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Research Article

Impact of Adulteration and Thermal Treatment on the quality of Ethiopian Honey

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ABSTRACT

The aim of this research was to evaluate quality and level of adulteration of honey collected from local markets and commercially recognized honey samples from the supermarket and comparing their quality to national and international standards. A total of 8 samples were collected, one directly from the beekeeper, 6 from the local market and the processed sample, from a supermarket. These samples were analyzed for 13 parameters to test the quality and level of adulteration. The obtained minimum and maximum values for the parameters were refractive index(1.4745-1.4886), moisture content (18.7-25%), conductivity (0.2-0.8 mS/cm), pH value (3.77-4.09), free acidity (28-72 meq/Kg), ash (0.05-0.74), diastase activity (0.31-18.7 DN), level of (HMF) (25.5-1036 mg/kg), proline content (19.3-580.9 mg/kg), reducing sugar (55-68.9%), sucrose content (2.35-17.3%), TSS (74.5-79.60 Brix) and TS (75-81.3%) respectively. From the analyzed 6 trace and Heavy metals Pb, Cd & Cr were not detected in all, but Cu, Zn and Co were detected in all samples and their values were in the range 0.02-0.1, 0.02-0.16 and 0.031-0.12 ppm respectively. The sample collected directly from a beekeeper fulfills international and national honey quality standards, whereas the remaining did not fulfill the standards.

Keywords: Adulteration; Beekeeper; Honey; Heavy metal; Hydroxy methyl furfural

INTRODUCTION

Honey is one of the most widely sought products due to its unique nutritional and medicinal properties, which are attributed to the influence of the different groups of substances it contains. Codex Alimentarius Commission defined honey as the natural sweet substance produced by honey bees, Apis mellifera, from the nectar of plants (blossoms) or from the secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honey bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature. Honey is one of the popular and primordial food products. It contains several nutritionally important substances that support good health and recovery. It is a characteristic sugary foodstuff; according to current regulations, apart from other forms of honey, no other substances or additives can be added to it.

Ethiopia has a favorable natural resource endowment for the bee keeping. Due to its varied ecological and climatic conditions, Ethiopia is among the major producer of honey both in Africa and in the world. In 2013 the country produced about 45,000 tons of honey accounting about 27% and 3% of African and World honey production respectively, making the country the largest producers in Africa and the tenth in the world. According to the report presented in the 5thApi Expo in Kigali, Rwanda Ethiopian honey production estimated to 53,000 tons in 2016.

Southern Ethiopia is one of the potential areas of honey production which accounts for 18% of the total honey production in the country. Southwest parts of the country particularly Kafka, Sheka and Bench-Maji zones are gifted with very diverse and dense natural forests. This favors the existence of dense honeybee population and production of a large volume of honey.

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A research conducted on the adulteration of different natural food items revealed that it has become a common problem in many parts of the world regardless of economic status of the nation.Honey has a high potential to be deliberately or unknowingly adulterated because of its cost, being produced in large fluctuations of weather and its harvesting is particularly weather sensitive. The act of honey adulteration is causing several economic impacts on the domestic and export opportunities of the product and may result in nutritional and health problems in consumers.According to a survey conducted in seven zones of Oromiya Region and Adis Abeba to assess honey adulterating materials several substances have been used as adulterant solely or blended with one another. Some of these are sugar, molasses, banana, candy, soil and stone, sugar and some plant root, Sugar and molasses, sugar and maize flour, sugar and candy, and sugar and banana.

When honey is adulterated with extraneous substance, the biochemical composition can get altered by chemical reactions that can occur during storage. This ultimately results in the consumer getting cheated or often become the victim of disease. The quality of honey produced in different countries, is mainly determined by its sensory analysis, physicochemical characteristics, and microbiological characteristics. The main quality criteria include the moisture content, electrical conductivity, ash content, reducing sugar, sucrose content, free acidity, level of Hydroxymethylfurfural (HMF), mineral content, water soluble solid content, total solid, the pH level, proline content, invertase activity and specific rotation.

Heavy metal analysis is important not only to determine the quality of honey, but also to evaluate environmental pollution, especially Cd and Pb levels. Higher Pb and Cd concentration is toxic for human. Higher Cd and Pb concentrations lead to cardiovascular problems and brain defection, hypertension, hearing difficulty, anemia, kidney disease and loss of intelligence. Although other elements found in the human diet (like Cu, Zn, Co and Cr) are important in human metabolism, but should not exceed the permissible levels.

MATERIALS AND METHODS

Sample collection and preparation

A total of 8 honey samples were collected, one sample directly from beekeeper (S1), six from local markets (S2-S6), and one sample was purchased from a super market (S8). These samples were analyzed for various quality parameters and for the parameter used for detection of adulteration.

All honey samples were analyzed using the same methods to ensure uniform conditions and comparability.

Determination of HMF

The level of Hydroxymethylfurfural (HMF) was determined using the standard Harmonized Method of the International Honey Commission IHC 2009.The samples were prepared by weighing 5g of honey in previously weighed 50 ml beaker. Each sample was dissolved with 25 ml of distilled water and then transferred into a 50 ml volumetric flask. 0.5 ml of Carrez I solution (150 mg/ml potassium ferrocyanide) was added to each of the flasks and thoroughly mixed. Similarly, 0.5 ml of Carrez II solution (30 g/100 ml zinc acetate) was added and thoroughly mixed. After mixing, distilled water was added up to the mark of each volumetric flask. The above solutions were then filtered with filter paper, and the first 10 ml was discarded. 5 ml of the remaining filtrate of each solution was transferred into each of two test tubes. The same procedure was used for all samples.

A reference solution was prepared by adding 5 ml of 0.20% metabisulphite ($Na2S2O5$) to one test tube containing 5ml of the above filtrate. A test sample solution was prepared by adding 5 ml of distilled water to the other test tube of filtrate. Both samples were mixed using a mixer.

The absorbance of the test sample solution and the reference solution were measured at 284 and 336 nm respectively. Finally, HMF was determined by subtracting the absorbance measured at 284 nm for HMF in the test sample solution (honey sample solution) against the absorbance of reference solution. The level of HMF in mg/kg was calculated using the following formula.

Determination of diastase activity

Diastase activity was determined by the method described in IHC 2009 (Phadebas method). One gram of honey sample was weighed in a pre-weighed beaker (100 gm) and then dissolved in 30ml distilled water. The solution was transferred into100ml volumetric flask with some amount of previously prepared acetate buffer solution and filled up to the mark. 5.0 ml of the solution was poured in to a test tube and heated at 40°C in water bath for 15 minutes. 5.0 ml aliquot of the acetate buffer (blank solution) was added into another test tube which was treated exactly as the sample solution. Phadebase tablet was added to both test tube and the mixture was stirred until the tablet disintegrated and then the test tubes were placed again in water bath. After 15 minutes 1ml of sodium hydroxide was added. Immediately the solution was filtered through filter paper and finally the absorbance of both the sample and blank were measured in the same wavelength at 620nm. The diastase activity was calculated by the following equation, the first one for higher diastase activity (8 to 40) and the second equation for lower diastase activity (0 to 8).

Apparent reducing sugar and sucrose content

Apparent reducing sugar content was determined by the modified Lane–Eynon procedure involving the reduction of Soxhlet's modification of Fehling's solution by titration at boiling point as described by IHC (2009) and sucrose content was determined by the method described in IHC 2009.

Evaluation of analytical method

To assess the validation of the method, 7 blank solutions were treated in a similar manner as the sample analysis procedure rather than the sample itself and triplicate digestion was performed. Finally, the absorbance was recorded and then the method detection limit (MDL) was calculated as three times the Standard Deviation (SDV) of seven blanks (MDL=3σ blank). The method detection limit of the analyzed metal in the samples were calculated as three times the Standard deviation of the blank (MDL=3σ blank). Figure 1 summarizes the method detection limits in this experiment. As described in the table all values were lower than the detected concentration of Cu, Zn, and Co in the honey samples.

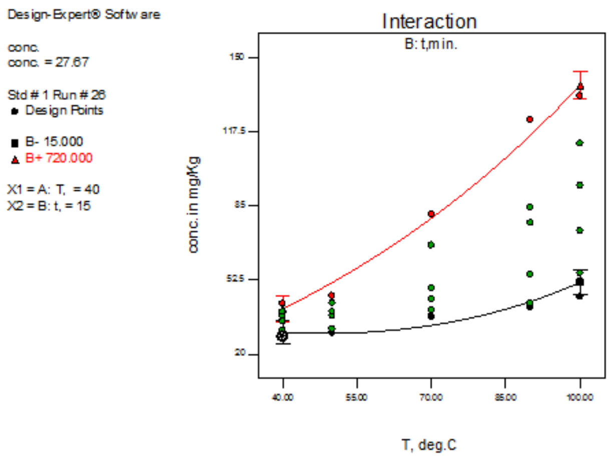

Figure 1: Response surface plots representing the interactive Effect of temperature and time on the concentration of HMF.

RESULTS AND DISCUSSION

Physicochemical quality of honey

The mean scores of the 8 samples and basic statistics obtained for refractive index, moisture content, total solids, total soluble solids, pH and free acidity, moisture content, electrical conductivity, color, ash, Hydroxymethylfurfural (HMF), proline content and diastase activity are summarized. There was significant difference ($p<0.05$) in the physicochemical parameters of all the honey samples.

Refractive index

The refractive index of honey is said to be a rapid, accurate and simple measure of its moisture content. The measured value of refractive index in the analyzed samples ranged from 1.4745-1.4886. The higher the moisture the lower the refractive index. The lower value of the refractive index was obtained from the industrially processed honey sample 1.4745 and the higher value was obtained for honey sample collected directly from the beekeeper 1.4886.

Moisture content

The moisture content of the honey samples ranged from 18.7 ± 0.1 to 25 ± 0.00%. A significant difference ($p<0.05$) was observed among the samples collected directly from the beekeeper, local market and commercial honey. The maximum limit of moisture content of honey set by Codex Alimentarius standard and EU directive is 20%. And, according to Ethiopian standard the moisture content of honey is grouped into three grades: Grade A (17.50 –19.00 g/100 g), Grade B (19.10–20.00 g/100 g) and Grade C (20.10–21.00 g/100 g)

Metals	Standard deviation (σ)	MDL(mg/Lit)
Cu	0.00221	0.01
Zn	0.00023	0.001
Co	0.00276	0.01

Table 1: Method Detection Limit.

The maximum moisture content obtained from this experiment was for the industrially processed sample 25±0.00% and the minimum value of 18.7±0.1% was obtained for sample directly collected from the beekeeper. This value fits Codex Alimentarius standard, Ethiopian standard and agrees with the moisture content of honey reported, their values were 18.88 ± 0.01 and 18.56 ± 1.45 respectively. This value was different from the average moisture content reported in the honey of southwestern parts of Ethiopia (22.86 ± 1.03%). In this study the fifth sample (S5) fulfill the second grade 'B'; S2 and S4 fulfill the third grade and S3, S6, S7, and S8contained above the maximum allowable limits set by Ethiopian standard; and, Codex Alimentarius and EU directive.

pH level and free acidity

Naturally honey contains higher acid content; this is due to the presence of wide range of organic acid. Most of organic acids are present in honey in the form of esters, which contributes to its characteristic flavor and aroma. The level of pH in the honey samples were acidic with pH value ranging from 3.78 ± 0.02 to 4.09 ± 0.12. These results agreed with the previous study reported on the pH value in Harenna forest (Ethiopia) honey produced in traditional hives (3.74–3.99). This parameter has great importance during the extraction and storage of honey, as it influences the texture, stability and shelf life of honey.

The mean free acidity of honey samples in this investigation ranged from 28 ± 0.00-72 ± 0.00 meq/kg. The minimum acidy was obtained in honey samples collected directly from beekeeper, this result was within the reported values by Getache which is in between (15 to 56.7 meq/kg) and (3.99 to 45.17 meq/kg) in honey of Tigraiy reported by Tadess G. The higher acidity value was observed in the samples collected from market,it was estimated to be 72 ± 0.00 meq/kg. As described in Table 1: S1, S3, S4, S5 and S8 are below the maximum permissible acidity value allowed by IHC and Codex Alimentarius it is 40 meq/Kg. On the other hand, the samples S2, S6 and S7 have greater acidity value than the maximum allowable limits. These values are most probably due to undesirable fermentation of honey sugar, the reason might be the higher moisture content and/or the presence of some extraneous substance in the samples.

CONCLUSION

In this work an attempt was made to evaluate the effect of adulteration of honey on its quality, and the effect of temperature on the HMF content. All the samples were analyzed using standard procedure and the results were compared with each other and with national and international standards. The result revealed that honey sample collected directly from beekeeper has relatively good quality. Some of the samples (S2 and S6) collect from local market and the processed honey sample contained higher level of HMF, percent sucrose, moisture content and lower content of proline. This confirms the samples might be adulterated with sugar as well as exposed to a higher temperature for a long period of time. The processed honey sample (S8) had higher content of HMF and it indicates the product was heated for longer times. Moreover, the sample contains a very low amount of proline, this confirms the sample adulterated with extraneous substance such as commercial sugar. According to the result obtained from this research there were a significant difference in the quality of honeys in the market.

The findings indicate the presence of Cu, Zn, and Co in all honey sample, the values were lesser than the maximum permissible limits. Pb, Cd, and Cr were not detected in all honey samples.

Based on the results, the content of HMF was highly influenced by exposure to heat treatment and duration of exposure. A noticeable increase in the concentration of HMF was evident, when the honey sample was heated above 700C. However, in the temperature range between 40 and 700C, the concentration exceeds the maximum permissible limit when the time of treatment was increased. Thus, the duration for heat treatment during processing of honey in the industry should be determined based on the original level of HMF before heat treatment. Therefore, for the processing of honey at industrial level there shouldn't be exposure to higher temperatures and time duration of exposure should be minimum even at lower temperature.

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