

Effect of storage on hydroxymethylfurfural (HMF) and color of some Algerian honey

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Abstract

In this study, the quality evaluation of six honey samples was carried out by the analysis of some physico-chemical parameters. All the samples showed water content within limits (20%), except for the sample H01. This may be the result of a premature harvest. Values of ash content, Electrical conductivity and pH prove that the samples were most likely of floral origin. However, the samples H01, H02 and H04 may be elaborated from honeydew, because of their high ash content and electrical conductivity. The very high level of sucrose of H06 can be due to an adulteration by an addition of sucrose. The total acidity of all the samples was within limits, indicating absence of undesirable fermentation. The highest HMF level and the lowest invertase activity of H06, suggesting that this sample has undergone a heat treatment. In the other hand, the impact of storage at different temperature (4, 20, and 35°C) on HMF and color was also investigated. Storage at 4 and 20°C had no considerable effect on these parameters. However, storage at 35°C caused an increase of HMF and the results exceed largely the allowed limit (40 mg/Kg). In the same time, the color of the samples is accentuated because of Maillard reaction.

Keywords

Honey
Algerian
Storage
HMF
Color

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Introduction

Honey is a natural substance which is used as a medicine since ancient time. It is made by Apis mellifera bees from nectar of blossoms (floral honey) or from secretions of living parts of plants or excretions of plant sucking insects (honeydew honey) (Sanz *et al.*, 2004; Belay *et al.* 2013). Honey is essentially a concentrated aqueous solution of inverted sugar, but it also contains a very complex mixture of other saccharides, enzymes, amino and organic acids, polyphenols, carotenoids, Maillard reaction products, vitamins, and minerals (Blasa *et al.*, 2006). Honey types differ from one country to another and in different regions in the same country due to the floral origin, soil composition and other factors consequently (Alqarni *et al.*, 2012). Honey quality can undergo some changes with duration and temperature of storage, which leads essentially to loss of enzymatic activities and formation of hydroxymethylfurfural (HMF), a cyclic aldehyde that is produced by degradation of sugars (Gidamis *et al.*, 2004). The presence of excessive amounts of HMF in honey has been considered as evidence of overheating and implies loss of freshness (Serra Bonvehi *et al.*, 2000).

The purposes of the present study is to estimate

the quality of some Algerian honeys by the analysis of some physico-chemical parameter and to investigate the effect of storage at 4°C, 20°C and 35°C, during 9 months, on HMF formation and honey samples color.

Material and Methods

Honey samples

The present study was carried out on six honey samples; samples H01, H02, H03 and H04 were harvested between June and November 2012 and collected directly from beekeepers in different regions of Bejaia, Algeria. Samples H05 and H06 were purchased from a local market in the same year (2012). The samples were stored in glass bottles.

Physico-chemical analyses

Moisture, ash, proline contents, and electrical conductivity were determined by the methods of Bogdanov *et al.* (1999). Moisture was evaluated by refractometry using Abbe-typ refractometer (RF 490, Euromexholland). Ash content was determined by heating 5 g of honey at 625°C in a muffle furnace. Proline content was determined by the measuring the absorbance at 510 nm of the resulting product between proline and ninhydrin in an acidic medium. Electrical conductivity was measured in a 20%

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(w/v) solution of honey in deionized water with low electrical conductivity using Leibohld model conductimeter.

Reducing sugars were determined by the titrimetric method using Fehling reagent (Journal Officiel Français, 1977).

Protein content was determined by the method of Azeredo *et al.* (2003). A volume of 0.1 ml of honey solution (50 % w/v) was added to 5 ml of Coomassie Brilliant Blue. After 2 min of incubation, the quantity of proteins was estimated at 595 nm in relation to bovine serum albumin standard curve.

pH, acidity, invertase activity and HMF content were determined according to the Harmonised Methods of the International Honey Commission (Bogdanov, 1999). pH was assessed in a 10% (w/v) solution of honey in distilled water by mean of pH meter (Crison micro pH 2000, Germany). Free, lactic and total acidities were determined by the titrimetric method. The addition of 0.05 M NaOH was stopped at pH 8.50 (free acidity); immediately, the remained solution of NaOH was added and, without delay, a back-titration was realised with 0.05 M H₂SO₄ (lactic acidity). Total acidity was obtained by adding free and lactic acidities. Invertase activity was determined basing on the spectrophotometric measurement of 4-nitrophenol, which is formed by the reaction of honey invertase with 4-nitrophenyl-D-glucopyranoside, used as a substrate. Results were expressed in Unit per Kg. Hydroxymethylfurfural (HMF) was quantified after clarifying the samples with Carrez reagents (I and II) and the addition of sodium bisulphate; absorbance was determined at 284 nm and 336 nm (UV-Vis 1601 spectrophotometer, Shimadzu).

Color was estimated by measuring the absorbance of honey solutions at 420 nm according to Bath and Singh (1999), after diluting 1.25 g of honey to 5 ml with hot distilled water and filtration.

Storage

Each honey sample (500 g) was divided into 3 aliquots in hermetically closed glass containers. The first aliquot was stored at 4°C, the second at 20 °C and the third at 35°C; the samples were stored during 9 months.

Statistical analysis

Statistical analysis of the data was carried out with STATISTICA 5.5 Fr. Analysis of variance (ANOVA) followed by LSD test (Least Significant Difference) was performed to estimate the statistically significant differences between honey samples for each parameter. In the other hand, statistical correlations

were determined by the same softwar.

Results and Discussion

Physico-chemical analyses

Table 1 shows the results expressed as mean (\pm SD) obtained from the physicochemical analysis of honey samples. Moisture is regarded as an important parameter of quality used to determine the maturity degree of honey and to estimate its shelf life (De Rodriguez *et al.*, 2004). Values obtained for this parameter ranged between 17.28 and 21.34%. They are higher than those obtained by Ibrahim Khalil *et al.* (2012) (11.59-14.13%). One sample (H01) presented water content over the maximum allowed by the Codex Alimentarius (20%); this can be the consequence of a premature harvest of honey. Values of ash content (0.137- 0.564%) fell within the limit allowed for floral honeys (0.6%); these results were similar to those found by Mendes *et al.*, (1998) who investigated Portuguese floral honeys (0.1-0.5%). Ash content depends on the material collected by bees during foraging on the flora. The soil type, in which the original nectar-bearing plant was located, also influences the quantity of minerals present in honeys (De Rodriguez *et al.*, 2004; Felsner *et al.*, 2004).

Proline content serves as an additional determinant of quality and in some cases also as a criterion for estimating the maturity of honey and as an indicator for detecting sugar adulteration (Meda *et al.*, 2005). The studied honey samples have good proline levels (551.88–890 mg/Kg); they were higher than the limit proposed by Bogdanov *et al.* (1999) (>180 mg/kg), indicating the maturity of the honeys and absence of adulteration.

Electrical conductivity (EC) shows a great variability according to the floral origin. Floral honeys should have conductivity values below than 0.8 mS/cm, while honeydew should have values over 0.8 mS/cm (Codex Alimentarius, 2001). EC values of the tested honeys ranged from 0.417 to 1.412 mS/cm; H01, H02 and H04, which have the highest values of ash content, have also EC values higher than 0.8 mS/cm, suggesting that these honeys were elaborated from honeydew. Our results are superior to those obtained by Khalil *et al.* (2012) (0,419-0,809 mS/cm).

In this study, we confirmed the existence of a linear relationship ($r = 0.93$, $P < 0.01$) between ash content and electrical conductivity of honeys obtained by Člechovská and Vorlová (2001), Downey *et al.* (2005) and Ouchemoukh *et al.* (2007). Sugar content present values varying from 62.75 to 76% (reducing

Table 1. Physico-chemical characteristics of the honey samples

Sample	H1	H2	H3	H4	H5	H6	SSD
Moisture (%)	21.34 ± 0.02 ^d	18.49 ± 0.04 ^c	17.28 ± 0.07 ^a	17.60 ± 0.32 ^{ab}	17.43 ± 0.08 ^a	18.08 ± 0.14 ^{bc}	***
Ash (%)	0.56 ± 0.02 ^d	0.54 ± 0.02 ^d	0.17 ± 0.01 ^a	0.51 ± 0.01 ^d	0.14 ± 0.02 ^a	0.14 ± 0.01 ^a	***
Proteins (µg/g)	0.84 ± 0.01 ^d	0.163 ± 0.03 ^d	0.96 ± 0.03 ^c	0.78 ± 0.04 ^{ab}	0.67 ± 0.02 ^b	0.80 ± 0.05 ^d	***
Proline (mg/kg)	620.75 ± 10.6	828.38 ± 15.86 ^{bc}	852.0 ± 9.90 ^c	581.42 ± 18.55 ^a	551.88 ± 10.50 ^a	644.22 ± 14.74 ^a	***
Total reducing sugar (%)	62.75 ± 1.06	65.75 ± 3.18	66.50 ± 0.0	76.00 ± 0	70.50 ± 0	75.00 ± 1.41	ND
Reducing sugar (%)	61.35 ± 0.0	63.61 ± 2.12	62.94 ± 1.06	73.39 ± 1.06	69.55 ± 1.41	61.46 ± 1.06	ND
Sucrose (%)	1.40	2.14	3.56	2.61	0.95	13.54	ND
Electrical Conductivity (mS/cm)	1.41 ± 0.01 ^e	0.91 ± 0.01 ^c	0.55 ± 0 ^d	0.99 ± 0.02 ^d	0.41 ± 0.01 ^a	0.42 ± 0.01 ^a	***
pH	4.58 ± 0 ^e	4.23 ± 0.03 ^c	3.97 ± 0.01 ^a	4.41 ± 0.01 ^d	4.11 ± 0.01 ^d	4.21 ± 0.01 ^c	***
HMF (mg/kg)	15.63 ± 0.85 ^{bc}	29.13 ± 0.47 ^d	17.87 ± 0.59 ^c	11.04 ± 0.66 ^b	82.00 ± 0.58 ^f	34.53 ± 0.33 ^e	***
Free Acidity (meq/Kg)	11.07 ± 1.36 ^{bc}	25.13 ± 2.53 ^e	18.23 ± 0.45 ^{cd}	20.63 ± 1.20 ^{de}	11.60 ± 0.79 ^{bd}	10.27 ± 0.65 ^a	***
Combined acidity (meq/Kg)	26.70 ± 0.50 ^d	14.90 ± 1.83 ^{cd}	13.73 ± 1.84 ^{cd}	15.53 ± 2.30 ^d	08.30 ± 1.82 ^a	10 ± 0.72 ^{bd}	**
Total acidity (meq/Kg)	75.97 ± 0.02	40.03 ± 1.26 ^d	31.97 ± 1.79 ^{bc}	36.17 ± 2.96 ^{cd}	19.90 ± 1.41 ^a	20.27 ± 1.25 ^a	***
Invertase (U/Kg)	33.70 ± 0.05	30.04 ± 0.01	51.18 ± 0.02	58.97 ± 0.01	12.39 ± 0.45	11.46 ± 0.01	ND
Color (Abs _{420nm})	0.87 ± 0.01 ^c	0.144 ± 0.03 ^e	0.101 ± 0.0 ^d	0.80 ± 0.02 ^d	0.77 ± 0.01 ^d	0.66 ± 0 ^a	***

Values with the same letter in each line do not differ significantly. The results are arranged in ascending order; a<b<c<d<e<f. SSD : statistical significance of the differences ; ** : highly significant (P<0,01), *** :very highly significant (P<0,001), ND : not determined

sugar) and 61.35 to 73.39% (total reducing sugar). The sugar composition depends highly on the type of flowers used by bees, as well as regional and climatic conditions. Sucrose content for all the samples fell within the limits of the Codex Alimentarius (5%), except the sample H06, which present a very high level of sucrose (13.54%); this can be due to an adulteration by an addition of sucrose to honey or an early harvest of honey responsible for the no full transformation of sucrose into glucose and fructose.

The analysed samples showed that protein contents ranged from 669.93 to 1635.48 µg/g; they are lower than those obtained in our previous study (3700-9400 µg/g) (Ouchemoukh *et al.*, 2007). Variations of the protein content can be attributed to the floral origin. During extraction and storage of honey, pH is of great importance, as it influences the texture, stability and shelf-life of honey (Bath and Singh, 1999). Values recorded for this parameter in this study ranged from 3.97 and 4.58. They indicate that the honeys tested were most likely of floral origin, since honeydew honeys generally have higher ash content than floral, resulting in honey with less active acidity and therefore a higher pH (Downey *et al.*, 2005). Bath and Singh (1999) reported that honey obtained during spring, is often more acidic than in autumn, when it usually contains more honeydew. Samples H01 and H04, which were collected in autumn, have the highest pH values, 4.58 and 4.41, respectively.

Values of free acidity are ranged between 6.80 and 12.50 meq/kg; lactonic acidity is ranged between 8.50 and 18.50 meq/kg, while total acidity varied from 16 to 30.50 meq/kg. Total acidity was within limits (below 50 meq/kg of honey), indicating absence of undesirable fermentation. The presence of organic acids, particularly gluconic acid in equilibrium with

lactones or esters, and inorganic ions, contribute to the honey acidity. The variation in acidity among different honey types may be attributed to variation in these constituents (Bath and Singh, 1999; De Rodriguez *et al.*, 2004).

Invertase activity of the honey samples varies from 5.45 to 75.97 U/Kg. These results are higher than those obtained by Serrano *et al.* (2007). The samples H05 and H06 purchased from the market have the lowest invertase activity (12.39 and 11.46 U/Kg, respectively); this can be the consequence of an eventual heating of these honeys. A significant correlation was observed between invertase activity and electrical conductivity ($r = 0.84$, $P < 0.05$). A similar correlation was also observed by Tsigouri and Passaloglou-Katrali (2000).

HMF measurement is used to evaluate the quality of honey; generally not present in fresh honey, its content increases during conditioning and storage. Then it can be useful to estimate the freshness of honey (Gonnet, 1993; Gidamis *et al.*, 2004; Zappala *et al.*, 2005) and since it is formed during acid hydrolysis of sucrose. The presence of high levels of this compound suggests the possibility that honey has been adulterated with invert syrup (Azeredo *et al.*, 2003; Sanz *et al.*, 2003). HMF was undetectable in the sample H01 indicating its high degree of freshness. H02, H03, H04 and H05 have an HMF content ranged between 11.04 and 34.53 mg/kg. These values were lower than the allowed maximum limit of 40 mg/kg as recommended by the Codex Alimentarius (2001). However, the commercial sample H06 has the highest HMF level (82 mg/kg), which confirm the heating of this honey. Our results are different from those reported by Makhloufi *et al.* (2007) (0, 5-124 mg/kg)

The color of honey is one of the factors

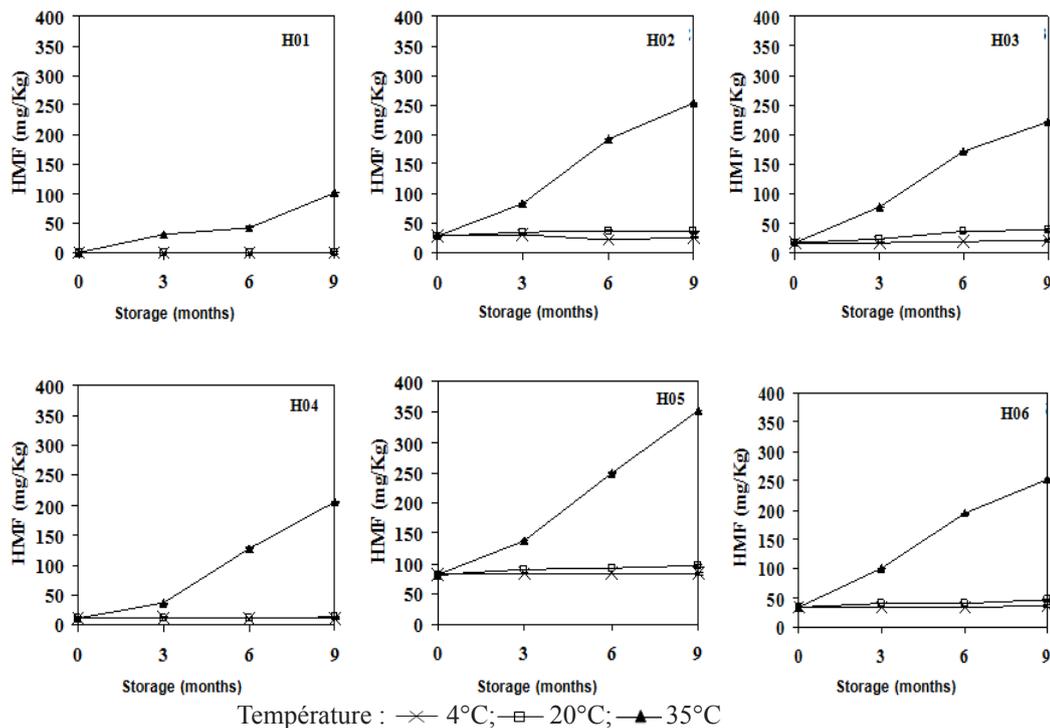


Figure 1. Effect of storage temperature and duration on HMF formation in the honey.

determining its price on the World market, and also its acceptability by the consumer (Gonzales *et al.*, 1999). The results obtained for this parameter varied from 0.663 to 1.443. Many studies (Alvarez-Suarez *et al.*, 2010 ; Terrab *et al.*, 2004) reported that honey color is an indicator of its mineral content, the higher the mineral content, the darker would be the color and vice versa. The light color of samples H05 and H06 was coherent with their low ash content, but this relation was not observed for the other samples. The color of honey can also reflect the pigments content (carotenoids, flavonoids, etc) (Amiot *et al.*, 1989).

Effect of storage

Several factors influence the formation of HMF, such as temperature, time of heating, storage conditions (Cordella *et al.*, 2005), and some chemical properties of honey (pH, total acidity, mineral content, quantity and type of reducing sugars) which are related to the floral source of honey (Ramirez Cervantes *et al.*, 2000; Gidamis *et al.*, 2004). According to Janzowski *et al.* (2000), at high concentrations, HMF can be cytotoxic, causing irritation to eyes and upper respiratory tract. The effects of storage duration on HMF formation at different temperature are illustrated in Figure 1. Storage at 4°C had no considerable effect on HMF formation.

Cherchi *et al.* (1997) did not observe significant changes in HMF in three types of honey even after a storage period of 24 months at refrigeration and

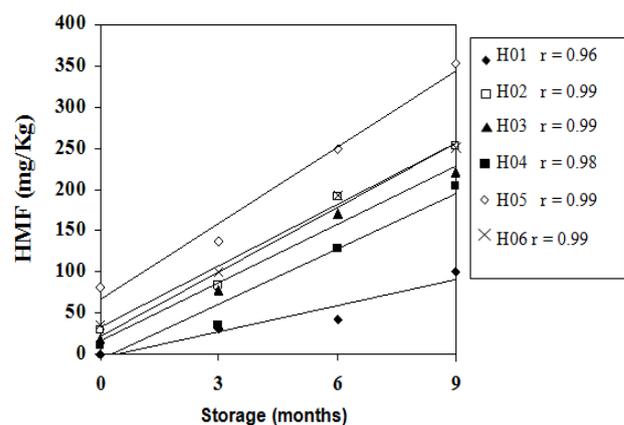


Figure 2. HMF formation as a function of storage duration at 35°C

ambient temperature. During the whole storage period at 20°C, the sample H01 had no detectable HMF content, while an important increase of HMF was noted in the sample H03 (17.87 to 38.98 mg/Kg), which was the most acidic sample (pH 3.97). Bath and Singh (1999) reported that honeys with low pH value produce more HMF during storage. The samples H02, H04, H05, and H06 presented a slight increase of HMF during storage at 20°C. At the highest storage temperature (35°C), a significant increase of HMF content was noted. After 9 months, HMF content varied from 100.84 (H01) to 353.09 mg/Kg (H05) and exceed largely the allowed limit (40 mg/Kg). The highest level of HMF observed in the sample H05 after storage, can be due to its high initial HMF content (82 mg/Kg) and its low pH

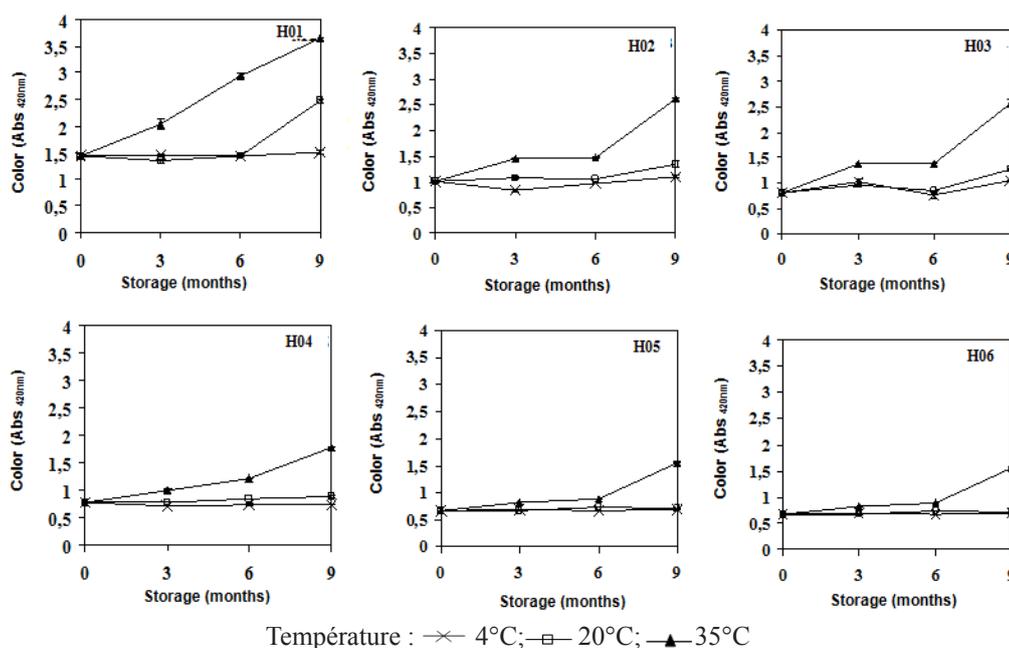


Figure 3. Effect of storage temperature and duration on the color of honey.

(4.11). Other factors such as high level of moisture and simple sugars (glucose and fructose) in honey can also offer favourable conditions for the HMF production (Fallico *et al.*, 2004). Linear regression models for the HMF production as a function of storage time at 35°C are reported in Figure 2.

The effects of storage duration on the color of honey at different temperature are shown in the Figure 3. Evolution of the samples color (at 20°C) was not very considerable, exception for the sample H02 which color value varied from 1.443 to 2.466. Storage at 35°C caused a high increase of the samples darkening, especially after 6 months of storage. Gonzales *et al.* (1999) who investigate the effect of storage at 37°C during 3 months on the color of some Argentinean honeys reported that the final color was strongly related to the initial color of honeys. In this study, the most pronounced darkening was observed in the sample H02, which was initially the darkest. A linear regression for the color evolution as a function of storage duration at 35°C is reported in the Figure 4.

Conclusion

The honeys investigated in this study are of acceptable quality standards as most of the quality parameters fall within the recommended limits. However, a few exceptions were observed. In fact, moisture values indicate that all the samples have a good degree of maturity, except the sample H01. Concerning the freshness of the samples, the high level of HMF and the low invertase activity of the two samples (H05 and H06) indicate that they have

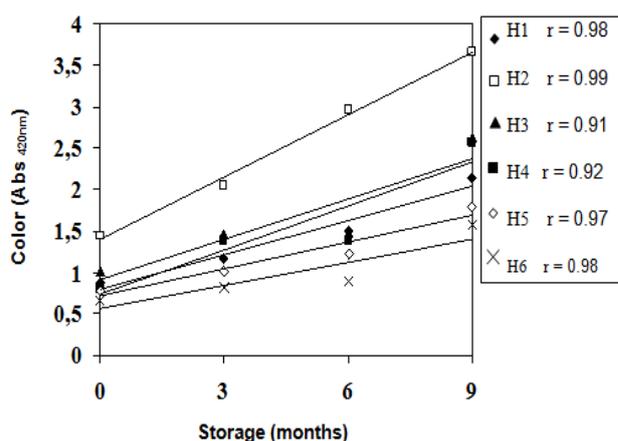


Figure 4. Color formation as a function of storage duration at 35°C

been probably heated. In addition to that, the sample H06 presents a very high level of sucrose (13.54%), which can be due to an adulteration. Ash content, pH and electrical conductivity indicate that the tested honeys were most likely of floral origin. The storage at 35°C accentuates HMF production, leading to loss of honey freshness. At the same time, the samples undergo a significant darkening due essentially to Maillard reaction. However, storage at 4 and 20°C didn't affect HMF content and color of honey. Storage temperature of honey should be carefully controlled (lower than 35°C) to preserve its chemical and sensory quality.

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