Botanical origin, colour, granulation, and sensory properties of the Harenna forest honey, Bale, Ethiopia

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ABSTRACT

In this study, the Harenna forest honey samples were investigated with respect to their botanical origin, granulation, colour and sensory properties. Sixteen honey samples were collected from two representative sites (Chiri, C, and Wabero, W) using random sampling techniques. Botanical origin was investigated using qualitative pollen analysis by counting 500 pollen grains using harmonised methods of melissopalynology. Granulation, colour, and sensory properties of honey were determined by visual observation, using Pufnd grader, acceptability and preference tests, respectively. Honey samples were also tested for tetracycline. Honey obtained from Wabero is originated dominantly from Syzygium guineense while Chiri was multifloral. The colour of honey ranged from 34 to 85 with light amber and extra light amber colours. The honey samples were free from tetracycline residue and form coarse granules slowly. Significant variation (p > 0.05) in sensory preference and acceptability tests not observed due to hive types and locations.

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1. Introduction

The Harenna forest is delineated in Bale zone, Ethiopia, mainly in the districts of Dello Mena, Harenna Buluk, Goba and Nensebo. This forest is one of the few remaining rainforest patches in the southeastern part of Ethiopia (Senbeta & Denich, 2006) and constitutes the largest part of Bale mountains national park. It differs from the southwestern rainforests in terms of dominant canopy tree species, and supports many vascular plant species (over 300 species) and endemic plant species than other rainforests (Senbeta, 2006). Some of the unique floristic composition and common climber species of the Harenna forest are reported elsewhere (Gole & Senbeta, 2008; Senbeta, 2006).

Honey is one of the well-traded product and important sources of livelihoods in the Harenna forest. The most dominate spp., which have apicultural value are Oncinotis tenuiloba, Polyscias fulva, Vernonia amygadalina, Vernonia auriculifera, Cordia africana, Ehretia cymosa, Diospyros abyssinica, Croton macrostachyus, Shirakiopsis elliptica, Ocotea kenynesis, Syzygium guineense, Olea welwitschii, Margaritaria discoidea, Gouania longispicata, Vepris dainelli and Pouteria adolfi-friederic (Senbeta, Gole, Denich, & Keibel, 2013). These species, other trees, shrubs and climbers provide nectar and pollen for honey bees (Genene, 2006).

The total number of bee hives in Ethiopia is 5,207,300 (95.96% traditional and 2.98% frame hives) (Central Statistical Agency., 2013). Bamboo, timber, bark, climber, mud, animal dung, grass, gourd, barrel, and clay pot are used to make traditional hives; and frame hives are made from timber, plywood, and chip-wood. In the Harenna forest the most common type of traditional hive is a hollow, cylindrical hive made from C. africana, which has a length of ~1 m and a diameter of ~25 cm. The bees fill the space with honeycombs from the top to down wards. The traditional hives hang on a long tree in the forest. The frame hives are Zander type, which is largely made from C. africana. The hive has mainly two rooms. The bottom box is used for brood rearing and the super box for honey storage. These hives were kept in the back yard.

The advantages of traditional hives include construction with locally available materials by the beekeepers; no need of skilled labour; hives help to trap colonies; and higher yield of beeswax than frame hives. The drawbacks of traditional beekeeping...
practices are production of lower quantity of honey than frame hive, no possibility of inspecting colony. In the case of frame hives, beekeeper has little fear of comb damage, the comb is fixed firmly to the four sides of the frame and thus facilitates easy harvesting, transportation easier, bees save time and energy to construct a replacement comb, easier to remove the honey without damaging the comb and easy of inspection. The disadvantages of frame hives are expensive to construct; a high degree of craftsmanship is required; beekeeper has to install a wired comb foundation; and requires importing centrifugal honey extractor, wax molder and other equipment.

The apicultural value of the Bale zone largely depends on the Harenna forest. According to Central Statistical Agency (2013), the Bale zone produces 1,349,801 kg honey per year from 139,306 bee hives. This volume is ~3% of the total honey production in Ethiopia, 45,095,201 kg. The Bale zone ranked 7th out of 63 zones of Ethiopia in volume of honey production.

Pollens is very important for honey bee nutrition. Honeybees collect pollen grains from plants to obtain protein for their survival and reproduction (Barth, Munhoz, & Luz, 2009; Escredo, Míguez, Fernández-González, & Carmen Seijo, 2013). The bees frequently collect a wide variety of pollen types, but they generally concentrate on a few species (Bauma, Rubink, Coulson, & Bryant, 2011). Botanical origin of honey can be verified by qualitative and quantitative microscopic pollen analyses based on the relative frequencies of the pollen types of nectariferous species.

Colour in liquid honey varies from clear and colourless to dark amber or black. The most important aspect of honey colour lies in its value for marketing and determination of its end use. Next to general quality determinations, colour is the single most important factor determining import and wholesale prices of honey (Krell, 1996). In many countries with a large honey market, consumer preferences are determined by the colour of honey. Honey colour is frequently given in millimeters on a Pfund scale or according to the U.S. Department of Agriculture classifications (White, 1975 and Crane, 1980). The Pfund scale has water white, extra white, white, extra light amber, light amber, amber and dark amber colour levels (Krell, 1996).

The colour of honey is characteristic of its floral source. Exposure to heat and the length of time that the honey stayed in the storage may also affect honey's colour. Honey appears lighter in colour after it has granulated. This depends upon the composition of the honey and its initial colour. Generally, the darkening of honey is temperature sensitive and occurs more rapidly when honey is stored at high temperatures. The determination of colour is a useful classification criterion for unifloral honeys. Honey colour is related with its flavour. Light coloured honey is mild whereas darker types have stronger flavours. Light honeys, like the Harenna forest honey, generally fetch the highest prices. Nevertheless, in Germany, Austria and Switzerland, dark honeys are especially appreciated. Dark coloured honeys are reported to contain more phenolic acid derivatives but less flavonoid than light coloured ones (Bogdanov, Ruoff, & Oddo, 2004).

Granulation is one of the characteristics for honey. Honey is a highly viscous sugar solution, often supersaturated and susceptible of time dependent crystallization, at a rate influenced by water content, presence of nucleation seeds, degree of supersaturation and viscosity (Venin, Spaziani, & Martini, 2010). Granulation of some honey kinds are faster and other kinds are slower. Speed of crystallization in honey is defined with a proportion and the content of carbohydrates in honey. It is known that carbohydrate glucose promotes the crystallization of honey, while carbohydrate fructose breaks crystallization of honey. It is necessary to note that honey can have a different speed of granulation (Dimins, Kuka, & Cakste, 2008). The origin of honey contributes to the rapid, medium and slow granulation process (Crane, Penelope, & Rosemary, 1984).

Many consumers still think that if honey has granulated it has gone bad or has been adulterated with sugar. Analysis of sensory properties of honey is used to evaluate flavours and identify certain defects such fermentation, impurities, and off-odors. It also plays an important role in defining product standards. Moreover, it is an essential part of consumer preference/aversion studies (Piana et al., 2004).

Natural crystallization of honey is usually an unwelcome process in honey industries. On the one hand, honey texture usually gets worse. On the other hand, an upper liquid phase poor in sugar content can lead to fermentation. In order to avoid these problems, induced granulation appears to be one of the alternatives. This process consists on seeding a liquid honey with finely crystallized honey at low temperature, so that crystals act as nuclei for growth. The resulting honey is creamy, smooth and very pleasant to taste (Cavia, Fernandez-Muino, Alonso-Torre, Huídobro, & Sancho, 2007).

During granulation water is freed. Consequently, the content of the liquid phase increases with increasing risk of fermentation. Granulated honey ferments more readily than the liquid honey, because when dextrose crystals are formed in the honey, the liquid phase has higher water content than the entire honey had when it was liquid and this lead to fermentation (krell, 1996; Nuru, 1991). Water in honey is mainly fixed to sugars via hydrogen bonding. During crystallization glucose is found as glucose monohydrate, each glucose molecule fixes only one molecule of water. The water fixed to glucose in solution is set free during the crystallization process which means that water activity (aw) increases. Therefore, less water is fixed in the crystallized state (Gleiter, Horn, & Isengard, 2006). Thus, partially crystallized honey may present preservation problems, which is why controlled and complete crystallization is often induced deliberately. In addition, partially crystallized or reliquified honey is not an attractive presentation for retail shelves.

The growing interest on issues associated with the variety related identification of products has been observed in the domain of nutritional science for several years. In a number of scientific centers, various honey identification procedures have been studied (Kowalski, Łukasiewicz, & Berski, 2013; Tuberoso et al., 2014). The rapid promotion of honey production and quality characterization are urgent needs for market development, because organic and fair trade honeys are fast growing market niches in the major honey consuming countries in the western part of the world. Introduction of fair trade and organic products will add to the value of Harenna forest honey and it will improve the income of farmers. These will convince the community to improve their beekeeping techniques and conserve the forest in a sustainable way for economic reasons.

Despite the large amount of honey produced and higher market demand for Ethiopian honey, there is little information about the botanical origin, colour, granulation and sensory properties of Ethiopian honey. Thus the objectives of this study were to investigate the Harenna forest honey based on botanical origin, colour, granulation and sensory property; and compare traditional and frame hive honeys with respect to these parameters.

2. Materials and methods

2.1. Study area

This study was conducted in Chiri and Wabero areas in Bale, Ethiopia, where the Harenna forest is delineated (Fig. 1). The Harenna Forest is one of the few remaining natural forests in the entire country of Ethiopia. It is located 550 km South East of Addis Ababa,
the capital of Ethiopia. The forest area is encompassed within geographical coordinates of 6°29′–7°10′N and 39°28′–39°57′E.

2.2. Sample collection

Sixteen colonies (four traditional and four frame hives from each study area) were selected using randomized lottery sampling methods, from beekeepers that are actively participated in different apicultural intervention in the forest area. Honey samples were harvested during the major honey flow season of the area between the 18th of January, 2010 and the 20th of February, 2010. Beekeepers were oriented about the methodology of honey sample collection. Similar materials of honey harvesting and handling were used for both traditional and modern hive honey sampling. These common materials include smoker, bee brush, brood free combs, rust free metallic honey containers and ½ a kg food grade glass jar, and dry and clean plastic honey containers.

Honey from traditional hives was comb honey. Late in the afternoon the traditional beekeeper mounts on the tree using a long rope (about 50 m). The traditional hive was tightened using rope and transferred to the ground. One or two beekeepers took the hive and set on stick bed, which has a length of 50–75 cm. The beekeeper puff smoke from the back (opposite to the entrance) and opens the hive. The vertically positioned fixed honey combs clip from the top, brushed and put in dry plastic bucket. The honey combs transported to temporarily arrange straining room. Straining and settling were used to take the liquid honey from the comb, and poured in ½ a kg air tight and moisture proof food grade glass jars. Then honey combs were broken into pieces and strained using honey sieve, and allowed to settle in a 50 kg metallic honey container. The strained and settled honey pours in a food grade glass jar from the outlet at the bottom.

Honey from frame hives was extracted honey. To harvest honey from frame hive, a beekeeper puff smoke at the entrance and then open the lid and smoke at the top. Honey containing frame combs were clipped from the super box using hive tool, and bees were swept from the comb using brush. The honey transported to temporary extraction places using empty super box. The sealed frame combs decapitated using uncapping fork and inserted into the honey extractor. Through centrifugation, the honey was drained from the cell and taken from the outlet of the honey extractor. The extracted honey was strained, settled, and later poured in a ½ kg food grade glass jar.

2.3. Colour determination

Colours of the honey samples were measured using a Pfund grader. Approximately 100 g of honey was poured into the sample holder of the Pfund grader. Determination was based on the matching of the honey sample colours with the colour indexes present in the glass Pfund grader.

2.4. Granulation test

The rate at which granulation took place and the nature of granulation were determined by keeping honey samples for a period of 120 days. Honey samples from traditional and frame hives were warmed and filtered in a clean cotton cloth and poured into a 0.5 kg glass container. Records were taken for the onset of granulation and nature of granulation visually. The data were taken in 2 days intervals on the status of granulation, extent of granulation and type of granulation (Dyce, 1975; Joseph et al., 2007).

2.5. Analysis of honey pollen

Determination of the botanical origin was performed using the harmonised methods of melissopalynology. Approximately 10 g of honey was weighed in a pointed glass centrifuge tube (capacity ca.
3. Results and discussion

3.1. Colour

The colour of honey is a useful parameter for the characterisation of the product. Besides to quality parameters stated by Codex and European Union, colour is the single most important factor determining import and wholesale prices (Krell, 1996). The colour of the Harenna forest honey ranged from 34 to 85 mm Pfund scale, grouped as extra light amber to light amber (Table 1). Six honey samples (C1, C2, C3, W14, W15 and W16) were found to be extra light amber and the remaining ten honey samples (C4, C5, C6, C7, C8, W9, W10, W11, W12 and W13) were light amber colours; out of the total honey samples, 62.5% light amber while 37.5% extra light amber.

3.2. Granulation test

Granulation test of the Harenna forest honey was undertaken for 120 days. Out of the sixteen honey samples only three honey samples were observed to granulate. The first onset of granulation was observed on traditional hive from Chiri, honey sample C6. The minimum time observed for the onset of granulation was at the 60th day. Honey plants enriching C6 honey sample were Trifolium spp., G. iongispicata, S. guineense and Hypoestes spp., accordingly. The second honey sample was again from Chiri traditional hive, honey sample C5. The minimum time for the onset of granulation for C5 was the 74th day. C5 was predominately produced from Guizotia spp. and enriched by Trifolium spp., Croton spp. and S. guineense, accordingly. Finally, the third onset of granulation was observed on Wabero traditional hive, honey sample W15. The minimum time for the onset of granulation was 103rd day. Honey sample W15 was produced and enriched by Hypoestes spp., Ekerbergia capensis, S. guineense and Unknown spp., accordingly. The results of this study showed that two honey samples from Chiri and one from Wabero tend to granulate faster, and the remaining samples stayed liquid.

Granulation is stimulated by some potential crystallization nuclei such as undissolved glucose crystals, air bubbles, pollen grains or any other water insoluble particles (Sanz, Gradillas, Jimeno, Perez, & Juan, 1995). Under this investigation, traditional hives were more prone to granulation than frame hives. This could be due to higher water insoluble solids of traditional hives, 0.14 ± 0.05, compared to frame hives, 0.09 ± 0.06 (Belay, Solomon, Bultossa, Adgaba, & Melaku, 2013).

3.3. Pollen analysis

Pollen analysis (melissopalynology) of honey is of great importance for quality control. Melissopalynology was an early branch of palynology (study of pollen and spores). Honey always includes numerous pollen grains (mainly from the plant species foraged

<table>
<thead>
<tr>
<th>Honey samples</th>
<th>Color name</th>
<th>Pfund scale (mm)</th>
</tr>
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<tbody>
<tr>
<td>C1,C2,C3,W14,W15,W16</td>
<td>Extra light amber</td>
<td>&gt;34–50</td>
</tr>
<tr>
<td>C4,C5,C6,C7,C8,W9,W10,W11,W12,W13</td>
<td>Light amber</td>
<td>&gt;50–85</td>
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<tr>
<td>C</td>
<td>Chiri; W = Wabero.</td>
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<tr>
<td>W</td>
<td>Water white</td>
<td>0–8</td>
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<tr>
<td>W</td>
<td>Extra white</td>
<td>&gt;8–17</td>
</tr>
<tr>
<td>W</td>
<td>White</td>
<td>&gt;17–34</td>
</tr>
<tr>
<td>W</td>
<td>Extra light amber</td>
<td>&gt;34–50</td>
</tr>
<tr>
<td>W</td>
<td>Light amber</td>
<td>&gt;50–85</td>
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<tr>
<td>W</td>
<td>Amber</td>
<td>&gt;85–114</td>
</tr>
<tr>
<td>W</td>
<td>Dark amber</td>
<td>&gt;114</td>
</tr>
</tbody>
</table>

Table 1
Color of honey samples grouped according to Pfund scale (n = 16).
by honeybees) and honeydew elements (like wax tubes, algae and fungal spores) that altogether provide a good fingerprint of the environment where the honey comes from. Pollen analysis can therefore be useful to determine the geographical and botanical origin of honeys (Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014; Marchini, Moretti, Otsuk, & Sodre, 2007; Owayss, 2005). Honey origin was verified by qualitative and quantitative microscopic pollen analyses. Sensory and physicochemical analyses are also needed for a correct diagnosis of botanical origin (Ohe et al., 2004; Owayss, 2005).

The determination of the botanical origin for Harenna forest honey samples was performed using the harmonised methods of melissopalynology (Kirs, Pall, Martverk, & Laos, 2011; Ohe et al., 2004; Silva et al., 2013). The relative frequency of nectariferous species in each sample and the results due to locations are presented in Tables 2 and 3, respectively.

The relative pollen distribution enriching honey samples indicated that S. guineense was a predominant honey plant in honey sample C3, C8, W9, W12, W13 and W16. G. longispicata was dominated in honey sample C7. Trifolium spp. was predominated in honey samples C4 and W14, while Guizotia spp. was predominant in honey sample C5 (Table 2). This could be due to the floral constancy behaviour of honeybees (Dadant, 1975). The floral consistency behaviour guide honey bees to persist on a single species of plants that reward food. This continues until the flower finish production of nectar and/or pollen.

Honey is considered as predominant from a given botanical origin (unifloral honey) if the relative frequency of the pollen of that taxon exceeds 45%. In addition to predominant frequency, different levels of abundance of given pollen type in nectar such as secondary, tertiary and quaternary enrichment are required for botanical description of honey (Ohe et al., 2004).

Honey plant coverage of honey sample from the study area was dominated primarily by S. guineense (Table 3). S. guineense is a dominant woody species in the natural forest and agro forestry practices of Dello Mena district, Bale, Ethiopia. According to Fichtl and Admass (1994) S. guineense foraged by bees vigorously for the abundant nectar and pollen from this tree is an important honey source for the country. The plant is recommended for planting to increase honey production. The secondary, tertiary and quaternary dominant species in enriching the Harenna honey samples in both locations were Trifolium spp., G. longispicata and Hypoestes spp., respectively.

The pollen analysis result, besides its role of identifying the botanical origin of honey, also contribute in signifying the dominate honey plants to be maintained and suggests the need for future investment for maintaining the biodiversity of the Harenna forest. Today the interest of honey consumers to unifloral honey worldwide inclined the honey processors to work with unifloral honey. The predominate nature of S. guineense, mainly from Wabero; can possibly able to produce unifloral honey.

### 3.4. Test for tetracycline

Tetracyclines are used to control bacterial diseases such as European and American foulbrood (Martel, Zeggane, Drajnudel, Faucon, & Aubert, 2006). This practice may cause contamination of beehive products and contributes to the problem of food safety.

The result for the tetracycline test showed more intensive deep colour on the test line than the control line. This intensive deep colour indicates that the Harenna forest honey samples were negative for tetracycline. Thus, the Harenna forest honey samples are free from residues of antibacterial drug and food safety related problem.

### 3.5. Sensory evaluation

#### 3.5.1. Preference test

Analysis of the data using the Chi-square test ($\chi^2$) (Resurreccion, 1998) showed that, the three attributes: colour, flavour and taste of honeys collected from traditional and frame hives did not show a significant difference ($p > 0.05$).

#### 3.5.2. Acceptability test

Effects of main factors on mean acceptance of the Harenna forest honey with respect to colour, flavour, taste and overall acceptability are presented in Table 4. The score of the colour acceptability ranged from 4.28 ± 0.86 to 4.42 ± 0.64 in a scale of 5. The mean value of colour acceptability was 4.35 ± 0.73. There were no significant ($p > 0.05$) differences in colour acceptance among the Chiri frame (CF), Chiri traditional (CT), Wabero frame (WF) and Wabero traditional (WT) honey samples. All the colour

### Table 2

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<tbody>
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<td>41</td>
<td>3</td>
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<td>70</td>
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<td>8</td>
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<td>7</td>
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</table>

C = Chiri; W = Wabero.

### Table 3

<table>
<thead>
<tr>
<th>Location</th>
<th>Syzygium guineense</th>
<th>Guania longispicata</th>
<th>Trifolium spp.</th>
<th>Hypoestes spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiri</td>
<td>34</td>
<td>13</td>
<td>28</td>
<td>8</td>
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<tr>
<td>Wabero</td>
<td>46</td>
<td>10</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Mean</td>
<td>40</td>
<td>11.5</td>
<td>22</td>
<td>9.5</td>
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</tbody>
</table>

Future investment for maintaining the biodiversity of the Harenna forest. Today the interest of honey consumers to unifloral honey worldwide inclined the honey processors to work with unifloral honey. The predominate nature of S. guineense, mainly from Wabero; can possibly able to produce unifloral honey.
scores of the honey samples were in between ‘moderately like’ and ‘extremely like’. Based on the scale indicated the colour of the Harenna forest honey was acceptable to the panelists. 

The score of flavour acceptance ranged from 4.08 ± 0.85 to 4.34 ± 0.75 in a scale of 5 with a mean value of 4.19 ± 0.80. There was no significant difference (p > 0.05) in flavour acceptance among the CF, CT, WF and WT honey samples. All the flavour scores of the honey were between ‘moderately like’ and ‘extremely like’, thus the colours of the Harenna forest honey were accepted by panelists.

The score of the taste acceptance values ranged from 4.34 ± 0.87 to 4.44 ± 0.61 in a scale of 5 with a mean value of 4.38 ± 0.77. There was no significant difference (p > 0.05) in taste acceptance among the CF, CT, WF and WT honey samples. All the taste scores of the honey were between ‘moderately like’ and ‘extremely like’, thus the taste of honey samples were acceptable by the panelists.

The effect of location and hive type on the overall acceptability of honey is presented in Table 4. The score of overall acceptability ranged from 4.48 ± 0.61 to 4.32 ± 0.62 in a scale of 5 with a mean value of 4.34 ± 0.67. Generally, the acceptance level of the Harenna forest honey was between ‘moderately like’ and ‘extremely like’. There was no significant difference (p > 0.05) in overall acceptance among the CF, CT, WF and WT honey samples. All the scores of overall acceptability were above ‘moderately like’ indicating high levels of the overall acceptability of the Harenna forest honey. 

The sensory assessment results of the Harenna forest honeys were correlated with the results of the physicochemical properties of honey published earlier (Belay et al., 2013) and the floristic, colour and granulation results gathered here. There were no significant (p > 0.05) difference in sensory preference test, colour, flavour, taste and overall acceptability of honey between traditional and frame hives. The results of this study indicated that traditional hive has no negative effect on sensory properties of honey, if honey harvesting and postharvest handlings are properly performed.

### 4. Conclusion

This study was conducted to assess the botanical origin, colour, granulation, tetracycline and sensory properties of the Harenna forest honey samples. Honey samples were harvested during the major honey flow season of the area between the 18th of January, 2010 and the 20th of February, 2010. The results of this study indicated that the Harenna forest honeys were extra light amber and light amber colours, 18.75% the honey samples form coarse granules slowly, and honey samples from traditional hives were more prone to granulation than frame hives. All honey samples were free from tetracycline residue. The Harenna forest honey originated dominantly from S. guineense from Wabero and multifloral honey from Chiri. Analysis of paired preference test using χ2 showed that honey samples from traditional hives were not significantly different (p > 0.05) from those of frame hives in colour, flavour and taste. All the acceptability scores of the honey samples were between ‘moderately like’ and ‘extremely like’, thus the honey samples were accepted by the panelists. There was no significant difference (p > 0.05) among the traditional honeys in all acceptability tests. The results of this study indicated that traditional hives which provides the largest share of honey for the country do not produce an inferior quality honey than frame hives, if honey harvesting and handling properly performed.

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