

## Detection of the Concentration of Some Metals in the Honey Product in Dawuro Zone, SNNPR, Ethiopia

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Received date: March 08, 2019; Accepted date: March 27, 2019; Published date: April 02, 2019

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### Abstract

Naturally honey contains a variety of mineral elements which mostly indicates the botanical origin of honey and contamination by environmental sources. This study was done to determine the concentration of metals and to investigate the effects of season and location on concentration of metals in honey produced in Dawuro zone. The ash content of honey was determined by muffle furnace and the content of metals was determined by flame atomic absorption spectrometry (AAS). The mean values of ash and different metals within the three honey production seasons in Dawuro zone were ranged as: ash content, 0.10-0.14 g/100 g and content of metals (mg/kg): Na, 12.66-29.92; K, 74.84-154.90; Mg, 17.00-24.21; Ca, 40.39-72.96; Mn, 0.37-0.49; Fe, 1.82-3.79; Co, 0.14-0.51; Cu, 0.08-0.27 and Cd, 0.055-0.063. The study area was not free of heavy metals however, the concentrations were below the maximum limits permitted and the study area was free of environmental pollution which conforms to international standards concerning the good quality of honey. Finally, further study on other physicochemical parameters of honey and regular follow up of hazardous substances in this study area were suggested.

**Keywords:** Dawuro; Honey; Metal concentration; Season

### Introduction

Honey is a thick, golden liquid produced by industrious bees and it is made using the nectar of flowering plants which is a sugary liquid and is saved inside the beehive for eating during times of scarcity [1,2].

Honey usually contains a variety of mineral elements naturally which mostly indicates the botanical origin or contamination by environmental sources [3,4]. Environmental, geographical and botanical factors have an impact on the trace element content of honey, and it is better to consider all of these factors when studying honey metal composition. The mineral content is about 0.04%-0.2% depending on if it is a light or dark honey type [3-6]. There are a large variety of elements in honey but the most abundant element was potassium [1,6,7].

Because honey is the ingredient of most industrial products for sweetening and improving food composition and has been used for a long time for other human health care, it must be free of any uncomfortable material content and should contain only the small amount of pollutants such as heavy metals [8-11]. Different physicochemical parameters were applicable to study the composition and quality of honey samples but in this study, the determination of ash content and elemental concentration has been performed to assess the levels of essential and trace elements [12,13]. Mineral content of honey is highly dependent on the soil type and the type of flower used by bees for nectar and the ash content of honey is also one criterion for the determination of the botanical origin of honey [3,14,15].

In Dawuro zone there are different apiculture areas with the high potential of honey production that is produced in different seasonal variation especially during October, December and around June.

However, currently, no study has been conducted about the content of metals and other physicochemical parameters. Specially investigating the content of trace metals in honey is important to know about pollution by environmental sources and thus it is useful to assess potential health risks associated with the intake of honey. Having information about honey quality characteristics would help the beekeepers and different unions to collect and store the honey samples in a proper manner. Therefore, this study was implemented to have a recorded data about the content of metals in honey that makes honey producers of different woredas of Dawuro zone, SNNPR, Ethiopia to be known in the honey international market.

### Materials and Methods

#### Description of the study area

Dawuro zone is located in southern nation nationalities and people regional (SNNPR) state of Ethiopia with an approximately 500 km from Addis Ababa the capital city of Ethiopia and it is found between 6°36' to 7°21' north latitudes and 36°68' to 37°52' east longitudes with an altitude of 500 to 3000 meters above sea level. herefore, the study was conducted in this zone with four woredas namely Loma, Mareka, Tocha and Isara that were selected purposefully depending on the areas where the high honey product is available.

#### Sample collection

The honey samples were collected in three different honey production seasons in a year; December (2015), June and October (2016) for each woreda. The collected honey samples were labeled and transported to Wolaita Sodo University and stored at room temperature for 5-6 months until sample preparation and analysis.

## Analysis of honey samples

Unwanted materials such as wax, sticks, dead bees and particles of combs were removed by straining the samples by clean cloth before analysis.

The ash content of samples was determined by taking 5 g of each honey sample in a preheated and pre-weighed quartz ash dish. In the weighed samples two drops of olive oil were added and the contents were charred in an oven until the samples are dry and smokeless. The samples then ignited in a muffle furnace at 550°C to constant weight [13].

$$\text{Ash content (\%)} = (W2 - W1) / M \times 100$$

Where W1 is weight of empty dish, W2 is weight of ash + dish after ignition and M is mass of honey sample taken.

The detection of content of selected metals was carried out after ash determination by dissolving the ash with 5 mL of 1 M nitric acid solution and the mixture was stirred on a heating plate to almost complete dryness. A stirring rod was used to transfer quantitatively through a funnel into a clean dry 25 mL volumetric flask. Then, 10 mL of the same acid was added, and the mixture was made up to 25 mL with distilled water. This solution of ash was then used for the determination of metals: Na, K, Ca, Fe, Mn, Cr, Co, Cu, Cd, and Pb by flame atomic absorption spectrometry (Perkin Elmer, USA) equipped with deuterium background corrector and hollow cathode lamps with air-acetylene flame. Na and K were analyzed by flame atomic emission spectrometry (FAES) while Ca, Mg, Cr, Mn, Fe, Co, Cu, Cd and Pb were analyzed by flame atomic absorption spectrometry (FAAS). The instrument response was periodically checked with known standards and the standard solutions of metals were prepared using appropriate stock solutions (1000 mg/L) for calibration [6,7,16]. All measurements during each season were carried out in triplicate to determine the total ash and content of metals.

## Statistical analysis

The data were collected, coded and entered in a Microsoft spreadsheet excel and analyzed in SPSS (version 16). ANOVA was used to assess the association of each season and location on quality parameters. Further analysis on differences among the means was determined using the Tukey test at 5% significant level.

## Results

The content of ash and metals was determined in four woredas of Dawuro Zone SNNPR of Ethiopia during the three honey production seasons in a year. The analysis results of ANOVA showed that there were significant differences ( $p < 0.05$ ) for ash and different metals analyzed between the different studied areas except for Mn ( $0.15 = p > 0.05$ ) during the third season.

According to the Post hoc multiple comparison test (Tukey HSD) at 0.05 levels analysis of ash values showed that there was no significant difference in ash contents between Loma and Tocha ( $0.09 = p > 0.05$ ) and between Mareka and Tocha ( $0.11 = p > 0.05$ ) for the first season, there was significant difference between Isara and Mareka ( $0.01 = p < 0.05$ ) and between Isara and Tocha ( $0.003 = p < 0.05$ ) for the second season and during the third season there was a significant difference ( $p < 0.05$ ) only between Isara and other three honey production areas.

Season one in four place (December 2015)				
Parameter	Loma	Maraqa	Tocha	Isara
Ash	0.14 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.22 ± 0.03
Na	31.27 ± 0.40	26.60 ± 0.32	21.45 ± 0.27	40.38 ± 0.64
K	143.06 ± 0.77	127.36 ± 0.30	162.49 ± 0.67	186.70 ± 0.95
Mg	25.78 ± 0.89	17.28 ± 0.58	21.51 ± 0.45	32.26 ± 0.41
Ca	74.88 ± 0.75	57.83 ± 0.44	67.85 ± 0.72	91.22 ± 0.53
Mn	0.49 ± 0.01	0.34 ± 0.03	0.76 ± 0.04	0.37 ± 0.03
Fe	4.64 ± 0.25	3.02 ± 0.05	3.15 ± 0.10	4.33 ± 0.12
Co	0.07 ± 0.01	0.15 ± 0.01	0.10 ± 0.01	1.74 ± 0.02
Cu	0.13 ± 0.01	0.15 ± 0.0	0.12 ± 0.01	0.26 ± 0.02
Cd	0.09 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.08 ± 0.01

**Table 1:** Ash (g/100 g) and content of metals (mg/kg) of honey in season one: mean ± SD.

Season one in four place (June 2016)				
Parameter	Loma	Maraqa	Tocha	Isara
Ash	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.13 ± 0.02
Na	14.38 ± 0.08	9.98 ± 0.09	8.12 ± 0.13	18.14 ± 0.09
K	64.60 ± 0.4	74.39 ± 0.93	62.65 ± 0.49	97.74 ± 0.55
Mg	19.12 ± 0.12	16.21 ± 0.06	11.38 ± 0.13	21.29 ± 0.09
Ca	41.58 ± 0.14	32.26 ± 0.14	36.85 ± 0.34	50.86 ± 0.53
Mn	0.39 ± 0.06	0.43 ± 0.09	0.48 ± 0.01	0.37 ± 0.04
Fe	2.01 ± 0.02	1.26 ± 0.03	1.57 ± 0.02	2.44 ± 0.02
Co	0.20 ± 0.02	0.05 ± 0.01	0.16 ± 0.01	0.18 ± 0.01
Cu	0.26 ± 0.04	0.20 ± 0.02	0.16 ± 0.01	0.46 ± 0.04
Cd	0.06 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.04 ± 0.01

**Table 2:** Ash (g/100 g) and content of metals (mg/kg) of honey in season two: mean ± SD.

Season one in four place (October 2016)				
Parameter	Loma	Maraqa	Tocha	Isara
Ash	0.12 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.15 ± 0.01
Na	27.76 ± 0.21	24.58 ± 0.13	19.62 ± 0.36	31.19 ± 0.10
K	93.99 ± 0.29	71.62 ± 0.33	83.07 ± 0.23	118.87 ± 0.48
Mg	22.26 ± 0.80	18.64 ± 0.67	13.94 ± 0.23	27.25 ± 0.58
Ca	54.44 ± 0.39	44.55 ± 0.53	39.42 ± 0.92	63.55 ± 0.58
Mn	0.23 ± 0.02	0.36 ± 0.04	0.39 ± 0.03	0.50 ± 0.03
Fe	2.31 ± 0.25	2.05 ± 0.16	2.09 ± 0.14	3.20 ± 0.23
Co	0.09 ± 0.01	0.08 ± 0.01	0.20 ± 0.02	0.19 ± 0.01
Cu	0.03 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.14 ± 0.01
Cd	0.08 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01

**Table 3:** Ash (g/100 g) and content of metals (mg/kg) of honey in season three: mean ± SD.

Results of the mineral contents of honey are mentioned in Tables 1-3 above.

During the first season the Tukey HSD tests at 0.05 levels proved that there was no significant difference in manganese concentration between Mareka and Isara (0.73=p>0.05), iron concentrations between Loma and Isara (0.12=p>0.05) and between Mareka and Tocha (0.7=p>0.05) and concentrations of cadmium between Loma and Isara (0.34=p>0.05) and between Mareka and Tocha (0.52=p>0.05) while for the content of copper there was significance difference (p<0.05) only between Isara and other places.

In the second honey production season the Tukey HSD tests indicated that there was no significant difference (p>0.05) between all honey production areas in manganese concentration, for the content of cobalt there was significant difference (p<0.05) only between Mareka and other places, for the content of copper there was insignificant difference between Loma and Mareka (0.23=p>0.05) and between Mareka and Tocha (0.42=p>0.05) and for cadmium concentration there was insignificant difference between Loma and Tocha (0.22=p>0.05) and between Tocha and Isara (0.48=p>0.05).

During the third honey production season the Tukey HSD showed that there was insignificant difference in manganese concentration between Mareka and Tocha (0.80=p>0.05), cobalt content between Loma and Mareka (0.93=p>0.05), while for iron there was significant difference (p<0.05) only between Isara and other three places and for cadmium concentration there was significant difference (p<0.05) only between Loma and other three places. The elements of Cr and Pb were not detected in all examined honey samples.

Note: the statistically significant values which did not mentioned here are the opposite of explanation.

## Discussion

The ash content of honey samples investigated in the four woredas of Dawuro zone was varied from 0.09-0.17 g/100 g (Table 4). The highest ash content observed in Isara would be due to the dark color of the honey samples which contributed to the high mineral content [3,17]. The Tukey tests evidenced that there was a significant difference in ash content only between Isara and other studied woredas in Dawuro zone.

Minerals		Ash	Na	K	Mg	Ca	Mn	Fe	Co	Cu	Cd
Places	Loma	0.12	24.47	100.55	22.39	56.98	0.37	2.99	0.12	0.14	0.08
	Mareka	0.09	20.39	91.12	17.38	44.88	0.38	2.11	0.09	0.14	0.06
	Tocha	0.09	16.4	102.73	15.61	48.04	0.54	2.27	0.16	0.12	0.05
	Isara	0.17	29.91	134.44	26.93	68.55	0.41	3.33	0.71	0.29	0.06

**Table 4:** Mean values of ash (g/100 g) and metal content (mg/kg) of honey in all four places of Dawuro district, SNNPR, Ethiopia in a year.

The ash content of honey in Dawuro district through the three honey production seasons ranged between 0.10%-0.14% (Table 5). The lowest ash value was observed during the second honey production season (around June 2015) and the highest was during the first honey production season (December 2015). The ash values of honey during the three production seasons showed a slight significant difference (0.038=p<0.05) only between season one and season two. All the honey samples during the three production seasons showed very low ash values which mostly depends on the nectar composition of predominant plants [3,18]. This range of ash content of honey was in agreement with ash content of honey produced in the Homesha district of western Ethiopia with ash value of 0.17%, honey produced in Sekota district, northern Ethiopia, with mean value of 0.14 %, Hareenna forest honey, Bale, Ethiopia with a mean of 0.19% and Venezuelan honey in the range of 0.03-0.13 g/100 g [19-22]. Studies on honey produced in West Shewa zone, Oromia region Ethiopia reported fewer ash values, 0.03-0.095 %, then our study area [23]. But high ash value, 0.23-2.33%, of honey from different origins was reported compared to our study [24]. Therefore, the ash values of honey in our study were below 0.6 g/100 that confirmed the botanical origin of the honey samples were floral (blossom) types [13,25].

Minerals	Ash	Na	K	Mg	Ca	Mn	Fe	Co	Cu	Cd
Season one	0.14	29.92	154.9	24.21	72.95	0.49	3.79	0.51	0.16	0.063
Season two	0.1	12.66	74.84	17	40.39	0.42	1.82	0.15	0.27	0.055
Season three	0.11	25.79	91.89	20.52	50.49	0.37	2.41	0.14	0.08	0.058

**Table 5:** Seasonal mean values of ash (g/100 g) and content of metal (mg/kg) of honey samples in Dawuro district.

Honey also contains different types of elements which contributed to ash content and electrical conductivity values [26-28]. As shown in Table 4 the metals studied in this zone were Na, K, Mg, Ca, Mn, Fe, Co, Cu, and Cd. The average content of these metals in the different studied areas of Dawuro zone (Loma, Mareka, Tocha and Isara) was ranged from; 16.40-29.91 mg/kg, 91.12-134.44 mg/kg, 15.61-26.93 mg/kg, 44.88-68.55 mg/kg, 0.37-0.54 mg/kg, 2.11-3.33 mg/kg, 0.09-0.71 mg/kg, 0.12-0.29 mg/kg and 0.05-0.08 mg/kg respectively. In the studied areas, it was observed that the honey samples collected in Isara woreda were close to black in color, which contributed to the highest ash levels and the highest concentration of metals, except manganese that showed the highest level in Tocha, than other areas in Dawuro zone. In contrast, the honey samples from Mareka area showed the lowest levels of K, Ca, Fe, and Co while Na, Mg, Cu, and Cd showed the lowest concentration in Tocha. The metal contents in Table 4 indicated that potassium being quantitatively the highest; calcium is the second predominant while cadmium showed the lowest contents in the four different studied areas.

The Tukey HSD tests at 0.05 levels (Table 6) showed that there were both significant and insignificant differences of the content of metals between the different woredas of Dawuro zone. The considerable variation in metal composition in the four studied woredas in Dawuro zone (Loma, Mareka, Tocha, and Isara) was because of difference in soil composition, floral type or environmental conditions [3,14,29].

Element	p < 0.05 (significantly different)	p > 0.05 no significant difference)
Na	Isara and Tocha	others
K		All
Mg	Others	Loma and Isara, Mareka and Tocha
Ca	Mareka and Isara, Tocha and Isara	Others
Mn	Loma and Tocha, Mareka and Tocha	Others
Fe	Mareka and Isara	Others
Co	Isara and Others	Others
Cu	Isara and Others	Others
Cd	Loma and Others	Others

**Table 6:** Tukey HSD tests at 0.05 levels for metals between different places.

The average contents of metals during the three honey production seasons in Dawuro zone (Table 5) was ranged as (mg/kg); Na (12.66-29.92), K (74.84-154.90), Mg (17.00-24.21), Ca (40.39-72.96), Mn (0.37-0.49), Fe (1.82-3.79), Co (0.14-0.51), Cu (0.08-0.27) and Cd (0.055-0.063). Cr and Pb were also analyzed but their amount was below the detection limit which indicated these study areas were free of environmental pollution. In this study, the average amount of metals showed significant differences (p < 0.05) between some honey production seasons in Dawuro zone that confirmed the influence of seasonal changes on the concentration of metals in honey [30].

Based on the Tukey HSD tests those which could not show significant differences between different productions seasons are summarized in Table 7 below.

Element	p-value	p > 0.05 no significant difference)
Na	0.17	Season one and three
K	0.09	Season one and three
Mg	0.18, 0.21	Season one and three (0.18), season two and three (0.21)
Ca	0.051	Season two and three
Mn	p > 0.05	Between all seasons
Fe	0.051	Season two and three
Co	p > 0.05	Between all seasons
Cu	0.052	Season one and three
Cd	p > 0.05	Between all seasons

**Table 7:** The Tukey HSD tests at 0.05 levels for metals between the three production seasons.

It was noticeable that for the elements Na, K, Ca, Mg, Fe, and Cd the highest concentration was detected during the first and the lowest has during the second honey production seasons. The highest amount of Mn and Co has been observed during season one and the lowest content was in season three, while for Cu the highest content was observed during season two and the lowest was during season three. As observed in Table 5 till potassium has the highest and calcium showed the second largest concentration while cadmium showed the lowest values in all honey production seasons. Most of the results reported in other study areas also indicated that potassium was quantitatively the highest detected element [16,26]. Comparable reports to our study were given in other geographical areas such as Argentinean honey [31]. Honey samples collected in Saudi Arabia showed the highest content of metals than our detected metals except for the comparable concentration of sodium [32]. Lazio region (central Italy) honey showed the highest concentration of minerals except for the comparable content of Calcium [7]. Kinds of honey from different regions of Turkey also illustrated the highest quantity of minerals including chromium and a large amount of lead [33]. In our study lead could not be detected, which might be due to impossible transportation of it by plants [34]. In contrary to our study low content of Mg, Mn, Fe, and Cu were reported in Kahramanmaraş city, Turkey honey samples [35]. The high abundance of trace metals was detected in the studied areas of Dawuro district, but these values could be safe for human consumption [36].

## Conclusion

The present study on ash content showed that the honey produced in Dawuro zone was floral types. Regarding seasonal effect the highest mineral content was found during the first season and the lowest was seen in the second season. In all the studied seasons and honey



areas potassium had the highest and cadmium showed the lowest concentration. Chromium and lead were not detected at all which prevail the honey production areas are free from environmental pollution. The levels of metals assessed in honey for its quality comparing with permissible limits showed that the concentrations were in a safety baseline level for human consumption. There was no health risk to consumers through the intake of honey in the studied agricultural areas, nevertheless further study on other physicochemical parameters of honey, regular follow up of harmful substances and training of beekeepers about good beekeeping practices should be carried out.

## Acknowledgments

The Authors would like to thank Wolaita Sodo University for financial support and Mr. Merga H/Mariam from Arba Minch University who participated during sample analysis.

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