

The Influence of Thermal Treatment on the Concentration of HMF in Honey

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Abstract: Honey is sweet, thick, syrup like, aromatic, semifluid or crystallized substance produced by bees after processing the flower nectar or nectar of living plants or other secretions (honeydew). Some insects collect it as food, enrich their secretions and transmit it in the honeycomb cells that cover the wax caps, and so preserve it of absorption, moisture and decay. According to the chemical composition, honey is a natural product that contains natural antioxidants. Many plants synthesize phytochemical substances with antioxidant activity, which are very important for the defence of the human body of radicals. Collecting nectar from such plants bees transferred bioactive components in between. Dark honey is richer in content of antioxidants. Honey is a convenient base for the formation of 5-hydroxymethylfurfuraldehyde (HMF), as it contains high concentrations of saccharide (mainly hexoses), has a low pH value (containing organic acid) and a low water content. Fresh honey has a low content of HMF, almost equal to zero. However, its concentration increases when honey is heated and is in its storage. If you expose samples of honey at the temperature of 60°C for a longer period of time, a significant increase in the concentration of HMF is present. Noticeable increase of HMF concentration is observed by heating the samples at 90°C for 60 minutes where the results show that the average concentration of HMF was 48.8 mg/kg. Therefore, the content of HMF in honey is an important parameter in determining the quality of honey, his age, antioxidant activity, as well as the loss of its nutritional value.

Keywords: HMF-Hydroxymethylfurfural, Aldehydes, Vitamins, Antioxidants, Fructose, Glucose

1. Introduction

Honey is the primary bee product that has the widest use. There are a number of other bee products, but most are used as additives to food, pharmaceutical and other products in order to improve the quality, reputation and price of these secondary bee products [1]. Honey is one of the few foods of pure natural food that has mineral ingredients in quantities that are sufficient to preserve the organic balance. Honey is also one of the few foods whose ingredients are not lost for a long time, which is not the case with fruits and vegetables.

He is also an outstanding broker-transmission link between the various vitamins [2]. The main ingredients of honey are: honey sugars, enzymes, minerals, water / in natural honey, alkaloids, organic acids, polyphenols, Maillard reaction products, hormones, vitamins, proteins, nitrogen compounds, various minor components [3]. There are several ways to classify honey. The quality of honey depends on the number

of different species of plants from which the nectar was harvested. Meadow and forest honey are among the best, because they are picked from more than hundreds of different plants [4]. The origin of honey in relation to plant species from where it has originated, is the most reliably determined through the analysis of the present pollen that is in more or less always in every honey. Certain characteristics, such as colour and flavour vary from year to year, or depend on the type of soil or other conditions [5]. The colour of honey may vary from almost colourless or pale yellow to dark brown or black honey. The most common colour is of amber and of different shades. Honey can be liquid, viscous, partially or fully crystallized. Smell and taste are different and are mostly conditioned by the botanical origin of honey. Processing and storage of honey can produce a series of reactions which lead to compounds that can affect health [6].

HMF is a substance is well known in food and chemical industries for many years, but the proportion of these

substances has become an indicator of heating and improper storage of honey. HMF is naturally present in honey, but its share in the fresh honey is very small and is below 1 mg/kg [7]. However, this share is growing rapidly, if the ambient temperature is above 20°C. HMF is not a harmful substance in amounts which are found in food. Many products based on sugar (syrup like juices) have levels of HMF that are 10-100 times higher than in honey. Many food products sweetened with fructose, e.g. soft drinks, caramels and other, can have HMF level between 100 and 1000 mg/kg. However, this share is growing rapidly if the ambient temperature is above 20°C. HMF is not a harmful substance in amounts which are found in food. Many products based on sugar (syrup like Occurrence and share of HMF in honey also depend on the type of honey, its pH-value, proportion of acids and moisture, and on exposure to light. HMF forms slowly in honey with a high pH. Forest honey has a higher pH than the meadow types of honey, so that this type of honey has a longer date of use with regard to the level of HMF. HMF is formed by dehydration of simple sugars (such as glucose and fructose) in acidic medium (pH 5 and below). HMF is broken down into levulinic and formic acid. The speed reaction itself is higher at an elevated temperature, and the speed increase is proportional to the temperature rise. The mechanism of formation of HMF from fructose is shown in Figure 1:

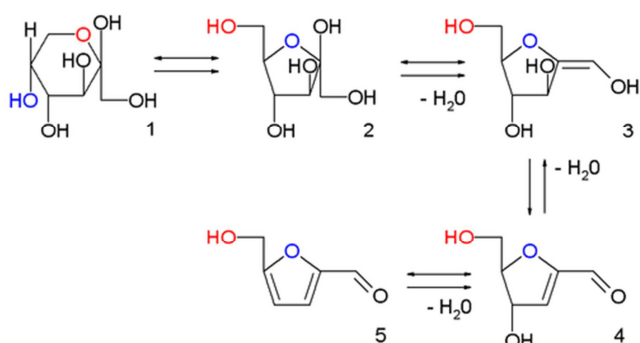


Figure 1. The mechanism of HMF from fructose: fructopyranose 1, fruktofuranose 2, two intermediate states that occur as a result of dehydration 3, 4 and the final HMF 5.

The activity of the enzyme diastase and HMF content are used as indicators of freshness and thermal processing of honey. HMF is a cyclic aldehyde widely present in the diet and has been identified in a variety of products. Can be converted to the 2, 5-dimethylfuran (DMF), which is a liquid biofuel, and in some cases better than ethanol [8]. With the oxidation of HMF furandicarboxylic acid is formed, which is suggested to replace the manufacture of terephthalic acid in polyester production. HMF is quickly produced during heating and storage of food rich in carbohydrates, and in some cases may be greater than 1 g/kg, for example, in nuts and candies. Larger amounts of HMF are naturally found in coffee and dried fruit [9].

Several types of roasted coffee contains between 300 - 2900 mg/kg of HMF. Prunes contain up to 2200 mg/kg of HMF, dark beer 13.3 mg/kg, bakery products between 4.1-151 mg/kg of HMF [10]. During toasting bread, the amount

increases to 14.8 (5 min) to 2024.8 mg/kg (60 min) [11].

HMF can be found in low amounts in honey, fruit juices, milk, as well as the breads, alcoholic drinks, vinegar, or biscuits. For example, fresh honey contains less than 15 mg/kg - depending on the pH value, temperature, age, type of honey, the proportion of acid, moisture, and light for exposure. Codex standard requires that honey has less than 40 mg/kg of HMF to guarantee that honey did not pass any kind of warming during processing. Except honey originating from countries or regions with tropical climate and temperature, it must be below 80 mg/kg [12].

HMF formation depends on the pH value of honey. In general, nectar honey has lower pH values. In the case of honey with a lower pH value, HMF is formed faster than those with higher pH-values. [13]. It can be formed into high fructose corn syrup (HFCS), the level of about 20 mg/kg of HMF, and increases during storage or warming. With many flavours, colours and other substances HMF may be formed with Maillard reactions, as well as during the crystallization time [14].

2. Materials and Methods

There are three main methods used for measuring HMF. The oldest of these is the method according to Winkler. This method is the spectrophotometric method used mainly in Germany. Another method is the White and the third method is the latest (HPLC), which is more popular and has been added to the list AOAC (Association of Official Analytical Chemists) [15]. Determination of hydroxymethylfurfural (HMF) in honey was done by the method according to White UV / VIS spectrophotometer "UVmini-1240V Shimadzu". The method according to White-in is widely used to determine HMF in honey samples. This method is recommended after HPLC analysis as the method of choice by IHC to determine the concentration of 5- (hydroxymethyl-) furan-2-karbaldehyda. Results are expressed in mg/kg [16]. Collected samples are of different botanical and geographical origin, from the Tuzla Canton and the wider area of Bosnia and Herzegovina and are available in retail in B&H. From the moment of taking up to analysis, samples of honey were stored in dark glass bottles with plastic closures. Before the analysis, samples of honey are well mixed or homogeneous. To analyze the content of HMF it was weighed 5 ± 0.001 g of honey in a glass and dissolved with 25 mL of distilled water. Honey solution is quantitatively transferred to a 50 mL flask. In such solution, 0.5 ml of Carrez I solution was added. The solution was homogenized, and then 0.5 ml of Carrez II and again homogenized by mixing. One drop of ethanol to prevent foaming was added, and then the residue is distilled with water supplement. Filtration of the solution was conducted through filter paper (blue bar). The first 10 ml of filtrate is discarded, then 5 ml pipette drops of the filtrate are added to two test tubes, in a one test tube 5 mL of distilled water is added (sample solution), and in the second tube 0.2 mL of 5% NaHSO_3 (reference solution). After that subsequent determination of absorption of the sample is performed relative to the reference solution at 284 nm

wavelength and 336 nm in a 10 mm quartz cell in 10, 15 and 30 minutes at 10°C, 40°C, and 60°C and 90°C. Samples whose absorbance at 284 nm exceeds a value of 0.6 is diluted with distilled water to a reference solution of 0.2% NaHSO₃ in the same way to obtain a lower absorbance value. The budget is based on the following routes:

$$HMF [mg/kg] = (A_{284} - A_{336}) \cdot 149,7 \cdot 5 \cdot D/W$$

Where is:

A₂₈₄- absorbance at a wavelength of 284 nm

A₃₃₆- absorbance at a wavelength of 336 nm

The value 149,7 is constant and is obtained on the basis of the next calculation:

$$149,7 = \frac{126 \cdot 1000 \cdot 1000}{16830 \cdot 10 \cdot 5}$$

Where is:

126- molecular mass of HMF-a

16 830-molar absorptivity of HMF at a wavelength of 284 nm

1000-converting g into mg

10-converting 5 into 50 mL

1000- converting g of honey into kg

5-conditional theoretical sample weight

D-dilution factor in case dilution is needed

W-sample weight of honey in g.

3. Discussion

The heat treatment of honey is used to facilitate loading and retention of the crystallization process. Speed crystallization of honey depends on temperature oscillations inside the room in which the honey is located. Hydroxymethylfurfural (HMF) is considered the most important degradation product of heating honey [17].

Since the temperature is a major factor that exerts its influence on the quality and chemical composition of honey, this study examined the effect of temperature on the content of hydroxymethylfurfural analyzed samples of clover honey from the territory of Bosnia and Herzegovina. The results showed that concentrations of HMF are different at different temperatures.

It is also important to take into account the time period during which the honey is exposed to a certain temperature. Therefore, analysis is performed of exposed honey or activity of the different temperatures and in different time intervals. In accordance with the pre-specified, the concentration of HMF was researched in the samples that were exposed to the temperature of the actions of 10, 40, 60 and 90°C at the intervals of 15 min, 30 min and 60 min.

Temperature between 10 to 14°C are considered as the most favourable temperatures which are important for extracting honey so it was necessary to examine the concentration of HMF at temperatures indicated, and then examine the effect of elevated temperature on the formation

of this compound.

Crystallization of honey is one of the indicators that the honey is pure, because it is not possible to imitate nature in this way. The period that passes from extracting honey to the first signs of crystallization varies depending on with which plants the bees collected the nectar and pollen. However, many people do not like to eat candied honey. To avoid this it is sufficient to heat to a temperature greater than 40°C on a water bath. After a short time honey will again become liquid and ready to use. From this aspect it is important to test the influence of the temperature 40 and 60°C at the HMF concentration, because the heating with the breaking of crystals may develop additional concentration of HMF. Honey is often used in addition to hot beverages (e.g., tea), where is exposed to high temperatures (about 90°C). This is one reason why it is important to examine the effect of temperature of 90°C on the concentration of HMF in honey. Changes of HMF in honey samples analyzed on standing at different temperatures at intervals of 15, 30 and 60 minutes are shown in figures 2, 3, 4 and 5.

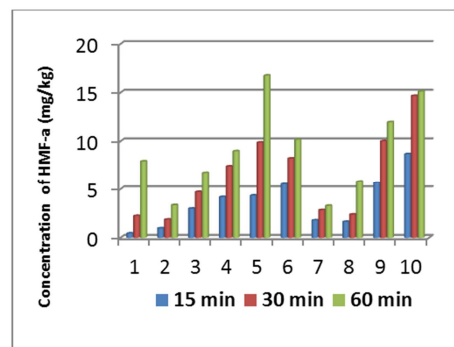


Figure 2. Changes of HMF concentration at the temperature of 10°C.

Initial concentrations of HMF in analyzed honey sample are less than the maximum permissible limit of 40 mg/kg-l. The lowest concentration of HMF was 0.449 mg/kg, while the highest concentration was 15.11 mg/kg in 15 min at 10°C. If honey is standing at 10°C for the time interval from 15 to 30 minutes the concentration of HMF increased to 44.93%. This increase of HMF concentration is slightly less for an interval of 30-60 minutes and is 31.55%, while for the time interval of 0-60 minutes the concentration of HMF increased by 60.65%.

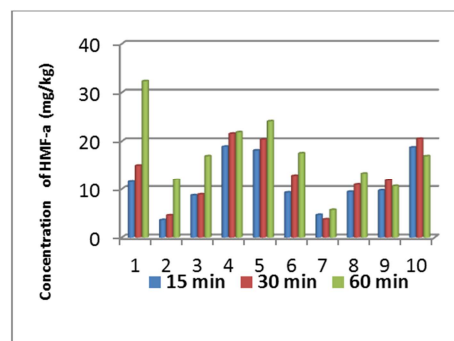


Figure 3. Changes of HMF concentration at the temperature of 40°C.

By exposing the honey at 40°C temperature over a period of 15 to 30 minutes the concentration of HMF increased by 11.38%. Increasing concentrations of HMF for a time interval of 30-60 minutes is slightly lower and is 22.62%. Total increase in the concentration of HMF for a period of 60 minutes at 40°C is 32.36%.

So, it takes a longer period of time, at 40°C, for concentration of HMF to be changed significantly.

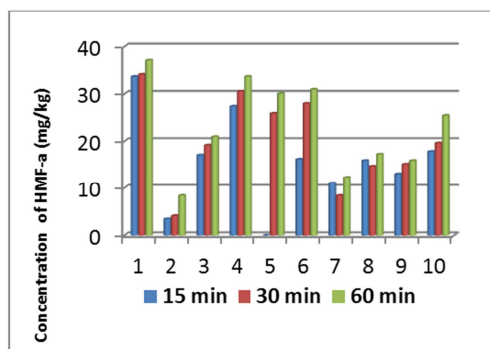


Figure 4. Changes of HMF concentration at the temperature of 60°C.

The highest concentration of HMF was 37.12 mg/kg in 60 minutes. Standing honey at the temperature of 60°C for a time interval of 15-30 minutes the concentration of HMF increased by 6.97%. For a time interval of 30-60 minutes the concentration of HMF increased by 17.46%. For the time interval of 0-60 minutes the concentration of HMF increased by 23.77%. So, only if you expose samples of honey at the temperature of 60°C for a longer period of time leads to a significant increase in the concentration of HMF.

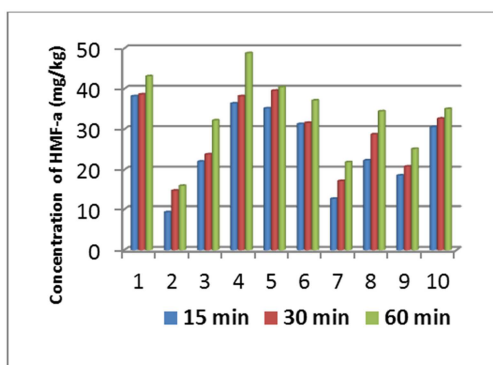


Figure 5. Changes of HMF concentration at the temperature of 90°C.

Noticeable increase of HMF concentration is observed by heating the samples at 90°C for 60 minutes where the results show that the average concentration of HMF was 48.8 mg/kg. This increase in the concentration of HMF to the time interval of 15-30 minutes is 5.78%, while the time interval of 30-60 minutes, the HMF concentration is increased to 14.46%. For the time interval 0-60 minutes the concentration of HMF increased by 25.60%.

4. Conclusion

Exposure of honey to high temperatures for a long period

favours the high content of HMF, which occurs as a result of dehydration of fructose. In addition to temperature even the time of storage of honey should be as small as possible, since the increase of HMF may be influenced by other factors than exposure of honey to adverse temperatures. Noticeable increase of HMF concentration is observed by heating the samples at 90°C for 60 minutes.

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