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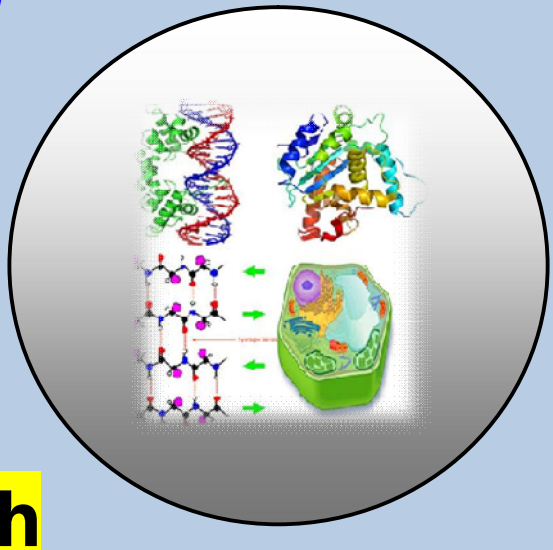
ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Index Copernicus International Value
IC Value of Journal 4.21 (Poland, Europe) (2012)
Global Impact factor of Journal: 0.587 (2012)
Scientific Journals Impact Factor: 2.597

J. Biol. Chem. Research
Volume 31 (2) 2014 Pages No. 891-900



Journal of Biological and Chemical Research
(An International Journal of Life Sciences and Chemistry)

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 31, No. 2: 891-900 (2014)
An International Journal of Life Sciences and Chemistry

ISSN 0970-4973 Print
ISSN 2319-3077 Online/Electronic

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RESEARCH PAPER

Received: 25/03/2014 Revised: 10/07/2014 Accepted: 14/07/2014

Some Physico-Chemical Properties and Microbiological Quality of Honey Sold in Jimma Town, Southwestern Ethiopia

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ABSTRACT

Honey is a natural sweet substance produced by honeybees from the nectar of blossoms or secretion of living parts of plants or excretions of plant-sucking insects living on parts of plants. Microbiological quality of honey being sold in Jimma town was evaluated using standard methods. A total of 30 honey samples were evaluated for some physico-chemical (pH, moisture, metal content, electrical conductivity, ash content) and microbiological quality. Accordingly, the average pH value was 4.98 and the mean moisture contents before and after drying at 105 ^{0}C were 11.1g and 7.85 g, respectively. The highest average metal content was iron (37.4 mg/kg) followed by cobalt (7.1 mg/kg). The mean electrical conductivity (µS/cm) and ash content (g ash/100 g honey) were 343.9 and 0.04, respectively. The mean Aerobic Mesophilic Bacterial count was found to be 2.2x10^4 CFU/g where as count of Staphylococci was 4.11x10^3 CFU/g and that of Enterobacteriaceae was 2.67x10^3 CFU/g. Aerobic bacterial spore count of 7.33x10^3 CFU/g was recorded while counts of yeast and mold were 4.65x10^2 and 5.9x10^2 CFU/g, respectively. No coliform count obtained, indicating that intolerance of acidic condition by the bacteria and/or absence of fecal contamination with the honey. From The present microbial profile study, the honey being sold in Jimma town is unsatisfactory in terms of microbiological quality.

Key words: Indicators, Metal content, Physico-Chemical Properties, Microbiology of honey and Microbial Profile.

INTRODUCTION

Honey is a sweet and viscous fluid produced by bees from the nectar of flowers. The nectar is ripened into honey by inversion of its sucrose sugar into fructose and glucose. It is somewhat acidic and has mild antiseptic properties, being sometimes used in the treatment of burns and lacerations. The definition of honey stipulates a pure product that does not allow for the addition of any other substance (Al-Aakhir, 2012).

According to Anon (2012) honey is as old as history is itself. One of the earliest evidence of honey harvesting is on a rock painting dating back 8000 years, this one found in Valencia, Spain shows a honey seeker robbing a wild bee colony. The bees were subdued with smoke and the tree or rocks opened resulting in destruction of the colony. Humans have eaten it, bathed in it, fixed their wounds with it and traded with it since history was recorded. Archaeologists discovered honey comb in Egypt that had been buried with the pharaohs in their tombs, the honey was preserved and was still edible. Honey is most commonly consumed in its unpreserved state, i.e. liquid, crystallized or in the comb. In these forms, it is taken as medicine, eaten as food or incorporated as an ingredient in various food recipes (Burgget, 1985). In confectionery production, honey is still included in many traditional products, which are consumed locally in considerable quantities and also exported. In gelatinous or gum product, honey can be used as flavoring agent. In industrial sector, some honey milk products exist such as pasteurized and homogenized sweetened with honey for long time storage e.g. yoghourt with honey (Ebisu et al.,1988). Africa is blessed with numerous types of wild honeybee (Adjare, 1990). Ethiopia is one of the countries of the continent which own big honey production potential and the largest honey producer in Africa and 10th largest honey producer all over the world with considerable amount of wax are produced in the country (Deffar, 1998). Ethiopia, having the highest number of bee colonies and surplus honey sources of flora, is the leading producer of honey and beeswax in Africa. Owing to its varied ecological and climatic conditions, it is home to some of the most diverse flora and fauna in Africa. Its forests and woodlands contain diverse plant species that provide surplus nectar and pollen to foraging bees (Deffar, 1998). Beekeeping is one of the oldest farming practices in the country. There is an ancient tradition for beekeeping in Ethiopia which stretches back into the millennia of the country's early history (Deffar, 1988). Of all countries in the world, probably no country has a longer tradition of beekeeping than Ethiopia (Hartmann, 2004). It has been practiced traditionally. Moreover, beekeeping is an appropriate and well-accepted farming technology and it is best suited to extensive range of ecosystems of tropical Africa. To date, over 10 million of bee colonies exist, which include both feral, and hived ones (Ayalew, 2001). Ethiopia and Tanzania produce about 2.5% and 1.15% of total world honey production, respectively. The total honey production of Ethiopia is estimated up to 24000 metric tonnes; only a small amount of this is marketed. Besides poor marketing conditions the main reason is that about 80% of the total Ethiopian honey production goes in to the local Tej-preparation, a honey wine, which consumed as national drink in large quantities (Hartmann, 2004).

Microorganisms in honey may influence quality or safety. Due to the natural properties of honey and control measures in the honey industry, honey is a product with minimal types and levels of microbes. Microbes of concern in post-harvest handling are those that are commonly found in honey are yeasts and spore-forming bacteria. Honey also support microorganisms like coliforms and those that under certain conditions could cause human illness. Primary sources of microbial contamination are likely to include pollen, the digestive tracts of honey bees, dust, air, earth and nectar, sources which are very difficult to control. The post harvest sources of microorganisms that influence any food product are also sources of contamination for honey because it is a ready-to-eat food. These include air, traditional way of handling, processing starting from harvesting process to consumption, materials used, storage condition, cross-contamination and selling buildings.

To this effect the present study is aimed at investigating the physicochemical properties and microbial quality of honey being sold in Jimma town.

MATERIAL AND METHODS

Description of Study Site and period

The study was conducted at Jimma town, located 353 km southwest of Addis Ababa, and an average altitude of 1, 780 m above sea level (Alemu *et al*., 2011).

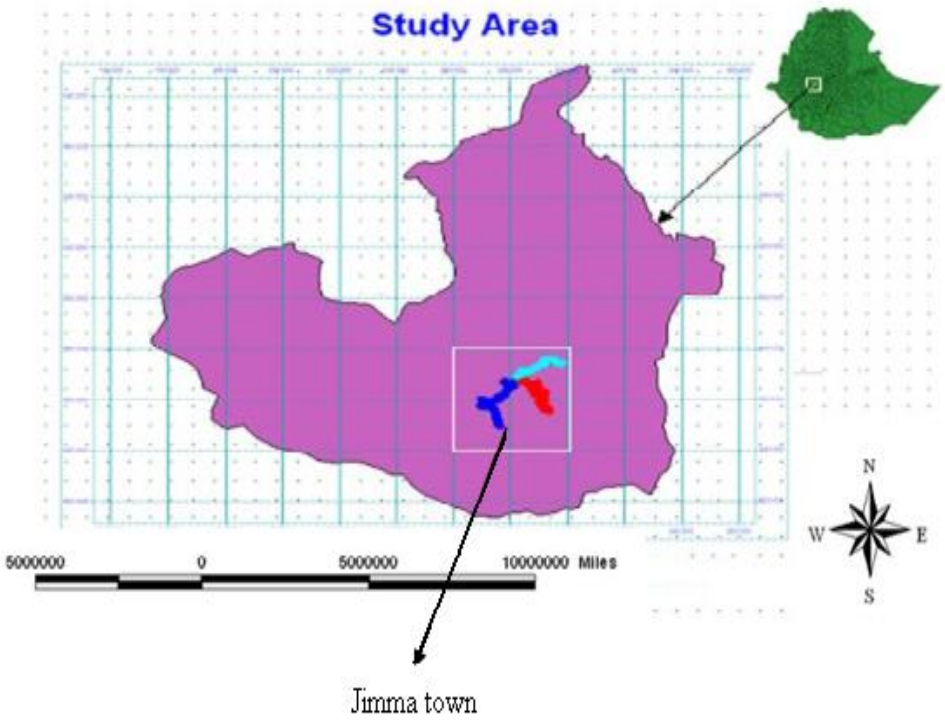

Figure 1. Map of Study Site (Alemu et al., 2011).

Microbiological analysis

Sample preparation

About 250 g of honey was collected from each seller of Jimma town using sterile plastics. The collected honey samples were analyzed for microbiological quality, including the enumeration of total aerobic mesophilic count, Enterobacteriaceae, Coliforms, yeast and molds, Aerobic bacterial Spore, and Staphylococci for the evaluation of sanitary practices. Accordingly, 25 g of honey each sample was mixed with 225 ml sterile peptone water and homogenized in a flask for five to ten minutes using orbital shaker at 110 rpm. After homogenization, 1 ml of each sample was transferred aseptically into 9 ml of sterile peptone water and mixed thoroughly by using vortex. The homogenates were serially diluted (10^{-1}-10^{-5}) and 0.1 ml aliquot of appropriate dilutions were spread-plated in duplicates on pre-solidified plates and incubated at appropriate temperature and time as indicated under methodology for counts of different microbial groups. The colonies were enumerated from countable plates and then expressed as colony forming units (CFU/ g).

Some physico-chemical characteristics of honey samples

The pH of 30 honey samples was measured using a pH meter from the homogenized mixture of 25g of honey and 225ml of peptone water. The moisture content was obtained by drying 10g of honey sample in a hot air oven at 105°C until a constant weight was attained.

Electrical conductivity of a solution of 20 g dry matter of honey in 100 mL distilled water was measured using anelectrical conductivity cell at 20°C (EC Tester 11, Multirange,Malaysia). The result is expressed in Micro Siemens per centimeter (($\mu S.\ cm^{-1}$) (AOAC, 1995). Determination of electrical conductivity. A 10% w/v (about 1 g of honey and about 9 mL of boiled warm water) solution of honey in freshly warm distilled water was dissolved using glass rod in a 50 mL beaker [18]. The conductivity meter was calibrated using standard calibration solution at 20 ^{o}C. The result is expressed in Micro Siemens per centimeter ((µS. cm-1) (AOAC, 1995).

Ash content was determined by ignition at 550°C in a furnace to constant mass (AOAC, 1990).

Determination of total ash content: Stainless steel crucibles (FB1400, Model Furnace, US) were washed, rinsed with distilled water and oven dried at 105^{o}C. About 5-10 g of honey samples were weighed and placed in a furnace first at 110^{o}C for half an hour and then at 550 oC for two hours to constant weight. Care was taken during heating so that no excess foaming took place. Then the honey samples were kept in open air for cooling and the constant masses after heating were measured. Finally ash content was calculated as g ash/100 g of honey (AOAC, 1995).

Determination of mineral content: Twenty honey samples were used for determination of the content of selected metals (copper, nickel, iron, manganese, zinc, chromium, cobalt and cadmium was determined at 324.8, 232.0, 248.3, 279.5, 213.9, 357.9, 240.7 and 228.8 nm, respectively and using air-acetylene flow where, the acetylene flow was made 1.5) was done in triplicate using flame atomic absorption spectroscopy, Analytik Jena NOVAA 300 AAS (Germany).

Microbial Enumeration

From appropriate dilution (1:10), 0.1ml of aliquot were spread Plated in duplicate on pre-dried surfaces of plates of Plate Count Agar ,MacConkey agar, Violet Red Bile Agar ,Nutreint Agar and Manitol Salt Agar for the counts of Aerobic Mesophiles, Enterobacteriaceae, Coliforms, Aerobic spore formers and *Staphylococci* ,respectively. Colonies were counted after the plates were incubated under aerobic condition at 30-37 ^{0}C for 24-48 hours. For fungul enumeration from appropriate dilutions (1:10), 0.1ml aliquot were spread-plated on pre-solidified surfaces of Potato Dextrose agar supplemented with 0.1g Chloramphenicol and incubated at 25-28 ^{0}C for 5-7 days. Smooth (non-hairy) colonies without extension at periphery (margin) were counted as yeasts. Hairy colonies with extension at periphery were counted as molds.

Microbial Analysis (floral assessment)

After enumeration of colony of aerobic mesophilic bacteria, 10-15 colonies with distinct morphological differences such as color, size and shape were randomly picked from countable plates and inoculated in to tubes containing about 5 ml nutrient broth . These were incubated at 30–32^{0}C over-night. Cultures were purified by repeated plating and maintained on appropriate slants at 4^{0}C for further characterization.

Characterization of the isolates

The preliminary tests included microscopic observation for cell morphology (cell shape, cell arrangement and presence/absence of endospore), Grams reaction, KOH-test, production of Catalase test, Cytochrome Oxidase test and O/F test (oxidative or fermentative utilization of glucose) following standard microbiological methods.

RESULTS

Physico-chemical analysis

From the total of 30 samples the highest (5.22) and lowest (4.81) pH values were obtained in Ag8 and Ag 9 samples with an average of 4.98 pH value. From samples taken and dried in dry air oven at 105^{o}C, the maximum moisture content was observed in M11(11.39) and the lowest moisture content was observed in M5 and Ag 1 (10.7 each) before oven-drying of the samples. On the other hand, the maximum and minimum moisture contents were observed in M4 and M9 (8.22) and M12 and Ag 8 (7.09), respectively after dry in oven (Table 1).

From the twenty honey samples considered for analysis of five selected metals (Fe, Cr, Co, Zn and Cu) as depicted in table 2, the contents (mg/Kg) of Fe and Co were found to be higher in all samples.

Microbial count

The mean of different microbial count from hone samples is summarized in Table 3. Accordingly, aerobic mesophilic count was found to be 2.2x10^4 CFU/g followed by *Staphylococci* (4.11x10^3 CFU/g) and that of Enterobacteriaceae (2.67x10^3 CFU/g). Generally, Counts of yeasts and moulds were less than counts of bacteria.

Table 1. Some Physico-chemical characteristics of honey samples sold in Jimma Town.

Sample	pH	Moisture cont		Electrical Conductivity (μS/cm)	Total ash (g ash/100 g honey)
		Before oven-dried	After oven-dried		
M1	4.95	11.36	7.96	422	0.01
M2	4.93	11.25	7.83	341	0.05
M3	4.97	11.234	8.072	451	0.06
M4	4.94	11.25	8.22	220	0.02
M5	4.89	10 .7	7.25	420	0.05
M6	4.9	11.21	7.93	440	0.05
M7	5.00	10.98	7.98	329	0.05
M8	5.11	11.174	7.754	402	0.05
M9	4.96	11.25	8.22	298	0.03
M10	5.15	11.36	7.78	314	0.02
M11	4.95	11.39	8.21	270	0.02
M12	4.99	11.02	7.09	410	0.03
M13	4.93	11.41	8.1	238	0.03
Ag1	5.01	10.7	7.858	298	0.06
Ag2	4.89	10.8	7.23	354	0.04
Ag3	4.97	11.05	8.072	263	0.06
Ag4	4.94	11.56	7.98	228	0.03
Ag5	5.1	10.98	7.83	420	0.05
Ag6	4.92	11.01	8.002	250	0.06
Ag7	4.83	11.23	8.1	312	0.04
Ag8	5.22	11.02	7.09	422	0.01
Ag9	4.81	11.05	8.02	341	0.05
Ag10	5.19	11.01	7.574	451	0.06
K1	4.99	10.91	7.4	220	0.02
K2	4.87	11.36	7.78	420	0.05
K3	5.02	11.03	8.004	440	0.05
D1	5.09	10.79	7.75	329	0.05
D2	5.00	10.85	7.41	402	0.05
H1	4.99	10.98	7.98	298	0.03
H2	4.89	11.151	8.101	314	0.02

Table 2. Final metal content (mg/kg) of honey sold in Jimma Town.

Sample	Fe	Cr	Co	Zn	Cu
Ag10	30.04	0.04	3.54	0.46	0.86
Ag1	16.87	0.65	6.71	6.31	0.48
D3	29.96	1.25	7.46	0.05	0.76
K2	49.84	7.84	8.04	0.38	0.93
M8	25.65	2.50	9.51	0.91	1.22
M13	38.27	8.55	8.88	2.84	0.93
Ag2	50.99	4.88	8.16	1.38	0.90
M2	32.10	6.05	4.93	1.63	0.95
M3	51.94	2.75	3.95	3.72	0.68
M4	45.55	4.93	7.08	5.36	0.70
M5	54.60	5.93	6.92	0.25	0.86
M6	34.81	3.86	8.00	0.12	0.83
M10	44.94	8.53	8.04	0.96	0.98
M11	28.84	1.46	6.93	0.14	0.70
Ag3	50.60	2.90	4.90	0.79	0.70
Ag4	47.30	4.00	5.10	0.22	0.83
Ag5	26.41	3.71	9.00	0.26	0.90
Ag7	31.96	0.93	8.80	0.38	0.19
Ag8	25.20	1.21	7.21	0.05	0.62
Ag9	32.39	3.10	8.09	0.11	0.62

Table 3. Mean counts of 30 samples in CFU/g in Jimma Town.

Mean counts of 30 samples (CFU/g)					
Aerobic mesophilic count	*Staphylococci*	Enterobacteriaceae	Aerobic bacterial spore count	yeast	Moulds
$2.2x10^4$	$4.11x10^3$	$2.67x10^3$	$7.33x10^3$	$4.65x10^2$	$5.9x10^2$

Morphology and Biochemical tests

The morphology and biochemical tests of 78 isolates from 30 samples revealed that the isolates displayed diverse morphological and biochemical features (Tables 4 and 5). Of the total 78 isolates, most of the isolates (92%) were Gram positive, of which about 23% were sporing (Table 4). Biochemically, the majorities (89.7%) of the isolates were oxidative and the majority (about 92%) of the isolates showed catalase positive. The cytchrome oxidase test also confirmed that the majority of the isolates (%) were positive for the test (Table 5).

Table 4. Morphological features of the 78 isolates from honey sold in Jimma Town.

Morphological features		No. of colonies
Cell shape	Rod	32
	Cocci	46
Colony arrangement	Diplobacilli	24
	Chain	24
	Grape like/clustered	22
	Single bacillus	8
Endospore	Present	21
	Absent	57
Gram reaction	+	72
	_	6

Table 5. Biochemical tests of the isolates in Jimma Town.

Biochemical tests of the isolates		No. of colonies
O/F test	Oxidative	70
	Fermentative	8
Catalase test	+	72
	_	6
Cytochrom test	+	27
	_	51
KOH test	+	72
	_	6

DISCUSSIONS

The Physico-chemical property of honey sold in Jimma town revealed that it is of quite acidic as the pH values were in the range of 4.81-5.22 with an average pH value of 5.01. Similarly, Joseph *et al* (2007) and Malika *et al*, (2005) reported pH values of honey between 3.4 and 6.1 and 4 and 4.5, respectively. Such acidic nature of the honey could be responsible to slow down or prevent the growth of many species of bacteria as relatively low level of microbes were detected. However, few acid tolerant groups such as yeasts can manage growth in such environment. Furthermore, the low moisture content of honey has a potential for reduction of many microbial growth as water activity plays significant role in controlling microbial growth. On the other hand, Malika *et al* (2005) have reported variation of moisture in honey samples from different origins could be due to the composition and floral origin. The strong interaction of sugar with water molecules may decrease the water available for microorganisms. The low moisture content of honey also forms an important part of the system which protects honey from attack by microorganisms.

The moisture content of honey may be widely related to the harvest season in Jimma and the level of maturity released in the hive. This parameter was highly important for the shelf life of the honey during storage. The metal content analysis from honey samples in the current study could be due to various factors such as emission of automobile exhaust gases, botanical origin, geographical conditions (Nigussie *et al*., 2012). The highest mean iron content (37.4 mg/kg) in the current study is also in agreement with a report by Nigussie *et al*. (2012) which could partly be due to the frequent metallic containers used during storage and transportation as observed during sample collection. Ash content and electrical conductivity are among the important parameters related to honey mineral content. From the present study, values of electrical conductivity are in acceptable range (≤ 0.7 mS/cm) as reported by Malika *et al*., (2005). The mean Aerobic mesophilic count ($2.2x10^4$ CFU/g), *Staphylococci* ($4.11x10^3$ CFU/ g) and Enterobacteriaceae ($2.67x10^3$ CFU/g) in the present study is unsatisfactory in terms of microbiological quality as described by Gilbert *et al*., (2000). Such overall microbial load per gram of honey was a signal regarding possibility of pathogen presence as the detection of microorganisms such as enterobacteriaceae reveal poor quality (if not safety) of honey probably during processing, handling or storage. Such poor processing and or handling/ storage could also have significant implication in affecting the shelf-life of honey. In contrast to the present study, Malika *et al*, (2005) obtained higher number of aerobic mesophilic count with a minimum count of $2.6x10^3$ CFU/ g and a maximum count of $4.54 x10^4$ CFU/g. This variation may be because of different reasons like air condition of the area, traditional way of handling, processing starting from harvesting process to consumption, materials used, storage condition, cross-contamination and selling buildings. However, the present study was higher than Malika *et al*, (2005) report, where aerobic bacterial spore counts were found in a minimum count of $1.8x10^3$ CFU/ g and a maximum count of 1.68x104 CFU/g. Similar to the present study, *Staphylococci* and Enterobacteriaceae had minimum count of $2x10^2$ CFU/g and a maximum count of $1.12x10^4$ CFU/g and with a minimum count of $2x10^2$ CFU/ g and a maximum count of $1.2x10^4$ CFU/g, respectively. Total coliforms were not detected in any sample similar to (Malika *et al*, 2005). Regarding the load of yeasts, current study indicated higher load/g ($4.65x10^2$) in contrast to previous report by Malika *et al* (2005) who reported $1x10^2$ to $1.9x10^3$ CFU/g. The presence of high amount aerobic mesophilic bacteria, *Staphylococci* and aerobic spore former could reduce the quality of honey and may have potential risk as honey is ready-to-eat food. The higher aerobic counts could be due to the poor general sanitation of the seller and/or environment. On the other hand, the presence of Staphylococci could be an indicators poor handling and contact with bare hand. Existence of aerobic bacterial spore count revealed the contamination of honey with spore forming bacteria such as *Bacillus* in adverse condition. It is therefore necessary that close supervision of such ready-to-eat food should be carried out by relevant authorities to reduce the risk of food poisoning.

ACKNOWLEDGEMENTS

The authors would like to extend their gratitude to the Department of Biology, College of Natural Sciences, Jimma University for supporting this research.

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