Original Research Article

Influence of floral origin and storage conditions on physicochemical properties of Libyan honeys

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UNDER PEER REVIEW

Abstract

Aim: The effect of botanical origin and storage conditions on the quality of two Libyan monofloral honey samples (thyme and ziziphus honeys) were assessed during prolonged storage (12 months) at room temperature.
Methodology: Physicochemical properties (moisture, viscosity, electrical conductivity, acidity pH, 5-(hydroxymethyl) furfural (HMF), diastase activity, sugars, and color) were monitored.
Results: Generally, moisture, acidity, diastatic activity, and color values were significantly higher in thyme honey, whereas viscosity, electrical conductivity, pH, HMF, and sugars content were higher in ziziphus honeys. In comparison with the initial values, viscosity, acidity, and HMF values of all honey samples increased during storage. Storage period and containers did not affect the electrical conductivity and sucrose contents for the two honey types, which were below the stipulated limits. Moisture content, pH, diastatic activity, color (L*), fructose, and glucose content decreased during storage. Honey samples stored in opaque container showed significantly lower changes in all parameters during the storage period compared to those stored in transparent bottles. The results showed that both honey types stored for 12 months at room temperature could be considered safe for human consumption according to maintaining physicochemical parameters.

Keywords: Flora, Honey, Nectar, Packaging, Physicochemical properties, Storage.

1. INTRODUCTION

Honey is a natural sweet product produced by *Apis mellifera* bees from nectar of plants (nectar honey). The honey composition (sugars, organic acids, enzymes, vitamins, proteins, and phytochemicals) is largely influenced by the botanical and geographical origin, environmental and climatic conditions, and postharvest handling practices [1, 2, 3]. The quality of honey varies greatly all over the world; it is evaluated based on chemical, physicochemical, and sensory properties. Organoleptic qualities such as flavor and taste are the most significant attributes affecting consumer preference. Honey aroma is produced by its volatile compounds which depend on nectar origin, processing, and storage conditions. In general, honey is either monofloral or multifloral depending on the source of the plant. Physicochemical and organoleptic properties are usually useful to assess the floral origin of honey [4]. The minimum and maximum limits for these parameters were established by Codex Alimentarius, European Union and some national laws [5,6,7].

It is well known that quality of the honeys decreases during storage. Several types of honeys are produced in Libya and largely consumed due to its nutritional, medicinal, and cosmetic properties. However, the information available on their floral origin**,** chemical and physical properties, and changes during storage is limited. Considering that honey is a product that can be stored for long periods until consumption, this study was conducted to assess the physicochemical properties of two Libyan honey samples from different botanical origins **(***Thymus capitatus*, Laimaceae family; *Ziziphus lotus***,** Rhamnacae family) as influenced by storage period at room temperature in opaque or transparent containers.

2. MATERIALS AND METHODS

2.1. Samples collection

The present study was performed on two honey types (Table 1) from reputed commercial brands in Libya (both monofloral). The honey samples were collected during July-2020 from beekeepers. Fresh honey samples (1 kg) were packed and sealed in glass bottles.

Table 1. Types of honey used in the current study

Type	Local name	Scientific name	Family	Location
1	Thyme	*Thymus capitatus*	Laimaceae	El-gagab
2	Sidr	*Ziziphus lotus*	Rhamnacae	El-abiar

2.2. Storage conditions

The samples were put in clean opaque and transparent glass containers, hermetically sealed, and kept at room temperature (20-26°C) for 12 months. The samples were evaluated every four months in terms of moisture, pH, acidity, 5-(hydroxymethyl)furfural, diastase activity, color, viscosity, electrical conductivity, and sugars.

2.3. Physicochemical properties

2.3.1. Moisture content

Moisture content was determined using a digital refractometer (Boeco, Germany) according to the method described earlier [8].

2.3.2. Viscosity

Viscosity of honey samples were measured by viscometer (Viscometer, V60002, FUNGIL AB, Spain) under the following conditions: (Spindle R7) 12 rpm, torque was maintained at 100% of instrument capacity [8].

2.3.3. Electrical conductivity

The electrical conductivity of honey was determined by conductivity meter (five easy, mettler, Switzerland). The result was expressed in millisiemens per centimeter (mS/cm) [8].

2.3.4. Free acidity

Ten grams of honey were dissolved in 75 mL of distilled water in a 250 mL beaker, and 100 µl of 1 % alcoholic solution of phenolphthalein was added. The solution was

UNDER PEER REVIEW

titrated to equivalent point with 0.1 N NaOH. Free acidity was determined based on the volume of NaOH used in titration and expressed as milliequivalent of acid/kg of honey [9].

2.3.5. pH

The pH of honey samples was determined using a digital pH-meter (Sartorius Ag, Germany) at room temperature according to [9]. Before determinations, the device was calibrated using different buffer solutions.

2.3.6. Hydroxymethyl furfural (HMF)

The HMF was determined according to the method described by [8] using spectrophotometer (UV/Vis Spectrophotometer, Jenway, England)

2.3.7. Diastase activity determination

Diastase activity of honey samples was determined using spectrophotometer (UV/Vis Spectrophotometer, Jenway, England) by following the method outlined by [8].

2.3.8. Determination of sugars

Sugars (Fructose, Glucose, and Sucrose) were determined by HPLC (Knauer, Germany). The column used was Phenomenex luna NH2 250 x 4.6 mm, column temperature was kept constant at 30°C, mobile phase was Acetonitrile: water (HPLC grade) 80:20 (v/v), the detection was carried out by RI detector and data were integrated by claritychrom software [8].

2.3.9. Color analysis

Colour parameters were measured with a tristimulus colorimeter (Smart Color Pro, USA) according to [10]. The colour was expressed in terms of L* (100 = white, 0 = black), a* (positive = redness, negative = greenness), and b* (positive = yellowness, negative = blueness) values.

2.4. Statistical analysis

The results were statistically analyzed using a completely randomized design (CRD) by analysis of variance (ANOVA) and were subjected to mean separation by least significant difference (LSD) at the level of significance 0.05 using CoStat software.

3. RESULTS AND DISCUSSION

3.1. Moisture content

The results showed that native thyme honey had significantly higher moisture content (18.66 g/100g) than ziziphus honey (17.16 g/100g) (Table 2). It is well known that honey's water content depends on various factors such as harvesting season, the degree of maturity reached in the hive, and the geographical and environmental factors [11, 12]. Evidently, the moisture contents of these honey samples are within the range of the required standards (less than or equal 20%) according to the international regulations of quality [6]. These results were in agreement with those reported for monofloral honey with moisture content ranged from 15.20 to 20.60% [13,14,15]. However, in this study moisture content decreased gradually and significantly for both types of honey during 12 months of storage. It is interesting to note that, during storage, most honey samples packed in transparent bottles significantly ($P<0.05$) had higher moisture content compared with honey samples stored in opaque ones. These results were similar to those previously reported for multifloral honeys stored in

UNDER PEER REVIEW

different containers [16]. It has been reported that higher water content might cause undesirable microbial fermentation during storage and formation of acetic acid [13].

Table 2. Changes in moisture content, viscosity, and electrical conductivity of Libyan thyme and ziziphus honey stored in transparent and opaque bottles for 12 months at room temperature.

Parameters	Treatments		Storage (months)			
	Honey type	Package type	0	4	8	12
Moisture content (g/100g)	Thyme	Transparent	18.66	18.40	17.03	16.66
		Opaque	18.66	17.91	16.51	16.04
	Ziziphus	Transparent	17.16	16.60	16.17	15.49
		Opaque	17.16	16.50	15.40	15.31
LSD			**0.27**			
Viscosity (cp)	Thyme	Transparent	330.26	336.03	352.04	363.90
		Opaque	330.26	340.21	354.38	366.49
	Ziziphus	Transparent	455.33	462.42	476.24	490.55
		Opaque	455.33	466.31	485.21	500.22
LSD			**1.50**			
Electrical conductivity (mS/cm)	Thymus	Transparent	0.38	0.36	0.38	0.44
		Opaque	0.38	0.36	0.36	0.41
	Ziziphus	Transparent	0.56	0.55	0.57	0.62
		Opaque	0.56	0.55	0.56	0.58
LSD			**ns**			

LSD, least significant difference ($P < 0.05$).
ns; not significant

3.2. Viscosity

At zero time, the results showed that ziziphus honey significantly had higher viscosity (455.33 cp) than thyme honey (330.26 cp) (Table 2). However, the viscosity values increased significantly in both honey types through the storage period which could be due to reduction in the moisture content and consequently increase in the concentration of the components the honey [17]. No significant differences among viscosity values of thyme honey samples stored in opaque and transparent bottles were observed during prolonged storage. In contrast, viscosity of ziziphus honey stored in opaque bottles generally increased significantly compared to those stored in transparent bottles.

3.3. Electrical conductivity (EC)

The EC of honey is a parameter that shows great variation according to the honey's floral origin and it is closely related to the concentrations of minerals or total ash, salts, organic acids, and proteins in honey [18] [13]. As shown in Table (2), regardless type of honey or container, EC values for all samples showed insignificant increase during storage period. The slight increase in ash content and EC can be due to the decrease in

UNDER REVIEW

the moisture content of honey during storage [19]. The values ranged from 0.35 to 0.62 mS/cm (average = 0.48 ± 0.10 mS/cm). The conductivity values found in the present study were close to the values reported by [20, 21, 22] respectively. Results of present study were within the limits of International Honey Standards and Directive 2001/110/EC from council of European Union for blossom honey (0.8 mS/cm) [7].

3.4. Free acidity

The results showed that thyme honey had significantly higher free acidity content (20.03 meq/kg) than Ziziphus honey (17.10 meq/kg) at zero time (Table 3). In the two Libyan honey samples, the free acidity of honey increased significantly ($P < 0.05$) during storage period. It could be noted that there was a significant influence of floral type and type of package on the total acidity of honey. The lower acidity values were observed in Ziziphus honey kept in opaque bottles compared with transparent ones, that may be related to the effect of light [16]. At the end of storage period (12 months), the highest acidity value was observed in thyme honey kept in transparent bottle (25.00 meq/kg) and the lowest (20.64 meq/kg) was reported in ziziphus honey kept in opaque bottles (Table 3). Acidity increasing may be due to partly fermentation of sugars during storage.

Table 3. Changes in free acidity, pH, hydroxymethyl furfural (HMF), and diastase activity of Libyan thyme and ziziphus honey stored in transparent and opaque bottles for 12 months at room temperature.

Parameters	**Treatments**		**Storage (months)**			
	Honey type	**Package type**	**0**	**4**	**8**	**12**
Free acidity (meq/kg)	Thyme	Transparent	20.03	23.17	24.16	25.00
		Opaque	20.03	21.53	22.13	23.53
	Ziziphus	Transparent	17.10	18.85	22.01	24.91
		Opaque	17.10	17.66	18.81	20.64
LSD			**1.16**			
pH	Thyme	Transparent	4.30	3.95	3.69	3.66
		Opaque	4.30	4.02	3.90	3.73
	Ziziphus	Transparent	4.56	4.15	3.86	3.27
		Opaque	4.56	4.25	4.15	3.97
LSD			**0.25**			
HMF (mg/kg)	Thyme	Transparent	15.26	19.20	25.43	29.05
		Opaque	15.26	17.65	19.68	21.96
	Ziziphus	Transparent	17.06	21.06	24.16	29.84
		Opaque	17.06	19.10	21.50	28.25
LSD			**1.74**			
Diastase activity (D.N)	Thyme	Transparent	35.03	23.10	20.21	15.80
		Opaque	35.03	27.10	25.10	22.15
	Ziziphus	Transparent	18.25	12.25	9.65	7.83
		Opaque	18.25	14.10	12.23	8.44
LSD			**1.74**			

LSD, least significant difference ($P < 0.05$).

UNDER PEER REVIEW

Generally, all honey samples had low free acidity values, which were still lower than the permissible limit (50 meq/kg) according to the international honey standards and the directive 2001/110/EC from the Council of European Union [7]. A similar trend of increase in acidity has been observed by [23].

3.5. pH

The pH value of honey is an important quality parameter applied at the production and during storage. It influences honey texture, stability, and shelf life [13]. As shown in Table (3), no significant differences were observed in the pH values of the two honey samples at zero time with pH values of 4.30 and 4.56 for thyme and ziziphus honey, respectively. Generally, the pH values insignificantly decreased in all samples stored in different containers during storage. However, pH values of samples stored in transparent bottles were lower than those stored in opaque ones which could be attributed to the exposure of honey to light and heat in transparent bottle [16]. The pH values in the current study were similar to those previously reported in Tunisia, Algerian, Spanish, and Portugal that varied between 3.50 and 4.58 [13] [24, 25, 26]. In general, a low pH of honey inhibits the growth and proliferation of microorganisms.

3.6. HMF content

It has been demonstrated that the HMF is an important indicator for honey freshness, quality, and heat processing [27] but not for its origin [28]. According to the International Trade Guidelines [7], the honey's HMF content should not exceed 40 mg/kg. In this study, there was no significant difference between the two honey samples in terms of HMF at zero time. However lower value was reported for thyme honey (15.26 mg/kg) compared to ziziphus honey (17.06 mg/kg). In general, HMF contents of the two honey types increased significantly ($P < 0.05$) during 12 months of storage. As illustrated in table (3), honey samples stored in transparent bottles revealed significantly higher HMF contents than those stored in opaque ones during storage. However, storage container did not affect the HMF contents of Ziziphus honey at the end of storage period. It is worth to note that during all storage periods, the HMF values of all honey samples did not exceed the above-mentioned maximum stipulated limits (40 mg/kg). Generally, several factors influence the formation of HMF, such as storage conditions (e.g. temperature), floral sources, the sugar type present in honey itself, and fructose: glucose ratio [29, 30, 31]. Similarly, it is well known that temperature and duration of heating process led to the formation of HMF, which is produced during acid-catalyzed dehydration of sugars such as fructose and glucose [13].

3.7. Diastase activity

Diastase activity is an important parameter in the evaluation of honey freshness and quality. In the current study, the initial diastase activities values of ziziphus honey were significantly higher (35.03 D.N) than that reported for thyme honey (18.25 D.N) (Table 3). In general, diastase activity decreased over the study period for all honey types and in different containers. However, it can be observed that honey samples packed in opaque bottles had higher diastase activity than those kept in transparent containers during 12 months of storage. Diastase activity is also considered as an indicator of aging because its activity may be reduced during storage, which was also observed in the studies conducted previously [16, 32, 33].

UNDER PEER REVIEW

3.8. Total sugar

The predominant sugar of the two investigated honeys was fructose followed by glucose, while sucrose was present in low amounts in all samples (Table 4). Initially, fructose content of ziziphus honey was significantly higher (42.02%) than that of thyme (39.23%). These results were similar to those previously reported by other researchers [4]. In the present study, fructose content of thyme and ziziphus honey decreased during storage at room temperature. White et al. [34] found that when untreated honey is stored for 2 years at temperatures between 23 and 28°C, an average of 18.5% of the free monosaccharide content is lost, the active enzymes in honey such as α-glucosidase play an important role in the formation of reducing monosaccharide. Regarding package type, the fructose content remains significantly higher in opaque container compared to transparent ones during storage. Glucose content of native thyme honey was higher (29.47%) than that of ziziphus honey **(**28.33%). Generally, glucose contents of both honey types decreased during storage. However, less reduction in glucose content was observed in honey samples stored in opaque containers compared to transparent ones that can be due to the exposure of honey to light and heat in transparent bottle. Regarding sucrose content, no significant changes ($P > 0.05$) were observed in both honey types at zero time and during storage and it was below the maximum limit (5%) recommended by the European Community. A high sucrose concentration in honey usually means a premature harvest of honey as sucrose has not been fully converted to fructose and glucose by the action of invertase [35].

Table 4. Changes in sugars content (fructose, glucose, and sucrose) of Libyan thyme and ziziphus honey stored in transparent and opaque bottles for 12 months at room temperature.

Parameters	**Treatments**		**Storage (months)**			
	Honey type	**Package type**	**0**	**4**	**8**	**12**
Fructose (%)	Thyme	Transparent	39.23	37.63	38.82	35.08
		Opaque	39.23	38.23	38.75	37.48
	Ziziphus	Transparent	42.02	40.52	39.42	36.32
		Opaque	42.02	41.52	41.71	39.70
LSD			**0.75**			
Glucose (%)	Thyme	Transparent	29.47	27.08	27.43	25.00
		Opaque	29.47	27.70	28.50	28.43
	Ziziphus	Transparent	28.33	26.26	27.30	26.08
		Opaque	28.33	27.20	28.07	27.31
LSD			**0.76**			
Sucrose (%)	Thymus	Transparent	3.38	3.77	3.65	3.53
		Opaque	3.38	3.67	3.50	3.20
	Ziziphus	Transparent	3.31	3.65	3.17	3.61
		Opaque	3.31	3.45	3.22	3.34
LSD			**ns**			

LSD, least significant difference ($P < 0.05$).
ns; not significant

3.9. Color

UNDER PEER REVIEW

Honey quality is greatly influenced by color which varies significantly depending on the botanical origin, heat treatments, and the storage time. However, the honey color varies from light to dark amber or black shades. Previous studies reported that there is a strong relation between honey color and flavor. Thus, light colored honey has mild flavor whereas darker types have stronger flavors. It is one of the parameters mostly taken into consideration by consumers in terms of quality and acceptability [36]. In this study, the color parameters of the two honeys are illustrated in Table (5). In terms of luminosity (lightness values), a significant variation was observed between thyme and ziziphus honey samples at zero-time where thyme honey showed higher L^* value (80.50) than ziziphus honey (66.38). The high values of L^* indicates clearness, while low values of L∗ indicates darkness. The lightness of ingredients has a major role in the honey assessment due to consumer preferences. The examined honey samples had orange, yellow, and green components. In the current study and during storage period there was a significant decrease in lightness for both types of honey. da Silva et al. [32] reported that the decrease in the lightness value may be related to the storage temperature and the components of honey such as the ratio of fructose/ glucose, free amino acids and moisture content. Several authors suggested that the main cause of honey browning may be due to the Maillard reaction [37]. The decrease in L^* value was significantly higher in honeys kept in transparent bottles than those stored in opaque containers.

Table 5. Changes in colour values (L*, a*, and b*) of Libyan thyme and ziziphus honey stored in transparent and opaque bottles for 12 months at room temperature.

Color parameters	**Treatments**		**Storage (months)**			
	Honey type	**Package type**	**0**	**4**	**8**	**12**
L*	Thyme	Transparent	80.50	65.40	63.19	60.00
		Opaque	80.50	70.34	68.30	64.70
	Ziziphus	Transparent	66.38	59.20	56.87	53.23
		Opaque	66.38	61.05	59.05	57.45
LSD			**1.28**			
a*	Thyme	Transparent	-1.18	-1.04	1.20	1.95
		Opaque	-1.18	-1.08	1.10	1.55
	Ziziphus	Transparent	-3.67	-3.33	1.34	1.80
		Opaque	-3.67	-3.31	0.44	1.43
LSD			**0.28**			
b*	Thymus	Transparent	7.10	6.70	6.60	5.83
		Opaque	7.10	6.96	6.70	6.23
	Ziziphus	Transparent	3.96	3.41	3.01	2.76
		Opaque	3.96	3.70	3.40	3.03
LSD			**ns**			

LSD, least significant difference ($P < 0.05$).
ns; not significant

The green components (-a* values) were present in thyme and ziziphus honeys. During storage, the two honey types showed a slight redness particularly those kept in transparent containers. The yellow hue (+b*) had a great variation, but no significant

UNDER PEER REVIEW

differences between honey samples were found ($P > 0.05$), the b* values ranged from 2.76 to 7.10 during storage period. Generally, the differences in terms of color between the honey types are due to the chemical composition and variety [38].

4. CONCLUSION

Physicochemical properties of two Libyan honeys (thyme and ziziphus) were investigated during storage for 12 months in opaque and transparent containers at room temperature. In general, changes in moisture, pH, acidity, HMF, diastase activity, color, viscosity, electrical conductivity, and sugars content of honey stored in opaque containers were less than those stored in transparent bottles under the same conditions. Likewise, electrical conductivity, sucrose, and b* value of all samples showed insignificant changes during storage. The results obtained in this study showed that honey stored for one year could be considered safe for consumption according to the evaluated physicochemical parameters and international standardizations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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