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Effect of Agro-Ecology, Source, and Beehive Type on Beeswax Quality

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Abstract

Beeswax is produced by honeybees used for various purposes. This experiment was conducted to evaluate the effect of agro-ecology, hive type and beeswax source on beeswax quality. A total of 18 samples were collected from different sources, altitudes, and hive types. Data were collected on 7 major quality parameters (melting point, refractive index, ash content, total volatile content, acid content, saponification value, and ester content). We followed the Ethiopian laboratory standard to analysis the beeswax quality parameters. The study evaluated beeswax quality in comparison with the national and international standards. The mean values of the parameters were 62.42 ± 0.44 ^{0}C, 1.44 ± 0.01, 0.14 ± 0.07%, 1.44 ± 0.3%, 25.43 ± 2.15 mg/g, 95.42 ± 5.1 mg/g, and 70.9 ± 6 mg/g for melting point, refractive index, ash content, total volatile matter, acid value, saponification value, and ester value, respectively. Majority of the parameters were within the acceptable limits set by Ethiopia, Kenya, Tanzania, and European standards. However, ester value from crude honey extract (68.68 mg/g) and lowland (66.7 mg/g) were lower than the lower limit (70 mg/g) set by the standards. Hive type did not show difference in quality among the samples ($p \geq 0.05$); however, agro-ecology and beeswax sources had effects on some of the quality parameters ($p \leq 0.05$). Beeswax of the study area generally was good quality, meeting most of the national and international standards. The sub-standard in ester value indicated quality threat requiring further investigation using advanced techniques.

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Introduction

Beeswax is a substance produced by bees to use as construction material for their combs. Beeswax is one of the most valuable and oldest bee products to be used by mankind (Kassaye, 2008; Adgaba, 2007) and still being used in various fields such as cosmetics, foods, pharmaceuticals and industry (Yadeta, 2014).

Beeswax is produced by young bees through specialized abdominal wax glands that metabolize sugars from honey into liquid wax, these wax scales are then removed with the legs and chewed with the mandibles, where they are mixed with enzymes and saliva to form a comb for brood rearing and long-term food storage within the hive (Hepburn, 1991; Xu *et al.,* 2024). Globally, beeswax is commonly classified into two major types: yellow beeswax, obtained directly from melted honeycombs and retaining natural pigments and aromatic compounds, and white beeswax, produced by bleaching yellow beeswax using sunlight or peroxide to remove color-causing components, this classification underpins widely accepted international standards for food, cosmetic, and pharmaceutical applications, with both forms recognized as safe and broadly utilized across industries (Kuznesof *et al.,* 2005; FR, 2026).

In Ethiopia, about 1.9 million farm households are involved in beekeeping and there are about 10 million colonies (Tezera, 2013). It is estimated that the country has the potential to produce 50,000 tonnes of beeswax per annum, which puts the country 4^{th} in the world in beeswax production (Kassaye, 2008; Adgaba, 2007).

Beeswax extraction is still very traditional in Ethiopia; old combs are collected from *'Tej'* leftovers are cleansed and formed into blocks using local materials. On the other hand, to get quality beeswax, rendering methods like solar-wax-melters and steam-wax-melters can be employed (Bradbear, 2009).

Beeswax should contain minimal amounts of contaminants, as it is used for in cosmetics, food and pharmaceutics (Bogdanov, 2016). Adulteration of beeswax with animal fats and petroleum has become a great threat to industry. The quality of beeswax is now challenging Ethiopian beekeepers, particularly beekeepers of the study area due to poor postharvest practices (like poor rendering method and storage) and intentionally adulterating beeswax with paraffin and animal fat (Alemu and Girma, 2019; Alemu *et al.,* 2013; Abera and Alemu, 2023). To meet the international beeswax market, assessing the quality of beeswax using the recognized laboratory standard parameters, including melting point, ester, acid, and saponification should be employed to confirm adulteration.

Beeswax quality varies notably with its source, i.e., wax from crude honey generally yields cleaner, higher-quality than from old combs, which often contains contaminants accumulated through repeated use (Gemeda and Kebebe, 2019). Hive type also influences beeswax quality, with traditional hives producing more contaminated wax than transitional and modern hives due to less controlled comb management (Bradbear, 2009). According to Tesfaye *et al.* (2016), agro-ecological conditions across lowland, midland, and highland zones further showed a difference in beeswax composition, who indicated that beeswax ester value of Dellomenna was significantly lower than that of Adaba and Dinsho districts, suggesting that lowland beeswax lower in saponifiable matter. In this regard, regional studies in Ethiopia have shown variable compliance with the country's standard (Addisu *et al.,* 2017; Bekele *et al.,* 2016). However, no study had yet focused on the current study area (Gazgibla district), which is a known beekeeping area in northern Ethiopia.

Thus, this study addressed a critical knowledge gap by systematically evaluating the physico-chemical quality of Gazgibla district beeswax across different sources of beeswax (honey extract, old comb, processed), hive type (traditional, transitional, modern), and agro-ecology (lowland, midland, highland). The study provided evidence-based recommendations for quality improvement and market positioning by comparing the beeswax quality of the district with the national and international standards.

Materials and Methods

Study Area

The study was conducted in Gazgibla district, Ethiopia, during 2024. From the district, six kebeles were purposively selected (Debrewoyila, Miyo, Asketema, Zarota, Bela, and Taba) based on their relative beekeeping potential.

Gazgibla extends between 12°7′8″ N to 12°15′7″ N and 38°52′19″ to 39°16′5″ E, covering 103,740 hectares. The district experiences a bimodal rainfall pattern, with Kiremt (June-September) as the main rainy season and Belg (March-May) as the smaller rainy season. The districts' high and rugged topography strongly influences local climate, creating variations in rainfall distribution and temperature. Higher elevations (range from 989 to 4043 m) tend to receive relatively more rainfall and experience cooler temperatures, while low lying areas are drier and hotter (GDPD, 2024).

Experimental Design

The experiment was conducted using a Completely Randomized Design, with samples collected from six distinct sites. Three experimental factors (agro-ecology, hive type and beeswax source) were considered in the study, and the design allowed for independent assessment of factor effects while minimizing experimental error.

Sample Size and Sampling Method

Purposive sampling technique was used to collect samples across agro-ecologies (highland, midland, lowland), hive types (traditional, transitional, modern) and beeswax sources. The study collected beeswax from crude honey wax, crude beeswax stored by beekeepers, and old combs, covering different hive types and agro-ecologies. The sampling method was according to Bogdanov (2016); Bonvehi and Bermejo (2012) who indicated beeswax composition can vary with floral availability, processing methods and environmental conditions. Samples were immediately placed in clean, dry, airtight containers and labeled following Ethiopian standard ET-1203-2005 to avoid contamination from honey, pollen, or dust. Clean tools were used to prevent quality loss.

A total of 18 beeswax samples were collected across agro-ecologies, hive types and beeswax sources. To represent the key variables, six samples were collected from different altitudes, six samples from the hive types, and six samples from the different beeswax processing methods.

The samples collected were stored in sealed glass containers and kept in cool, dry conditions before laboratory analysis. Temperature and environmental conditions were noted due to beeswax becoming brittle when cold and softening near 35-40°C, which can affect analysis.

The beeswax specifications of Ethiopian and the European standards for the major beeswax parameters are indicated in Table 1, (Bogdanov, 2016; QSAE, 2005).

Table 1. Beeswax specifications of Ethiopian and the European standard

Property specification	Ethiopian standard	European pharmacopeias
Melting point, oC	61-66	61-65
Refractive index at 75oC	1.4400-1.4450	1.440-1.445
Ash % by mass max.	0.20	-
Total volatile matter % mass, max	0.75	-
Acid value max	17-24	18-23
Saponification value min.	85-105	87-104
Ester value	70-80	70-80

Laboratory Analysis

We followed the Ethiopian standard laboratory protocols set for Ethiopian beeswax specification procedures (QSAE, 2005). The laboratory analysis was conducted at Sekota dry-land agricultural research center. Seven different parameters were analyzed, viz, melting point, and refractive index, ash content, total volatile content, acid content, saponification value, and ester content. melting point, and refractive index are used to detect adulteration of beeswax with paraffin (Tulloch, 1980); acid content, saponification value, and ester content used to assess hydrolysis (Bogdanov, 2016; Krell, 1996).

The procedures used to analyze the above parameters are indicated below (QSAE, 2005; Tulloch, 1980; Bogdanov, 2016; Krell, 1996).

- **Melting Point**: the method used for this parameter was capillary tube. When a transparent drop was formed the temperature was recorded.
- **Refractive Index**: At the temperature of 75 ± 1 °C, it was measured using a digital Abbe refractometer.
- **Ash Content**: Sample was incinerated in a muffle furnace at 550-650 °C until constant weight. Ash % = (Mass of ash / Mass of sample) × 100.
- **Total Volatile Matter**: Sample was dried in an oven at 105 °C to constant weight. TVM% = [Loss in weight) / (Mass of sample)] × 100.
- **Acid Value**: Sample was dissolved in a benzene: ethanol mixture and titrated with standard 0.5N KOH using phenolphthalein indicator. Acid Value (mg KOH/g) = (56.1 × V × N) / M where *V*=volume of KOH, *N*=normality, *M*=mass of sample
- **Saponification Value**: Sample was refluxed with excess alcoholic KOH, and the unreacted KOH was titrated with standard hydrochloric acid (HCl).

  Saponification Value (mg KOH/g) = [56.1 × (B - S) × N] / M

  where *B*=HCl for blank, *S*=HCl for sample.
- **Ester Value**: Calculated as Ester Value = Saponification Value – Acid Value.

Statistical Analysis

Data were analyzed using SAS software. ANOVA assumptions were checked using the Shapiro-Wilk test for normality and Levene’s test for homogeneity of variances, and assumptions were satisfied. The mean separation was conducted at a 95% confidence level, and LSD was used with appropriate caution due to the small sample size. Similar approaches have been used in a study from Spain (Bonvehi and Bermejo, 2012) to evaluate the effects of geographic and processing variables on beeswax quality.

Results and Discussion

Effect of Beeswax Source on Physico-chemical Properties

The effect of beeswax source variation on the quality of beeswax in the Gazgibla district is indicated in Table 2. The source or processing method of beeswax showed significant effect on one of the quality parameters of the beeswax ($p \le 0.05$). Majority of the parameters (melting point, and refractive index, ash content, total volatile content, acid content, saponification value) did not show variation across the sources of beeswax ($p \ge 0.05$), however, ester content showed significance variation ($p \leq 0.05$). The mean ester value of beeswax collected from the crude-honey extract (68.62 mg KOH/g) had significantly lower than ($p \leq 0.05$) the value obtained from the old comb (73.42 mg KOH/g) and processed wax (70.66 mg KOH/g). The mean ester value from the crude-honey extract (68.62 mg KOH/g) was lower than the lower limit set by Ethiopian, Kenyan, Tanzanian and European standards (70 mg KOH/g).

The lower values of ester of beeswax of the study area collected from the crude honey extract (68.62 mg KOH/g) could be due to hydrolyzing of the ester of the beeswax into free fatty acids. The beeswax quality parameter, ester, is a good indicator on the composition of beeswax. This lower value might be also due to an accelerated process by prolonged storage under unfavorable conditions, and excessive usage of heat during processing. The finding is like the report of overheating during melting hydrolyzed esters into free fatty acids (Bogdanov, 2016), and the report of traditional boiling methods often degrade quality of Ethiopian beeswax (Adgaba, 2007). According to Adgaba (2007), the process of crude wax extraction is by boiling of the honeycombs directly in which the high temperature deteriorates the esters. Similar reports were indicated as overheating beeswax could darken and change the physico-chemical properties (Bogdanov, 2015; Chatzipanagis, 2024). So, this finding calls for improvements on beeswax quality focusing on the beeswax processing techniques of the study area to maintain the ester values.

Moreover, the mean value of the total volatile matter of the study area, 1.44%, calls for special attention for the future as it indicates contaminated with a small amount of moisture or other volatile substances. Luckily, this value is yet within the acceptable limits set by the national and international standards.

Table 2. Effect of source variation on beeswax quality

Parameter	Source of beeswax (Mean ±SD)			
	Crud Honey (N=6)	**Producer (N=6)**	**Old (N=6)**	**Overall (N=18)**
Melting point	62.16 ±0.26	62.25±0.42	62.75 ±0.42	62.39 ± 0.44^{NS}
Refractive Index	1.44±0.00	1.44±0.00	1.44±0.00	1.44 ± 0.0^{NS}
Ash content	0.13±0.04	0.20±0.07	0.10±0.046	0.14 ± 0.07^{NS}
Total Volatile Matter	1.52±0.33	1.44±0.31	1.36±0.26	1.44 ± 0.3^{NS}
Acid content	25.03±1.04	25.84±2.68	25.43±2.66	$25,43 \pm 2.15^{NS}$
Saponification Value	95.65±6.65	94.31±4.63	96.30±4.49	95.42 ± 5.1^{NS}
Ester value	68.62 ± 6.10^{a}	$70.66.22^{b}$	73.42 ± 4.74^{b}	$70.90 \pm 6.09^{*}$

NS = Not significant; *=P≤0.05; N = Sample number

Effect of Hive Type on Beeswax Physio-chemical Properties

The effect of hive type on the physio-chemical properties of beeswax produced in Gazgibla district is reported in Table 3. There were no significant differences ($p \geq 0.05$) on the beeswax quality produced in the study area by the types of hives, namely modern, transitional and traditional hives. All the tested parameters (melting point, and refractive index, ash content, total volatile content, acid content, saponification and ester values) did not show statical variation across the hive types ($p \geq 0.05$).

Hive type had no effect on the physio-chemical properties of beeswax ($p \geq 0.05$) in Gazgibla district. The inherent quality of beeswax did not compromise by the promotion of modern hive for honey production. The study indicated that the cleaner beeswax produced by modern hives were chemically similar to the composition of beeswax produced from traditional hives. This finding confirms the misperceived traditional thought that the beeswax produced from traditional hive is inferior to the modern hive and confirms the concept that upgrading hive technology does not compromise beeswax quality. Similar result was reported by Getachew (2015) who reported that the purity of beeswax produced by traditional and modern hives did not show significant difference. Besides, the consistent value of beeswax total volatile matter, 1.44%, across the different hive types further suggests that the design of hive does not intricacy affect the quality of beeswax. This would be important the extension program adopting modern hive does not hive negative effect on the quality of beeswax.

Table 3. The effect of hive type on the beeswax quality parameters

Parameter	Hive type (Mean ±SE)			
	Modern (N=6)	Transitional (N=6)	Traditional (N=6)	Overall (N=18)
Melting point	62.33±0.41	62.42±0.49	62.42±0.49	62.39±0.44^{NS}
Refractive Index	1.44±0.00	1.44±0.00	1.44±0.00	1.44±0.0^{NS}
Ash content	0.15±0.07	0.17±0.09	0.12±0.03	0.14±0.07^{NS}
Total Volatile Matter	1.6±0.34	1.31±0.23	1.41±0.26	1.44±0.29^{NS}
Acid Value	26.31±0.34	24.82±1.35	25.16±0.93	25.43±2.15^{NS}
Saponification Value	97.67±5.72	93.63±3.24	94.96±5.92	95.421±5.1^{NS}
Ester value	69.94±7.52	69.77±5.11	73.99±5.01	70.90±6.08^{NS}

NS = Not significant; N = Sample number

Effect of Altitudinal Variation on Beeswax Physio-chemical Properties

The effect of variation of altitude on the physico-chemical characteristics of beeswax of Gazgibla district is reported in Table 4. The agroecological variations had significant effect ($p \leq 0.05$) on the quality of beeswax produced in the study area. The mean values of beeswax from the lowland area for the total volatile matter (1.64%) and the ester content (66.68mg KOH/g) in the study area had higher value ($p \leq 0.001$), and lower value compared with the highland ($p \leq 0.01$), respectively. However, there were no significant differences ($p \geq 0.05$) between the lowland and midland in both parameters.

The ester value from the lowland area was below the limits set by the national and international standards. This low value also pinpoints the environmental stress conditions on the honeybee colonies or bee forages or the local processing techniques. On the other hand, the higher total volatile matter value (1.64%) in the lowland agro-ecology could be due to the residual moisture or other volatile substances due to the hotter climatic conditions on the postharvest management of the beeswax. The lowland's higher total volatile matter and lower ester value could be extrapolated to the increased temperature and low humidity, resulting in the escalation of oxidation processes and retention of moisture. The reports from TBS (2010) were similar with this finding, which indicated the beeswax from lowlands had higher impurities.

According to Bogdanov (2016), the hotter temperature in the lowland areas may influence the chemical composition of the produced beeswax. This may also affect the postharvest handling practices of beeswax. According to this study, environmental factors affected the final physico-chemical properties of beeswax, which involves tailoring zone-specific postharvest beeswax protocols. This calls for the need for location specific beeswax rendering and storing protocols.

Table 4. The effect of altitude variation on the quality parameters of beeswax

Parameter	Location (Mean ±SE)			
	Lowland (N=6)	Midland (N=6)	Highland (N=6)	Overall (N=18)
Melting point	62.33±0.52	62.75±0.28	62.08±0.2	62.39±0.44^{NS}
Refractive index	1.44±0.00	1.44±0.0	1.44±0.0	1.44±0.0^{NS}
Ash content	0.12±0.07	0.15±0.06	0.16±0.07	0.14±0.0 ^{NS}
Total volatile matter	1.64±0.28^{a}	1.49±0.27^{ab}	1.19±0.08^{b}	1.44±0.29***
Acid Value	26.59±3.20	25.11±1.58	24.60±0.66	25.43±2.15^{NS}
Saponification value	93.74±5.56	96.95±4.80	95.58±5.31	95.42±5.1^{NS}
Ester value	66.68±4.34^{b}	70.99±5.87^{ab}	75.03±5.53^{a}	70.90±6.08**

NS = Not significant **=P≤0.01; *** = (P≤0.001); N = Sample number

Beeswax Quality of Study Area Compared with the Standard Limits

Table 5 indicates the mean beeswax values of the analyzed quality parameters as compared with the relevant standards, namely, Ethiopian, Kenyan, Tanzanian and European standards. The overall mean value for melting point in the study area was 62.39±0.44 ^{0}C. This value is within the range of the standards indicated in table 4 (QSAE, 2005; TBS, 2010; KBS, 2013; EP, 2023). This finding is in line with the reports of Bogdanov (2015) who indicate such low values showed the absence of adulterants like paraffin. The other four parameters (refractive index (1.44±0), acid content (25.43±0.2.15 mg KOH/g), total volatile content (1.44±0.3%), and saponification value (95.42±5.1 mg KOH/g) also comply with the national and international standards.

The overall mean ester value complies with the minimum limits set for national and international standards (Table 5) although it showed significant variation across beeswax sources and altitudes, sometimes below the standard limits (see 3.1. and 3.3). This finding is consistent with the reports of Bekele *et al.* (2016) and Addisu *et al.* (2017). On the other hand, the ash content of the study area (0.14%) was lower than the limits set by the Ethiopian, Kenyan and Tanzanian standards.

The mean values of the beeswax produced in the district for melting point, acid content, and saponification were found in within the minimum values set by the national and international standards. According to Adgaba (2007); Bonvehi and Bermejo (2012), these results suggest that beeswax produced in the study area is free from the common adulterants, including paraffin and animal fats that lower the melting point, and alter the acid and saponification values, respectively. Consequently, the beeswax quality challenge of the study area appears not to be deliberate adulteration by the beekeepers, rather probably due to sub-optimal beeswax processing method and the effect of altitudinal or agro-ecological. The total volatile matter of the beeswax produced in the district indicates improper drying or storage.

On the other hand, the beeswax ester values in some of the samples collected from the district highpoint deterioration of quality, which limits the export market (Bogdanov, 2016). Similarly, even though the total volatile matter value was found within the acceptable limits set by the national and international standards, authors like to suggest that this parameter should be closely monitored by the concerned institutions working in the study area.

Generally, the overall beeswax produced in the study area was competitively good quality (pure). Most of the values for the analyzed parameters were following the national (Ethiopian), African (Kenyan and Tanzanian) and European (European Pharmacopeias) standards (Table 5).

Table 5. Beeswax quality of the study area compared to the standards

Parameter	Current Study		Standard			
	Range	Mean±SD	Ethiopian	Kenya	Tanzanian	EP
Melting Point	62-63	62.39±0.44	61-66	62-65	62-65	61-65
Refractive Index	1.44-1.44	1.44±0.0	1.44-1.45	1.44-1.45	1.44-1.45	1.44-1.45
Ash Content	0.04-0.27	0.14±0.07	0.20	0.6	0.6	-
Total Volatile Matter	1.12-1.88	1.44±0.29	0.75	-	1	-
Acid Content	22.66-30.82	25.43±2.15	17-24	17-24	17-24	18-23
Saponification	88.08-102.3	95.42±5.1	85-105	-	87-104	87-104
Ester Value	60.14-79.64	70.9±6.08	70-80	70-79	70-79	70-80

SD = Standard Deviation; EP = European Pharmacopeias.

The small sample size used for beeswax quality analysis, although samples were collected from six distinct sites and assessed across three experimental factors (agro-ecology, hive type, and beeswax source), represents a limitation of this study. Budget constraints restricted the number of samples that could be collected and processed, ultimately reducing the statistical power of the analysis and limiting the ability to detect subtle but meaningful differences among the factors. Consequently, some variations in beeswax quality may remain undetected, and the generalizability of the findings across wider production systems may be constrained.

Conclusions

The beeswax produced in Gazgibla district generally met the major national and international quality standards, indicating that most samples were within acceptable natural quality ranges. However, statistically significant reductions in ester values were observed in beeswax originating from crude honey extraction practices and from lowland agro-ecological zones. Further analytical work using advanced techniques such as gas chromatography is also warranted to identify the underlying botanical, environmental, or postharvest factors contributing to reduced ester values.

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Conflicts of Interest

Regarding the publication of this manuscript, the authors declare that this work does not conflict with the interests of authors.

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Author Contribution

The first author performed the experiments, collected the data, and wrote the original draft. The second and third authors reviewed and critically edited the manuscript for intellectual content. All authors have read and approved the final version of the manuscript for submission to the journal.

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