ASSESSMENT OF PROCESSING METHODS, SENSORY ATTRIBUTES, NUTRITIONAL QUALITY AND SAFETY OF CASSAVA LEAVES PRODUCT (*ISOMBE*) IN RWANDA

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN FOOD SCIENCE AND TECHNOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

Cassava (*Manihot esculenta* Cranz) leaves are cherished as a vegetable in Africa, but contain a toxic compound, cyanide. Studies were conducted to assess utilization, cyanide and nutritional value of cassava leaves after different preparation procedures in Rwanda. After a survey, leaves from bitter, sweet and wild cassava were: (1) pounded and cooked, (2) dried, pounded and cooked, and (3) pounded, dried and cooked. Drying was done to brittleness in a solar dryer after leaves were blanched. Sensory evaluation was done using a five point hedonic scale, where 5= like very much, 4= like moderately, 3= neither like nor dislike, 2= dislike moderately and 1= dislike very much. Cyanogens, vitamin C, β-carotene, crude protein, iron, calcium, phosphorus, potassium, zinc and moisture content (dry weight basis) were determined in: (i) un-dried, (ii) dried, (iii) un-dried and cooked, and (iv) dried and cooked. The chemicals of dry stored samples were also monitored after three, six, nine and twelve months. Results showed that cassava leaves from the three species were consumed as food and sun-drying was a single method used by farmers to extend the storage life. Colour, taste, aroma, texture and overall acceptability were principally affected by processing procedures. Fresh and dry leaves were preferred as vegetable except when they were pounded after drying. After boiling for 30 minutes, cyanide level (40 mg HCN/kg) was above FAO/WHO recommendation (10 mg HCN equivalent/kg) in the relish, but was judged as safe for the fact that it is served in small quantities as side food, reducing the HCN by serving to minor levels in comparison to documented acute oral lethal dose of HCN for an adult (30-210 HCN/60 kg bodyweight). Except vitamin C, amounts of β-carotene, iron, calcium, phosphorus, potassium and zinc were considerable, averaging 340, 153, 4264, 3531, 8426 and 54 mg/kg, respectively, and protein (34%) was high and valuable for cyanide human body detoxification. Stored, moisture increased significantly by 6.8% and shelf life was estimated at six months in

water, air and light proof material. Further studies in Rwanda on cassava cyanide disorders and approximate safe quantities of cassava leaves relish are recommended.

DECLARATION

I, Marie Goretti UMUHOZARIHO, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

In loving memory of my parents, Jean Intwaza and Thérèse Kamaziga, for building the groundwork of my achievements and unending prayers and benedictions,

To my beloved husband Antoine Ruzindana Nyirigira, and our lovely children Digne Brigitte Kamugire and Rodrigue Gisabo, for providing constant support, encouragement, prayers and unconditional love,

To all my brothers, sisters, nieces, nephews and Jennifer Keza, for encouragement, prayers and endless love,

To my true friends, for encouragement and prayers,

To all persons who are struggling for peace and development of our beautiful Africa,

My PhD thesis is dedicated.

TABLE OF CONTENTS

EXTENDED ABSTRACT ii

DECLARATION iv

COPYRIGHT v

ACKNOWLEDGEMENTS vi

DEDICATION viii

TABLE OF CONTENTS ix

LIST OF APPENDICES xi

LIST OF ABBREVIATIONS xii

LIST OF PAPERS INCLUDED IN THE THESIS xiv

1.0 INTRODUCTION 1

1.1 Background Information 1

1.2 Cassava Leaves: An Overview 5

1.2.1 Species 6

1.2.2 Cyanide as a major drawback in cassava leaves utilization 8

1.2.2.1 Cyanogenic glucosides: synthesis 8

1.2.2.2 Cyanogenesis and cyanide toxicity 9

1.2.3 Major nutrients of cassava leaves 11

1.2.3.1 Protein 11

1.2.3.2 Micronutrients 11

1.2.4 Other contributions of cassava leaves as a food to human health 12

1.2.5 Other contributions of leafy vegetables to human health 13

1.3 Drying as a Simple and Valuable Food Preservation Method 15

1.3.1 Principles and methods involved in drying 15

1.3.2 Blanching as a treatment prior to drying 17

1.3.3 Appropriate methods in drying and storage of leafy vegetables 17

1.4 Problem Statement and Justification of the Study 18

1.5 Objectives of the Study 20

1.5.1 Overall objective 20

1.5.2 Specific objectives 20

2.0 CONCLUSIONS AND RECOMMENDATIONS 21

2.1 Conclusions 21

2.2 Recommendations 23

REFERENCES 25

PAPER ONE 39

PAPER TWO 53

PAPER THREE 62

PAPER FOUR 71

APPENDICES 84

LIST OF APPENDICES

Appendix 1: Paper 4 Acceptance Certificate 84

Appendix 2: Baseline Survey Questionnaire 85

Appendix 3: Sensory Evaluation Form 92

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometer
ANOVA	Analysis Of Varience
AOAC	Association of Official Analytical Chemists
CABI	Commonwealth Agriculture Bureau International
CCDN	Cassava Cyanide Diseases and Neurolathyrism
CIAT	Centro Internacional de Agricultura Tropical
CMD	Cassava Mosaic virus Disease
CRC	Chemical Rubber Company
CRD	Completely Randomized Design
D.C.	Daily Climatology
dSPE	dispersive Solid Phase Extraction
EDPRS	Economic Development and Poverty Reduction
EIAR	Ethiopian Institute of Agriculture Research
EN	European Norms
FAO	Food and Agriculture Organization
GPS	Geographical Position System
HCN	Hydrogen Cyanide
HDPT	High Density Polyethylene
HPLC	High Performance Liquid Chromatography
HNL	Hydroxynitrile Lyase
IDD	Iodine Deficiency Disorders
IFAD	International Fund for Agriculture Development
IITA	International Institute of Tropical Agriculture
ISAR	Institut des Sciences Agronomiques du Rwanda

ISO	International Organization for Standardization
LSD	Least Significant Difference
MINAGRI	Ministry Of Agriculture and Animal Resources
MINECOFIN	Ministry of Finance and Economic Planning
MINITERE	Ministry of Lands Resettlement, Environment, Forestry, Water and Mines
MMA	Match Makers Associate
PDA	Photodiode Array
RAB	Rwanda Agriculture Board
RAE	Retinol Activity Equivalence
RBS	Rwanda Bureau of Standards
RDA	Recommended Dietary Allowance
REMA	Rwanda Environment Management Authority
SAS	Statistical Analysis System
SIDA	Swedish International Development Agency
SUA	Sokoine University of Agriculture
TAN	Tropical Ataxic Neuropathy
T&R	Transmitting and Receiving
TCARC	Technical Centre for Agriculture and Rural Cooperation
TFDA	Tanzania Food and Drug Authority
UK	United Kingdom
UNEP	United Nations Environment Programme
UR	University of Rwanda
USA	United States of America
UV	Ultraviolet
WHO	World Health Organization

LIST OF PAPERS INCLUDED IN THE THESIS

(i) Umuhozariho, M. G., Shayo, N. B., Msuya, J. M. and Sallah, P. Y. K. (2011). Utilization of cassava leaves as a vegetable in Rwanda. *Rwanda Journal: Agriculture Sciences* 24 (Series E):15-27.

(ii) Umuhozariho, M. G., Shayo, N. B., Msuya, J. M. and Sallah, P. Y. K. (2013). Sensory evaluation of different preparations of cassava leaves from three species as a leafy vegetable. *African Journal of Biotechnology* 12 (46): 6452-6459.

(iii) Umuhozariho, M. G., Shayo, N. B., Msuya, J. M. and Sallah, P. Y. K. (2014). Cyanide and selected nutrients content of different preparations of leaves from three cassava species. *African Journal of Food Science* 8 (3): 122-129.

(iv) Umuhozariho, M. G., Shayo, N. B., Msuya, J. M. and Sallah, P. Y. K. (2014). Assessment of over time changes of moisture, cyanide and selected nutrients of stored dry leaves from three cassava species. *African Journal of Biotechnology* (Accepted on 4/09/2014).

1.0 INTRODUCTION

1.1 Background Information

Cassava (*Manihot esculenta* Crantz) is one of the staple food crops in Africa. The estimated total world cassava production in 2010 was 230 million tonnes, with an increase of 25% since 2000, and Africa was the continent with the largest cassava production (53% of world production) (FAO, 2012). Data of cassava production per capita reported by FAO (2012) show how cassava is important in Africa. In 2010, Angola led the production per capita with 726 kg/person followed by Ghana with 563 kg/person. Cassava is a rain-fed crop mainly in humid and sub-humid regions, but being particularly suited to conditions of low soil nutrients and able to survive drought, it has become one of the most important food crops almost entirely in countries within the tropics (Huzsvai and Rajkai, 2009). Cassava is therefore an important famine reserve crop in countries with unreliable rainfall. Another important property that makes cassava popular is that it can remain in the soil after maturity for up to three years without decaying (Tumuhimbise *et al.*, 2012). This is a practical solution to food insecurity for smallholder farmers as cassava harvesting can be delayed until needed for use (piecemeal harvesting, sell, or processing).

In Rwanda, although the country is not known among the largest producers, cassava is important and is the third among main food crops after banana and sweet potato. It is one of the priority crops that are being promoted for economic development and poverty reduction in the agricultural sector (Rwanda Ministry of Finance and Economic Planning, 2007). Rwanda is characterized by dramatic contrasts in rainfall as the elevation changes from the lowland areas of east and south (900 mm) to the mountain chains in the north and west (1500 mm). As the economy of Rwanda is heavily dependent on rain-fed agriculture, crops are being prioritized by regions based on physical factors such as rainfall and soil. Therefore, as cassava tolerates long drought and poor soils, it is intensively grown in the semi-arid regions of the lowland areas of Rwanda, experiencing low rainfall, averaging 900 mm per annum as reported by Rwanda Environment Management Authority (2007). It has been revealed that most of the cassava produced in Nigeria, one of the largest cassava producers, still comes from peasant farmers who depend on manual tools for their field operations (Kolawole *et al.*, 2010). According to FAO (2013), cassava is one of the few staple crops that can be produced efficiently without the need for mechanization or purchased inputs, and in marginal areas with poor soils and unpredictable rainfall. In Rwanda, cassava is described as "classic food security crop" because it offers the advantage of a harvest even in situations of erratic rainfall and infertile soils (Mushiyimana *et al.*, 2011).

As human food, cassava produces bulky roots with a heavy concentration of carbohydrates, and leaves that constitute a good source of proteins and micronutrients (IITA, 1990). Cassava roots are rich in digestible carbohydrates, principally starch, being mainly composed of two polysaccharides, amylose (20%) and amylopectin (80%) (Sandoval *et al.*, 2008). According to Kabawila *et al.* (2005), cassava root constitutes energy-rich food, having higher energy density (610 KJ/100 g fresh weight bases) than other root crops such as sweet potatoes (460 KJ/100 g) and taro (490 KJ/100 g). Cassava leaves consumption as a vegetable is also well known and widespread in most countries in tropical Africa. Cassava leaf meals have different names in different African countries such as, *pondu, sakasaka, matamba* and *sombe* in Democratic Republic of Congo (DRC), *nkwen* in Cameroon, *ravitoto* in Madagascar, *mathapa* in Mozambique (Achidi *et al.*, 2005), *kisamvu* in Tanzania and *isombe* in Rwanda and Burundi. In some African countries, especially in Central Africa and some countries of West Africa, cassava leaves are the basic vegetables (more than 60% of all vegetables eaten there), being the cheapest and richest source of protein (Achidi *et al.*, 2005). Nutritionally, cassava leaves are more contributing than roots by providing protein, minerals and vitamins (Akinwale *et al.*, 2010). For protein in cassava leaves, the ratios (g/100 g crude protein) of all amino acids, except methionine, exceed the FAO's recommended reference protein (Ayodeji *et al.*, 2005). Other nutrients include minerals and vitamins. According to IITA (1990), raw cassava leaves contain 7 g of protein, 303 mg of calcium, 119 mg of phosphorous, 7.6 mg of iron, 11.775 µg of ß-carotene and 8 mg of vitamin C per 100 g edible portion, fresh weight basis. They are more nutritious if compared with other vegetables such as raw spinach and sweet potato leaves. Spinach contains 2.4 g of protein, 62 mg of calcium, 39 mg of phosphorous, 3.9 mg of iron, 3.640 µg of ß-carotene and 56 mg of vitamin C. Sweet potato leaves contain 117 mg of calcium, 1.8 mg of iron, 7.2 mg of vitamin C per 100 g fresh weight of leaves (Shahidul, 2014).

Cassava leaves were reported to be among Rwanda's high value vegetables that have potential on domestic, regional and international markets (Austin *et al.*, 2009). The leaves are widely consumed in Rwanda but there is little documented evidence on their consumption. Nevertheless, according to Umuhozariho *et al.* (2011), human cassava leaves consumption is greatest in low and middle land areas of Rwanda for their more availability than other leafy vegetables. According to Rwanda Ministry of Agriculture and Animal Resources (2011), 700,000 agricultural households in Rwanda grow cassava, 42, 25 and 22% of the national cassava production are principally produced in three ecological-zones: Mayaga which covers eastern parts of Kamonyi, Ruhango, Nyanza and Gisagara Districts; Imbo which includes western parts of Rusizi and Nyamasheke Districts, and Bugeserara which extends on Bugera and Ngoma Districts. Umuhozariho *et al.* (2011) pointed out that all farmers in Ruhango District (Ecological zone of Mayaga) grow cassava as main crop and almost of them harvest cassava leaves as vegetables. In addition, the authors noted a significant trade of fresh cassava leaves in different markets such as principal and super markets of Kigali city.

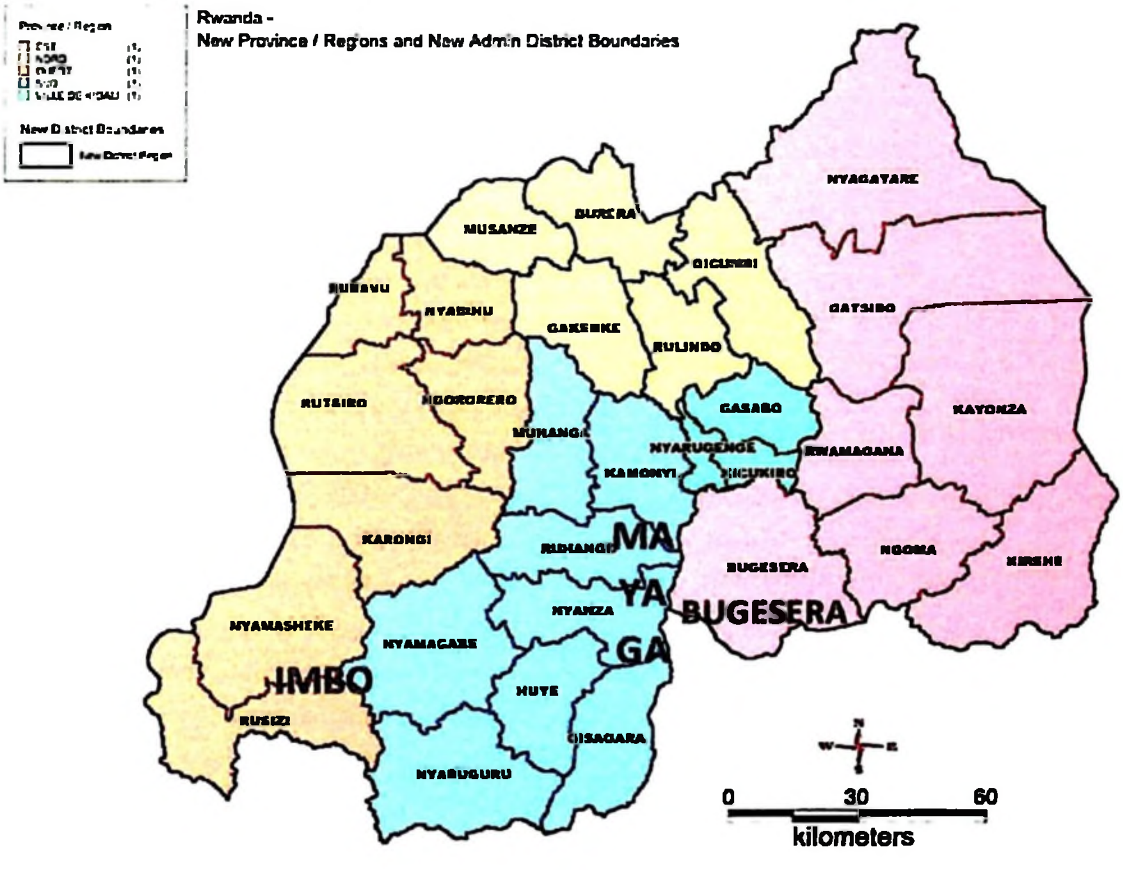

Figure 1: Most important areas of cassava production in Rwanda

Source: Rwanda Ministry of Agriculture and Animal Resources (2011)

However, cassava products contain cyanide, in the form of cyanogenic glucosides, primarily linamarin and small amounts of lotaustralin (in the ratio 93:7) (Uyoh *et al.*, 2007). The cyanogenic glucosides are distributed throughout the cassava plant, with highest levels in leaves (Etonihu *et al.*, 2011). When they are hydrolysed by linamarase, a naturally occurring enzyme in cassava, a poisonous chemical compound known as hydrogen cyanide (HCN) is liberated (White *et al.,* 2003). The potential toxicity due to the presence of endogenous cyanogenic glucosides constitutes a major drawback of cassava and cassava products utilization. Therefore, before consumption, cassava roots and leaves are processed to reduce the cyanogenic glucosides. As found by Cliff *et al.* (2011), poorly processed cassava products can be a source of cyanide-associated health disorders such as Tropical Ataxic Neuropathy (TAN) and *konzo*. These problems have been reported in many countries of Africa such as the Democratic Republic of Congo, Mozambique, Nigeria, Tanzania (Mlingi *et al.*, 2011; Ciglenecki *et al.*, 2011; Nhassico *et al.*, 2008) and Ethiopia (CCDN, 2012).

1.2 Cassava Leaves: An Overview

Experiences from several scientists such as Umuhozariho *et al.* (2011) and Achidi *et al.* (2005) indicate that millions of tonnes of cassava leaves are harvested and used as vegetables by many families, especially in Africa, and provide protein, vitamins and minerals (Umuhozariho *et al.* 2014; Akinwale *et al.*, 2010; Data and Owuru, 2010; Priadi *et al.*, 2009). The harvesting is done by selecting tender leaves, growing shoots and growing stems (Umuhozariho *et al.*, 2011; Achidi *et al.*, 2005). The potential contribution of plant foods to micronutrient status depends upon the retention of the nutrients after handling, processing and preparations (Bradbury and Denton, 2014; Essiett and Akpan, 2013; Oguche Gladys, 2011; Thompson and Amoroso, 2011; Shackleton *et al.*, 2009). Particularly for cassava products, which require more preparations for detoxification, the cyanide reduction is accompanied by nutrient losses (Anhwange *et al.*, 2011; Abah Idah *et al.*, 2010; Eze, 2010; Udofia *et al.*, 2010) and therefore information is needed, not only on the residual cyanide to ensure consumer safety, but also on nutritional composition to value their quality and contribution to consumer's health.

As it applies to other vegetable products, cassava leaves price varies much according to season or market location. Leaves are available as seasonal surpluses during certain parts of the year (rainy season) and go to waste due to improper processing, pre-packaging, handling, distribution and marketing. During the peak season, vegetables in general are sold at very low prices and some are simply wasted (TCARC, 2007). This reduces income to farmers, adding to their poverty.

Preparation of cassava leaves is more involving and time consuming than other leafy vegetables that are simply chopped and boiled for a few minutes (Prabhu and Barret, 2009; Achidi *et al.*, 2005). However, as noted by Gould (1999), food convenience includes ease of preparation and storage, satisfactory shelf life, nutritionally healthier and safer product. This puts consumption of cassava leaves in a less advantageous situation unless actions are taken to research and identify the key issues involved in its processing and utilization.

1.2.1 Species

A total of three cassava species, namely *Manihot utilissima* (bitter), *Manihot dulcis* (sweet) and *Manihot glasiovii* (wild) have been recognized by earlier researchers (Nassar, 2006). The height of the plant ranges from about 1 to 3.5 metres depending on the species, variety and growing conditions. The mature stems and branches are rounded and generally of uniform thickness, not more than 5-7 centimetres of diameter and the leaves are palmate (Sugino and Mayrowani, 2009). The three species, bitter, sweet and wild, respectively named as *Gitaminsi*, *Imiribwa* and *Igicucu* in local language, are grown in Rwanda and from all the three, leaves are consumed as a green vegetable (Umuhozariho *et al.*, 2011).

Figure 2: Leaves from sweet (left), wild (middle) and bitter (right) cassava species

Based on farmer's ethno-classification. Mkumbira *et al.* (2003) categorized cassava into two cultivars: sweet and bitter. Farmers regard roots from sweet cultivars as non-toxic. Boiling is the more general method employed for preparing cassava roots as food. In contrast, roots from bitter cultivars must be processed to reduce cyanide and toxicity prior to consumption. According to CODEX STAN 300 (2010), hydrogen cyanide content for sweet cultivars should be less than 50 mg/kg (fresh weight basis) while bitter cultivars are those containing more than 50 mg/kg of cyanides expressed as hydrogen cyanide (fresh weight basis). According to Vieira *et al.* (2011), sweet cassava includes cultivars with hydrogen cyanide concentrations of less than 100 mg/kg in fresh roots. However, it has been reported that cassava toxicity varies according to potassium level in soil (Endris, 2009) and carbon monoxide in the environment (Gleadow *et al.*, 2009). Drought also increases the cyanogenic glucoside content, hence the toxicity of cassava (Cliff *et al.*, 2011; Mlingi *et al.*, 2011). Bourdoux (1988) suggested that cassava classification based on cassava toxicity cannot be useful, as the environment has an effect on the root toxicity. So, the tendency is to regard all cassava as varieties of *Manihot esculenta* Crantz. Wild species vary in growth habit from short shrubs to tree-like crop. It has been used as a source of useful characteristics in agricultural improvement (Nassar *et al.*, 2008; Nassar, 2006). In Rwanda, *Manihot glaziovii* is planted in yards and gardens providing shades. They are also used in fields to protect soil against erosion. Their leaves are a precious food as green leafy vegetable and more available than leaves from bitter and sweet cultivars (Umuhozariho *et al.*, 2011).

1.2.2 Cyanide as a major drawback in cassava leaves utilization

All cassava cultivars contain cyanogenic glucosides. However, a wide variation in the concentration of cyanogens exists among different cultivars and can range from 1 to 2000 mg/kg (CIAT, 2007; Cardoso *et al.*, 2005). The concentrations of cyanogens vary also in different varieties, between tissues in the same plant and even between compartments of the same tissue (Burns *et al.*, 2012; Bradbury and Denton, 2011). Etonihu *et al.* (2011) reported the cyanide concentrations in different parts of cassava plant to be in the order of: leaves > peel > root (fresh) > stem. It is assumed that the glucosides are synthesized in the young tender leaves. Cassava leaves, however, contribute more than roots to improve human nutrition due to their high content of protein, minerals and vitamins (IITA, 1990).

1.2.2.1 Cyanogenic glucosides: synthesis

Cassava produces two cyanogenic glucosides, linamarin and lotaustralin. Linamarin is the predominant one. According to Etonihu *et al.* (2011) and White *et al.* (2003), the two cyanogenic glucosides are present in all organs of the plant. The cyanogenic glucosides are synthesized from amino acid valine and isoleucine respectively and may be synthesized only in leaves and distributed to all parts of the plant where they accumulate (Siritunga and Sayre, 2004).

1.2.2.2 Cyanogenesis and cyanide toxicity

Cyanogenesis exists in over 2500 plant species and is regarded as having an important role in plant defence against herbivores (Zagrobelry *et al.*, 2008). Cyanogenesis is the ability of plants to produce, under some circumstances, the toxic hydrogen cyanide (HCN) (Zagrobelry *et al.*, 2008). The plants produce complex compounds, mainly glucosides, which may break down to produce HCN. Those compounds are therefore known as cyanogenic compounds. Plants also produce enzymes that break down the cyanogenic compounds but they are always stored separately inside plant cells. It is only when the plant is damaged, and the structural integrity of the plant cells is destroyed that the enzyme acts on the cyanogenic compounds to produce cyanide (Zagrobelry *et al.*, 2008; McMahon *et al.*, 1995; Hughes *et al.*, 1992). In cassava plant cells, the cyanogenic glucosides are stored inside the vacuoles in the cytoplasm while the linamarase enzyme capable of degrading them is located in the cell wall outside the cytoplasm (McMahon *et al.*, 1995; Hughes *et al.*, 1992). When cassava tissues are bruised and the cellular structures are disrupted, linamarin and lotaustralin come in contact with linamarase, acetone cyanohydrin from linamarin and 2- butanone cyanohydrin from lotaustralin are produced. These cyanohydrins are unstable and decompose spontaneously to corresponding ketones and hydrogen cyanide (HCN) at pH values above 5 and temperatures above 35°C. This breakdown may also be catalysed by the enzyme α-hydroxynitrile lyase (HNL), located in apoplastic space (Siritunga and Sayre, 2004).

Consumption of cassava products that are not sufficiently processed may contain cyanogens that can cause cyanide poisoning. Chronic, low level cyanide exposure has been associated with the development of goitre, Tropical Ataxic Neuropathy (TAN) and cretinism (Nhassico *et al.*, 2008; CCDN, 2007). TAN is a collective term for disorders which include, lesions in the skin, mucous membranes, optic and auditory as well as peripheral nerves, while cretinism is a condition of stunted growth in children (Katumbas and Spencer, 2007). *Konzo*, a paralytic disorder, manifested by an irreversible paralysis of legs and other locomotive disorders, is also linked to prolonged consumption of insufficiently processed cassava products (CCDN, 2007; Cardoso *et al.*, 2005). *Konzo* occurs mainly among children and women of child bearing age in contrast to TAN which is a progressive disorder that mainly affects old people (CCDN, 2008; CCDN, 2007). Consumption of cassava products containing large amounts of cyanogens can cause cyanide poisoning sometimes known as acute intoxications with symptoms of headaches, nausea, dizziness, diarrhoea, vomiting, muscular paralysis and respiratory stress as cyanide affects the cytochrome oxidase system, which may result into coma and sometimes death (Nhassico *et al.*, 2008; CCDN, 2007).

Some of the prerequisites for occurrence of cyanide poisoning are insufficient cassava processing that leaves high residual levels of cyanogens in the foods and a protein deficient diet. The universal mechanism in human (animal) to detoxify cyanide (CN) is its conversion, catalysed by an enzyme rhodanese, to produce thiocyanate (SCN), which is water soluble and removed in urine (Katumbas and Spencer, 2007). The synthesis of thiocyanate requires sulphur-containing amino acids (cysteine and methionine). These are essential amino acids, meaning that they cannot be synthesized in the human body and they must be obtained from the eaten food. If the diet is with insufficient intake of sulphur-containing amino acids, it increases the effects of cyanide poisoning (CCDN, 2007). One effect of the poisoning can be the increased demand of sulphur- containing amino acids to be used up in detoxifying ingested cyanide, which reduces their availability to protein synthesis, limiting the amount of protein available for growth, leading to stunting of children (CCDN, 2007). This can be the explanation of the reason that, in the countries where cassava constitutes a staple food, cyanide poisoning occurs during crisis when protein-rich foods are not accessible, such as in war, droughts and floods, in very deprived areas and among very poor rural people (Banea *et al.*, 2012; Cliff *et al.*, 2011; Mlingi *et al.*, 2011; Allen, 2010; Burns *et al.*, 2010).

1.2.3 Major nutrients of cassava leaves

1.2.3.1 Protein

Proteins are made up of amino acids and each protein has a particular sequence of amino acids. Proteins can be classified as: (1) Complete when it contains all the essential amino acids, (2) Incomplete when lacking one or more essential amino acids. Essential amino acids are those which the body cannot synthesize on its own to meet all the body's amino acid requirements for tissue maintenance and growth (Marieb and Hoehn, 2007). They must be supplied in the body by foods as the body cannot synthesize them. Cassava leaves are well balanced in essential amino acids, although they contain lower proportions of methionine and cysteine, but the levels (g/100g crude protein) of all other amino acids exceed the FAO's recommended reference protein (Ayodeji *et al.*, 2005).

1.2.3.2 Micronutrients

Vitamins and minerals are referred to as micronutrients because the body needs them in very small quantities (milligrams and micrograms) for growth, development and maintenance (Thompson and Amoroso, 2011). Human body cannot synthesize them and, therefore they are supplied through diet. Cassava leaves are among the richest African indigenous vegetables in micronutrients, especially vitamin C and E, β-carotene (source of vitamin A), iron and zinc (Shackleton *et al.*, 2009). Vitamin A, iron and zinc deficiency disorders are among the most common forms of micronutrient malnutrition (Dairo and Ige, 2009). There are several different approaches to combating micronutrient malnutrition at the level of population, including supplementation or provision of single micronutrient, food fortification and natural rich-foods diversification (Harrison, 2010). Food-based strategy is intake of micronutrient rich-foods. Among all those strategies, natural rich-foods diversification strategy seems to be sustainable because fortification has been associated with increased price and supplementation is necessary for groups at high risk as a short-term emergency, and micronutrient deficiencies rarely occur in isolation (Thompson and Amoroso, 2011; Gibson, 2004; FAO/WHO, 2003). Enhanced consumption of vegetables has been known to alleviate micronutrient malnutrition (Prabhu and Barret, 2009). However vitamins are easily destroyed by light and oxygen as well as through poor food preparation, storage, handling and cooking (Gould, 1999). Therefore, foods, especially fruits and vegetables must be processed carefully for enhancing vitamin availability and consumption and care must be taken even for preliminary preparations and storage.

1.2.4 Other contributions of cassava leaves as a food to human health

Cassava leaves are among the top three African indigenous vegetables rich in nutrients. As reported by Shackleton *et al.* (2009), cassava leaves are the second in β-carotene after *Moringa oleifera*, the second in vitamin C after *Moringa stenopetala*, the third in vitamin E after *Moringa stenopetala* and *oleifera*, the third in zinc after *Pterocapus mildbraedii* and *Moringa oleifera*, the third in antioxidant activity after *Adansonia digitata* and *Rorippa madagascariensis*, and the third in total phenolic after *Rorippa madagascariensis* and *Adansonia digitata*. Considering the qualities, the popularity and cultivation of cassava, efforts may be done to improve storage and transport for engaging cassava leaves in business as it is for roots.

1.2.5 Other contributions of leafy vegetables to human health

Green leafy vegetables are rich in pro-vitamin A, iron, folates and other micronutrients (Essiett and Akpan, 2013; Shackleton *et al.,* 2009). African leafy vegetables can make significant contributions to the diets of populations in provision of elements, particularly iron, zinc, and vitamins A, B complex and C, which have been reported as deficient in many parts of Sub-Saharan Africa (United Nations University, 2005). Among the leafy vegetables currently consumed in East and West Africa, some are notably nutritious and high in ß- carotene and iron contents: cowpea leaves (*Vigna unguiculata*), baobab leaves (*Adansonia digitata*), amaranth (*Amaranthus viridis*), spider plant (*Cleome gynandra*), jute mallow (*Corchorus olitorius*), moringa leaves (*Moringa oleifera*), African nightshade (*Solanum scabrum*), cassava leaves (*Manihot esculenta*), pumpkin leaves (*Cucurbita spp.*) and sweet potato leaves (*Ipomoea batatas*) (Weinberger and Swai, 2006; Grubben and Denton, 2004). Nutritional components in plants include 90 to 98% macro-nutrients such as protein, carbohydrate and oil, and 1 to 10% micro-nutrients, including at least 17 essential minerals, 13 essential vitamins and an abundance of secondary metabolites estimated at greater than 200 000 compounds, and among the metabolites, some phytochemicals have positive physiological effects on humans and act as protective agents for health maintenance and promotion, or have medicinal properties. Beneficial phytochemicals include flavonoids, carotenoids, glucosinolates and sulphur compounds, which function as antioxidants, anti-cancer, anti-inflammatory or anti-microbial compounds or influence blood component profiles (Essiett and Akpan, 2013). According to Hughes (2006), oxidative stress is an underlying factor in health and disease in humans and a proper balance of oxidants and antioxidants is important to maintain health and improve longevity.

Vegetables, especially green vegetables, are served as side relish with starchy foods. The traditional combination of starchy staples with a green vegetable sauce plays an important role in balancing dietary quality. It was reported by Laker (2005) that the staple cereal and leguminous crops common in Sub-Saharan Africa (maize, sorghum, rice and beans) do not absorb zinc when growing and therefore cannot provide zinc in the diet. The combination with green vegetables is an important way to supplement the diet with different micronutrients.

Insufficient vegetable and fruit consumption causes 2.7 million deaths annually worldwide and belongs to the top ten risk factors contributing to high mortality rate (Ezzati *et al.,* 2002). The minimum per capita vegetable supplies recommended per person per year are 73 kg (Yang and Keding, 2009). In Sub-Saharan Africa, per capita vegetable supplies are only 43% of what is needed, leading to widespread malnutrition and micronutrient deficiencies, mainly vitamin A and iron deficiencies and chronic diseases such as cardiovascular, cancer, chronic respiratory and diabetes (Yang and Keding, 2009).

Adequate processing and handling of cassava leaves can be a sustainable solution to combating micronutrient malnutrition by making the food available throughout the year and preserving its components. Different preservation methods can be applied to avoid vegetable deterioration as well as physical losses. The simplest method, inexpensive and thus affordable even to rural communities, and for larger production is drying, especially using natural solar energy.

1.3 Drying as a Simple and Valuable Food Preservation Method

The major purpose of food preservation is to protect it against deterioration and spoilage. The rate of deterioration of raw food commodities may be very high. The spoilage is caused by microorganisms that decompose food constituents, enzymes that break down proteins, lipids, carbohydrates and vitamins, and substances that chemically react with others or with the ambient oxygen. Bad smelling, toxic substances, discoloration and alteration of flavour and nutrients can be the results (Thomas, 2008). Oxygen in air is also an important factor in the spoilage of some foods by promoting the action of some types of enzymes, chemical reactions and growth of some types of spoilage micro-organisms. Some components of foods, including fat and vitamins (riboflavin, vitamins A and C) are susceptible to losses when exposed to light (Fellows and Axtell, 2001).

Different factors need to be controlled for preserving physical and nutritional quality of foods. Water content is one of the most important factors that contribute to food deterioration. All micro-organisms, chemical reactions and enzymes require moisture to act and to be sources of changes (Emebu and Anyika, 2011; Fellows and Axtell, 2001). Among the techniques designed to control the factors and causes of food deterioration, drying is simple.

1.3.1 Principles and methods involved in drying

Drying is one of the oldest methods of food preservation. Drying preserves foods by removing excess moisture from food to prevent decay and spoilage. Water content of properly dried food varies from 5 to 25% depending on the type of foodstuff. Successful drying depends on enough heat to draw out moisture without cooking the food, not seriously affecting the flavour, texture and colour of the food. It also depends on air to absorb the released moisture and adequate air circulation to remove the moisture (Kendall *et al.*, 2010; James and Kuipers, 2003).

Foods are dried when the water contained within them (intracellular and extracellular) is removed into the surrounding air. It must first move to the surface of food and then be evaporated as water vapour. For effective drying, the air should be relatively hotter than the food item, dry and moving. Air can only remove water from foods if it has the capacity to hold extra water vapour, meaning at lower humidity, and the humidity is dependent on the temperature of the air. At high temperatures, the humidity is reduced and air can carry more water vapour and vice versa (Azam-Ali *et al*, 2003). Different methods of drying have systems to provide heat (energy) to move the water as vapour and heat the surrounding air. Direct sun, solar and oven drying use sun or electricity as a source of energy. Wind or fans are used to increase the speed of the air movement and the dryness of the environment. Among the methods, two are discussed, open sun and solar drying as they use solar energy, which are appropriate for rural areas.

For the sun drying method, no costs are incurred to heat or to circulate the air because solar energy is natural. It is the cheapest, involving simply laying the products in the open sun on mats, roofs or drying floors. Unfortunately, this type of drying has its limitations. The product is open to contamination by dust, the process is very dependent on good weather, and the very slow drying rates of the process create the danger of mould growth. The direct exposure to sunlight reduces the quality (colour and vitamin content) of the final product (MMA, 2008). It performs well in warm and dry conditions. At night and during the rainy periods, this type of drying cannot be used. The temperature of the food during sun drying is usually 5-15°C above ambient temperature (Chakraverty *et al.*, 2003). Nevertheless, sun drying remains an economically viable method for preserving foods for the rural poor although the products obtained may be of poor quality.

Solar drying also uses the solar energy and has some advantages over the open air sun drying when correctly designed. Solar dryers give faster drying rates by heating the air to 10-30°C above ambient temperature, preserve nutrients and colour of the final products, and prevent insects. They are made of solar energy concentrators or collectors with natural or forced airflow inside the dryers. Solar dryers can eliminate the negative effects of the open air sun drying method and can be more efficient in making use of solar energy (Almuhanna, 2010; Banout and Ehl, 2010; Lotfalian *et al.*, 2010; Medugu, 2010; MMA, 2008; Chakraverty *et al.*, 2003). Solar dryers can use solar energy both in the thermal form for the drying process and the electrical form for driving the fans, by means of the solar collector and solar module respectively.

1.3.2 Blanching as a treatment prior to drying

Blanching is an indispensable treatment carried out prior to processing vegetables such as drying for slowing or stopping enzyme activity before processing. It also shortens the drying and rehydration time by relaxing the tissue walls so that moisture can escape and later re-enter more rapidly (Kendall *et al.*, 2010; Azam-Ali *et al.*, 2003; James and Kuipers, 2003). It protects nutrients such as vitamins by inactivating enzymes, and contributes to reduction of microbial influence by killing some microbial load. Blanching is done by submersion of vegetables in boiling water for some minutes or circulating steam freely around the vegetables for some minutes depending on the type of vegetables (Kendall *et al.*, 2010).

1.3.3 Appropriate methods in drying and storage of leafy vegetables

To ensure safe storage of dried vegetables, the final moisture content should be less than 10% (Fellows, 2009). At this moisture level, microbial and enzymatic activities are limited by unavailability of water. According to Boyer and Mckinney (2009) and

TCARC (2007), in general, vegetables that are dried until they are brittle, packed in airtight, light and moisture proof packaging material, can be stored for six months at room temperature in a dry place. The stability of dried foods during storage depends on its ability to pick up moisture from air that can rehydrate the food and be in contact with oxygen and light. Therefore, a special packaging material that is water and air impermeable and opaque to light is required. According to Azam-Ali (2003), dried products are storable without refrigeration and this storage offers the following advantages: (i) simplification of distribution, (ii) energy saving during storage, (iii) extending market and (iv) extending shelf-life.

1.4 Problem Statement and Justification of the Study

Hunger and malnutrition can exist in spite of adequate food production. These can be the result of uneven distribution, deterioration and losses of available food resources. Hence, optimum utilization of available food or reduction of physical and nutritional post-harvest food losses by adequate processing and handling are essential for sustainable food security.

As mentioned earlier, cassava leaves are highly concentrated with cyanogenic glycosides (White *et al.* 2003). For reducing the poisonous cyanide before their consumption as food, cassava leaves undergo involving preparations which can affect negatively the nutrients levels. Therefore, a better evaluation of the relish to nutritional contribution should be done after the different stages of preparation such as sorting, cleaning, pounding and cooking. To ensure consumers' safety, the residual cyanide level should be of concern.

Prepared cassava leaves as human food, locally called *isombe* is widespread and highly valued in Rwanda, in terms of preference compared to many other vegetables such as bean, pumpkin, sweet potato and moringa leaves, spider plant, African nightshade, African eggplant and cabbage. Cassava leaves are commonly used freshly harvested or stored in refrigerators as fresh food in some families and big super-markets. Similarly, experiences from several countries indicate that millions of tons of cassava leaves have been harvested and used as vegetables by many African families (Achidi *et al.*, 2005). Despite its extended use and value in several countries of Africa, cassava leaves have received minimum attention in food research, especially in Rwanda.

Another problem is price variation according to season or market location. In Rwanda, a price by bunch of ten to twenty Rwandan Francs during the rainy season becomes five to ten times higher during the dry season in local markets and even higher in towns and cities. To stabilize cassava leaves availability and price in different markets and seasons as response to national food security concerns, preservation methods need to be improved for extended storage and distribution throughout the year.

In Rwanda, cassava is one of the priority crops that are being promoted for development and poverty reduction in the agricultural sector (Rwandan Ministry of Finance and Economic Planning (2007). Consequently, production of cassava leaves is increasing and these leaves need to be processed to optimize the utilization of surplus. Improvement of cassava leaves processing methods is urgently needed to promote storability, food security, marketability and suitable income generation to farmers. In addition, cassava leaves are known to be good source of nutrients such as protein, minerals and β-carotene, and if promoted they can play potential role in reducing nutrient deficiencies, especially protein, iron and vitamin A deficiencies in African countries.

To address the problems, a study was undertaken in Rwanda from 2011 to 2014. The study assessed different processing procedures of cassava leaves in Rwanda. This has considered the different cassava species from which leaves are eaten as vegetable in Rwanda as well as the methods of preparation, to determine the potential contribution to nutrition and safety after undergoing the different steps of preparation. This is intended to improve and promote cassava leaves product called *isombe* in Rwanda and eaten as side food after being pounded and boiled in water with addition of oil, salt and groundnut paste. The research was therefore an endeavour to address seasonal scarcity and surplus losses, convenience and price fluctuations by processing cassava leaves, using drying method that can preserve quality of the final product.

1.5 Objectives of the Study

1.5.1 Overall objective

To investigate processing and preservation methods of cassava leaves as green vegetables in Rwanda and the effect of these methods on the nutritional quality and safety of the processed cassava leaves as a vegetable product locally known as *isombe*.

1.5.2 Specific objectives

(i) To identify the preparation methods and consumption frequency of cassava leaves in Rwanda (Paper One).

(ii) To evaluate sensory attributes of the prepared leaves from different cassava species and by different processing procedures (Paper Two).

(iii) To assess the effects of different processing procedures on cyanide, protein, and micronutrient levels of leaves from different cassava species (sweet, bitter and wild) found in Rwanda (Paper Three).

(iv) To evaluate the effect of storage time on moisture, cyanide, protein, and micronutrient levels of leaves from different cassava species (Paper Four).

2.0 CONCLUSIONS AND RECOMMENDATIONS

This thesis is a compilation of Papers One, Two, Three and Four. Methods, materials, discoveries and relevant conclusions for the four different studies are described in each paper. The following conclusions and recommendations are therefore general to the overall research.

2.1 Conclusions

Cassava leaves are among the basic vegetables in Rwanda. As cassava is the most cultivated crop in low altitude regions of Rwanda, for its tolerance to drought, its leaves are more available than other leafy vegetables such as amaranth and constitute a very common meal for almost all cassava producers. At farm gates and in all the markets of Ruhango and Kigali city, cassava leaves are sold in fresh form, un-pounded or pounded depending on market. Sun-drying is a single processing method to extend cassava leaves shelf life in Ruhango and it is practiced by very few farmers. Production is very high during the rainy seasons and postharvest losses become very high. Leaves from studied cassava species (i.e., bitter, sweet and wild) are all consumed as vegetable, but leaves from wild cassava are more available in markets. Prices are very different according to market location and season and are very low at farm gate that many farmers do not engage in selling these cassava leaves.

Dry cassava leaves from all the three species are acceptable and liked as green vegetable in Rwanda. Processing procedures were strong sources of differences in sensory evaluation, and fresh leaves were the best, followed by the pounded before drying.

For marketability and preservation issues, cassava leaves can be dried in solar dryers and preferably after they are pounded for organoleptic qualities.

The studied leaves, after preparation (blanching, drying, cooking and storage) provide considerable amounts of β-carotene, protein and minerals, but these treatments reduce extensively vitamin C content. Fresh and dry leaves from the bitter, sweet and wild cassava cultivated in Rwanda are rich in nutrients and their potential to contribute to human nutrition is enormous, especially for β-carotene, minerals and protein. Optimum utilization of cassava products (roots and leaves) can positively contribute to value of cassava as cash crop and thus, producer profit, sustainable availability throughout the year and price stability. Consequently, cassava may improve not only food security and poverty reduction, but also nutritional status of consumers.

Cyanide levels in the relishes from both fresh and dry leaves are above the recommended level by FAO/WHO (1991) for food. But considering that leafy vegetables are served in small quantities as side relish, reducing the HCN by serving to minor levels in comparison to documented acute oral lethal dose of HCN for an adult (30-210 HCN/60 kg bodyweight), the leaves relishes can be judged to be safe with regards to toxicity. Besides, cassava leaves are reported good sources of protein, which is an important nutrient for cyanide human detoxification. However, sufficient cooking, at least for 30 minutes is necessary.

At ambient conditions of Morogoro, for physical and nutritional qualities, the solar dried leaves can be stored for six months using water, air and light proof packaging materials. This period is sufficient for distribution and marketing through national, regional and international markets. Therefore, market oriented cassava leaves can be produced in Rwanda and appropriately dried for human food.

2.2 Recommendations

The Government of Rwanda is promoting cassava as one of the priority crops for economic development and poverty reduction in Rwanda. The Government can also promote solar dryer utilization, through cooperatives or individual processors. Installing solar dryers in low altitude regions of Rwanda may be beneficial as the sunny periods are long and cassava leaves production is high in the semi-arid areas of the country. For regular and sufficient raw material, cultivation of wild cassava, which does not give roots, may be promoted as regular harvesting of leaves from bitter and sweet cassava plants tends to affect the root yields.

For the community services attribution, High Education and Research Institutes such as the University of Rwanda (UR) and Rwanda Agricultural Board (RAB) can contribute to educating processors in appropriate drying of vegetable, particularly cassava leaves for food to preserve physical and nutritional quality. The Government of Rwanda is recommended to avail different type of food grade packaging materials, including air and water tight and light proof in addition to suitability to environment as regulated by the Government, for extended storage of dry *isombe* in affordable conditions (ambient temperatures and humidity).

Considering the consumption rate of cassava leaves in Rwanda, Rwanda Bureaux of Standard (RBS) needs to develop standards for *isombe* product and boiling time may be highlighted on the labels (not less than 30 minutes) for reduction of cyanide toxicity.

In regard with the findings emanated from this study, the researcher recommends further studies, especially in low and middle land areas of Rwanda, where cassava root and leaves constitute basic foods, sometimes together as it is said "Costume", to focus on assessment of public health disorders due to high cyanide intake so that control measures can be taken.

Similarly, a thorough study on safe quantities in regard to cyanide in cassava leaves relish, considering different age groups such as children (low bodyweight), adults and elders, should be done for guiding consumers on potential toxicity of cassava food products, *isombe* included.

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PAPER ONE

Utilization of cassava leaves as a vegetable in Rwanda

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Utilization of Cassava Leaves as a Vegetable in Rwanda

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Abstract

Cassava (Manihot esculenta Crantz) leaves is an important vegetable in Rwanda. The objectives of this study were to determine cassava species from which leaves are harvested as vegetable and identify leaf preparation methods, consumption rate, price variation, storability and perception of post-harvest losses. A pre-tested structured questionnaire with closed and open-ended questions was administered to stratified groups of cassava leaves producers and consumers in four purposely selected sectors of Ruhango District, and retailers in the main markets of Ruhango and Kigali city. In total, 171 respondents were interviewed from 11 to 26 August 2011. Cassava leaves are highly consumed in Ruhango District as 96.0 % of families harvested leaves for food. On average, 17.5 % of farmers sell cassava leaves that are retailed in markets of towns and cities including Kigali City. Sweet (Manihot dulcis), bitter (Manihot utilissima) and wild (Manihot glaziovii) are the cassava species from which leaves are consumed as vegetable, but the leaves of wild cassava are preferred by 66.0 % of consumers. Prices of cassava leaves varied significantly (p=0.0182) according to season with higher prices in the dry than rainy season. Prices of leaves at farm gate and retail levels were highly different (p= 0.0016), averaging 32 and 65 Frw by bunch in the rainy season, respectively. Despite the high consumption and trade of cassava leaves, post-harvest losses were high, especially in the rainy season. Cassava leaves were mainly cooked fresh, but 14.4 % of households processed leaves by sun drying. The storage period was extended to two months by sun-drying. In each case and, prior to cooking, cassava leaves were pounded. Cassava leaves are considered as a favourite and nutritive vegetable and technologies to improve storability, value and trade are needed. Assessment of effects of processing on nutritional quality and safety is also important.

Key words: *cassava species, leaves, vegetable, preparation methods, Rwanda*

1. Introduction

Cassava (*Manihot esculenta* cranz) is one of the most important staple food crops grown in tropical Africa. It is a food security crop and source of income in many tropical African countries, including Rwanda. Tolerance to extreme stress makes cassava adapt to different agro-ecological zones of Rwanda (MINAGRI, 2002). Cassava plays a major role in efforts to alleviate the African food crisis because of its efficient production of cheap food energy, year-round availability, tolerance to unsuitable ecological and soil conditions in comparison with other crops, and suitability to present farming and food systems in Africa (Cock, 1985). Nutritionally, the starchy root of cassava is poor in nutrients, but leaves are a good source of proteins and micronutrients (Ayodeji, 2005).

Cassava leaves dishes, known as *isombe* in Rwanda and Burundi; *pondu*, *sakasaka*, *matamba* and *sombe* in different languages of Democratic Republic of Congo; *nkwen* in Cameroun, *kisamvu* in Tanzania, *chigwada* in Malawi, *ravitoto* in Madagascar, *mathapa* in Mozambique, and so on, are favorite green vegetable and thus constitute a major part of the family's daily food in almost all tropical Africa (Achidi et *al.*, 2005). They are used as side-dishes or soup to accompany rice, cassava or maize paste. Elsewhere in the world, young cassava leaves are used as a vegetable. In Indonesia, the leaves are used to reduce the prevalence of protein deficiency and anemia because of their high protein and micronutrient contents, and are cheap and readily available in rural, remote and marginal communities (Hidayat *et al*, 2002).

Despite its nutritional quality, cassava leaves, just as the roots, contain cyanogenic glucosides, linamarin and lotaustralin that are hydrolyzed by endogenous enzyme, linamarase, to hydrogen cyanide (HCN). The hydrogen cyanid is responsible for potential toxicity associated with inadequately processed cassava foods (CCDN, 2007). Siritunga and Sayre (2004) reported cyanogens levels of 200-1.300 mg HCN equivalents/kg dry weight in leaves and 10-500 mg HCN equivalents/kg dry weight in roots. Generally, HCN levels in leaves are higher than maximum levels of 10 mg HCN equivalents/kg dry weight recommended for foods by the FAO

(1991) and CCDN (2005). Therefore, cassava leaves must be processed to remove cyanogens prior to consumption. Various traditional methods according to local customs and preferences to improve palatability and reduce the toxicity are used.

This study was conducted to assess the extent of cassava leaves utilization as green vegetable in Rwanda, determine processing and conservation methods from leave-picking to serving the cooked dish, price variation, perception of postharvest losses, as well as cassava species from which leaves are harvested.

2. Materials and Methods

2.1. Location and description of the study area

The study was undertaken in Rwanda, a country that is located between latitudes 1° 04'S and 2° 51' S, and longitudes 28° 53' E and 30° 53' E (Fig. 1). The country is characterized by dramatic contrasts in temperature and rainfall as the elevation changes from the lowland savannah areas of the east to the mountain chains in the west (MINITERE, 2004; REMA, 2007). The temperature ranges between 16 to 24 °C with the highest temperatures in the lowland regions of the eastern and south-western parts of the country and the rainfall varies from about 900 mm in the east and south to 1500 mm in the north and north-western volcanic highlands (Twagiramungu, 2006).

The economy of Rwanda is heavily dependent on rain-fed agriculture and its variability leads to decrease in agricultural productivity due to droughts in eastern and southern parts and floods or landslides in areas experiencing heavy rains such as Northern and Western Provinces (REMA, 2007). Based on physical factors (temperature, rainfall and soil), agro-climatic regions have been defined, crops prioritized by regions and crop regionalization have been implemented throughout Rwanda (MINECOFIN, 2007). Cassava is produced all over the Eastern and Southern Provinces of Rwanda as a priority crop, but the major production area is located in Ruhango District and was purposely chosen as the study site. As typical of Rwanda, altitude decreases from west to east in Ruhango and the lowland areas experience low rainfall, averaging 900 mm per annum (Twagiramungu, 2006; REMA, 2007). As cassava tolerates a relatively long period of drought once the crop is established (El-Sharkawy, 2007), it is grown more extensively in the semi-arid regions of Rwanda including some sectors of Ruhango District. Households of producers and consumers were systematically sampled in four eastern sectors of the district and these were Ruhango, Mbuye, Ntongwe and Kinazi. During the survey, it was observed that cassava leaves from the sampled sectors were sold in the main markets of Ruhango and Kigali city, usually by intermediaries. Therefore, retailers were randomly selected in these markets to estimate the final price of the leaves.

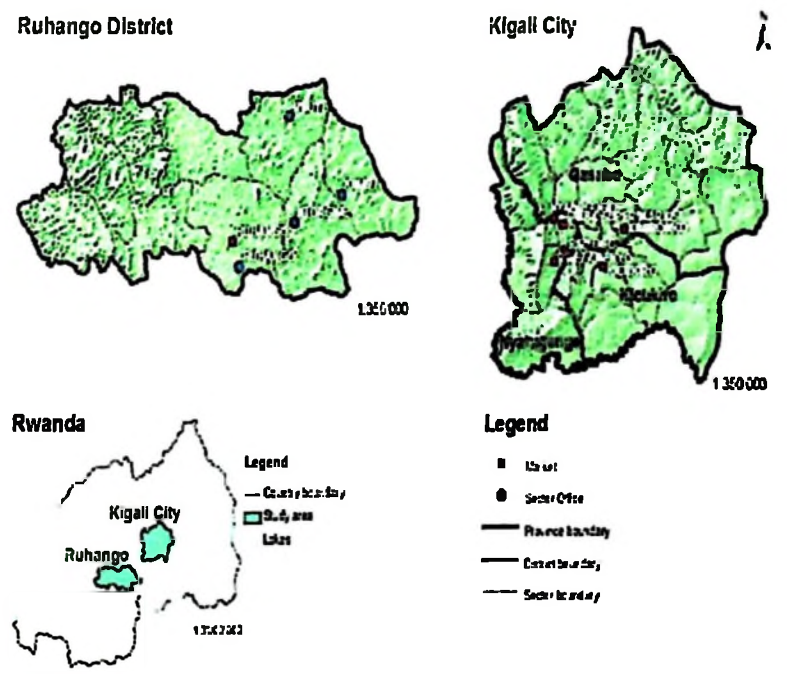

Figure 1: Map showing the location of the study areas in Rwanda

2.2. Data collection

Geographical Position System (GPS) instrument (RINO 130-GARMIN) was used to locate the selected sectors (Ruhango, Mbuye, Ntongwe and Kinazi). The geographical coordinates of markets of

Ruhango, Kicukiro, Kimironko, Nyabugogo, Nyamirambo, Rwezamenyo and Simba Super market were also determined and maps were drawn to show the location of sectors and markets in Ruhango District and Kigali City (Fig. 1).

A cross-sectional design was used in the survey and a pre-tested structured questionnaire with closed and open-ended questions was administered to respondents in the four sectors and Kigali markets. The population was stratified in three groups, namely cassava leaves producers, consumers and retailers. In each sector, 25 producers and 13 consumers were interviewed, except in one sector where only 11 consumers were questioned. A total sample size of 100 producers and 50 consumers were, therefore, interviewed in Ruhango District. A list of households within each sector was used to systematically choose the required number of individuals in each stratum. Price of cassava leaves was assessed at farmer level and randomly in the main markets of Ruhango and Kigali City. Therefore, a total sample size of 21 retailers, 50 consumers and 100 producers was used as the population was homogenous.

2.3. Statistical analysis

Data collected on species of cassava from which leaves are harvested for food, rate of consumption and post-harvest losses of the cassava leaves were coded and analyzed using Statistical Analysis Systems (SAS) software, version 9.2 (SAS Institute, 2008). Prices at different levels (farm and market) and during different seasons (dry and rainy seasons) were compared using student test (t-test). Storage techniques and storage periods were cross-tabulated.

3. Results and Discussion

Cassava crop is very important in Ruhango Disrtict as all the 100 households surveyed grow cassava as staple crop (Table 1). More than half (58 %) of households produce *Manihot dulcis* with sweet roots, *Manihot utilissima* with bitter roots and *Manihot glaziovii* or wild cassava for which roots are not consumed (Table 1).

Table 1: Distribution of households for cassava, cassava species, reasons and frequency of harvesting, preparing and eating cassava leaves

Factors	% Households
Cassava cultivation	100
Cassava species	
Sweet alone	10.20
Bitter alone	1.02
Sweet and Bitter	26.53
Sweet, Bitter and Wild	58.16
Sweet and wild	4.09
Cassava leaves harvesting	96.00
Reasons for harvesting cassava leaves	
Selling	3.03
Selling and cooking at home	13.13
Selling, cooking at home and feeding livestock	1.01
Cooking at home	75.76
Cooking at home and feeding livestock	7.07
Frequency of harvesting cassava leaves	
One time a week	29.59
Two times a week	26.53
Three times a week	13.27
More than three times a week	1.02
Frequency of preparing cassava leaves	
One time a week	71.43
Two times a week	12.24
Three times a week	4.08
Frequency of eating cassava leaves	
One time a week	14.00
Two times a week	42.00
Three times a week	28.00

Sweet cassava was mostly produced in the area as it was cultivated by 99 % of households (Table 1). In 2006 and 2009, eight sweet cassava varieties (TIME 14, 192/0057, 95/NA/000, MH95/0414, MM96/36, MM96/5280, MM96/0287 and MM96/7204) that are resistant to CMD (Cassava Mosaic virus Disease) and Green Mite were released in the low and middle altitude ecological zones of Rwanda (MINAGRI, 2005). For this reason, pest resistant sweet cassava varieties have been widely adopted by all farmers.

Ninety six percent of the surveyed households harvested cassava leaves as vegetable (Table 1). The reasons for cassava leaves harvesting were different from household to household and included cooking at home, selling as fresh vegetables and feeding livestock (Table 1). The results of the survey revealed that cassava leaves were harvested principally for meals preparation at home (97 %) and selling as fresh vegetables (17 %). Cassava leaves were not much used as livestock feed except for pigs after the leaves have been cooked. Consumption of cassava leaves was high in the study area and most families prepare leaves for food once a week to be served more than two times per week, as food can be kept cooked and re-heated before consumption (Table 1).
Cassava leaves were harvested from all three species (sweet, bitter and wild) and in harvesting, young leaves were plucked or branches were cut according to the species (Table 2). Farmers reported that branches were not cut from sweet and bitter cassava that are still growing as this would negatively affect productivity and quality of roots. Hence, for sweet and bitter cassava species, it is only at the root harvesting stage that branches with leaves were cut. For wild cassava species, branches were cut regularly to promote growth of new branches and leaves.

At farmer's level, prices by bunch of cassava leaves varied significantly ($p=0.0182$) according to seasons with higher prices in dry seasons (Table 3). In addition, prices at production and retail levels (final price) differed significantly ($p=0.0016$) in the rainy season, averaging 32.9 and 65.0 Frw by bunch, respectively (Table 4). In the dry season, prices were not different ($p=0.197$), averaging 65.0 and 86.8 Frw by bunch for farmer and retailer, respectively (Table 4). Farm gate and retail prices were both high in the dry season because of scarcity of leaves during this period.

Table 2: Mode of harvesting cassava leaves and proportion of used mode according to cassava species

Determinants	% Households
Mode of harvesting	
Leaf selection	23.47
Branch cutting	31.63
Leaf selection and branch cutting	44.90
Mode of harvesting by cassava species	
Sweet cassava	
Leaf selection	89.66
Branch cutting	10.34
Biter cassava	
Leaf selection	79.01
Branch cutting	20.99
Wild cassava	
Leaf selection	1.96
Branch cutting	98.04

Table 3: Comparison of prices of cassava leaves at farmer and retailer levels by season

Prices		Mean	t value	P value
Farmers' price	Dry season	65.0000	2.52	0.0182*
	Rainy season	32.9412		
Retailers' price	Dry season	81.4286	1.96	0.0578
	Rainy season	51.8750		

*, ** = Significantly different at 0.05 and 0.01 levels of probability, respectively

Most consumers (80.0 %), retailers (88.9 %) and farmers (79.4 %) indicated that wild cassava leaves were preferred over leaves from other species. They stated good taste, easier pounding, nutritive and constant availability as the reasons for preferring leaves from wild species (Table 5).

Table 4: Comparison of prices of cassava leaves between farmers and retailers in different seasons.

Prices	Dry season			Rainy season		
	Mean	t value	p-value	Mean	t value	p-value
Farmers' price	65.0000	-1.32	0.1967	32.9412	-3.44	0.0016**
Retailers' price	86.8421			65.0000		

*, ** = Significantly different at 0.05 and 0.01 levels of probability, respectively

Table 5: Reasons for preferring wild cassava leaves and perception of losses.

Determinants	Proportion in %		
	Farmers	Consumers	Retailers
Preference by groups	79.4	80.0	88.9
Reason of preference			
Easy to pound	6.67	5.00	-
Liked by consumers	6.67	-	15.79
Nutritive	5.00	5.00	5.26
Year- round availability	13.33	32.50	36.84
Good taste (not bitter)	26.67	45.00	15.79
Losses of leaves per year			
None	17.05	-	10.00
A quarter	4.55	-	35.00
Between a quarter and a half	9.09	-	25.00
A half	4.55	-	15.00
More than a half	55.68	-	15.00

Despite the high rate of consumption and trade of cassava leaves, post-harvest losses were not negligible, especially in the rainy season (Table 5). Losses were higher at farmer than retailer level because retailers purchased leaves according to present demand.

Table 6: Main constraints in cassava leaves utilization

Strata/Constraints	% of Households
Farmers	
Low price in rainy seasons	8.2
Low price in rainy season and lack of buyers	2.35
Low price in rainy season, lack of buyers and lack of technology for storing	5.88
Low price in rainy season and lack of technology for storing	5.88
Lack of buyers	49.41
Lack of buyers and technology for storing	12.94
Lack of technology for storing	8.24
Retailers	
Scarcity in dry season	10.53
Scarcity in dry season and lack of buyers in rainy season	10.53
Scarcity in dry season, lack of buyers in rainy season and lack of technology for storing	5.26
Scarcity in dry season and lack of technology for storing	10.53
Lack of buyers in rainy season	52.63
Lack of buyers in rainy season and technology for storing	10.53
Consumers	
Scarcity and high price in dry season, and hard and time consuming preparation	22.24
Scarcity and high price in dry season	8.16
Scarcity, high price in dry season and bitterness in rainy season	6.12
Scarcity in dry season and time consuming preparation	8.16
High price in dry season, hard and time consuming preparation	8.16
Hard to prepare	12.24
Time consuming preparation	10.20
Hard and time consuming preparation	16.32

Methods of preparation and storage of cassava leaves

Many recipes from cassava leaves were identified in the surveyed area. The preparation method did not vary much and commonly consisted of four main steps. The first step was selection and harvesting of tender cassava leaves, tender growing shoots and soft growing stems. This step was similar to that reported by Katz and Weaver (2003). The second step was pounding, usually in a woody mortar with pestle, but vegetable grinders made by local manufacturers were popular and were usually used in markets. Cooking was the following step and was done by boiling in water with spices, oil and salt. The last step was serving cooked leaves with rice, maize/cassava paste or with roots/tuber foods. Before pounding, cassava leaves were sometimes blanched.

Un-pounded cassava leaves were kept for one to two days by frequent sprays of water, one to two days in cool place, and two to three days, depending on demand, in refrigerators after leaves are pounded. In dry seasons, most consumers and retailers experienced problems of scarcity of cassava leaves (Table 6). At farmer's level, few households preserved cassava leaves by sun drying; whole leaves were dried by 7.7 % of households and pounded leaves also by 7.7 % before prolonged storage. In contrast to recommendation of Kendal et *al.* (2010), cassava leaves were not blanched before drying and drying was done before or after pounding. Lack of technology for preserving cassava leaves was the most crucial constraint reported by farmers and retailers (Table 6).

4. Conclusion and Recommendations

Cassava leaves are a major vegetable in Ruhango District and most families consumed it as vegetable at least once per week. Three species of cassava are cultivated, namely sweet, bitter and wild cassava and leaves from all the three are consumed. There was variation in the preference for particular species as vegetable and, wild cassava is believed to taste good and easy to pound. Mode of harvesting cassava leaves varied for species. Branches were cut from wild cassava that do not produce edible roots, but for sweet and bitter cassava, leaves were picked from branches to preserve quantity and quality of roots. Cassava leaves are a source of income in Ruhango

District, but prices varied significantly according to season, even though cassava leaves are said to be available all year-round.

Despite the high consumption, post-harvest losses of cassava leaves are considerable because of their high perishability. Sun-drying to extend the shelf life is practiced by very few families. Processing methods need to be improved to preserve cassava leaves for prolonged storage and value addition. Assessment of the effects of processing methods on nutritional quality and food safety is also recommended.

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PAPER TWO

Sensory evaluation of different preparations of cassava leaves from three species as a leafy vegetable

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Sensory evaluation of different preparations of cassava leaves from three species as a leafy vegetable

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Cassava leaves are largely consumed in Africa and are among the top three African indigenous vegetables rich in nutrients. Leaves from bitter (*Manihot utilissima*), sweet (*Manihot dulcis*) and wild (*Manihot glaziovii*) species of cassava were cooked by boiling in salted (sodium bicarbonate and table salt) water with the addition of palm oil and ground-nut paste, following processing by "pounding", "pounding and then drying" and, "drying and then pounding". The drying was done in tunnel solar drier at temperature of 65°C on average. Nine samples (three species x three processing methods) were evaluated by 31 panelists, using a five point hedonic scale, where 5 = like very much and 1= dislike very much. Cassava species affected significantly (p = 0.0047; 0.0206) scoring for texture and overall acceptability, respectively, but not for colour, aroma and taste. Processing method highly significantly (p< 0.0001) affected all the sensory attributes scoring. Leaves from all three species were liked as leafy vegetable, except when pounded after drying.

Key words: Cassava leaves, cassava species, sensory characteristics, tunnel solar drying, processing methods, Rwanda.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the staple food crops in Africa, it is a rainy fed crop grown mainly in humid and sub humid regions, but being particularly suited to conditions of low nutrients availability of soils and able to survive drought, Cassava became one of the most important food crops in almost entire countries within the tropics (Huzsvai and Rajkai, 2009; El-Sharkawy, 2007). For instance in Rwanda, cassava is the third among main food crops after banana and sweet potatoes, and is one of the priority crops that are being promoted for economic development and poverty reduction in the agricultural sector (MINECOFIN, 2007). In some countries of West and Central Africa, cassava roots and leaves are the basic foods. The Congolese consider the cassava as "all sufficient" because the cassava starchy roots are complemented nutritionally by cassava leaves, which are good source of protein, vitamins and mineral (Bradbury and Denton, 2011). Austin et al. (2009) after realizing the value and utilization of cassava leaves as green vegetable, he placed them among Rwanda's high value vegetables that have potential on domestic, regional and international markets.

Cassava leaves are among the top three African indigenous vegetables rich in nutrients. They are the second in β-carotene after *Moringa oleifera*, the second in vitamin C after *Moringa stenopetala*, the third in vitamin E after *M. stenopetala* and *M. oleifera*, the third in zinc after *Pterocarpus mildbraedii* and *M. oleifera*, the third in antioxidant activity after *Adansonia digitata* and *Rorippa madagascariensis* and the third in total phenolic after

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R. madagascariensis and *A. digitata* (Shackleton et al., 2009). Micronutrients, as well as the many non-nutrient phytochemicals in vegetables are associated with health maintenance and prevention of chronic diseases (Yang and Keding, 2009; Steinmetz and Potter, 1996). For that healthy and nutrition importance, cassava leaves consumption may be improved by preservation methods that can increase its availability and quality.

Cassava leaves, like many other leafy vegetables, are generally seasonal with surpluses in the rainy season and scarcity with high costs in the dry season. Their perishability causes a considerable amount of post-harvest losses in rainy season. To minimize the losses and stabilize availability and price in different markets and seasons, appropriate preservation methods, affordable to rural communities, are needed. According to the study of Thomas (2008), spoilage of food is due to three main causes: Microorganisms, enzymes and chemical reactions. Drying, especially sun-drying is among the oldest and cheapest preservation methods that slow down or completely stop food deterioration by removing available water, the principal factor of food deterioration. As reported by Umuhozariho et al. (2011), in some rural areas of Rwanda, especially in dry regions, where cassava is a main food crop, leaves are dried on mats in open air, either pounded or un-pounded, for increasing shelf life. However, only fresh cassava leaves are sold in different local markets in villages, cities and super markets, the dry products being consumed at family levels. To stimulate people to process market oriented product, the dry cassava leaves need to be tested for physical quality and acceptability.

The direct exposure to sunlight is known to reduce the quality (colour and vitamin contents) of the final product (MMA, 2008). The product is open to various contaminations such as dust, insects, wetness and rain. Moreover, the process is very dependent on good weather, and the very slow drying rates of the process create the danger of mould growth. In contrast, solar dryers are simple installations that can eliminate the negative effects of open air sun drying and thus seems to be the most promising. Solar drying offers the following advantages over sun drying when correctly designed: Faster drying rate, greater retention of vitamins, especially vitamins A and C, minimizing damage from rain, protection against infection among others, and also some advantages over the conventional drying with respect to cost and adaptability to small scale farmers (Eze, 2010; Ferreira et al., 2008). According to literatures, solar drying gives faster drying rates by heating the air to 10-30°C above ambient temperature, using solar energy collectors with natural or forced airflow inside the dryers, and thus increasing their efficiency (Eze, 2010; Lotfalian et al., 2010; Ferreira et al., 2008). In addition, solar dryers are a promising means for tropical countries to meet their requirements as the available amount of solar energy in most cases is sufficient to cover the energy requirements for small dryers (Eze, 2010).

The present study was conducted to assess the usefulness of tunnel solar drying for preserving sensory qualities of cassava leaves and determine which cassava species and processing procedures are preferable for better physical properties and acceptability, as food relishes after leaves are cooked.

MATERIALS AND METHODS

Materials

Collection of cassava leaves

In April 2012, tender cassava leaves were harvested from three species of cassava, bitter (*Manihot utilissima*), sweet (*Manihot dulcis*) and wild (*Manihot glaziovii*). Varieties named Igicucu, Seruruseke (5280) and ISAR 1961 were chosen for wild, sweet and bitter cassava species respectively (Figure 1). In order to minimize the effects of age, environment and soil types on sensory characteristics, leaves samples of the same age were selected from the same field, Rwanda Agricultural Board (RAB)'s field at the Karama Research Station, in Bugesera District of Eastern Province of Rwanda.

Experimental design of sample preparation

The three cassava species as source of vegetable and three processing methods in a completely randomized design (CRD) were evaluated for sensory attributes in one session. The preparation procedures used to prepare leaves from each cassava species before cooking were: Pounding fresh leaves, drying pounded leaves, and drying un-pounded leaves (Figure 2). In this study, both fresh and dried leaves were pounded using traditional woody mortar and pestle. In addition, leaves were blanched before drying and blanching was carried out according to the method described by Kendall et al. (2010). Thus, leaves were submerged in boiling water for 4 min, and then immediately cooled in tap water at ambient temperature. Before drying, pounded and un-pounded leaves were kept in closed polyethylene bags and were stored on ice in an ice-chest for direct transportation to the solar drying station at Sokoine University of Agriculture, Morogoro, Tanzania.

Drying procedures

Samples of pounded and un-pounded leaves from bitter, sweet and wild cassava were dried to brittle using a tunnel solar dryer (Figures 3 and 4). The complete drying was when sample leaves became entirely brittle. Green vegetables contains less sugar, and can be dried to brittle and water content 4-8%, depending on the type of vegetable (James and Kuipers, 2003). The time taken to complete drying for each sample was noted. The temperature inside the tunnel solar dryer was recorded at 8 a.m., noon and 8 p.m. each day. After drying, samples were packed in plastic materials, sealed and stored in opaque cartons at ambient temperature before cooking for sensory evaluation.

Cooking procedures

Similar cooking procedures were followed for all the nine samples. Thus, processed samples were boiled in salted (sodium bicarbonate and table salt) water, with the addition of palm oil and ground-

Figure 1. Sample leaves from sweet (left), wild (middle) and bitter (right) cassava species.

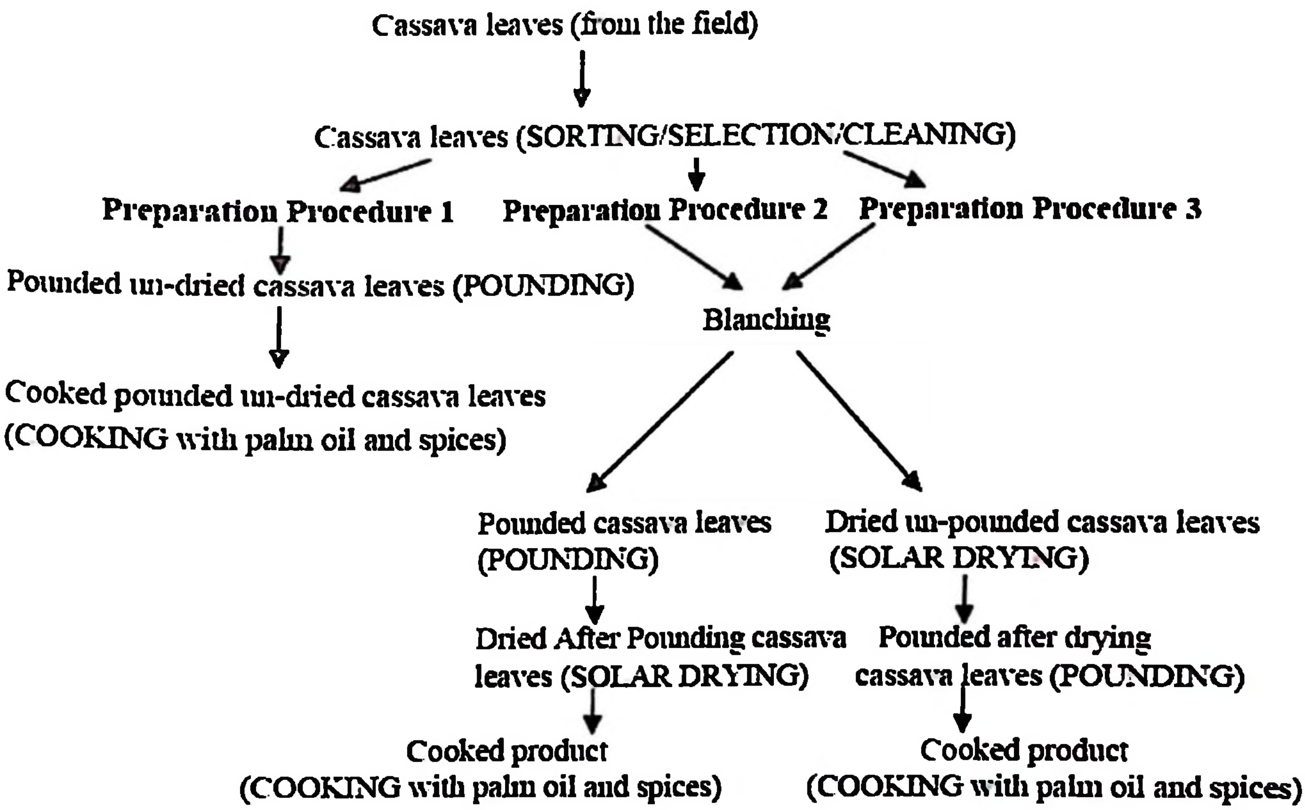

Figure 2. Flow diagram illustrating preparation procedures of cassava leaves.

Figure 3. Tunnel solar dryer covered after placing samples for drying.

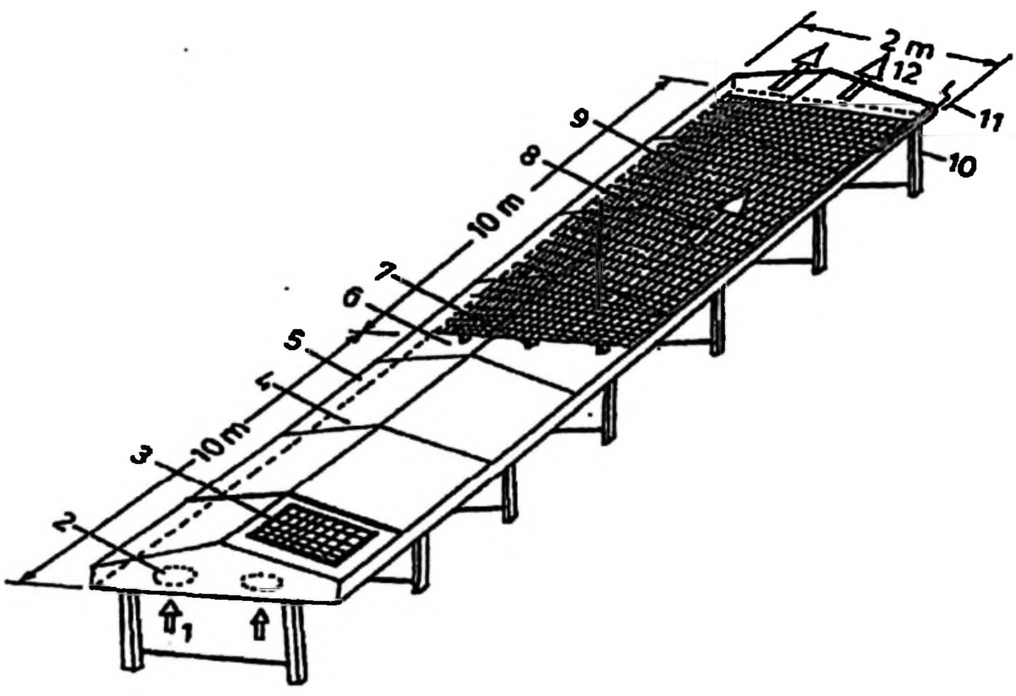

Figure 4. Diagram of the tunnel solar drier. 1, Air inlet; 2, fan; 3, solar module; 4, solar collector; 5, side metal frame; 6, outlet of the collector; 7, wooden support; 8, plastic net; 9, roof structure for supporting the plastic cover; 10, base structure for supporting the tunnel drier; 11, rolling bar; 12, outlet of the tunnel drier.

Table 1. Significance levels from the analyses of variance and for main factors and their interactions on sensory characteristics of cooked cassava leaves as vegetable.

Parameter	Sensory attributes and their p-values				
	Color	Taste***	Aroma	Texture	Overall acceptability
Cassava species	1.1308	0.4890	0.0860	0.0047	0.0206
Processing methods	<.0001	<.0001	<.0001	<.0001	<.0001
Species x methods	0.7526	-	0.0312	0.0733	0.0502
Age group	0.0010	0.1581	<.0001	0.0013	0.0007
Gender	0.9012	0.5898	0.8476	0.0003	0.9040
Age group x Gender	0.1246	-	0.0081	0.0597	0.0908
R^2	0.78	-	0.85	0.90	0.88
CV	9.36	-	10.70	6.65	9.50

**R^2: Coefficient of determination; CV: coefficient of variation; ***Kruskal Wallis test rather than ANOVA and thus no R^2 nor CV.

nut paste, for about 45 min.

Sensory evaluation

A panel of 31 adults (aged above 21 years old), were purposively selected for sensory evaluation. All the participants were familiar with cassava leaves meals even if they were oriented on making inferences and recording the scores for each sample. The panel comprised of females and males of age ≥ 21 years old. A five point hedonic scales as described by Larmond (1977) were used, where 5 = like very much, 4 = like moderately, 3 = neither like nor dislike, 2 = dislike moderately and 1 = dislike very much. The nine samples were served in identical containers, coded with 3 digit random numbers and presented to panellists in one session. The sensory attributes of interest were colour, taste, texture or mouth feel, aroma and overall acceptability. Necessary precautions were taken to reduce crossover effects by selecting greater number of interested panellists rather than motivating panellists and using small number and repeating preparations. In addition, panellists rinsed their mouths with water before tasting the next sample. Out of the 31 panellists, 61% were females and 39% were males. Their ages ranged between 21 and 57 years. Out of 19 females, 47% were young (< 35years) and 53% aged (≥ 35 years) while among males, 75% were young and 25% aged. The session was held in one of the laboratories of the Faculty of Agriculture, National University of Rwanda (NUR), from 11 a.m. to 14 p.m., in a uniform and natural lighting environment.

Statistical analysis

Data analysed included time of drying, temperature inside the tunnel solar dryer and scores for different sensory attributes per sample. After averaging the scores across the five sensory attributes, the "Kolmogorov-Smirnov" test to assess the normality of the averages was carried out as suggested by Kutner et al. (2005). Multiple ways analysis of variance (ANOVA) was applied to sensory attributes with normally distributed averages and, Kruskal Wallis nonparametric test was done for sensory characteristics with strong departure from normal distribution (Kutner et al., 2005). Statistical Analysis Systems (SAS) software, version 9.2 (SAS Institute, 2008) was used to compute ANOVA (F-test statistics), Fisher's Least Significant Difference (LSD) to separate means, and Kruskal Wallis test. The treatments were judged significantly different and highly significantly different at $p < 0.05$ and $p < 0.01$, respectively.

RESULTS AND DISCUSSION

On average, the temperature inside the tunnel solar dryer was 65°C even if the mean monthly temperature of the area was 25°C. The duration of drying was 14 and 16 h under sunny conditions for un-pounded and pounded cassava leaves, respectively. These times of drying of less than one day under the sunny conditions concurred with reports from several studies that solar dryers improve efficiency and quality of the dried food products by increasing temperature of drying and decreasing period of drying. For a tunnel solar dryer, products receive energy both from hot air supplied from the collectors and from incident solar radiation. This increases the temperature inside the dryer and accelerates the drying process (Almhanna, 2012; Banout and Ehl, 2010; Lotfalian et al., 2010; Medugu, 2010; Ferreira et al., 2008).

The effect of processing methods, cassava species, panellists' gender and ages, and their pair wise interactions on various sensory attributes are shown in Table 1. Kolmogorov-Smirnov test indicated that only taste score averages showed a strong departure from normality and Kruskal Wallis test was done. Processing method had high ($p< 0.0001$) significant effects on all the sensory characteristics (colour, taste, aroma, texture and overall acceptability). Cassava species did not significantly influence colour, aroma and taste. In contrast, the species significantly ($p = 0.0206$) influenced overall acceptability and highly significantly ($p = 0.0047$) influenced texture of cooked relishes. Cassava species and processing methods interaction effects were significant for aroma with $p = 0.0312$. Age group of panellists had considerable effects on scoring of different sensory attributes with p-values of 0.0010, < 0.0001, 0.0013 and 0.0007 for color, aroma, texture and overall acceptability, respectively. Gender did not have any significant effect on the averages of the colour, taste, aroma and overall acceptability scoring but highly

Table 2. Means for sensory characteristics for leaves from different cassava species and processing methods.

Parameter		Sensory attributes and their scores				
		Colour	Taste	Aroma	Texture	Overall acceptability
Species	Bitter	4.04[a]	3.72[a]	3.71[a]	3.79[a]	3.77[a]
	Sweet	4.03[a]	3.95[a]	3.95[a]	3.86[a]	3.85[a]
	Wild	3.76[a]	3.57[a]	3.57[a]	3.64[b]	3.46[b]
Processing methods	Dried and then pounded	3.40[c]	2.90[c]	2.90[c]	3.06[c]	2.87[c]
	Fresh	4.58[a]	4.36[a]	4.38[a]	4.28[a]	4.45[a]
	Pounded and then dried	3.86[b]	3.94[b]	3.95[b]	3.65[b]	3.76[b]
Age groups	Aged (≥35)	4.17[a]	3.91[a]	4.07[a]	3.81[a]	3.91[a]
	Young (<35)	3.71[b]	3.55[b]	3.41[b]	3.52[b]	3.48[b]
Gender	Female	3.93[a]	3.75[a]	3.73[a]	3.83[a]	3.70[a]
	Male	3.95[a]	3.71[a]	3.76[a]	3.49[b]	3.69[a]

For each sensory characteristic, within column, values with the same letter were not significantly different ($p<0.05$).

influenced texture scoring with a p-value of 0.0003. Age group and gender interaction effects were highly significant ($p = 0.0081$) for only aroma.

Cassava species were equally liked for colour, taste and aroma by panellists (Table 2). Texture and overall acceptability were rated differently with sweet and bitter species being equally liked and the wild species the least preferred (Table 2). During pounding, it was observed that fresh wild cassava leaves were juicier when compared to sweet and bitter. The crude water made pounding hard and particles larger. The less liked texture has been attributed to the larger particles after pounding and cooking. The study showed that leaves from sweet and bitter cassava species were liked more than those from the wild species, although Umuhozariho et al. (2011) reported earlier that leafy vegetable of wild cassava species were more utilized as human food in Rwanda. The reason may be that authors were concerned with consumer habits with respect to cassava leaves which were more subjective, and one of the reported reasons was the availability of wild cassava leaves as the farmers are more interested in root production than leaves for bitter and sweet species. This is the first study of sensory comparison of leaves from different species of cassava in Rwanda, clearly showing leaves from wild cassava species are less preferred by panellists compared to those from cultivated species.

Panellists above 35 years of age liked all preparations of the vegetables for all the sensory attributes more than those less than 35 years (Table 2). This was not surprising as Larmond (1977) mentioned age and sex of panellist among important factors that can influence result in sensory test.

Colour of the different vegetable preparations differed significantly according to processing methods, but not to species (Table 2). In this study, all samples were cooked in salted (sodium bicarbonate and table salt) water, but blanching had been done in un-salted water before drying. Cooked fresh leaves became bright-green while dried ones were dark-green. Heating green vegetables in an alkaline solution such as sodium bicarbonate ($NaHCO_3$) make the cooking water slightly basic, the magnesium ion is retained in the chlorophyll and the colour is a bright-green. In contrast, when vegetables are heated without the alkaline, such as blanching before drying as in the present study, part of their cells are disrupted and some organic acids are released and react with chlorophyll. The reaction with the acids replaces the magnesium atom (Mg) of chlorophyll with a hydrogen atom (H) to form an unattractive dark-green pigment pheophytin (FAO, 1995). The unlikeable colour was probably due to the formation of pheophytin in dry cooked cassava leaves.

Fresh cassava leaves were the most liked, followed by the "pounded and then dried". In another investigation, Mepba et al. (2007) found that panellists preferred fresh to dry vegetable soup. "Dried before pounding" was the least liked for all sensory attributes (Table 2). The poor rating of "dried before pounding" products for all sensory characteristics were attributed to their bitterness as commented by panellists.

Taste and aroma were highly correlated with overall acceptability, with $r = 0.93$, $p<0.0001$ and $r = 0.91$, $p<0.0001$, respectively. Taste and aroma were also much correlated ($r = 0.90$, $p<0.0001$) (Table 3). High correlation between taste, aroma and overall acceptability is not surprising. Taste perception has been suggested to play a key role in determining individual food preferences and dietary habits (IUFoST, 2012; Garcia- Bailo et al., 2009). Equally, Clark (1998) reported a similar relation of a strong influence of taste and aroma (odour and flavour) on food acceptability, and that the two sensory characteristics are considered the key of food choice. The high positive correlation among the sensory characteristics, especially taste, aroma and overall acceptability (Table 3), suggests that the unlikeable bitter

Table 3. Linear correlation among sensory characteristics of processed leafy vegetables from three cassava species.

Sensory attribute		Colour	Taste	Aroma	Texture	Overall acceptability
Colour	r	1	0.8198	0.7764	0.7619	0.8881
	Prob > |r|		<.0001	<.0001	<.0001	<.0001
Taste	r		1	0.9059	0.8673	0.9393
	Prob > |r|			<.0001	<.0001	<.0001
Aroma	r			1	0.7949	0.9109
	Prob > |r|				<.0001	<.0001
Texture	r				1	0.8569
	Prob > |r|					<.0001
Overall acceptability						1

r, Linear correlation coefficient; Prob > |r|, probability of having a correlation factor equal to or larger than the obtained r.

taste could be the only source of "dried before pounding" vegetables rejection. Also when leaves were pounded after they were dried, particles were very fine and gave a soup-like texture after cooking, which was less liked by panellists.

Bitterness in the "dried before pounding" cassava leaves may possibly be attributed to high levels of residual cyanogens. Karltun et al. (2004) demonstrated a strong correlation between bitter taste and cyanogen (HCN) potential in cassava. Awoyinka et al. (1995) reported that blanching does not change HCN-potential, but pounding or grinding reduces both HCN-potential and tannins. As in the present study, the leaves were blanched to limit degradation of nutrients and colour, endogenous enzymes, linamarase and hydroxynitrile lyase, important in linamarin and acetone cyanohydrin hydrolysis, were also deactivated. But significantly, linamarin and the break down product of linamarin, cyanohydrin can decompose spontaneously at high temperatures or pH 4 and above, to release HCN, harmful to human health, but volatile during preparations (Cereda and Mottos, 1996; Mkpong et al., 1990). Cyanogen levels were not analysed in this study, an additional study for safety of the products is necessary.

Conclusion

Leaves from all the three species of cassava found in Rwanda (bitter, sweet and wild) were liked for food as green vegetables. However, leaves from wild species were less preferred for texture and overall acceptability than those from sweet and bitter species. Processing methods were a strong source of differences in sensory attributes and fresh leaves were the best, followed by "pounded before drying" leaves for all the sensory attributes. Therefore, for marketability and preservation issues, cassava leaves can be processed by solar drying, and preferably pounded before they are dried for a better taste and texture. Blanching in alkaline water is appropriate to preserve the light-green colour, preferred by consumers. Though un-pounded leaves dried faster and pounding after drying was easier, they were poorly rated, especially for taste, aroma and overall acceptability. An additional study on cyanide and nutrients of the processed cassava leaves is highly recommended to ensure nutritional quality and safety of the products for human consumption.

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PAPER THREE

Cyanide and selected nutrients content of different preparations of leaves from three cassava species

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Cyanide and selected nutrients content of different preparations of leaves from three cassava species

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Cassava leaves are largely consumed as vegetable in African, but contain a toxic compound, cyanide. To ascertain their safety and contribution to human nutrition, after a number of pre-treatments preceding their boiling in water, cyanide, vitamin C, β-carotene, crude protein, iron, calcium, phosphorus, potassium and zinc contents were assessed in leaves from bitter, sweet and wild cassava species, boiled for 15 and 30 min after differently processed by: (1) pounding un-dried (UND), (2) drying before pounding (DBP) and (3) drying after pounding (DAP). Blanching headed drying was done in a tunnel solar dryer. Results showed that cassava species, processing procedures, and boiling time significantly ($p < 0.05$) reduced cyanide and the nutrients. However, except vitamin C, eliminated to almost nil, other nutrients were retained at considerable levels. Sensibly decreased by drying and/or boiling, cyanide levels ranged from 32 - 50 mg HCN/kg (dry matter basis) after boiling for 30 min. These levels, above the recommended level (10 mg HCN/kg) for foods, were safe with regard to cyanide toxicity based on the fact that the vegetable is served in small quantities as side food. consumed quantities of relishes as side foods. Nevertheless, it was advisable not to make them the everyday foods, especially to lower body weight such as children, and to extend time of cooking.

Key words: Cassava leaves, cassava species, processing procedures, cyanide and nutrients, Rwanda

INTRODUCTION

Cassava is a very important crop in the tropics and a staple food for over 800 million people (Nassar et al., 2007), growing over a range of climates and altitudes and on a wide variety of soils (FAO/IFAD, 2005). In Africa, Rwanda included, cassava is primarily used as food, consuming roots as starchy food (Nweke et al., 2002), but leaves are also largely consumed as green vegetables (Achidi et al., 2005).

Producing a valuable and safe food from cassava and cassava leaves involve certain challenges. In fact, cassava and cassava leaves contain cyanide, in the form of cyanogenic glucosides, primarily linamarin and small lotaustralin (Uyoh et al., 2007). The cyanogenic glucosides are distributed throughout the cassava plant, with highest levels in leaves (Etonihu et al., 2011). Under high temperature, pressure, and use of enzyme (linamarase), or mineral acids, cyanogenic glucosides are decomposed into acetone cyanohydrins which, at pH above 5 or temperatures above 35°C, is broken down, spontaneously, into poisonous compound, hydrogen cyanide (HCN) (Siritunga and Sayre, 2004). Cyanohydrins are the most dangerous form of the cyanide because at the elevated pHs and temperatures present in the human body, it rapidly decomposes to release the poisonous

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hydrogen cyanide. The continued consumption of high dietary cyanogens has been linked with a number of chronic health disorders, and occasionally death, depending on the level of cyanogens, frequency of cyanogens exposure and, quality and quantity of protein intake status (Cliff et al., 2011; Nhassico et al., 2008; CCDN, 2007). For the human body detoxification, unbound cyanide is converted to less toxic thiocyanate (SCN) and is excreted in the urine. The synthesis of thiocyanate requires sulphur-containing amino acids, as a consequence of protein intake (CCDN, 2007).

Despite the presence of the poisonous component, numerous publications have provided evidence on potential contribution of cassava leaves in human nutrition by providing protein, minerals and vitamins, depending on preparation techniques (Akinwale et al., 2010; Mulokozi et al., 2007; Ayodeji, 2005). Faber and Van Jaarsveld (2007) revealed that improved handling, such as optimizing time of thermal treatment, drying process and preliminary preparations can preserve quality of treated food. In Rwanda, leaves from three species of cassava, bitter (*Manihot utilissima),* sweet (*Manihot dulcis)* and wild (*Manihot glaziovii)* are valued and highly utilized as green vegetables. They are usually cooked freshly harvested, but preservation by sun-drying to extend their shelf life is also reported by Umuhozariho et al., (2011). The direct exposure to sunlight is known to reduce the quality (colour and vitamin contents) of the final product (MMA, 2008). However, solar drying is reported among strategies to combating nutrients losses in processed food stuffs and extending the availability of the nutrient-rich foods, beyond the season in which they are in abundance (Oguche Gladys, 2011; Thompson and Amoroso, 2011).

The present study was undertaken to improve drying of cassava leaves by using tunnel solar dryer and evaluate levels of cyanide and nutrients in the dried and un-dried leaves, from the three cassava species and after preparation as human food.

MATERIALS AND METHODS

Collection of cassava leaves

In June, 2012, tender cassava leaves, the first matured up to leaf position five were harvested from three species of cassava, bitter (*Manihot utilissima*), sweet (*Manihot dulcis*) and wild (*Manihot glaziovii*). Varieties named "Seruruseke" (5280), ISAR 1961 and "Igicucu" were chosen for sweet, bitter and wild, respectively. In order to minimize the effects of age, environment and soil type on chemical composition, leaves samples of the same age were selected from the same field, Rwanda Agricultural Board (RAB)'s field at the Karama Research Station, in Bugesera District of Eastern Province of Rwanda.

Sample preparation

Samples were collected in the field and transported in closed polyethylene bags, which were stored in a cool box containing ice. Each sample was divided into two portions, first portion was analyzed in fresh condition and for the second portion analysis was done after blanching and drying. Blanching was done by submersion in boiling water for 4-5 min, and then immediately cooled in tap water at ambient temperature as described by Kendall et al. (2010).

Three different preparation procedures were conducted, namely: (1) Un-dried (UND) (2) dried before pounding (DBP) and (3) dried after pounding (DAP) leaves. Pounding was done using wooden mortar and pestle, while drying was done using a tunnel solar dryer at Sokoine University of Agriculture. The products obtained by the three different preparation procedures (Figure 1) were chemically analyzed, un-boiled and boiled for 15 and 30 min. Moisture, cyanide, protein and minerals (Ca, Fe, K, P and Zn) were determined. The first four analyses were conducted at Sokoine University of Agriculture laboratories, while vitamins (Ascorbic acid and β-carotene) analyses were done at the Tanzania Food and Drug Authority (TFDA), in Dar-Es-Salaam. All chemical analyses were carried out in quadruple.

Drying procedures

After blanching, pounded and un-pounded leaves from bitter, sweet and wild cassava were dried using a tunnel solar dryer. Temperatures inside the dryer were recorded at 8 a.m., noon and 8 p.m. each day, averaging 38°C. The complete drying was when the samples were dried until they became brittle. The dried samples were immediately packed in plastic materials, sealed and transported to laboratories, in opaque cartons to avoid light effect before analysis.

Cooking procedures

The cooking consisted of boiling for 15 and 30 min, in distilled water (1:2) and (1:9) respectively for un-dried and dried samples as volume of sample by volume of water, in stainless steel and without cover. The dried samples were first soaked in water for about 5 min before starting the fire. Un-dried and cooked samples were kept frozen before analysis.

Cyanide (HCN) and nutrients determination

Cyanide (HCN) levels in the samples were determined by alkaline titrating method as described by AOAC (1995), official method 915.03B. Moisture content of samples was determined as outlined by AOAC (1995), official method 934.01. For minerals, sample ashes and solutions were obtained respectively by official methods 965.09 and 982.23 described by AOAC (1995). Total phosphorus (P) was obtained using ascorbic acid blue color procedure and by reading the absorbance at a wavelength of 884 nm on a UNICAM 5625 UV/visible spectrometer (Okalebo et al., 1993). Calcium (Ca) and potassium (K) were measured by flame photometry, reading their absorbance at 422.7 and 766.5 nm respectively on a Cole-Parmer instrument, model 2655-00 Digital flame Analyzer. Iron (Fe) and zinc (Zn) were determined by reading their absorbance at 248.3 and 213.9 nm, respectively on a UNICAM 919 Atomic Absorption Spectrometer (AAS) using Hollow Cathode lamps (Okalebo et al., 1993). Crude protein content was determined by using the micro-Kjeldahl method (AOAC, 1995), official method 920.87. Vitamin C (ascorbic acid) content was determined as outlined by ISO (1984), method 6557/2. B-carotene was measured using a high performance liquid chromatography (HPLC), equipped with a Photodiode Array (PDA) detector fitted with a 436 nm wavelength. For sample preparation, aliquots were extracted by solvent n-Hexane (Priadi et al., 2009; Tee Siong and Lam, 1992). Further extraction and clean-up was done using a dispersive Solid Phase Extraction (dSPE) technique as described in AOAC (2007), official method 2007 0.1.

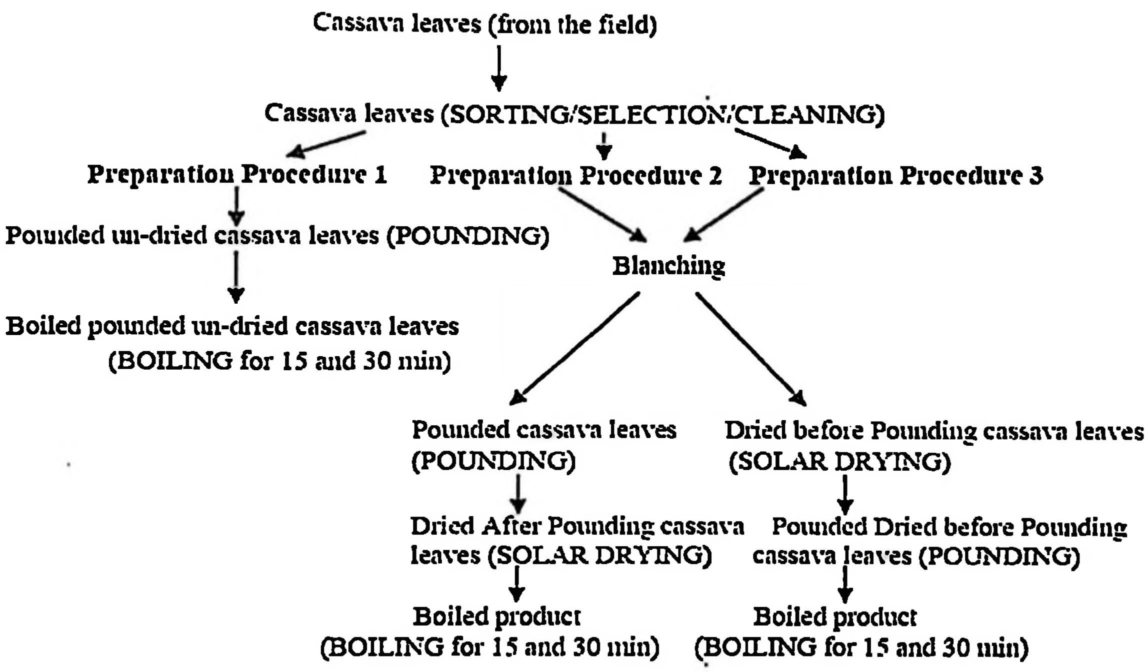

Figure 1. Flow diagram illustrating preparation procedures of cassava leaves.

Statistical data analysis

Data from the results of chemical analyses of samples were subjected to statistical analysis, using SAS 9.2 (SAS Institute, 2008). Kolmogorov-Smirnov test was first carried out to assess the normality of the data (Kutner et al., 2005). Multiple ways (species with three levels specifically bitter, sweet and wild, processing methods with three levels namely un-dried, dried before pounding and dried after pounding, boiling time with 2 levels, that is, 15 and 30 min) analysis of variance (ANOVA) was applied after assuming the normal distribution of the data. Where the treatments had statistical significant effect on the response variables of interest, Fisher's least significant differences (LSD) test was used to separate the means. The treatments were judged statistically significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Cyanide, ascorbic acid, β-carotene, protein, iron, calcium, phosphorus, potassium and zinc were chemically determined in cassava leaves from three species (bitter, sweet and wild). Overall effect of species and processing procedures on contents are shown in Table 1. From the results in the table, it was noticeable that for un-dried and dried samples, before boiling, wild species had the highest concentrations in all determinations (cyanide, ascorbic acid, β-carotene, protein, iron, calcium, phosphorus, potassium and zinc). Bitter species had also high protein content as the wild. Sweet species was less concentrated in cyanide, β-carotene, crude protein, calcium, phosphorus and potassium than wild and bitter. Bitter and sweet species had similar concentrations of ascorbic acid and iron. Bitter species was less concentrated in zinc than wild and sweet.

The presence of cyanide in all the studied leaves confirmed the earlier reports that all cassava cultivars contain cyanogenic glucoside, in a wide variation according to varieties (CIAT, 2007). The levels of cyanide in fresh leaves of the studied species were 1905, 1480 and 2179 mg HCN/kg respectively, for bitter, sweet and wild. The values were in the ranges reported by earlier researchers, from 189 to 2466 mg HCN/kg fresh weight basis by Fukuba et al. (1982) and 800 to 3200 mg HCN/kg dry matter by Ravindran (1995). The high values in the studied leaves were not surprising as cyanide is known to be distributed throughout the cassava plant, but with highest levels in leaves (Etonihu et al., 2011). However, depending on the varieties, moderate and low cyanide content cassava leaves have been discovered by Bums et al. (2012). As continued consumption of high dietary cyanide has been linked with a number of chronic health disorders (Nhassico et al., 2008; CCDN, 2007), it is evident that, cassava leaves in the present study need to be properly processed to reduce their cyanide before consumption by humans.

Nutrients varied significantly ($p < 0.05$) according to cassava species. Sarkiyayi and Agar (2010) revealed significant differences in protein and mineral contents when investigating sweet and bitter roots and it may be the same phenomenon in the leaves. In un-dried samples, the average values of crude protein (35-36%), iron (230-278 mg/kg), calcium (7373-8822 mg/kg), phosphorus (4413-4907 mg/kg), potassium (15110-17119 mg/kg) and

Table 1. Mean levels of cyanide and selected nutrients of cassava leaves according to species and processing procedure.

Parameter		Cyanide (mg/kg)	Vitamin (mg/100g)		Crude protein (%)	Mineral (mg/kg)				
PP	CS	HCN	AA	B-C	CP	Fe	Ca	P	K	Zn
UND	Bitter	1905.0±25.0^{b}	8.41±0.03^{b}	51.2±0.5^{b}	36.6±0.5^{a}	230.4±1.6^{b}	7441.0±60.3^{b}	4607.7±48.8^{b}	16309.9±102.9^{b}	64.2±0.7^{c}
	Sweet	1480.5±18.9^{c}	8.39±0.02^{b}	40.6±0.3^{c}	35.2±0.8^{b}	230.4±1.3^{b}	7373.5±63.3^{c}	4413.3±45.6^{c}	15110.4±116.8^{c}	67.0±0.6^{b}
	Wild	2179.7±29.1^{a}	13.27±0.09^{a}	80.4±0.3^{a}	36.6±0.2^{a}	278.3±2.1^{a}	8222.5±61.8^{a}	4907.4±50.2^{a}	17119.9±109.5^{a}	76.2±0.7^{a}
DBP	Bitter	562.8±24.2^{b}	0.00075±0.00^{b}	43.7±0.8^{b}	36.8±0.7^{a}	222.5±0.6^{b}	6956.1±65.0^{b}	4590.4±43.5^{b}	15520.6±139.4^{b}	61.9±0.7^{c}
	Sweet	467.8±27.6^{c}	0.00075±0.00^{b}	39.8±0.2^{c}	35.1±0.9^{b}	221.8±1.9^{b}	6587.0±60.3^{c}	4212.0±49.7^{c}	14358.1±150.2^{c}	65.5±1.2^{b}
	Wild	873.3±27.3^{a}	0.00090±0.00^{a}	65.9±0.2^{a}	36.1±0.1^{a}	243.0±0.3^{a}	8070.2±62.1^{a}	4877.4±48.6^{a}	16157.4±133.0^{a}	73.2±0.8^{a}
DAP	Bitter	413.6±21.7^{b}	0.00070±0.00^{b}	39.1±0.1^{b}	36.6±0.1^{a}	215.2±0.1^{b}	6360.2±68.7^{b}	4454.6±48.6^{b}	14693.3±156.2^{b}	59.7±1.3^{c}
	Sweet	352.4±23.6^{c}	0.00070±0.00^{b}	29.1±0.0^{c}	35.2±0.1^{b}	215.3±0.2^{b}	6124.7±69.9^{c}	3957.2±52.3^{c}	12922.2±151.0^{c}	62.1±0.2^{b}
	Wild	684.9±21.6^{a}	0.00080±0.00^{a}	63.5±0.2^{a}	36.3±0.1^{a}	228.9±0.5^{a}	7898.8±63.6^{a}	4607.2±48.7^{a}	15765.7±118.7^{a}	69.5±1.6^{a}

Values are means and SE of nine independent determinations, dry matter basis in quadruple. Means within columns superscript by similar letter are not significantly different from each other ($P < 0.05$) by Fisher's least significant difference (LSD). PP = Processing procedures, CS = cassava species, HCN = hydrogen cyanide, AA = ascorbic acid, β-C = β-carotene, CP = crude protein, Fe = iron, Ca = calcium, P = phosphorus, K = potassium, Zn = zinc, UND = un-dried, DBP = dried before pounding and DAP = dried after pounding

zinc (64-76 mg/kg) agreed with values in earlier reports (Dada and Owuru, 2010; Ravindran, 1995). Values of β-carotene (406-804 mg/kg) were in agreement with values mentioned by Priadi et al. (2009). Considering these nutrient levels, it can be said that cassava leaves are good source of β-carotene, protein and minerals and similar observation has been written by Achidi et al. (2005) and Ayodeji (2005). Mulokozi et al. (2007) and Akinwale et al. (2010) noticed also the potential contribution of cassava leaves, especially in vitamin A and suggested to properly prepare them for more profit from their present nutrients.

The cassava leaves were differently processed before cooking: (1) "Un-dried (UND)", (2) "Dried before pounding (DBP)" and (3) "Dried after pounding (DAP)". DBP and DAP leaves were blanched before solar drying, principally for inactivating potentially deleterious enzymes. The leaves were dried to brittle and on average, water content of the leaves varied from 83.5% for fresh to 4.7% for dried. As it is mentioned by James and Kuipers (2003), green vegetables contains less sugar, and thus, they can be dried to brittle and water content, 4-8%, depending on the type of vegetable. Un-dried samples were more concentrated in cyanide, ascorbic acid, β-carotene, iron, calcium and potassium than dried, but crude protein seemed not to be sensibly affected by drying. Dried before pounding and dried after pounding samples were the first in protein and second in iron and potassium. Dried un-pounded (DBP) samples retained more cyanide and nutrients than the dried pounded leaves (DAP). Drying reduced deeply the ascorbic acid content of the samples. The differences between un-dried and dried leaves were due to the combination effects of blanching and drying, because before drying leaves were blanched. It has been revealed that blanching and drying reduce the poisonous compound, cyanide, but unluckily accompanied by nutrients losses (Oguche Gladys, 2011; Anhwange et al., 2011; Eze, 2010; Abah Idah et al., 2010; Udofia et al., 2010). From the same table (Table 1), it was observable that pounding promoted cyanide and nutrients removal. The decrease, probably due to leaching or solubility in evaporated or drained water, was facilitated by small sized particles of dried after pounding products.

After the solar drying, the residual cyanide was still high (684-873 mg HCN/kg dry matter). An additional treatment was necessary for safety of the foodstuffs. Cooking by boiling in water is well known to reduce sensibly cyanide (Gernah et al., 2012; Ubi et al., 2008). Therefore, the UND, DBP and DAP were boiled, in distilled water, using stainless materials, for 15 and 30 min. In addition to cyanide reduction, findings in this study showed a significant ($p < 0.05$) decrease in protein, vitamins and minerals with cooking time and it has been the same observation in earlier study of Gernah et al. (2012). The concern was to assess a state of the cooked cassava leaves in regards to cyanogens and nutrients. Means of cyanide, ascorbic acid, β-carotene, protein, iron, calcium, phosphorus, potassium and zinc of the vegetables,

Table 2. Mean levels of cyanide and selected nutrients of boiled cassava leaves for 15 minutes.

Parameter		Cyanide (mg/kg)	Vitamin (mg/100g)		Crude protein (%)	Mineral (mg/kg)				
PP	CS	HCN	AA	B-C	CP	Fe	Ca	P	K	Zn
UND	Bitter	571.2±5.6^b	5.78±0.33^b	34.1±1.9^b	36.8±0.7^b	144.7±0.4^b	4230.4±11.9^c	3644.0±30.6^c	8917.0±87.9^b	45.8±0.8^c
	Sweet	501.9±1.4^c	5.39±0.05^c	33.1±2.6^c	35.5±0.4^c	142.5±1.4^c	4622.7±34.1^b	3872.9±33.9^b	8905.2±149.4^c	58.8±0.9^b
	Wild	696.5±3.3^a	11.49±0.04^a	49.1±3.1^a	36.9±0.8^a	210.4±1.8^a	6729.3±19.8^a	4533.0±31.6^a	8984.3±119.6^a	68.7±0.8^a
DBP	Bitter	532.8±4.2^b	-	35.9±0.2^b	36.8±0.7^b	92.5±0.6^c	4186.2±25.0^c	3199.4±23.5^b	8520.6±139.4^b	45.7±2.3^c
	Sweet	467.8±4.6^c	-	33.0±4.8^c	36.7±0.2^b	97.8±1.9^b	4487.0 ±20.3^b	3212.0±20.7^b	8858.1±150.2^a	58.1±0.2^b
	Wild	543.3±4.3^a	-	39.8±0.2^a	37.2±0.9^a	113.0±0.3^a	6370.2±32.1^a	4346.3±33.4^a	8857.4±133.0^a	63.5±1.6^a
DAP	Bitter	303.6±3.7^b	-	34.2±0.1^b	35.3±0.2^b	92.2±0.1^c	4160.2±28.7^b	3177.4±28.6^b	8493.3±156.2^c	45.1±0.1^c
	Sweet	292.4±3.6^c	-	29.1±0.1^c	35.2±0.1^b	95.3±0.3^b	4424.7±16.9^b	3157.2±42.3^b	8822.2±151.0^b	58.3±0.2^b
	Wild	314.9±3.6^a	-	35.5±0.2^a	36.7±0.1^a	112.9±0.5^a	6298.8±36.6^b	4307.2±38.7^a	8855.7±118.7^a	86.3±0.1^a

Values are means and SE of nine independent determinations, dry matter basis in quadruple. Means within sub-columns superscript by similar letter are not significantly different from each other ($P < 0.05$) by Fisher's least significant difference (LSD). PP = processing procedures, CS = cassava species, HCN = hydrogen cyanide AA = ascorbic acid, βC = β-carotene, CP=Crude protein, Fe = iron, Ca = calcium, P = phosphorus, K = potassium, Zn = zinc, UND = un-dried, DBP = dried before pounding and DAP = dried after pounding.

boiled for 15 and 30 min are given respectively in Tables 2 and 3.

After boiling for 15 min (Table 2), depending on species and processing procedure, the residual cyanide levels ranged from 209 to 696 mg HCN/kg and remained high so that an extension of cooking time was highly indispensable. After boiling for 30 min (Table 3), the remaining levels of cyanide, across species and processing procedures, varied from 32 to 50 mg HCN/kg. From Tables 2 and 3, processing procedure that excluded blanching (UND) was more effective in removing cyanide by heating than those procedures that included blanching (DBP and DAP). This was attributed to the action of endogenous linamarase on cyanogenic glucosides, following the intimate contact in the finely-divided tissues, during pounding, between linamarin and the hydrolyzing enzyme, linamarase, which promotes rapid breakdown of cyanogens glucosides into a free form, hydrogen cyanide (HCN) (White et al., 2003), while blanching inactivated enzymes in the dried samples and limited easier hydrolysis of cyanogenic glucosides into hydrogen cyanide. The hydrogen cyanide is known to be easily removed by heat during boiling. Similar to drying, after boiling, it was visible that pounding and then drying (DAP) improved cyanide reduction. The reason may be the same for drying, small sized particles of boiled products.

Cassava leaves as a safe human food

After cooking for 30 min, moisture content of the called "relishes" was on average 87%. Therefore, one kilogram (dry weight basis) is equivalent to about 4 kg of the cooked vegetable as it is eaten (relishes). Under normal circumstances, this volume is shared by many persons in one meal considering an adult person can eat up to 100-200 g of the vegetable relish. Furthermore, different studies reported that an acute oral lethal dose of hydrogen cyanide (HCN) is proportional to body weight (WHO, 2004). But a large variation of the doses showed a lack of precision. For example, levels ranging from 30-210 mg of HCN for a 60 kg adult have been recorded by Montgomery (1980). Committee of experts in codex standards concluded that a cyanide level of up to 10 mg HCN/kg of cassava flour is not associated with acute toxicity (FAO/WHO, 1993) and the level became recommended by FAO/WHO (1991) as safe for human foods. Therefore, the cassava leaves in this study, un-dried and solar dried, after being boiled for 30 min, can be said to be safe for human consumption in regards to cyanide toxicity, based on the acute oral lethal doses, but also by considering that the quantities of green vegetables are usually small by serving, as a side relish for the starchy based food. Besides, relish from the leaves was found as source of protein, and the nutrient is known to be helpful in cyanide human body detoxification (Nhassico et al., 2008; CCDN, 2007).

Potential contribution of cassava leaves to human nutrition

For the nutrients, the nine vegetable relishes

Table 3. Mean levels of Cyanide and selected nutrients of boiled cassava leaves for 30 minutes.

Parameter		Cyanide (mg/kg)	Vitamin (mg/100g)		Crude Protein (%)	Mineral (mg/kg)				
PP	CS	HCN	AA	B-C	CP	Fe	Ca	P	K	Zn
UND	Bitter	35.4±2.0^{b}	0.24±0.01^{b}	33.0±1.0^{c}	33.7±0.6^{b}	199.4±1.4^{b}	3939.8±27.8^{c}	3564.9±16.5^{c}	8849.3±102.6^{b}	45.4±0.4^{c}
	Sweet	32.8±1.5^{c}	0.20±0.03^{c}	32.6±0.4^{c}	33.8±0.4^{b}	182.7±0.8^{c}	4391.4±24.7^{b}	3795.5±28.2^{b}	8834.1±159.1^{b}	57.9±0.1^{b}
	Wild	40.7±1.5^{a}	0.30±0.01^{a}	43.2±1.9^{a}	34.3±0.2^{a}	219.6±2.5^{a}	5939.3±27.5^{a}	4321.7±23.2^{a}	8960.3±173.5^{a}	68.4±1.2^{a}
DBP	Bitter	47.7±1.1^{b}	-	33.5±0.6^{b}	35.8±0.1^{a}	144.5±2.8^{b}	3505.7±26.3^{c}	3120.9±35.5^{c}	8216.6±124.3^{c}	45.1±0.2^{c}
	Sweet	45.2±0.8^{c}	-	32.6±0.8^{c}	35.0±1.2^{b}	143.9±1.4^{c}	3654.3±28.3^{b}	3200.4±23.6^{b}	8521.8±169.7^{b}	54.4±0.7^{b}
	Wild	50.4±0.4^{a}	-	37.7±0.3^{a}	35.9±0.6^{a}	205.9±3.5^{a}	5436.0±25.6^{a}	4166.9±4.2^{a}	8861.1±180.9^{a}	63.0±1.2^{a}
DAP	Bitter	38.0±2.9^{b}	-	33.2±0.2^{b}	35.3±0.9^{a}	87.1±3.6^{c}	3221.6±33.3^{c}	2982.9±25.0^{b}	7840.7±181.6^{b}	44.5±0.7^{c}
	Sweet	36.4±1.8^{c}	-	29.0±1.8^{c}	35.4±0.5^{a}	95.0±0.6^{b}	3400.2±22.6^{b}	2737.8±25.1^{c}	7008.9±171.6^{c}	51.9±0.4^{b}
	Wild	41.8±2.1^{a}	-	34.3±0.1^{a}	35.0±0.4^{b}	103.9±1.1^{a}	4886.4±18.9^{a}	3898.3±16.9^{a}	8746.5±168.0^{a}	61.7±0.6^{a}

Values are means and SE of nine independent determinations, dry matter basis in quadruple. Means within sub-columns superscript by similar letter are not significantly different from each other ($P < 0.05$) by Fisher's Least Significant Difference (LSD). PP = Processing procedures, CS = cassava species, HCN = hydrogen cyanide AA = ascorbic acid, βC = β-carotene, CP = crude protein, Fe = iron, Ca = calcium, P = phosphorus, K = potassium, Zn = zinc, PM = processing method, SP = species, UND = un-dried, DBP = dried before pounding and DAP = dried after pounding.

retained appreciable levels of the studied nutrients, except vitamin C, for which levels were too small to be considered as traces in relishes from dried and cooked cassava leaves. The severe reduction of ascorbic acid may be related to the fact that it is thermo-labile at mild heating and very sensitive to blanching, drying and cooking (Faber and Van Jaarsveld, 2007). To understand the contribution of the relishes to human nutrition, their content levels of β-carotene, protein, iron, calcium, phosphorus, potassium and zinc were compared with Recommended Dietary Allowances (RDAs). For β-carotene, because the body converts all dietary sources of vitamin A into retinol, it is explained as retinol activity equivalent (RAE) and believing that 1 µg of retinol is equal to 6 µg of β-carotene (Food and Nutrition Board, 2001), the β-carotene mean values were calculated into RAE before being compared to RDA. Results of the comparison are shown by Table 4.

From the Table 4, the amounts in grams (dry matter basis) of relish from un-dried (UND) leaves (128, 50, 190, 205, 202, 263, and 14 g) to meet respectively protein, iron, calcium, phosphorus, potassium, zinc and β-carotene RDAs, were less than the amounts of relishes from DBP (128, 60, 208, 228, 210, 272 and 15 g), and DAP (132, 105, 228, 249, 228, 287 and 16 g), needed to meet the respective nutrients (protein, iron, calcium, phosphorus, potassium, zinc and β-carotene) RDAs.

The results showed that relish from un-dried leaves (UND) provides more nutrients than relish from dried leaves. This was attributed to blanching and drying, indispensable treatments for quality and storability (Oguche Gladys, 2011; Anhwange et al., 2011; Eze, 2010). Moreover, comparing the dried samples, drying before pounding (DBP) procedure provides more nutrients than pounding before drying treatment (DAP), but the latter contains less cyanide and then is safer for human consumption. B-carotene and iron are adequately contributed by the cassava leaves, considering the slighter required quantities of the greens to meet their RDAs (Table 4).

Conclusion and recommendations

Cassava leaves of bitter, sweet and wild species, when un-dried or solar dried, have potential to contribute to vitamin A, protein and mineral requirements. Vitamin C is very low in cooked (un-dried and dried) cassava leaves that a complement vitamin C rich-food is necessary to accompany cassava leafy meal. Leaves from wild species are the richest in nutrients, followed by bitter while sweet is the least. Fresh (un-dried) leaves give the more safe and rich foods, but for preservation purposes, solar drying is efficient, and pounding cassava leaves before they are dried is a more recommended drying method for cyanide reduction. Cyanide levels are significantly different in leaves from different cassava species, but after sufficient cooking, the difference is small that vegetable relishes from all the cassava species can be judged to be safe for human consumption. Blanching, drying and prolonged cooking treatments (30 min) reduce sensibly cyanide levels

Table 4. Average levels of nutrients in processed and cooked (30 min) cassava leaves and required amount of the relishes to meet the RDAs.

PP	CP (%)	Fe (mg/kg)	Ca (mg/kg)	P(mg/kg)	K (mg/kg)	Zn (mg/kg)	B-C (µgRAE/g)
UND	35.3±0.4	200.7±3.9	4756.5±59.9	3893.0±37.2	8881.2±180.4	56.6±1.1	61.5
DBP	35.5±1.3	164.1±2.5	4199.4±24.8	3495.5±28.8	8532.4±162.5	54.8±0.8	58.1
DAP	33.3±1.0	95.3±1.4	3836.7±32.6	3205.3±33.5	7864.7±165.4	52.5±1.3	54.4
RDA(mg)	45,000	10	800	800	1800	15	900 (µg RAE)
Required amount of the vegetable relishes (g) to meet the RDAs (dry matter basis)							
UND	128.5	50.2	190.5	205.4	202.6	253.6	14.6
DBP	128.3	60.7	208.0	228.7	210.5	272.3	15.4
DAP	132.3	105.2	228.6	249.6	228.6	287.7	16.5

Values are means and SE of six independent determinations, dry matter basis in quadruple. CP = crude protein, Fe = iron, Ca = calcium, P = phosphorus, K = potassium, Zn = zinc, PP = processing procedures, UND = un-dried, DBP = dried before pounding, DAP = dried after pounding, RDAs = Recommended Dietary Allowances by day and RAE = retinol activity equivalent.

(32-50 mg HCN/kg dry matter basis), but not at recommended level for human foods (10 mg HCN/kg dry matter basis). However, considering the small quantities by serving of green vegetables as side food, the protein level in cassava leaves, important nutrient in cyanide human body detoxification, and the acute oral lethal doses of hydrogen cyanide by bodyweight, the cassava leaves food can be said to be safe for human consumption. However, it is not advisable to consume cassava leaves as an everyday vegetable relish or in large quantities. In addition, cassava leaves meals may be limited for lower body weights such as children. The frequency may be reduced by promoting other greens such as amaranths, spinach and cabbage, even in arid areas, where cassava leaves are highly utilized as human food because other leafy vegetables cannot grow well in the present conditions. Cassava varieties with low levels of cyanide in leaves should be released for leaves consumption purposes to alleviate nutrient deficiencies, especially β-carotene and iron.

In general, amounts of nutrients retained after cooking un-dried and dried cassava leaves (after 30 min) are significant that cassava leaves as food are judged to contribute nutritionally to human health, especially vitamin A and iron.

Protein content is also of interest, and as cassava leaves are affordable by even poor people whose access to protein rich-foods such as milk, meat and fish is hard, the leaves can be helpful. But time of cooking may be extended to at least 30 min to improve reduction of cyanide level in the cassava leaves relishes.

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PAPER FOUR

Assessment of over time changes of moisture, cyanide and selected nutrients of stored dry leaves from three cassava species

ASSESSMENT OF OVER TIME CHANGES OF MOISTURE, CYANIDE AND SELECTED NUTRIENTS OF STORED DRY LEAVES FROM THREE CASSAVA SPECIES

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ABSTRACT
Most fresh agricultural products are quickly perishable and various methods of preservation are necessary. Cassava leaves from different types of cassava (bitter, sweet and wild) were (1) dried un-pounded and (2) dried pounded in a tunnel solar dryer, filled in High Density Polyethylene material, sealed and placed into opaque cartons. The packing materials were purposively chosen to limit water, oxygen and light access. The complete drying was when samples were completely brittle. The storage was done at room temperature at Sokoine University of Agriculture, Morogoro, Tanzania. The main purpose of the study was to estimate shelf life by evaluating satisfactory quality in terms of nutritional values, dryness and organoleptic parameters. Water, cyanide, ascorbic acid, β-carotene, protein, iron, phosphorus, potassium and zinc were chemically analyzed at zero, three, six, nine and twelve months of storage. Dryness and organoleptic parameters were also evaluated at these different storage lengths. Processing procedure had significant effect only on water ($p=0.0358$), cyanide ($p=0.0189$) and β-carotene ($p=0.0214$) contents. Storage time affected water, cyanide, ascorbic acid, β-carotene, protein, iron, phosphorus, potassium and zinc significantly ($p<.0001$). Water content increased by 6.8% and ascorbic acid decreased to zero while β-carotene, protein, minerals and cyanide showed slight decline during the storage period. The optimum storage time under the conditions was judged to be six months for nutrients and organoleptic parameters stability.

Key words: Cassava, leaves, solar drying, storage, Rwanda

INTRODUCTION
Agriculture is a substantial food source for rural and urban populations, and also a reliable source of income through selling fresh or processed products (Legg and Tresh, 2000). However, most fresh agricultural products are usually seasonal and quickly perishable. Hunger and malnutrition can exist in spite of adequate food production because of uneven distribution, deterioration and losses of available resources. To make foods available

throughout the year, humans have developed various methods of preservation to keep food produced in one harvest for gradual consumption until next harvest. Microorganisms and enzymes that promote spoilage in foods thrive well in foods with high moisture contents and thus drying works as a preservation method simply by reducing the water content of the products and making it unavailable for chemical reactions and growth of microorganisms (Emebu and Anyika, 2011). Dry food products can be distributed and stored at ambient temperatures and this is affordable and common system in rural world, where cooling facilities are not available. As examples, maize, rice and bean are usually dried for extending storage period. According to Mills (1989), their respective equilibrium moisture contents for a safe storage are 13.5, 13.0 and 15.0 %, in controlled storage conditions of temperature (27°C) and relative humidity (70 %). In rural world, storage of the dry products at uncontrolled ambient temperature and relative humidity is common, especially in developing countries, but their storing lives are not stable. Among food preservation methods by reducing water to equilibrium levels, sun drying is the simplest, inexpensive and commonly adequate for rural and poor communities. However, solar drying offers the following over sun drying: faster drying rate, greater retention of nutrients and organoleptic qualities (Eze, 2010). In addition, minimizing exposure to rain, dust and insects by solar dryers reduces contamination and biological hazards. A sensory evaluation of solar dried cassava leaves in Rwanda showed a greater retention of colour, taste, aroma and texture (Umuhozariho *et al.*, 2013). Solar drying has also some advantages over the conventional drying with respect to cost and adaptability to small scale farmers. In reality, solar dryers are promising means for tropical countries to meet their requirements as the available amount of solar energy in most cases are sufficient to cover the required heat for small dryers.

Cassava (*Manihot esculenta* Cruntz) is a staple root crop in many countries of the tropics and particularly in sub-Saharan Africa (Huzsvai and Rajkai, 2009; Legg *et al.*, 2006). According to FAO (2013), cassava can be produced efficiently without the need for mechanization or purchased inputs, and in marginal areas with poor soils and unpredictable rainfall. In fact, ccassava is known to tolerate prolonged drought conditions and low nutrient soils (Leihner, 2002). In Rwanda, cassava is described as "classic food security crop" because it offers the advantage of a harvest even in situations of erratic rainfall and infertile soils (Mushiyimana *et al.*, 2011). In low altitude regions of Rwanda, cassava is among the main crop plants and one of the priority crops that are being promoted for economic development and poverty reduction in the agricultural sector (MINECOFIN, 2007).

Achidi *et al.* (2005) indicate that millions of tonnes of cassava leaves are harvested and used as vegetables by many families, especially in Africa, and provide protein, vitamins and minerals (Akinwale *et al.*, 2010; Priadi *et al.*, 2009). They are usually utilized freshly harvested. As it applies to other vegetable products, cassava leaves price varies much according to season and market location. Leaves are available as seasonal surpluses during certain parts of the year (rainy season) and go to waste due to improper processing, pre-packaging, handling, distribution and marketing. During the peak season, vegetables in general are sold at very low prices and some are simply wasted (TCARC, 2007). This reduces income for farmers, adding to the people's poverty.

For preservation issues in rural communities, cassava leaves are sun dried and consumed at family level during the off-season. For example in low land areas of Rwanda, where cassava is the principal crop, cassava leaves are sun dried to brittle, stored in different types of containers, without any concern about water vapour, air and light access for gradual consumption in long dry season.

However, shelf life of dry food products is for finite period, depending on the type of the product, final moisture content, packaging material and storage conditions (Boyer and Mckinney, 2009; Fellows, 2009). James and Kuipers (2003) and Thomas (2008) mentioned that optimization of storage conditions, specifically by controlling moisture, temperature, oxygen and light, is very important to postpone rotting and spoilage of food products. Therefore, containers would be not only for containing, but also for protecting the food products from outside influences, precisely from water, gases and light entry (Marsh and Bugusu, 2007).

In the present study, leaves from different varieties of *Manihot esculenta* (bitter, sweet and wild) have been processed by drying, using a tunnel solar dryer, and packed in opaque, water and air proof material for storage at ambient temperature. The main purpose of the study was to estimate shelf life by periodically evaluating satisfactory quality in terms of nutritional values, dryness, smell and appearance (colour) of the improved solar dried cassava leaves product called *isombe* and *kisanvu* respectively in Rwanda and Tanzania.

MATERIALS AND METHODS

Collection of cassava leaves

Tender cassava leaves, the first matured up to leaf position five were harvested from three different cassava varieties, named as "Seruruseke" (5280), ISAR 1961 and "Igicucu" were chosen for sweet, bitter and wild respectively. In order to minimize the effects of age, environment and soil type on chemical composition, leaves of same age were selected from similar plot at Rwanda Agricultural Board (RAB)'s field at the Karama Research Station, in Bugesera District of Eastern Province of Rwanda. The preparation procedures are shown in Fig. 1.

Sample preparation

Samples were collected in the field and transported in closed polyethylene bags, which were stored in a cool box containing ice. Each sample was divided into two portions after blanching, first portion was dried un-pounded and for the second portion drying was done after pounding. Blanching was done by submersion in boiling water for 4-5 minutes, and then immediately cooled in tap water at ambient temperature as described by Kendal *et al.* (2010).

Two different preparation procedures were conducted, namely: (1) Drying un-pounded and (2) Drying pounded leaves. Pounding was done using wooden mortar and pestle, while drying was done using a tunnel solar dryer at Sokoine University of Agriculture. The products obtained by the two different preparation procedures (Fig. 1) were assessed for dryness, color and smell/odor, and chemically analyzed for moisture, cyanide, protein, vitamins and minerals (Ca, Fe, K, P and Zn) at 0, 3, 6, 9, and 12 months of storage. The first four chemical analyses were conducted at Sokoine University of Agriculture laboratories, while vitamins (Ascorbic acid and β-carotene) analyses were done at the Tanzania Food and Drug Authority (TFDA), in Dar-es-Salaam. All chemical analyses were carried out in quadruple.

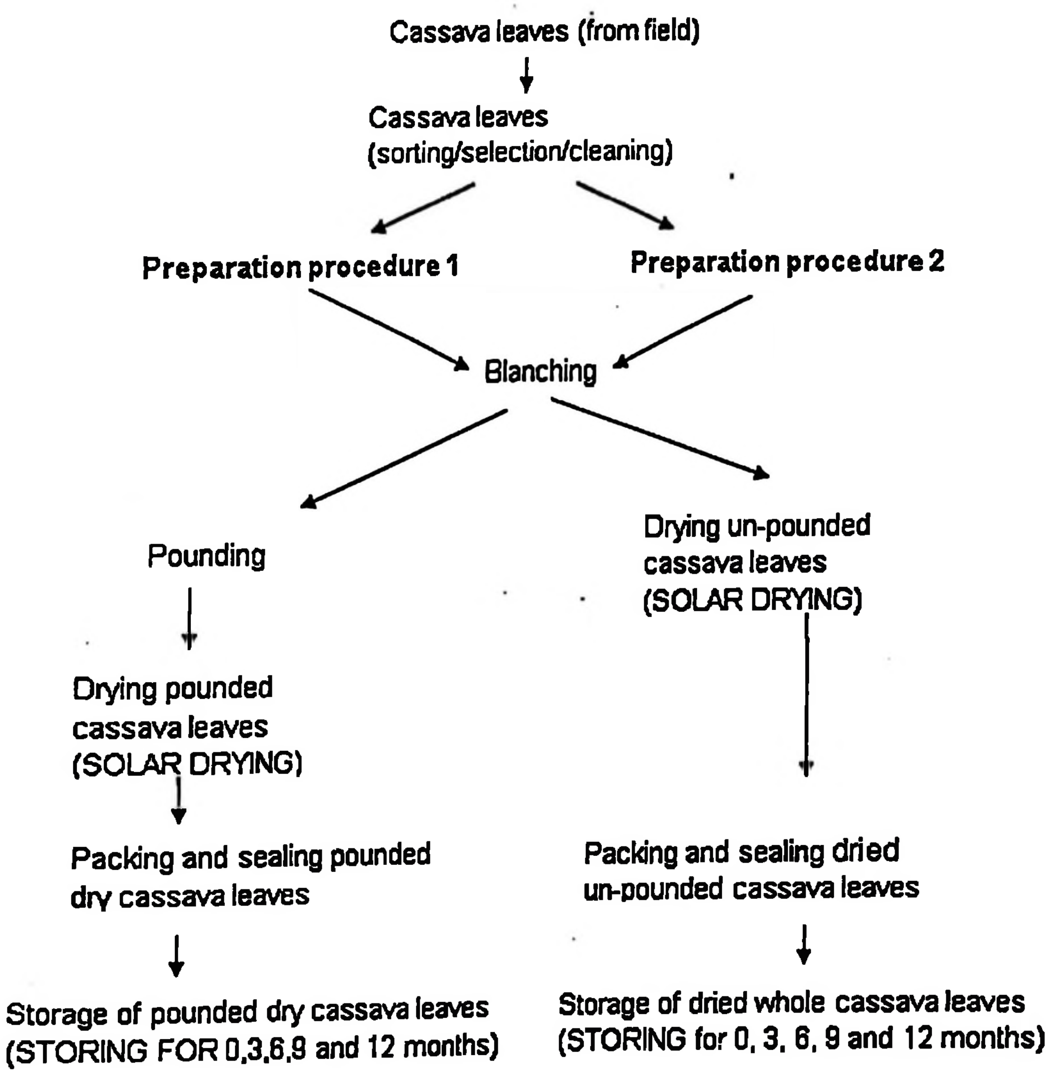

Figure 1: Flow chart of preparation procedures of cassava leaves

Drying, packing and storing

Temperatures inside the dryer were recorded at 8 a.m., noon and 8 p.m. each day, averaging 38°C. The complete drying was reached when the samples became entirely brittle. The dried samples were immediately filled in plastic bags. To avoid absorption of water, each bag was sealed in high density polyethylene (HDPT) material and placed into opaque cartons. HDPT and opaque carton were purposively chosen to limit oxygen, water and light access. Storage was done at room temperature, at Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Each of the three test cassava species/varieties (bitter, sweet and wild) was treated separately.

Moisture, cyanide (HCN) and nutrients determination

Moisture content of samples was determined as outlined in AOAC (1995), official method 934.01. Cyanide (HCN) levels in the samples were determined by alkaline titrating method as described by AOAC (1995), official method 915.03B. Minerals, sample ashes and solutions were obtained respectively by official methods 965.09 and 982.23 described by AOAC (1995). Total phosphorus (P) was obtained using ascorbic acid blue color procedure and by reading the absorbance at a wavelength of 884 nm on a UNICAM 5625 UV/visible spectrometer (Okalebo *et al.*, 1993). Calcium (Ca) and potassium (K) were measured by flame photometry, reading their absorbance at 422.7 and 766.5 nm respectively on a Cole-Parmer instrument, Model 2655-00 Digital flame Analyzer. Iron (Fe) and zinc (Zn) were determined by reading their absorbance at 248.3 and 213.9 nm respectively on a UNICAM 919 Atomic Absorption Spectrometer (AAS) using Hollow Cathode lamps (Okalebo *et al.*, 1993). Crude protein content was determined by using the micro-Kjeldahl method (AOAC, 1995), official method 920.87. Vitamin C (ascorbic acid) content was determined as outlined by ISO (1984), method 6557/2. B-carotene was measured using a High Performance Liquid Chromatography (HPLC), equipped with a Photodiode Array (PDA) detector fitted with a 436 nm wavelength. For sample preparation, aliquots were extracted by solvent n-Hexane (Priadi *et al*, 2009; Tee Siong and Lam, 1992). Further extraction and clean-up was done using a dispersive Solid Phase Extraction (dSPE) technique as described in AOAC (2007), official method 2007 0.1.

Statistical analysis

Data from the chemical analysis of the samples were subjected to statistical analysis, using SAS 9.2 (SAS Institute, 2008). Longitudinal analysis techniques which account for correlation among observations over time and equally spaced measurements were used as suggested by Agresti (2007) and, Tiwari and Shukla (2011). The effect was judged significant at $p<0.05$.

RESULTS AND DISCUSSION

As revealed by Mills (1989), agricultural products change physically and chemically and need to be managed. For "Dried pounded" and "Dried un-pounded" cassava leaves from bitter, sweet and wild, the final moisture content was on average 4.6 % and were completely brittle at packing time.

As it is mentioned by James and Kuipers (2003), green vegetables contains less sugar, and thus, dryness to brittle can be considered as safe moisture content levels. In general, at brittle, water contents of green vegetables are between 4-8 %, depending on the type of vegetable. Thus, dried at 4.6 %, cassava leaves were at safe moisture content level or in equilibrium with a present temperature and relative humidity of the air. But in ambient conditions, storage temperature and relative humidity were uncontrolled and could not be kept for keeping the leaves quality. In the conditions, over time physical and chemical changes were to be evaluated because stored agricultural products are influenced by many factors that determine their keeping quality, including product condition, storage container, length of storage and type of handling (Mills, 1989).

At nine and twelve months of storage, the dryness changed from brittle to pliable. The appearance did not noticeably change during the storage time of one year while the odor characteristic became more pronounced at pliable than at brittle dryness.

Results of chemical analyses of the samples (just after drying the un-pounded and pounded leaves) were statistically analyzed and effects of cassava type, processing procedure and storage period are shown in Table 1. From the table, cassava types did not have significant influence on the overtime changes of moisture, cyanide, ascorbic acid, β-carotene, protein, Iron, calcium, phosphorus, potassium and zinc contents ($p>0.05$). Processing procedures had significant effect only on moisture ($p=0.0358$), cyanide ($p=0.0189$) and β-carotene ($p=0.0214$) while storage time affected all the chemical contents significantly ($p<.0001$).

Table 1: Effect of cassava species, processing procedures and storage time on water, cyanide and nutrients of stored dry cassava leaves

Chemical contents	Cassava types	Processing procedures	Storage times
		P-values	
Water	0.9086	0.0358*	<.0001**
HCN	0.2420	0.0189*	<.0001**
β-carotene	0.1504	0.0214*	<.0001**
Protein	0.0662	0.1704	<.0001**
Fe	0.0604	0.0639	<.0001**
Ca	0.1484	0.0797	<.0001**
P	0.1645	0.4232	<.0001**
K	0.3440	0.5625	<.0001**
Zn	0.1505	0.7478	<.0001**

* Significant effect ($p<0.05$), ** highly significant effect ($p<0.01$)

Mean concentrations of moisture, cyanide, ascorbic acid, β-carotene, protein, iron, calcium, phosphorous, potassium and zinc contents of stored un-pounded and pounded dry cassava leaves, at different period of storage (0, 3, 6, 9 and 12 months) are given in Table 2. From the table, for each cassava type and processing procedure, only moisture content increased over time, while cyanide, protein, β-carotene and minerals slightly decreased as time increased. The influence of processing procedure on sample contents during storage was not surprising as some samples were pounded while others were un-pounded. Their pieces had different sizes and it is known that pounding and slicing in small pieces increase the surface available for water evaporation or absorption depending on relative humidity of the storage room (FAO, 1995). In this study, water increased with time. The increase of water during dry food storage has also been reported by Gupta *et al.* (2012). The increase may be attributed to water vapor absorption through packaging material. In fact, all packaging materials may be permeable to water vapor and air at a certain extent (Marsh and Bugusu, 2007; Brody *et al.* 2002). According to the same authors, the best barriers may have low permeability which is said to be increased by elevated and variable temperatures of storage room and a recommended storage temperature for dry foods is 21°C/70°F. However, under ambient conditions, temperatures are not controlled. In Morogoro, where the dry products were stored, the average annual temperature varies between 25 and 30°C according to altitude and season (Tanzania Minister of State, Planning and Parastatal sector Reform 1997). The temperatures are high and not stable during the year. Higher ambient temperatures also accelerate oxidative degradation of foods, and the oxidative rate is promoted when the cellular integrity is destroyed (Boon *et al*, 2010). Besides, considerable temperature differences within container are a major driving force for moisture translocation and condensation and microorganisms and enzymes that promote spoilage in foods thrive well in foods with high moisture contents (Mills, 1989). Taga *et al.* (2008) noted cyanide liberation from residual linamarin after foods are processed and therefore, concluded the noticeable increase of cyanide characteristic smell during storage period may be caused by the liberated free cyanide.

Nutrient contents deterioration of stored foods is inevitable to some extent. Among deteriorative reactions that cause food components decomposition are enzymatic and non-enzymatic oxidations. All these reactions are known to take place in the presence of oxygen, favoured by water, and promoted by light (Bonilla *et al.*, 2010; Kim *et al.*, 2005; Gibis *et al.*, 2011). Enzymatic reaction was excluded by blanching before drying, light was limited by opaque carton, but as reported by Brody *et al.* (2002), increase of water in stored dry foods through the package is inevitable. Also, despite the hermetic sealing before storing, residual oxygen is unavoidably present in the package headspace and product interstices (Kim *et al.*, 2005). As mentioned by Brody *et al.* (2002), all packaging materials may be permeable, not only to water vapour, but also to air that contain oxygen. Ascorbic acid, β-carotene, protein, iron, calcium, phosphorus, potassium and zinc decreases may be attributed to the deteriorative reactions. The continuous decline of nutrient contents, especially vitamin C of dried green leaves during storage has been also reported by Negi and Roy (2001).

For physical and nutritional quality, dry green vegetables may be consumed before losing their dryness and for the dried cassava leaves in this study, at six months of storage, the products were still brittle, but at nine months, the structure changed to pliable and moisture content to 8.3 and 11 percent at nine and twelve months of storage, respectively. The observation was in agreement with what was reported by Boyer and Mckinney (2009) that, in general, vegetables dried until they are brittle, packed in airtight, light and moisture-proof packaging materials can be stored for 6 months at room temperature and dry place.

Table 2: Mean levels of cyanide, moisture and selected nutrients of cassava leaves according to cassava type, drying procedure and storage time

ST length	BITTER, SWEET AND WILD CASSAVA AND PROCESSING PROCEDURES																			
	Dried and stored un-pounded cassava leaves										Dried and stored pounded cassava leaves									
	BITTER										BITTER									
Month	% MC	mg/kg HCN	mg/100 g AA	B-C	% CP	Fe	Ca	mg/kg P	K	Zn	mg/kg HCN	% MC	mg/100 g AA	B-C	% CP	Fe	Ca	mg/kg P	K	Zn
0	4.7	562.8	75×10^{-5}	43.7	36.8	222.5	6956.1	4590.4	15520.6	61.9	413.6	4.6	70×10^{-5}	39.1	36.6	215.2	6360.2	4454.6	14693.3	59.7
3	5.4	562.5	-	43.7	36.7	221.5	6956.1	4588.2	15518.8	60.8	413.1	5.6	-	38.7	36.2	213.9	6359.4	4453.3	14691.1	59.1
6	6.3	562.1	-	43.1	36.5	220.7	6955.3	4586.6	15515.6	58.9	412.5	6.8	-	38.2	35.9	212.2	6358.1	4451.8	14689.9	58.4
9	8.1	561.8	-	42.5	35.7	219.9	6955.1	4584.4	15513.8	56.6	412.1	8.4	-	37.8	35.5	211.3	6356.6	4450.4	14688.9	56.1
12	10.8	560.9	-	41.6	35.2	218.6	6954.5	4580.4	15511.7	55.7	411.7	11.1	-	37.1	34.9	210.4	6354.7	4448.7	14687.7	55.2
	SWEET										SWEET									
0	4.7	467.7	75×10^{-5}	39.8	35.2	221.8	6587.0	4212.0	14358.1	65.5	352.4	4.6	70×10^{-5}	29.1	35.1	215.3	6124.7	3957.2	12922.2	62.1
3	5.5	467.7	-	39.7	35.2	220.7	6587.0	4211.8	14356.9	64.3	352.1	5.7	-	28.4	34.7	214.2	6123.5	3955.6	12920.8	61.5
6	6.2	467. 4	-	39.1	34.8	219.6	6587.0	4209.2	14353.4	62.5	352.0	6.7	-	27.5	34.4	213.7	6122.2	3954.1	12919.4	60.9
9	7.8	467. 1	-	38.4	34.4	218.1	6586.1	4207.7	14350.1	60.7	351.8	8.4	-	26.5	33.8	212.5	6121.1	3952.7	12917. 6	59.6
12	10.5	466. 3	-	37.5	33.8	217.2	6585.2	4205.8	14348.3	59.8	351. 4	11.1	-	25.3	33.1	211.3	6119.5	3950.8	12915.7	58.6
	WILD										WILD									
0	4.8	873.3	90×10^{-5}	65.9	36.3	243.0	8070.2	4877.4	16157.4	73.2	684.9	4.6	80×10^{-5}	63.5	36.1	228.9	7894.8	4607.2	15765.7	69.5
3	5.8	873.1	-	65.7	36.2	242.6	8070.1	4875.7	16155.8	72.3	684.4	5.9	-	62.8	35.7	227.7	7893.9	4605.4	15763.8	68.4
6	6.5	871.8	-	65.1	36.0	241.5	8068.2	4873.5	16153.2	70.5	684.1	6.7	-	62.2	35.3	226.8	7892.5	4603.1	15761.9	67.8
9	7.8	871.4	-	64.3	35.6	240.8	8067.6	4870.7	16151.6	68.7	683.7	8.2	-	61.6	34.9	224.2	7890.4	4601.3	15759.8	67.4
12	10.5	870.7	-	63.5	36.1	238.9	8067.4	4868.8	16149.8	65.7	683.2	11.1	-	60.8	34.3	223.8	7888.9	4598.9	15757.9	65.2

Values are means of ten independent determinations in quadruple. ST= Storage, MC= Moisture content, HCN= hydrogen cyanide, AA=Ascorbic Acid, β-C=β-Carotene, CP=Crude protein, Fe=Iron, Ca=Calcium, P=Phosphorus, K=Potassium, Zn=Zinc.

CONCLUSION AND RECOMMANDATIONS

Storage time affected water, cyanide, ascorbic acid, β-carotene, protein, iron, phosphorus, potassium and zinc significantly. Water content increased by 6.8% and ascorbic acid decreased to zero while β-carotene, protein, minerals and cyanide showed slight decline during the storage period. The optimum storage time under the conditions was judged to be six months for nutrients and organoleptic parameters stability.

Storage at uncontrollable high and variable ambient temperatures such as these of Morogoro (25-30□C), promotes deteriorative reactions which are the main causes of nutritive and sensory quality losses of the dry *isombe*, even when stored in opaque, water and air proof, and hermetically sealed containers.

When the adequate drying, storage and packing conditions are not combined, storage life can be very limited, less than six months. Therefore, food quality control of dry foods in rural areas, where appropriate packaging materials are not available, is recommended to ensure nutritious and safe foods.

At industrial level, promoting dry food staffs such as dry cassava leaf products could include water absorber use for safe and stable storage because water inevitably increase within containers.

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APPENDICES

Appendix 1: Paper 4 Acceptance Certificate

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African Journal of Biotechnology

Acceptance Certificate

Date:	*10-Sep-2014*
Manuscript Number	*AJB/09.07.14/14036*
Manuscript Title:	*ASSESSMENT OF OVER TIME CHANGES OF MOISTURE, CYANIDE AND SELECTED NUTRIENTS OF STORED DRY LEAVES FROM THREE CASSAVA SPECIES*
Corresponding Author:	*M.G. Umuhozariho*
Corresponding Author Email	*mg_umuhozariho@yahoo.com*
Author(s):	*M.G. UMUHOZARIHO arie Goretti*
Date Accepted:	*04-Sep-2014*

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Appendix 2: Baseline Survey Questionnaire

I. Key Informants / In depth Interview

Name of the interviewer..

Date of interview...

Name of respondent..

Responsibility of respondent..

Questions

1. What are the cassava leaves outputs (utilization) in Rwanda?
2. Can all cassava species (Sweet, Bitter, and Wild) give *isombe*? Yes ( ) No ( )
3. Which are the factors that can affect cassava leave or *isombe* quality?
4. Which are the cassava leaves (*isombe*) harvesting techniques in Rwanda?
5. Can the cassava leaves harvesting techniques affect yield production (leaves and root)?
6. Which are the *isombe* quality preparation procedures in Rwanda?
7. Which are the factors affecting cassava leaves price fluctuation in Rwanda? Can cassava species affect this?
8. What are the cassava leaves storage methods used in Rwanda, if any?

II. Questionnaire for producers (100)

Name of the interviewer..............................

Date of interview....................................

Name of respondent.................................

Name of District.......Sector.......................

1. What is your age?...........(years)
2. Sex of the producer (tick)
   (a) Female ( ) (b) Male ( )
3. Educational level (Tick)
   (a) Not formal schooling ( ) (b) Primary school ( ) (c) Secondary school ( )
   d) Post-secondary school ( )
4. Marital status of the farmer
   (a) Single ( ) (b) Married ( ) (c) Divorced ( ) (d) Widowed ( ) separated ( )
5. What is your household size?
   (a) Less than 3 (b) Between 3 and 7 (c) Between 7 and 10 (d) More than 10
   (a)
6. Do you grow cassava?
   Yes ( ) No ( )
7. If yes, which cassava species do you grow? (Tick)
   (a) Bitter ( ) (b) Sweet ( ) (c) Wild ( )
8. Do you ever harvest cassava leaves?
   (a) Yes ( ) (b) No ( )

9. I f yes, for which purposes? (Please, number them from NO 1 according to the main purpose)
   (a) For selling ( ) (b) For cooking yourself ( ) (c) For animal feed ( )
10. How often do you harvest it? (Tick)
   (a) Once a week ( ) (b) Two times a week ( ) (c) More than two times a week ( )
11. How do you harvest it?(Please number them from NO 1 according to the)
   (a) By cutting growing shoots( ) (b) by selecting some leaves ( ) (c) Both
12. If both, when cutting the growing shoots? (Explain)..
   When selecting some leaves? (Explain)...
13. Have you ever sold cassava leaves last rainy season?
   Yes ( ) No ( )
14. Have you ever sold cassava leaves last dry season?
   Yes ( ) No ( )
15. How much did you sell by bunch
   - Last rainy season? (.... Frw)
   - Last dry season? (.... Frw)
16. Do you always sell all the harvest once a day? Yes ( ) No ( )
17. If no, how do you manage the remaining ones? (Please number them starting from what happens more often)
   (a)Take back home and give to animals ( ) (b) Take back home and prepare it yourself ( )
   (c)Throw away ( ) (d) Store for selling it next day ( )
18. If stored, how do you keep them
   - In rainy season in order to avoid spoilage/deterioration? Explain)
     ....
     ....
     ....
   - In dry season in order to avoid spoilage/deterioration? Explain)
     ....
     ....
     ....
     ....
19. Is the keeping period depending on the season? Yes ( ) No ( )
20. How long can you keep them?
   - In rainy season (a) 1 day ( ) (b) 2 days ( ) (c) 3 days ( ) (d) more than 3 days ( ).
   - In dry season (a) 1 day ( ) (b) 2 days ( ) (c) 3 days ( ) (d) more than 3 days ( ).
21. What do you compare is the thrown quantity to the sold one?
   - In rainy season?
   (a) None (b) A quarter ( ) (c) Between a quarter and a half ( ) (d) A half ( ) (e) More than a half
   - In dry season?
   (a) None (b) A quarter ( ) (c) Between a quarter and a half ( ) (d) A half ( ) (e) More than half
22. Do you sell different species (sweet, bitter, wild) of cassava leaves? (a) Yes ( ), (b) No ( )
23. If yes, what one do you sell? (Mention them please).

- (1) ....
- (2)..
- (3)..

24. If yes (Q_{28}), how do you sell them? (Tick)
    (a) Mixture (b) separately
25. If mixture, how does it affect the price? (Tick)
    (a) Affect the price positively (b) Affect the price negatively (c) Do not change the price
26. If separately, which cassava leaf species (sweet, bitter, wild) is more preferred? (Please number them according to the preference from No1).
    (1) Sweet
    (2) Bitter
    (3) Wild
27. What do you think are the reasons for that preference?
    ....
    ....
    ....
28. Does the price change according to the cassava leaf species? (a) Yes ( ) (b) No ( )
29. If yes, which one is more expensive? (Please rank them according to the high price from No 1)
    (1) Sweet
    (2) Bitter
    (3) Wild
30. Do you face any challenge in entire process of *isombe* selling? (Please rank them according to priority from No 1 to 3)
    - Price fluctuation
    - Storage
    - Post-harvest losses
31. Do you have any other challenges apart from the mentioned above? (Please mention them)

III. Questionnaire for retailers (21)

Name of the interviewer.............................

Date of interview....................................

Name of respondent................................

Name of District.......Sector.....................

1. What is your age?..........(years)
2. Sex of the producer (tick)
    (b) Female ( ) (b) Male ( )
3. Educational level (Tick)
    (b) Not formal schooling ( ) (b) Primary school ( ) (c) Secondary school ( )
    e) Post-secondary school ( )
4. Marital status of the farmer
    (b) Single ( ) (b) Married ( ) (c) Divorced ( ) (d) Widowed ( )
5. What is your household size?

(b) Less than 3 (b) Between 3 and 7 (c) Between 7 and 10 (d) More than 10

6. Where do you usually get the retailed cassava leaves?
   (a) From your own cultivation ( ) (b) Bought from wholesalers or farmers ( ) (c) Both
7. If from your cultivation, how do you harvest it?
   (b) By cutting growing shoots ( ) (b) by selecting some leaves ( ) (c) Both ( )
8. If both,
   - When cutting growing shoot? (Explain)……………………………………………………………
   - When selecting some leave? (Explain)……………………………………………………………
9. How often do you harvest?
   (a) Once a week ( ) (b) Two times a week ( ) (c) More than two times a week ( )

16. If purchased from wholesaler, for how much did you buy by a bunch?

(a) Last rainy season (…….. Frw) (b) Last dry season (………..Frw)

17. How much did you sell them?

(a) Last rainy season (…….. Frw) (b) Last dry season (………..Frw)

18. Are you supplied with the leaves for both seasons? (Explain)

……………………………………………………………………………………………
…..

19. Have you ever sold them pounded?

Yes ( ) No ( )

20. How much is 1 kg for each season?

(a) Dry season (…….. Frw) (b) Rainy season (…….. Frw)

21. Do you sell all the supplied once a day
   - In rainy season? Yes ( ) No ( )
   - In dry season? Yes ( ) No ( )
22. If no in Q_{21}, how do you manage the remaining ones? (Please number them starting by which happened more often).
   (a) Take back home and give to animals ( ) (b) Take back home and prepare it yourself ( )
   (b) Throw away ( ) (c) Store for selling it next days ( )
23. If stored, how do you keep them in order to avoid spoilage/deterioration? Explain)
   (a) Sweet……………………………………………………………………………
   ……………………….
   (b) Bitter……………………………………………………………………………
   ……………………….
   (c) Wild……………………………………………………………………………
   ……………………….
24. Is the keeping period depending on the season (rainy or dry)? Yes ( ) No ( )
25. If yes, how long can you keep them

(b) Less than 3 (b) Between 3 and 7 (c) Between 7 and 10 (d) More than 10

6. Where do you usually get the retailed cassava leaves?
   (a) From your own cultivation ( ) (b) Bought from wholesalers or farmers ( ) (c) Both
7. If from your cultivation, how do you harvest it?
   (b) By cutting growing shoots ( ) (b) by selecting some leaves ( ) (c) Both ( )
8. If both,
   - When cutting growing shoot? (Explain)……………………………………………………………
   - When selecting some leave? (Explain)……………………………………………………………
9. How often do you harvest?
   (a) Once a week ( ) (b) Two times a week ( ) (c) More than two times a week ( )

16. If purchased from wholesaler, for how much did you buy by a bunch?

(a) Last rainy season (…….. Frw) (b) Last dry season (…………Frw)

17. How much did you sell them?

(a) Last rainy season (…….. Frw) (b) Last dry season (…………Frw)

18. Are you supplied with the leaves for both seasons? (Explain)

……………………………………………………………………………………………………
…..

19. Have you ever sold them pounded?

Yes ( ) No ( )

20. How much is 1 kg for each season?

(a) Dry season (…….. Frw) (b) Rainy season (…….. Frw)

21. Do you sell all the supplied once a day
   - In rainy season? Yes ( ) No ( )
   - In dry season? Yes ( ) No ( )
22. If no in Q_{21}, how do you manage the remaining ones? (Please number them starting by which happened more often).
   (a) Take back home and give to animals ( ) (b) Take back home and prepare it yourself ( )
   (b) Throw away ( ) (c) Store for selling it next days ( )
23. If stored, how do you keep them in order to avoid spoilage/deterioration? Explain)
   (a) Sweet…………………………………………………………………………………
   ………………………
   (b) Bitter…………………………………………………………………………………
   ………………………
   (c) Wild…………………………………………………………………………………
   ………………………
24. Is the keeping period depending on the season (rainy or dry)? Yes ( ) No ( )
25. If yes, how long can you keep them

- In rainy season? (a) 1 day ( ) (b) 2 days ( ) (c) 3 days ( ) (d) more than 3 days ( ).
- In dry season? (a) 1 day ( ) (b) 2 days ( ) (c) 3 days ( ) (d) more than 3 days ( ).

26. What do you think is the thrown quantity compared to the sold one
    - In rainy season?

    (a) None (b) A quarter ( ) (c) Between a quarter and a half ( ) (c) A half ( ) (d) More than a half ( )
    - In dry season?

    (a) None (b) A quarter ( ) (c) Between a quarter and a half ( ) (c) A half ( ) (d) More than a half ( )
27. Do you sell different species (sweet, bitter, wild) of cassava leaves? (a) Yes ( ), (b) No ( )
28. If yes, what one do you sell? (Mention them please).
    - (1) ....
    - (2)...
    - (3)...
29. If yes (Q_{28}), how do you sell them? (Tick)

    (b) Mixture (b) separately
30. If mixture, how does it affect the price? (Tick)

    (b) Affect the price positively or increase the price (b) Affect the price negatively or decrease the price (c) Do not change the price
31. If separately, which cassava leaf species (sweet, bitter, wild) is more preferred? (Please rank them according to the preference from No1).

    (1) Sweet

    (2) Bitter

    (3) Wild
32. What do you think are the reasons of that preference?

    ....

    ....

    ....
33. Does the price change according the cassava leaf species? (a) Yes (b) No
34. If yes, which one is more expensive? (Please rank them according to the high price from No 1)

    (1) Sweet

    (2) Bitter

    (3) Wild
35. Do you face any challenge in entire process of *sombe* retailing? (Please rank them according to priority from No 1 to 3)
    - Season availability
    - Price fluctuation
    - Storage
36. Do you have any other challenges apart from the mentioned above? (Please mention them).

IV. Questionnaire for consumers (50)

Name of the interviewer..............................

Date of interview.....................................

Name of respondent.................................

Name of District.......Sector......................

1. What is your age?...........(years)
2. Sex of the producer (tick)
   (c) Female ( ) (b) Male ( )
3. Educational level (Tick)
   (c) Not formal schooling ( ) (b) Primary school ( ) (c) Secondary school ( )
   f) Post-secondary school ( )
4. Marital status of the farmer
   (c) Single ( ) (b) Married ( ) (c) Divorced ( ) (d) Widowed ( )
5. What is your household size?
   (c) Less than 3 (b) Between 3 and 7 (c) Between 7 and 10 (d) More than 10
6. Have you ever heard about *isombe*?
   Yes ( ) No ( )
7. If yes, have you ever eaten it?
   Yes ( ) No ( )
8. If yes, do you prepare it yourself?
   Yes ( ) No ( )
9. If yes, how do you prepare it? (sequence of preparation techniques)
   ....
   ....
   ....
   ....
10. How often do you prepare *isombe* at home?
    (a) Once a week ( ) (b) Twice a Week ( ) (c) More than twice a week
11. How often do you eat *isombe* at your home?
12. Do you prepare different species (sweet, bitter, wild) of cassava leaves? (a) Yes ( ), (b) No ( )
13. If yes, what one do you prepare? (Mention them please).
    - (1) ....
    - (2)..
    - (3)..
14. If yes (Q_{12}), how do you prepare them? (Tick)
    (c) Mixture (b) separately
15. If mixture, how does it affect the taste? (Tick)
    (c) Affect the taste positively (b) Affect the taste negatively (c) Do not change the taste
16. If separately, which cassava leaf species (sweet, bitter, wild) do you prefer the most? (Please rank them according to the preference from No1).
    - Sweet
    - Bitter
    - Wild

17. What are the reasons for the preference?

....

....

Does the price change according the cassava leaf species? (a) Yes (b) No

18. If yes, which one is more expensive? (Please number them according to the high price from No 1)
    (a) Sweet
    (b) Bitter
    (c) Wild
20. Does the price of raw cassava leaves vary according to season?
21. Yes ( ) (b) No ( )
22. If yes, how much is a bunch?
    (a) Rainy season (........ Frw) (b) Dry season (........ Frw)
23. Have you ever bought pounded *isombe*?
    (a) Yes ( ) (b) No ( )
24. If yes, how much is 1 kg (pounded)?
    (a) Rainy season (.......Frw) (b) Dry season (........Frw)
25. Is there any constraint in *isombe* preparation?
    (a)Yes ( ) (b) No ( )
25. If yes in Q_{24}, what are those constraints? (Please mention them).

26. Do you face any challenge in entire process of *isombe* preparation procedures? (Please rank them according to priority from No 1 to 6)
    - Season availability
    - Price fluctuation
    - Storage
    - Poisoning
    - Preparation
    - Time consuming
27. Do you have any other challenges apart from the mentioned above? (Please mention them).

....

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THANK YOU FOR YOUR COOPERATION!

Appendix 3: Sensory Evaluation Form

Sex ....
Age ....
Time ....
Date ....

Please look at and taste each nine coded samples. Indicate how much you like or dislike each of samples by checking the appropriate sample attribute and indicate your references in the column against each attribute. Put the appropriate number against each attribute.

5= Like very much, 4= Like moderately, 3= Neither like nor dislike, 2= Dislike moderately, 1= Dislike very much.

Sample codes / Characteristics	450	452	454	451	459	457	454	456	453
Color									
Taste									
Aroma (Odor and flavor)									
Texture (mouth feel)									
Overall Acceptability									

Comments...
....
....
....

WE THANK YOU VERY MUCH FOR YOUR KIND COLLABORATION!