

## Effectt of Heating and Storage on Honey Hydroxy Methylfurfural and Diastase Activity

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**Abstract:** The purpose of this research was to assess the effect of temporary heating the quality characteristics of honey during storage. Honey samples obtain from Hatay were heated at 55, 60, 65°C for 6, 9, 15, 20, 30 minutes. Honey samples were left to room temperature (21 °C) and stored this ambient temperature for 29 weeks to be assessed. The changes in the diastase activity, hydroxyl methylfurfural (HMF) content were observed. Heating treatment applied to honey did not effect on diastase number except the effect storage time. Also, HMF content of honey samples were affected significantly from storage time and heating.

**Key words:** Honey, Diastase, HMF, Heating, Storage, Quality

### Introduction

The composition of honey is depend on produced season, origin of nectar and climatic conditions. The most important factor affecting honey composition is plant origin. The honey contains proteins, enzymes, water, carbon hydrate, acids, dextrin, ash, vitamins, pollen and substance of aroma (Crane, 1975; Yaniv and Rudich, 1996; Silici, 2002 and Fallico *et al.*, 2004). The contents of these components in honey are the most important quality criteria of honey and indicate some important deterministic quality properties of the honey sample (Table I). Honey having high water content is more likely to ferment. The mineral content of honey indicates the botanical origin. The blossom honeys have lower mineral content than honeydew honey. Honey storage has remarkable influence on increase in invert sugar content. A prolong storage period of honey causes invert sugar to increase (White *et al.*, 1961). Honey fermentation caused an increase in acidity because of this a maximum acidity value.

HMF levels and diastase testing, for measuring honey quality, have been in use for over 75 years (Fallico *et al.*, 2004 and White, 1994). Honey processing frequently requires heating both to reduce viscosity and to prevent crystallization or fermentation (Singh *et al.*, 1988 and Fallico *et al.*, 2004). The less HMF in honey is the better honey quality. Also, effects of HMF in honey depend on heat process after harvesting, storage time and pH of honey and storage temperature. Honey diastase activity is a quality factor influenced by honey storage, heating and thus, an indicator of honey freshness and overheating. Conductivity is a good criterion of the botanical origin of honey. Today it is determined in routine honey analysis instead of the mineral content. This measurement depends on the mineral and acid content of honey; when their content increases conductivity also increases (CODEX, 1993). There is a linear relationship between the mineral content and the electrical conductivity (Piazza *et al.*, 1991). A honey quality standards according to the draft CL 1998/12-S of the CODEX Alimentarius, to the draft 96/0114 (CNS) of the EU and TSE 90/3036 were given in Table I.

The main quality factors that are used in the honey international trade with its sensorial characteristics (that is flavour, colour and taste) are moisture, HMF content and diastase activity as well. The latter two being strongly influenced by heating and storage duration of this produce. The one that had the bigger importance during recent years in the international trade was the HMF content (Ramirez Cervantes *et al.*, 2000)

In the present study, HMF content and diastase level, moisture, invert sugar, sucrose, ash, proteins and pH were determined. The changes in the diastase activity and HMF content during heating at different temperatures. The changes in HMF contents and diastase activity of the samples were also examined after 29-week storage.

### Materials and Methods

**Honey Samples:** Samples of honey were used taken from Hatay provinces, during harvesting season. In fresh honeys, moisture, mineral content, acidity, diastase number, HMF, invert sugar, sucrose, proteins, pH and electrical conductivity were determined.

**Heating Treatment:** Honey was divided into 15 portions, each of those being then submitted to one of the treatments under study heating at 55, 60, 65 °C during 6, 9, 15, 20 and 30 minutes, respectively. The heated samples were then left room temperature. Periodically, that is once every 3-month, small samples were taken for analysis, for 29 weeks. All experiments were conducted in duplicated. The effect was assessed, of heating on changing the main quality characteristic during storage; diastase activity and HMF content.

**Analytic Methods:** The samples of honey were analysed by TSE (Institute of Turkish Standards)' s and the standard

methods of Association of Official analytical Chemists (AOAC, 1990). Moisture in honey was determined in a refractometer (Anonymous, 1990), proteins in honey were determined by using kjeldahl apparatus (AOAC, 1990) and ash percentage was measured by calcinations at 600 °C to ash furnace. Electrical conductivity depends on the ash and acid content of honey; the higher their content the higher the resulting conductivity. There is linear relationship between the ash content and the electrical conductivity (Piazza *et al.*, 1991):  $C = 0.14 + 1.74 A$  Where C is the electrical conductivity in mili Siemens  $\text{cm}^{-1}$  and A the ash content in g/100g.

Hydroxy methylfurfural (HMF) was determined calorimetrically after dilution with distilled water and addition of p-toluidine solution. Absorbance of the solution was determined 550 nm using 1 cm cell in an LKB-Biochrom Spectrophotometer. The acidity, 10 g of the honey samples were accurately dissolved in 75 ml  $\text{CO}_2$  free distilled water and titrated with 0.1 N NaOH, pH of the honey solution was measured by a pH meter. The diastase activity was determined according to Anonymous, 1990. Parameters obtained from the chemical analysis were compared to TSE, Codex and EU standards limits (Table I).

**Data Analysis:** Statistical analysis was made using repeated measure design and it was used SPSS 9.05 Statistical package in analysis of data (Bek and Efe, 1998 and Tabachnick and Fidell, 1996).

## Results

The means and ranges of values for each characteristic in honey are summarized and compared to TSE, CODEX and EU standards given in Table I.

Table 1: Biochemical characteristic of the honey.

Quality Criteria	Result of Analysis	TSE	CODEX	EU
Mineral Content (%)	0.131	$\leq 0.6$ (blossom) $\leq 1.0$ (honeydew)	$\leq 0.6$ (blossom) $\leq 1.0$ (honeydew)	$\leq 0.6$ (blossom) $\leq 1.2$ (honeydew)
Moisture Content (%)	15.23	$\leq 21\text{g}/100\text{g}$	$\leq 21\text{g}/100\text{g}$	$\leq 21\text{g}/100\text{g}$
Acidity ( $\text{meq kg}^{-1}$ )	32.3	$\leq 40$ $\text{meq kg}^{-1}$ (blossom) $\leq 40$ $\text{meq kg}^{-1}$ (honeydew)	$\leq 50$ $\text{meq kg}^{-1}$ (blossom) $\leq 50$ $\text{meq kg}^{-1}$ (honeydew)	$\leq 40$ $\text{meq kg}^{-1}$ (blossom) $\leq 40$ $\text{meq kg}^{-1}$ (honeydew)
HMF ( $\text{mg kg}^{-1}$ )	5.73	$\leq 40$ $\text{mg kg}^{-1}$ (blossom) $\leq 40$ $\text{mg kg}^{-1}$ (honeydew)	$\leq 80$ $\text{mg kg}^{-1}$ (blossom) $\leq 80$ $\text{mg kg}^{-1}$ (honeydew)	$\leq 40$ $\text{mg kg}^{-1}$ (blossom) $\leq 40$ $\text{mg kg}^{-1}$ (honeydew)
Diastase Level	17.9	$\geq 8$ (blossom) $\geq 8$ (honeydew)	$\geq 8$ (honeydew) $\geq 8$ (blossom)	$\geq 8$ (blossom) $\geq 8$ (honeydew)
Invert Sugar Content (%)	65.87	$\geq 65$ (blossom) $\geq 60$ (honeydew)	$\geq 65$ (blossom) $\geq 60$ (honeydew)	$\geq 65$ (blossom) $\geq 60$ (honeydew)
Sucrose (%)	6.54	$\leq 5$ (blossom) $\leq 10$ (honeydew)	$\leq 5$ (blossom) $\leq 10$ (honeydew)	$\leq 5$ (blossom) $\leq 10$ (honeydew)
Electrical conductivity ( $\text{mS}/\text{cm}^{-1}$ )	0.36	Blossom honeys excepted the honeys listed below and blends of honeydew and blossom honey Honeydew and chestnut honey, excepted the honeys listed below and blends with those	$\leq 0.8$ $\leq 0.8$	

Average moisture (%), mineral content (%), acidity ( $\text{meq kg}^{-1}$ ), diastase number, HMF ( $\text{mg kg}^{-1}$ ), invert sugar (%), sucrose (%), protein, pH and electrical conductivity ( $\text{mS}/\text{cm}^{-1}$ ) were found to be 15.3(%), 0.131 (%), 32.3 ( $\text{meq kg}^{-1}$ ), 17.9, 5.73 ( $\text{mg kg}^{-1}$ ), 65.87 (%), 6.4 (%), 6.54, 0.62, 0.36, respectively. The average moisture, mineral content, HMF, diastase number, acidity, invert sugar, sucrose and ash were in line with those given in TSE, CODEX, and EU standards.

**The Effect of Heating on Diastase Number :** The values of diastase number in different temperatures and different storage time are summarized in Table 2 and Fig. 1. During the storage, the changes of the diastase number in honey samples, according to fifteen treatments had similar behaviors as diminished continuously (Table 2). Diastase number was decreased from 18.53 to 14.53 at the end of third month. In addition, it was decrease from 18.53 to 4.53 (75.55 %) at the end of 29 weeks, according to storage time (Fig. 1)

Table 2: The effect of heating on Diastase Number

Temperature	27.08.2001 Diastase Number	21/12/2001 Diastase Number	18.03.2002 Diastase Number
55 °C – 6 Min.	17,9	13,9	5
55 °C – 9 Min.	17,9	17,9	2,5
55 °C – 15 Min.	17,9	13,9	2,5
55 °C – 20 Min.	23,0	13,9	5
55 °C – 30 Min.	17,9	13,9	8,3
Mean ( $\bar{x} \pm S_x$ )	18.92 $\pm$ 1.02	14.7 $\pm$ 0.8	4.66 $\pm$ 1.06
60 °C – 6 Min.	17,9	13,9	2,5
60 °C – 9 Min.	17,9	13,9	2,5
60 °C – 15 Min.	17,9	13,9	8,3
60 °C – 20 Min.	23,0	13,9	5
60 °C – 30 Min.	17,9	13,9	5
Mean ( $\bar{x} \pm S_x$ )	18.92 $\pm$ 1.02	13.9 $\pm$ 0	4.66 $\pm$ 1.067
65 °C – 6 Min.	17,9	13,9	2,5
65 °C – 9 Min.	17,9	13,9	2,5
65 °C – 15 Min.	17,9	13,9	5
65 °C – 20 Min.	17,9	13,9	2,5
65 °C – 30 Min.	17,9	13,9	2,5
Mean ( $\bar{x} \pm S_x$ )	17.9 $\pm$ 0	13.9 $\pm$ 0	3 $\pm$ 0.5
Unheated	17,9	13,9	10,9
Mean	18,5375	14,15	4,53125
% Decreased	-23,668	-67,977	-75,556

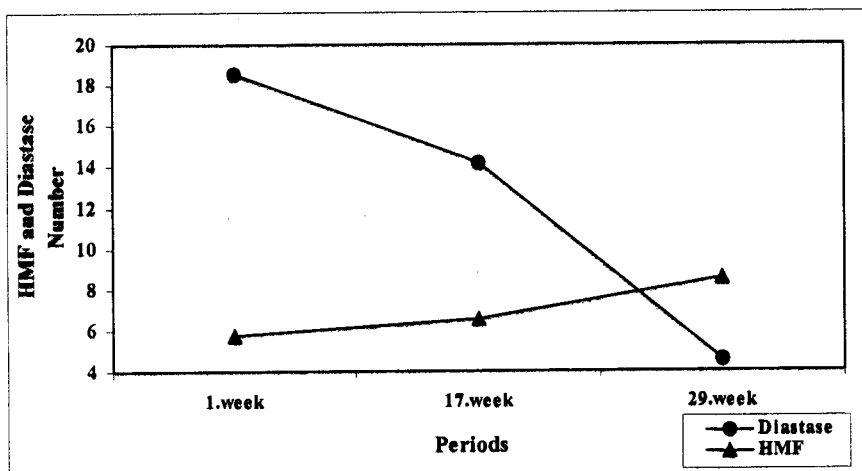


Fig. 1: Variation of HMF and diastase number by storage time (week).

When the mean diastase number of the honey samples from different storage time was found to be statistically significant ( $P < 0.005$ ) On the other hand, there was no significant ( $P > 0.005$ ) difference among the temperature of heating on diastase number.

**The Effect of Heating on HMF Content:** The initial content of HMF was low in the samples of the honey and the heating to which honey samples were growths depend on the storage time. During storage, the changes of the HMF content in the honey samples according fifteen treatments followed similar global behaviors, as HMF raised continuously during storage, although according to different ratios (Table 3; Fig. 1).

However, there was no significant difference among the temperature of heating on HMF. When the mean HMF content of the honey samples from different storage time and difference among the temperature of heating were found to be statistically significant ( $P < 0.05$ ).

HMF content was increased from 5.73 to 6.55 compare to initial value at the end of the third month. In addition it was increased from 5.73 to 8.52 (48 %) compared to the initial value at the end of 29 weeks according to storage time (Table 3; Fig. 1)

Analyzing the increasing dynamic of the HMF concentration during storage a first degree dynamics could be

established, taking into account a ratio of the type:

$$Kt = C / Co$$

**Table 3: The effect of heating on HMF**

Temperature	27. 08 .2001 HMF	21 . 12. 2001 HMF	18 .0 3.2002 HMF
55 °C – 6 Min.	5,6	4,99	6,4
55 °C – 9 Min.	4,4	4,8	7,2
55 °C – 15 Min.	4,9	5,76	8
55 °C – 20 Min.	5,9	6,91	8,48
55 °C – 30 Min.	5,2	5,76	8,8
Mean ( $\bar{x} \pm S_x$ )	5.2± 0.262	5.64± 0.372	7.77 ±0.437
60 °C – 6 Min.	5,2	5,95	8,15
60 °C – 9 Min.	5,6	6,34	8,64
60 °C – 15 Min.	5,8	6,53	9,28
60 °C – 20 Min.	7,3	6,72	9,6
60 °C – 30 Min.	5,2	7,29	9,6
Mean ( $\bar{x} \pm S_x$ )	5.82 ± 0.387	6.56± 0.221	9.054 ±0.286
65 °C – 6 Min.	6,5	6,72	8,48
65 °C – 9 Min.	5,8	7,29	8,8
65 °C – 15 Min.	6,5	7,48	9,6
65 °C – 20 Min.	6,3	8,06	9,6
65 °C – 30 Min.	5,2	7,88	10,4
Mean ( $\bar{x} \pm S_x$ )	6.06 ± 0.250	7.44± 0.221	9.376 ±0.337
Unheated	5,3	6,3	6,64
Mean	5,73125	6,5575	8,520625
% Increased	14,416	29,93	48,67

**Table 4: Correlation coefficients and constants ( of the first degree) of HMF forming during honey storage**

Temperature(°C) and Heating time (min)	Correlation Coefficient	Dynamic Constant, K
55°C-6 Min	0,495968	0,011430
55°C-9 Min	0,890007	0,015470
55°C-15 Min	0,944784	0,014633
55°C-20 Min	0,97866	0,012493
55°C-30 Min	0,895679	0,014421
Mean ( $\bar{x} \pm S_x$ )	0.8410± 0.0878	0.0137± 0.0007
60°C-6 Min	0,936317	0,013912
60°C-9 Min	0,918843	0,013245
60°C-15 Min	0,698534	0,010683
60°C-20 Min	0,698534	0,010683
60°C-30 Min	0,993829	0,014998
Mean ( $\bar{x} \pm S_x$ )	0.8959± 0.0510	0.01321± 0.0007
65°C-6 Min	0,875486	0,011340
65°C-9 Min	0,996292	0,012929
65°C-15 Min	0,957813	0,011998
65°C-20 Min	0,99904	0,012379
65°C-30 Min	0,994073	0,015529
Mean ( $\bar{x} \pm S_x$ )	0.9645± 0.0235	0.01282± 0.0007
Unheated	-0,65815	0,009128

Where C, is the concentration in HMF (in mg kg<sup>-1</sup>) after the storage time t, in weeks; Co, is the initial HMF concentration at the beginning of the storage; K, is the constant of first degree.

According to obtained K constants (Table 4) there is high correlation coefficients obtained. The increase of the HMF concentration might be related to the diminution of the fructose content (Ramirez Cervantes *et al.*, 2000), temperature and time of heating (Fallico *et al.*, 2004; Bath and Singh, 1999; Karabournioti *et al.*, 2001 and White, 1978) storage conditions; use of metallic containers (Fallico *et al.*, 2004; Cherchi *et al.*, 1997; Kubis and Ingr, 1998; Papoff *et al.*, 1995 and White, 1964) and chemical properties of honey, which are attributed to the floral source from where the honey has been extracted, these indicate pH, total acidity, mineral content (Anam and Dart, 1995; Bath and Singh, 1999; Singh and Bath, 1997, 1998) however, no information on the correlation between chemical characteristics and HMF level of honey

is available (Fallico *et al.*, 2004).

## Discussion

During the storage, the changes of the diastase number in honey samples, according to fifteen treatments had similar behaviors as diminished continuously (Table 2); the initial content of HMF was low in the samples of the honey and the heating to which honey samples were growth depend on the storage time (Table 3). This general qualitative data is similar to that found in other studies (White, 1964; Skowronek *et al.*, 1994; Ramirez Cervantes *et al.*, 2000 and Karabournioti *et al.*, 2001).

Yılmaz and Küfrevioğlu (1999), reported as the mean diastase level in honey samples following one-year storage ( $20 \pm 5$  °C) decreased from 14.6 to 10.7 in their study. Tharasyvoulou (1986) found that the average decrease in diastase level for 20 samples stored for one year at  $25 \pm 4$  °C was %40; while Sancho *et al.* (1992) reported a decreased of 33% in 115 samples after one year storage at 15-25 °C. In the present study, the 75 % average decrease in diastase activity found after storage for twenty-nine week was higher than those found for other honeys.

Yılmaz and Küfrevioğlu (1999), Tharasyvoulou (1986) and Sancho *et al.* (1992) reported as the means HMF contents (mg kg<sup>-1</sup>) increased (from 3.3 to 19.1; a from 0.0 to 8.8 and from 4.7 to 13.1) after one-year storage respectively in their study. The contents of HMF in the honey samples in our study showed similarity to those mention above.

In conclusion, heat treatment at 55 °C, 60 °C, 65 °C, during five studied temporary heating times (that is, 6,9,15,20 and 30 minutes), applied to honey exerted no significant effect on the diastase number except storage time (twenty-nine weeks). Also HMF content of honey samples were found statistical significant according to storage time and temperature heating. However, diastase number was decreased about 75%, HMF contents was increased about 48 % at the end of the 29 weeks.

Beekeepers strongly are advised not to do the faulty processes in harvest and storage of honey such as heating of honey and unsuitable storage conditions; use of metallic containers. In this way the better quality of honey appropriate to TSE, CODEX and EU standards would be produced by training the beekeepers on the importance of biochemical contents of honey for human health and export.

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