Research Article

Changes in HMF content and diastase activity in honey after heating treatment

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Abstract

Like any other food items which are placed for sale, honey is one of them; therefore, its freshness is a matter of concern. Beekeepers usually use heat crystallized honey to make it more captivating to consumers. In this process honey is heated to degrade fructose to hydroxymethylfurfural (HMF). Heating also deactivate diastase enzyme in honey. This research is focused on enzyme degradation and estimation of changes in HMF content of honey (multi-floral & acacia) due to thermal treatment performed at 4°C, 25°C, 35°C, 45°C and 55°C temperatures for 4 and 8 hours. Both these quality parameters are one of the important indicators for freshness, storage and proper heating of honey. The samples of Multi-floral honey were collected from beekeepers of distinct areas of Punjab. Results were subjected to Statistical analysis. It was found that except 4°C and 25°C, the other temperatures caused rising of HMF content and lowering in diastase activity with different ratio depending upon duration of heating time.

Keywords: Diastase activity; European bee; Pakistani honey; Thermal treatment

Introduction

Honey is a supersaturated liquid of sugar, particularly glucose and fructose, with small amounts of other compounds like enzymes, dextrin, volatile oils, waxes, organic acids, albuminoidal gum substances, ethers and other minerals [1, 2]. Vital quality parameters include Hydroxymethylfurfural or HMF which is a cyclic aldehyde (C₆H₆O₃) and is formed by the removal of water molecule from fructose and glucose in acidic medium and increases with temperature and storage time [3, 4]. HMF is present only in small amounts in freshly prepared honey more or less reaching to zero. However, it further

increases during the heating or storage processes. The formation of HMF mainly be influenced by the pH such as in the acidic nectar originated type honey it built more than in darker honeys with higher pH [5, 6]. Short time heat treatment causes only slight increase in HMF content even at higher temperature [7]. Additionally, a steady temperature dependent increase of HMF takes place upon storage of honey. Codex Alimentarius and EU standards set the maximum level of HMF is 40 mg/kg, while honey taken from the tropics and blends with them should not have HMF level more than 80 mg/kg. Beekeeping organizations of some

countries have set a maximum limit of 15 mg/kg for specially labelled “quality” or “virgin” honeys like Germany, Italy, Finland, and Switzerland. HMF can be used as a sign of honey quality and in all over the world recognized due to its capability to indicate the freshness or lack of freshness of honey [6].

Natural honey has various enzymes, which are either secreted directly by bee’s salivary glands or found in the nectar or pollen. They are responsible for the conversion of nectar and honeydew to honey. The enzymes are amylases, invertases, glucosidases, catalases, phosphatases and other [8]. Among them, diastase is one of the important enzymes. It is thermally stable, starch-digesting α and β -amylase enzymes. The α -amylase splits the starch chain and make dextrin’s, and the β -amylase separates the reducing sugar maltose from the ends of the starch chain [9]. This enzyme helps in conversion of starch to maltose and is added by bees during honey production [10]. Diastase is relatively resistant to heat and storage [11]. It is measured as Diastase Number (DN), which may be defined as “amount of enzyme, which will convert 0.01 gram of starch to the prescribed end-point in one hour at 40°C under the conditions of the test” [12]. It also serves as a sensitive indicator of the honey treatment and storage [4]. This research is aimed on studying the effect and variations caused due to heating treatment on thermo-labile compounds in honey (HMF and diastase) as honey freshness parameter.

Materials and methods

A total of 25 fresh Pakistani *Apis mellifera* honey samples were collected directly from beekeepers from different areas i-e Sargodha, Faisalabad, Toba tak singh and Shahkot of Punjab, Pakistan during September to December 2017. 200g samples were packed in air-tight jars and kept at 5 different temperatures (4°C, 25°C, 35°C, 45°C and

55°C) for 4 and 8 hours in a water bath to study the effect of temperature on its HMF content and diastase activity. After and during experiment samples were kept at room temperature (25°C). No preservative was added at any stage. Samples were diluted in distilled water at room temperature according to the requirements of the test. Both biochemical tests were performed in triplicate. Estimation of hydroxymethylfurfural in honey was followed by Winkler method [13]. In this method honey solutions are mixed with p-toluidine and barbituric acid solutions and the end color is measured against a blank in 1cm cuvettes at 550nm. The content of HMF was calculated as $HMF = 192 \times A \times 10 / \text{weight of honey in grams}$. The results were expressed as mg/Kg. The method used to determine diastase activity is based on actual work of Schade [14]. Results are stated in Schade units per gram of honey. The method based on the principle “the enzyme in the sample under standard conditions acts upon a standard solution of starch, capable of developing, with iodine, a colour in a defined range of intensity”. The decrease in the blue colour was measured at intervals. A plot of absorbance against time is used to determine the time t_x required to reach the specified absorbance, 0.235. The diastase activity is calculated as diastase number (DN) using this formula:

$$DN = \frac{60 \text{ minutes}}{t_x} \times \frac{0.10}{0.01} \times \frac{1.0}{2.0} = \frac{300}{t_x}$$

Statistical analysis

The data was statistically analyzed and tests were presented as mean $\pm$ SD. The results of these biochemical parameters were compared with standard values ANOVA (Table 1 & 2). Difference between means at 95% confidence level ($p < 0.05$) were considered statistically significant (Steel and Tori, 1981).

Table 1. Analysis of variance for HMF content

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Temperature	4	111768.354	27942.089	141.18**
Error	120	23750.680	197.922	
Total	124	135519.034		

** = Highly significant (P<0.01)

Table 2. Analysis of variance for Diastase number

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Temperature	4	467.175	116.794	62.01**
Error	120	226.003	1.883	
Total	124	693.177		

** = Highly significant (P<0.01)

Results and discussion

Hydroxymethylfurfural (HMF) content

For measuring honey quality HMF levels and diastase testing have been in use for over 75 years [15]. It is important to understand the effects of heat of pasteurization on aging and HMF levels due to importance of honey [16]. In current study HMF was well within the prescribed International limit at 4°C and 25°C after four and even eight hours heating. While increased in 35°C after eight hours heating and was closer to the prescribed standard. Rest of the two temperatures caused HMF content accumulation either close or beyond the set limit (Fig.1). The HMF content of 25 samples of *Apis mellifera* honey at 4°C ranged between 2.88-28.99mg/kg with a mean value of 14.43 ± 1.85 mg/kg lie within the Codex Draft value and had fresh honey characteristics. At 25°C the HMF content was between 26.7 to 49.6mg/kg with a mean value of 33.28 ± 1.05 mg/kg compared to 18% increase from 35°C and 200% from 4°C. All honey samples at 45 °C indicate raise of 77.7% from 35°C and 434% from 4°C. However, highest HMF content (96.83mg/kg ± 4.12) was found at 55°C among all the temperature ranges that was more 25.5% from 45°C, 190% from 25°C, 122% from 35°C, and 571% from 4°C. The result

obtained from the present study regarding HMF content accumulation with increasing temperature has also been reported by other authors. Microwave heating slightly influenced on hydroxymethylfurfural content in the tested honey samples in comparison with conventional heating approach depending upon heating time and temperature ranges [17]. [18] Found 2.3mg/kg to 17.8mg/kg kept at 20-25°C in Brazilian Pampa biome honey, 12.07 to 27.43 mg/kg by [19] in six Tunisian honeys kept at 20°C and 15.14mg/kg to 54.06mg/kg at 45 °C by [20]. These findings are in agreement with present studies. Changes in HMF were recorded about 9 times higher than non-heated honey after heating *acacia* honey at 100°C by [21, 22] reported HMF content of honey samples heated at 35°C ranged between 79.2mg/kg to 238.4mg/kg higher than observed in present study at same temperature. [15] also observed gradual increase in HMF level during heating treatment at 50°C, 70°C and 100°C. [23] determined HMF content in *Apis mellifera* honey samples ranged between 4-9mg/kg much lower than found in present study at same temperature. The low (2.63-2.85mg/kg) HMF content were reported by [3]. The overall increase of HMF content at

25°C is 130% from 4°C but did not exceeds the prescribed limit. So, honey samples at

room temperature seem to be of good quality i.e. $\leq 60\text{mg/kg}$ (Table 3).

Figure 1. Variation of HMF Content at different temperatures

Table 3. International standard for HMF content and diastase activity of honey

S. No.	Parameters	Codex draft 1999	Directive 2001/EC	EU draft 1999
1	HMF content (mg/kg)	≤ 60	≤ 60	≤ 40
2	Diastase Number (DN)	≥ 8	≥ 8	≥ 8

Diastase activity

The honey proteins consist of mainly enzymes, reviewed by White [24]. One of them is diastase which digests starch to form maltose. Its heat resistant property makes it important for indicating of honey quality and is used as meter of honey freshness. Value of 8 diastase units is set in the Codex Alimentarius and the European honey directive. Its deterioration depends upon storage and heat [25]. This enzyme activity in honey samples kept at 4°C recorded in the range between 10.7DN to 23DN with mean value of $14.07\text{DN} \pm 0.464$ (Fig. 2). [26] found diastase activity from 9°C to 23.3°C. Decline of 16% in enzyme activity has been recorded from 4°C to 25°C in present study (10DN-13.6DN) which was lower as compared to [23] at same temperature. Moreover, a few honey samples either didn't show any change or small loss in diastase activity as also

noticed by [27] at 25°C. The value of diastase activity was within the Codex Standards at 25°C i.e. $\leq 8\text{DN}$. [28] found DA in strawberry samples of honey with the mean value of 3.58 Go the degrees stored at room temperature ($20 \pm 3^\circ\text{C}$). Enzymatic activity mainly depends upon geographic and floral origins of the product, also on its freshness [29]. The DA range was 8.7-12.5DN, 8.5-10DN and 6.9-8.82DN at 35°C, 45°C and 55°C, respectively. So, the loss of enzymatic activity was 25%, 34% and 37% more compared to 4°C after 4 hour's thermal treatment. [23] reported 23-32DN range at 30°C. The declining behavior in activity expressed by diastase enzyme at higher temperature (55°C) in the present study has also been reported [3] where 61-70% loss of diastase activity was observed in honey samples heated at 55°C for 3-15 minutes. [30] compared the microwave heating and

conventional thermal approach to study the inactivation of diastase activity in Polish Honey. [31] Did not found that short term heating at 55, 65, 75°C for 5,15,20,25 minutes does not affect the diastase activity. [32] Found 5.73 to 16.64 DN kept at 4°C being lowest in citrus and highest in *zizipus* honey collected from Punjab and KPK

provinces of Pakistan. These DN values are lower than present investigation at same 4°C. [33] documented less DA (14.50DN) in *A. mellifera* honeys. Sajid *et al.* [34] quantified Diastase activity 34.39(DN) at room temperature in Pakistani honey from various floral sources.

Figure 2. Variation of DN at different temperatures

Conclusion

Many of the components of honey are unstable over time and are thermolabile. Therefore, it can be concluded that temperatures causes drop in enzymatic activity significantly. Moreover, high temperature also resulted in increase in HMF content making honey possibly unfit to consume.

Authors' contributions

Conceived and designed the experiments: S Qamer, Performed the experiments: M Sajid, Analyzed the data: T Yasmin, Contributed reagents/ materials/ analysis tools: F Asad & S Qamer, Wrote the paper: M Sajid & S Qamer.

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