

Evaluation of Physico Chemical Qualities of Beeswax in Selected Districts of Kafa Zone, Southern Nations Nationalities and Peoples Region (SNNPR), Ethiopia

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Abstract

The study was conducted in selected districts of Kafa Zone of Southern Nations Nationalities and Peoples Region (SNNPR), Ethiopia with an intention to identify the physico chemical quality status of beeswax produced in the areas. Three districts; Chena, Gimbo and Gesha and three peasant associations (PAs) from each district were purposively selected based on their potentialities for production and marketing of honey and beeswax. Before collecting sample beeswaxes, a preliminary survey was conducted to identify the major actors taking part in production, processing and marketing of beeswax. Accordingly, beeswax samples were collected from fresh combs, old combs, local mead houses and cooperatives for detail quality analysis. The Mean \pm SE value for melting point, refractive index, ash content, total volatile matter, acid value, Saponification value, ester value, and ester to acid ratio of beeswax of the area is found to be 62.35 \pm 0.15, 1.4416 \pm 0.0001, 0.0857 \pm 0.0144, 0.4156 \pm 0.0385, 22.689 \pm 0.3793, 94.7540 \pm 0.8617, 72.065 \pm 0.4766, 3.201 \pm 0.0625 respectively. All the samples passed for paraffin and other waxes, and fats and fatty acid tests. The result shows no significant variation among studied districts in all parameters. However, significantly higher value of refractive index, total volatile matters and ash contents were recorded in samples from local mead houses at $p < 0.05$. This might be due to poor handling practices and an intentional addition of foreign materials such as salt for preserving beeswax from wax moths' attacks and 'kocho' for increasing its volume which are commonly practiced by some individuals of 'tej' houses. The overall results revealed that all the beeswax samples collected from various sources are found within the acceptable ranges of national and international standards.

Keywords: beeswax, physico-chemical qualities, Kafa zone

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1. INTRODUCTION

Beeswax is a valuable natural product obtained from honeybees secreted from four pairs of glands located underside of the abdomen of young worker bees which are fully developed during 12 to 18 days of age and starts diminishing as their age increases and during feed shortages (Brown, 2010; Carillo *et al.*, 2015). However, it will be reactivated in times of emergency or during shortages of younger bees (Bogdanov, 2016a). Honeybees produce wax for constructing their combs and cell capping for storing honey, pollen and rearing broods (Bradbear, 2009). Despite its uses for making foundation sheets, beeswax is also widely used in various fields including cosmetics, foods, pharmaceuticals, engineering and industries (Bogdanov, 2004b; 2016b; Hilmi *et al.*, 2011).

Naturally beeswax is composed of esters of fatty acids including mono ester (35%), diester (14%), triester (3%), hydroxyl monoester (4%), hydroxyl polyester (8%), various hydrocarbons (14%), free acids (12%), long chain alcohols (1%) and other substances (Brian, 2015; CBI, 2015). Pure beeswax is whitish in color. However, presence of pollen in the combs and contamination with other substances during processing and storage triggers it to become yellowish or yellow brownish (Bogdanov, 2016a). As beeswax has a wide range of uses for human beings, it should contain minimum level of contaminations as much as possible (Bogdanov, 2009). Nowadays, various plagiaristic products and toxic contaminants are becoming the major threats impairing its quality status (Bogdanov, 2004a; EPOPA, 2006; Gemechis Legesse, 2014). Identifying the quality status of this product along each actor will ease the progress for further intervention so as to produce internationally compatible product which is the very issue of beeswax trading. According to various studies such as Nuru (2007); Bekele, *et al.*, (2016); Addisu, *et al.*, (2017); Yeshitela *et al.*, (2018) indicated that the physico-chemical quality status of beeswax in Ethiopia is varying from place to places or actors mainly due to the variation in its management practices and intentional adulteration processes. Consequently, identifying the physico-chemical quality status of this product across different areas of the country and market actors is very imperative to undertake further intervention. Hence, the current study was principally aimed with evaluating the physico chemical quality status of beeswax in the study areas obtained from different sources.

Beeswax is a very complex natural product owing its own physical properties which could be distinguished

using various sensory quality testing mechanisms such as color, odor, consistence, breakability, chewing test, cutting test, splinters testing and rubbing tests. The color of beeswax immediately produced by honeybees is whitish. However, tends to yellowish or yellow brown upon staying and contaminations with pollen and other impurities. Pure beeswax has pleasant, honey like aromas and shows grainy, blunt, not crystalline structure up on breaking the block. It shouldn't stick to teeth when chewed and it should not stick to fingers when rolled between fingers. It should not stick to knife up on cutting and has uniform consistency. However, this is not the case if contaminated with paraffin, fats and other oils (Bogdanov, 2004b). Though sensory quality detection methods seem to be the simplest and quickest methods, sometimes it might be difficult to clearly detect the beeswax qualities based on the level of contaminations or adulterations. Hence, detail lab analysis for various physico chemical parameters such as melting point, specific gravity, refractive index, acid value, ester value, saponification value, paraffin and other waxes tests are paramount for clearly detecting its quality status. For our study case the analysis was done for the above listed parameters except specific gravity due to lab equipment.

2. MATERIALS AND METHODS

2.1. Description of the study areas

The study was conducted in Kafa zone of Southern Nations Nationalities and Peoples Region (SNNPR). Kafa zone is situated at 6°14'28" to 8°7'11" N latitude and 35°26'37" to 36°47'28" E longitude covering an area of 10,602.7 sq. km (Wikipedia, 2017). The agro ecology of the zone includes 11.64% (Highlands), 59.45% (mid land) and 28.91% (low lands). As the area is covered with moist afro mountain rain forests, it has opportunities to receive nearly year-round rain fall with minimum and maximum rain falls ranging from 1000 to 2200 mm annually. The minimum and maximum temperature of the zone is 10.1 and 27.5°C respectively (Friis, 1992, USAID, 2005). According to CSA (2017), Kafazone is expected to have a population of 1,102,278 (541,682 male and 560,596 female); of whom 963,852 (87%) are rural inhabitants.

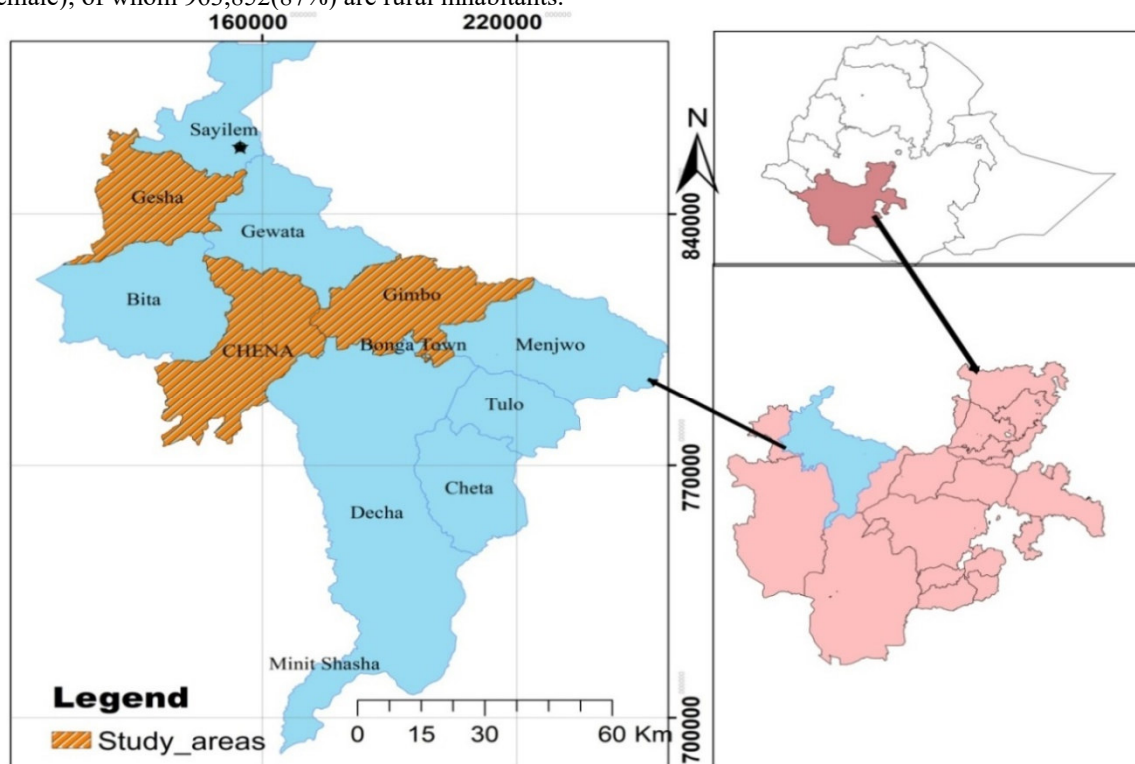


Figure 1. Map of Kafa zone

2.2. Methods of sample collection and Lab analysis

The available beeswax samples were collected following the market channels of the three districts (Chena, Gesha and Gimbo). Accordingly, the samples were collected from fresh combs, old combs, local mead/ 'tej' houses and cooperatives. Beeswax samples from fresh combs were obtained by collecting crude honey from beekeepers producing in bulky amounts and 3- samples were collected from each district. Beeswaxes from old combs were obtained through collecting each available small amount of broken and discarded combs from beekeepers compound/ apiaries. Accordingly, a total of five samples (i.e. 2-from Gesha, 2-from Chena and 1 from Gimbo district) were collected. During the time of sample collection, about 14- local mead houses were counted in the studied areas (i.e. 7- in Chena, 4- in Gesha and 3- in Gimbo districts). In the studied areas only 2- cooperatives (1-

in Chena and 1-in Gesha districts) practicing collecting, processing and selling beeswax to central markets. Hence, the beeswax samples were collected from both. Hence, a total of 24 beeswax samples from different sources were considered for the study. From each sample sources a minimum of 500gm of beeswax samples were taken by keeping increasing the amount based on the amount of available beeswax source. The collected samples were purified using hot water extraction method, by boiling the beeswax in water and allowing passing through tight clothes/“*abujedi*”-*Amharic*/ and putting in clean, dry and sealable containers and labeled before being sent to lab analysis. The lab analysis was under taken at Sekota Dry land Agricultural Research Center (SDARC) laboratory for its detail physico-chemical qualities. The physico-chemical qualities including melting point, saponification values, refractive index, acid values, ester values, paraffin and other waxes, total volatile matters, fats and fatty acids were investigated based on the detail procedures of Ethiopian beeswax quality specifications ET-1203-2005(QSAE, 2005).

2.2.1. Melting point

To determine the melting point of the beeswax, first the wax was melted by warming it in a water bath at a temperature just sufficient to melt it. Then the thermometer was dipped and withdrawn so as to get the bulb thinly coated with the wax and it was left for 24 hours. After 24 hours, the thermometer was inserted into the test tube through the bored cork and then the test tube was placed in the water bath and the temperature was gradually raised at the rate of 1°C in 3 minutes and observing for the first drop of melted wax occurs and recorded as the melting point of the beeswax(QSAE, 2005).

2.2.2. Refractive index at 75°C

The collected wax samples were melted and filtered through fast filter paper in order to remove any impurities and last traces of moistures. The temperature of the refractometer was adjusted at $75 \pm 1^\circ\text{C}$ by circulating the water from the water path. A few drops of the sample were placed on the lower prism and the prism was closed and tightened firmly and allowed to stand for 1-2 minutes. Finally, the refractive index was read and recorded after the sample attained the test temperature(QSAE, 2005).

2.2.3. Ash content, (%) by mass, max

The platinum dish was heated to redness, cooled to room temperature in desiccators and weighed. Then, about 50g of the wax was taken in a watch glass and weighed accurately. About 3/4 of this quantity was transferred to the platinum dish and heated on a Bunsen burner so the wax burns gently at the furnace. When about half of the material burnt, we stopped heating and cooled the sample; we added the remaining material. The material was heated again until it was completely charred. Finally, it was incinerated in a muffle furnace at 550°C - 650°C for an hour and then cooled to room temperature in desiccators and weighed. Incineration, cooling and weighing process until was repeated until the difference between successive weightings was less than one milligram(QSAE, 2005). Then, the ash content was determined using the formula;

$$\text{Ash, percent by mass} = 100M_2/M_1 \quad (1)$$

Where; M_2 = mass in g of the ash; M_1 = mass in g of the material taken for the test.

2.2.4. Total volatile matter, % by mass, max.

A 10 g of wax was accurately measured in a suitable dish, previously dried and weighed, and placed in an oven maintained at 105°C for 6 hours. Then, we cooled the dish in a desiccator and weighing with the lid on and we heated the dish again in the oven for thirty minutes repeating the process until the loss in mass between two successive weightings are less than one milligram and then recording the lowest mass obtained(QSAE, 2005).

$$\text{Total volatile matter at } 105^\circ\text{C, percent by mass} = \frac{100(M_1 - M_2)}{M_1 - M_3} \quad (2)$$

Where; M_1 - mass in gram of the dish with the material before heating; M_2 - mass in gram of the dish after heating;

M_3 - mass in gram of the empty dish

2.2.5. Acid value, max.

We thoroughly mixed the wax until entirely becoming liquid before weighing. Then, a 5 g of wax was weighed in a 250 ml conical flask and 75 ml of a mixture of two parts of benzene and one part of rectified spirit was added and heated under reflux until the sample is dissolved and allowed to cool to room temperature. Then, titrated with standard potassium hydroxide (KOH) solution using phenolphthalein as indicator(QSAE, 2005).

$$\text{Acid value} = \frac{56.1VN}{M} \quad (3)$$

Where; 56.1 – is equivalent weight of Potassium hydroxide(KOH), V = volume in ml of standard potassium hydroxide solution used; N = normality of standard potassium hydroxide solution and M = mass in gram of the material taken for the test.

2.2.6. Saponification cloud point, min.

About 2.0 g of the wax was weighed in a tarred conical flask and 25 ml of methylketone was added followed by addition of 25 ml of alcoholic potassium hydroxide solution. Then a few pieces of pumice stone were added and reflux condenser was connected to the flask. The flask was heated on a water bath for 2 hours and boiled steadily but gently. The flask and condenser were cooled, and the inside of the condenser was washed with about 10 ml of rectified spirit. Finally about 1 ml of phenolphthalein indicator solution was added and titrated with standard

hydrochloric acid. Blank determination was carried out at the same time.

$$\text{Saponification value} = \frac{56.1(B-S)N}{M} \quad (4)$$

Where, 56.1 – is equivalent weight of Potassium hydroxide (KOH); B = volume in ml of standard hydrochloric acid required for the blank; S = volume in ml of standard hydrochloric acid required for the material; N = normality of standard hydrochloric acid (HCL), and M = mass in g of the beeswax (QSAE, 2005).

2.2.7. Ester value

The Ester value can be calculated by subtracting the acid value determined from the saponification value (QSAE, 2005).

$$\text{Ester value} = \text{Saponification value} - \text{Acid Value} \quad (5)$$

2.2.8. Fats and fatty acids

To detect the fats and fatty acids content, first 5g of the wax was boiled for about 10 minutes with 80 ml of sodium hydroxide solution while adding the water during boiling to replace the water lost due to evaporation. Then, after cooling and filtering the solution through glass wool, the filtrate was acidified by diluting with hydrochloric acid. Then, the material was checked for passing the test. Accordingly, it will pass the test if the solution doesn't become turbid after acidification (QSAE, 2005).

2.2.9. Paraffin and other waxes

To test the paraffin and other wax in beeswax sample, 1g of beeswax was weighed and placed in a conical flask fitted with a water-cooled reflux condenser and boiling under reflux for an hour after adding 10 ml of alcoholic KOH solution. Detaching the flask from the condenser, inserting suitably a thermometer into the liquid in the flask and allow to cool while stirring constantly. Then, the wax samples were checked for passing the test (QSAE, 2005). Accordingly, each sample had passed the test as it doesn't become cloudy at a temperature higher than 61°C but became cloudy at a temperature between 59°C and 61°C.

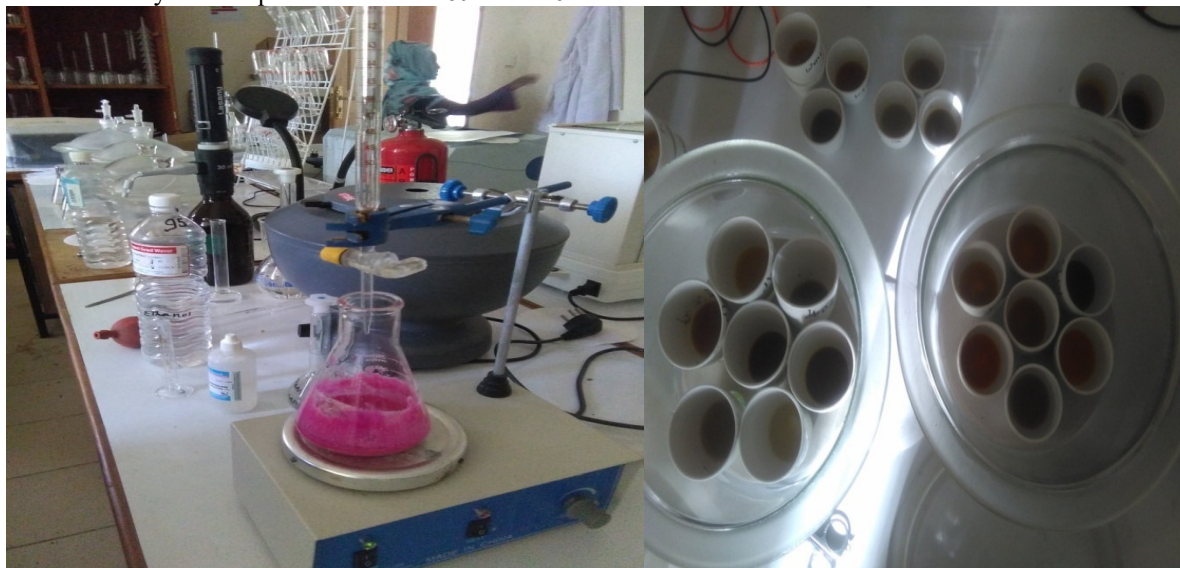


Photo 1: Laboratory analysis

2.2.10. Data Management and Statistical Analysis

The available qualitative data were analysed using Statistical Package for Social Science (SPSS-version 20) software.

Presences of associations between different quality parameters of samples were tested. The Correlation among different quality tests were used to justify the standards of beeswax in the study areas. Generalized Linear Model (GLM) at $p < 0.05$ level of significances was used to separate the means whenever ANOVA showed statistically significant variations.

The following analysis of variance (ANOVA) model was used for data analysis.

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \quad (6)$$

Where: Y_{ij} = quality of beeswax; μ = overall mean; α_i = the effect of the i^{th} location or source of beeswax; ϵ_{ij} = random error

3. RESULT AND DISCUSSION

3.1. Melting point

The melting point is one of the most important quality parameters used to detect the adulteration of beeswax. The overall melting point of beeswax ($\text{Mean} \pm \text{SE}$) in the studied area was measured to be $62.35 \pm 0.15^\circ\text{C}$, ranging from 61 to 64°C . It is found within the acceptable range of national standards ($61-66^\circ\text{C}$) as well as Kenyan, Tanzanian

and European standards (Table 4). The melting point of the beeswax in the studied areas doesn't have significant variation among the studied districts as well as different sources at $p < 0.05$ (Table 2 and Table 3).

3.2. Refractive Index, at 75°C

The refractive index of a substance is the ratio of velocity of light in the air to that of an object/substance. It is measured by an apparatus called refractometer. The refractive index of beeswax in the studied areas (Mean \pm SE) was found to be 1.4416 ± 0.0001 , ranging from 1.4409 to 1.4427. It satisfies the national standard which is in the range of 1.4400 to 1.4450. The refractive index of beeswax in the studied areas doesn't have significant variation among studied districts (Table 2). However, there is a significant variation based on its source at $p < 0.05$ (Table 3). Accordingly, significantly higher refractive index value was recorded on beeswax sourced from local mead/'tej' houses. This might be due to some impurities found in waxes from 'tej' houses due to contaminations with dusts or other impurities.

3.3. The ash content, % by mass, max.

The ash content is used to determine either the wax is contaminated with soils and other impurities or not. The ash content of beeswax produced in the current studied area (Mean \pm SE) was found to be 0.0856 ± 0.0144 , ranging from 0.0115 to 0.2354 (Table 1). It is within the acceptable national standards (0.2%). The ash content of the beeswax in the studied area was significantly vary based on its source at $p < 0.05$. Accordingly, the beeswax sample from local mead/'tej' houses has significantly higher ash contents than beeswax from fresh combs, cooperatives and old combs (Table 3). This might be due to the fact that beeswax from local mead/'tej' house is more likely prone for contamination with soils or other impurities during storages and processing. Similarly, higher ash contents, 0.051 was recorded for beeswax samples collected from tej houses found at different regions of the country (Oromia, Amhara, Tigray and SNNPR) (Yeshitela *et al.*, 2018). The current result is somehow greater than the ash content report from South Wollo zone which is reported to be 0.0345 (Addisu *et al.*, 2017). The higher ash content might be resulted from the addition of table salt (NaCl) on beeswax at 'tej' houses as a preservation mechanism from wax moth attacks, contamination with other impurities which are added for 'tej'/local beer making such as 'Gesho/Rhamnus prinoides' (Photo:2) and the poor management practices.

3.4. Total volatile matter, % by mass, max.

The total volatile matter in beeswax includes all substance other than moisture which is lost in the form of vapor during combustion in dry oven. The maximum limit for acceptable total volatile matter of beeswax in Ethiopian Standard is 0.75. The total volatile matter for beeswax in the studied areas (Mean \pm SE) was found to be 0.4156 ± 0.0385 ranging from 0.1919 to 0.8721 (Table 1).

The current result revealed that the total volatile matter of beeswax in the study areas is within the acceptable range of national standard. Similar results 0.3335 and 0.5491 were reported by Bekele *et al.*, (2016) and Addisu *et al.*, (2017) respectively. The total volatile matter of the beeswax has no significant variations among study districts (Table 2). However, there is significant variation between sample sources. Accordingly, the beeswax samples collected from 'tej' houses have significantly higher value (0.6265) of total volatile matters than other sources (Table 3). Similarly, higher total volatile matter 0.8077 and 1.5 were obtained from 'tej' houses by Addisu *et al.*, (2017) and Yeshitela *et al.*, (2018) respectively. The higher total volatile matter at 'tej' houses might be due to its mishandling and exposures for prolonged sun lights during storages.

3.5. Acid value, max.

The acid value in beeswax is determined by measuring the amount of KOH consumed to neutralize the free acids in 1 gram of wax sample. The acid value (Mean \pm SE) of the beeswaxes in the studied area was 22.689 ± 0.379 , ranging from 18.894 to 26.361 (Table 1). The obtained result shows that it falls within the acceptable national and international ranges (Table 4). Similar acceptable range of acid values 21.66 ± 2.26 , 22.33 ± 0.39 and 18.92 ± 2.7735 were reported by Nuru (2007b); Bekele *et al.*, (2016) and Addisu *et al.*, (2017) respectively. The acid value does not have significant variation based on the source and studied districts, at $p < 0.05$ (Table 1 and 2). According to Nuru (2007b), of the total 75 beeswax samples collected from various actors, 8 failed to meet the required standards. 3 of them were from intermediary collector and the remaining 5 were from final processors and exporters.

3.6. Saponification cloud point, min, (mg KOH/g)

The Saponification cloud point is the amount of Potassium Hydroxide (KOH) required for hydrolyzing 1g of sample beeswax. It is one of the simple and preferable parameters used to detect the adulteration of beeswax. However, this method is limited to detect quantities greater than 1 % of higher melting point (i.e. 80-85°C) paraffin waxes and more than 4-5 % of lower melting points (50-55°C) paraffin (Bonvehi and Bermejo, 2012). The mean Saponification cloud point of the beeswax in the studied areas (Mean \pm SE) was 94.7540 ± 0.8617 mg KOH/g ranging from 82.1233 to 102.1266 mg KOH/g (Table 1). Similar acceptable

results;98.04,91.19 and 93.6were reported byBekeleet *al.*,(2016), Addisuet *al.*, (2017) and Yeshitelaet *al.*, (2018) respectively.No significant variations were found among studied districts and sources of samples (Table 2 and 3).

3.7. Ester value,(mg KOH/gm)

Ester value is a measure of Potassium Hydroxide (KOH) required to saponify the ester in 1g ofbeeswax sample and it is determined by calculating the difference between Saponification value and acid value.The variation in ester value of beeswax could result from prolonged heat exposures of the beeswax (Nuru, 2007b).

The mean ester value of beeswax samples of the studied areas was 72.065 ± 0.477 , ranging from 67.9702 to 83.233(Table 1).Similar results which are within acceptable ranges (70-79) were reported by Addisu *et al.*,(2017); Bekeleet *al.*,(2016) and Nuru (2007b).The mean ester value of the beeswax does not vary significantlyamong studied districts at $p<0.05$. However, the ester value of beeswax sourced from ‘*tej*’ houses has significantly lower values than fresh combs, old combs and processors. The result obtained from current study satisfies both the national and international standards.

3.8. Ester to acid ratio

The ester to acid ration is a value obtained when the ester value is divided by its acid value. It indicates whether the wax was stored during longer period or not; as the storage time increased, the ester to acid value will be decreasegradually (Tulloch, 1980). It is not taken as a major quality parameter in Ethiopia and has no acceptable ranges. However, according to Kenyan standards, an acceptable ester to acid ratio should be 3 to 4.3(KBS, 2013). According to the current result, the ester to acid ratio of the studied areas (Mean \pm SE) isobtained to be 3.201 ± 0.0625 ranging from 2.493 to 4.405(Table 1). Equivalent results,3.38 and 3.7995 was obtained by Bekele ,2016 andAddisuet *al.*, 2017 respectively. The beeswax sampled from ‘*tej*’ houses had significantly lower values of ester to acid ratio at $p<0.05$ than fresh combs, processors and old comb sources. This might be due to prolonged stays of crude beeswax at ‘*tej*’ houses with less management. Sometimes some ‘*tej*’housesmay purposively expose it to sunlight to reduce wax moth and small hive beetle attacks. The result shows there is no significant variation of ester to acid values among studieddistricts (Table 2).

3.9. Fats and fatty acids test

All sample solutions have passed the test for fats and fatty acids by boiling with NaOH.Sample solutions become clear after acidifying with 4N HCl(Table 1).

3.10. Paraffin and other waxes

The paraffin and other waxes indicate the contamination or adulteration of natural beeswax with other waxes sources. According to the test result for paraffin and other waxes, all the samples collected in the studied areas have passed the test that the liquid should become cloudy at a temperature lower than 61°C.



Photo 2: Wax processing at local mead houses (mixed with some ‘*Rhamnus prinoides*’ leaves)

Table1. Physico chemical quality characteristic of beeswax in the study areas

Sample (N = 24)		
parameters	Range	Mean \pm SE
Melting point ($^{\circ}$ C)	61.00 – 64.00	62.35 \pm 0.15
Refractive index	1.4409-1.4427	1.4416 \pm 0.0001
Ash content ,% by mass)	0.0115-0.2354	0.0856 \pm 0.0144
Total volatile matter (% by mass)	0.1919-0.8721	0.4156 \pm 0.0385
Acid value, max	18.894-26.361	22.689 \pm 0.3793
Saponification value ($^{\circ}$ C)	82.1233-102.1266	94.7540 \pm 0.8617
Ester value	67.9702-83.233	72.065 \pm 0.4766
Ester to Acid ratio	2.4930-4.405	3.201 \pm 0.0625
Fats and Fatty acid	-	Passed
Paraffin and other waxes	-	Passed

NB- N= is number of samples, SE= Standard error of Mean

Table 2. Mean comparison of physicochemical qualities of beeswax by districts

Parameters	Districts(Mean \pm SE)			
	Chena (N= 9)	Gimbo(N=8)	Gesha (N=7)	Overall (N=24)
Melting point ($^{\circ}$ C)	62.17 \pm 0.25	62.63 \pm 0.25	62.29 \pm 0.32	62.35 \pm 0.15 ^{NS}
Refractive index	1.4416 \pm 0.0002	1.4416 \pm 0.0002	1.4417 \pm 0.0002	1.4416 \pm 0.0001 ^{NS}
Ash content,% by mass	0.0809 \pm 0.0245	0.0811 \pm 0.0188	0.0970 \pm 0.0366	0.0856 \pm 0.0144 ^{NS}
Total volatile matter	0.4151 \pm 0.0619	0.4357 \pm 0.0791	0.4105 \pm 0.0674	0.4207 \pm 0.0385 ^{NS}
Acid value, max	23.069 \pm 0.533	22.504 \pm 0.7923	22.393 \pm 0.717	22.684 \pm 0.379 ^{NS}
Saponification value ($^{\circ}$ C)	95.397 \pm 0.666	93.939 \pm 2.020	94.858 \pm 1.8282	94.754 \pm 0.862 ^{NS}
Ester value	73.284 \pm 0.612	72.686 \pm 0.963	74.037 \pm 0.971	73.304 \pm 0.477 ^{NS}
Easter to Acid ratio	3.191 \pm 0.080	3.258 \pm 0.121	3.332 \pm 0.137	3.254 \pm 0.062 ^{NS}

Table 3. Mean comparison of physicochemical properties of beeswax by sources

Parameters	Sources (Mean \pm SE)				
	Freshcombs; N=9	'Tej'houses; N=8	cooperatives; N=2	Old combs; N=5	Overall
Melting point($^{\circ}$ C)	62.33 \pm 0.204	62.38 \pm 0.363	62.75 \pm 0.750	62.2 \pm 0.255	62.35 \pm 0.15 ^{NS}
Refractive index	1.4413 \pm 0.0001 ^b	1.4422 \pm 0.0001 ^a	1.4413 \pm 0.0002 ^b	1.4415 \pm 0.0002 ^b	1.4416 \pm 0.0001
Ash content, % by mass	0.0224 \pm 0.0022 ^b	0.1450 \pm 0.0147 ^a	0.0133 \pm 0.0018 ^b	0.1334 \pm 0.0333 ^{ab}	0.0856 \pm 0.0144
Total volatile matter	0.3334 \pm 0.0244 ^b	0.6265 \pm 0.0678 ^a	0.3168 \pm 0.0042 ^b	0.2899 \pm 0.0160 ^b	0.4207 \pm 0.0385
Acid value, max	21.861 \pm 0.579	23.194 \pm 0.7611	21.867 \pm 0.633	23.674 \pm 0.6179	22.684 \pm 0.379 ^{NS}
Saponification value ($^{\circ}$ C)	96.7403 \pm 1.1474	91.3960 \pm 1.6761	95.6710 \pm 1.3278	96.1847 \pm 0.9108	94.7540 \pm 0.8617 ^{NS}
Ester value	75.324 \pm 0.573 ^a	70.952 \pm 0.496 ^b	74.304 \pm 0.195 ^{ab}	73.033 \pm 0.478 ^{ab}	73.304 \pm 0.477
Easter to Acid ratio	3.463 \pm 0.0879 ^a	3.082 \pm 0.1026 ^b	3.401 \pm 0.0895 ^{ab}	3.095 \pm 0.0984 ^b	3.254 \pm 0.0625

NB: -Letters with different superscripts within a row are significantly difference among sources ($p < 0.05$), N= Number of sample,

SE= Standard error of Mean, NN=Non significant

Table 4. The mean comparison of wax quality of the study areas with national and international standards

Characteristics/Specification	Current result (Mean ±SE)	Ethiopian Standard (ET 1203/2005) *	Kenyan Standard (CD /05-1279:2013) **	Tanzanian Standard (TBS/ AFDC 17/4049) ***	European Pharmacopeias (ET 1203/2005) *
Melting point (°C)	62.35±0.15	61 – 66	62 - 65	62 – 65	61 - 65
Refractive index, at 75°C	1.4416±0.0001	1.4400-1.4450	1.4398 - 1.4455	- 1.4398 - 1.4455	- 1.440-1.4450
Ash, % by mass, max.	0.0808±0.0144	0.20	0.6	0.6	-
Total volatile matter, max	0.4207±0.0385	0.75	-	1	-
Acid value, max	22.684±0.379	17-24	17-24	17-24	18-23
Saponification value, min.	94.7540±0.8616	85-105	-	87-104	87-104
Ester value	73.304±0.477	70-80	70-79	70-79	70-80
Ester to Acid ratio	3.254±0.0625	-	3.0 - 4.3	-	-
Fats and Fatty acids	Passed	To pass test	To pass test	To pass test	-
Paraffin and other waxes	Passed	To pass test	To pass test	To pass test	Absent

Notice: N=Number of sample, SE= Standard error of Mean, -= not available; Source: * (QSAE, 2005), ** (KBS, 2013), *** (TBS, 2010)

4. CONCLUSION AND RECOMMENDATIONS

In the study areas, local retailers, whole sellers, 'tej' houses, local collectors, cooperatives and private companies are identified as the major honey and beeswax market chain actors. However, 'tej' houses and cooperatives are identified to be the major actors taking part in processing and marketing of beeswax. In spite of its less production, the ways of managing beeswax in general and 'tej' houses in particular is very poor which is prone for contaminations and some intentionally added preservatives basically 'NaCl' which is used for preserving the beeswax from waxmoth attacks might have also its own impacts resulting for some deviations in some parameters of waxes from local mead houses. According to the laboratory results, all the beeswax samples in the area taken from different sources (from 'tej' houses, honey combs, old combs and cooperatives) are found within the acceptable range of both national and international standards in all parameters which indicates the beeswax product in the studied areas is mostly natural.

Based on the present study findings, the following points can be forwarded and recommended:

- Awareness creation and follow-ups on appropriate management aspects of beeswax products should be given for beeswax producers and market actors in general and 'tej' house owners in particular.
- The inclusion rates of preservatives (NaCl) and other adulterants especially at local mead houses need further investigation, and their effects on the quality of beeswax require follow up studies.
- Moreover, local quality assurance entities for beeswax should be established for regular quality monitoring.

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