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Short Communication

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Proximate Value of Honey within Ashanti Mampong Market Enterprises in Ghana. A Short Communication

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Abstract *Background:* Nutritionally, honey has many derived health benefits as a constituent of sugars and other materials. Objective: Proximate composition analysis (PCA) of honey was conducted in the Ashanti Mampong Municipality. *Materials and Methods:* Five honey sample types (A, B, C, D, E) purposively collected stored in clean tightly sealed glass bottles from local markets enterprises in the Ashanti Mampong Municipality screening. Prior to analyses, bottles containing crystalized honey were placed in a water bath. This was done to liquefy the honey for easy handling and analysis. The PCA was run for moisture, crude protein, ether extract (crude fat), crude ash, carbohydrates, crude fibre contents which depict honey quality. *Results:* Comparatively ascertained average moisture content (MC = 18.0 %); crude fibre (CF = 0.7% to 1.5%), confirms the honey's marketable safety, even though CF of samples A, D (1.23±0.279 %; 1.46±0.279%, were respectively above the 1.04±0.279%) expected. Although the honey fat contents fell within 0.2 to 0.5%, ash contents were generally above ≤ 0.6%.The carbohydrate (79.778 %), protein (0.2-0.5% averagely 0.33%) levels conformed to 60-80%, 0.2-0.5%respective guidelines stipulated by the Codex Alimentarius Commission's International honey acceptable standards. Conclusion: Honey distributers along market value chains should be properly educated on safe handling/processing to protect the health of public consumers by the Municipality Environmental Health Department. Further honey quality analyses can thoroughly screen trace elements, pesticides, Antibiotic Resistant Bacteria (ARBs), Antibiotic Resistant Genes (ARGs) as contaminants from different engineered or natural hives constituting honey storage devices.

Keywords Honey, proximate composition, safety, Codex Alimentarius Commission

Introduction

Honey is a natural product considered as an unsalted, high viscosity, nutritious substance that is produced by honeybees [1]. This occurs when the sugary fluid secreted within flowers and other sweet accumulated from plants in the form of nectar are converted and stored in the honeycombs by the honey bees [2]. Comb honey and extracted honey are the two commonly patronized types found in commercial markets. Comb honey is classically presented in its original comb portions thereof, whereas, extracted honey is the type usually removed from the comb and presented in several forms. Originality of honey firmly depicts its unique characteristics features such aroma, colour, composition, flavour in food value chains [3].

The composition of honey which is highly variable, but determines its sweetness. Honey taste depends upon the environment the original nectar is gathered which includes plants source, seasonal and geographical differences [4]. Its sweetness is also stemming from distinct constituents of carbohydrates or sugars (glucose, fructose), and water which forms most part of the honey. The mass ratio carbohydrates content of pure honey is at least 60% [5]. The moisture content of honey is air-dependently affected by relative humidity and temperature of an area. It is internationally recognized that high-quality honey should be have a moisture content under 20%. Beekeepers must be able to control the moisture level with a good degree of accuracy. As the extraction period draws closer, beekeepers will use a honey refractometer to measure the concentration of water in honey. Once the moisture content reaches a desirable level, it can be removed from the comb for processing [6]. Other minor constituents of honey include vitamins, minerals, dietary antioxidants, proteins, biologically active compounds found in plants such as organic acids, vitamins, enzymes [7].

Honey, anutritional substance has many health benefits which may derive from the arrangement and constituents of the types of sugars present and other materials, mostly from the activities of the bees in the honey production [8]. These health benefits include the treatment of minor illness such as fight acne, colds, fatigue treat burns, clear bladder infection and sinuses, stop arthritis pain, relieve toothache, aided fertility, digestion, weight loss, and strengthening of the immune system [9-10]. According to Jegede, China is the leading producer of 650,000 metric tonnes of honey among the top ten countries worldwide followed by Turkey (115000 MT), USA 165million pounds, Iran (> 79,000 MT), Russian federation (> 95000 MT), India (38177.08 MT), Mexico (57000-62000 MT), Brazil (country with the purest honey source), Ukraine (65000-75000 tonnes), and New Zealand (> 20000 tonnes) annually when the world honey production increased by 10-15% within10 years (2010-2020) [11]. Ethiopia remains the leading producer of honey in Africa with >50,000 MTs annually as of December, 2020 even though Nigeria is touting to soon be leading [12-13].

The tropical climate with respect to the dynamic agroecological conditions of Ghana endows the country for honey production [14]. Humid weather conditions favour nectar production from various flowering plant species and sustains the functions of honey bees. Although, Ghana seldom meets the local demand for honey, this is achievable through the importation of bottled honey distributed in supermarkets and shops countywide [15]. Total income generated from honey was about1,076,378 U.S.A.$ in Ghana when production increased from 236,795kg in 2007 to 428,836kg in 2008. The Volta Region recorded the highest income of 235,940 U.S.A.$, Brong-Ahafo Region recorded 185,961 U.S.A.$, Central Region of 137,548 U.S.A.$ and the Upper East (26,935) and Upper West Regions (30,677) recorded the lowest income from honey production in the same year [14]. Though honey is consumed by majority of Ghanaians, there is inadequate systematic research done to determine whether honey from different areas has the same properties and conform to international standards. In this study, different honey samples from different market centres were investigated for its proximate values. Parameters screened included percentage ash, moisture, mineral, fibre and carbohydrate (sugar).

Materials and Methods

Five honey samples used in this study were obtained from local markets designated A, B, C, D and E in Mampong Municipality were collected and stored in clean glass bottles and sealed. The tightly sealed bottles containing the samples were analysed. Prior to analysis, bottles containing crystalized honey were placed in a water bath. This was done to liquefy the honey for easy handling and analysis. Analysis of the honey samples was done for the presence of moisture, crude protein, ether extract (crude fat), crude ash, carbohydrates, and crude fibre.

Determination of Moisture Content

The five honey samples were dried in the oven for 72 hours at 60^{o}C to determine their moisture contents as fresh. The moisture contents were determined to ascertain the shelf lives of the honey samples. The moisture content was computed by the difference in weight before and after drying in the oven and the value expressed in % in terms of the initial weight taken.

$$\text{Moisture (\%)} = \frac{(W1 - W2)}{W1} \text{ x } 100$$

Where, W1 = weight (g) of sample before drying; W2= weight (g) of sample after drying.

Dried residues of the honey samples were then milled into uniform particle size and used for the determination of the other proximate values namely; crude protein, crude fat, crude fibre, crude ash and carbohydrate.

Determination of Crude Protein

The determination of crude protein was by the Kjeldahl procedure. The Kjeldahl procedure can be basically divided into three parts: (1) digestion, (2) distillation, (3) titration. Two (2.0) grams of each of the five samples was digested with 25ml of concentrated H_2SO_4 and boiled for about 60mins with intermittent swelling to get every particle digested.

$$\text{Protein} \xrightarrow[\text{Heat, catalyst}]{\text{Sulfuric acid } (NH_4)_2SO_4}$$

After the digestion, the digest was allowed to cool and diluted with 80ml of distilled water. The diluted digest was then subjected to a distillation process by the addition of 40% NaOH and collected over 5% Boric acid with mixed indicator using Khejdahl nitrogen distillation apparatus.

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_3 + Na_2SO_4 + 2H_2O$$

$$NH_3 + H_3BO_3 \text{ (Boric acid)} \longrightarrow NH_4 + H_2BO_3^-$$

The distillate was then titrated against a standard acid of known concentration. This analysis determines total nitrogen and not usable nitrogen and this is the reason it is call a crude protein analysis. The total nitrogen in the honey samples were computed and the crude protein obtained by multiplying the total nitrogen by 6.25 which is a protein factor. The crude protein is then expressed in %.

Determination of Ether Extract (Crude Fat)

Two (2.0) grams of each of the honey samples were weighed into an enveloped filter paper and stapled. Each of the enveloped samples was subjected to a Soxhlet extraction process using petroleum ether as the extraction solvent. An initial weight of the Soxhlet flask was taken before the start of the process after drying in the oven for 30 minutes to obtain a constant weight. The extraction process was left uninterrupted for two hours after which the flask was re-dried in the oven for 30 minutes to expel the any ether, allowed to cool and re-weighed. The weight of crude fat was then obtained from the difference of the initial and final weight of the flask and the value expressed in % in terms of the initial sample weight taken. The total fat content (w) in g/100 g (corresponds to %) of the sample is calculated using the following formula:

$$W = \frac{(m_2 - m_1)}{m_0 \, x \, 100}$$

Where: m_1: Mass of the empty Soxhlet extraction flask with boiling stones in g

m_2: Mass of the Soxhlet extraction flask with fat after drying in g

m_0: Weight at the start of the analysis in g

Determination of Crude Fibre

The determination of the crude fibre is dependent on the residue obtained from the ether extraction. Each of the residues obtained from the ether extraction was dried in the oven for 30 minutes at 110 ^oC to expel any ether in the residue. The weight of the residual content was then weighed into a Lab conical flask and subjected to acid and alkali digestion. The content was firstly digested with dilute H_2SO_4 followed by digestion with dilute NaOH and subsequent washing of the residue with ethanol. The acid digestion and the alkali digestion eliminate the protein portion and the soluble carbohydrate portion of the material, which is referred to as nitrogen- free extracts (NFEs) respectively. The residue was then dried in the oven for two hours at 135^oC. The residue after drying is composed of

mainly the non-digestible carbohydrate, which is termed crude fibre and minerals. Hence, the ashing of the residue eliminates the organic matter thus the crude fibre of the material leaving the inorganic portion, which is the mineral. The crude fibre of the material was therefore obtained by the difference between the dried residue and the ash. This was expressed in % in terms of the initial weight thus the weight after ether extraction. The %age of crude fibre (wet weight basis) is calculated as follows:

$$\% \text{ crude fibre} = \frac{(W2 - W1)}{W1} \text{ x } 100$$

Where; W1 = ash and, W2 = dried residue.

Determination of Ash Content

Two (2.0) grams of each of the dried honey samples were weighed into already weighed crucibles in fivefold which were dried in the oven for 30 minutes to obtain constant weight. The fivefold crucible containing the samples was placed in a muffle furnace for two hours at 600^{o}C at constant heat. After the two-hour period, the fivefold crucible was removed from the furnace, allowed to cool and re-weighed. The weight of ash of the various samples was obtained by subtracting the weight of the empty crucible from the weight of crucible with the ash. The ash content of the various samples was expressed in %age in terms of the initial weight of sample taken. The %age ash values were obtained from averages of the fivefold determination.

$$\% \text{ ash content on dry basis,}$$
$$(C) = \frac{(W2 - W1)}{(B - A)} \text{ x } 100$$

Where: A= weight of the crucible; B= weight of crucible and sample after evaporation; C= weight of crucible and sample after ashing

Determination of Carbohydrate Content

The total %age carbohydrate content was determined using the difference method as reported by the AOAC method. The total protein, crude fat, moisture and ash content of the sample were added and subtracted from 100%. The value obtained is the %age carbohydrate content. Carbohydrate contents of the honey samples were determined by calculation (by difference) as follows:

% Carbohydrate=100 % – (%Moisture+%Crude Fat+%Crude Protein+%Ash)

Results and Discussion

The analytical proximate values and % differences in honey nutritional value of honey samples components relative to international standards are summarily presented (Tables 1, 2).

Table 1: Analytical proximate values of honey samples

Samples	% Carbohydrate	Crude Protein	Crude Fibre	Crude Fat	Crude Ash	Moisture Content
A	80.40±1.109	0.24±0.084	1.23±0.279	0.47±0.103	1.67±0.146	17.22±1.045
B	79.08±1.109	0.35±0.084	0.79±0.279	0.39±0.103	1.83±0.146	18.35±1.045
C	80.15±1.109	0.32±0.084	0.94±0.279	0.45±0.103	1.43±0.146	17.65±1.045
D	81.02±1.109	0.28±0.084	1.46±0.279	0.36±0.103	1.58±0.146	16.76±1.045
E	78.24±1.109	0.46±0.084	0.87±0.279	0.21±0.103	1.66±0.146	19.43±1.045
Mean	79.778	0.33	1.058	0.376	1.634	17.882
STD	1.109	0.084	0.279	0.103	0.146	1.045
CV (%)	1.39	25.36	26.41	27.35	8.92	5.84

Table 2: Percentage differences in honey nutritional value components relative to international standards

Parameter	Mean ± SD	(%) differences between maximum and minimum values	International standard recommended Value	Samples with values above expected international standard.
Carbohydrate (Glucose+Fructose)	79.778±1.109	80.887-78.669 =2.218	60-80%	A, C, D
Crude Protein	0.330± 0.084	0.414-0.246 = 0.168	0.1-0.4%	E
Crude fibre	1.058±0.279	1.337-0.779 = 0.558	-	
Crude fat	0.376±0.103	0.479-0.273 = 0.206	-	
Crude ash	1.634±0.146	1.780-1.488 = 0.292)	≤ 0.6%	None
Moisture content	17.882±1.045	18.927-16.837=2.090	≤ 21%	None

Proximate analysis (PA) is carried out to ascertain for the nutritional value contents of food products [5]. It practically offers for advanced application of Classical, novel approaches to the analysis of honey and detection of adulterants in order to safeguard public health [16]. Principally, moisture content (MC) is necessary parameter to check in determination of honey shelf-life which influences other the principal characteristics (viscosity, weight, flavour, preservation, and palatability) of honey Analytical MCs of the honey samples ranged from 16% to almost 20%. This result may be related to the relatively low moisture levels of the ambient air at which honey is displayed for sale in shops within the Mampong Municipality. The Codex Alimentarius International Food Standard has approved MC of not > 21% for marketed honey acceptability for distribution or consumption purposes [17]. The mean moisture level of almost 18.0 % obtained for honey samples confirms its marketable safety based on its conformity to the Codex Alimentarius requirement.

Comparatively, the crude fibre content ranged from 0.7% to 1.5%. Sample A, D, contained higher(1.23±0.279%, 1.46±0.279%, respectively above the 1.04±0.279%) standard average crude fibre level of the entire marketed honey samples examined. Ash content (AC), an important aspect of honey proximate value is issued to examine the floral origins apart from denoting the mineral content during nutritional evaluation [18]. An acceptable standard deviation (SD) of 0.15% for the AC has been stated by the National Honey Board [2]. Detective SD = 0.146 %, of the analysed honey samples AC categorically falls within the 2003 NHB standard acceptability margin. The ACs were above the ≤ 0.6% guidelines stipulated by the Codex Alimentarius Commission. Honey normally has low ash content and the variability among different samples depends upon the sources of materials and propolis garnered by the bees during foraging [7]. High AC of the analysed honey samples may be due to factors such as soil and atmospheric conditions and the type of plant species from which the nectar propolis were obtained [19].

Additionally, the fat contents of the honey samples fell within 0.2 to 0.5%. Honey is undoubtedly a crude source of carbohydrate at varying concentrations. Also, da Silva et al clarified that honey contains at least 60% carbohydrate [16]. However, average value (79.778 %) in the analyses was closer to the ranged absolute concentrations/standard deviations (78-81%±1.109) per individual samples screened. Higher carbohydrate content of honey suggests its potential to impart more energy into human body upon consumption to boost performance of higher energy demanding psychomotor activities such as athletics, driving, dancing, boxing apart from promoting dissipation of antioxidants in the blood, heart health, wound healing among others [20-22].

About 40–65% of the total amount of nitrogen in honey is in protein with the remaining part been amino acids. Honey protein content varies depending on the honey bees species. *Apis mellifera* honey contains between 0.2% and 1.6% protein while *Apiscerana* honey contains from 0.1% to 3.3% protein [23-24]. Amount of honey protein detected in the analyses were generally between 0.2-0.5%, although about 0.33 % was averagely ascertained. Despite that, pollen constitutes the main source of protein, amino acids, alongside proteins enrichment in honeys are attributed to both vegetable and animal sources, including nectar secretions, fluids of the salivary glands and

pharynx of honeybees [25-27]. Except the honey carbohydrate and moisture contents which were slightly > 2 %, the actual differences between highest and lowest proximate values were < 1 %, and acceptable within the 2001CODEX Alimentarius Standards.

Conclusion & Recommendation

Summarily, the honey samples randomly collected, analysed from sellers in Mampong Municipality were found to almost conform to international standard. Imperatively, extractors and sellers should be well educated on approved honey processing and handling standard to safeguards the health of public consumers of honey in the Municipality. However, further studies are recommended on the physicochemical, trace elemental, pesticides, microbial and antibiotic resistant genes traces in the honey harvested from different wood hives, natural hives and honey storage devices in order to comparatively determine the quality characteristics for marketing and consumption purposes.

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