1	Physicochemical Properties and Pesticide Residue in Market Honey Collected from the
<mark>2</mark>	Major Honey Producing Zones in South Western Ethiopia
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23	ABSTRACT
24	The study aimed to evaluate the physicochemical composition and pesticide residues in honey.
25	Sixty honey samples were collected from three main honey producing zones; Jimma, Kefa and
26	Bench Maji, from which the physicochemical composition and the levels of 11 pesticide residue
27	was determined. The mean Hydroxy methyl furfural (HMF) content for the Jimma, Kefa, and
28	Bench Maji samples respectively were 81.37±2.32, 104±3.12, and 74.5±1.72. Of all the 60
29	honey samples determined, the levels of pesticide residue were ranging from 8.67 - $4305\mu g/kg$.
30	Out of all the pesticide residue positive samples, the majority contained pesticide residue above
31	the EU limit of $10\mu g/kg$. Overall, nearly five out of 11 pesticide residue was detected from the
32	honey samples and the mean level of the each pesticide residue was above the EU limit.
33	Therefore, it is imperative to improve the quality and safety of honey from the standpoints of
34	trade and health.
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36	Keywords: Honey, Physicochemical properties, Pesticides residues, Ethiopia
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1. Introduction

Honey is a naturally occurring product produced by bees from plant nectar, plant secretions, or the excretions of insects that feed on live plants. These materials are gathered by bees, which then blend them with their unique compounds, dehydrate them, and allow them to develop and ripen within honeycombs (Turkey Ministry of Food, Agriculture and Livestock, 2012). According to Turkish Standards Institution (2010), there are three primary types of it produced:

comb honey, honeydew, and blossom or nectar honey.

The composition of honey is significantly influenced by the type of plant used to make it, as well as by environmental and meteorological factors (Cimpoiu *et al.*, 2013). Ethiopia is thought to be home to the majority of honeybees in Africa. It is estimated that there are 10 million honeybee colonies in the country, of which 7.5 million are maintained in hives by about 2 million small-scale beekeepers, and the remaining 500,000 exist as wild colonies in the forests (Sahle *et al.*, 2018). The nation could yield 50,000 tons of wax and 500,000 tons of honey, respectively (MoARD, 2008). But the 2017 study found that 6,000 tons of wax and 66,221.82 tons of honey were produced annually (CSA, 2018), which amounts to 13.24% and 12%, respectively, of the predicted potential for beeswax and honey. As a result, the country produces more honey than any other country in Africa and ranks ninth in the world (Bogdanov, 2011).

Nearly every region in Ethiopia produces honey. Of the potential honey production, the most important producing regions are Oromia (38%), Amhara (26%), SNNPR (18%), and Tigray (7%). This is due to regional variations in honey production capacity. The nation's northwestern, western, and south-western regions are frequently viable sites for honey production (Gezahegn, 2001).

The sensory, physical-chemical, and microbiological characteristics of honey all play a significant part in defining its quality. Clear guidelines for the physicochemical quality requirements for honey are provided by the European Community Guidelines 2001/110 (Council Directive of the European Union, 2002). The main variables of interest are moisture content, electrical conductivity, ash, reducing and non-reducing sugars, free acidity, diastase activity, and the amount of hydroxyl methylfurfural (HMF) (Belay et al., 2013).

Beyond physicochemical considerations, however, an increasing number of experts are turning their attention to the problem of pesticide contamination of animal-sourced food. Pesticides are biological agents that are applied to crops to either kill or inhibit pests that are interfering with human food production. They offer benefits as well as drawbacks (FAO, 2002). Pesticides applied to plants may be toxic to bees if they come into contact with the treated plants or if they fly over a contaminated area and inhale harmful airborne particles. By contaminating pollen and nectar, they can also kill the entire colony (Dively & Kamel, 2012). However, there could be other reasons why pesticides are present in hives as well. There could be legacy contamination (Karise et al., 2017). Pesticide residues from non-agricultural areas can also be absorbed by beehives (Kode, 2018).

Beginning in the 1940s, organochlorine pesticides (OCPs) were widely used in agriculture and pest management. However, in the 1970s, due to their long-lasting effects on the environment, OCPs were banned in many countries. Nonetheless, OCPs were widely used in Ethiopia to manage insect pests for both public health and agricultural reasons (Negatu, et al., 2016). The Food and Agriculture Organization's study on the obsolete pesticide inventory lists Ethiopia as one of the many African countries impacted by the problem of obsolete pesticide stockpiles. The most dangerous pesticides are found in these landfills (Abiye & Hadera, 2005).

One prospective location for beekeeping in Ethiopia is in the southwest, where honey production is a significant source of revenue for local farmers. There is a dearth of information regarding the quality perspective based on national and international standards, despite the areas' potential and the volume of honey produced. Thus, the purpose of this study was to present data regarding the physicochemical characteristics and pesticide residue of honey samples that were gathered from Ethiopia's southwest.

2. Materials and methods

2.1 Sampling area and sample collection

The honey samples were collected purposively from the most potential honey producing zones namely Jimma (Jimma city), Kefa (Bonga city) and Bench Maji (Mizan city), South western Ethiopia. A total of 60 honey samples of each weighing 0.5 kg (20 samples from each cities)

were collected randomly from local retailer stores in November 2021 and then samples were transferred to Animal products and Input Quality Testing Center, Laboratory under appropriate conditions for conducting physicochemical and pesticide residue analysis. Honey samples were purified of all foreign materials and strained with great precautions not to be contaminated and exposed for heat (Pavelkova *et al.*, 2013). The honey samples were labelled with numbers, site of honey sources and date of collection. Before being examined, the honey samples were kept at room temperature in a dry, odor free space away from ants and other undesired objects.

2.2 Physicochemical analysis

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- The physicochemical quality properties (electrical conductivity, moisture and Hydroxy methyl
- furfural (HMF) of the honey samples were evaluated according to the principles and procedures
- of the international honey commission (IHC, 2009).

2.2.1 Electrical conductivity

- To determine electrical conductivity 10 g of anhydrous honey sample mixed with deionized
- water that makes 50 ml of solution was prepared. After inserting the conductivity cell in a
- solution at 20 °C, the conductance was measured in mille Siemens by conductivity meter. The
- electrical conductivity was measured by means of a conductivity meter after calibrating with a
- 127 0.01 N KCl solution (IHC, 2009).

128 2.2.2 Moisture content

- The moisture content of the honey samples was determined by measuring the refractive index at
- 20 °C according to the harmonized method for honey developed by the International Honey
- 131 Commission (IHC). The determination of moisture was done using Digital Refractometer
- 132 (Kruss, Germany). All measurements were performed at 20°C. The samples were directly
- smeared on the surface of the prism evenly; after two minutes the reading of refractive index
- was recorded. Each sample was measured twice and averages of two readings recorded and
- corresponding value for moisture content recorded.

136 **2.2.3 HMF**

- 137 A honey sample (5 gram) was dissolved in 25 ml deionized water and transferred quantitatively
- to a 50ml volumetric flask and made up to 50 ml with deionized water, then centrifuged for 10
- minutes at 3500 rpm before injection on the HPLC for HMF analysis (Terrab et al., 2003).

- HPLC Agilent 1260 Infinity II system with diode array detector, software Open Lab CDS was
- used for sample analysis. The column used was a ZORBAX Eclipse XDB-C18, reverse phase
- 142 (Supelco, Bellefonte, PA) stainless steel column (150mm *4.6 mm i.d.; film thickness 5
- 143 µm). The mobile phase was water: methanol (70:30, v/v), and the flow rate was 1 ml/min with
- an injection volume of 10 μl.

2.3 Pesticides residue analysis

- Eleven pesticides were identified and quantified through analysis of the honey samples. From
- organochlorine pesticides: Hexachloro benzene (HCB), Heptachlor, Endosulphan, Aldrin,
- Dichloro diphenyl dichloroethane (p,p-DDE), Dichloro diphenyl trichloroethane(p.p-DDT),
- 149 Dichloro diphenyl dichloroethylene (p,p-DDD) and Lindane; from Artificial
- 150 Pyrethroids:Cypermethrin and Deltamethrin and from Organophosphorus: Chloropyrifos
- residues were analysed.

152 2.3.1 Chemicals and reagents

- Reference standards of HCB (96.6%; Lot No.VKV-007245), Lindane (85.7%, Lot No.VIH-
- 154 115002), Aldrin (91.3%, Lot No.VKV-003060), Heptachlor (96.6%, Lot No.VKV-006111),
- 155 Chlorpyrifos (99.6%, Lot No.VIH-165271), Endosulfan (99.2%, Lot No.VKV-007245),
- 156 Deltamethrin (96.6%, Lot No.VIH-114990), DDE (99.7%, Lot No.KVV-000760), DDD
- 157 (96.6%, Lot No.VIH-114750), DDT (100%, Lot No.UKV-001008), Cypermethrin (85.7%, Lot
- No.WK-000207) were supplied from Fluka Goldie chemika. Chemical reagents such as
- 159 Acetonitrile were supplied from Sisco research laboratories (Maharashtra, India). Hexane was
- procured from Merck Company. Ultra-pure water (18.2M Ω cm⁻¹) was produced in-house using
- thermo scientific BARNSTEAD Genpure UV-TOC/UF, water purification system
- 162 (Hungary).
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- All pesticide reference standards were stored according to manufacturer's recommendations
- until use. Pesticide stock solutions were prepared in acetonitrile at 1 µg/ml and stored in amber
- screw-capped glass vials at -20 °C.

2.3.2 Equipment and instrumentation

- 168 The major equipment that were used for the sample preparation include analytical balances
- 169 (±0.01 mg) (sartorious AG, Germany), Refrigerated Centrifuge (HERMLE, Z446K; Germany),

- Nitrogen concentrator (Multi Vap 54 Lab Tech, USA), Wrist action mechanical shaker (model
- 75, Burrell Scientific, LLC/ Pittsburgh, PA, USA), Vortex mixer (Bio cote Sturt, Italy), Micro
- pipettes, Duran Bottles, Bottle top dispenser, beakers, various class A volumetric flasks and
- measuring cylinders, pH meter (Bibby scientific Ltd, U,K). All glassware and other apparatuses
- were scrupulously cleaned and dried in a drying cabinet (LEEC, Limited, UK).

2.3.3 Preparation of standard solution

- Stock standard solutions were prepared at concentrations corresponding to 1 mg/mL (1000
- μg/ml) taking in to account purity level, stability and solubility of the pesticide in the solvent.
- 178 The standard solutions were prepared in acetonitrile for each pesticides by transferring 10 mg
- equivalent of the base materials quantitatively in to a 10 ml class A volumetric flasks separately
- and diluted to volume and stored in amber vials at \leq -20 °C. A working standard was made from
- stock solution by taking the required amount from each separate pesticide stock to make mixed
- standard by taking into consideration the maximum residue limit (MRL) for each pesticide in
- 183 honey matrix.

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2.3.4 Extraction methods

- AOAC official method 2007.01 was used for analysis of pesticide in honey samples. All reagents
- used were of analytical grade. Samples were extracted using the Quick, Easy, Cheap, Effective,
- Rugged, Safe (QuEChERS) multi residue method for the analysis of pesticide residues in low fat
- matrix (Calatayud et al., 2016). QuEChERS is chosen for the analysis of pesticides in honey
- based on the description of several studies in the literature demonstrating its efficiency and good
- 190 performance for extraction of pesticides in this matrix (Kujawski et al., 2014). Another criterion
- used to choose the sample preparation technique was acceptable recoveries for all analytes. A
- 192 10g sample of honey sample was weighed in falcon tube and spiked for some quality control
- sample with the pesticide standard solution and mixed with 10ml of water and homogenized by
- shaking by vortex mixer. The sample was mixed with 10ml of acetonitrile and submitted to
- extraction by agitating for 1 min. Extraction salts 4g MgSo₄, 1g Na Acetate) was added and
- vortexed for 2 minutes and shake for 5 minutes and then Centrifuged for 15 minutes at 4500 rpm.

- 198 Cleanup: Then, the organic phase was separated by centrifugation at 4500 rpm for 15 min.
- organic phase was collected, Transfer 6 ml of extract to QUECHERS containing 900 mg
- 200 MgSo₄, 300 mg PSA and 150 mg C18 for clean-up.
- 201 Drying: Four ml was transferred to clean 15 ml falcon tube and evaporated in a rotary
- 202 evaporator under reduced pressure at 6°C and dried under a gentle stream of pure nitrogen.
- Finally the residue was reconstituted by 0.5 ml of hexane and passed through 0.50μm sized
- pore PTFE (poly tetrafluoro ethylene) filter and brought to GC-ECD Auto sampler for analysis.

2.3.5 GC/ECD analysis

- 206 Confirmatory run analysis was done on Agilent 7890A gas chromatography and a packed
- 207 column HP-5-5% phenyl methyl siloxan (30m x 320μm i.d., film thickness 0.25 μm). The GC
- was operated under following conditions: The initial temperature of oven was 75°C, gradually
- increased at 25°C/min to up to 150°C held for 0 min, then increased at 5°C/min to 280°C held
- 210 for 10 min. The total run time was 39 minutes. Nitrogen (99.995% purity) was used as carrier
- 211 gas at a flow rate of 60 ml/min. From an aliquot of 0.5 ml, 2 micro liters was injected in
- splitless mode. Chem-station software was used for instrument control and data analysis.

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2.3.6 Method validation

- 215 The validation parameters known as specificity, linearity, limit of detection (LOD), limit of
- 216 quantification (LOQ), recovery and precision were determined in compliance with the
- European Commission guidance document SANTE/2017/11813. In order to verify the method
- 218 specificity and confirm the absence of potential interfering compounds around the retention
- 219 time of each pesticide, 20 blank extract of the matrix and 20 fortified/spiked extracts were
- analyzed and then, checked for the presence of significantly interfering peak at each retention
- time of targeted pesticides within 2.5% margin of the retention time. Linearity of the method
- was evaluated by constructing a matrix-matched calibration curves (MMC) of aliquots obtained
- from samples spiked with pesticide standards of interest. Calibration standards were prepared
- by spiking honey matrices with a known quantity of target analytes of calibration range
- comprising the maximum residue limit (MRL). The spiked concentrations were 0.75, 1.0, 1.5,
- 226 2.0, 2.5 and 3.0 times of the corresponding MRLs, each in duplicate, with expected
- concentration equidistantly ranging from 7.5 to 150µg/kg. The plot of the MMC for each
- compound was based on the peak areas of each analyte at various concentrations. Linear spiked

calibration curves for all the interest pesticides were obtained with correlation coefficient (r²) >0.990. LOD and LOQ varied from 1.62-9.57 and 5.40-29.69, respectively which is lower than maximum residue limit set for each pesticide and recoveries of all the pesticides were found to be acceptable ranging from 88.7- 125.6% with an associated repeatability, RSD <20 %, for most of the compounds within the scope of the method. Matrix-matched calibration standards were used to calculate recoveries as this helped in compensating for any matrix effects arising from matrix interferences.

2.4 Statistical analysis

Data were entered and analyzed using SPSS version 20 statistical software. All the measurements were carried out in triplicates and the data were presented as means \pm standard deviations. Analysis of variance (ANOVA) was used to compare the quantified variables in the samples of honey. When p values less than 0.05 (p < 0.05) considered as statistically significant.

3. Results and discussion

3.1 The physicochemical properties of honey

The physicochemical quality properties (moisture, electrical conductivity and HMF) of the honey samples are presented in (Table 1).

Table 1. Physicochemical properties of honey samples collected from South Western Ethiopia, 2021

Physicochemi cal	Sam	Overall mean	Standards			
properties (Mean <u>+</u> SD	Jimma (n=20)	Kefa (n=20)	Bench Maji (n=20)		Ethiopian standard	EU standard
Moisture (g/100g)	18.19±1.71a	17.00±2.16a	16.88±2.16 a	17.35	≤22	≤20
Electrical conductivity (mS/cm)	0.68±0.45 a	0.61±0.35 a	0.71±0.28 a	0.66	< 0.8	< 0.8
HMF (mg/kg)	81.37± 2.32 b	104±3.12 a	74.5±1.72c	86.62	40	40

^{*}Means with different letters across a row are significantly different. SD: Standard deviation, n: number of samples collected, EU: European, HMF: Hydroxymethylfurfural.

3.1.1 Moisture content

Bench Maji, Kefa, and Jimma had average moisture contents of 16.88%, 17%, and 18.19%, respectively, of the honey samples in our study. These moisture contents were all within the range that was allowed for the global honey market. Since moisture content has a major impact on how quickly honey ferments, how long it lasts, and how it processes, it is the most significant quality factor (Gebremedhin, et al., 2013). The varied moisture content of honey is influenced by several factors, including the type of hive used, the time of year it is harvested, the amount of maturity the honey reaches in the hive, the outside weather, and the moisture content of the plant it comes from (Finola et al., 2007; Nanda et al., 2003).

The moisture content of this study is higher than that of Kebede & Adgaba (2011), who discovered a mean moisture content of 15.94% in the Silte district, Southern Ethiopia. In contrast to the current finding, Getachew et al. (2014) reported a higher mean moisture content (22.86%) of honey samples collected from Southern Ethiopia's Gesha, Masha, and Sheko areas. Our samples' moisture contents fall between the range of 14.21% and 16.59% reported by Samira & Nebia (2017), suggesting ideal harvesting and a good level of maturity. A different study conducted by Ouchemoukh et al. (2007) also revealed that the moisture content of eleven honey samples from Bejaia, Algeria, varies from 14.6 to 19%.

In this investigation, the moisture content of the honey samples gathered from the three locations did not differ significantly (P > 0.05). Furthermore, none of the samples had a moisture content higher than 20%, which is the maximum permitted by EU Directive (European Commission, 2002). A crucial criterion for overall honey quality (blossom honey) is the maximum of 20%, as stated by the EU and FAO/WHO (Codex Alimentarius, 2001; The Council of the European Union, 2002). This suggests that most of the honey samples used in this investigation were collected when ripe, or capped, and then stored for a predetermined period of time under ideal conditions (Fredirick et al., 2013).

3.1.2 Electrical conductivity

A close relationship exists between the concentration of mineral salts, organic acids, and amino acids and electrical conductivity. The parameter is crucial for differentiating honeys from various floral sources because the concentration of these compounds in nectar source plants

varies significantly (Terrab et al., 2004). According to our study's findings, the three locations' mean electric conductivity ranged from 0.61 to 0.71 mS/cm overall (Table 1). The electric conductivity of honey did not differ significantly (P > 0.05) between locations. With 75% of the electric conductivity measurements being less than 0.8 mS/cm, this study fully satisfies both European and Ethiopian requirements (IHC, 2002).

Our research's findings generally concur with those of an earlier study conducted in Ethiopia. According to a study by Tesfaye (2015), honey samples taken from the Bale Oromia region of Ethiopia had an average electric conductivity of 0.65 mS/cm with a range of 0.22-1.34 mS/cm. In a different study, Mulugeta (2017) also found that honey samples taken from the Oromia region's Ambo district had a mean electrical conductivity of 0.55 mS/cm. A different study by Belay et al. (2013) found that honey samples taken from the Bale Harenna forest had a mean of 0.70 mS/cm, which is higher than the current result. Based on electrical conductivity it is possible to generalize that honey of the study area is of good quality and fulfill the Ethiopian and European standards. In contrast to our findings, honey samples from Yemen and Egypt were reported to have high electrical conductivity (4.18 and 1.98 ms/cm, respectively) (E1

3.1.3 Hydroxy methylfurfural (HMF)

Sohaimy et al., 2015) (El Sohaimy et al., 2015).

The honey samples examined in this study had an average HMF value of 86.62 mg/kg, with a range of 74.5–104 mg/kg (Table 1). The results of our study indicated a higher mean HMF content, which is unacceptable according to global honey market standards and exceeds both the EU and Ethiopian standard limits of 40 mg/kg and 40 mg/kg, respectively. Furthermore, our findings surpass those of Getachew et al. (2014), who reported a mean HMF content of 19.52 mg/kg in honey samples collected from the Southern Ethiopian districts of Gesha, Masha, and Sheko.

Honey's concentration of HMF rises with storage and extended heating (Bogdanov, 2011). Higher HMF content has been reported in other nations, which is consistent with our findings. In Kenya, research conducted by Fredrick et al. (2013) and Muthui (2012) found that honey samples obtained from supermarkets contained 85.4 mg/kg and 3.7–389.4 mg/kg of HMF.

respectively. A related study conducted in Uganda by Kugonza & Dorothy (2008) found that honey samples taken from supermarkets had an HMF content of 103.2 mg/kg (mean).

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3.2 Pesticide residue levels in honey samples

This study was conducted to determine the levels of 11 pesticide residues in honey samples collected from South Western Ethiopia. The levels of pesticide residue detected in the honey samples are described in (Table 2).

Table 2.The levels of pesticide residues determined in honey samples collected from South Western Ethiopia, November 2021.

Types of	Jimma (20)			Kefa (20)			Bench Maji (20)		
pesticid	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
es	$(\mu g/kg)$	(µg/kg)	$(\mu g/kg)$	$(\mu g/kg)$	$(\mu g/kg)$				
HCB	191.05	4305.0	1012.5	BDL	BDL	BDL	1068.4	1068.4	1068.4
LDN	92.13	92.13	92.13	196.43	431.75	314.09	9.92	9.92	9.92
HEP	9.57	70.56	27.33	BDL	BDL	BDL	10.74	31.08	22.25
ALD	9.45	78.36	17.04	9.16	71.61	38.08	8.67	25.17	16.92
CHL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
END	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
DDE	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
DDD	59.39	59.39	59.39	BDL	BDL	BDL	BDL	BDL	BDL
DDT	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
CYP	BDL	BDL	BDL	119.48	119.48	119.48	BDL	BDL	BDL
DEL	BDL	BDL	BDL	36.71	75.28	55.99	36.05	36.05	36.05

^{*}BDL: Below Detection Limit, HCB: Hexachlorobenzene, LDN: Lindane, HEP: Heptachlor, ALD:

325 Deltamethrine

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Nearly all of the mean concentrations of pesticide residue found in the honey samples taken from the three locations exceeded the EU limit $(10\mu g/kg)$ for pesticide residue in honey, as shown in (Table 2). The use of the aforementioned pesticides in agricultural fields may be the

Aldrine, CHL: Chlorpyrifos, END: Endosulfan, DDE: Dichlorodiphenyldichloroethylene, DDD:

Dichlorodiphenyldichloroethane, DDT: Dichlorodiphenyltrichloroethane, CYP: Cypermethrine, DEL:

cause of the pesticide residue found in the honey samples used in our investigation. Another explanation for their presence in the honey samples is the persistent nature of organochlorine pesticides in the environment from their prior use in public health and agriculture. These organochlorine pesticides bioaccumulate at higher rates in plant tissues than in ambient environmental media like water and air. Organochlorine pesticides enter the food chain through these activities and end up in flower plants (Eissa et al., 2014).

- In contrast to our results, previous Ethiopian studies that reported low mean pesticide residue levels. According to a study by Mulugeta et al. (2017), honey samples taken from the West Shewa zone in the Oromia region contained endosulfan (mean: 11.7µg/kg), DDD (mean: 35μg/kg), and DDT (mean: 5μg/kg). All of the honey samples that were taken from the Walmara district of the Oromia Special Zone had pesticide residue levels that were below the detection limit, according to a different study by Kebede (2019). A different study by Beyene et al. (2023) in the East Shewa and West Arsi Zones of Oromia revealed that the most prevalent pollutant was chlorpyrifos (16.7%), followed by endosulfan sulphate (12.5%). The residues varied from 0.1 µg/kg to 14.7 µg/kg chlorpyrifos, 0.2 µg/kg to 106.2 µg/kg Endosulfan sulphate in Dugda, 0 to 16.4 µg/kg DDT in Shashemene, 2.1 µg/kg to 51 µg/kg Chlorpyrifos, and 4 μg/kg to 31 μg/kg Endosulfan sulphate in Negele Arsi.
- Similarly, low pesticide residue level has been also reported in other countries. In a Ghanaian study, Godfred et al. (2017) found that honey samples taken from forested areas contained endosulfan and aldrin (mean: 10µg/kg). In a different study, Blasco et al. (2004) found that honey samples from Spain and Portugal had mean HCB concentrations of 300 µg/kg and 390 µg/kg, respectively. In addition, a study by Erdogrul (2007) found that honey samples taken from Kahramanmaras, Turkey, contained 300µg/kg (mean) HCB, 40µg/kg (mean) heptachlor, 60µg/kg (mean) Aldrin, and 360µg/kg (mean) endosulfan.
 - Additionally, 92.13μg/kg, 314.09μg/kg, and 9.92μg/kg of lindane were found on average in the honey samples that were collected from Jimma, Kefa, and Benchm Maji, respectively, for our study. Compared to France (8.5 μg/kg) (Chauzatet al., 2006), Egypt (9.4 μg/kg) (Malhat et al., 2015), Turkey (3.7 μg/kg) (Yavuzet al., 2010), and Serbia (4.45 μg/kg) (Kartalovic et al., 2015), these mean concentrations were higher. Different nations and governments reacted differently to the inclusion of lindane on the list of persistent organic pollutants (POPs) in the

360 Stockholm Convention, which has prohibited its production and use since 2010 (Madaj et al.,

361 <u>2016</u>).

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4. Conclusion

- This study found that the physicochemical characteristics, like electrical conductivity and 363 moisture content, are within permissible limits for both Ethiopian and EU standards. 364 Nevertheless, the Hydroxy methylfurfural surpasses the 40 mg/kg Ethiopian and 40 mg/kg EU 365 366 limits, indicating deterioration, length of storage, and condition of the honey. The study's findings also verify that, out of 11 pesticide residues, five were found in the honey samples, 367 368 with concentrations ranging from 8.67 µg/kg to 4305 µg/kg. The majority of the mean concentrations of pesticide residue found in the honey samples exceeded the European Union's 369 370 threshold of 10µg/kg. Therefore, pesticide residues in tested honey samples could be harmful to the health of the consumer. Thus, it should be necessary to stop the illegal pesticide trade, 371 guarantee farmers' access to safe and less toxic chemical varieties, establish new regulations 372 regarding the use of agrochemical crops and pesticides, and foster goodwill among beekeepers 373 374 and crop growers during the spraying season. Moreover, to enhance food safety and safeguard consumer health, agricultural crops should not be sprayed with pesticides while they are in 375 bloom. 376
- 377 Declaration of competing interest
- No potential conflict of interest was reported by the author(s)
- 379 Authors' Contributions
- FF and AA were writing the protocol, participated in data collection and experimental work. FF
- drafted and prepared the manuscript. FF and AA contributed to editing the manuscript. All
- authors contributed to the manuscript and approved the submitted version.

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