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DETERMINATION OF SHELF LIFE OF CHASTE TREE (*Vitex agnus castus*) HONEY AND PINE HONEY

Aytül Uçak Koç[1]*, Mete Karacaoğlu[2], Hilal Şahin Nadem[3], Mustafa Doğan[4]

[1]Adnan Menderes University, Koçarlı Vocational School, Aydın, Turkey
[2]Adnan Menderes University, Faculty of Agriculture, Department of Animal Science, Aydın, Turkey
[3]Adnan Menderes University, Engineering Faculty, Food Engineering Department, Aydın, Turkey
[4]Republic of Turkey Ministry of Food Agriculture and Livestock, Denizli Food Control Laboratory Directorate, Denizli, Turkey

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Abstract

Chaste tree and pine honeys were bought from three different producers (natural) and three local markets (commercial). The honey samples were stored for 30 months at room temperature. The shelf life of the samples was estimated for indices of HMF and diastase activity. The values for HMF formation and diastase deactivation fitted to a zero-order reaction. The highest rate of HMF formation was observed in the chaste tree honey as 2.674±0.013 mg/kg/month while the least value was observed in the pine honey as 0.435±0.037 mg/kg/month. The maximum diastase deactivation was determined in the natural chaste tree honey as 0.613±0.000 DU/kg/months and the minimum was 0.318±0.002 DU/kg/month for the commercial chaste tree sample. In terms of HMF, all honeys except commercial chaste tree honey, have exhibited longer shelf life than 30 months of storage. The results showed that the shelf life of the commercial honeys depend on the botanical origin, pH, electrical conductivity, temperature and storage.

Keywords: Chaste tree, pine honey, shelf life, HMF, diastase.

HAYIT (*Vitex agnus castus*) VE ÇAM BALININ RAF ÖMRÜNÜN BELİRLENMESİ

Öz

Hayıt ve çam balları üç farklı üretici (doğal) ve üç farklı yerel marketten (ticari) satın alınmış ve bal örnekleri 30 ay oda sıcaklığında depolanmıştır. Bal örneklerinin raf ömrü HMF miktarı ve diastaz aktivite indeksleri üzerinden tahmin edilmiştir. HMF oluşumu ve diastaz deaktivasyonu sıfırıncı dereceden kinetik modele uyumlu bulunmuştur. En yüksek HMF oluşum hızı hayıt ballarında 2.674±0.013 mg/kg/ay olarak hesaplanırken en düşük hız çam balında 0.435±0.037 mg/kg/ay olarak belirlenmiştir. Diastaz sayısı bakımından ise, en yüksek (0.613±0.000 DU/kg/ay) ve en düşük (0.318±0.002 DU/kg/ay) deaktivasyon hızı doğal ve ticari hayıt ballarında belirlenmiştir. Balların 30 ay depolanması sonunda HMF bakımından ticari hayıt balı hariç diğer tüm ballar daha uzun raf ömrüne sahip olmuştur. Bu sonuçlar ticari balların raf ömrünün botanik orjin, pH, elektriksel iletkenlik, sıcaklık ve depolamaya bağlı olduğunu göstermektedir.

Anahtar kelimeler: Hayıt balı, çam balı, raf ömrü, HMF, diastaz.

* *Corresponding author* / Yazışmalardan sorumlu yazar
aucak@adu.edu.tr, (+90) 2567727348, (+90) 7727460

INTRODUCTION

Consumer expectations and legislation address the highest guarantees for safety, authenticity and quality. Honey is always considered as a healthy and natural food. It contains several enzymes, water, carbohydrate, acids, dextrin, ash, vitamins, pollen and substance aroma (1-3). The amounts of these components are the most important characteristics that can be used to assess the quality of a honey sample.

The composition and properties of honey are dependent on the season of production, origin of nectar, the flowers visited by the bees and the local climate from which the honey is harvested (4). In addition, the processing, handling and storage of honey may further influence its composition (5, 6). Some honey samples also contain potential toxic compounds such as 5-hydroxymethylfurfural (HMF), which is produced by the Maillard reactions during heat treatment or storage, and commonly observed in foods such as milk, fruit juices and grape molasses (7, 8).

HMF concentrations are used to determine the freshness and shelf life of honey. HMF is usually absent in fresh and untreated honey (9) but its concentration increases because of heating or long-term storage (10, 11). It has been highlighted that the chemical composition plays an important role in the final level of HMF in honey (10, 12-16).

Several factors influence the formation of HMF in honey during storage: humidity, thermal and photochemical stress (17), the use of metallic containers (18) and the physicochemical properties (the pH, total acidity and mineral content) of the honey, related to the floral sources from which the honey has been collected (12). Under dry and pyrolytic conditions, an alternative pathway has been proposed to HMF formation from fructose and sucrose. In acidic conditions, HMF can be formed at low temperatures (19), however, its concentration may also be drastically increase with an increase in temperature and storage. In addition to temperature, the rate of HMF formation in honey is also reported to be dependent on the pH of honey samples (20) as well as the water content (21, 22).

The kinetics of HMF development in unifloral honeys, and its dependence from pH of samples, has been investigated (11). The negative effects of too restrictive HMF standard in trading, some unifloral honeys have been highlighted and estimated the most probable shelf life for each honey (4, 23).

Diastase and HMF evolution were determined in Spanish honeys after their extraction up to 28 months of storage (24). The evolution of invertase has been studied in honeys of different origin (25, 26) and the effect of heating and filtration on antioxidant activity during storage of unifloral honeys have been studied (27).

The Codex Alimentarius (Alinorm 01/25 2000) has established the HMF concentration in honey after processing and/or blending and the acceptable HMF concentrations in honey samples should be lower than 80 mg/kg. However, the European Union (2002) recommends a lower limit of 40 mg/kg with the following exceptions: 80 mg/kg is allowed for honey that originates from countries or regions with tropical temperatures, while a lower limit of only 15 mg/kg is allowed for honey with low enzymatic levels. HMF standards in Turkey (Turkish Alimentarus Codex 2003) have been adopted from the European Union (EU) (Directive 2001/110 EC 2001), guaranteeing the limits up to the "Sell by" date, usually in 36 months.

Turkey, one of the most important honey producers and exporters in the world, has suitable geographical and climatic conditions for apiculture where approximately 7.5 million hives resided and led to a production of 105000 tons of honey in 2016 (28). Anatolian honeys are rich in pollen types per sample and 85% of the world's floral types can be found in Turkish honeys. Despite the great diversity of honeys produced in Anatolia, there have been limited studies for the characterization and classification by botanical or geographical origin. In addition, most of the studies are limited for the compositional analysis (29-38).

Pine honey is a kind of honeydew. This type of honey is produced by honey bees from excretions of plant-sucking insect (*Marchalina hellenica*) feeding on pine trees (39). Pine honey is a specific endemic product and can be found only in Turkey and Greece. In Turkey, as one of the main pine honey producers, pine honey constitutes most the total production of the country as well as floral honey. It was reported that the compositions of pine honey and floral honey differ depending on pH, mineral content and sugar profile (40).

Majority of Turkish honey is harvested and marketed by the beekeepers. Therefore, they are fresher and exposed to less heat load than the commercial honeys which undergo subsequent heat treatments for shelf life and filling purposes. Therefore, the purpose of this investigation was to determine the HMF and diastase levels in both natural and commercial pine honey and chaste tree honeys during storage for up to 30 months to develop models to estimate the most probable shelf life for each honey.

MATERIALS AND METHODS

Samples

Pine honey and chaste tree (*Vitex agnus-castus*) honey samples were obtained from three different beekeepers located in Aydin province in the Aegean Region of Turkey. In addition, processed pine honey and chaste tree honey and samples were bought from three different markets in Aydin. Next, the honey samples were analyzed to determine the moisture, glucose, fructose, pH, free acids, electrical conductivity, ash, diastase activity and HMF content.

Analyses

Moisture content of honey samples were determined by using Abbe refractometer (Pleuger 2WA) reading at 20°C and obtained corresponding percentage moisture according to the TS 13365 (41). For pH, 10g of honey sample was weighed and dissolved in 75 ml of distilled water (TS 1728). Next, the solution was taken into a beaker and the pH value was recorded by a pH meter (WTW, pH 330/SET1).

Acidity of the samples was determined according to the TS 13360. For this, 10 g of honey added into 75 ml distilled water and honey solution was neutralized by using 0.1N NaOH till pH reaches to 8.3. Acidity of the samples was calculated by the following equation:

Acidity (meq formic acid/kg honey) = (FxNxV)/m x 1000

Where F, N and V are the factor, normality and used volume of NaOH, respectively and m is the sample amount.

The electrical conductivity was determined by conductivity meter (WTW COND311). Electrical conductivity meter was first calibrated with water and then conductance cell was dipped into honey solution (10.0%) and reading was noted after the instrument was stabilized. Ash content (%) was determined according to the method of TS 2131-ISO928. For this, 2 g of honey was put into a porcelain crucible and the sample was burned at 550±25 ºC in the muffle furnace. Brix of honey samples were determined by Abbe refractometer (Pleuger 2WA). The dry matter scale was prepared according to pure sucrose solution at the 20 °C. Diastase in honey was measured using a UV-spectrometer (Shimadzu UV-160A) according to the method of Harmonised Methods of the International Honey Commission, Schade Diastase Determination Method, 34-37, 2002. Harmonised Methods of The International Honey Commission was used to determine HMF contents of the samples. According to this method, 5 g of honey was diluted into 100 mL distilled water, filtered through 0.45 µm and then it was immediately injected into a high-pressure liquid chromatography (HPLC) with a diode array detector (Agilent HP 1100 UV-DAD). The HPLC column used for the measurements was ACE-5 (250 X 4,6 mm, C18). The HPLC conditions were as following: isocratic elution of water: methanol (90:10) mixture, flow rate of 1 mL/min and injection volume of 20 µl. All the solvents used for the measurements were HPLC grade (Merck). The wavelength range was 190–660 nm and the chromatograms were monitored at 285 nm. The compound was identified by spiking the honey peak with an HMF standard (Sigma-Aldrich, H 40807), and by comparing the HMF standard's spectrum with those of the honey samples. HMF was determined from an external calibration curve with the signal at 285 nm. Table 1 shows retention time for HMF peak, correlation coefficient belonging to calibration curve, dedection (LOD), limit of cantitation (LOQ) and %R.

Table 1. Retention time (R_T) for HMF peak, correlation coefficient belonging to calibration curve (r^2), dedection (LOD), limit of cantitation (LOQ) and %R

R_T	8.96
r^2	0.9999
LOD (mg/kg)	0.95
LOQ(mg/kg)	1.00
% R	98.4

Shelf Life Estimation through HMF and diastase

Honey samples were stored at room temperature (23-25°C) for 30 months in the laboratory and every month small portions of samples were taken for analysis. The kinetic data analysis for HMF formation and diastase deactivation during storage were performed. Average kinetics equations were obtained using the data from Tables 2 and 3. The self-life of the samples was estimated using regression analysis of 30 months long term data which are represented by the zero-order kinetics (Eq. 1) as below;

$$C_t = C_o + k_o\, t \quad \text{(zero-order kinetics)} \qquad (1)$$

Where C_o is the initial concentration of HMF (mg/kg) or diastase activity (DU/kg), k_o is the rate constant (1/month), C_t is HMF concentration or diastase activity after t months of storage at room temperature, t is the storage time of honey samples (42).

Regression analysis (curve fitting) and the calculation of kinetic rate constants were performed using the Microsoft Excel 2013 software. To verify the validity of the kinetic model and to measure linearity, regression coefficients (R^2) were calculated. The maximum storage period at 95% confidence was also estimated using a lower diastase (<8 DU/kg) and upper HMF (40 mg/kg) levels.

RESULTS AND DISCUSSION

The characterization of the chaste tree and pine honeys from local beekeepers (natural) and local market (commercial) are given in Table 2 and 3. In this study, the moisture values were between 14.86±0.066% (chaste tree natural-3) and 19.9±0.066% (pine honey commercial-1). These results were like the some of the studies reported (4, 43-47). Belitz et al. (48) offered 17.1% of moisture gradient as a threshold value for the fermentation of honey. Depending on the characteristics of honeys, there is a risk of fermentation between 17.1% and 20% of moisture contents. However, the risk of fermentation is high over 20% of moisture (48).

In this study, except for one natural pine honey, moisture contents of pine honeys were over 17% (Table 2 and 3) probably due to the harvest season. Chaste tree honeys were harvested towards the end of July (during the highest seasonal temperatures), but pine honeys were harvested from September to December. HMF formation and diastase deactivation values during 30 months of storage are given in Table 4, 5, 6, 7.

Table 2. Characterization of the Chaste tree honey samples

Parameters	Chaste tree honey (natural)			Chaste tree honey (commercial)		
	1	2	3	1	2	3
Moisture %	15.40±0.003	16.72±0.128	14.86±0.066	16.32±0.133	16.45±0.133	15.93±0.066
Glucose %	34.8±0.233	31.1±0.901	31.3±0.081	35.5±0.680	32.4±0.088	34.3±0.594
Fructose %	40.7±0.290	43.0±0.233	39.6±0.326	39.8±0.120	39.0±0.120	42.0±0.348
Sucrose %	0.54±0.005	n.d	n.d	n.d	0.96±0.0120	n.d
Fruc + Gluc %	75.6±0.524	74.1±1041	70.9±0.408	75.3±0.561	71.4±0.066	76.3±0.638
Fruc / Gluc %	1.16±0.003	1.38±0.0226	1.26±0.008	1.11±0.024	1.2±0.005	1.22±0.043
Dry matter %	84.6±0.00	83.2±0.129	85.1±0.066	83.67±0.133	83.5±0.133	84.0±0.066
pH	3.83±0.042	3.63±0.006	3.83±0.062	3.77±0.008	3.77±0.022	3.64±0.018
Acidity meq/kg	34.0±0.45	28.8±0.62	17.2±0.24	34.1±0.23	22.5±0.06	22.5±0.09
Ash %	0.24±0.004	0.21±0.038	0.24±0.010	0.21±0.017	0.16±0.005	0.24±0.003
HMF mg/kg	5.27±0.120	4.73±0.088	4.77±0.088	7.1±0.115	6.83±0.120	7.9±0.057
Conductivity (ms/cm)	0.633±0.002	0.493±0.006	0.264±0.002	0.493±0.022	0.443±0.003	0.271±0.011
Diastase (DU/kg)	27.2±0.10	26.9±0.05	27.45±0.05	24.1±0.1	21.4±0.15	20.95±0.05

n.d.: not detected.

Table 3. Characterization of the pine honey samples

Parameters	Pine honey (natural)			Pine honey (commercial)		
	1	2	3	1	2	3
Moisture %	15.80±0.115	17.62±0.000	17.9±0.057	19.9±0.066	17.3±0.133	17.8±0.033
Glucose %	25.7±0.133	26.4±0.233	25.2±0.233	27.3±0.120	28.0±0.531	27.9±0.472
Fructose %	33.7±0081	34.3±0.122	37.4±0.033	33.9±0.1	37.4±0.327	36.0±0.041
Sucrose %	n.d	n.d	n.d	0.54±0.005	0.96±0.012	n.d
Fruc + Gluc %	59.4±0.346	60.7±0.100	62.6±0.260	61.2±0.219	65.4±0.204	63.9±0.405
Fruc / Gluc %	1.31±0.005	1.29±0.017	1.48±0.012	1.24±0.003	1.33±0.036	1.28±0.024
Dry matter %	84.2±0.115	82.4±0.133	82.1±0.057	80.1±0.066	82.7±0.133	82.2±0.033
Ph	4.13±0.033	4.02±0.005	3.91±0.0115	4.25±0.028	4.02±0.008	3.91±0.037
Acidity meq/kg	23.3±0.25	26.3±0.1	32.3±0.15	20.9±0.1	25.25±0.05	32.25±0.15
Ash %	0.53±0.005	0.68±0.025	0.37±0.019	0.438±0.010	0.648±0.010	0.357±0.0107
HMF mg/kg	0.56±0.064	0.54±0.024	0.93±0.0305	1.24±0.034	3.27±0.218	10.33±0.185
Conductivity (ms/cm)	1.405±0.001	1.269±0.001	0.827±0.001	1.175±0.005	1.255±0.005	1.134±0.001
Diastase (DU/kg)	31±0.70	19.05±0.15	22.1±0.20	17.55±0.05	16.8±0.175	16.18±0.175

n.d.: not detected.

Table 4. Evolution of HMF (mg/kg) in Chaste tree honey samples during storage at room temperature

Months of storage	Chaste tree honey (natural)			Chaste tree honey (commercial)		
	1	2	3	1	2	3
0	5.3±0.12	4.7±0.09	4.8±0.09	7.1±0.12	6.8±0.12	7.9±0.05
2	5.9±0.03	5.5±0.17	5.4±0.12	7.8±0.26	7.7±0.20	9.5±0.30
4	6.5±0.15	6.9±0.12	7.1±0.15	9.5±0.25	9.2±0.05	10.5±0.15
6	7.3±0.18	7.4±0.01	8.2±0.20	12.3±0.06	11.1±0.17	12.8±0.35
8	10.3±0.17	8.1±0.20	10.7±0.37	16.8±0.35	11.9±0.25	17.5±0.24
10	15.4±0.40	14.6±0.60	14.3±0.11	21.6±0.23	19.4±0.15	23.9±0.00
12	20.4±0.33	17.7±0.23	17.5±0.40	29.9±0.11	22.0±0.20	25.7±0.57
16	24.4±0.45	22.2±0.66	21.6±0.13	40.2±0.18	36.4±0.33	32.6±3.76
20	29.4±0.06	28.3±0.11	26.4±0.28	53.6±0.42	44.3±0.55	41.5±2.74
24	33.9±0.11	32.0±0.02	30.2±0.36	65.5±0.05	58.9±0.20	53.8±5.07
30	33.7±0.11	36.4±0.11	35.0±0.43	82.6±0.59	68.8±0.39	65.6±3.24

Table 5. Evolution of HMF (mg/kg) in pine honey samples during storage at room temperature

Months of storage	Pine honey (natural)		Pine honey (commercial)			
	1	2	3	1	2	3
0	0.6±0.06	0.5±0.02	0.9±0.03	1.2±0.03	3.3±0.22	10.3±0.19
2	0.8±0.04	0.7±0.04	1.2±0.03	1.3±0.02	4.0±0.18	12.6±0.50
4	1.0±0.07	0.8±0.02	1.5±0.04	1.5±0.03	4.9±0.28	14.4±0.57
6	1.2±0.59	1.1±0.05	2.6±0.03	1.8±0.02	6.7±0.03	15.8±0.27
8	2.2±0.14	2.6±0.02	5.4±0.01	2.5±0.15	6.9±0.05	19.2±0.40
10	2.6±0.08	4.5±0.20	6.5±0.23	3.5±0.14	8.4±0.26	21.6±1.65
12	4.3±0.09	6.1±0.15	8.3±0.23	5.6±0.09	10.7±0.17	28.9±0.15
16	6.5±0.67	8.4±1.21	9.2±0.57	7.5±0.70	13.6±1.08	31.3±0.07
20	8.5±0.89	9.9±1.36	10.2±1.12	8.6±0.95	16.5±1.19	34.1±1.01
24	10.1±0.67	11.5±1.37	11.9±1.87	11.9±1.11	18.8±1.00	37.5±0.99
30	12.5±0.5	12.9±1.17	13.8±2.58	14.9±1.37	23.0±1.81	40.0±1.01

The initial HMF amounts of the chaste tree natural honeys were higher than the pine honey natural honeys. In addition, the initial HMF values of the commercial chaste tree and pine honeys were higher than those of the local producer's honeys. This situation showed that the chemical characeteristics of the honeys are mainly related to the the botanical origin, storage period and temperature (11).

Commercial pine honey -3 had the highest HMF content of 10.3 mg/kg however this value did not exceed the allowable limits in 30 months of storage time. Although the initial HMF contents of the commercial chaste tree honeys (7.1, 6.8

Table 6. Evolution of diastase in honeys during storage at room temperature

Months of storage	Chaste tree honey (natural)			Chaste tree honey (commercial)		
	1	2	3	1	2	3
0	27.3±0.20	27.7±0.30	27.3±0.25	24.3±0.05	21.0±0.05	21.3±0.05
2	26.6±0.05	26.5±0.20	26.4±0.15	23.3±0.15	20.5±0.10	20.3±0.20
4	26.2±0.15	25.8±0.05	25.3±0.05	22.2±0.20	19.5±0.30	19.4±0.15
6	25.7±0.05	23.9±0.10	24.4±0.10	21.4±0.15	18.4±0.20	18.4±0.25
8	23.7±0.05	21.8±0.05	22.8±0.10	19.0±0.10	17.5±0.10	16.7±0.12
10	22.8±0.05	19.7±0.10	21.8±0.10	16.6±0.30	16.5±0.30	15.2±0.18
12	21.2±0.05	17.7±0.15	18.9±0.10	15.9±0.10	15.7±0.10	14.3±0.04
16	19.9±0.07	19.7±0.10	16.0±0.20	13.4±0.10	14.1±0.10	13.3±0.10
20	18.5±0.36	17.9±0.03	14.0±0.05	10.9±0.05	13.6±0.15	12.0±0.03
24	16.7±0.11	15.9±0.10	12.3±0.08	9.4±0.20	13.1±1.41	10.9±0.37
30	15.6±0.09	13.9±0.10	10.6±0.24	7.4±.0.10	11.2±1.41	9.6±0.37

Table 7. Evolution of diastase in honeys during storage at room temperature

Months of storage	Pine honey (natural)			Pine honey (commercial)		
	1	2	3	1	2	3
0	31.7±0.05	19.2±0.15	22.3±0.1	17.6±0.05	16.7±0.03	16.3±0.05
2	30.0±0.11	18.1±0.08	21.1±0.15	16.9±0.05	14.6±0.23	14.9±0.06
4	28.5±0.17	16.6±0.10	20.0±0.25	15.6±0.08	12.8±0.10	13.2±0.13
6	27.2±0.14	15.4±0.28	18.0±0.04	14.1±0.30	11.3±0.05	12.0±0.25
8	25.6±0.15	13.9±0.15	17.6±0.15	13.3±0.30	10.4±0.30	11.5±0.20
10	24.5±0.26	13.5±0.25	16.0±0.13	12.0±0.24	8.4±0.03	11.0±0.49
12	23.7±0.09	12.1±0.10	14.9±0.20	11.8±0.10	7.9±0.13	8.9±0.05
16	21.8±0.45	10.4±0.41	13.9±1.03	11.2±0.60	7.2±0.26	8.2±0.22
20	20.5±0.90	8.88±0.13	11.3±0.74	10.1±0.55	6.15±0.35	6.9±0.11
24	17.9±8.95	7.69±0.19	9.11±1.09	8.0±0.05	5.1±0.10	5.9±0.37
30	17.1±1.78	5.05±0.45	7.4±1.1	6.19±0.59	4.3±0.29	4.2±0.7

and 7.9 mg/kg, respectively) were in the limit (< 40mg/kg) of the standard, these values exceeded the limit after 20 months of storage. Natural chaste tree and pine honeys had the lower HMF contents than the commercial corresponding samples. Fallico et al. (11, 49) reported that the chestnut honey did not exceed the HMF limit (40 mg/kg) during 18 months of storage at room temperature and reported pH values of the chestnut honey between 4.98 and 6.5. In terms of pH, conductivity and ash content, natural and commercial pine honeys had the higher values than those of the chaste tree honey samples. The pH values of the pine honeys were between 3.9 and 4.3 and these values are in agreement with the values reported by Turhan et al. (50). The results showed that there is a strong relationship between pH and HMF contents of the honeys. The initial HMF contents of the chaste tree honeys were higher than the values of pine honeys probably due to the lower pH levels of the chaste tree honeys than the pine honeys. As, Souza et al. (51) reported that HMF formation is favored at pH 2.7-3.9.

On the other hand, O'Brien (52) has stated that fructose is much more effective than glucose for the formation of HMF. In this respect, the higher fructose contens of the chaste tree honeys might also caused to the more HMF formation during storage. In addition, heating of the commercial honey samples during packaging caused to increased HMF contents of these samples than the natural honeys. In addition, HMF content of the samples increased linearly by storage. Al-Diab and Jarkas (53) indicated that strong heating and long storage increase the HMF content of the honeys. According to these results, the chaste tree honeys should not be stored longer than 36 months with respect to the HMF values. Table 8 and 9 show the kinetic constants of HMF formation and diastase deactivation during storage.

Table 8. Regression analysis of HMF formation in honey samples at room temperature[a]

Samples	k_o (mg/kg/months)	Linear equation	R^2
CTN-1*	1.235±0.009	t = (C_t-3.050)/1.235	0.9704
CTN-2	1.199±0.002	t = (C_t -2.333)/1.199	0.9718
CTN-3	1.099±0.010	t = (C_t -3.286)/1.099	0.9893
CTC-1**	2.674±0.013	t = (C_t -0.560)/2.674	0.9798
CTC-2	2.258±0.013	t = (C_t -0.140)/2.258	0.9673
CTC-3	1.993±0.002	t = (C_t -0.3.51)/1.993	0.9830
PHN-1+	0.435±0.037	t = (C_t -0.645)/0.435	0.9798
PHN-2	0.479±0.032	t = (C_t -0.379)/0.479	0.9636
PHN-3	0.466±0.116	t = (C_t -0.903)/0.466	0.9530
PHC-1++	0.486±0.026	t = (C_t -0.324)/0.486	0.9686
PHC-2	0.684±0.001	t = (C_t -2.390)/0.684	0.9933
PHC-3	1.079±0.055	t = (C_t -10.71)/1.079	0.9737

[a] t: months of storage; C_t:HMF concentration after t months of storage; *CTN: Chaste tree honey-natural; **CTC: Chaste tree honey- commercial; + PHN: Pine honey-natural; ++ PHC: Pine honey-commercial

Table 9. Regression analysis of diastase deactivation in honey samples at room temperature[a]

Samples	k_o (DU/kg/months)	Linear equation	R^2
CTN-1*	0.424±0.002	t= (Ct -27.3)/-0.424	0.9761
CTN-2	0.449±0.001	t = (Ct -26.34)/-0.449	0.8899
CTN-3	0.613±0.000	t = (Ct -27.33)/-0.613	0.9745
CTC-1**	0.605±0.008	t = (Ct -23.94)/-0.605	0.9752
CTC-2	0.318±0.002	t = (Ct -20.33)/-0.318	0.9456
CTC-3	0.402±0.005	t = (Ct -20.39)/-0.402	0.9523
PHN-1+	0.478±0.058	t = (Ct -30.98)/-0.478	0.9636
PHN-2	0.470±0.014	t = (Ct -18.51)/-0.470	0.9838
PHN-3	0.510±0.043	t = (Ct -21.77)/-0.510	0.9862
PHC-1++	0.373±0.010	t = (Ct -16.91)/-0.373	0.9761
PHC-2	0.396±0.006	t = (Ct -14.36)/-0.396	0.8869
PHC-3	0.394±0.017	t = (Ct -15.03)/-0.394	0.9578

[a] t: months of storage; C_t:Diastase activity after t months of storage; *CTN: Chaste tree honey-natural; **CTC: Chaste tree honey- commercial; + PHN: Pine honey-natural; ++ PHC: Pine honey-commercial

The k_o values for HMF formation ranged between 0.44 and 2.67 ppm/month. These findings agreed with the results of Fallico et al. (4), who determined the K values of some honeys between 0.7 and 2.8 ppm per month. The highest value of k_o (2.674 ppm per month) for HMF formation was calculated for the chaste tree commercial honey while the minimum k_o (0.435 ppm per month) was determined for the natural pine honey. All chaste tree honeys had the higher k_o values than those of the pine honeys. In addition, all commercial samples had the higher k_o values than those of the natural honeys probably due to heat treatment for the commercial samples. Table 9 presents the k_o values of diastase deactivation in honey samples. These values (DU/kg/months) changed in 0.318-0.613 DU/kg per month, which were like the findings (0.258-0.515 DU/kg per month) of Fallico et al. (4). In general, the chaste tree honeys had the higher rate of diastase deactivation than the pine honeys during storage at room temperature.

In the present research, the shelf lives of the chaste tree natural honeys with respect to HMF level were calculated as 30.1, 30.5 and 33.5 months while those values were determined as 15.1, 17.6, 18.5 months for the commercial chaste tree honeys (Table 10). Estimated shelf lives for the natural pine honeys were 94.1, 87.1 and 89.3 months whereas commercial pine honey samples had 84.5, 55.7 and 26.8 months (Table 10). In keeping with EU and CA criteria, the shelf life of honey is determined to be 36 months. However, the self lives of some honeys (specifically the chaste tree honeys) in this study were determined to be shorter than 36 months. Therefore, the criteria of EU and CA need to be revised depending on the botanical origin of the honeys.

Table 10. Shelf life comparison of honeys using 40 ppm of HMF as limit

Sample	Estimated shelf life (months)			Scheduled shelf life (months)	Difference (months)
	Most likely value	Min	Max		
CTN-1*	30.1	30.0	30.2	36	-5.8
CTN-2	31.5	31.4	31.5	36	-4.5
CTN-3	33.5	33.4	33.7	36	-2.5
CTC-1**	15.1	15.1	15.2	36	-20.9
CTC-2	17.6	17.5	17.6	36	-18.5
CTC-3	18.5	16.9	21.5	36	-17.5
PHN-1+	94.1	84.2	103.4	36	+58.1
PHN-2	87.1	70.1	103.9	36	+51.03
PHN-3	89.3	62.8	109.1	36	+53.30
PHC-1++	84.5	72.1	99.0	36	+48.47
PHC-2	55.7	21.2	64.5	36	+19.67
PHC-3	26.8	26.1	28.5	36	-9.24

*CTN: Chaste tree honey-natural; ** CTC: Chaste tree honey-commercial; + PHN: Pine honey-natural; ++ PHC: Pine honey commercial

According to Table 11, the shelf lives of the natural chaste tree honeys with respect to diastase levels were calculated to be 45.5, 40.8 and 31.5 months while the shelf lives of the commercial chaste tree honeys were 26.5, 38.8, 30.9 months. On the other hand, the shelf lives of the natural pine honeys were estimated as 46.9, 22.4 and 27.2 months whereas the commercial pine honeys had 24.1, 16.0 and 17.9 months.

These results showed that there is a big variation among the commercial honeys (chaste tree and pine honey) depending on the initial HMF and the diastase levels. These findings were in aggreement with the previously reported studies (49, 54-55). The shelf life of the commercial honeys depends on the degree and period of heating applied during packaging.

CONCLUSION

This study showed that there are significant differences between natural and commercial processed honeys in terms of quality criteria. The HMF content and diastase level can be sufficiently used to determine the freshness of the honey. HMF formation and diastase deactivation fitted to a zero-order reaction during storage at room temperature. The highest rate of HMF formation and diastase deactivation was determined for the chaste tree honeys. All honeys, except commercial chaste tree honey, have exhibited longer shelf life than 30 months of storage with respect to HMF formation. The present study showed that the shelf life of the commercial chaste tree honeys should be less than 36 months as they have higher initial values of HMF and also, they probably are subjected to high heat treatment before packaging.

Table 11. Shelf life comparison of honeys using 8 DU of diastase as limit

Sample	Estimated shelf life (months)			Scheduled shelf life (months)	Difference (months)
	Most likely value	Min	Max		
CTN-1*	45.5	45.1	45.9	36	+9.45
CTN-2	40.8	40.6	41.1	36	+4.79
CTN-3	31.5	31.3	31.7	36	-4.49
CTC-1**	26.4	26.1	26.6	36	-9.64
CTC-2	38.8	38.6	39.1	36	+2.82
CTC-3	30.9	30.2	31.5	36	-5.15
PHN-1+	46.9	42.1	51,8	36	+10.93
PHN-2	22.4	22.1	22.6	36	-13.64
PHN-3	27.2	25.1	29.3	36	-8.80
PHC-1++	24.1	24.1	24.2	36	-11,87
PHC-2	16.0	15.8	16.3	36	-19.98
PHC-3	17.9	17.2	18.5	36	-18.13

*CTN: Chaste tree honey-natural; ** CTC: Chaste tree honey-commercial; + PHN: Pine honey-natural; ++ PHC: Pine honey-commercial

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