06	92.57^{b} ± 0.00	1.38^{f} ± 0.12	2.95^{b} ± 0.03
9	60	2	92.96^{bc} ± 0.13	0.54^{a} ± 0.03	5.80^{e} ± 0.09	5.83^{e} ± 0.09	93.41^{a} ± 0.13	1.61^{d} ± 0.03	2.32^{c} ± 0.06
10	60	0	91.37^{bc} ± 0.34	-0.06^{f} ± 0.03	7.26^{a} ± 0.15	7.26^{a} ± 0.15	92.07^{c} ± 0.32	1.92^{a} ± 0.04	-

DT - Drying temperature; COP - Concentration of pre-treatment, CCW - Cassava from white landrace pre-treated with calcium chloride, *L** - Lightness/darkness, *a** - redness/greenness, *b** - yellowness/blueness, WI - whiteness index, BI - brownness index, ΔE - total colour change. Values are mean ± standard deviation (n = 3). Means in the same column with different superscripts are significantly different at 95% confidence level.

Table 5.5. Regression models relating model parameters and colour properties of cassava flour

Dependent variables	Models	R^2
	Cassava flour from red landrace pre-treated with citric acid	
*L**	+92.33+0.0353A-0.0679B+0.1900AB-0.1469A^2+2.156B^2	0.6328
*a**	+0.9700-0.2147A+0.0913B-0.1200AB-0.0962A^2-0.3862B^2	0.9334
*b**	+6.68+0.3138A-0.0449B+0.1900AB+0.0788A^2-0.0162B^2	0.6968
Chroma	+6.75+0.2912A-0.0394B+0.1775AB+0.0681A^2-0.0469B^2	0.6784
WI	+92.89-0.0617A-0.0950B+0.1900AB-0.0381A^2-0.1206B^2	0.4832
BI	+1.94+0.0446A+0.0060B+0.0250AB+0.0063A^2-0.0837B^2	0.7884
ΔE	+1.56+0.0844A+0.0084B+0.1175AB-0.0937A^2-0.0062B^2	0.4241
	Cassava flour from red landrace pre-treated with calcium chloride	
*L**	+93.54-0.0350A-0.0165B-0.0600AB-0.3438A^2+0.1912B^2	0.8888
*a**	+0.2200-0.1470A+0.0051B-0.0957AB-0.1330A^2-0.2137B^2	0.8420
*b**	+4.91+0.2930A-0.1797B+0.1450AB+0.1994A^2+0.1819B^2	0.8311
Chroma	+4.91+0.2955A-0.1772B+0.1500AB+0.1994A^2+0.1869B^2	0.8294
WI	+93.89-0.2137A-0.0125B+0.0250AB-0.4262A^2-0.5412B^2	0.6756
BI	+1.30+0.0524A-0.0462B+0.0200AB+0.0331A^2+0.0056 B^2	0.8697
ΔE	+2.61-0.1596A+0.0754B-0.0325AB-0.3881A^2+0.0869 B^2	0.9576
	Cassava flour from white landrace pre-treated with citric acid	
*L**	+91.80-0.2968A+0.0786B-0.2475AB+0.1265A^2+0.2981B^2	0.9850
*a**	+1.12-0.1753A-0.0347B+0.0750AB-0.1794A^2-0.4919B^2	0.9660
*b**	+7.07+0.7643A+0.0567B+0.6125AB-0.1706A^2+0.02844B^2	0.9361
Chroma	+7.16+0.7462A+0.0542B+0.6175AB-0.1944A^2+0.2406B^2	0.9341
WI	+92.44-0.1697A+0.0712B-0.1425AB+0.0781A^2+0.1431B^2	0.6464
BI	+2.09+0.1838A+0.0090B+0.1850AB-0.0854A^2-0.0232B^2	0.9374
ΔE	+9.57-0.0291A+0.0278B-0.0200AB+0.0244A^2+0.0019B^2	0.8870
	Cassava flour from white landrace pre-treated with calcium chloride	
*L**	+95.96-0.3029A+0.1645B-0.0700AB-0.2275A^2+0.1875B^2	0.9078
*a**	+0.5400-0.0741A+0.0130B-0.1492AB-0.1021A^2-0.3429B^2	0.8626
*b**	+5.80+0.5275A-0.1704B+0.3825AB+0.0075A^2+0.0150B^2	0.9981
Chroma	+5.83+0.5255A-0.1666B+0.3750AB-0.0015A^2+0.0035B^2	0.9972
WI	+93.41-0.2614A+0.0287B-0.0575AB-0.3169 A^2-0.2919B^2	0.6450
BI	+1.61+0.1320A-0.0476B+0.0750AB-0.0150 A^2-0.0650B^2	0.9592
ΔE	+2.32-0.5670A+0.2595B-0.3100AB-0.1744 A^2+0.1031B^2	0.9803

*L** - Lightness/darkness, *a** - redness/greenness, *b** - yellowness/blueness, WI - whiteness index, BI - brownness index, ΔE - colour difference, A - Linear effect of drying temperature; B - Linear effect of concentration of pre-treatment; AB - Interactive effect of drying temperature and concentration of pre-treatment; A^2 - Quadratic effect of drying temperature, B^2 - Quadratic effect of concentration of pre-treatment.

This is buttressed by the significance of the quadratic effect of COP on the *L** values (not reported) in all experimental groups. The contour plots for *L** values show an increase as COP increased. These findings are in line with the reports of Nemaungani *et al.* (2019) on citric acid and calcium chloride pre-treated flour processed from solar-dried cassava chips. The measure of redness/greenness (*a** values) of the samples varied significantly ($p < 0.05$). Analysis of variance shows that the *a** values of the control sample in citric acid pre-treated flours were significantly lower ($p < 0.05$) than the experimental samples within the respective groups (Tables 5.6 and 5.7). Control samples CCR and CCW exhibited significantly higher *b** values, chroma and browning index within their respective experimental groups. This implies that calcium chloride may have a reducing effect on the *b** value, chroma and brownness index of the flours. Change in colour (ΔE) is a function of the difference between the *L**, *a**, *b** values of the experimental samples and the control. Colour change was highest (9.54 to 9.69) in cassava flour processed from the white landrace pre-treated with citric acid (CAW) and lowest (1.11 to 1.81) cassava flour processed from the red landrace pre-treated with citric acid. This gives a hint on the effect of landrace on the colour properties of cassava flour. The trend of the whiteness index (WI), the trend was similar to that of *L** discussed above, the control in all experimental groups had the least WI. The values of *L** (87.67-93.57), *a** (-0.27-1.1), *b** (8.4-11.83), chroma (8.4-11.87) and WI (82.88-89.42) reported in the work of Omolola *et al.* (2017) for cassava flour are similar to those in this study. The findings of Falade and Ayetigbo (2015) on the effect of citric acid modification on colour properties of yam starch agree with the trend in this study. The authors (Falade and Ayetigbo, 2015) reported that *L**, *a**, WI increased while *b** values decreased with citric acid modification of yam starches from four different cultivars.

5.3.6 Multi-response optimisation of cassava flour processing

Multi-response optimisation of proximate components and colour properties of cassava flour was conducted, separately, using the design expert software. Optimisation goal for the former was set as; starch content in range, minimised moisture content, maximised ash and crude fibre content. Overlay plots for optimal processing conditions for cassava flour from all four experimental groups with the desired qualities (Figure 5.5). Optimal conditions (70ºC/3%w/v) for all four experimental samples were the same. However, the responses and desirability differed; CAR (Ash – 4.17; Crude fibre – 4.88; Moisture – 7.70%; Desirability - 0.912); CCR (Ash - 3.37; Crude fibre – 4.86; Moisture – 7.73; Desirability - 0.955); CAW (Ash - 3.93; Crude fibre – 4.78; Moisture – 7.70; Desirability - 0.992); CCW (Ash - 2.96; Crude fibre – 4.57; Moisture – 7.92; Desirability - 0.997).

Table 5.6. Experimental runs and colour properties of cassava flour (CAR) under different processing conditions

CAR	Independent variables		Dependent variables						
	DT (ºC)	COP (%w/v)	*L**	*a**	*b**	Chroma	WI	BI	ΔE
1	50	1	92.94^{a} ± 0.11	0.69^{c} ± 0.03	6.30^{d} ± 0.12	6.34^{d} ± 0.12	93.43^{a} ± 0.10	1.77^{de} ± 0.03	1.81^{a} ± 0.10
2	70	1	92.15^{bc} ± 0.40	0.34^{e} ± 0.02	6.85^{bc} ± 0.04	6.86^{bc} ± 0.04	92.57^{c} ± 0.00	1.87^{c} ± 0.01	1.11^{e} ± 0.32
3	50	3	92.14^{bc} ± 0.16	0.97^{a} ± 0.01	5.91^{e} ± 0.04	5.99^{e} ± 0.04	92.67^{c} ± 0.14	1.73^{e} ± 0.01	1.46^{c} ± 0.09
4	70	3	92.11^{bc} ± 0.14	0.14^{f} ± 0.04	7.22^{a} ± 0.04	7.22^{a} ± 0.04	92.57^{c} ± 0.00	1.93^{b} ± 0.02	1.23^{de} ± 0.12
5	45	2	91.91^{c} ± 0.11	0.92^{b} ± 0.05	6.78^{bc} ± 0.19	6.85^{bc} ± 0.20	92.57^{c} ± 0.00	1.97^{ab} ± 0.06	1.34^{cd} ± 0.06
6	74	2	92.29^{b} ± 0.04	0.54^{d} ± 0.01	7.24^{a} ± 0.04	7.26^{a} ± 0.03	92.90^{b} ± 0.03	2.01^{a} ± 0.01	1.52^{bc} ± 0.02
7	60	0.6	92.72^{a} ± 0.05	-0.08^{g} ± 0.03	6.94^{b} ± 0.09	6.94^{b} ± 0.09	92.57^{c} ± 0.00	1.80^{d} ± 0.03	1.50^{bc} ± 0.03
8	60	3.4	92.93^{a} ± 0.07	0.38^{e} ± 0.02	6.70^{c} ± 0.03	6.71^{c} ± 0.03	92.57^{c} ± 0.00	1.82^{cd} ± 0.01	1.71^{ab} ± 0.07
9	60	2	92.33^{b} ± 0.09	0.97^{a} ± 0.05	6.68^{c} ± 0.10	6.75^{c} ± 0.10	92.89^{b} ± 0.08	1.94^{b} ± 0.04	1.56^{bc} ± 0.03
10	60	0	91.37^{d} ± 0.16	-0.20^{h} ± 0.02	6.30^{d} ± 0.14	6.31^{d} ± 0.14	91.99^{d} ± 0.15	1.63^{f} ± 0.03	-

DT - Drying temperature; COP - Concentration of pre-treatment, CAR - Cassava from red landrace pre-treated with citric acid, *L** - Lightness/darkness, *a** - redness/greenness, *b** - yellowness/blueness, WI - whiteness index, BI - brownness index, ΔE - colour difference. Values are mean ± standard deviation (n = 3). Means in the same column with different superscripts are significantly different at 95% confidence level.

Table 5.7. Experimental runs and colour properties of cassava flour (CAW) under different processing conditions.

CAW	Independent variables		Dependent variables						
	DT (ºC)	COP (%w/v)	*L**	*a**	*b**	Chroma	WI	BI	ΔE
1	50	1	$92.19^{bc} \pm 0.06$	$0.88^{b} \pm 0.00$	$6.95^{c} \pm 0.14$	$7.00^{c} \pm 0.13$	$92.78^{b} \pm 0.06$	$1.99^{cd} \pm 0.04$	$9.56^{cd} \pm 0.00$
2	70	1	$92.12^{cd} \pm 0.14$	$0.34^{d} \pm 0.15$	$7.17^{c} \pm 0.16$	$7.18^{bc} \pm 0.15$	$92.57^{c} \pm 0.00$	$1.96^{d} \pm 0.05$	$9.54^{d} \pm 0.00$
3	50	3	$92.91^{a} \pm 0.07$	$0.57^{c} \pm 0.05$	$5.57^{d} \pm 0.06$	$5.60^{d} \pm 0.05$	$93.35^{a} \pm 0.06$	$1.55^{f} \pm 0.01$	$9.69^{a} \pm 0.01$
4	70	3	$91.85^{de} \pm 0.23$	$0.33^{d} \pm 0.04$	$8.24^{a} \pm 0.27$	$8.25^{a} \pm 0.27$	$92.57^{c} \pm 0.00$	$2.26^{a} \pm 0.09$	$9.59^{b} \pm 0.03$
5	45	2	$92.45^{bc} \pm 0.02$	$0.90^{b} \pm 0.02$	$5.79^{d} \pm 0.07$	$5.86^{d} \pm 0.07$	$92.57^{c} \pm 0.00$	$1.68^{e} \pm 0.02$	$9.66^{a} \pm 0.01$
6	74	2	$91.57^{ef} \pm 0.08$	$0.46^{d} \pm 0.03$	$8.07^{a} \pm 0.23$	$8.08^{a} \pm 0.23$	$92.31^{d} \pm 0.09$	$2.24^{a} \pm 0.07$	$9.58^{bc} \pm 0.02$
7	60	0.6	$92.29^{bc} \pm 0.17$	$0.04^{f} \pm 0.01$	$7.57^{b} \pm 0.33$	$7.57^{b} \pm 0.33$	$92.57^{c} \pm 0.00$	$2.01^{cd} \pm 0.09$	$9.56^{cd} \pm 0.01$
8	60	3.4	$92.42^{bc} \pm 0.23$	$0.07^{f} \pm 0.06$	$8.11^{a} \pm 0.42$	$8.11^{a} \pm 0.42$	$92.57^{c} \pm 0.00$	$2.16^{ab} \pm 0.13$	$9.59^{b} \pm 0.03$
9	60	2	$91.80^{e} \pm 0.08$	$1.12^{a} \pm 0.02$	$7.07^{c} \pm 0.09$	$7.16^{c} \pm 0.08$	$92.44^{cd} \pm 0.08$	$2.09^{bc} \pm 0.02$	$9.57^{bcd} \pm 0.00$
10	60	0	$91.37^{f} \pm 0.34$	$-0.06^{g} \pm 0.03$	$7.26^{bc} \pm 0.15$	$7.26^{bc} \pm 0.15$	$92.07^{e} \pm 0.32$	$1.92^{d} \pm 0.04$	-

DT - Drying temperature; COP - Concentration of pre-treatment, CAW - Cassava from white landrace pre-treated with citric acid, *L** - Lightness/darkness, *a** - redness/greenness, *b** - yellowness/blueness, WI - whiteness index, BI - brownness index, ΔE - colour difference. Values are mean ± standard deviation (n = 3). Means in the same column with different superscripts are significantly different at 95% confidence level.

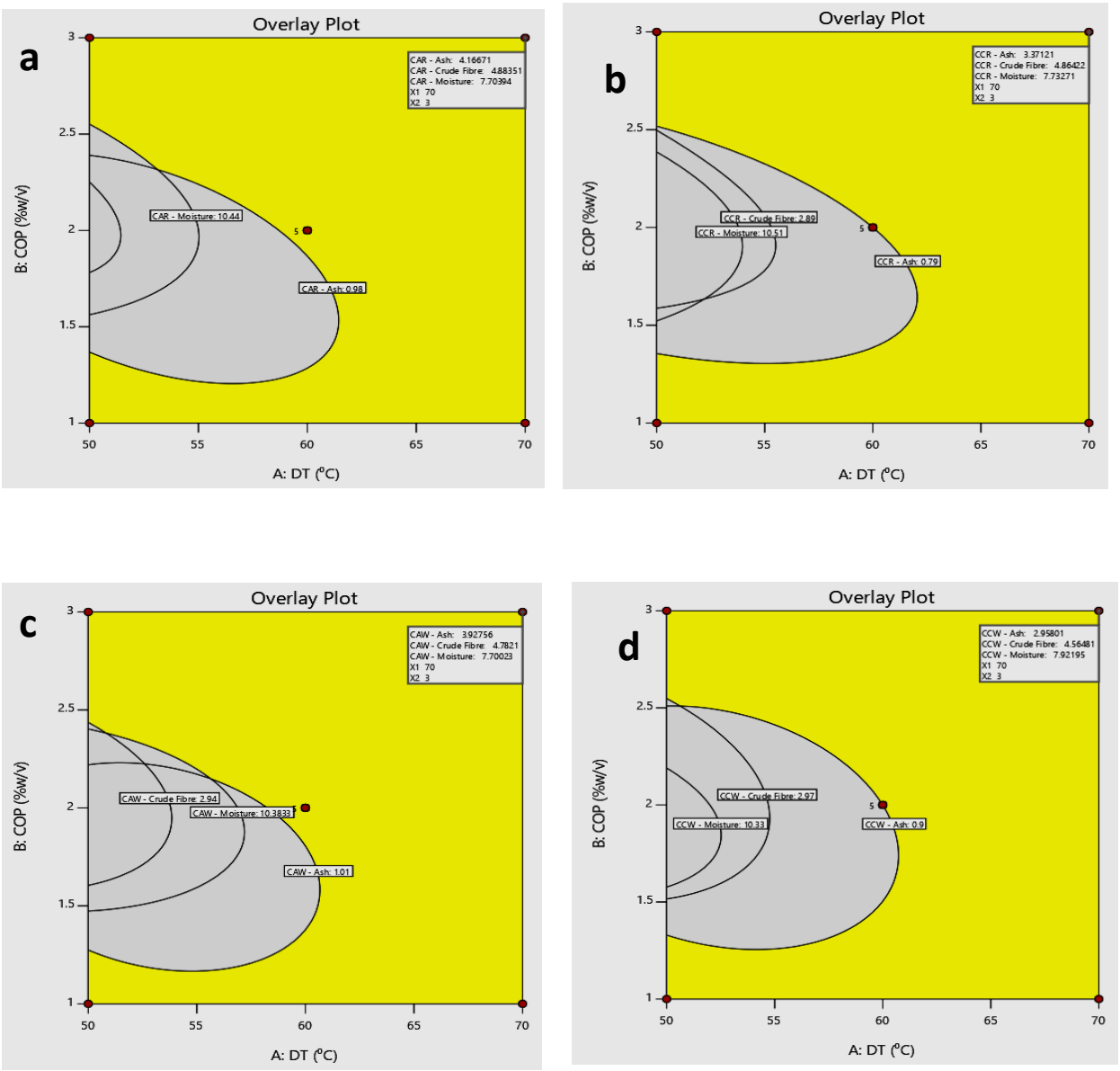

Figure 5.5 Multi-response optimisation overlay plots of processing conditions and major components of cassava flour. **a** - Cassava flour from red landrace pre-treated with citric acid; **b** - Cassava flour from red landrace pre-treated with calcium chloride; **c** - Cassava flour from white landrace pre-treated with citric acid; **d** - Cassava flour from white landrace pre-treated with calcium chloride. DT - drying temperature (ºC), COP- Concentration of pre-treatment (%w/v).

Optimisation of colour properties had the targets: minimised BI and maximised WI and *L** values. The two colour parameters maximised correspond to consumers' preference for cassava flour. Omolola *et al.* (2017) state that whiteness is the characteristic and acceptable colour property associated with cassava flour by consumers. The RSM overlay plots generated from the design expert software (Figure 5.6) show the processing conditions for obtaining cassava flour with these desired properties for all four experimental groups. The optimal processing conditions are CAR- 50ºC/1%w/v and 0.795 desirability; CCR-

57.31ºC/2.45%w/v and 0.546 desirability; CAW- 50ºC/3%w/v with desirability of 0.846 and CCW- 51.87ºC/2.95%w/v and 0.821 desirability. Low *a** and *b** values with relatively high *L** and WI values recorded for all flour samples indicate that the samples are white. Whiteness is an advantageous consumer property especially for substitution of wheat flour.

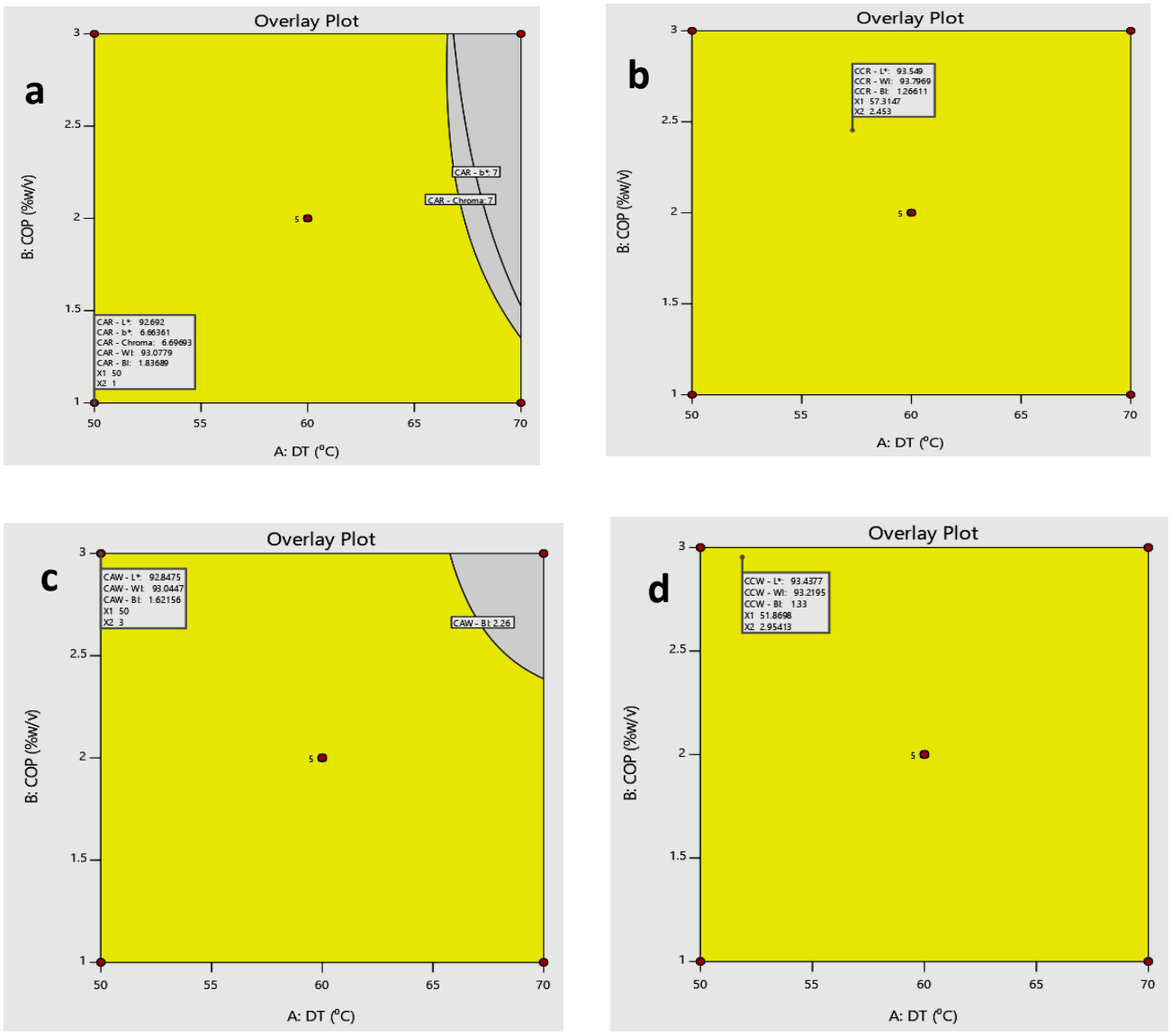

Figure 5.6. Overlay plots for optimisation of processing condition and colour properties of cassava flour. **a** - Cassava flour from red landrace pre-treated with citric acid; **b** - Cassava flour from red landrace pre-treated with calcium chloride; **c** - Cassava flour from white landrace pre-treated with citric acid; **d** - Cassava flour from white landrace pre-treated with calcium chloride. DT - drying temperature (ºC), COP- Concentration of pre-treatment (%w/v).Whiteness is an advantageous consumer property especially with respect to substitution of wheat flour.

5.4 Conclusion

This study reveals that the interactive effects of chemical pre-treatment and drying temperature results in cassava flours with good shelf stability, ncreased ash content and high fibre content which is good for the health of consumers. An increase in lightness and whiteness index of the flour shows that both calcium chloride and citric acid pre-treatment have the mitigating efficacy against enzymatic browning associated with postharvest physiological deterioration and processing of cassava flour. Information obtained in this study on optimisation of proximate composition and colour properties will be useful for cassava flour processing and enhancing its utilisation.

CHAPTER SIX: GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 General Conclusion of Research

It was hypothesised that cassava landraces used in this study may or may not differ in physical, chemical and structural characteristics. However, morphological, physical, chemical and structural characterisation of both landraces proves the alternative hypothesis to be valid. Morphological characterisation shows that the leaves stalk of the red and white cassava landraces possessed distinct virtual features. The stalk of the red landrace appeared brown while that of the white landrace appeared green in colour. This difference in colour of their leaves stalks aids the differentiation and easy identification of the cassava landraces at the point of harvest. Visual assessment of the periderm and cortex of both landraces shows that the red landrace was more reddish in appearance than the white landrace. The redness of the stalk and peels of the red landrace may be responsible for the nomenclature (red) given to the landrace. The parenchyma of both landraces appeared creamy upon visual assessment. However, instrumental assessment and samples paired t-test analysis showed that *L*, a*, b** values, whiteness and brownness index of both landraces significantly ($p < 0.05$) differed. It therefore implies that virtual assessment maybe misleading with regards to the colour properties of the root. Firmness and stiffness of the red landrace were significantly higher ($p < 0.05$) than the white landrace in their peeled form.

The root peel thickness influenced percentage flour yield. Results showed that the peel of the red landrace was significantly thicker ($p < 0.05$) than that of the white landrace. This result aligns with the percentage flour yield of both landraces with the yield of the white landrace significantly higher than the yield of the red landrace. Drimie and McLachlan (2013) stated that cassava grown in South Africa is mostly of the sweet variety. Values of cyanide obtained in this study for root and flour of both landraces are below 10 mg/kg, which implies that the landraces can be classified as sweet cassava. Cyanide content of the root was not significantly different ($p > 0.05$) in both landraces but the cyanide content of the flours was significantly ($p < 0.05$) different. Therefore, the root and flour of both landraces are safe for human consumption with minimal processing. The roots could be suitable for minimally processed foods such as chips.

Moisture content fresh cassava root of the red and white landraces were not significantly different ($p > 0.05$). Processing the root to flour allowed for sufficient loss of moisture. The moisture content of flours from both landraces was below 12% which makes them suitable for long storage. The pH value of the root was higher than that of their corresponding flour which was attributed to moisture loss during processing. As moisture

decreased, acidity increased. The ash, crude fibre and starch content of both landraces were not significantly ($p > 0.05$) different. The high starch content, approximately 80% dry weight basis, in these landraces makes them suitable for starch production.

Metabolic profiling of both landraces involved the identification and quantification of phenolic acids, FAMEs and sugars in the roots. Identified phenolic acids are vanillic, protocatechuic, syringic and gallic, trans-cinnamic, m-coumaric, p-coumaric, ferulic and caffeic acid. Data obtained in this study shows that the relative abundance of all the phenolic acids identified was higher in the white landrace. Hexadecanoic acid methyl ester, octadecanoic acid methyl ester, 9-octadecanoic acid methyl ester, (Z.Z) 9,12-octadecanoic acid methyl ester and alpha-Linolenic acid methyl ester are the FAMEs identified in the landraces. The amount of FAMEs was higher in the red landrace than the white and they were all significantly different ($p < 0.05$). All sugars identified were higher in the red landrace than the white landrace. The concentration of sucrose in both landraces was the highest in comparison to other sugars identified. High amounts of sucrose and low cyanide in the roots of both landraces confirm that they are of the sweet variety which is advantageous end-use properties. The scanning electron micrographs of flour from both cassava landraces revealed that most of the starch granules are spherical and truncated which is similar to reports in the literature that cassava starch had mostly oval and truncated shape. No conspicuous distinctions were observed in the granular morphology of flour from both landraces, with the granules dispersed yet clustered in no regular pattern. X-ray diffractometry shows that both landraces exhibit the A-type of starch crystallinity, just like wheat, which makes them a suitable substitute for wheat flour.

X-ray fluorescence spectrometry employed in determining the elemental composition, identified K, Mg, Ca, Al, P, Fe, Cr and Ti in flours of both cassava landraces. Independent samples t-test at equal variance shows that there was no significant difference ($p > 0.05$) in the elemental composition of both samples except for their K, Ca and P contents. These elements play major and essential roles in food chemistry and nutrition although they generally represent a small portion of food composition (Danbaba et al., 2015). The elemental composition shows that the flours contain essential elements vital for proper functioning of the human body.

Assessment of the effect of pre-treatment and drying temperature on the physicochemical properties of flours showed that processing variables did not influence the percentage starch content of the flours. Ash content significantly ($p < 0.05$) increased by drying temperature and concentration of pre-treatment. The increase in ash content as COP increased could be attributed to elements from pre-treatment solutions used in soaking fresh

cassava chips in water, before drying, which may be retained in the samples after processing. This observation was buttressed by ANOVA of the model parameters F- and p- values which indicate that the linear, interactive and quadratic effect of the experimental factors significantly influenced the ash content of cassava flour. Regression models generated by RSM and the coefficient of determination (R^2) of the ash content were relatively high between 0.9446 and 0.9710. The least and highest crude fibre was exhibited by samples of experimental run 1 (50ºC/1%w/v) and 4 (70ºC/3%w/v) in all the experimental groups It gives a clue that the crude fibre content increased with DT and COP. The response surface plots for all experimental groups had similar shapes.

This study revealed that pre-treatment of cassava flour with citric acid and calcium chloride influences the bulk density, water holding capacity and thermal properties of the flour. It was observed that the gelatinisation temperatures and enthalpy of flours from both landraces were high. This was attributed to the nature of starch (A-type) and non-starch components. The control samples exhibited low values of enthalpy within each experimental group. This indicates that the processing conditions may have an increasing effect on the enthalpy of the starch granules in the flour. Variation in the shapes of the response surface plots of the experimental groups for gelatinisation temperatures implies that the type of landrace and processing variables influenced the thermal properties of the flours. The optimal processing conditions for minimised gelatinisation temperatures and enthalpy are 50DT/1COP with a desirability of 0.805 for CAR, 57.23DT/1.75COP with 0.841 desirability for CCR, 70DT/1COP with 0.754 desirability for CAW and 50DT/1COP with 0.732 desirability for CCW.

A comparison of both pre-treatments shows that citric acid had a significant decreasing effect on WHC of the flour. This implies that citric acid may have a more compacting effect on the molecular structure of starch in the flour samples than calcium chloride. This observation is buttressed by the ANOVA of model parameters about the WHC of the samples generated by RSM. The decreasing effect of citric acid and significance of its quadratic effect on the WHC of flour from both landraces is further buttressed by the coefficient of determination (R^2) values obtained in the study. The coefficient of determination (R^2) of citric acid-treated experimental group was higher than calcium chloride treated experimental group. The trend observed for WHC of cassava flour was similar to the LBD. The LBD of all the CCR samples was higher than the CAR under the same concentration of pre-treatment and drying temperature. Results from the study infer that drying temperature influences the bulk density of the flours. Common to all experimental groups were the least LBD exhibited at 45DT/2COP) while the highest LBD was exhibited at 70DT/3COP for CAR and 74DT/2COP for CCR, CAW

and CCW. It can be observed that the least LBD was below the lower limit at drying temperature (45°C) while the highest LBDs were at the other extreme, 70 and 74°C.

Control samples had significantly lower ($p < 0.05$) *L** values than the experimental samples in all four groups. This shows that pre-treatment of cassava flour from both landraces with citric acid or calcium chloride enhances the lightness of the flour. This is buttressed by the significance of the quadratic effect of COP on the *L** values in all experimental groups. The contour plots for *L** values showed an increase as COP increased. Control samples of citric acid-treated flour exhibited significantly higher *b** values, chroma and browning index within their respective experimental groups. This implies that calcium chloride may have a reducing effect on the *b** value, chroma and brownness index of the flours. Change in colour (ΔE) is a function of the difference between the *L**, *a**, *b** values of the experimental samples and the control. Colour change was highest in cassava flour processed from the white landrace pre-treated with citric acid (CAW) and lowest in cassava flour processed from the red landrace pre-treated with citric acid. This gives a hint on the effect of landrace on the colour properties of cassava flour. Low *a** and *b** values with relatively high *L** and WI values recorded for all flour samples indicate that the samples are white. Whiteness is an advantageous consumer property especially for substitution of wheat flour.

This study reveals that the interactive effects of chemical pre-treatment and drying temperature results in cassava flours with good shelf stability, increased ash content and high fibre content which is good for the health of consumers. An increase in lightness and whiteness index of the flour shows that both calcium chloride and citric acid pre-treatment have the mitigating efficacy against enzymatic browning associated with PPD and processing of cassava flour. Information obtained in this study on optimisation of processing conditions for desired quality in cassava flour is valuable for processors. It maybe deduced that the concentration of pre-treatment and drying temperature applied were not adverse and the roots and flours maybe suitable for various food applications. The study proves the second research hypothesis that chemical pre-treatment and drying temperature may not influence the physical, chemical, thermal, and functional properties of cassava flour to be invalid

6.2 Recommendations for Further Research

In the course of this research, some areas that require further investigations were noticed and are highlighted:

1. Dietary fibre and resistant starch content of the cassava landraces.
2. Ascertain the amylose and amylopectin content of the flours and how processing influences their content.

3. The application of the pre-treated cassava flour for bakery and other food applications.
4. Consumer acceptance survey of cassava flour bakery products in South Africa.
5. The effect of chemical pre-treatment on the hydrocyanogenic content of the flour should be investigated.
6. A concrete mathematical relationship may be established between the bulk densities (LBD, PBD, and ΔBD) and particle size of cassava if further investigation is conducted.

6.3 Award Received, Consortium, Workshop, and Conferences Attended

Some research output and academic outings were birthed from this PhD study. They include attendance of a consortium, workshop, conferences, and receipt of an award of excellence.

6.3.1 Award of excellence

Udoro, E. O. & Anyasi, T. A. (2018). Cassava (*Manihot esculenta* Crantz) flour - classification, application and quality attributes as a function of processing variables. 5th International ISEKI_Food Conference Germany, 3 - 5 July 2018. Foods (MDPI Journal) award for best poster/short oral presentation.

6.3.2 Conference presentations

Udoro, E. O., Anyasi, T. A., & Jideani, A. I. O. (2020). Response surface optimization of cassava flour colour properties with varying chemical pre-treatment and drying temperature. AB 118, p. 115-116. Proceedings of the 44th NIFST Conference & Annual General Meeting, Nigerian Institute of Food Science and Technology (NIFST), Nigeria, 14th -15th October 2020. Theme: Agro and Food-Processing for Wealth Creation – The Nigerian Experience. Okafor G I, Oluwole O B, Alamu E A, Okolie N P, Alagbe E E, Ojo T I, Okafor J N, Agu H O, Okpala L C, Nicholas-Okpara V A N, Ogunji A & Shitty T A (Eds).

Nemaungani, P., Udoro E. O., Anyasi, T. A., & Jideani, A. I. O. (2019). Physico-functional changes associated with chemical pre-treatment of solar-dried flour from South African *Manihot esculenta* Crantz landraces. Proceedings of the 43rd Conference and annual general meeting, Nigerian Institute of Food Science and Technology, Awka, Nigeria, 14th -18th October, 2019. pp:199-200.

Udoro, E. O., Anyasi, T. A., & Jideani, A. I. O. (2018). Safety concerns of a major African staple: cassava (*Manihot esculenta* Crantz). 19th IUFoST World Congress, Mumbai, India. 23 -27 October, 2018. *(Poster presentation)*

Udoro, E. O., Anyasi, T. A., & Jideani, A. I. O. (2018). Comparative physicochemical profiling of alkaline and acid pretreated cassava (*Manihot esculenta* Crantz) flour from South African landraces. 19th IUFoST World Congress, Mumbai, India. 23 -27 October, 2018. *(Poster presentation)*

6.3.3 Consortium meeting

Long Term EU-Africa research and innovation Partnership on Food and Nutrition Security and Sustainable Agriculture (LEAP-Agri) NUTRIFOODS consortium, University of Pretoria South Africa. 4th – 6th September, 2019.

6.3.4 Workshop meeting

Udoro E. O. (2017). Cassava root (*Manihot esculenta* Crantz) characterisation and evaluation of process-induced changes on the functionality of its flour. Presentation at Trilateral workshop: United Kingdom, South Africa, Kenya, Increased food security through improved postharvest systems using renewable and sustainable energy for rural communities. Protea Hotel, Stellenbosch, South Africa. 4th to 8th September, 2017.

6.4 Publications

Udoro, E. O., Anyasi, T. A., and Jideani, A. I. O. (2020). Characterization of the root and flour of South African *Manihot esculenta* Crantz landraces and their potential end-use properties. *International Journal of Food Properties*, 23(1): 820-838. DOI: 10.1080/10942912.2020.1759625

Udoro, E. O., Anyasi, T. A., and Jideani, A. I. O. (2020). Interactive effects of chemical pretreatment and drying on the physicochemical properties of cassava flour using response surface methodology. *International Journal of Food Science*, Article ID 7234372. DOI:10.1155/2020/7234372

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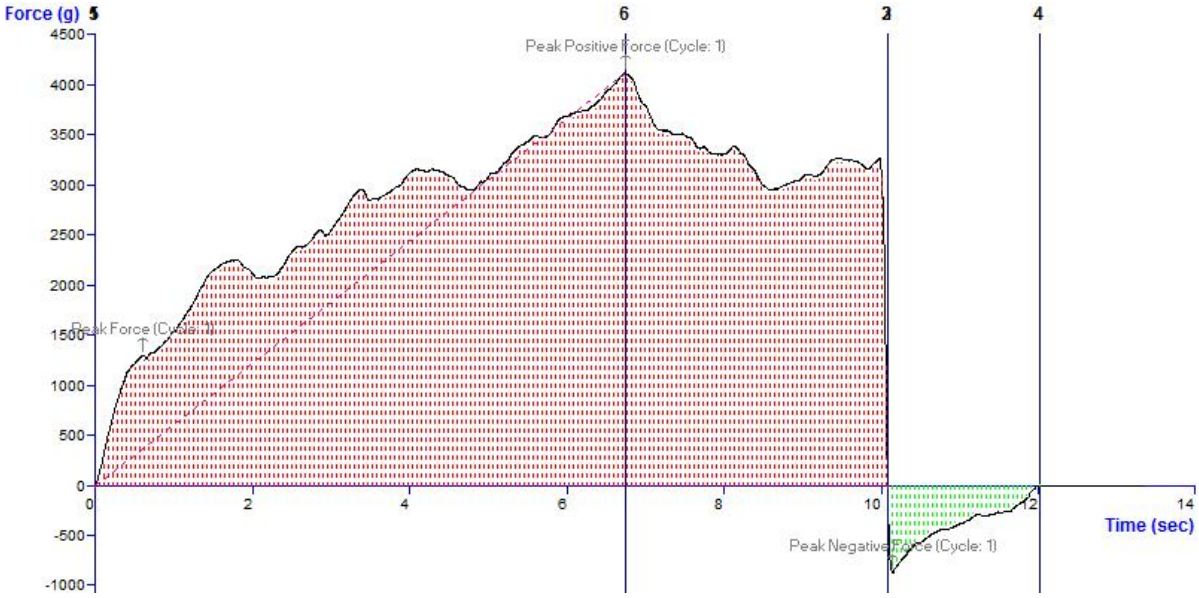
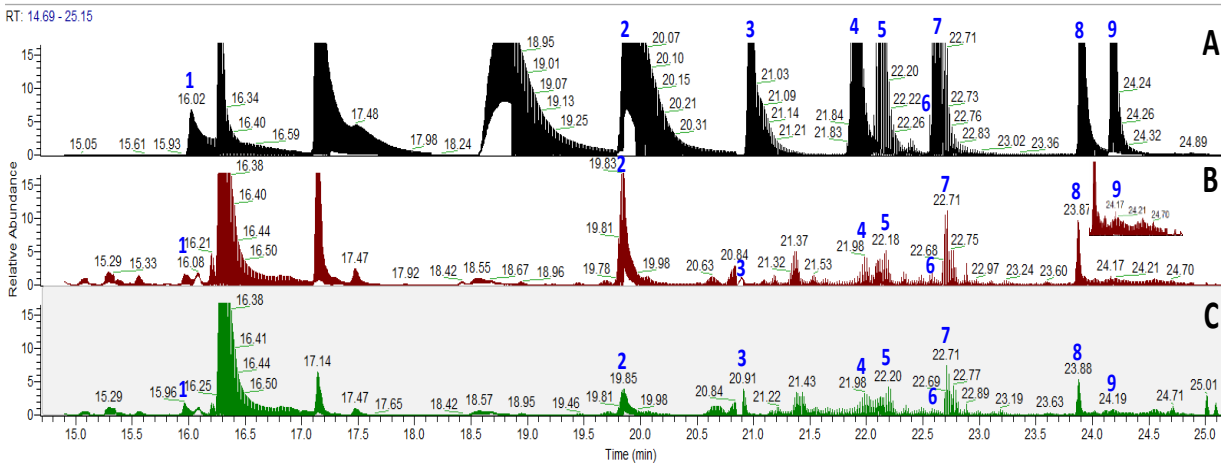
APPENDICES

Appendix A: A typical force-time curve for texture analysis of cassava root.

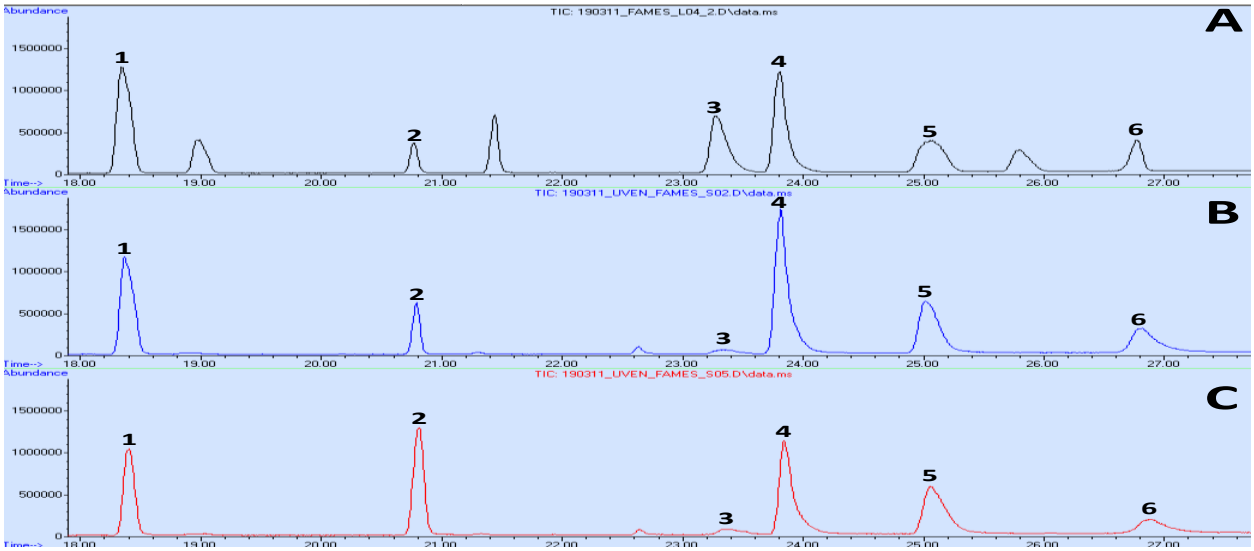


Appendix B: Overlayed chromatograms of phenolic acids in cassava root



A - 100 ppb phenolic standard mix, **B -** white cassava landrace, **C -** red cassava landrace. **1** = trans-cinnamic acid, **2** = vanillic acid, **3** = protocatechuic acid, **4** = m-coumaric acid, **5** = syringic acid, **6** = p-coumaric acid, **7** = gallic acid, **8** = ferulic acid and **9** = caffeic acid.

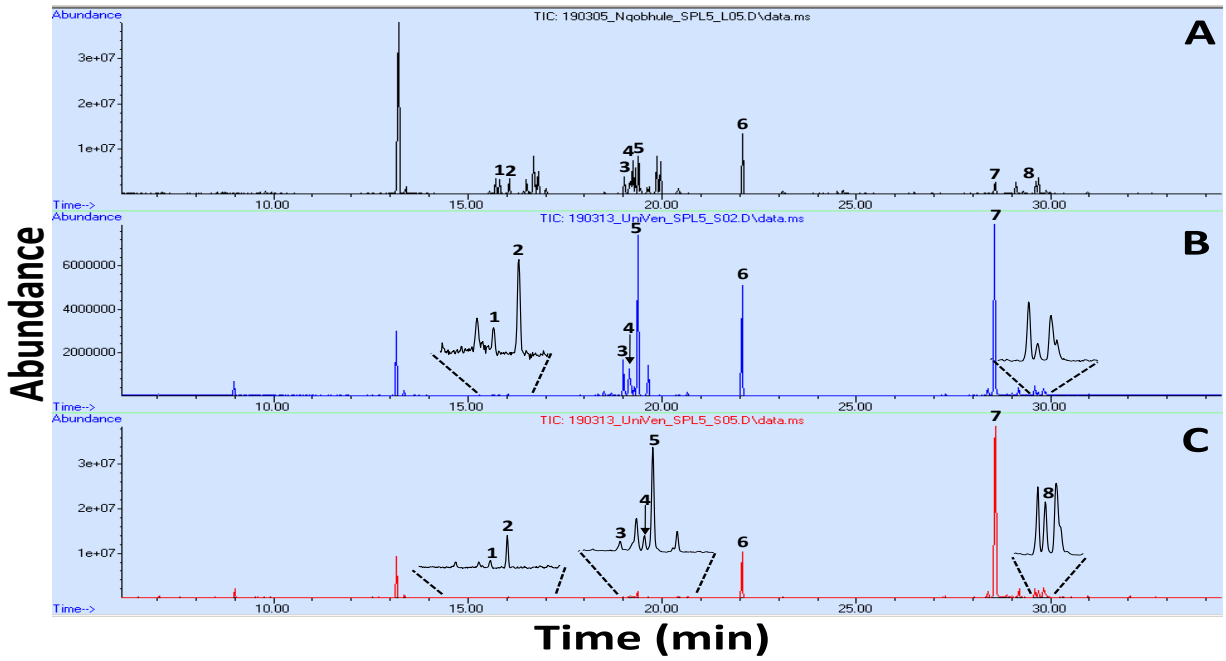
Appendix C: Overlayed chromatograms of fatty acid methyl esters in cassava root



A - 20 ppm standard FAME mix, **B** - red cassava landrace, **C** - white cassava landrace. **1** = hexadecanoic acid methyl ester (C16:0), **2** = heptadecanoic acid methyl ester (C17:0) (internal standard), **3** = octadecanoic acid methyl ester (C18:1), **4** = 9-Octadecanoic acid methyl ester (C18:1), **5** = (Z.Z) 9,12-Octadecenoic acid methyl ester (C18:2) and **6** = α-linolenic acid methyl ester (C18:3n3).

‘

Appendix D: Overlayed chromatograms of sugars in cassava root



A - 25 ppm sugar mix, **B** - red cassava landrace, **C** - white cassava landrace. **1** = L-arabinose, **2** = ribose, **3** = fructose, **4** = mannose, **5** = glucose, **6** = myo-inositol, **7** = sucrose and **8** = maltose.