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

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***Abstract*— By comparing honey collected from local markets and commercially recognized honey samples from supermarkets to national and international standards, this study aimed to determine the quality and degree of adulteration. A total of eight samples were acquired, consisting of one supplemented sample from a grocery store, six supplements from the neighborhood market, and one supplement from the beekeeper. In evaluating the quantity and grade of adulteration in these samples, thirteen criteria were applied. Ash (0.05–0.74), diastase activity (0.31–18.7DN), HMF (25.5–1036mg/kg), proline content (19.3-580.9mg/kg), moisture content (18.7–25 percent), conductivity (0.2–0.8ms/cm), Ph (3.77–4.09), free acidity (28–72meq/Kg), reducing sugar (55–68.9 percent), sucrose content (2.35–17.3 percent), TSS (74.5–79.6 0Brix), and TS (75-81.3 percent) were the parameters that were measured within the specified ranges. Copper, zinc, and iron were all found in every sample, with concentrations ranging from 0.02-0.1 ppm, 0.02-0.16 ppm, and 0.031-0.12 ppm, respectively, among the six trace and heavy elements examined. In contrast to the other samples, the one taken straight from a beekeeper is of high enough quality to pass both national and international honey quality tests.**

***Index Terms*— Adulteration, Beekeeper, Honey, Heavy metal, Hydroxy methyl furfural.**

I. Introduction

Due to the obvious remarkable nutritional and therapeutic benefits that are related with the action of the several chemical groups that it contains, honey is one of the items that is in high demand. In order to produce the sweet delicacy that is known as honey, the honey bee, also known as Apis mellifera, collects naturally occurring sweet compounds from a variety of sources. The Codex Alimentarius Commission states that honey can be found in flowers, secretions, or even in the waste products of insects that consume plants. The bees get nectar from these sources and alter it by incorporating their own materials. After that, they place it in a honeycomb, dry it off, store it, and allow it to ripen and mature. (Codex Alimentarius Commission, 2001aand Codex Alimentarius Commission 2001b). One of the most ancient and widely consumed foods is honey. It helps with health and recuperation because to its nutritious content. Current restrictions prohibit the addition of any ingredients or additions to this characteristically sweet food item, with the exception of other types of honey. (Codex Alimentarius Commission, 2001a).

Mo A and ILRI (2013) found that Ethiopia's natural resource endowment is favorable for bee rearing. Ethiopia is one of the world's and Africa's leading honey producers because of its diverse ecosystems and climates. In 2013, the largest producers produced 45,000 tons of honey, which is equivalent to approximately 27 percent of all honey produced in Africa and 3 percent of all honey produced worldwide.

In southern Ethiopia, which makes up 18% of all the honey made in the country, there are places where honey production could be successful. Rich and diverse forest habitats may be found across the southwest of the nation, particularly in the Kafka, Sheka, and Bench-Maji zones. This promotes the honeybee colony's density and yield of a large quantity of honey. (Getachew, A., et al., 2012).

The prevalence of natural food adulteration has grown globally, according to studies that looked at a variety of countries' economies (Fairchild, G.F et.al, 2000). Honey is susceptible to intentional or unintentional adulteration because to its high price, subject production to huge weather swings, and especially weather-sensitive harvesting. Honey adulteration is having a negative effect on the product's local and international markets, and it might even lead to health and nutrition issues for consumers (Oroian, M., 2012). According to the findings of a study that examined honey adulterants in seven different zones around Adis Abeba and the Oromiya Region, a variety of chemicals have been utilized as adulterants, either on their own or in combination with one another. There are many other combinations that may be found, such as sugar and molasses, sugar and banana, sugar and dirt, sugar and specific plant roots, sugar and corn flour, sugar and candy, and sugar and banana combination. (Meseret G., Taye N, 2017).

Chemical processes that might happen during storage can change the biological content of honey when it is contaminated with foreign substances. The customer is either taken advantage of or becomes sick as a consequence of this. Analysis of sensory data, physicochemical properties, and microbiological traits are the key determinants of honey quality among nations. Wetness, electrical conductivity, ash content, sucrose concentration, free acidity, degree of hydroxymethylfurfural (HMF), mineral composition, water solubility, total solids, pH, proline activity, invertase activity, and specific rotation are among the most major factor of quality. (Bogdanov, et.al., 2015 and EU, 2001).

Assessing environmental contamination, particularly levels of Cd and Pb, and honey quality are both aided by heavy metal analysis. Lead and cadmium at higher concentrations are harmful to humans. Numerous deleterious health outcomes, such as heart disease, stroke, hypertension, hearing loss, anemia, renal illness, and cognitive decline, are linked to elevated levels of Cd and Pb. In despite the fact that other elements such as copper, zinc, cobalt, and chromium are

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essential for human metabolism, it is essential that the quantities of these elements do not exceed what is regarded to be suitable for a human diet. (Maiyo, W.K, et.al., 2014 and Chukwujindu M. et.al., 2015).

II. MATERIALS AND METHODS

Sample Collection and Preparation

One honey sample was obtained from the beekeeper (S1), six samples were obtained from neighboring markets (S2-S6), and one sample was obtained from a store. In all, there were eight honey samples collected (S8). "The samples were examined using a number of different quality indicators, one of which was the indication for its ability to identify adulteration. To make sure everything was consistent and comparable, we used the same methodology to examine all of the honey samples.

Physicochemical Characterization

Determination of Refractive Index and Moisture

All of the samples' moisture contents were determined using the IHC-recommended technique and an Abbe refract metre (60/DR, UK). (Bogdanov. S, 2009).

A. Determination of Electrical Conductivity

A conductivity metre (Sensodirect 12/6800, Germany) was used in order to evaluate the electrical conductivity of each and every one of the samples. This was done in line with the Harmonized procedure that is adhered to by the International Honey Commission (IHC). (Bogdanov. S, 2009).

Determination of Ash Content

In accordance with the AOAC (1990) method 920.181, the amount of ash that was present in the honey was determined. (White, J. W, 1984).

B. Determination of Total Soluble Solids (TSS) and Total Solids (TS)

The total soluble solid content of honey samples was determined using an Abbe refractometer (60/DR, UK), and the AOAC standards were adhered to in order to accomplish this task (AOAC, 1990).

C. Determination of pH level

Using a digital pH metre (JENWAY 3220, Germany) in compliance with IHC 2009, the pH level of the honey samples was detected (Bogdanov. S, 2009).

D. Determination Free Acidity

According to IHC, acid-base titration was used to evaluate the free acid level in the samples, which was then expressed as meq/kg of honey.

E. Determination of Proline content

Acid-base titration was used to estimate the free acid content of the samples, and the results were represented as measured units per kg of honey in accordance with IHC.

F. Determination of HMF

According to Bogdanov et al. (2009), the International Honey Commission's standard Harmonized Method (IHC 2009) was used to quantify the amount of Hydroxymethylfurfural (HMF). Using a beaker with a capacity of fifty millilitres, five grammes of honey were metered out for each sample. In a volumetric flask with a capacity of fifty millilitres, each sample was dissolved in twenty-five millilitres of distilled water. Each flask received 0.5 millilitres of Carrez I solution, which contained 150 milligrams per millilitre of potassium ferrocyanide, and was carefully mixed. Following that, a solution of Carrez II, which included thirty grammes of zinc acetate for every one hundred millilitres of zinc acetate, was gradually added and well mixed. Following the completion of the mixing process, the distilled water was then poured into each volumetric flask to the correct amount. The first 10 millilitres of the aforementioned solutions were thrown away after they had been filtered via filter paper. The residual filtrate from each solution was divided into two test tubes, with 5 ml each. Every sample went through the same procedure.

One test tube that already held five millilitres of the previously stated filtrate was filled with five millilitres of 0.20 percent metabisulphite (Na2S2O5) to create a reference solution. The second test tube holding the filtrate was filled with five millilitres of distilled water to create a test sample solution. The two samples were mixed together using a mixer.

When compared to the absorbance of the reference solution, which was 336 nm, the absorbance of the test sample solution was 284 nm. This was the last step in the process of determining the concentration of HMF. The absorbance of the reference solution and the test sample solution (the honey sample solution) were compared at a wavelength of 284 nm. It was determined that the HMF level in mg/kg could be calculated using the following formula.

$$HMF\ mg/Kg\ = \frac{(Abs284 - Abs336) \times\ 149.7\ \times\ 5\ \times D}{W}$$

Where: -

A284 = absorbance at 284 nm, A336 = absorbance at 336 nm, 5 = theoretical nominal sample weight and D = dilution factor, in case dilution is necessary.
W = Weight in g of the honey sample, 149.7 is constant.

G. Determination of Diastase Activity

IHC 2009 provides a description of the Phadebas technique, which was used in order to measure the diastase activity measured (Bogdanov. S, 2009). Within a beaker that had been pre weighed to 100 grammes, a sample of honey weighing one gramme and thirty millilitres of distilled water were dissolved After that, the solution was transferred and a little amount of the

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previously made acetate buffer solution were added until the 100 ml volumetric flask was completely filled.

TABLE 1
RECOVERY TEST RESULT IN HONEY SAMPLES

Metals	Detected conc. in the sample (mg/L)	Added Conc. (mg/L)	Conc. In Spiked sample (mg/L)	Amount recovered (mg/L)	Recovery (%)
Cu	0.1	0.05	0.146	0.046	92
Zn	0.16	0.08	0.238	0.078	97.5
Co	0.12	0.06	0.177	0.057	95

Five milliliters of the solution were placed in a test tube and heated for fifteen minutes at 40 degrees Celsius in a water bath. Five milliliters of the acetate buffer were added to a different test tube to duplicate the sample solution (blank solution). Each test tube was filled with the Phadebase tablet, and then the liquid was swirled until the tablet broke apart. Afterward, the test tubes were again immersed in water. Sodium hydroxide, 1 milliliter, was added after 15 minutes. The sample and blank were both tested for absorbance at 620 nm, the same wavelength used for the immediate filtering of the solution using filter paper. To calculate the diastase activity, two equations were used: one for diastase activity between 8 and 40, and another for diastase activity between 0 and 8.

$DN = 28.2 \times \Delta A\ 620 + 2.64$

$DN = 35.2 \times \Delta A620 - 0.46$

The best straight line created by linearly regressing A 620 (x-axis) on DN (y-axis) has an intercept of 2.64 and a slope of 28.2. By linearly regressing A 620 (x-axis) on DN (y-axis), the optimal straight line is generated; its intercept is 0.46 and its slope is 35.2.

H. Apparent Reducing Sugar and Sucrose Content

The modified Lane-Eynon technique was used to ascertain the sucrose concentration and apparent reducing sugar content, per IHC (2009) (Bogdanov, S., 2009). Through titration at boiling point, Soxhlet us modification of Fehling's solution was reduced in this procedure.

Determination of Heavy Metals

The determination of heavy metals was carried out using microwave-assisted sample digestion, following the method published by Mohamed et al. (2014) with a few alterations.

1) Recovery test

In order to assess the efficacy of the digestive process, honey samples were fortified with predetermined amounts of the metals, while the digestion itself was rigorously observed. The recovered values were measured and determined using the following formula (Mohammed M, et. al., 2014):

$$\text{Percent recovery} = \frac{S - NS}{\textit{The spiked metal content}} \times 100$$

2) Where: For a spiked sample, the metal content is denoted by S, and for a non-spiked sample, by NS.

The accuracy of the digestion method in the honey samples was assessed by adding with the known concentration of the standard metals (spiking). As described in the Table 1. The recovered amounts of Cu, Zn and Co were 92, 97.5 and 95%, these confirms the validity of the proposed methods.

3) Evaluation of Analytical Method

In order to evaluate the method's validity, triplicate digestion was carried out on seven blank solutions that were handled in a way identical to the sample analysis technique, instead of the sample itself. After the absorbance was recorded, the method detection limit (MDL) was determined by increasing the standard deviation (SDV) of seven blanks by three. This was done because the MDL was computed. The formula MDL = 3σ blank arose as a consequence of this. It was discovered that the MDL of the metal that was analyzed in the samples was three times the standard deviation of the blank, which included the 3σ blank. Table 2 summarizes the detection limits of the experimental approach. Each number was below the honey samples' observed content of Cu, Zn, or Co, as shown in the table.

TABLE II
METHOD DETECTION LIMIT

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

Analysis of Data

The outcomes were provided as the mean plus standard deviation, and all measurements were done in triplicate. Statistical methods used in the research changed according to the variables and data collected. Utilizing analysis of variance (ANOVA), the measured parameter in the honey samples was compared. The significance was assessed using $P < 0.05$. The programme used for these analyses was SPSS version 20. The data was organised using Excel, and the ANOVA (response surface cubic model) was performed on the HMF concentration as a function of time and temperature in design-expert -7 software. The correlation between temperature (A: T) and time

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(B: t) of treatment and the response (The yield of HMF/concentration of HMF) was analyzed using ANOVA.

III. RESULTS AND DISCUSSION

A. Physicochemical quality of honey

Table 3 presents the average findings and fundamental data for the eight samples, organised according to the following categories: refractive index, total solids, total soluble solids, electrical conductivity, color, ash, hydroxymethylfurfural (HMF), proline concentration, diastase activity, and moisture content. Statistically significant differences ($p < 0.05$) were observed in the physicochemical parameters of every single honey sample.

TABLE III
MC, ASH CONTENT, PH, FREE ACIDITY, EC AND TS VALUES (MEAN ± SD) TSS, HMF, R. SUGAR, SUCROSE, PROLINE AND DIASTASE ACTIVITY VALUES (MEAN ± SD) OF HONEY IN THE ANALYZED SAMPLES OF HONEY IN THE ANALYZED SAMPLES

Honey Sample	MC (%)	Ash	pH	F. Acidity meq/Kg	EC (mS/cm).	TS (%)
S1	18.7 ± 0.10^{a}	0.56 ± 0.003^{h}	4.003 ± 0.02^{yr}	28 ± 0.00^{d}	0.37 ± 0.002^{c}	81.3 ± 0.10^{e}
S2	20.7 ± 0.9^{b}	0.54 ± 0.002^{i}	3.9 ± 0.1^{yn}	72 ± 0.00^{e}	0.8 ± 0.1^{e}	79.6 ± 0.10^{f}
S3	21.9 ± 0.2^{c}	0.74 ± 0.002^{j}	4.01 ± 0.1^{yt}	35 ± 00^{f}	0.22 ± 0.03^{bd}	78.1 ± 0.10^{g}
S4	20.7 ± 0.1^{d}	0.05 ± 0.001^{k}	4.09 ± 0.1^{yzn}	39.7 ± 0.6^{g}	0.39 ± 0.01^{a}	79.3 ± 0^{f}
S5	19.6 ± 0.03^{a}	0.36 ± 0.002^{l}	4.06 ± 0.1^{yq}	34.3 ± 0.3^{f}	0.27 ± 0.02^{b}	80.4 ± 0.10^{h}
S6	23.6 ± 0.1^{e}	0.64 ± 0.001^{m}	3.96 ± 0.04^{y}	70.3 ± 0.3^{h}	0.4 ± 0.01^{ac}	76.4 ± 0.10^{i}
S7	21.3 ± 0.1^{f}	0.14 ± 0.001^{n}	3.9 ± 0.1^{y}	44.3 ± 0.4^{i}	0.24 ± 0.04^{bd}	78.7 ± 0.40^{j}
S8	25 ± 0.00^{g}	0.18 ± 0.001^{o}	3.77 ± 0.02^{p}	35 ± 0.3^{f}	0.20 ± 0.01^{d}	75 ± 0.10^{k}

Honey samples	TSS (%)	HMF (mg/Kg)	R. sugar (%)	Sucrose (%)	Proline mg/Kg	D. activity (DN)
S1	79.6 ± 0.6^{a}	25.55 ± 0.1^{g}	68.96 ± 0.9^{p}	2.35 ± 0.2^{d}	580.4 ± 0.3^{i}	18.7 ± 0.1^{c}
S2	78 ± 0.2^{ab}	145.73 ± 0.5^{h}	63.92 ± 0.6^{ou}	15.27 ± 0.04^{g}	177.6 ± 1.8^{j}	5.5 ± 0^{d}
S3	77 ± 0.4^{bc}	61.23 ± 0.1^{i}	66.1 ± 0.7qt	7.58 ± 1.1^{e}	223.3 ± 0.1^{k}	12.4 ± 0.1^{e}
S4	77.5 ± 0.2^{ac}	71.4 ± 1.2^{j}	64.79 ± 0.6^{ot}	6.18 ± 0.9^{ef}	386.8 ± 1.01^{l}	15.9 ± 0.03^{f}
S5	77.5 ± 1.6^{ad}	49 ± 0.2^{k}	66.92 ± 0.6^{t}	2.28 ± 0.1^{d}	340.39 ± 0.4^{m}	10.5 ± 0.04^{g}
S6	74.5 ± 0.6^{e}	217.96 ± 1.3^{l}	62.47 ± 0.1^{u}	13.74 ± 0.6^{g}	179.3 ± 1.03^{i}	4.1 ± 0.04^{h}
S7	77.7 ± 0.1^{ad}	40.96 ± 0.4^{m}	66.52 ± 0.7^{t}	5.62 ± 0.7^{f}	247.9 ± 1.1^{n}	13.1 ± 0.04^{i}
S8	73.4 ± 0.4^{e}	1036.43 ± 0.1^{n}	55.28 ± 0.28^{v}	17.37 ± 0.7^{h}	19.3 ± 1.01^{o}	0.3 ± 0.07^{j}

Note: MC – moisture content, F. Acidity – free acidity, EC – Electrical conductivity, TS- total soluble solid. S1 – sample collected from the beekeeper, S2 to S7 – Samples collected from the local market and S8- sample purchased from the supermarket (the processed sample). Results are reported as mean ± SD. In each column, values with different letters (superscripts) indicate significant differences ($p < 0.05$).

Note: TSS- total soluble solid, HMF- content of hydroxyl methyl furfural, R. sugar- reducing sugar D. activity-diastase activity. S1 – sample collected from the beekeeper, S2 to S7 – Samples collected from the local market and S8- sample purchased from the supermarket (the processed sample). Results are reported as mean ± SD. In each column, values with

B. Refractive index

According to White (1975a), honey's refractive index is a quick, accurate, and easy way to determine how much moisture is in the honey. The samples that were examined had refractive indices that varied between 1.4745 - 1.4886. The refractive index decreases as the relative humidity rises. Honey that was obtained directly from the beekeeper had a refractive index value of 1.4886, whereas honey that has undergone industrial processing had a lower value of 1.4745.

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different letters (superscripts) indicate significant differences ($p < 0.05$).

Moisture Content

The moisture contents of the honey samples ranged from 18.7 ± 0.1 to 25 ± 0.00 %. The beekeeper's samples, those from the neighbourhood market, and samples of commercial honey all shown a significant variance ($p < 0.05$). Honey must contain more than 20% moisture, as stated in the Codex Alimentarius standard and the EU legislation (Codex Alimentarius Commission 2001b and Getachew, A, et. al., 2014). And there are three classes of honey based on their moisture content, as per Ethiopian standards: 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). (Belay, A, et. al., 2013)

In this experiment, the highest moisture level of 25±0.00% was found in the industrially processed sample, whereas the lowest moisture content of 18.7±0.1% was found in the beekeeper's direct sample. The moisture content of honey as reported by Surh Y.J. et. al. (1994) and Prica, N. et.al. (2014), with values of 18.88 ± 0.01 and 18.56 ± 1.45 respectively, is consistent with this value, which also matches the Codex Alimentarius standard and the Ethiopian standard. Compared to the average moisture content of 22.86 ± 1.03% found in honey from southern Ethiopia, this figure was different (Getachew, A., et. al., 2014). Results indicate that S5, S2, and S4 all meet second grade 'B' standards, S3 and S6 all meet third grade standards, and S3, S7, and S8 all included levels that were higher than what is allowed by Ethiopian standard, Codex Alimentarius, and EU regulation. These values are summarized in Table 3.

C. pH level and free acidity

Due to the presence of a broad variety of organic acids, honey naturally possesses a greater acid content. Prica et al. (2014) found that esters, which make up the majority of honey's organic acid content, are responsible for the sweet scent and flavor of honey. The honey samples had an acidic pH level, with values range from 3.78 ± 0.02 to 4.09 ± 0.12. In line with these results, honey produced in traditional hives from Ethiopia's Harenna woodland has a pH of 3.74 to 3.99. (Belay, A., et. al., 2013). The processing and storage of honey, as well as the product's consistency, stability, and shelf life, are all significantly influenced by this feature. (Prica, N, et.al., 2014).

The honey samples used in this study had an average free acidity level ranging from 28 ± 0.00 to 72±0.00meq/kg. Getachew, A., et al. (2014) discovered that the acidity of honey samples obtained directly from beekeepers ranged from 15 to 56.7 meq/kg, whereas Tadess G. (2014) discovered that Tigraiy honey had an acidity range of 3.99 to 45.17 meq/kg. The samples from the market were judged to have a higher acidity value of 72 ± 0.00 meq/kg. According to Table 3: According to IHC and Codex Alimentarius, the acidity levels of S1, S3, S4, S5, and S8 are within the permitted limit of 40meq/Kg (Bogdanov. S, 2009 and Codex Alimentarius Commission, 2001a). The acidity readings of samples S2, S6, and S7, on the other hand, exceed the acceptable limits. These findings are most likely the result of an unintended fermentation of honey sugar, which occurred as a result of the greater moisture level and/or the presence of any foreign object in the samples.

D. Conductivity

Mineral and acid content has a significant impact on electrical conductivity, one of the most essential physical properties; a greater concentration indicates a higher electrical conductivity. The lowest electrical conductivity of the sample that was exposed to the experiment was found to range from 0.22 ± 0.032 to 0.800 ± 0.08, as per Table 3. The electrical conductivity value obtained from the investigated samples highly agrees with the value reported in Herzegovinian honey (Prica, N, et.al. 2014), the values were 0.13 mS to 2 mS/cm. The electric conductivity values that were obtained are all below the suggested maximum limit of 0.8 mS/cm, as stated in the international quality norms (Codex Alimentarius Commission, 2001a). The higher value (0.8 mS/cm) was obtained for sample two (S2) collected from the market. The rest of the samples had relatively lower electric conductivity values S8 (0.204±0.0061), S3 (0.218 ± 0.0321), S5 (0.239 ± 0.0425) and S7 (0.269 ± 0.015) respectively (shown in Table 3), except S8 all others are collected from the market. S8 is the processed honey sample collected from a supermarket. We found that the electrical conductivity values of the honey samples that we studied, which ranged from 0.17 to 1.35 mS/cm, were in agreement with the values that Gangwar, S.K. (2016) reported for Ethiopian honey.

E. Total Soluble Solid (TSS) and Total Solid (TS)

Table 3 shows that the total soluble solids in the sample varied between 73.4 ±0.36 and 79 ±0.00. There was a statistically significant difference between the two samples, with sample S1 containing the highest total soluble solids (TSS) value (79.6±0.56), which was obtained directly from the beekeeper, and sample S8, which was obtained from the market, containing the lowest TSS value (73.4 ±0.36). According to the grading system utilized by the United States Department of Agriculture, honey that has a total solids concentration (TSS) of 81.4 percent or higher is classified as high-grade honey (A and B). Honey that has a TSS that falls between 80 percent and 81.3 percent is classified as lower grade honey (C). This study's honey samples, with the exception of sample (S1), were all deemed to be of lower grade quality since they fell below this range. Sugars make up the bulk of honey's total soluble solids. This study's results were in agreement with those of commercial honey products sold in Bangladesh, which range from 73% to 80% (Krishnasree et. al., 2017). Krishnasree et al. (2017) also found that TSS varied from 75.5% to 79.0% Brix, therefore our findings are consistent with theirs. Generally, the analyzed sample had lower total soluble solid

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compared with the standard value recommended by IHC.

The total solids of honey linked to moisture content. Moistness decreases as total solid content rises and moisture rises as total solid content falls. From 75 ± 0.1 to 81 ± 0.1% of the total solid was derived from this investigation. According to Table 3, the sample taken directly from a beekeeper yielded a greater value of 81±0.1%, whereas processed honey and commercial honey from the market yielded lower values of 75±0.1% and 76.4±0.1%, respectively. According to Kayode and Oyeyemi (2014), the total solid value of Nigerian honey ranges from 76.6 to 90.73%. This study's results were in agreement with their findings. Generally, except the sample S1 and S5 all samples had lower TS value compared with the standard.

Reducing Sugar

In this study, the honey samples with reducing sugar levels ranging from 55.276±0.28 to 68.96±0.94 were studied. As described in (Table 3) the highest value (68.96 ±0. 94) of reducing sugar was recorded for the sample collected directly from the beekeeper. Reducing sugar value of commercial honey samples analyzed in this study were S2 (63.92 ±0.61), S3 (66.1 ±0.66%), S4(64.79 ± 0.63%), S5 (66.92 ±0.55%), S6 (62.47 ±0.06%) and S7 (66.52 ± 0.67%) respectively. The lowest value of 55.28±0.28% was observed for S8. The value that was reported for honey from southern Ethiopia, which was in the range of 54.45-79.99, was in agreement with these findings (Getachew, A., et. al., 2012). A statistically significant difference ($p < 0.05$) was seen between samples obtained directly from the beekeeper, samples obtained from the adjacent market, and commercial honey samples (Table 3). On the other hand, every value saves the one that was directly collected from the beekeeper disagreed with the findings of B. Zabrodska and L. Vorlová (2015). The results of this study, as indicated in Table 3, were in good agreement with the values reported by Tadess and Gebregziabher (2014) regarding the purposeful addition of commercial sucrose to honey (32.90–51.65 percent) and the stated values of reducing sugar in honey purchased from the local market (65.68–75.96 percent). A total of four samples (S1, S3, S5, and S7) met both the EU and Ethiopia's standards. According to the Ethiopian Standard (2005), the lowest permissible concentration of reducing sugar in honey is 65 g/100g. This might mean that some of the honey samples obtained from the neighbourhood market had artificial ingredients, such as sugar, in addition to the natural honey component.

F. Sucrose Content

In the sample that was tested, the levels of sucrose varied between 2.35 ± 0.2 and 17.368 ± 0.7%, as shown in Table 3. The beekeeper's honey (S1) and honey purchased from a local store (S5) both had the smallest amounts of sugar (2.35 ± 0.2, 2.275 ± 0.05%). There are 7.58 ±1.14, 6.179 ±0.9, and 5.624% ±0.7% in S3, S4, and S7, respectively. These values agreed with the reported amount of sucrose in honey of southwestern Ethiopia which rangedfrom1.32 % to 10.98 % (Gangwar, S. K.2010). The highest amount (17.4 ±0.7%) of sucrose was found in the processed honey sample (S8) purchased from the supermarket. The rest of the samples that contain highest sucrose content (S2 and S6) were collected from the local market, recorded value were 15.27 ± 0.04 and 13.74 ±0.58% respectively. This analysis's results showed that, with a few significant exceptions, the vast majority of samples sourced from the local market do not meet the extreme limits set by the European Union and Ethiopia. A maximum of five grammes per one hundred grammes of honey, or five percent, is permitted per regulations imposed by Ethiopia and the European Union (EU, 2001 and Ethiopian standards, 2005). The higher the sucrose contents of samples, higher the chance of the sample being adulterated.

G. Proline Content

As described in Table 3 the amount of proline in the present investigation ranged from 580.39 ± 0.26 to 19.34 ± 1.0 mg/Kg. The sample collected directly from beekeeper had a greater amount (580.39mg/Kg) compared to others. The proline content of the samples taken from the local market (S2 to S7) was found to be the lowest at 19.34 ±1.01. $P < 0.05$ indicates that there was a statistically significant difference in the values across each sample. The recorded values were 177.58 ±1.76, 223.25 ±0.09, 386.81 ±1.01, 340.39 ±0.36, 179.3 ±1.033 & 247.9 ±1.07 mg/Kg, respectively. Results for proline content ranged from 178 to 726 mg/kg in every sample, with the processed honey being the outlier. Truzzi, Cristina, et al. (2014). The outcome showed that the three samples—S2 and S6, which were gathered from the neighbourhood market—as well as Sample S8, which was processed honey, did not meet the upper level established by the EU and Ethiopian regulations (EU, 2001 and Ethiopian standards, 2005). These values show the samples were adulterated most probably with commercially available sugar in order to maximize the profit margin".

H. Diastase Activity

Table 3 displays the Diastase activity of the honey samples that were analyzed. According to Table 3, the processed honey sample bought from the store had the lowest diastase activity (0.314 ±0.07 DN), whereas the sample received directly from the beekeeper had the greatest diastase activity (18.7 ±0.127 DN). These findings corroborated those of Ahmed et al. (2012) and Gangwar et al. (2016). However, three samples S8 (0.314 ±0.07 DN), S7 (4.08 ±0.035 DN) and S2 (5.524 ±0.00 DN) from the samples had lower diastase activity. Each sample's value difference was statistically significant ($p \sim 0.05$). (Table 3). The lower values of diastase activity might be due to high heat treatment and the samples might be adulterated with other extraneous substance like commercial sucrose and/or molasses.

I. HMF Content

According to Table 3, the honey samples that were tested had

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levels of HMF ranging from 25.55±0.055 to 1036±0.115 mg/Kg. In compliance with the EU, Ethiopian quality standards (QSAE), and Codex requirements for honey(Kayode, J. and Oyeyemi, S.D., 2014; EU, 2001; Codex Alimentarius Commission, 2001a), the honey sample obtained directly from the beekeeper (from a traditional hive) had a level of HMF of 25.55 mg/kg. The findings, which varied from 2.2 to 36.15 mg/kg, on the physicochemical characteristics of honey in southwest Ethiopia were consistent with it (Getachew, A., et. al., 2012). Every honey sample from the neighbourhood market, except for S1, exhibited higher HMF levels. The values were S2 (145.73±0.527), S3 (61.23±0.058), S4 (71.42±1.167), S5 (49±0.17), S6 (217.96±1.38), S7 (40.96±0.35) and S8 (1036.43±0.115). S8, the processed honey sample had the maximum value.

The statistical significance of the value difference among the samples was established ($p < 0.05$) according to Table 3. The findings were in agreement with the findings of the research on the levels of HMF in honey and other foods conducted by Bogdanov et al. in 2015. The reported result was 986.57–1131.76mg/Kg and negligible amount, 5mg/Kg to 922 mg/Kg in Pakistan and Indonesian honey samples respectively. The findings showed that sample 2 and sample 7 (S2 and S6) in particular may have been subjected to high temperatures and interfered with table sugar. The processed sample (S8) had highest HMF level when compared to other, this could probably be because of high temperature for long times. The other important reason is that the samples could be stored for a long period in bad storage condition. The industrially processed honey sample had a surprisingly higher HMF concentration than any of the other samples.

Ash Content

There are a variety of factors that could influence the quantity of ash that is present in honey, according to Belay (2013). These factors include the kind of soil, the mineral concentration in the nectar, the apiary, and the botanical origin of the honey samples. It is thought of as a quality indicator that may reveal the potential botanical source of honey. All eight honey samples had an average percent ash concentration that varied between 0.05 ± 0.001 and 0.74 ± 0.002. The ash content of honey reported in Nigerian honey was similar to these values. (Abel A. A. and Adedoyin D. B. 2011). The results of ash content obtained from this study were comparable with the results (0.09 - 0.54 %) of Adigrat honey (Tadess G., Gebregziabher B. 2014). The Codex Alimentarius Commission 2001b and Nyau. V, et.al., 2013 both state that the maximum mineral level in honey is 0.6 g/100g. The study's results demonstrated that all samples, with the exception of the local market sample (S3), fall within an acceptable range and are below the lower limit.

Trace and Heavy Metals

Honey samples were collected from a variety of sources, including beekeepers in Bonga's Kaffa zone, processed honey samples from supermarkets and local markets, and honey samples from local markets. The quantities of six heavy metals, namely zinc, cobalt, lead, cadmium, and cadmium, are represented in Table 4. The findings of this study offer a concise description of the levels of these heavy metals that were found in various samples of honey. The elements lead, cadmium, and cadmium were not found in all of the honey samples that were tested.

Cu, Zn, and Co heavy metal tests on all of the honey samples came back positive. The amounts of heavy metals in the honey samples ranged between 0.02 and 0.1 for Cu, 0.02 and 0.16 for Zn, and 0.03 and 0.12 for Co. The concentration of Cu and Zn obtained in this research agreed with the reported result in Kenyan honey, the maximum and the minimum values were 0.012 and 0.25, and, 0.032 and 0.123 respectively (Maiyo, W.K. et. al., 2014). The concentration of Co obtained in this research was also in agreement with the reported result in Nigerian honey (less than 0.25) (Chukwujindu M. et. al., 2015). The amounts of these metals in none of the samples were higher than the permissible limits, which are 250µg/g for zinc and 5µg/g for silver, established by the Codex Alimentarius Commission. (Codex Alimentarius Commission Standards, 2002).

Thermal Treatment of honey and its Effect on the Formation of HMF Within the time extend of 30–180 minutes at 400C, the findings show that the concentration of HMF increased to a high of 36.67 mg/kg, with a very small rise in concentration of 42%. When the time extended to 720 minutes at the same temperature, the value was 42mg/kg which means 63.36% rise in HMF level. This value exceeded by small amount from the maximum permissible level allowed by ESA, EU & IHC (Nyau. V et.al., 2013). The result agreed with that reported for Herzegovinian honey, (Ethiopian Standard, 2005).

When the temperature was increased to 500C, the level of HMF showed slightly higher value (15-180 minutes), while at 400C, the value of HMF was less (49.39%). But the level exceeded the permissible limit (40mg/Kg) allowed by ESA, EU and IHC. (Bogdanov, S. 2009).

When honey samples were heated at 700C the level of HMF increased significantly after exposure above 60 minutes, the values were 44.165, 48.95, 54.49, and64.98 within the time interval of 60, 180, 360 and720 minutes respectively. Compared to the initial level of HMF it increased by 215.37%. This result also agrees with those reported in Brazilian Honey (Temamogullari F. et.al., 2012).

The results obtained from the experiment indicated that the significant increase in concentration of HMF depends on temperature variation and time intervals. It was observed that on exposure to temperature of 900C for 60, 180, 360 and 720min the level of HMF increased to 54.79, 77.47, 84.21 and 122 mg/kg respectively. The 3-D plots (Figure 1 'A' & 'B') through response surface models indicate the interaction of both independent variables such as time and temperature. The amount of HMF recorded in samples at 1000Cexposed for 15, 30, 60, 180, 360 and 720min ranged from 45.13-133.08mg/Kg, this result also agrees with that reported for Brazilian honey.

The above results show an increase in the value of HMF with the rise in temperature.

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The treatment time also significantly affect the increase of the concentration of HMF.

increased. The highest permitted concentration of HMF in honey samples, as stated by Nyau et al. (2013), is 40 mg/kg; this is less than the honey sample's initial value.

TABLE IV
CONCENTRATION OF TRACE AND HEAVY METALS IN HONEY SAMPLES (mg /L)

Samples	Conc. Metals (mg/L) or ppm					
	Cu	Zn	Co	Cr	Pb	Cd
S1	0.02±0.002	0.05±0.004	0.031±0.02	ND	ND	ND
S2	0.03±0.01	0.04±0.003	0.05±0.01	ND	ND	ND
S3	0.05±0.003	0.03±0.002	0.062±0.03	ND	ND	ND
S4	0.05±0.002	0.04±0.001	0.07±0.003	ND	ND	ND
S6	0.06±0.003	0.02±0.003	0.08±0.01	ND	ND	ND
S7	0.08±0.002	0.16±0.004	0.11±0.01	ND	ND	ND
S8	0.1±0.003	0.03±0.003	0.12±0.01	ND	ND	ND

Note: ND - Not detected

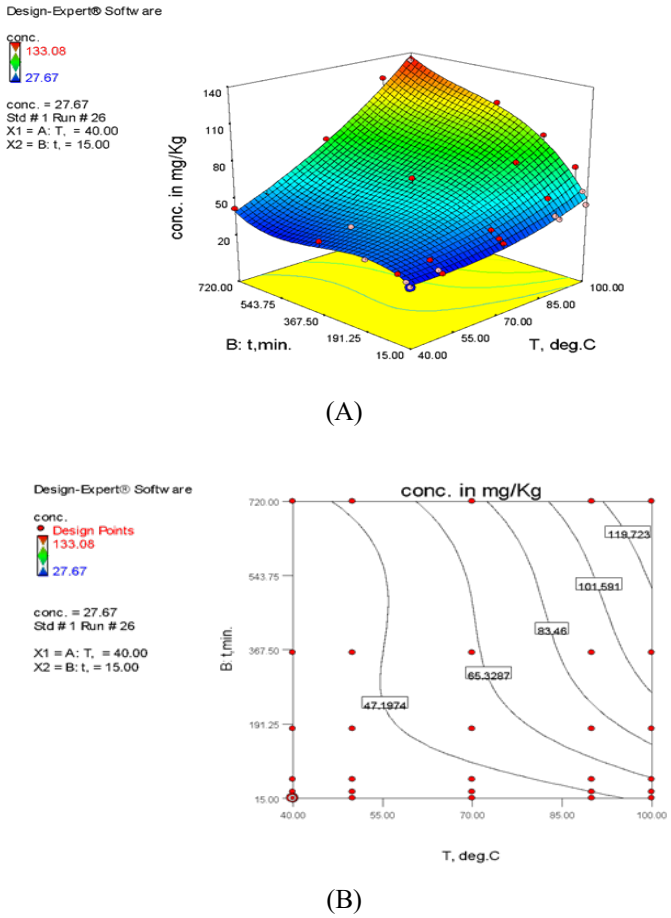

Fig.1. (A and B).Response surface plots representing the Effect of temperature and time on the concentration of HMF

Figure (2) showed the interaction between treatment temperatures and time intervals and their influence on HMF concentration. It indicates with increase in treatment temperature and time interval, yield of HMF also rapidly

With an F-value of 101.49, the ANOVA analysis demonstrates the significance of the model. Only 0.01 percent of instances might have a "Model F-Value" this large due to noise. Model terms of significance are denoted by "Prob > F" values less than 0.0500. The words A, AB, A2, B2, AB2, and B3 have significant meaning in this model. In the event that the values exceed 0.1000, the model terms are deemed not significant. "Adeq Precision" states that the signal-to-noise ratio is quantified. Ideally, the ratio should be higher than 4. The ratio of 37.25 indicates a strong enough signal. The findings demonstrated that the model was statistically valid and could be used to explore the design space. The final equation may be written as follows in terms of the coded factors:

$$Y = 63.76 + 37.38 \times A + 5.6 \times B + 18.99 \times A \times B + 8.54\ A2 - 7.68 \times B2 + 0.72 \times A2 \times B - 8.45 \times A \times B2 + 1.09 \times A3 + 17.89 \times B3$$

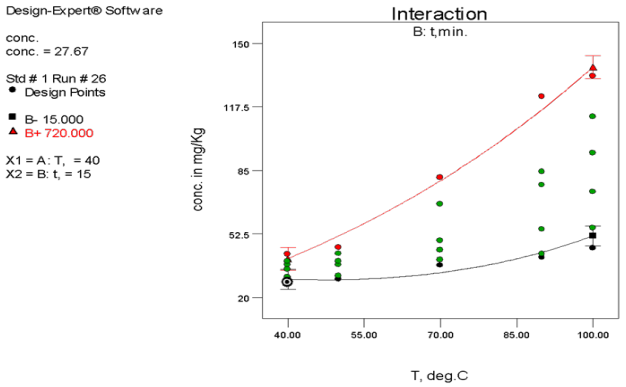

Fig .1. Response surface plots representing the interactive Effect of temperature and time on the concentration of HMF

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CONCLUSION

The aim of this work was to examine the effects of adulteration and temperature on the concentration of HMF and its quality. We compared the findings from each sample to those from the others and to national and international standards after we analyzed them all using standard method. The findings demonstrated that honey samples procured directly from beekeepers exhibit a high level of quality. The honey sample that underwent processing exhibited high levels of HMF, sugar percentage, moisture content, and reduced proline concentration. In contrast, samples S2 and S6 were purchased from the nearby market. This shows that the samples were likely heated for an extended amount of time and maybe contaminated with sugar. Sample S8, which was processed, had a greater concentration of HMF, suggesting that the product

was heated for an extended period of time. It is also confirmed that the sample was tainted with an extraneous ingredient, such commercial sugar, because of the very low concentration of proline. The findings of this study indicated that different honeys on the market had significantly different quality levels.

Although all of the honey samples tested positive for Cu, Zn, and Co, the concentrations were within the regulatory acceptable levels. Not all honey samples included Pb, Cd, or Cr. According to the findings, the amount of HMF was significantly affected by how long it was exposed to heat treatment. When the honey sample was heated over 700C, there was a clear rise in the concentration of HMF. But as the treatment period is prolonged, the concentration goes over the maximum allowable limit in the temperature range of 40 to 700C. The quantity of HMF that was initially present before to heat treatment should be used to determine how long honey in the industry will be heat processed.

Thus, even at lower temperatures, there should be no exposure to temperatures more than a certain point, and the length of exposure should be kept to a minimum, when processing honey at an industrial level.

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