UNIVERSITY OF RWANDA

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DEPARTMENT OF CHEMISTRY

OPTION OF BIO-ORGANIC CHEMISTRY

CONTRIBUTION TO PHYSICO-CHEMICAL AND BACTERIOLOGICAL ANALYSIS OF HONEY PRODUCED BY MIG AND ABEILLE LTD.

A dissertation submitted in partial fulfillment of academic requirements for the award of Bachelor's degree in chemistry.

Option: Bio-organic chemistry

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Huye, May, 2016

DECLARATION

We, Jean Aimé Méthode NDAGIJIMANA and Florence KANTENGWA, students in University of Rwanda, College of Sciences, department of Chemistry, Option of Bio-organic Chemistry, hereby declare that this dissertation entitled "CONTRIBUTION TO PHYSICO-CHEMICAL AND BACTERIOLOGICAL ANALYSIS OF HONEY PRODUCED BY MIG AND ABEILLE LTD" is our original work and has never been submitted elsewhere for the award of any academic degree.

Jean Aimé Méthode NDAGIJIMANA

Signature.....

Date.....

Florence KANTENGWA

Signature.....

Date.....

DEDICATION

We dedicate this book to our parents who have been unceasingly helping and compassionate to us since our existence,

To our beloved brothers and sisters who supported us a lot in our studies,

To our beloved grandmother who has been trying her best to care about us,

To our classmate who have been supported us in our studies.

This work is dedicated to all fellow friends.

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LIST OF ABBREVIATIONS AND ACRONYMS

MIG: Multisector Investment Group.

HMF : Hydromethylfurfural.

LADAMET : Laboratoire d'Analyse de Denrées Alimentaire Médicament.

%: Percentage.

FDA: Food and Drugs Administration.

pH: Hydrogen Potential.

µm: Micrometer.

WHO: World Health Organisation.

ABSTRACT

The study was conducted to determine the physicochemical and bacteriological analysis of honey produced by MIG ltd and Abeille ltd from NYAMAGABE and HUYE District respectively.

Honey is a natural sweet substance produced by honey bees, from the nectars of plant flowers and honey dew. Honey has an antimicrobial activity which is linked to hydrogen peroxide which is produced by glucose oxidize especially when honey is diluted.

During this studies, we have done the analysis of honey samples with the aid of pH meter for determination of pH, Abbe refractometer for determination of refractive index, Titration method for determination of total acidity, Luff-schoorl method for determination of reducing sugar and sucrose, Indirect method for determination of moisture content, Gravimetric method for determination of ash content, Indirect method for determination of electrical conductivity and culture media method for identification of total coliforms, E.coli, Salmonella, Staphylococcus aureus and Faecal coliforms bacteria.

The results obtained show that the analyzed parameter has interdependence. The ash content has linear relationship with electrical conductivity, moisture content and refractive index has linear relationship. Both acidity and moisture content have the effect on the absence and growth of microorganism. Both honey produced by MIG ltd and Abeille ltd are of good quality because they meet the range of standard and rich in energetic substances.

Key words: Physico-chemical parameters, Moisture content, Luff-schoorl method, Honey, Bacteriological parameters.

CHAPTER I. GENERAL INTRODUCTION

I.1 INTRODUCTION

Honey is heterogeneous mixture of proteins, flower nectar sugars and glandular secretions produced by honey bees [1]. Honey bees convert the thin watery nectar they collect from flowers into honey by evaporating some of the moisture and adding an enzyme that breaks down the sugar into laevulose and dextrose [2]. Honey is rich source of natural sugar like fructose and glucose along with essential vitamins, minerals and antioxidants contents including ascorbic acid, flavonoids, phenolic acids, Amino and proteins. Generally, the darker the honey is, the higher its phenolic content and its antioxidant power is contained [3]. Honey is natural sweetener and boasts its wonderful health benefits [4].

Honey has been used as natural remedies for centuries to treat several ailments. It has been all time favorite of nutritionist and dieticians. Honey has antibiotic properties and provides an effective dressing for wounds and burns. Honey is being used as an ingredient in cosmetics and toiletries [2]. Honey has an Antimicrobial activity which is linked to hydrogen peroxide which is produced by glucose oxidase especially when honey is diluted. In addition, hydrogen peroxide has antibacterial activities and at the same time it is not tissue damaging. In the diluted form of honey, produced hydrogen peroxide is an important stimulant of the growth of tissues and has the potential for wound healing. The hydrogen peroxide of most of the honeys can be destroyed by heat or by the presence of catalase [5].

Although honey has an anti-microbial properties that discourage the growth of many microorganisms typically, honey can be expected to contain low number and a limited variety of microbes. It can hold microorganisms present in pollen, dust, air, soil and nectar which are very difficult sources to control. However, microbial contamination can also be originated from food handlers, equipment and cross contamination which can be easily controlled by standard sanitation and good manufacturing practices during harvest and honey processing [6].

The organic honey is free from artificial substances, such as coloring agents and preservatives, or, contamination from chemical sprays. Honey may become contaminated with pesticides if

bees visit flower that have been sprayed and it is not safe to feed to infants because it can contain spores of the bacterium clostridium botulinum.

These spores can become the bacteria that cause fatal foodborne illness especially in children under 1year old [2, 7]. In Rwanda, little is known about the safety of a particular type of honey. Prior to bacteriological analysis, the physico-chemical properties (such as pH, color, proteins, moisture content, total sugar content and vitamins) of honey sample will be analyzed to assess the quality of these honey samples.

I.2. PROJECT DESCRIPTION

A. Problem statement

The greatest health risk from food today is contamination by viruses, bacteria and to a lesser extent by various forms of fungi and parasite. These microorganisms are able to grow rapidly in food generally rich in moisture; protein and have neutral pH can cause food borne illness. And you cannot usually tell by taste, smell or sight that a particular food contains harmful microorganism. You may not even be aware that food has caused you distress [8, 9]. Therefore, our research project will be focused on physical, chemical and bacteriological analysis of honey to assess its quality in order to be consumed by people without causing them harmful effect.

B. Research objectives

1. Main objective

The aim of this work is to assess the quality of the honey produced by MIG ltd and Abeille ltd.

2. Specific objectives

- To do the physical and chemical analysis of honey sample
- To do the bacteriological analysis of honey sample

C. The research questions

Our works will emphasize on the following research questions

1. What are the degree of physical and chemical parameters existing in the analyzed honey;

2. What are the degree of contamination by harmful microorganism in the analyzed honey;
3. What are the effect of that microorganism on human body;
4. What are the standard of honey to be consumed by people;

D. Research methodology

In order to respond the research questions, the following methodologies will be used:

- Collection of honey sample
- Physico-chemical analysis
- Bacteriological analysis
- The theoretical background and literature review are done by consulting and analysing different types of documentation related to the same field of research.

E. Scope of the project

This project will be limited to physico-chemical and bacteriological analysis of honey produced by MIG and Abeille Ltd.

F. Expected outcome

After completion of this project effectively, the obtained result will allow us to be aware if the honey produced by MIG and Abeille Ltd is of good quality or not and people will benefit to get honey which has no harmful effect on their life; MIG and Abeille Ltd will benefit to produce the honey of good quality which lead them to expansion of market with hope that their product will be accepted by food control organization.

G. Layout of the project

The project is subdivided into three chapters:

Chapter I: General introduction

Chapter II: Material and Methods.

Chapter III: Results and discussion.

At the end we will give conclusion and recommendations**.**

The first chapter gives the introduction, problem statement, objectives of the study and the methodology approach of the project and the review of the literature related to the honey.

The second chapter will be dealing with data collection and data analyses of honey sample.

The third chapter will cover the result and discussion by focusing on the relationship between that result and the effect of the parameter analyzed on human life.

At the end, we will give specific conclusion according to the obtained result and give some recommendations.

I.3. LITERATURE REVIEW

I.3.1. Definition of honey

According to Codex Alimentarius, honey is the natural sweet substance produced by honey bees 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 honey comb to ripen and mature [10].

I.3.2. Honey categories

Honey may be categorized according to its origin, the way it has been harvested and processed, and its intended use.

I.3.2.1. Honey categories concerning the origin

- **Blossom honey** is obtained predominantly from the nectar of flowers (as opposed to honeydew honey).
- **Honeydew honey** is produced by bees after they collect 'honeydew' – secretions of insects belonging to the genus *Rhynchota*, which pierce plant cells, ingest plant sap and then secrete it again. Honeydew honey colour varies from very light brown or greenish to almost black [2].
- **Monofloral honey** is where the bees have been foraging predominantly on one type of plant, and is named according to that plant. Common monofloral honey types are clover, *Acacia*, lime (linden) and sunflower honey. Monofloral honey is priced more highly than polyfloral honey. Light, monofloral honeys like orange blossom or *Acacia* – because they look so attractive – always obtain higher prices then blends of honeys.
- **Multifloral honey** (also known as polyfloral) has several botanical sources, none of which is predominant, for example, meadow blossom honey and forest honey [11].

I.3.2.2 Honey categories concerning processing

- **Comb honey** is pieces of honeycomb, as produced by the bees, where the beekeeper has done no processing to separate the honey from the beeswax. The beeswax comb, as well as the honey, is edible. Comb honey always fetches a very good price, as the consumer can be sure that the honey has not been contaminated in any way. Ironically, this can be one of the easiest forms of honey to harvest and prepare for sale
- **Strained honey** is honey obtained by straining honeycombs, to separate the honey from the beeswax [11].
- **Chunk honey** is a jar of liquid honey inside which is placed a piece of comb honey. This can look very attractive. It is important that the liquid honey is a type that is very light and clear, and will not granulate over a long period. Honeys from *Acacia* and *Robinia pseudoacacia* are often used for this. This type of product depends on the right type of honeys and excellent packaging, and can achieve a very good price.
- **Extracted honey** is honey obtained by centrifuging honeycombs.
- **Pressed honey** is extracted by pressing honeycombs with or without the application of moderate heat.
- **Crystallized or granulated honey** is strained honey that has crystallized
- **Creamed honey** is strained honey that has been seeded to start crystallization and then stirred to produce a honey of uniform, soft consistency [2].

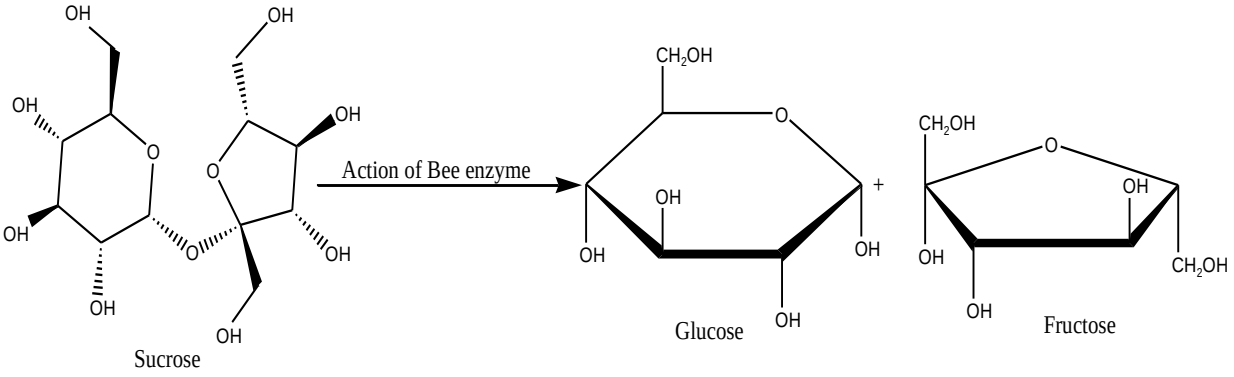
I.3.2.3 Honey categories concerning intended use

- **Table honey** means honey intended for consumers, to be eaten directly or as a natural sweetener for drinks or in cooking.
- **Industrial or bakers' honey** is honey that does not meet fully all the criteria for table honey, for example, the hydroxymethylfurfural (HMF) content may be higher than 40 mg/kg, although the regulations allow some exceptions. This may be because it has been heated too much, or it naturally has a high HMF, and is therefore regarded, according to the EU criteria, to be of lower quality than table honey. In this case, it still qualifies for use in the food industry, for the manufacture of bakery goods, confectionery, breakfast cereals, sauces, tobacco, and products such as honey-roasted nuts and pharmaceutical products.

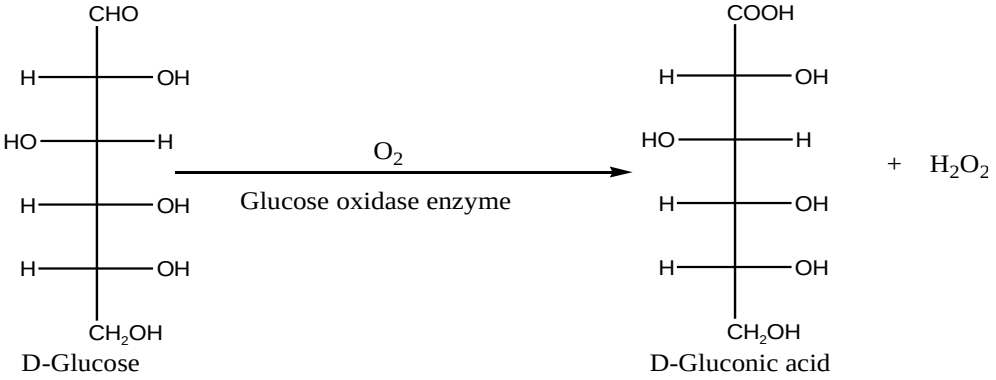
About 20 percent of honey on the world market is classified as bakers' honey. Major substitutes for industrial honey are sugar, invert sugar, and corn syrup, but honey is valued because it conveys a message of 'natural value' to manufactured products [12].

I.3.3. ESSENTIAL COMPOSITION OF HONEY

Honey consists of a mixture of sugars, mostly glucose and fructose which comes from the action of bee enzyme to the nectar sucrose [13].



In addition to water (usually 17-20 percent), honey also contains very small amounts of other substances, including minerals, vitamins, proteins and amino acids. The dominant acid is gluconic acid, produced by the action of bee enzymes on some of the glucose molecules in honey. This reaction is accompanied by the production of hydrogen peroxide [14].



A minor, but important component of most honey is pollen. Pollen is carried to the bees' nest (hive) and stored inside it quite separately from nectar, but a few pollen grains find their way into nectar, and eventually into honey. The pollen in honey can be identified using a microscope, and

gives guides to the plants from which bees have been collecting nectar and pollen. Experts are able to determine the geographical origin of honey by the pollen it contains.

This science of melissopalynology requires only an optical microscope for seeing the pollens in honey, and knowledge of the characteristic shapes of pollens that should be present in particular honeys. In many countries, pollen analysis of the locally produced honeys is regularly carried out and the pollen specialists have a precise knowledge of the pollen spectrum of the honeys of their region. The color of honey varies from nearly colorless to dark brown. The consistency can be fluid, viscous or partly to entirely crystallized. The flavor and aroma vary, but are derived from the plant origin.

Honey sold as such shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. Honey shall not have any objectionable matter, flavor, aroma, or taint absorbed from foreign matter during its processing and storage. The honey shall not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter. Honey shall not be heated or processed to such an extent that its essential composition is changed and/ or its quality is impaired. Chemical or biochemical treatments shall not be used to influence honey crystallization. The 'ash' content of honey is mainly mineral trace elements. Minerals present are calcium, copper, iron, magnesium, manganese, potassium, sodium, chlorides, phosphates, silicates and sulphates. Dark honeys are often very rich in minerals, but variation in the mineral content of different honeys is great. These trace amounts of minerals may be important for human nutrition [15].

Table 1: Composition of fully ripened honey

Substance	**Percentage**
Levulose(fructose)	41.0
Dextrose(glucose)	35.0
Sucrose	1.9
Dextrins	1.5
Minerals	0.2
Undetermined	3.4
Water	17.0

I.3.4 THE USES OF HONEY

- **For bees**

Bees produce honey to act as a food store for the colony for periods when there are no flowers, or the climate is adverse. For example, during the winters of northern, temperate countries, few plants are flowering between October and March, and bee colonies need honey stores to survive throughout this flowering dearth period, and when it may be too cold to leave the nest. In tropical countries, bees need to survive through seasons when there are no flowers, periods of drought, or when bees are not able to forage because of rain or other adverse weather.

- **As food for humans**

Honey is a useful source of high-carbohydrate food, and usually contains a rich diversity of minor constituents (minerals, proteins, vitamins and others), adding nutritional variety to human diets [4].

Table 2: Energy value of honey

Energy value (kcal/kg)	3,040
Sweetness	High
Sugar content (%)	80
Minerals, protein, enzymes	Very little, but valuable

- **As a medicine**

Honey also has medicinal use, for example, honey mixed with hot water, lemon juice or hot milk is a well-known remedy for coughs and sore throats. Honey has antibiotic properties and provides an effective dressing for wounds and burns [2].

CHAPTER II. MATERIAL AND METHODS

II.1 MATERIALS AND REAGENTS

Materials	Reagents
Analytical balance	Sodium hydroxide
Muffle furnace	phenolphthalein indicator
Spatula	acetone
Erlenmeyer flask	hydrochloric acid
Abbe refractometer	sulphuric acid
pH meter	luff reagent
Beakers	Sodium thiosulphate
Tissue paper	copper sulphate
Filter paper	potassium ferrocynide
Graduated cylinder	potassium iodide
Desiccator	Lauryl sulfate broth
Silica crucible	peptone water
Picnometer	kovac's reagent
Petri dishes	monnital salt Agar
Bunsen burner	Salmonella S Agar
Incubator	Agar
Test tube	Buffer solution and Distilled water

II.2. METHODS

II.2.1 Honey samples

Two different packed types of honey samples were collected from MIG ltd located at Nyamagabe District and Abeille ltd located at Huye District at the date of 05/04/2016 and 06/04/2016 respectively and the experiment work was conducted in LADAMET alimentary food laboratory in University of Rwanda

(a) **(b)**

Figure 1: Honey sample from MIG ltd (a) and Abeille Ltd (b)

II.2.2. Physicochemical parameter analysis of honey

II.2.2.1. Determination of ash content

Ash or mineral content is the portion of the food or any organic material that remains after it is burned at very high temperature. The ash constituents include potassium, sodium, calcium and magnesium which are present in larger amounts as well as smaller quantities of aluminum, iron, copper, manganese or zinc, arsenic, iodine, fluorine and other elements present in traces. The ash content represents the total mineral content in food. Although minerals represent a small portion they play an important role from a physicochemical, technological, and nutritional point of view [16].

Procedure

For determination of ash content; we weighed 10g of each sample in silica crucible. We heated the crucible containing the sample in muffle furnace about 4hours at 650^0C. We cooled it in desiccators and weighed. To ensure completion of ashing, we reheated it again in furnace for half an hour more, we cooled and weighed it. We have calculated the ash content by the following formula:

$$A\ (\%) = \frac{m1}{m0} \times 100$$ [17].

Where: m_1 is weigh of sample after ashing

m_0 is weigh of fresh sample taken

In our work, we have used the values below:

For MIG Ltd	For Abeille
$m_{1=}$0.0036g	$m_{1=}$0.0044g
$m_{0=}$2.4175g	$m_{0=}$2.8541g

After calculation the obtained results have been recorded in table 3.

II.2.2.2. Electrical conductivity

Conductivity of a substance is defined as 'the ability or power to conduct or transmit heat, electricity or sound'. When an electrical potential difference is placed across a conductor, its movable charges flow, giving rise to an electric current. This property is called conductivity. Since the charge on ions in solution facilitates the conductance of electrical current, the conductivity of a solution is proportional to its ion concentration.

The conductivity depends on the value of the pH, on the temperature of measurement and on the amount of CO_2 which has been dissolved in the honey to form ions. The conductivity is also affected by the concentration of ions already present in the honey such as iron, sodium and potassium. Chemical composition of water determines its conductivity. Hence this becomes the most widely used measure of the purity of water. Conductivity is a good criterion of the

botanical origin of honey and today it is determined in routine honey control instead of the ash content. This measurement depends on the ash and acid content of honey; the higher their content, the higher the resulting conductivity. There is a linear relationship between the ash content and the electrical conductivity

C = 0.14+1.74A

Where **C,** is the electrical conductivity in millisiemens cm^{-1} and **A** is the ash content in g/100g [17]. The results are recorded in table 3.

II.2.2.3. Determination of pH

For determination of pH in the honey, digital pH meter is to be used.

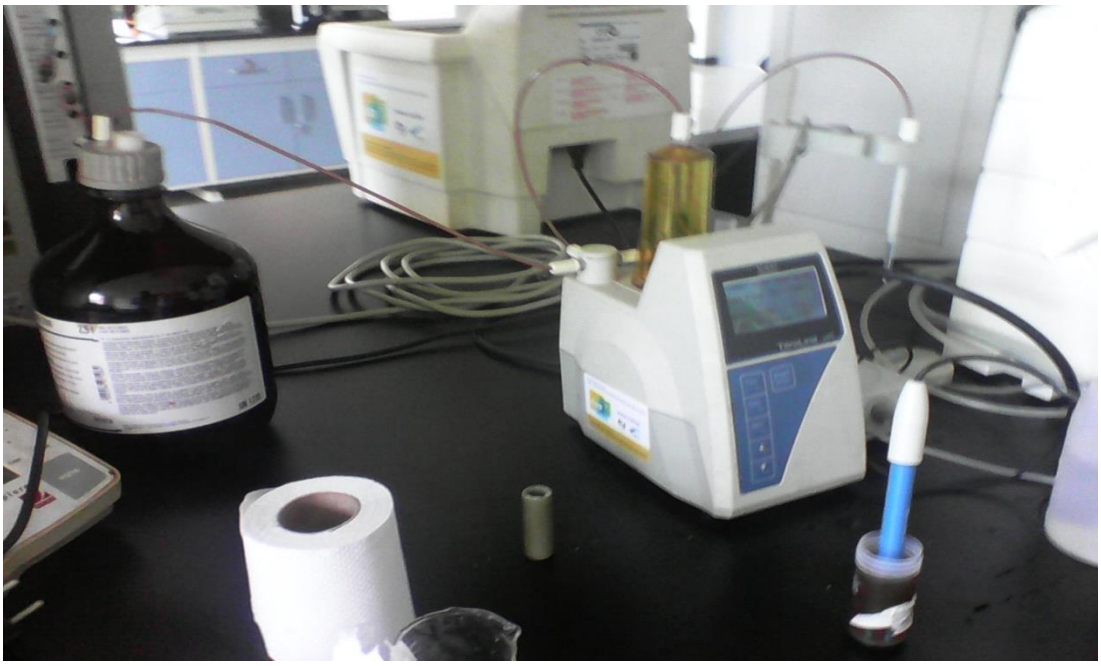


Figure 2: pH meter

Procedure

We have calibrated the pH meter by using a buffer solution at pH 4 and 7. Then, we poured the portion of honey sample from MIG ltd into the beaker and we inserted the pH meter in it. Finally, we have read the pH of that sample on the screen of pH meter. After reading of pH of honey produced by MIG ltd is completed, we have washed the electrode with distilled water and

dried-up with tissue paper. Similarly, as continue series, the pH of honey produced by Abeille ltd was determined accordingly.

II.2.2.4. Determination of the total acidity of honey

The acidity of any honey is directly related to the floral sources that created it. Honey contains a number of different acids, including about 18 amino acids, many different organic acids, as well as aliphatic and aromatic acids. The aromatic acids greatly contribute to the flavor of honey [18].

In order to determine the acidity of honey we have used titration method.

Procedure

Firstly, we have weighted 10g of honey sample and poured it in 75ml of distilled water. We took 10ml of that solution with 3 drops of phenolphthalein indicator and we have titrated the above solution with solution of 0.1N NaOH until the coloration become pink. The volume of alkali used was noted and calculated by using the following formula:

Total acidity in millequivalent/kg = V×C×10

Where V: the volume of NaOH used

C; the concentration of NaOH

The obtained volume at the end of titration is 18ml and 17 ml for MIG ltd and Abeille ltd respectively at 0.1N concentration of NaOH

After calculation the obtained result are recorded in table 3.

II.2.2.5. Determination of refractive index

The speed of light in vacuum is a universal constant, but when light travels through any other medium its speed slows down as it gets constantly absorbed and reemitted by the atoms in the material. The ratio of the speed of light in vacuum to its speed in another medium/material is called as the refractive **index** of the medium/material and is denoted by 'n' [19]. Refractive index of a transparent solid or liquid, which is a measure of its interaction with electromagnetic radiation, can be determined by various methods [20]. For us we have used refractometers in chemical analysis to determine the refractive indices of honey.

Figure 3: Refractometer

Procedures

1. We opened the prism and thoroughly cleaned its surface.
2. With a pipette, we dropped 4 drops of honey sample produced by MIG Ltd on the prism, and we closed the prisms together.
3. We rotated the eyepiece in order to obtain good visibility of the filament intersection and scale in the eyepiece field of view. If the boundary between the dark and bright fields is unclear or colorful, rotate the compensator knob and obtain a sharp boundary line. Then bring the intersection of the eyepiece threads onto the boundary line between the dark and light fields by rotating the knob then we read the refractive index on screen of refractometer and we recorded the result in the table 3
4. We cleaned the prism with filter paper and acetone and we repeated steps1-3 for solution of honey sample produced by Abeille ltd.

II.2.2.6. Determination of moisture content

The terms "moisture content" and "water content" have been used interchangeably to designate the quantity of water contained in food. It is important to food manufacturers for a variety of reasons. Moisture is an important factor in food quality, preservation, and resistance to deterioration [12].

Procedure

We weighted rapidly 10g of honey sample in beaker of 100ml by using analytical balance and we mixed it with 50 ml of hot water then we poured that solution in Erlenmeyer flask at 100ml. we have cleaned beaker by using a portion of hot water in order to avoid the loss of any quantity of honey sample and we cooled the solution at ordinary temperature then we completed the Erlenmeyer flask by using distilled water and mixed in order to make homogenize solution. We took the density of that solution at 15^0C within a pichnometer and we looked on the table of Plato the percentage of solution then we putted it at 100ml of sample. Finally, we made the difference between 100 and percentage of water contained in sample.

II.2.2.7. Determination of sugar content

Glucose and fructose have the identical reducing power toward the alkaline solution of copper sulfate ($CuSO_4$). It is calculated by using the luff-schoorl table. The dosage by using Luff-schoorl method gives the total of reducing sugar. Sucrose does not intervene within dosage but by chemical or enzymatic hydrolysis the transformation of sugar permit the transformation to inverted sugar. It means that after hydrolysis by luff-schoorl method it gives the sum of glucose, fructose and invert sugar from sucrose. Therefore, the difference between the two dosages, before and after hydrolysis gives the quantity of inverted sugar contained within the sample [21, 22].

Procedures

We have weighted 5g of homogenized honey and introduced it within beaker of 100ml and added a hot water then we put that solution to the flask of 100ml. We cleaned beaker clearly by using a small quantity of hot distilled water and pour that water in solution contained in flask. After turning the solution to ordinary temperature. We added 1ml of potassium ferrocynide at 15% and 1ml of zinc at 30%. We added also two drops of phenolphthalein and drops to drops

sodium hydroxide to the appearance of resistant pink color. After that we have made the solution to 100ml and we made the filtration of it. The solution after filtration contains the solution A. The dilution of solution A 10 time give the solution B, in order to get the sum of glucose and fructose, we took 10ml of solution B within 250 ml Erlenmeyer flask with 15 ml of distilled water and 25ml of luff reagent. We have preceded the luff-schoorl method and luff-schoorl table, and we calculated the quantity of the sum of glucose and fructose in mg then to its quantity in 100mg of honey.

Method luff-schoorl

We took 10ml of the hydrolyzed solution and 15ml of distilled water and we placed them in a 250 ml Erlenmeyer flask; we added 25ml of luff reagent exactly measured. After addition of pumice stones, in the flask with reflux condenser, we brought to a boil 2 minutes exactly. The solution is cooled, then we added 3 g KI dissolved in 5 ml of water and then 25 ml of sulfuric acid 25 % and we introduced carefully by the small portions. We titrated iodine released by $Na_2S_2O_3$ N/10 in the presence of 5 ml of starch. We titrated reagent operating under the same conditions but replacing the sugar solution with 25 ml of distilled water.

Expression of results

By subtracting the number of milliliters of sodium thiosulphate corresponding to pure reagent (white), the number of milliliters of sodium thiosulphate resulting dosage of the sugar solution, we have the number of milliliters of sodium thiosulphate corresponding to the sugar sample (= carbohydrates reducer). The luff - schoorl table (appendix 2) directly gives the weight of sugar corresponding to the volume of $Na_2S_2O_3$ N / 10 used.

Determination of sucrose

Sucrose content (%) = sugars after hydrolysis (%) - sugars before hydrolysis (%) x 0.95

Vp = Vr - Vs

The amount of glucose, fructose and sucrose (%) = 100 * Ps / Pm*10

Where:

Pm, weight of homogenized honey.

Vs, the number of milliliters of $Na_2S_2O_3$ resulting dosage of the sugar solution

Vr , the number of milliliters of $Na_2S_2O_3$ corresponding pure reagent (white).

Vp, the number of milliliters of $Na_2S_2O_3$ corresponding to the sugar sample.

Ps, the number of mg of glucose sum, fructose and sucrose corresponding to the number of milliliters of $Na_2S_2O_3$ corresponding to the sugar sample.

Where the values that have been used, are below:

From MIG Ltd	**From Abeille,**
After hydrolysis	**After hydrolysis**
Pm=5g	Pm=5g
Vs=9.6ml	Vs=10ml
Vr=24.6ml	Vr=24.6ml
Vp=15ml	Vp=14.6ml
Ps=38.5mg	Ps=37.38mg
Before hydrolysis	**Before hydrolysis**
Vs=9.7ml	Vs=10.2ml
Vp=14.9ml	Vp=14.4ml
Ps=38.22mg	Ps=36.82mg

After calculation, the obtained results are recorded in table 3.

II.2.3 BACTERIOLOGICAL ANALYSIS

II.2.3.1 Identification of Total Coliforms

- 100mL of well homogenized water have been filtered aseptically on membrane of cellulose ester with a porosity of 0.45µm, and we prepared four plates containing 10 ml sterile Lauryl sulfate broth and agar (a solid media) growth. Then, we added 10 ml of honey to each plate.
- We incubated those plates at 37^0C for 24h, and then we removed the plates from incubator to count the number of colonies formed by total coliforms. Then, the results are recorded in table 4.

II.2.3.2 Identification of Faecal coliform

Faecal coliform bacteria are bacteria that are associated with human or animal wastes as they usually live in human or animal intestinal tracts. Faecal coliforms bacteria are predominantly E-Coli. The group of faecal coliform and E-coli bacteria are more closely related with faecal pollution. The presence of faecal coliform and E-coli bacteria is not acceptable and must be eliminated from honey.

Procedure

- We have filtered 100mL of well homogenized water aseptically on membrane of cellulose ester with a porosity of 0.45µm, we prepared four plates containing 10 ml sterile Lauryl sulfate broth and agar (a solid media) growth. Then, we added 10ml of honey sample to each plate.
- We incubated those plates at 44^0C for 24h, those four plates and therefore, we removed the plates from incubator to count the colonies and we founded that there are no colonies formed. Then, the results are recorded in table 4.

II.2.3.3 Identification of Escherichia coli

- We prepared four tubes containing 5ml of peptone water (solid media.
- We added 5 ml of honey sample.
- Using hence platinum, we picked up at least one bacteria from fecal coliforms colony and we inserted into the tube.
- We repeated the previous procedures for the three remaining tubes.
- We incubated those tubes at 44^0C for 24h.
- We removed the tubes from incubator and add a kovac's reagent to each tube.
- Indole test is positive if there is appearance of pink layer at top of the solution.
  The results are recorded in table 4.

II.2.3.4. Identification of staphylococcus aureus at 37^0C

Procedure

- 100mL of well homogenized water have been filtered aseptically on membrane of cellulose ester with a porosity of 0.45µm, and we prepared four plates containing 10 ml sterile mannitol salt Agar. Then, we added 10 ml of honey to each plate.
- We incubated those plates at 37^0C for 24h, and then we removed the plates from incubator to count the number of colonies formed by staphylococcus aureus (we found there is no colonies formed).

Then the results are recorded in table 4.

II.2.3.5. Identification of salmonella at 37^0C

Procedure

- 100mL of well homogenized water have been filtered aseptically on membrane of cellulose ester with a porosity of 0.45µm, and we prepared four plates containing 10 ml sterile salmonella S. Agar. Then, we added 10 ml of honey to each plate.
- We incubated those plates at 37^0C for 24h, and then we removed the plates from incubator to count the number of colonies formed by Salmonella (we found there is no colonies formed).

  Then the results are recorded in table 4.

CHAP TER III. RESULTS AND DISCUSSION

III.1. RESULTS FOR THE PHYSICOCHEMICAL ANALYSIS

Table 3: Results of physico-chemical parameters

PARAMETER	MIG Ltd	Abeille Ltd	FDA Standard [11].
Ash content(g/100g)	0.14	0.15	<0.6
Electrical conductivity(ms/cm)	0.3836	0.401	<0.8
pH	3.81	3.76	3-6
Total acidity(meq/kg)	18	17	<50
Refractive index	1.4906	1.4905	1.474-1.504
Moisture content (%)	15.6	16.7	<20
Sum of glucose and fructose (%)	77	74.76	>60
Sucrose (%)	0.532	1.064	<5

During our experiments, several physiochemical quality parameters employed in routine honey quality control purposes were analyzed in two honey samples, one from MIG Ltd, other from Abeille Ltd. Those parameters are: pH, ash content, moisture content, electrical conductivity, total acidity, refractive index, sucrose and sum of glucose and fructose as shown in above table.

1. Ash content and electrical conductivity

The honey produced by Abeille ltd has high ash content and electrical conductivity compared to the honey produced by MIG ltd, but all quantities meet the range of FDA standard. Honey contains electrolytes in the form of acids and minerals, which cause the variation of electrical conductivity.

2. pH and Total Acidity

For the pH, it meets the FDA standard range and it shows that all sample of honey we have tested are acidic but the honey from Abeille Ltd is more acidic than honey from MIG Ltd. The total acidity of the tested samples is 18meq/kg and 17meq/kg all meet the range, this acidity

come from the oxidation of glucose by the action of gluco-oxidase enzyme to form gluconic acid which is dominant in honey with the other 18 amino acid and organic acid. Therefore the low acidity founded in tested sample contribute to its quality as it inhibits the presence and growth of microorganisms [14].

3. Moisture content and Refractive index

The moisture contents of MIG and Abeille ltd honey samples are 15.6% and 16.7% respectively. Therefore, MIG Ltd honey sample has low water quantity compared to Abeille honey sample and this difference is due to the source of honey and climatic condition. The maximum water content for honey according to FDA Standards is <20%. Moisture content is used to assess the likelihood of honey to ferment; honey with high water content is more likely to ferment. Honey produced by MIG ltd has more refractive index than the honey produced by Abeille ltd because the samples from Abeille ltd has high water content which allow the light to pass easily in the sample and the refractive index becomes lower.

4. The sum of glucose and fructose

The results obtained for the sum of glucose and fructose are in range and the honey produced by MIG ltd has more quantity compared to that produced by Abeille ltd. This difference is due to the high action of enzyme which break down the sucrose of nectar into fructose.

5. Quantity of sucrose

The quantities of sucrose obtained on both samples are in range but the honey produced by Abeille ltd has more sucrose compared to the one from MIG Ltd. We cannot conclude that this quantity is from the addition sucrose but it may due to the low activity of enzyme on sucrose which lead to the existence of unbroken sucrose [14].

III.2. RESULTS FOR BACTERIOLOGICAL ANALYSIS

Table 4: Results of bacteriological parameters

Parameters	Culture Medium	MIG Ltd	Abeille Ltd	WHO standard
Staphylococcus aureus at 37^0C in 24h	Monnital salt Agar	0 Cfu/100ml	0 Cfu/100ml	Absence/100ml
Total coliforms at 37^0C in 24h	Lauryl sulfate broth and Agar	0 Cfu/100ml	0 Cfu/100ml	Absence/100ml
Faecal coliforms at 44^0C in 24h	Lauryl sulfate broth and Agar	0 Cfu/100ml	0 Cfu/100ml	Absence/100ml
Escherichia coli at 44^0C in 24h	Peptone water & Kovac's Reagent	0 Cfu/100ml	0 Cfu/100ml	Absence/100ml
Salmonella at 37^0C in 24h	Salmonella S. Agar	0 Cfu/100ml	0 Cfu/100ml	Absence/100ml

Due to the obtained result as shown in the above table, both tested honey samples don't contain bacteria; it cannot cause the food borne illness therefore this honey resistance is due to observed acidity and moisture content with hydrogen peroxide which contribute to the existence of high antimicrobial activity. The water content of honey is a key factor in inhibiting the existence and grow of microorganism. At 17%, its water content is much lower than that of bacteria or fungi. Honey also has a low water activity; this is a measure of the amount of water in substance that is available to support microbial growth. Water activity is on a scale of 0 to 1, with most moulds and bacteria being unable to grow under a water activity of 0.75. Honey has a water activity of 0.6. This combined with the fact that its low water content dehydrates bacteria [14].

Another factor that helps honey being resistant to microbes is its acidity. This acidity contributed by a number of acids; including formic acid and citric acid but dominant acid is gluconic acid; produced by the action of bee enzymes on some of the glucose molecules in the honey. This further boosts honey's antibacterial properties, as many bacterial thrive in neutral rather than acidic. Hydrogen peroxide is also produced during the production of gluconic acid. This too can inhibit the growth of bacteria [14].

CONCLUSION AND RECOMMENDATIONS

1. CONCLUSION

The aim of our work was to assess the quality of honey produced by MIG ltd and Abeille ltd by analyzing the physicochemical and bacteriological parameters by comparing them to the standard in order to appreciate those products.

In order to achieve that objective and answering the research question we have done the analysis of honey samples with the aid of pH meter for determination of pH, abbe refractometer for determination of refractive index, titration method for determination of total acidity, luff-schoorl method for determination of reducing sugar and sucrose, indirect method for determination of moisture content, gravimetric method for determination of ash content, indirect method for determination of electrical conductivity and culture media method for identification of bacteria.

During our work the analyzed parameters meet the standard range of honey, but honey produced by MIG ltd has more sugar compared to honey produced by Abeille ltd. The ash content has linear relationship with electrical conductivity, moisture content and refractive index has linear relationship. Both acidity and moisture content have the effect on the absence and growth of microorganism. Both honey produced by MIG ltd and Abeille ltd are of good quality but we appreciate more the honey produced by MIG ltd because it meets the range of standard and rich in energetic substances.

2. RECOMMENDATIONS

After concluding the research, the following recommendations were addressed.

To the consumer

We advise people to take honey as part of their food because it contains sugar and other nutrients needed by our body to well function.

To University of Rwanda

We recommend the university of Rwanda especially Department of chemistry to introduce the module of microbiogical course in order to give to the students sufficient the knowledge about

microbiological analysis and have enough materials and reagents in order to perform all necessary analyses.

To the researcher

We recommend the researcher to do further studies about honey in order to light the other hidden properties of honey

We advise the researcher to do the analysis of clostridium botulinum bacteria because it is harmful to children.

To MIG Ltd and Abeille ltd

- We advise them to have their own laboratory to assess the quality of their produced honey before distributing them because quality changes with climatic season and the source of honey.
- For good quality of the honey they produce, we advise them to increase their production quantity.

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APPENDICES

Appendix 1

Preparation of Luff –schoorl reagent

We dissolved separately

50g of pure citric acid ($C_6H_8O_7.H_2O$) into 50ml of water;

25g of pentahydrate copper sulphate ($CuSO_4.5H_2O$) free from iron, in 100ml of water;

388g of pure anhydrous sodium carbonate ($Na_2CO_3.10H_2O$) in 400ml of warm water. We leaved it to cool, we poured slowly the citric acid solution in sodium carbonate solution then to the solution of copper sulfate solution and we completed it to 1l of solution by using distilled water

Calculation of coefficient of inversion

0.95 is the coefficient of inversion to take into count due to the difference.

Molecular weight of sugars (g/mol)

Sucrose ($C_{12}H_{22}O_{11}$):342

Glucose ($C_6H_{12}O_6$):180

Fructose ($C_6H_{12}O_6$):180

So, the molecular weight is 342 correspond to 360 or the ratio 342/360 = 0.95

Appendix 2

Table 5: Values for 25ml of Luff-Schoorl Reagent

$Na_2S_2O_3$ 0.1 mol/litre	Glucose, fructose invert sugars $C_6H_{12}O_6$		Lactose $C_{12}H_{22}O_{11}$		Maltose $C_{12}H_{22}O_{11}$	
Ml	mg	difference	mg	difference	mg	difference
1	2.4	2.4	3.6	3.7	3.9	3.9
2	4.8	2.4	7.3	3.7	7.8	3.9
3	7.2	2.5	11.0	3.7	11.7	3.9
4	9.7	2.5	14.7	3.7	15.6	4.0
5	12.2	2.5	18.4	3.7	19.6	3.9
6	14.7	2.5	22.1	3.7	23.5	4.0
7	17.2	2.6	25.8	3.7	27.5	4.0
8	19.8	2.6	29.5	3.7	31.5	4.0
9	22.4	2.6	33.2	3.8	35.5	4.0
10	25.0	2.6	37.0	3.8	39.5	4.0
11	27.6	2.7	40.8	3.8	43.5	4.0
12	30.3	2.7	44.6	3.8	47.5	4.1
13	33.0	2.7	48.4	3.8	51.6	4.1
14	35.7	2.8	52.2	3.8	55.7	4.1
15	38.5	2.8	56.0	3.9	59.8	4.1
16	41.3	2.9	59.9	3.9	63.9	4.1
17	44.2	2.9	63.8	3.9	68.0	4.2
18	47.1	2.9	67.7	4.0	72.2	4.3
19	50.0	3.0	71.7	4.0	76.5	4.4
20	53.0	3.0	75.7	4.1	80.9	4.5
21	56.0	3.1	79.8	4.1	85.4	4.6
22	59.1	3.1	83.9	4.1	90.0	4.6
23	62.2		88.0		94.6	

[21, 22].