Application Note: 51864

The Determination of HMF in Honey with an Evolution Array UV-Visible Spectrophotometer

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Key Words

- Equation Calculator
- Food Quality
- Honey
- Hydroxymethyl-furfural (HMF)
- UV-Visible Spectroscopy

Introduction

Hydroxymethylfurfural, or HMF, is an aldehyde that is often used as an indicator for the quality of honey. HMF, as shown in Figure 1, is generated by the decomposition of fructose in acidic conditions. It occurs naturally over time in most honeys; however, high levels of HMF may be the result of inadequate storage, adulteration with sugar additives, or severe heat treatment.[1] Although HMF is not thought to be a harmful substance, food standards in many countries regulate the levels of HMF in honey. Korean and European Union regulatory standards are shown in Table 1. In this application note, the HMF content of honey is determined by the White Method using a Thermo Scientific Evolution Array UV-Visible spectrophotometer.[2]

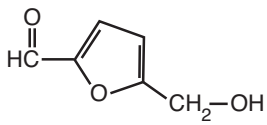

Figure 1: Chemical Structure of Hydroxymethylfurfural (HMF)

Regulatory Directive	Maximum Levels of HMF
EU Council Directive 2001 (2001/110/EC)	40 mg/kg, or 80 mg/kg for honey from tropical climates
Korean Food Code	80 mg/kg

Table 1: Regulatory standards for HMF levels in honey

Experiment and Results

The absorbance of a clarified aqueous honey solution was measured against a reference solution of the same honey in which the 284 nm chromophore of HMF was destroyed by bisulfite. The HMF content of honey was then calculated using the following equation:

$$\text{HMF (mg/100 g of honey)} = \frac{(A_{284} - A_{336}) \times \text{Factor}}{W}$$

Where: W = wt of sample in grams

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

And: 126 = the molecular weight of honey
16830 = the molar absorptivity of HMF at 284 nm

Five grams of honey sample was dissolved in 25 ml of deionized water. 0.5 ml of Carrez Solution I (150 mg/ml potassium ferrocyanide) was added to the sample and mixed well. 0.5 ml of Carrez Solution II (300 mg/ml zinc acetate) was then added to the sample and mixed well. The sample was brought to a final volume of 50 ml with deionized water using a drop of alcohol to suppress surface foam. The sample was filtered and the first 10 ml of filtrate was discarded. 5 ml of the remaining filtrate was transferred into each of two test tubes. A reference sample was prepared by adding 5 ml of 0.20% sodium bisulfite to one test tube of filtrate. A test sample was prepared by adding 5 ml of deionized water to the other test tube of filtrate. Both samples were mixed well with a vortex mixer. The absorbance of the test sample was measured against the reference sample at 284 nm and 336 nm using the experimental method parameters as shown in Figure 2.

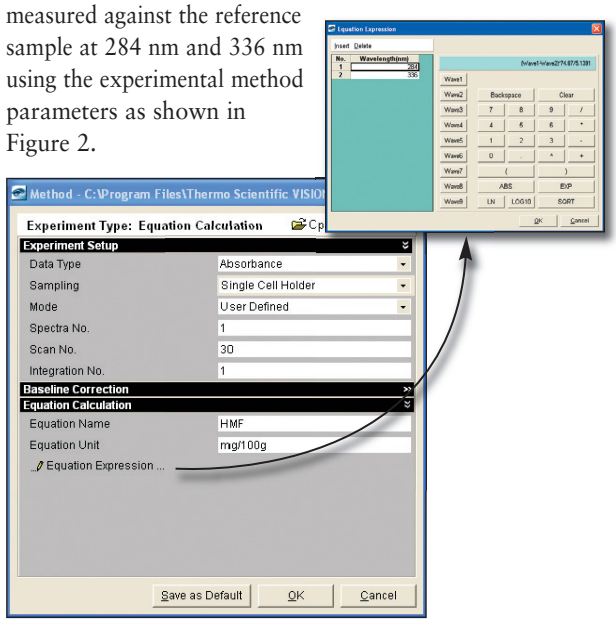

Figure 2: Method Parameters

Two different samples of honey were measured using this procedure. The spectra of both honey samples are shown in Figure 3. The HMF content of honey was found to be 3.3635 mg/100 g for Honey 1 and 1.6981 mg/100 g for Honey 2 as shown in Table 2.

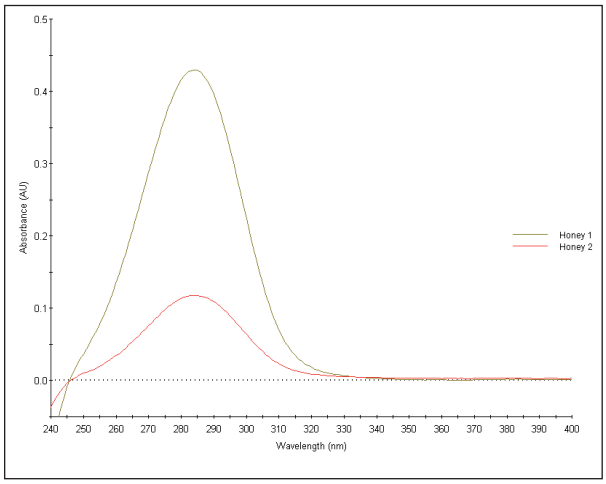

Figure 3: Spectra of honey samples

Sample	HMF (mg/100 g)	284 nm	336 nm
Honey 1	6.3625	0.4295	0.0046
Honey 2	1.6981	0.1177	0.0043

Table 2: Calculated HMF content in honey samples

Conclusion

The equation calculation mode in Thermo Scientific VISION*collect* software enables the user to calculate the HMF content of honey quickly and easily. The HMF content of Honey 2 was found to be lower than that of Honey 1. Both honey 1 and 2 meet the Korean directive for the maximum level of HMF, while only Honey 2 meets the EC directive.

References

1. Moralles, V., et al. (2009) *Combined use of HMF and furosine to assess fresh honey quality*. Journal of the Science of Food and Agriculture, Vol. 89, 8:1332-1338.
2. White, J.W. (1979) *Spectrophotometric method for hydroxymethylfurfural in honey*. Journal of the Association of Official Analytical Chemists, Vol. 62, 3:509-514

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