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Assessment of physicochemical, microbiological, mineral and heavy metal parameters of honey

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Abstract

The qualities of the honey were evaluated using the following attributes Physico-chemical, Microbiological, minerals and metallic contaminants respectively. The Physico-chemical analysis revealed a moisture content of 17.45%, Ash 0.64%, Total solid 81.25%, Fat 0.64%, Protein 0.25%, Fibre 0.03%, Total carbohydrate81.22%, Energy value of 329.5 Kcal, pH@24.2oC, Sucrose content 5.48%, Nitrogen content 0.004%, Total acidity 3.45%, Reducing sugar 61.82%, insoluble water content 0.06%, Refractive index of 1.477, and Specific gravity of 1.234 respectively. The microbiological analysis showed a total bacterial count TBC of 7x102 cfu/g, Yeast count 2x101cfu/g and Mould count 2x101cfu/g, which was within the national standards range by SON as 1x102 cfu/g, 5x101 cfu/g, and 5x101 cfu/g respectively while the Coliform count, Escherichia count, Salmonella count, Staphylococus count and Clostridium count were not detected this research. Generally, honey may contain organisms from bees, soil, air and dust that may be introduced during post-harvest handling. This is evidence that honey is well preserved against bacteria so that these organisms would not survive unfavourable conditions. The mineral analysis showed the presences of potassium{K), Calcium (Ca), Sodium (Na), Magnesium (Mg), Zink (Zn), Iron (Fe) Arsenic (As) respectively with values of 70.5, 21.0, 95.0,12.0, 1.3, 1.0, and 1.0 (Mg/100g) while Cupper and Lead were not detected.

Keywords: Physicochemical; Microbiological; Honey; Mineral

1. Introduction

Honey has a long history of human consumption and is used in various products like foods and beverages as a sweeteners and flavouring. Flavours of honey depend on the nectar source of various types and grades of honey that are available [1] Honey is classified by the floral source of the nectar from which it was made. [2]

Honey is a sugary and flavourful product which has been consumed as a high nutritive value food [3]. It is basically composed of a complex mixture of sugars, of which fructose and glucose account for nearly 85–95%, and other minor substances, such as amino acids, proteins, vitamins, and lipids [3] as well as Phytochemicals (such as organic acids, vitamins, and enzymes etc),

Honey naturally contains small amounts of enzymes that are introduced by the bees during various phases of the honey manufacturing process. The predominant enzymes are diastase (amylase), invertase (a-glycosidase) and glucose oxidase. Other enzymes such as catalase and acid phosphatase are generally present in lesser amounts. [4] as well as other substances that may serve as sources of dietary antioxidants. Each of these minor constituents was known to have

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distinctive nutritional or medicinal properties and the unique blend account for the varied different applications of natural honeys. [5] Honey contains varying amount of mineral substances ranging from 0.02 –1.03 g/ 100 g. The main element found in honey is potassium with an average of about one-third of the total [6]. Several investigations have shown that the trace element contents of honey depend mainly on the botanical origin of the honey [7].

The specific composition of any batch of honey will depend largely on the mix of flowers available to the bees that produced the honey. Typical honey analysis shows the following: Fructose: 38.0%, Glucose: 31.0%, Sucrose: 1.0%, Water: 17.0%, other sugars (maltose, melezitose): 9.0%, Ash: 0.17%, others: 3.38%. [8]

The quality of honey relied to a great extent on the art of the producer in storing and blending the product [9] as well as its sensorial, taste and consistency, chemical, physical, microbiological characteristics and floral source and region of origin [2&3] a quality product will enhance and developed the confidence that encourages return of customers due to efficient production of the product. [10].

The changes in the proportions of the original sugars is due to the enzymes present in honey, presence of over 5% of sucrose may be due to artificial feeding of the bees with the sugar during the winter or during periods of drought. [11]

The procedure for processing and storage method of a given sample of honey depends greatly on its moisture content, the probability of undesired fermentation caused by osmophilic yeasts. Honey samples with carbohydrate content < 83% and moisture content > 17.1% are easily prone to fermentation especially when they are kept at temperatures > 11°C. A well processed honey is expected to have a long shelf life and should not ferment. The viscosity of honey is essential to its processing and it has an important link to its technological applications as a super cooled aqueous sugar solution. Honey of high quality is usually thick and viscous. The viscosity of a honey sample is a function of the composition of its sugars, water and colloid content. If the concentration of water is increased, honey becomes less viscous. Proteins and other colloidal substances increases honey viscosity, but their amount in honey may be insignificant. The percentage of fructose content in honey has also been found to affect is viscosity and rheological properties. Honeys become less viscous with increase in fructose content. The viscosity of various honey samples from different sources around the globe had been reported [12&13].

2. Material and methods

2.1. Sample Collection

The honey sample was purchased at Mile 12 Market, close to Izala central mosques Mile 12. The sample was stored in a refrigerator prior to the analysis.

2.2. Physico-Chemical Analysis

2.2.1. Proximate parameters

(Moisture, protein, carbohydrate, fats, crude fibre and ash) were determined using the Association of Official Analytical Chemists, 15th edition [14] method. Nitrogen content of the samples was determined by the Kjedhal method. The weight difference method was used to determine moisture and ash content while crude fat was determine using the above method with petroleum ether as solvent. The carbohydrate content was determined by calculation using the difference method %Carbohydrate = [100-%(protein + fat + moisture + ash+ fiber)]. Digestible crude protein was calculated using the equation: %DCP = 7.87 x log CP – 3.06 (Hagger and Ahmed, 1970) while energy value was estimated using the equation, Energy Value = (4 x protein + 9 x fat + 4 x Carbohydrate).

2.2.2. pH

The PH is the activity of Hydrogen ions in the water and expressed by negative logarithm to the base 10 of the H+ ion activity in moles/L. pH was measured using portable pH meter on sample. The pH meter was first calibrated using buffer solutions as follows

pH 4.7 and 9.2. pH = - log H+; or H+ = (10)-pH.

2.3. Determination of diastase activity

A standard solution of starch, capable of developing with iodine a colour in a defined range of intensity, is acted upon by the enzyme in the honey under standard conditions. The diminution in the blue colour is measured at intervals. A

plot of absorbance against time is used to determine the time tx required to reach absorbance 0.235nm. The diastase number is calculated as 300 divided by tx.

2.3.1. Minerals composition

This was measured by spectrometric methods using Shimadzu AA-7000 Atomic Absorption Spectrophotometer

2.4. Microbiological Analysis

Salmonella, Shigella agar, Violent Red bile agar, Nutrient agar, *Staphylococcus* agar, Potato Dextrose Agar each were prepared according to manufacturer specification. 1g of sample were taking and inoculated in to 9ml of sterile distilled water, this was use for serial dilution (10^{-1}, 10^{-3} and 10^{-5}) and was inoculated in to petri dishes using pour plate. The media of isolation were prepared on each plate and were incubated at 370c for 24-48hrs. [15].

3. Results

Table 1 Physico-chemical characteristics

S/N	Physicochemical Parameters	Unit	Result
1	Moisture content	%	17.445
2	Ash content	%	0.64
3	Total solid	%	81.25
4	Fat content	%	0.64
5	Protein	%	0.25
6	Crude fibre	%	0.03
7	Total carbohydrate	%	81.22
8	Energy Value	Kcal	29.57
9	pH@24.2^{o}C	%	6.09
10	Sucrose content	%	5.48
11	Nitrogen content	%	0.04
12	Specific gravity	%	1.2343
13	Total acidity	%	3.45
14	Reducing Sugar	%	61.82
15	Insoluble water content	%	0.06
16	Refractive index	NS	1.447
17	Viscosity	mpa	962
18	Diastase activity	DN	11
19	Lund reaction	mL	2.6

Physico-chemical characteristics

Table 2 Microbiological Assessment of the sample

S/N	Microbial Parameters	UNIT	RESULT
1	Total Viable Count	Cfu/g	7.0x103
2	Yeast Count	Cfu/g	2.0x101
3	Mould Count	Cfu/g	2.0x101
4	Total Coliform Count	Cfu/g	ND
5	Escherishia Coli Count	Cfu/g	ND
6	Salmonella - Shigella	Cfu/g	ND
7	Staphylococus	Cfu/g	ND
8	Clostridium Count	Cfu/g	ND

Key, ND. Not detected

Table 3 Mineral and heavy metals Composition

S/N	Minerals and Metallic Contaminants	Unit	Result
1	Potassium (K)	(mg/100g)	70.5
2	Calcium (Ca)	(mg/100g)	21.0
3	Sodium (Na)	(mg/100g)	95.0
4	Magnesium (Mg)	(mg/100g)	12.0
5	Zink (Zn)	(mg/100g)	1.3
6	Iron (Fe)	(mg/100g)	1.0
7	Arsenic (As)	(mg/100g)	<1.0
8	Copper (Cu)	(mg/100g)	ND
9	Lead (Pb)	(mg/100g)	ND

Key, ND. Not detected

4. Discussion

The Moisture content of 17.45% was recoded which was within the range of SON standards this variable depends on climatic factors, season of production and maturity of honey. The low moisture content helps to protect and preserved honey for longer periods of time from microbiological action. The pH 6.09% of the sample is within the national regulations by SON range from 3.42-6.10

The Ash content was 0.67% which was maximum range of acceptable limit, the flora origin of honey has been responsible for the variability in ash content as reported by Molan, [16]. Ash content presents the total inorganic minerals available in the sample after incineration [17]. The Codex Alimentarius Commission [18] specified that an ash content of not more than 0.6% for normal honey. The average acidity of honey sample analyzed in the present study was 3.45% was in the range but very low for acceptable range in the world honey market as it ranges between 15.05 - 56.7% [19-20]. Differences in honey acidity could be caused by differences in geographical condition, harvesting, and procedure storage conditions [21] which could be the reason in the case of the studied sample. The total soluble solids of the honey were 81.25 % Most of the total soluble solids for honey are sugars. These account for about 80% or more of solids by weight. The percentage reducing sugars value of honey sample analyzed in the present study was 61.82% as contained in Table 1, which fulfilled the requirements of Quality and Standards Authority of Ethiopia (QSAE) [22]. The percentage sucrose content of the sample analyzed in the present study was 5.48% which was in the ranges between 1.32 % to 10.98 % acceptable ranges in the world honey market. The sucrose content of honey depends on botanical origin of nectar [23]. The specific gravity value of 1.234g/cm were obtained these values were in within the range finding of the values by El-Aab and Al-Amrony [24] (1.2105g/cm 1.2081g/cm and 1.2270g/cm investigated for four different samples from Benghazi in Libya. The specific gravity property of honey has not been legislated by the European legislation [25]. The sucrose content of the honey sample analyzed in the present study was 5.48 which is acceptable range in the world honey market. The sucrose content of honey depends on botanical origin of nectar [26] its level should not exceed 5% according to an International Regulatory Standards. The result of this study reveals that sucrose content of honey samples does not qualify the requirement of an International Regulatory. The higher sucrose content of honey could be the result of an early harvest of honey, in which sucrose has not been converted to fructose and glucose [27] which probably attributed to higher sucrose content

4.1. The diastase activity

The analysis shows an activity of 11DN. Note, that the use of enzyme activities as indicators of honey freshness is often criticized as the enzyme activities in honey depend on the intensity of the nectar flow and the amount of nectar processing by the honey bees [28]. Therefore, honey from very rich nectar sources often show high natural enzyme activities. The law diastase activity is as a result of poor and less rich nectar source.

4.2. Microbiological analysis

Of the honey is shown in Table 2: The total bacterial count TBC was $7x10^2$ cfu/g, Yeast count $2x10^1$cfu/g and Mould count $2x10^1$cfu/g. This was within the national standards range by SON which was $1x10^2$ cfu/g, $5x10^1$ cfu/g, and $5x10^1$

cfu/g, respectively while the Coliform count, *Escherichia* count, *Salmonella* count, *Staphylococus* count and *Clostridium* count were not detected this research. Generally, honey may contain organisms from bees, soil, air and dust that may be introduced during post-harvest handling [29]. This is the evidence that honey is well preserved against bacteria so that these organisms would not survive unfavourable conditions. This result is in agreement with the report that honeys are very low in bacterial and coliform counts.

4.3. Mineral analysis

Showed the presences of potassium{K), Calcium (Ca), Sodium (Na), Magnesium (Mg), Zink (Zn), Iron (Fe) Arsenic (As) with values of 70.5, 21.0, 95.0,12.0, 1.3, 1.0, and 1.0 (Mg/100g) respectively, while Cupper and Lead were not detected. The honey is rich in minerals. The percentage mineral content is considered as a quality criterion indicating the possible botanical origin of honey [30]. The differences in mineral content majorly depend on the type of soil in which the original nectar bearing plant was located [31, 32].

5. Conclusion

This study reveals that honey possesses some nutritional quality that can be used as supplement for human daily needs. The quality and nutritional composition defend on the honey origin, and vegetation type.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declared that, no conflict of interest regarding the publication of this paper.

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