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Research Article

Effects of Various Adulterants Materials on Properties of Beeswax

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Abstract

Beeswax is one of the best valued bee products and its demand is high these times in the world market. Adulteration of beeswax with inexpensive materials has become a challenge for its quality and marketing. The objective of this study was to characterize the physicochemical properties of adulterated beeswax and then identify better methods to detect adulterated beeswax. In this paper, the properties of beeswax-adulterant mixtures, such as beeswax with candle, beeswax with animal tallow, beeswax with soap, beeswax with maize flour, beeswax with lime stone, beeswax with salt, and beeswax mixed with powder of "kocho" in different ratios were prepared based on previous survey study results and quality parameters were analyzed using the official methods. The results indicated that the density of beeswax adulterated with different adulterants such as soap, animal tallow, limestone, "kocho", salt, candle and maize flour for different proportions were significantly different at $p<0.05$. Parameters such as acid value of animal tallow, saponification point, ratio number and ester value of maize flour, ratio number, acid value and saponification point of "kocho", melting and saponification point of candle, ash content of lime stone, melting point, ash value and saponification point for salt, and ash content and ratio number for soap were found to be better methods to identify beeswax adulterated with the respective adulterants mentioned above. It is important to characterize beeswax quality parameters using more sensitive analytical instruments. Pure beeswax is important for bee's colony health and to attract good market.

Keywords

Adulterants, Adulterated Beeswax, Demand, Kocho, Pure Beeswax

1. Introduction

A natural wax that worker bees secrete from glands under their abdomen is called beeswax. Beeswax is secreted by the honey bee workers in the form of wax scales and it is further being chewed by their mandibles which results in the transformation of texturally anisotropic scale wax into isotropic comb wax [1]. The term beeswax is often limited to wax produced by honeybees (*Apis species*) and many would specify *Apis mellifera* L. as a source [2]. It is one of the most valuable bee products and it is also one of the oldest items used by mankind [3]. Beeswax, with its unique characteristics, is now being used in the development of new products in various fields such as cosmetics, foods, pharmaceuticals, engineering and industry [4]. Specifically, most of the wax produced nowadays are used in the manufacture of cosmetics, such as hand and face creams, lipsticks and depilatory wax and many other uses. Moreover, the pharmaceutical industry

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uses the wax in various ointments, for coating pills and suppositories and other miscellaneous industrial products [5]. Bee wax is widely used in many branches of the national economy [6, 7], as well as in metallurgical, electrical, chemical, pharmaceutical [8-10] food industry, in particular in the production of cheese and sweets, as well as the coverage of fruits and vegetables in order to increase their shelf life [11, 12].

Some physico-chemical parameters used to analyze oils and fats and described in the official methods of analysis from many pharmacopoeias and countries are commonly applied to evaluate the beeswax quality and detect possible adulterations [13]. Melting point, acid, saponification, ester and ratio number values are the most commonly determined parameters, whereas the peroxide value and iodine absorption number have also been considered in a few works [14, 15]. The proposed value ranges for the parameters in pure beeswax differ from one country to another [16, 17]. These differences could be related to the point of origin of the beeswax because the environmental and geographical factors play a significant role in the bee adaptation and, as a result, in beeswax composition [14, 15].

The relatively high cost of beeswax in comparison with other industrial waxes and lower-price products, such as microcrystalline wax or paraffin, tallow, stearine and stearic acid, promotes its adulteration with these mentioned fatty products, which is also favored by a lack of regulations [2]. Adulteration of beeswax with these cheaper materials has become a problem for beeswax quality and its marketing, especially adulteration of beeswax with paraffin [2, 18]. In Ethiopia, beeswax adulteration has been reported repeatedly and is becoming a critical problem [19, 20]. These investigations indicated that materials used to adulterate beeswax include animal tallow/fat, candle/paraffin, soap, maize flour and lime stone [20].

The presence of adulterants in beeswax affects the values of some physicochemical parameters of the wax [2, 14, 15, 21]. Thus, several authors have discriminated the adulterated beeswaxes according to the anomalous values of some analytical parameters, for instance the melting point, the saponification value or the iodine value [15]. Physicochemical measurements like density, melting and saponification values, ester value, ratio number and iodine value have helped to detect adulteration of beeswax with paraffin, stearic acid and animal tallow [22]. Similarly, a melting point and saponification cloud point could equally serve in the detection of as little as 2% animal tallow used in beeswax adulteration [19]. However, as reported by [20], more adulterant materials have been utilized by the actors and the techniques of adulteration are also ever changing. Therefore, physical and chemical characterization of the adulterated beeswax using different adulterants at different proportions is important to identify which measurements are more appropriate to be employed for a given beeswax adulterant material and at what proportion level it should be applied. In this study, adulterant materials identified in earlier survey works were used to adulterate beeswax deliberately in different proportions of mixing and beeswax physical and chemical parameters were quantified to see how the adulterants were affect their measurements. The objective of this study was to characterize the physicochemical properties of pure and adulterated beeswax, then identify better methods to detect adulterated beeswax.

2. Materials and Methods

2.1. Beeswax Sample Collection and Purification

Crude beeswax were collected directly from beekeepers found in West, Southwest and Central zones of Oromia region in Ethiopia in two years, from 2022 to 2023 based on the time of availability. Pure and natural beeswax samples were obtained by extracting the crude beeswax using submerged beeswax extracting method by purifying by boiling in water and filtering using cheese cloth, method of beeswax processing.

2.2. Adulterant Material Collections

The adulterant materials were those reported by [20]. These include animal tallow, candle, soap, maize flour and lime stone will be acquired from the local market and other available sources.

2.3. Preparation of the Adulterant-beeswax Mixtures

The mixtures of pure beeswax and adulterants were prepared in proportions identified by survey work earlier. The adulterants were candle, maize flour, animal tallow, limestone, soap, salt, and “kocho”. For each adulterant material, five levels of mixtures, 1:1, 2:1, 3:1 and 4:1, including pure beeswax were prepared in the respective orders of pure beeswax for the adulterant and analyzed for each parameter indicated. To achieve a good mixture and homogeneity in the sampling, the beeswax-adulterant mixtures were heated in an aluminum vessel up to the total melting of the mixture, which required a temperature of about 70 °C and a time close to 5 min. After that, the mixtures were allowed to cool at room temperature for 24 hours and kept at room temperature and in darkness until their analysis.

2.4. Determining the Physicochemical Parameters of Pure Beeswax, and Beeswax Adulterated with Different Adulterants

2.4.1. Density

A calibrated pycnometer was weighed empty first and then full of water. Both determinations were made at 25 ℃ and in triplicate to calculate the mass mean values. Then, an amount of 10.0 + 0.1 mg of beeswax was placed into a beaker containing water. As beeswax is less dense than water, the first one floats on the surface of the liquid. Next, small volume of methanol was added from a burette until beeswax was suspended in the liquid. At that moment, the density of the liquid matched up with that of the beeswax sample, so the beeswax density (in g/mL) at a temperature of 20 ℃ could be determined with the pycnometer by means of the expression:

$$\text{Density}= 0.9982\,\frac{[(m1-m2)+A]}{m3-m2+A}$$

Where, A = 0.0012 (m1-m2), with m1 being the mass, in g, of the pycnometer containing the hydroalcoholic mixture; m2 the mass, in g, of the empty and completely dry pycnometer; and m3 the mass, in g, of the pycnometer containing ethanol. Three different portions of the beeswax sample was weighed to calculate the corresponding density values and an average for each sample.

2.4.2. Saponification Value

A beeswax amount of 0.300 + 0.001g was weighed and placed into a 10-mL glass vial. Then, 4 mL of a 4 M NaOH aqueous solution was added to the vial. The vial will be closed and put into an oven to carry out the saponification at 100 ℃ for 1hour. The solution, still hot, was titrated with a 0.5 M HCl solution, using phenolphthalein as an indicator and constant manual shaking. A blank assay was also conducted. The determinations were made in triplicate. The saponification value, expressed as mg KOH/g, was calculated as follows:

$$\text{Saponification value}=\ 56.1\,\frac{M(V-V')}{W}$$

where V is the volume, in mL, of HCl solution required by the blank; V' is the volume, in mL, of HCl solution required for the sample; M is the molarity of the HCl solution; and w is the mass, in g, of the beeswax sample.

2.4.3. Acid Value

A beeswax amount of 1.00 + 0.01g was dissolved in 50mL of chloroform with the help of an ultrasonic bath. Then, two drops of phenolphthalein were added and the solution was titrated with 0.05 M NaOH in methanol with continuous manual shaking until the indicator turns. A blank assay was also titrated to correct solvent acidity. The acid value (in mg NaOH/g) was calculated by the formula:

$$\text{Acid value}=56.1\,\frac{M(V-V')}{W}$$

where V is the volume, in mL, of NaOH solution in methanol required by the sample; V' is the volume, in mL, of NaOH solution required for the blank; M is the molarity of the NaOH solution; and w is the mass, in g, of the beeswax sample. The NaOH solution was previously normalized against an aqueous solution of 0.05 M potassium acid phthalate with phenolphthalein as an indicator. The analyses were also done in triplicate.

2.4.4. Ester Value and Ratio Number

The ester value was determined by subtracting the acid value from the saponification value, and the ratio number was calculated as the ester value divided by the acid value.

2.4.5. Melting Point

This index was determined following the “capillary tube method.” Melted beeswax was introduced in a 10 cm long x 2 mm internal diameter thin-wall hollow capillary tube, until reaching a height of about 1 cm. Once the beeswax solidified, the tube was kept at room temperature for about 24 h. After that, the capillary tube containing the beeswax was introduced into a water bath that was slowly warmed at 1-2 ℃/min; the temperature was checked with a thermometer, whose bulb had to be as close as possible to the beeswax column introduced in the capillary tube. The melting temperature was that at which the beeswax was completely molten; the beeswax liquid is entirely transparent without turbidity.

2.4.6. Ash Content

To tare the porcelain capsules, they were kept in the muffle at 600 ℃ for 30 minutes then cooled within a desiccator until they reached a constant weight. An amount of 2.000+ 0.001g was placed in the capsules, heated prudently until inflammation point in the muffle port and finally heated at 600 ℃ in the muffle for 1.5 h. After that, the capsules with the ashes were cooled in a desiccator until they reached a constant weight. The ash percentage will be determined as follows:

$$\text{Ash content}=\ 100\,\frac{(w1-w2)}{W}$$

Where W1 is the mass, in g, of the porcelain capsule with the ash content; W2 is the mass, in g, of the empty capsule; and w is the mass, in g, of the beeswax sample. Sample analyses will be done in triplicate.

2.5. Data Management and Analysis

Data was entered into Microsoft excel sheet and transported to analysis software. Multiple variance analysis (MANOVA) was run with SPSS software. Each type of beeswax-adulterant mixture was considered an independent variable.

3. Results and Discussion

In this paper physicochemical properties of the beeswax-adulterant mixtures (beeswax with soap, beeswax with maize flour, beeswax with lime stone, beeswax with salt, beeswax with “kocho”, beeswax with animal tallow and beeswax with candle in different ratios were prepared and analyzed for density, ash content, melting point, ester value and ratio number, acid value and saponification value. The results are shown in Tables 1, 2, 3, 4, 5, 6 and 7 for beeswax-candle, beeswax-animal tallow, beeswax-soap, beeswax-lime stone and beeswax-“kocho” adulterated material laboratory analysis.

Table 1. *Results of physicochemical properties of beeswax-candle mixtures (Mean ±SD).*

Beeswax-candle ratio	**ρ (g/cm^3)**	**AC (%)**	**MPt (℃)**	**EV**	**RN**	**AV**	**SV (℃)**
1:1	0.85 ±0.003^{c}	0.148 ±0.04^{abcde}	62.00 ±0.00^{cbd}	87.33 ±28.50^{ab}	2.58 ±1.39^{c}	15.43 ±0.84^{bcd}	102.85 ±28.05^{ab}
2:1	0.322 ±0.001^{e}	0.367 ±0.14^{abc}	59.75 ±0.25^{e}	99.48 ±9.35^{a}	5.80 ±2.44^{a}	13.37 ±0.00^{e}	28.05 ±9.35^{d}
3:1	1.135 ±0.004^{a}	0.49 ±0.28^{a}	63.00 ±0.00^{bc}	57.33 ±18.50^{d}	1.41 ±0.45^{e}	16.27 ±1.40^{b}	74.80 ±18.70^{abc}
4:1	0.44 ±0.003^{d}	0.44 ±0.02^{ab}	63.00 ±0.00^{b}	27.00 ±9.00^{e}	5.08 ±1.19^{ab}	18.51 ±0.56^{a}	46.75 ±9.35^{de}
Natural beeswax	0.96 ±0.001^{b}	0.33 ±0.13^{abcd}	64.75 ±0.25^{a}	33.00 ±4.04^{abc}	2.28 ±1.61^{cd}	15.29 ±0.98^{bc}	125.62 ±29.04^{a}
ESQA	-	-	61-66	70-80	-	17.0-24.0	-

Different letters down the column show significant difference (p =0.05). SD=standard deviation, ρ = density, AC= Ash content, Mpt = melting point, EV=Ester value, RN=ratio number, AV=acid value, SV= Saponification value. ESQA =Ethiopian Standard and quality Authority.

Table 2. *Results of physicochemical properties of beeswax- animal tallow mixtures (Mean ±SD).*

Beeswax-tallow ratio	**ρ (g/cm^3)**	**AC (%)**	**MPt (℃)**	**EV**	**RN**	**AV**	**SV (℃)**
1:1	0.473 ±0.003^{e}	0.34 ±0.003^{b}	59.50 ±0.50^{e}	98.00 ±9.00^{ab}	4.4925 ±3.13^{ab}	47.70 ±2.80^{a}	102.85 ±9.35 ^{abcd}
2:1	0.699 ±0.005^{d}	0.22 ±0.04^{bcde}	62.00 ±0.00^{bc}	99.33 ±9.50^{a}	2.7210 ±2.11^{abc}	23.42 ±0.14^{b}	102.85 ±9.35^{abcd}
3:1	0.662 ±0.004^{c}	0.24 ±0.08^{bcd}	62.75 ±0.75^{b}	67.67 ±29.50^{abcde}	4.6246 ±0.36^{a}	14.59 ±0.56^{e}	105.75 ±19.20^{abc}
4:1	0.756 ±0.001^{b}	0.76 ±0.73^{a}	61.75 ±0.25^{bcd}	93.67 ±19.50^{abc}	1.0544 ±0.39^{abcde}	15.43 ±0.28^{c}	112.20 ±18.70^{ab}
Natural beeswax	0.959 ±0.001 ^{a}	0.33 ±0.13^{bc}	64.75 ±0.25^{a}	86.33 ±4.04^{abcd}	2.28 ±1.61^{abcd}	15.29 ±0.98^{d}	125.62 ±29.04^{a}
ESQA	-	-	61-66	70-80	-	17.0-24.0	-

Different letters down the column show significant difference (p =0.05).

Table 3. *Results of physicochemical properties of beeswax-soap mixtures (Mean ±SD).*

Beeswax- soap ratio	**ρ (g/cm^3)**	**AC (%)**	**MPt (℃)**	**EV**	**RN**	**AV**	**SV (℃)**
3: 1	0.76 ±0.001^{c}	2.77 ±0.21^{b}	63.08 ±0.38^{abc}	20.33 ±4.51^{c}	6.63 ±1.56 ^{ab}	22.53 ±1.86^{a}	28.46 ±14.93^{c}
4: 1	0.84 ±.00153^{b}	5.62 ±1.88^{a}	64.42 ±3.56^{ab}	64.67 ±10.21^{ab}	11.74 ±7.66^{a}	21.22 ±1.91^{ab}	84.16 ±9.35^{b}
Natural beeswax	0.959 ±0.001 ^{a}	0.33 ±0.13^{c}	64.75 ±0.25^{a}	86.33 ±4.04^{a}	2.28 ±1.61 ^{c}	15.29 ±0.98^{c}	125.62 ±29.04^{a}
ESQA	-	-	61-66	70-80	-	17.0-24.0	-

Different letters down the column show significant difference (p =0.05).

Table 4. Physico-chemical properties of beeswax- maize flour adulterated materials (mean ±SD).

Beeswax- maize flour ratio	ρ (g/cm^3)	AC (%)	MPt (℃)	EV	EV to AV	AV	SV (℃)
1:1	1.01 ±0.002^{cde}	0.46 ±0.09^{bc}	64.50 ±0.50^{b}	120. 33 ±46.50^{a}	5.93 ±1.92^{a}	19.78 ±0.14^{bc}	15.41 ±2.86^{e}
2:1	1.48 ±0.003^{c}	0.38 ±0.07^{bcd}	63.50 ±0.50^{ab}	107.33 ±37.50^{b}	0.81 ±0.50^{e}	18.23 ±0.28^{d}	80.41 ±58.15^{d}
3:1	1.35 ±0.003^{cd}	1.62 ±1.29^{a}	63.50 ±0.50^{abc}	106.00 ±13.00^{bc}	2.96 ±2.78^{c}	20.20 ±0.01^{b}	140.25 ±28.05^{ab}
4:1	2.12 ±0.006^{a}	0.68 ±0.21^{b}	61.50 ±0.50^{f}	61.00 ±47.00^{e}	4.59 ±1.63^{ab}	21.60 ±0.28^{a}	144.88 ±36.120^{a}
Natural beeswax	0.99 ±0.004^{b}	0.33 ±0.13^{bcde}	64.75 ±0.25^{a}	86.33 ±4.04^{d}	2.28 ±1.61^{cd}	15.29 ±0.98^{e}	125.62 ±29.04 ^{abc}
ESQA	-	-	61-66	70-80	-	17.0-24.0	-

The value was significantly different from that of pure beeswax (p =0.05)..

Table 5. Results of physicochemical properties of beeswax- lime stone mixtures (Mean ±SD).

Beeswax- lime stone ratio	ρ (g/cm^3)	AC (%)	MPt (℃)	EV	EV to AV	AV	SV (℃)
1:1	1.26 ±0.003^{a}	42.81 ±2.13^{a}	65.50 ±0.00^{abcd}	47.67 ±18.50^{ab}	1.83 ±0.81^{abcde}	14.59 ±0.00^{de}	62.20 ±18.70^{abc}
2:1	1.09 ±0.003^{c}	25.05 ±2.70^{b}	64.75 ±0.25^{ab}	33.00 ±14.00^{e}	3.27 ±0.85^{abc}	21.88 ±0.00^{bc}	51.425 ±14.02^{e}
3:1	1.19 ±0.003^{b}	16.31 ±1.63^{c}	64.50 ±0.50^{abc}	73.33 ±18.50^{abcd}	4.18 ±0.67^{a}	23.84 ±1.40^{a}	93.50 ±18.70^{abcd}
4:1	0.94 ±0.003^{e}	10.83 ±0.83^{d}	62.00 ±0.00^{abcde}	100.00 ±9.00^{a}	3.30 ±1.07^{ab}	22.72 ±0.28^{b}	121.55 ±9.35^{ab}
Natural beeswax	0.959 ±0.001^{d}	0.33 ±0.13^{e}	64.75 ±0.25^{a}	86.33 ±4.04 ^{abc}	2.28 ±1.61^{abcd}	15.29 ±0.98^{d}	125.62 ±29.04^{a}
ESQA	-	-	61-66	70-80	-	17.0-24.0	-

Different letters down the column show significant difference (p =0.05).

Table 6. Physicochemical properties of beeswax- salt adulterated materials (mean ±SD).

Beeswax- salt ratio	ρ (g/cm^3)	AC (%)	MPt (℃)	EV	RN	AV	SV (℃)
1:1	1.175 ±0.002^{b}	7.53 ±.28^{a}	88.50 ±0.50^{b}	41.67 ±19.50^{abcd}	5.75 ±2.19^{b}	20.76 ±1.12^{abc}	56.10 ±18.70^{c}
2:1	1.57 ±0.004^{a}	6.25 ±2.48^{bc}	96.50 ±3.50^{a}	57.33 ±51.16^{abc}	18.27 ±3.97^{a}	22.44 ±0.56^{ab}	35.53 ±21.56^{e}
3:1	0.96 ±0.0025^{d}	6.57 ±3.84^{b}	64.50 ±0.50^{cd}	60.33 ±29.50^{ab}	1.16 ±0.40^{d}	20.76 ±0.56^{abcd}	67.48 ±14.75^{b}
4:1	0.65 ±0.0015^{e}	4.35 ±1.60^{bcd}	63.25 ±0.25^{cde}	24.00 ±9.00^{e}	0.53 ±0.01^{e}	22.72 ±0.28^{a}	46.75 ±9.35^{d}
Natural beeswax	0.99 ±0.004^{c}	0.33 ±0.13^{e}	64.75 ±0.25^{c}	86.33 ±4.04^{a}	2.28 ±1.61^{bc}	15.29 ±0.98^{e}	125.62 ±29.04^{a}
ESQA	-	-	61-66	70-80	-	17.0-24.0	-

Different letters down the column show significant difference (p =0.05).

Table 7. Results of physicochemical properties of beeswax-"kocho" adulteration (Mean ±SD).

Beeswax-"kocho" adulterants ratio	ρ (g/cm^3)	AC (%)	MPt (℃)	EV	EV to AV	AV	SV (℃)
1:1	1.61±0.01[a]	0.41±0.02[abcd]	63.25±0.25[abc]	86.33±8.50[abc]	4.2981±2.19[ab]	16.41±1.26[d]	102.85±9.35[abcd]
2:1	0.76±0.001[c]	0.49±0.10[abc]	63.00±0.00[abcd]	83.33±2.52[ab]	2.8829±1.43[d]	17.39±0.00[c]	112.20±0.00[abc]
3:1	0.55±0.00[e]	0.68±0.01[a]	62.75±0.25[abcde]	79.33±47.50[a]	5.7211±0.99[a]	19.35±0.28[b]	114.02±4.67[ab]
4:1	0.70±0.001[d]	0.61±0.20[ab]	62.25±0.25[ab]	52.00±37.00[abcde]	3.6296±0.93[abc]	22.86±0.42[a]	74.80±37.40 [e]
Natural beeswax	0.99±0.004[b]	0.33±0.13[abcde]	64.75±0.25[a]	80.33±4.04[abcd]	2.28±1.61[e]	15.29±0.98[e]	125.62±29.04[a]
ESQA	-	-	61-66	70-80	-	17.0-24.0	-

Different letters down the column show significant difference (p =0.05).
SD=standard deviation, ρ = density, AC= Ash content, Mpt = melting point, EV=Ester value, RN=ratio number, AV=acid value, SV= Saponification value. ESQA =Ethiopian Standard and quality Authority.

3.1. Beeswax-candle Adulterated Materials

Adulterated beeswax with candle only and equal amount it have uniform appearance, yellow in color, breakage is like beeswax and good smell. The density of beeswax adulterated with the lowest ratio of candle (4 parts of natural beeswax with 1 part of candle) was 0.44±.003 g/cm^3, which is less than that of natural beeswax. This value indicated that beeswax adulterated with candle can be detected by the density parameter. The density of beeswax adulterated with candle in ratio of 1:1, 2:1, 3:1 and 4:1 (beeswax to candle) was 0.848±0030, 0.322±.001, 1.135±0.004, 0.44±0.003 and 0.959±0.001 g/sm^3, respectively. The density of beeswax adulterated with candle was significantly different from that of natural pure beeswax (p=0.05). The mean melting points of beeswax mixed with candle in ratios of 1:1, 2:1, 3:1 and 4:1 were 62.00 ±0.00, 59.75±.25, 63.00±.00, and 63.00±0.00 ℃, respectively. The melting points also showed a significant difference between the adulterated beeswax at different ratios and pure beeswax (p=0.05). The melting points of all candle adulterated beeswax were less than those of pure beeswax, which is similar to what has been reported [23]. The mean saponification values of the beeswax-candle mixed in the ratios of 1:1, 2:1, 3:1 and 4:1 were 102.85±28.05, 46.75±9.35, 74.80±18.70 and 28.05±9.35 ℃, which indicated that samples with a high beeswax proportion had the highest saponification value. Although the mean acid values increased with the increased ratios of the candle, the differences were not significant among the treatments as well as with the control (pure beeswax). This suggests that the acid value of beeswax is worthless to detect candle adulteration of beeswax. Ester value to acid values ratio or ratio number of beeswax-candle mixtures in the ratios of 1:1, 2:1, 3:1 and 4:1 were 2.58±1.39, 5.80±2.44, 5.08±1.19, 1.41±45, respectively. This result showed that the values decreased as the proportion of beeswax increased (Table 1). The current values are outside the permitted range set by the Ethiopian Standard and Quality Authority, which ranges from 70 to 80. Thus, the ester value to acid value ratio or ratio number might be a better method to detect beeswax adulterated with candle than other beeswax quality parameters.

3.2. Beeswax- animal Tallow Adulterated Materials

Beeswax and animal tallow: Natural beeswax adulterated with different percent of animal tallow color gradually changed from light yellow to less light yellow as the amount of beeswax increased. The smell of beeswax adulterated with animal tallow was different from that of pure beeswax. The above picture shows beeswax adulterated with animal tallow and the color became dark yellow, which is similar to that of pure beeswax. Adulterated beeswax with animal tallow has a tallow smell only when an equal amount of tallow added, and the dark yellow color, smoothly breakable and its appearance it seems beeswax.

Beeswax with candle: when an equal amount of candle and beeswax were mixed and fewer amounts of pure beeswax were mixed, the mixture produced a smell of animal tallow, a light yellow color, did not form a uniform appearance inside and easily or smoothly breakable. The density of beeswax adulterated with the lowest ratio of animal tallow 4:1 (natural beeswax to animal tallow) was 0.76±.001 g/cm^3. The value of the density of beeswax samples adulterated with animal tallow was slightly lower (Table 2), which was lower by 0.2 g/cm^3 than the lower limit of pure. Beeswax samples adulterated with animal tallow were melted at a slightly lower temperature at 61.75±0.25℃, which was lower by 1.25℃ than the lower limit of pure beeswax melting point standards.

Beeswax samples mixed with a lower quantity of animal tallow tended to melt at a lower temperature (Table 2), indicating that the increased proportion of animal tallow in beeswax decreased the melting point of the mixture. According to a previous study the melting point of pure animal tallow is 46℃ while that of saponification points is 44℃ [24]. Contrarily, the saponification value followed the opposite trend, in which beeswax samples mixed with 1:1 (one part animal tallow one part beeswax) saponified at 102.85±9.35℃ and beeswax samples adulterated with 4:1 (natural beeswax to animal tallow) saponified at a further higher temperature of 112.20±18.70℃ (Table 2). The presence of adulterants in beeswax affects the values of some physicochemical parameters of the wax [15].

3.3. Beeswax- soap Adulterated Materials

When beeswax was adulterated with a ratio of 1:1 and 1:2 (beeswax to soap) or a higher percentage of soap, it was moistened. The pure beeswax could be easily identified from the beeswax-soap adulterated material without physicochemical property parameter determination. However, beeswax adulterated with ratios of 3:1 and 4:1 (beeswax to soap) physically similar to pure beeswax, with mean densities of 0.76±0.001 and 0.84±00153 g/cm^3, respectively, which was significantly different from unadulterated beeswax (Table 3), but there was no significant difference between the adulterated beeswax and pure beeswax at p=0.05. The acid value ranged from 16.41±1.26 to 22.86±42 that showed an increase as the percentage of beeswax content in the mixture increased. The results indicated that the acid values, ash content, ester values, ratio number, saponification and density of beeswax-soap mixtures were significantly different from pure beeswax (p=0.05). The presence of adulterants in beeswax affects the values of some physicochemical parameters of the wax [2]. According to these results, ash content, density and ratio number could be used as methods to detect beeswax-soap adulterated materials good quality beeswax.

3.4. Beeswax-maize Flour Adulterated Materials

The density of beeswax adulterated with maize flour in ratios of 1:1, 2:1, 3:1 and 4:1 (beeswax to maize flour) was 1.51±002, 1.48±003, and 1.35±003 and 1.12±006 g/sm^3, respectively. The density of beeswax adulterated with maize flour is not significantly different from that of natural beeswax (p=0.05). The means of melting points ranged of beeswax mixed with maize flour in different ratios were 64.50±0.50, 63.50 ±0.50, 63.50±0.50, and 61.50±0.50℃, respectively. The melting point also shown a little decrease as the content of the pure beeswax in the beeswax-maize flour mixture increased. The acid value ranged from 16.41±1.26 to 22.86±42, which showed an increase as the percentage of pure beeswax content in the mixture was increased (Table 4). The result indicated that the acid values of beeswax-maize flour mixed materials significantly different from each other and pure beeswax. The presence of adulterants in beeswax affects the values of some physicochemical parameters of the wax [14]. A saponification value of the beeswax-maize flour mixture was ranged from 74.80±37.40 to 114.02±4.67 ℃, which indicated the increment as the percentage of beeswax in the mixture increased. Ester value to acid values ratio or ratio number of 1:1, 2:1, 3:1 and 4:1 beeswax to maize flour ratios were 4.59±1.63, 0.81±0.50, 2.96±2.78 and 5.93±1.92, respectively. The ester values were 120.33±46.50, 107.33±37.50, 106.00±13.00, 2.96±2.78 and 61.00±47.00, respectively (Table 4). This result indicates that ester value decreases as the beeswax percentage in the mixture increases, showing that the ester value could be used as one method to detect beeswax adulterated with maize flour.

3.5. Beeswax-lime Stone Adulterated Materials

The density of beeswax adulterated with lime stone in the ratios of 1:1, 2:1, 3:1, 4:1 (beeswax to lime stone) was 1.26±0.003, 1.09±0.003, 1.19±0.003 and 0.94±0.003 g/sm^3, respectively (Table 5). The density of beeswax adulterated with lime stone (4 portion of beeswax, 1 of lime stone) was significantly different from that of natural beeswax ($p<0.05$). The result indicated addition of little lime stone increased the detection of lime stone adulterated beeswax. The melting point also showed a decrease as the content of the bees wax in the beeswax- lime stone adulterated material increased, but there were no significant differences between the adulterated beeswax and pure beeswax at p=0.05 (Table 5). The acid values showed an increase as the percentage of beeswax content in the mixture increased. The results indicated that the acid values of the adulterated materials (beeswax to lime stone ratio) were statistically different from pure beeswax (p=0.05). However, ester values and ash contents were showed significant differences (Table 5). These results suggest that the ester value and ash content of beeswax adulterated with lime stone could be used for the quality detection of beeswax adulterated with a lime stone).

3.6. Beeswax- salt Adulterated Materials

The density of beeswax adulterated with salt was ranged from 0.654±0.002 to 1.57±0.003 g/sm^3. The density of the beeswax adulterated with salt was significantly different from that of natural beeswax (p=0.00). The density of the sample was decreased in density as the percent of natural beeswax increased in the mixture of beeswax and salt. The melting point ranged from 63.25±0.25 to 96.5±3.50 ℃. The melting point also showed a decreasing trend as the content of the beeswax in the beeswax-salt adulterated material increased. The acid value of the mixtures was ranged 20.76 ±0.56 to 22.72 ±0.28 with the highest acid for the mixtures that contained more percentage of the beeswax (Table 6). The presence of adulterants in beeswax affects the values of some physicochemical parameters of the wax [21].

The acid values found was not different from that of natural beeswax, which ranged from 17.0-24.0. A saponification value of the beeswax-salt material was ranged from 35.53±21.57 to 67.48 ±14.75 ℃, indicating that the increment in the percentage of beeswax in the mixture increased the saponification value of the mixture. Ester value to acid values ratio or ratio number ranged from 0.53±0.01 to 18.27±3.97, indicating the reduction as the beeswax percentage in the mixture increases.

3.7. Beeswax- "kocho" Adulterated Materials

'Kocho' is bread-like fermented food and product made from the decortication and fermentation of enset (Ensete Ventricosum) parts [25]. The price is cheaper than beeswax. It is sometimes used as adulterant material with beeswax to increase profit from beeswax sale. When equal amount of "kocho" is adulterated with beeswax it was simple to detect the adulterated beeswax from the pure beeswax by smell. However, the smell changed and became similar to that of pure beeswax as the percentage of the adulterant decreased. Moreover, the two materials could not have a uniform structure when mixed together in a one-to-one ration.

The density of beeswax adulterated with "kocho" was ranged from 0.55±0.00 g/cm^3 to 1.61±0.01 g/sm^3. The density of beeswax adulterated with it was significantly different from that of natural beeswax (p=0.05). The result indicated decrease in density as the percent of natural beeswax increased in the mixture of beeswax and this adulterant material. The melting point ranged from 62.75±0.25℃ to 64.75±0.25℃ (Table 7). According to Ethiopian Standard and quality Authority the melting point, acid value, and saponification cloud point are from 61-66℃, 17.0-24.0 and 70-80℃ while American wax importers and refiners Association permit 62-65℃ for melting point, 17.0-24.0 for acid value, 72-79 for ester value, 3.3-4.2 for ratio of ester value to acid value and saponification cloud point less than 65℃ [14], respectively. According to this study beeswax adulterated with "kocho" in ratio of 1:1, 2: 1, 3:1 and 1:4 beeswax to "kocho" (mass/mass) of "kocho" in beeswax) better methods were density and acid value to identify the adulterated beeswax from pure one. If an adulterant is found in beeswax the values of some physicochemical parameters of the wax are affected [15].

4. Conclusion and Recommendation

Natural beeswax is one of the highly valuable products produced by *Apis mellifera* bees. The adulteration of inexpensive materials has created problems with their quality and marketing in recent times. In this study, adulterant materials with beeswax in different proportions showed different values of physical and chemical quality parameters. The results indicated how the adulterants affect the physical quality of natural beeswax, like cracking during melting and forming a block of wax and color changes in wax when forming blocks. During the characterization of pure beeswax and adulterated beeswax, adulterated beeswax showed several variations in physicochemical properties, which fell out of the standard quality range. Therefore, parameters like acid value for animal tallow, saponification for maize flour, ester value for 'kocho', ash content for lime stone, melting point for salt, and density for soap are found to be the best methods to identify adulterated beeswax with the respective adulterants mixed in large proportions. In a small percentage of adulterants, modern digital technologies should be used to identify adulterated beeswax from pure natural beeswax. Moreover, as adulterating beeswax with other cheaper materials becomes more complex, it is important to create awareness and strengthen monitoring on beeswax quality control because the problem may affect the health of bees and the market for their products.

Abbreviations

M	Molarity
NaOH	Sodium Hydroxide
HCl	Hydrochloric Acid
mg	Milligram
KOH	Potassium Hydroxide
V	Volume
mL	Milliliter
GC-MS	Gas Chromatography-mass Spectroscopy
SD	Standard Deviation
ρ	Density
AC	Ash Content
Mpt	Melting Point
EV	Ester Value
RN	Ratio Number
AV	Acid Value
SV	Saponification Value
ESQA	Ethiopian Standard and Quality Authority
℃	Degree Celsius

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Conflicts of Interest

The authors declare no conflicts of interest.

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