EFFECT OF TIME-BASED OVEN-DRYING ON THE NUTRITIONAL QUALITY OF COWPEA (*VIGNA UNGUICULATA*) LEAVES

BY

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MINI-DISSERTATION SUBMITED IN PARTIAL FULFILMENT FOR THE REQUIREMENTS OF THE DEGREE OF

MASTER OF SCIENCE

IN

AGRICULTURE (HORTICULTURE)

IN THE

FACULTY OF SCIENCE AND AGRICULTURE (SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES)

AT THE

UNIVERSITY OF LIMPOPO

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2019

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DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Horticulture) has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

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DEDICATION

This study is dedicated to my mother, Julia Morongoa Mafokoane.

ACKNOWLEDGEMENT

Firstly, I would like to thank the almighty God for the great strength, wisdom, guidance and courage he bestowed upon me to be able to carry through this research. Secondly, I would like to thank my parents, Mr M.B. and Mrs M.J. Mafokoane, my sisters, Hunadi, Pascalis, Sophy and Regina, and also my brothers, Lambert and Mahlane. I would also like to acknowledge my nephew Phineas and my daughters Mahlogonolo and Keneilwe. I will forever remain in debt of your support and kindness. I wish to express my sincere gratitude and appreciation to my supervisor Doctor M.S. Mphosi and my co-supervisor Doctor K.G. Shadung who encouraged and inspired me, made insightful comments, productive criticism and useful suggestions during the research. Thank you very much. I am greatly honoured to have worked under Limpopo Agro-Food Technology Station at University of Limpopo. Special thanks to Zama Ngubane, Morongoa Mathipa and Vutlhare Mahlale for all the training and assistance needed for analysis of nutritional qualities and my fellow post-graduate students for their inputs in my research project. I am also grateful for the assistance I received from the employees of Limpopo Agro-Food Technology Station, Mrs E. Thobejane, Ms M. Legodi and Ms M. Ramakgolo, your assistance is appreciated and will forever be remembered. My warmest appreciation goes to the National Research Foundation (NRF) of South Africa for funding my Master of Science in Agriculture.

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ABSTRACT

Innovative methods of preserving the quality of traditionally processed green leafy vegetables are underway in Africa. Improvement of processing and preservation methods of leafy vegetables is another way of overcoming perishability restrictions and guaranteeing continued quality food supply in rural areas. The objectives of this study were: to determine the effect of time-based oven-drying on (1) mineral composition, (2) proximate composition and (3) microbial profiling of cowpea (*Vigna unguiculata*). Separate experiments were conducted for raw and cooked cowpea leaves with four treatments, *viz* 0 (sun dried), 24, 48 and 72 hours of oven-drying, arranged in a randomised complete block design with 5 replications. In raw cowpea leaves, relative to control (sun-drying), oven-drying period 48hrs, significantly decreased Potassium (K), Manganese (Mn), and Sodium (Na) content in raw cowpea leaves by 6, 9 and 13%, respectively. Similarly, oven-drying period 72hrs significantly decreased Ca, Fe, Mg, Zn, P and S by 5, 11, 16, 18 and 57%, respectively. In cooked cowpea leaves, relative to control (sun-drying), oven-drying period 24hrs significantly increased Na by 18%. Similarly 48hrs oven-drying periods increased Fe and K by 6 and 8%, respectively. Similarly, oven-drying period 72hrs significantly increased Ca, Mg and Mn by 8, 8 and 3%, respectively. In contrast, oven-drying period 72hrs significantly reduced Zn, P and S by 16, 10 and 39%, respectively. Relative to control (sun-drying), oven-drying period 24hrs significantly increased fat by 46% in raw cowpea leaves, however oven-drying period 72hrs significantly decreased protein, moisture, ash, fibre and carbohydrate by 10, 29, 18, 0.5, and 7% respectively. In contrast, relative to control (sun-drying), 72hrs increased energy by 3%. In cooked cowpea leaves, relative to control (sun-drying), oven-drying period 24hrs significantly increased energy by 1%. In contrast, relative to control (sun-drying) 72hrs oven-drying period decreased energy by 1%. Similarly, oven-drying 72hrs significantly decreased protein, moisture, ash, fat, fibre and carbohydrate by 8, 14, 13, 19, 0.4 and 10% respectively. Relative to control (sun-drying), oven-drying periods 24hrs significantly increased *Staphylococcus* spp. in raw cowpea leaves by 6%, respectively. Relative to control (sun-drying) 72hrs oven-drying period significantly decreased *Shigella* spp. by 92%, respectively. In cooked leaves, relative to control (sun-drying), 72hrs drying periods decreased both *Shigella* spp. and *Staphylococcus* spp. by

99 and 21%, respectively. Total coliforms unit of *Salmonella* spp, *Escherichia coli*, *Pseudomonas* spp, and *Bacillus cereus* were absent and/or at an undetectable level according to the Tempo Biomerieux system results. In conclusion, cooked leaves retained most essential mineral elements as compared to raw when subjected to 72hrs of oven-drying as there was an improvement in the concentration of Ca, Fe, K, Mg, Mn and Na. However, in both raw and cooked cowpea leaves proximate composition was negatively affected as there was a decrease in protein content. Oven-drying period of 24 hours can be used to minimize the loss of protein. Cooked cowpea leaves subjected to 72hrs of oven-drying had least total coliforms for both *Shigella* spp and *Staphylococcus* spp, therefore have potential to serve as an alternative to sun-drying to reduce microorganism causing spoilage in leafy vegetables. Drying raw cowpea leaves under oven-drying periods less than 72 hours should be avoided as it reduces the mineral concentration and increase microbial count of microorganisms responsible for spoilage.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Leafy vegetables play a crucial role in many African homes as relish. They are an important sources of protein, minerals, vitamins, fibre and other nutrients which are usually in short supply in daily diets (Asaolu *et al*., 2012). Consumption of leafy vegetables as sources of food could enhance food security in developing countries where poverty and climate is problematic to the rural populace. In many developing countries the supply of minerals is not enough to meet the mineral requirements of domesticated animals and rapidly growing population. Minerals cannot be produced by animals and must be provided from plants or mineral-rich water (Asaolu *et al*., 2012).

Cowpea *(Vigna unguiculata* (L.) Walp.) is a warm-season, annual, herbaceous legume (Kabas *et al*., 2007; Peyrano *et al*., 2015), which is widely adapted, tolerant and nutritious (Ehlers and Hall, 1997) and is commonly intercropped with maize and utilized in various forms in Africa (Abu *et al*., 2005). It is a good source of foliates, minerals, proximate, protein and oxalic acid (Antova *et al*., 2014; Faboya and Aku, 1996) therefore is an important constituent in the traditional starch based diet to alleviate malnutrition and nutrient deficiencies and subsequently food insecurity (van der Walt *et al*., 2009).

However, like many leafy vegetables, cowpea is highly perishable and as a results, it is susceptible to mechanical, physical, chemical and microbial effect that are major causes of deterioration and spoilage therefore require preservation (Potter and Hotchkiss, 1995). Preservation increase storage life through lowering the water activity of the product, consequently inhibiting chemical deterioration and microbial growth and improve nutritional, functional, convenience and sensory properties (Rahman, 2007). Preservation is achieved through processing and storage method that involves controlling water structure and atmosphere, packaging, use of heat and energy, using chemicals and microbes depending on resources and available infrastructure at hand (Rahman, 2007).

Generally, cowpea predominantly has been processed through traditional processing methods for improving its shelf life (Muchoki *et al*., 2007). Usually the processing of cowpeas in the marginal areas of Africa involves mainly traditional methods such as cooking, sun and solar drying (Muchoki *et al*., 2007). However during traditional processing great losses of nutritional quality occur due to leaching and oxidation which increases food insecurity. South Africa is among the countries with mineral deficiencies and malnutrition challenges in Africa (Wawire *et al*., 2012). Solar and sun drying are the earliest methods used in preserving leafy vegetables, the challenges arising with these methods are the adverse effects on the nutritional composition of these leafy vegetables (Muchoki *et al*., 2007).

1.1.1 Description of research problem

In many rural areas different processing methods such as traditional cooking, sun drying and appropriate storage are used to process leafy vegetables (Mduma *et al.*, 2010). These methods have been the most commonly used techniques for predominantly achieving the processing of cowpea leaves. However, according to Njoroge *et al*. (2015) these methods have challenges such as hygiene through contamination by microorganisms, other debris and lack of temperature control that renders these methods inefficient. Therefore, are casual due to levels of nutritional losses as their effect and increase prevalence of micronutrient deficiencies resulting in food insecurities (Negi and Roy, 2000).

1.1.2 Impact of research problem

Traditional means of drying to preserve food contributed to poor nutritional products that increased nutrient deficiencies and malnutrition related diseases/disorders such as poor brain development, stunted growth, increased risks of chronic diseases and age-related generative diseases (Belane and Dakora, 2011; Mduma *et al.*, 2010, Schonfeldt and Pretorius, 2011). There has been increased expenses as the South African government formed a policy of adding trace elements such as selenium (Se), iron (Fe), and zinc (Zn) in food materials in order to meet the dietary levels recommended by the World Health Organization (Belane and Dakora, 2011) to alleviate nutrient deficiencies. While the approach by the South African government may address the problem in temporarily, it is not sustainable in a long term (Belane and Dakora, 2011). In 2006 the South African Department of Health published statistics relating to *Escherichia coli* food poisoning resulting in health issues and death rate that was mainly associated with consumption of edible plants in the Eastern Cape, KwaZulu-Natal and Limpopo provinces of South Africa, between 2001 and 2005 (Otun, 2015).

1.1.3 Possible causes of research problem

Traditional processing is known to have variable effects on the quality of leafy vegetables (Otun, 2015). Although these methods of preservation are cheap, they are linked with problems like pollution by foreign materials, dirt, dust and wind- blown debris and insect infestation as well as uneven drying (Nicanuru, 2016). Sun drying has been practiced due to insufficient resources particularly energy and technology required for conventional methods of processing (Nahar, 2009). Therefore, this resulted in poor quality of the dried product that does not meet the nutritional requirements of rural people nor have any health benefits rendering these processing methods ineffective (Belane and Dakora, 2011).

1.1.4 Possible solutions of research problem

Innovative methods of preserving the quality of traditionally processed green leafy vegetables are underway in Africa. Oven-drying consistently retained nutritional quality and extended the shelf life of food products. Ayodeji (2005), suggested that this method could be used as an alternative to traditional processing. Oven-drying was tested and reduced perishability of leafy vegetables and retained considerable amount of essential nutrient elements, proximate components and microorganism responsible for spoilage. At the shortest time exposure to heat during drying most nutritional components were retained while at higher time of exposure many were lost. Results of the study by Ayodeji (2005) demonstrated that oven-drying could serve as potential processing method at the shortest time interval.

1.1.5 General focus of the study

The study focused on the effects of time-based oven-drying on essential nutrient elements, proximate composition and microbial profiling of cowpea leaves.

1.2 Problem Statement

Traditional processing methods have an impact on nutritional quality of leafy vegetables. This impact may result in nutrient losses of cowpea processed leaves thereby leading to malnutrition and subsequently food insecurities.

1.3 Motivation of the Study

Modernisation in the agricultural system has resulted in more innovative and advanced technologies that are convenient and rather easy to adopt in order to optimise the nutritional retention and extended preservation period of leafy vegetables such as cowpea leaves.

1.4 Purpose of the study

1.4.1 Aim

To investigate effect of time-based oven-drying on the nutritional quality of cowpea leaves.

1.4.2 Objectives

The objectives of the study were to:

1. Determine the effect of time-based oven-drying on mineral composition of cowpea leaves.
2. Determine the effect of time-based oven-drying on the proximate composition of cowpea leaves.
3. Determine the effect of time-based oven-drying on the microbial profiling of cowpea leaves.

1.5 Reliability, validity and objectivity

Reliability was ensured by using appropriate statistical levels of significance ($P \leq 0.05$). A set of treatments was used to increase the range of validity. Validity was ensured by conducting the experiment at the same location during one season. Objectivity was achieved by discussing the findings on the basis of empirical evidence as shown by statistical analyses, with findings checked for similarities and differences with findings in other studies.

1.6 Bias

In this study, bias was minimized by ensuring that the experimental error in each experiment and trial was reduced through increased replications and randomization.

1.7 Scientific significance of the study

Findings of this study would improve the preservation of leafy vegetables and retaining nutritional value of these leafy vegetables, thus maintaining nutritional quality of cowpea leafy vegetable.

1.8 Structure of mini-dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the three subsequent chapters (Chapter 3, 4 and 5) addressed each of the three objectives, sequentially. In the final chapter (Chapter 6), findings in all chapters were summarized and integrated to provide recommendations with respect to future research, culminating in a conclusion which tied the entire study together. Literature citation and referencing followed the Harvard style using author-alphabets as prescribed by the relevant University of Limpopo Senate-approved policy framework.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Leafy vegetables are identified as crops that can help in ensuring food security, nutrition and ultimately good health (Weinberger and Msuya, 2004). Such crops require less intense care, can be grown without use of synthetical fertilizers or herbicides, are fast growing, and are resilient than many other conventional crops. Leafy vegetables have long been recognised as the cheapest and most available potential sources of minerals because of their ability to synthesize them from a wide range of virtually unlimited and readily available primary materials such as water, carbon dioxide, and atmospheric nitrogen and sunlight (Aletor *et al*., 2002). However, like many vegetables such as *Cucumis sativas* (cucumber), *Lycopersican esculentum* (tomato) and *Solanum tubersum* (potato), leafy vegetables are highly perishable therefore require preservation (Potter and Hotchkiss, 1995). Preservation is achieved through traditional processing which include cooking, sun and solar drying (Muchoki *et al*., 2007). However during traditional processing great losses of nutritional quality occur which increases food insecurity that is already endemic in South Africa (Wawire *et al*., 2012).

2.2 Work done on the research problem

2.2.1 Processing methods of leafy vegetables

<u>Traditional cooking</u>: Traditional cooking include the boiling of leafy vegetables for about or more than 30 minutes until leaves are tender and soft with the lid on before drying (Njoroge *et al*., 2015). Many leafy vegetables show significant increase in moisture content when cooked while protein, fat and ash content significantly decreases during cooking process (Schonfeldt and Pretorius, 2011). Some have a high iron content which in nutritional terms it might play a role in reducing iron deficiency in South Africa (Schonfeldt and Pretorius, 2011). For most species the young growing shoots and tender leaves are the plant parts that are used in the preparation of vegetable dishes. Petioles and in some cases young tender stems are also included, but old, hard stems are usually discarded (Njoroge *et al*., 2015). Cooking methods differ from boiling, which may include the replacement of the first cooking water with fresh water in the case of bitter-tasting

species, such as *Solanum retroflxum* (Van Rensburg *et al*., 2007), to steaming that uses very small amount of water and short cooking times, as in the case of pumpkin leaves and flowers. According to Van Rensburg *et al*. (2007), the recipes used to prepare these varied leafy vegetables tend to be fairly homogeneous within particular cultural groups limiting culinary diversity

<u>Blanching method</u>: Blanching is a temporary heat treatment to inactivate enzymes catalase and peroxidase present in the vegetables. A portion of the leaves are plunged into boiling water at 94°C for 5-15 minutes, then removed and passed through cold water to halt the blanching process (van Rensburg *et al*., 2007). Conventional blanching for up to 15 minutes results in a significant increase in riboflavin content of many leafy vegetables whereas thermal processing results in significant decrease in vitamins (Mosha *et al*., 1995). Blanching is a heat pre-treatment that inactivate enzymes before processing in order to inhibit activities that cause food deterioration. The indigenous knowledge system-based drying methods of households in places like Limpopo sometimes involve blanching vegetables before drying; however, this is not always the case (Nyembe, 2015). In studies by Schonfeldt and Pretorius (2011), researchers found that blanching various vegetables, for 5 or 10 minutes, caused the antioxidant activity to decrease, increase or remain, depending on the type of vegetable. In addition, Nyembe (2005) reported that blanching vegetables before drying them improved the retention of some vitamins. Even though the nutritional value is expected to be the highest in raw vegetables, Nyembe (2005) reported that there are some vegetables that experience an increase in carotene and antioxidant activity as a result of blanching compared to the raw counterparts. Blanching also has a positive impact on sensory attributes as it has a positive effect on colour, texture and flavour retention (Schonfeldt and Pretorius, 2011).

<u>Oven-drying</u>: Oven-drying falls under the category of air-drying methods and under this process; atmosphere is used as the drying medium and heat at varying temperatures different modes. The drying medium which is the hot air, is allowed to pass over and through the product that has been placed in open trays. The rate of drying under oven-drying depends on temperature, humidity, air velocity and distribution pattern, air

exchange, product geometry and properties and thickness (Porter and Murray, 2002). In general, the higher the air temperature, the faster the rate at which drying occurs. And similarly, the higher the air velocity the higher the drying rate; the lower the air humidity, the higher the drying rate. Relative humidity (measure of dryness) is lower when the temperature is raised (Rahman, 2007). The disadvantages of oven-drying method is that it (i) changes food product quality and is limited by how the product react to heat and expected nutritional quality of the final product, (ii) at times takes a longer period of time even at high temperature, which may then cause serious damages to the flavour, colour, texture and nutrients in dried products (Porter and Murray, 2002) and (iii) it exposes the product externally to the drying temperatures for a longer time than the inferior of the food product material (Rahman, 2007).

Sun-drying: Under sun drying process, foods are directly exposed to the sun rays by placing them on the ground or left hanging in the air. The main disadvantages of this type of drying are (i) contamination from the environment, (ii) product losses and contaminations by wind, insects and birds, (iii) large space requirements, (iv) difficulty in controlling the process, and (v) bad odour (Njoroge *et al*., 2015). When the climate is not particularly suitable for air drying or better quality is desired, conentional air drying is primarily used (Muchoki *et al*., 2007). However, sun drying is the cheapest method of drying foods. Preservation through sun drying is the logical option for rural households that have limited resources because of low the cost of such preservation (Negi and Roy, 2000). However, several research studies have identified that sun drying method leads to high nutrient losses in the dehydrated vegetables while it also requires a longer drying period for appropriately reduced moisture content (Muchoki *et al*., 2007). Since there is no heat regulator, vegetables can be over-dried or under-dried (Njoroge *et al*., 2015). Sun drying also exposes vegetables to contaminants like dust and insects (Njoroge *et al*., 2015). Lastly, direct ultraviolet (UV) exposure causes the vegetables to discolour and lose nutrients excessively (Muchoki *et al*., 2007).

Solar-drying: Solar drying is similar to sun drying however utilizes radiation energy derived from the sun. It is a process that utilizes renewable energy. The main disadvantages of solar drying are (i) the rate of drying cannot be easily controlled, (ii) contamination by microorganisms and insect infestation, (iii) the need for large areas of space and (iv) high labour inputs (Mosha *et al*., 1995). Solar drying is being studied as an alternative to sun drying. This is because the cover which prevents direct sun exposure has potential to reduce nutrient losses and other effects of direct UV exposure, and it is a more hygienic method. Nyembe (2005) found that it took 3 to 5 days to dry indigenous vegetables using solar panels, which is a relatively long period. It would be anticipated that due to a relatively good heat circulation in solar panels, the constant even distribution of heat would facilitate for more rapid drying. Schonfeldt and Pretorius (2011) found that oven drying, just like solar drying, retains more carotene than sun drying, it also reduces drying time, allows for even heat distribution and improves some sensory attributes like colour and texture. However, due to unaffordable inputs required with some of the preservation technologies, sun drying is the simplest, affordable and easily accessible means for poor households to preserve seasonal foods (Nyembe, 2005).

2.2.2 Nutrient contents of South African leafy vegetables.

Nutrient contents of traditional South African leafy vegetables were evaluated (Schonfeldt and Pretorius, 2011). Raw amaranth, pumpkin leaves and cat's whiskers had a high iron content and wild jute which in nutritional terms it might play a role in reducing iron deficiency in S.A (Schonfeldt and Pretorius, 2011). The minerals of these leafy vegetables decreased during cooking but had a good index of nutritional quality for protein (Schonfeldt and Pretorius, 2011). Both unprocessed and processed leafy vegetables contained high levels of beta-carotene but with low levels of vitamin B2 (Schonfeldt and Pretorius, 2011). The indigenous leafy vegetables, *Dracaena gracilis*, *Scorodocarpus borneensis*, *Gnetum gnemon*, *Pangium edule* and *Helminthostachys zeylanica* were selected and analysed to determine their nutritional contents (Ogbuji *et al*., 2016). The result showed that *S. borneensis* and *D. gracilis* contain high concentration of P, *P. edule* contains high Ca, Cu and Mg, *H. zeylanica* had highest Zn, Fe, K and Na and *G. gnemon* showed high Mn (Ogbuji *et al*., 2016; Asyira *et al*., 2016). The study of mineral contents of the indigenous leafy vegetables can help to enhance the efficiency of nutrient intake by local people and further information is required to enable the vegetables to be processed effectively (Ogbuji *et al*., 2016; Asyira *et al*., 2016).

2.2.3 Nutritional quality of leafy vegetables.

Proximate composition of leafy vegetables includes protein, ash, moisture, carbohydrates, fibre and fat (Antova *et al*., 2014). The oil content tends to be relatively low, but with an extremely high content of biologically active compounds such as tocopherols in the oils and phospholipids (Antova *et al*., 2014). According to Mosha *et al*., (1995) the traditional processing practices of sun/shade drying and storing in ventilated containers resulted in a significant decrease in ascorbic acid, riboflavin, thiamine and proximate composition of peanut, amaranth, pumpkin and sweet potato leaves (Mosha *et al*., 1995). Conventional blanching and cooking for up to 15 minutes resulted in a significant increase in riboflavin content of peanut and pumpkin greens while in amaranth and sweet potato leaves thermal processing resulted in significant decrease in vitamins (Mosha *et al*., 1995). However, the vegetables were good dietary sources of minerals, carbohydrate and protein. Proximate composition and phytochemical constituents of leaves of *Acalypha hispida*, *Acalypha marginata* and *Acalypha racemosa* were also investigated. Proximate composition of leaves of *Acalypha hispida* showed that it contained an average moisture of 11.02%, crude fat (6.15%), ash (10.32%), crude protein (13.78%), crude fibre (10.25%) and carbohydrate (44.48%) (Iniaghe *et al*., 2009). Similarly, *Acalypha marginata* showed that it contained moisture (10.83%), crude fat (5.60%), ash (15.68%), crude protein (18.15%), crude fibre (11.50%) and carbohydrate (38.24%); while *Acalypha racemosa* contained moisture (11.91%), crude fat (6.30%), ash (13.14%), crude protein (16.19%), crude fibre (7.20%) and carbohydrate (45.26%) (Iniaghe *et al*., 2009). All these results indicate that the leaves of these *Acalypha* species contain nutrients and mineral elements that may be useful in nutrition.

2.2.4 Microbial plate analysis of leafy vegetables.

Contamination of leafy vegetables is amongst the challenges that cause microbial spoilage in leafy vegetables (Adegoke and Odesla, 1996). Microorganisms responsible for leafy vegetables spoilage include fungi such as *Aspergillus* spp, *Botrytis cenera, Fusarium* spp., *Penicillium*, *Phytophthora*, *Pythium* and *Rhizopus* and bacteria such as *Erwinia*, *Carotovora*, *Clostridium* spp., *Pseudomonas* spp., *Corynebacterium* and *Xanthomonas*, *campestris*, *Escherichia coli*, *Bacillus cereus*, *Salmonella* spp., *Listeria monocytogenes*, *Shigella* spp. and *Staphylococcus* spp. (Otun, 2015). These microorganisms cause huge economic losses and are capable of producing harmful metabolites in the affected areas, constituting a potential danger to humans (Adegoke and Odesla, 1996). Microbial plate analysis observed in Otun (2015) study on the effect of processing techniques on the microbial and nutritional qualities of leafy vegetables showed the presence of yeasts and bacteria such as *Pseudomonas*, *Klebsiella*, *Staphylococci*, *Streptococci*, and *Enterobacteria* including enteropathogens such as *Salmonella* spp., *Shigella dysenteriae* and *E. coli*. The spore formers were abundant with average log coliforms per unit per gram greater than 9, several species of Bacillus were isolated (Otun, 2015). The most effective processing method which reduced microbial count to below South African Bureau of Standard’s range without reducing the nutritional quality was the washing of the leaves twice with tap water thereafter steam tunnel blanch at 94°C for 12 minutes (Otun, 2015).

2.3 Work not yet done on research problem

Cowpea leaves have been reported to have nutritional composition of minerals, amino acids and proximate composition of protein, fat, dietary fibre, carbohydrates, ash content and moisture content but are still prone to spoilage by microorganism and nutritional deterioration due to processing (Antova *et al*., 2014). Throughout the literature reviewed, more work is cited on nutritional composition of these leaves, but little scientific comparison exists between indigenous processing methods and the modern conventional processing and their effects on the nutritional composition of cowpea leaves. Therefore, effect of time-based oven-drying on the nutritional quality of cowpea leaves will be investigated.

2.4 Addressing the identified gaps

In order to address the identified gaps, the study focused on reviewing the effects of time-based oven-drying on nutritional quality of cowpea leaves. Although nutritional studies involving time-based oven-drying were still limited, most of the work had been focusing on the efficacy of oven-drying with a single period used alone.

CHAPTER 3

EFFECT OF TIME-BASED OVEN-DRYING ON THE ESSENTIAL MINERAL ELEMENTS OF COWPEA (*VIGNA UNGUICULATA*) LEAVES

3.1 Introduction

Edible leafy vegetables are rich in vitamins and essential minerals (micro and macro-nutrient) elements (Mella, 2000; Ruel *et al*., 2005). In addition to their high concentration of nutrients, leafy vegetables provide dietary energy, making them valuable in energy limited diets (Mella, 2000; Ruel *et al*., 2005). Leafy vegetable's concentrations of essential elements vary and may even exceed the critical concentrations, which is the minimum concentration required for growth. The concentrations of essential mineral elements vary from species to species (Ogbuji *et al*., 2016). Essential mineral elements are arbitrarily divided into macronutrients (elements required in higher quantities) and micronutrients (elements required in smaller quantities (Asyira *et al*., 2016). Leafy vegetables have been identified to ensure continued supply of these essential elements, therefore ensuring food security, nutrition and ultimately good health in low resourced areas (Weinberger and Msuya, 2004).

Leafy vegetables are highly perishable when stored in fresh form and therefore require preservation techniques. Majority of people in resource poor communities in South Africa predominantly achieve preservation through traditional cooking and sun drying (Njoroge *et al*., 2015). However, these methods are known to cause nutritional losses due to leaching, oxidation, radiation, and/or volatilisation (Muchoki *et al*., 2007). Gradual development and adoption of various preservation methods to make leafy vegetables available all year round are underway. However nutrient retention during preservation of cowpea is still a challenge among most rural communities of Limpopo Province. The objective of the study was to investigate the effect of time-based oven-drying on essential mineral elements of cooked and raw cowpea leaves.

3.2 Materials and methods

3.2.1 Experimental sites

Fresh cowpea leaves were harvested at Ga-Mothapo, Nobody village (23°52”0’S, 29°43”0’E) in Limpopo Province, South Africa. The location had summer rainfall with mean annual rainfall of 478 mm, while minimum/maximum temperatures average 16°C/ 36°C. The field contain Hutton soil. Samples were transported to Limpopo Agro-Food Technology Station (LATS) laboratory in polythene bags for analysis.

3.2.2 Research design and treatments

The leaves were divided into two equal portions (one portion raw and one portion cooked), each portion per drying method was laid in a randomised complete block design with 4 treatments, *viz*, 0 (sun dried), 24, 48 and 72 hours of oven-drying with 5 replications.

3.2.3 Planting and cultural practices

Cowpea seeds were directly planted in plots of 5m × 5m in late November with inter-row spacing of 60 cm and 30 cm intra-row spacing. The plants were rain-fed and weeding achieved through hand-hoeing. The plants were not fertilized and no chemicals were applied. After 5 weeks of emergence 10 plants were sampled by uprooting the entire plant and transported to the lab in polythene bags.

3.2.4 Preparation

Leaves were divided into two equal portions, the first portion of the leaves was left raw and uniformly spread on plastic trays. The second portion was cooked at 95°C in 1000 ml of water in a stainless steel pot using a DEFY 600 series stove for 3 hours. Tender and edible leaves were plated on similar plastic trays. Both portions were subjected to oven-dying at 52°C in an air-forced oven (EcoTherm, Labotech) for 24, 48 and 72 hours and sun dried for 3 days at maximum temperatures of 27°C. Dried samples were finely ground through an IKA WERIKE MAF 10 basic grinder to pass through a 1 mm sieve.

3.2.5 Method

Approximately 0.10 g dried materials was digested in 40 ml of 4% nitric acid (HNO_3), followed by placing the container on a vortex to allow for complete wetting of the mixture. The materials were magnetically stirred, thereafter incubated in a 95°C water-bath for 90 minutes, allowed to cool down at room temperature, filtered, decanted into 50 ml tubes which were covered with a foil and then selected nutrient elements were analysed using the inductively coupled plasma optical emission spectrometry (ICPE-9000).

3.2.6 Data collection

Selected essential mineral elements; P, K, Se, Ca, Mg, S, Cu, Na, Zn, Mn and Fe in mg/L data were collected.

3.2.7 Data analysis

Data were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute Inc., 2008). Least Significant Difference test was used to compare means at the probability of 5%.

3.3 Results

Mineral concentrations were significantly influenced in both raw and cooked cowpea leaves by oven-drying periods (Table 3.1 and 3.2). Relative to control (sun-drying), oven-drying period 48hrs significantly decreased K, Mn, and Na contents (Table 3.1) in raw cowpea leaves by 6, 9 and 13%, respectively. Similarly, oven-drying period 72hrs significantly decreased raw cowpea Ca, Fe, Mg, Zn, P and S content (Table 3.1) by 5, 11, 16, 18 and 57%, respectively . In cooked cowpea leaves, relative to control (sun-drying), oven-drying period 24hrs significantly increased Na content (Table 3.2) by 18%. Similarly 48hrs oven-drying periods increased Fe and K content (Table 3.2) by 6 and 8%, respectively. Similarly, oven-drying period 72hrs significantly increased Ca, Mg and Mn contents (Table 3.2) by 8, 8 and 3%, respectively. In contrast, oven-drying period 72hrs significantly reduced Zn, P and S contents by (Table 3.2) by 16, 10 and 39%, respectively.

Table 3.1 Response of essential minerals on raw cowpea leaves subjected to different oven-drying periods.

	Ca mg/ L	Fe mg/ L	K mg/L	Mg mg/ L	Mn mg/ L	Na mg/L	Zn mg/L	P mg/L	S mg/L
Treatment	Y-Value	Y-Value	Y-Value	Y-Value	Y-Value	Y-Value	Y-Value	Y-Value	Y-Value
Sun-drying	14,660^{a}	1,386^{ay}	19,100^{a}	3,4920^{a}	0,732^{a}	3,890^{a}	0,6900^{a}	0,6600^{a}	2,1088^{a}
24hrs	14,600^{a}	1,306^{ab}	18,640^{ab}	3,2460^{a}	0,691^{ab}	3,794^{a}	0,6600^{a}	0,5800^{bc}	0,9812^{b}
48hrs	14,080^{a}	1,262^{ab}	17,960^{b}	3,2080^{a}	0,663^{b}	3,394^{b}	0,6000^{b}	0,6100^{ab}	0,9230^{b}
72hrs	13,980^{a}	1,240^{b}	18,440^{a}	3,1420^{a}	0,676^{ab}	3,514^{ab}	0,5800^{b}	0,5400^{c}	0,8964^{b}

[Z]Relative Impact = [(treatment/control – 1) × 100].

[Y]Column means the same letter were not different ($P \leq 0.05$) according to Least Significant Difference test.

Table 3.2 Response of essential minerals on cooked cowpea leaves subjected to different oven-drying periods.

	Ca mg/ L	Fe mg/ L	K mg/ L	Mg mg/L	Mn mg/L	Na mg/ L	Zn mg/L	P mg/L	S mg/L
Treatment	Y-Value	Y-Value	Y-Value	Y-Value	Y-Value	Y-Value	Y-Value	Y-Value	Y-value
Sun-drying	11,680[b]	1,140[b]	14,820[b]	2,676[b]	0,562[a]	3,140[c]	0,695[a]	0,6370[a]	1,476[a]
24hrs	12,000[ab]	1,186[ab]	15,660[a]	2,820[a]	0,570[a]	3,712[a]	0,646[ab]	0,6252[a]	0,950[b]
48hrs	12,380[a]	1,206[a]	15,960[a]	2,852[a]	0,573[a]	3,346[bc]	0,585[b]	0,6162[ab]	0,913[b]
72hrs	12,600[a]	1,170[ab]	15,820[a]	2,894[a]	0,580[a]	3,584[ab]	0,582[b]	0,5746[b]	0,898[b]

[Z]Relative Impact = [(treatment/control – 1) × 100].

[Y]Column means the same letter were not different ($P \leq 0.05$) according to Least Significant Difference test.

3.4 Discussion

The increment of Ca, Fe, K, Mg, Mn and Na contents in cooked cowpea leaves content observed in the current study has been attributed to the heat-stability of these essential mineral elements during cooking process in other studies (Beruk *et al*., 2015) (Table 3.2). Iron values in raw and cooked sun-dried cowpea leaves were 1,386 mg/L and 1,140 mg/L respectively (Table 3.1 and 3.2). Raw cowpea leaf Fe Content was significantly decreased to 1,240 mg/L after 72 hrs of drying (Table 3.1) while cooked cowpea leaf Fe content was increased to 1,206 mg/L after 48hrs of drying (Table 3.2). The results of the current study can be attested to that observed by different studies (Ogbuji *et al*., 2016 and Gupta *et al*., 2002). Iron is crucial in the diet especially for pregnant and nursing mothers as well as infants. It is also needed by the elderly to reduce cases of diseases associated with deficiency of iron such as anemia (D'Mello, 2003) and is also needed for haemoglobin formation (Fasuyi, 2006). K values of sundried raw and cooked cowpea leaves were 19,100 mg/L and 14,820 mg/L (Table 3.1 and 3.2). The increase in dietary K has been reported to lower blood pressure and reduces the risk of stroke in humans (Sawka, 2005). The results of the current study can be attested to that observed by different studies (Aletor *et al*., 2002).

Mg values of sundried cooked and raw cowpea leaves were 2,676 and 3,4920 mg/L respectively (Table 3.1 and 3.2). Mg is needed in treating of diarrhoea and other gastrointestinal defects when taken in about 470 mg/day. It also has the ability to treat duodenal cancers when 1200 mg/day is ingested, secondary coronary heart diseases and congested heart failure when about 384 mg/day is taken. The Mg RDAs ranges between 26 and 260 mg/day for the various human categories (FAO, 2001). The values of magnesium are different from those reported in this work; the difference might due to soil compositions and the rate of uptake of minerals by individual vegetables (Anjorin et al., 2010). Magnesium is good by human health as it is known to reduce blood pressure (Song *et al*., 2004). Ca values of sundried raw and cooked cowpea leaves were 14,660 mg/L and 11,680 mg/L (Table 3.1 and 3.2). The decrease in Ca has been reported to cause hypocalcaemia subsequently poor bones and teeth strength and development (Osborne and Voogt, 1978). The results of the current study were similar to that observed by the study of Njoroge *et al*., (2015). P values of sundried raw and cooked cowpea leaves were 0,6600 mg/L and 0,6370 mg/L (Table 3.1 and 3.2). P content was significantly decreased to 0,5400 mg/L after 72 hrs of drying in raw cowpea leaves (Table 3.1) while cooked cowpea leaf P content was increased to 0,5746 mg/L after 72hrs of drying (Table 3.2). Low concentrations could be attributed by the chemical forms of the element and their concentration in the environment, as South African soils have been reported to have the poorest availability of P in the soil (Sobiso *et al*., 2017). Calcium, phosphorus and magnesium minerals are involved in the building and maintaining of rigid structures to support the body in appreciable quantity, are essential for the proper formation of bones and teeth (Osborne and Voogt, 1978). For example, in calcium, 99% of the total amount (i.e. 1000–1200 g in adult) occurs in bones and teeth while about 600–700 g of phosphorus is also present in bones and teeth. The two elements, together with a much smaller quantity of magnesium (20–80 g), form a crystal lattice which is largely responsible for the rigidity and strength of bones and teeth (Osborne and Voogt, 1978).

Na values of sundried cooked and raw cowpea leaves ranged from 3,140 to 3,890 mg/L (Table 3.1 and 3.2). Raw cowpea leaf Na content was significantly decreased to 3,394 mg/L after 48hrs of drying (Table 3.1) while cooked cowpea leaf Na content was increased to 3,712 mg/L after 24hrs of drying (Table 3.2). The results of the current study can be attested to that observed by a different study of Belane and Dakora, (2011). Na is an important element, although it is often maligned as a cause of high blood pressure, it also plays several roles in the body. Sodium helps control blood pressure and regulates the function of muscles and nerves (WHO, 2012; Whelton *et al*., 2012). Deficiency of Na leads to the disturbance of tissue-water and acid-base balance that is important to good nutritional status (WHO, 2012; Whelton *et al*., 2012).

Mn values of sundried raw and cooked cowpea leaves were 0,732 mg/L and 0,562 mg/L, respectively (Table 3.1 and 3.2). The decrease in Mn raw cowpea leaves has been reported to reduce creation of essential enzymes for building bones, forming connective tissues, reduced regulation of blood sugar levels and metabolism of fats and carbohydrate amongst other things (Crossgrove and Zheng, 2004). The results of this study are similar to those reported in the study of Asaolu *et al*., (2012) in leafy vegetables. Zn values of sundried cooked and raw cowpea leaves were 0,695 and 0,6900 mg/L respectively (Table 3.1 and 3.2). Zn is important in treating ulcers, acne and sickle cell anaemia when taken in about 34 mg/day. It also has the ability to treat herpes, high cholesterol and rheumatoid arthritis when 40 mg/day is ingested (Bhowmik and Chiranjib, 2010).

S values of sundried cooked and raw cowpea leaves ranged from 2,1088 to 1,476 mg/L (Table 3.1 and 3.2). Raw cowpea leaf S content was significantly decreased to 0,8964 mg/L after 72 hrs of drying (Table 3.1) while cooked cowpea leaf S content was decreased to 0,898 mg/L after 72hrs of drying (Table 3.2). The results of the current study can be attested to that observed in the study of Medoua and Oldewage-Theron, (2014). S is important in making vital amino acids used to create protein for cell, tissues, hormones, enzymes and antibodies (WHO, 2012). Sulphur is believed to build flexible cells in the arteries and veins to allow oxygen and nutrient to pass through their walls for ease breaths (WHO, 2012).

3.5 Conclusion

Time-based oven-drying is researched to develop an efficient drying method of retaining nutrient elements in cowpea leaves. In the current study, in cooked leaves, time-based oven-drying promoted and retained most essential elements, except for Zn, P and S. therefore it could be recommended for cooked leaves.

CHAPTER 4

EFFECT OF TIME-BASED OVEN-DRYING ON THE PROXIMATE COMPOSITION OF COWPEA (*VIGNA UNGUICULATA*) LEAVES

4.1 Introduction

Food demands have been increased with the increasing human population growth resulting in high demands for growing food crops especially leafy vegetables (Aletor *et al*., 2002). Vegetables being the rich source of carbohydrates, fats and proteins which form major part of human diet, are the cheapest source of energy (Aletor *et al*., 2002; Hussain *et al.,* 2009; Kwenin *et al*., 2011). The importance of this biochemical has been recorded by various scientists (Aletor *et al*., 2002). Besides the bio-chemicals, moisture, fibre and ash contents as well as the energy values of individual vegetable and plant species have also been regarded crucial to human health and soil quality (Hussain *et al.,* 2010). Most developing countries depend on starch based food and leafy vegetables as the staple food for the supply of both energy and protein (Onwordi *et al*., 2009). This accounts in part for protein, vitamins, minerals and essential amino acids deficiencies which prevail among the population (Onwordi *et al*., 2009). However the challenge is the perishability of these foods and contamination by vegetable spoiling microorganisms.

The preservation of foods is achieved through pasteurization and/or drying. Pasteurization requires food to be sterilized and sealed after treatment to avoid any new/re-contamination, whereas drying methods decrease the water activity of the product and consequently, reduce growth of microorganisms and decrease chemical reactions in order to extend the shelf life of the product at room temperature (Rahman, 2007). However, these methods affect the nutritional value of leafy vegetables through modification or direct loss of proximate (Medoua *et al*., 2014). Proximate composition of food namely; carbohydrates, fats, proteins, moisture, fibre, ash contents and the energy are also important biochemicals which are required in the body and should form major portion of human diet. However, the biochemicals are adversely affected by different processing methods. Therefore, the study intends to investigate the effect of time-based oven-drying on proximate composition of cowpea leaves.

4.2 Materials and methods

The experimental sites, research design and treatments and data analysis were as described in chapter 3.

4.2.1 Method

Ash was determined by heating samples at 550°C for 6 hours in a muffle furnace and moisture content was determined using RADWAG max 50 (Lasec SA, Cape Town SA). An Allihan Condenser Soxhlet extraction apparats was used to determine fat content with petroleum ether as an solvent, which was evaporated at 60-80°C and the fat left inside the beaker. Weight gained was used to calculate the fat content. Nitrogen was determined using Dumas method (Leco Truspec N, Michigan USA) and the quantity of protein calculated as 6.25 × N. Fibre content was determined as described in Association of Official Analytical Chemists 1995 and carbohydrates determined by difference method.

4.2.2 Data collection

The proximate composition data collected was ash content, moisture content, fat %, protein content, energy content, carbohydrate content and fibre content.

4.3 Results

Proximate composition was significantly influenced in both raw and cooked cowpea leaves by different oven-drying periods (Table 4.1 and 4.2). There was a significant decrease in protein, ash, moisture and carbohydrate content in raw cowpea leaves whereas in cooked cowpea leaves a decrease in protein, moisture, ash, fat, fibre and carbohydrate content was observed. In both raw and cooked cowpea leaves, protein content and ash was significantly reduced under 72hrs oven-drying period by 10% and 8%, respectively as well as 18 and 13%, respectively, similarly moisture content of both raw and cooked cowpea leaves was significantly decreased by 7% and 14% at 72hrs oven drying period, respectively (Table 4.1 and 4.2).

Both raw and cooked cowpea leaf energy content was significantly increased by 3% and 1%, respectively, under 48 hours of oven-drying period (Table 4.1 and 4.2). Relative to sun-drying, 24hrs, 48hrs and 72hrs oven-drying periods increased raw cowpea fat content by 46%, 28%, 34%, respectively (Table 4.1), whereas 24hrs, 48hrs and 72hrs oven-drying periods decreased cooked cowpea leaf fat content by 3%,12%, and 19%, respectively (Table 4.2). Relative to sun-drying, 72hrs oven-drying periods significantly decreased both raw and cooked carbohydrates by 7% and 10%, respectively (Table 4.1 and 4.2). Relative to sun-drying, 72hrs oven-drying periods decreased raw cowpea fibre content by 0,5%, whereas 24hrs oven-drying periods decreased cooked fibre content by 0,4% (Table 4.1 and 4.2).

Table 4.1 Response of proximate composition on raw cowpea leaves subjected to different oven-drying periods.

	Protein	Moisture	Energy	Ash	Fat	Fibre	Carbohydrates
Treatment	Y-value %	Y-value %	Y-value %	Y-value %	Y-value %	Y-value %	Y-value %
Sun-drying	16,634[a]	7,388[a]	16,926[b]	7,942[a]	2,794[c]	14,152[a]	56,410[a]
24hrs	16,632[a]	7,578[a]	17,224[ab]	7,704[a]	4,078[a]	14,132[a]	53,760[b]
48hrs	15,814[b]	6,876[a]	17,266[ab]	7,762[a]	3,586[b]	14,106[a]	52,532[b]
72hrs	14,902[c]	5,230[b]	17,424[a]	6,488[b]	3,730[ab]	14,086[a]	52,526[b]

[Z]Impact = [treatment/control – 1) × 100].

[Y]Column means the same letter were not different ($P \leq 0.05$) according to Least Significant Difference test.

Table 4.2 Response of proximate composition on cooked cowpea leaves subjected to different oven-drying periods.

	Protein	Moisture	Energy	Ash	Fat	Fibre	Carbohydrates
Treatment	Y-value %	Y-value %	Y-value %	Y-value %	Y-value %	Y-value %	Y-value %
Sun-drying	15,324[a]	7,200[a]	17,488[ab]	6,814[a]	4,006[a]	15,948[a]	53,760[a]
24hrs	15,296[a]	7,578[a]	17,724[a]	6,564[a]	3,902[a]	15,900[b]	50,062[b]
48hrs	15,074[a]	7,002[a]	17,660[a]	6,494[ab]	3,538[ab]	15,892[b]	49,294[b]
72hrs	14,130[b]	6,166[b]	17,372[b]	5,956[b]	3,252[b]	15,890[b]	48,116[b]

[Z]Impact = [treatment/control – 1) × 100].

[Y]Column means the same letter were not different ($P \leq 0.05$) according to Least Significant Difference test.

4.4 Discussion

The decrease in protein, moisture, ash, fibre and carbohydrates contents in raw and cooked cowpea leaves observed in the current study has been linked to denaturing, evaporation and instability of certain proximate, browning during cooking process and diffusion in other studies (Aletor *et al*., 2002) (Table 4.1 and 4.2). Protein values in raw and cooked sun-dried cowpea leaves were 16,634% and 15,324%, respectively (Table 4.1 and 4.2). Raw cowpea leaf protein content was significantly decreased to 14,902% after 72 hrs of drying (Table 4.1) while cooked cowpea leaf protein content was decreased to 14,130% after 72hrs of drying (Table 4.2). The results of the current study are similar to that observed by Kubmarawa *et al*., (2009). Protein is important for performing a number of functions within in the human body, these functions include catalysing metabolic reactions, deoxyribonucleic acid replication, responding to stimuli and transporting molecules (Saldanha, 1995).

Moisture values of sundried raw and cooked cowpea leaves were 7,388% and 7,200% (Table 4.1 and 4.2). The decrease in moisture has been reported to extent the shelf life period of leafy vegetables by lowering the water activity therefore inhibiting microbial growth and chemical reaction that lead to deterioration. However, low water in food is critical as water is an important constituent for transporting oxygen, fat and glucose to muscles, regulate body temperature and ease digestion of food and excretion of waste (Rahman, 2007; Kalapo and Sanni, 2007). The results of the current study are similar to those observed by Asaulo *et al*., (2002). Energy values of sundried cooked and raw cowpea leaves were 16,926 and 17,488 Kj/g respectively (Table 4.1 and 4.2). Energy is required for metabolic functions, muscular activity, physiological functions, heat production, growth and synthesis of new tissues (Pearson, 1976). Energy can be obtained from many foods, however the recommended calorie intake is 10% from added sugars, 10% from saturated fats and 2300 mg of sodium, this recommendation help promote health and reduce the risk of chronic diseases (WHO, 2012). The values of ash in the study of Onyeike and Oguike (2003) on groundnut (*Arachis hypogaea*) are significantly different from those reported in this work; the difference might be due to evolution of water and other volatile constituents as vapours. Ash is a good indicator of the total amount of minerals within food, important component for preservation and nutritional evaluation and its content is a widely accepted index of refinement of foods (Lewu *et al*., 2009).

Fibre and carbohydrates are important for a healthy system and metabolism; maintain a healthy weight and lowering a risk of diabetes and heart diseases (Hussain *et al*., 2010). Fibre is a type of carbohydrate that the body cannot digest. Thirty grams of fibre in adults prevent and relieve constipation while 325g of carbohydrate in adults help lower blood cholesterol and help lower glucose levels (WHO, 2012). The two proximate, together reduces the risk of developing various conditions including heart disease, diabetes, diverticular diseases and constipation (Badifu, 2001; Lintas, 1992).

Fat values in raw and cooked sun-dried cowpea leaves were 2,794% and 4,006% respectively (Table 4.1 and 4.2). Raw cowpea leaf fat content was significantly increased to 6,488% after 72 hrs of drying (Table 4.1) while cooked cowpea leaf fat content was decreased to 3,252% after 72hrs of drying (Table 4.2). The results of the current study are similar to that observed by Badifu (2001). Fat is important for absorbing fat-soluble vitamins such as vitamin A, D, E and K and fat deficiency subsequently cause deficiencies associated with these vitamins; skin problems, cognitive problems and vision problems (Lintas, 1992). The change in fat contents in raw and cooked cowpea leaves observed in the current study has been attributed to unavailability due to strong double bond within the fatty acid chain, heat sensitivity and solubility in water, denaturing, evaporation and instability of certain proximate, browning during cooking process and diffusion in other studies (Pearson, 1976) (Table 4.2).

4.5 Conclusion

Proximate composition as affected by oven-drying period is researched to review the impact of this processing method on the nutritional quality to develop a regime that retains the nutritional quality of leafy vegetables. In the current study, oven-drying periods were observed to reduce the proximate components of cooked cowpea leaves. The oven-drying periods also had positive effects on some proximate composition on raw cowpea leaves and this could affect nutrition and subsequently food security. Therefore it is advisable to dry leafy vegetables raw at 24 hours of oven-drying for optimum proximate.

CHAPTER 5

EFFECT OF TIME-BASED OVEN-DRYING ON THE MICROBIAL PROFILING OF COWPEA (*VIGNA UNGUICULATA*) LEAVES

5.1 Introduction

Leafy vegetables are readily available as food all year and, thus, help to promote food security (Okonya *et al*., 2010). However, research has shown that around a quarter of all fresh harvested leafy vegetables are rotten prior to reaching consumers as a result of spoilage through microbial contamination (Otun, 2015). Microorganisms are natural contaminants of fresh produce and minimally processed fresh-cut products, and contamination arises from a number of sources, including postharvest handling and processing (Mpuchane and Gashe, 1998). Due to the nature of the treatments applied to this type of product, a conducive environment and time for growth of spoilage organisms and microorganisms of public health significance is created (Mpuchane and Gashe, 1998). Microorganisms found on vegetable leaves include bacteria or fungi that have grown on and colonized the leaf surface by utilizing nutrients exuded from plant tissues (Barth *et al*., 2009). These could be toxin producing microorganism or spoilage-causing microorganisms. Besides causing huge economic losses, some fungal species could produce harmful metabolites in the affected areas, constituting a potential health concern for humans (Barth *et al*., 2009). Therefore, these perishable leafy vegetables require special processing methods to reduce microbial count, risk of food poisoning and to prevent post-harvest losses thus prolonging their shelf-life (Otun, 2015).

It is well-known that processing of vegetables without lowering the water activity enhances a faster physiological deterioration, biochemical changes and microbial deterioration of the product even when only minimal processing techniques can be used (Otun, 2015), which may result in deterioration of the colour, texture and flavour (Otun, 2015). While conventional food-processing techniques extend the shelf-life of fruit and vegetables, the minimal processing to which vegetables are subjected renders products still with some contamination (Syne *et al*., 2013). Therefore, the objective of the study was to investigate the effect of time-based oven-drying on microbial profiling of cowpea leaves.

5.2 Materials and methods

Experimental sites, research design and treatments and data analysis were as described in chapter 3.

5.2.1 Method

The total viable count (TVC) enumerating the total population of viable microorganisms was used to determine the microbial organism responsible for microbial spoilage. Ten (10) g of each dried sample was weighed and transferred into Tempo polyethylene bag, diluted with peptone water and placed into Tempo Biomerieux system for preparation and analysis used according to manufacturer's instructions.

5.2.2 Data collection

Data collected for microbial profiling was total coliforms unit of *Shigella* spp, *Salmonella* spp, *Escherichia coli*, *Pseudomonas* spp, *Staphylococcus* spp and *Bacillus cereus* per gram of each sample.

5.3 Results

Relative to control (sun-drying), oven-drying periods 24hrs significantly increased *Staphylococcus* spp. in raw cowpea leaves by 6%, respectively (Table 5.1). Relative to control (sun-drying) 72hrs oven-drying period significantly decreased *Shigella* spp. by 92%, respectively (Table 5.1). In cooked leaves, relative to control (sun-drying), 72hrs drying periods decreased both *Shigella* spp. and *Staphylococcus* spp. by 99 and 21%, respectively (Table 5.2). Total coliforms unit of *Salmonella* spp, *Escherichia coli*, *Pseudomonas* spp, and *Bacillus cereus* were absent and/or at an undetectable level according to the Tempo Biomerieux system results.

Table 5.1 Response of Total coliforms of micro-organisms responsible for spoilage on raw cowpea leaves subjected to different oven-drying periods.

	Shigella spp cfu/ g	*Staphylococcus* spp cfu/ g
Treatment	Y-value	Y-value
Sun-drying	16,80[a]	4,760[c]
24hrs	3,000[b]	5,060[a]
48hrs	2,600[bc]	5,040[a]
72hrs	1,400[c]	4,920[bc]

[Z] Impact = [(treatment/control – 1) × 100].

[Y]Column means the same letter were not different ($P \leq 0.05$) according to Least significant difference test.

Table 5.2 Response of Total coliforms of micro-organisms responsible for spoilage on cooked cowpea leaves subjected to different oven-drying periods.

	Shigella spp cfu/ g	*Staphylococcus* spp cfu/ g
Treatment	Y-value	Y-value
Sun-drying	84,80[a]	4,280[a]
24hrs	2,800[b]	4,260[a]
48hrs	2,000[b]	4,060[a]
72hrs	1,000[b]	3,400[b]

[Z]Impact = [(treatment/control – 1) × 100].

[Y]Column means the same letter were not different ($P \leq 0.05$) according to Least Significant Difference test.

5.4 Discussion

The decrease in the count of total coliforms of *Shigella* spp. in raw and cooked cowpea leaves in 72 hours oven-drying and decrease in the count of total coliforms of cooked *Staphylococcus* spp. observed in the current study has been attributed to lowered water activity that inhibit the growth and proliferation of microorganism in other studies (Aletor *et al*., 2002) (Table 5.1 and 5.2). *Shigella* spp. values in raw and cooked sun-dried cowpea leaves were 16,80 and 84,80 cfu/ g respectively (Table 5.1 and 5.2). Raw cowpea leaf *Shigella* spp. total coliform count was significantly decreased to 1.400 cfu/ g after 72 hrs of oven-drying (Table 5.1) while cooked cowpea leaf protein content was decreased to 1.000% after 72hrs of oven-drying (Table 5.2). The results of the current study are similar to that observed by Otun (2015) in moringa leaves. *Shigella* spp. is one of the leading bacterial causes of diarrhoea worldwide. Mpuchane and Gashe (1998) explained that *Shigella* spp is soil borne and is transmitted through the faecal-oral route and enter the human body via the ingestion of food or water contaminated with the bacteria and poor hygiene and lack of access to clean water and sanitation promote the spread of this enteric disease.

Total coliform counts of Staphylococcus spp. of sundried raw and cooked cowpea leaves were 4,760 cfu/ g and 4,280 cfu/ g (Table 5.1 and 5.2). The increase in *Staphylococcus* spp. in raw cowpea leaves has been reported to accelerate the deterioration of leafy vegetables and reduce the shelf life period of leafy vegetables and increase food poisoning caused by the pathogen (Lowy, 1998). Intoxication is caused by the ingestion of enterotoxins within food left usually at room temperature (Balaban and Rasooly, 2000). *Staphylococcus* spp is a common cause of community-acquired urinary tract infections (Lowy, 1998). Relative to control, raw cowpea leaf *Staphylococcus* spp. total coliform count was significantly increased to 4,920 cfu/ g after 72 hrs of oven-drying (Table 5.1) similarly, cooked cowpea leaf leaf *Staphylococcus* spp. was decreased to 3,400 cfu/ g after 72hrs of oven-drying (Table 5.2). The results of the current study are similar to that observed by Mpuchane and Gashe (1998).

5.5 Conclusion

Leafy vegetables are good sources of nutrients (vitamins, minerals, and dietary fiber) and water, but also for microorganism growth under ambient conditions leading to produce deterioration. Microbial spoilage can occur in the field production or any stage of the supply chain, which includes postharvest handling, packaging, and in certain cases during processing operations. Thus, safety is considered to be the most important component of quality and it can be compromised by pathogenic microorganisms. In the current study, oven-drying periods were observed to reduce microorganisms. Cooking followed by oven-drying had negative effects on microorganism that are responsible for spoilage on cowpea leaves. Therefore, it is advisable to cook then dry leafy vegetables for longer (72 hours of oven-drying) to reduce the total count of microorganism; however, this could be crucial to nutritional quality.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The study was primarily involved in research and development of processing method specifically oven-drying of leafy vegetable to improve and retain their nutritional quality during preservation. The study was carried out to determine the effect of time-based oven-drying on the essential nutrient elements, proximate composition and microbial profiling of cowpea leaves. Oven-drying periods demoted the nutritional quality of raw cowpea leaves, with essential nutrient elements decreased and increased in cooking by 72hrs oven-drying periods, proximate components also was reduced by oven-drying except for energy and microbial plate count also reduced. Oven-drying periods in the current study were rather unbeneficial, since the nutritional quality was reduced and there were negative effects on the nutritional qualities tested. Empirical research demonstrated that the form which leafy vegetables should be dried is when cooked than raw.

6.2 Conclusion

Oven-drying periods showed undesirable effects on the nutritional quality of cowpea leaves, when leaves used during trials were in raw conditions. The leaves showed potential in retaining essential mineral elements Ca, Fe, K, Mg, Mn and Na when cooked and oven-dried at 72hrs and also reduction in total coliforms of microbe responsible for spoilage when cooked prior oven-drying at 72hrs than when dried raw. However, there was undesirable results in proximate composition as oven-drying periods greater than 24hrs in both raw and cooked leaves reduced the protein content of cowpea leaves by significant amounts which is important in rural areas where leafy vegetables are a source of protein. Oven-drying period of 24 hours while cooked can be used to minimize the loss of protein in cowpea leafy vegetable. Cooked cowpea leaves subjected to 72hrs of oven-drying had least total coliforms for both *Shigella* spp and *Staphylococcus* spp, therefore have potential to serve as an alternative to sun-drying to reduce microorganism causing spoilage in leafy vegetables. Drying raw cowpea leaves under oven-drying periods less than 72hrs should be avoided as it reduces the mineral concentration and increase microbial count of microorganisms responsible for spoilage. Findings in this study provide preliminary nutritional composition of cowpea leaves after processing for consideration when processing to attain optimal nutrient retention.

6.3 Recommendations

In the study, the concentrations of minerals were retained when cowpea leaves were raw and sundried and oven-dried cooked, whereas proximate compositions were retained when raw cowpea leaves were oven-dried at 24hrs of oven-drying. The study was aimed at addressing two issues: (a) preservation of cowpea leaves while raw or cooked, it would be necessary to test all nutritional parameters on cowpea to verify whether cooking increase/ retain the nutritional quality of cowpea leaves. (b) time-based oven-drying did not minimise mineral losses, therefore for more preservation of minerals raw leaves can be sundried, whereas raw oven dried leaves had retained more proximate composition and cooked oven-drying leaves reduced total coliforms of micro-organism responsible for spoilage.

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APPENDICES

3.1 Analysis of variance for Ca in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,65700	0,16425		
Treatment	3	1,83400	0,61133	2,09	0,1551
Error	12	3,51100	0,29528		
Total	19	6,0020			

3.2 Analysis of variance for Fe in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,07623	0,01906		
Treatment	3	0,06234	0,02078	2,23	0,1370
Error	12	0,11169	0,00931		
Total	19	0,25025			

3.3 Analysis of variance for K in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,54800	0,13700		
Treatment	3	3,34950	1,11650	9,25	0,0019
Error	12	1,44800	0,12067		
Total	19	5,34550			

3.4 Analysis of variance for Mg in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,26452	0,06613		
Treatment	3	0,35036	0,11679	1,51	0,2631
Error	12	0,93044	0,07754		
Total	19	0,1,54532			

3.5 Analysis of variance for Mn in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,00700	1,749		
Treatment	3	0,01325	4,417	2,18	0,1436
Error	12	0,02434	2,028		
Total	19	0,04459			

3.6 Analysis of variance for Na in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,17487	0,04372		
Treatment	3	0,81176	0,27059	3,59	0,0464
Error	12	0,90409	0,07534		
Total	19	1,89072			

3.7 Analysis of variance for Zn in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,02726	0,00681		
Treatment	3	0,04055	0,01352	8,60	0,0026
Error	12	0,01886	0,00157		
Total	19	0,08667			

3.8 Analysis of variance for P in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,01612	0,00403		
Treatment	3	0,04083	0,01361	5,53	0,0128
Error	12	0,02954	0,00246		
Total	19	0,08649			

3.9 Analysis of variance for S in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	1,14220	0,28555		
Treatment	3	5,19850	1,73283	6,30	0,0082
Error	12	3,30021	0,27502		
Total	19	9,64091			

3.10 Analysis of variance for Ca in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	1,07200	0,26800		
Treatment	3	2,40400	0,80133	3,73	0,0418
Error	12	2,57600	0,21467		
Total	19	6.052			

3.11 Analysis of variance for Fe in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,00587	1,468		
Treatment	3	0,01166	3,885	2,54	0,1058
Error	12	0,01837	1,531		
Total	19	0,03590			

3.12 Analysis of variance for K in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,36300	0,09075		
Treatment	3	3,92550	1,30850	5,74	0,0113
Error	12	2,73700	0,22808		
Total	19	7,02550			

3.13 Analysis of variance for Mg in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,03377	0,00844		
Treatment	3	0,13437	0,04479	5,65	0,0119
Error	12	0,09515	0,00793		
Total	19	0,26329			

3.14 Analysis of variance for Mn in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	8,370	2,092		
Treatment	3	1,166	3,888	1,70	0,2197
Error	12	2,743	2,285		
Total	19	4,746			

3.15 Analysis of variance for Na in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,09582	0,02396		
Treatment	3	0,96717	0,32239	6,56	0,0071
Error	12	0,58990	0,04916		
Total	19	1,65289			

3.16 Analysis of variance for Zn in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,00812	0,00203		
Treatment	3	0,04398	0,01466	6,58	0,0070
Error	12	0,02673	0,00223		
Total	19	0,07884			

3.17 Analysis of variance for P in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,00295	7,371		
Treatment	3	0,01105	3,682	2,77	0,0873
Error	12	0,1594	1,329		
Total	19	0,02994			

3.18 Analysis of variance for S in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,14333	0,03583		
Treatment	3	1,16591	0,38864	12,20	0,0006
Error	12	0,38222	0,03185		
Total	19	1,69146			

4.1 Analysis of variance for Protein in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,4971	0,12428		
Treatment	3	10,2075	3,40250	22,63	0,0000
Error	12	1,8039	0,15032		
Total	19	12,5085			

4.2 Analysis of variance for Moisture in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,0805	0,02013		
Treatment	3	17,0880	5,69601	15,61	0,0002
Error	12	4,3780	0,36483		
Total	19	21,5465			

4.3 Analysis of variance for Energy in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,12510	0,03127		
Treatment	3	0,64892	0,21631	1,79	0,2032
Error	12	1,45278	0,12107		
Total	19	2,22680			

4.4 Analysis of variance for Ash in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	3,7046	0,92616		
Treatment	3	6,6353	2,21177	3,50	0,0497
Error	12	7,5847	0,63206		
Total	19	17,9247			

4.5 Analysis of variance for Fat in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,31492	0,07873		
Treatment	3	4,41990	1,47330	14,47	0,0003
Error	12	1,22180	0,10182		
Total	19	5,95662			

4.6 Analysis of variance for Fibre in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,25943	0,06486		
Treatment	3	0,12580	0,00419	0,87	0,4830
Error	12	0,05777	0,00481		
Total	19	0,322978			

4.7 Analysis of variance for carbohydrates in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	10,6607	2,6607		
Treatment	3	50,2220	16,7407	14,14	0,0009
Error	12	18,0300	1,5025		
Total	19	78,8948			

4.8 Analysis of variance for Protein in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	1,30308	0,32577		
Treatment	3	4,73612	1,57871	6,70	0,0066
Error	12	2,82848	0,23571		
Total	19	8,86768			

4.9 Analysis of variance for Moisture in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,23948	0,05987		
Treatment	3	5,34457	1,78152	5,29	0,0148
Error	12	4,04040	0,33670		
Total	19	9,62445			

4.10 Analysis of variance for Energy in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,06948	0,01737		
Treatment	3	0,54869	0,18290	5,89	0,0104
Error	12	0,37268	0,03106		
Total	19	0,99085			

4.11 Analysis of variance for Ash in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	1,38977	0,34744		
Treatment	3	1,95634	0,65211	3,36	0,0550
Error	12	2,32631	0,19386		
Total	19	5,67242			

4.12 Analysis of variance for Fat in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	1,02037	0,25509		
Treatment	3	1,79394	0,59798	4,80	0,0201
Error	12	1,49399	0,12450		
Total	19	4,30830			

4.13 Analysis of variance for Fibre in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,48350	0,12088		
Treatment	3	0.01121	0,00374	3,28	0,0584
Error	12	0.01366	0,00114		
Total	19	0,50838			

4.14 Analysis of variance for Carbohydrate in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	3,380	0,8450		
Treatment	3	89,049	29,6831	14,12	0,0003
Error	12	25,227	2,1022		
Total	19	117,656			

5.1 Analysis of variance for *Staphylococcus spp* in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,10300	0,02575		
Treatment	3	0,56550	0,18850	12,78	0,0005
Error	12	0,17700	0,01475		
Total	19	0,84550			

5.2 Analysis of variance for *Shigella spp* in raw cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	12,200	3,050		
Treatment	3	791,750	263,917	243,62	0,0000
Error	12	13,000	1,083		
Total	19	816, 950			

5.3 Analysis of variance for *Staphylococcus spp* in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	0,20500	0,05125		
Treatment	3	2,54800	0,84933	27,77	0,0000
Error	12	0,36700	0,03058		
Total	19	3,12000			

5.4 Analysis of variance for *Shigella spp* in cooked cowpea leaves subjected to different oven-drying periods.

Source	DF	SS	MS	F	P
Replication	4	32,3	8,07		
Treatment	3	25759,0	8586,32	925,75	0,0000
Error	12	111,3	9,27		
Total	19	25902,6			