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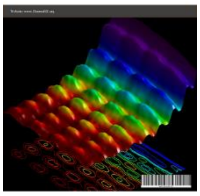

Phytocompounds, Heavy Metal and Mineral Contents in honey Samples from Selected Markets in the Kumasi Metropolis

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Abstract

The present study sought to determine the content of calcium (Ca), magnesium (Mg), lead (Pb), iron (Fe), zinc (Zn) and eight phytocompounds in honey collected from twenty–three markets in the Kumasi Metropolis of Ghana. The mineral and heavy metal contents were analysed using the Atomic Absorption Spectrometry (novAA® 400P) and phytoconstituents by standard qualitative procedures. This study revealed that Mg and Ca concentrations ranged from 3.61–15.93 mg/kg and not detected–3.24 mg/kg, respectively. The concentrations of Ca and Mg were within the Recommended Dietary Allowance and Nutrient Reference Value respectively; an indication that the honey can be used as food source rich in Mg and Ca. The levels of Fe, Zn and Pb were lower than the limit of detection, indicating their safety from metal contaminants. A considerable number of phytoconstituents were detected. In general, the results obtained indicate that the honey samples collected from the Kumasi metropolis are of good quality.

Keywords:

Kumasi Metropolis;
Phytochemical Analysis;
Mineral Contents;
Heavy Metals;
Honey.

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1- Introduction

Honey is a natural product obtained from the excretions or secretions of the living parts of plants by bees (Apis mellifera). Under circumstances where the climate is adverse with no flowers on plants, Apis mellifera make honey as a nutrient reservoir for the colony [1]. In recent times, due to the perceived nutritional value of honey, its production and the art of beekeeping have seen an enormously increase in Africa [2, 3]. Honey cannot be regarded as a complete food by human dietary standards, however, it has the potential as a nutritional supplement. Honey is a more palatable carbohydrate food compared to sucrose for infants since it is easily digested. Honey mostly contains monosaccharides or simple sugars with 65% of glucose and fructose as the main components [4]. In the range of 0.02 to 1.03 %, honey contains several micro and macro–minerals [1]. Honey is rich in non–enzymatic and enzymatic antioxidants, such as proteins, amino acid, organic acid, carotenoid derivative, phenolic acid, flavonoids, ascorbic acid, catalase and glucose oxidase [5]. The mineral composition of honey is influenced by the beekeeping practices, environmental conditions, climate, geographical origin and plant species [6, 7]. Generally, honey can be multi– floral or mono–floral based on their sources [4, 7].

Based on its components, honey is regarded as a nutritional supplement and a natural product with different therapeutic properties, such as fungicidal, antidiabetic, radical scavenging, bactericidal, antiviral, anti–inflammatory, wound healing and antioxidant activities. Several environmental matrices (e.g. soil, air and water) which can contribute to the entire chemical composition of honey are exposed to inorganic and organic pollutants [8]. Insect induced pollution, particularly by bio–monitoring has received much interest due to the increased concern on health and environmental problems. The compositions of heavy metals in honey can affect consumer's health. These heavy metals emanate from different sources including leaded petrol from busy highways, non–ferrous metallurgy, industrial smelter pollution, factory emissions, and agrochemicals including arsenic–based pesticides, organic mercury and cadmium–containing

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fertilizers [9, 10]. Heavy metals are considered as the main inorganic pollutants in honey due to their physiological functions via the food chain [11].

Since honey bees are permanently exposed to pollutants [12], different plant species are used in bio–monitoring [13] with the needle–shaped leaves and thick epicuticular wax layer as the most frequent indicator for checking airborne pollution [8, 14]. Due to their direct contact and high mobility with the beehives environment, honey bees take pollutants into the hive via digestion and inhalation, from the environment [13, 15]. These features make the honey bee a multi contaminant collector. Honey bees are considered a potential biosensor of the genetically modified organisms and insecticides because of their mortality rate [16].

Recently, the levels of heavy metals in honey have been reported [10, 13, 17-21]. These studies mainly focused on the evaluation of heavy metals in honey as a vital bio–indicator of environmental contamination. In Ghana, it is projected that about 60% of the locally produced honey is of poor quality [22]. Despite several scientific studies into the levels of metals and minerals in honey, there is limited data on the quality and therapeutic properties of honey in Ghana. Therefore, this study was conducted to measure the therapeutic properties, metals and mineral composition of honey samples from the Kumasi Metropolis for the first time.

2- Materials and Methods

2-1- Sampling

In this study, twenty–three (23) different honey samples were purchased from twenty different market places within the Kumasi metropolis. The names of the various market places and the corresponding labels assigned to each sample are illustrated in Table 1.

Table 1. Summary of samples and coding.

Names of market	Labels of samples
Kotei	AH1
Ayeduase	AH2
New site	AH3
Tech junction	AH4
Ayigya Zongo	AH5
High school junction/Atonsu	AH6
Atonsu-Agogo	AH7
Yelewa	AH8
Aboabo–station	AH9
Dr Mensah	AH10
Kejetia	AH11
Adum	AH12
Asawase	AH13
Anloga	AH14
Asafo	AH15
Accra town	AH16
Aboabo	AH17
Oforikrom	AH18
Roman Hill	AH19
Kumasi Central market	AH20

Before storage, sample containers were washed thoroughly with hot–soapy water and were made to dry under the sun for 4 hours. Samples were then transferred into the sample containers. The samples were then kept at 4^{o}C in a refrigerator prior to analysis.

2-2- Sample Preparation

About 1 g of each honey sample was weighed into 24 different digestion tubes. About 10 ml of aqua regia was then measured with the aid of a measuring cylinder and transferred into the digestion tubes containing the honey samples. The above mixture was gently shaken to obtain a uniform mixture. The digestion tubes containing the aqua regia and the honey samples were then arranged on digestion blocks and placed on a hotplate. The temperature of the set–up hotplate was adjusted to 100 ^{o}C. The digestion tubes were inserted into the blocks when the temperature of the thermometer rose to 100 ºC. Sample solutions were then allowed to heat for an hour where the solutions change from deep yellow to pale yellow. Digested sample solutions were then allowed to cool to room temperature. Finally, the solution was filtered through a Whatman filter paper. The digested sample solutions were topped up to a final volume of 50 mL with deionised water.

2-3- Determination of Mineral Elements

The mineral contents (Calcium and magnesium) and heavy metals (Fe, Pb and Zn) were measured by Atomic Absorption Spectrometry (novAA® 400P) with D2 background correction and air/acetylene flame. The instrument was calibrated and standardised with different working standards. After confirming that the instrument was correctly calibrated, the concentrations of the minerals and heavy metals in each honey sample were measured. The standard solution of each element used for the calibration was prepared by diluting a standard stock solution (1000 mg/L) of each metal. The serial dilution of each analysed metal was prepared during the day of analysis. All the reagents used were of a high analytical grade. Due to the absence of suitable certified reference materials of honey, the recovery rate was obtained using a known spiked concentration of all the studied metal. The percentage (%) recovery of the analysed heavy metals ranged from 99.3 to 103 % (Table 2), signifying good precision, validity and accuracy of the method used.

Table 2. Recovery analysis of the analysed heavy metals in the spiked honey samples.

Trace element	Spiked value (mg/kg)	Measured value (mg/kg)	Recovery (%)
Mg	10	9.94	99.3
Ca	10	9.93	98.5
Fe	10	10.3	103
Pd	10	10.1	102
Zn	10	9.98	99.8

2-4- Statistical Analysis

All statistical analysis was performed using the IBM Statistical Package for the Social Sciences 20 program suite. The Pearson's correlation was used to access the significant difference ($p < 0.05$) among the mineral compositions in the honey samples.

3- Results and Discussion

3-1- Mineral Content

Minerals are naturally occurring inorganic substances in the environment from a nutritional perspective followed by the mineralisation of animal and plant tissues [23]. Minerals are formed by geological processes and they are vital for the regulation of the metabolic pathway in the human body [24]. The mineral contents is a significant index of a potential indicator of geographical origin and possible ecological pollution of honey. Minerals are chemical elements required by the body for normal functioning. The body makes use of minerals for several different functions, such as regulating the heartbeat, making hormones and building bones. Table 3 displays the mean concentrations of mineral content and heavy metals in twenty–three types of honey.

Calcium (Ca) is an essential nutrient which aids in calcification of bone and teeth [25]. Ca is a macronutrient essential for animals and plants, and occupy the exchange sites in calcareous and neutral soils. Calcium is required to supports the immune system, regulating blood pressure, blood clotting, nerve functioning and aid muscles contract and relax. The Ca concentrations in the honey samples ranged between N.D. and 3.24 mg/kg in Ayeduase market Table 3. The levels of Ca in this study was lower than those recorded in Malaysian honey (183.67 mg/kg) [26], Ireland (111 mg/kg) [27], Italy (47.7 mg/kg) [28], honeydew honey from Italy (356 mg/kg) [29], Spain (113 mg/kg) [30] and Southeast Anatolia, Turkey (51 mg/kg) [31].

Table 3. The concentrations of heavy metals and mineral contents in honey samples in mg/kg

Sample	Ca	Mg	Fe	Pb	Zn
AH1	2.014	13.189	*N.D.*	*N.D.*	*N.D.*
AH2	3.244	11.609	*N.D.*	*N.D.*	*N.D.*
AH3	0.114	14.299	*N.D.*	*N.D.*	*N.D.*
AH4	1.059	7.529	*N.D.*	*N.D.*	*N.D.*
AH5	1.134	5.789	*N.D.*	*N.D.*	*N.D.*
AH6	*N.D.*	6.649	*N.D.*	*N.D.*	*N.D.*

AH7	1.434	9.384	*N.D.*	*N.D.*	*N.D.*
AH8	*N.D.*	9.594	*N.D.*	*N.D.*	*N.D.*
AH9	*N.D.*	15.554	*N.D.*	*N.D.*	*N.D.*
AH10	*N.D.*	3.614	*N.D.*	*N.D.*	*N.D.*
AH11	0.809	10.164	*N.D.*	*N.D.*	*N.D.*
AH12	*N.D.*	15.049	*N.D.*	*N.D.*	*N.D.*
AH13	0.229	7.929	*N.D.*	*N.D.*	*N.D.*
AH14	*N.D.*	15.934	*N.D.*	*N.D.*	*N.D.*
AH15	*N.D.*	11.694	*N.D.*	*N.D.*	*N.D.*
AH16	*N.D.*	14.819	*N.D.*	*N.D.*	*N.D.*
AH17	*N.D.*	7.069	*N.D.*	*N.D.*	*N.D.*
AH18	*N.D.*	6.214	*N.D.*	*N.D.*	*N.D.*
AH19	*N.D.*	14.514	*N.D.*	*N.D.*	*N.D.*
AH20	*N.D.*	11.694	*N.D.*	*N.D.*	*N.D.*
AH21	*N.D.*	11.064	*N.D.*	*N.D.*	*N.D.*
AH22	*N.D.*	11.399	*N.D.*	*N.D.*	*N.D.*
AH23	*N.D.*	11.404	*N.D.*	*N.D.*	*N.D.*

Magnesium (Mg) exists in several enzymes involved the metabolism of lipids, carbohydrates and proteins. Magnesium is found in bones and it is vital for nerve impulse transmission, muscle contraction, protein production, and also supports the immune system. The lack of Mg in the human body may cause muscle spasms, osteoporosis, diabetes, high blood pressure and cardiovascular diseases [32]. The most abundant mineral composition in the analysed honey samples was Mg, with concentrations ranging between 3.61 mg/kg in Anloga market and 15.93 mg/kg in the Dr. Mensah Market. The concentrations of Mg in this study was lower than the Italian honey (56.7 mg/kg) [29], Malaysia honey samples (21.83–199.33 mg/kg) [26], and honeys from Spain (136 mg/kg) [33]. There was no significant difference ($p > 0.5$) between Ca and Mg. This indicates that the results obtained in this study are vital for characterising the properties of honey, but not to be used to differentiate the origin of the mineral components [34].

The concentrations of mineral contents in honey depends on soil composition and botanical origin [35]. According to Bagchi [36], the Recommended Dietary Allowance (RDA) for calcium in mg/100g is 450. The RDA value for calcium in the present study was mg/100g to 4.364 and this was very low as compared to the recommended value of 450 mg/100g. The Nutrient Reference Value (NRV) of magnesium has been recorded as 300 mg/100g [36]. The calculated value of magnesium in this study was also found to be 261.141 mg/100g, which is also lower than the recommended value. This implies that the honey samples can be targeted as some food source rich in magnesium and calcium. Thus, individuals who consume a teaspoon of honey are capable of meeting their daily magnesium and calcium requirements.

3-2- Heavy Metal Content

Since heavy metals can be transported either via the surfaces of leaves or the root system of plants into nectar, it is vital to analyse their levels in the honey samples because of their toxic nature. The heavy metal content of all the analysed honey samples were low and similar to concentrations in honey from other unpolluted sampling sites [17, 37]. The concentration of Fe, Zn and Pb in all the honey samples were all lower than the limit of detection, and this could be attributed to the absence of their vast contaminant source in the target region. The concentrations of macro – and micro– elements in the honey samples rely on factors, such as bee colonies, health and physiological status of bee specimens, the age of worker bees, the method of rearing bee colonies, ecological status, and area of apiary (including Nectar source plant and types of soils) [38]. Similar studies also recorded the absence of Pb in honey [5]. The samples had statistically zero means and did not present significant correlation values, contrary to results observed by other studies [39]. The present study reveals that there is no evidence of contamination of the honey samples collected from the various marketplaces and this is an indication of quality.

A hierarchical clustering was used to classify the sampling sites and the results has been shown in Figure 1.

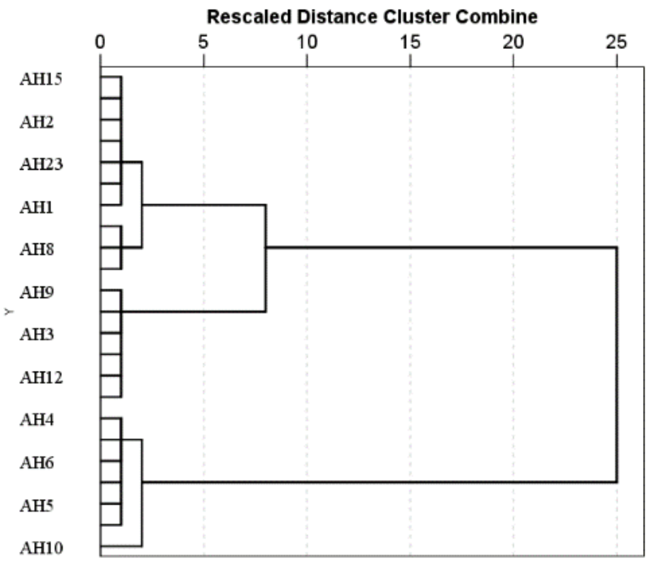

Figure 1. Hierarchical cluster analysis

Three main groups were observed based on the cluster analysis results. From this study, it was observed that cluster A (honey from AH1, AH2, AH8, AH15 and AH23) has higher levels of compositions compared to the other sampling markets. The honey purchased from AH3, AH9 and AH12 were grouped in the B cluster, while those from AH4, AH5, AH6 and AH10 were associated with cluster A at the last phase of the iterative accumulation process. Cluster A showed lower levels of minerals and trace metals than the other groups.

3-3- Phytochemical Analysis

The outcome of the screening of honey samples obtained from the various markets in Kumasi for phytocompounds is presented in Table 4.

Table 4. Results of phytochemical analysis of the analysed honey samples.

Samples	Cardiac glycoside	Carotenoids	Coumarins	Flavonoids	Saponins	Steroids	Tanins	Terpenoids
AH1	+	+	–	–	+	–	+	+
AH2	–	+	–	–	+	–	+	+
AH3	–	+	–	+	+	–	+	+
AH4	–	+	–	+	–	–	+	+
AH5	–	+	–	+	–	–	+	+
AH6	–	+	–	+	–	–	+	+
AH7	–	+	–	+	–	–	+	+
AH8	–	+	–	+	–	–	+	+
AH9	–	+	–	+	+	–	+	+
AH10	–	+	–	+	+	–	+	+
AH11	–	+	–	–	–	–	+	+
AH12	–	+	–	–	+	–	+	+
AH13	–	+	–	–	–	–	+	+
AH14	–	+	–	–	+	–	+	+
AH15	–	+	–	+	+	–	+	+
AH16	+	+	–	–	+	–	+	+
AH17	–	+	–	+	–	–	+	+
AH18	+	+	–	+	+	+	+	+
AH19	+	+	–	+	+	+	+	+
AH20	+	+	–	+	+	+	+	+
AH21	+	+	–	+	+	+	+	+
AH22	+	+	–	+	+	+	+	+
AH23	–	+	–	+	+	+	+	+

(+) – presence, (–) – absence

The study confirmed the presence of phytochemicals, such as saponins, flavonoids, terpenoids, cardiac glycosides, tannins, steroids, coumarins, carotenoids in honey Table 4. Out of the 23 samples that were analysed for the presence of phytochemicals, 23 revealed the presence of tannins, 23 for terpenoids, 15 for Saponins, 15 for flavonoids, 23 for carotenoids, 1 for Cardiac glycosides and 7 for Steroids, while none showed the presence of coumarins. Coumarins are a group of natural compounds with multi–biological activities including anti–HIV, anti–hypertension, anti–tumour, pain relief and others. Tannins, terpenoids and carotenoids were the highest occurring phytochemical in honey being present in all the honey samples. Saponins were found in 65.22% of the samples and they are known to offer protection against the effects of attack by pests and pathogens. Flavonoids and phenolic are said to be the most important groups of secondary bioactive compounds and metabolites in plants [40]. In this study, 65.22% of the samples showing the presence of flavonoids which confirms that consumption of the honey can benefit from their anti–inflammatory and analgesic effects [41]. Anti–microbial activity of terpenoids in honey has been studied previously [42]. Henceforth, honey can be used as a potential anti–microbial agent since terpenoids were present in all the honey samples. About 10 % of the samples showed the presence of cardiac glycosides and those samples can help in the management of cancers, such as bladder cancer, lung cancer, colon cancer and breast cancer. Steroids, which occurred in 30.43 % of the samples are one of the most widely used groups of drugs with or without indication in anaesthetic practice [43].

4-Conclusion

Since the characterisation of honey from the Kumasi Metropolis is not yet available in literature, this study offers an initial comprehensive and detailed assessment of phytochemical analysis, mineral and trace metal composition of the available honey samples. The statistical analysis shows that none of the honey samples showed significant correlation values between Ca and Mg. Also, there is a high level of terpenoids, carotenoids and tannins in all the studied samples and thus, the honey in the study area can be used as a potential food supplement. The results of this study showed that the different types of honey had different mineral compositions. Cluster analysis of the mineral contents showed a clear distinction between the different honey samples. In summary, the investigated honey was abundant in minerals, which are vital for human health and growth. The concentrations of the heavy metals were below the detection limit for all the heavy metals. This indicates that there was no evidence of contamination of the analysed honey. Therefore, the investigated honey from the Kumasi Metropolis can be said to be safe for consumption.

5- Acknowledgments

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6- Conflict of Interest

The authors declare no conflict of interest.

7- Funding

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