INFLUENCE OF BOTANIC AND GEOGRAPHIC ORIGIN ON QUALITY OF HONEY FROM TANZANIA

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EXTENDED ABSTRACT

In a view of the expanding global market, characterization of floral and geographic origins of honey has become a more important issue than ever. In general, prices of honey at the international market are set according to floral and geographic origin of the nectar, which relate to the chemical composition. This study was conducted to assess and characterize Tanzania honey from different floral origins and geographic zones based on physicochemical properties and minerals content, sugar profile, total phenol and antioxidant activities as well as sensory properties and consumer acceptability. Samples from two floral origins, *Miombo* and *Acacia* were collected from five different zones in Tanzania namely Central, Coastal, Lake, Northern and Southern zones and subjected to chemical and sensory analyses. Complete randomized block design with floral origin and geographic zones as main principal factors were used to assess the effect of these factors on stated parameters.

Moisture content, ash, pH, acidity, viscosity and colour were determined as per methods described in AOAC (2005). Phenolic content was determined using modified Folin Ciocalteau method and antioxidant activity using Ferric Reducing Antioxidant Power (FRAP) assay. Sugar profile and quantification was performed using Shimadzu high-performance liquid chromatograph equipped with refractive index detector (RID-10A) as per method 977.20 in AOAC (2005). Minerals: copper, zinc, lead, iron, calcium and magnesium were determined using Atomic Absorption Spectophotometer (AAS), sodium and potassium using flame photometer as described by AOAC standard methods (2005). Quantitative Descriptive sensory analysis was done using a panel of 8 trained judges using 9 point an unstructured line scale for rating the intensity of an attribute while consumers acceptability study was done using a 9 point hedonic scale as described by Lawless and

Heyman (2010) to evaluate acceptability for the attributes of colour, aroma, viscosity and general acceptability. Data were analysed by R software for univariate Analysis of Variances (ANOVA) to determine significant variations between the main factors. Means were separated by Tukey's Honest significant differences at $p < 0.05$. Multivariate Principal Component Analysis (PCA) was done by Latentix software and Cluster analysis was done by Latentix and R software to determine the systematic variations in the study variables.

Most properties of honey varied significantly ($p < 0.05$) between geographical zones and floral origins with values of honey from *Miombo* floral origin being higher compared to honey samples from *Acacia* origin. Northern zone honey samples within *Miombo* origin had significantly ($p < 0.05$) higher moisture content, ash and acidity while Lake zone honey samples had significantly ($p < 0.05$) higher viscosity. Southern highland *Miombo* honey samples had higher colour value on pfund scale. PCA results showed that colour and viscosity had more effect on the variability of physico chemical properties. Phenolic content (mg gallic acid equivalent/100g) and antioxidant activities as μM Fe^{2+}/100g were significantly ($p < 0.05$) higher in the Northern zone than in other zones. Honey samples from *Miombo* had significantly higher values of 127.9 – 395.2 μM Fe^{2+}/100g than *Acacia* honey samples with values of 119.5 – 168.8 μM Fe^{2+}/100g between floral origins. Bi plot from principal component analysis, PC1 explained 99.2% of variations and showed that both parameters (total phenol and antioxidant activity) had higher effect on the variability. A strong correlation ($R^2 = 0.929$ and $R^2 = 0.869$) between phenolic contents and antioxidant activities were observed in both *Miombo* and *Acacia* honey samples, respectively.

There were significant differences ($p < 0.05$) in fructose, sucrose and total sugars between zones and floral origins. Fructose was found to be the most dominant sugar in the honey samples in each zone and floral origin with the highest contents observed in *Acacia* honey samples (44.7 – 47.0 g/100g) than their *Miombo* counterpart (39.5 – 42.0 g/100g). Glucose, the second dominant sugar was not significantly ($p > 0.05$) different between floral origins and among zones. Sucrose occurred in small amounts in honey samples from all zones and floral origins. The variation of each sugar between zones within each origin were also significant ($p < 0.05$) with Northern zone had the highest content of total sugar. PC1 bi plot explained 90.7% of total variations with high contribution from fructose and total sugars. Potassium was observed to be the most abundant macro minerals in the honey samples (380.2 – 3488.1 ppm) followed by magnesium (128.1 – 2409.5 ppm), calcium (86.3 – 336.6 ppm) and sodium (78.1 – 165.3 ppm) while iron was the most abundant micro minerals (24.5 – 36.0 ppm) followed by zinc (2.5 – 8.7 ppm) and copper (0.2 – 0.5 ppm). The northern zone honey samples from both floral origins had lead contents above maximum allowed limits as per Tanzania honey standard. The variations in macro minerals between zones were significant with Northern zone having higher contents of Ca, Mg, K and Na in samples originating from *Miombo* floral origin. Furthermore, the variations in mineral contents between floral origins were also significant with honey samples from *Miombo* floral origin having significantly much higher content of minerals than *Acacia* honey samples. Multivariate cluster analysis revealed grouping/similarities of zones according to the mineral they contain.

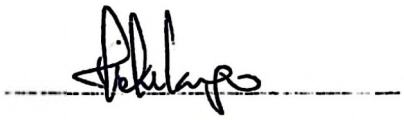
In quantitative descriptive analysis, variations in mean intensity scores of attributes between zones were significant ($p < 0.05$) in aroma, clarity, hue, viscosity and whiteness, respectively for honey samples from *Miombo* origin. Southern zone showed high values in aroma and hue, Central zone had high viscosity values while Coastal zone had highest

values in clarity and whiteness compared to other zones. In sample from *Acacia* origin. Northern zone had significantly ($p < 0.05$) highest scores in aroma. viscosity and whiteness where Lake zone had high values in clarity and Central zone had high values in hue.

Therefore, the study has revealed that fructose was the most abundant sugar in the honey samples while potassium and iron are the most abundant macro and micro minerals in honey samples under the study. Furthermore, geographical zones and floral origins have significant influences on physical chemicals properties, total phenols and antioxidant activities sugar, minerals and sensory properties of honey samples from five geographical zones and two floral origins in Tanzania. It is therefore recommended that. honey be included in human diet due to its high nutritional and antioxidant properties. In addition, further research be undertaken to create a data base which will successfully enable characterization of Tanzania honey, thus increasing its competitiveness in the local and international market.

DECLARATION

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



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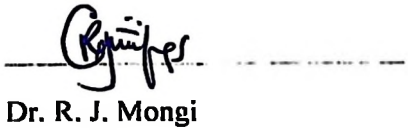

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DEDICATION

This dissertation is lovingly dedicated to my mother, Mrs. Zippora Lukuyia Shekilango.

Her support, encouragement, and constant love have sustained me throughout my life.

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	Atomic Absorption Spectrometer
AOAC	Association of Official Analytical Chemists
CRBD	Complete Randomized Block Design
DFTNCS	Department of Food Technology, Nutrition and Consumer Sciences
dPas	Decipascalsecond
EC	Electrical conductivity
EPOPA	Export Promotion of Organic Products from Africa
EU	European Union
F/G	Fructose/Glucose ratio
FAOSTAT	Food and Agriculture Organization Startistics
Fe	Iron
FRAP	Ferric Reducing Ability Power
G/W	Glucose/Water ratio
GAE	Gallic acid Equivalent
HCl	Hydrochloric acid
ISO	International for Standardization
ITC	International Trade Centre
Kg	Kilogramme
M	Molarity
mM	Millimolar
NaOH	Sodium hydroxide
NHB	National Honey Board
PC	Principal Component

PCA	Principal Component Analysis
PLSR	Partial Least Square Regression
QDA	Quantitative Descriptive Analysis
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
TPC	Total Phenolic Content
TPTZ	Tris(2-pyridyl)-*s*-triazine
TZS	Tanzania Standard
URT	United Republic of Tanzania
USA	United States of America
µM	Micromolar

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Honey is one of the main products of beekeeping, the other major product being beeswax. It is defined as the natural sweet substance produced by honey bees (*Apis mellifera*), from the nectar of plants or from secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants. The honey bees collect and transform the nectar by combining it with specific substrate of their own. The transformed nectar is then deposited and stored in honeycombs where it is dehydrated and left to ripen and mature (European Council, 2002).

The ripened honey is a complex product containing mixture of numerous substances with sugars. Carbohydrate mainly fructose and glucose ranging from 85 to 95% constitute the largest portion of the dry matter. Other substances are small amounts of protein mainly enzymes and free amino acids, vitamins, minerals and trace compounds (Aronne *et al.*, 2010). The composition and properties of honey are influenced by the type of flowers visited by the bees, geographical area, weather condition and soils (Mohamed *et al.*, 2010; Conti and Botrè, 2001).

Worldwide, China is the leading producer of honey with 26% of world production share of honey (FAOSTAT, 2010). It is followed by Europe, Turkey, Ukraine and Argentina. In Africa, Ethiopia is the largest producer of honey with a total production amounting to 41,000 tons (FAOSTAT, 2005). Tanzania is one of the countries with substantial production of honey. Its production has increased from 4,860 tons (2001) to 9, 380 tons in (2012), with more than half of its product being consumed locally (Mwakatobe and

Mlingwa, 2006). This is due to the increased awareness on the benefits of honey as food and as a product with healing qualities (ITC, 2015; URT, 2009).

The current trend has shown that the consumption of honey is higher in developed countries where their domestic demand does not meet the internal market demand. The European Union (EU) is the world's largest consumer of honey and consumes approximately 22% of the world's honey production. The majority of honey is imported from the developing world and this has provided an opportunity for Tanzania honey to enter international market (EPOPA, 2006).

The demand for Tanzania honey is very high in the world market, mainly because of it being organically produced (Mwakatobe and Mlingwa, 2006). Organic production is one of the requirements in the market especially in Europe though other factors should be adhered to by all producers as far as quality control and assurance is concern (EPOPA, 2006). In the European markets, honey produced by EU countries is classified in terms of the botanical and geographical origin so as to compete more effectively with cheap honey appearing in EU market from China and developing countries (Diego *et al.*, 2005). Various studies by Moussa *et al.* (2012) and others have indicated that, floral source, i.e., the flowers from which bees gathered nectar to produce the honey determines many of the quality attributes of honey, such as composition and colour only to mention few.

Therefore, identifying the quality factors of Tanzania honey based on its floral and geographical location is essential for appropriate labelling of the produced honey, and enhancement of its local, regional and international marketability. Furthermore, understanding the product genuinity in terms of floral and geographical origin is essential to avoid unfair competition that can be created and possible destabilize the

market which may disrupt the international/regional and even the national economy. A major concern of food control is to ensure that honey is genuine in respect of the legislative requirements as it is a product of limited supply and of relatively high price, hence its quality assurance becomes extremely important.

1.2 Problem Statement and Justification

In recent years, the world has seen an increase in international honey trade and this has led to the requirement of universal standards for the protection of consumers. Moreover, since quality, origin and colour of the honey are the major factors in price setting in global market, there is an increased attention in the determination of the floral and geographic origin of honey (EPOPA, 2006).

It has been reported that, different floral sources foraged by honey bees situated at different geographical location have a major influence on the quality of honey in term of composition and acceptability (Kumar and Mandal, 2009; Gidamis *et al.*, 2004; Foldhazi *et al.*, 1996). This is because the floral origin of honey is one of the most important parameters of honey quality and depends on the species of plants that bees use in their nourishment (Tucak *et al.*, 2007). The quality of Tanzania honey differs because of various factors like floral source, geographical, seasonal, and processing conditions. Based on floral sources, Tanzania honey has been classified as monoflora or polyflora depending on the floral source (Gidamis *et al.*, 2004). Despite adequate literature review, information on the influence of floral origin and geographical location on the quality of honey from Tanzania such as physico-chemical characteristics, sugar profile (glucose, fructose, and maltose), total phenols content and antioxidant activity, mineral content, sensory properties and consumer acceptability is limited. Usually, for many consumers of honey, only sensorial characteristics such as colour and texture (the state of crystallization)

are the main quality parameters (Moise *et al.*, 2007). They mostly prefer light coloured honey which is a bit expensive while regarding those in crystallized state as of inferior quality.

Guaranteeing honey quality is becoming increasingly important for consumer, producers and regulatory authorities like TBS and TFDA. This study therefore seek to generate the missing information which in turn will serve as basis for establishing the variation of Tanzania honey based on geographical and floral origin, appropriate classification of honey so as to compete in the domestic and global market as well as for control of genuinity of honey with respect to floral and geographic origin by regulatory authorities so as to prevent product mislabeling by unfaithful producers and businessmen.

1.3 Objectives of the Study

1.3.1 Overall objective

The general objective of the study was to determine how quality of honey is influenced by different floral and botanical origins in Tanzania

1.3.2 Specific objectives

i. To determine the physico-chemical characteristics of honey samples.
ii. To determine sugar profile of honey samples.
iii. To determine the total phenols content and antioxidant activity of the honey samples.
iv. To determine heavy metals, micro and macro mineral content of honey samples.
v. To assess the sensory properties and consumer acceptability of honey samples.

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CHAPTER TWO

MANUSCRIPT ONE

2.0 PYHSICO-CHEMICAL PROPERTIES OF HONEY SAMPLES FROM DIFFERENT BOTANIC AND GEOGRAPHIC ZONES IN TANZANIA

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Abstract

The effect of variations in floral and geographical location on physicochemical properties of Tanzania honey was investigated. Samples were collected from two floral sources (*Miombo* and *Acacia*) in five different zones (Central, Coastal, Lake, Northern and Southern zones) and evaluated for moisture content, ash, pH, acidity, viscosity and colour as per methods described in AOAC (2005). Most parameters of honey from *Miombo* origin had higher values compared to honey from *Acacia* origin. The moisture content, ash, pH, acidity, viscosity and colour ranged from 16.60 - 20.30%, 0.05 - 0.96%, 3.55 - 4.59, 22.34 - 40.31 meq/kg, 26.00 - 59.00 dpas and 31.55 - 143.98 mm on pfund scale, respectively. Honey samples varied significantly ($p < 0.05$) in different physico-chemical properties between geographical zones and flora origins. Northern zone honey samples within *Miombo* origin had significantly ($p < 0.05$) higher moisture content (20.30 ± 0.40%), ash (0.96 ± 0.03%) and acidity (40.31 ± 0.29 meq/kg) with lower pH value of 3.56 ± 0.14 and 26.00 ± 21.20 dPas for viscosity than samples from other zones and origin. Lake zone honey had significantly higher viscosity in both *Miombo* and *Acacia* type with respective value of 59.00 ± 27.51 and 51.33 ± 1.15 dPas. Southern Highland *Miombo* honey samples were darker (143.98 ± 41.64 mm – dark amber) while *Acacia* honey from Central zone had the lowest colour value of 62.28 ± 42.61mm – light amber. All physicochemical properties differed significantly ($p < 0.05$) with exception of moisture content, pH and viscosity between flora origins. The PCA result showed that colour and viscosity had more effect on the variability of physical chemical properties. Therefore it can be concluded that geographical zones and floral origin had significant effect on the physicochemical properties of honey produced in Tanzania.

Keywords: *Floral, geographic, physicochemical, moisture content, honey*

2.1 Introduction

Honey is one of the main products of beekeeping and it is defined as the natural sweet substance produced by honey bees (*Apis mellifera*), from the nectar of plants or from secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants (European Council, 2002). It is a natural food with high nutritional value composed of readily available sugars (mainly fructose and glucose), amino acids, enzymes, protein, vitamins, minerals, organic acids and phenol compounds (Ouchemoukh *et al.*, 2007).

In recent years, beekeeping and honey production in Tanzania are considered as important livelihoods and income generating activities for survival of people and also conserving natural resources. This is evidently seen in the current efforts by the government to encourage and support beekeeping industry in Tanzania (Msemo, 2013). In the country, honey is widely used as food, medicine, raw materials for industrial beer production and as a source of income (Muruke, 2011).

The global demand for honey and beeswax and other products has been increasing over the past 10 years due to increased levels of awareness and health consciousness among the consumers (ITC, 2015). This in turn, has led to an increase in international trade in honey, hence giving opportunity for developing countries like Tanzania to enter into the international market. However, nevertheless, information on quality, origin and colour of the honey are important major factors in price setting in global market. Moreover, information on the characteristics of honey according to their botanical/floral and geographic origins is also important factor in price setting which is recently gaining an increased attention (EPOPA, 2006).

It has been reported that the quality of honey is dependent on the floral source from which the bees collect the nectar from, geographic origin, the climatic conditions under which plants grow and the processing and storage conditions of honey (Abdulkhaliq and Swaileh, 2016; Ciappini *et al.*, 2016; El-Metwally, 2015, Kas˜koniene *et al.*, 2010). Due to these variations, honey differs in physicochemical properties compositions and sensory perception (Satyapal, 2015). This has caused physicochemical characteristics such as pH, sugar content, electrical conductivity, proline, enzymatic activity, water content, ash content, diastase activity and mineral content (Zn, Na, Mg, Fe) to be used in many studies to assess quality and characteristics of honey based on their floral/geographical origins complemented by pollen analysis (Khalafi *et al.*, 2016; Soria *et al.*,2004, Furkan and Gumus, 2010; Moise *et al.*, 2007; Tucack *et al.*, 2004). Several authors (Diego *et al.*, 2005; Cordella *et al.*, 2002) have reported the use of some of physicochemical parameters in the identification of the botanical and geographical origin of honey. To establish the significance of parameters closely related to the origin of honey, quality control methods, multivariate statistical analysis have been applied (Naab *et al.*, 2009).

However, information on physicochemical properties of honey produced in Tanzania is limited. Unfortunately, study on physicochemical properties of honey in Tanzania by Muruke (2014) was not wide enough to cover characterization according to floral origin and geographical zones. This study therefore sought to establish the missing information, which may serve as basis for initiation of compiling data base that may be used to characterize honey from different flora and geographic origin in Tanzania. In addition, proper identification in terms of floral and location will increase Tanzania's honey penetration into the international market.

2.2 Materials and Methods

2.2.1 Study area

The study was conducted in five zones of Tanzania namely: Lake, Central, Coastal, Northern and Southern highland zones. The analytical work was conducted at the Department of Food Technology, Nutrition and Consumer Sciences (DFTNCS) laboratory, Sokoine University of Agriculture (SUA) and at Tanzania Bureau of Standards (TBS) Food Laboratory.

2.2.2 Materials

Honey samples were picked purposively depending on availability and distribution of floral/botanic origin, and they include *Acacia* honey from *Acacia spp* and honey from *Miombo* woodland. Twenty four honey samples (15 *Miombo* and 9 *Acacia* samples) were purchased directly from the beekeepers from different regions in the respective zones; Lake (Kigoma and Simiyu), Northern (Manyara), Central (Tabora and Dodoma), Coastal (Morogoro) and Southern Highland (Katavi). Analytical grade reagents and chemicals were obtained from TBS, DFTNC laboratory and/or purchased from suppliers in Dar es Salaam.

2.2.3 Research design

Complete Randomized Block Design (CRBD) with replication was used in this study. The principal factors were floral source with two levels (*Miombo* and *Acacia*) and geographical location with five levels (the zones). The effect of these factors on analyzed parameters was determined. The design mathematical model is depicted in Equation 1.

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad \ldots\ldots(1)$$

Where μ is the overall (grand) mean. α_i is the effect due to the i^{th} treatment (floral).

β_j s the effect due to the j^{th} block (geographical) and ε_{ij} is the error term

2.2.4 Chemical analyses

2.2.4.1 Moisture content

Moisture content of the samples was determined by refractometric method as described in method 44.4.04 in AOAC (2005) by measuring the refractive index using an Abbe refractometer at 20°C. Prior to analysis, the samples were homogenized by placing in water bath at 40°C to dissolve sugar crystals and make handling of the sample easier. The refractometer was calibrated with distilled water before use and after every 12 sample readings. The readings were converted to moisture content (% m/m) using reference table of estimation for moisture content (appendix 3).

2.2.4.2 pH and Acidity

pH and acidity of honey samples were determined as described in method 44.4.20 in AOAC (2005). 10 g of honey was weighed and dissolved in 75 ml of CO_2 free distilled water. The pH of the samples was measured by a pH meter (JENWAY 4330, UK) which was calibrated before use with buffer solution of pH 4.0. 7.0 and 10.0. The acidity of sample solutions were determined by titrating with 0.1M NaOH solution using neutralized phenolphthalein as indicator to pH 8.3.

2.2.4.3 Ash content

Ash content of honey samples was determined by method 44.4.05 as described in AOAC, (2005). Five grammes of honey was weighed into a pre-weighed crucible and gently heated on a hot plate until the sample is black and dry. Then the crucible was transferred to the muffle furnace and ignited at 600°C to a constant weight. The % ash was calculated using equation 2.

$$\%Ash = \frac{(B-C)}{A} x100 \quad \text{.....(2)}$$

Where: A = sample weight in g, B = weight in g of crucible and contents after drying and C = weight in g of empty crucible

2.2.4.4 Colour

Colour of honey samples was measured using UV/Vis spectrophotometer (Labomed Inc, USA) as adopted from Ferreira *et al.* (2009) and Aazza *et al.* (2013). Honey samples were warmed in a water bath at 40°C to dissolve sugar crystals. The samples were rapidly cooled to room temperature and the absorbance was read from honey solution (50% (w/v) at 635 nm. Honey samples were converted and classified according to the Pfund scale as given by White (1984). The conversion of the absorbance values (*A* 635) was done using equation 3.

$$(\text{mm Pfund}) = -38.70 + (371.39.A_{635}) \quad \text{.....(3)}$$

2.2.4.5 Viscosity

Viscosity measurements were carried out using a rotational viscometer (Haeke – viscometer 2 plus) according to Bakier (2007). About 50 mls of sample was placed in a test cup and viscosity was measured using rotor 1 (R1) at room temperature.

2.2.5 Statistical data analysis

Data obtained were analyzed by using the R statistical package (R Development Core Team, Version 3.0.0 Vienna, Austria). Analysis of variance (Anova) was used to determine the significant differences between the main factors. Means were separated using Tukey's Honest Significant difference ($p < 0.05$). Principal Component Analysis (PCA) was used to determine the systematic variations in data (Martens and Martens, 2001) using Latentix Software (LatentiX Aps Team, version 2.12, Frederiksberg Denmark). Results were presented as arithmetic mean and standard deviation in tables and graphs as well as in PCA bi plots.

2.3 Results and Discussion

2.3.1 Effect of geographical zones and floral origin on physico-chemical quality

2.3.1.1 Moisture content

Results for moisture content between zones in each floral origin are presented in Figure 2.1a. There were significant differences ($p < 0.05$) in moisture content between zones in *Miombo* floral origin. The Northern zone samples had significantly higher moisture content of 20.30 ± 0.40g/100 g while Central and Southern Highland zones had statistically lower values ranging from 16.87 - 16.99g/100 g.

No significant ($p > 0.05$) variation was observed in moisture contents between zones in *Acacia* floral origin.

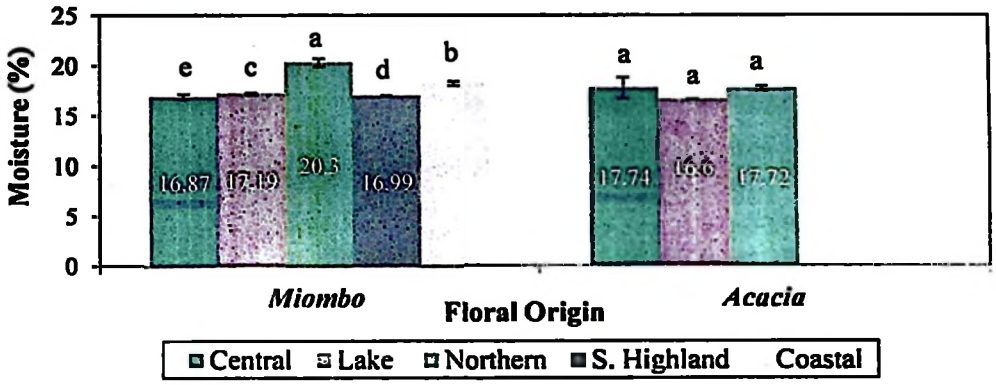

Figure 2.1a: Moisture content of honey samples from different zones within the same floral origin. Bars with different letters are significantly different at $p < 0.05$

Figure 2.1b shows variation of moisture content between floral origins in each zone. The northern zone samples only showed significant variation in moisture content between *Acacia* and *Miombo* floral origin. *Miombo* had higher value of 20.3 ± 0.4 than *Acacia* samples with values of 17.72 %. No significant ($p > 0.05$) variations in moisture contents observed between floral origin in Central and Lake zones.

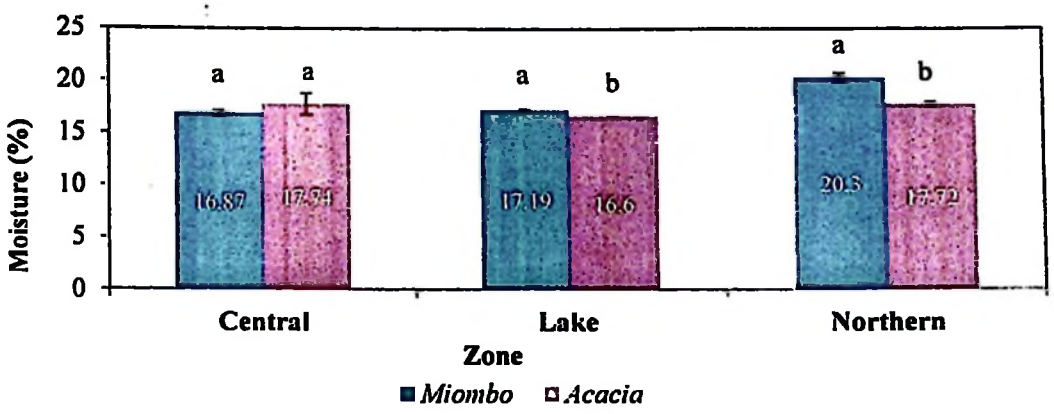

Figure 2.1b: Moisture content of honey samples between floral origins within a zone

Bars with different letters are significantly different at $p < 0.05$

The moisture content in evaluated honey samples ranged from 16.87-20.30% in *Miombo* honey and from 16.60-17.74% in *Acacia* honey. This finding is in agreement with Mendes *et al.* (1998) who reported moisture content of honey to range from 13 to 20%. This implies that honey samples under study met the requirements for maximum allowed limit for honey as stipulated in the Codex Alimentarius Standard for honey (2001) and Tanzania Honey Standard (2006). Moisture content is one of the quality criteria that determines the capability of honey to remain stable (shelf life) and ability to resist spoilage by yeast fermentation (Erez *et al.*, 2015; Singh and Bath, 1997). In addition, moisture content is also of great importance because it is considered to be a useful parameter for describing moistness and viscosity of honey (Feás *et al.* (2010). The moisture contents below 22% is highly important and is an indicative of good storage ability or the shelf-life of the honey during storage as high moisture content could lead to fermentation during storage (Zerrouk *et al.*, 2011; Gomes *et al.*, 2010). This implies that samples from this study had high shelf stability.

The significant variation in moisture content between zones observed in this study could be due to different locations from which the honey samples were obtained and also due to

different floral source, i.e, *Miombo* and *Acacia*. Apart from the level of maturity reached within the hive, the moisture content of honey depends on several factors such as the harvest season, climatic conditions, floral origin, geographical location and the moisture content of the original plant (Nanda *et al.*, 2003; Ouchemoukh *et al.*, 2007; Kurkova *et al.*, 2006; Gulfraz *et al.*, 2011). However, water content can be artificially altered during honey processing (Bogdanov *et al.*, 2004). Similar moisture content values to this study were also reported by Ciappini *et al.* (2016) who obtained a value of 17.1% (clover) and 17.3% (*eucalyptus*) in chemometrics classification of unifloral honey study. However, the values are higher than 14.5-19, 13.9-29.9, 14.3-20.2 and 14.3-16 reported by Abdulkhaliq and Swaileh (2016) in Palestine honey, Muruke (2014) in Tanzania honey, Chakir *et al.* (2011) in honey from different plants in Morocco and Khalafi *et al.* (2016) in Iranian honey.

The relatively higher moisture contents in *Miombo* honey samples from Northern zone indicate greater susceptibility to fermentation, spoilage and flavor loss leading to a decrease in quality (Costa *et al.*, 1999). High moisture contents can be an indicator of premature extraction or extraction under high humidity conditions (Ajlouni and Sujirapinyokul, 2010) and it causes undesirable fermentation in honey during storage caused by the action of osmotolerant yeasts and resulting in the formation of ethyl alcohol and carbon dioxide (Saxena *et al.*, 2010; Al *et al.*, 2009).

2.3.1.2 Ash content

Figure 2.2a shows the ash contents in honey samples between different zones in each floral origin. In *Miombo* floral origin, Northern zone had higher ash content of 0.96 % followed by Lake zone with 0.5 % and Coastal zone with 0.27 %. Central zone had significant ($p < 0.05$) lowest value of 0.19%.

In *Acacia* samples, Northern zone had again significant highest value of 0.22 % followed by Central zone with 0.12% and lowest in Lake Zone with value of 0.05 %. When compared to *Miombo*, *Acacia* honey samples had generally lower values in ash content.

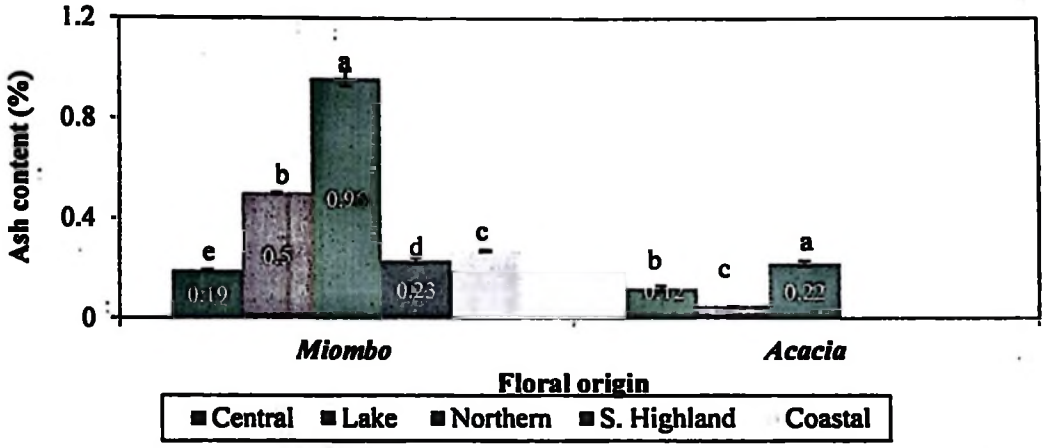

Figure 2.2a: Ash content of honey samples from different zones within the same floral origin. Bars with different letters are significantly different at $p < 0.05$

Figure 2.2b shows variation of ash content between floral origins in each zone. Significant variations ($p < 0.05$) in ash contents were observed between *Acacia* and *Miombo* in all zones (Central, Northern and Lake zones). *Miombo* honey samples had higher ash value of 0.96, 0.5 and 0.19% than *Acacia* honey samples with values of 0.22, 0.05 and 0.12% for Northern, Lake and Central zones, respectively.

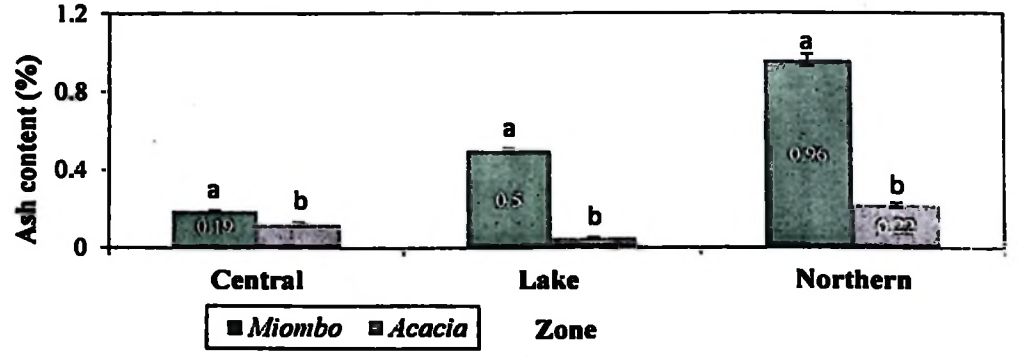

Figure 2.2b: Ash content of honey samples between floral origins within a zone

Bars with different letters are significantly different at $p < 0.05$

Ash content is one of the parameters associated with botanical and geographical origins of honey samples and generally depends on nectar composition of predominant plants in their formation (Erez *et al.*, 2015; Al-Khalifa and Al-Arify, 1999). The obtained results of ash varied widely and significantly between zones and ranged from 0.19 - 0.96% in *Miombo* honey and 0.05 - 0.22% in *Acacia* honey. The obtained values for most honey samples were in the acceptable range as given by National honey Standard (TZS 851:2006) with exception of honey from *Miombo* in Northern zone. Similar ash contents were reported by Muruke (2014) in Tanzania honey, (0.07 - 1.24%). Parviz *et al.* (2015), Mondragón-Cortez *et al.* (2012) and Nanda *et al.* (2009) obtained comparable results to this study in ash content at a range of 0.17 to 0.8%,0.18 - 0.41% and 0.13 - 0.35%, respectively when studying variety of honeys from Iran, Mexico and India. However, lower values than the observed results in the study were reported for Pakistan honey by Abdulkhaliq and Swaileh (2016). This variability observed in the results could be due to difference in soils, geographic location, atmospheric conditions, the harvesting processes, beekeeping techniques as well as the materials collected by the bees during the foraging on the flora (Finola *et al.*, 2007).

The significantly higher value of ash content in Northern zone honey samples could be due to the flora visited and nectar collected by the bees (Moniruzzaman *et al.*, 2014). Saxena *et al.* (2010) reported that, ash content in honey reflects its richness in minerals and is determined by the floral origin. Furthermore, minerals ash are introduced into honey primarily with pollen and its contents depends on the predominant pollen present in honey (Erez *et al.*, 2015). The reported observation can be related to the collected samples from this zone as they originally contained a lot of visibly pollens.

2.3.1.3 pH

Figure 2.3a shows the pH in honey samples between different zones in each floral origin. *Miombo* honey samples differed significantly ($p < 0.05$) between zones with Coastal zone

having the highest value of 4.5 followed by Lake zone with 4.2 and lowest in Northern with 3.56. For the three zones of *Acacia* origin, Lake zone had significantly ($p < 0.05$) higher pH value of 4.07 followed by Central and Northern zones which were statistically similar ($p > 0.05$) with values that ranged from 3.51.- 3.65.

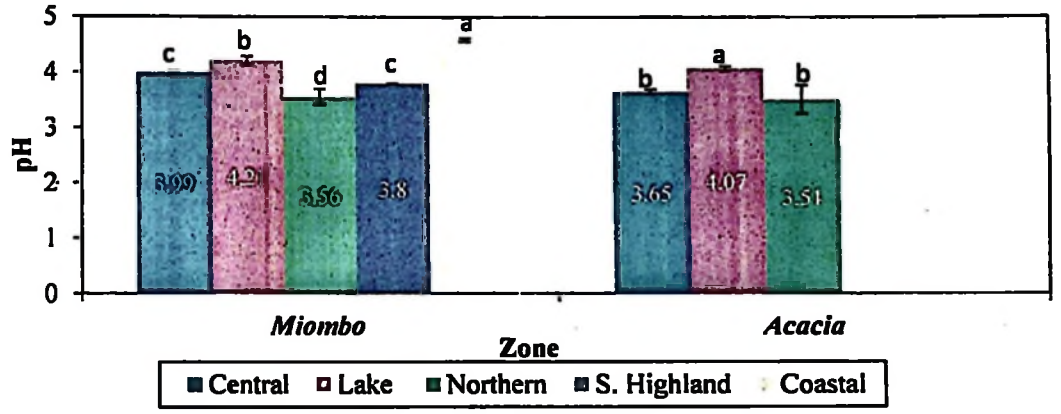

Figure 2.3a: pH of honey samples from different zones within the same floral origin

Bars with different letters are significantly different at $p < 0.05$

Results for pH content between floral origins in each zone are presented in Figure 2.3b. Honey samples differed significantly ($p < 0.05$) only in the Central zone with *Miombo* sample having higher value of 3.99 than *Acacia* with pH value of 3.65.

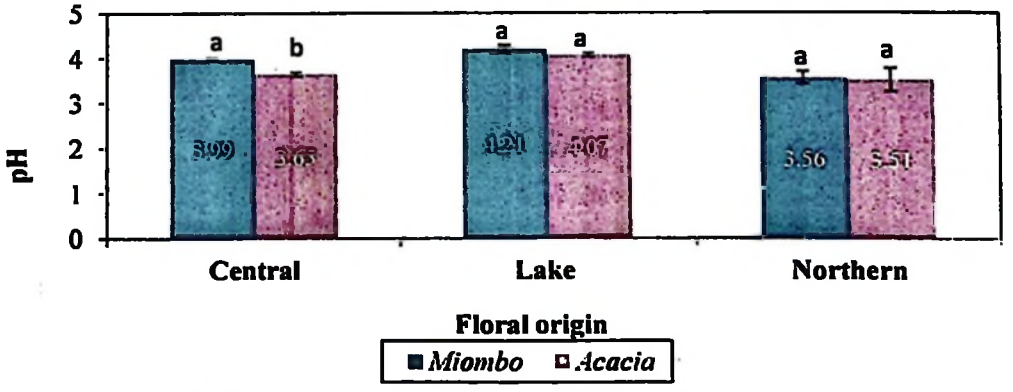

Figure 2.3b: pH of honey samples between floral origins within a zone

Bars with different letters are significantly different at $p < 0.05$

The pH of the honey is of great importance during the extraction and storage of honey as it influences the texture, stability and shelf life of honey (Terrab *et al.*, 2004). The obtained pH values in this study varied from 3.56 - 4.59 in *Miombo* samples and 3.51 - 4.07 in *Acacia* samples and were in accordance to the given pH values that ranged between 3.2 and 4.5 reported by Bogdanov *et al.* (1999). The observed variations in pH obtained could be ascribed to the different contents of honey acids and minerals (Kamal *et al.*, 2002). The low pH of honey inhibits the presence and growth of microorganisms thus giving honey a good keeping quality and extends its shelf life during storage (Feás *et al.*, 2010). Comparable results of 3.29 - 4.06 and 3.5 – 4.4 were reported in studies by Feás *et al.* (2010) (3.29 - 4.06) and Ouchemoukh *et al.* (2007) in Algeria honey. Higher results than the observed values were obtained by Abdulkhaliq and Swaileh (2016) in Palestine (3.0 – 6.0). The values of pH obtained were against the observation done by Khalafi *et al.* (2016) who observed that the honey sample with more ash content compared to other samples possessed high pH. This could be seen in the pH value of *Miombo* and *Acacia* honey from the Northern zone which had the highest ash contents but had the lowest pH in their respective floral groups.

2.3.1.4 Acidity

Results for acidity in honey samples between zones in each floral origin are presented in Figure 2.4a. All *Miombo* honey samples differed significantly between zones with Northern zone having highest value of 40.31 meq/kg followed by 32.91 meq/kg in Lake zone and Southern Highland zone with 28.65 meq/kg. Furthermore, there were significant differences ($p < 0.05$) in acidity between zones in *Acacia* origin with Northern zone honey sample having again a higher value of 29.17 meq/kg than Central and Lake zones that had statistically similar ($p > 0.05$) values ranging from 22.34 - 22.48 meq/kg. Generally, *Miombo* honey samples had higher values of acidity than *Acacia* conterpart.

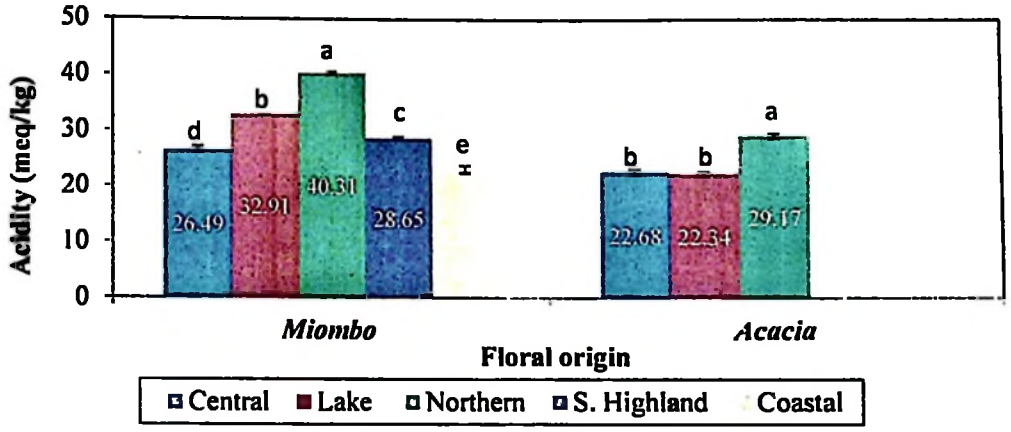

Figure 2.4a: Acidity of honey samples from different zones within the same floral origin. Bars with different letters are significantly different at $p < 0.05$

Figure 2.4b shows acidity content of honey from different floral origins within the same zone. There were significant variations in acidity between floral origins within each zone with *Miombo* honey samples having higher values of 24.49, 32.91 and 40.31 meq/kg for Central, Lake and Northern zones respectively than respective values of 22.68, 22.34, and 29.17 in *Acacia* honey samples.

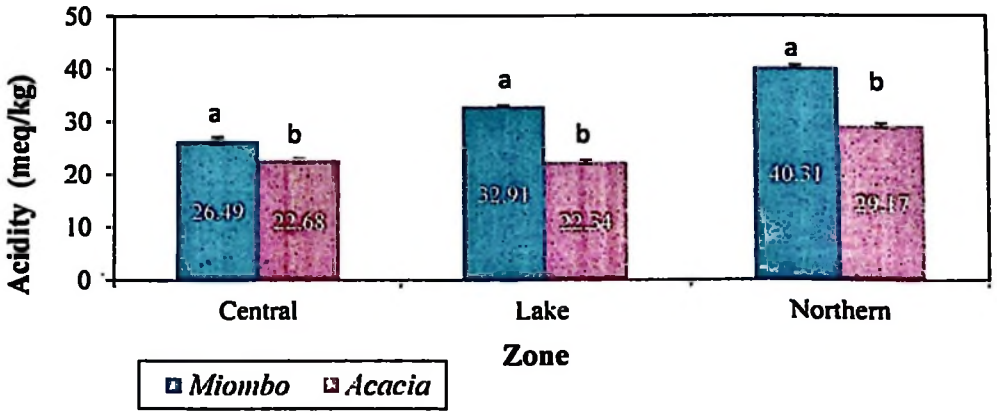

Figure 2.4b: Acidity of honey samples between floral origins within a zone Bars with different letters are significantly different at $p < 0.05$

The observed acidity values were in the allowed limit of 40 meq/kg for Tanzanian standard (2006). Almeida (2016) in Brazil honey observed similar values that ranged from 12.77 to 55.72 meq/kg but Awad and Elgornazi. (2016) and Muli *et al.* (2007) reported slightly higher values than those observed in this study. These variations could be attributed to floral origin or harvest season (Ojeda de Rodri'guez *et al.*, 2004, Perez-Arquillue' *et al.*, 1994). Free acidity in honey may be explained by taking into account the presence of organic acids, which are proportional to the corresponding lactones, or internal esters, and some inorganic ions such as phosphate or sulphate (Finola *et al.*, 2007). It normally contributes to its flavor, improves antioxidant activity and influences against the action of microorganisms (Cavia *et al.*, 2007).

The markedly higher acidity content in *Miombo honey* samples from the Northern zone than other zones was still in the allowable limit by the Tanzania honey standard and in international trade requirement (40 meq/kg). High level of acidity in honey is an indicative sign of fermentation occurrence (Zerrouk *et al.*, 2011). Similarly, Liberato *et al.* (2013) observed high free acidity above 50 meq/kg had corresponding high moisture content. Chemical properties of the organic and inorganic acids and their ionization rate in the honey samples together with the amino acids content provided by the nectar and salivary enzymes from bees tend to influence the acidity content (Vieira, 2005). The content of gluconic acid produced from glucose by the action of the glucose-oxidase enzyme tends to increase during the storage of honey because this enzyme remains active even after processing. This causes an increase in honey acidity during the storage and as a result the pH decreases (Pamplona, 1989).

2.3.1.5 Viscosity

Figure 2.5a shows viscosity scores between zones in each floral origin. Some honey samples differed significantly between zones in each floral origin. In *Miombo* samples.

Lake zone had highest value of 59 dPas followed by central and Southern Highland honey samples with values of 40.33 - 43.67 dPas and lowest in Northern zone with 26 dPas.

Lake zone had again higher value of 51.53 followed by Northern zone with 39.67 dPas and lowest in central zone with 29.67 dPas in *Acacia* floral origin.

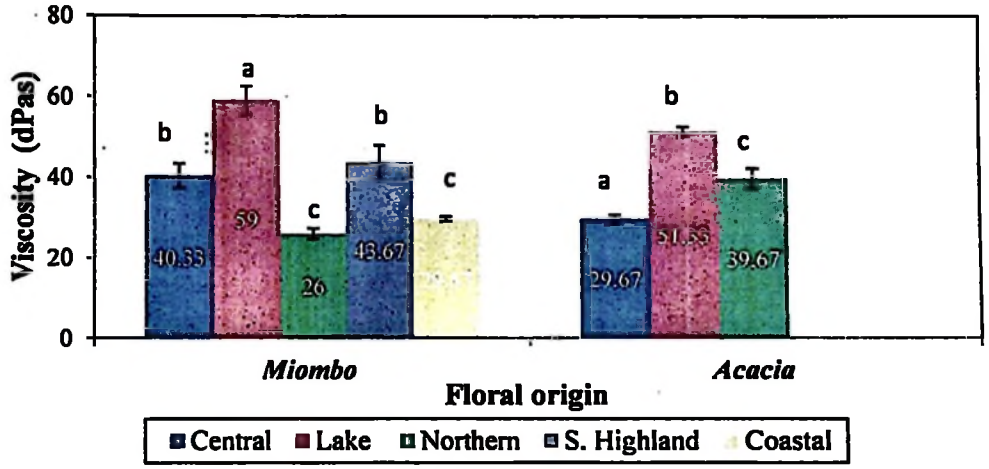

Figure 2.5a: Viscosity of honey samples from different zones within the same floral origin. Bars with different letters are significantly different at $p < 0.05$

Results for viscosity content of honey samples between floral origins within each zone are presented in Figure 2.5b. There were significant variations in viscosity between the two origins with *Miombo* samples dominating in Central and Lake zones with respective higher values of 40.33 and 59 dPas than values of 29.67 and 51.53 dPas respectively observed in *Acacia samples*. On the other hand, *Acacia* samples had significantly higher value of 39.67 than 29.33 dPas observed in *Miombo* samples in the Northern zone.

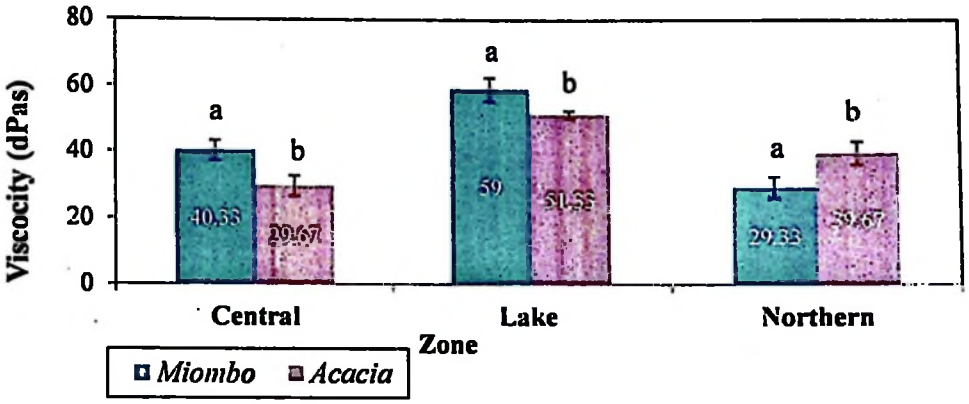

Figure 2.5b: Viscosity of honey samples between floral origins within a zone

Bars with different letters are significantly different at $p < 0.05$

The rheological properties of honey are important qualities that influence the sensory quality of the product and also affect a number of technological operations, such as honey heating, mixing, filtering, hydraulic transport and bottling (Yanniotis *et al.*, 2006). Honey viscosity also plays a significant role in the crystallization process (Rüegg and Blanc. 1981). The significant variation in viscosity between zones and geographical zones could be associated to different temperature and moisture content levels. It has been reported that, honey, in its liquid state, behaves like a Newtonian fluid whose viscosity is mainly dependent on temperature (Sopade *et al.*, 2002) and water content (Zaitoun *et al.*, 2001; Yanniotis *et al.*, 2006). At room temperature honey viscosity is equal to 9.9 Pas with water content of 18.9% and up to 61.1 Pas with 13.9% of water (Lazaridou *et al.*, 2004). A temperature drop to 0°C will cause further increase of honey viscosity – as high as 450 - 2400 Pas (Bhandari *et al.*, 1999; Bakier, 2006). This observation was in agreement with Yanniotis *et al.* (2006) and Zaitoun *et al.* (2001) who reported that like temperature, moisture content of honey has a significant influence on its viscosity, that is, the increase in moisture content results in an exponential drop of viscosity. In addition to moisture and temperature, carbohydrates (sugars) are also a determinant of physicochemical properties

of honey such as viscosity, hygroscopicity and granulation. It should be noted that the presence of a trisaccharide – melezitose – considerably increases honey viscosity (Lazaridou *et al.*, 2004).

2.3.1.6 Colour

There were significant variations in colour between zones in the studied honey samples and the colour ranged from white to dark amber in *Miombo* honey and from extra white to light amber in *Acacia* honey (Figure 2.6a). In samples obtained from *Miombo* origin, the highest Pfund value was observed in Southern Highland zone samples with value of 143.98 mm followed by Central zone with 121.37 mm and Northern zone with 100.32 mm. The lowest Pfund value was observed in Coastal zone samples with 31.55 mm. In *Acacia* honey the highest pfund was observed in Central zone with 62.28 mm while the lowest value was in Lake zone samples with value of 17.38 mm.

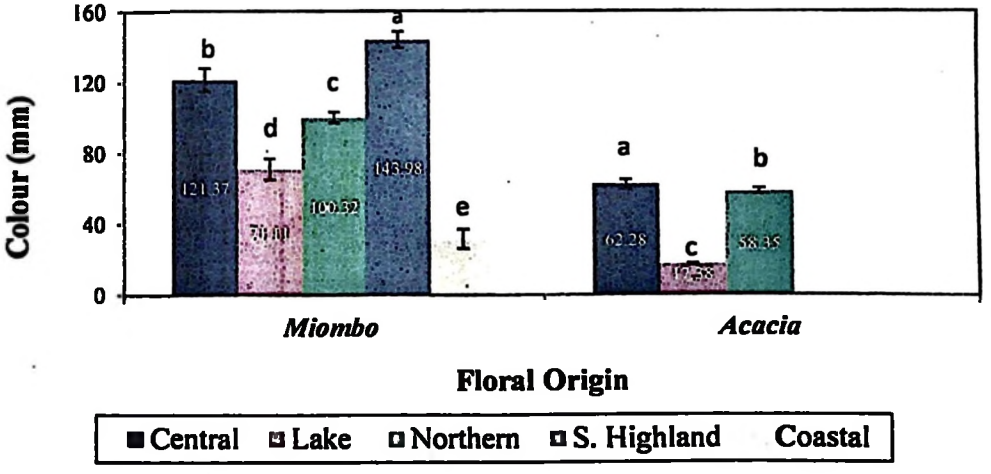

Figure 2.6a: Colour of honey samples from different zones within the same floral origin. Bars with different letters are significantly different at $p < 0.05$.

Figure 2.6b shows variation of pfund between floral origins within each zone. *Miombo* samples varied significantly ($p < 0.05$) from *Acacia* samples within each zone evaluated. Respective higher values of 121.37, 71.11 and 100.32 mm were observed in Central, Lake and Northern zones than 62.28, 17.38 and 58.35 mm observed in *Acacia* counterparts.

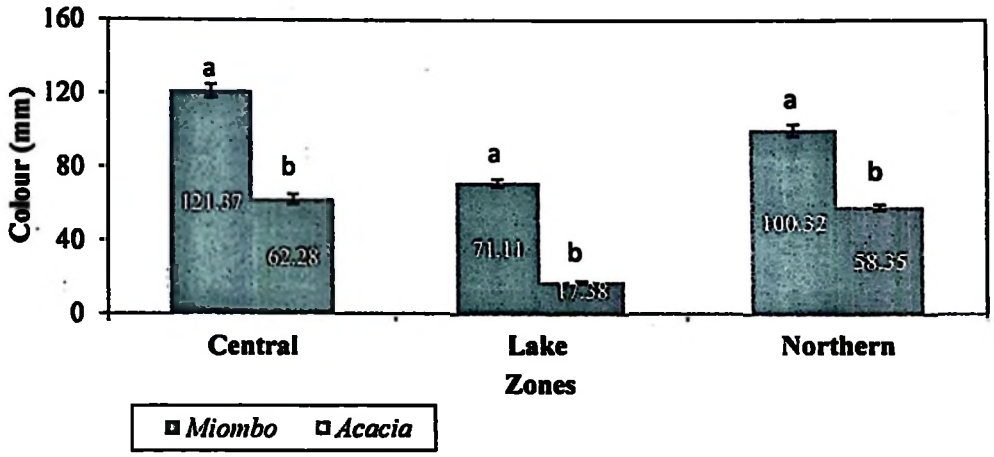

Figure 2.6b: Colour of honey samples between floral origins within a zone

Bars with different letters are significantly different at $p < 0.05$

Honey colour is one of the factors that determine honey quality, price as well as its acceptance in the world market (Viuda-Martos *et al.*, 2010). Honey from different origins were studied and found to have pfund value of 73.88 ± 2.29 mm (Egypt), 56.40 ± 2.32 mm (Yemen) and 56.40 ± 2.32 mm (Saudi) (El Sohaimy *et al.*, 2015). Varied pfund values observed in this study could be due to the reasons that, honey from different floral sources consist of different compositions and concentrations of pigments mainly polyphenols and carotenoids (Ram, 2011). Usually dark-colored honeys are associated with high ash content while light-colored honeys with low ash content (Alvarez-Suarez *et al.*, 2010). This was in contrast with the observation from our results where the darkest honey did not have high ash content. This discrepancy could be due to beekeeper's handling practices of the combs such as the use of old wax combs for producing honey (El Sohaimy *et al.*,

2015). On the other hand, contamination of honey with heavy metals, presence of high minerals ash content and storage of honey at high temperature has been linked to darker colour of honey (El-Metwally, 2015).

2.4 Systematic Variation of Samples by Principal Component Analysis

Figures 2.7a shows PCA bi-plot with two first significant principal components on average physicochemical properties. Principal Component 1 (PC1) accounts for 91.84% of total variations and showed a contrast between some *Miombo* samples and all *Acacia* samples in physical chemical properties between flora origins while PC2 accounted 7.22% of total variations. Colour and viscosity had high loading than other parameters implying high contribution to variability in the physical chemical properties.

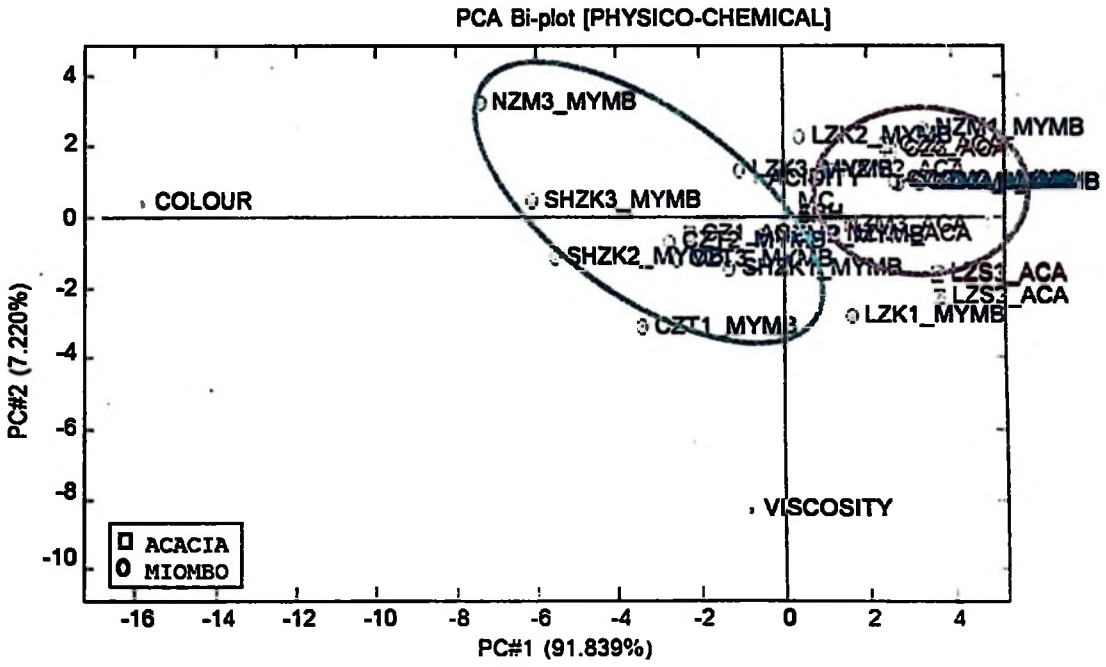

Figure 2.7a: PCA Bi-plot showing variation in physicochemical properties between honey floral origins (*Miombo* and *Acacia*)

Key: MYB - *Miombo* honey, ACA - *Acacia* honey, Coastal zone - COZM, Central zone – CZT, Lake zone – LZK, Northern zone – NZM, Southern highland zone – SHZK

Furthermore, *Miombo* samples from Central and Southern zones were positively correlated with colour along PC 1 and clearly separated from *Miombo* (Coastal) and *Miombo* and *Acacia* (Lake) samples. The *Acacia* honey samples correlated negatively with colour. *Miombo* samples from Central and Southern zone were clearly separated from each other on the positive side of PC 1.

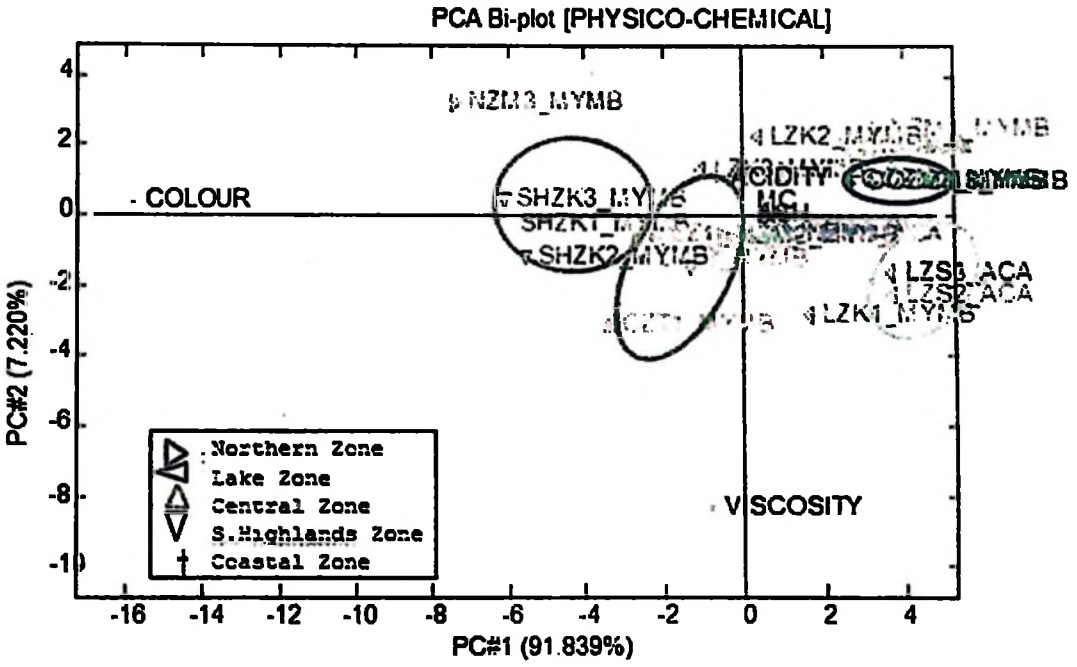

Figure 2.7b: PCA Bi-plot showing variation in physicochemical properties between honey floral origins and between zones

Key: MYB - *Miombo* honey, ACA - *Acacia* honey, Coastal zone - COZM, Central zone – CZT, Lake zone – LZK, Northern zone – NZM, Southern highland zone – SHZK

The same was observed on the negative side of PC1 where *Acacia* honey samples from the same zone (Lake, Northern and Central zone) were grouped together to form a clear separation from one another but were crowded with overlapping *Miombo* samples. Similarly, same visible separation pattern between zones along PC1 is depicted in Figure

7b. Southern Highland zone and Central zone were separated Coastal zone along PC1 while Central zone was separated from Coastal zone along PC2. The loadings of the physicochemical variables indicate that colour dominates the first component for separation of honeys according to floral and geographic origin along PC1 while viscosity is responsible for separation along PC2. Moisture content, ash, pH and acidity did not have much loading/effect to the separation samples as they were situated close to the origin.

2.5 Conclusions

This study observed that difference in flora and geographic origins had an effect on the physicochemical properties of Tanzania honey. Northern zone *Miombo* honey samples were observed to have high moisture content, ash and acidity while Coastal zone had high pH and Lake zone was high in viscosity. In *Acacia* samples, Central zone had high value of moisture content and colour with Northern zone being high in acidity and ash content. Lake zone *Acacia* honey samples were found to be more viscous and had high pH value. Biplots of PCA showed a contrast between samples from *Miombo* origin on one side and some *Miombo* and all *Acacia* honey samples on the other side. Also, Southern Highland, Central, Lake zone and Coastal zone samples were grouped together to form separation from each other. Colour and viscosity were seen to have higher loadings to the separation of samples and therefore, these parameters can be used as quality indicator when characterizing honey from different floral and geographic origin in the local and international market.

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CHAPTER THREE

MANUSCRIPT TWO

3.0 TOTAL PHENOLIC CONTENTS (TPC) AND ANTIOXIDANT ACTIVITIES OF HONEY FROM DIFFERENT GEOGRAPHICAL ZONES AND FLORAL ORIGIN IN TANZANIA

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Abstract

Honey samples from different floral origins and zones in Tanzania were screened for total phenolic content and for potential antioxidant activity. Samples were collected from two floral sources (*Miombo* and *Acacia*) and from five different zones (Central, Coastal, Lake, Northern, Southern Highland zone). Phenolic content of the collected honey samples was determined using modified Folin Ciocalteau method and antioxidant activity using ferric reducing antioxidant power (FRAP) assay. Statistical analysis was carried out to assess if there is any significant differences in phenolic content and antioxidant activity between zones and floral origins using analysis of variance. Phenolic content expressed as mg Gallic Acid Equivalent/100g ranged from 119.5 - 168.8 in *Acacia* honey and from 127.9 - 395.2 in *Miombo* honey. Antioxidant activity for *Miombo* honey samples ranged from 402 - 956.3 μM Fe^{2+}/100g while *Acacia* honey ranged from 253 -- 368 μM Fe^{2-}/100g. Among evaluated zones, Northern zone had the highest value for phenolic content and antioxidant activity with *Miombo* honey showing higher values than *Acacia* honey between floral origins. The variations observed were a result of different floral source visited by bees to collect the nectar and geographic locations where honey was collected from. Principal component analysis showed that 99.2% of variations were explained and there was a clear separation of zones and floral origin (*Miombo* honey and *Acacia* honey) along PC1. Strong correlations were observed between total phenol and antioxidant activity of both *Miombo* and *Acacia* honeys indicating its reliability in characterization of honey.

Keyword: *Phenol, antioxidant, floral, geographic location, honey*

3.1 Introduction

Honey is a natural substance produced by honeybees (*Apis mellifera*) from the nectar of blossoms and secretions of plants. It is a remarkably complex natural liquid that is reported to contain many beneficial substances with its composition being rather variable and primarily depending on the floral source (White, 1975). Honey has been used both in medical and domestic needs since ancient times, but of recent its uses is becoming increasingly popular due to its potential role in contributing to human health attributed by its antioxidant properties (Sime *et al.*, 2015; Khalil *et al.*, 2010). In addition to floral source, certain external factors also play a role in composition, such as seasonal and environmental factors and processing (Shahnawaz *et al.*, 2013).

Honey is a highly supersaturated solution of a complex mixture of sugars, mainly fructose and glucose as the main contributors. It also contains small amount of other constituents which are known to have antioxidant properties such as enzymes (e.g. catalase, peroxidase, glucose oxidase), minerals, proteins, vitamins, organic acids, flavonoids, phenolic compounds, organic acids, carotenoin-like substances, maillard reaction products, amino acids, proteins and other phytochemicals (Eleazu *et al.*, 2013; Andrade *et al.*,1997).

It is an important and unique food product containing bioactive compounds derived from plant nectar or added by bees which acts as natural antioxidants (Al-Mamary *et al.*, 2002). The antioxidants play an important role in food preservation and human health through combating damage caused by oxidizing agents. Various types of phytochemicals with antioxidants have been reported in honey such as vitamins C and E, flavonoids and phenolic compounds (Khalil *et al.*, 2010). Phenolic compounds or polyphenols are one of the most important groups originating from plants as secondary products (Bravo, 1998)

and their contents vary greatly according to the geographical and climatic conditions which contribute to different characteristic colors, flavors, aromas, and bioactivities (Molan, 1996: Abu-Tarboush *et al.*, 1993).

Recent studies on honey indicated that the biological actions of honey can be ascribed to its polyphenolic contents, which are elucidated by its antioxidant, anti-inflammatory, anti-proliferative and antimicrobial actions (Alvarez-Suarez *et al.*, 2013). The antioxidant activity of phenolics is mainly due to their redox properties, which allows them to act as reducing agents (Mihai *et al.*, 2011). However, composition of active compounds in honey differ with locations (Estevinho *et al.*, 2008) and leading to samples originating from similar floral origins have different antioxidant contents (Moniruzzaman *et al.*, 2013).

Despite adequate literature review, information on the phytochemical composition of Tanzania honey from different geographical and floral sources ranging from savannah to *miombo* woodland in Tanzania is missing. Therefore, this study was designed to investigate total phenolic contents and antioxidant activities of honey samples from different floral and geographical sources of Tanzania.

3.2 Materials and Methods

3.2.1 Study area

The study was conducted in five zones of Tanzania namely, Lake, Central, Coastal, Northern and Southern highland zones. The analytical work was conducted at the Department of Food Technology, Nutrition and Consumer Sciences (DFTNCS) laboratory, Sokoine University of Agriculture (SUA), Morogoro and at Tanzania Bureau of Standards (TBS) Laboratory Dar-es Salaam.

3.2.2 Materials

Honey samples were picked purposively depending on availability and distribution of floral/botanic origin, and they include *Acacia* honey from *Acacia spp* and honey from *Miombo* woodland. Twenty four honey samples (15 *Miombo* and 9 *Acacia* samples) were purchased directly from the beekeepers from different regions in the respective zones; Lake (Kigoma and Simiyu), Northern (Manyara), Central (Tabora and Dodoma). Coastal (Morogoro) and Southern Highland (Katavi). Analytical grade reagents and chemicals were obtained from TBS, DFTNCS laboratory and/or purchased from suppliers in Dar es Salaam.

3.2.3 Research design

Complete randomized block design (CRBD) with replication was used in this study. The principal factors were floral source with two levels (*Miombo* and *Acacia*) and geographical location with five levels (the zones). The effect of these factors on analyzed parameters were determined. The design mathematical model is depicted in

Equation 1.

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad \text{.....} \quad (1)$$

Where μ is the overall (grand) mean. αi is the effect due to the i^{th} treatment (floral),

β_j s the effect due to the j^{th} block (geographical) and ε_{ij} is the error term

3.2.4 Chemical analyses

3.2.4.1 Sample extraction

Three grammes of honey sample was mixed with 30 ml of methanol and sonicated for 15 minutes at 0°C and left overnight. The mixture was centrifuged at 9000 rpm using Universal 320R centrifuge (Hettich Zentrifugen, German) and the supernatant was decanted and stored at 20°C prior to analyses.

3.2.4.2 Total phenol

Determination was done by using the Folin-Ciocalteu reagent (FCR) method as described by Singleton *et al.* (1999) and ISO 14502-1(2005). About 0.5 mls of extracted sample was transferred into a separate tube and then 2.5 mls of diluted Folin-Ciocalteu phenol reagent and 2 mls of 7.5% sodium carbonate solution were added and mixed thoroughly. The mixture was allowed to stand at room temperature for two hours and the absorbance was read at 765 nm against blank using UV-VIS spectrophotometer (Labomed Inc. USA). All determinations were performed in triplicate. Gallic acid was used as a standard and concentration of 0.01 - 0.05 mg/ml of gallic acid were prepared in methanol. The total phenolics were expressed as gallic acid equivalents GAE (mg GAE/100 g) of honey

3.2.4.3 Antioxidant activity

Antioxidant activity in samples was measured using the Ferric Reducing Ability of Plasma (FRAP) method by Benzie and Strain (1996) and modification by Halvorsen *et al.* (2002). The assay was based on the absorbance change when iron (III) 2,4,6-tripyridyl-s-triazine $(Fe[TPTZ])^{3+}$ is reduced to $(Fe[TPTZ])^{2+}$ (intense blue) at 595nm. The FRAP reagent was prepared by mixing 2.5 mL of a solution of 10 mmol/L TPTZ in 40 mmol/L HCl, 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 0.30 mol/L acetate buffer (pH 3.6). About 0.5ml of sample was placed in a tube and 4.5 mls of FRAP solution were added. The mixture was incubated at 37°C for 10 minutes and absorbance was measured at 595 nm using UV VIS spectrophotometer (Labomed Inc, USA). A calibration curve was constructed using $FeSO_4$ solutions (concentrations from 0.1 to 1mM, in 0.2 mM increments) and the absorbance was measured at the same wavelength. The results were expressed as μM $Fe2^{+}$/100 g of honey.

3.2.5 Statistical data analysis

Data obtained were analyzed using the R statistical package (R Development Core Team, Version 3.0.0 Vienna, Austria). Analysis of variance (ANOVA) was used to determine the

significant differences between the main factors. Means were separated using Tukey's Honest Significant difference ($p < 0.05$). Principal Component Analysis (PCA) was used to determine the systematic variations in data (Martens and Martens, 2001) using Latentix Software (LatentiX Aps Team, version 2.12, Frederiksberg Denmark). Results were presented as arithmetic mean and standard deviation in Tables and graphs as well as in PCA bi plots.

3.3 Results and Discussion

3.3.1 Effect of zones and floral origin on total phenol contents

Figure 3.1 shows the concentration of total phenolic compounds in honey samples between different zones within each floral source. The total phenolic contents differed significantly ($p < 0.05$) between zones in each floral source with Northern and Coastal zone samples having highest and lowest values of 395.2 and 127.9 mg GAE/100g respectively in *Miombo* floral origin. Among *Acacia* honey samples, Central zone had significantly ($p < 0.05$) higher TPC value of 168.8mg GAE/100g) than Lake and Northern zone, which had statistically similar ($p > 0.05$) values of 119.5 - 126.2 mg GAE/100g.

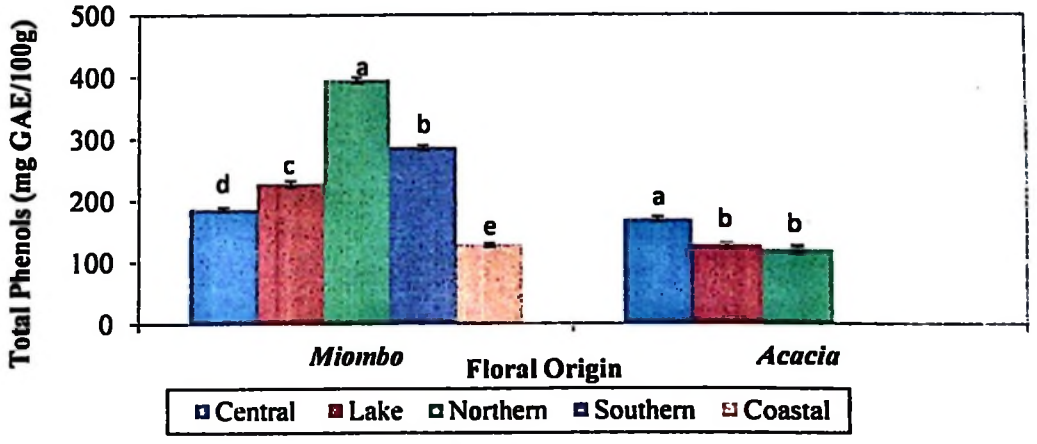

Figure 3.1: Total Phenol contents (mg GAE/100g) of honey samples of similar floral origin between different zones. Bars with different letters are significantly different at $p < 0.05$

The results showing the effect of floral origin in TPC within each zone are depicted in Figure 3.2. There was a significant difference ($p < 0.05$) in total phenol content between the *Miombo* and *Acacia* honey samples within each evaluated zones. *Miombo* samples had higher values of 185.9, 227.3 and 395.2 mg GAE/100g in Central, Lake and Northern zones respectively, than respective values of 168.2, 126.2 and 119.5 mg GAE/100g in *Acacia* floral source. No data obtained for *Acacia* in coastal and southern highland zones.

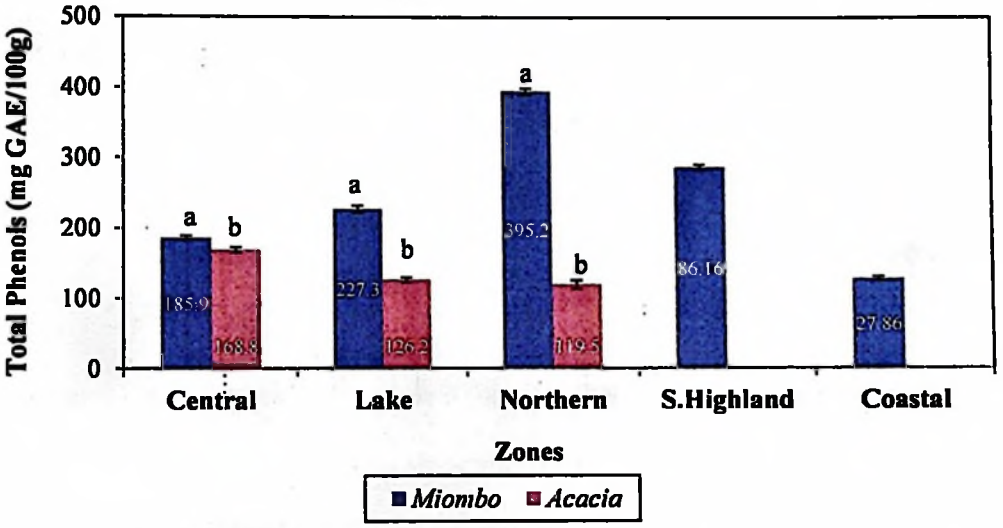

Figure 3.2: Total Phenol contents (mg GAE/100g) of honey samples between floral origins within zones. Bars with different letters are significantly different at $p < 0.05$

Phenolic compounds are one of the most important groups of compounds occurring in plants. The observed variation in total phenolic contents between flora and geographical zones could be explained by the fact that different types of honey contain a wide range of phytochemicals including flavonoids, polyphenols and phenolic acids which act as antioxidants which depend on geographical and climatic conditions (Jaganathan and Mandal, 2009). These results were in agreement with findings by Muruke (2014) who studied total phenolic content of Tanzania honey and obtained a range of 31 – 618 mg GAE/100g, which was similar to 119.5-395 mg GAE/100 g obtained in this study.

However, the observed findings were lower than 330-610 mg GAE/100 g reported by Sime *et al.* (2015) in his study on total phenols content of natural honey from different geographical locations in Ethiopia. These differences could be attributed to difference in honey samples as they were of different floral source and geographic location. Honey collected from different nectar of plant flower species contains different levels of phenolics that possess antioxidant activity (Rababah *et al.*, 2014; Bertoncelj *et al.*, 2007). Al-Mamary *et al.* (2002); Cheynier (2005) and Mu_noz *et al.* (2007) reported that biotic and abiotic stresses caused by environmental factors are able to trigger changes in the plant's metabolism. These changes may affect the polyphenol biosynthesis, especially phenolic acids, which represent the evolutionary response to plants adaptation to different environmental characteristics.

Furthermore, the observed variation in TPC between *Miombo* and *Acacia* could be ascribed to the fact that TPC differs with floral origin as reported in other studies by Bertoncelj *et al.* (2007) and Azad *et al.* (2016). Bertoncelj *et al.* (2007) found out that phenolic content differ with floral sources with *Acacia* honey samples showing low total phenolic content and consequently lower antioxidant capacity than other honey types. This was also shown by Azad *et al.* (2016) who observed that the total phenolic content showed significant differences among the different floral sources of Chinese honey samples.

The higher TPC contents in Northern zone sample from *Miombo* source could be associated to the presence of high quantity of flavonoid compounds (Ayoub *et al.*, 2009). This can be supported by Muruke (2014) whose results showed that high phenolic content corresponded with high flavonoid content in Tanzania raw honey and stingless bees honey. Similar observation was also seen in a study by Moniruzzaman *et al.* (2013) who

reported that the honey samples high flavonoid content also tended to have correspondingly high phenolic content. Also, the strongest positive significant correlation was found to be between total phenolics and total flavonoids (r =0.9590) (Islam *et al.*, 2012). Flavonoids is one of the most important class of antioxidant together with polyphenols whose amount and type also depend largely upon the floral source/variety of the honey. They have a great effect on many biological activities (e.g., antibacterial, anti-inflammatory and antiallergic) (Khalil *et al.*, 2010: Ayoub *et al.*, 2009). This suggests for further studies in Northern zone honey samples for more exploration on antioxidant compounds of Tanzanian honey.

3.3.2 Effect of zones and floral origins on total antioxidant activity

Total antioxidant activities of honey samples from different zones are presented in Figure 3.3. It showed a significant difference ($p < 0.05$) in total antioxidant contents between all zones with Northern zone having higher value of 956.3 µM Fe^{2+}/100g and coastal zone with lowest value 401.68 µM Fe^{2+}/100g within the *Miombo* floral origin. Similarly, *Acacia* samples showed significant differences ($p < 0.05$) in antioxidant activity between zones with Central zone having the highest value of 368.3 µM Fe^{2+}/100g followed by Northern zone with values of 279.3 µM Fe^{2+}/100g and least value of 252.6 µM Fe^{2+}/100g in the Lake Zone. Generally, the results showed that *Miombo* had higher antioxidant activity values than their *Acacia* counterparts (Figure 3.3).

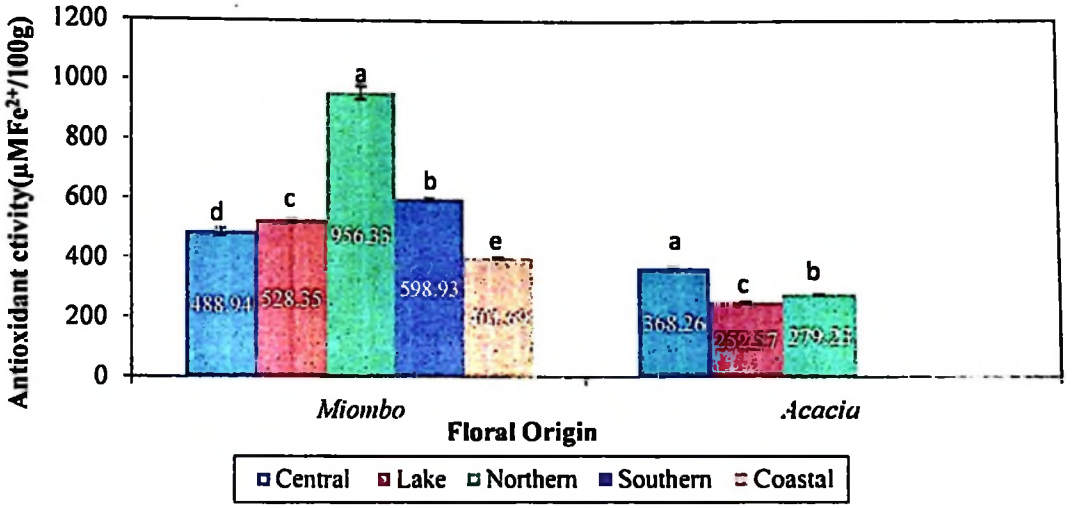

Figure 3.3: Antioxidant activities (µM Fe^{2+}/100g) of honey samples of similar floral origin between different zones

Bars with different letters are significantly different at $p < 0.05$

Figure 3.4 shows the results of the effect of floral origin in antioxidant activity of honey samples within each zone. The antioxidant activities differed significantly ($p < 0.05$) between floral origins in each zone. Similar to TPC, *Miombo* samples had higher values of antioxidant activity of 488.9, 528.35 and 953. 3 µM Fe^{2+}/100g in Central, Lake and Northern zones respectively than respective values of 368.26, 252.6 and 279.2 µM Fe^{2+}/100g g in *Acacia* floral origin.

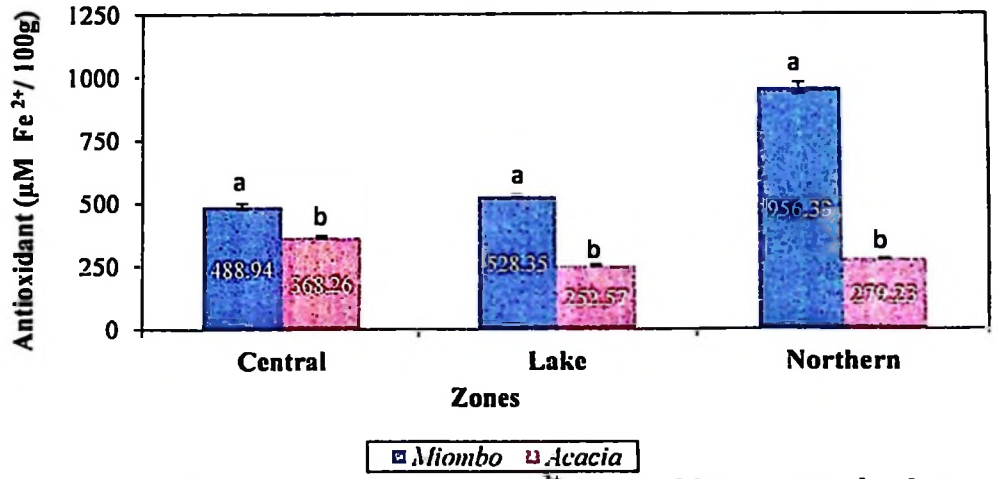

Figure 3.4: Antioxidant activities (µM Fe^{2+}/100g) of honey samples between floral origins within zones. Bars with different letters are significantly different at $p < 0.05$

The higher antioxidant activities for *Miombo* honey samples from Northern zone could be attributed to the high phenolic content observed in the samples. Estevinho *et al.* (2008) and Ferreira *et al.* (2009) have demonstrated that the antioxidant activity of honey is due to the large amount of phenolics present. This is also true for the *Acacia* honey sample from Central zone, which showed the highest antioxidant activity coupled with highest value of phenolic content. The overall observation could be seen that honey from *Miombo* floral have high antioxidant activity due to high phenolic content compared to *Acacia* honey which showed less antioxidant activity and low phenolic content. Therefore. the differences found among the honey samples of different types strengthen the widely accepted theory that the antioxidant activity of honey varies greatly depending on the floral source and on external factors, such as season and environment, as well as the processing method used (Go´mez-Caravaca *et al.*, 2006).

The FRAP assay gives a direct estimation of the antioxidants or reductants present in a sample based on its ability to reduce the Fe3+/Fe2+ couple (Islam *et al.*, 2012). It is considered to be a good indicator of antioxidant activity due to its reducing power test. in which the capacity of breaking radical chain reactions is reflected (Dong *et al.*, 2011). The main factor which has a key role and responsible for this action is phenolic substances of honey (Aljadi and Kamaruddin, 2004; Al-Mamary *et al.*, 2002). The determined antioxidant activity in this study measured by FRAP corresponds well to that determined by others such as Penna *et al.* (2013) who evaluated Italian honey and obtained a range of 216.57 to 695.64 µM Fe^{2+}/100g; Das *et al.* (2013) who observed antioxidant properties of honey from India (101-622 µM Fe (II) equivalence) and Moniruzzaman *et al.* (2014) who obtained a range of 116.00 to 786.22 µM Fe^{2+}/100g of honey. Salgueiro *et al.* (2014) reported higher values (23 000 – 11 6000 µM Fe^{2+}/100g). The higher values in *Miombo*

than in *Acacia* sample is similar to the findings by Bertoncelj *et al.* (2007) and Krpan *et al.* (2009).

3.3.3 Principal Component Analysis

Figure 3.5a shows bi-plot with two first significant principal components on average total phenol and antioxidant properties. Principal component 1 (PC1) accounted for 99.187% of the variations while PC2 accounted 0.813% of total variations. PC 1 is a clear contrast between *Miombo* and *Acacia* samples. Furthermore, PCI separated Northern samples from other samples. All *Miombo* honey samples positively correlated with total phenol and antioxidant activity along PC1 apart from *Miombo* samples from Coastal zone which were on the negative side of PC1. The *Acacia* honey samples correlated negatively with total phenol and antioxidant activity. Similarly, visible separation between zones is depicted in Figure 3.5b. All zones with samples from *Miombo* floral origin were clearly separated from each other on the positive side of PC 1 with Northern zone being expressed more with total phenol and antioxidant capacity. The same was observed on the negative side of PC1 where *Acacia* samples from the same zone were grouped together to form a clear separation from one another.

Furthermore, the loadings of the TPC and antioxidants variables indicates that antioxidant activity dominate the first component for separation of honeys according to floral and geographic origin along PC1 while total phenol is responsible for separation along PC2. *Miombo* honey from Northern zone was observed to have higher correlation with total phenol content and antioxidant capacity of the samples (Abdi and Williams, 2010).

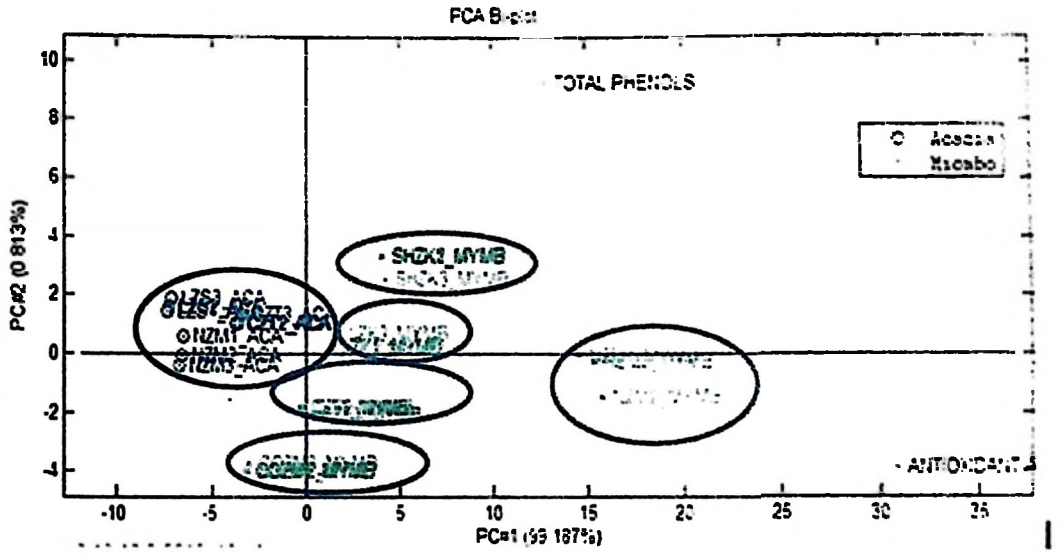

Figure 3.5a: PCA Bi-plot showing variation in antioxidant activities between honey floral origins (*Miombo* and *Acacia*)

Key: MYB - *Miombo* honey. ACA - *Acacia* honey. Coastal zone - COZM. Central zone – CZT. Lake zone – LZK. Northern zone – NZM. Southern highland zone – SHZK

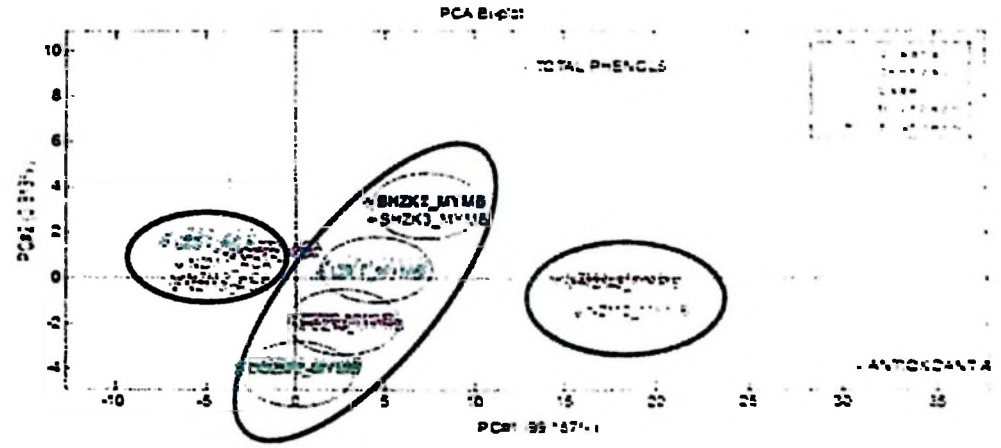

Figure 3.5b: PCA Bi-plot showing variation in antioxidant activities between honey floral origins and between zones

Key: MYB - *Miombo* honey. ACA - *Acacia* honey. Coastal zone - COZM. Central zone – CZT. Lake zone – LZK. Northern zone – NZM. Southern highland zone – SHZK

3.3.4 Correlation analysis between total phenolic contents and antioxidant activities

The correlation analysis between total phenolic and antioxidant activity of honey samples is shown in Figure 3.6. There was a strong positive correlation between the TPC and antioxidant activities within *Miombo* floral origin (R^2=0.9293) as depicted in Figure 3.6A and within *Acacia* samples (R^2= 0.8691) as depicted in figure 3.6B. This finding shows that, the antioxidant activity of honey is strongly correlated to the TPC contents (Gambacorta *et al.*, 2014; Krpan *et al.*, 2009; Giorgiana *et al.*, 2008). Similar correlations between total phenols and antioxidant activity in plants have been reported (Sreeramulu *et al.*, 2010; Mao *et al.*, 2010; Blasa *et al.*, 2006; Beretta *et al.*, 2005; Meda *et al.*, 2005; Gheldof and Engeseth, 2002).

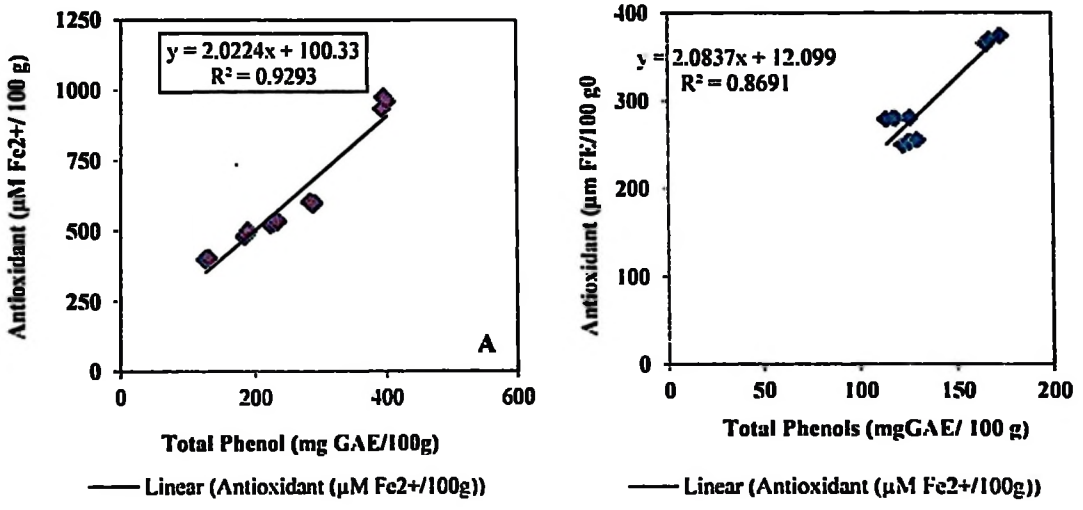

Figure 3.6: Correlation between total phenols and antioxidant activities (µM Fe^{2+}/100g) of honey samples of *Miombo* (A) and *Acacia* (B) floral origin

3.4 Conclusions

This study evaluated honey samples from different floral sources (origin) and locations (geographical) in Tanzania for total phenol content and antioxidant capacity. Higher value in phenolic content and antioxidant activity of *Miombo* samples were observed in Northern zone. In *Acacia* samples, Central zone was observed to have high phenolic and

total antioxidant activity. Between floral origins, samples from *Miombo* were found to have higher total phenolic content and antioxidant activity. Biplots of PCA clearly separated *Acacia* samples from *Miombo* samples between flora origins and zones were clearly grouped together and separated from each other. Total phenolics were observed to have a strong correlation with antioxidant activity especially in *Miombo* samples. From the results of this study, it can be observed that difference in floral and geographic origins has an effect on total phenolic and antioxidant activity of Tanzanian honey. Therefore, these parameters can be used as an indicator of honey quality.

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CHAPTER FOUR

MANUSCRIPT THREE

4.0 DETERMINATION OF SUGARS IN HONEY FROM DIFFERENT FLORAL/GEOGRAPHIC ORIGIN IN TANZANIA

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Abstract

Sugar characteristics of honey from different floral sources and geographic locations in Tanzania were evaluated in this study. Samples were collected from two floral sources (*Miombo* and *Acacia*) and from five different zones (Central, Coastal, Lake, Northern, Southern zone). Sugar profile and quantification were performed using Shimadzu high-performance liquid chromatograph equipped with refractive index detector (RID-10A) as per method 977.20 in AOAC (2005). Complete randomized block research design with floral and geographic zone as main principal factors was used. The results showed significant differences ($p < 0.05$) in fructose, sucrose and total sugars between zones and floral origins. Fructose ranged from 39.5 – 47 g/100g and was found to be the dominant sugar with the highest contents observed in *Acacia* samples. Glucose, the second dominant sugar ranged from 32.0 – 35.0g/100g and was not significantly different between floral origins and among zones. Sucrose varied significantly and ranged from 5.1 – 7.3 g/100g. Northern zone had the highest content of total sugar of 79.7 g/100g. PCA bi plots explained 90.7% of total variations and gave a clear contrast between floral origins and zones. All *Acacia* samples were correlated with high content of fructose and total sugar. *Miombo* from Central, Coastal and Lake zones were correlated with glucose content while *Miombo* from Southern highland and Northern zones correlated with lower content of fructose, glucose and total sugars. Therefore, sugar profile can be considered as one of the useful ways that can be used to effectively characterize honey according to floral and geographical origins.

Keywords: *Fructose, glucose, honey, floral, geographic*

4.1 Introduction

Sugars are simple carbohydrates and are important for everyday life biological functions such as providing energy for running vital roles of the living body (Kamal and Klein. 2011). Honey has very old history for human life since ancient times mainly as a sweetening agent, and its consumption has grown drastically during the last few decades due to its high nutritional value and unique flavour (Kivrak *et al.*, 2016). The composition of honey consists primarily simple sugars mainly glucose and fructose and may contain low levels of sucrose and/or maltose (Moussa *et al.*, 2012; Kamal and Klein. 2011). It also consists of other minor substances such as organic acids, mineral, vitamins and lipids (Finola *et al.*, 2007).

As a result of mentioned natural properties, honey has become expensive than any other sweetener, and it can therefore, be a target of adulteration (Sivakesava and Irudayaraj, 2001). Adulteration has become a very important authenticity issue of late, and it is increasingly important for consumers, producers, and regulatory authorities (Boussaid *et al.*, 2013). Currently in honey global markets, quality of honey is done in order to verify its authenticity regarding adulteration and authenticity regarding honey labeling which attest to its floral or geographic origin (Madas *et al.*, 2014). Having knowledge on the chemical characteristics and sugar composition of honey as one of the respected health-promoting natural products, is of general interest in terms of their protection against adulteration (Arvanitoyannis *et al.*, 2005).

The properties and composition of honey are very well known to vary depending on floral origin, geographical, environmental and seasonal conditions. In consideration of all these factors, sugar composition variations of honey would for that reason be influenced by the variations in nectar content together with other factors such as climatic conditions, soil

type and beekeeper activities (Pires *et al.*, 2009; Bogdanov *et al.*, 2008). According to Bogdanov *et al.* (2004), the relative amount of the two monosaccharides, fructose and glucose can be useful for the classification of botanic origin of honeys, as well as the fructose-glucose and glucose-water ratios.

Many authors have illustrated the influence of flora and geographic origin on honey in different countries such as Boussaid *et al.* (2013); Kirs *et al.* (2011) and Marghitas *et al.* (2009) in Tunisia, Estonia and Romania, respectively. In Tanzania, very few studies have been done on the quality of honey including sugars but none of them had determined its characterization based on floral and geographic origin. This study aimed at determining sugar profile (fructose, glucose and sucrose) of Tanzania honey from different floral and geographic origin.

4.2 Materials and Methods

4.2.1 Study area

The study was conducted in five zones of Tanzania namely, Lake, Central, Coastal, Northern and Southern highland zones. The analytical work was conducted at Tanzania Food and Drugs Authority (TFDA) Laboratory in Dar es Salaam.

4.2.2 Materials

Honey samples were picked purposively depending on availability and distribution of floral/botanic origin, and they include *Acacia* honey from *Acacia spp* and honey from *Miombo* woodland. Twenty four honey samples (15 *Miombo* and 9 *Acacia* samples) were purchased directly from the beekeepers from different regions in the respective zones: Lake (Kigoma and Simiyu), Northern (Manyara), Central (Tabora and Dodoma), Coastal

(Morogoro) and Southern Highland (Katavi). Analytical grade reagents and chemicals were obtained from TFDA laboratory.

4.2.3 Research design

Complete randomized block design (CRBD) with replication was used in this study. The principal factors were floral source with two levels (*Miombo* and *Acacia*) and geographical location with five levels (the zones). The effect of these factors on glucose, fructose and sucrose content of samples was determined. The design mathematical model is depicted in Equation 1.

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad \ldots\ldots(1)$$

Where μ is the overall (grand) mean, αi is the effect due to the i^{th} treatment (floral), β_j s the effect due to the j^{th} block (geographical) and ε_{ij} is the error term

4.2.4 Glucose, fructose and sucrose determination

Determination of sugar profile and quantification was performed using Shimadzu high-performance liquid chromatograph equipped with refractive index detector (RID-10A) as per method 977.20 in AOAC (2005). A 5 g sample was dissolved in 100 ml volumetric flask with water and 25 mls of methanol. The mixture was filtered through a membrane filter into a 100 ml volumetric flask and the solution was topped to the mark with distilled water. The separation was performed using carbohydrate analysis column (4.6 mm in diameter, 250 mm length) with a particle size diameter of 5-7 µm. The column was kept at 30°C throughout the analysis. The mobile phase was composed of 85% acetonitrile in water. The injection volumes of the samples were 20 µl, with a flow rate of 2 ml/min. Peaks were identified on the basis of their retention times. Quantification was performed according to the external standard method on peak areas (Bogdanov, 2009b). Duplicate injections were performed and average peak areas were used for the peak quantification.

Glucose, fructose and sucrose were used as standards to determine the sugar content of honey.

4.2.5 Statistical data analysis

Data were analyzed using the R statistical package (R Development Core Team, Version 3.0.0 Vienna, Austria). Analysis of variance (Anova) was used to determine the significant differences between the main factors. Means were separated using Tukey's Honest Significant difference ($\alpha = 0.05$). Principal Component Analysis (PCA) was used to determine the systematic variations in the data (Martens and Martens. 2001) using LatentiX software (LatentiX Aps. Team. version 2.12. Frederiksberg. Denmark). Results were presented as arithmetic mean and standard deviation in tables and graphs as well as in PCA bi plots.

4.3 Results and Discussion

4.3.1 Sugar profile between and within zones

Table 4.1 shows variation of sugars within and between zones in the floral origins. The findings showed that honey samples were composed primarily of the simple sugars fructose and glucose as well as little amount of sucrose which is composed of fructose and glucose linked together. In all zones, fructose dominated significantly higher with values ranged from 39.5 to 42 g/100 g than glucose with values ranged from 32.8 - 33.7 g/100 g and sucrose with lowest values of 5.1 - 7.1 g/100 g. The total sugar ranged from 72.6 to 75.8 g /100 g.

The variation of sugars between zones in each floral origin were not significant ($p > 0.05$) except for *Acacia* samples whereby Lake zone had significantly higher fructose value of

47g/100g followed by Central zone with 45 g/100 g and least value in the Northern zone with 44.7 g/100 g.

Table 4.1: Sugar profile of honey samples within and between zones in *Miombo* and *Acacia* floral zones

Floral origin	Zone	Sugars (g/100g)			
		Fructose	Glucose	Sucrose	Total
Miombo	Central	40.6 ± 0.89^{a}	33.2 ± 512^{a}	$6.0 \pm 0.72b^{c}$	73.8 ± 5.20^{a}
	Lake	41.9 ± 0.81^{a}	33.5 ± 2.88^{a}	5.1 ± 0.50^{c}	75.4 ± 3.45^{a}
	Northern	40.8 ± 2.64^{a}	32.8 ± 3.65^{a}	7.1 ± 2.511^{a}	73.6 ± 5.15^{ab}
	Southern	39.5 ± 2.17^{b}	33.13 ± 4.5^{a}	6.50 ± 1.15^{b}	72.6 ± 3.49^{b}
	Highland				
	Coastal	42.04 ± 4.34^{a}	33.73 ± 0.57^{a}	$5.69 \pm 0.51b^{c}$	75.8 ± 4.70^{a}
Acacia	Central	45.3 ± 8.97^{ab}	33.0 ± 1.46^{a}	7.3 ± 0.7^{b}	78.3 ± 9.45^{a}
	Lake	47.0 ± 0.00^{a}	32.0 ± 0.00^{a}	6.7 ± 0.00^{a}	78.99 ± 0.00^{a}
	Northern	$44.7 \pm 1.04b$	35.0 ± 1.32^{a}	6.75 ± 0.28^{a}	79.7 ± 2.25^{b}

Mean values are expressed as mean ±SD (n=9)
Mean values with different superscript letters along the columns are significantly different at $p < 0.05$

4.3.2 Sugar profile between floral origins within zones

The results for sugars profiles between floral origins in each zone presented in Table 4.2 showed significant differences in some of evaluated zones. In Central and Lake zones, total sugar, fructose and sucrose contents differed significantly between the two origins with *Acacia* samples having higher total sugar values of 78.3 and 78.99 g/100g respectively than respective lower values of 74.9 and 75.4 g/100g observed in *Miombo* samples within the same zone. Similarly, *Acacia* samples observed to have significantly higher fructose values of 45 and 47 g/ 100 g in Central and Lake Zones respectively. No *acacia* honey data was collected from Southern highland and Coastal zones as *Acacia* spp is not dominant in Southern Highland zone and no sample could be obtained from Central zone due to seasonality.

Furthermore, in the same zones (Central and Lake). *Acacia* samples had higher sucrose values of 7.25 and 6.67 g/100 g respectively than *Miombo* samples with respective values of 6.04 and 5.14 g/100g. No significant ($p > 0.05$) variation was observed between the same within the Northern zone. There was no significant ($p < 0.05$) variations in glucose contents between *Miombo* and *Acacia* origin within each zone (Table 4.2).

Table 4.2: Sugar profile between *Miombo* and *Acacia* floral origins in each zone

Zone	Floral origin	Sugars (g/100g)			
		Fructose	Glucose	Sucrose	Total
Central	*Acacia*	45.3 ± 0.89^a	33.0 ± 1.46^a	$7.25 \pm .75^a$	78.3 ± 9.45^a
	Miombo	40.6 ± 8.97^b	33.2 ± 5.12^a	6.04 ± 0.72^b	74.9 ± 5.20^b
Lake	*Acacia*	46.98 ± 0.0^a	32.01 ± 0.0^a	6.67 ± 0.0^a	78.99 ± 00^a
	Miombo	41.89 ± 0.81^b	33.49 ± 2.88^a	5.14 ± 0.50^b	75.4 ± 3.45^b
Northern	*Acacia*	44.7 ± 1.04^a	35.0 ± 1.32^a	6.75 ± 0.28^a	72.8 ± 2.25^a
	Miombo	40.8 ± 2.64^a	32.8 ± 3.65^a	7.1 ± 2.51^a	73.6 ± 5.15^a
S. Highland	*Acacia*	-	-	-	-
	Miombo	39.5 ± 2.17	33.1 ± 4.46	6.50 ± 1.15	72.6 ± 3.49
Coastal	*Acacia*	-	-	-	-
	Miombo	$42.04 \pm 4.3.4$	33.73 ± 0.57	5.69 ± 0.51	75.8 ± 4.70

Mean values are expressed as mean±SD (n=9)
Mean values with different superscript letters along the columns are significantly different at $p < 0.05$

The results showed that, fructose was the dominating sugar in analyzed honey samples from all zones and floral origins followed by glucose. Fructose and glucose have been found to be the major constituents of honey (Kucuk *et al.*, 2007). These findings agree with many other studies on honey such as Kirs *et al.* (2011); Madas *et al.* (2014) and Adriana *et al.* (2012). However, the observed values from the study were higher and lower than those observed by Velásquez Giraldo *et al.* (2013) and Chua and Adnan (2014), respectively.

The high fructose content observed in the evaluated samples of honey was merely expected as fructose has been mentioned to be the predominant sugar. The sweet taste of honey has been associated with fructose content as some honey rich in fructose were found to taste sweeter (NHB, 2016). Moreover, predominance of fructose over glucose is one way in which honey is differentiated from commercial sugars and is one of the quality signs (Buba *et al.*, 2013). Therefore, the high content of fructose over glucose in analyzed samples could only mean that the samples collected for the study were in good quality. Variations observed in fructose contents between floral origins could be due to the differences in sugars present in the nectar collected, and the invertase enzymes present in the bee (Bogdanov, 2009a, Zafar *et al.*, 2008). In addition, the variation between zones could be explained by the fact that composition of sugars is said to be affected by geographical location where honey was produced and harvesting time (Chua and Adnan, 2014).

The sugars of honey are responsible for many of the physicochemical properties such as viscosity, hygroscopic and granulation characteristics of honey (Gobessa *et al.*, 2012). All of the investigated honey samples contained recommended amount of total sugars (fructose+glucose) as per requirements of the international standard for honey by Codex Alimentarius Commission (2001) and Tanzania honey Standard (TZS 851) for honey of > 60 g/100 g. The higher total sugar in *Acacia* samples than *Miombo* samples is similar to results observed in Malaysian honey where *Acacia* honey was found to have higher total soluble solids (fructose, glucose, sucrose) than other types of honey (A-rahaman *et al.*, 2013). Contrary results were seen in a study by Marghitas *et al.* (2010) who reported lower values of total sugars than the observed results. The chemical composition of the nectar collected from floral source in different zones could be a reason for the significant

variations in total sugar that were observed in total sugar. Therefore, this may explain the lower fructose contents in the Southern highland zone samples compared to other zones.

Despite the observed significant differences ($p < 0.05$) in the sucrose content between honey samples from some different geographical zones all samples had sucrose content above the maximum limit of 5% allowed by Codex Alimentarius Commission (2001) and Tanzania national standard (2006). Though, there are some exception of some kind of honey from nectar with naturally higher sucrose like false *Acacia* (*Robinia pseudoaccacia*), high sucrose content could suggest a number of things. The high content could have been caused by the sugar feeding of bees as a supplement food (Abdulkhaliq and Swaileh, 2016; Abu Tarboush *et al.*, 1993). Its content can indicate that the honey was harvested before its "ripening", causing an incomplete transformation of sucrose into fructose and glucose by action of the invertase enzyme secreted by bees (Azeredo *et al.*, 1999). Likewise, sucrose content is also used to monitor honey quality and adulteration as honey adulterated with sugar is usually high in sucrose level (Chua *et al.*, 2014, Dos Santos *et al.*, 2014). Similar results in high sucrose content were reported in a study by Dos Santos *et al.* (2014). However, a lower range of 0.23 – 3.41% of sucrose for Algerian poly-floral honey was reported by Ouchemoukh *et al.* (2007).

4.3.3 Sugar ratios between zones and floral origins

In *Miombo* origin samples, the fructose/glucose ratio ranged from 1.2 to 1.3 while glucose/water ratio ranged 1.6 to 2.0 (Figure 4.1a). Significant ($p < 0.05$) variation was observed in glucose/water ratio only between zones with Central, Lake and Southern Highland zones scoring higher value of 2 than lowest value of 1.6 in the Northern zone. As for *Acacia* origin samples, the fructose/glucose ratio ranged from 1.1 to 1.5 while glucose water ratio ranged 1.9 to 2.0 (Figure 4.1b). Significant ($p < 0.05$) variation was

observed in both parameters between the zones where Lake zone had highest fructose/glucose ratio value of 1.5 while northern zone had the lowest value. Furthermore, significantly higher glucose/water ratio was observed in Northern and Southern Highland zones and lower value of 1.9 in Central zone.

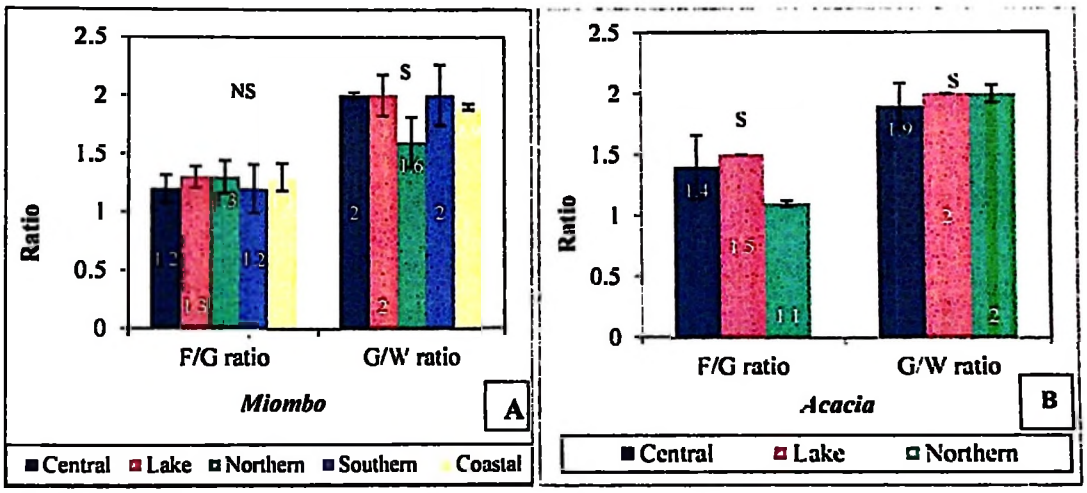

Figure 4.1: Variations of F/G and G/W ratio between zones origin in each floral type where A – *Miombo* floral samples; B – *Acacia* floral samples

Key: F/G – fructose/glucose ratio, G/W – glucose/water ratio, S – significant different, NS – not significant different

The variation of fructose/glucose ratio and glucose water ratio between flora origins are shown in Figure 4.2 A and B. The fructose/glucose ratios differed significantly ($p < 0.05$) between the floral origins within each zone with *Acacia* samples having higher ratio values than in *Miombo*. The glucose/water ratios differed significantly ($p < 0.05$) between the floral origins within each zone with *Miombo* samples having higher ratio values except in the Northern zone.

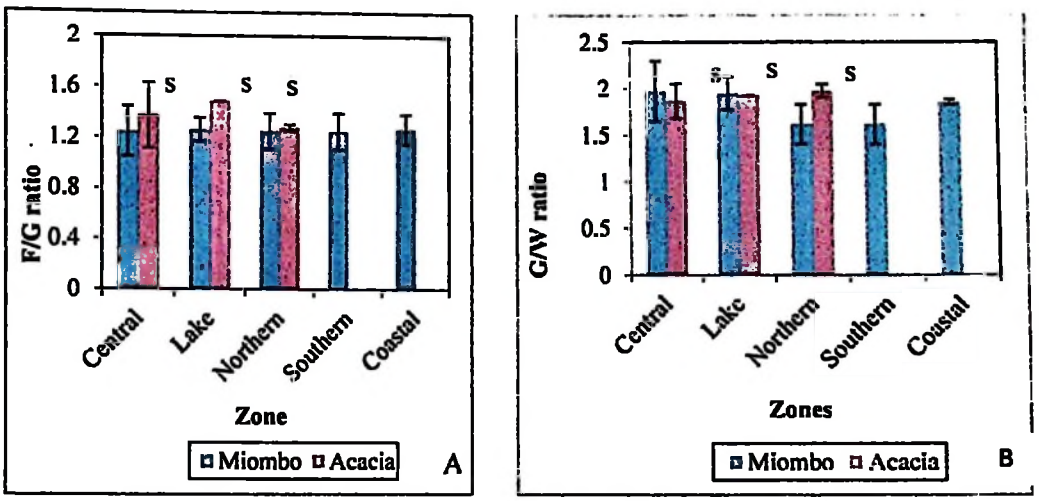

Figure 4.2: Variations of F/G and G/W ratio between floral types in each zone where A – F/G ratio variations and B – G/W ratio variations

Key: F/G – fructose/glucose ratio, G/W – glucose/water ratio, S – significant, NS – non significant

Fructose/glucose ratio and glucose/water ratios are parameters that are used to predict the tendency of honey to crystallize. The speed of honey to crystallize depends on the relative amount of each sugar (Ouchemoukh *et al.*, 2010). High F/G ratios more than 1.5 indicates that honeys would remain liquid for longer periods due to modification of the saturated level of glucose by the presence of the larger amount of fructose while ratio of 1.1 or less, would enhance crystallization (Mondragón-Cortez *et al.*, 2012). Similar fructose/glucose ratio ranged from 1.2 - 1.3 and 1.1 - 1.5 for *Miombo* and *Acacia* honey respectively obtained from this study were also reported ratio by Buba *et al.* (2013) who observed a range of 1.18 - 1.29. The ratios obtained in this study were above one and this means that fructose content was higher than glucose in the evaluated samples. Also, the F/G ratios of *Acacia* samples were higher compared to *Miombo* samples. Relating to actual samples, some *Miombo* samples could have started crystallization process as samples were very viscous though with no clear separation of sugars crystals especially in samples from Central, Southern Highland and Lake zone which is also supported by their low F/G ratios.

In addition, the fructose/glucose ratio is said to have an impact on honey flavour, since fructose is sweeter than glucose (Ojeda de Rodríguez *et al.*, 2004).

In addition to fructose and glucose, honey contains others sugars such as maltose, furanose and insoluble substances like dextrin, colloids which also influence the crystallization process. Due to this influences, the glucose/water (G/W) ratio is considered to be more appropriate than the fructose/glucose (F/G) ratio for the prediction of honey crystallization (Buba *et al.*, 2013; Baroni *et al.*, 2009). It has been stated that when the glucose/water ratio is < 1.3 honey crystallization is very slow or even zero, and it is complete and rapid when the ratio is close or > 2.0 (Amir *et al.*, 2010). The observed G/W ratio ranged from 1.6 - 2.0 and 1.9 – 2.0 between zones within *Miombo* and *Acacia* samples. The high ratio observed means the evaluated honey are in crystallization stage but this could be correlated to honey samples from Central, Lake and Southern Highland zones which were very viscous either due to crystallization or having low moisture contents. This is in agreement with literature as the samples showed low F/G ratios with high G/W ratios.

4.3.4 Principal component analysis

Bi-plot with two first significant principal components on honey sugars between floral and among geographical zone origin is given in Figure 4.3 (a and b). Principal component 1 (PC1) accounted for 90.662% of the variations while PC2 accounts for 5.269% of the total variations. PC 1 is a clear contrast between *Miombo* and *Acacia* samples with *Miombo* samples on the left side and *Acacia* samples on the right side of PC (Figure 4.3a). All *Miombo* honey samples positively correlated with glucose while *Acacia* honey samples correlated positively with fructose, sucrose and total sugars along PC1. Similarly, visible separation pattern between zones was depicted in Figure 4.3b. All zones with samples from *Miombo* flora were clearly separated from each other and from zones with *Acacia*

samples on the negative side of PC 1. The same was observed on the positive side of PC1 where *Acacia* samples from the same zone were grouped together to form a clear separation from one another.

Furthermore, the loadings of the sensory variables indicate that fructose and total sugars dominate the first component for separation of honeys according to floral and geographic origin along PC1. Along PC2, sucrose and glucose were responsible for separation with fructose and total sugars to a little extent Figure 4.3 (a and b).

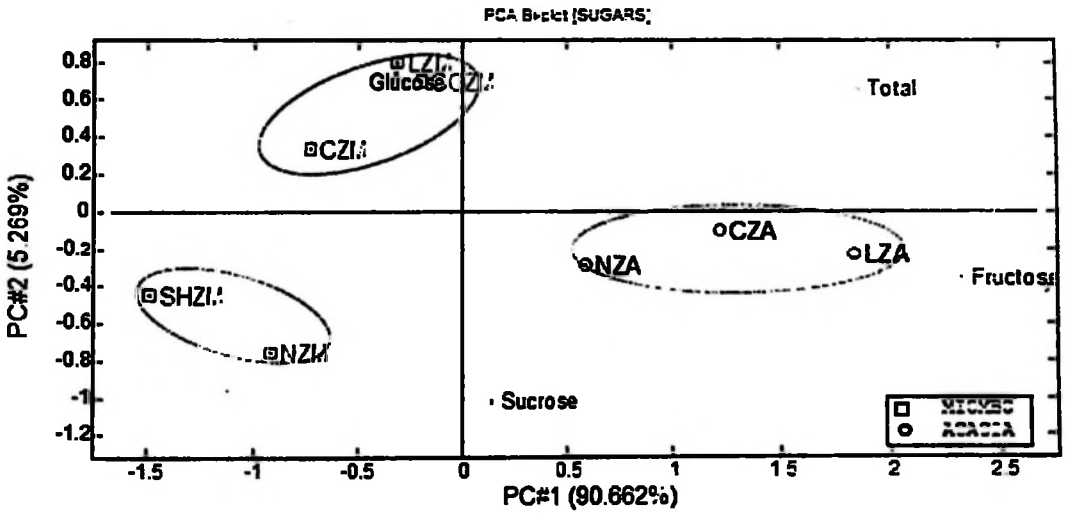

Figure 4.3a: PCA Bi-plot showing systematic variation of sugars in honey samples in between floral origins (*Miombo* and *Acacia*)

Key: M - *Miombo* honey, A - *Acacia* honey, Coastal zone - COZ, Central zone – CZ, Lake zone – LZ, Northern zone – NZ, Southern highland zone – SHZ

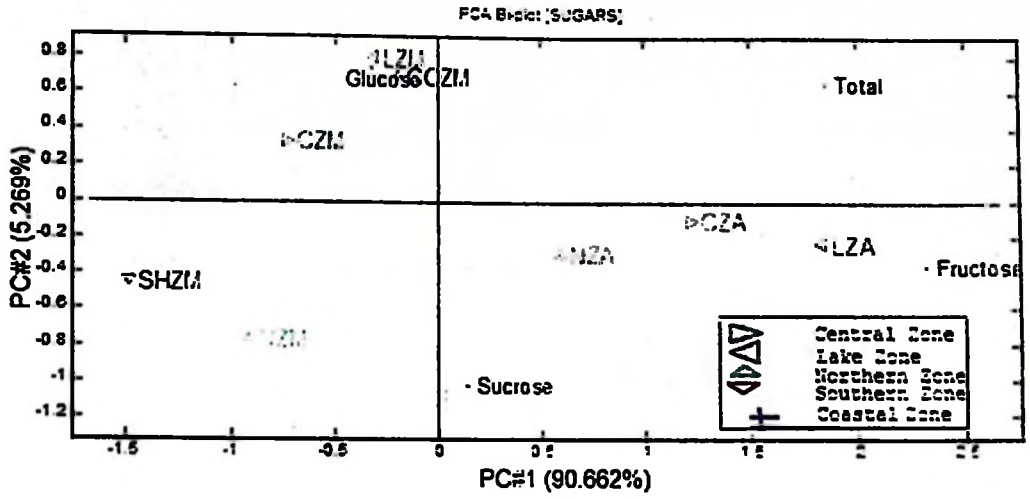

Figure 4.3b: PCA Bi-plot showing variation in honey sugars between different zones

Key: M - *Miombo* honey, A - *Acacia* honey, Coastal zone - COZ, Central zone – CZ, Lake zone – LZ, Northern zone – NZ, Southern highland zone – SHZ

4.4 Conclusions

Honey floral origin and geographical origin was found to have effect on the sugar contents of honey. Fructose was the dominant sugar in all evaluated honey followed by glucose. In *Miombo* floral origin, samples varied from each other in sugar contents with highest values in fructose, glucose and total sugar being found in Coastal zone while sucrose was higher in Northern zone. Highest values in glucose and total sugar were found in Northern zone, fructose in Lake zone while Central zone had the highest value in sucrose among samples from *Acacia* origin. Between the floral origin, most *Acacia* samples were found to contain high content of fructose, sucrose and total sugars than *Miombo* honey with the exception of Northern zone which showed high value in sucrose and total sugars from *Miombo* samples. Glucose content was observed to be higher in *Miombo* samples with the exception of Northern zone.

Fructose/glucose and G/W ratios were lowest and high in Lake, Central and Southern Highland zones respectively among *Miombo* samples while in *Acacia* samples, Northern

zone showed the lowest and highest values in the same ratios. From the results, it was observed that F/G ratio was highest in *Acacia* samples while G/W ratio was high in *Miombo* samples with exception of the Northern zone. A clear contrast between floral and geographical zones was obtained in bi plots indicating that fructose and total sugars can be reliable variable in characterization of honey from different sources and locations.

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CHAPTER FIVE

MANISCRIPT FOUR

5.0 EVALUATION OF MINERAL PROPERTIES OF HONEY FROM DIFFERENT GEOGRAPHICAL AND BOTANIC ORIGIN IN TANZANIA

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Abstract

The effect of geographical zones and floral origins on mineral contents of honey samples in Tanzania was investigated in this study. Honey samples were two floral sources (*Miombo* and *Acacia*) in Central, Coastal, Lake, Northern and Southern zones and determined for copper, zinc, lead, iron, calcium and magnesium using AAS, sodium and potassium using flame photometer as described by AOAC standard methods (2005). Complete randomized block design with floral and geographic zone as principal factors was used in this study. Potassium was observed to be the most abundant macro minerals in the honey samples with 1177.54 ± 49.68 - 3488.1 ± 87.17 and 380.19 ± 89.30 - 746.48 ± 0.00 ppm in *Miombo* and *Acacia* origins respectively. Iron was the most abundant micro minerals with values of 24.47 ± 3.91 - 36.04 ± 0.5 ppm and 24.73 ± 3.91 - 27.50 ± 3.13 ppm in *Miombo* and *Acacia* floral origins respectively followed by zinc and copper. Lead contents varied from 0.16 ± 0.02 - 1.02 ± 1.00 ppm and 0.03 ± 0.0 - 1.92 ± 1.42 ppm in *Miombo* and *Acacia* floral origin respectively with higher values more pronounced in Northern zone. The variations in macrominerals between zones were significant with Northern zone having higher contents of Ca, Mg, K and Na with values of 336.61, 24099.46, 3488.13 and 116.74 respectively in samples originating from *Miombo* floral origin. Lake and Coastal zones showed lower value in Ca and Mg while Central zone had lower values in potassium and sodium. Contents of minerals varied significantly ($p < 0.05$) in the order of K > Mg > Ca > Na in both floral origins. Furthermore, the variations in mineral contents between floral origins were also significant with samples from *Miombo* floral origin having significantly much higher content of minerals than *Acacia* samples. *Miombo* honey samples had higher potassium and magnesium values of 760.27 ± 16.58 and 541.24 ± 35.09 ppm, 3488.13 ± 87.17 and 2409.46 ± 503.20, 2572 ± 23.9 and 750.26 ± 16.74 ppm in Central, Northern and Lake zones respectively than respective lower values of 380.20 ± 89.30 and 439.574 ± 10.33 ppm, 418.32 ± 1.86 and 128.08 ± 10.43

ppm as well as 746.45 ± 0.00 and 177.07 ± 0.00 ppm. Multivariate cluster analysis revealed grouping/similarities of zones according to the mineral they contain. Therefore, from the results it has been observed potassium and iron are the most abundant macro and micro minerals in the honey samples with geographical zones and floral origins having significant influence on the mineral contents of the honey samples in this study. It is therefore recommended for inclusion of honey in human daily diet in order to get these important minerals. However, further studies are needed to ascertain the sources of higher minerals and lead content in the Northern zone honey samples.

Key words: *Miombo, Acacia, Macrominerals, microminerals, Lead, Potassium, honey*

5.1 Introduction

Current trend of health consciousness is making people search for more natural products which have no side effects to satisfy their quench for their requirements. Honey, among many, is one of the product that is regarded as natural and has high nutritional and medicinal value (Hemalatha and Satyanarayana, 2015). It is a natural substance produced by honeybees (*Apis mellifera*) from flower nectar or from honeydew and acts as a source of readily available sugar, organic acids, some amino acids, minerals (macro and microminerals), and biologically active compounds that have antibacterial and antioxidant properties whose chemical composition and amount is influenced by the floral source, climate and environmental conditions (Ulloa *et al.*, 2015; Brudzynski and Miotto, 2011; Rosa *et al.*, 2011; Bogdanov, 2007).

Honey is intrinsically connected to the territory in which it is produced, that is, it is closely tied to the flora visited as well as the trace elements that the plants receive from the ground, water and air. Honey is recognized to be a biological indicator of environmental quality where with mineral content as one of the indicators of the environment pollution (Przybylowski and Wilczynska, 2001). Environmental pollution factors may contribute to the presence of metals in honey and they include industrial factories, leaded petrol, pesticides and agrochemicals such as cadmium-containing fertilizers (Rasheda, 2009; Bratu, 2005).

Various minerals found in honey such as chromium, copper, iron, manganese, zinc are present in very low amount. Some have been found to be essential for human body but when in excess amount, the same elements can become very toxic (Pisano *et al.*, 2008). Other elements such as Fe, Cu, Mn, Zn and Mg play very important role in biochemical reactions of which their presence in enzymes acts as metal activators and helps in group

transfer (Hemalatha and Satyanarayana, 2015). Potassium is usually the most abundant element in honeys, others being sodium, iron, copper, manganese, silicon, calcium, and magnesium (Belouali *et al.*, 2008; Downey *et al.*, 2005).

It is of environmental importance at global level that the determination of trace elements is done as part of the quality control of honey as the world's total production is increasing (Vanhanen *et al.*, 2011). Despite literature review, information on microminerals, macro minerals and heavy metals in Tanzania honey from different geographical zones and floral origins is missing. Therefore, this study aimed at seeking the missing information.

5.2 Materials and Methods

5.2.1 Study area

The study was conducted in five zones of Tanzania namely, Lake, Central, Coastal, Northern and Southern highland zones. The analytical work was conducted at the Department of Food Technology, Nutrition and Consumer Sciences (DFTNCS) laboratory, Sokoine University of Agriculture (SUA), Morogoro and at Tanzania Bureau of Standards (TBS) Laboratory Dar-es Salaam.

5.2.2 Materials

Honey samples were picked purposively depending on availability and distribution of floral/botanic origin, and they include *acacia* honey from *acacia spp* and *miombo* honey from *miombo* woodland. They were purchased directly from the beekeepers from different regions in the respective zones; Lake (Kigoma and Simiyu), Northern (Manyara), Central (Tabora and Dodoma), Coastal (Morogoro) and Southern Highland (Katavi). Analytical grade reagents and chemicals were obtained from TBS, DFTNC laboratory and/or purchased from suppliers in Dar es Salaam.

5.2.3 Methods

5.2.3.1 Research design

Complete randomized block design (CRBD) with replication was used in this study. The principal factors were floral source with two levels (*Miombo* and *Acacia*) and geographical location with five levels (zones). The effect of these factors on minerals was determined. The design mathematical model is depicted in Equation 1.

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad \text{.....(1)}$$

Where μ is the overall (grand) mean, αi is the effect due to the i^{th} treatment (floral).

$ß_j$ s the effect due to the j^{th} block (geographical) and ε_{ij} is the error term

5.2.3.2 Determination of mineral content

The analysis of minerals was done according to the AOAC (2005) procedures. One gramme of test portions were dried, ashed at 450°C and digested with 10 mls of HCl (6M). The solutions were filtered and levels of minerals content were determined. Copper, zinc, lead, iron, calcium and magnesium were determined by Unicam 919 Atomic Absorption Spectrometer (AAS). Sodium and potassium was determined using flame photometer.

5.2.3.3 Statistical data analysis

Data obtained were analyzed by using the R statistical package (R Development Core Team, Version 3.0.0 Vienna, Austria). Analysis of variance (Anova) was used to determine the significant differences between the main factors. Means were separated using Tukey's Honest Significant difference ($\alpha = 0.05$). Multivariate cluster analysis was used to determine the grouping of zones and floral origins according to their similarity and differences (Martens and Martens, 2001). Data were presented in tabular form and graphical as dendrogram.

5.3 Results and Discussion

5.3.1 Effect of geographical zone and floral origin in macro minerals of honey

The macro minerals in honey samples presented in Table 1 differed siginificantly ($p < 0.05$) between zones within both *Miombo* and *Acacia* origins for some minerals. In honey samples originating from *Miombo* origin, Northern contained the largest amount of calcium, magnesium, potassium and sodium corresponding to values 336.6 ± 15.0, 2409.5 ± 503.2, 3488.1 ± 87.2 and 116.7 ± 11.1 ppm, respectively. Lowest values of calcium and magnesium, with values of 86.3 ± 10.9 and 350.1 ± 27.8 ppm were observed in Lake and Coastal zones, respectively. Central zone had the lowest potassium and sodium values of 760.3 ± 16.6 and 78.1 ± 18.5 ppm, respectively.

Lake zone had significantly ($p < 0.05$) higher potassium content with values of 746.5 ± 0.0 ppm in *Acacia* honey samples. The lowest content of potassium was observed in Central zone (380.19 ± 89.3 ppm) which was not statistically different from Northern zone (418.3 ± 1.9 ppm). Lake and Central zone were significant similar ($p > 0.05$) in sodium contents but both differed significantly ($p < 0.05$) from the Northern zone which had the lowest value of 105.0 ± 1.5 ppm of sodium. Magnesium content was significantly ($p < 0.05$) higher in Central zone with value of 439.6 ± 10.3 ppm and the lowest value of 177.1 ± 0.0 ppm was observed in Lake zone. No significant difference ($p > 0.05$) was observed in calcium content in all evaluated zones (Table 5.1).

Generally, samples from both floral origins had highest potassium contents of 760.3 ± 16.6 to 3488.1 ± 87.2 ppm followed by magnesium with 350.1 ± 27.8 to 2409.5 ± 503.2 ppm, calcium with 86.3 ± 11.0 to 179.1 ± 11.0 ppm for samples from *Miombo* floral origin (Table 5.1). As for *Acacia* honey samples, potassium content ranged from 380.2 ± 89.3 to

746.5±0.0 ppm, magnesium from 128.1 ± 10.4 to 439.6 ± 10.3 ppm, calcium from 153.1 ± 0.0 to 155.9 ± 26.6 ppm and sodium from 105.0 ± 1.5 to 165.3 ± 0.0 ppm.

Table 5.1: Mineral contents of honey from different zones within *Miombo* and *Acacia* floral origins

		Macro element (ppm)			
Floral	**Zone**	**Calcium**	**Magnesium**	**Potassium**	**Sodium**
Miombo					
	Central	147.9 ± 26.0^{bc}	541.2 ± 35.1^{b}	760.3 ± 16.6^{d}	78.1 ± 18.5^{b}
	Lake	86.3 ± 10.9^{d}	750.3 ± 16.7^{b}	2572.1 ± 424.0^{b}	112.8 ± 7.4^{a}
	Northern	336.6 ± 15.0^{a}	2409.5 ± 503.2^{a}	3488.1 ± 87.2^{a}	116.7 ± 11.1^{a}
	S. Highland	179.1 ± 11.0^{b}	453.2 ± 10.7^{b}	1534.8 ± 455.3^{c}	83.3 ± 7.5^{ab}
	Coastal	135.3 ± 3.3^{c}	350.1 ± 27.8^{b}	1177.5 ± 49.7^{cd}	116.6 ± 14.5^{a}
Acacia					
	Central	155.4 ± 43.7^{a}	439.6 ± 10.3^{a}	380.2 ± 89.3^{b}	152.3 ± 13.3^{a}
	Lake	153.1 ± 0.0^{a}	177.1 ± 0.0^{b}	746.5 ± 0.0^{a}	165.3 ± 0.0^{a}
	Northern	155.9 ± 26.6^{a}	128.1 ± 10.4^{c}	418.3 ± 1.9^{b}	105.0 ± 1.5^{b}

Values are expressed as arithmetic mean ±standard deviation (n=9)

Mean values with different superscripts letters along the columns are significantly different at $p < 0.05$.

Furthermore, variations of macro minerals in honey samples originating from *Miombo* and *Acacia* were significant ($p < 0.05$) with exception of calcium in Central zone and sodium in Northern zone. In Central zone, samples from *Miombo* floral origin had higher magnesium and potassium values of 541.2 ± 35.1 and 760.3 ± 16.6 ppm respectively than respective values of 439.6 ± 10.3 and 380.2 ± 89.3 ppm found in samples originating from *Acacia* floral origin with similar trend being observed in Northern and Lake zone. Contrary observation was seen in *Acacia* honey samples from Central and Lake zones which had significantly higher calcium and sodium values of 153.1 ± 0.0 ppm and 165.3 ± 0.0 ppm respectively than their *Miombo* counterparts (Table 5.2).

Table 5.2: Macro mineral contents of honey from different floral origins

Zone	Floral	Macro element (ppm)			
		Calcium	Magnesium	Potassium	Sodium
Central	*Miombo*	146.9 ± 26.0^{a}	541.2 ± 35.1^{a}	760.3 ± 16.6^{a}	78.1 ± 18.5^{b}
	Acacia	155.4 ± 43.7^{a}	439.6 ± 10.3^{b}	380.2 ± 89.3^{b}	152.3 ± 13.3^{a}
Northern	*Miombo*	336.6 ± 15.0^{a}	2409.5 ± 503.2^{a}	3488.1 ± 87.2^{a}	116.7 ± 11.1^{a}
	Acacia	155.9 ± 26.6^{b}	128.1 ± 10.4^{b}	418.3 ± 1.9^{b}	105.0 ± 1.5^{a}
Lake	*Miombo*	86.3 ± 10.9^{b}	750.3 ± 16.7^{a}	2572.1 ± 424.0^{a}	112.8 ± 7.4^{b}
	Acacia	153.1 ± 0.0^{a}	177.1 ± 0.0^{b}	746.5 ± 0.0^{b}	165.3 ± 0.0^{a}

Values are expressed as arithmetic mean ±standard deviation (n=9)

Mean values with different superscripts letters along the columns are significantly different at $p < 0.05$.

The multivariate results by cluster analysis using Partition Clustering (K means) and agglomerative hierarchical clustering methods to ascertain grouping of honey samples according to their differences and similarities in macro element are shown in Table 5.3 and dendrogram (Figure 5.1). The finding show three main different clusters of zonal honey according to mineral contents they possess and labeled as A, B and C (Table 5.3). The group A comprised of *Acacia* honey samples from all zones and one sample originated from *Miombo* floral origin in Central zone. These were characterized by similar amount of calcium ranged from 146.9 to 1.55.9 ppm.

Group B comprised of Southern Highland and Coastal zones and were mainly characterized by similar amount of magnesium and potassium while group C comprised of Lake and Northern *Miombo* samples were characterized by higher similar magnesium and potassium levels (Table 5.3 and Figure 5.1).

Table 5.3: Clusters of honey samples according to their similarities and differences in macro minerals by K mean method

Zone	Calcium (ppm)	Magnesium (ppm)	Potassium (ppm)	Sodium (ppm)	Kmeans	Cluster label
CZA	155.4	439.6	380.2	152.3	1	A
LZA	153.1	177.1	746.5	165.3	1	A
NZA	155.9	128.1	418.3	105.0	1	A
CZM	146.9	541.2	760.3	78.1	1	A
SHZM	179.1	453.2	1534.8	83.3	2	B
COZM	135.3	350.1	1177.5	116.6	2	B
LZM	86.3	750.3	2572.1	112.8	4	C
NZM	336.6	2409.5	3488.1	116.7	3	C

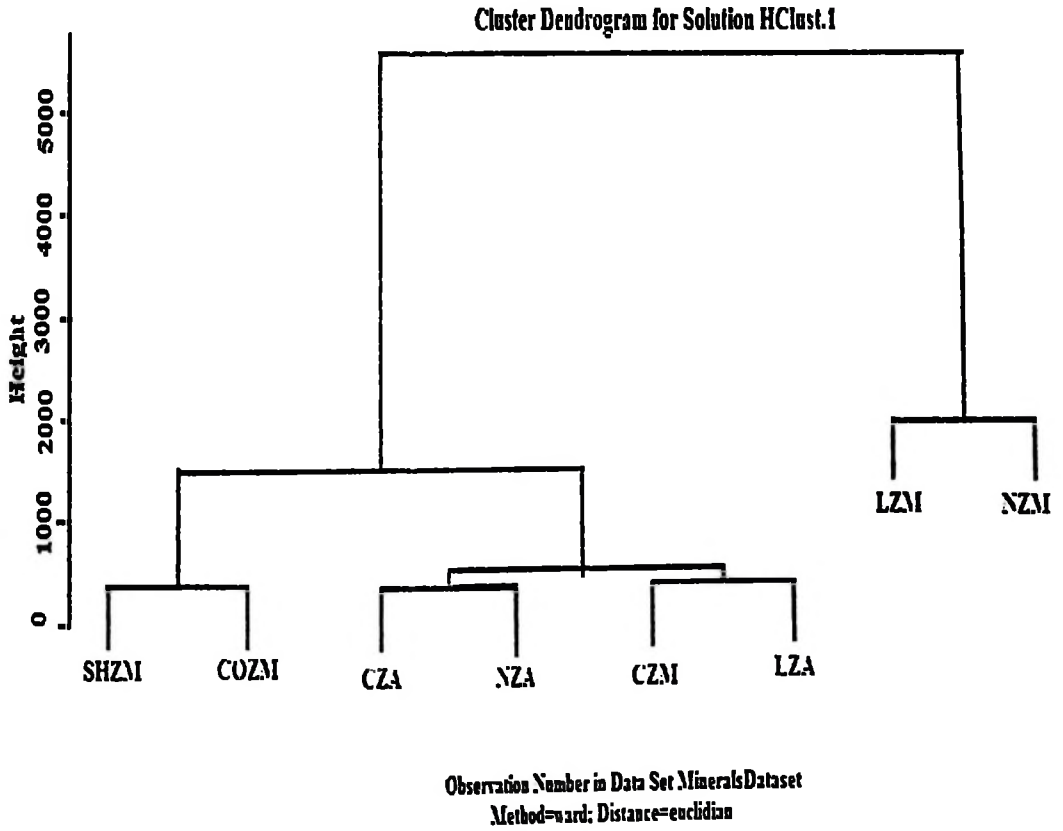

Figure 5.1: Dendrogram showing clusters of honey samples according to their macro mineral contents

Key: M - *Miombo* honey, A - *Acacia* honey, Coastal zone - COZ, Central zone – CZ, Lake zone – LZ, Northern zone – NZ, Southern highland zone – SHZ

The findings of this study imply that, potassium was the most abundant mineral in the honey samples followed by magnesium, calcium and then sodium. This is in agreement with the study by Fernández-Torres *et al.* (2005) which reported potassium to be the most abundant metal in honey. Adebiyi *et al.* (2004) found similar findings among elements investigated in Nigerian honey. However, a study by Liberato *et al.* (2013) in Palestinian honey observed slightly different mineral trend from that of this study as the order of abundant minerals in their study was K, Na, Ca and Mg with values 183.86, 104.67, 90.99 and 22.74 mg/kg, respectively. Potassium values obtained in Tanzania honey were comparable to those obtained by Awad and Elgornazi (2016) and Boussaid *et al.* (2013) who obtained a range of 1567 - 1747ppm in Libyan honey from different regions and a range of 172 - 976 ppm in Tunisian honey. Contrary to our study, much lower values of potassium of 312 pmm and 667 - 821.91ppm were respectively reported by Olga *et al.* (2012) and Nanda *et al.* (2009) in Spain and India.

Potassium is a very significant body mineral, important to cellular functions. It is one of the main blood minerals called "electrolytes" (the others are sodium and chloride), which means it carries a tiny electrical charge (potential). Epidemiological and clinical studies show that a high potassium diet lowers blood pressure in individuals with raised blood pressure and reduces cardiovascular disease mortality. Furthermore, a high potassium diet may also prevent or at least slow the progression of renal disease. An increased potassium intake has been associated with lowering urinary calcium excretion and plays an important role in the management of hypercalciuria and kidney stones. It is also likely to decrease the risk of osteoporosis (He and MacGregor, 2008).

Moreover, the observed significant variations in mineral contents between zones in both floral origins could be the content of soil minerals as plants obtain minerals in soils, the

content of which can be reflected in honey (Hemalatha and Satyanarayana, 2015). It should be remembered that study samples were collected from different geographic locations which have different climatic conditions and soils types. Some authors have reported that ash content in honey reflects its richness in minerals and is usually determined by its floral origin (Saxena *et al.*, 2010). This was consistently similar with the ash results observed in this study where Northern zone samples showed high content of ash.

5.3.2 Effect of zones and floral origin on micro minerals and heavy metals content of honey

5.3.2.1 Iron, copper and zinc

Micro mineral and heavy metal contents of honey samples from different zones within each floral origin are presented in Table 5.4. It showed that iron was the most abundant micro mineral in the *Miombo* honey samples with values of 24.5 ± 2.0 to 36.0 ± 0.5 ppm followed by zinc with values of 4.3 ± 0.1 to 8.7 ± 0.2 ppm and lowest amount of copper of 0.4 ± 0.0 to 0.5 ± 0.1 ppm. The micro mineral content differed significantly ($p < 0.05$) between zones in each floral origin with Northern zone having the highest lead content of 1.0 ± 1.0 ppm and the lowest copper value of 0.4 ± 0.0 ppm in honey samples originating from *Miombo* floral origin. Significantly high contents of copper, iron and zinc were observed in Lake, Southern Highland and Central zones with values of 0.5 ± 0.1, 36.0 ± 0.5 and 8.7 ± 0.2 ppm respectively compared to other zones (Table 5.4).

Table 5.4: Micro mineral and heavy metal contents of honey samples from different zones within each floral origin

Floral	Zone	Heavy metal (ppm) Lead	Micro minerals (ppm) Copper	Iron	Zinc
Miombo					
	Central	0.5 ± 0.0^{b}	0.5 ± 0.1^{a}	31.4 ± 0.7^{b}	8.7 ± 0.2^{a}
	Lake	0.4 ± 0.1^{b}	0.5 ± 0.1^{a}	33.0 ± 1.1^{ab}	6.2 ± 0.3^{b}
	Northern	1.0 ± 1.0^{a}	0.34 ± 0.0^{b}	30.7 ± 2.4^{b}	5.4 ± 0.5^{bc}
	S. Highland	0.2 ± 0.0^{c}	0.5 ± 0.0^{ab}	36.0 ± 0.5^{a}	5.3 ± 0.9^{c}
	Coastal	0.1 ± 0.1^{d}	0.4 ± 0.0^{b}	24.5 ± 2.01	4.3 ± 0.1^{bc}
Acacia					
	Central	0.4 ± 0.01^{b}	0.2 ± 0.0^{a}	27.5 ± 3.1^{a}	6.3 ± 3.0^{a}
	Lake	0.03 ± 0.00^{c}	0.2 ± 0.0^{b}	23.7 ± 0.0^{a}	2.7 ± 0.0^{a}
	Northern	1.9 ± 1.42^{a}	0.3 ± 0.0^{a}	24.7 ± 3.9^{a}	2.5 ± 0.3^{a}

Values are expressed as arithmetic mean ±standard deviation (n = 9)

Mean values with different superscripts letters along the columns are significantly different at $p < 0.05$.

Similarly, iron was the most abundant micro minerals in *Acacia* samples with values of 23.7 ± 0.0 to 27.5 ± 3.1 ppm, followed by zinc with values of 2.5 ± 0.3 to 6.3 ± 3.0 and lowest values of 0.2 ± 0.0 in copper. Northern and Central zones had similar significant ($p < 0.05$) higher contents in copper with values of 0.2 ± 0.0 to 0.3 ± 0.0 ppm than Lake zone with values of 0.2 ± 0.00 ppm. No significant ($p > 0.05$) variation were observed in iron and zinc between zones.

ii. Lead

Lead contents in honey samples ranged from ranged 0.1 ± 0.1 to 1.0 ± 1.0 ppm in honey samples from *Miombo* origin while values ranging from 0.03 ± 0.0 to 1.9 ± 1.4 ppm were observed in *Acacia* honey samples. Regardless of the floral origin of honey, the level of lead found in Northern zone was significantly higher than levels in other zones. In honey samples from *Miombo* floral origin, it was 1.0 ± 1.0 ppm while in *Acacia* honey samples it was 1.9 ± 1.4 ppm.

Variations in micro minerals and lead contents between floral origins were also significant in each zone with *Miombo* honey samples showing higher content of minerals than honey from *Acacia* flora origin (Table 5.5).

Table 5.5: Mineral contents (Mg/ 100 g) of honey samples from at different floral origin within each zone

		Heavy metal (ppm)	Micro minerals (ppm)		
Zone	**Floral**	**Lead**	**Copper**	**Iron**	**Zinc**
Central					
	Miombo	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	31.4 ± 0.7^{a}	8.7 ± 0.2^{a}
	Acacia	0.4 ± 0.0^{b}	0.23 ± 0.0^{b}	27.5 ± 3.1^{a}	6.3 ± 3.0^{a}
Northern	*Miombo*	1.0 ± 1.0^{b}	0.4 ± 0.0^{a}	30.7 ± 2.5^{a}	5.41 ± 0.5^{a}
	Acacia	1.9 ± 1.4^{a}	0.3 ± 0.0^{b}	24.7 ± 3.9^{a}	2.48 ± 0.3^{b}
Lake	*Miombo*	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	33.0 ± 1.1^{a}	6.18 ± 0.3^{a}
	Acacia	0.03 ± 0.0^{b}	0.2 ± 0.0^{b}	23.7 ± 0.0^{b}	2.66 ± 0.0^{b}

Values are expressed as arithmetic mean ±standard deviation (n = 9)

Mean values with different superscripts letters along the columns are significantly different at $p < 0.05$.

Table 5.6 and Figure 5.2 show the multivariate cluster analysis results of honey samples grouping according to their differences and similarities in micro minerals and lead. Four major clusters were observed each related to micro minerals and lead contents. The group A comprised of Central zone *Acacia* honey sample characterized by similar moderate amount of 0.37 ppm.

Group B comprised of honey samples from *Miombo* floral origin in Central, Lake and Northern zones mainly characterized by higher contents of micro minerals and lead contents as compared to the other zones while group C comprised of honey samples from Southern Highland zone characterized by high iron content. Group D comprised of Acacia

honey samples from Lake and Northern zones with similar iron and zinc levels (Table 5.6 and Figure 5.2).

Table 5.6: Clusters of honey samples accoring to their similarities and differences in micro minerals and heavy metal by K mean method

Zone	Lead (ppm)	Copper (ppm)	Iron (ppm)	Zinc (ppm)	Kmeans	Cluster label
CZM	0.5	0.5	31.4	8.7	2	B
LZM	0.4	0.5	33.2	6.2	2	B
NZM	1.0	0.4	30.7	5.4	2	B
SHZM	0.2	0.5	36.0	5.3	3	C
COZM	0.1	0.4	24.5	4.3	4	D
CZA	0.4	0.2	27.5	6.3	1	A
LZA	0.03	0.2	23.7	2.7	4	D
NZA	1.9	0.3	24.7	2.5	4	D

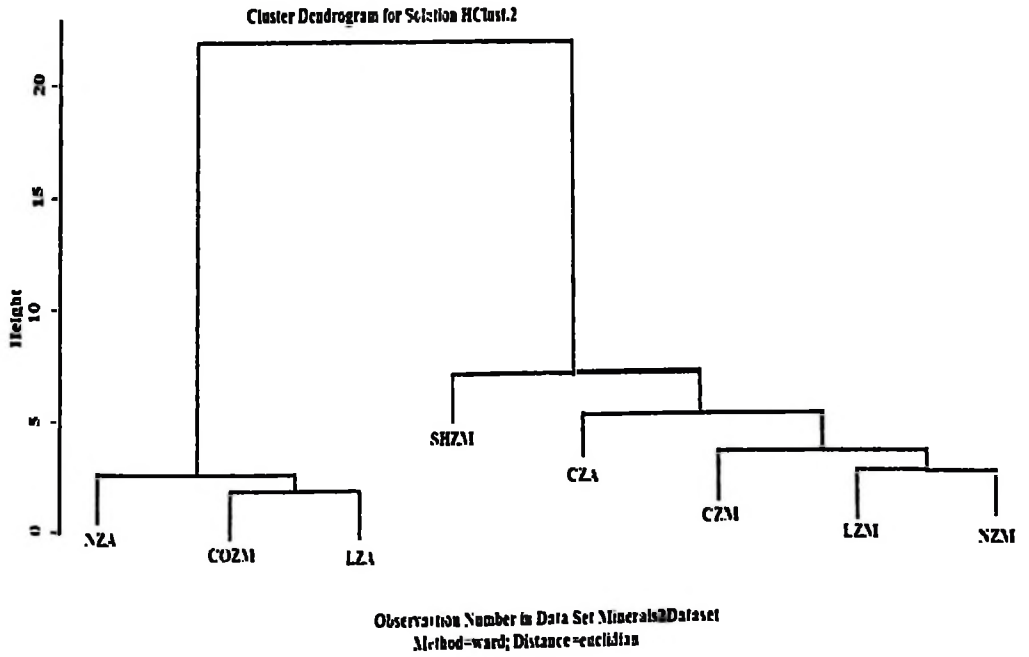

Figure 5.2: Dendrogram showing clusters of honey samples according to their micro mineral and heavy metal contents

Key: M - *Miombo* honey, A - *Acacia* honey, Coastal zone - COZ, Central zone – CZ, Lake zone – LZ, Northern zone – NZ, Southern highland zone – SHZ

As in macro minerals, the observed significant variations in micro mineral contents between zones in both floral origins could be the content of soil minerals as plants obtain minerals in soils the content of which can be reflected in honey (Hemalatha and Satyanarayana, 2015). This is in agreement with a study by Pohl (2009) that the levels of minerals in honey vary according to the botanical origin and soil composition. From the results, this can be observed in different mineral contents between the floral origins within a particular zone. The significantly higher values of lead observed in the Northern zone honey samples were above maximum allowed limit as stipulated by National Standard (TZS 851) of 0.5 ppm. High content of heavy metals has been associated with environmental contamination. Lead is said to be one the most severe contaminant mainly caused by industrial activities or automobile exhaust gas emission (Przybylowski and Wilczynska, 2001). Since honey from both flora indicated high levels of lead, there is a possibility that the beehive were set in an area which was near industrial activities or activities that pose contamination to environment.

5.4 Conclusions

In a view of the findings of this study, it was concluded that potasium was the most abundant macromineral in the honey samples followed by magesium, calcium and sodium. Iron was the most abundant micro minerals followed by zinc and copper. Northern zone samples from each floral origin had heavy metal, lead which was found to be above maximum allowed limits as per Tanzania honey standard.

Furthermore the observed significant varaiations in honey macro and micro mineral and heavy metal contents between geographical zones and floral origins under the study indicate these factors have influence on honey mineral contents. Northern zone was observed to have high contents of all minerals while *Miombo* honey samples had highest

scores in mineral than its *Acacia* counterpart. Therefore, inclusion of honey in our daily diet is highly recommended in order to get these improtant minerals. However, the high mineral and lead contents in Northern zone samples suggest for a further studies to establish the major scources of these variations and then to design a mitigation stratety for the contaminants.

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CHAPTER SIX

MANUSCRIPT FIVE

6.0 SENSONRY PROPERTIES, CONSUMER ACCEPTABILITY AND PREFERENCE MAPPING OF HONEY FROM DIFFERENT GEOGRAPHICAL AND BOTANIC ORIGIN IN TANZANIA

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Abstract

Honey samples from different floral origins and geographical zones in Tanzania were evaluated for sensory properties. Samples were collected from two floral sources (*Miombo* and *Acacia*) and from five different zones (Central. Coastal. Lake. Northern and Southern zones). Consumers study was done using a 9 point hedonic scale as described by Lawless and Heyman (2010) to evaluate acceptance for the attributes of colour. aroma. viscosity and general acceptability. Descriptive sensory profiling was done using trained panelists and an unstructured line scale for rating the intensity of an attribute. A statistical analysis was carried out to identify significant differences in sensory attributes. In quantitative descriptive analysis, variations between zones were significant and scores ranged from 5.8 - 7.5, 5.1 - 6.9, 4.6 - 8.4, 5.4 - 7.8 and 2.1 - 5.7 in aroma. clarity. hue. viscosity and whiteness respectively for *Miombo* honey samples. Southern zone showed high scores in aroma and hue, viscosity in Central zone while Coastal zone had high score in clarity and whiteness. In *Acacia* honey samples, variations were significant and aroma ranged from 5.3 - 5.8, 6.9 - 82. 4.3 - 5.6, 6.1 - 6.96 and 5.1 - 6.4 in clarity. hue. viscosity and whiteness respectively. Northern zone was seen to have high scores in aroma, viscosity and whiteness, clarity in Lake zone and hue in Central zone. PCA bi plots gave clear contrast between *Miombo* and *Acacia* samples and samples from a single zone were grouped together to form clear separation from each other. It was observed that *Miombo* samples correlated more with aroma, hue and viscosity while *Acacia* honey samples were correlated with clarity and whiteness. Similar significant variations were seen between zones. In *Miombo* honey, samples from Southern Highland zone were more correlated with hue and aroma, Coastal zone with whiteness and clarity while lake and Central zone were correlated with viscosity. *Acacia* honey samples from Central. Northern and Lake zone were correlated with clarity and whiteness. Honey samples from Coastal zone (*Miombo*) and Lake zone (*Acacia*) were more accepted by consumers and both were

correlated with clarity and whiteness. Therefore, from the results, it is concluded that clear honey with fair colour is more accepted by consumers and sensory attributes can be used to characterize honey according to geographical and floral origins.

Keyword: *Consumer acceptability, quantitative analysis, honey*

6.1 Introduction

Honey is the most important product of bees consisting basically of a complex mixture of carbohydrates, especially glucose and fructose, organic acids, amino acids, minerals, vitamins, enzymes, pollens, and pigments (Fallico *et al.* 2004). The country and global trends are moving towards a clearer understanding of the honey geographical zone, botanical origin, nutraceutical properties, health and sensory properties. Many studies have emphasized the importance of honey sensory analysis in order to identify and differentiate honeys by botanical origin and quality (Castro-Vázquez, 2009). It is an important tool for determining its floral origin (characterization), for subsequent quality control practices and which ultimately will determine consumer preferences towards the product (Ciappini *et al.*, 2013). In such tests, honey properties are scored and described using the senses of human beings as analytical tool (Kaakeh and Gadelhak, 2005). Furthermore, it has been reported that honeys from similar locations could also differ in sensory profiles. The anticipated variations are said to be greatly influenced by the geographic origin, an important quality factor closely correlated with the chemical and sensory characteristics of honeys (Belay *et al.*, 2015).

The sensory quality is what consumers perceive directly and is the ultimate measure of the product while sensory analysis is the examination of a product through the evaluation of the attributes perceptible by the five sense organs (organoleptic attributes), such as colour, odour, taste, touch, texture and noise (Drake, 2007; Piana, 2004). It is made possible through employment of basic techniques such as descriptive sensory analysis, consumer test and preference mapping (Mongi *et al.*, 2013). Descriptive analysis provides quantitative descriptions of all the sensory attributes of a food or products based on perceptions of a group qualified assessors while consumer test is an assessment to test whether the consumers like the product, accept or prefer it over another product (Lawless

and Heyman, 2010). Preference mapping is said to be a perceptual map that describes which attributes contributed to consumer liking by using the relationship distances of consumers' hedonic judgements and /or matrix of descriptive sensory data (Tenenhaus *et al.* (2005). It has been reported that, variety of floral origins, geographical region of production, climatic conditions of the producing area and processing and storage methods are usually the driving factors for each honey to possess unique combination of components resulting into different organoleptic properties (Turhan *et al.*, 2008). However, nevertheless, information on the sensory properties and acceptability of honey samples from different geographical zones and botanical origins in Tanzania is limited. Therefore, this study aimed at evaluating descriptive sensory analysis, consumer acceptability and preference mapping of honey from different geographical zones and botanical origins.

6.2 Materials and Methods

6.2.1 Study area

The study was conducted in five zones of Tanzania namely, Lake, Central, Coastal, Northern and Southern highland zones. Sensory evaluation exercise was conducted at the Department of Food Technology, Nutrition and Consumer Sciences (DFTNCS) laboratory, Sokoine University of Agriculture (SUA), Morogoro.

6.2.2 Materials

Honey samples were picked purposively depending on availability and distribution of floral/botanic origin, and they include *Acacia* honey from *Acacia spp* and honey from *Miombo* woodland. Twenty four honey samples (15 *Miombo* and 9 *Acacia* samples) were purchased directly from the beekeepers from different regions in the respective zones: Lake (Kigoma and Simiyu), Northern (Manyara), Central (Tabora and Dodoma), Coastal

(Morogoro) and Southern Highland (Katavi). Materials for sensory evaluation were purchased from the local markets and supermarkets in Morogoro.

6.2.3 Sensory evaluation

6.2.3.1 Quantitative Descriptive Analysis (QDA)

A total of 8 trained panelists comprising of 5 male and 3 female with age ranging from 22 to 27 years participated in descriptive sensory analysis of honey samples according to method described in Lawless and Heyman (2010). The test was conducted at the Department of Food Technology, Nutrition and Consumer Sciences (DFTNCS) sensory laboratory and the assessors were selected and trained according to ISO 8586 (2012). During training panelists developed descriptors describing differences between samples and they agreed on the following attributes clarity, whiteness, colour hue, aroma and viscosity (Table 6.1). They also developed and agreed on an unstructured 9 line scale for rating the intensity of an attribute (appendix 2). The left side of the scale corresponded to the lowest intensity of each attribute (value 1) and the right side corresponded to the highest intensity (value 9). The samples were coded with 3-digit random numbers and were served to each panelist in a randomized order. The obtained average responses were used in the univariate and multivariate analyses. Both pre trial test and panel performance assessment to ascertain agreement of panelist in discriminating samples and their reproducibility were done.

Table 6.1: Definitions of sensory attributes used in descriptive sensory analyses

Attribute		Definition	Anchor
Colour	Colour hue	Brown colour like the colour of beer bottle	Light brown - deep brown
	Whiteness	Degree of whiteness , away from brown colour (loss of natural colour)	Deep brown – light brown
Aroma	Honey aroma	Aromatics associated with honey, a representative sample was chosen from samples and referred to as honey aroma	Less honey smell – more honey smell
Clarity	transparency	Clarity was associated with clarity of sunflower oil, no presence of any particles	Less clear – more clear
Viscosity	Fluidity/resistance to flow freely	The lowest viscous value was related to water fluidity (resistance to flow)	Watery – viscous

Source: Study panelists

6.2.3.2 Consumer acceptability test

The sensory test was conducted at the DFTNCS by 72 untrained consumers of both sexes aged between 20-45 years. About 20 g of honey was placed in cups randomly coded with 3-digit numbers and the cups were served to the panelists in a randomized order on the day of evaluation. A 9 point hedonic scale (where 1 = dislike extremely and 9 = like extremely) as described by Lawless and Heyman (2010) was used in this test to evaluate acceptance for the attributes of colour, aroma, viscosity and finally expressing judgment on overall acceptability of sample (appendix 1).

6.2.4 Statistical data analysis

Data were analysed by using the R statistical package (R Development Core Team, Version 3.0.0 Vienna, Austria) for Analysis of variance (Anova) to determine the significant differences in sensory attributes and consumer acceptability between geographical zones and botanical origins. Means were separated using Tukeys Honest Significant Difference ($p < 0.05$). Principal Component Analysis (PCA) was used to

determine the systematic variations in sensory data (Martens and Martens. 2001) using Latentix Software (LatentiX Aps Team, version 2.12, Frederiksberg Denmark). Results were presented as arithmetic mean and standard deviation in tables and graphs as well as in PCA bi plots.

6.3 Results and Discussion

6.3.1 Quantitative descriptive analysis of honey

Mean intensity ratings of descriptive attributes between zones are shown in Table 6.2. There were significant differences ($p < 0.05$) in mean intensity scores of attributes between different geographical zones in each floral origin. In *Miombo* floral origin, Southern Highland zone had the highest mean aroma and hue intensities scores of 7.5 and 8.4 respectively and lowest of mean scores in clarity and whiteness with values of 5.1 and 2.1 respectively. Coastal zone had the highest mean score in whiteness with 5.7 and similar clarity value of 6.9 as in Lake zone but with lowest mean scores of 5.8 and 4.6 in aroma and hue respectively. Significant ($p < 0.05$) highest viscous samples were observed in Central zone with score of 7.8 while lowest samples observed in the Northern zone with intensity score of 4.8.

Similarly, there were significant ($p < 0.05$) variations in intensity scores between different zones in *Acacia* samples. Higher mean aroma, viscosity and whiteness scores were observed in the Northern zone while the highest clarity and lowest hue values were found in Lake zone with values of 8.2 and 4.3 respectively.. Central zone had the highest mean hue score 5.6 of and lowest mean scores in viscosity and whiteness of 6.1 and 5.1 respectively (Table 6.2).

Table 6.2: Mean intensity scores of honey samples with floral origins

Floral origin	Sample	Aroma	Clarity	Hue	Viscosity	Whiteness
Miombo	CZM	6.8 ± 1.57^{abc}	6.5 ± 1.3^{ab}	7.6 ± 1.21^{b}	7.8 ± 1.02^{a}	2.8 ± 1.4^{c}
	COZM	5.8 ± 1.42^{d}	6.9 ± 1.41^{a}	4.6 ± 1.59^{d}	5.9 ± 1.30^{c}	5.7 ± 1.67^{a}
	LZM	6.8 ± 1.4^{cd}	6.9 ± 1.76^{a}	7.0 ± 1.20^{c}	6.7 ± 1.44^{b}	3.6 ± 1.38^{b}
	NZM	6.9 ± 1.74^{ab}	6.1 ± 1.77^{b}	6.6 ± 1.20^{c}	5.4 ± 1.76^{c}	3.9 ± 1.42^{b}
	SHZM	7.5 ± 1.09^{1}	5.1 ± 2.16^{c}	8.4 ± 0.64^{a}	7.2 ± 0.97^{b}	2.1 ± 1.00^{d}
Acacia	CZA	5.4 ± 1.89^{cd}	7.0 ± 1.71 b	5.6 ± 1.80^{a}	6.1 ± 1.43^{b}	5.1 ± 1.5^{b}
	LZA	5.3 ± 1.89^{b}	8.2 ± 0.89 a	4.3 ± 1.50^{b}	6.1 ± 1.87^{b}	5.9 ± 1.26^{a}
	NZA	5.8 ± 1.45^{a}	6.9 ± 1.30 b	4.69 ± 1.68^{b}	6.96 ± 1.3^{a}	6.4 ± 1.64^{a}

Values are expressed as mean ± SD (n = 48)
Mean values with different superscript letters along the columns are significantly different at $p < 0.05$
Key: M - *Miombo* honey, A - *Acacia* honey, Coastal zone - COZ, Central zone – CZ, Lake zone – LZ, Northern zone – NZ, Southern highland zone – SHZ

Observed aroma and hue variations between zones imply that zones had significant effect on the sensory properties of honey. Southern Highland zone had high mean score in aroma and hue (colour) while Coastal zone had the lowest values in the mentioned attributes. Aroma and hue attributes of honey are subjective and honey is often judged according to its colour (Bradbear, 2009). This was further elaborated by NHB (2016) and Nudi (2013) who stated that colour, flavour, and even the scent of honey varies widely depending on the source of nectar with light colored honey having a delicate or mild taste/scent while dark-coloured honey is more intensely flavored/scented. Moreover, colour (hue) has been used to form quick opinions of other characteristics of honey, and it is therefore believed that the strength or desirability of flavor/scent can be inferred from its colour (Popov-Raljića *et al.*, 2015; Ciappini *et al.*, 2013). This observation is consistent with our results as it can be seen that samples with the highest score in colour hue also scored high in aroma in *Miombo* floral origin.

The different scores in 'hue' and 'whiteness' attributes in different samples from different zones indicates that honey in the study samples varied significantly ($p < 0.05$) in colour. It is known that honey varies tremendously in color depending largely on its floral source and geographical location and ranges from very pale yellow through ambers to a darkish red amber to nearly black (NHB, 2016). In this study, samples from *Miombo* woodland ranged from light amber to dark amber in contrast to sample form *acacia* honey which ranged from white to extra light amber. This could be explained by the fact that honey sensory properties such as colour, aroma and flavour, are differentiated by several factors, including bee species, botanical source, and climatic and soil conditions at the location where the honey was produced (Carvalho *et al.*, 2009).

6.3.2 Variations of attributes between floral origins within a zone

The differences in mean intensity scores of attributes of the honey between floral origins in each zone were significant at $p < 0.05$ as presented in Table 6.3. *Miombo* honey samples had the highest aroma and hue intensity scores in Northern and Central zones with values of 6.8 and 7.6 respectively. In Central zone, higher aroma and hue intensity scores of 6.8 and 7.6 were observed in *Miombo* samples than respective lower values of 5.4 and 5.6 in *Acacia* samples while in Lake zone significantly higher values of 6.8 and 7.0 for the same attributes were observed in *Miombo* samples than respective values of 5.3 and 4.3 in *Acacia* counterpart samples. Furthermore, the higher aroma and hue values of 6.9 and 6.6, in *Miombo* samples than 5.8 and 4.7 in *Acacia* samples respectively were observed in the Northern zone. On the other hand *Acacia* samples had higher mean clarity, viscosity and whiteness scores than their *Miombo* counterparts as depicted in Table 6.3.

Table 6.3: Mean intensity scores of honey samples within each zone

Zone	Floral Origin	Aroma	Clarity	Hue	Viscosity	Whiteness
Central	*Acacia*	5.4 ± 1.89^{b}	7.0 ± 1.71^{a}	5.6 ± 1.80^{b}	7.8 ± 1.02^{a}	5.08 ± 1.50^{a}
	Miombo	6.8 ± 1.57^{a}	6.5 ± 1.29^{a}	7.6 ± 1.21^{a}	6.1 ± 1.43^{b}	2.8 ± 1.42^{b}
Lake	*Acacia*	5.3 ± 1.89^{b}	8.2 ± 0.90^{a}	4.3 ± 1.53^{b}	6.1 ± 1.87^{b}	6.0 ± 1.26^{a}
	Miombo	6.8 ± 1.40^{a}	6.9 ± 1.76^{b}	7.0 ± 1.20^{a}	6.7 ± 1.44^{a}	3.6 ± 1.38^{b}
Northern	*Acacia*	5.8 ± 1.45^{b}	6.9 ± 1.30^{a}	4.7 ± 1.68^{b}	7.0 ± 1.30^{a}	6.4 ± 1.64^{a}
	Miombo	6.9 ± 1.74^{a}	6.1 ± 1.77^{b}	6.6 ± 1.20^{a}	5.4 ± 1.76^{b}	3.9 ± 1.4^{b}

Values are expressed as mean ± SD (n = 48)
Mean values with different superscript letters along the columns are significantly different at $p < 0.05$
Key: M - *Miombo* honey, A - *Acacia* honey, Coastal zone - COZ, Central zone – CZ, Lake zone – LZ, Northern zone – NZ, Southern highland zone – SHZ

The results indicated that samples from *Miombo* flora origin were characterized by deep colour and aroma attributes compared to samples from *Acacia* floral origin which were characterized by clarity and whiteness attributes. Similar observations were seen by Marcazzan *et al.* (2014) who reported low scores of aroma in *Acacia* honey compared to other types of honey. The variations of the investigated honey properties can be closely correlated to the environmental conditions, floral and soil changes due to the climatic conditions and altitude as specific characteristics of honey were observed from different locations (Stolzenbach *et al.*, 2011). Besides, honeys from similar locations could also differ within their characteristics (Popov-Raljić *et al.*, 2015). This was evident in the difference scores observed between floral origins within the same zone.

6.4 Consumer Study

6.4.1 Consumer panel characteristics

The consumer panel was comprised of 72 panelists whereby 57% were male 43% were female. Out of these, 95.83% were undergraduate students in the age group of 18 - 25 years and 4.17% were postgraduate students falling in the age group of 26 - 35 years.

Twenty two of the panelists were frequent user of the honey on daily basis, 16.66% once a week, 26.39% once per month and 34.72% seldom uses honey. Those who preferred raw honey were 84.72% while the ones preferring pasteurized honey were 15.28% (Table 6.4).

Table 6.4: Characteristics of the consumer acceptability panel (n=72)

Attribute	Category	Frequency (n)	Percentage (%)
Gender	Male	41	56.94
	Female	31	43.06
	Total	**72**	**100**
Age group	18 - 25	69	95.83
	26 - 35	3	4.17
	Total	72	100
Education group	Undergraduate	69	95.83
	Postgraduate	3	4.17
		72	100
Consumption	Daily	16	22.22
	Once/week	12	16.66
	Once/month	19	26.39
	Seldom	25	34.72
	Total	**72**	**100**
Honey preference	Pasteurized	11	15.28
	Raw	61	84.72
	Total	**72**	**100**

6.4.2 Hedonic test

Acceptability of evaluated honey between zones in each floral origin is shown in Table 6.5. In *Miombo* samples, zones varied significantly ($p < 0.05$) in acceptability with Coastal zone having higher overall acceptability of 7.5 point, followed by Central and Lake zones with 6.8, Southern Highland zone with 6.1 with the least value of 5.6 in Northern zone. Similarly, the zones differed significantly in acceptability among *Acacia*

samples with Lake zone having highest value of 7.3, followed by Central zone with value of 6.9 and Northern zone having again the lowest acceptability values of 5.1 by consumers.

Table 6.5: Mean hedonic scores for honey samples between zones in *Miombo* and *Acacia* floral origins

	Overall acceptability	
Zone	***Miombo***	***Acacia***
Central	6.8 ± 1.54^{b}	6.9 ± 1.13^{b}
Coastal	7.5 ± 1.0^{a}	-
Lake	6.8 ± 1.53^{b}	7.3 ± 1.2^{a}
Northern	5.6 ± 2.0^{c}	5.1 ± 2.13^{c}
Southern	6.5 ± 1.89^{b}	-

Values are expressed as mean ± SD (n = 48)
Mean values with different superscript letters along the columns are significantly different at $p < 0.05$

In product development, consumer testing is considered to be one of the most important tests and its primary purpose is to assess the personal response by current and potential customers of a product or specific product characteristics (Soma, 2013). The observed differences in acceptability between honey samples from different geographical zones and botanical origins suggest that these factors have influence on the overall acceptability of honey and could be associated again to different composition due to bee species, botanical source, and climatic and soil conditions at the location where the honey was produced (Carvalho *et al.*, 2009). Similar variations were previously reported by Carvalho *et al.* (2009) and Ferreira *et al.* (2009) in their studies. The acceptability of samples by consumers could have been driven by presence of high intensity of some attributes to some samples and these were clearly revealed by multivariate technique, the preference mapping which showed the relationship between sensory and consumer/ acceptability results.

6.5 Relationship Between Sensory and Consumer Data (Preference Mapping)

6.5.1 Principal component analysis of sensory data

Figures 6.1a shows bi-plot with two first significant principal components on sensory attributes of honey. Principal component 1 (PC1) accounted for 86.27% of the variations while PC2 accounted 8.181% of total variations. PC 1 is a clear contrast between *Miombo* and *Acacia* floral origins samples with the exception of *Miombo* samples from Coastal zone (COZM) which overlapped with *Acacia* samples. All *Miombo* samples positively correlated with aroma, hue and viscosity attributes along PC1 while the *Acacia* honey samples correlated positively with clarity and whiteness attributes. PC2 was mainly a contrast between aroma on one side and the rest of the attributes on the other side.

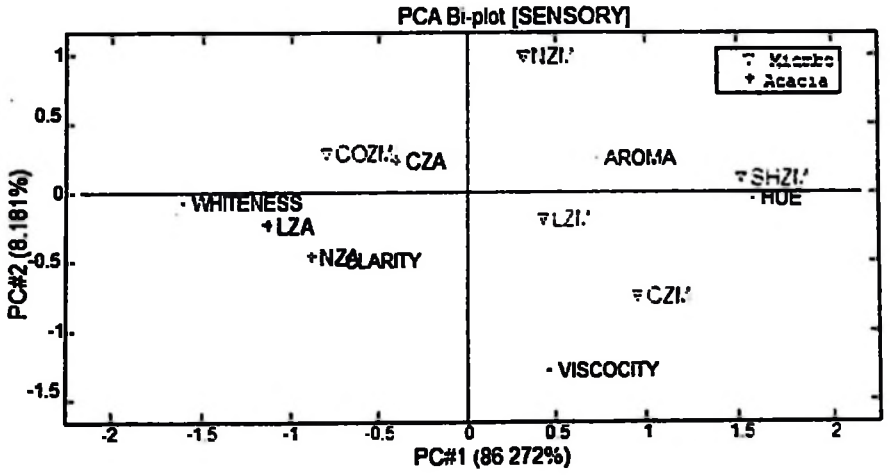

Figure 6.1a: PCA Bi-plot showing honey sample variations in sensory properties between *Miombo* (Red colour) and *Acacia* (Blue Colour) origins

Key: M - *Miombo* honey, A - *Acacia* honey. Coastal zone - COZ. Central zone – CZ. Lake zone – LZ. Northern zone – NZ, Southern highland zone – SHZ

Figure 6.1b shows visible separation pattern between zones with similar proportions as in figure 6.1a. All zones with samples were clearly separated from each other along PC 1 and

PC 2. Southern, Lake and Central zones were associated with hue, aroma and viscosity and contrasted from Coastal and Lake zones which were associated with whiteness and clarity along PC1

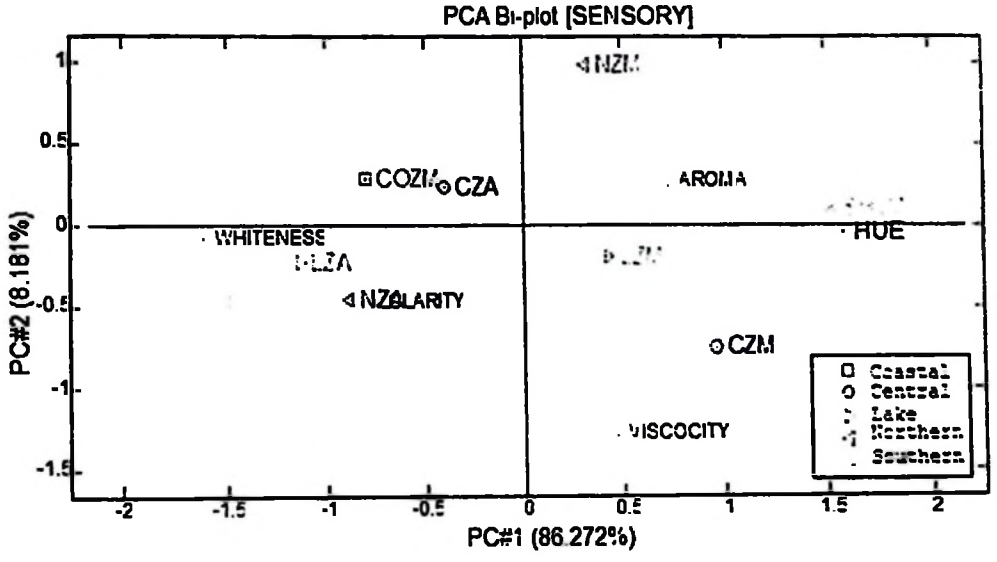

Figure 6.1b: PCA Bi-plot showing honey sample variations in sensory properties between zones

Key: M - *Miombo* honey, A - *Acacia* honey, Coastal zone - COZ, Central zone – CZ, Lake zone – LZ, Northern zone – NZ, Southern highland zone – SHZ

Furthermore, the loadings of the sensory variables indicate that hue and whiteness dominate the first component for separation of honeys according to floral and geographic origin along PC1 with little contribution from clarity and viscosity attributes. Along PC2, viscosity was responsible for separation with clarity and aroma to a little extent. Honey from Coastal zone, though comes from *Miombo* woodland was grouped on the left side of PC1. This may be contributed by its high scores in whiteness and clarity. The samples from Coastal zone evaluated for colour were found to be water white to white on pfund scale. Therefore, from the PCA bi plots, it can be concluded that *Miombo* honeys were

correlated with aroma. hue and viscosity while *Acacia* honeys were correlated with clarity and whiteness.

6.5.2 Relationship between descriptive data and hedonic liking by PLSR

Results from a partial least square regression (PLSR) using descriptive data as X-variables and liking rated by the consumers as Y-variables are given in Figure 6.2. The first two significant components indicate 49% of the variations in X and 51% in Y. It was observed that the consumers fall to the right of the vertical Y-axis around and outside 50% explained circle. The varied consumer's preferences provide insight into the sensory attributes that are important to individual's consumer acceptability. The acceptance values of consumers were inclined toward the direction of *Miombo* honey samples from Coastal zone which was associated with clarity and whiteness. This means that honey consumers showed a strong preference for light and clear honey than dark, aromatic and viscous honey as these variables were situated in the opposite direction of liking. In the X-axis, similar preference for *Acacia* samples from Central, Lake and Northern zone was seen due to their high association with clarity and whiteness. *Miombo* honye samples from Central, Lake, Northern and Southern highland zones were least preferred due to high intensity in hue, aroma and viscosity.

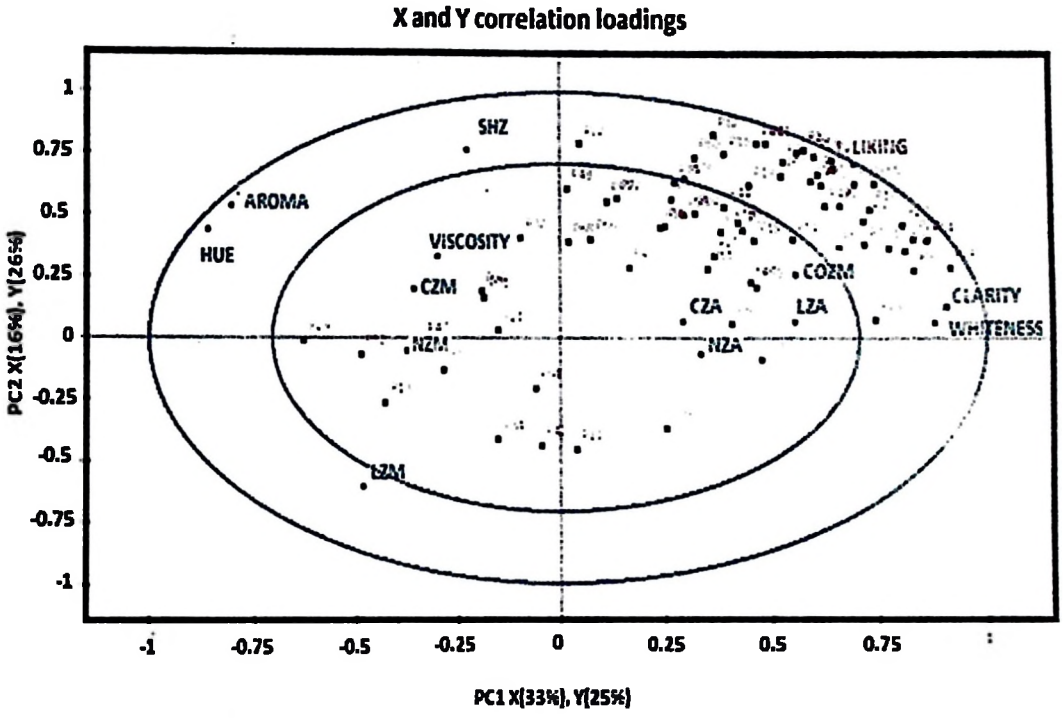

Figure 6.2: Correlation loadings from a partial least squares regression of honey samples from different geographical zones and floral origins with descriptive data as X variables and hedonic rating as Y variables

Key: M - *Miombo* honey, A - *Acacia* honey, Coastal zone - COZ, Central zone – CZ, Lake zone – LZ, Northern zone – NZ, Southern highland zone – SHZ

The findings suggest that, clarity and whiteness attributes were the driver for consumer liking of honey samples in this study. This implies that most consumers preferred honey that has moderate aroma, not deep in colour and should be clear.

6.6 Conclusions

From the findings, it is concluded that geographical zones and botanical origins have significant influence on the sensory properties and consumer acceptability of honey samples. In honey samples from *Miombo* floral origin, Southern highland had significantly

higher mean aroma and hue intensity scores. Central zone had higher viscosity, Coastal zone had higher clarity and whiteness scores. As for *Acacia* honey sample. Northern zone had higher aroma, viscosity and whiteness intensity scores compared to honey samples from other zones while Lake and central zones had higher clarity and hue scores. respectively.

Moreover, it was found that Costal zone was the most accepted honey sample among the *Miombo* honey samples while samples from the Lake zone were the most accepted samples among samples from *Acacia* origin. The preference mapping results indicated that, clarity and whiteness are the major drivers for consumer liking of honey samples from different geographical and floral origins in this study. It is therefore recommended that, honey sample of highest clarity and appeal be produced for marketing and human consumption.

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CHAPTER SEVEN

7.0 OVERALL CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Therefore, this study has revealed that fructose was the most abundant sugar in the honey samples while Potassium and Iron are the most abundant macro and micro minerals in honey samples respectively. Furthermore, it can be concluded that flora source of nectar and geographic location where honey is produced have significant impact on the quality properties and sensory attributes of the produced honey. *Miombo* honey samples were observed to have higher values than *Acacia* honey in the evaluated quality properties. Effect of geographic location was also observed as, Northern zone honey was found to have characteristic high content of moisture, acidity, ash, total sugars, phenols and antioxidant activity. Lake zone had viscous honey while Southern Highland was found to have more dark coloured honey. The Northern zone honey high content of phenol and antioxidant activity.

7.2 Recommendations

Recommendations made based on the study findings are as follows:

i. A national database of honey quality needsto be established in order to properly cluster and characterize Tanzania honey according to geographical and floral origin so as to compete effectively in the local and international market.

ii. A study to ascertain high macro mineral and Lead contents in the Northern zone honey with Lead values above maximum limits is needed.

iii. Due to their observed nutritional quality of honey, inclusion of honey in the daily diet is recommended.

iv. High antioxidant activities could indicate high potency of honey and thus recommended to be used in medicinal formulations. however. this should be coupled with further research to ascertain the findings and come up with a rigid conclusion.

v. *Miombo* honey samples from Coastal zone were seen to overlapped in parameters with *Acacia* honey samples in Principal Component Analysis throughout the study. This could be an indication of mispresentation of honey samples. i.e.. *Acacia* honey samples as *Miombo* honey sample. It is recommended that authenticity and characterization of samples is important in order to detect and remove such ambiguous samples sent to the international market. Also. regulatory authorities should educate producers on the importance of proper labeling and genuinity of the product.

APPENDICES

SOKOINE UNIVERSITY OF AGRICULTURE

COLLEGE OF AGRICULTURE (CoA)

DEPARTMENT OF FOOD TECHNOLOGY, NUTRITION AND CONSUMER SCIENCES

Appendix 1: Consumer Test for Honey Samples

P.No.....

.....Date.....Time.....

Please evaluate each of the fifteen (15) coded honey samples from left to right. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your degree of liking (9-1) in the column against each attribute. Put the appropriate number against each attribute.

Key: 9- Like extremely, 8-Like very much, 7- Like moderately, 6-Like slightly, 5-Neither like nor dislike 4-Dislike slightly, 3- Dislike moderately, 2-Dislike very much, 1-Dislike extremely.

	Sample code														
Attribute	**313**	**417**	**527**	**555**	**645**	**211**	**884**	**577**	**467**	**924**	**712**	**918**	**066**	**214**	**131**
Colour															
Aroma															
Viscosity															
Overall Acceptability															

Last we would be happy if you will answer some additional questions.

We need some information about our consumers. and would appreciate it if you could answer the following questions:

1. Gender:

- ☐ Female
- ☐ Male

2. Age

- ☐ 15-30
- ☐ 31-45
- ☐ 46-60

3. Which group do you fit

- ☐ Undergraduate
- ☐ Postgraduate
- ☐ Staff

3. How often do you eat/consumer product/s?

- ☐ Daily
- ☐ Once in a week
- ☐ Once in month
- ☐ Seldom

4. What kind of honey do you prefer?

- ☐ Raw
- ☐ Pasteurized

5. Which brand do you prefer?

- ☐ 313
- ☐ 417
- ☐ 527
- ☐ 555
- ☐ 645
- ☐ 211
- ☐ 884
- ☐ 577
- ☐ 467
- ☐ 924
- ☐ 712
- ☐ 918
- ☐ 066
- ☐ 214
- ☐ 131

Thank you for your cooperation!

Appendix 2: Quantitative descriptive analysis

Quantitative Descriptive Sensory Evaluation form

Sensory Evaluation Form

Quantitative descriptive Analysis (QDA) of Honey Samples

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

Please evaluate each coded ample in the order they are listed. Choose appropriate number in a scale from 1 to 9, where 1 is low intensity and 9 is high intensity. How do you find the following characteristics for different honey? Put the appropriate number against each characteristic.

Sample number

Clarity

Not clear 1 2 3 4 5 6 7 8 9 Very clear

Whiteness

Brown 1 2 3 4 5 6 7 8 9 Very faint brown

Colour Hue

Brown 1 2 3 4 5 6 7 8 9 Dark Brown

Viscosity

Not viscous 1 2 3 4 5 6 7 8 9 Very viscous

Aroma

Not aromatic 1 2 3 4 5 6 7 8 9 Very aromatic

Thank you for your cooperation!

Appendix 3: Estimation of moisture content

Refractive index (20°C)	Moisture content (percent)	Refractive index (20°C)	Moisture content (percent)	Refractive index (20°C)	Moisture content (percent)
1.5044	13.0	1.4935	17.2	1.4830	21.4
1.5038	13.2	1.4930	17.4	1.4825	21.4
1.5033	13.4	1.4925	17.6	1.4820	21.8
1.5028	13.6	1.4920	17.8	1.4815	22.0
1.5023	13.8	1.4915	18.0	1.4810	22.2
1.5018	14.0	1.4910	18.2	1.4805	22.4
1.5012	14.2	1.4905	18.4	1.4800	22.6
1.5007	14.4	1.4900	18.6	1.4795	22.8
1.5002	14.6	1.4895	18.8	1.4790	23.0
1.4997	14.8	1.4890	19.0	1.4785	23.2
1.4992	15.0	1.4885	19.2	1.4780	23.4
1.4987	15.2	1.4880	19.4	1.4775	23.6
1.4982	15.4	1.4875	19.6	1.4770	23.8
1.4976	15.6	1.4870	19.8	1.4765	24.0
1.4971	15.8	1.4865	20.0	1.4760	24.2
1.4966	16.0	1.4860	20.2	1.4755	24.4
1.4961	16.2	1.4855	20.4	1.4750	24.6
1.4956	16.4	1.4850	20.6	1.4745	24.8
1.4946	16.8	1.4845	20.8	1.4740	25.0
1.4940	17.0	1.4840	21.0		
		1.4835	21.2		

Thank you for your cooperation!

Appendix 4: Tables for analysis of variances

1. ANALYSIS OF VARIANCES TABLES FOR PHYSICOCHEMICAL PROPERTIES BETWEEN ZONES WITHIN A FLORAL ORIGIN

A) Miombo

Analysis of variance table: Acidity (meq/kg)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	526.36	131.591	631.55	5.699e-12 ***
Residuals	10	2.08	0.208		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Ash (%)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	1.24251	0.310627	1513.2	7.315e-14 ***
Residuals	10	0.00205	0.000205		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Colour (mm pfund)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	23132	5783.0	2.986	0.07314
Residuals	10	19367	1936.7		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: MC (%)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	24.7356	6.1839	110.45	3.128e-08 ***
Residuals	10	0.5599	0.0560		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: pH

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	1.86083	0.46521	81.812	1.341e-07 ***
Residuals	10	0.05686	0.00569		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Viscocity (dPas)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	2030.9	507.73	1.263	0.3466
Residuals	10	4020.0	402.00		

B) Acacia

Analysis of variance table: Acidity (meq/kg)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sample	2	88.972	44.486	308.34	8.947e-07 ***
Residuals	6	0.866	0.144		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Ash (%)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sample	2	0.047564	0.0237822	492.81	2.215e-07 ***
Residuals	6	0.000290	0.0000483		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table: Colour (mm pfund)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sample	2	3708.7	1854.37	3.0599	0.1213
Residuals	6	3636.2	606.03		

Analysis of variance table: Moisture content (%)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sample	2	2.5696	1.2848	3.4189	0.1021
Residuals	6	2.2548	0.3758		

Analysis of variance table: pH

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sample	2	0.50978	0.254892	11.031	0.009774 **
Residuals	6	0.13864	0.023106		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Viscocity (dPas)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	705.56	352.78	3.5278	0.09707
Residuals	6	600.00	100.00		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

2. ANALYSIS OF VARIANCES TABLES FOR MACRO MINERALS BETWEEN ZONES WITHIN SAME FLORAL ORIGIN

A) Acacia

Analysis of variance table: Calcium (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	13.8	6.91	0.0079	0.9921
Residuals	6	5238.6	873.10		

Analysis of variance table: Magnesium (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	168325	84163	1171.8	1.665e-08 ***
Residuals	6	431	72		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Potassium (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	243271	121636	45.738	0.0002332 ***
Residuals	6	15956	2659		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Potassium (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	243271	121636	45.738	0.0002332 ***
Residuals	6	15956	2659		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Sodium (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	6038.3	3019.16	50.794	0.0001734 ***
Residuals	6	356.6	59.44		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

B) Miombo

Analysis of variance table: Calcium (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	109063	27266	118.55	2.216e-08 ***
Residuals	10	2300	230		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Magnesium (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	8794825	2198706	43.009	2.864e-06 ***
Residuals	10	511218	51122		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Potassium (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	14783825	3695956	46.502	1.986e-06 ***
Residuals	10	794799	79480		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Sodium (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	4398.2	1099.56	6.9949	0.005927 **
Residuals	10	1571.9	157.19		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3. ANALYSIS OF VARIANCES TABLES FOR MICRO MINERALS BETWEEN ZONES WITHIN FLORAL ORIGIN

A) Acacia

Analysis of variance table: Copper (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	0.014875	0.0074374	10.927	0.009996 **
Residuals	6	0.004084	0.0006807		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table: Iron (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	23.249	11.6244	1.3922	0.3187
Residuals	6	50.100	8.3499		

Analysis of variance table: Lead (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	6.1374	3.06870	4.571	0.06222 .
Residuals	6	4.0280	0.67133		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Zinc (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	2	28.184	14.0919	4.5967	0.06159 .
Residuals	6	18.394	3.0656		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

B) Miombo

Analysis of variance table: Copper (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	0.039994	0.0099986	5.7926	0.01119 *
Residuals	10	0.017261	0.0017261		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Iron (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	217.156	54.289	23.351	4.666e-05 ***
Residuals	10	23.249	2.325		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance table: Lead (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	1.7004	0.42510	2.1795	0.1449
Residuals	10	1.9505	0.19505		

Analysis of variance table: Zinc (ppm)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Zone	4	33.529	8.3824	38.054	5.067e-06 ***
Residuals	10	2.203	0.2203		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1