# PHYSICO-CHEMICAL PROPERTIES OF BEESWAX FROM FOUR DIFFERENT HONEY BEE (*Apis*) SPECIES

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## ABSTRACT

**Physico-chemical parameters such as melting point, density, acid value, iodine absorption number, and saponification value were determined for different beeswax samples extracted from the honey combs of *Apis mellifera* Linneaus, *A. cerana* Fabricius, *A. dorsata* Fabricius, and *A. breviligula* Maa taken from three different locations in the Philippines. The average mean values calculated for *A. mellifera, A. cerana, A. dorsata,* and *A. breviligula*, respectively, are as follows: melting point (in °C): 62.81 ± 0.18, 64.35 ± 0.17, 59.28 ± 0.18, and 59.44 ± 0.24; density (in g/mL): 0.9546 ± 0.0035, 0.9611 ± 0.0092, 0.9838 ± 0.0167, and 0.9691 ± 0.0109; acid value (in mg KOH/g sample): 16.22 ± 0.23, 5.90 ± 0.32, 5.21 ± 0.28, and 4.68 ± 0.25; iodine absorption number (in g/100 g sample): 4.35 ± 0.39, 4.70 ± 0.42, 4.52 ± 0.38, and 5.41 ± 0.23; and saponification value (in mg KOH/g sample): 94.20 ± 2.30, 99.40 ± 4.92, 99.2 ± 2.2, and 94.9 ± 3.9.**

## INTRODUCTION

Beeswax is a valuable hive product secreted by bees from four pairs of wax glands on the underside of the abdomen. Its production is influenced by nectar flow, brood rearing, presence of queen, temperature, and presence of pollen as protein source (Yañez et al., 2013; Bogdanov, 2014). Beeswax is composed primarily of alkanes, wax esters, and free fatty acids. The bees use beeswax in building combs that are also used to house larvae and store honey and pollen. Among Philippine bee species, the giant honey bee, *Apis dorsata* Fabricius,

builds the largest honeycomb. A single comb of the giant honey bee may be up to one meter square and can contain up to 10-25 kg of honey. The bees are stimulated to produce beeswax whenever there is more of honey to be stored and there is a lack of honeycomb to which the other bee products could be stored. During the production of one kilogram of wax, around eight kilograms of honey are being consumed by the bees (Lynn & Cooney, 2003; Bradbear, 2009).

Humans take advantage of these bee products and utilize them in medicine, food, technology, etc. Aside from its use to the bees, beeswax is also used in making candles, soaps and many other products which can be sources of income (Blomquist et al., 1980; Bogdanov, 2014; Cassier & Lensky, 1995; Hepburn et al., 1991). The wide range of uses of beeswax correlates to its wide temperature range between its becoming plastic (32oC) and melting (61-66oC), which plays the most practical importance among the properties of beeswax. Since there are a lot of uses of beeswax for bees alone, it can be considered as storage for contaminants in the beehive and these could be passed on to other bee products and other bees in the honeybee colony as well. Having this problem would put the agricultural sector at risk, particularly apiculture. Nowadays, beekeeping industry has also become modern with low expenditures but high cost and demand of bee products (Ministry of Natural Resources and Tourism, Tanzania, 2007). Thus, it is important to keep and monitor the quality of the beeswax to be in its best state in order to preserve the natural properties of other beekeeping products (Yañez et al., 2013; Maia & Nunes, 2013). The beeswax used for commercial purposes should be of the best quality especially if it is locally produced so that it could be exported to other countries as well.

This study aimed to determine and compare the physico-chemical properties of beeswax from four different species, namely *Apis mellifera* Linnaeus, *A. cerana* Fabricius, *A. dorsata*, and *A. breviligula* Maa.

## MATERIALS AND METHODS

### Preparation of beeswax sample

Honeycombs of *A. mellifera*, *A. cerana*, *A. dorsata*, and *A. breviligula* were cleaned, first by removing bee broods or other bee products from compartments of each honeycomb. After this, crude beeswax was extracted directly by submerging the cleaned honeycombs in a water bath which was heated gradually until the wax floats on top of the water layer. The mixture with the floating wax was then filtered using silk screen cloth to separate the honeycomb residues from the wax and water. The cloth was pressed until all the waxes were recovered. The mixture of wax and water was then cooled to room temperature. The crude beeswax obtained was further purified by melting it again in water and filtering it using cheese cloth to remove small particles that remained from the first part of extraction. The purified beeswax was air dried before storage.

## Physico-chemical Parameters

All analyses/determinations of physico-chemical parameters were done in triplicates.

Melting point. Melting point range of beeswax samples was determined using the capillary tube method cited by Bernal et al. (2005). Melted beeswax of *A. mellifera, A. cerana, A. dorsata*, and *A. breviligula* were introduced separately in a 7.5 cm long x 1.0 mm internal diameter thin-wall hollow capillary tube, until a height of about one cm was achieved. The beeswax samples were allowed to solidify in the capillary tubes. The capillary tube containing the beeswax sample was placed on a water bath that was slowly warmed at 1-2 °C/min. The temperature range at which the beeswax starts to melt until it completely melts was recorded using a thermometer that was attached to the capillary tube.

Density. The density of beeswax samples was determined based on the method cited by Bernal et al. (2005). A clean, dry and sterilized one-ml syringe was weighed first and filled to capacity with distilled water. Approximately 10 mg beeswax was placed in a vial followed by the addition of 50 drops of distilled water. A small volume (approximately 30 drops) of methanol was then added gradually until the beeswax was suspended into the liquid. Afterwards, the hydroalcoholic mixture was filled into the calibrated syringe and was weighed. The beeswax density was determined by using the formula:

$$\text{Density} = 0.9982 \times \frac{(m_1 - m_2) + A}{(m_3 - m_2) + A}$$

where $A = 0.0012$; $m_1$ is the mass in grams of the syringe containing the hydroalcoholic mixture; $m_2$, the mass in grams of the empty and completely dry syringe; and $m_3$, the mass in grams of the syringe containing water.

*Acid value.* The acid value of beeswax samples was determined based on the method cited by Bernal et al. (2005). Approximately 0.5 grams of beeswax sample was dissolved in 25 mL of dichloromethane. The solution was soaked overnight followed by addition of three drops of phenolphthalein indicator. The solution was titrated using standardized 0.05 M sodium hydroxide in methanol until a faint pink color was achieved as its endpoint. A blank solution containing 25 mL of dichloromethane was allowed to stand overnight and also titrated using the same standardized 0.05 M NaOH in methanol solution to correct the solvent acidity. The acid value (in mg KOH/g of sample) was calculated using the formula:

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

where V is the volume (in mL) of NaOH solution required by the sample; V', the volume (in mL) of NaOH solution required by the blank; and w, the mass (in g) of the beeswax sample used.

*Iodine absorption number.* The iodine absorption number of the beeswax samples was determined using the Hanus method cited by Bernal et al. (2005).

The first part was to weigh $0.300 \pm 0.005$ g of beeswax, which was dissolved in 10 mL dichloromethane. Afterwards, five mL of Hanus reagent (a solution prepared by dissolving two g of IBr in 100 mL acetic acid) was added to the beeswax placed in a conical flask. The mixture was softly shaken for 30 sec and kept in darkness at room temperature for one hour to complete the addition of $I_2$ to the double bonds. Subsequently, five mL of an 8% KI aqueous solution was added and titrated with a 0.1 M $Na_2S_2O_3$ solution, with constant shaking and using starch solution as indicator. The indicator was added when the titration is close to a final point.

A blank was also conducted under the same conditions to correct for the possible influence of the reagents. The iodine absorption number (in g/100 g of sample) was calculated by using the equation:

$$\text{Iodine value} = 12.69 M \frac{(V' - V)}{w}$$

where V is the volume (mL) of 0.1 M $Na_2S_2O_3$ solution required by the sample; V', the volume (mL) of 0.1 M $Na_2S_2O_3$ solution required for the blank; and w, the mass (g) of the beeswax sample. The $Na_2S_2O_3$ was normalized with a 0.05 M $KIO_3$ solution to which 10 mL 2M HCl and 20 mL KI was added as well. Iodine values for each sample were averaged from three measurements.

*Saponification number.* The saponification number of beeswax samples was determined using the method cited by Bonhevi & Bermejo (2012). Precisely 1.0000 gram of beeswax was dissolved in 25 mL of 1 M potassium hydroxide-ethanol solution. The solution was refluxed for one hour at $100^{o}C$. Immediately, the hot solution (around $65-70^{o}C$) was titrated using the standardized 0.5 M hydrochloric acid solution and phenolphthalein as indicator. A blank solution containing 25 mL of 1 M potassium hydroxide – ethanol solution was refluxed for one hour at $100^{o}C$ and was also titrated using the same 0.5 M hydrochloric acid with the faint pink end point in order to determine the total amount of acid needed to neutralize the base. The saponification number (in mg potassium hydroxide per g fat) was calculated using the formula:

$$\text{Saponification number} = 56.1 M \frac{(V - V')}{w}$$

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

## RESULTS AND DISCUSSION

The mean values and the ranges obtained for the determination of different physico-chemical parameters are shown in Table 1. The melting points of all the beeswax from *A. mellifera* and *A. cerana,* from three different locations, fall

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**Table 1.** Physico-chemical parameters of beeswax samples from four different honey bee species obtained at three different locations.

| Species | Location | Parameter | | | | |
|---|---|---|---|---|---|---|
| | | Melting Point (°C) | Density (g/mL) | Acid Value (mg KOH/ g sample) | Iodine Absorption Value (g/100 g sample) | Saponification Value (mg KOH/ g sample) |
| *A. mellifera* | Palawan | 62.72± 0.31 | 0.9528± 0.0010 | 16.04± 0.12 | 4.69± 0.66 | 96.07±  4.52 |
| | Los Baños (Laguna) | 62.94± 0.43 | 0.9531± 0.0051 | 16.09± 0.23 | 4.29± 0.93 | 90.44±  3.13 |
| | Laguna | 62.78± 0.30 | 0.9578± 0.0034 | 16.54± 0.18 | 4.08± 0.62 | 96.10±  4.52 |
| *A. cerana* | Albay | 64.17± 0.36 | 0.9549± 0.0059 | 6.33± 0.60 | 4.40± 0.85 | 103.27±  8.49 |
| | Lipa (Batangas) | 64.68± 0.35 | 0.9638± 0.0301 | 5.88± 0.60 | 5.01± 1.00 | 103.59±10.58 |
| | Los Baños (Laguna) | 64.31± 0.33 | 0.9647± 0.0071 | 5.51± 0.54 | 4.70± 0.55 | 91.34±  6.97 |
| *A. dorsata* | Palawan 1 | 59.41± 0.29 | 0.9743± 0.0302 | 5.21± 0.53 | 4.49± 1.14 | 100.00±  2.9 |
| | Palawan 2 | 59.11± 0.39 | 1.0040± 0.0418 | 5.16± 0.48 | 4.29± 0.36 | 99.40±  6.2 |
| | Palawan 3 | 59.31± 0.39 | 0.9732± 0.0197 | 5.28± 0.69 | 4.79± 0.47 | 98.20±  3.5 |
| *A. breviligula* | Palaui (Cagayan) | 59.06± 0.43 | 0.9896± 0.0130 | 4.89± 0.55 | 5.21± 0.50 | 98.60±  5.6 |
| | UPLB (Laguna) | 59.47± 0.55 | 0.9523± 0.0097 | 4.89± 0.45 | 5.62± 0.43 | 99.10±  8.0 |
| | Mindoro | 59.78± 0.22 | 0.9655± 0.0270 | 4.26± 0.28 | 5.41± 0.42 | 87.10±  5.9 |

within the range (61-65°C) that was set for the quality criteria for the melting point of routine beeswax testing followed by Bogdanov (2009). The observed melting points of beeswax from *A. dorsata* and *A. breviligula* were found to be lower than that from *A. mellifera*. According to Tulloch (1980) and Bogdanov (2009), beeswax of Asian origin have shorter carbon chain length compounds, simpler monoesters, and fewer free fatty acid methyl esters than that of *A. mellifera*, which could account for their lower melting points. Among the species, the beeswax from *A. cerana* showed the highest melting point. The thermal characteristics of beeswax could also affect its important structural features such as stiffness, strength, and toughness, depending on the temperature variations from its environment (Buchwald et al., 2006). A higher melting point is usually preferred since the wax becomes softer upon reaching the melting point. This characteristic has an advantage in terms of making the wax flexible and bendable, enabling them to be molded easily such as in candle-making (Mutsaers et al., 2005).

The density of the beeswax from *A. mellifera* and *A. cerana* fall within the

range of 0.95-0.96 g/mL as set by Bogdanov (2009), with the exception of *A. cerana* from Lipa, Batangas and Los Baños, Laguna.  The density of the beeswax from *A. cerana* has a relatively higher density compared to that of *A. mellifera*. On the other hand, the beeswax from *A. dorsata* and *A. breviligula* have relatively higher densities compared to the previously known (literature) values, except for the beeswax of *A. breviligula* from UPLB (also in Laguna) and Mindoro which were within the literature value range.  Since the density of the beeswax is affected by the proportion of compounds present in it, differences in the density values obtained may be due to the variation in the botanical origin of the beeswax samples (Tesfaye et al.*,* 2017).

The determined acid values of the beeswax from *A. mellifera* and *A. cerana* were lower than the range (17-22 mg KOH/g sample) set by Bogdanov (2009). The acid value of beeswax of *A. cerana* is also lower than that from *A. mellifera*. On the other hand, the obtained acid values for the beeswax of *A. dorsata* and *A. breviligula* at three different locations were lower than the range of *A. mellifera* wax which is 17-22 mg KOH/g sample. This may be due to some of the disadvantages of the method for acid value determination, such as incomplete solubility of the oil/fat and the possibility of error due to bias on the determination of the endpoint of the solution upon titration.  Another possible reason may be the origin of the beeswax since environmental and geographical factors could affect bee adaptation, as well as their products (Bernal et al.*,* 2005). Having an acid value lower than that of *A. mellifera* beeswax could also mean that there is a much lower amount of fatty acids present in the beeswax of *A. dorsata* (Tulloch, 1980).  For soap-making, low acid value is preferred because it indicates longer chain fatty acids. The shorter the chains of fatty acid, the more soluble it is in water. Also, the longer the chains of fatty acid, the more prone it is to oxidation in its free form and formation of salts. Hence, lower acid value is desired (Kurdash & Turyan, 2005).

The iodine absorption number of beeswax from *A. mellifera* and *A. cerana* were found to be lower than the values set by Akoh & Min (2002), which is 7-16 g/100 g sample. The same trend was observed for the beeswax from *A. dorsata* and *A. breviligula*. This difference from the iodine values from the literature may be due to the manner of beeswax preparation wherein oxidation may have occurred, thereby reducing the unsaturated part resulting to lower iodine value. A lower value of iodine absorption value could also mean that there is a low degree of unsaturated fatty acids present in the beeswax samples.  A different method could be employed in order to determine if the actual iodine value obtained was that of the true value for conjugated double bonds. In soap-making, hard bars of soap are produced when the present fatty acid chains of beeswax are saturated. In contrast, soft bars of soap are produced when there are more of unsaturated fatty acid chains of beeswax since they have lower melting points, thus they are more easily molded and shaped (Hughes & Bond, 2013).

Saponification value was the last parameter determined. The beeswax of *A. mellifera* and *A. cerana* were within the range (87-102 mg KOH/g sample) set by Bogdanov (2009).  Moreover, the obtained saponification values for the beeswax from *A. dorsata* and *A. breviligula*, from three different locations, were all within

the range set by Bogdanov (2009). Saponification value provides information about the characteristic average molecular weight. A low saponification value corresponds to a large average molecular weight that may come from the long chains of fatty acids.  A high chain length indicates that there are fewer carboxylate functional groups per mass fat (Darpan, 2000; Chaudhari, 2013).  A low saponification value would produce a soft soap that can be used in producing skin care products. On the other hand, a high saponification value would correspond to a hard soap that can be used in producing cleansing agents (Browning, 2002).

For the trends observed, the higher the melting point, the lower the acid value, which indicates the presence of longer fatty acid chains that would break at higher temperature. Theoretically, low iodine absorption values correlate to a low degree of unsaturation, thus a higher melting point on beeswax samples. However, this was not the trend observed for the correlation of the melting point to the iodine absorption number. This may be due to the incomplete absorption of the halogen reagents used that may have affected the obtained iodine values (Chowdhury & Mukherjee, 1955). For saponification value, a lower value corresponds to longer chains of fatty acids, thus the larger the molecular weight and higher the melting point of the beeswax.

Figure 1 shows the relative ratios of the average mean values of these parameters relative to that of the species with the highest corresponding value. From the observed values, it is recommended to use beeswax with high melting point to produce a more flexible and more bendable type of candles. Among the samples studied, the beeswax from *A. cerana* is the best type of wax which can be used for candle-making.  For making soap, it is recommended to use beeswax with low acid value.  Thus, among those studied, it is recommended to use beeswax from *A. breviligula*. In terms of iodine absorption number, the higher the value, the softer bars of soap would be produced. Thus, the iodine absorption number would matter depending on the desired quality of soap. Lastly, the saponification value recommended for skin care products is low. Among the species, the beeswax from *A. breviligula* would be the best source of wax for this type of production. On the other hand, a high saponification value is required for cleansing agents. Thus, the beeswax from *A. cerana* would be the best type for this purpose. Generally, the type of beeswax that would be used is determined depending on its purpose.

## CONCLUSION

In this study, the physico-chemical properties of beeswax from *A. mellifera, A. cerana, A. dorsata*, and *A. breviligula* from different locations were determined. The melting points were found to be lower in beeswax of *A. dorsata* and *A. breviligula* than that from *A. mellifera.* On the other hand, *A. cerana* beeswax has the highest melting point among the species/beeswax samples studied. The density of the beeswax samples fall within the range of 0.95-0.96 g/mL for both species of *A. mellifera* and *A. cerana*, while the densities of the beeswax samples
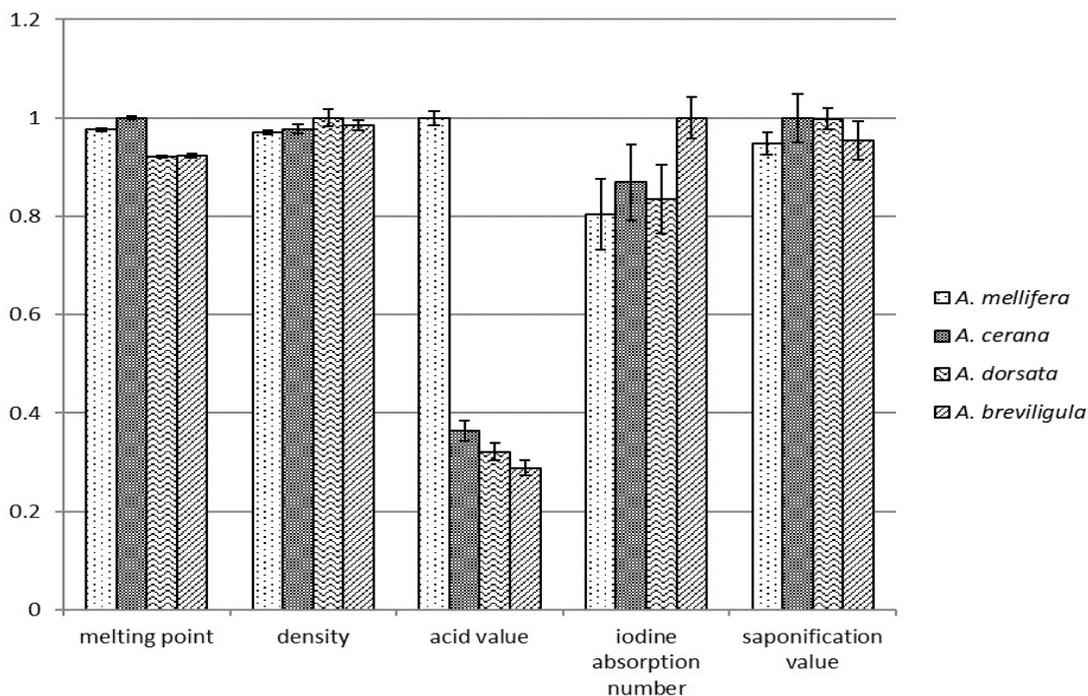
**Figure 1.** Relative ratios of the average mean of the physico-chemical parameters. For each parameter, values are calculated relative to that of the bee species with the highest corresponding average mean.

from *A. dorsata* and *A. breviligula* were higher compared to the other two, except for the beeswax of *A. breviligula* from UPLB and Mindoro which also fall within the range. The obtained acid values were found to be lower than the range of 17-22 mg KOH/g sample for all four species studied. The beeswax of *A. mellifera* showed the highest acid value. The iodine absorption values for the beeswax samples of all four species studied were lower than the range of 7-16 g/100 g sample. Lastly, the saponification values determined for all beeswax samples of species from *A. mellifera*, *A. cerana, A. dorsata,* and *A. breviligula* fall within the range of 87-102mg KOH/g sample. Among the species, the beeswax of *A. breviligula* from Mindoro has the lowest saponification value, while the highest saponification value comes from the beeswax of *A. cerana* from Albay and Lipa.

## ACKNOWLEDGEMENT

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