

EVALUATING THE SHELF LIFE OF *APIS MELLIFERA* HONEY COLLECTED IN SOUTH PUNJAB, PAKISTAN

SAMINA QAMER^{1*}, ABIR ISHTIAQ², BILAL AHMAD², ZULFIQAR HAIDER³, NAVEED AHMAD KHAN⁴, SHAKILA NAZ⁵, HAZRAT USMAN SHERANI³, MUHAMMAD SHAHJAHAN⁵, MUHAMMAD ADRESS⁵

^{1*}Department of Zoology, Rawalpindi Women University, Satellite Town, Rawalpindi

²Department of Zoology, Islamia University Bahawalpur, Bahawalnagar Campus.

³Department of Zoology, Government College University, Faisalabad

⁴Institute of Pure and Applied Biology, Zoology Division, Bahauddin Zakriya University, Multan

⁵Department of Zoology, Government College University Faisalabad, Layyah Campus

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ABSTRACT

The study aimed to assess the shelf life of multi-floral honey collected from the southern region of Punjab Province, Pakistan, over 12 months. Fifty honey samples were analyzed for various physiochemical parameters every four months at room temperature. Initial values included pH, free acidity, electrical conductivity, diastase number, invertase number, HMF, proline content, fructose, glucose, and sucrose percentages. After 12 months, the pH, free acidity, electrical conductivity, diastase, invertase, glucose, and fructose values decreased, while HMF content, proline content, and sucrose percentage increased. These findings suggest that honey composition changes during storage, shortening its shelf life. Fresh *A. mellifera* honey is recommended for consumption within 6 to 8 months to maintain its quality.

1. INTRODUCTION

Honey is a nutritious substance which is obtain from floral sources by bees (da Silva et al., 2016; Dong et al., 2018). It's chemically composed of mono and dissacrrides carbohydrates like fructose, glucose, maltose and sucrose (Sakač et al., 2019; Sajid et al., 2022). However, minerals, Protein, organic acid, amino acid, vitamins and aromatic substance are minor components found in honey (Amiry et al., 2017; Čanadanović-Brunet et al., 2014; Sakač et al., 2019). A further two hundred more substances are testified in honey (Ferreira et al, 2009). Due to presence of these components, that can act as antibacterial and anti-inflammatory chemicals (Liu et al, 2013). The quality of honey depends on various components including floral sources, climate condition, processing, Post harvesting handling and conditions for storage (Khan et al., 2016; Kaygusuz et al., 2016; Ansari et al, 2018).

Different types of chemical reactions caused changes in honey, like HMF is formed during sugar dehydration in acidic medium formed HMF, which is also used as honey freshness indicator parameter. The extracted fresh honey is usually present in liquid form, however, with the passage of time during storage honey may start crystallizing depending upon the nectar source and Glucose %. It is less valuable in market, so heat is used to liquefy the crystalline honey. As a result, honey lose their original chemical composition. The limited sources and excesses amount of honey price has provided an increased interest in adulteration (Barra et al., 2010; da Silva et al., 2016). Honey quality parameters are valuable for identifying these likely adulterations and to confirm hygiene conditions for handling and storing honey (Puscas et al., 2013). The aim of the present research was to evaluate the shelf life of fresh *A.mellifera* honey samples stored at room temperature for one year as per the International Honey Commission.

*Corresponding Author: samina.qamer@f.rwu.edu.pk

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2. MATERIALS AND METHODS

Honey Collection

Fifty samples of honey were directly collected from beekeepers from the different areas/ districts such as Muzaffargarh, DG Khan, Multan and Bahawalpur of South Punjab Pakistan during the year 2021-2022. Plastic bottles and sealed glass were used to store fresh honey samples, then labeled and dated. In the laboratory samples were stored at room temperature, till analysis was completed. No preservatives or heating agents are done at any stage.

Honey quality tests

All physicochemical parameters were determined according to the European Honey Commission methods [Bogdanov et al., \(1999\)](#). pH and EC meter were used to determine Honey pH and Electrical Conductivity, 10g honey in 75ml deionized water. Same solution was used for the titration of free acidity [AOAC \(1975\)](#). Proline content was estimated according to [Ough, \(1969\)](#). For HMF determination Winkler method was used ([Winkler, 1955](#)). Honey Diastase and Invertase activity were determined according to the procedure of [Siegenthaler \(1977\)](#) and [Schade et al. \(1958\)](#). Reducing sugar (fructose and glucose) and apparent sucrose was determined by using Lane and Eynon procedure as described in [AOAC \(2000\)](#).

Statistical analysis

One way Analysis of variance (ANOVA) with P value at 0.05 significance level was used to analyze the data by using latest version of statistical software (SPSS-2001).

3. RESULTS AND DISCUSSION

Fig:1 shows the effect of one year storage on various quality parameters of 50 honey samples. According to [Terrab et al., \(2002\)](#) pH is an important factor that enhances honey quality and shelf life ([Carreck, 2012](#); [Dietemann et al., 2013](#)). The current results showed that honey samples were more acidic as the average value of pH lies between 3.69-5.15. [Sajid et al., \(2022\)](#) also observed acidic pH (4.28) in *A.mellifera* Pakistani honeys. [Laredj and waffa \(2017\)](#) and [Lokossou et al., \(2017\)](#) reported Algerian and Burkina Fasan honey's pH in the range of 4.17-4.20 and 5-5.8, respectively. The upper value of 5-5.8 by [Lokossou et al., \(2017\)](#) and lower 3.67 by [Boussaid et al., \(2018\)](#) (3.67- 4.11) in Tunisian honey, 4.35 in Pakistani honey from same species ([Sajid et al., 2020](#)) seems related to present determined pH. More acidic honey or low pH of honey significantly support to the antimicrobial property of honey ([Sajid et al 2020](#)). Initially average free acidity was 21.54, which amplified to 40.80 meq/Kg by the end of 12 months, although was still within international limits (≤ 50 meq/kg). Acidity

reported by [Ciappini et al., \(2016\)](#) 19.5-22.3 meq/Kg in Argentine honey, [Alqarni et al. \(2016\)](#) 55.5–145.5 meq/kg, [Azonwade et al., \(2018\)](#) 35.7-40.5 meq/kg in Benin honey, [El-Haskoury et al. \(2018\)](#) 16.50–59.50 meq/kg and was 33–46.5 meq/kg in Pakistani honey by [Sajid et al., 2020](#). The honey acidity varies due to different floral sources and yielding seasons.

Similarly, EC ranges found by [Gular et al. \(2017\)](#) 0.11-0.25mS/cm in Tunisian honey, 0.11–0.61 mS/cm by [Sajid et al., \(2020\)](#) and 0.27mS/cm in 2022 in Pakistani honeys, seems related to the current research values of 0.13-0.27 meq/kg. [Anhwange, et al., \(2015\)](#) 0.86- 0.91mS/cm in Nigerian honey, [Bousaid et al. \(2018\)](#) (0.39–0.89 mS/cm), [Lokossou et al. \(2017\)](#) (0.37–1.43 mS/cm) reported higher EC than the present study. Variation in Electrical conductivity of honey depends on different botanical sources explored by honeybees as nectar.

According to the European Union Honey Directive, certain constituents must be determined for honeys proposed for human consumption. Diastase enzyme activity (diastase number) is one of them. In general diastase activity should not be less than 8. Heating the honey degrades the enzyme, which is why the EU directive states minimum values. Diastase in honey converts starch to short-chain sugars and the enzymes' activity hints at possible heating and/or poor storage conditions. It originated both from plant and honeybees salivary and hypopharyngeal gland secretions. It is quite a stable enzyme so considered as storage indicator parameter. It naturally deteriorates over time and also with storage. A decline from 39.05 DN to 19.19DN was observed for 12 months storage period, the limit was within the Codex range (≥ 8 DN) yet. [Moloudian et al. \(2018\)](#) also recorded reduction in diastase activity from 28.68DN to 17.75DN in Iranian honey, this trend is similar to the current study. [Sajid et al., \(2020\)](#) measured 26.97–43.47(DN) in Pakistani fresh honeys from various floral origin. [Anhwange, et al., \(2015\)](#) determined diastase range below (7.61DN) and just above the minimum limit (10.18 (DN) in Riyom, Plateau State honey.

Invertase is another essential enzyme which is liable to convert nectar and honeydew into honey. It has high sensitivity towards temperature increase. Therefore, in some European countries invertase activity determination is also used as a parameter related to the freshness of honey, to its warming or storing condition. [Boussaid et al., \(2018\)](#) and [Lichtenberg-Kraag, \(2012\)](#) stated invertase range of 46.25-184.68 (IN) and 33.76-86.95(IN) in Tunisia and German honey, respectively, which is closely related to the current IN values (27.70-67(IN). Below the IHC limit, invertase activity (23.91-0.02(IN) was found by [Parvanov et al., \(2012\)](#) in Bulgarian honey.

Honey freshness and purity depends on HMF (Al-Ghamdi et al., 2019) which is an aldehydic compound formed by the dehydration of some sugars, mostly fructose (Rosatella et al., 2011; Chris & Cornelia, 2014) and its levels should not exceed 40mg/kg. It is also temperature and storage conditions sensitive (Al-Ghamdi et al., 2019). In the present study higher (43.62mg/kg), yet with IHC limit, HMF content were measured after 12 months storage compared to initial 14.68mg/kg. Ciappini et al., (2016) was described HMF range from 12.07 to 27.43 mg/kg in Tunisia honey. Comparative to current findings low HMF values have been recorded in Argentine honey (6.7-7.2meq/Kg) and Indian honey (3.87-4.64mg/kg), respectively, by Boussaid et al., (2018) and Kirvak & Karababa (2017). Chris and Cornelia (2014) treated *Acacia* and *lime* honey under various storage conditions and later tested its freshness by measuring HMF content. Similarly, Sajid et al. (2019) kept multi-floral honey at different temperatures and then checked its freshness by estimating HMF content. Sajid et al., in 2020 and 2022 found averagely 30.85mg/kg and 29.2mg/kg HMF in fresh Pakistani honeys, respectively.

Proline is predominant non-essential amino acid making up almost 50% to 85% of total amino acid content. Its origin is mainly honeybee's salivary gland secretion mixed with nectar to convert it in honey. Proline content are usually applied as honey maturation criterion and it should not be less than 180mg/kg in honey as per IHC standard (Qamer et al., 2007; de Slvia et al., 2016). In present study Proline content after 4 months storage at room temperature was 469.82mg/kg, however, it dropped up to 148.30mg/kg level after 12 months storage which was less than minimum limit set by IHC. Sajid et al., (2022) reported an average of 235.32mg/kg Proline content in *A.mellifera* honey without storing or any other treatment. Aazza et al. (2018) and Nayik and Nanda (2015) investigated the proline content between 256.46-924.98mg/Kg and 205.3-551.74mg/Kg.

As far as monosaccharides and sucrose are concerned, Fructose (42%), Glucose (38%) and Sucrose (2.25%) were found well within limits set by IHC even after 4-month storage. Anyhow, lowering in monosaccharides and raising in sucrose (6.76%) beyond the standard limits was observed in next 8 months. Sajid et al., (2022) determined fructose (38.97%), glucose (32.10%) and sucrose (2.25%) values fairly below the maximum border line set by IHC in fresh *A.mellifera* honey collected from Punjab province. Guler et al., (2017) and Khaliq & Swaileh, (2017) were reported reducing sugar concentration range from 68.41-68.77g/100g and 68.9-83.6g/100g in Turkish and Palestine honeys without storage, respectively. Khan et al., 2016 documented minimum 32.45% in *Zizpus spp* honey and maximum 38.03% Fructose in *Clover* honey from Punjab and

Khyber Pakhtunkhwa, respectively, from Provinces of Pakistan. Similarly, lowest (24%) from *Zizpus spp* and highest (32%) glucose in *Clover* and *Sunflower* was found from Punjab and Khyber Pakhtunkhwa honey samples, respectively. In the same way least sucrose (1.12%) was recorded in *Acacia* honey collected from Punjab and Khyber Pakhtunkhwa. Although he found more than 6% and 7% in *clover* and *Eucalyptus* honeys from Punjab Province and 9.7% sucrose in currant bush honey collected from Khyber Pakhtunkhwa. The maximum level of sucrose should not exceed 5% in honey as per recommendation of IHC. Khaliq and Swaileh, (2017) reported the sucrose% between 1.1-6.9g/100g in Palestine honey. While Guler et al., (2017) had sucrose concentration (0- 3.05g/100g) well within limits in Turkish honey.

4. CONCLUSION

From the above results it is evident that there took place some irreversible changes after one year storage at room temperature that shortens the freshness and shelf life of Pakistani honey produced by *A.mellifera*. Therefore, it is safe to consume honey within six months without compromising its quality.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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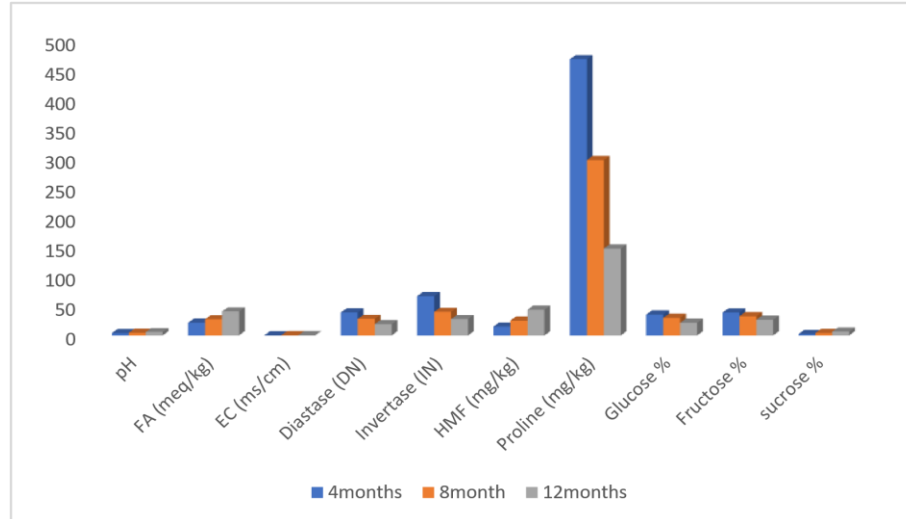


Fig.1. Graphic representation of Change in quality parameters of honey during 12th month's storage