DETERMINATION OF PROXIMATE COMPOSITIONS OF HONEY SOURCED FROM DIFFERENT LOCATIONS IN TWO STATES IN NIGERIA

BY

ATURU, OLUWABUKOLA BELINDA

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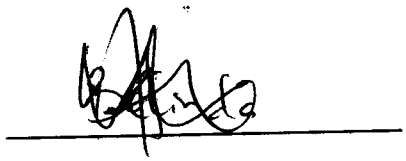
FEBRUARY, 2012.

DECLARATION

I hereby declare that this project is a record of a research work that was undertaken and written by me. It has not been presented before for any degree or diploma or certificate at any University or Institution. Information derived from personal communications, published and unpublished works of others were duly referenced in the text.



28/02/2012

Aturu, Oluwabukola Belinda

2006/24054EA

Date

CERTIFICATION

This is to certify that this project entitled "Determination of Proximate Compositions of Honey Sourced from Different Locations in Two States in Nigeria" meets the regulations governing the award of the degree of Bachelor of Engineering (B. ENG.) of the Federal University of Technology, Minna, and it is approved for its contribution to scientific knowledge and literary presentation.

Engr. Dr. O. Chukwu
Supervisor

27/02/2012
Date

Engr. Dr. P. A. Idah
Head of Department

27/02/2012
Date

External Examiner

22 02 2012
Date

DEDICATION

This project is dedicated to Almighty God, the sustainer of my life.

ACKNOWLEDGEMENTS

My sincere gratitude goes to God Almighty who sustained and kept me alive during the course of my programme in school. Without him by my side, I wonder where I would have been at this moment.

I would also like to appreciate the effort of my supervisor, Engr. Dr. O. Chukwu who with his tight schedule still took time to go through every piece of this project carefully and corrected me when I ought to be corrected.

I also like to show my appreciation to the following people: My level adviser in the person of Mrs. B. A. Orhevba for the love she showed to me. Also the H.O.D, Engr. Dr. P. A. Idah and all the Lecturers in the Agricultural and Bioresources Engineering Department, Federal University of Technology, Minna, for their tireless effort in delivering sound lectures to me and for their love and care especially Dr. G. Agidi and Engr. Dr. I. E. Ahaneku.

I would like to express my appreciation to my parents Mr. and Mrs. Charles Aturu who through their financial support and prayers have made me who I am today and also my siblings: Betty, Victor, Dora and King-Charles who showered me with their love and care.

Finally, my sincere appreciation goes to my friends and colleagues Andrew, Adaora, Felicia, Mary, Terry, Mayokun, Wale, Justice, Jennifer and others not mentioned, who gave me supports in various forms throughout my stay on campus. I pray that God will bless and reward you all abundantly. Amen.

ABSTRACT

This study was carried out to determine the proximate compositions of honey sourced from different locations in two states Nigeria. These locations were Bida, Minna, Suleja, Kontagora in Niger State and Obollo-Afor in Enugu State. The various honey samples were analysed for proximate compositions and physicochemical properties under standard laboratory conditions using the Association of Official Analytical Chemists nutritional guidelines. It was observed that the pH values of honey from Minna and Suleja were within the range of standard but those from Obollo-Afor, Bida and Kontagora were slightly above the standard. The specific gravity of honey from Bida, Minna, and Suleja were within the range of the standard value while that of Obollo-Afor was below and Kontagora was above the standard values. Though the refractive index of the honey obtained from Kontagora and Obollo-Afor were not significantly different from each other, they were significantly higher than those obtained from Suleja, Bida, and Minna respectively. The viscosity at 20°C of the five samples decreased in the order: Minna > Kontagora > Obollo-Afor > Suleja > Bida. The sensory evaluation of flavour, taste, colour and consistency of the five (5) samples of honey indicated that they were not significantly different from each other ($P > 0.5$). The moisture content of honey from all the locations fell below that of the standard. Also, the protein content of all the samples were higher than that of the standard while carbohydrate content of honey from Bida, Suleja, Minna, and Kontagora were below that of the standard value but that of Obollo-Afor was above the standard. The ash content and reducing sugar in the honey from the five locations were far above that of the standard except that from Obollo-Afor while the free fatty acid of the honey from Suleja, Kontagora, Bida and Obollo-Afor were lower than the standard value and that of Minna was higher. Honey from Bida and Kontagora had higher Vitamin C than those of Suleja, Minna, and Obollo-Afor while the maltose and fructose concentration in the honey obtained were above and below the standard values respectively. Glucose concentrations of honey from the five locations were within the standard value. From the results obtained, it was observed that the honey from Bida has good quality attribute followed by the one from Obollo-Afor, while the ones from Kontagora, Minna and Suleja have the least quality attribute. As a result, honeys from Bida and Obollo-Afor are recommended for consumption in their current form while those from Kontagora, Suleja and Minna are recommended for further processing before consumption.

TABLE OF CONTENTS

Cover page	i
Title page	ii
Declaration	iii
Certification	iv
Dedication	v
Acknowledgements	vi
Abstract	vii
Table of Contents	viii
List of Tables	xii
List of Figures	xiii
List of Plates	xiv
CHAPTER ONE	
1.0 INTRODUCTION	1
1.1 Background to the Study	1
1.2 Statement of the Problem	4
1.3 Objectives of the Study	5

1.5 Scope of the Study 5

CHAPTER TWO

2.0 LITERATURE REVIEW 6

2.1 History and Formation of Honey 6

2.2 Extraction of Honey from Honeycomb 8

2.3 Nutritional Properties of Honey 8

2.4 Indicators of Quality 10

2.5 Identification of Adulterated Honey 11

2.6 Physical Properties of Honey 12

2.6.1 Chemical Compositions of Honey 13

2.7 Classification of Honey Based on Source 16

2.7.1 Classification of Honey Based on Processing and Packaging 18

2.8 Modern Uses of Honey 21

CHAPTER THREE

3.0 MATERIALS AND METHODS 24

3.1 Materials 24

3.1.1 Collection of Honey 24

3.1.2 Apparatus Used in the Proximate Composition Analysis of the Honey 25

3.1.3 Reagents Used for the Proximate Compositions of Honey 28

3.2. Determination of Physical Properties of Honey 29

3.2.1 Determination of Specific Gravity 29

3.2.2 Determination of Viscosity 30

3.2.3 Determination of Refractive Index 30

3.2.4 Determination of pH Value 31

3.3 Determination of the Proximate Composition of Honey 32

3.3.1 Determination of Percentage Moisture Content of Honey 32

3.3.2 Determination of Percentage Crude Protein of Honey 33

3.3.3 Determination of Percentage Ash Content of Honey 34

3.3.4 Determination of Carbohydrate Content of Honey 34

3.3.5 Determination of Vitamin C Content of Honey 35

3.3.6 Determination of Reducing Sugar of Honey 35

3.3.7 Determination of Maltose Content of Honey 35

3.3.8 Determination of Free Fatty Acid in Honey 36

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION 38

4.1 Results 38

4.1.1 Physical Characteristics of Honey 38

4.1.2 Means of Sensory Evaluation of five Samples by 16 Respondents 39

4.1.3 Proximate Analysis of Honey 40

4.2 Discussion of Results 41

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS 47

5.1 Conclusions 47

5.2 Recommendations 49

REFERENCES 50

APPENDICES 54

LIST OF TABLES

Table	Page
2.1 The Average Composition of Floral Honeys Produced in Italy	14
2.2 Some Chemical Components of Brands of Honey on Sale in Qatar	14
2.3 Some Important Components in Honey that are Usually Specified in International or Local Standards	16
4.1 Physical Characteristics of Honey	38
4.2 Sensory Evaluation of Honey	39
4.3 Proximate Compositions of Honey	40

LIST OF PLATES

Plate		Page
3.1	Collected Samples of Honey	24
3.2	Soxhlet Apparatus	26
3.3	Muffle Furnace	26
3.4	Electronic Air Oven	26
3.5	Kjeldahl Digestion Block	27
3.6	Refractometer	27

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Honey is a sweet food made by bees using nectar from flowers. The variety produced by honey bees (the genus *Apis*) is the one most commonly referred to and is the type of honey collected by beekeepers. Honey is produced by bees as a food source. In cold weather or when fresh food sources are scarce, bees use their stored honey as their source of energy. By contriving for bee swarms to nest in artificial hives, people have been able to semi-domesticate the insects, and harvest excess honey. In the hive (or in a wild nest), there are three types of bee: a single female queen bee, a seasonally variable number of male drone bees to fertilize new queens, and some 20,000 to 40,000 female worker bees (Val, 2007). The worker bees raise larvae and collect the nectar that will become honey in the hive. Leaving the hive, they collect sugar-rich flower nectar.

Honey has a long history of human consumption, and is used in various foods and beverages as a sweetener and flavoring. It also has a role in religion and symbolism. Flavours of honey vary based on the nectar source, and various types and grades of honey are available. It is also used in various medicinal traditions to treat ailments. The study of pollens and spores in raw honey (melissopalynology) can determine floral sources of honey (Vaughn and Bryant, 2001). Because bees carry an electrostatic charge and can attract other particles, the same techniques of melissopalynology can be used in area environmental studies of radioactive particles, dust or particulate pollution (Tonelli *et al.*, 1990).

Honey collection is an ancient activity. Eva Crane's, *The Archaeology of Beekeeping,* states that humans began hunting for honey at least 10,000 years ago (Wikipedia, 2011). She

evidenced this with a cave painting in Valencia, Spain. The painting is a Mesolithic rock painting, showing two female honey-hunters collecting honey and honeycomb from a wild bee nest. The two women are depicted in the nude, carrying baskets, and using a long, wobbly ladder to reach the wild nest.

In ancient Egypt, honey was used to sweeten cakes and biscuits, and was used in many other dishes. The fertility god of Egypt, Min, was offered honey. The Maya used honey from the stingless bee for culinary purposes, and continue to do so today. The Maya also regard the bee as sacred (Wikipedia, 2011).

The physical properties of honey vary, depending on water content, the type of flora used to produce it, temperature, and the proportion of the specific sugars it contains. Fresh honey is a supersaturated liquid, containing more sugar than the water can typically dissolve at ambient temperatures. At room temperature, honey is a supercooled liquid, in which the glucose will precipitate into solid granules. This forms a semi-solid solution of precipitated sugars in a solution of sugars and other ingredients.

The melting point of crystallised honey is between 40 and 50 °C, depending on its composition. Below this temperature, honey can be either in a metastable state, meaning that it will not crystallise until a seed crystal is added, or, more often, it is in a "labile" state, being saturated with enough sugars to crystallise spontaneously. The rate of crystallisation is affected by the ratio of the main sugars, fructose to glucose, as well as the dextrin content. Temperature also affects the rate of crystallisation, which is fastest between 13 and 17 °C. Below 5 °C, the honey will not crystallise and, thus, the original texture and flavour can be preserved indefinitely (Wikipedia, 2011).

Honey is a mixture of sugars and other compounds. With respect to carbohydrates, honey is mainly fructose (about 38.5%) and glucose (about 31.0%), making it similar to the synthetically produced inverted sugar syrup, which is approximately 48% fructose, 47% glucose, and 5% sucrose. Honey's remaining carbohydrates include maltose, sucrose, and other complex carbohydrates. As with all nutritive sweeteners, honey is mostly sugars and contains only trace amounts of vitamins or minerals. Honey also contains tiny amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinocembrin. (Martos *et al* 2000). The specific composition of any batch of honey depends on the flowers available to the bees that produced the honey.

High-quality honey can be distinguished by fragrance, taste, and consistency. Ripe, freshly collected, high-quality honey at 20 °C should flow from a knife in a straight stream, without breaking into separate drops. After falling down, the honey should form a bead. The honey, when poured, should form small, temporary layers that disappear fairly quickly, indicating high viscosity. If not, it indicates excessive water content (over 20%) of the product. Honey with excessive water content is not suitable for long-term preservation (Wikipedia, 2011).

In jars, fresh honey should appear as a pure, consistent fluid, and should not set in layers. Within a few weeks to a few months of extraction, many varieties of honey crystallise into a cream-coloured solid. Some varieties of honey, including tupelo, acacia, and sage, crystallise less regularly. Honey may be heated during bottling at temperatures of 40–49°C to delay or inhibit crystallisation. Overheating is indicated by change in enzyme levels, for instance, diastase activity, which can be determined with the Schade or the Phadebas methods. A fluffy film on the

surface of the honey (like a white foam), or marble-coloured or white-spotted crystallisation on a container's sides, is formed by air bubbles trapped during the bottling process.

The main uses of honey are in cooking, baking, as a spread on bread, and as an addition to various beverages, such as tea, and as a sweetener in some commercial beverages. According to The National Honey Board (a USDA-overseen organization), "honey stipulates a pure product that does not allow for the addition of any other substance.This includes, but is not limited to, water or other sweeteners". Honey barbecue and honey mustard are common and popular sauce flavours. Honey is the main ingredient in the alcoholic beverage mead, which is also known as "honey wine" or "honey beer". Historically, the ferment for mead was honey's naturally occurring yeast. Honey is also used as an adjunct in some beers.

1.2 Statement of the Problem

Honey has a wide range of applications; ranging from its use in cooking, baking, as spread on bread to as a sweetener in some commercial beverages. Amidst all these uses, little is known by most consumers about its physical and nutritional compositions. As such, consumers end to consume any honey that comes their way without knowing if it is adulterated or pure oney because most honey sellers claim to sell pure honey whereas is mostly adulterated. As uch, this work is geared towards obtaining honey from different parts of Niger State and etermining its proximate compositions in order to ascertain if they meet the standard ompositions of pure honey needed for human consumption.

1.3 Objectives of the Study

The objectives of this study are:

i. To determine some physical and nutritional properties of honey obtained from different locations in two states in Nigeria.

ii. To compare the results with the standard for the physical and nutritional properties.

1.4 Justification of the Study

Honey is of great importance to man in the religious, medicinal and beverage sector. This work will help in comparing the proximate compositions of honey sourced from different locations in two states in Nigeria. The differences in proximate compositions, if any, will now serve as an important nutritional data. Also the differences in physical properties, if any, will help consumers to distinguish between adulterated and pure honey.

1.5 Scope of the Study

This study is limited to the determination of proximate compositions of honey sourced from Bida, Minna, Suleja and Kontagora in Niger State and Obollo-Afor, Nsukka in Enugu State. The honey obtained will be subjected to chemical analysis using the Association of Official Analytical Chemists so as to determine some nutritional values of the honey which include: carbohydrate, sugar, protein, fat, water, vitamins, and mineral contents. Physical properties like colour, odour, viscosity, specific gravity and refractive index will also be determined.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and Formation of Honey

Honey is one of the oldest and best-loved sweetening agents for foods and, over the centuries, it has still retained a "natural" image (Aparna and Rajalakshmi, 1999). Honey collection is an ancient activity. Eva Crane's, *The Archaeology of Beekeeping,* states that humans began hunting for honey at least 10,000 years ago (Wikipedia, 2011). The author evidenced this with a cave painting in Valencia, Spain. The painting is a Mesolithic rock painting, showing two female honey-hunters collecting honey and honeycomb from a wild bee nest. The two women are depicted in the nude, carrying baskets, and using a long, wobbly ladder to reach the wild nest.

In ancient Egypt, honey was used to sweeten cakes and biscuits, and was used in many other dishes. Ancient Egyptian and Middle Eastern peoples also used honey for embalming the dead. Pliny the Elder devoted considerable space in their book '*Naturalis Historia*' to the bee and honey, and its many uses. The fertility god of Egypt, Min, was offered honey (Wikipedia, 2011).

The art of beekeeping appeared in ancient China for a long time and hardly traceable to its origin. In the book "Golden Rules of Business Success" written by Fan Li (or Tao Zhu Gong) during the Spring and Autumn Period, there are some parts mentioning the art of beekeeping and the importance of the quality of the wooden box for bee keeping that can affect the quality of its honey. Honey was also cultivated in ancient Mesoamerica.

The Maya used honey from the stingless bee for culinary purposes, and continue to do so today. The Maya also regard the bee as sacred. Some cultures believed honey had many practical

health uses. It was used as an ointment for rashes and burns, and to help soothe sore throats when no other medicinal practices were available. Whilst a number of species of bee, such as *Apis dorsata* in Nepal (Joshi *et al.*, 2000), may be important. Locally, most honey is produced by two species of bee, namely *Apis mellifera* (the so-called honey bee) and *Apis cerana* which has been "domesticated" in parts of Asia (Crane, 1975). The raw material for the production of "floral" honey is nectar, a dilute solution of sugars found in the nectaries of flowering plants, while "honeydew" honey is made by bees that extract sugars from the living tissues of plants or fruits, and/or scavenge the excretions of insects that tap the veins of higher plants.

In general, floral honey is superior to the honeydew variant and, in theory, the nectar from any plant can be used by bees to make honey. However, there are massive differences between plant species with respect to their potential to support populations of bees and in the compositions of their nectars. The dominant components in all nectars are carbohydrates and, while some nectars contain mainly sucrose, in others, the sugars are confined to glucose and fructose; some minerals and vitamins may be present at low levels (Crane, 1975). It is the transformation of this sugary nectar into honey that is important from a human standpoint. In essence, the nectar is collected by foraging bees in honey sacs and, on returning to the hive, the nectar is passed to another bee(s) before finally being stored in a cell in the comb. After deposition in the cell, the evaporation of water gradually raises the solids content to around 80 per cent over a period of three to four days and, during this stage, invertase secreted by the bees converts most of the sucrose into hexoses to give the final sugar spectrum of the honey. Additional changes in carbohydrate composition and a degradation of pollen accompany the further manipulation of the nectar by the bees during the filling of the cells of the comb

(Vonderohe, 1994), and the air-tight seal of wax ensures that the honey cannot absorb water and,perhaps, spoil.

2.2 Extraction of Honey from Honeycomb

Extraction of the honey from the combs at the end of the season involves breaking the waxy seal and removing the honey by centrifugation. The honey is then strained and, in some cases, filtered and heated to eliminate yeasts that could cause spoilage. Once bottled, some products can be prone to hardening due to the crystallisation of the sugars. The presence of "nuclei" in the honey can be relevant but, as all natural honeys contain pollen grains, the presence of particulate matter is inevitable unless it has been finely strained (*Codex Alimentarius*, 2001). Consequently, control of crystallisation depends on keeping the glucose to water ratio in the region of 1.50:1.75, for it is this ratio that governs the extent and rate of the process (White, 1975). If this aspect can be controlled, then honey should remain a viscous, fluid material during ambient temperature storage.

2.3 Nutritional Properties of Honey

Honey is a mixture of sugars and other compounds. With respect to carbohydrates, honey is mainly fructose (about 38.5%) and glucose (about 31.0%) (National Honey Board, 2010), making it similar to the synthetically produced inverted sugar syrup, which is approximately 48% fructose, 47% glucose, and 5% sucrose. Honey's remaining carbohydrates include maltose, sucrose, and other complex carbohydrates (National Honey Board, 2010). As with all nutritive sweeteners, honey is mostly sugars and contains only trace amounts of vitamins or minerals. Honey also contains tiny amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinocembrin (Martos *et al.*, 2000). The

specific composition of any batch of honey depends on the flowers.available to the bees that produced the honey. A typical composition of honey is presented below:

- Fructose: 38.2%
- Glucose: 31.3%
- Sucrose: 1.3%
- Maltose: 7.1%
- Water: 17.2%
- Higher sugars: 1.5%
- Ash: 0.2%
- Others/undetermined: 3.2%

Source: Rainer, (1996).

The glycemic index of honey ranges from 31 to 78, depending on the variety. Honey has a density of about 1.36 kilograms per litre (36% denser than water) (Rainer, 1996). Isotope ratio mass spectrometry can be used to detect addition of corn syrup or sugar cane sugars by the carbon isotopic signature. Addition of sugars originating from corn or sugar cane (C4 plants, unlike the plants used by bees, which are predominantly C3 plants) skews the isotopic ratio of sugars present in honey, but does not influence the isotopic ratio of proteins; in an unadulterated honey, the carbon isotopic ratios of sugars and proteins should match. As low as 7% level of addition can be detected (Wikipedia, 2011).

2.4 Indicators of Quality

High-quality honey can be distinguished by fragrance, taste, and consistency. Ripe, freshly collected, high-quality honey at 20 °C should flow from a knife in a straight stream, without breaking into separate drops. After falling down, the honey should form a bead. The honey, when poured, should form small, temporary layers that disappear fairly quickly, indicating high viscosity. If not, it indicates excessive water content (over 20%) of the product. Honey with excessive water content is not suitable for long-term preservation. In jars, fresh honey should appear as a pure, consistent fluid, and should not set in layers. Within a few weeks to a few months of extraction, many varieties of honey crystallise into a cream-coloured solid. Some varieties of honey, including tupelo, acacia, and sage, crystallise less regularly.

Honey may be heated during bottling at temperatures of 40–49°C to delay or inhibit crystallisation. Overheating is indicated by change in enzyme levels, for instance, diastase activity, which can be determined with the Schade or the Phadebas methods. A fluffy film on the surface of the honey (like a white foam), or marble-coloured or white-spotted crystallisation on a containers' sides, is formed by air bubbles trapped during the bottling process.

A 2008 Italian study determined that nuclear magnetic resonance spectroscopy can be used to distinguish between different honey types, and can be used to pinpoint the area where it was produced. Researchers were able to identify differences in acacia and polyfloral honeys by the differing proportions of fructose and sucrose, as well as differing levels of aromatic amino acids phenylalanine and tyrosine. This ability allows greater ease of selecting compatible stocks (Wikipedia, 2011).

2.5 Identification of Adulterated Honey

Many foods and food ingredients have the potential to be adulterated. Those that are expensive (e.g. vanilla, extra virgin olive oil) (Downey *et al.*, 2002), and those compositions or yields may vary as a result of fluctuations in weather during growth and harvest seasons (e.g. coffee, oranges) (Briandlet *et al.*, 1997) may be particularly susceptible to this practice. Economic adulteration, i.e., the extension of a food or food ingredient by a cheaper and inferior product or component, is of considerable concern to food manufacturers, regulatory agencies, and consumers alike. Honey is highly prized by consumers as a natural sweet substance. It is defined as "the natural sweet substance produced by honeybees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store, and leave in the honeycomb to ripen and mature". While demand for honey is increasing, production is in decline for a variety of socio-economic factors. Extension of honey by addition of other sweet substances such as sugars or industrial syrups at some stage during production or processing could be an attractive means of economic adulteration. Identifying this type of adulteration is important for financial reasons.

Many different analytical techniques are employed in authenticity testing of honey. Among them are NMR (Nuclear Magnetic Resonance) spectroscopy (Lindner *et al.*, 1996) HPLC (High Performance Liquid Chromatography) (Swallow and Low, 1990), GC (Gas Chromatography), and carbon isotope ratio analysis. These techniques, while reported to be successful, are costly and require considerable analytical skill. With the exception of NMR spectroscopy, these techniques are also time-consuming and will destroy samples under test. A

need therefore exists for a rapid, nondestructive, and less expensive method suitable at least for screening honey samples for authenticity confirmation. Vibration spectroscopic methods (near and Mid Infrared) have previously been applied to a range of authenticity problems. In combination with multivariate data analysis, they possess the speed, simplicity, and low cost per analysis required for screening techniques. MIR spectroscopy (2500 - 25 000 nm) may have particular benefits since it contains more spectral information than its NIR counterpart and the fundamental vibration absorption bands in the MIR are better resolved than the broad overtone and combination absorption bands which arise in the Near Infrared spectral region (750 - 2500 nm).

The availability of ATR (Attenuated Total Reflectance) crystals simplifies sample handling in the MIR region. Previous reports have described the use of these vibration spectroscopic techniques to determine the chemical compositions of honey samples and also for detecting added sugar or syrups in honey (Sivakesava and Irudayaraj, 2001). However, the experimental design used in the latter reports facilitated classification on the basis of alteration of the solids content of the honey or by adulteration with syrups possessing a very different chemical compositions to that of honey.

2.6 Physical Properties of Honey

The physical properties of honey vary, depending on water content, the type of flora used to produce it, temperature, and the proportion of the specific sugars it contains. Fresh honey is a supersaturated liquid, non-Newtonian fluid and containing more sugar than the water can typically dissolve at ambient temperatures. At room temperature, honey is a super-cooled liquid,

in which the glucose will precipitate into solid granules. This forms a semi-solid solution of precipitated sugars in a solution of sugars and other ingredients.

The melting point of crystallised honey is between 40 and 50 °C, depending on its composition. Below this temperature, honey can be either in a meta-stable state, meaning that it will not crystallise until a seed crystal is added, or, more often, it is in a "labile" state, being saturated with enough sugars to crystallise spontaneously (Root *et al.*, 2005). The rate of crystallisation is affected by the ratio of the main sugars, fructose to glucose, as well as the dextrin content. Temperature also affects the rate of crystallisation, which is fastest between 13 and 17 °C. Below 5 °C, the honey will not crystallise and, thus, the original texture and about 31.0%, making it similar to the synthetically produced inverted sugar syrup, which is approximately 48% fructose, 47% glucose, and 5% sucrose.

2.6.1 Chemical Compositions of Honey

The characteristic aroma and flavour of honey, often associated with the dominant source of pollen, such as "heather honey" in England, "lotus tree honey" in the Arabian Gulf or "buckwheat honey" in North America (Zhou *et al.*, 2002) is one of the most attractive features of the product, and (Castro- Vazquez *et al.* (2003) identified over 120 volatile compounds that may contribute to the unique aroma of rosemary honey. However, many retail brands are non-specific, blended products identified only by their country of origin and, in an attempt to check on authenticity, most attention has centred on the major components. Although a variable natural product, Mincione and Leuzzi (1993) suggested the most floral honeys produced in Italy would have an average composition along the lines shown in Table 2.1, but the contrast with specific floral types is quite marked with respect to both the reducing and total sugars.

Table 2.1: The Average Composition of Floral Honeys Produced in Italy, and the Compositions of some Specific Types

Component (gkg^{-1})	Average	Eucalyptus	Thistle	Lavender	Citrus
Water	176.0	160	166	144	167
Reducing sugars	719.0	757	744	765	748
Total sugars	745.0	772	777	784	757
Sucrose	25.0	13.7	17.7	19.3	15.7
Ash	2.14	1.8	2.4	0.5	2.1
Insoluble substances	0.12	0.16	0.12	0.12	0.08

Source: Mincione and Leuzzi (1993)

Table 2.2: Some Chemical Components of Brands of Honey on Sale in Qatar

Brand	Water content	Reducing sugars	Total sugars	Glucose	Fructose
A)	164	686	757	278	408
B)	174	724	748	366	357
C)	178	688	740	310	377
D)	164	550	722	246	306
E)	164	571	614	222	349

Notes: All figures as gkg^{-1} and the means of duplicate analyses on bulked samples of a single brand

Source: Al-Jedah *et al.* (2003)

However, with some commercial brands the variations can as shown in Table 2.2, be even greater. Anupama *et al.* (2003) noted a similar pattern amongst commercial honeys on sale in India, with total reducing sugars varying from 613 to 726g/kg. In an attempt to limit variability and the possible marketing of sub-standard or adulterated products, *Codex Alimentarius* (2001) has proposed the compositional standards shown in Table 2.3. There are, of course, differences between the standards of different countries or regions and, in the Middle East for example, the *Gulf Standards* (1993) suggested a minimum level of apparent reducing sugars of 650g/kg instead of the minimum of 600g/kg for fructose + glucose (*Codex Alimentarius*, 2001). It is noticeable that brands (D) and (E) in Table 2.2 would be rejected against both standards, as would at least one of the samples from India (Anupama *et al.*, 2003).

There is a widely accepted maximum of 50g/kg for sucrose in floral honeys, but some entirely authentic honeys, such as citrus honey, can have sucrose concentrations up to $100gkg^{-1}$ due to the source of the nectar (International Honey Commission, 2002). In honeydew honeys, in particular, a range of oligosaccharides can contribute up to $170gkg^{-1}$ to the figure for total sugars and, in even in floral honeys, the contribution can be up to $80gkg^{-1}$ (Weston and Brocklebank, 1999). Aside from their nutritional value, it is relevant that these carbohydrates contribute to the low water activity of honey (0.75), a value that makes it a "safe" product to store in the home (Corry, 1979). Honey is reported to contain little or no fat, but free fatty acids like palmitic, (16:0), oleic (18:1) and linolenic (18:3) were easily detected in white clover honey (*Trifoliumrepens*) (Tan *et al.*, 1988; Singh and Bath, 1997). The protein content varies between around 1.0 to $4.0gkg^{-1}$, and the higher values are most notable for their impact on the thixotrophic properties of the product.

Table 2.3: Some Important Components in Honey that are Usually Specified in International or Local Standards

Water	Max. 200, except for specified types, e.g. clover or heather honey – 220
Glucose + fructose	Min. 600, except for specified types, e.g. honeydew honeys – 450
Sucrose	Max. 50, except for specified types, e.g. citrus honey – 100
Ash	Max. 6.0
Electrical conductivity ($mScm^{-1}$)	< 0.8
Acidity (milli-equivalents)	Max. 5.0
Insoluble material	Max. 1.0

Notes: All figures as gkg^{-1} unless otherwise stated

Source: Al-Jedah *et al.* (2003)

2.7 Classification of Honey Based on Source

Honey is classified by its floral source, and there are also divisions according to the packaging and processing used. There are also regional honeys. Honey is also graded on its colour and optical density by USDA standards, graded on a scale called the Pfund scale, which ranges from 0 for "water white" honey to more than 114 for "dark amber" honey (Wikipedia, 2011).

Floral Honey

Generally, honey is classified by the floral source of the nectar from which it was made. Honeys can be from specific types of flower nectars, from indeterminate origin, or can be blended after collection.

Blended Honey

Most commercially available honeys are blended, meaning it is a mixture of two or more honeys differing in floral source, colour, flavour, density or geographic origin.

Poly-floral Honey

Poly-floral honey, also known as wildflower honey, is derived from the nectar of many types of flowers. The taste may vary from year to year, and the aroma and the flavour can be more or less intense, depending on which bloomings are prevalent.

Mono-floral Honey

Mono-floral honey is made primarily from the nectar of one type of flower. Different mono-floral honeys have a distinctive flavour and colour because of differences between their principal nectar sources. To produce mono-floral honey, beekeepers keep beehives in an area where the bees have access to only one type of flower. In practice, because of the difficulties in containing bees, a small proportion of any honey will be from additional nectar from other flower types. Typical examples of North American mono-floral honeys are clover, orange blossom, sage, tupelo, buckwheat, fireweed, and sourwood. Some typical European examples include thyme, thistle, heather, acacia, dandelion, sunflower, honeysuckle, and varieties from lime and chestnut trees. In North Africa, such as Egypt, examples include clover, cotton, and citrus (mainly orange blossoms).

Honeydew Honey

Instead of taking nectar, bees can take honeydew, the sweet secretions of aphids or other plant sap-sucking insects. Honeydew honey is very dark- brown in colour, with a rich fragrance of stewed fruit or fig jam, and is not sweet like nectar honeys (Wikipedia, 2011). Germany's Black Forest is a well known source of honeydew-based honeys, as well as some regions in Bulgaria and Northern California in the United States. In Greece, pine honey (a type of honeydew honey) constitutes 60–65% of the annual honey production. Honeydew honey is popular in some areas, but in other areas beekeepers have difficulty selling the stronger flavoured product.

The production of honeydew honey has some complications and dangers. The honey has a much larger proportion of indigestible than light floral honeys, thus causing dysentery to the bees, resulting in the death of colonies in areas with cold winters. Good beekeeping management requires the removal of honeydew prior to winter in colder areas. Bees collecting this resource also have to be fed protein supplements, as honeydew lacks the protein-rich pollen accompaniment gathered from flowers.

2.7.1 Classification of Honey Based on Processing and Packaging

Generally, honey is bottled in its familiar liquid form. However, honey is sold in other forms, and can be subjected to a variety of processing methods.

- Crystallised honey is honey in which some of the glucose content has spontaneously crystallised from solution as the monohydrate. This is also called "granulated honey." Honey that has crystallised over time (or commercially purchased crystallised) in the

home can be returned to a liquid state if stirred in a container sitting in warm water at 49°C.

- Pasteurised honey is honey that has been heated in a pasteurisation process 71.7°C or higher. Pasteurisation destroys yeast cells. It also liquefies any micro-crystals in the honey, which delays the onset of visible crystallisation. However, excessive heat exposure also results in product deterioration, as it increases the level of hydroxymethylfurfural (HMF) and reduces enzyme (e.g. diastase) activity. Heat also affects appearance (darkens the natural honey colour), taste, and fragrance (Subramanian *et al., 2007*).
- Raw honey is honey as it exists in the beehive or as obtained by extraction, settling or straining, without adding heat (although some honey that has been "minimally processed" is often labelled as raw honey). Raw honey contains some pollen and may contain small particles of wax. Local raw honey is sought after by allergy sufferers as the pollen impurities are thought to lessen the sensitivity to hay fever.
- Strained honey has been passed through a mesh material to remove particulate material (pieces of wax, propolis, and other defects) without removing pollen, minerals or valuable enzymes.
- Ultra-filtered honey is processed by very fine filtration under high pressure to remove all extraneous solids and pollen grains. The process typically heats honey to 65–77°C to more easily pass through the fine filter. Ultra-filtered honey is very clear and has a longer shelf life, because it crystallises more slowly because of the high temperatures breaking down any sugar seed crystals, making it preferred by the supermarket trade.

- Ultra-sonicated honey has been processed by ultra-sonication (a non-thermal processing alternative for honey). When honey is exposed to ultra-sonication, most of the yeast cells are destroyed. Those cells that survive sonication generally lose their ability to grow, which reduces the rate of honey fermentation substantially. Ultra-sonication also eliminates existing crystals and inhibits further crystallization in honey. Ultrasonically aided liquefaction can work at substantially lower temperatures of approximately 35°C and can reduce liquefaction time to less than 30 seconds.
- Whipped honey, also called creamed honey, spun honey, churned honey, candied honey, honey fondant, and set honey (in the UK), has been processed to control crystallisation. Whipped honey contains a large number of small crystals in the honey. The small crystals prevent the formation of larger crystals that can occur in unprocessed honey. The processing also produces a honey with a smooth, spreadable consistency.
- Dried honey has the moisture extracted from liquid honey to create completely solid, non-sticky granules. This process may or may not include the use of drying and anti-caking agents. Dried honey is commonly used to garnish desserts.
- Comb honey is honey still in the honeybees' wax comb. It traditionally is collected by using standard wooden frames in honey supers. The frames are collected and the comb is cut out in chunks before packaging. As an alternative to this labour intensive method, plastic rings or cartridges can be used that do not require manual cutting of the comb, and speed packaging. Comb honey harvested in the traditional manner is also referred to as "cut-comb honey". In India, honey is harvested from forests in bee's natural habitat. It is said that honey will be consumed by the bees on the new moon day, so it is cultivated the day before (Wikipedia, 2011).

- Chunk honey is packed in wide-mouthed containers consisting of one or more pieces of comb honey immersed in extracted liquid honey.

2.8 Modern Uses of Honey

As a food and in cooking

The main uses of honey are in cooking, baking, as a spread on bread, and as an addition to various beverages, such as tea, and as a sweetener in some commercial beverages. According to the National Honey Board (a USDA-overseen organization), "honey stipulates a pure product that does not allow for the addition of any other substance. This includes, but is not limited to, water or other sweeteners". Honey barbecue and honey mustard are common and popular sauce flavours.

Medicinal Uses and Health Effects of Honey

Honey is the main ingredient in the alcoholic beverage mead, which is also known as "honey wine" or "honey beer". Historically, the ferment for mead was honey's naturally occurring yeast. Honey is also used as an adjunct in some beers. For at least 2700 years, honey has been used to treat a variety of ailments through topical application, but only recently have the antiseptic and antibacterial properties of honey been chemically explained. Wound Gels that contain antibacterial honey and have regulatory approval for wound care are now available to help conventional medicine in the battle against drug resistant strains of bacteria (Wahdan, 1998).

As an antimicrobial agent honey may have the potential for treating a variety of ailments. One New Zealand researcher says a particular type of honey may be useful in treating MRSA infections (Wahdan, 1998). Antibacterial properties of honey are the result of the low water

activity causing osmosis, hydrogen peroxide effect, and high acidity. Honey may also be used to alleviate the effects of a sore throat. It is mixed with lemon juice and consumed. The mixture coats the throat alleviating discomfort, and the antibacterial, antiseptic properties are good for the throat as well.

When used topically (as, for example, a wound dressing), hydrogen peroxide is produced by dilution with body fluids. As a result, hydrogen peroxide is released slowly and acts as an antiseptic (http/Worldwidewounds.com/2006). In diabetic ulcers, topical honey has been used successfully in a comprehensive treatment of diabetic ulcers when the patient cannot use other topical antibiotics. The pH of honey is commonly between 3.2 and 4.5. This relatively acidic pH level prevents the growth of many bacteria (Waikato Honey Research Unit, 2011).Antioxidants in honey have even been implicated in reducing the damage done to the colon in colitis. Such claims are consistent with its use in many traditions of folk medicine (Bilsel *et al.*, 2002).

Other Medical Applications of Honey

Some studies suggest the topical use of honey may reduce odours, swelling, and scarring when used to treat wounds; it may also prevent the dressing from sticking to the healing wound (Waikato Honey Research Unit, 2011). Honey has been shown to be an effective treatment for conjunctivitis in rats (Al-Waili, 2004).

Unfiltered, pasteurised honey is widely believed to alleviate allergies, though neither commercially filtered nor raw honey was shown to be more effective than placebo in a controlled study of 36 participants with ocular allergies (American Academy of Allergy, 2010). Nearly 1 in 3 of the volunteers dropped out of the study because they couldn't tolerate eating one tablespoon of honey every day due to the overly sweet taste. The official conclusion: "This study does not

confirm the widely held belief that honey relieves the symptoms of allergic rhinoconjunctivitis." A more recent study has shown pollen collected by bees to exert an antiallergenic effect, mediated by an inhibition of IgE immunoglobulin binding to mast cells. This inhibited mast cell degranulation and thus reduced allergic reaction (Ishikawa, 2008). The risk of experiencing anaphylaxis as an immune system reaction may outweigh any potential allergy relief.

A review in the Cochrane Library suggests honey could reduce the time it takes for a burn to heal-up to four days sooner in some cases. The review included 19 studies with 2,554 participants. Although the honey treatment healed moderate burns faster than traditional dressings did, the author recommended viewing the findings with caution, since a single researcher performed all of the burn studies.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection of Honey

The honey used as samples for analysis were obtained from different sales outlet in Nigeria which are Bida, Minna, Suleja, Kontagora all in Niger State and Obollo-Afor in Nsukka, Enugu State of Nigeria. The sample from Bida (350 ml) was collected on the 15th of August, the sample from Suleja (150 ml) was collected on the 19th of August, while that of Minna (123 ml) and Kontagora (60 ml) were collected on the 21st of August, 2011. The sample (70 ml) from Obollo-Afor was collected on the 30th of September, 2011. The pictures of the collected samples are shown in Plate I.

Plate I: Collected Samples of Honey

3.1.2 Apparatuses Used for the Determination of Proximate Compositions of Honey

The following apparatuses were used in the determination of proximate compositions.

Viscometer

Pipette (Pyrex, England)

Measuring Cylinder (Pyrex, England)

Soxhlet apparatus(Plate II)

Kjeldahl digestion block (Model No. 451699, England) (Plate III)

Burette (Pyrex England)

Flat bottom flask (Pyrex, England)

Refractometer (Plate IV)

Silica dishes

Petri dishes

Weighing balance (Analytical balance machine by Salter, Model 250)

Spatula

Beaker (Pyrex, England)

Electric air oven (Gallen kamp furnace, Model No. 2346AA) (Plate V)

Test tube (Pyrex England)

Dessicator

Muffle furnace (Plate VI)

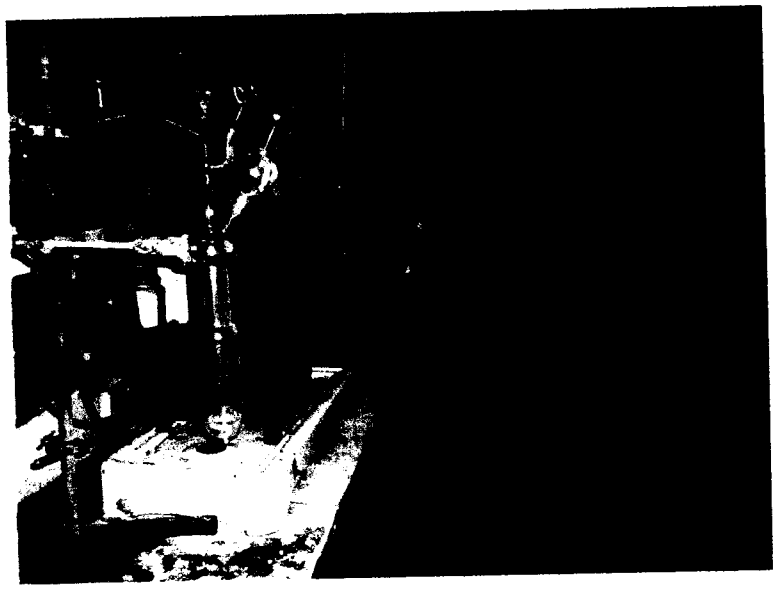


Plate II: Soxhlet Apparatus

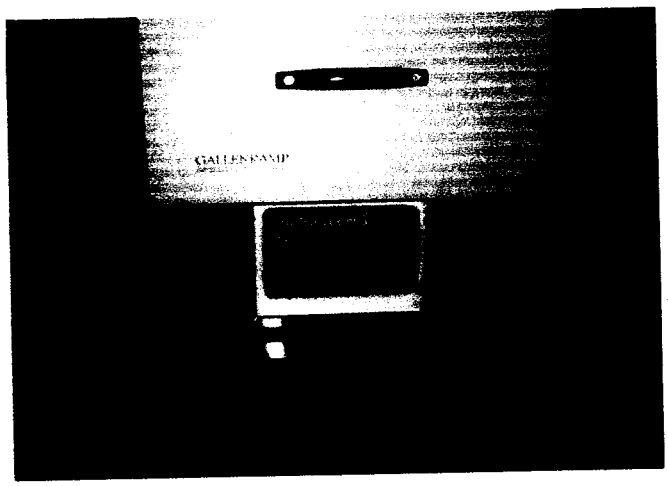


Plate VI: Muffle Furnace

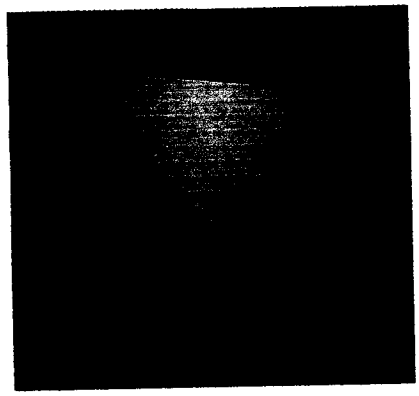


Plate V: Electronic Air Oven

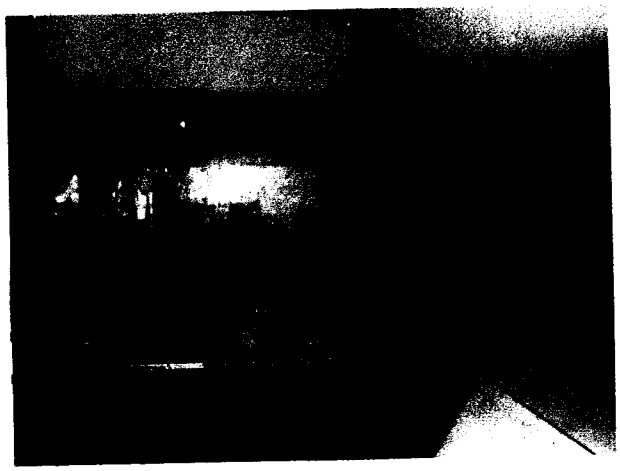


Plate III: Kjeldahl Digestion Block.

Plate IV: Refractometer

.3 Reagents Used for the Proximate Compositions of Honey

The following reagents were used in the proximate compositions of the samples of honey.

Distilled water

Potassium hydroxide (KOH) solution

Tetraoxosulphate (VI) acid (H_2SO_4)

Boric acid (H_3BO_3)

Oxalic Acid ($(COOH)_2$)

Saturated potassium Iodide solution (KI)

Sodium Hydroxide (NaOH) solution

Copper Sulphate ($CuSO_4$)

Phenolphthalein indicator

Hydrochloric acid (HCl)

Ammonium Sulphate $(NH_4)_2SO_4$

Aqueous potassium Iodide solution, (KI)

Sodium Sulphate (Na_2SO_4)

Sulphuric Acid (H_2SO_4)

Acetic Acid (CH_3COOH)

3.2 Determination of Physical Properties of Honey

The physical properties of Honey were carried out at National Cereals Research Institute, Badeggi – Bida, Niger state, Nigeria. The followig physical properties of honey were determined using the methods of AOAC (2004) Nutritional guidelines.

1. Specific Gravity
2. Viscosity
3. Refractive Index
4. pH
5. Colour
6. Taste
7. Flavour

3.2.1 Determination of Specific Gravity

Specific gravity is the ratio of the density of a substance to the density of a saturated substance under specified conditions. For liquids and solids the standard is usually water at 4°C or some other specified temperature. For gases the standard is often air or hydrogen at the same temperature and pressure as the substance. A pycnometer bottle was used to determine the density of the honey. A clean and dry pycnometer bottle of 50 ml capacity was weighed (w_0) and then filled with the honey; a stopper was inserted and reweighed to give (w_1). The honey was substituted with water after washing and drying the bottle and weighed to give (W_2). The expression for specific gravity (Sp.gr) is:

$$\text{Specific Gravity} = (W_1 - W_2) / (W_2 - W_0)$$

$$= \text{Mass of the substance / Mass of equal volume of water.}$$

3.2.2 Determination of Viscosity

The viscosity of honey is an indication of the degree of flow (fluidity) at different temperature. A clean, dried viscometer with a flow time above 200 seconds for the fluid to be tested was elected. The sample was filtered through a sintered glass (fine mesh screen) to eliminate dust and other solid material in the liquid sample. The viscosity meter was charged with the sample by inverting the tube's thinner arm into the liquid sample and suction force was drawn up to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometer was placed into a holder and inserted to a constant temperature bath set at 29°C and allow approximately 10minutes for the sample to come to the bath temperature 29°C. The suction force was then applied to the thinner arm to draw the sample slightly above the upper timing mark. The afflux time by timing the flow of the sample as it flow freely from the upper timing mark to the lower timing mark was recorded.

3.2.3 Determination of Refractive Index

Refractive index is the ratio of the speed of light at a definite wave length in a vacuum to ts speed in the medium and this varies with the wave length of light and temperature. Refractometer was used in this determination. Few drops of the sample were transferred into the glass slide of the refractometer. Water at 20°C was circulated round the glass slide to keep its emperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was djusted to be in line with intersection of the cross. At no parallax error, the pointer on the scale ointed to the refractive index. This was repeated and the mean value noted and recorded as the efractive index.

3.2.4 Determination of pH value

About 2g of the sample was poured into a clean dry 25 ml beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. It was then cooled in a cold water bath to 25°C. The pH electrode was standardized with buffer solution and the electrode immersed into the sample and the pH value was read and recorded.

The physical properties of honey would be presented in a pro-forma as shown in Table 3.1

Table 3.1 Physical Properties of Honey

Properties	Value
Specific Gravity	
Viscosity	
Refractive Index	
Ph	
Colour	
Taste	
Flavour	

3.3 Determination of the Proximate Compositions of Honey

The proximate composition analysis was carried out at National Cereals Research Institute, Badeggi – Bida, Niger State, Nigeria. The proximate compositions of honey were determined using the methods of AOAC (2004) and they include:

1. Moisture Content
2. Crude protein
3. Ash Content
4. Carbohydrate
5. Vitamin C
6. Reducing Sugar
7. Glucose
8. Maltose
9. Fructose
10. Free Fatty Acid

3.3.1 Determination of Percentage Moisture Content

Moisture content was determined by the direct air oven method of Association of Analytically Chemist (AOAC, 2004). A clean dry flat bottom silica dish was weighed and 2g of the sample pipetted into it. The sample was evaporated on a water bath and with the aid of forceps: it was then transferred into the air oven previously set at 105°C. After three hours, it was then removed from the oven, cooled in desiccators and reweighed again. The process was repeated until constant weight was obtained.

$$\text{Moisture content} = \frac{(A - B) \times 100}{A}$$

3.3.2 Determination of percentage Crude protein

Amino acids are the building blocks of protein. Proteins are therefore polymers of amino acids, most of which are α-amino acids having the general formulae NH_2CHR_2COOH. It is the only macronutrient in food that contains nitrogen. Crude protein was determined by the kjeldahl method (AOAC, 2004). The process involved three stages; digestion, distillation and titration.

Digestion: About 2g of the sample formulated was measured into a 50 ml kjeldahl flask, digestion tablets were then added to the flask followed by 20 ml of concentrated sulphuric acid (H_2SO_4) which was poured slowly down the side of the flask while holding the flask in a slant position. The flask was then placed on a digestion block in a fume cupboard at about 300°C until a clear solution was obtained. The flask was then made up to 100 ml with distilled water after cooling at room temperature.

Distillation: About 5 ml of the diluted digest of each sample was transferred into a separate Kjeldahl flask and 20 ml of 40% sodium Hydroxide (NaOH) was added and rinsed with few drops of distilled water, the flask was then placed at the heating end of the distillation unit. 5 ml of 4% boric acid solution was placed in 100 ml Erlenmeyer flask and 2 – 3 drops of mixed indicator was added (Bromo cresol green + methyl red indicator 5 : v/v ratio) before placing from the receiving end of the distilling unit. Ammonia was distilled into Boric acid solution until 75 ml mark was reached. The boric acid distillate was then treated with 0.1N hydrochloric (HCl) to a pink end point, which persist for about 15minutes. Percentage protein was calculated by multiplying the percentage of nitrogen with suitable factor (6.25)

%Nitrogen = A/B x 0.1 x 1/E

Where: A = Volume of acid used to neutralize the distillate

B = Volume of sample taken for distillation

C = Volume made after distillation (100ml)

D = Volume of sample

E = Acid factor

Crude Protein (%) = Nitrogen x factor (6.25)

3.3.3 Determination of percentage Ash content

Ash in food constitutes the residue remaining after all the moisture have been removed as well as the organic materials (fats, proteins, carbohydrates, vitamins, organic matter, etc.) have been burnt away by igniting at a temperature of around 500°C (Ihekoronye *et al.*,1985). Ash was determined by the method of AOAC (2004). Two grammes of sample was weighed and placed in a water bath to be evaporated. It was then placed in a separate porcelain crucible (England) and its content placed in a Gallenkamp furnace and ashed to 105°C for about 10 hours. The sample was then removed from the furnace, cooled in desiccators and weighed. The percentage ash was calculated as:

$$\text{Ash (\%)} = \frac{\text{weight of crucible + ash} - \text{weight of crucible}}{\text{Initial sample}}$$

3.3.4 Determination of Carbohydrate

Carbohydrate sample of 0.1g was dissolved in 2 ml of water. The dilute solution was poured into a test tube. Two drops of molisch's reagent was added and it was then shaked, 2 ml of conc. H_2SO_4 was carefully poured into the solution in the test tube and was allowed to stand for 2 minutes. A red violet purple ring develops at the interface of the two solutions.

By Difference: In this method, carbohydrate content is obtained by the calculation having estimated all the other fractions by proximate composition analysis i.e.

% Available carbohydrates = 100 – (% Moisture + % Ash + % Protein + % Fibre)

3.3.5 Determination of Vitamin C Content

The method which is used here is the filtration of method of Vogel, (1978). Two grammes of the sample was weighed. About 100 ml of distilled water was added to the weighed sample in a volumetric flask. It was then filtered to get a clear solution. 50 ml of the unconcentrated sample was pipette into 100 ml volumetric flask in triplicate. 25 ml of 20% metaphosphoric (0.5% oxalic acid) is added as a stabilizing agent and diluted to 100 ml volume. 10 ml of it was then pipette into small flasks in which 2.5 ml acetone was then added. This was then titrated with indophenols solution (2, 6-dichlorophenolindophenol) to a faint pink colour which persists for 15 seconds. The formula for a calculation of milligramme (mg) of vitamin C is

Vitamin C = mg/100ml juice = 20 (v) (c) where

V = ml indophenol solution in titration

C = Mg vitamin C/ml indophenols

3.3.6 Determination of Reducing Sugar

About 7.5g 0f the sample was homogenized with 200 ml distilled water and washed quantitatively into a 500 ml volumetric flask with distilled water. This was now made up to the mark and then filter. With few exceptions (such as for soluble in preserves) it was necessary to clear solution of sugars before they are estimated using zinc ferrocyanide.

3.3.7 Determination of Maltose

Five grammes of honey and a teaspoonful of ignited quartz sand were introduced into a 100 ml flask and mixed well by rotating the flask. 46 ml of buffer solution was then added to the mixture and the entire mixture was then mixed by a rotating flask until all the honey was thoroughly in suspension. The honey sand mixture and the buffer solution were then separately and individually brought to 30°C before the two were mixed. This was now digested for 1hr at

30°C followed by shaking the flask by rotation every 15mins. At the end of the 1hr, 2 ml was added to 10% sulphuric acid solution and it was mixed thoroughly. 2 ml of sodium tungstate solution was then added and mixed and allowed to stand for 2mins. This was now filtered through a fluted No. 4 whatman paper, discarding the first 8 or 10 drops and pippetting 5ml of filtered extract into a test tube of approximately 50 ml capacity. By pipette, exactly 10 ml of alkaline ferrocyanide was added to the 5 ml extract in the test tube and the test tube was immersed in vigorously boiling water bath. The test tube was allowed to remain in the boiling water for 20 mins and cooled under running water and poured at once into a 100 ml flask. It was now rinsed out with 25 ml of acetic acid and added to the content of the flask, mixing thoroughly. 1ml of potassium iodide solution followed by 2 ml soluble starch solution was added and mixed. This was now titrated with 0.05M of sodium thiosulphate to the complete disappearance of the blue colour using 10 ml burette.

3.3.8 Determination of Free Fatty Acid

The free fatty acid of the honey is the number of milligram of potassium hydroxide required to neutralize 1gramme of the sample (Ewing, 1971). The acid value measures the extent to which the glycerides in the oil have been decomposed by lipase action. 25 ml of diethyl ether and 25 ml of alcohol was mixed with 1ml of phenolphthalein and this was carefully neutralized with 0.1M sodium hydroxide. Two gramme of the oil was dissolved in the mixed neutral solvent and this was titrated with aqueous 0.1M sodium hydroxide shaking constantly until a pink colour which persists for 15seconds was obtained.

$$\text{Acid value} = \frac{\text{titre (ml)} \times 5.61}{\text{Weight of sample used}}$$

The proximate composition of honey would be presented in a pro-forma as shown in Table 3.2

Table 3.2: Proximate Compositions of Honey

Properties	**1**	**2**	**3**	**Mean**	**Standard Deviation**
	X_1	X_2	X_3	$\bar{X}$	S.D
Moisture Content					
Crude Protein					
Ash Content					
Carbohydrate					
Vitamin C					
Reducing Sugar					
Glucose					
Maltose					
Fructose					
Free Fatty Acid					

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Physical Characteristics of Honey

The physical characteristics of honey are presented in Table 4.1.

Table 4.1: Physical Characteristics of Honey

Parameters	Bida	Minna	Suleja	Kontagora	Obollo-Afor	Standard range*
pH	$4.62^{b}\pm0.03$	$4.45^{c}\pm0.03$	$4.45^{c}\pm0.10$	$4.79^{a}\pm0.05$	$4.55^{bc}\pm0.01$	3.2 – 4.5
V 20°C	43.00±0.00	65.00±0.00	54.00±0.00	62.00±0.00	58.00±0.00	40 – 60
S G	$1.27^{b}\pm0.03$	$1.26^{b}\pm0.01$	$1.27^{b}\pm0.02$	$2.32^{a}\pm0.04$	$0.84^{c}\pm0.00$	1.40 – 1.45
R I	$1.47^{b}\pm0.02$	$1.43^{c}\pm0.01$	$1.48^{b}\pm0.01$	$1.52^{a}\pm0.02$	$1.51^{a}\pm0.00$	1.4 – 1.5

Table 1 show that the physical properties of honey from the five locations are significantly differences. ($p \leq 0.05$)

Key: S.G = Specific Gravity, R.I = Refractive Index, V=Viscosity

*Source: (Codex Alimentarius, 2001)

4.1.2 Means of Sensory Evaluation of Five Samples by 16 Respondents

The sensory evaluation of the physical characteristics of honey are represented in Table 4.2

Table 4.2: Sensory Evaluation of Honey

Parameter	Mean ± SD				
	Bida	**Minna**	**K/gora**	**Suleja**	**Obollo-Afor**
Colour	$6.13^{a} \pm 1.09$	$5.38^{bc} \pm 0.62$	$4.81^{c} \pm 0.75$	$4.81^{c} \pm 0.66$	$5.88^{ab} \pm 0.72$
Flavour	$5.19^{a} \pm 1.17$	$5.50^{a} \pm 1.21$	$5.19^{a} \pm 1.28$	$5.38^{a} \pm 1.09$	$5.44^{a} \pm 1.21$
Taste	$4.94^{a} \pm 1.53$	$5.69^{a} \pm 1.08$	$4.9^{4a} \pm 1.61$	$4.63^{a} \pm 1.31$	$5.13^{a} \pm 0.81$
Mouth feel	$5.38^{ab} \pm 1.15$	$5.63^{a} \pm 1.15$	$4.63^{bc} \pm 1.36$	$4.06^{c} \pm 1.34$	$5.00^{ab} \pm 1.16$
Consistency	$5.13^{a} \pm 0.89$	$5.25^{a} \pm 1.07$	$4.63^{a} \pm 1.31$	$4.56^{a} \pm 1.41$	$5.06^{a} \pm 1.18$
Overall acceptability	$5.55^{a} \pm 1.04$	$5.64^{a} \pm 0.81$	$5.09^{ab} \pm 0.83$	$4.27^{b} \pm 1.62$	$5.09^{ab} \pm 1.30$

Means with different superscript are significantly different from each other ($P \leq 0.05$) while those with the same superscript are not significantly different from each other.

Key: S.D = Standard Deviation, K/gora = Kotangora

4.1.3 Proximate Analysis of Honey

The proximate compositions of honey are shown in Table 4.3

Table 4.3: Proximate Composition of Honey

Parameter	Bida	Minna	Suleja	Kontagora	Obollo-Afor	Standard range*
MC (%)	$8.83^{b}\pm0.76$	$15.00^{a}\pm 5.00$	$16.83^{a}\pm0.58$	$9.50^{b}\pm1.00$	$7.79^{b}\pm0.26$	20.00
CP (%)	$4.13^{a}\pm0.12$	$2.32^{b}\pm0.21$	$0.36^{d}\pm0.06$	$1.14^{c}\pm0.19$	$1.06^{c}\pm0.01$	0.30
CHO (%)	$69.58^{b}\pm1.51$	$57.18^{d}\pm1.66$	$64.20^{c}\pm0.67$	$53.07^{e}\pm1.76$	$87.05^{a}\pm0.17$	82.40
Ash (%)	$1.33^{d}\pm0.29$	$2.33^{c}\pm0.29$	$5.83^{b}\pm0.76$	$8.67^{a}\pm0.58$	$1.24^{d}\pm0.24$	0.6 – 1.2
R S(%)	$13.78^{c}\pm1.45$	$23.51^{b}\pm1.48$	$20.48^{b}\pm0.73$	$2.95^{c}\pm3.51$	$9.14^{d}\pm0.03$	4.5 – 6.5
FFA	$1.66^{d}\pm0.02$	$6.03^{a}\pm0.03$	$4.51^{b}\pm0.08$	$2.65^{c}\pm0.04$	$0.25^{e}\pm0.00$	5.00
Vitamin C	$21.41^{a}\pm0.21$	$10.50^{b}\pm0.12$	$12.40^{b}\pm0.16$	$20.76^{a}\pm0.09$	$4.03^{c}\pm3.46$	2.22-4mg
Maltose(%)	$16.51^{c}\pm0.33$	$19.45^{a}\pm0.37$	$15.51^{d}\pm0.52$	$18.56^{b}\pm0.28$	$20.00^{a}\pm0.50$	32.00
F (%)	$0.012^{d}\pm0.001$	$0.015^{cd}\pm0.001$	$0.017^{bc}\pm0.002$	$0.021^{a}\pm0.001$	$0.018^{ab}\pm0.003$	32.00
G (%)	$0.019^{b}\pm0.001$	$0.022^{a}\pm0.001$	$0.017^{b}\pm0.001$	$0.021^{a}\pm0.001$	$0.015^{c}\pm0.001$	38.00

Values are Mean ± standard deviation

Values on the row with different superscript are significantly different from each other ($p\leq0.05$) while those with the same superscript are not ($p>0.05$)

Key: MC = Moisture Content, CP = Crude Protein, F= Frutose, G=Glucose, RS = Reducing Sugar, CHO = Carbohydrate, EC = Electrical Conductivity, FFA = Free Fatty Acid

*Source: (Codex Alimentarius, 2001)

4.2 Discussion of Results

Table 4.1 shows the physical characteristics of honey obtained from Bida, Minna, Suleja, Kontagora and Obollo-Afor. The pH of honey from Kontagora was significantly higher than those from Bida, Obollo-Afor, Minna and Suleja. Bida's was not significantly different from Obollo-Afor's but significantly higher than Minna's and Suleja's, which were not significantly different from each other and from Obollo-Afor's. The pH of Honey from Minna and Suleja were within the Range of the standard pH values but those of Obollo-Afor, Bida and Kontagora were slightly above that of the standard which implies that they will have higher mineral content. This is in line with the assertion that honeys of a higher pH (alkaline value) has been found to have higher mineral content (Echigo and Takenaka, 1974).

The specific gravity of the honey from Kontagora was significantly higher than those from Bida, Suleja, Minna and Obollo-Afor. Bida, Suleja and Minna were not significantly different from each other but were significantly higher than that from Obollo-Afor, with the lowest specific gravity. The specific gravity of honey from Bida, Minna, and Suleja were within the range of the standard value, that of Obollo-Afor was below and Kontagora was above the standard values. This shows that honeys from Bida, Minna, and Suleja are thicker, thus better for consumption.

The refractive index of the honey obtained from kontagora and Obollo-Afor were not significantly different from each other but they were significantly higher than those obtained from Suleja, Bida, and Minna respectively. Those obtained from Suleja and Bida were not significantly different from each other, but were significantly higher than that obtained from Minna, which had the lowest refractive index. The refractive index of all the samples falls within the range of the standards.

The viscosity at 20°C of the five samples decreased in the order: Minna > Kontagora > Obollo-Afor > Suleja > Bida. The viscosity of honey from Minna was higher than those from Kontagora, Obollo-Afor, Suleja and Bida. That of Kontagora was higher than those of Obollo-Afor, Suleja, and Bida. Obollo-Afor was higher than Suleja and Bida and Suleja was higher than Bida. The viscosity of those from Bida, Suleja, and Obollo-Afor were within the standard range while those of Minna and Kontagora were above the standard range. Similar to that of specific gravity, higher viscosity implies higher specific gravity and hence are thicker. As a result the honey from Bida, Suleja, and Obollo-Afor are within the limit of thickness and more preferred for consumption.

Table 4.2 shows the mean sensory evaluation scores of 16 respondents for the five (5) samples of honey tested.

Based on the means obtained from the scores of the 16 respondents, the flavour, taste and consistency of the five (5) samples of honey were not significantly different from each other (P> 0.5). This showed that the flavour, taste and consistency of the samples were equally preferred. The approximate score for flavour falls between 5 and 6 (liked slightly and liked moderately) while the score of consistency was approximately 5 (like slightly). The taste, flavour and consistency of all the samples were liked. There was significant difference in the mean score of colour between the samples ($P < 0.05$). The colour score of samples from Bida and Obollo-Afor were not significantly different from each other and they had approximate score of 6 (like moderately) but Bida sample was more preferred to sample Minna, Kotangora and Suleja with approximate scores of 5 (like slightly). The colour of Obollo-Afor sample was preferred as sample and that of sample Minna, as samples Kotangora and Suleja. The colour of all the samples was liked. The mouthfeel of the five samples showed some level of significant

difference (P<0.05). The Mouth feel of samples from Minna, Bida and Obollo-Afor were not significantly different from each other. They had an approximate score of 5 (like slightly) but that of sample from Minna was preferred significantly more than samples from Kotangora and Suleja. The mouthfeel of samples from Bida and Obollo-Afor was preferred like sample from Kotangora, which also has an approximate score of 5 (like slightly) but were more preferred significantly to sample from Suleja, which had an approximate score of 4 (Neither like' nor dislike).

The Overall acceptability of the five samples showed some level of significance at 5% confidence limit as revealed by Duncan Multiple Range Test (DMRT). The acceptability of samples from Bida, Minna, Kotangora and Obollo-Afor were not significantly different from each other but the acceptability of samples from Bida and Minna with approximate score of 6 (like moderately) were preferred significantly more to that of sample Suleja, with an approximate score of 4 (neither like nor dislike). Although sample Suleja was preferred as samples Kotangora and Obollo-Afor with approximate scores of 5 (like slightly) since there was no significant differences between their scores.

Table 4.3 shows the proximate compositions of honey collected at different locations in two states in Nigeria and the standard range (*Codex Alimentarius* Honey Standard).

A one way ANOVA revealed that there were significant differences in the mean proximate compositions of the honey from the five (5) locations considered ($p \leq 0.05$).

The moisture content of honey from Suleja and Minnna were not significantly different from each other but were significantly higher than those from Kontagora, Bida and Obollo-Afor, which were not significantly different from each other. The moisture content of honey from all the locations fall below that of the standard and thus could preserve longer as moisture could

mean high water activity that could encourage growth of microorganism and bait some chemical reaction that could cause spoilage.

The protein content of the honey from Bida was significantly higher than those from Minna, Kontagora, Suleja, and Obollo-Afor. That from Minna was significantly higher than those from Kontagora, Suleja and Obollo-Afor. Kontagora was significantly higher than Suleja and Obollo-Afor and Suleja was not significantly different from that from Obollo-Afor, having the lowest protein content. The protein content of the honey from those five locations were higher than that of the standard which shows that they contain more nutrient in terms of the protein content.

The carbohydrate content of the honey from Obollo-Afor was significantly higher than those from Bida, Suleja, Minna, and Kontagora. That of Bida was significantly higher than those of Suleja, Minna, and Kontagora; Suleja was significantly higher than Minna and Kontagora and Minna was significantly higher than kontagora. The Carbohydrate content of Honey from Bida, Suleja, Minna, and Kontagora were below that of the standard value but that of Obollo-Afor was above the standard. Since they contain reasonably high carbohydrate, this shows that they can supply energy for biological activities.

The Honey from kontagora had significantly higher ash content than those from Suleja, Minna, Bida, and Obollo-Afor. That of Suleja was significantly higher than those of Minna, Bida and Obollo-Afor, which were not significantly different from each other compared with the standard. The ash content of honey from the five locations was above standard value. This shows that they have higher approximate inorganic content or mineral content than that of the standard.

The reducing sugar of honey from Kontagora was significantly higher than those from Minna, Suleja, Bida, and Obollo-Afor. Those from Minna and Suleja were not significantly

different from each other but were significantly higher than those from Bida and Obollo-Afor. The reducing sugar in the honey from the five locations was far above that of the standard except that from Obollo-Afor that was close but higher.

The free fatty acid of honey from Minna was significantly higher than those from Suleja, Kontagora, Bida, and Obollo-Afor. That from Suleja was significantly higher than those from Kontagora, Bida, and Obollo-Afor. Honey from Kontagora had significantly higher free fatty acid than those from Bida and Obbollo-Afor and that from Bida was significantly higher than that from Obollo-Afor, with the lowest amount of free Fatty acid. The free Fatty acid of the honey from Suleja, Kontagora, Bida and Obbolo-Afor were lower than the standard value of honey but that of Minna was higher than the standard value. Generally free fatty acid is an index of level of cholesterol in food, which implies that lower FFA values in honey are more desirable medically.

Honey from Bida and Kontagora had Vitamin C value that were not significantly different from each other but were significantly higher than those of Suleja, Minna, and Obollo-Afor. Those of Suleja and Minna were not significantly different from Obollo-Afor. One gramme of the honey from the five locations contains Vitamin C higher than that of the Standard value range.

The maltose concentration of honey from Obollo-Afor and Minna were not significantly different from each other but were significantly higher than those from Kontagora, Bida and Suleja Respectively. That from Kontagora was significantly higher than those from Bida and Suleja, while that from Suleja, has the lowest maltose concentration. The values of maltose concentration in the honey obtained from the five locations were within the standard value. Since

they contain reasonable amount of reducing sugar, this contributes to all the sweetening of honey.

The fructose concentration of the honey from kontagora and Obollo-Afor were not significantly different from each other but that from Kontagora was significantly higher than those from Suleja, Minna, and Bida. Those from Obollo-Afor and Suleja were not significantly different from each other but significantly higher than that from Bida. The honey from Bida and that from Minna were not significantly different in their fructose concentration. The fructose concentrations of honey from the five locations were within the standard value.

The glucose concentration of honey from Minna and kontagora were not significantly different from each other but were significantly higher than those from Bida, Suleja, and Obollo-Afor respectively. Bida and Suleja were not significantly different from each other but were significantly higher than Obollo-Afor, with the lowest glucose concentration. These concentrations were within the standard value. Frutose and glucose are reducing sugar that contributes to the sweeten of honey, so values indicates there acceptability for consumption.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The following conclusions were made after a thorough analysis of the honey samples obtained from different sources: Bida, Minna, Suleja, Kontagora and Obollo-Afor.

(i) The honey from Bida and Obollo-Afor has high quality characteristics. For instance, the mean free fatty acid value of (1.66 mgKOH/g) and (0.25 mgKOH/g) for the Bida and Obollo-Afor samples respectively, which are the least are good index of the low value of cholesterol in the two honey samples. The pH for all the samples were within the acidity range as indicated by Souza *et al.,* (2006) which indicates that they have good sweetening ability. However, the pH and free fatty acids values of the samples from Minna and Suleja are among the highest which indicates the high probability of the honey becoming acidic.

(ii) From the proximate analysis, crude protein levels of 4.13%, carbohydrate of the honey from Bida are among the highest values. These are essential nutrients that the human body needs for the growth of difference cells. The samples from Kontagora have lowest crude protein levels and carbohydrate values of 1.14% and 53.07% respectively.

(iii) The low electrical conductivity and ash contents of the samples from Bida and Obollo-Afor indicate their high degree of purity compared to others from Kontagora and Suleja.

(iv) The overall acceptability of the honey from Bida covering colour, flavour, taste, mouth feel and consistency had the highest acceptability while those of Kontagora and Suleja had the lowest overall acceptability ratings.

From these results, it was concluded that the honey from Bida has the best quality attributes followed by that from Obollo-Afor, while the ones from Kontagora and Suleja have the least quality attributes. As a result, honey from Bida and Obollo-Afor are recommended for consumption while those of Kontagora and Obollo-Afor are recommended for further examination and probably to be refined further before consumption.

5.2 Recommendations

The following recommendations have been made from this study.

1. Honey should be purchased only from NAFDAC recognised sales outlet. This is because most of the honeys sold in the market are adulterated honey.
2. The public needs to be sensitized on the importance of honey. This is because honey has many medicinal uses mostly in the treatment of diabetic and ulcer. It can also serve as a sweetener in various beverage drinks.
3. There is a need for the setting up of "Honey Regulatory Board" so that the production of impure and adulterated honey can be checked and properly handled with consequent closure of companies that are engaged in the production of honey.
4. More research should be carried out to determine more properties and uses of honey.

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APPENDICES

Determination of Specific Gravity

$$\text{Specific Gravity} = \frac{\text{Weight of Honey}}{\text{Weight of Water}}$$

Weight of empty pycnometer bottle = 25.10g

Volume of pycnometer bottle = 50ml

Determination of Acid Value and Free Fatty Acid

$$\text{Acid Value} = \frac{\text{Titre Value (ml)} \times 5.61}{\text{Weight of sample used}}$$

Weight of Sample used = 2g

Acid Value = FFA x 2

$$\text{FFA} = \frac{\text{Acid Value}}{2}$$

Determination of Moisture Content of the Honey

$$\% \text{ Moisture content} = \frac{\text{Weight of moisture in the honey} \times 100}{\text{Weight of honey before oven drying}}$$

Weight of empty petri dish = 41.81g

Determination of Crude Protein

Crude Protein (%) = Nitrogen x factor (6.25)

%Nitrogen = A/B x 0.1 x 1/E

Where: A = Volume of acid used to neutralize the distillate

B = Volume of sample taken for distillation

C = Volume made after distillation (100ml)

D = Volume of sample

E = Acid factor

Determination of Percentage Ash Content

$$\text{Ash (\%)} = \frac{\text{weight of crucible + ash} - \text{weight of crucible}}{\text{Initial sample}}$$

Determination of Carbohydrate

% Available carbohydrates = 100 – (% Moisture + % Ash + % Protein + % Fibre)

Determination Vitamin C Content

Vitamin C = mg/100ml juice = 20 (v) (c) where

V = ml indophenol solution in titration

C = Mg vitamin C/ml indophenols

CENTRAL SERVICES LABORATORY

NATIONAL CEREALS RESEARCH INSTITUTE
BADEGGI, P.M.B. 8, BIDA, NIGER STATE

CHEMISTRY LABORATORY RESULT OF ANALYSIS ON (HONEY)

Sample description	Moisture Content %	Crude protein %	Maltose %	Fructose %	CHO %	Glucose %	Ph	Reducing Sugar %	Free Fatty Acid %	Vit. C mg/g	% Ash Content	Viscosity At 20°C	Specific Gravity	Refractive Index
O.Afor 1	7.93	1.05	20.5	0.02	87.22	0.016	4.54	9.15	0.25	6.034	1.00	58°C	0.840	1.5094
O. Afor 2	7.94	1.06	19.5	0.02	86.88	0.014	4.55	9.10	0.25	0.032	1.47	58°C	0.841	1.5094
O. Afor 3	7.485	1.055	20.0	0.015	87.04	0.0145	4.545	9.155	0.25	6.033	1.235	58°C	0.840	1.5094
BID 1	9.5	4.00	16.87	0.011	68.10	0.018	4.60	15.10	1.68	21.60	1.50	43°c	1.29	1.50
BID 2	9.0	4.22	16.42	0.013	69.54	0.020	4.65	14.00	1.66	21.44	1.50	43°c	1.23	1.46
BID 3	8.0	4.17	16.23	0.011	71.11	0.018	4.62	12.23	1.64	21.18	1.00	43°c	1.28	1.46
MIN 1	20.0	2.32	19.68	0.016	55.28	0.023	4.43	25.10	6.06	10.48	2.50	65°c	1.27	1.42
MIN 2	10.0	2.11	19.02	0.014	57.95	0.021	4.44	23.27	6.00	10.63	2.00	65°c	1.25	1.44
MIN 3	15.0	2.53	19.64	0.014	58.32	0.022	4.48	22.17	6.04	10.40	2.50	65°c	1.27	1.42
SUL 1	16.5	0.31	15.23	0.015	64.97	0.016	4.34	20.00	4.56	12.22	6.00	54°c	1.26	1.48
SUL 2	17.5	0.42	16.11	0.017	63.73	0.018	4.52	20.13	4.42	12.53	6.50	54°c	1.27	1.48
SUL 3	16.5	0.35	15.18	0.018	63.89	0.017	4.50	21.32	4.54	12.45	5.00	54°c	1.29	1.47
KONT 1	8.50	1.13	18.75	0.021	51.21	0.021	4.75	30.15	2.68	20.66	9.00	62°c	2.33	1.52
KONT 2	9.50	0.95	18.24	0.020	54.72	0.020	4.85	27.50	2.60	20,82	8.00	62°c	2.28	1.50
KONT 3	10.50	1.33	18.68	0.022	53.28	0.022	4.78	28.20	2.66	20.80	9.00	62°c	2.35	1.53

Key: O.AFOR = Obollo – Afor, BID = Bida, MIN = Minna, SUL = Suleja, KONT = Kontagora

Sample analyzed by: Abdul B. Kudu

Head of Central Services Lab: .....

OFFICE OF THE HEAD
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National Cereals Research Institute
Badeggi, Niger State
Sign ..... Date .....