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The change in physicochemical characteristics of coffee flower honey (Coffea robusta) in Vietnam during storage

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ABSTRACT

Honey was reported numerous benefit containing nutritional ingredients and phytochemical such as polyphenol, flavonoid was widely used around the world. In this study, the physicochemical characteristics on the quality of coffee flower honey has been evaluated during storage. The honey was collected from Dak Lak, Buon Me Thuot, and Kon Tum province in Vietnam and stored at room temperature (30-32°C) for 12 month. Total sugar, reducing sugar, hydroxymethylfurfural (HMF), acid value, total polyphenol content (TPC) and total flavonoid content (TFC) were evaluated for each two month to determine the quality of sensory quality and nutritional value. After 12 month storage time, the content of total sugar, and reducing sugar gradually decreased along with the increase of HMF and acid content in the coffee flower honey samples a lead to sweetness of honey is decrease, acidity, and color increase, which is a signal indicating a decline in sensory quality and nutritional value. The content of TPC and TFC increased during the first 6-10 months of storage and then also tended to decrease, indicating that coffee flower honey should not be stored for too long (more than 10 months) because of phytochemicals reducing.

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1- Introduction

Honey is a complex of compounds, from plants, that bees have harvested from pollen. The compound of honey: 95% carbohydrates, of which 95% is glucose and fructose. In addition to carbohydrates, honey contains water, enzymes, amino acids, vitamins, minerals, polyphenols, antioxidants, fatty acids, and volatile compounds. As a food that is interested in its high nutritional value and versatility in fields such as medicine, food, cosmetics, … In medicine, there have been studies and applications of antibacterial and therapeutic properties of honey with wounds of different origins from pediatric patients, and the conclusion is that covering the wound with honey (MGH) inhibits the entry of pathogens and has antibacterial properties. Furthermore, MGH keeps the wound moist and has powerful restorations, such as autolysis of non-critical tissue and restoration of vascular structure. The anti-inflammatory and antioxidant effects of MGH together with added vitamins C and E may inhibit scar formation. This suggests that MGH is safe, easy to apply, and can be recommended for all types of wounds, as well as being safe and easy to use. In addition, honey also plays the role of a medicinal herb, an important component of traditional medicine remedies and health foods such as reducing dry eyes, and mucositis, ...

In the functional food and health food industry now, honey is indispensable, it is added as an excellent nutritional supplement, providing energy, vitamins, minerals, and antioxidants for the body. Because honey contains many antioxidants active substances such as polyphenols and flavonoids as well as many vitamins also antibacterial properties. Honey is increasingly used in the beauty industry such as cosmetics, masks, ...

The quality of honey is concerned with organoleptic properties as well as physical, chemical, and microbiological criteria. According to the international honey trade, the quality of honey refers to its organoleptic properties (taste, consistency, and color) and chemical composition (moisture and HMF content, diastase index, pH), acidity as well as carbohydrate content and ratio) [1]. It is widely agreed that the quality of honey depends on the source of nectar and honeydew collected by the bees, the climate of the honey's vegetative origin, and the conditions during storage and processing [2-9].

Storage has a significant influence on the maintenance of honey quality. Honey, after being left for a long time, produces unpleasant odor compounds, reducing nutritional value.

Therefore, with the desire to contribute to preserving the source of honey with nutritional value, we have conducted a study on the change in physicochemical properties of coffee flower honey in Vietnam over time preservation method to create a premise for research on methods of preserving coffee flower honey to preserve the nutritional value of this honey in Vietnam.

2-Materials and Methods

2.1. Chemicals and reagents

Chemicals used for the physicochemical analysis were purchased from Merck (Darmstadt, Germany). Glucose, gallic

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acid, quercetin and folin – ciocalteu's reagent were purchased from Sigma-Aldrich (USA). All chemicals used were of analytical grade unless specified. All samples were stored in sealed glass jars and at room temperature (20–30°C) until analysis.

2.2. Sample

Coffee honey samples were collected at the end of the coffee blossom (March 2021) at five beekeeping establishments in Cu Kuin district (CoffeeHC 1, CoffeeHC 2, CoffeeHC 3), Buon Me Thuot City (CoffeeHB 1, CoffeeHB 2) of Dak Lak province, Vietnam. Two coffee honey samples in Gia lai province (GL) and Kon Tum province (DH), Vietnam.

2.3. Total sugar contents.

Total sugar contents were determined by the phenol-sulfuric method Nielsen (2003) [9]. Glucose was used to construct the calibration curve with a concentration scale of 0-100 ppm.

Honey was weighed 1g and dissolved it with 20 ml of water in a beaker, then transferred to a 100 ml rated jar, filled to level mark by distilled water. The solution is done through 11 mm filter paper. Take 1 ml of filtrate solution made up to 100 mL with distilled water - which is solution B. Next, 1 ml of solution B was added 1ml of phenol 5% and 5ml H_2SO_4. After leaving the reaction for 10 min, we continued to cool the solution in a thermostatic bath at 25°C for 10 min and then measured it photometrically at 490 nm.

2.4. Free reducing sugar contents.

The free reducing sugars contents were determined by the DNS method and glucose was used to construct the calibration curve with a concentration scale of 0-100 ppm. Honey (1 g) was dissolved in water made up to 100 mL. The solution was filtered through an 11 µm filter paper. The filtrate solution (1ml) was made up to 25ml with distilled water. The DNS (1 mL) was added to 1mL diluted solution. The reaction solution was kept in a thermostatic bath at 90°C until a reddish-brown color appeared and cooled for 10 min. Then, the reaction solution was added distilled water (7 mL). The absorbance was determined with a spectrophotometer at 540 nm.

2.5. Hydroxymethylfurfural (HMF)

Honey (5 g) was dissolved in 25 mL of distilled water. The absorbance was measured at 284 and 336 nm against a filtered solution treated with $NaHSO_3$ [10].

2.6. Free acid contents

Honey (10 g) was added to 75 mL of distilled water, and then the solution was stirred with a magnetic stirrer until the solution was completely dissolved. The solution was titrated with 0.1 N NaOH to pH = 8.30, recording the volume of NaOH consumed [10].

2.7. Total polyphenol Content (TPC) and Flavonoid Content (TFC)

The polyphenol content (TPC) was assessed at room temperature with the Folin–Ciocalteu phenol reagent [11]. The gallic acid was used to the standard. The sample honey (0.05 mg/ml) was reaction with Folin-Ciocalteu's reagent and Na_2CO_3 in 30 min. The absorbance was read at 765 nm. Results were expressed in terms of µg of gallic acid (used as standard) equivalents (GAE)/g of honey ± standard deviation (SD). The experiments were performed in triplicate.

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The flavonoid content (TFC) was determined according to the method described by Zhishen et al. (1999). The honey solution (0.05 mg/ml) was mixed with 0.15 ml $NaNO_2$ for 5 min. Then, added 0.3 ml $AlCl_3$ 10% for 6 min. Lastly, 1 ml NaOH 1M and 0.55 ml of distilled water was added. The mixture was determined by a spectrophotometer at 510 nm. The quecrtin was used to the standard and results were expressed in mg of quercetin equivalents (mg QE/g of honey) [12].

2.8. Data Analysis

Differences were assessed using one- and two-factor analysis of variance (Statgraphics Centurion XV). The results were reported as mean ± standard deviation (SD). The samples are compared specifically based on the tukey method to correct the P- value. Linear regression analysis method to find out the correlation between factors.

3- Results and Discussion

3.1. Total sugar and free reducing sugar contents.

Before storage, total sugar content in coffee flower honey samples ranged from 755.47 - 825.39 g/kg. (**Figure 1.**), the total sugar content of the sample decreased gradually with the storage time. From the 6th month onwards, the content decreased sharply. From the time before storage to the 6th month, the total sugar content decreased by about 1.6 - 6% compared to the original total sugar content. Samples HC1, HB2, and DH decreased more than 20% in total sugar content after 12 months of storage at room temperature in the dark. The total sugar content decreases when storage for a long time could be the reason for dereasing the sweetness and affecting the quality of honey. For explaning these results, the sugar composition changes during storage due to the action of enzymes and temperature [13].

The reducing sugar content gradually decreased during storage (**Figure 2.**), which decreased 39 - 67% compaing with the original honey samples. After the storage time, the reducing sugar content keenly decline, which effecting nutrition value significantly. The appearance of amino acids and reducing sugar facilitate for Maillard reaction leading to change of color variation swarthy. This is also the reason fo total sugar content of the above samples has decreased. The ratio of high levels of reducing sugars in honey is supposedly good impact to diabetics due to relate low GI (glycemic index). Therefore, the deduction of reducing sugars during storage time could derease nutritional value of honey [14].

3.2. Hydroxymethylfurfural (HMF)

HMF increased steadily over each month of storage, showing that after 12 months of storage (**Fiigure 3.**), an increase of 23 - 67% compared to the original HMF. HMF is also a product of the Maillard reaction, so when the reducing sugar content is reduced as much as above, it means that the HMF content in the coffee flower honey samples in this study will also increase much after the storage time. The hydroxymethylfurfural (HMF) is one of the important parameters to determine the freshness of honey, as well as the storage duration and conditions, and it tends to increase during processing and/or aging of the product [15, 16]. Several factors have been reported to influence the levels of HMF, such as temperature and time of heating, storage conditions, pH, and floral

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sources, therefore the content of HMF provides an indication of overheating and storage in poor conditions [17].

3.3. Free acid contents

Free acid content in coffee flower honey samples before storage (**Figure 4.**) ranged from 12.9 - 38.9 mg/g. Most of the samples had high free acid values after 12 months of storage. This change increases sharply from the 8th to the 12th month. The acid content of honey is influenced by many factors, such as the time and manner of honey being stored, the presence of different organic acids in the flower, geographical origin, harvest season, etc. The free acids in honey contribute to the flavor and are partially responsible for the antimicrobial activity [18]. Similarly our study, the increase of acid value in honey is also found in the study of Cavia, the authors evaluated the free acidity of 35 unheated Spanish honeys stored at room temperature and for more than 30 months, the results showed that, after 20 months of storage, most of the honey samples showed a steady increase in free acidity. The other study of Mehdi Zarei reported a tendency of slight increase acid value in flower of Alfalfa, Milkvetch, Multifloral, Thyme and Lotus honey [19]. Some previous study reported that, a rise of free acid value could be fermentation of honey sugar [20].

3.4. Total phenolic and flavonoid contents

The results in **Figure 5** show that the phenolic content in all samples increased more than the baseline (11-41%) after 12 months of storage. TPC increased to the highest level at the 10th month of storage from 29-59% of the original content. From the 10-12th month, TPC of all sample tend to decrease. The TPC value of 12th month showed that decreased (13-19%) compared to the results at the 10th month. TPC of primary honey samples ranged from 0.255 - 0.705 mg/kg, high phenolic content in coffee flower honey samples suggests potential health benefits for humans, as according to studies previously showed that total phenolic content is correlated with the bioactivity of honey including antimicrobial, antioxidant, anti-inflammatory, antineoplastic, antiulcer, and cardiovascular disease [21]. In our study, TPC value was observed obtaning the highest at 10th month, then decreased. The resuls indicated that the biological activity of honey may also be reduced when the storage time gradually increase

The TFC content increased gradually from the beginning to the 6th month during storage, and increased to hightest about 50-76% compared to the initial value (**Figure 6.**). From the 6th to the 12th month after the storage time, the TFCs of the samples began to decrease gradually, the obvious loss is shown at 6th month when TFC decreased 28-73%. Some of the initial TFC and after 12 months of storage increased 17-50%. While, the others are equal or lower such as HC1 and HB2 – these are also the two samples with the largest decrease in TFC from the 6th month of storage. As in total phenolic content, the tend to decrease was observed in total flavonoid content. This was predicted because flvonoid was one of subclass in polyphenol. This TFC change is consistent with the results previously studied by Šarić et al. (2020) on flavonoid changes during honey storage. This study reported that the TFC of honey will be increased during the first 6-9 months of storage and then gradually decreased with longer storage [22].

4- Conclusion

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The content of total sugar, reducing sugar, HMF, acid, TPC and TFC are indicators to assess the quality of honey after storage time. The content of total sugar, reducing sugar gradually decreased along with the increase of HMF and acid content in the coffee flower honey samples after 12 months of storage, which is a signal indicating a decline in sensory quality and nutritional value. Significantly nourishes honey as sweetness decreases, acidity, and color increase. The content of TPC and TFC increased during the first 6-10 months of storage and then also tended to decrease, indicating that coffee flower honey should not be stored for too long (more than 10 months) as it will affect the activity. biology of this honey, reducing the value of honey's health benefits. Honey should not be stored for too long.

5-Acknowledgement

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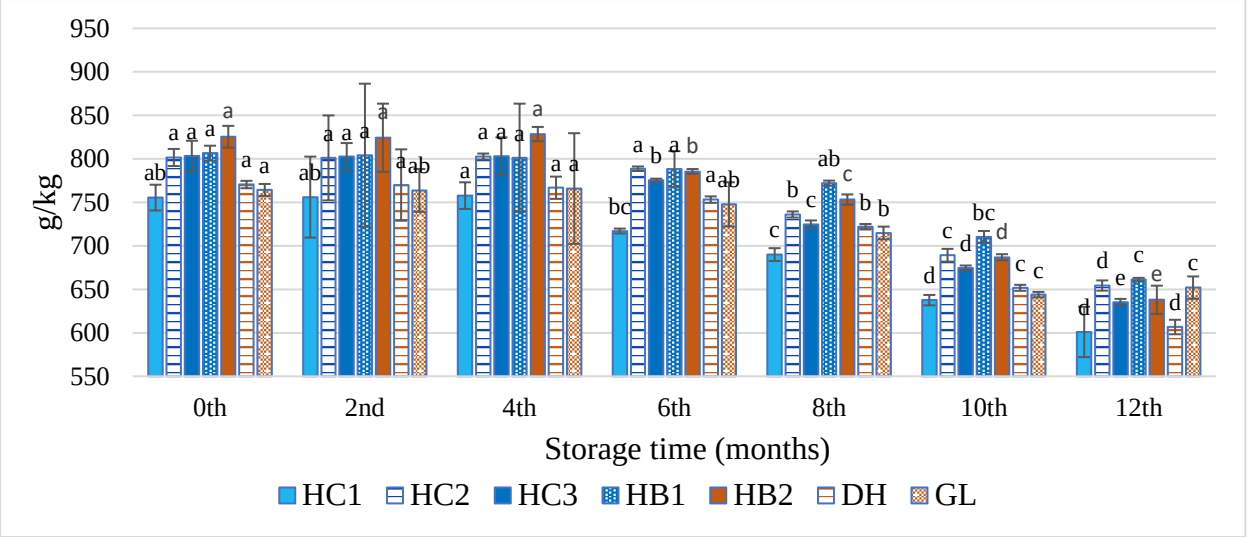

Values are expressed as Mean ± SD, letters a, b, c, d and e represent the difference between samples ($p < 0.05$)

Figure 1: Total sugar contents of Coffee honey in during 12 months of storage (g/kg)

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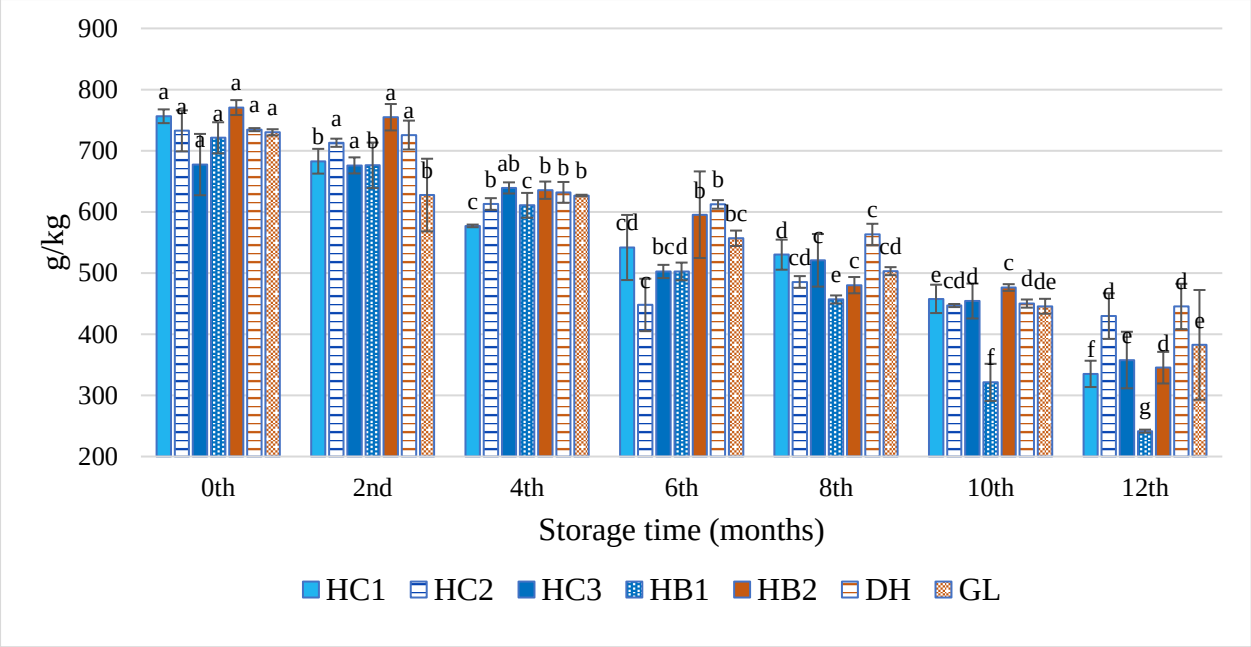

Values are expressed as Mean ± SD, letters a, b, c, d, e and f represent the difference between samples ($p < 0.05$)

Figure 2: The reducing sugar contents of Coffee honey in during 12 months of storage (g/kg).

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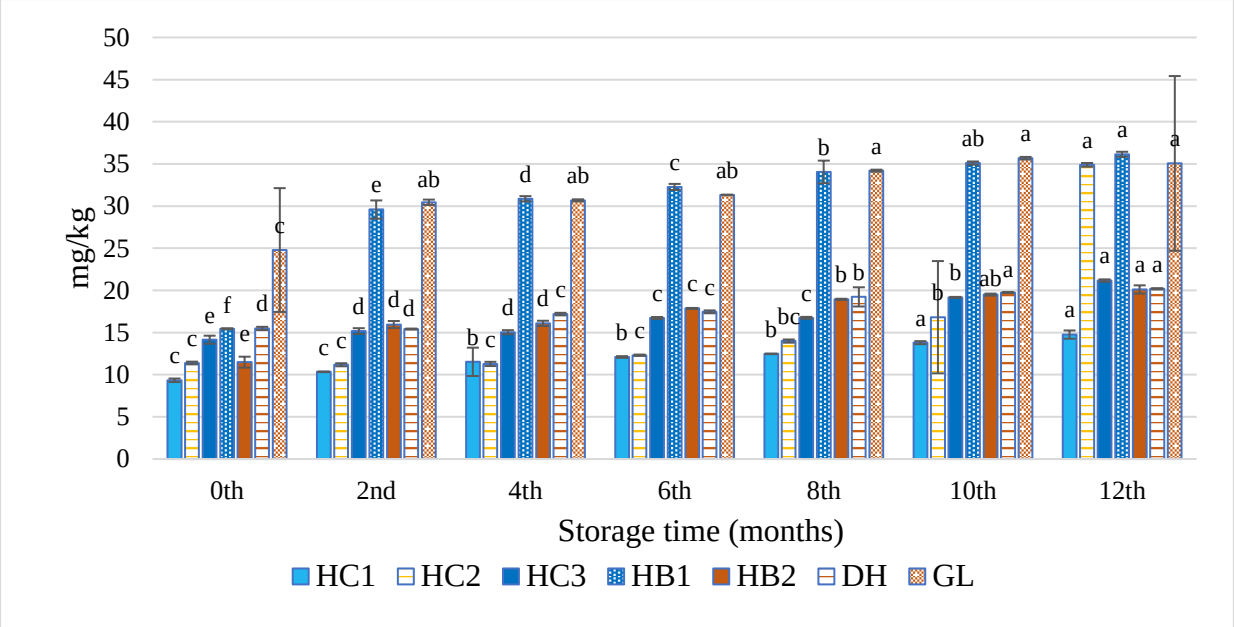

Values are expressed as Mean ± SD, letters a, b, c, d, e and f represent the difference between samples ($p < 0.05$)

Figure 3: Hydroxymethylfurfural contents of Coffee honey in during 12 months of storage (mg/kg).

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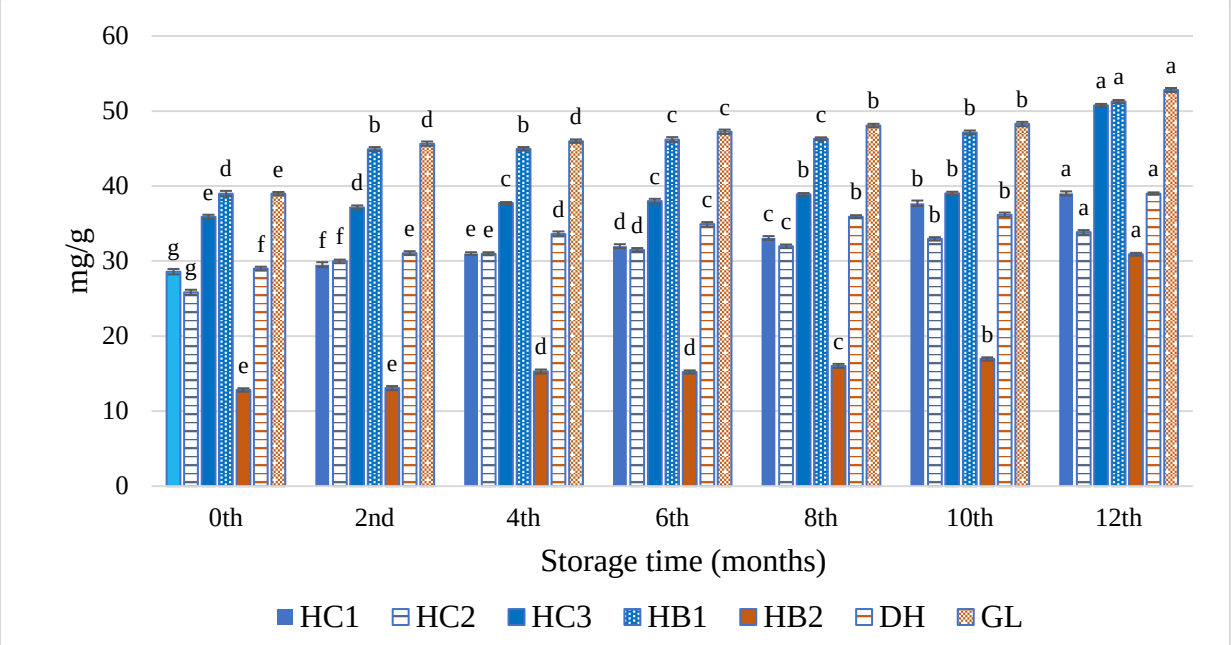

Values are expressed as Mean ± SD, letters a, b, c, d, e, f and g represent the difference between samples ($p < 0.05$)

Figure 4: Free acids contents of Coffee honey in during 12 months of storage (mg/g).

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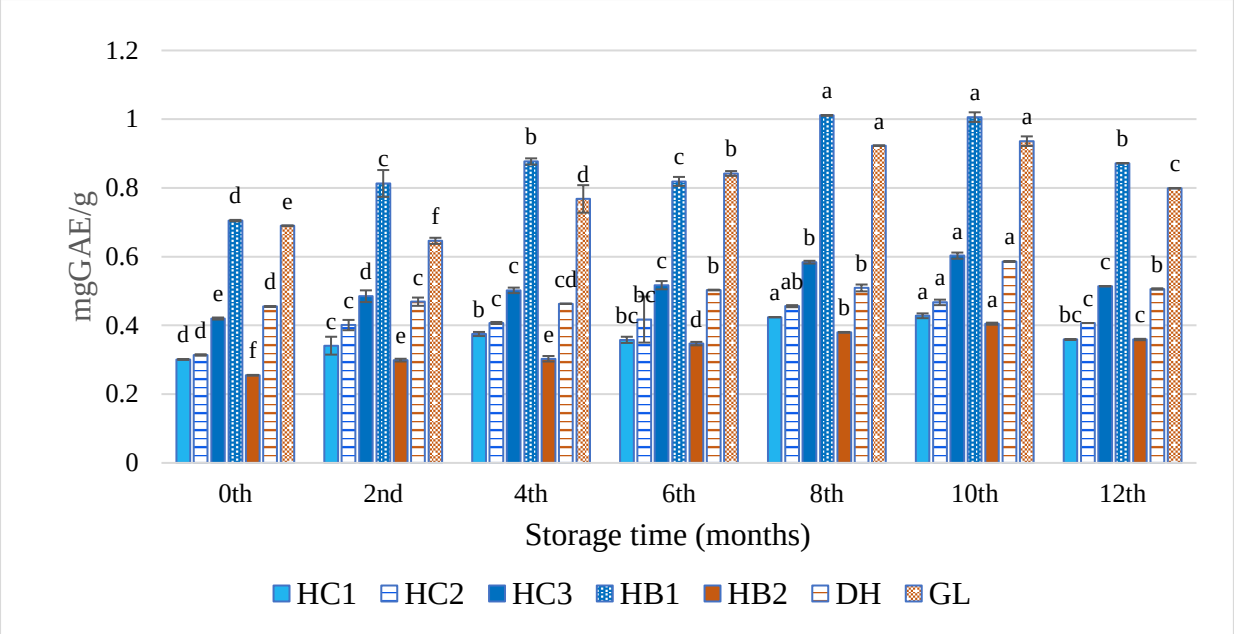

Values are expressed as Mean ± SD, letters a, b, c, d, e, and f represent the difference between samples ($p < 0.05$)

Figure 5: TPC of Coffee honey in for 12 months storage (mg GAE/g)

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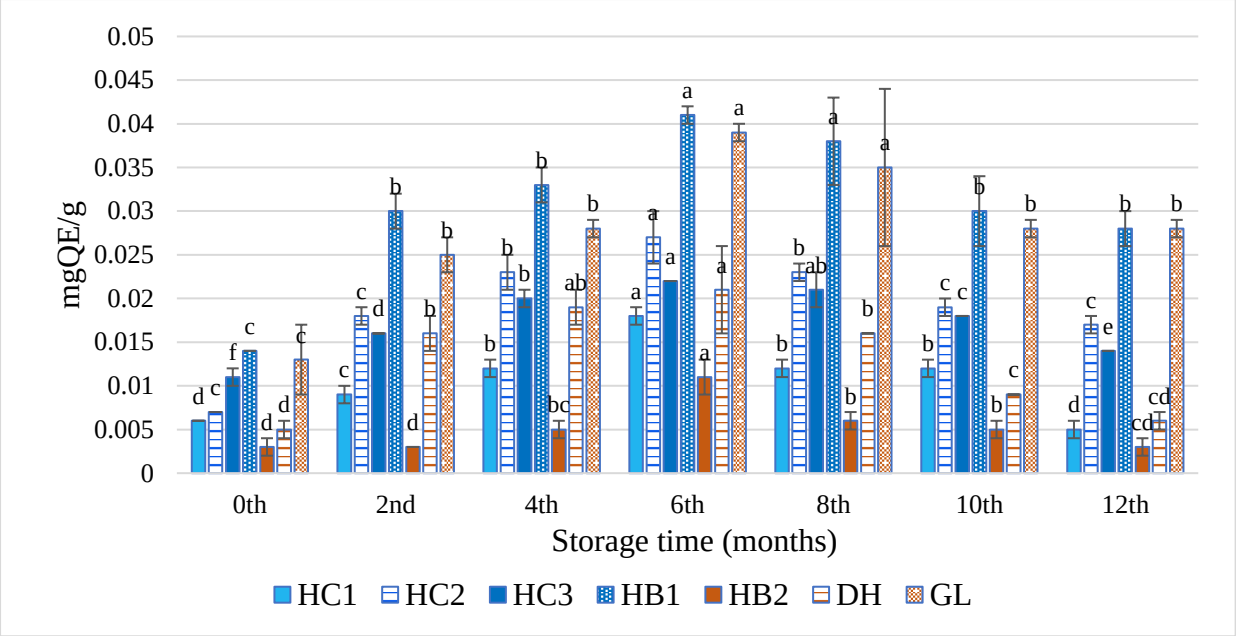

Values are expressed as Mean ± SD, letters a, b, c, d, e, and f represent the difference between samples ($p < 0.05$)

Figure 6: TFC of Coffee honey in during 12 months o

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