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Physicochemical properties of honey produced in Sekota district, northern Ethiopia

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Abstract

This study was conducted to characterize honey produced in Sekota district in northern Ethiopia and to assess the effects of location (lowland, midland and highland) and hive type (modern zander-frame and traditional tube basket) on the quality of honey produced in the area. A total of 20 honey samples were collected from four locations in Sekota district. Reducing sugars, apparent sucrose, pH, moisture, ash, hydroxymethylfurfural, acidity and water-insoluble solids contents of the honey samples were analyzed. The pH of honey samples collected from the midland of the district was significantly higher than ($p < 0.05$) the pH of honey samples collected from lowland areas. Hive type significantly ($p < 0.05$) influenced the reducing sugars contents of the honey samples. The water-insoluble solids content of the honey samples analyzed in this study is above the maximum limit set by national and international standards for water-insoluble solids content of honey. Although honey produced in Sekota district is generally of good quality, efforts need to be made to reduce the water-insoluble solids content of the honey.

Introduction

Beekeeping is an important agricultural activity in Ethiopia. Owing to its varied ecological and climatic conditions, the country is home to some of the most diverse flora and fauna in Africa, making it highly suitable for sustaining a large number of bee colonies (Adgaba, 2007). The country has the largest bee population in Africa of over 10 million bee colonies, out of which about 7.5 million are confined in hives and the remaining exist in the forest (Adgaba, 2007). This makes the country one of the largest honey producers and the third largest beeswax producer worldwide. Ethiopian honey production accounts for approximately 2.5% of the world production and 21.7% of African honey production (MoARD, 2007).

Honey is the major product of honeybees which has important nutritional value and provides significant economic contributions. Quality control of honey is important to determine its suitability for processing and to meet the demand of the market. Honey shall not have foreign taste, begun to ferment, heated to the extent of destroying its natural enzymes and a substance that endanger human health (Official Journal of the European Communities, 2002). The types of beehives, the materials from which beehives are made, origin of queen of bees (*Apis mellifera* subspecies *monticolla*) and the botanic origin of honey influence the quality of honey of which the botanic origin of honey has the major effect on the physical and chemical properties of honey (Tucak *et al*., 2007). Jones *et al.* (2011) reported that the composition and quality of honey are greatly influenced by geographical and environmental factors. Despite the large number of honeybee colonies and diversified honey floral resources, production of honey is far below its potential in the country. Moreover, the apiculture sector has received little research and development attention and the honey produced in the different agro-ecologies of the country has not been characterized to date. Sekota district which is located in Amhara Region in northern Ethiopia is identified as one of the potential areas for beekeeping in the country and honey is an important source of income for farmers in the area. The district has three major agro-ecological zones: hot to warm sub-moist agro-ecology which accounts for 27.1%, moderate or tepid sub-moist agro-ecology which accounts for 71.7% and cold sub-moist agro-ecology which accounts for 1.2% of the district. The annual rainfall, which is erratic in distribution, varies between 350 and 650

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mm.

To date no study has been conducted to assess the quality and physicochemical properties of honey produced in Sekota district. In order to increase income of beekeepers and marketability of honey produced in the study area, it is important to determine the physicochemical properties of the honey vis-à-vis national and international standards set for honey. This study was, therefore, designed to characterize honey produced in Sekota district of the Amhara Regional State in northern Ethiopia.

Materials and Methods

Description of the study district

Waghimra zone is one of the 11 administrative zones in Amhara National Regional State of Ethiopia and comprises of six districts (Figure 1). Sekota is one of the six districts in the zone and is located between 12° 23' and 13° 16' north latitude and 38° 44' and 39° 21' east longitude (Adefress *et al.*, 2000). Sekota district which is located in the eastern part of the Amhara Region is identified to be potential area for beekeeping but the area is getting degraded and deforested from time to time which negatively affect the apicultural subsector. Despite this, the bees are producing quality honey against the harsh environmental conditions. The sources of nectar in Sekota district are annual plant species (herbaceous plants and grasses) which account for 39% followed by shrubs (33.9%) and trees (27.1%), respectively (Jemberie, 2008). The most important sources of honeybee flora in the lowlands of Sekota district are *Acacia asak*, *Terminalia glaucescens* and *Sorghum bicolor.* On the other hand, the most important sources of honeybee flora in the highlands and midlands of the study district are *Becium grandiflorum*, *Euclea shimperi* and *Vicia faba*. The major honeybee plants, floral types and flowering calendar in Sekota district are indicated in Table 1.

Sampling technique and sample size

Sekota district comprises a total of 33 peasant associations (PAs) which were classified into three highland, 24 midland and six lowland areas. From these two PAs from the highland and three PAs each from lowland and midland areas of the district were randomly selected for honey sampling. All the honey samples were harvested in the same period, following the main rainy season between 15 October and 15 November, 2009. A total of 20 honey samples were collected from four locations (midland, highland, lowland and local markets). Six honey samples from three PAs of the midland, four samples from three PAs of the lowland, four samples from two PAs of the highland and six samples from local market were collected. The honey samples from the highland, midland and lowland areas were collected at farm gate from randomly selected beekeepers of the PAs in October 2009. On the other hand, the honey samples from the local market were purchased from randomly selected honey vendors from local markets in the district. Out of the 20 honey samples, 10 samples (2 from highland, 2 from lowland, 3 from midland and 3 from market) which were extracted by centrifugation

Table 1. Major honeybee plants, floral types and flowering calendar in Sekota district

Local name (Agewugna)	Scientific name	Floral type	Flowering calendar
Mentesie	*Becium grandiflorum*	Shrub	August 15-September 20
Dedho	*Euclea shimperi*	Shrub	Year round
Mashila	*Sorghum bicolor*	Crop	September to October
Kushashle	*Echinops* spp.	Herb	January to February
Abika	*Acacia tortolis*	Tree	March to June
Keyi girar	*Acacia seyal*	Tree	March to June
Tsalwa	*Acacia asak*	Tree	Year round
Ekima	*Terminalia glaucescens*	Tree	Year round
Qundoberbere	*Schinus molle*	Tree	Year round
Teji matebiya	*Hypoestes trifolia*	Herb	September
Aba tsemare	*Ocimum bacilicum*	Herb	August 15-September 20
Sibkana	*Albezia amara*	Tree	May to August
Kenteftafa	*Pterolobium stellatum*	Shrub	March
Wanza	*Cordia africana*	Tree	October to December
Eret	*Aloe* spp.	Shrub	September to October
Agam	*Carissa edulis*	Shrub	October to December
Yeferenji suf	*Helianthus annuus*	Crop	September to October
Adey Ababa	*Bidens* spp.	Herb	August 15-September 20
Beles	*Opuntia* spp.	Shrub	April to June
Bahirzaf	*Eucalyptus camaldlensis*	Tree	May
Giba	*Ziziphus spinachristi*	Tree	September to February
Kalkalda	*Euphorbia* spp.	Shrub	Year round
Goza (bedana)	*Balanite aegyptica*	Tree	January to February
Noug	*Guizotia abyssinica*	Crop	September
Bakela	*Vicia faba*	Crop	August 15-September 20
Dikuan tilla	*Verbena officinalis*	Herb	July 15 to December
Bisana	*Croton macrostachyus*	Tree	January to February
Ambacho	*Rumex nervosus*	Shrub	March
Selit	*Sesamum indicum*	Crop	August
Sesbania	*Sesbania sesban*	Shrub	January
Maluza	*Asparagus* spp.	Shrub	March
Kessie	*Lippia adoensis*	Herb	September
Kinchib	*Euphorbia tirucalli*	Shrub	Year round
Firtata	*Adansonia digitata*	Tree	June

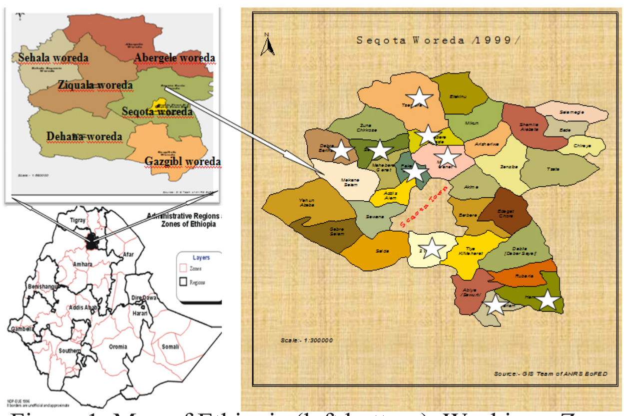

Figure 1. Map of Ethiopia (left bottom), Waghimra Zone (left upper) and Sekota district (right). Areas marked by star symbols in the right side of the map are the sampling sites (selected Peasant Associations).

A) Traditional beehives used in Sekota district B) Inspection of the traditional hive before harvesting

Figure 2. Pictures of traditional beehives used in Sekota district

were obtained from modern hives and 10 samples (2 from highland, 2 from lowland, 3 from midland and 3 from market) which were extracted by pressing were obtained from traditional hives.

In Sekota district, beehives are exclusively placed in shelters at backyards. The major types of bee hives used in Sekota district are traditional and modern hives. There are also transitional hives but they only account for 1% of the honeybee colonies of the district (Alemu, 2010). As a result the hive types used for honey sampling in the present study were traditional and modern hives. The traditional hives (tube form) (Figure 2) are made from locally available materials such as bamboo, tree branches and grass whereas the modern (Zander-frame) hives are made from timbers of *Cordia africana*.

Physicochemical properties

The honey samples (250 g) were amassed in clean plastic container (1 kg capacity) and analyzed for reducing sugars, apparent sucrose, moisture, ash, hydroxymethylfurfural (HMF), acidity, pH and water-insoluble solids contents. Moisture content of the honey samples was determined using Refractometer (Leica Abbe Mark II Plus Refractometer). After correction for temperature, the refractive index values were converted to moisture content (%) using the conversion table reported by White *et al.* (1962). The reducing sugars content of the honey samples was determined by the modified procedure of Lane and Eynon (1923), involving the reduction of Soxhlet's modification of Fehling's solution by titration with copper sulphate at boiling point (60°C) against a solution of reducing sugars in honey using methylene blue as an internal indicator (Lane and Eynon, 1923). The apparent sucrose content of the honey samples was determined according to the procedure of Lane and Eynon (1923). Titration was done following similar procedure as for the determination of reducing sugars. Total ash content of the honey samples was determined by calcinations of 5 g of honey, overnight, in muffle furnace at 550°C until constant mass (Bogdanov, 2002). The honey samples were warmed using hotplate before calcinations. The residue was then weighed after cooling to room temperature in desiccator. Total ash content (%) was calculated according to Quality and Standards Authority of Ethiopia (QSAE, 2005).

Free acidity of honey samples was determined according to the procedure described by Bogdanov (2002). Hydroxymethylfurfural (HMF) content of the honey samples was determined spectrophotometrically according to White (1979 as reported by Bogdanov, 2002). pH of the honey samples was measured by a pH-Meter (WTW inoLab pH 720, GmbH, Germany) after preparing a solution containing 10 g honey in 75 ml of CO_2 free distilled water (Bogdanov, 2002). The water-insoluble solids content of the honey samples was determined according to the procedures described by Bogdanov (2002) by filtering honey solution through the sintered glass crucible (pore size 30 microns). The residue that remained after filtering (water-insoluble solids) was weighed after the crucible was dried at 135°C for an hour and cooled in a desiccator. The water-insoluble solids content was expressed as g/100 g of the honey sample used. Each of the honey samples was analyzed in duplicate for the parameters considered.

Statistical analysis

The data generated were analyzed by the analysis of variance technique using SAS (2002) software. Comparison of the physicochemical properties was made between honey samples obtained from the four locations and between honey samples obtained from the two hive types. Duncan's multiple range test was used for mean separation when ANOVA showed significant difference between mean values and significant differences were declared at 5% significance level.

Results and Discussion

Moisture content of honey depends on season of harvest and the degree of maturity reached in the hive (Asif *et al.*, 2002). Moisture content is one of the factors that determine the shelf life of the honey during storage (Peŕez-Arqillue *et al.*, 1994). The minimum, maximum and mean moisture contents of honey from Sekota district are reported in Table 2. According to the Ethiopian standard, the maximum limit for moisture content of honey is 23% (Adgaba, 1999). The average moisture content of honey obtained from traditional hives (16.6%) was significantly higher ($p < 0.05$) than the average moisture content of honey obtained from modern hives (15.3%) (Table 3). This result agrees with the findings of Adgaba (1999)

Table 2. Physicochemical properties of honey produced in Sekota district (n = 20)

Parameters	Range	Mean ± SD
Moisture (% by mass)	13.9-17.7	16.0 ± 1.25
Total ash (% by mass)	0.01-0.52	0.14 ± 0.13
Acidity (meq/kg)	10-38.98	23.54 ± 7.74
pH	3.55-4.75	4.05 ± 0.34
HMF (mg/kg)	0-2.5	0.9 ± 0.71
Reducing sugars (% by mass)	63.4-71.7	67.3 ± 2.42
Apparent sucrose (% by mass)	1.0 -5.2	3.1 ± 0.98
Water-insoluble matter (g/100 g)[a]	0.003-2.77	0.62 ± 0.79

HMF = hydroxymethylfurfural; n = number of samples; SD = standard deviation.

Table 3. Comparison of physicochemical properties of honey samples collected from modern and traditional hives in Sekota district

Parameters	Hive type (Mean ± SD)	
	Modern (n = 10)	Traditional (n = 10)
Moisture (% by mass)	15.3^{a} ± 1.03	16.6^{b} ± 1.14
Total ash (% by mass)	0.13 ± 0.15	0.15 ± 0.12
Acidity (meq/kg)	19.72 ± 8.28	27.34 ± 5.06
pH	3.97 ± 0.36	4.13 ± 0.32
HMF (mg/kg)	0.88 ± 0.61	0.91 ± 0.84
Reducing sugars (% by mass)	68.3^{a} ± 2.17	66.4^{b} ± 2.39
Apparent sucrose (% by mass)	2.9 ± 1.01	3.3 ± 0.95
Water-insoluble solids (g/100 g)	0.53 ± 0.63	0.70 ± 0.95

HMF = hydroxymethylfurfural; n = number of samples; means followed by different superscript letters in a row are significantly different ($p < 0.05$); SD = standard deviation.

Table 4. Comparison of physicochemical properties of honey samples collected from three locations (agro-ecologies) and the market in Sekota district

Parameters	Location (Mean ± SD)			
	Lowland (n = 4)	(n = 6)	Highland (n = 4)	Market (n = 6)
Moisture (% by mass)	15.3 ± 0.70	16.1 ± 1.09	16.9 ± 0.74	15.7 ± 1.74
Total ash (% by mass)	0.15 ± 0.16	0.19 ± 0.19	0.14 ± 0.12	0.09 ± 0.06
Acidity (meq/kg)	18.45 ± 8.48	26.5 ± 4.52	22.17 ± 11.34	24.89 ± 7.22
pH	3.9^{ab} ± 0.27	4.35^{c} ± 0.31	3.98^{abc} ± 0.21	3.98^{abc} ± 0.32
HMF (mg/kg)	1.05 ± 0.58	0.93 ± 0.92	0.76 ± 0.73	0.85 ± 0.73
Reducing sugars (% by mass)	67.7 ± 2.09	69 ± 2.38	65.3 ± 1.32	66.8 ± 2.45
Apparent sucrose (% by mass)	3.6 ± 0.51	2.6 ± 1.08	3.1 ± 0.80	3.3 ± 1.17
Water-insoluble solids (g/100 g)	0.28 ± 0.34	0.5 ± 0.66	1.02 ± 1.24	0.7 ± 0.82

HMF = hydroxymethylfurfural; n = number of samples; means followed by different superscript letters in a row are significantly different ($p < 0.05$); SD = standard deviation.

who reported that honey collected from traditional hives of Ethiopia had higher moisture content than honey samples collected from improved hives. But there was no significant difference in moisture content between honey samples obtained from the different locations ($p > 0.05$) (Table 4). The variation in moisture content of honey samples collected from the two hive types might be due to differences in honey harvesting practices.

Majority of the honey samples collected from the study area (95%) had a moisture content of less than 17.5%. The mean moisture content of the study area's honey is lower than the country's average (20.6%) for moisture content of honey (Adgaba, 1999). According to the standards of the Ethiopian Quality and Standards Authority, moisture contents of honey of the study area falls under Grade 'A' category (QSAE, 2005). According to the Ethiopian standard, honey is graded into three categories based on moisture content, that is, Grade A: 17.5-19%, Grade B: 19.1-20% and Grade C: 20.1-21%. The maximum acceptable moisture content of honey reported by the International Honey Commission is 20% (Bogdanov, 2002).

Generally, the moisture content of honey produced in Sekota district is within the acceptable range vis-à-vis national and international standards. The water content of honey can naturally be as low as 13% or as high as 23% depending on the source of the honey and climatic conditions (Bradbear, 2009). Honey produced during rainy season has higher moisture content than honey produced during drier periods (Ordóñez *et al.*, 2004). The mean ash content of the study area's honey is indicated in Table 2. No significant difference ($p > 0.05$) was observed between the ash content of honey collected from traditional hives and ash content of honey collected from modern hives (Table 3). Honey from the different localities also did not show significant difference ($p > 0.05$) in their ash content (Table 4). The mean ash content of honey produced in the study area is lower than the country's average (0.23%) for mineral content of honey (Adgaba, 1999). The maximum acceptable mineral content of honey is 0.6% (Bogdanov, 2002; QSAE, 2005). The result of this study shows that the mean ash content of the study area's honey (0.14%) is within the acceptable national and International limits for honey's mineral content. The mineral content of honey is related to the geographical and botanical origin of the honey and it is an important indicator of possible environmental pollution plus soil types of the area (Anklam, 1998).

Acidity contributes not only to the flavor of honey but also to its antimicrobial property. Although the acidity of honey is desirable, when the acidity increases very much, the honey becomes sour. The average free acidity value of the honey samples analyzed in this study is indicated in Table 2. The acidity of honey samples collected from traditional hives and honey obtained from modern hives was not statistically significant ($p > 0.05$) (Table 3). Similarly, no significant difference ($p > 0.05$) in acidity was observed between honey samples collected from different localities (Table 4). The average free acidity of the study area's honey is below the national average (39.9 meq/kg) reported by Adgaba (1999). The mean acidity of the study area's honey is also lower than the maximum limit set for honey of tropical origin, i.e., 50 meq/kg reported by Bogdanov (2002). The low acidity of honey samples analyzed in the present study suggests absence of unwanted fermentation in the honey samples.

The mean pH value of the honey samples analyzed is indicated in Table 2. There was no significant difference ($p > 0.05$) in pH between honey samples obtained from traditional and modern hives (Table 3). But the pH of honey collected from different locations showed significant difference ($p < 0.05$) (Table 4). Honey obtained from the midland of the district had significantly higher pH (4.35) value ($p < 0.05$) than honey obtained from the lowland (3.9). This difference observed might be due to the variations in vegetation sources and harvesting practices. Both active acidity (pH) and total acidity are parameters used to characterize quality of honey. But pH of honey is not directly related to the free acidity because of the buffering action of the various acids and minerals present in honey. Bogdanov *et al.* (1999) reported that pH of honey should be between 3.2 and 4.5. The mean pH value of honey of the study area lies within the reported pH range for honey.

The mean hydroxymethylfurfural (HMF) content of the study area's honey is reported in Table 2. No significant difference ($p > 0.05$) in HMF content was observed between honey samples obtained from traditional and modern hives (Table 3) and also between honey samples obtained from different locations (agro-ecologies) (Table 4). The mean HMF content of the study area's honey is much lower than the national average (32.4 mg/kg) (Adgaba, 1999). According to QSAE (2005) and Bogdanov (2002), the maximum limit of HMF content in honey is 40 and 60 mg/kg, respectively. The amount of hydroxymethylfurfural in honey is one of the important indicators of honey's quality indicating whether the honey is aged or over-heated (Mairaj *et al.*, 2008). The very low HMF content of the honey samples analyzed in the present study implies that the honey collected from the study area was fresh. Bogdanov (2002) reported that HMF is generally not present in fresh honey and its content increases during conditioning and storage, depending on the pH and storage temperature. Moreover, Tharasyvoulou (1986) reported that the average HMF content of honey produced in Greek increased from an initial value of zero to 8.8 mg/kg after one year storage.

Honey is a mixture of principally two reducing sugars namely glucose and fructose, giving it similar properties to invert syrup. This gives it the ability to remain liquid for long periods of time. The minimum, maximum and mean reducing sugars content of honey samples collected from the study area are indicated in Table 2. Honey collected from modern hives of the district had significantly higher ($p < 0.05$) reducing sugars content than honey samples collected from traditional hives (Table 3). The difference in the reducing sugars content observed between the honey samples analyzed might be attributed to moisture content since honey collected from modern hives had significantly lower moisture content as compared to honey collected from traditional hives. On the other hand, there was no significant difference ($p > 0.05$) in the amounts of reducing sugars between honey samples collected from the different localities (Table 4). The reducing sugars content of honey of the study area is higher than the minimum limits 65% set by QSAE (2005) and 60% set by Bogdanov (2002). Adgaba (1999) reported a mean reducing sugars content of 65.5% for honey produced in Ethiopia. Sugars are the main constituents of honey comprising of about 95% of honeys dry weight (Bogdanov, 2011). Reducing and non-reducing sugars together account for 85-95% of honey's carbohydrate and their amount depend on the source of nectar (Cavian, 2002).

The average apparent sucrose content of the study area's honey is reported in Table 2. The mean sucrose content of the study area's honey is lower than the maximum limit 10% set by QSAE (2005) and 5% set by Bogdanov (2002). The mean sucrose content of the study area's honey is also lower than the national average of 3.6% which was reported by Adgaba (1999). Sucrose content of honey is used to detect adulteration of honey by addition of cane or beet sugars. The result indicates that honey produced in Sekota district is natural and not adulterated. The sucrose content between honey samples collected from traditional and modern hives was not significantly different ($p > 0.05$) (Table 3). The amount of sucrose in the honey samples obtained from the different localities also did not show significant difference ($p > 0.05$) (Table 4). Sucrose content of honey mainly depends on botanical origin of nectar and according to International Regulatory Standards it should not exceed 5% (g/100 g) except for some kind of honey from nectar with naturally higher content of this compound - false accacia (*Robinia pseudoaccacia*), alfalfa (*Medicago sativa*), Banksia (*Banksia menzes*), French honeysuckle (*Hedysarum*), red gum (*Eucaluptus camandulensis*), leatherwood (*Eucrypis lucida*, *Eucryphia milliganii*), lavender (*Lavendula* spp.), borage (*Borago officinalis*) (Codex Alimentarius Commission, 2001; Official Journal of the European Communities, 2002). The maximum sucrose content criterion set at 5% helps but little to assess the authenticity and to estimate the degree of adulteration of the product (Rybak-Chmielewska and Szczęsna, 2003; Rybak-Chmielewska *et al.*, 2006).

The mean water-insoluble solids content of the study area's honey is indicated in Table 2. No significant difference ($p > 0.05$) in water-insoluble solids content was observed between honey samples obtained from traditional and modern hives (Table 3). The amount of water-insoluble solids of honey samples obtained from different locations also did not show significant difference ($p > 0.05$) (Table 4). The maximum acceptable level of water-insoluble matter in honey is 0.1% according to Ethiopian standard (QSAE, 2005) and it is 0.1% for extracted honey and 0.5% for pressed honey as reported by Codex Alimentarius Commission (1989). According to Bogdanov (2002), even 0.1% of water-insoluble matter in honey is a very high value. The result shows that the average amount of water-insoluble solids of the study area's honey (0.62%) is much higher than the aforementioned standards. This might be attributed to lack of straining equipment, poor hygienic conditions and improper harvesting of honey combs together with pollen and brood in honey samples produced in the area. Honey's water-insoluble matter (solids) includes wax, pollen, honey-comb debris, bee and filth particles. So honey's water-insoluble matter is used as a criterion of honey cleanliness.

Conclusion

Except for pH, no significant difference ($p > 0.05$) was observed for the other quality parameters considered between honey samples collected from the different locations of Sekota district. Reducing sugars and moisture contents of honey samples were significantly influenced ($p < 0.05$) by the type of hive used. However, hive type did not affect the pH, acidity, apparent sucrose, ash, hydroxymethylfurfural and water-insoluble solids contents of the honey samples. Apart from the high water-insoluble solids content, honey produced in Sekota district complies with both the national and international standards for the quality parameters considered. In order to reduce the water-insoluble solids content and improve the quality of honey produced in the study area, provision of training to beekeepers on honey straining and harvesting techniques, and pre- and post-handling of honey is required.

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