"Adulteration On Natural Honey Quality Properties: A Case Study Of Mashonaland East, Mashonaland Central And Manicaland Province Of Zimbabwe, On Street Honey Sellers' Behavior."

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Abstract: Honey is a natural substance of high commercial value and limited supply. It is considered as natural complex food product produced from nectar of plants by bees' worldwide. It is an outstanding sweetening product that can be used by humans without processed and possessed. Honey adulteration is a common problem across the world in general and in Zimbabwe in particular, and has a significant economic impact, it can be done by the addition of different cheap foreign materials which may harm the body. The issue of food adulteration is a global concern and developing countries are at higher risk associated with it due to lack of monitoring and polices on food production. Adulteration to honey product in Zimbabwe can be done by adding substances such as sugar, banana, molasses, water, maize syrup and wheat flour to the honey. Honey being a natural product, the composition of honey is highly variable because of adulteration from one country to another. Adulteration of honey in most cases, when takes place, it alters physicochemical and rheology of original honey quality, resulting in reduction in its original nutritive and medicinal value. The methods of discrimination of honey adulterant, accurate quantification of the adulterants, must have been made use for producing high quality of honey and free from any foreign material addition. Honey is becoming popular as a reputable and effective therapeutic agent by medical doctors of conventional medicine and by the general public across the world. It has been effectively used in the treatment of many diseases, such as gastrointestinal diseases, skin diseases, cancer, heart diseases, and neurological degeneration in many countries. This product is an excellent source of energy such as carbohydrates and also contains water, small amounts of organic acids, vitamins, minerals, flavonoids, and enzymes required by the body to function well. As a natural product on high demand, honey has been targeted for adulteration. The authenticity of honey is of great importance to save human life from unhealthy food. The current work focusses on studying the authenticity of honey sold on the streets of Zimbabwe. For this purpose, physical properties such as moisture content, pH, and total ash, reducing sugars, free acidity, diastase activity, electrical conductivity and hydroxymethylfurfural (HMF) were measured from the collected samples. Laboratory tests and simple tests were carried out. The obtained results were compared with the European Union (2001) standards, which are the harmonized methods of the international honey commission.

Keywords: honey adulteration, Results, Honey physicochemical properties, rheology of original honey quality, Standard preparation and simple tests.

1. Introduction.

Different authors have different definitions of honey (Anidiobu, et al., 2019). One of the authors, Merriam Webster (Merriam, 2021) defined honey as a sweet, sticky yellowish-brown fluid made by bees and not excluding other insects from nectar collected from flowers. This product has been used by human kind as food for immemorial years (Afonso, et al., 2018; Agila & Barringer, 2012; Frew, et al., 2013). Honey has been scientifically proven to contain a number of nutritionally important substances that support good health and recovery from ill bodies. Science and laboratory test results have proved that honey is a sugary foodstuff. Current regulations state that apart from other forms of honey no other substances or additives can be added to honey bees (Codex, 2011). Research has approved that the composition and properties of honey vary with the floral and honeydew sources used by honeybees to manufacture that honey, as well as regional and climatic conditions of the apiary (Tavakolipour & Ashtari, 2020). Natural honey syrup preserves human health and give it a strong protection from several diseases. The (Wikipedia, 2022) defined as a pure and natural product that does not include any other additive substances besides the original substances used by the bees to manufacture the honey on their own, without the assistance of the humankind (Anklam, 2021). Adulteration of honey across the world takes place in countries with less technology and knowledge to detect adulterated honey, which results in reduction in nutrition and medicinal value of the adulterated honey (Zappala, et al., 2020). Honey in most countries from producers is not tested. The reason for testing honey is for quality control purposes and to verify the authenticity of the honey and to find out if there is no possible presence of artificial components or adulteration. It also helps to address good processing and packaging methods of honey and market's needs (Abdulrhman, 2020; Woodcock, et al., 2009). Properties of honey is helpful for comparison of natural honey samples from different sites. It serves as important indicator that can help to distinguish natural honey from manmade honey (Belobrajdic, et al., 2021). Adulteration changes quality characteristics of natural honey. Honey adulterated by sugar addition changes in some chemical and/or biochemical parameters (Zappala, et al., 2020). Some reactions affected are enzymatic activity, electrical conductivity, and contents of specific compounds (HMF, glucose, fructose, sucrose, maltose, isomaltose, proline as well as ash when compared to a control (Merriam, 2021). Chemical parameters, such as HMF content, which is suggested as a test to detect the addition of invert syrups, may be not the best because HMF and enzymatic activity vary in different honeys and can quickly change in honeys when exposed to heat or poor storage in warm conditions (Belobrajdic, et al., 2021).

2. Statement of the problem

The number of beekeepers in Zimbabwe is not increasing. The number of beehives, beekeepers own does not warrant the quantity of honey found in the streets being sold by vendors. The quantity of honey being sold in streets has increased and yet the place where the honeys are produced is not known. Most of the honeys being sold in streets does not have indication of their origin. A number of honey sellers in veranda of shops in town and long distance highway roads has necessitated the research of the quality of honey being sold in these roads.

3. Objective

To test the physicochemical, rheological properties and nutritional properties of honey sold along the highway roads and streets of towns in Zimbabwe.

3.1 Materials and methods

Twenty processed and bottled honey samples were collected for honey quality testing. Five samples (sample A) were bought at Chaseyama in Chimanimani, along Birchenough Bridge – Mutare Highway road which is in Manicaland, five samples (sample B) were bought in Mutare town (Manicaland) where vendors are selling bottled honey in verandas of shops, five bottles (Sample C) were bought in Mashonaland East, at Marondera, along the Marondera – Harare highway road and the last five bottles (sample D) were bought in the city of Harare which is Mashonaland Central. The five bottles from each location were bought from five different seller, this means one bottle was bought from each of the five honey sellers. The five bottles for each location were mixed thoroughly to form one single sample. We ended up with four bottle jars of honey. Each bottle jar sample representing the location, the honey was collected. At each location, one sample of unprocessed honey was bought, since these street honey sellers, they sell both the unprocessed honey and processed honey. The results of processed and unprocessed honey for each location were compared after testing. The unprocessed sample (honey in combs) was used as a control.

a) Moisture content

The moisture content of honey samples was determined by measuring the refractive index of the sample using an Abbe Refractometer. The method is based on the principle that refractive index of honey increases with honey solids content. The refractive index of distilled water (1.3330) was used as a reference to measure moisture content in honey. The surface of the prism was covered with drops of homogenized honey sample and the prism was closed for four minutes to stabilize. The refractive index was adjusted to read at a temperature of 20°C Measurements were done in duplicate and an average value was computed. The mean refractive index was converted to moisture content using the following formula: Moisture content = [–log10 (Corrected Refractive Index–1) – 0.2681]/0.002243 (QSAE, 2005).

b) pH

Twenty grams of honey sample was dissolved in 75 ml of distilled water in a 500 ml beaker and stirred using a magnetic stirrer. Then the pH was measured with a digital pH-meter (Orion Star J257), which was calibrated using pH 4.0 and 7.0 buffer solutions (QSAE, 2005). The work was done in the laboratory.

c) Total ash

Ash content of honey samples was also determined according to the procedures of QSAE (2005). A quartz dish was heated in an electric furnace at 600°C and subsequently cooled in a desiccator to room temperature and the dish was weighed (M2). Five grams of honey sample was weighed to the nearest 0.001g (M0) and added to the dish. Two drops of olive oil was added to the dish to prevent frothing and then the dish was placed in preheated furnace and heated for 1.5 hour at a temperature of 600°C The dish with the ash was then cooled in a desiccator and weighed. The ashing procedure was continued until constant weight was reached (M1). Ash (% by mass) was calculated using the following formula:

Ash (% by mass) = (M1 – M2)/Mo x100 (QSAE, 2005). The work was done in the laboratory.

3.2 Determination of Sugars in Honey

The reducing sugars (fructose and glucose) and non-reducing sugar (sucrose) were determined by HPLC Knauer Instrument. Two pumps, R1 detector, column oven, and clarity-chrome software was used to determine sugars. Instrument condition: Column: The flow rate was adjusted at 1.5 mL/min, the column used was Luna NH2 column for carbohydrate analysis, the column oven temperature kept constant at 40°C, the RI detector operated at room temperature, the mobile phase was acetonitrile: HPLC grade: water (80:20,v:v). Sample preparation: 5 g of sample dissolved in 12 mL methanol HPLC grade, Quantitatively transferred to measuring flask 50mL completed to the mark with HPLC grade water, sonicated for 20 min, Filtering through PTFE filter (0.2mm), kept at 0°C until analysis. Standard preparation: Pipette 25mL methanol into a 100mL calibrated flask. Depending on the sugars to be analyzed, dissolve the amounts detailed below in approximately 40mL water and transfer quantitatively to the flask and fill to the mark with water. Fructose: 2.000g; glucose: 1.500g; sucrose: 0.250g; maltose: 0.150g. (Codex, 2011). The work was done in the laboratory.

a) Free acidity

Free acidity of honey samples was determined according to the procedures of QSAE (2005). Honey sample (20 g) was dissolved in 150 ml distilled water in a 500 ml beaker and stirred with a magnetic stirrer. The solution was titrated with standardized 0.1 M NaOH solution to a final pH of 8.30. Then the amount of NaOH solution used for titration was recorded. The results were expressed in milliequivalent (meq) of acid per kg of honey using the following equation.

Acidity =10V (QSAE, 2005)

Where V = the volume of 0.1M NaOH used and 10 is the amount of honey sample used.

All this was done in the laboratory.

3.3 Determination of Diastase Activity

Determination of diastase activity was evaluated spectrophotometrically based on the method of Schade, et al.(1958) using the Shade method (UVA/IS Spectometer Lambda ll, Perkin Elmer, USA). The diastase activity is calculated as diastase number (DN). DN expresses units of diastase activity (Gothe unit). One unit is defined as the amount of enzyme that will convert 0.01 g of starch to the prescribed end-point in 1 h at 40°C, (Bogdanov, et al., 1997). The diastase activity is calculated as diastase number (DN). DN expresses units of diastase activity (Gothe unit).

a) Electrical Conductivity

The electrical conductivity at 1/5 was determined according to the method described by Bogdanov, et al (2004) using a conduct meter (Cond 3210 WTW). The measurements were carried out at 20°C in a 20% aqueous solution with respect to the dry matter of the honey. The value of the conductivity was directly determined by the cell in the solution after immersion. The results were expressed in micro-Siemens per centimeter (µS/cm).

Data related to honey quality were analyzed using GLM of SAS software (SAS, 2002).

b) Hydroxymethylfurfural

Five grams of honey sample was weighed into a small beaker. The honey sample was dissolved in 25 ml of water and transferred into a 50 ml flask. Half ml of Carrez solution was added and thoroughly mixed. Then half ml of Carrez solution II was added into the 50 ml flask and thoroughly mixed and then diluted with distilled water up to the volumetric mark of the flask. A drop of ethanol was added to the mixture to suppress foam. The mixture was filtered through general purpose filter paper and the first 10 ml of the filtrate was discarded. Five ml of the remaining filtrate was pipetted into each of two test tubes. Then five ml of water was added to one of the test tubes and thoroughly mixed and five ml of sodium bisulphite solution (0.2%) was added to the second test tube and mixed (the reference solution) using a Vortex mixer. The absorbance readings of the sample solution against the reference solution at 284 and 336 nm, respectively were taken using the UVVisible Evolution 201 spectrophotometer within one hour of preparation. The result was calculated as follows: HMF in mg/kg = (A284 – A336) x 149.7 x 5 x D/W (QSAE, 2005)

Where A284 = absorbance at 284 nm, A336 = absorbance at 336 nm, 149.7 = constant, 5 = theoretical nominal sample weight, W = weight of the honey sample in gram, D = dilution factor. The work was done in the laboratory.

3.4 Simple honey tests

Simple honey tests were performed to find out if the simple tests results agree with the laboratory tests results. The simple tests performed were:

a) Flame Test: A candle was used to find out if the honey give smokeless flame when ignited to show that its pure honey (Nyuk & Kandhasamy, 2020). Adulted honey is confirmed smoky flame and cracking sound during flame testing (Teferi, 2021).

b) Heating Effect: light heating of honey samples to dissolve crystallized substances was carried out. This was done to find out if the sample was pure honey. When pure honey is heated, it melts to clear transparent viscous solution and the wax floats on top (Zielinski, et al., 2018). If it is an adulted honey, the mixture of honey with starch from potato, banana, wheat flour or maize flour melts to form dispersed non-transparent liquid. A mixture of honey with sugar which is more than 50 % w/w melts to form thicker, dispersed and partially transparent liquid during heating (Teferi, 2021).

c) Water test: one table spoon of honey sample was added into a glass of water. Pure honey honey settle at the bottom of the glass of water with water. Adulterated honey dissolve in water when added to the water glass with water (Teferi, 2021).

d) Light a Fire: Matchstick test was carried out to find out if it is pure honey (Ruiz-Matute, et al., 2019). The tip of the matchstick was dipped in honey and strike to light it. If it is pure honey, the match lights easily and the flame burn the honey easily. Artificial honey give smock or fail to burn due to high moisture content (Teferi, 2021).

e) Thumb Test: A drop of the honey was put on the thumb and spread. If the honey spreads and spills, the honey is not pure. If it keeps intact, it's pure (Teferi, 2021).

f) Coca cola test: Coca cola test method was carried out. If coca cola is added into honey, fermentation takes place during mixing. Artificial honey does not ferment (Teferi, 2021).

Physicochemical properties of honey collected from road highways and verandahs of shops were analyzed and compared to European Union (EU) standards.

Table 1: *European Union (EU) standards (2001) of honey quality*

Parameters	**Unit**	**European Union Standards (2001)**
Moisture content	%	Maximum 20.0
pH	%	Maximum 5.0
Total ash	%	Maximum 0.6
Reducing sugar	%	Minimum 60.0
Saccharose	%	Maximum 5.0
Hydroxymethyl furfural (HMF)	mg kg^{-1}	Minimum 60.0
Free acidity	meq kg^{-1}	Maximum 50.0
Diastase activity	Goethe	Minimum 8.0
Electrical conductivity	µS cm^{-1}	Maximum 0.8

Source: *Codex Alimentarius and to the EU Draft 96/0114 (CNS).*

4. Laboratory results of honey samples

Table 2: *Honey from Chaseyama, Birchenough Bridge – Mutare Highway (sample A)*

No	Variable	Unit	Bottled & processed honey bought from vendors	Honey in combs (unprocessed) honey bought from vendors
1	Moisture content	g/100g	19.2%	19.3%
2	pH		3.3	3.2
3	Total ash	g/100g	0.43%	0.42
4	Reducing sugar	%	71.6.6%	72.3%
5	Saccharose	%	3.9%	4.1%
6	Hydroxymethylfurfural (HMF)	mgkg -1	41.1mg/kg	39.4mg/kg
7	Free acidity	meqkg -1	36.7 meq acid/kg	35.2 meq acid/kg
8	Diastase activity	Goth scale	8.9goth scale	9.4 goth scale
9	Electrical conductivity	µS cm $/cm^{-1}$	0.26 / µS cm^{-1}	0.28 /µS cm^{-1}

Table 3: Honey from Mutare town (sample B)

No	Variable	Unit	Bottled & processed honey bought from vendors	Honey in combs (unprocessed) honey bought from vendors
1	Moisture content	g/100g	24.8%	19.9%
2	pH		4.9	3.2
3	Total ash	g/100g	0.87%	0.41
4	Reducing sugar	%	49.7%	72.2%
5	Saccharose	%	6.1%	3.2%
6	Hydroxymethylfurfural (HMF)	mgkg -1	73mg/kg	49.2mg/kg
7	Free acidity	meqkg -1	64.3 meq acid/kg	20.3 meq acid/kg
8	Diastase activity	goth scale	4.4 goth scale	11.8 goth scale
9	Electrical conductivity	ms/cm^{-1}	1.154 ms/cm^{-1}	0.285 ms/cm^{-1}

Tabele 4: Honey from Marondera – Harare highway road (sample C)

No	Variable	Unit	Bottled & processed honey bought from vendors	Honey in combs (unprocessed) honey bought from vendors
1	Moisture content	g/100g	23.7%	19.8%
2	pH		6.1	3.8
3	Total ash	g/100g	1.82%	0.42
4	Reducing sugar	%	48.7%	73.2%
5	Saccharose	%	6.4%	3.4%
6	Hydroxymethylfurfural (HMF)	mgkg -1	86mg/kg	39.2mg/kg
7	Free acidity	meqkg -1	69.6 meq acid/kg	20.2 meq acid/kg
8	Diastase activity	goth scale	19.7 goth scale	15.8 goth scale
9	Electrical conductivity	ms/cm^{-1}	1.748 ms/cm^{-1}	0.285 ms/cm^{-1}

Table 5: Honey from the city of Harare (sample D)

No	Variable	Unit	Bottled & processed honey bought from vendors	Honey in combs (unprocessed) honey bought from vendors
1	Moisture content	g/100g	23.7%	18.7%
2	pH		6.5	3.2
3	Total ash	g/100g	1.62%	0.42
4	Reducing sugar		48.7%	78.2%
	Hydroxymethylfurfural (HMF)	mgkg -1	87.2mg/kg	39.2mg/kg
5	Free acidity	meqkg -1	69.6 meq acid/kg	23.3 meq acid/kg
6	Diastase activity		5.1 goth scale	13.35 goth scale
7	Electrical conductivity	ms/cm^{-1}	1.15 ms/cm^{-1}	0.285 ms/cm^{-1}

5. Results from simple honey sample tests

5.1 Honey from Chaseyama, Birchenough Bridge – Mutare Highway (sample A)

a) Flame Test

The processed and bottled sample and the unprocessed honey sample gave smokeless flame when ignited using candle flame. There was no cracking sound during flame test. This was a sign that the honey was not tempered with.

b) Heating Effect

Both samples, the unprocessed honey and processed honey melted and cleared transparent viscous solution and wax materials floated on top.

c) Water test

When one table spoon of honey samples was added into a glass of water and observation of the added honey showed settled at the bottom of the glass. The processed and the unprocessed honey settled at the bottom of the glass.

d) Light a Fire

When the tip of a matchstick dipped in honey and stricken to light it. It gave light easily and the flame burnt off the honey for both processed and unprocessed samples.

e) Thumb Test

A drop of each of the processed and unprocessed honey was put on the thumb. The honey drops did not spread around the thumb or spill. The honey stayed intact on the thumb.

f) Coca cola test

A table spoon of honey from unprocessed honey and processed honey were put into two different beakers. A teaspoon of coca cola was added into a beaker with processed honey and another teaspoon was added into the beaker with unprocessed honey. The content of coca cola and honey caused fermentation during mixing. Both the processed honey and the unprocessed honey fermented.

6. Results from samples B, C and D

The results from samples B, C and D were showing similar results on flame test, heating effect, water test, fire lighting, thumb test and coca cola test.

a) Flame Test

The processed and bottled sample and the unprocessed honey sample reacted differently. Presence of adulterants was confirmed by observation of smoky flame and cracking sound during flame test on processed and bottled honey. Smokeless flame and uncracking sound was observed on unprocessed honey.

b) Heating Effect

Confirmation of sugar added to honey was observed by the mixture, melting to form thicker, relatively dispersed and partially transparent liquid after heating the mixture for processed and bottled honey. For unprocessed honey, melted and cleared transparent viscous solution and wax materials floated on top.

c) Water test

One table spoon of processed and bottled honey sample was added into a glass of water and observation of the added honey dissolved before reaching the bottom of the glass of water. The unprocessed honey settled at the bottom of the glass of water.

d) Light a Fire

The tip of a matchstick was dipped in an unprocessed honey and stricken it to light and it gave the flame easily and burnt off the honey. When it was dipped in processed and bottled honey, it gave smokes instead of burning because of the high moisture content it contain.

e) Thumb Test

A drop of the processed and bottled honey was put on the thumb. The honey spread around the thumb right away and spilled. Another drop of honey from unprocessed honey from the honey combs was purt on the thumb and it stayed intact.

f) Coca cola test

The coca cola test method was carried out by mixing processed honey with coca cola and no fermentation took place. When the unprocessed honey was mixed with honey, fermentation took place.

7.0 Results discussion.

Sample A has moisture content level which is within the European Union Standard (2001), which states that good quality honey should have a maximum moisture content of 20%. The laboratory moisture content of sample A results, for processed and bottled honey is 19.2% and the moisture content of honey in combs (unprocessed honey) is 19.3%. This shows that this honey has no adulteration done to it, which affect its water content. The laboratory results are supported by the Light a Fire and Flame Test. Simple honey test. When matchstick was dipped in an unprocessed honey and stricken it to light and it gave the flame easily and burnt off the honey. It also gave the same results with the processed and bottled honey. The processed and bottled sample and the unprocessed honey sample gave smokeless flame when ignited using candle flame. This simple test supported the laboratory test that there is no water added to the honey. This shows that there is no water added to the honey at Chaseyama, along the Birchenough –Mutare highway where the vendors sell honey at this site. The pH, total ash percentage, reducing sugar percentage, Saccharose percentage, hydroxymethylfurfural (HMF), free acidity, diastase activity and electrical conductivity of honey at Chaseyama, a site along Birchenough Bridge – Mutare Highway (sample A**)**, has street vendors, selling honey which meet the European Union standards (2001). The beekeepers at this site are being supported by a non-governmental organization known as Germ Star in beekeeping. Selling honey on the road side was considered as an alternative way of marketing their produce. These beekeepers are very honest in giving dates when their honey was harvested and processed. This site where they sell their honey is in rural area and closer to the selling point, they have a honey processing plant, where the honey is processed and bottled. Street honey sellers of samples B, C and D were found to be selling adulterated honey. The only honey which was found to be good was the unprocessed honey for the three sites. All the processed honey was found to be adulterated. The honey was not meeting the European Union standards (2001). People living in towns and closer to big town can do anything which can give them money, which includes dirty activities.

8. Conclusion

Several studies prove that pure honey is not toxic to the body, instead it exhibits medicinal effects by reducing excess body weight gain and obesity. Adulterated honeys display effects that are harmful to the body and induce diseases (Abhirami S & R., 2019; Abdulrhman, 2020; Anthony & Balasuriya, 20221). Pure honey contains not only simple sugars (fructose and glucose) it also contains other nutrients such as proteins, antioxidants and minerals which are essential to human health (Ambaw, et al., 2018). Because of that, it is commonly used as a natural sweetener and nutritional food. Its benefit to human health has been known for thousands of years (Anklam, 2021; Belobrajdic, et al., 2021). Adulterated honey lead to diabetics. In addition adulteration negatively influences market growth by damaging customer confidence in honey from unknown sources.

9. Recommendations

(a) Zimbabwe should develop strict national legislation on apiculture sector to avoid unnecessary mix of adulterant materials in honey to protect human health.
(b) Training beekeepers and other stakeholders on how to handle the quality of honey and how to identify the adulterated honey using the simple honey test will help to reduce honey adulteration.
(c) Honey sellers should be registered and their products regularly monitored to control honey adulteration.
(d) Developing simple and cost-effective kits to detect honey adulteration which could be afforded by any member of the society for the people to be able to test the honey quality before consuming the honey.

10. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper

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Author Profile

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I received the certificate in agriculture from Kushinga Phikelela Agriculture Institute in 1991, which is in Zimbabwe. I went to Gweru Teachers' College where I received a diploma in Agriculture Education in 1997. From 1998, I worked in the ministry of education as a secondary school teacher, teaching Agriculture up to 2004. In January 2005 I left teaching and went to the University of Zimbabwe where I graduated with a B.S. Animal Production in December 2007. In January 2008, I joined the Ministry of Agriculture, in the Department of Livestock Production and Development as a livestock Specialist. In 2017, I applied for a study leave and went to Chinhoyi University and Technology where I graduated with a M.S. Applied Entrepreneurship in Agriculture in 2018. In 2020 I applied for a PhD research in beekeeping at Chinhoyi University and Technology which I am doing now. I am very much interested in training the youths in livestock projects, so that one day they will make a living out of these projects when Covid 19 is over