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Effect of storage on the quality evaluating parameters of *Apis mellifera* and *Apis cerana* honey from Punjab and Khyber Pakhtunkhwa, Pakistan

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Abstract

Honey as a sweet ingredient that is produced by bees. The freshness, quality and storage of honey is of great importance for consumers demand. The current research was aims to determine the storage effect of quality parameters in *Apis cerana* and *Apis mellifera* honey. A total of 200 fresh honey samples of honey bee species were directly collected from bee keepers in different areas of Punjab and Khyber Pakhtunkhwa, Pakistan. At initial phase of physicochemical parameters determined for *A.mellifera* and *A.cerana* are Color 12-95, 30-80 mmPfund, Moisture 14, 13.5%, pH 3, 3.7, EC 0.1, 0.018mS/cm, Free acidity 28.5, 15.6meq/kg, Lactone 4, 3.81meq/kg, Total acidity 37.5, 25.72meq/kg, Ash 0.05, 0%, HMF 10.08, 5.88mg/kg, Proline 153.84, 189.44mg/kg, Diastase 11.1, 18.23DN, Invertase 9.08, 17.5IN and Total phenol 43.2, 16.92mgGA/100g respectively. After one year storage, Darking in color (19, 43mmPfund), increased in moisture content (17.2, 15%), pH (4.2, 3.8), EC (0.24, 0.03-mS/cm), FA (39.5, 26.6meq/kg), LA (8, 5.02meq /kg), TA (51.5, 41.06meq/kg), ash (0.2, 0%), HMF (56.3, 23.08mg/kg) and TP (74, 42.81mgGA/100g). While decreased in Proline (40.38, 155.31mg/kg), DI (2.2, 8.18DN) and IN (2.2, 7.48IN). The former and later values belong to *A.mellifera* and *A.cerana* honeys. Results indicated that the moisture, ash content, total acidity, HMF, proline and invertase number in *A.mellifera* and *A.cerana* are quite different and can be used as indicator to distinguished honeys of these two honey bee species. Over all, it was concluded that the effect of storage on *A.cerana* honey was minimum as compared *Apis mellifera* honey and is more recommend and fit for human consumption.

Key Point; Moisture, Acidity, HMF, Proline, Diastate, Proline and Total Phenol.

INTRODUCTION

In world, dominant honey bee diversity were present in Asian region. There are three native (*Apis cerana, Apis dorsata, Apis laboriosa)* and one exotic *(Apis florea*) honey bee species present in this area (Crane, 1999; Oldroyd 2006; Chantawannakul et al., 2016). The presence of rich fauna indicate that it is not only the floral diversity but also the potential for beekeeping that make valuable economic industry (Theisen-Jones and Bienefeld, 2016).

Honey is a sweet ingredient produced by bees from the nectar of flower (Da Silva et al., 2016; Dong et al., 2018; Sajid et al., 2020). The major components in all natural honey are almost same, but their physicochemical properties are mainly dependent on the geographical regions floral sources, enviormetal conditions, the processing and the storage of honey (Lazarević et al., 2012; Boussaid et al., 2018; Sakac et al., 2019). Colour, moisture, pH, EC, acidity, ash content, Hydroxymethylfurfural (HMF), proline content, enzymes, sugar percentage, and total phenolic and flavonoids contents are all these quality parameters (Adgaba et al., 2017; Ansari et al., 2018; Sajid et al., 2019).

During storage, there are some predictable changes that usually occur because of different chemical reactions like HMF. In honey, Adulteration has been seen due to the limited availability and the high price of honey (Barra et al., 2010; da Silva et al., 2016).

Materials and methods

200 fresh honey samples of both honey bee species were directly collected from bee keepers and apiaries in different areas of Khyber Pakhtunkhwa and Punjab Province Pakistan during the year 2017-2019. All these honey samples were stored at room temperature in plastic bottles with proper labeling and dating till completion of analysis.

Determination of honey color

According to Wilczynska, (2014) honey color was measured by a color refractometer name as Minolta Chromameter® CR 410.

Determination of Moisture Content (%)

Moisture content were determined through Honey refrectometer (Wedmore 1955).

Determination of pH

In honey solution pH meter was used to determine pH value (AOAC official 1990).

Determination of Electrical Conductivity (EC)

According to Vorwohl (1964) method EC meter was used to determine EC. Results were shown in micro-Siemens per centimeter (µS/cm).

Determination of Acidity

AOAC Official 1990 method was used to determine free acidity, lactone and Total acidy in honey. T.A. = F.A. + L.A

Determination of Ash Content

Ash content in honey determined by Hamburg 1988 as recommended by International Honey Commission (2009). $\text{Ash} = \frac{(m1-m2)}{m0} \times 100$

Determination of HMF Content

Winkler (1995) method was used for determination of HMF contents. Absorbance was noted at 550nm. $\text{HMF} = \frac{192 \times A \times 10}{\text{weight of honey in grams}}$

Determination of Proline Content

In case of proline, Ough (1969) method recommended by International Honey standard method was used. After preparation of samples, absorbance was taken at 510nm.

$$\text{Proline} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 40 \times \frac{16}{5}$$

Determination of Diastase with Phadebas

Bogdanov et al, 1997 method was used to determination diastase with Phadebas tablets. All samples test tube absorbance was done at 620nm. $DN = 28.2 \times \Delta A + 2.64$

Determination of Invertase activity

Invertase acitivity was judgment at 400nm absorbance by using Siegenthaler (1977) method as recommended by Bogdanov et al, (1997). $IN = 21.64 \times \Delta A\ 400$

Determination of Total Phenols

Moreira et al. (2003) method was used to determine total phenols and phenolic content. Absorbance was read after two hours at 765nm. Result were expressed in mg GAE/100.

Statistical analysis

SPSS-2001 software was used to analyze the data. The statistical difference in honey samples were tested with ANOVA at $p < 0.05$

Results and discussion

Color

Honey color is first indictor parameter that depends on consumer demands. The change in coloration of *A.mellifera* and *Apis cerana* honey was observed from Extra light to Amber after one year storage. Same trend were seen in 45% Algerian honey samples storage at 37°C for three months and in 48% Croatia honey at 25°C for 104 days has been reported by Mouhoubi-Tafinine et al. (2018) and Kedzierska-Matysek (2016), respectively. The variation in color

showed aging effect on honeys.

Moisture Content (%)

After one year storage, the moisture content of *A.mellifera* honeys were beyond the Codex limit as compared *A.cerana* honey samples. Al-Ghamdi et al. (2019), Ahmed et al. (2016) and Moniruzzaman et al. (2013) showed significant increase in moisture content of 40% in Saudi honey at 80°C for 30 minutes for 6th months, 44% in Pakistani honey at 25°C for one year and 30% in Malaysian honey at 4-5°C after four months. Overall increase in moisture content is due to floral sources.

pH

pH is another important parameter that can increase the quality, sustainability and shelf life of honey (Terrab et al., 2002). Results indicated that before and after storage both honeys pH were in acidic in nature. Azonewade et al., (2018) and lokossou et al., (2017) reported pH of 5.08 and 5.00-5.48 respectively, showing almost the same range as found in the present research. However, Laredj and waffa (2017) and Mohammed et al., (2017) recorded acidic pH values (2.97 and 3.60) in Algerian and Saudi honeys lower than present pH values.

Electrical Conductivity (EC)

On the whole, a significant (24% and 26%) increase in the EC after six month of storage but beyond the limits during twelve months storage in *A.mellifera* honey as compared *A.cerana* respectively, as an impact of aging in the honey. Radtke and Lichtenberg-Kraag (2018), Laredj et al. (2017) and Can et al. (2015) stated EC value of 0.61mS/cm, 0.580mS/cm and 0.80mS/cm after storage of twenty four, six and eight months, respectively, near the present results.

Acidity

According to Codex method acidity in honey not more than 50meq/kg. After storage *A.cerana* honey acidity concentration were within honey standard method than *A.mellifera.* da Silva et al. (2020) and Chou et al (2020) showed significant increase total acidity value in Taiwani and Brazalian honey after eight and sixteen month of storage.

Ash Content

This quality parameter depends on the type of soil used to grow flowering plants from which honey bees collect nectar (Karabagias et al. 2014). An average of 12% (0.08%-0.14%) and 25% (0.14%-0.21%) increment in ash content of *A.cerana* honey samples was measured after 6 and 12 months of storage, respectively than *A.mellifera* honey samples. Mouhoubi-Tafinine et al. (2018) and Hasan (2013) after nine and eight months of storage showed higher (53% and 64%), while Šarić et al. (2012) showed less increase in ash content than present results.

HMF Content

Honey purity and its freshness depends upon the concentration of HMF (Codex Alimentarius, 2001). After Twelve months storage the HMF content of *A.mellifera* honeys showed increase of 80% (30.4-183.68mg/kg) and 73% (56.3-298.43mg/kg) respectively. Like *A.mellifera*, *A.cerana* honey samples HMF was increase 55% (30.3-47.87mg/kg), respectively. Similar trend of increased in MHF were reported by Chou et al (2020), (75%) for six months at 35°C, Czipa et al. (2019) (77%) for 2 year at 40°C, Al-Ghamdi et al. (2019) (81%) for 6th month at 80°C. Storage and floral sources are the major causes of higher HMF (Terrab et al., 2002; Meda et al., 2005).

Proline Content (PC)

During storage, declining trend were seen in both *A.mellifera* (42% and 38%) and *Apis cerana* (35% and 30%) respectively, honeys proline content. Same situation were seen in Algerian, Nepali and Iraqi honey after sixteen, nine and four months of storage given by Mouhoubi-Tafinine et al. (2018), Ahmed et al. (2016) and Hasan (2013).

Diastase Number (DN)

Honey diastase act as indicator of honey freshness but their activity is influenced during storage and heating (Bogdanov et al. 2004). Storage for twelve months produced 76% and 50% lower diastase number in both honeys were similar results shown by Hassan (2013) after 24 months storage. Whereas, Chou et al (2020) (20%) at 4°C for three months in Taiwan honey, da Silva et al. (2020) (12%) at 12-21°C for two year respectively, reported decline of diastase number than present study.

Invertase Number (IN)

On the whole, a significant (43.11% and 82.50%) decrease in invertase activity was found in *Apis mellifera* honey samples as compared *Apis cerana* after storage of one year. About, 84% at 35°C for nine months, 70.5% at 20-30°C for twenty-four months and 66% at 25-29°C for sixteen months decline in Algerian, Nepali and Pakistani honey showed by Mouhoubi-Tafinine et al. (2018), Hassan (2013) and Qamer et al. (2013). However, the lowering trend in IN number due to aging effect.

Total Phenols (TP)

A significant influence of storage was also shown on total phenolic content (97.45% and 110%) in *Apis mellifera* and (55% and 40%) respectively *Apis cerana* honeys after six and twelve months storage. Chou et al (2020) and da Silva et al. (2020) showed (75%) and (80%) total

phenolic content in Taiwani and Brazilian honey, whereas Czipa et al. (2019), Šarić et al. (2012) were recorded decline of 76% and 91.8 % TP value than present study.

Conclusion

It can be concluded that *A.cerna* (Native honey bee specie of Pakistan) honey is comparatively better than *A.mellifera* honey both in term of quality and its shelf life. So that Pakistani honeys are good exportable quality as per International Honey Standard.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper

Table 1. Effect of aging on shelf life of quality evaluating parameters in *A.cerana* and *A.mellifera* honey

Sr. No	Parameters	Source	0 month	After 6months	After12months	Codex draft	Eu draft
1	Color (mmPfund)	*A.mellifera*	12-95	16-107	19-123	-----	-----
		A.cerana	30-80	36-87	43-102		
2	Moisture (%)	*A.mellifera*	14-21.5	16.7-21.9	17.2-23.2	≤21	≤21
		A.cerana	13.5-19.44	14.5-19.7	15-20.02		
3	pH	*A.mellifera*	3-5.3	3.6-6	4.2-6.3	-----	-----
		A.cerana	3.7-5.1	3.8-5.45	3.8-5.85		
4	EC (mS/cm)	*A.mellifera*	0.1-0.6	0.17-0.87	0.24-0.96	≤0.8	≤0.8
		A.cerana	0.018-0.49	0.03-0.49	0.03-0.54		
5	Free acidity (meq/kg)	*A.mellifera*	28.5-45	35-58.5	39.5-63.5	≤50	≤50
		A.cerana	15.6-37.4	18.63-38.14	26.6-39.29		
6	Lactone (meq/kg)	*A.mellifera*	4-13.5	5-15.5	8-19.7	-----	-----
		A.cerana	3.81-13.41	4.48-17.18	5.02-19.2		
7	Total acidity (meq/kg)	*A.mellifera*	37.5-53	43-70	51.5-80.5	≤50	≤50
		A.cerana	25.72-46	28.25-48.11	41.06-49.52		
8	Ash content (%)	*A.mellifera*	0.05-0.6	0.09-0.7	0.2-0.9	≤0.6	≤0.6
		A.cerana	0-0.25	0-0.31	0-0.39		
9	HMF content (mg/kg)	*A.mellifera*	10.08-69.12	30.4-183.68	56.3-298.43	≤60	≤40
		A.cerana	5.88-46.72	20-50.13	23.08-59.28		
10	Proline (mg/kg)	*A.mellifera*	153.84-581.1	85.66-256.15	40.38-235.4	≥180	≥180
		A.cerana	189.44-46	170.62-42	155.31-378.2		
11	Diastase Number (DN)	*A.mellifera*	11.1-49.6	4.9-33.05	2.2-20.7		≥8
		A.cerana	18.23-48.92	12.7-37.6	8.18-29.12	≥8	
12	Invertase Number (IN)	*A.mellifera*	9.08-87.15	3.4-59.8	2.2-28.8	≥10	-----

		A.cerana	**17.5-64.17**	**13.03-56.29**	**7.485-42.01**		
13	**Total phenol (mgGA/100g)**	***A.mellifera***	**43.2-168.9**	**67.1-221.3**	**74-270.4**	**≤200**	**≤200**
		A.cerana	**16.92-152.8**	**29.1-173.27**	**42.81-196.26**		

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