APPLICATION NOTE

UV/Visible Spectroscopy

Determination of Hydroxymethylfurfural (HMF) in Honey using the LAMBDA Spectrophotometer

Introduction

The level of Hydroxymethylfurfural (HMF) present is a key indicator as to the quality of honey. In this application note, the HMF content has been determined using the LAMBDA™ 465 UV/Vis Spectrophotometer and the Equation Calculation mode in the UV Lab™ software.

Principle

Normally, HMF is generated by the decomposition of fructose in acid conditions. It occurs naturally in most honeys and increases rapidly with heat treatment of honey. Therefore, it can be used as an indicator of heating and storage time at elevated temperatures. Good quality honey has a lower amount of HMF. Even though HMF is not a harmful substance, many countries restrict the maximum allowable amount of HMF in honey:

Korean Food Code: 80 mg/kg
EU Council Directive 2001: 40 mg/kg, or 80 mg/kg for honey sourced from hot climates

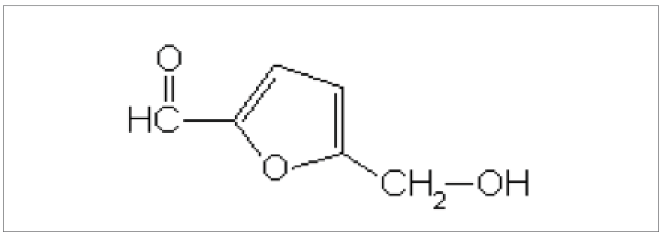

Figure 1. Hydroxymethylfurfural (HMF)

HMF can be determined by a method in which the absorbance of a clarified aqueous honey solution is determined against a reference solution of the same honey in which the 284 nm chromophore of HMF has been destroyed by bisulfite[1]. HMF content of honey is calculated using following equation:

$$\text{HMF (mg/100g honey)} = \frac{(\text{A284-A336}) * 74.87}{\text{W}}$$

Where :
W = wt of sample (g)
A284, A336 = absorbance reading

$$\text{Factor} = \frac{126 * 100 * 1000 * 100}{16830 * 1000} = 74.87$$

126 = MW of HMF
16830 = molar absorptivity of HMF at 284 nm

Reagents and Apparatus

1. Carrez solution I
   - Dissolve 15 g potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) in D.I water and dilute to 100 ml
2. Carrez solution II
   - Dissolve 30 g zinc acetate ($Zn(CH_3CO_2)_2 \cdot 2H_2O$) in D.I water and dilute to 100 ml
3. Sodium bisulfite ($NaHSO_3$) solution
   - 0.20% in D.I water
4. Honey 1, Honey 2
5. D.I water
6. LAMBDA 465 (PDA UV/Vis Spectrophotometer)
7. UV Lab Software
8. Cuvettes (10 mm pathlength)

Procedure

1. Dissolve honey (5 g: weighed to the nearest 1 mg in a small beaker) in D.I water (25 ml) and transfer all of the solution to a 50 ml volumetric flask (including washing the residue from the beaker with small amount of D.I water).
2. Add 0.5 ml Carrez solution I and mix.
3. Add 0.5 ml Carrez solution II and mix.
4. Fill the flask with D.I water (a drop of alcohol may be added to suppress surface foam).
5. Filter through paper rejecting the first 10 ml of filtrate.
6. Pipette 5 ml of filtrate into each of two test tubes.
7. Pipette 5 ml D.I water into one (sample) and 5 ml 0.20% bisulfite into the other (reference).
8. Mix well using vortex mixer.
9. Measure the absorbance of the sample against the reference at 284 nm and 336 nm.

Instrument Parameters

The instrument parameters of the LAMBDA 465 are as follows. Figure 2 shows experimental method.

Experiment Setup

Data type:	Absorbance
Sampling:	Single cell
Mode:	Scan no.: 30, Integration no.: 1

Equation Calculation

Equation Name:	HMF
Equation Unit:	mg/100 g
Wavelength:	284 nm, 336 nm
Equation Expression:	(Wave1 - Wave2) * 74.87 / 5

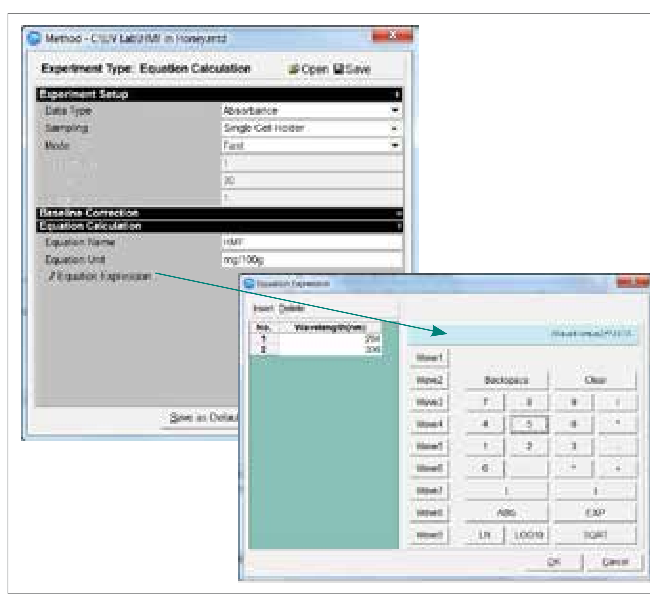

Figure 2. Experimental method

Result

Figure 3 shows the spectra of honey samples. The absorbance values and HMF content of each sample are shown in Table 1. The calculated HMF content per 100 g of honey sample is 6.3625 mg (Honey1) and 1.6981 mg (Honey2).

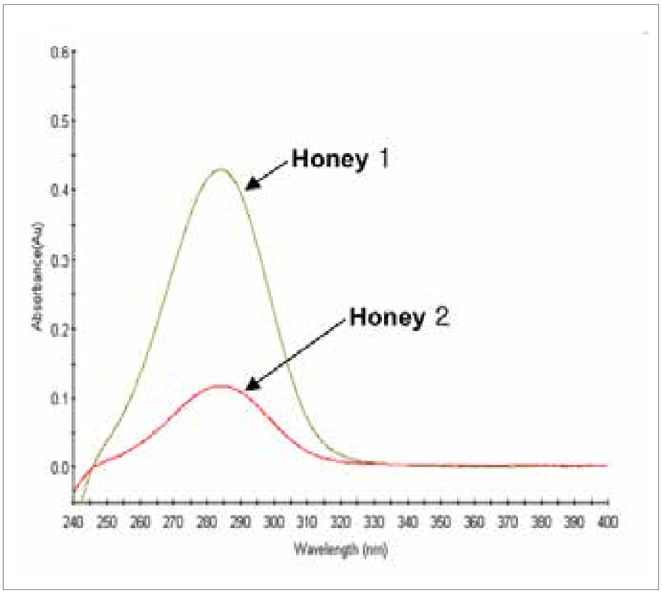

Figure 3. Spectra of Honey

Table 1. HMF contents of honeys in mg/kg.

Name	HMF (mg/kg)	284 nm	336 nm
Honey 1	63.625	0.4295	0.0046
Honey 2	16.981	0.1177	0.0043

Conclusion

Honey quality was determined by measuring the HMF content using the LAMBDA 465 spectrophotometer and UV Lab software. The test showed that Honey 2 shows lower levels of HMF compared to that of Honey 1. Honey 1 exceeds the EU limits for HMF.

The Equation Calculation mode in UV Lab allows the HMF content to be determined easily and rapidly. Using the LAMBDA 465 allows data to be quickly collected over the full wavelength range from 190 nm to 1100 nm.

Reference

1. White, J W (1979) Spectrophotometric method for hydroxymethylfurfural in honey. J Assoc Off Anal Chem Vol 62 509-514

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