VARIATION IN β-CAROTENE CONTENT AND PHYSICOCHEMICAL PROPERTIES OF ORANGE-FLESHED SWEET POTATO (*Ipomoea batatas* (L.) Lam) CULTIVARS GROWN IN LIMPOPO PROVINCE, SOUTH AFRICA

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ABSTRACT

In recent years, there has been a lot of interest in orange fleshed sweet potato (OFSP) [*Ipomoea batatas* (L.) Lam] cultivar, as a cheaper source of vitamin A. In Southern Africa, reports have shown that one in every three children has deficiency in vitamin A. This study investigated the variations in β-carotene (vitamin A precursor) content among selected OFSP cultivars grown in Limpopo Province, South Africa. In addition, the study investigated the effects of location on functional properties, total carotenoids and antioxidant profile of the sweet potato flour as well as their physicochemical, functional and nutritional properties. Four (4) sweet potato cultivars [*Dagga, Bophelo, Impilo* (orange fleshed) and *Mvuvhelo* (cream fleshed)] were obtained from two locations, namely, University of Venda (Univen) agricultural experimental farm and Tshiombo irrigation scheme (Tshiombo) and analysed for pasting, functional, physicochemical and nutritional properties. The sweet potato flours from these two locations were also analysed by computed tomographic (CT) scans for flour particle density. Mineral and microstructure of OFSP flours were analysed by scanning electron microscope (SEM). All measurements, except the tristimulus L* a* b* and other colour parameters from five replicates, were performed in triplicate. Statistical analysis was performed using a one-way analysis of variance and means were separated using Duncan's multiple range test; $P < 0.05$ was considered to be statistically significant. Pearson's correlation coefficients were used to determine the correlation among functional, physicochemical, nutritional and pasting properties of sweet potato flour. β-carotene content varied significantly between the two locations from 70.98 ± 0.8 µg/g (Tshiombo) to 86.09 ± 2.0 µg/g (Univen), among cultivars from 1.71 ± 0.0 µg/g (*Mvuvhelo*) to 201.50 ± 1.0 µg/g (*Dagga*) and among orange fleshed (*Dagga, Bophelo* and *Impilo*) flours from 28.38 ± 0.2 µg/g (*Impilo*) to 201.50 ± 1.0 µg/g (*Dagga*). There was a similar trend in the contents of α-carotene to that of β-carotene from the two locations, which ranged from 0.63 ± 0.0 mol/L (Tshiombo) to 1.01 ± 0.0 mol/L (Univen). *Mvuvhelo* had significantly the lowest α-carotene content (0.50 ± 0.0 mol/L) and *Impilo* the highest (1.28 ± 0.0 mol/L). The total carotenoids was highest for *Impilo* (7.56 µg/g) and lowest for *Mvuvhelo* (1.33 ± 0.1 µg/g) but did not vary significantly ($P > 0.05$) between

the locations. The antioxidant activity, based on 2, 2-diphenyl-1picrylhydrazyl (DPPH) free radical scavenging activity, was significantly highest in *Bophelo* (63.37 ± 0.8 mMAAE $ml^{-1)}$ and lowest in *Dagga* (26.93 ± 1.4 mMAAE ml^{-1}) flours. The ferric reducing antioxidant power (FRAP) varied significantly across the locations and among the cultivars and ranged from 14.45 ± 0.3 µMTE ml^{-1} (Tshiombo) to 17.40 ± 1.1 µMTE ml^{-1} (Univen). FRAP was significantly highest in *Impilo* (21.12 ± 2.1 µMTE ml^{-1}) and lowest in *Mvuvhelo* (8.16 ± 2.0 µMTE ml^{-1}). Total carotenoid content positively correlated with DPPH (0.486*), FRAP (0.830**), β-carotene (0.370*), and α-carotene (0.424*). The antioxidant content and the radical scavenging activity were significantly correlated (0.563**). Vitamin A content was significantly lowest (6.88 ± 0.38 µg/g retinol) in *Dagga* flour and highest (7.97 ± 0.25 µg/g) in *Impilo* flour. Protein content varied significantly across the two locations and ranged from 4.23% (Tshiombo) to 7.16% (Univen). *Dagga* had significantly the lowest (3.47%) protein content while *Impilo* had the highest (7.53%). The fat content of the flours varied significantly between the two locations ranging from 0.63% (Tshiombo) to 1.01% (Univen). The protein content was positively correlated 0.594** and 0.421* to vitamin A and fat contents respectively. The *Bophelo* colour luminosity was significantly lower (L* 76.2) than the other two OFSP, *Dagga* (L* 83.51) and *Impilo* (L* 82.07). *Bophelo* a* (14.09) also differed significantly across all sweet potato cultivars. All cultivars differed significantly in b* values. Colour intensity (ΔE*) ranged from 15.87 to 38.48 for *Mvuvhelo* and *Bophelo,* respectively. Chroma ranged from 87.52 to 84.95 for *Dagga* and *Mvuvhelo* respectively. Total starch content was significantly different in all sweet potato cultivars and ranged from 244.07 µg/100 g (*Bophelo*) to 325.04 mg/g (*Dagga*). Resistant starch differed significantly in all sweet potato cultivars ranging from 1.21 µg/100 g dwb (*Bophelo*) to 7.07 µg/100 g dwb (*Impilo*). Ash content was significantly different in all cultivars and ranged from 3.17% (*Dagga*) to 3.79% (*Bophelo*). Water holding capacity (WHC) varied significantly across the two locations and ranged from 1.44 g H_2O/g DM (Tshiombo) to 1.72 g H_2O/g DM (Univen). *Bophelo* had significantly the lowest (1.27 g H_2O/g DM) WHC and *Impilo* the highest (1.99 g H_2O/g DM). The ash content negatively correlated to total starch (-0.928**) but positively

correlated to WHC (0.654**). Peak viscosity differed significantly across all cultivars under study and ranged from 126.54 RVU (Bophelo) to 302.36 RVU. (*Dagga*). There was significant difference in trough viscosity across all cultivars ranging from 55.97 RVU (*Impilo*) to 280.78 RVU (*Dagga*). There were significant differences in the final viscosity across all cultivars ranging from 78.99 RVU (*Impilo*) to 319.26 (*Dagga*). Peak time viscosity varied significantly across all cultivars ranging from 3.87 min (*Impilo*) to 8.47 min. (*Bophelo*). *Dagga* cultivar had significantly the highest pasting temperature (94.16^{o}C) and *Bophelo* the lowest (77.75^{o}C). The setback viscosity was significantly highest 51.10 RVU (*Mvuvhelo*) and lowest 23.01 RVU (*Impilo*). All cultivars from the two locations consistently showed high potassium content ranging from 0.46% (*Impilo*) to 1.38% (*Dagga*) as compared to other minerals such as magnesium, calcium and chlorine which ranged from 0.16% (*Impilo*) to 0.34%. (*Dagga*). The CT scans revealed that there was a possibility of evaluating the effect of location on the density of granules of sweet potato flour and also the inherent differences in granular distribution of various sweet potato cultivars. SEM revealed high amounts of potassium, magnesium and chlorine. A profile of physicochemical and functional properties of flour of some of the OFSP cultivars produced in Limpopo province, South Africa has been drawn. This study also highlights the nutritional quality of the flour from four sweet potato cultivars and provides a ranking of flour to help the producers in the selection process. *Bophelo* flour was ranked the highest in terms of β- and, α-carotenes, total carotenoids and antioxidant capacity while *Impilo* was ranked highest in terms of functional properties and ash content. This study has highlighted significant differences in a number of parameters measured among the sweet potato cultivars. Therefore, in order to derive the maximum benefit from a given cultivar careful selection of sweet potato cultivars would be necessary.

Keywords: *Ipomoea batatas*; β-carotene; vitamin A, flour; carotenoids content; colour; functional properties; antioxidants; physicochemical; sweet potato cultivars.

DECLARATION

I, **Henry Silungwe**, hereby declare that this thesis for the degree of Doctor of Philosophy submitted to the Department of Food Science and Technology, School of Agriculture at the University of Venda has not been previously submitted at this or any other University. It is original in design and in execution, and all reference materials and work of others contained therein have been duly acknowledged.

Signature…….. **Date** ……

Henry Silungwe

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DEDICATION

To Nangale, Kalizya, Kambole, Niza and Mambwe
For their inspiration and company.

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LIST OF ACRONYMS

AMG	Amyloglucosidase
AOAC	Association of Analytical Chemists
ARC	Agricultural Research Council
ATP	Adenosine Triphosphate
ΔC	Chroma colour value
CIE	Commission Internationalé Eclairage
CIP	International Potato Centre
CRD	Complete Randomized Design
CSIR	Centre for Science and Industrial Research
DMSO	Dimethyl Sulfoxide
DPPH	Diphenyl-1-picrylhydrazyl
Δ	Change in measured attribute
ΔE*	Total colour difference
FAO	Food and Agriculture Organization
FRAP	Ferric Reducing Antioxidant Power
GOPOD	Glucose Oxidase-Peroxidase reagent
HPLC	High Performance Liquid Chromatography
IMS	Industrial Methylated Spirits (denatured ethanol)
MRC	Medical Research Council
NFCS	National Food Consumption Survey
OFSP	Orange Fleshed Sweet Potato
RAE	Retinol Activity Equivalent
RE	Retinol Equivalent
RGB	Red, green, blue
RS	Resistant Starch
RS_3	Type III Resistant Starch
RVA	Rapid Viscosity Analyzer
SAVACG	South African Vitamin A Consultative Group
SEM	Scanning Electron Microscope
SPSS	Statistical Package for the Social Sciences
TDF	Total dietary fibre
VAD	Vitamin A Deficiency
VITAA	Vitamin A for Africa
WFSP	White fleshed Sweet Potato
WHO	World Health Organization

WI	Whiteness index
YI	Yellowness index

LIST OF UNITS

E_a	Activation energy (watts per gram or kilojoules per mole)
cP	Centipoise (cP), 12 cP = 1 Rapid Viscosity Unit (RVU)
IU	International Units
ISU	International Standard Units
mMAAE	Millimole Ascorbic Acid Equivalent
nm	Nanometer
nA	Polarizing current
RVU	Rapid Viscosity Unit (1 RVU = 12 cP (cent poise)
TE	Trolox Equivalent

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CHAPTER 1: INTRODUCTION

1.1 Background Information

Sweet potato *(Ipomoea batatas* (L.) Lam) is a dicotyledonous plant that belongs to the family of *Convolvulaceae* (Woolfe, 1992). It is the fifth most important crop in developing countries (Rumbaoa *et al*., 2009) and rich in dietary fibres, minerals, vitamins, antioxidants such as phenolic acids and β-carotene (Ishida *et al*., 2000).

The sweet potato is a nutritious and generous source of food for humans and animals and therefore, an important and valuable staple crop for the whole world (FAO, 1997). It is also a source of starch, sugar and alcohol (Kozai *et al*., 1998). The crop grows throughout the tropics and subtropics (Scott, 1992), especially in Asian and African countries which account for about 95% of the world's sweet potato production (Mok *et al*., 1997) and can be harvested throughout the year.

In 2005, Harvest plus launched the breeding of crops to increase their nutritive value in China and some of the target crops included sweet potato. Since then, there has been an increase in the number of sweet potato cultivars that have been breed to increase their nutritive value such as high starch, dry matter and β-carotene contents as well as resistant to disease, high yielding and good taste. In addition to having economic benefits in the production of starch and snack foods (Wu *et al*., 2008), they are used as staple in poor areas.

Bio-fortification is a process of breeding nutrients into food crops, and this is a cost effective way of delivering micronutrients to sections of the population who do not have access to various diets (Saltzman *et al*., (2015). In this way, the rural poor people who have no access to commercially fortified foods have guaranteed access to the essential micronutrients in their diet. Commercial fortification on the other hand, refers to the addition or restoration of micronutrients to food which are lost during processing (Hoffpauer and Wright, 1994).

Sweet potato provides food security to many households in Africa and elsewhere. Reports from East Africa suggest that a large number of poor resourced farmers produce the crop as part of their house-hold food (Bashaasha *et al*., 1995). Recently, sweet potato

genotypes that have good amounts of Vitamin A, frequently referred to as orange fleshed sweet potato (OFSP) have been recognized as a potential means for saving thousands of children in Africa from Vitamin A deficiency-related diseases such as night blindness (van Jaarsveld *et al.,* 2005).

A number of strategies to combat vitamin A deficiency (VAD) are being promoted in developing countries and they include fortification (Dary and Mora, 2002); dietary supplementation (Beaton *et al*., 1993), diet diversification (Gibson and Hotz, 2001), and the use of bio-fortified staple foods (Welch, 2002). These strategies alter the quality of food ingested by having added essential nutrients. OFSP is one of the few foods that has high amount of highly bioavailable β-carotene which is a precursor of vitamin A (Faber and Laubscher, 2008). Bioavailability refers to part of the food that is consumed and becomes available to the body to utilize for physiological functions or storage in the body (Fraser and Bramley, 2004).

Some of the carotenoids are vitamin A precursors since they are absorbed and converted into vitamin A in the human body. β-carotene, an orange, red, yellow or purple coloured pigment found in plants and fruits is an organic compound and chemically, it is classified as a hydrocarbon and specifically as a terpenoid that reflects its derivation from isoprene units (Gao *et al.,* 2012). Carotenoids are responsible for imparting the orange colour in fruits and vegetables. β-carotene is generally regarded as the commercially most important and widely used carotenoid (Schierle *et al*., 2004).

Woolfe (1992), reported that OFSP cultivars contain high amounts of dietary fibre, starch, β-carotene, vitamin C, B6 and folate, as well as antioxidants which include; anthocyanins, tocopherol and phenolic acids. The chemical structure of β-carotene in OFSP is shown in Figure 1.

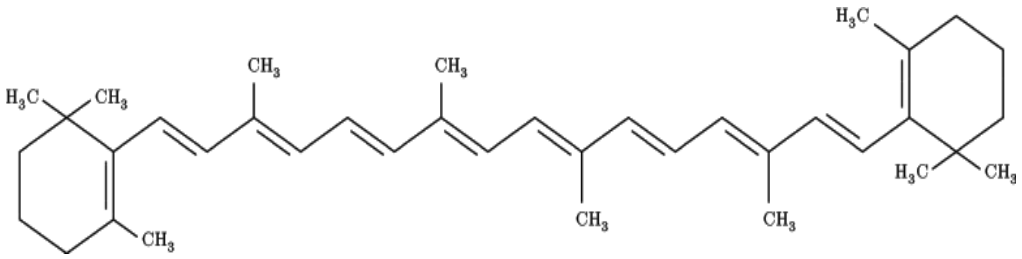
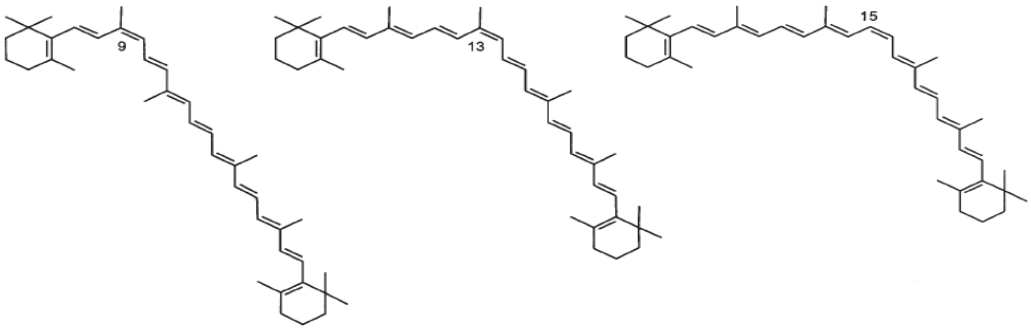


Figure 1: Chemical structure of β-carotene.
Adapted from Rodriquez-Amaya (2011).

Sweet potatoes have genes (1bMYB1 and 1bMYB2s) that code for the production of anthocyanins in the tuberous roots of sweet potato (Mano, *et al*., 2007). Mano *et al*., (2007) have also reported that the expression of 1bMYB1 is adequate to induce all structural anthocyanin genes and anthocyanin accumulation in the flesh of tuberous roots.

According to Schierle *et al*., (2004), β-carotene has three predominant isomers, 9-*cis*, 13-*cis* and 15-*cis*. The predominant geometrical isomers of β-carotene are shown in Figure 2.



9- *cis*- β-carotene, 13- *cis*- β-carotene and 15-*cis*- β-carotene.

Figure 2: Predominant geometrical isomers of β-carotene: *all- trans*-carotene.
Source: Schierle *et al*. (2004).

The limited financial resources of many poor rural households dictated for alternative strategies to food supplementation and fortification (Hagenimana and Louw, 2000). FAO (1997) suggested the use of varieties that were breed for higher mineral content as a sustainable food-based strategy to increase food security for large rural populations that are remote from other food intervention strategies. According to a Medscape report, edited by Ansstas, (2014), VAD in the USA is prevalent among malnourished, elderly and chronically sick populations, but more prevalent in developing countries. Vitamin A is important for a health skin, immune system and good eye and

health vision. However, excess vitamin A is toxic, but β-carotene from food is good because the excess β-carotene is stored in the body and only converted when needed for physiological processes (Ansstas, 2014).

1.2 General profile of sweet potato cultivars under study

Four sweet potato varieties, three OFSP (*Dagga, Bophelo and Impilo*) and one CMFSP *(Mvuvhelo*), were selected from among the commonly grown varieties in Limpopo Province. The flour from the selected varieties were analysed for functional, pasting, nutritional and physicochemical properties.

The four (4) sweet potato cultivars used in this study (*Dagga, Bophelo, Impilo and Mvuvhelo*) are shown in Figure 3. Their general morphological and physiological descriptions are shown in Table 1.

Figure 3: Profile of sweet potato under study.
(A) *Bophelo*; (B) *Impilo*; (C) *Dagga* (199062.1); (D) *Mvuvhelo.*
Source: ARC, (2015).

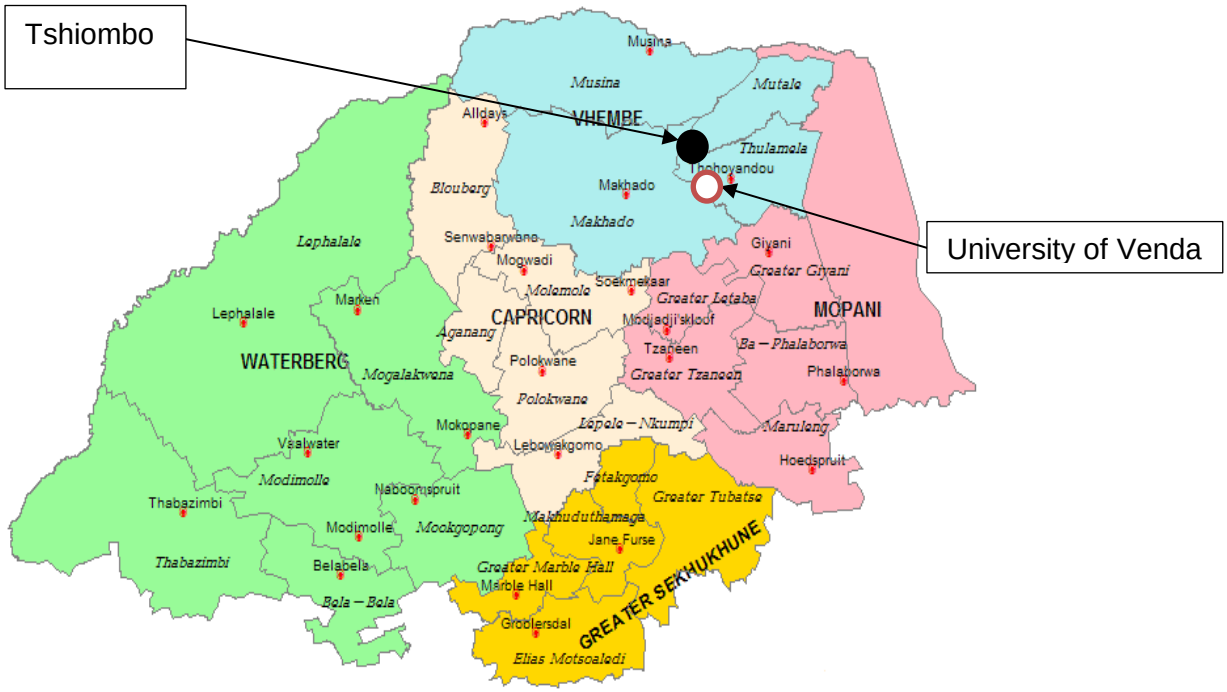
Table 1: General profiles of sweet potato cultivars under study

Sweet potato cultivar	Shape	Skin colour	Flesh colour	Advantages	Disadvantages	Origin
Dagga (199062.10)	Heavy oblong-elliptic	Cream, slight orange	Yellow orange	Very high yield, medium – good test. Quick maturing.	Few constrictions, veins, medium dry (21.4%)	International potato centre (CIP)
Bophelo	Round elliptic	Pale orange	Orange	Good yield, uniform tuber, Medium β-carotene, medium dry (22.5 %), good test.	Few grooves and constrictions	ARC- Roodeplaat; meaning, life
Impilo	Round to round elliptic	Yellow orange	Pale orange to orange	Good yield, uniform tubers,	Medium dry (21.4%), Medium to low beta carotene	ARC- Roodeplaat; meaning health (Xosa)
Mvuvhelo	Round – round elliptic	Pale cream	Pale cream	High yield. Dry (22.4%), Average to good taste, quick maturing	Some grooves	ARC- Roodeplaat; Meaning, calabash (Venda)

Source: Agricultural Research Council (ARC, 2015).

Map 1 shows that location of South Africa's nine provinces and Map 2 shows the location of sweet potato sample sites.

Map 1: South Africa and its nine provincial demarcations. Source: www.Local government.co.za (22/04/2016)



Map 2: Location of sweet potato sample sites in Vhembe District, Limpopo province. *Source: www.Local government.co.za (22/04/2016)*

1.3 Problem statement

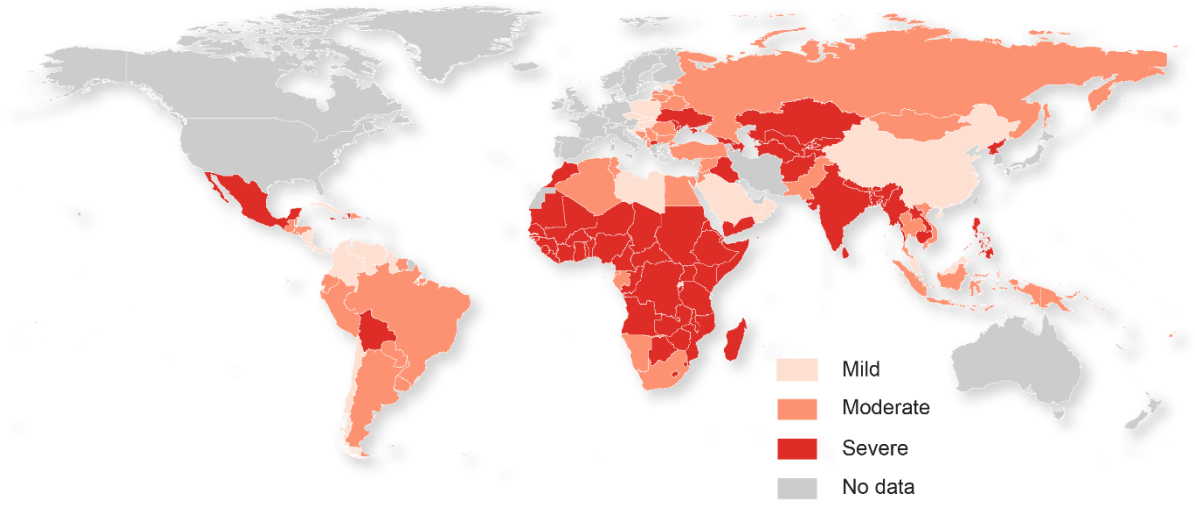
Vitamin A deficiency (VAD) is of great health importance in Africa, it affects a large number of people, mainly children and women in poorly resourced areas (Faber and Laubscher, 2008). Current data obtained from developing countries indicate that about 48 - 70% of children are vitamin A deficient, with 200 out of 1000 babies born dying from malnutrition and more than 600 out of 100 000 mothers dying during child birth. All these deaths are related to VAD (Yanggen *et al.,* 2006).

Although most households have an abundance of plant sources rich in β-carotene, children in developing countries still suffer from VAD. This may be due to lack of knowledge, lack of care and the apparently lower vitamin A activity of the pro-vitamin (β-carotene rich foods (Louw, 2001). South Africa, has one in three children with a low vitamin A status; with the rural areas being most affected (SAVACG, 1996). During the early 1960s, the WHO stated that South Africa had a clinical VAD problem as VAD in South Africa was suspected of being responsible for as many as one (1) out of every four (4) children's deaths (Department of Health, 2002). The effect of VAD include poor nutrition; poor health: morbidity and mortality, lost productivity and hence persistent poverty and reduced economic growth. Labadarios *et al.,* (2000), report that five of the ten most seriously affected provinces by VAD include Limpopo, KwaZulu Natal, Mpumalanga, North West and Eastern Cape.

1.4 Rationale

Animal origin foods which include; chicken liver, kidney, fish and fish oils, butter, animal milk, beef liver, and egg yolk contain vitamin A in its active forms for example retinol which can be used directly and easily by the human body (van Jaarsveld *et al*., 2005). However, children and women in poorly resourced developing countries have only limited access to vitamin A rich animal foods, including milk (Hagenimana and Low, 2000). OFSP cultivars have emerged as a promising plant source with a high β-carotene content that can make a significant contribution to vitamin A intake by individuals at risk of vitamin A

deficiency (Leighton, 2007). The problem of VAD is more prevalent in Africa, South America and Asia. Map 3 shows the vitamin A status of populations in various continents.



Map 3: World-wide vitamin A deficiency profile.
Source: http://www.harvestplus.org/content/vitamin *25/04/2016*

However, studies have shown that there is a wide variation in β-carotene content among the OFSP cultivars. Sweet potatoes, like other living organisms are also affected by environmental factors. Laurie *et al*. (2012), reported variations in β-carotene content as affected by irrigation and fertilizer levels. Bovell-Benjamin (2007) suggested genetic variation among the OFSP cultivars as the other factor that influences the level of β-carotene content in sweet potato cultivars.

1.5 Hypotheses

(i) There is no significant difference in the variation of β-carotene content among the OFSP grown in two different locations in Limpopo Province.

(ii) There is no difference in the physicochemical properties of OFSP cultivars grown in two different locations in Limpopo Province.

(iii) There is no difference in the functional properties of OFSP cultivars grown in two different locations of Limpopo province.

1.6 Importance of the study

The information on the extent of β-carotene content variation in sweet potato cultivars grown in Limpopo province is scarce. This study, will therefore, provide information that can be used for the selection of sweet potato cultivars with superior β-carotene content as a provitamin precursor. It is also important to have an in depth understanding of the physicochemical characteristics of these sweet potato cultivars for purposes of processing and utilization. Sweet potatoes, like other root crops, are highly perishable and hence, the production of sweet potato flour may provide scope for longer shelf-life. This would make sweet potato with its nutritional benefits available to both urban and rural household communities.

1.7 Aims of the study

(i) To determine the level of variation in β-carotene content among five selected orange fleshed sweet potato cultivars grown in different locations in Limpopo Province.

(ii) To compare the physicochemical, nutritional and functional properties of selected OFSP cultivars grown in different locations in Limpopo Province.

1.8 Specific objectives

(i) To determine the variation in β-carotene content in the flour of four sweet potato cultivars grown in Limpopo province, South Africa for purposes of selection and for specific food applications.

(ii) To determine the total carotenoid content and antioxidant profile of OFSP cultivar flour

(iii) To profile the physicochemical and nutritional properties of OFSP cultivar flour.

(iv) To determine the effect of location on the functional properties of OFSP cultivar flour.

1.8.1 Envisaged outcomes

The following outcomes are envisaged from this study:

(i) Knowledge of total carotenoid content and antioxidant levels of orange sweet potato cultivars under study will be generated and disseminated.

(ii) Functional properties of the four sweet potato cultivars elucidated.

(iii) Ranking of the orange-fleshed sweet potato cultivars understudy in terms of overall functional, nutritional and physicochemical properties for selection purposes.

1.9 Experimental design

A two by four (2 x 4 x 3) factorial experiment in a complete randomized design (CRD) was applied. Three orange fleshed (*Dagga, Bophelo, Impilo*) sweet potato cultivars and one cream fleshed (*Mvuvhelo)* sweet potato cultivar were obtained from two sites, namely, Tshiombo irrigation scheme and University of Venda experimental farm. The university of Venda experimental farm is located about 2 km west of Thohoyandou town in the Limpopo Province of South Africa, at approximate latitude 22º58'S and longitude 30º26' E; altitude 596 m above sea level. Tshiombo irrigation scheme is situated approximately 30º 45’ East longitude and 22º 79’ South latitude; altitude 650 m above sea level and 25 km from Thohoyandou town.

All measurements (except tristimulus L* a* b* colour in five replicates) were conducted in triplicate and the results obtained are expressed as mean ± standard error (SE). Statistical analysis was performed using a one-way analysis of variance (ANOVA) and means of results for each experiment were separated using Duncan’s multiple range test (Teow *et al.,* 2007). P values < 0.05 were considered to be statistically significant. Pearson’s correlation was used to determine correlation properties of sweet potato flour. All statistical analyses were conducted using IBM Statistical Package for Social Sciences (SPSS version 22.0, IBM Inc., New York, USA.) and STASTICA version 10 (Stat Soft)..

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam) is one of the food security crops for resource poor farmers of the world and feeds millions of people in developing countries (Ewell and Mutuura, 1994). It is a native of central and South America and belongs to the morning-glory family, *Convolvulaceae* (Ware, 1980). The crop is able to adapt to tropical, subtropical and warm temperate regions and provides high amounts of starch to staple diets (Martin and Delshpande, 1988). Sweet potato is one of the most efficient food crops in terms of calorific value per land area (Ndangui *et al*., 2014). In poor resourced areas, sweet potato is often cultivated in marginal soils under low agricultural input conditions, with limited water for irrigation (Laurie *et al*., 2012; Lebot. 2009; Woolfe, 1992). Sweet potato roots are consumed for carbohydrates, dietary fibre and vitamin A and C.

Among the staple food crops of the world, sweet potato production ranks seventh (7^{th}) after wheat, rice, maize, potato, barley and cassava, based on weight, according to documents of Food and Agriculture Organization and ranks fourth (4^{th}) in the tropics (FAO, 2008). Several attributes of sweet potato account for its prominence and the recent resurgence in interest in the crop (Clark *et al*., 2013) and these include the breeding of sweet potato varieties with high β-carotene content.

Sweet potato is a very important market vegetable which fits into a rotation with basic 3 - 6 month crops (Williams, 1991). It is among the most under-exploited of the developing world's major crops (Tomlins *et al*., 2012). Its wide adaptation to various climatic and soil conditions, coupled with its easy to propagate, makes it a good candidate crop for the resource poor households to provide the much needed nutritional benefits.

2.2 Botanical classification of sweet potato

Sweet potato belong to the plantea (plants) kingdom and has 9 subclasses. *Ipomoea batatas* species is the most important of the *Ipomoea* genus. Table 2 shows the sweet potato classification based on Carolus Linnaeus classification.

Table 2: Sweet potato classification

Kingdom	Plantea - Plants
Sub kingdom	Tracheobionta – Vascular plants
Super division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida - Cotyledons
Sub class	Asteridae
Order	Solanales
Family	Convolvulaceae – Morning glory family
Genus	*Ipomoea* (L.) – Morning glory
Species	*Ipomoea batatas* (L.) – Sweet potato

Source: *www.Botanical- online.com/English/sweet_ potato.htm* (23/03/2016).

2.3 Climatic requirements

Sweet potato is a tropical herbaceous dicot vine fairly and a drought resistant vegetable. A light frost kills the leaves and a soil temperature of 10°C or below results in a chilling damage. It is better secured when the rainfall is abundant in the early part of growth. The best growth takes place at 22°C - 25°C; and this temperature is also good for maximum starch content (Bhardwaj *et al.,* 2004). It is adapted to many agro-ecological zones and can give a good yield with few inputs (Ewell, 2001). It is widely grown throughout the tropics and warm temperate regions of the world between 40^{o} N and S of the equator and between sea level and 2300 m (Bourke, 1982; Shukla, 1976).

Sweet potato which is a long-night plant requires maximum light for development, even though, the tuber growth seem to be not only influenced by photoperiod but also by other factors such as temperature fluctuations, and short-days favouring folia growth (Boggess *et al.,* 1970).

2.4 Sweet potato types

There are two broad categories of sweet potatoes that are used as food source for human. The two types are staple and dessert types. The staple type is most widely grown in the tropics. It generally has white to cream coloured flesh and higher content of dry matter, starch and protein than the dessert type. The dessert type generally has orange flesh and a higher content of β-carotene and simple sugar than the staple type (Clark, 1998). The dessert type sweet potatoes grown in Limpopo Province includes; *Impilo*, *Vhupilo/Bophelo*, *blessbok*, *Ndou*, *Mvuvhelo*, *Deureguard* and *Resisto* (ARC, 2011). Figure 4 shows some examples of staple and dessert types.

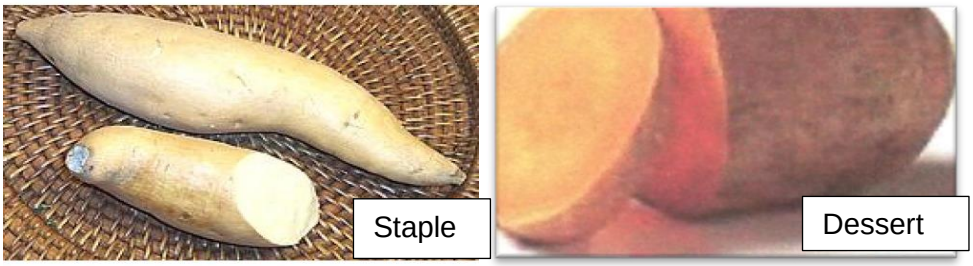


Figure 4: Staple and dessert type sweet potatoes.
Source: ARC, (2015); Clark (1998).

2.5 Utilisation

The tubers are consumed in various food forms including: Fresh, boiled, fried, sun-dried, and oven dried. The leaves especially the tender ones are picked and fried or boiled as relish.

2.6 Forms in which sweet potatoes are currently processed

In Southern Africa, sweet potatoes have been traditionally consumed in boiled form. However, in recent times, sweet potatoes are also consumed in various forms including fried sweet potato chips (Figure 5), and dried forms (Figure 6). In Zambia, in addition to boiling, sweet potatoes are sliced and dried forming a processed product known as “vitonya” in Lungu language (Zambia). These dried sweet potatoes known as “vitonya”, are consumed during off

season by re-boiling with peanut butter to form a sweet potato stew, or roasting. Figure 7 shows the forms in which sweet potato is processed.

Figure 5: Boiled, sliced and dried, fried sweet potato chips.
Picture taken by author.

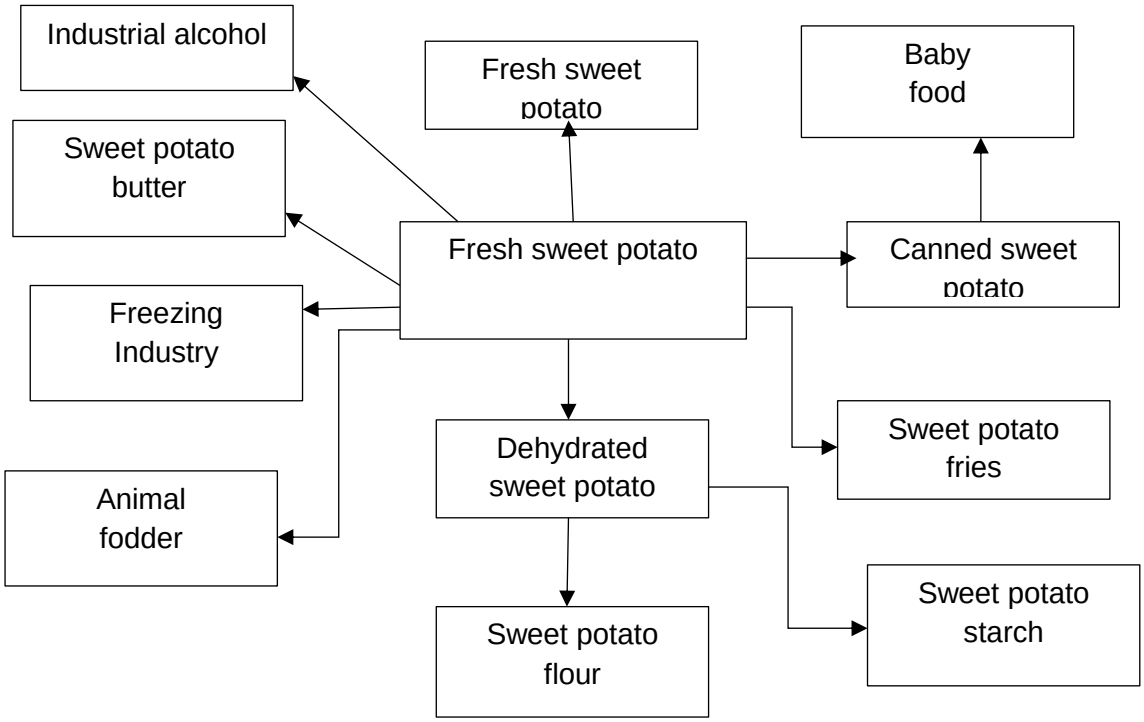


Figure 6: Forms in which sweet potato is currently processed.
Source: DAFF (South Africa), 2011.

Owori *et al*. (2007) describes products developed from OFSP, soya and maize flours by Kasawo Grain millers, Kampala. Figure 8 shows sweet potato products from Eastern and central Africa.

Figure 7: Sweet potato processed products from Eastern and Central Africa.
Top left; boiled fresh roots, bread and *Mandazi*. Bottom left; cookies, crisps, juice.
Adapted from Owori *et al*. (2007).

2.7 Annual sweet potato production in South Africa

Over 95% of the global sweet potato crop is produced in developing countries, where it is the fifth (5^{th}) most important food crop in terms of fresh weight (FAOSTAT, 2009), In 2004, approximately 129,536,275 million tons were produced in more than 100 countries (CIP, 2005).

The main producing regions in South Africa are Northern Cape, Western Cape, Limpopo, Free State, Eastern Cape and Gauteng. The production in 1999 and 2000 was 52,000 tons and increased in 2001 to 57,000 tons. The production decreased in 2002 to 51,000 tons and in 2003 decreased to 50,000 tons and then increased significantly in 2004 to 55,000 tons. The production dropped to its lowest in 2006 to 48,000 tons (DAFF, 2011).

The drop in production of sweet potato can be attributed to climatic conditions and increased cost of production. In 2008 (49,000 tons), there was also a 4% decline in production compared to 2007 (50,000 tons). The production of sweet potatoes increased in 2009 (63,000 tons) (NDA, 2008 and 2010). Figure 9 shows South Africa's annual production of sweet potato over a ten year period from 2001 to 2010.

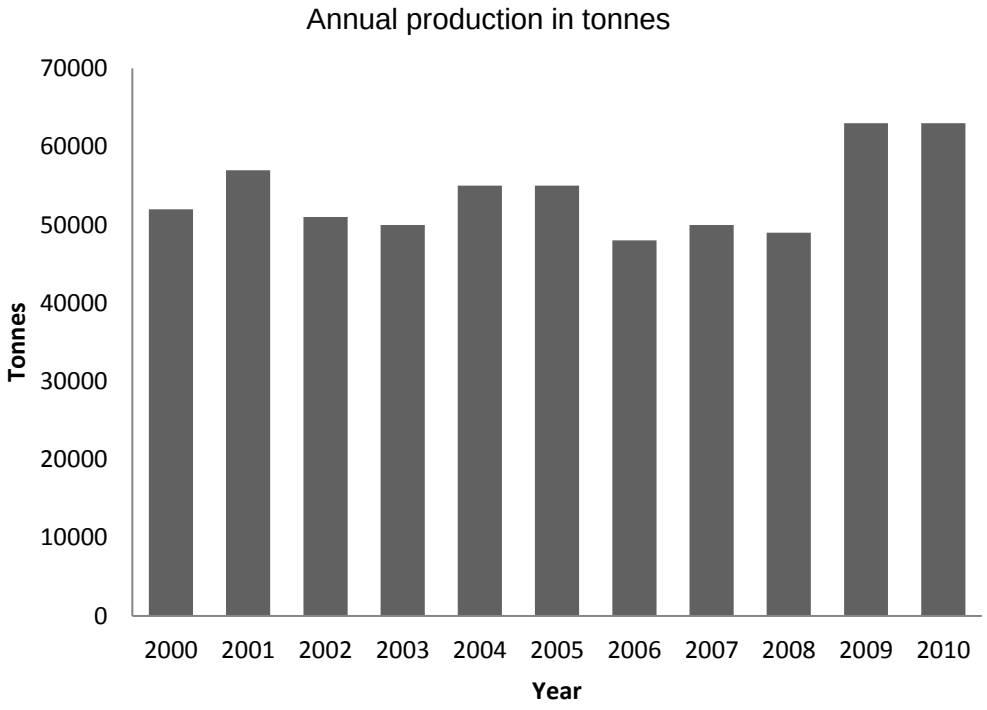


Figure 8: South Africa's annual sweet potato production statistics.
Source: DAFF (South Africa), (2011).

Even though the production at household level is comparatively low, some households produce adequate amounts of sweet potato with extra for sale to other households. In Limpopo province, the production at household level is predominantly managed by women.

2.8 Sweet potato value chain.

Sweet potato value chain process includes the producers of sweet potatoes, pack house owners for cleaning, grading and quality control, cold storage and transportation facilities, value additions. The potato value chain depicting the above processes is shown in Figure 10.

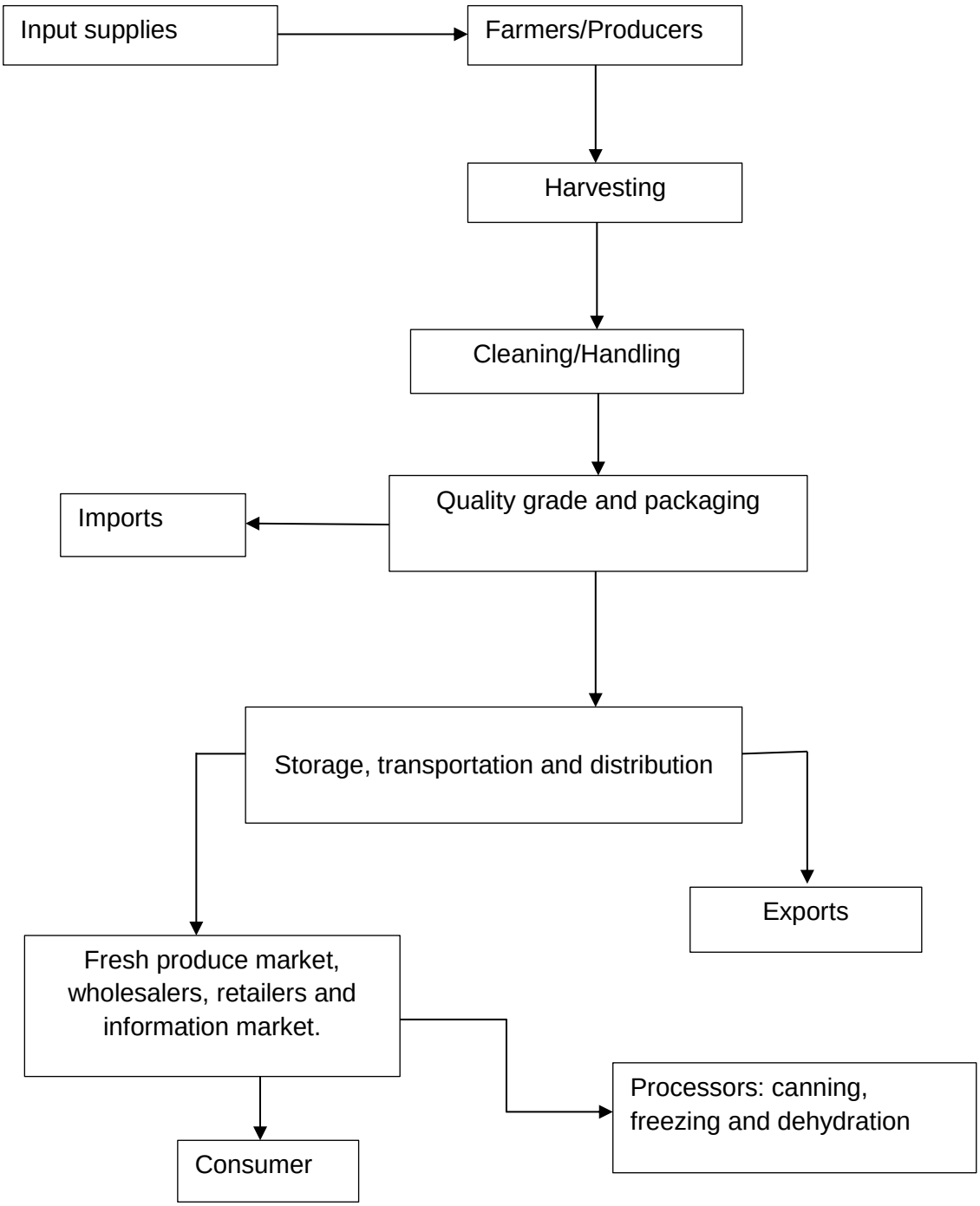


Figure 9: Summarised sweet potato value chain in South Africa.
Source: DAFF, South Africa, (2011).

2.9 Nutritional and health benefits of sweet potato

Sweet potato is a high energy food. Its storage roots have a total carbohydrate content ranging from 25 - 30% of which 98% is considered easily digestible (Bovell-Benjamin, 2007). Sweet potato, especially the orange fleshed sweet potato cultivars, are excellent sources of pro-vitamin A carotenoids (Clark, 1998). Sweet potatoes contain high levels of phenolic compounds that have been reported to have a potential for use as functional food for improving human health.

Sweet potato phenolics were found to inhibit the growth of human colon, leukaemia, and stomach cancer cells (Kurata *et al.,* 2007). It has been reported to ameliorate diabetes in humans (FAO, 1997), and inhibit *in vitro* growth of viruses and fungi (Chandler *et al.,* 1988). Sweet potato are rich in starch, carbohydrates, β-carotene, dietary fibre vitamin A and vitamin B6 (Austin, 1988).

The nutritive value of sweet potato compared to other vegetable is considerably higher (Woolfe, 1992). The nutritive value considered by the centre includes, vitamin A, complex carbohydrates, Iron, Vitamin C, protein calcium and iron.

Hossain *et al*.(1987) reported sweet potato tubers to contain several constituents including two mono amino phosphatides, lecithin, and cephalin, organic acids such as oxalic acid, others include methoxyl, uronic acid, phytin, colouring matter especially for coloured cultivars, resins, tannins, phytosterol and phytosterolin. Besides the antioxidant capacity of OFSP, sweet potato contains some essential mineral. Table 3 shows a summary of some of the essential minerals found in sweet potatoes and their functions.

Table 3: Essential minerals in sweet potatoes and functions

Mineral	Function
Copper	Aids the body in the storage of Iron and maintains healthy joints and supple skin.
Manganese	Helps the body to form the bone and metabolism of energy from carbohydrates, protein and fats.
Magnesium	Helps the body to produce energy, makes protein and signals muscles to relax and contract.
Phosphorus	Helps generate energy, forms major component of bones and teeth. Promotes cell growth and repair.
Potassium	Ensures proper hydration by helping in maintaining proper electrolyte balance in the body and health maintenance.

Source: Gibson *et al.* (2001).

2.9.1 β-carotene

Mangles *et al*., (1993), and Mdziniso *et al*., (2006) reported β-carotene as a member of the carotenoid family, a group of antioxidants that includes lycopene, zeaxanthin, lutein and α-carotene. β-carotene and α-carotene are the only ones among the carotenoids to be converted to significant amounts of Vitamin A in the human body and are by far the largest quantities found in vegetables and fruits.

Rodriquez-Amaya *et al*. (2011) reported that carotenoids were among the important food constituents imparting food quality and human health. Besides pro-vitamin A activity, some of these compounds, have also been credited with other functions beside the provitamin A activity, especially β-carotene. Recent studies (George *et al*., 2004; Ruxton *et al*., 2006; Saura-Calixito and Goñi, 2006; Wang *et al.*, 2011; Wu *et al*., 2004a and Wu *et al*., 2004b) have postulated health promoting effects including, immune enhancement and

reduction of the risk of developing degenerative diseases, cancer, type II diabetes, cardiovascular diseases, cataracts and macular degeneration.

Carotenoids are prone to geometrical isomerisation, so they can exist as all-*trans* (all-E) or *cis* (Z) isomers (Melendez-Martinez *et al*., 2013). The formation of the latter as analytical artefacts is as a result of light, heat and other conditions, both in extracts and in foods (Aman *et al*., 2005; Marx *et al.,* 2003; Pott *et al*., 2002; Updike and Schwartz, 2003; Vásquez-Caicedo *et al*., 2005). The chemical structures of carotenoids that are important to human nutrition are shown in Figure 12.

Beta-carotene as a carotenoid found naturally in plants and serves as an accessory to photosynthesis. β-carotene is not only responsible for the pigmentation in orange coloured vegetables and, but also contributes to the pigment in red, yellow, and green coloured fruits and vegetables. Though some food sources are rich in β-carotene including cantaloupe, broccoli, spinach, and palm oil, carrots are the major supplier of β-carotene in most human diets (Beam, 2011). β-carotene as an antioxidant is also vital for normal functioning of eye structures, acting as a filter, protecting the fibre portion of the lens against light-induced damage. Deficiency in vitamin, as well as vitamins A and zinc; leave the lens of the eye susceptible to free radical damage and the formation of cataract (Welch, 2002).

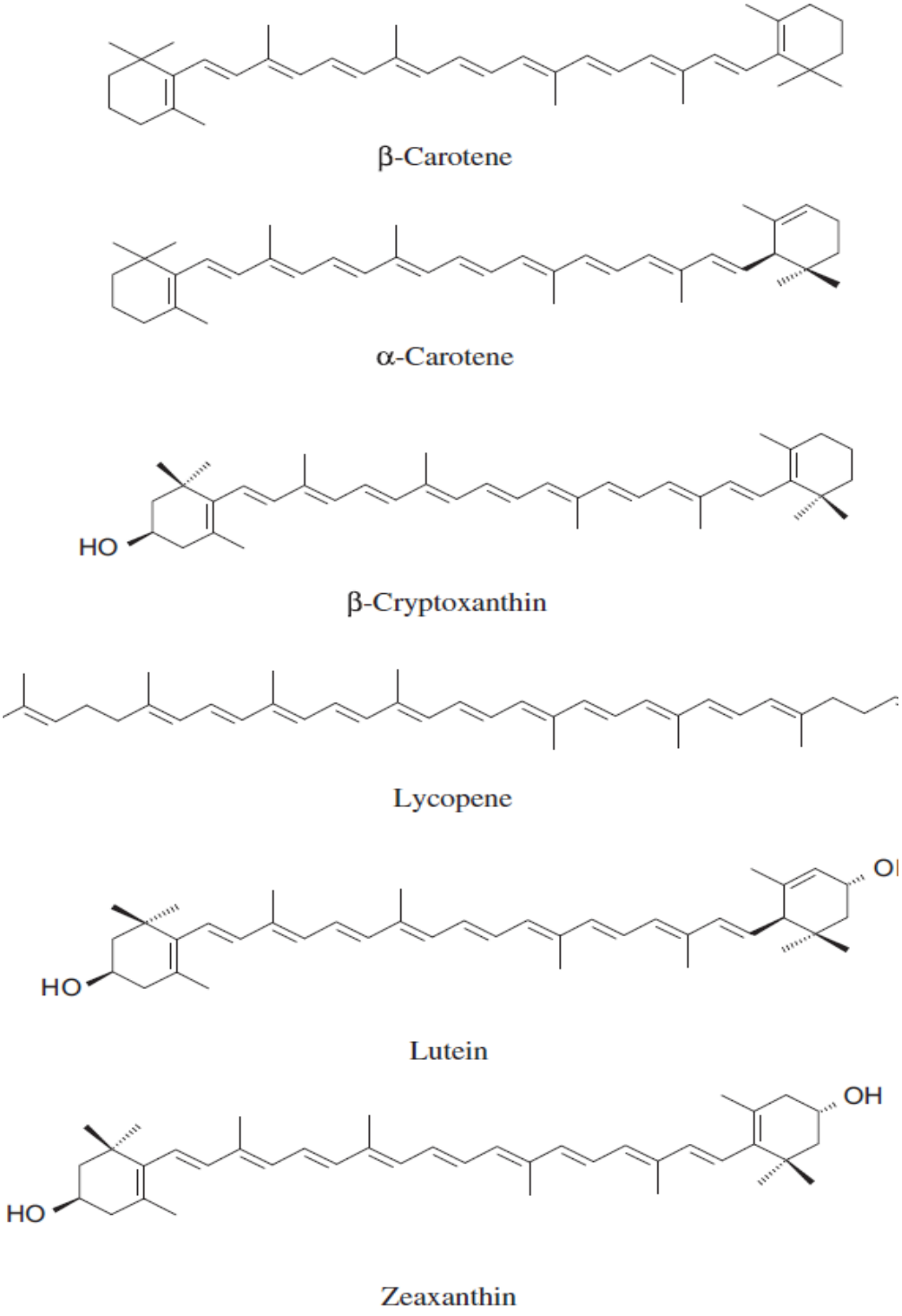


Figure 10: Structure of carotenoids shown to be important to human nutrition. Source: Rodriquez-Amaya *et al.,* (2011).

2.9.2 Vitamin A

Vitamin A (retinol) as an essential nutrient is needed in small amounts by human body for normal functioning of the eyes, growth and development and maintenance of epithelial cellular integrity, immunity and reproduction (FAO/WHO, 1998). FAO, (1997) defines VAD as tissue concentration of vitamin A low enough to have adverse health consequences even if there is no evidence of xerophthalmia.

Over 600 carotenoids have been characterized and at least 30 have the ability to be converted into vitamin A once ingested. β-carotene is one of them and has been termed the most active of the carotenoids because of this. While vitamin A can have toxic effects if taken in large doses, β-carotene only coverts itself into vitamin A when needed by the human body. As β-carotene, gets converted into vitamin A, it helps in the vision, particularly night blindness (Cooper, 2011). Some literature have reported that people who frequently live on diets rich in β-carotene (vitamin A precursor), lower risks of suffering from arteriosclerosis, cancer and degenerative eye diseases than those that consumed less (YanHu, 2011).

The FAO/WHO (1998) defines dietary requirements for micronutrients as an intake level which meets specific criteria for adequacy thereby minimizing risk of nutrient deficiency or excess. The mean requirement and safe levels are: for infants to 6 months (325 µg vitamin A/day; pre-school to 6 years (200 – 400 µ RE/day) and adults (4.8 µg RE/day) to 9.3 µg RE/day).

2.9.3 Leukoplakia

Leukoplakia are precancerous lesions on the lips or mouth and high doses of vitamin A have been used to treat Leukoplakia. Recently, β-carotene has shown to be just as effective as vitamin A though much safer and is now the preferred choice for treatment of Leukoplakia (Garewal and Schantz, 1995).

2.9.4 Human Immunodeficiency Virus (HIV)

Studies by Omene *et al.,* (1996) on two stratified groups of AIDS and non AIDS children showed a 50% decrease in β-carotene levels in AIDS patients compared to non AIDS patients. Their results supported those reported earlier by Murata *et al*., (1994) which concluded that the changes of lymphocyte subsets in HIV infections were related to β-carotene levels. However, some studies showed very little or no benefits, there have been a few that produced positive results on immune function in patients with HIV and AIDS (Omene *et al.,* 1996).

2.9.5 Side effects of β-carotene intake

Tanvetyanon and Bepler (2008) and Stahl *et al.,* (1998) reported, the frequent side effect of excessive β-carotene intake as carotenodermia, which is a harmless condition that presenting itself as an orange skin tint as a result of the deposition of carotenoids in the outermost layer of the epidermis. Chronic, high doses of β-carotene supplements have been associated with increased rate of lung cancer among those who smoke (Tanvetyanon and Bepler, 2008). In addition, supplemental β-carotene may increase the risk of prostate cancer, intra-cerebral haemorrhage, and cardiovascular disease and total mortality in people who smoke cigarettes or have a history of high-level exposure to asbestos (Johnson (2002); Russell, (2002).

Studies on rats have shown that the body cannot convert the beta-carotene stored in the liver into vitamin A, even if a deficit develops (Koushik *et al.,* 2006). A result of heavy consumption of synthetic β-carotene from a great variety of foods to which it is added, and from natural sources, may result in saturating the liver's storage capacity for fat soluble vitamins, so that reserves of other fat soluble vitamins, for example, vitamin D and vitamin A, are not created (Koushik *et al.,* 2006).

2.9.6 Effects of β-carotene deficiency

Diets low in vitamin A or its precursors such as β-carotene and carotenoids renders the human body susceptible to damage by free radicals as well as increased risk of chronic diseases such as heart disease and cancers (Ruxton *et al*., 2006; Wang *et al.*, 2011).

Symptoms of a β-carotene deficiency mimic those of a vitamin A deficiency: dry skin, night blindness, dry hair, broken finger nails, susceptibility to infection (Ansstas, 2014).

2.9.7 Foods rich in β-carotene content

Foods from animals, such as eggs, butter fish oils, liver, and milk, contain vitamin A (retinol) which can be used directly and easily by the human body. But the rural and urban poor in developing countries have only limited access to these expensive vitamin A rich animal food products. Table 4 shows some plant foods rich in β-carotene.

Table 4: Rankings of the top 10 food sources rich in β-carotene

Food	Ranking	Total β-carotene content in µg per 100 g serving
Sweet potato (Orange fleshed)	1	9442
Kale	2	9226
Carrots	3	8255
Turnip greens	4	6952
Mustard greens	5	6300
Spinach	6	6288
Dried herbs	7	5584
Butter nut	8	4570
Lettuce	9	4443
Collards (a cousin of cabbage)	10	3842

Source:http://www.healthaliciousness.com/articles/natural-food-sources-of-beta-carotene.php (20/09/2015).

Although an abundance of plant sources rich in β-carotene is available to many households, children in developing countries still suffer from vitamin A deficiency. This may be caused by lack of knowledge, lack of care and the apparently lower vitamin A activity of the pro-vitamin A rich foods (Louw, 2001). School-age children are considered to be at the greatest risk of developing vitamin A deficiency because they are preparing for the impending adolescent growth spurt, while some are already experiencing it (Department of Health, 2002).

2.9.8 Absorption and storage of β-carotene in human body

Clinx (2009) suggested that between 10 to 50% of the total β-carotene consumed is absorbed in the gastrointestinal tract and that increased intake of dietary fibre decreases the absorption of carotenoids. Within the intestinal wall (mucosa), β-carotene is partially converted into vitamin A by the enzyme dioxygenase. Excess β-carotene is stored in the fat tissues of the body and the liver (Clinx, 2009). The excess β-carotene is only converted by the body into vitamin A when needed.

2.9.9 Bioavailability and bioconversion of pro-vitamin A carotenoids

Bioavailability is a fraction of an ingested nutrient that becomes available to the body for utilization in physiological functions or for storage (Fraser and Bramley, 2004). Bioconversion refers to the proportion of bioavailable carotenoids converted to retinol (Jackson, 1997; Castenmiller and West, 1998). The factors that influencing the bioavailability of carotenoids include the size of the food granules eaten, food matrix, food preparation methods that disrupt the food matrix to different degrees, the presence of fibre (inhibits carotenoid absorption), dietary fat (enhances absorption) and of bile salts and pancreatic enzymes in the intestinal lumen (enhances digestion), and the nutritional status of the individual (Fraser and Bramley, 2004). The food matrix and the presence of dietary fat are of particular importance (Faber and Wenhold, 2006).

2.10 α-carotene

Alpha-carotene belongs to a group of powerful antioxidants that include β-carotene, lycopene, zeaxanthin and lutein. α-carotene as natural pigment, confers the pleasant orange, yellow, or red colour of many vegetables, fruits, egg yolk, crustaceans, and some fish (Rodriquez-Amaya *et al*., 2011). α-carotene and β-carotene are converted to significant amounts of vitamin A in the human body, and β-carotene is by far the most plentiful carotenoid found in fruits and vegetables (Mangles *et al.,* 1993).

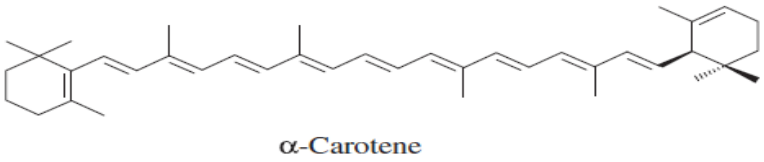


Figure 11: Chemical structure of α-carotene.
Source: Rodriquez-Amaya, (2011).

2.11 Starch

Starches are polysaccharides, that are made up of a number of monosaccharides or sugar (glucose) molecules linked to gether with α-D-(1-4) and/or α-D-(1-6) linkages (Sajilata *et al*., 2006). Starch is the commonest storage carbohydrate in plants and also the largest source of carbohydrates in human food (Singh *et al*., 2010). The starch comprise two main structural components, the amylose which is essentially a linear polymer in which glucose residues are α-D-(1-4) linked, typically consitituting 15 % to 20% of starch and amylopectin which is a more branched molecule with α-D-(1-4) and α-D-(1-6) linkages is a major component of starch (BNF, 1990). Figure 12 shows the two main structural components of starch. Singh *et al*. (2010), presented a wide variation in the rate of starch hydrolysis among different food products, from the lowest for lentils to the highest for boiled potatoes. Table 5 shows *in vitro* digestibility of starch in various food products.

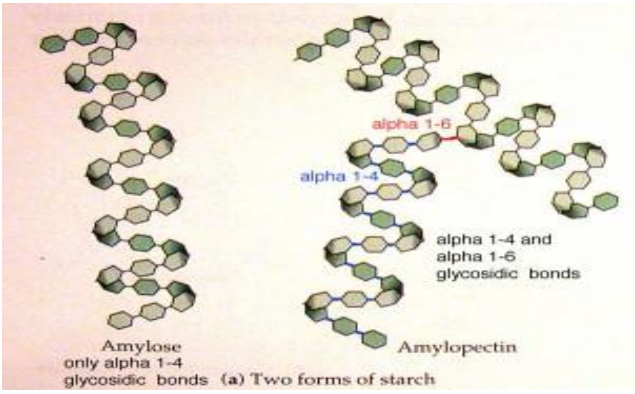


Figure 12: Two main forms of starch.
Source: BNF (1990).

Table 5. *In vitro* digestibility (%)of starch in various food products

Foods	rapidly digestible starch	Slowly digestible starch	Resistant starch 1	Resistant starch 2	Resistant starch 3
Flour, white	38	50	-	3	Traces
Short bread	56	43	-	-	Traces
Bread white	94	4	-	-	2
Bread whole meal	90	8	-	-	2
Spaghetti, white	55	36	8	-	1
Biscuits made with 50% raw banana flour	34	27	-	39	Traces
Biscuits made with 50% raw potato flour,	36	29	-	35	Traces
peas, chick canned	56	24	5	-	14
Beans, dried, freshly cooked	37	45	11	Traces	6
Beans, red kidney canned	25	-	-	15	60

Source: BNF (1990).

Based on the time of *in vitro* digestion of starch fractions, three types of starch namely; (1) rapidly digestible starch (RDS), (2) slowly digestible starch (SDS) and (3) resistant starch (RS), the resistant starch could be explained by the equation (1) of Sajilata *et al*., (2006) as follows:

$$RS = TS – (RDS + SDS) \quad [1]$$

Hence, total starch (TS) can be expressed as:

TS = RDS +SDS + RS [2]

2.12 Resistant starch

Resistant starch (RS) as the name implies, is the type or form of starch that resists digestion and is not fully digested and absorbed but rather turned into short-chain fatty acids by intestinal bacteria. RS is the sum of starch and starch degradation products that pass into the large intestine, which makes the distinction between starch that is hydrolyzed and the products absorbed in the human small intestine and starch that reaches the human large intestine either intact or partly hydrolyzed (RS) (Englyst and Hudson, 1996). Resistant starch is further divided into 4 fractions namely; Rs_1, Rs_2, Rs_3 and Rs_4 (Champ, 2004). The characteristics of different starches are shown in Table 6.

Table 6: Classification of types of resistant starch (RS) and food sources

Type of RS	Description	Food source	Resistance minimized by
Rs_1	Physically protected	Whole-or partly milled grain and seeds, legumes	Milling, chewing
Rs_2	Un-gelatinized resistant granules with type B crystallinity, slowly hydrolyzed by α-amylase	Raw potatoes, green bananas, some legumes, high amylase corn	Food processing and cooling
Rs_3	Retrograded starch	Cooked and cooled potatoes, bread, cornflakes, food products with repeated moist heat treatment	Processing conditions
Rs_4	Chemically modified starches due to cross-linking with chemical reagents	Foods in which modified starches have been used (e.g. Breads, cakes).	Less susceptible to digestibility in vitro

Source: Nugent (2005).

2.12.1 Functional properties of resistant starch

Resistant starch which assists in the control of diabetes is also reported to positively influence the functioning of microbial flora, glycemic index (GI), and the digestive tract (Fuentes-Zaragoza *et al*., 2010). The GI concept is an extension of the fibre hypothesis, suggesting

that fibre consumption reduces the rate of nutrient influx from the gut (Jenkins *et al.,* 2002). They further suggest that consumption of low GI diets were associated with higher – high density lipoprotein (HDL) - cholesterol concentrations, resulting in decreased risk of developing diabetes and cardiovascular disease. The key role of HDL as a carrier of excess cellular cholesterol in the reverse cholesterol pathway is believed to provide protection against atherosclerosis (Brewer, 2004). The desirable physicochemical properties of resistant starch such as water binding capacity, swelling, viscosity increase and gel formation makes it useful in a variety of food products (Fausto *et al.*, 1997). Sajilata *et al*. (2006) concluded that starch is ideal for use in ready-to-eat cereals, snacks, pasta/noodle, baked and fried foods and permits easy labelling as simply starch conferring nutraceutical benefits.

2.12.2 Potential food application of resistant starch

Due to its low water holding capacity, resistant starch provides good handling and improves texture in the final product (Martinez-Cervera and Fiszman, 2008). Fuentes-Zaragoza *et al*. (2010), suggested that careful control of processing conditions such as moisture content, pH, temperature duration of heating, repeated heating and cooling may increase resistant starch by as much as 30%.

2.13 Dietary fibre

Dietary fibre is a heterogeneous group of components for which several definitions and analytical methods were developed (Westenbrink *et al*., 2013). Codex Alimentarius commission (2009) defines dietary fibre as carbohydrate polymers with ≥ 10 monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestines of humans. It has now been generally accepted that dietary fibre plays an important role in the prevention of several diseases (Rodríquez *et al*., 2006). The importance of dietary fibre is derived from its role of decreasing the risk of diseases such as diabetes, cardiovascular diseases, colon cancer, constipation and diverticulosis (Ramulu and Rao, 1997).

2.14 Effect of processing methods on β-carotene content of sweet potato

2.14.1 Introduction

Beta-carotene content as one of the vital components of carotenoids of OFSP is affected by a number of factors including; variety, growing environment, postharvest handling such as processing and storage, light and oxygen (Dincer *et al*., 2011). Qiu *et al*. (2009) suggest that the geometry of β-carotene maybe changed from *trans* to *cis* forms. They suggest that the *all-trans*-β-carotene, mainly converts to 9-*cis* and 13-*cis* β-carotene by thermal treatment and exposure to light. However, other forms of isomers, such as 15-*cis* and 13, 15-di-*cis* are produced. As the *cis*- isomers increase, there is a decrease in the activity of provitamin A and colour intensity (Chen *et al*., 1995).

The importance of sweet potato in food security, health and global well-being has been well recognized worldwide (Bovell-Benjamin, 2007; van Hall, 2007; Hagenimana *et al.,* 1999; Waramboi *et al*., 2011; van Jaarsveld *et al.,* 2005). Apart from the consumption, and health benefits derived thereof, sweet potato is also utilized in the production of industrial starches and alcohol (Li *et al*., 2015; Ferrari *et al*., 2013; Zaidul *et al*., 2007; Anastácio and Carvalho, 2013; Peng *et al*., 2013; Hashem and Darwish, 2010.).

Sweet potato is a major starch staple food in Africa and other lower income countries in Asia and Latin America. In these regions the crop is widely grown and it provides household food security to many poor resourced farmers (Bashaasha *et al*., 1995). Recently, sweet potato genotypes that have good amounts of vitamin A popularly known as OFSP have been recognized as a potential means for saving thousands of children in Africa from vitamin A deficiency-related diseases such as night blindness (van Jaarsveld *et al.,* 2005*).*

A number of different intervention strategies to address vitamin A deficiency are being promoted in developing countries. The interventions include; diet supplementation, fortification of foods, bio-fortification of staple foods, and the making of sweet potato-wheat flour blends (Beaton *et al*., 1993; Dary and Mora, 2002; Welch, 2002; Srivastava *et al.,* 2012).

Faber and Laubscher, (2008), reported the OFSP as one of the foods that can provide highly bio-available β-carotene, a precursor to vitamin A. β-carotene is a

strongly-coloured red-orange pigment abundant in plants and fruits. It is an organic compound and chemically, it is classified as a hydrocarbon and specifically as a terpenoid, reflecting its derivation from isoprene units. Carotenoids are responsible for giving the orange colour in fruits and vegetables in which they are present.

Some of the carotenoids are vitamin A precursors since they are absorbed and converted into vitamin A in the human body. β-carotene is generally regarded as an important and commercially most widely utilized carotenoid (Schierle *et al*., 2004). OFSP varieties, contain appreciable amounts of β-carotene, starch, dietary fibre, minerals, vitamins (especially Vitamins C, B6 and folate), as well as antioxidants, such as phenolic acids, anthocyanins, and tocopherol (Woolfe, 1992).

Carotenoids are a group of antioxidants which includes; lycopene, lutein, α-carotene, β-carotene, and zeaxanthin. However, of all the carotenoids, only α-carotene and β-carotene are converted to significant amounts of vitamin A in the human body, and beta-carotene is by far the most plentiful carotenoid found in fruits and vegetables (Mangles *et al,* 1993). Rodriquez-Amaya *et al*. (2011), suggest that carotenoids are among the most valuable food constituents in terms of food quality and human health effects.

Recent studies (Ruxton, *et al*., 2006; Saura and Goňi, 2006; Wang *et al.*, 2011; Wu *et al*., 2004a; and Wu *et al*., 2004b) have postulated health promoting effects of β-carotene including, immune enhancement and reduction of the risk of developing degenerative diseases, cancer, type II diabetes , cardiovascular diseases, cataracts and mascular degeneration.

2.14.2 Optimisation of β-carotene content

Carotenoids are prone to geometrical isomerisation, so they can exist as all-trans (all-E) or *cis* (Z) isomers (Melendez-Martinez *et al*., 2013). The formation of the latter as analytical artefacts is as a result of light, heat and other conditions, both in extracts and in foods (Aman *et al*., 2005; Aman *et al*., 2005; Marx *et al*., 2003; Pott, Marx *et al*., 2002; Updike & Schwartz, 2003; Vásquez-Caicedo *et al*., 2005).

Bengtsson *et al*., (2008), reported moderately low losses in *all-trans*-β-carotene due to degradation and isomerisation; however they reported lower retention in *all-trans*-β-carotene content in OFSP in open-air sun drying. Their work also showed that *all-trans*-β-carotene was the major provitamin A content of improved OFSP cultivars ranging from 108 to 315 µg (dry matter). Bengtsson *et al*. (2008), concluded that solar and oven-drying are considered to be appropriate drying methods resulting in high retention values of *all-trans*-β-carotene. Experiments on controlled storage of OFSP by Bechoff *et al.* (2009), showed that there is a linear relationship between β-carotene degradation rate and water activity, and between β-carotene degradation rate and oxygen concentration.

2.15 Effect of storage on β-carotene content of sweet potato

A number of scholars have demonstrated that β-carotene content was affected by the processing method, genetic, location and farming practices among others (Wu *et al*., 2008; Laurie *et al*., 2012; van Jaarsveld *et al.,* 2006; Dutta *et al*., 2006). Grace *et al*. (2014), suggest that the phytochemical content of the produce can be affected by post-harvest handling procedures such as temperature, curing time, irradiation time and exposure to light due to tissue biochemical responses. Their results on purple-fleshed sweet potato cultivar showed a decline in total phenolic content due to anthocyanin degradation during storage.

Bechoff *et al.* (2010) suggest that temperature and oxygen were the main parameters that need control for the reduction of carotenoid degradation during storage. They also report that light and water activity have negligible and limited effect on carotenoid degradation respectively. Karabulut *et al.* (2007), while working on the effect of drying conditions on colour values and β-carotene content of apricots (*Prunus armenica* L.), found that sulphur treatment decreases the drying time for all conditions and that colour values and β-carotene content of hot air dried samples were favourable in comparison to air dried apricots (*P. armenica* L). They also found that sulphur treatment decreased the drying time for all drying conditions.

van Jaarsveld *et al.,* (2006), found true retention of β-carotene to be 92% when medium sized OFSP were covered with water and boiled for 20 min in a pot with the lid on but, it took longer time (30 min) to cook when boiled without a lid and consequently, true retention was lower (88%). Bengtsson *et al*., (2008), report that solar drying of OFSP slices to moisture content of ≤10% result in the retention of *all- trans β*-carotene by 91% and that deep frying of OFSP roots resulted in the retention of β-carotene by 78%.

2.16 Some food sources of β-carotene

β-carotene can be obtained from the consumption of various plant foods. Table 7 shows some plant sources high in β-carotene content.

Table 7: Food sources of β-carotene and quantity in µg per 100 g serving.

General Food Sources of β-carotene	Total β-carotene quantity in µg per 100 g serving
Spices (Paprika, Cayane, Chilli)	26162
Sweet Potato (Orange fleshed)	11509
Kale	8823
Carrots	8332
Turnip greens	6952
Mustard greens	6300
Spinach	6288
Dried Herbs	5584
Butter nut	4570
Lettuce	4443
Cos or Romaine lettuce	5226
Parsley	5054
Dried herbs	4806
Butternut squash	4570
Garden peas	4150
Sun dried chilli peppers	14844
Sweet potato chips	14205
Carrot juice	9303
Leeks	1000
Mixed vegetables canned	5670
Canned pumpkin	6940
Watercress	1914
Cantaloupe Melon	2020
Dried apricots	2163
peas	1250
Broccoli	929

Adapted from: *www.healthaliciousness.com/articles*.28/02/2015

2.17 Future research direction

Future research direction about sweet potato should be the continuation of the current breeding of sweet potato with higher β-carotene content (especially orange flashed). This will ensure availability of cultivars with higher carotenoid content. Future research should also focus on processing methods that seek to retain as much carotenoids (especially β-carotene) as possible (Picouet *et al*., 2015; Rawson *et al*., 2011; D'Evoli *et al*., 2013; Karabulut *et al*., 2007; Bechoff *et al*., 2009; van Jaarsveld *et al*., 2006).

Current research on the rapid determination of sweet potato carotenoids, and the effect of processing methods on the availability of β-carotene, retention of β-carotene, β-carotene yield and antioxidant efficacy of β-carotene content in sweet potato cultivars should continue to be explored (Oloo, *et al*., 2014; Bengtsson *et al*., 2008; Teow *et al.,* 2007; Huang *et al*., 1999; Laurie *et al*., 2012; van Jaarsveld, 2006; Liu *et al*., 2006).

There is likely to be an increase in the consumption of sweet potatoes particularly the orange fleshed due to increased awareness campaigns on the benefits of the consumption of OFSP cultivars especially among the rural communities and children. This will give rise to the research to determine the effect of bioactive compounds and other micro elements present in sweet potatoes.

As postharvest handling and processing improves, there is likely to be more sweet potato products on the shelves of super markets than before. This will lead to increased consumption of sweet potatoes and sweet potato food products as ingredient in established food products and novel food products by the urban and peri-urban communities.

From global a perspective, under nutrition, food insecurity issues, droughts and limited agricultural technologies are major problems (Bovelle-Benjamin, 2007). From this perspective, future research should continue to be directed on the breeding of sweet potato cultivars with higher β-carotene content, higher yield, more pest and drought resistant cultivars. Kidmose *et al*. (2007) suggest that in developed countries, focus should be on innovation of new sweet potato products with a good taste and high health value. In contrast

with the developing countries, the researchers suggest a focus on content and stability of β-carotene in sweet potato.

2.17.1 Good processing methods to conserve β-carotene

There is general consensus that consumption of fruit and vegetables containing adequate micronutrients and bioactive compounds is highly essential to meet the body's needs and to prevent degenerative diseases such as cardiovascular diseases, diabetes, cancer, hypertension as well as improving fitness and in the delay of aging (Mohamed *et al*., 2005 and Sulaiman *et al*., 2011).

Various researchers (Eastwood, 1999; WHO, 2004; Lui, 2003; Kaur, and Kapoor, 2008; Jaganath and Crozier, 2008; Hung *et al*., 2004; Horbowicz and Saniewski, 2000; Teow *et al*., 2007; Ahmed *et al*. 2010; Sulaiman *et al*., 2011 and Mohamed *et al*., 2005) have reported the importance of phytochemicals and antioxidants in human health and nutrition. Tomato consumption which is associated with decreased risk of some cancer types is a reservoir of diverse antioxidant molecules, such as ascorbic acid, vitamin E, carotenoids, flavonoids and phenolic acids (George *et al*., 2004).

Rawson *et al.,* (2011) suggested the need to streamline the processes through a combinations of technologies including, the optimization of practical applications, process optimisation of thermal processes in combination with non-thermal technologies, such as high pressure, ultrasound and pulsed electric field. Picouet *et al*. (2015), studied the effect of thermal and high-pressure treatments on the carotene content, microbiological safety and sensory properties of acidified and of non-acidified carrot juice and found that while major physicochemical parameters remained stable during the entire sampling period, major changes occurred in the processed samples for both high pressure and mild heat treatments.

2.17.2 Non-thermal processing methods to conserve β-carotene

Conventional thermal processing which relies essentially on the generation of heat outside the product to be heated is the most widely used process technology (Rawson *et al.*, (2011). Thermal processing technology ensures microbiological safety of the products. The current knowledge on heat treatment of food products is that it can affect levels of bioactive compounds and their products. Zepka and Mercadante (2009), studied the effect of organic acid and heating treatments on carotenoid degradation on simulated cashew, apple and juice. The authors found that the levels of *all-trans*-carotenoids decreased with increase in amounts of *cis* isomers and oxidation products as time and heating temperature increased.

However, consumer demand for nutritious foods, (bioactive and compound retention) has led to increased interest in non-thermal processing techniques. Non thermal processing technologies include among others, pulsed electric field (PEF), consisting of high intensity fields such as 15 - 40 kV/cm, 5 -100 pulses, 40 to 700 μs 1.1 to 100Hz (Zulueta *et al*., 2010), ultrasound energy generated by sound waves of 20,000Hz or more (Rawson *et al.*, 2011), ultraviolet (UV-C) light exposure of 400J/m^2, intense and short – duration pulses of broad spectrum (Fonseca and Rushing, 2006; Lopez-Rubira *et al*., 2005; González- Aguilar *et al*., 2007).

2.18 Conclusion

Sweet potatoes are highly perishable owing to their high water content. Sweet potato flour, which contains appreciable amounts of β-carotene, is a more stable sweet potato product that can be stored for a longer period than fresh roots. Therefore, research on the effect of processing methods on β-carotene content in sweet potato flour will continue to take centre stage. This is mainly due to the fact that sweet potato flour is one of the forms in which the shelf-life of the product can be extended there by contributing to food security in developing countries.

CHAPTER 3: CAROTENOIDS AND ANTIOXIDANT PROFILE OF OFSP FLOUR

3.1 Introduction

The objective of this study was to quantify the total carotenoids and antioxidant capacity of flour from 8 sweet potato cultivars from two different locations. Carotenoids are a group of antioxidants that include α-carotene, β-carotene, lycopene, zeaxanthin, and lutein. However, of all the carotenoids, only α-carotene and β-carotene are converted to large amounts of vitamin A in the human body, and β-carotene is by far the most plentiful carotenoid found in fruits and vegetables (Mangles *et al.,* 1993). Humans can assimilate carotenoids from the foods that they eat but many other animals cannot.

Carotenoids are prone to geometrical isomerisation, so they can exist as all-*trans* (all-E) or *cis* (Z) isomers (Melendez-Martinez *et al*., 2013). The formation of the latter as analytical artefacts is attributed to the light, heat and other conditions, both in extracts and in foods (Aman *et al*., 2005; Marx *et al*., 2003; Pott *et al*., 2002; Updike & Schwartz, 2003; Vásquez-Caicedo *et al*., 2005).

While free radicals, superoxide radicals (O_2), hydrogen peroxide (H_2O_2) and other reactive oxygen species are constantly formed in the human body (Okezie, 1998), phytochemicals especially polyphenols are known to have a high free-radical scavenging activity that helps to reduce the risk of chronic diseases such as cardiovascular disease and cancer. Cardiovascular conditions and cancer are examples of degenerative conditions (Wootton-Beard and Ryan, 2011; Ruxton *et al*., 2006; Sies and Jones, 2007; Ames *et al*., 1993; Saura and Goňi, 2006). Fruits and vegetables are a rich source of phytochemicals, such as carotenoids, flavonoids and other phenolic compounds (Teow *et al*., 2007).

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic-lipophilic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma and the lipid-soluble antioxidants protect cell membranes from lipid peroxidation (Sies, 1997). Oxidation reactions can produce free radicals which can start chain reactions resulting in the death of the cell.

Antioxidants are substances that are capable of counteracting the damaging, effects of the physiological process of oxidation in cells or living organisms. Natural antioxidants present in fruits and vegetables, such as vitamins and phenolic compounds, are considered to be responsible for these chemopreventive effects (Ruxton *et al*., 2006; Saura-Calixto and Goñi, 2006). Many studies have shown that fruit and vegetable consumption correlates with reduced cardiovascular disease risk (Wang *et al*., 2011). This chapter deals with the quantification of total carotenoids and antioxidant capacities of flour from three OFSP and one CFSP cultivars and one cream fleshed cultivar grown in two locations in Limpopo province. It also highlights the variations in β- and α-carotenes and mineral contents in the flour of sweet potato cultivars selected for this study.

3.2 Materials and Methods

Fresh OFSP and cream fleshed tubers used in this study were harvested from two locations in Limpopo Province namely; University of Venda experimental farm (Univen) and Tshiombo Irrigation Scheme (Tshiombo). The tubers were washed peeled, sliced in approximately 2 cm rings and 2 mm thick and dried in an oven at 55^{o}C. The tried slice where packed in ziploc plastics and stored at -20^{o}C and later milled into flour. The analyses, materials and equipment used are shown in Appendix 1.

3.2.1 Determination of total carotenoids

Total carotenoids were determined by the method of Opara and Al-Ani (2010) with some modifications. Approximately 1 g of sweet potato flour was mixed with 14 ml of n-hexane-acetone solution in the ratio of 3:2 respectively. The mixture was vortexed for 2 min and then centrifuged at 3000 rpm for 30 min at 4^{o}C. The samples were allowed to stand for 20 min in the dark place. The absorbance (Optical density, OD) of the clear solution was measured at 450 nm wavelength using a spectrophotometer (Thermo Fisher scientific, Helios Omega UV-Vis, US). The carotenoids in sweet potato samples were calculated using a linear equation Y = 0.0034x +3.2166 (R^2. = 0.9526) Appendix 5. Total carotenoids content in sweet potato was corrected using the formula (3) given by Opara and Al-Ani (2010).

Carotenoid content (g/ml) = $\frac{\mathbf{OD_{450}\ X\ 4}}{\text{Mass of sample (g)}}$ [3]

3.2.2. Determination of antioxidant capacity by DPPH assay

One gram of OFSP flour was accurately weighed into a 15 ml centrifuge test tube. Antioxidant contents were extracted by adding 10 ml of 50% methanol and the sample was vortexed for about 30 s. The sweet potato sample was centrifuged at 4000 rpm for 20 min at 4°C to precipitate particulates.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Samples of sweet potato cultivar flour were tested against a stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution using Wong *et al*. (2006) method with minor modifications. The absorbance of a solution of 0.1 mM of DPPH in methanol was measured at 515 nm and this did not change throughout the assay period. In triplicates, a dilute sweet potato sample (15 µl) was mixed with methanol (735 µl) and then with DPPH solution (750 µl, 0.1 mM). The mixture was incubated for 30 min in the dark at room temperature. The change in absorbance was measured at 515 nm. Antioxidant activity based on the DPPH free-radical scavenging activity was calculated using a standard curve (Appendix 6). The radical scavenging activity was expressed as mean ± standard error mMol of ascorbic acid equivalent per ml crude sweet potato sample (mMAAE ml $^{-1}$).

3.2.3 Determination of antioxidant capacity by FRAP assay

The extract was collected and total antioxidant (hydrophilic and lipophilic) capacity (TAC) was determined by the use of ferric reducing/antioxidant (FRAP) assay using Benzie and Strain (1996) method with some minor modifications. According to (Khanizadeh *et al*., 2008), the assay is used to measure the ability of antioxidants in the sample to reduce ferric-tripyridyltriazine (Fe $^{3+}$-TPTZ) complex to the blue –coloured ferrous form (Fe^{2+}) which absorbs light at 593 nm.

Triplicate sweet potato samples were diluted with 2850 µl FRAP reagent (300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution, 20 mm ferric chloride) and incubated in the dark for 30 min. Absorbance was measured at 593 nm using a spectrophotometer (Helios UV-vis, Thermo fisher scientific, USA).

The ferric reducing/antioxidant power in sweet potato samples was calculated using a standard curve (Appendix 7). Total antioxidant capacity was expressed as means ± SE mM of Trolox equivalent (TE) per millilitre of crude sweet potato sample extract (µMTEml^{-1}).

3.2.4 Determination of β-carotene content

β-carotene extraction

Oven dried OFSP flour (2 g) was mixed with approximately 2 g of calcium carbonate, 1 g of diatomaceous earth, and 25 ml of methanol. A hexane–acetone (1:1) mixture (50 ml) was added and stirred. The mixture was filtered under vacuum through a funnel with a fritted disk. The residue in the funnel was washed two more times with 25 ml of methanol and then by 50 ml of the hexane–acetone mixture. All of the extracts were combined in a 250 ml separating funnel and washed with water. A few drops of saturated sodium chloride solution were added to the funnel to facilitate phase separation. The aqueous phase was discarded and the upper layer (yellow colour) was transferred to a 50 ml volumetric flask and made to volume with hexane (Chandler & Schwartz, 1998). Samples were stored in dark vials at 20˚C until analysis.

Measurement of β-carotene

The instrument was first calibrated with high purity (98%) β-carotene standard. The absorbance (A) of solution was determined as soon as possible with a spectrophotometer at 436 nm. β-carotene content was calculated using formula (4).

$$C = \frac{A\ x\ 454}{196\ x\ L\ x\ W} \qquad [4]$$

Where, C = concentration carotene (mg/lb) in original sample,

L = cell length in cm (1 cm), and

W = g product / ml final dilution (2.000 g / 100 ml).

Results reported as mg β-carotene/lb, multiply by 2.2 to give ppm (µg/g).

3.2.5 Determination of α- carotene content

Oven dried OFSP and cream fleshed sweet potato flour (2 g) was mixed with approximately 2 g of calcium carbonate, 1 g of diatomaceous earth, and 25 ml of methanol. A hexane–acetone (1:1) mixture (50 ml) was added and stirred. The mixture was filtered under vacuum through a funnel with a fritted disk. The residue in the funnel was washed two more times with 25 ml of methanol and then by 50 ml of the hexane–acetone mixture. All of the extracts were combined in a 250 ml separating funnel and washed with water.

A few drops of saturated NaCl solution were added to the funnel to facilitate phase separation. The aqueous phase was discarded and the upper layer (yellow colour) was transferred to a 50 ml volumetric flask and made to volume with hexane (Chandler & Schwartz, 1998). Samples were stored in dark vials at 20˚C until analysis. The absorbance (A) of above extracted solution was determined as soon as possible using a spectrophotometer at 422 nm. The α-carotene concentration was calculated using formula [5].

A spectrophotometer reading at 422 nm was used to measure absorbance and the concentration of α-carotene, was calculated using the equation [5]

$$A = \varepsilon cL \qquad [5]$$

Therefore, C = A/ ε L (also considered the dilution factor and w.t of samples)

Where:

A = Absorbance of sample,

ε = Molar absorptivity of α-carotene = 2800,

C = Concentration carotene (mol/L) in original sample,

L = Cell length in cm (1 cm).

3.2.6 Determination of mineral content by scanning electron microscope

Imaging of the samples and analysis of the mineral compositions was accomplished using a method described by Ahmed *et al.* (2010) by using a Zeiss EVO® MA15 scanning electron microscope (SEM) at the Stellenbosch University. Quantitative analysis and Secondary Electron images require 15 µm of gold coating, on the surface. Samples were identified with Secondary electron images, and compositions were quantified by EDX analysis using an Oxford Instruments® X-Max 20 mm^2 detector and Oxford INCA software. Beam conditions during the quantitative analyses were 20 KV, with a working distance of 8.5 mm and approximately beam current of – 20 nA. The counting time was 10 seconds live-time. Pure Co were used periodically to correct for detector drift.

3.3 Statistical analysis

All measurements were performed in triplicates, the results obtained were expressed as mean ± standard error (SE). Statistical analysis was performed using a one-way analysis of variance (ANOVA) and means of results for each experiment were separated using Duncan's multiple range test (Duncan, 1995). P values < 0.05 were considered to be statistically significant. Pearson's correlation was used to determine correlational properties of sweet potato flour. All statistical analyses were conducted using an IBM Statistical Package for Social Sciences (SPSS version 22, IBM Inc. New York, USA) and STASTICA version 10 (StatSoft) and Microsoft Excel version 10.

3.4 Results and discussion

3.4.1 Total carotenoids

The results of analysis of variance for total carotenoids and antioxidant capacity of OFSP flour from two locations are shown in Table 8. The results showed significant differences ($P < 0.05$) in total carotenoids between the OFSP cultivars (*Impilo, Dagga and Bophelo*) and the cream fleshed sweet potato cultivar (*Mvuvhelo*). However, there was no significant difference in the amounts of total carotenoids among the OFSP cultivars. There was also no significant difference in the amounts of total carotenoids between the two locations. The follow-up tests for main effects of carotenoids, antioxidant capacity and β, - α-carotene in flour of sweet potato cultivars are shown in Table 10. The results show that cream fleshed *Mvuvhelo* and orange fleshed *Dagga* flour have significantly the lowest (1.33 g/ml) and highest (6.91 g/ml) total carotenoids respectively. It has been long established that the OFSP cultivars contain significantly higher amounts of carotenoids which give the yellow to orange colour on OFSP cultivars (van Jaarsveld *et al.*, 2006). Fernandez-Orozco, *et al*. (2013), found a wide variation in total carotenoids of *solanum* sp cultivars with varying flesh colours. Bechoff, *et al*. (2009), found that carotenoid losses during storage were more of a nutritional constraint to the utilisation of dried sweet potato than the losses occurring during drying.

3.4.2 *2, 2-Diphenyl-1-picrylhydrazyl* (DPPH) assay

The radical scavenging activity was determined based on the ability of OFSP cultivar flour extract to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The results (Table 8) showed that there was a significant difference ($P < 0.05$) in the DPPH radical scavenging activity in the flour of OFSP cultivars. The results of the follow-up tests for main effect of radical scavenging activity are shown in Table 10. *Bophelo* and *Dagga* showed the highest (63.37 $mMAAEml^{-1}$) and lowest (26.93 $mMAAEml^{-1}$) radical scavenging activity, respectively (Table 10). However, there was no significant difference in the radical scavenging capacity between OFSP cultivar *Dagga* cultivar (26.93 $mMAAEml^{-1}$) and the cream fleshed cultivar

Mvuvhelo (28.17 mMAAEml^{-1}). Oki *et al.* (2006), suggested that the β-carotene in OFSP contributed an estimated 36 to 79% to total radical scavenging activity.

3.4.3 Ferric reducing antioxidant capacity (FRAP)

The ferric reducing antioxidant capacity (FRAP) measures the ability of antioxidants in the sample to reduce ferric- tripyridyltriazine (Fe_3– TPTZ) complex to the blue-coloured ferrous form (Fe_2) which absorbs light at 593 nm (Khanizadeh *et al*., 2008). The results of analysis of variance for carotenoids and antioxidant profile are shown in Table 8. The results showed significant differences ($P < 0.05$) in FRAP between location, cultivar and location x cultivar interactions of the sweet potato flour from different cultivars.

The results of the follow-up tests for main effects on FRAP are shown in Table 10. The University of Venda (Univen) experimental farm OFSP cultivar flour show significantly higher (17.40 µMTEml^{-1}) FRAP than that of Tshiombo irrigation scheme (14.45 µMTEml^{-1}). FRAP was significantly highest (21.12 µMTEml^{-1}) in *Impilo* flour and lowest (16.15 µMTEml^{-1}) in *Dagga* flour. These results are comparable to those reported by Teow *et al*., (2007), on the dark OFSP clone 11-20 (18.2 µmol TE/g).

Table 8: Analysis of variance for carotenoids and antioxidant profile of sweet potato flour.

Variable	df	MS	F	P- Level	Sig
Carotenoids					
Location	1	0.053	0.154	0.699	Ns
Cultivar	3	53.955	157.213	0.000	*
Location X cultivar	3	0.174	0.506	0.683	Ns
Error	16	0.343			
DPPH					
Location	1	6.761	0.599	0.450	Ns
Cultivar	3	1713.006	151.718	0.000	*
Location X cultivar	3	21.427	1.898	0.171	Ns
Error	16	11.291			
FRAP					
Location	1	22.792	10.285	0.005	*
Cultivar	3	210.308	94.901	0.000	*
Location X cultivar	3	33.055	14.916	0.000	*
Error	16	2.216			

Note: * Denote statistically significant at P < 0.05; Duncan's multiple range tests. Ns: Not significant at P < 0.05; Sig = Significancy; DPPH = 2, 2-diphenyl-1-picrylhydrazyl; FRAP = Ferric reducing antioxidant capacity.

3.4.4 β-carotene and α-carotene

The results of analysis of variance for β- and α-carotene profiles of OFSP flour are shown in Table 9. The results showed a significant difference (P < 0.05) between cultivar, location and location x cultivar interactions in the β-carotene content of all the flours. The results of the follow-up tests (Table 10) show that OFSP flour from Univen experimental farm had significantly higher (86.09 µg/g) β- carotene content than that from Tshiombo irrigation scheme (70.98 µg/g). All OFSP cultivar flours differ significantly among cultivars and ranged from 28.37 µg/g for *Impilo* to 201.50 µg/g for *Dagga*. The results showed significant differences in β-carotene content between location x cultivar interactions and ranged from 1.40 µg/g in *Mvuvhelo* from Univen experimental farm to 241.89 µg/g *Dagga* from *Tshiombo* irrigation scheme. There is was a significant variation in β-carotene among the cultivars.

The results of analysis of variance for α-carotene are shown in Table 9. The results showed a similar trend to that of β-carotene in that there was a significant (P < 0.05) difference across the two locations. The follow-up tests (Table 10) show significant differences between α-carotene content from Tshiombo irrigation scheme, and that from

Univen experimental farm and ranged from 0.63 mol/L to 1.01mol/L respectively. The results also showed significant differences in α-carotene content among OFSP cultivar flours and ranged from 0.50 mol/L in *Mvuvhelo* to 1.28 mol/L in Impilo. However, there was no significant difference between location X cultivar interaction.

Table 9: Analysis of variance for β, α-carotene profile of OFSP flour

Cultivar	df	MS	F	P-Level	Sig
β-carotene					
Location	1	1370.175	932.083	0.000	*
Cultivar	3	47112.420	32049.000	0.000	*
Location X cultivar	3	8959.997	6095.180	0.000	*
Error	16	0.191			
α-carotene					
Location	1	5.1E-05	3.592	0.0	*
Cultivar	3	0.0033	231.800	0.000	*
Location X cultivar	3	0.0008	53.446	0.338	NS
Error	16	1E-05			

Note: * Statistically significant at P < 0.05; Duncan's multiple range test.
Ns: Not statistically significant at P < 0.05; OFSP = Orange fleshed sweet potato; Sig = Significancy.

Table 10: Carotenoids, antioxidant, and β/ α-carotene profile of OFSP flour.

Main effect		Carotenoids (µg/g)	β-carotene (µg/g)	α-carotene (µg/g)	Antioxidant activity	
					DPPH (mMAAE ml-1)	FRAP (µMTE ml-1)
Location	Tshiombo	7.42 ± 0.10^{a}	70.98 ± 0.8^{b}	0.03 ± 0.02^{a}	41.12 ± 4.4^{ns}	14.45 ± 2.1^{b}
	Univen	7.22 ± 0.15^{a}	86.09 ± 2.0^{a}	0.02 ± 0.01^{b}	40.10 ± 2.8^{ns}	17.40 ± 1.1^{a}
Cultivar	*Dagga*	6.91 ± 0.5^{a}	201.50 ± 1.0^{a}	0.57 ± 0.0^{b}	26.93 ± 1.4^{c}	16.15 ± 1.0^{b}
	Bophelo	7.43 ± 0.0^{a}	82.55 ± 0.4^{b}	0.22 ± 0.0^{c}	63.37 ± 0.8^{a}	20.26 ± 0.3^{a}
	Impilo	7.56 ± 0.0^{a}	28.37 ± 0.2^{c}	0.013 ± 0.0^{a}	40.05 ± 2.0^{b}	21.12 ± 0.3^{a}
	Mvuvhelo	1.33 ± 0.1^{b}	1.71 ± 0.0^{d}	0.003 ± 0.0^{c}	28.17 ± 1.4^{c}	8.16 ± 2.0^{c}
Location X Cultivar						
Tshiombo X *Dagga*		7.42 ± 0.10^{ns}	241.89 ± 1.4^{a}	0.07 ± 0.01^{b}	23.40 ± 1.36^{d}	16.59 ± 2.0^{b}
Tshiombo X *Bophelo*		7.43 ± 0.01^{ns}	30.32 ± 0.6^{e}	0.01 ± 0.00^{d}	62.07 ± 0.7^{a}	20.78 ± 0.2^{a}
Tshiombo X I*mpilo*		7.83 ± 0.14^{ns}	9.68 ± 0.3^{f}	0.01 ± 0.00^{c}	42.70 ± 0.8^{b}	20.71 ± 0.4^{a}
Tshiombo X *Mvuvhelo*		1.35 ± 0.04^{ns}	2.02 ± 0.0^{g}	0.01 ± 0.00^{f}	30.40 ± 1.50^{c}	3.72 ± 0.4^{d}
Univen X *Dagga*		7.22 ± 0.15^{ns}	161.12 ± 0.4^{b}	0.04 ± 0.002^{c}	27.13 ± 0.11^{d}	15.72 ± 1.2^{b}
Univen X *Bophelo*		7.24 ± 0.03^{ns}	134.79 ± 0.1^{c}	0.03 ± 0.002^{b}	65.21 ± 0.11^{a}	19.75 ± 0.1^{a}
Univen X *Impilo*		7.68 ± 0.02^{ns}	47.05 ± 1.2^{d}	0.02 ± 0.002^{a}	35.57 ± 0.00^{b}	21.53 ± 0.2^{a}
Univen X *Mvuvhelo*		1.33 ± 0.01^{ns}	1.40 ± 0.1^{g}	0.00 ± 0.00^{e}	28.30 ± 0.11^{c}	12.60 ± 0.5^{c}

Note: Values are means ± standard error. Values with same letter superscript in the same column are not significantly different at P < 0.05, Duncan's multiple range tests. (Location n =12; cultivar n = 8).
ns: Not significantly difference at P < 0.05. Univen = University of Venda, DPPH = *2, 2-Diphenyl-1-picrylhydrazyl;* FRAP = Ferric reducing antioxidant power; AAE = Ascorbic acid equivalent; TE = Trolox equivalent; = OFSP = Orange fleshed sweet potato.

Correlation analysis

Pearson correlation was carried out to determine the relationships between carotenoids, FRAP, DPPH radical scavenging activity, β-carotene and α-carotene (Table 11). The results showed that free radical scavenging activity was significantly positively ($P < 0.05$) correlated with the total carotenoids (0.486*) while FRAP showed strong positive correlation to total carotenoids (0.830**). Both β-carotene and α- carotene were significantly correlated to total carotenoids, 0.37* and 0.424* respectively. As the total carotenoids increase so are the β-/α-carotene contents. However, both β-carotene and α- carotene were insignificantly negatively correlated to DPPH but not correlated to FRAP (Table 11). The higher the total carotenoids the greater the free radical scavenging and activity.

Table 11: Correlation of carotenoids, antioxidants, and β, α-carotene contents of OFSP flour

Variables	Total	Antioxidant			
	Carotenoids	DPPH	FRAP	β-carotene	α-carotene
Total carotenoids	1				
DPPH	0.486*	1			
FRAP	0.830**	0.563**	1		
β-carotene	0.370*	-134	0.197	1	
α-carotene	0.424*	-179	0.173	0.974**	1

* Denotes significant correlation at $P < 0.05$. ** Denote significant correlation at $P < 0.001$; DPPH = 2, 2-diphenyl-1-picrylhydrazyl; FRAP = Ferric reducing antioxidant capacity, OFSP = Orange fleshed sweet potato.

The rank order of cultivar flour according to total carotenoids, antioxidant activity, β-/α-carotene content is shown in Table 12. Flour from *Dagga* cultivar was ranked highest in β-/and α- carotene contents while *Impilo* flour was ranked highest in total carotenoids and ferric reducing antioxidant power. Flour from *Bophelo* cultivar was ranked highest in the ability to reduce free radicals and second highest in all other parameters measured, such as FRAP, β-carotene and α-carotene and best ranked overall.

Table 12. Rank order of OFSP flour based on carotenoids, antioxidants, β- and α- carotenes.

Cultivar	Carotenoids (µg/g)	Antioxidants		β-carotene (µg/g)	α-carotene (mol/L)
		DPPH (mMAAE ml^{-1})	FRAP (mMTE ml^{-1})		
Dagga	3	4	3	1	1
Bophelo	2	1	2	2	2
Impilo	1	2	1	3	3
Mvuvhelo	4	3	4	4	4

1 = Ranked highest and 4 = Ranked least in terms of parameters measured; DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = Ferric reducing antioxidant capacity, OFSP = Orange fleshed sweet potato.

Impilo cultivar was ranked highest in total carotenoids content and ferric reducing antioxidant activity. *Dagga* cultivar with highest β- and α-carotene contents had the lowest free radical scavenging activity.

3.4.5 Mineral content by scanning electron microscope

The results of mineral content as measured by scanning electron microscope (SEM) are shown in Figures 16 and 17. The results showed that potassium was predominantly higher in all cultivars and in both locations in comparison to magnesium, Chlorine and calcium. *Mvuvhelo* flour showed the highest amounts of potassium followed by *Dagga* and *Bophelo* flour. *Dagga* flour from the University of Venda experimental farm showed the highest amounts of magnesium among the four cultivars while *Mvuvhelo* flour from Tshiombo irrigation scheme flours contained the highest amount of magnesium among the four cultivars.

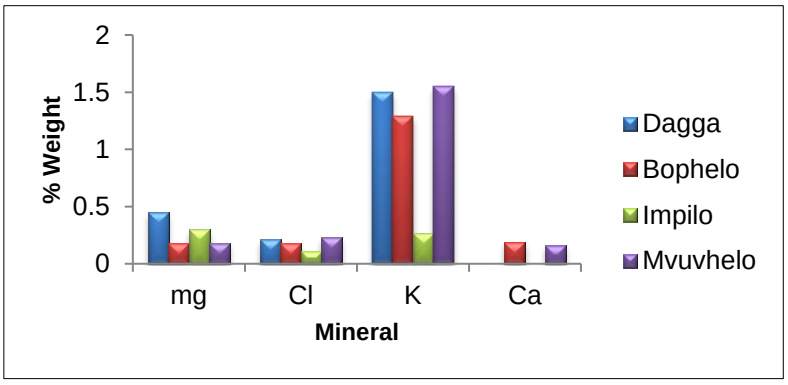


Figure 13: Mineral in sweet potato flour from University of Venda experimental farm.

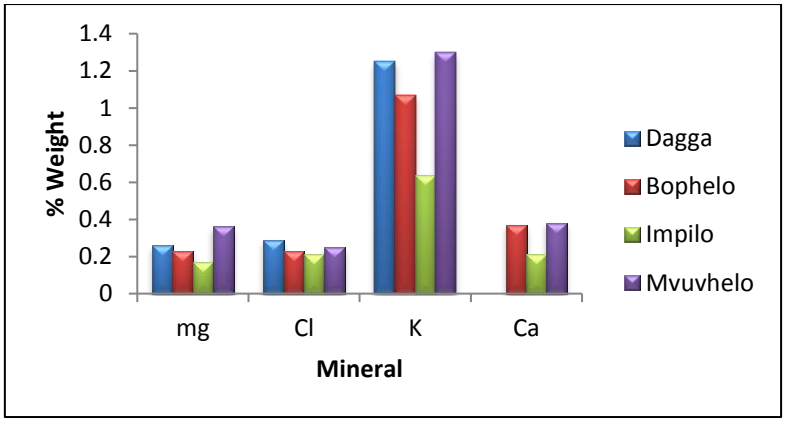


Figure 14: Mineral in sweet potato flour from Tshiombo irrigation scheme.

Mvuvhelo had the highest potassium and magnesium concentration and *Impilo* cultivar had the lowest among the four cultivars. Dagga cultivars had very low calcium concentration compared with the other three cultivars.

Summary of results

β-carotene varied significantly between the two locations, between OFSP cultivars and the interactions. Laurie *et al*., (2012) and Bovell-Benjamin (2007) reported variation in β-carotene to be affected by irrigation, fertilizer application and genetic variation respectively. There was no significant difference in α-carotene content between the two locations but differed among the OFSP flours.

There were no significant differences ($P > 0.05$) in the total carotenoids of OFSP flour from the two locations. As expected, there were significant differences between the OFSP flour and the cream sweet potato flour. However, there was no significant difference in total carotenoids among the OFSP flour. The radical scavenging activity of OFSP flour differed significantly ($P < 0.05$) but no significant differences between the location X cultivar interaction.

3.5 Conclusion

This study revealed significant variations in the β-carotene content of OFSP flour between locations, cultivars and location-cultivar interactions. α-carotene showed a similar trend to β-carotene, but showed no significant difference in location-cultivar interactions. The study revealed no significant differences in the amount of total carotenoids in the flour of OFSP cultivars from the two locations. The FRAP of OFSP showed variability across the locations and cultivar-location interactions. Flour from *Bophelo* cultivar had overall superior characteristics namely total carotenoids, antioxidant activity, β- and α-carotene contents. All sweet potato cultivar flours predominantly contained high amounts of potassium and this is in line with the mineral composition of sweet potato roots (Woolfe, 1992). *Bophelo* cultivar ranked the best among the 4 sweet potato cultivars in terms of their mean total carotenoids, antioxidant, β- and α-carotene contents. Total carotenoids had a strong positive correlation with β and α-carotene, FRAP and DPPH. Hence *Bophelo* cultivar is superior to the other OFSP cultivars in this study. For industry intending to process sweet potato flour on large scale for purposes of combating VAD, *Bophelo* should be considered first among the four sweet potato cultivars in this study. As the name suggests, *Bophelo* (Life, in Sepedi) should be recommended for production among the poor resourced communities.

CHAPTER 4: PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES OF OFSP FLOUR.

4.1 Introduction

There has been renewed interest in the production of OFSP flour to extend the shelf-life of sweet potato. The last 10 years or so has seen the development and commercialisation of a number of food products into flour (Murrieta-Pazos *et al*., 2012). From the consumer's stand point, quick and complete reconstitution of the flour/flour products are among the main quality indicators (Forny *et al.,* 2011). In the food industry, the interest with flour is linked mainly to their stability (chemical and microbiological), reduced transport costs and general convenience as well as intermediate products between several industries (Murrieta-Pazos *et al*., 2012).

Sweet potato (*I. batatas* (L.) Lam) is a relatively easy crop to produce, even by poorly resourced household communities in developing countries. The challenge has been the processing of sweet potato into a form that can easily be reconstituted and provide the necessary nutritional and functional benefits derived from the consumption of sweet potatoes. Processing of sweet potatoes into flour is a very promising venture in this regard in order to extend the shelf–life of the product and mitigate the prevalence of vitamin A deficiency, especially in children 0 - 6 years in developing countries. The objective of this investigation was to examine the effect of processing on the physicochemical and nutritional properties of OFSP cultivars grown in two locations, within Thulamela municipality in Vhembe District, Limpopo province.

4.2 Materials and Methods

OFSP tubers used in this investigation were obtained from two locations in Vhembe District, Limpopo Province; namely University of Venda experimental farm and Tshiombo irrigation scheme. The tubers were washed in tap water, peeled, sliced and dried in an air oven dryer and later milled and sieved to 1 mm flour using a Retsch laboratory mill, Germany.

4.2.1 Determination of vitamin A (retinol)

Due to labile nature of retinol, samples were saponified under nitrogen atmosphere and in the presence of pyrogallic acid (Barua and Furr, 1988). Standards and samples were saponified in basic ethanol-water solution, neutralized, and diluted. This process converted fats to fatty acids, and retinyl esters to retinol and the corresponding fatty acids. Extract clean-up was carried out with a C18 cartridge and vitamin A concentrate eluted with a smaller volume of isopropanol. Retinol was quantified in an HPLC (Delta 600, Waters India Pvt.) system, using UV detection at 326 nm. The concentration was calculated by comparing the peak heights or peak areas of retinol in test samples with those of established standards.

Saponification and extraction of sample

The hot plate was preheated; the cooling water was adjusted to precool reflux condensers. Two millilitres of vitamin A working standard was pipetted into 125 mL Erlenmeyer flask, and 25 mL of 95% ethanol was added. Two grams (2 g) of sweet potato flour sample were accurately weighed into the flasks and 40 mL of 95% ethanol was added. A pea sized piece (approximately 50 mg) of pyrogallic acid (antioxidant) was added to each standard and sample flask, then a glass bead was added to promote boiling. All the flasks were swirled to ensure that all samples were thoroughly dispersed in the solution. Nitrogen flow was turned on to ensure a nitrogen atmosphere in all flasks while refluxing. Ten millilitre (10 mL) 50% KOH solution was pipetted into each flask and immediately, flasks were placed on a hot plate under reflux condenser and swirled. The flasks were refluxed for 45 min and

swirled every 10 min. Reflux flasks were removed from the hot plate and quickly cooled to room temperature using ice water. Ten millilitre (10 mL) glacial acetic acid solution were pipetted into each flask to neutralize the KOH, mixed well and allowed to cool to room temperature. The solution in each flask was transferred quantitatively to 100 ml volumetric flasks using 50:50 THF: ethanol and diluted to volume with the same solution. The volumetric flasks were fitted with stoppers and inverted 10 times. Samples were allowed to set for at least 1 hr at room temperature and overnight in refrigerator to allow fatty acid salts formed during saponification to precipitate. The higher the fat content in the sample, the more precipitate formed.

The optimised conditions for the analysis of vitamin A:

Mobile phase, methanol: water (89:11), Flow rate: 1.3 mL/min, Wave length: 323 nm. The HPLC system was started and allowed to warm up and equilibrate for a minimum of 30 min with mobile phase flowing. Vitamin A standard was injected into HPLC system and mobile phase was adjusted to achieve a solution of 1.5 or better for *cis* and *trans* forms. All-*trans*-retinol eluted in approximately 6 min. The standards were repeatedly injected until peak height(s) or areas were reproducible. Samples solutions were injected, interspersed with standard solutions after every nine samples to ensure accurate quantification. Where retinol peak height exceeded the one for the high standard by more than 25%, sample solutions were diluted using 10 mL 50% KOH solution, 40 mL 95% ethanol, 10 mL glacial acetic acid and 40mL 50:50 THF : ethanol solution. Vitamin A content was calculated using the formula 6.

$$\text{Vitamin A, (µg/g) (as retinol)} = \frac{RF_A \times pH_{samp} \times 100}{W} \quad [6]$$

Where:

pH_{samp} = total sample peak height or area of *all-trans*,

100 = dilution volume of sample,

W = weight of sample in g

4.2.2 Determination of fat content

The method of AOAC 2000, method 920.390, was used to extract the fat content from the OFSP flour samples. Fat was extracted using a 3 position configuration system Velp scientific solvent extractor series R148/3, Perten instruments, Australia. The system is suitable for the separation of a substance or a group of elements from solid and semi-solid samples according to the Rendall technique (Rendall, 1984). The system consists of immersion, washing and solvent recovery stages.

Approximately 1.5 g of finely milled sweet potato flour was weighed using a Mettler-Toledo digital balance (± 0.01 g) in an extraction thimble (33 x 80 mm); the thimble was then attached to the holder and securely attached to the Fat extraction unit. The initial weight of an extraction cup was taken and a 70 mL of petroleum ether was added to the extraction cup. The cup was then positioned for the immersion of the extraction thimble containing the sweet potato sample. The thimble with sample was then immersed in petroleum ether for 60 min at 80^{o}C, and then held for 60 min washing cycle and 60 min fat recovery. The extraction cup with recovered fat were placed in a desiccator to cool. The extraction cup with the recovered fat was reweighed on a digital balance (Mettler-Toledo) and the % fat was finally calculated using formula (7).

$$\% \ \text{Fat} = \frac{\text{Wf}}{\text{Ws}} \text{x } 100 \qquad [7]$$

Where:

Wf = weight of the fat extract

Ws = weight of the original sample

4.2.3 Determination of protein content

The AOAC (2000) method 960.52 with some modifications was used to determine the protein content in the sweet potato flour. One gram (1 g) of sweet potato flour was accurately weighed using a digital Mettler Toledo balance (± 0.01 g) and transferred to a digestion tube. One Kjeldahl tablet and 20 ml concentrated sulphuric acid from an automatic dispenser were added to the weighed sweet potato flour. The tube was then placed in a

preheated fully automatic digester (DKL8, Velp Scientific,) at 420°C for 60 min. until a clear solution was obtained.

After digestion, the tubes were cooled and diluted with 60 ml distilled water. The tubes with digested and diluted sample were placed in a distillation (UDK 129, Velp Scientific). A conical flask containing 25 ml of 4% boric acid (indicator), was placed under the condenser outlet. Eighty millilitres (80 ml) of 35% NaOH was dispensed and distilled for 4 min. The ammonium borate solution that formed was then titrated with 0.2 M hydrochloric acid to a light pink end point.

Total and crude proteins were calculated as follows:

$$\% \text{ N } = \frac{(\text{Vt}-\text{Vb})X\ 1.400xN}{\text{Mass of Sample (g)}} \quad [8]$$

$$\% \text{ Crude protein} = \frac{(\text{Vt}-\text{Vb})\ \text{X}\ 1.4007\text{XNX}6.25)}{\text{Mass of Sapmle (g)}} \quad [9]$$

Where:

Vt = Volume of acid required for Sample
Vb = Volume of acid required for blank
N = Normality of standard acid

4.2.4 Determination of pH

The pH was determined using the method of Dadzie and Orchard (1997). Approximately 2 g of OFSP flour was homogenized with 90 ml of distilled water for 2 min and filtered using Whatman filter paper No 1. Readings were recorded by inserting the pH electrode in the filtrate on stabilization of sample filtrate.

4.2.5 Determination of moisture content.

The moisture content was determined using methods of AOAC (2000) method 925.45. Empty crucibles were dried in the oven at 100°C for 30 min and weighed (W1). One and half grams of well mixed sample was placed in the crucible, accurately weighed and the combined weight recorded as W2. The crucible was kept in an oven at 100 - 105°C for 6 - 12 hrs until a constant weight was obtained before placing the sample in the desiccator to cool.

The crucible was weighed again after cooling (W3). The % moisture content was calculated using formula 10.

Moisture content (%) = $\frac{W2-W3}{W2-W1} \times 100$ [10]

Where:

W1 = initial weight of empty crucible
W2 = weight of crucible + sweet potato flour sample
W3 = final weight of crucible + (dried) sweet potato flour sample

4.2.6 CIE tristimulus (L* a* b*) colour measurement and other parameters

The colour of the food is the first vision impression to the consumer and influences consumer's choice and preferences. The consumer uses colour as one of the major factors to evaluate the quality of food products (Pathare *et al*., 2013). Yam and Papadakis (2004) suggested that in food engineering, it was necessary to analyze the surface colour of the food samples both subjectively (visual) and qualitatively (colour distribution and averages). Research institutes and universities have developed computer vision systems for product quality inspection, evaluation, sorting, prediction and grading (Sun, 2000; Blasco *et al.,*2009; Manickavasagan *et al.*, 2014; Grillo *et al*., 2014; Wang *et al.,* 2012 and Barbin *et al*., 2013).

The colour of milled sweet potato flour was evaluated visually and objectively using a hand held chroma meter model CR- 400, Konica Minolta sensing Inc., Japan. The L*, a*, b* values were analyzed from five values. *L** values range from 100 (white) to 0 (black), *a** values range from +*a** (green) to –*a** (red), and *b** values range from +*b** (yellow) to – *b** (blue). The chroma (ΔC), colour intensity (ΔE*) and hue angle (H) were calculated using equations of Hunt (1991).

$$\Delta C = \sqrt{(\Delta a*)^2 + (\Delta b*)^2} \quad [11]$$

$$\Delta \in = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad [12]$$

Hue angle = Tan^{-1} b/a [13]

Whiteness (WI) and Yellowness (YI) Indices

The Tristimulus (L* a* b*) values obtained from sweet potato flour samples were used to determine the whiteness and yellowness index. The whiteness indices (WI) are widely measured to yield numbers that closely correlate with consumers' preference for white colours (Pathare *et al*. 2013). The yellowness (YI) indices are associated with the general degradation of products by light, chemical and processing (Pathare *et al*. 2013). The whiteness index (WI) and yellowness index of sweet potato flour (YI) were determined by the methods of Rodriguez-Aguilera *et al.* (2011) and Pathare *et al*. (2013), equations (14) and (15)

$$WI = 100 - \sqrt{(100 - L*) + a*^2 + b*^2} \quad [14]$$

$$YI = \frac{142.86b*}{L*} \quad [15]$$

4.3 Statistical analysis

All measurements (except CIE colours in five replicates) were performed in triplicates and the results obtained are expressed as mean ± standard error (SE). Statistical analysis was performed using a one-way analysis of variance (ANOVA) and means of results for each experiment were separated using Duncan's multiple range test (Duncan, 1995). P values < 0.05 were considered to be statistically significant. Pearson's correlation coefficients were used to determine correlation among properties of sweet potato flour. All statistical analyses were conducted using IBM Statistical Package for Social Sciences (SPSS version 22, IBM Inc., New York, USA.) and follow-up analyses were analyzed using STASTICA version 10 (StatSoft).

4.4 Results and discussion

The results of analysis of variance on the effect of processing on physicochemical and nutritional properties of sweet potato flour are shown in Table 13. The results reveal significant (P < 0.05) differences in vitamin A content between cultivar and location-cultivar

interactions. There were also significant differences in fat content between the two locations and within the cultivars. Protein content showed significant differences between the locations, cultivar and location x cultivar interactions. The two locations showed significant differences in the pH of sweet potato flour. There was also a significant difference in the pH of the flour between the cultivar and location interactions. There was no significant difference in flour moisture content between the two locations, cultivar and location cultivar interactions. The results for the effect of processing on physicochemical and nutritional properties (vitamin A, fat, protein, moisture content and pH) are shown in Table 14.

4.4.1 Vitamin A (retinol) content

The results of the main effects for physicochemical and nutritional properties are shown in Table 14. There was a significant difference ($P < 0.05$) in vitamin A content among the cultivars and ranged from 6.88 µg/g retinol in *Dagga* to 7.97 µg/g in *Impilo*. According to WHO (2009) definition, a vitamin A plasma retinol concentration of < 0.70 µmol/L is of public concern in many countries and affects an estimated population of 190 million of pre-school children and 19.1 million pregnant women, and these are mostly in Africa and South–East Asian developing countries. The amounts of vitamin A in the OFSP under study, could supply sufficient amounts of vitamin A retinol to meet the required vitamin A intake by children under the age of 6. The vitamin A content in the flour of OFSP from the two locations did not differ significantly.

Table 13: Physicochemical and nutritional properties of OFSP flour.

Variable	df	MS	F	P-level	Significance
Vitamin A					
Location	1	0.084	0.3354	0.5706	Ns
Cultivar	3	1.535	6.1288	0.0056	*
Location X Cultivar	3	1.408	5.6189	0.0079	*
Error	16	0.251			
% Fat					
Location	1	0.883	35.817	0.000	*
Cultivar	3	0.827	33.532	0.000	*
Location X Cultivar	3	0.030	1.210	0.338	Ns
Error	16	0.025			
% Protein					
Location	1	51.381	268.596	0.00	*
Cultivar	3	17.178	89.799	0.00	*
Location X Cultivar	3	10.984	57.420	0.00	*
Error	16	0.191			
pH					
Location	1	1.046	9.344	0.008	*
Cultivar	3	0.183	1.637	0.220	Ns
Location X Cultivar	3	0.634	5.665	0.008	*
Error	16	0.112			
% Moisture	1	3.916	1.843	0.193	Ns
Location	3	3.338	1.571	0.235	Ns
Cultivar	3	4.725	2.224	0.125	Ns
Location X Cultivar	16	2.13			
Error					

Note: * Denotes statistically significant at P < 0.05. Ns: denotes not significant at P < 0.05, OFSP = Orange fleshed sweet potato

K'osambo *et al* (1998) reported that the farming site had no significant effect on pro-vitamin A β-carotene content. However, they reported root age to have greater influence on pro-vitamin A content. There was a significant difference in vitamin A content between cultivar and location interactions and ranged from 7.47 µg/g retinol in Tshiombo irrigation scheme in *Dagga* cultivar flour to 8.45 µg/g retinol in Univen *Impilo* cultivar flour. The differences in vitamin A content could be attributed to the genetic differences of OFSP cultivars. The percent fat differed significantly between the two locations and ranged from 0.63% in the flour from Tshiombo cultivar flour to 1.01% in the from University of Venda experimental farm.

Table 14: Physicochemical and nutritional properties of OFSP flour

Effect		Vitamin A (µg/g)	Fat content (%)	Protein content (%)	pH
Location					
	Tshiombo	7.54 ± 0.09^{a}	0.63 ± 0.09^{b}	4.23 ± 0.06^{b}	6.01 ± 0.11^{b}
	Univen	7.65 ± 0.09^{a}	1.01 ± 0.11^{a}	7.16 ± 0.06^{a}	6.48 ± 0.11^{a}
Cultivar					
	Dagga	6.88 ± 0.38^{b}	0.98 ± 0.15^{b}	3.47 ± 0.33^{d}	6.45 ± 0.05^{ns}
	Bophelo	7.63 ± 0.12^{a}	0.53 ± 0.09^{c}	5.56 ± 0.22^{c}	6.15 ± 0.03^{ns}
	Impilo	7.97 ± 0.25^{a}	1.28 ± 0.10^{a}	7.53 ± 1.57^{a}	6.40 ± 0.00^{ns}
	Mvuvhelo	7.93 ± 0.23^{a}	0.50 ± 0.07^{c}	6.22 ± 0.54^{b}	6.10 ± 0.02^{ns}
Location X Cultivar					
Tshiombo X *Dagga*		7.47 ± 0.09^{b}	0.87 ± 0.06^{b}	2.74 ± 0.06^{f}	6.29 ± 0.04^{bc}
Tshiombo X *Bophelo*		7.64 ± 0.22^{ab}	0.86 ± 0.04^{b}	5.08 ± 0.10^{d}	5.48 ± 0.07^{d}
Tshiombo X *Impilo*		7.48 ± 0.20 ^{b}	1.06 ± 0.01^{a}	4.09 ± 0.12^{e}	6.30 ± 0.15^{abc}
Tshiombo X *Mvuvhelo*		7.57 ± 0.15^{b}	0.37 ± 0.02^{c}	5.02 ± 0.06^{d}	6.19 ± 0.09^{bc}
Univen X *Dagga*		6.28 ± 0.61^{c}	1.24 ± 0.40^{b}	4.20 ± 0.06^{e}	6.60 ± 0.43^{ab}
Univen X *Bophelo*		7.62 ± 0.15^{ab}	0.60 ± 0.02^{c}	6.04 ± 0.06^{c}	6.82 ± 0.05^{a}
Univen X *Impilo*		8.45 ± 0.17^{a}	1.49 ± 0.03^{a}	10.97 ± 0.7^{a}	6.50 ± 0.04^{abc}
Univen X *Mvuvhelo*		8.29 ± 0.35^{ab}	0.67 ± 0.02^{c}	7.41 ± 0.15^{b}	6.01 ± 0.27^{cd}

Values are means ± standard error, means with same letter superscript in the same sub-column are not significantly different (P < 0.05), (Location n =12; cultivar n = 6; Location X Cultivar n = 3). Ns: no significantly difference at P < 0.05. OFSP = Orange fleshed sweet potato.

4.4.2 Fat content

The results of the analysis for fat content are shown in Table 14. The results showed that the fat content was significantly higher (1.01%) in Univen sweet potato flour compared to Tshiombo flour. This could be attributed to genotypic differences. Tshiombo cultivars have the lowest fat content (0.63%). *Impilo* flour had the highest (1.28%) fat content and *Bophelo* had the lowest (0.53%). These results are higher than those reported by Bovell-Benjamin *et al*., (2007) which ranged from 0.1% to 0.3%.

4.4.3 Protein content

Protein content (Table 14) was significantly the highest (7.16%) in Univen sweet potato cultivar flour and the lowest (4.23%) in Tshiombo sweet potato cultivar flour. The location - cultivar interaction showed that at Univen experimental farm *Impilo* cultivar flour had significantly the highest (10.97%) protein content and at Tshiombo the *Dagga* cultivar flour had significantly the lowest (2.74%). Li, 1982; Ishida *et al*., 2000 reported varietal differences and environmental influence on protein content of sweet potato. The protein

values reported in this study are higher than those reported by Ishida *et al*. (2000) which ranged from 1.3% to 2.1% for two potato cultivars. However, the results in this study compare well with those reported by Ravindran *et al*. (1995) which ranged from 3.0% to 7.2%. Therefore the production environment may influence the protein content in the sweet potato.

4.4.4 pH

The pH of Univen grown sweet potato flour was significantly the highest (6.48) while that of Tshiombo sweet potato flour was lowest (6.01). The location x cultivar interaction showed *Bophelo* flour grown at Univen to have significantly the highest (6.82) pH while Tshiombo grown *Bophelo* had the lowest (5.48). The differences in the pH of the flour from the two locations could be attributed to the growing environment of the sweet potatoes. The higher pH levels could favour availability of nutrients that become available to plants under higher pH levels.

Correlation of physicochemical and nutritional properties.

Mean values of physicochemical and nutritional properties were used to evaluate the Pearson's correlation coefficients (Table 15). The results revealed a significantly high (0.594**) correlation between protein and vitamin A contents. As the protein content increased so was the vitamin A and fat content. Protein was also significantly (0.421*) correlated with fat content. Fat content was significantly (0.454*) correlated with moisture content. Vitamin A content was negatively (-0.072) correlated with fat content. However, as the fat content increase, the vitamin A content decreased (Table 15). As the vitamin A content increased the protein content of cultivar flour also increased. However, there was negative correlation between vitamin A, moisture and fat contents.

Table 15: Correlation of physicochemical and nutritional properties of sweet potato flour.

Variables	Vitamin A	Fat	Protein	pH	MC
Vitamin A	1				
Fat	- 0.072	1			
Protein	0.594**	0.421*	1		
pH	-0.070	0.379	0.095	1	
MC	-0.310	0.454*	0.139	0.139	1

* Significant at P < 0.05; ** Significant at P < 0.01; MC = Moisture content.

4.4.5 CIE tristimulus (L* a* b*) and other colour parameters

The tristimulus (L* a* b*) colour space is a more visual uniform scale (Falade and Okafor, 2013). According to Commission Internationale de l' Eclairage (CIE) system, the human eye has three receptors namely; red, green and blue (Pathare *et al*., 2013). The visual colours of the four (4) cultivars from the two locations can be described as orange (*Bophelo, Dagga* and *Impilo*) and cream (*Mvuvhelo*). The CIE tristimulus (L* a* b*), chroma (C*), colour density (ΔE*) and hue angle (H^o) are shown in Tables 15, 16 and 17. The L* parameter was significantly highest (86.09) in the flour of *Dagga* cultivar and lowest (76.28) in *Bophelo.* The a* (14.09) and b* (35.59) values were significantly highest in *Bophelo* flour and significantly lowest 0.62 and 15.86 in *Mvuvhelo*, respectively. The colour density (ΔE*) was significantly highest 38.48 ± 7.55 in (*Bophelo*) and lowest (26.14 ± 0.79) in *Dagga*. There was no significant difference in the hue angle (H^o) across all sweet potato cultivar flours.

Table 16: Effect of OFSP cultivar on Tristimulus (L* a* b*) colour and other parameters.

Cultivar	L*	a*	b*	ΔE*	C*	Hue Angle (H^o)
Dagga	83.51 ± 0.88^{a}	4.84 ± 0.98^{b}	25.53 ± 0.28^{c}	26.14 ± 0.79^{c}	87.52 ± 0.32^{a}	-17.36 ± 1.76^{a}
Bophelo	76.28 ± 0.61^{b}	14.09 ± 1.11^{a}	35.59 ± 1.01^{a}	38.48 ± 7.55^{a}	85.47 ± 0.70^{bc}	-0.30 ± 17.88^{a}
Impilo	82.07 ± 0.62^{a}	4.56 ± 0.48^{b}	29.07 ± 0.61^{b}	29.45 ± 0.43^{b}	87.23 ± 0.67^{ab}	0.32 ± 1.51^{a}
Mvuvhelo	83.45 ± 0.75^{a}	0.62 ± 0.04^{c}	15.86 ± 0.14^{d}	15.87 ± 0.73^{d}	84.95 ± 10.93^{c}	5.19 ± 1.99^{a}

Values are means ± standard error, means in a column with the same letter superscript are not significantly different ($P < 0.05$), ($n = 10$). L* = lightness to darkness; a* = redness (+)/greenness (–); b* = yellowness (+)/ blueness (–); C* = Chroma; ΔE* = Colour difference; OFSP = Orange fleshed sweet potato.

Table 17: Tristimulus (L* a* b*) and other colour parameters of OFSP flours from Tshiombo.

Location	Cultivar	L*	a*	b*	ΔE*	ΔC*	Hue Angle(H^o)
Tshiombo	*Dagga*	86.09 ± 0.08^a	1.90 ± 0.04^b	25.68 ± 0.45^c	25.75 ± 0.46^c	89.86 ± 0.10^a	0.73 ± 0.20^a
	Bophelo	77.20 ± 0.75^d	16.30 ± 1.70^a	32.70 ± 0.49^a	36.69 ± 0.57^a	85.48 ± 0.87^c	-0.63 ± 0.22^a
	Impilo	83.56 ± 0.67^b	3.17 ± 0.18^b	27.57 ± 0.62^b	27.75 ± 0.63^b	88.06 ± 0.64^b	2.05 ± 3.2^a
	Mvuvhelo	81.49 ± 0.20^c	0.51 ± 0.02^b	15.70 ± 0.13^d	15.71 ± 0.09^d	82.99 ± 0.18^d	0.76 ± 3.18^a

Values are means ± standard error, means in a column with the same letter superscript are not significantly different (P < 0.05), (n = 10). L* = lightness to darkness; a* = redness (+)/greenness (–); b* = yellowness (+)/ blueness (–); C* = Chroma; ΔE* = Colour difference; OFSP = Orange fleshed sweet potato.

Table 18: Tristimulus (L* a* b*) and other colour parameters of OFSP flours from Univen.

Location	Cultivar	L*	a*	b*	ΔE*	C*	Hue Angle (H^o)
Univen	*Dagga*	80.93 ± 0.35^b	7.77 ± 0.17^b	25.38 ± 0.38^c	26.54 ± 0.41^c	85.18 ± 0.21^b	9.64 ± 1.99^a
	Bophelo	75.36 ± 0.82^c	11.88 ± 0.39^a	38.47 ± 0.42^a	40.27 ± 0.51^a	85.46 ± 0.54^{ab}	-34.10 ± 36.03^a
	Impilo	80.58 ± 0.42^b	5.95 ± 0.17^c	30.56 ± 0.40^b	31.14 ± 0.43^b	86.40 ± 0.25^{ab}	-0.48 ± 0.10^a
	Mvuvhelo	85.42 ± 0.73^a	0.74 ± 0.03^d	16.01 ± 0.25^d	16.03 ± 0.25^d	86.91 ± 0.68^a	-0.11 ± 2.75^a

Values are means ± standard error, means in a column with the same letter superscript are not significantly different (P < 0.05), (n = 10). L* = lightness to darkness; a* = redness (+)/greenness (–); b* = yellowness (+)/ blueness (–); C* = Chroma; ΔE* = Colour difference; OFSP = Orange fleshed sweet potato.

Whiteness (WI) and Yellowness (YI) index

The WI of food is used to measure the consumer preferences for white colour and therefore gives an indication of the level of discolouration during processing. The YI gives an indication of the general product degradation by processing, light and chemical exposure (Pathare *et al.* 2013). The whiteness (WI) and yellowness (YI) indices shown in Figures 15 and 16 revealed significant ($P < 0.05$) differences in the WI and YI of flour between cultivars from Tshiombo (Figure 15). The WI and YI of flour from Univen cultivars show little difference between the WI and YI (Figure 16).

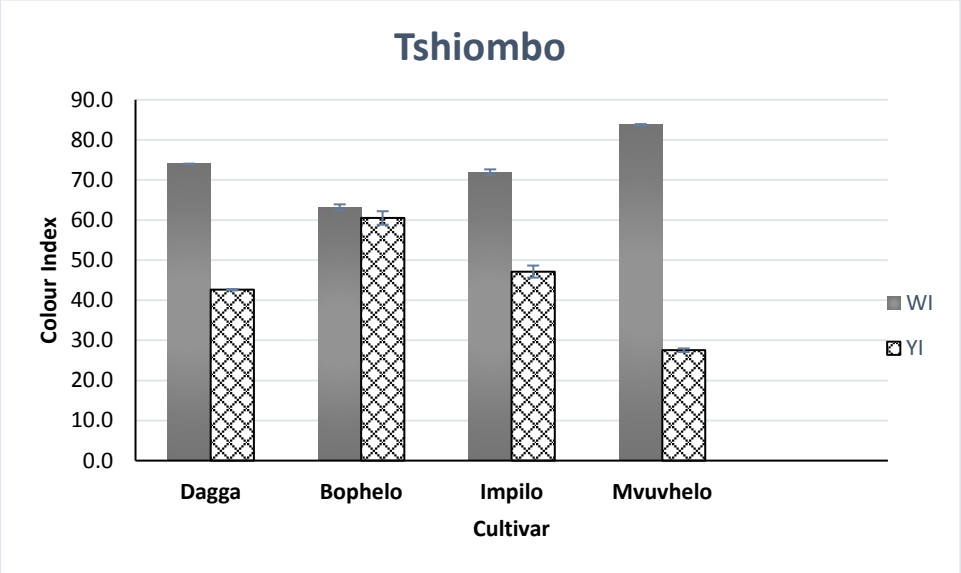


Figure 15: Whiteness and yellowness indices of sweet potato flour from Tshiombo.
YI = Yellowness index; WI = Whiteness index.

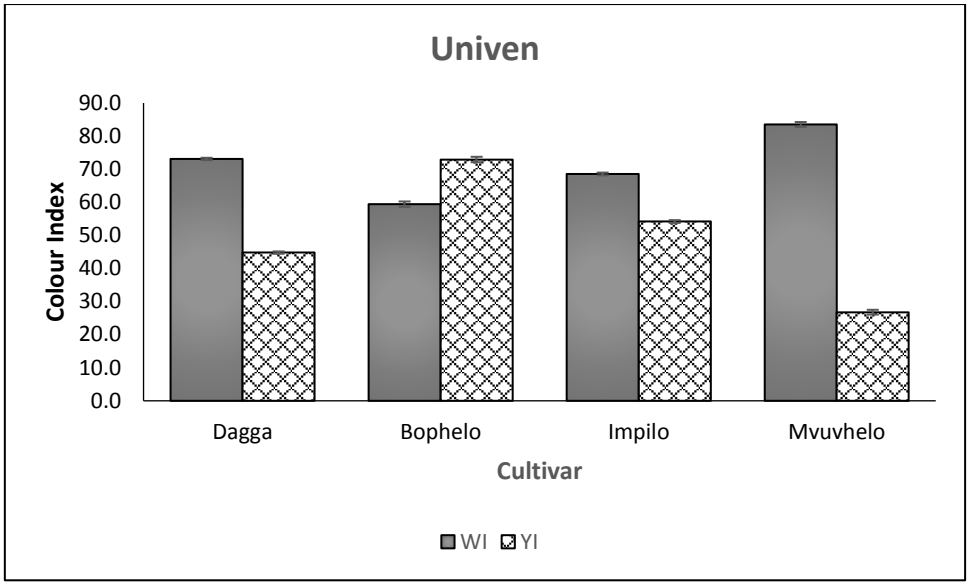


Figure 16: Whiteness and yellowness indices of sweet potato flour from Univen.

YI = Yellowness index; WI = Whiteness index.

4.5 Conclusion

Impilo flour contained the highest vitamin A (retinol), fat and protein contents. Fat varied in all cultivar flours. University of Venda experimental farm sweet potato flour contained higher fat and protein contents as well as a higher pH value. This could be attributed to different management practice levels between the two locations. The flour from *Bophelo* cultivar maintained higher WI and YI from both locations after processing. Protein content positively correlated with vitamin A as well as fat content. Both colour density and chroma varied between cultivar flour. *Impilo* had superior vitamin A, fat and protein contents.

CHAPTER 5: EFFECT OF LOCATION ON THE FUNCTIONAL PROPERTIES OF OFSP FLOUR.

5.1 Introduction

Due to high moisture content, sweet potatoes are normally dried to lower moisture content in order to extend product shelf-life. Bengtsson *et al*. (2008) reported hot-air drying and solar drying as the conventional drying methods of sweet potato to lower their moisture content. Hot-air drying has the disadvantage of taking long even at high temperature, which may result in serious damage to the flour, colour and nutrients in dried products (Sharma and Prasad, 2003; Ratti, 2001).

Vacuum freeze-drying technology which would be the best method of water removal with better quality final products compared to other drying methods is still practised on an industrial scale to dry coffee, spices, meats, food ingredients and other high-value foods (Zhang, *et al*., 2010; Ratti, 2001; Lin, *et al*., 1998). The aim of this study was to investigate the effect of location on functional properties of OFSP cultivar flour.

5.2 Materials and Methods

5.2.1 Determination of total starch

Starch was determined following the method described by Megazyme International. (2015). The hydrolysis of starch to glucose is catalysed by amyloglucosidase. Glucose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalysed by hexokinase glucose -6-phosphate (G6P) is then oxidized to 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) in a reaction catalysed by glucose -6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD is reduced to NADH. The consequent increase in absorbance at 340 nm is directly proportional to the glucose concentration. Table 18 shows the hydrolysis of starch to glucose.

Approximately 1 g sweet potato flour was transferred into a flask (100 – 250 ml). Twenty millimetres (20 ml) of dimethyl sulfoxide (DMSO) and 5 ml of 8 M HCL were added to the sample. The flasks containing the sample were covered to block light and incubated for

30 min at 60°C in a shaking water bath. Fifty millilitres deionized water was added to the flasks, the pH was adjusted to 4 – 5 with 5 ml of 8M HCl. The samples were then cooled to room temperature and diluted with 100 ml deionized water. One millilitre of the solutions were pipetted into appropriately marked test tubes and mixed as shown in Table 18. The test tubes were then incubated for 15 min at 60 °C in a shaking water bath and cooled to room temperature.

Starch assay

Table 19: Procedure for the starch assay.

Serial No	Starch assay reagent (ml)	Sample (ml)	D/W (ml)
1) Starch assay reagent blank	1	-	1
2) Sample blank	-	1	1
3) Glucose assay reagent blank	-	-	2
4) Test	1	1	-

Source: AOAC (2000) method 2002.02

Glucose assay

A solution corresponding to glucose of approximately 0.5 – 50 µg was pipetted into marked test tubes as indicated in Table 19.The assay was repeated to give a $\blacktriangle A_{340}$ between 0.03 and 1.6. The test tubes were incubated for 15 min at room temperature (18-35 °C). The absorbance was then measured at 340 nm.

Table 20: Procedure for the glucose assay.

Serial No	Glucose assay reagent (ml)	Sample volume in µl (Solution from Starch Assay)
1) Starch assay reagent blank	1.0	50
2) Sample blank	1.0	50
3) Glucose assay reagent blank	1.0	50
4) Test	1.0	50

Source: AOAC (2000).

Calculations

Total blank

The total blank took into account the contribution to the absorbance of the sample, the glucose assay reagent and the starch assay reagent. The absorbance of the glucose assay reagent was subtracted from the sample blank so that the absorbance of the glucose assay reagent was counted only once in the total absorbance since it was in both the sample and the starch assay reagent blank.

$$A_{\text{Total blank}} = (A_{\text{sample Blank}} - A_{\text{glucose Assay Reagent Blank}}) + A_{\text{Starch Assay Reagent Blank}}$$

Starch Concentration in (mg/ml) = *SC*

$$SC = \frac{(\blacktriangle A)\,(TVSA/SVSA)\,(TVGA/SVGA)\,(\text{Starch MW})\,(F)}{(\acute{\varepsilon})\,(d)\,(1{,}000)} \qquad [16]$$

Where:

▲A = $A_{\text{Test}} - A_{\text{total Blank}}$

TVSA = Total Assay Volume from Starch Assay in ml

SVSA = Sample Volume from Starch Assay in ml

TVGA = Total Assay Volume from glucose Assay in ml

SVGA = Sample volume from glucose Assay in ml Starch

MW = 162.1 g/mole or equivalently 162.1µg/µmoles

F = Dilution Factor from Sample Preparation

ε = Millimolar extinction coefficient for NADH at 340 nm Millimolar^{-1} cm^{-1} or equivalently (ml/µmoles) (1/cm)

d = Light path (cm) = 1 cm

1,000= Conversion Factor for µg to mg

$$SC\ (mg/ml) = \frac{(\blacktriangle A)\,(2)\,(TVGA/SVGA)\,(162.1)\,(F)}{(6.22)(1)\,(1{,}000)} \qquad [17]$$

$$\text{Starch Concentration (SC) (mg/ml)} = (\blacktriangle A)\,(TVGA/SVGA)\,(F)\,(0.052) \qquad [18]$$

5.2.2 Determination of resistant starch

The part of starch which is not broken down by human enzymes in the small intestine is referred to as resistant starch (RS). Resistant starch is generally considered to be part of the components that make up total dietary fibre (Sarawong *et al*., 2014). The shape and size of the pasting curve for a particular starch sample will depend on the properties of that starch. Each starch molecule is a large polymer consisting of glucose units. There are two distinct polymer types; amylose and amylopectin (BNF, 1990). Amylose is a relatively small polymer with a linear structure, whereas amylopectin is a very large polymer that exhibits substantial branching. Figure 17 shows a typical temperature profile and pasting curve for starch food products.

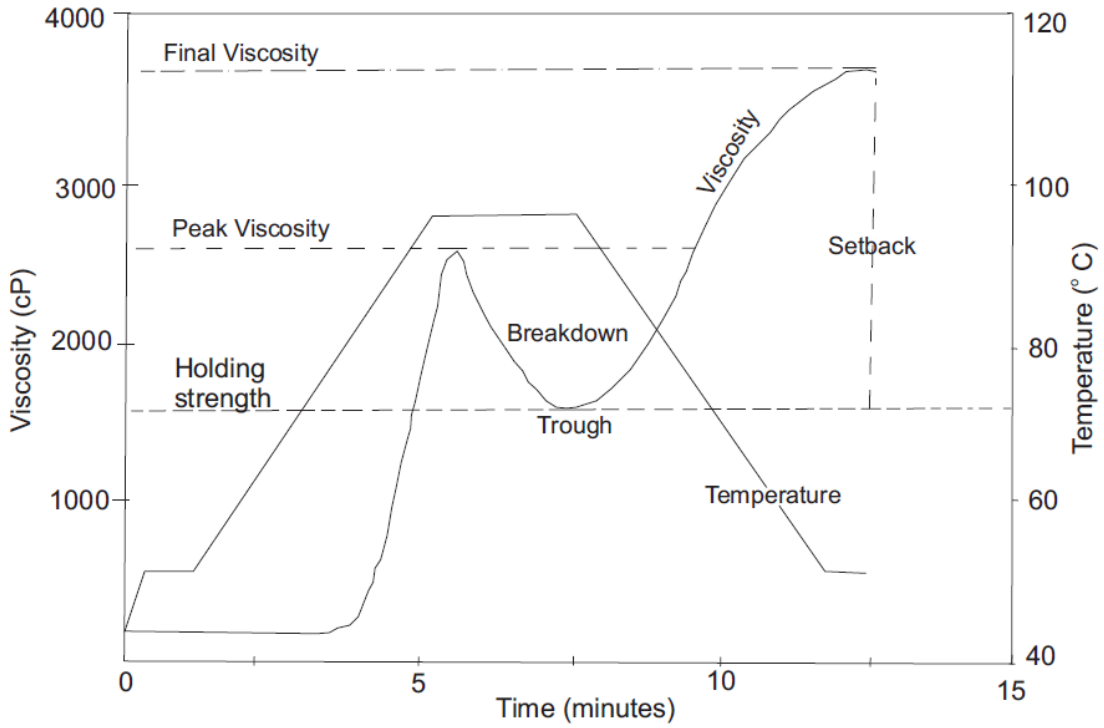


Figure 17: Typical pasting curve and temperature profile.
Perten Instruments of Australia, (2010). 12 cent poise (cP) = 1 Rapid viscosity unit (RVU).

Sample preparation

Hundred (100) mg of sweet potato flour samples were incubated in a shaking water bath with pancreatic α-amylase and amyloglucosidase (AMG) for 16 hrs at 37°C, during which time non-resistant starch was solubilised and hydrolysed to D-glucose by the combined action of the two enzymes. The reaction was terminated by the addition of an equal volume of ethanol or industrial methylated spirits (IMS, denatured ethanol), and the RS was recovered as a pellet on centrifugation. This was then washed twice by suspension in aqueous IMS or ethanol (50% v/v), followed by centrifugation. Free liquid was removed by decantation. RS in the pellet was dissolved in 2 M KOH by vigorously stirring in an ice-water bath over a magnetic stirrer following the method published by Megazyme International, (2015).

This solution was neutralised with acetate buffer and the starch was quantitatively hydrolysed to glucose with AMG. D-glucose was measured with glucose oxidase-peroxidase reagent (GOPOD), and this was a measure of the RS content of the sample. Non-resistant starch (solubilised starch) was determined by pooling the original supernatant and the washings, adjusting the volume to 100 mL and measuring D-glucose content with GOPOD.

The moisture content of the sweet potato flour was determined by the by AOAC (2000) Method 925.10. Hydrolysis and solubilisation of non-resistant starch was achieved by accurately weighing 100 mg of OFSP flour samples into screw cap tube (Corning culture tube; 16 x 125 mm) and tubes were gently tapped to ensure all contents fell at the bottom. Four (4.0) mL of pancreatic α-amylase (10 mg/mL) containing AMG (3 U/mL) (Solution 2) was added to each tube. The tubes were tightly capped and contents mixed on a vortex mixer. The tubes were later attached horizontally in a shaking water bath and incubated tubes at 37°C with continuous shaking (200 strokes /min) for exactly 16 hr (100 forward and 100 reverse).

The tubes were removed from the water bath and excess surface water was removed with paper towel, tube caps were removed and the contents treated with 4.0 mL of ethanol (99% v/v) with vigorous stirring on a vortex mixer, then the tubes were centrifuged at

1,500 g (approx. 3,000 rpm) for 10 min (non-capped). The supernatants were carefully decanted and the pellets re-suspended in 2 mL of 50% ethanol or 50% IMS with vigorous stirring on a vortex mixer. A further 6 mL of 50% IMS were added; tubes were mixed and centrifuged again at 1,500 g for 10 min. The supernatants were decanted and suspension were repeated and centrifuged once more. Finally the supernatants were decanted and tubes were inverted on absorbent paper to drain excess liquid.

Measurement of resistant starch

A magnetic stirrer bar (5 mm x 15 mm) and 2 mL of 2 M KOH were added to each tube, re-suspended the pellets and dissolved the RS by stirring for approximately 20 min in an ice/water bath over a magnetic stirrer. Eight (8) mL of 1.2 M sodium acetate buffer (pH 3.8) was added to each tube with stirring on the magnetic stirrer. Immediately 0.1 mL of AMG (solution1; 3300 U/mL) was added to each tube, mixed well, placed in a water bath at 50°C and the tubes were incubated for 30 min with intermittent mixing on a vortex mixer. For samples containing more than 10% resistant starch and those containing less than 10% resistant starch equations [19] and [20] were used respectively.

Calculations

The resistant starch, non-resistant (solubilised) starch and total starch content (%, on a dry weight basis) in test samples were calculated as follows:

Resistant Starch (g/100 g sample) (samples containing > 10% RS):

= ΔE x F x 100/0.1 x 1/1000 x 100/W x 162/180

= ΔE x F/W x 90. [19]

Resistant starch (g/100 g sample) (samples containing < 10% RS):

= ΔE x F x 10.3/0.1 x 1/1000 x 100/W x 162/180

= ΔE x F/W x 9.27. [20]

Where:

ΔE = absorbance (reaction) read against the reagent blank.

F = conversion from absorbance to micrograms (the absorbance obtained for 100 µg of D-glucose in the GOPOD reaction is determined, and F = 100 (µg of D-glucose) divided by the GOPOD absorbance for this 100 µg of D-glucose.

100/0.1 = volume correction (0.1 mL taken from 100 mL).

1/1000 = conversion from micrograms to milligrams.

W = dry weight of sample analysed "as is" weight x [(100-moisture content)/100].

100/W = factor to present RS as a percentage of sample weight.

162/180 = factor to convert from free D-glucose, as determined, to anhydro-D-glucose as occurs in starch.

10.3/0.1 = volume correction (0.1 mL taken from 10.3 mL) for samples containing 0-10% RS Where the incubation solution is not diluted and the final volume is ~ 10.3 mL.

5.2.3 Determination of water holding capacity of sweet potato flour

Water holding capacity was measured according to the method described by Mei *et al.,* (2010). One (1) g of sweet potato flour was weighed into pre-weighed 15 ml centrifuged tubes. Ten (10) ml of distilled water was added and vortexed for 2 min. The samples were thoroughly wetted and allowed to stand at room temperature for 30 min and centrifuged at 3000 rpm for 20 min at 4^{o}C using a 36 place Ependorf AG centrifuge model 5810R, Germany. The supernatant was decanted and the centrifuge tube containing sediment was weighed. Water holding capacity; gram of water per gram of dry matter was calculated using formula (21).

$$\text{WHC (g } H_2O\text{/g DM)} = \frac{(W2 - W1)}{W0} \quad [21]$$

Where:

W0 = the weight of the dry sample in grams

W1 = the weight of the tube plus the dry sample in grams

W2 = the weight of the tube plus the sediment in grams

5.2.4 Determination of pasting properties sweet potato flour

The pasting properties of sweet potato flour sample were determined in duplicates using a Rapid Visco-analyzer (RVA Series 4500, perten instruments of Australia Pty. Ltd., Unit 13, 2 Eden park drive Macquarie park, NSW 2102, Australia). The product weight and

water weight were both corrected using the base sample moisture content using the Thermocline for windows calculator. The samples' moisture content ranged from 3.3% to 7.4% and the corrected sample weight ranged from 3.3 g to 3.4 g. The corrected distilled water weight ranged from 25.1 g to 25.24 g. The samples were weighed in a plastic weighing boat and carefully poured in the RVA canister containing distilled water.

The standard Profile1 procedure (Mahasukhonthachat *et al*., 2010) was used to determine the pasting properties of sweet potato flour. A suspension containing 10% (w/w, solids) in distilled water was heated from 50°C and held at 960 rpm for 10 s, stirred at the same temperature and 160 rpm for an additional 50 s, heated to 95°C in 3 min 42 s, held at 95°C for 2 min 30 s before cooling to 50°C in 3 min 48 s, and held at 50°C for 2 min. The RVA Thermocline™ for windows (TCW) software (ver. 3.0) was used to obtain the pasting temperature, initial viscosity, peak viscosity (PV) and final viscosity (FV). A typical temperature profile and pasting curve is shown in Figure 17.

For best results, sample and water weights were corrected for the sample moisture content to give a constant dry weight. The typical moisture content is 11 or 14%. A sample weight calculator in the thermocline software (TCW) was used to calculate the corrected sample and water weights. Where sample and water weight were not fixed, the formulae for calculating the corrected sample weight and water weight are given in equations (22) and (23). The results were expressed in cP; where; 12 cP; = 1 Rapid Visco Analyser units (RVU).

$$S1 = \frac{So(100-Mo)}{(100-M1)} \quad [22]$$

$$W_1 = W_o + S_o - S_1 \quad [23]$$

Where: s_1 = corrected sample weight (g), w_1 = *corrected* water weight (g), m_1 = actual moisture content of sample (%), s_0 = standard sample weight (typically 3.5-4.0 g), w_0 = standard water weight (typically 25 g); m_0 = reference moisture content typically 11 or 14 %)

If water weight remains fixed, then the corrected sample weight can be calculated using the formula (24) and (25) respectively.

$$S1 = \frac{SoWo(100-mo)}{Wo(100-m1)+So(mo-m1)} \quad [24]$$

$$W1 = \frac{Wo(100-m1)+So(mo-m1)}{100-mo} \quad [25]$$

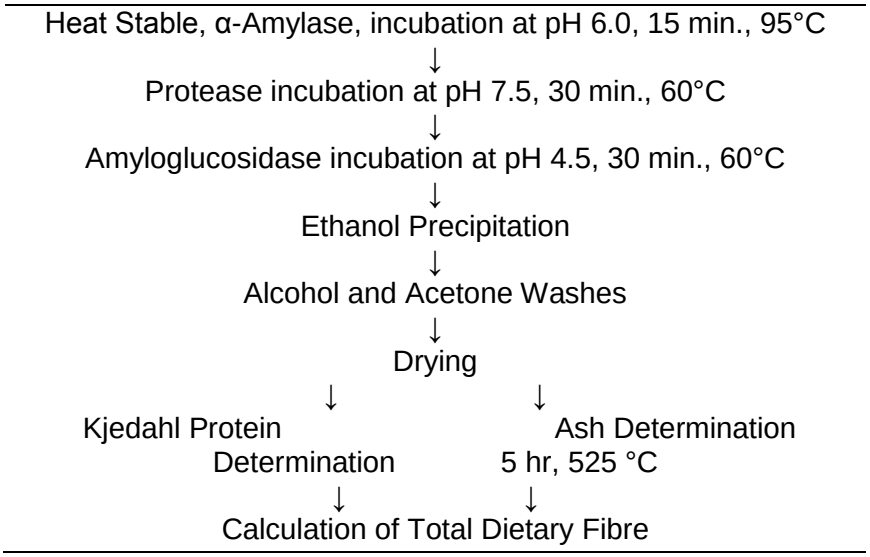
5.2.5 Determination of total dietary fibre

Introduction

Dietary fibre is a mixture of complex organic substances and was initially defined as remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man. Megazyme international, (2015), suggested that dietary fibre includes hydrophobic compounds, such as soluble and insoluble polysaccharides and non-digestable oligosaccharides as well as a range of non-swellable, more or less hydrophobic compounds, such as cutins, suberins and lignins.

This procedure for the determination of total dietary fibre is based on the methods published by Megazyme International (2015) based on Lee *et al*., (1992) and Prosky, *et al*., (1988), methods and is shown in Table 20.

Table 21: Procedure for determination of dietary fibre.



Source: Megazyme International (2015).

Crucibles were thoroughly heated for 1 hr at 525ºC, cooled soaked and rinsed in water and then air dried. Half a gram (0.5 g) of celite was added to each crucible and dried at 130 ºC to constant weight (1 hr or more). Crucibles were cooled in a desiccator and weighed to the nearest 0.1 mg. The weight was recorded as “Celite + Crucible “or W1 and stored in desiccators until needed.

The blanks were run along with samples through the entire procedure to measure any contribution to residue from the reagents. Samples to be tested for dietary fibre were run at least in quadruplicates so that duplicate protein and ash values were available for improved accuracy. Four 1 g samples of sweet potato flour were weighed into tall form beakers to the nearest 0.1 mg. To each beaker, 50 ml of pH 6.0 phosphate buffer, 0.1 ml α-amylase were added and mixed well. Each beaker was covered with an aluminium foil and placed in a boiling water bath.

The beakers were then gently agitated at 5 min intervals and incubated for 15 min after the internal beaker temperature reached 95 ºC. The solutions were allowed to cool to room temperature. The pH of the solution in each beaker was adjusted to 7.5 ± 0.2 by adding 10 ml of 0.275 N NaOH. Pipette 0.1 ml (5 mg Protease). Each beaker was covered

with aluminium foil and placed in 60 °C water bath. With continuous agitation, beakers were incubated for 30 min after the internal temperature of the beakers reached 60 °C.

The beakers were allowed to cool to room temperature. The pH of the solutions in each beaker was adjusted to between pH 4.0 and 4.6 by adding 10 ml of 0.325 M HCl. To each beaker, 0.1 ml of amyloglucosidase was added and covered with aluminium foil and placed in a 60°C water bath. With continuous agitation, beakers were incubated for 30 min after the internal temperature of the beakers reached 60 °C. To each beaker, 4 volumes of 95% ethanol was added. The solutions were set at room temperature overnight to allow complete precipitation. The precipitate was filtered wet and bed of Celite in each crucible was redistributed the using 78% ethanol. A gentle suction was applied to draw Celite onto frit as an even mat, maintain gentle suction and quantitatively transfer the precipitate and suspension from each beaker to its respective crucible. The residue was washed with three 20 ml portions of 78% ethanol, two 10 ml portions of 95% ethanol, and two 10 ml portions of acetone.

The crucibles containing the residues were dried overnight at 105 °C, cooled in desiccators and weighed to the nearest 0.1 mg and recorded as “Residue + Celite + Crucible” or W2. The residues from two samples and two blanks were analyzed for protein by Kjeldahl nitrogen analysis as specified by the AOAC (2000). Factor 6.25 was used to convert ammonia determined in the analysis to protein. The residues in the crucibles from the two samples and two blanks were ashed for 5 hr at 525°C, cooled in the desiccator, weighed to the nearest 0.1 mg and recorded as “Ash + Celite +Crucible weight” or W3.

Calculations

Residue weight = W2-W1; Ash Weight = W3-W1

B = R blank – P blank - A blank

$$\%TDF = \frac{\text{R sample} - \text{P sample} - \text{A sample} - \text{B}}{\text{SW}} \text{ X } 100 \qquad [26]$$

Where:

TDF = Total Dietary Fibre

R = Average Residue Weight (mg)

P = Average Protein Weight (mg)

A = Average Ash Weight (mg)

SW = Average Sample Weight (mg)

5.2.6 Scanning electron microscopy of sweet potato flour starch granules

Imaging of the sweet potato flour samples was accomplished using a Leo® 1430VP scanning electron microscope (SEM) according to the method by Yadav *et al.* (2006). Prior to imaging the samples were mounted on a stub with double sided carbon tape. The samples were then coated with a thin layer of gold in order to make the sample surface electrically conductive. Beam conditions during surface analysis were 7 KV and approximately 1.5 nA, with a working distance of 13 mm and a spot size of 150. Figures 25 and 26 show the SE images of the sweet potato flour granules.

5.2.7 Computed tomographic scans of OFSP flour

The scanning system comprised a computer tomography (CT) scanner, an X-ray inspection machine (General Electric Phoenix VTomex Model L240). A five- axis universal x-ray imaging computer Tomography system designed for the Digital x-rays and Computed Tomography inspection of small objects was used to inspect and anlyse the density of four sweet potato flour samples from Limpopo Province. An industrial Computer CT Scanner is an X-ray inspection machine that allows 2D X-ray inspection of materials. The CT scanner sends X-rays through the material being studied. Each rotation provides a picture of thin slices of the object/material being studied. These pictures are saved as a group on a computer.CT scan has been extensively described by Da-Wen (2016).

Samples of sweet potato flour were placed in CTscan test tubes and loaded into a CT scanner. The sweet potato samples were allowed to move: Vertical-12^{o,} Horizontal-12 ^{o}, Z axis -12 ^{o}, Tilt -+/- 20 Degrees, rotation – 360 Degrees continuous. The CT scanner was allowed to take thin picture images of sweet potato flour samples. The pictures where saved as a group on the computer for further analysis.

5.2.8 Determination of ash content

The total mineral content of a food may be estimated as the ash content, which is the inorganic residue remaining after the organic matter has been burnt away (James, 1996). Crucibles were heated in the oven to constant weight overnight and cooled in the desiccator; 3 g of sweet potato flour samples were accurately weighed into the crucibles. The crucibles were transferred to a muffle furnace at about 550°C and left there until a white or light grey ash is achieved. The samples were cooled in a desiccator and re-weighed.

Calculation

Calculation of the total ash as percentage of the original sample was calculated using formula 27.

$$\% \text{ Ash} = \frac{W3-W1}{W2-W1} X\ 100 \qquad [27]$$

Where:

W_1 = Weight of empty crucible,
W_2 = Weight of crucible + sweet potato flour samples
W_3 = weight of crucible + ash.

5.3 Statistical analysis

Statistical analysis was carried out as described in section 3.3.

5.4 Results and discussion

The results of analysis of variance on the effect of location on the functional properties of OFSP cultivar flour are shown in Table 21. The results showed significant differences ($P < 0.05$) in total starch, resistant starch, WHC and ash content for location, cultivar and location – cultivar interactions. The results of the follow-up tests are shown in Table 22. The results revealed significant differences in functional properties between the OFSP cultivar flours as well as location - cultivar interactions. The results of the correlation matrix among the functional properties of OFSP cultivar flour are shown in Table 23. The results revealed significant negative correction between total starch, WHC and ash contents. However, results showed a significant positive correlation between WHC and ash content.

5.4.1 Total starch

The results of effect of location on functional properties (Table 22) revealed significant differences in total starch of OFSP cultivar flour between the two locations and ranged from 226.08 µg/100 g in Univen experimental farm flour to 331.44 µg/100 g in Tshiombo irrigation scheme cultivar flours. The total starch also differed significantly among the OFSP cultivar flours and ranged from 244.04 µg/100 g in *Bophelo* cultivar to 325.04 µg/100 g in *Dagga* cultivar (Table 22). The location – cultivar interaction also shows significant differences in total starch ranging from 153.67 µg/100 g in Univen *Impilo* cultivar flour to 365.85 µg/100 g in Tshiombo *Mvuvhelo* cultivar flour. *Mvuvhelo* would be the cultivar of choice for starch production among the cultivars under this study.

Table 22: Effect of location on functional properties of OFSP flour.

Variable	df	MS	F	P-Level	Significance
Total starch					
Location	1	56542.30	26629.50	0.000	*
Cultivar	2	8696.11	4095.10	0.000	*
Location X cultivar	3	11389.67	5364.15	0.000	*
Error	16	2.12			
Resistant starch					
Location	1	18.872	11776.50	0.000	*
Cultivar	2	35.296	22025.90	0.000	*
Location X cultivar	3	1.488	928.44	0.00	*
Error	16	0.002			
WHC				0.000	*
Location	1	0.324	37.550	0.000	*
Cultivar	2	0.685	79.270	0.000	*
Location X cultivar	3	0.340	39.420		
Error	16	0.009			
Ash					
Location	1	18.743	4089.03	0.000	*
Cultivar	2	0.519	113.21	0.000	*
Location X cultivar	3	2.087	455.26	0.000	*
Error	16	0.005			

* Statistically significant at P < 0.05; Duncan's multiple range tests. WHC = Water holding capacity, OFSP = Orange fleshed sweet potato.

5.4.2 Resistant starch

The results of the analysis of variance for resistant starch (RS) are shown in Table 22. The results showed significant differences (P < 0.05) in RS in the flours from the two locations. Tshiombo sweet potato cultivar flours had significantly the highest (4.69 µg/100 g)

RS and Univen the lowest (2.92 µg/100 g). These differences in RS could be attributed to environmental effects (Tester and Karkalas, 2010). There were also significant differences in the RS amongst the OFSP cultivar flours ranging from 1.21 µg/100 g in *Bophelo* cultivar flour to 7.07 µg/100 g in *Impilo* flour. These differences could be due to genotypic variations. Perera *et al*. (2010) attributed the variation in resistant starch of foods to genetic factors. Location –cultivar interactions showed a significant variation ranging from 0.89 µg/100 g in Univen *Bophelo* flour to 8.43 µg/100 g in Tshiombo Impilo flour.

5.4.3 Water holding capacity (WHC)

The results of analysis of the effect of location on WHC are shown in Table 22. The results showed significant differences ($P < 0.05$) in WHC of OFSP flour from the two locations and ranged from 1.44 g H_2O/g DM in Tshiombo flours to 1.72 H_2O/g DM in Univen flours. Location- cultivar interactions significantly varied and ranged from 1.23 g H_2O/g DM in Univen *Dagga* flour to 2.46 g H_2O/g DM in Univen *Impilo* flour.

5.4.4 Ash content

The ash content analysis (Table 22) on the main effect of location shows a significant difference ($P < 0.05$) between two locations (Map 2). Univen flours show the highest (4.44%) ash content and Tshiombo flours the lowest 2.54%. Ash content also varied significantly across the OFSP cultivar flour and ranged from 3.17 % in *Dagga* cultivar flour to 3.80 in *Bophelo* cultivar flour. There was significant differences in ash content in the location-cultivar interactions and ranged from 1.90% in Tshiombo *Bophelo* flour to 5.41% in Univen *Impilo* flour. *Impilo* cultivar had higher nutritional content compared to other cultivars in this study.

Table 23: Main effect of location on functional and nutritional properties of OFSP flour.

Main Effect	Total starch (µg/100 g)	Resistant starch (µg/100 g)	WHC (g H2O/g DM)	Ash (%)
Location				
Tshiombo	331.44 ± 11.38^{a}	4.69 ± 0.77^{a}	1.44 ± 0.04^{b}	2.54 ± 0.15^{b}
Univen	226.08 ± 18.40^{b}	2.92 ± 0.55^{b}	1.72 ± 0.16^{a}	4.44 ± 0.20^{a}
Cultivar				
Dagga	325.04 ± 1.69^{a}	3.53 ± 0.56^{b}	1.29 ± 0.05^{c}	3.17 ± 0.23^{d}
Bophelo	244.07 ± 10.39^{d}	1.21 ± 0.14^{d}	1.62 ± 0.04^{b}	3.80 ± 0.24^{a}
Impilo	254.35 ± 45.03^{c}	7.07 ± 0.61^{a}	1.99 ± 0.21^{a}	3.65 ± 0.79^{b}
Mvuvhelo	299.29 ± 29.77^{b}	3.39 ± 0.28^{c}	1.27 ± 0.04^{c}	3.31 ± 0.33^{c}
Location X Cultivar				
Tshiombo X *Dagga*	328.75 ± 0.73^{c}	4.78 ± 0.01^{c}	1.36 ± 0.04^{c}	2.65 ± 0.01^{f}
Tshiombo X *Bophelo*	267.28 ± 1.18^{e}	1.54 ± 0.03^{g}	1.57 ± 0.01^{b}	3.26 ± 0.02^{e}
Tshiombo X *Impilo*	355.03 ± 1.53^{b}	8.43 ± 0.02^{a}	1.52 ± 0.02^{b}	1.90 ± 0.00^{g}
Tshiombo X *Mvuvhelo*	365.85 ± 0.56^{a}	4.01 ± 0.04^{d}	1.26 ± 0.06^{c}	2.58 ± 0.01^{f}
Univen X *Dagga*	321.33 ± 0.21^{d}	2.27 ± 0.01^{f}	1.23 ± 0.01^{c}	3.68 ± 0.05^{d}
Univen X *Bophelo*	220.87 ± 0.56^{g}	0.89 ± 0.01^{h}	1.67 ± 0.06^{b}	4.33 ± 0.02^{b}
Univen X *Impilo*	153.67 ± 0.77^{h}	5.72 ± 0.03^{b}	2.46 ± 0.02^{a}	5.41± 0.09^{a}
Univen X *Mvuvhelo*	232.74 ± 0.37^{f}	2.77 ± 0.01^{e}	1.28 ± 0.07^{c}	4.03 ± 0.02^{c}

Values are means ± standard error, different letter superscripts in a column are significantly different (P < 0.05), Location (n = 12), cultivar (n = 6), Location X cultivar interaction (n = 3), WHC = Water holding capacity, OFSP = Orange fleshed sweet potato.

Correlation analysis

Pearson's correlation analysis was carried out to determine the correlation between the functional and nutritional properties (Total starch, RS, water holding capacity and ash content) of sweet potato flour (Table 24). The results showed significantly negative correlation between total starch, ash content (-0.928**) and WHC (-0.741**). However, ash content was significantly positively correlated with WHC (0.654**). RS was negatively (-0.382) and positively (0.245) correlated to ash content and WHC, respectively. Augustin et al., (2008), reported that some resistant starches can be problematic due to lack of ability to hold moisture, they thicken or form gels. Resistant starches contribute to low glycemic Index and subsequently helps blood sugar in type II diabetic patients.

Table 24: Correlational analysis of the functional and nutritional properties of OFSP flour.

Variables	Total Starch	Resistant starch	WHC	Ash
Total Starch	1			
Resistant Starch	0.275	1		
WHC	-0.741**	0.245	1	
Ash	-0.928**	-382	0.654**	1

*Correlation is significant at (P < 0.05), ** Correlation is significant at 0.01 (1-tailed), WHC = water holding capacity, OFSP = Orange fleshed sweet potato.

5.4.5 Total dietary fibre

Results of total dietary fibre analysis are shown in Figures 21 and 22 for Tshiombo irrigation scheme cultivars and University of Venda experimental farm respectively. The results showed *Bophelo* flour the highest (18.0% w/w) and *Dagga* flour the lowest (12.7% w/w) dietary fibre for Tshiombo irrigation scheme. For Univen experimental farm cultivars, *Impilo* cultivar flour showed highest (19.6% w/w) and *Dagga* cultivar flour the lowest (16.4% w/w) dietary fibre. Huang *et al*. (1999) reported dietary fibre ranging from 2.01 g/100 g fresh weight to 3.87 g/100 g fresh weight.

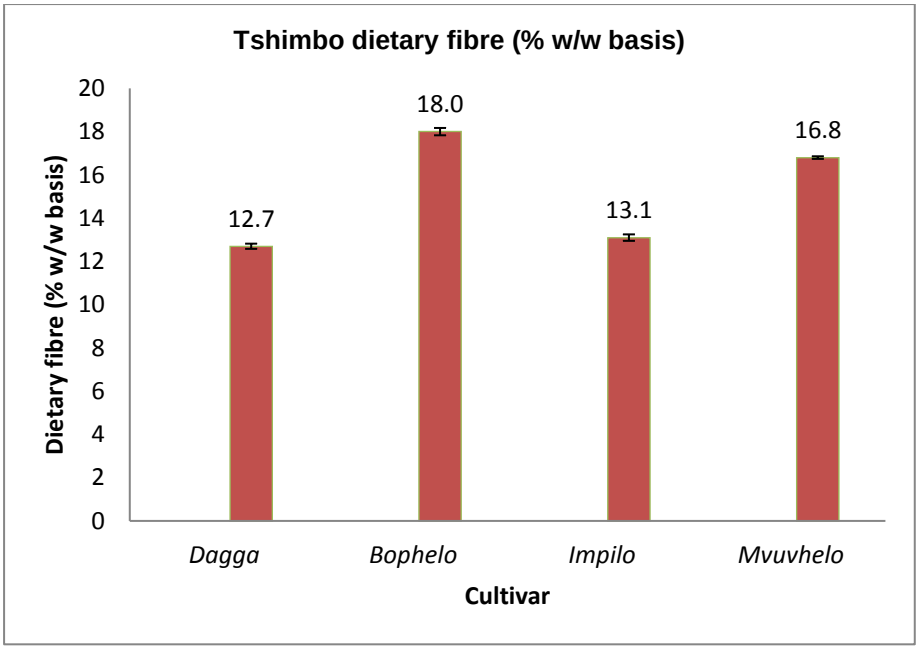


Figure 18: Total dietary fibre in Tshiombo sweet potato flour.

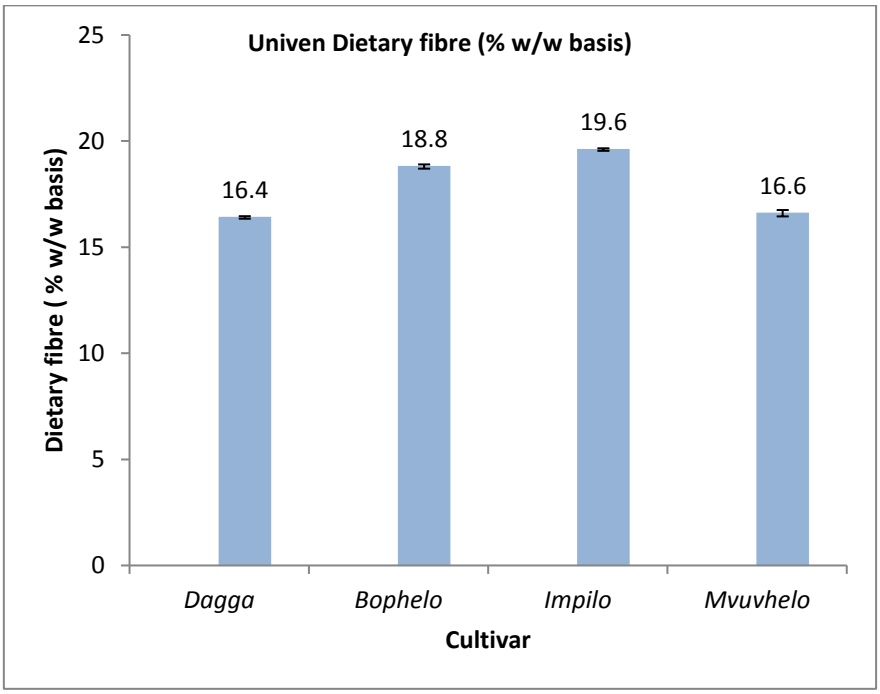


Figure 19: Total dietary fibre in Univen sweet potato flour.

Based on the parameters measured in this section which included total starch, RS, WHC and ash content, *Impilo* OFSP cultivar flour was ranked the highest and *Bophelo* the lowest. The ranking results are shown in Table 25.

Table 25: Rank order of sweet potato flour according to total starch, RS, WHC and ash.

Cultivar	Total Starch (µg/100 g)	Resistant Starch (µg/100 g)	WHC (g H20/DM)	Ash content (%)
Dagga	1	2	3	4
Bophelo	4	4	2	1
Impilo	3	1	1	2
Mvuvhelo	2	3	4	3

WHC = Water holding capacity; 1 = highly ranked; 4 = least ranked

5.4.6 Pasting properties of OFSP flour

The results of analysis of the pasting properties of OFSP flour are shown in Tables 25, 26 and 27. The results of the correlation matrix between pasting, functional and nutritional properties of OFSP flour are shown in Table 28. Representative rapid viscosity analysis plots of OFSP flour pasting properties are shown in Figures 24 and 25. The results of the pasting properties of OFSP cultivar flours revealed significant differences ($P < 0.05$) in the pasting properties of flour of cultivars. The rapid viscosity analysis plots revealed variation in the typical peak shapes of viscograms of starch from OFSP products. *Bophelo* flour from both representation, showed a somewhat flat peak compared to the flour from other OFSP cultivars (Appendix 3).

The ability of starch to imbibe water and swell is dependent on the pasting temperature. Starch granules swell and form paste in the presence of water and heat (Falade and Okafor, 2013). Peak viscosity (PV) is a measure of the point at which gelatinized starch reaches its maximum viscosity during heating in water (Tsakama *et al*., 2010). It also indicates the capacity of starch to bind water (Shimelis *et al.,* 2006).

PV (Table 27) varied significantly between OFSP cultivar flour from 126.54 RVU (*Bophelo*) to 302.36 RVU (*Dagga*). These results are comparable to PV obtained by Falade and Okafor (2013) on the cocoyam starch pasting properties which ranged from 191.3 RVU to 306 RVU. Waramboi *et al.,* (2011) reported much lower value range of 144 cP (12.08 RVU) to 1260 cP (105 RVU) in 25 sweet potato varieties from Papua New Guinea and Australia.

Table 26: Mean paste properties of OFSP flour.

Cultivar	Peak viscosity (RVU)	Trough viscosity (RVU)	Break down viscosity (RVU)	Final viscosity (RVU)	Peak time (min)	Paste temp (^{o}C)	Setback viscosity (RVU)
Dagga	302.36 ± 70.73^{a}	280.78 ± 67.55^{a}	21.58 ± 4.29^{b}	319.26 ± 75.37^{a}	5.67 ± 0.42^{c}	94.16 ± 0.60^{a}	38.49 ± 8.41^{b}
Bophelo	126.54 ± 2.33^{d}	109.89 ± 6.66^{c}	16.65 ± 4.59^{b}	140.89 ± 4.95^{c}	8.48 ± 1.68^{a}	77.75 ± 7.46^{c}	31.00 ± 5.18^{bc}
Impilo	137.74 ± 18.83^{c}	55.97 ± 7.96^{d}	81.77± 26.79 ^{a}	78.99 ±19.48^{d}	3.87 ± 0.02^{d}	86.80 ± 1.65^{b}	23.01 ± 8.53^{c}
Mvuvhelo	155.25 ± 40.45^{b}	133.38 ± 32.17^{b}	22.01 ± 8.22 ^{b}	182.83 ± 45.32^{b}	6.37 ± 0.46^{b}	93.93 ± 1.04^{a}	51.10 ± 12.65^{a}

Values are mean ± standard error (n = 5);, values with different letter superscript in the same column indicate statistically significant differences (P < 0.05). RVU = Rapid viscosity unit; Note: 1 RVU = 12 cP; OFSP = Orange fleshed sweet potato.

Table 27: Paste properties of OFSP flour from University of Venda experimental farm.

Cultivar	Peak viscosity (RVU)	Trough(Hot paste) viscosity (RVU)	Break down viscosity (RVU)	Final (Cold paste) viscosity (RVU)	Peak time (min)	Paste temp (^{o}C)	Setback viscosity (RVU)
Dagga	144.22 ± 1.60^{a}	129.89 ± 0.29^{a}	14.33 ± 1.37^{b}	150.86 ± 0.27^{a}	4.73 ± 0.03^{b}	94.57 ± 0.21^{a}	20.97 ± 0.49^{b}
Bophelo	131.14 ± 1.74^{b}	124.69 ± 1.19^{b}	6.44 ± 0.74^{c}	148.00 ± 0.17^{a}	4.71 ± 0.06^{b}	94.27 ± 0.26^{a}	23.31 ± 1.22^{b}
Impilo	95.69 ± 1.66^{c}	73.78 ± 0.21^{c}	21.92 ± 1.86^{a}	115.81 ± 0.84^{b}	3.87 ± 0.03^{c}	88.70 ± 2.23^{b}	42.03 ± 1.04^{a}
Mvuvhelo	64.80 ± 0.87^{d}	61.44 ± 0.60^{d}	3.64 ± 0.09^{c}	81.19 ± 2.93^{c}	5.40 ± 0.12^{a}	94.92 ± 0.04^{a}	23.03 ± 0.02^{b}

Values are mean ± standard error (n = 5)., values with different letter superscript in the same column indicate statistically significant difference (P < 0.05) RVU = Rapid viscosity unit; cP = Cent poise; RVU = Rapid viscosity unit; Note: 1 RVU = 12 cP; OFSP = Orange fleshed sweet potato.

Table 28: Paste properties of OFSP flour from Tshiombo irrigation scheme.

Cultivar	Peak viscosity (RVU)	Trough viscosity (RVU)	Break down viscosity (RVU)	Final viscosity (RVU)	Peak time (min)	Paste temp (°C)	Setback viscosity (RVU)
Dagga	245.69 ± 0.70^{b}	431.67 ± 4.83^{a}	28.83 ± 4.23^{c}	487.66 ± 4.66^{a}	6.61 ± 0.55^{c}	93.75 ± 0.88^{a}	56.00 ±4.80^{b}
Bophelo	126.95 ± 0.12^{d}	95.08 ± 0.00^{c}	26.86 ± 0.12^{c}	133.78 ± 6.00^{c}	12.24 ± 0.01^{a}	61.23 ± 0.03^{d}	38.70 ± 6.00^{b}
Impilo	179.78 ± 0.48^{c}	38.17 ± 0.00^{d}	40.39 ± 0.48^{b}	42.17 ± 0.24^{d}	3.87 ± 0.02^{d}	84.89 ± 0.13^{b}	4.00 ± 0.24^{c}
Mvuvhelo	460.50 ± 0.43^{a}	205.31 ± 0.14^{b}	141.61 ± 0.34^{a}	284.47 ± 2.55^{b}	7.34 ± 0.19^{b}	92.93 ± 1.48^{a}	79.16 ± 0.24^{a}

Values are mean ± standard error (n = 5), values with different letter(s) in the same column indicate statistically significant differences ($P < 0.05$)
RVU = Rapid viscosity unit; cP = Cent poise; Note: 12 cP = 1 RVU; OFSP = Orange fleshed sweet potato.

PV is an indication of the maximum swelling of starch granule before disintegration. The PV is also referred to as the equilibrium point between swelling and breakdown of the granules (Liu *et al.,* 2006). Peak viscosity correlated significantly positively with Trough (Hot paste) 0.94** and Final (Cold paste) 0.92** viscosity but correlated negatively (- 0.56**) with ash content of the flour (Table 29).

Trough (hot paste) viscosity- an indication of holding strength or the ability of paste to withstand breakdown during cooling (Julianti *et al.,* 2015) differed significantly ($P < 0.05$) among sweet potato cultivar flour and ranged from (55.97 RVU) in *Impilo* to (280.78 RVU) in *Dagga*. These results are comparable to those reported by Tsakama, Mwangwela, Manani and Mahungu (2010). Waramboi *et al*. (2011) reported much lower range 63 cP (5.25 RVU) to 1015 cP (84.58 RVU) of trough viscosity for sweet potato flour. Trough viscosity significantly negatively correlated (-0.52**) with protein content of sweet potato flour (Table 29).

Breakdown viscosity which is an indication of resistance of sweet potato flour to heat and shear, was significantly highest 81.77 RVU (*Impilo*) and lowest 16.65 (*Bophelo*). Breakdown is an estimation of paste resistance to disintegration in response to heat and, shear means that the lower break down viscosity in *Bophelo* flour shows greater resistance to breakdown. Tsakama *et al*. (2010), reported a breakdown range of 221.74 cP (18.48 RVU) to 889.34 cP (74.11 RVU) in 11 sweet potato varieties. Breakdown viscosity correlated significantly positively (0.67**) with ash content of sweet potato flour, but insignificantly negatively correlated with peak time (- 0.19), pasting temperature (- 0.18) and WHC (-0.03).

Setback viscosity, which measures the tendency of starch to undergo retrodegradation (Owuamanam *et al.,* 2010), varied significantly from 23.01 RVU *(Impilo*) to 51.10 RVU (*Mvuvhelo*). The results show a higher tendency of *Mvuvhelo* flour to retrogradation during cooling than that of *Impilo* (Table 26). Setback viscosity was significantly negatively correlated (- 0.43*) with fat content and significantly positively correlated 0.59**, 0.69** and 0.39* with trough viscosity, FV and peak time respectively (Table 29).

Peak time of sweet potato flour varied significantly from 3.87 min (*Impilo*) to 8.48 min (*Bophelo*). The mean peak time recorded in this study, were higher than those reported by Falade and Okafor, (2013), Nabubuya *et al*. (2012) and Aina *et al.* (2012) but comparable to those reported by Abegunde *et al*. (2013) and Tsakama *et al*. (2010). PV was negatively correlated -0.66** and -0.67** with pH and fat content respectively. As the flour pH and fat decrease, the peak viscosity increases.

Pasting temperatures for sweet potato flour varied significantly from 77.75^{o}C (*Bophelo*) to 94.16^{o}C (*Dagga*). Kaur *et al*. (2011) reported similar pasting temperature in wheat (94.9^{o}C) to that of *Bophelo* but lower (76.4^{o}C) for sweet potato. Nabubuya *et al*., (2010), reported a high (84.2^{o}C) pasting temperature for sweet potato variety new kawogo. The literature suggests dependency of pasting temperature on starch granule size with small granules being more resistant to rapture and loss of molecular order (Dreher and Berry, 1983). Waterschoot *et al*. (2015) reported a higher peak, trough and end viscosity for blends with smaller granules. The pasting temperatures reported in this study are relatively higher than those reported by Liu *et al*. (2006); Osundahunsi *et al*. (2003), and Aina *et al*. (2009). Pasting temperature significantly positively correlated (0.61**) with pH of the flour paste but significantly negatively correlate (-0.77**) with peak time.

Final (cold paste) viscosity (FV) is a measure of the ability of starch to form a paste or gel after cooling (Shimelis *et al*., 2006). The FV gives an indication of the behaviour of starch in the product when it is cooked. The increase of FV of all starches has been attributed to aggregation of amylose molecules on cooling (Kaur *et al*., 2007). FV varied significantly among all sweet potato flour ranging from 78.99 RVU (*Impilo*) to 319.26 RVU (*Dagga*). The results reported in this study have a higher variance compared to those reported by Tsakama *et al*. (2010) on the 11 sweet potato varieties flour which ranged from 2304.00 cP (192 RVU) to 3261.67 cP (271.75 RVU).

Location X cultivar interaction for University of Venda sweet potato cultivars

The results of the effect of location X cultivar interaction on pasting properties of flour from Univen experimental farm OFSP cultivars are shown in Table 26. The results show significant ($P < 0.05$) variations in peak trough, breakdown, final and setback viscosities. PV varied significantly ($P < 0.05$) among the sweet potato cultivar flour ranging from 64.80 RVU (*Mvuvhelo*) to 144.22 RVU *(Dagga*). Breakdown viscosity was significantly highest (21.92 RVU) in *Impilo* flour and lowest (3.64 RVU) in *Mvuvhelo*. FV was significantly highest 150.86 RVU (*Dagga*) and lowest 81.19 RVU *(Mvuvhelo*). Peak time was significantly highest (5.4 min) in *Mvuvhelo* and lowest (3.85 min) in *Impilo*. The PV results are within the range of those reported by Falade and Okafor, (2013) and Tsakama *et al*. (2010) on physicochemical properties of starch extracts from cocoyam and eleven sweet potato respectively, which ranged from 191.3 RVU to 306.29 RVU. Paste temperature was significantly lowest (88.70^{o}C) in *Impilo* and highest (94.92^{o}C) in *Mvuvhelo*. The setback viscosity was significantly highest (42.03 RVU) in *Impilo* and lowest (23.03 RVU) in *Mvuvhelo*.

Location X cultivar interaction for Tshiombo irrigation scheme sweet potato cultivars

The results of the effect of location X cultivar interaction on pasting properties of flour from Tshiombo irrigation scheme sweet potato cultivars are shown in Table 27. The results show significant ($P < 0.05$) variations in peak trough, breakdown, final and setback viscosities between the cultivar flours. PV varied significantly among the cultivar flour ranging from 126.95 RVU in *Bophelo* to 460.50 RVU in *Mvuvhelo*. Break down viscosity was significantly highest (141.61 RVU) in *Mvuvhelo* and lowest (26.86 RVU) *Bophelo*. Breakdown viscosity is a measure of the degree of disintegration of granules or paste stability (Tsakama *et al.,* 2010). Zaidul *et al.* (2007) suggested that at breakdown, swollen granules disrupt further and amylose molecules generally leach into solution.

Correlation matrix of pasting, functional and nutritional properties of OFSP flour.

The correlation matrix for pasting, functional and nutritional properties (Table 29) showed that peak and breakdown viscosities were both significantly negatively correlated with ash content by (- 0.56**) and (-0.67**) respectively. Setback viscosity was significantly positively correlated with trough (0.59**), final viscosity (0.69**) and peak time (0.39*). Fat content was significantly negatively correlated (-0.67**) with and setback (-0.43*). As the fat content increase the setback and peak time decrease. The pH showed significant positive correlation with pasting temperature (0.61**) but significantly negatively correlated with peak time (-0.66**). As the pH increases the temperature increases too but the peak time decreases.

Table 29: Correlation matrix of pasting, functional and nutritional properties of OFSP flour.

	Peak	Trough	Break down	Final viscosity	Peak time	Pasting temp	Setback	Vit A	Fat	Protein	pH	MC	WHC	Ash
Peak	1.00													
Trough	0.94**	1.00												
Breakdown	0.15	- 0.20	1.00											
Final viscosity	0.92**	0.99**	- 0.23	1.00										
Peak time	0.10	0.17	- 0.19	0.22	1.00									
Pasting temp	0.21	0.27	- 0.18	0.24	- 0.77**	1.00								
Setback	0.49	0.59**	- 0.30	0.69**	0.39*	0.52	1.00							
Vitamin A	0.21	- 0.18	- 0.07	- 0.14	- 0.17	- 0.07	0.15	1.00						
Fat	- 0.24	- 0.28	0.13	- 0.32	- 0.67**	0.25	- 0.43*	- 0.07	1.00					
Protein	0.64	- 0.52**	- 0.31	- 0.48*	- 0.28	0.05	- 0.02	0.59**	0.42	1.00				
pH	0.42	0.58	- 0.05	0.22	- 0.66**	0.61**	- 0.16	- 0.07	0.38*	0.10	1.00			
MC	-0.21	- 0.19	- 0.05	- 0.19	- 0.16	- 0.03	- 0.16	- 0.32	0.45*	0.12	0.16	1.00		
WHC	-0.32	- 0.31	- 0.03	- 0.28	- 0.24	- 0.16	- 0.04	0.51**	0.53**	0.77**	0.13	0.28	1.00	
Ash	-0.56**	- 0.32	- 0.67**	- 0.30	- 0.25	0.14	- 0.08	0.35*	0.42*	0.84**	0.25	0.26	0.65**	1.00

MC = Moisture content; WHC = Water holding capacity; Vit A = Vitamin A, * Significant at P < 0.05; ** Significant at P < 0.01; OFSP = Orange fleshed sweet potato.

Uniform PV by starches is an indication of uniform granular/particle size of the flour Liu *et al.* (2006). Appendix 3 shows a variation in PV and is an indication of variation in granular/particle size within the OFSP cultivars in both locations. These results are supported by the CT scans results which indicated a variation in the size of granules from similar cultivars grown in different locations.

5.4.7 Scanning electron microscopy (SEM) of OFSP flour granules

The results of scanned (SEM) sweet potato starch granules are shown in Figures 20 and 21. These results showed remarkable granule differences between similar cultivars from different locations. Whereas starch granules of flour for *Bophelo* cultivar from Tshiombo (Figure 20) show some diamond shape like, a similar cultivar (*Bophelo*) from Univen (Figure 21) shows a roundish structure. The roundish granule structures of Univen OFSP cultivars are similar to those reported by Shariffa *et al*. (2009) in tapioca and sweet potato starches.

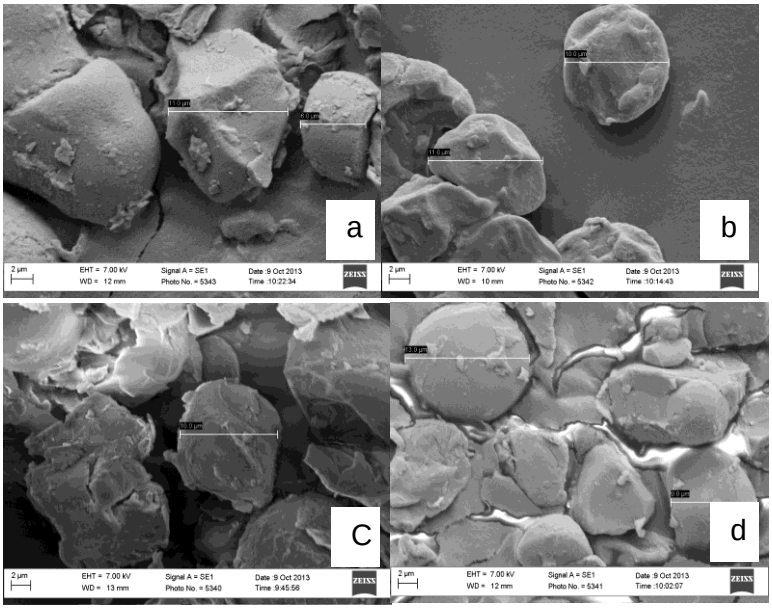


Figure 20: Scanning electron microscopy of OFSP flour starch granules from Tshiombo.
(a) Dagga; (b) Bophelo; (c) Impilo and (d) Mvuvhelo; OFSP = Orange fleshed sweet potato.

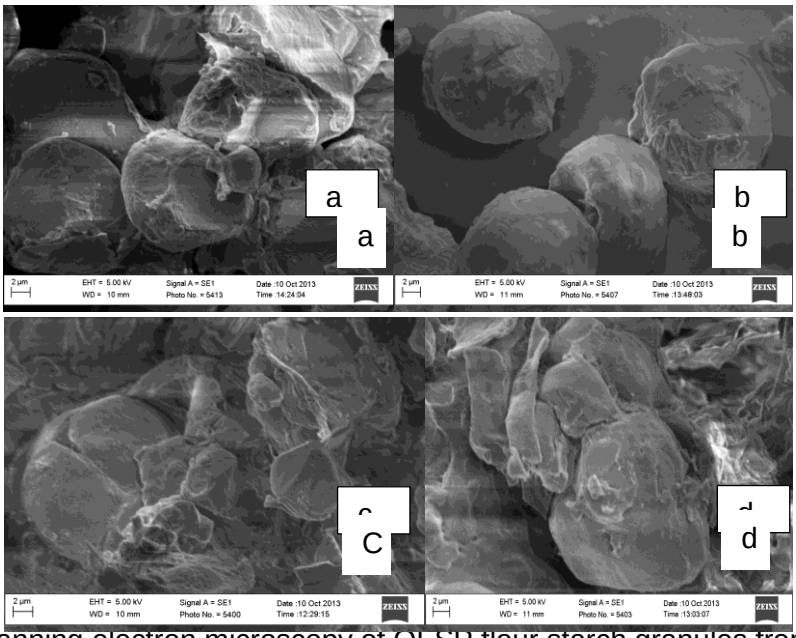


Figure 21: Scanning electron microscopy of OFSP flour starch granules from Univen.
(a) *Dagga; (b) Bophelo; (c) Impilo* and *(d) Mvuvhelo*; OFSP = Orange fleshed sweet potato

5.4.8 Computed tomographic scans of OFSP flour granules

Computed tomographic (CT) scans of sweet potato flour from OFSP flour are shown in Figures 22, 23, 24 and 25. The results revealed differences in the granule size of flours in the cross section CT images of the layered sweet potato flour. The green line indicates the barrier between the two flour samples: (A) is the flour from *Dagga* sweet potato with the top part originating from Tshiombo and the bottom from Univen, (B) is the flour of the *Bophelo* sweet potato cultivar with the top part originating from Univen and the bottom from Tshiombo, (C) is the flour of the *Impilo* sweet potato with the top power originating from Tshiombo and the bottom from Univen and (D) is the flour of the *Mvuvhelo* sweet potato with the top originating from Tshiombo and the bottom from Univen. CT scans in foods have been used mainly for non-destructive studies. This CT scan was used to investigate visible differences in granules of different OFSP cultivars from different locations.

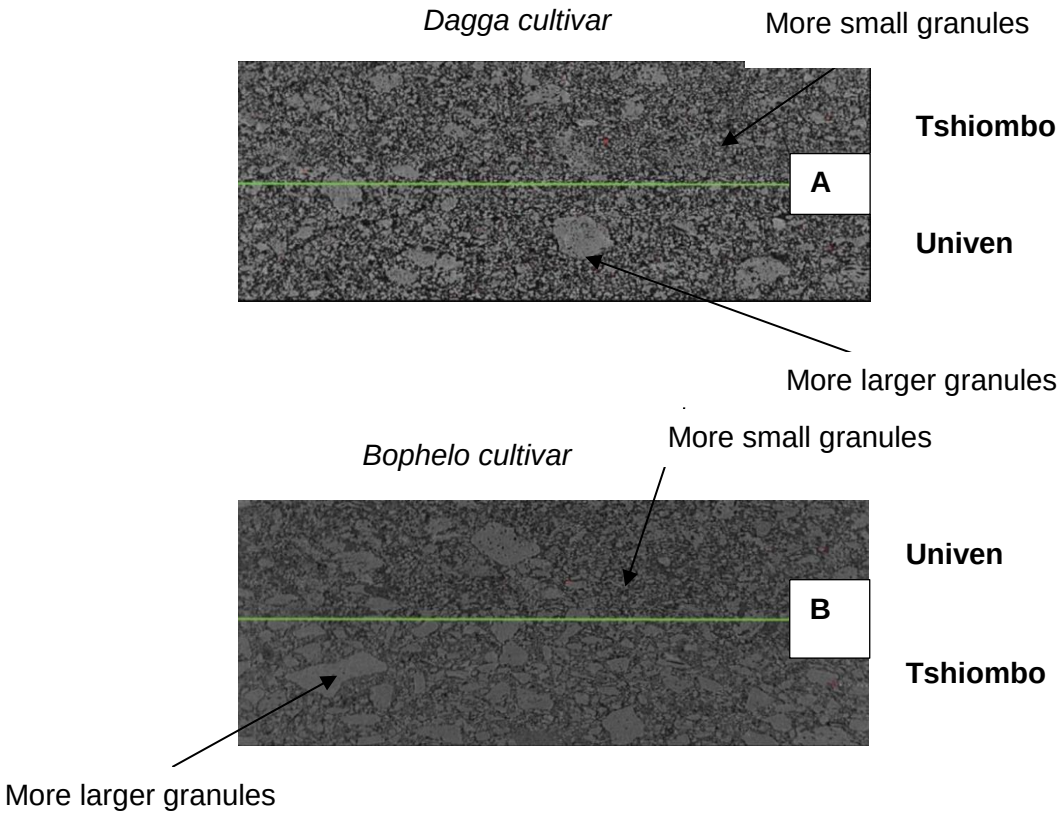


Figure 22: Computed tomographic scan of sweet potato flour granules from different locations.

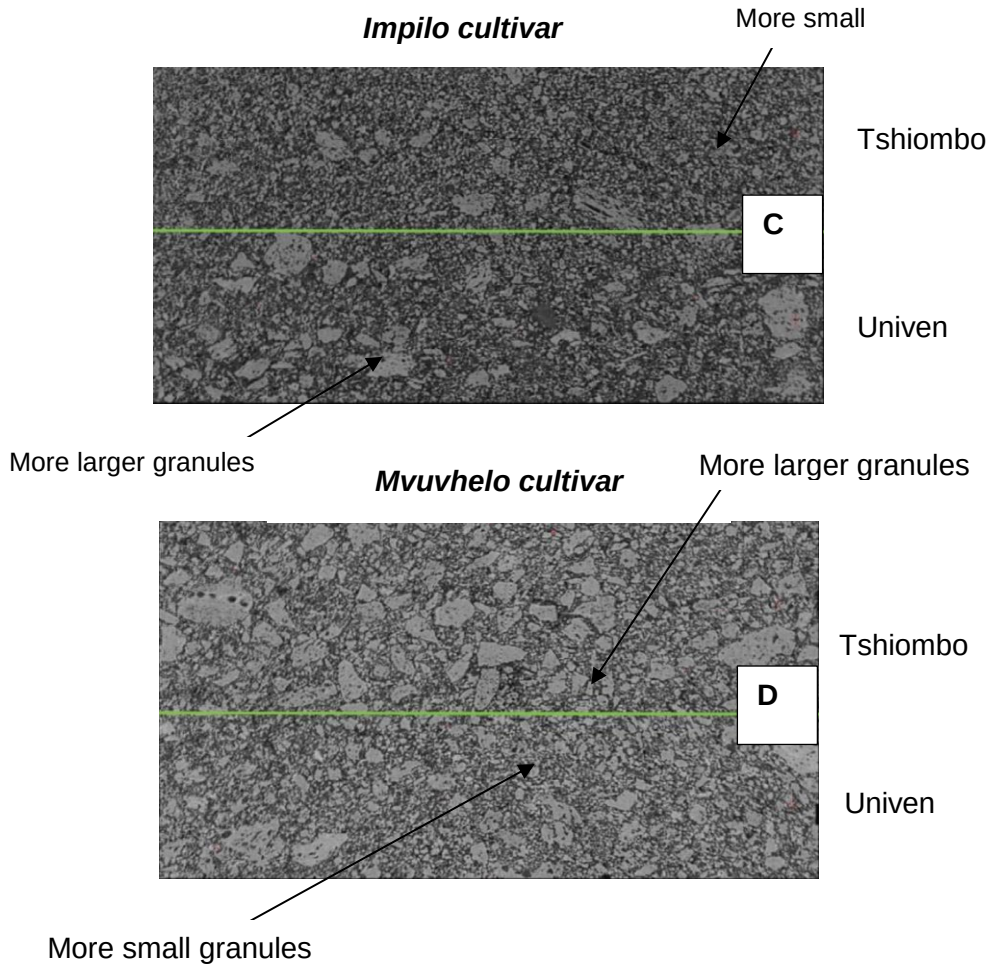


Figure 23: Computed tomographic scan of sweet potato flour granules from Different locations.

The CT scan indicates that in some cases, there was a visible difference in granule size between the sweet potato cultivar flours from Tshiombo and Univen. The CT scan on the *Dagga* flour (Figure 22), indicates that both the Univen and the Tshiombo have closely similar granule sizes; this is similar for the *Bophelo* cultivar (Figure 22). However, in the *Impilo* cultivar, the Univen sample seemed to have a smaller number of larger granules and in the *Mvuvhelo,* the Tshiombo sample has a large number of larger granule sizes. (Figure 23) and varietal differences can be visibly identified.

The CT data also indicates the difference in density of the scanned samples. The *Bophelo* cultivar (Figure 24) was colour coded to differentiate between the different densities on the cultivars. In this case, the *Bophelo* cultivar was contrasted to indicate the less dense material in purple and the more dense material in green. What is clear in this cultivar is that the larger grains are denser than the smaller grain sizes in the cultivar. A quick volumetric analysis of the cultivar indicated that the less dense material made up 48% of the cultivar and the more dense material made up 50% of the cultivar.

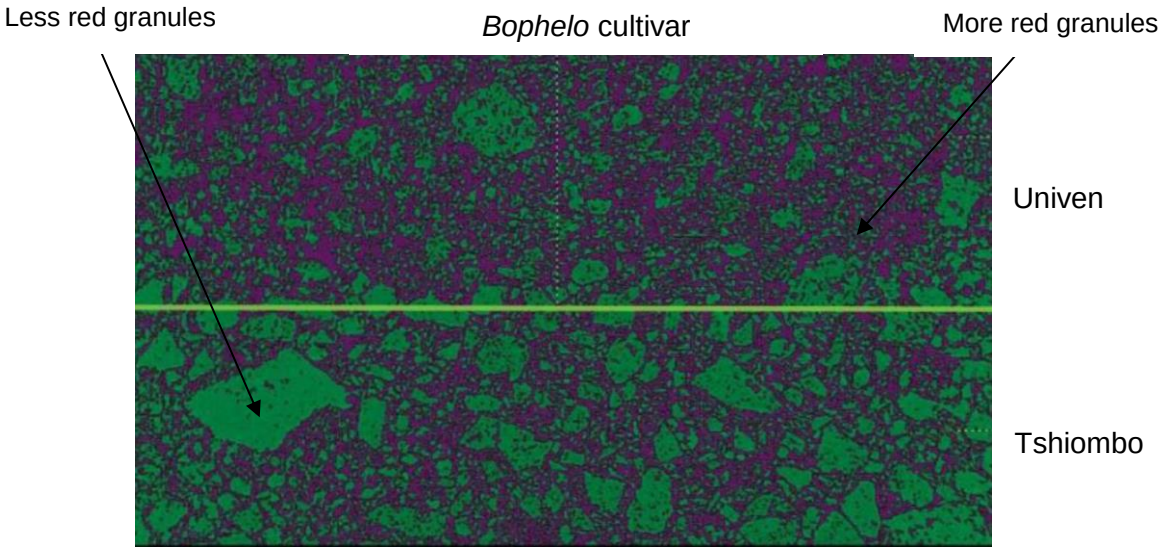


Figure 24: Computed tomographic scan of cross section of Bophelo cultivar flour.

The purple colour represents smaller less dense material and the green represents more dense material. A further investigation into what the two colour represent could shed more light on the usefulness of the CT scans in studying some OFSP flour constituents. Until recently CT scans have been used in the medical field. In the food industry CT scans are now frequently used in the non- destructive assessment of fruit quality (Magwaza and Opara, 2014; Arendse *et al.,* 2016).

In all the cultivars, very small, very dense granules were also observed which accommodate the last 2% of the sample volume. A transparent cross section of the *Dagga* cultivar (Figure 25), indicated that in some cases, the flours from the different sites contained different volumes of dense granules. In this image (Figure 25), it was evident that the Tshiombo contained more of the denser red grains than the Univen (Bottom) half which could indicate that the location had an effect on the density of granules of sweet potato flour.

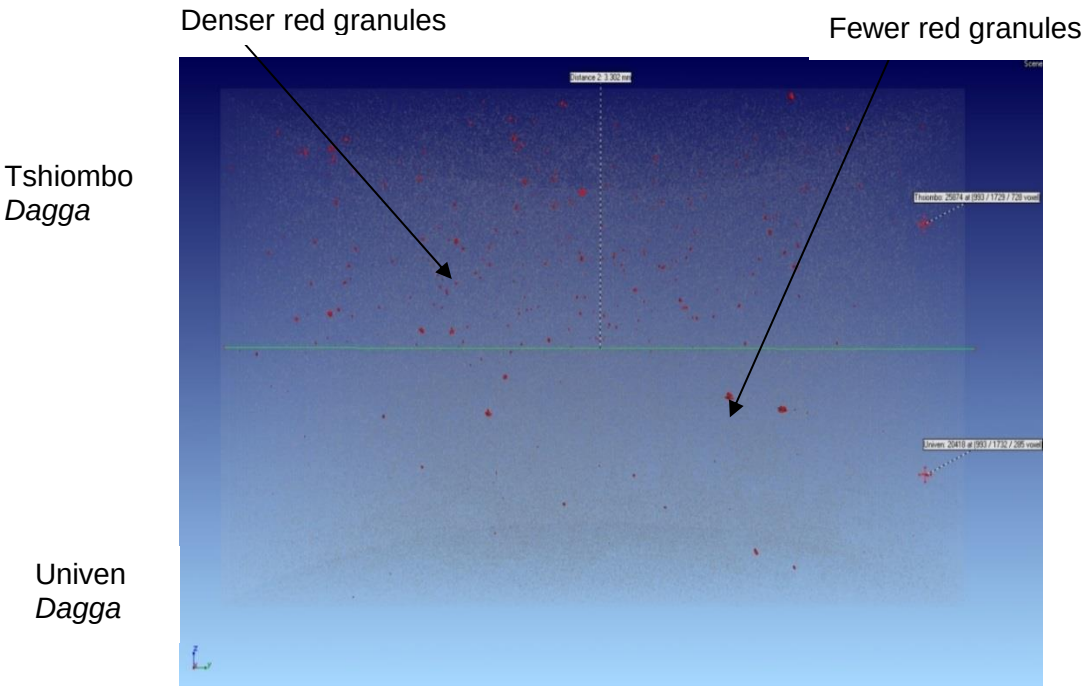


Figure 25: Transparent of the Dagga flour cultivar flours from different locations.

The analysis peformed on the OFSP flours indicated that it ispossible to perform CT scans on these low density flour samples with a very small particle size. The initial analyis indicated that there is a difference in praticle size of the flour from different locations as shown in the flours of *Dagga* and *Mvuvhelo* cultivars. The scans also pointed out that the larger granule sizes had a higher density than the bulk small granules.

5.5 Conclusion

A significantly wide variation in functional properties affected by location and cultivar interaction has been established in this study. Tshiombo location OFSP cultivar flours had higher total and resistant starch content while Univen OFSP cultivar flours had higher WHC and ash content. The correlation results showed that as total starch increased both WHC and ash content decreased. The point at which gelatinized starch reaches its maximum was different between the OFSP cultivar flours and *Impilo* flour had the highest resistant to heat. *Mvuvhelo* had a higher tendency towards retrodegradation.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

β-carotene varied significantly across the two locations, between OFSP cultivars and the interactions. Laurie *et al*. (2012) and Bovell-Benjamin (2007) reported that variation in β-carotene content is affected by irrigation, fertilizer application and genetic variation respectively. The variation in β-carotene content among all cultivars indicates the need for selection of cultivars with higher β-carotene content among the OFSP cultivars. *Impilo* cultivar was ranked highest in terms of physicochemical and nutritional parameters.

The OFSP cultivar flour showed significant variation in pasting properties such as peak, trough, breakdown, final, and setback viscosities as well as peak time and pasting temperatures. The viscosity graphs showed differences in the peak viscosiy (Appendix 3). The significant variation in pasting properties of the OFSP flour indicates the need for selection for a cultivar to be utilised for various processing requirements. Location among other environmental factors affects the nutritional and functional properties of OFSP flour. Total carotenoids, antioxidant power and α-carotenes were affect by both cultivar and location. Location had an effect on protein and fat contents and pH.

The SEM results for starch granules revealed differences in the granule shapes namely diamond and roundish. The CT scans showed that it is possible to identify some differences in the flour of OFSP culticars. The density of granules can easily be seen (Figures 22, 23 , 24, and 25). The ash content varied between the locations and among the OFSP cultivar flours.

Sweet potatoes are highly perishable owing to their high water content. Sweet potato flour, which contains appreciable amounts of β-carotene, is a more stable sweet potato product that can be stored for a longer period than fresh roots. (Van hal, 2007). Therefore, research on the effect of processing methods on β-carotene content in OFSP flour will continue to take centre stage.

6.2 Recommendations

A larger number of cultivars should be included in future studies to ensure that more cultivars are profiled and evaluated for physicochemical, nutritional and functional properties. This will widen the chances for better selection of sweet potato cultivars with desired properties. There is need to conduct shelf-life studies and microbial quality on the OFSP cultivar flour to ensure that the flour is utilised more efficiently before the expiry of its shelf life. Therefore, research on the effect of processing methods on β-carotene content in OFSP flour should include a variety of processing methods. This is mainly due to the fact that sweet potato flour is one of the forms through which the shelf-life of the product can be extended and there by contribute to food security in developing countries.

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APPENDICES

APPENDIX 1: SUMMARY OF ANALYSES, MATERIALS AND EQUIPMENT USED IN THIS STUDY.

Analysis	Materials	Equipment
Total dietary fiber	Sweet potato flour samples, α-Amylase, Heat Stable, Protease; Amyloglucosidase, Celite, Arabinogalactan, Casein, β-Glucan; Pectin; Starch, Corn, Starch, Wheat, Petroleum ether; Ethyl, alcohol; Acetone, Sodium Phosphate, Dibasic, anhydrous, deionized water, Sodium Phosphate, Monobasic, anhydrous, Sodium Hydroxide, Hydrochloric Acid, 1.0 M HCl.	A vacuum pump, Desiccator, Muffle furnace, Boiling water bath, Beakers, Analytical balance pH meter, Kjeldahl instrument.
Protein content	Sweet potato flour samples, distilled water,4% Boric acid, Kjeldahl tablet, Sulphuric acid, NaOH, 0.2 M HCl, Conical flask, Digestion tubes, Digital balance.	Automatic digester (DKL 8), Distillation unit UDK 129, Titrator.
Fat content	Sweet potato flour samples, Digital balance. Extraction thimbles, petroleum ether, desiccator	Extractor (Velp Scientific, R 148/3)
Mineral content by SEM	Sweet potato flour samples, 15 μm gold coating, Oxford INCA software.	Zeiss EVO® MA15 Scanning Electron Microscope, EDX X-Max 20 mm^2 detector,
Colour Analysis	Sweet potato flour samples,	chroma meter model CR- 400, Konica Minolta, PC

APPENDIX 1: ANALYSES, MATERIALS AND EQUIPMENT USED (Cont'd).

Analysis	Materials	Equipment
Total carotenoids	Sweet potato flour samples, n-hexene-acetone solution(3:2),	Spectrophotometer (Omega UV-Vis),
Antioxidant capacity by FRAP Assay	Sweet potato flour samples, Methanol, Sodium acetate, Ferric chloride, ferric-tripyridyltriazine (Fe^{3+}- TPTZ)	Weighing balance, centrifuge (Ependorf AG 5810R), Vortex, Spectrophotometer (Omega UV-Vis),
Antioxidant capacity by DPPH Assay	Sweet potato flour samples, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Solution, Methanol,	Weighing Balance ± 0.01 SE, centrifuge (Ependorf AG 5810R), Vortex, Spectrophotometer (Omega UV-Vis),
β-carotene	Sweet potato flour samples, Hexane –acetone (1:1), Funnel, deionized water, Methanol, calcium carbonate, Diatomaceous earth-Hyflo super-Cell., β-carotene standards, vials.	Spectrophotometer,
α - carotene	Sweet potato flour samples, Hexane –acetone (1:1), Funnel, deionized water, Methanol, calcium carbonate, Diatomaceous earth-Hyflo super-Cell., β-carotene standards, vials.	Spectrophotometer, HPLC
Vitamin A	Sweet potato flour samples, Certified vitamin A acetate concentrate (USP) or Retinyl, palmitate, all-trans, Acetic acid glacial, AR Acetonitrile, AR Isopropanol, AR Methanol, HPLC grade, Absolute ethanol, AR Tetrahydrofuran (THF), AR grade, transisomers of retinol, Hexane (n-Hexane 95% for HPLC), Pyrogallic acid, crystal, AR grade	HPLC, System; Reverse-phaseC18 column,5 µm (4.6x250mm, Photometric detector (PDA) monitoring absorbance at 326 nm, Injector, Pump operating continuously at 1.0-2.0 mL/min with a flow precision of ± 1%.
Ash content	Sweet potato flour samples, Crucibles electronic balance.	Muffle furnace,
Nano surface structure of sweet potato flour.	Sweet potato flour samples, carbon tape, thin layer of gold.	Leo® 1430VP Scanning Electron Microscope

APPENDIX 1: ANALYSES, MATERIALS AND EQUIPMENT USED.

Analysis	Materials	Equipment
Paste viscosity of sweet potato flour	Sweet potato flour samples, distilled water, plastic weighing boats, RVA canisters	Rapid Visco-Analyzer (RVA) Series 4500 Unit. PC with Thermocline for windows (TCW) software version 3.0
Moisture content of sweet potato flour	Sweet potato flour samples, crucibles,	Oven dryer, Desiccator, Weighing balance
Water holding capacity (WHC)	Sweet potato flour, centrifuge tubes, distilled water	Weighing balance, air oven dryer; Ependorf AG centrifuge 5810R.
Computer tomography (CT) scans of sweet potato flour	Sweet potato flour samples, CT scan test tubes.	CT scanner (Phoenix VTomex Model L240), PC with windows software image and video captures analysis
Total starch in sweet potato flour	Sweet potato flour samples, Deionized water, 5N NaOH, Test tubes, 8M HCl, Amyloglucosidase, Conical flasks (100-250 ml), DMSO,	Shaking water bath. Spectrophotometer, Incubator.
Resistant starch (R3)	Sweet potato flour samples, pancreatic α- amylase, p-nitrophenyl, β-maltoside, Amyloglucosidase (AMG), denatured ethanol, Resistant starch control, Glucose oxidase/peroxide reagent buffer (GOPOD),2 M KOH, Glucose oxidase/peroxide reagent Enzyme (GOPOD), D-glucose standard solution. Pipettor, Corning Culture Tubes, Thermometer, Volumetric flasks.	Shaking water bath, Centrifuge, Analytical balance, Magnetic stirrer bars, pH Meter, Vortex mixer, Spectrophotometer.

APPENDIX 2: LIST OF FORMULAE.

	Formula	Page
1	RS = TS – (RDS + SDS)	27
2	TS = RDS +SDS + RS	28
3	Carotenoid content (g/ml) = $\frac{\mathbf{OD_{450}\ X\ 4}}{\text{Mass of sample (g)}}$	40
4	$C = \frac{A\ x\ 454}{196\ x\ L\ x\ W}$	42
5	A = *εcL*	43
6	*Vita*min *A*, µg/*g* (*as retinol*) = $\frac{RF_A\ x\ PH_{samp}\ x100}{W}$	56
7	$\%\ Fat = \frac{Wf}{Ws} x\ 100$	57
8	% N = $\frac{(Vt-Vb)X\ 1.400xN}{\text{Mass of Sample (g)}}$	58
9	% Protein = $\frac{(Vt-Vb)X1.4007XNX6.25)}{\text{Mass of Sapmle (g)}}$	58
10	Moisture content (%) = $\frac{W2-W3}{W2-W1} \times 100$	59
11	Colour chroma $\Delta C = \sqrt{(\Delta a^*)^2+(\Delta b^*)^2}$	59
12	Colour density $\Delta E = \sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2}$	59
13	Hue angle: $\text{Hue angle} = \text{Tan}^{-1} b/a$	59
14	Whiteness Index: WI = 100 - $\sqrt{(100-L*)+a*^2+b*^2}$	60
15	Yellowness Index: $\text{YI} = \frac{142.86b*}{\text{L*}}$	60
16	Starch Conc. SC = $\frac{(\blacktriangle A)\ (TVSA/SVSA)\ (TVGA/SVGA)\ (Starch\ MW)\ (F)}{(ε)\ (d)\ (1,000)}$	72
17	Starch Conversion (mg/ml) = $\frac{(\blacktriangle A)\ (2)\ (TVGA/SVGA)\ (162.1)\ (F)}{(6.22)(1)\ (1,000)}$	72
18	Starch Concentration: (SC) (mg/ml) = (▲A) (TVGA/SVGA) (F) (0.052)	72
19	Starch Resistant for samples (> 10 % RS): ΔE x F/W x 90.	75
20	Starch Resistant for samples (< 10% RS): ΔE x F/W x 9.27	75

21 Water holding capacity: WHC (g H_2O/g DM) = $\frac{(W2 - W1)}{W0}$ 76

22 Corrected Sample weight: $S1 = \frac{So(100-Mo)}{(100-M1)}$ 77

23 Corrected water weight: $W_1 = W_o + S_o - S_1$ 77

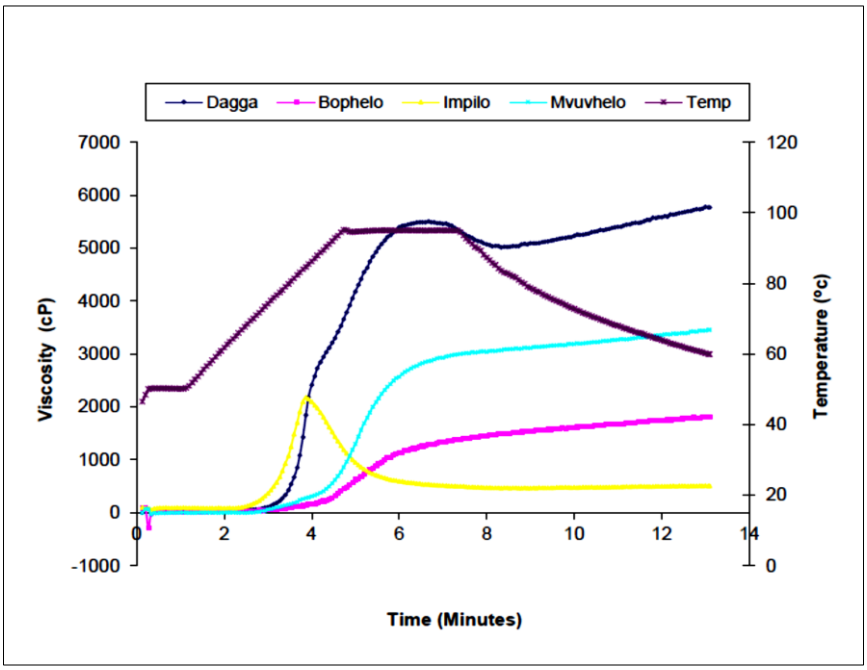
24 Constant moisture : $S_1 = \frac{s_o w_o (100 - m_o)}{w_o(100 - m_1) + s_o(m_o - m_1)}$ 78

25 Fixed weight: $W_1 = \frac{w_o(100 - m_1) + s_o(m_o - m_1)}{100 - m_o}$ 78

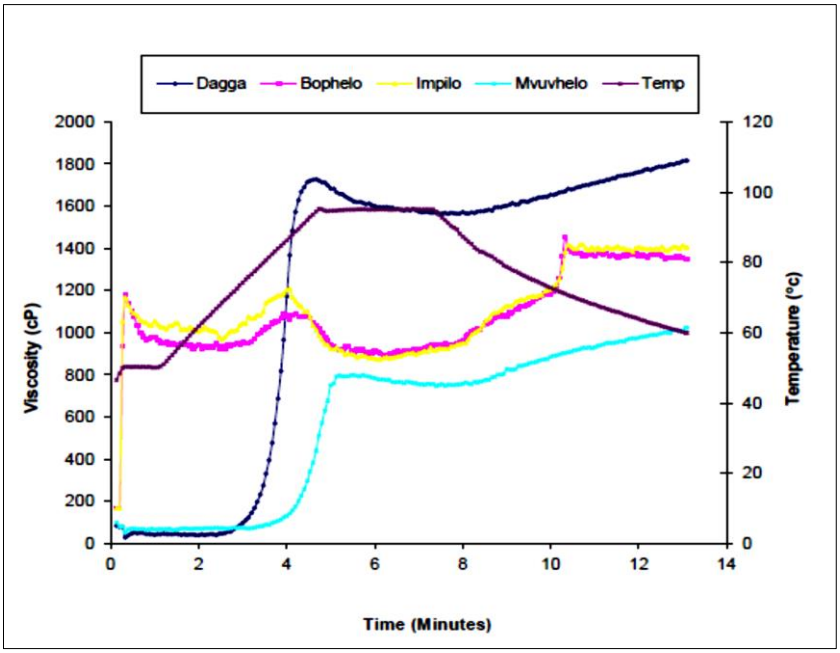
26 % TDF = [R sample – P sample – A sample - B)/SW] X 100 80

27 % Ash = $\frac{W3-W1}{W2-W1} X\ 100$ 82

APENDIX 3: REPRESENTATIVE RAPID VISCOSITY ANALYSIS PLOTS FOR TSHIOMBO AND UNIVEN CULTIVAR FLOURS.

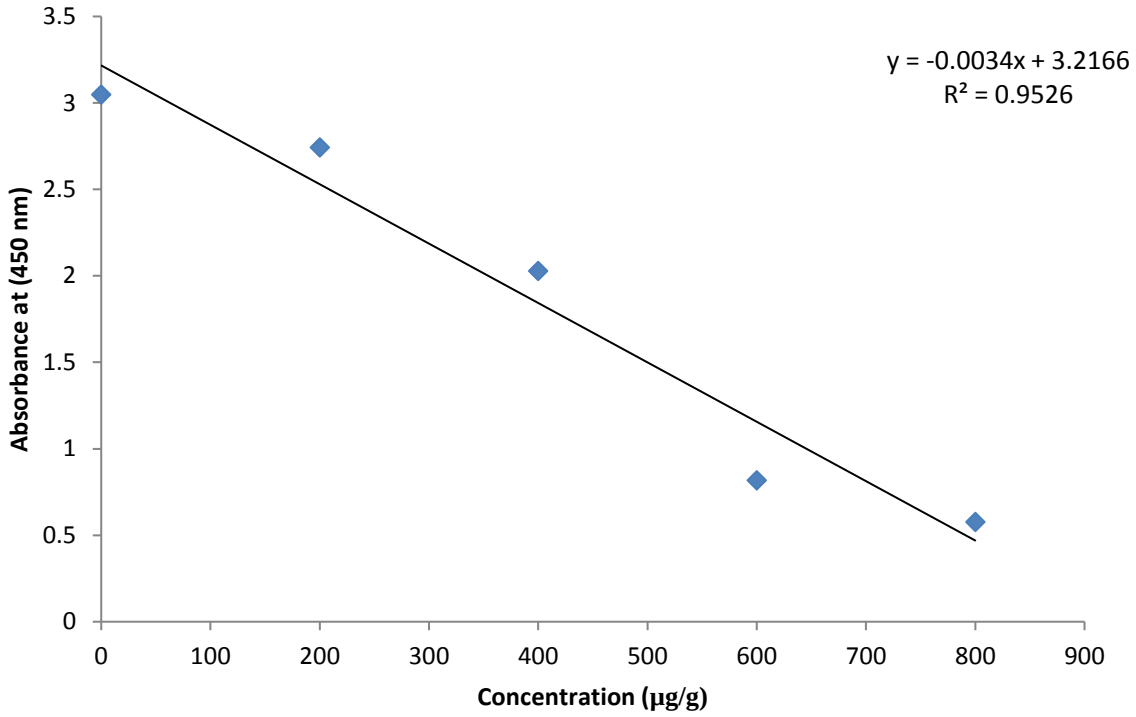


Plot of viscosity values across the different cultivars in Tshiombo location

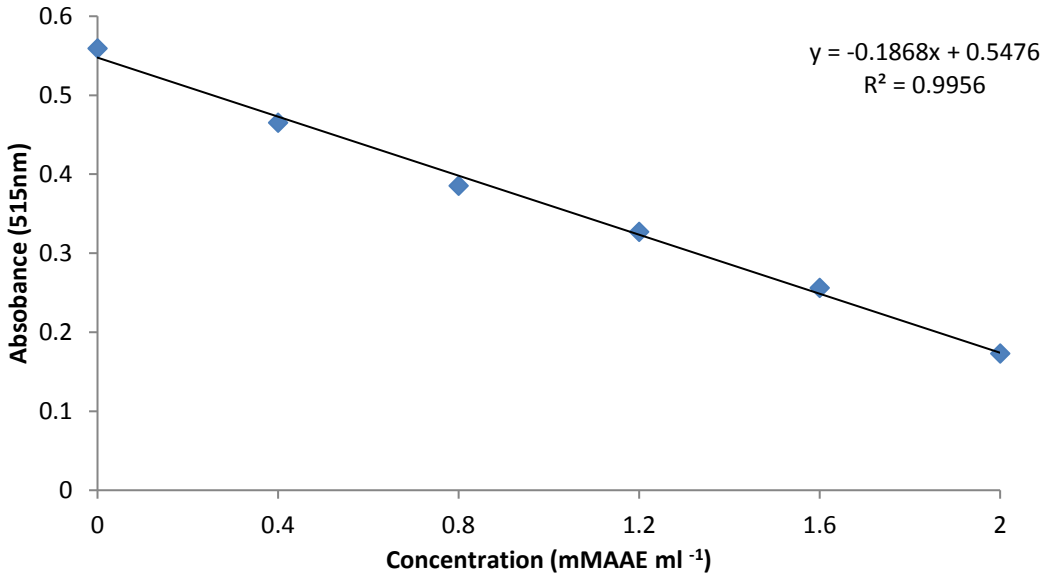


Plot of viscosity values across the different cultivars in Univen location.

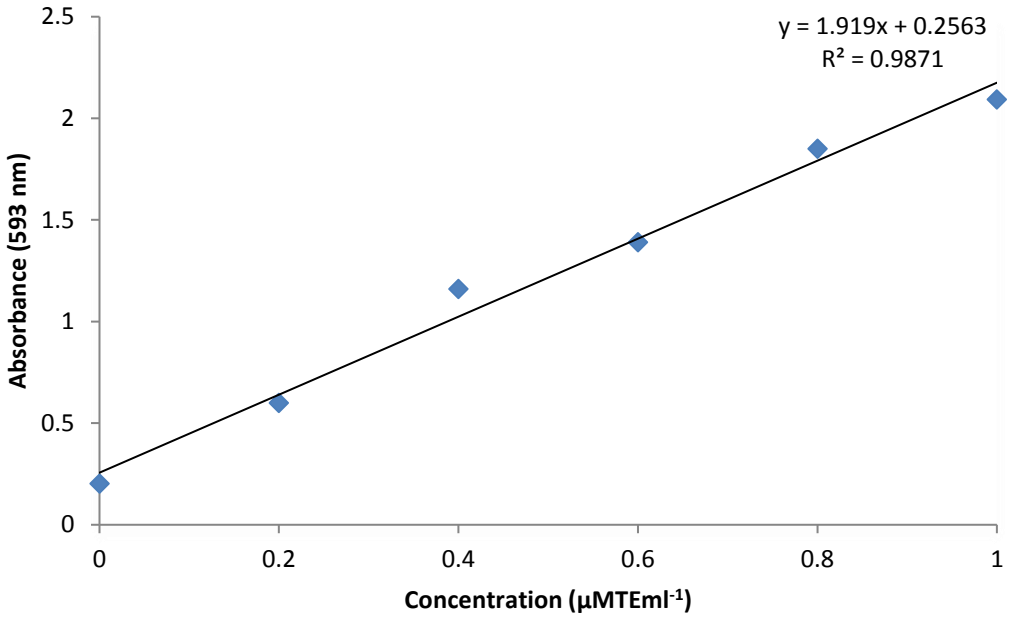
APPENDIX 4: STANDARD CURVE USED IN THE CALCULATION OF TOTAL CAROTENOIDS IN SWEET POTATO FLOURS



APPENDIX 5: STANDARD CURVE USED IN THE CALCULATION OF RADICAL SCAVENGING ACTIVITY BY 2, 2-DIPHENYL-1-PICRYLHYDRAZYL ASSAY.



APPENDIX 6: STANDARD CURVE FOR CALCULATION OF FERRIC REDUCING ANTIOXIDANT POWER BY FRAP ASSAY



APPENDIX 7: LIST OF ORAL PRESENTATIONS AT INTERNATIONAL CONFERENCE

1. **H. Silungwe**, G.R.A. Mchau and A.I.O. Jideani. (2015). Carotenoids and antioxidant profile of flour from four sweet potato (*Ipomoea batatas* (L.) Lam) cultivars from Limpopo Province, South Africa. 1st UNIVEN-WSU International Research conference "Research and Innovation for sustainable development and the transformation of society" 02 – 04 September 2015, International Convention Centre, East London, Eastern Cape, South Africa.

2. **H. Silungwe**, G.R.A. Mchau and A.I.O. Jideani. (2015). Effect of location on the functional properties of flour from four sweet potato *(Ipomoea batatas* (L.) Lam) cultivars from Limpopo Province, South Africa. 1st UNIVEN-WSU International Research conference "Research and Innovation for sustainable development and the transformation of society" 02 – 04 September 2015, International Convention Centre, East London, Eastern Cape, South Africa.