Analysis of Honey Color and HMF Content using a GENESYS UV-Visible Spectrophotometer

Daniel Frasco, Thermo Fisher Scientific, Madison, WI, USA

Introduction

Well known as a sweetener and baking ingredient, honey has been produced on an industrial scale in most countries around the world. Honey is made up of a complex mixture of carbohydrates, water, and a large number of minor components that can make it susceptible to adulteration. The components of honey can also be affected by excessive heating either during the manufacturing process or by inadequate storage conditions.[1–2] The determination of honey quality is an important factor for producers, regulatory authorities, and researchers. In this work, two key parameters for evaluating honey quality were examined using a Thermo Scientific™ GENESYS™ 50 UV-Visible Spectrophotometer: color and HMF content.

Honey color analysis

The color of honey is closely linked to its botanical origin and is an important parameter for evaluating honey quality. Honey color is generally related to its sensory properties such as flavor and odor and can give information on its floral source, mineral content, and storage conditions.[3] Honey color standard designations are expressed using the Pfund scale according to the USDA classification shown in Table 1.[4] The Pfund scale originated from the use of a color grader which measured honey based on the distance a wedge was moved to make a match, and is expressed in millimeters (mm). Honey color can be determined in the Pfund scale using a spectrophotometer by measuring the absorbance of a pure honey sample at 560 nm and multiplying by a factor of 3.15, using deionized water as a blank.

Table 1. Color designations of honey

USDA color standard designation	Color range Pfund scale (mm)	Sample result range
Water White	≤8	0 – 0.094
Extra White	> 8 and ≤ 17	0.094 – 0.189
White	> 17 and ≤ 34	0.189 – 0.378
Extra Light Amber	> 34 and ≤ 50	0.378 – 0.595
Light Amber	> 50 and ≤ 85	0.595 – 1.389
Amber	> 85 and ≤ 114	1.389 – 3.008
Dark Amber	>114	> 3.008

Honey samples measured against deionized water as a blank. The absorbance of the sample is measured at 560 nm and multiplied by a factor of 3.15 to compare to the Sample result range.

The color of various purchased honey samples was determined using a GENESYS 50 UV-Visible Spectrophotometer. A fixed method was configured in the GENESYS onboard control software with 560 nm as the wavelength and 3.150 as the Factor (Figure 1). With deionized water as a blank, five different honey samples were analyzed by transferring them directly into a 10-mm cuvette with care taken to avoid bubbles (Figure 2). The values obtained from each sample are presented in Table 2. The honey samples were then categorized by color based on where the sample result fell in the ranges described in Table 1. The purchased honey samples represented a range of colors from White to Light Amber.

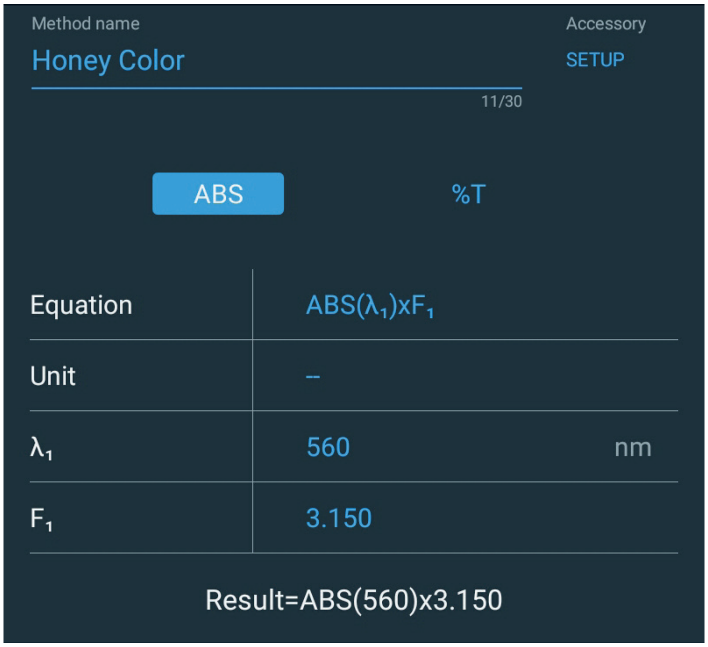

Figure 1. GENESYS honey color analysis method parameters

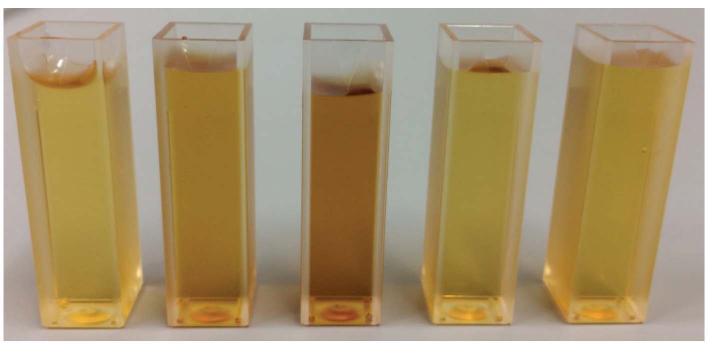

Figure 2. Honey samples in 10-mm cuvettes

Table 2. Calculated honey color designations

Honey sample	A_{560} x 3.150	Honey color
Honey 1	0.373	White
Honey 2	0.928	Light Amber
Honey 3	1.208	Light Amber
Honey 4	0.405	Extra Light Amber
Honey 5	0.512	Extra Light Amber

HMF content in honey

Hydroxymethylfurfural (HMF) occurs naturally over time in most honeys from the decomposition of fructose in acidic conditions (Figure 3). HMF content is often used as a measurement of honey quality as high levels in the honey may be a result of inadequate storage, adulteration with sugar additives, or severe heat treatments to decrease its viscosity.[5] Research has suggested HMF and its derivatives may have both positive and negative effects to human health.[2,6] The maximum amount of HMF that can be contained in honey is regulated internationally depending on the region (Table 3). The Codex Alimentarius Commission, an international organization formed to facilitate world trade, recommends the maximum amount of HMF to be 40 mg/kg, or 80mg/kg for honey from tropical climates. However this is intended for voluntary application by commercial partners and not enforced as a governmental regulation. The European Union established the same maximum amount of HMF as The Codex Alimentarius Commission. The United States currently has no legal limit for HMF concentration.

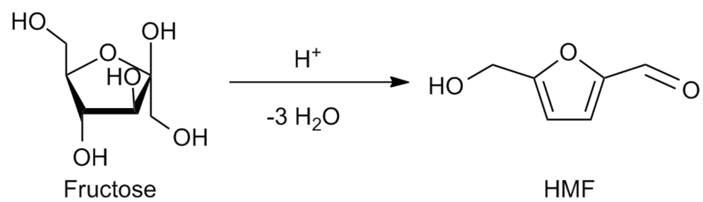

Figure 3. Formation of hydroxymethylfurfural from fructose

Table 3. Regulatory standards for maximum HMF in honey

Regulatory directive	Maximum value of HMF
Codex Alimentarius Commission (CODEX STAN 12-1981)	40 mg/kg, or 80mg/kg for honey from tropical climates
European Union (Directive 110/2001)	40 mg/kg, or 80mg/kg for honey from tropical climates
Korean Food Code	80 mg/kg

The amount of HMF in honey can be determined by the White Method established by the International Honey Commission as an acceptable HMF determination.[7] Using a GENESYS UV-Vis spectrophotometer with the White Method involves measuring the absorbance at 284 nm of a clarified aqueous honey solution against a reference solution of the same honey where the HMF chromophore was destroyed by bisulfite. The HMF content of honey is then calculated using the following equation:

$$\text{HMF (mg/kg of honey)} = \frac{(A_{284} - A_{336}) \times \text{Factor}}{W}$$

W = Weight in g of the honey sample

$$\text{Factor} = \frac{126 \times 1000 \times 1000}{16830 \times 10} = 748.66$$

126 = Molecular weight of HMF

16830 = Molar absorptivity of HMF at 284 nm

Honey samples were prepared by first weighing 5 g of honey into a 50-mL beaker, dissolving in 25 mL of water, and transferring quantitatively into a 50-mL volumetric flask. 0.5 mL of Carrez solution I (150 mg/mL potassium ferrocyanide) was added to the flask and mixed well. 0.5 mL Carrez Solution II (300 mg/mL zinc acetate) was then added and mixed. The sample was brought to a final volume of 50 mL with deionized water using a drop of ethanol to suppress foam formation. The sample was then filtered with the first 10 mL discarded. 5.0 mL of remaining filtrate was then transferred into each of two test tubes. 5.0 mL of water was added to one test tube and mixed. This served as the sample solution. 5.0 mL of a 0.2% sodium bisulfite solution was added to the second test tube and mixed. This served as the reference solution. Both solutions were transferred into individually labeled 10-mm quartz cuvettes.

A fixed method was selected in the GENESYS onboard control software and programed to measure each solution at 284 nm and 336 nm as displayed in Figure 4. Each sample solution was analyzed, making sure to blank the instrument with its corresponding reference solution before each analysis. The absorbance difference (284 nm – 336 nm) obtained by the spectrophotometer was then multiplied by the factor 748.66 and divided by the weight of the honey sample to determine the HMF content in honey (see above equation). Five honey samples were analyzed for their HMF content and the results are displayed in Table 4. Spectra of the samples were also obtained using the Scan method and are displayed in Figure 5. According to both the Codex Alimentarius Commission and European Union, only Honey 3 and Honey 5, which had HMF concentrations of 30.1 mg/kg and 38.3 mg/kg, met the HMF directive.

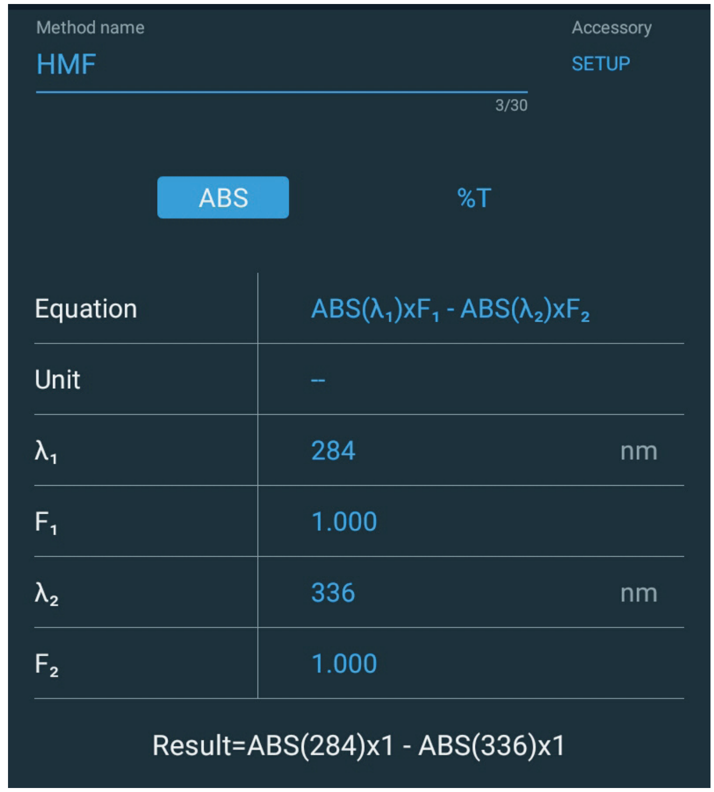

Figure 4. GENESYS HMF analysis method parameters

Table 4. Calculated HMF content in honey samples

Honey sample	Absorbance (284 nm – 336 nm)	HMF (mg/kg)
Honey 1	0.360	43.9
Honey 2	0.443	64.5
Honey 3	0.197	30.1
Honey 4	0.331	50.1
Honey 5	0.226	38.3

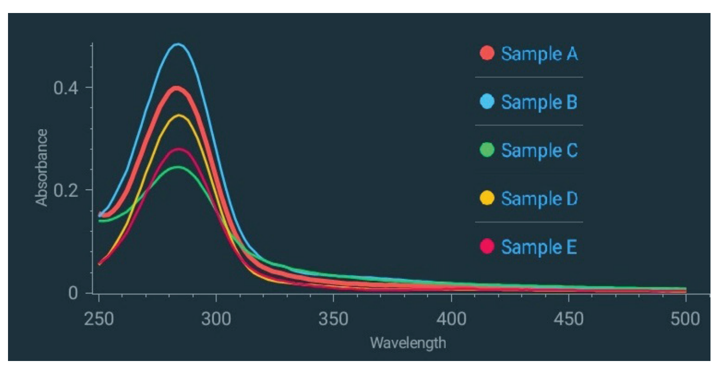

Figure 5. UV-Vis spectra of honey samples

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Conclusion

The Thermo Scientific GENESYS 50 UV-Visible Spectrophotometer is an effective tool to evaluate the quality of honey samples by determining both their color and HMF content. The onboard control software on the GENESYS spectrophotometer touchscreen enables the user to easily obtain absorbance values at multiple wavelengths and automatically apply calculations to simplify analysis.

Of the five honey samples analyzed, one was White, two were Extra Light Amber, and two were Light Amber according to the Pfund Scale. The same five samples were also analyzed using the White Method to determine their HMF content. Only two of the samples met the Codex Alimentarius Commission and European Union directive for maximum HMF content, which was likely due to heating or inadequate storage.

References

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