Karamanoğlu Mehmetbey Üniversitesi
Mühendislik ve Doğa Bilimleri Dergisi
Karamanoğlu MehmetbeyUniversity
Journal of Engineeringand Natural Sciences
e-ISSN: 2687-5071© KMUJENS

DOI: 10.55213/kmujens.1529151 Research Article (Araştırma Makalesi) 6(2): 41-45 (2024)

The Use of HPLC-DAD Method in Determining the Quality of Honeys: 5-Hydroxymethylfurfural (HMF) Analysis

Merve ÖZBAY[1*], Fatma Nur ARSLAN[2], Gazi GÖRÜR[3]

[1] University of Karamanoğlu Mehmetbey, Faculty of Applied Sciences, Department of Gastronomy and Culinary Arts, Karaman, Turkiye.
[2] University of Karamanoğlu Mehmetbey, Kamil Özdağ Faculty of Science, Department of Chemistry, Karaman, Turkiye
[3] University of Niğde Ömer Halisdemir, Faculty of Science and Art, Department of Biotechnology, Niğde, Turkiye

Alındı/Received: 06/08/2024; Kabul/Accepted: 19/09/2024;Yayın/Published: 04/12/2024

! This study is produced from a part of Dr. Merve OZBAY's PhD thesis data.

[*] *Corresponding author e-mail:mozbay@kmu.edu.tr*

Abstract

The recognition of 5-hydroxymethylfurfural (HMF) content in honeys has been the subject of many scientific studies as it is a significant indicator of the toxicity, its processing method, storage situations and age. In this research, the amount of HMF in 95 different flower and honeydew honey samples obtained from markets, factory sales offices and local beekeepers in Turkiye was determined. High performance liquid chromatography (HPLC) method was employed to recognize HMF contents. Before sample analysis, the standard HMF solutions were prepared at different concentrations and method validation studies were carried out. The HMF content limit value of flower and honeydew honey in the Honey Communiqué (TFC/Communiqué No:2020/7) is stated as 40 mg.kg^{-1}. According to the obtained results, the HMF contents in honey samples were found as 0.00-16.65 mg.kg^{-1}. It was determined that the amounts of HMF were at the highest level in monofloral milk vetch honey (MH_MV) samples coded MH_MV3 and MH_MV4. Therefore, it was concluded that all of the honey samples were compatible with the Honey Communiqué in terms of HMF and had appropriate honey quality values.

Keywords: Floral honey, Honeydew honey, 5-hydroxymethylfurfural, HPLC

Balların Kalitesinin Belirlenmesinde HPLC-DAD Metodunun Kullanımı: 5-Hidroksimetilfurfural (HMF) Analizi

Öz

Baldaki 5-hidroksimetilfurfural (HMF) içeriğinin tespiti balın toksititesinin, işlenme şeklinin, saklama koşullarının ve yaşının önemli bir göstergesi olduğu için birçok bilimsel araştırmanın konusu olmuştur. Bu çalışmada Türkiye'deki marketlerden, fabrika satış ofislerinden ve yerel bal üreticilerinden temin edilen 95 farklı çiçek ve salgı balı numunesinin HMF içeriği belirlenmiştir. HMF içeriklerinin tespitinde yüksek performanslı sıvı kromatografi (HPLC) yöntemi uygulanmıştır. Numune analizleri öncesinde farklı konsantrasyonlarda standart HMF çözeltileri hazırlanmış ve yönteme ait metot validasyon çalışmaları gerçekleştirilmiştir. Bal Tebliğinde (TGK/Tebliğ No:2020/7) çiçek ve salgı ballarında HMF içeriği sınır değeri 40 mg.kg^{-1} şeklinde belirtilmektedir. Gerçekleştirdiğimiz analizlerden elde edilen sonuçlara göre numunelerdeki HMF değerleri 0.00-16.65 mg.kg^{-1}şeklindedir. MH_MV3 ve MH_MV4 kodlu monofloral geven balı numunelerinde HMF değerinin en yüksek düzeyde olduğu belirlenmiştir. Sonuç olarak incelenen bal numunelerinin tümünün HMF açısından tebliğ ile uyumlu olduğu ve uygun bal kalite değerlerine sahip oldukları kanaatine varılmıştır.

Anahtar Kelimeler: Çiçek balı, Salgı balı, 5-hidroksimetilfurfural, HPLC

Atıf / To cite: Özbay M, Arslan FN, Görür G (2024). The Use of HPLC-DAD Method in Determining the Quality of Honeys: 5-Hydroxymethylfurfural (HMF) Analysis. Karamanoğlu Mehmetbey University Journal of Engineering and Natural Sciences, 6(2): 41-45.

1. INTRODUCTION

Honey is one of the most complicated nutritions fabricated by honeybees from the plants and nectar of diverse honeydews. It is a sweetener and could be employed by a human devoid of procedure (Das et al. 2022; Elhamdaoui et al. 2020). Honey is a mixture of many different types of components which mainly organic acids, sugars (fructose

and glucose), minerals, enzymes and water (International Honey Commission 2009; Lewkowski 2001; Kurtagić et al. 2021). Meteorological, climate, entomological and floral issues all impress the compound and feature of honey (El Sohaimy et al. 2015; De Almeida et al. 2016; Samarghandian et al. 2017). Also, the compound of honey is remarkably controlled by the time, temperature and other conditions of storage (Islam et al. 2012; Mehryar et al. 2013; Das et al. 2022; Chua et al. 2012; Sabireen et al. 2020). Physico-chemical features of the honeys like the contents of sugar, water, HMF, acidity, amino acid, electrical conductivity, mineral, vitamin, organic acid, proline, protein, enzyme activity and organoleptic features are established by common quality standards like EU regulation; however, as markers the contents of HMF, sucrose, the ratio of fructose to glucose draw attention for honey quality (Jandrić et al. 2017; Das et al. 2022).

HMF has a furan ring skeleton, and it has a carbaldehyde group at the site of the 5-hydroxymethyl skeleton (Kurtagić et al. 2021; Lewkowski 2001; PM da Silva et al. 2016). It is almost not existing in fresh foods; however, it is naturally forming in some types of foods including sugars (particularly ketosis and aldoses), and also in cooked and baked foods (Vorlová et al. 2006; Shapla et al. 2018; Kurtagić et al. 2021). HMF is derived from carbohydrates (like glucose, fructose and sucrose) during Maillard reaction based on acid catalytic hydrolysis and dehydration steps. Newly fabricated honey samples have extremely little HMF, and its limit should be 40 $mg.kg^{-1}$ to Codex Alimentarius Commission (Bastos et al. 2012; Das et al. 2022; Ruiz Matute et al. 2010).

The temperature range of 32-40°C during fabrication process doesn't affect the quality of honey. However, HMF tends to increase when honey is heated above 60°C (Shapla et al. 2018). The higher amount of HMF depicts the dishonesty of honey quality owing to fabrication faults mostly heating over 60°C to make stronger viscosity and get rid of fermentation/solidification, inappropriate storage situations, the adding of adulterants like sugar solution and aging (Shapla et al. 2018; Das et al. 2022).The content of HMF in honeys have an indicator role for storage or shelf life of them (Kurtagić et al. 2021).

Recent studies have proven the carcinogenic, genotoxic and cytotoxic impacts of HMF, disclosing the need of recognizing its amount in honeys (Sabireen et al. 2020; Glatt et al.2012; Pastoriza et al. 2017). For the recognition of HMF amount in foods, different methods are used (International Honey Commission 2009; Shapla et al. 2018; Zappala et al. 2005; Makawi et al. 2009; Kurtagić et al. 2021). Herein, the HMF content of 95 different flower and honeydew honey samples obtained from markets, factory sales offices and local honey producers in Turkiye was determined by the validated HPLC method.

2. MATERIAL AND METHOD

2.1. Chemicals and Standards

Methanol (HPLC-Grade), ultrapure water (HPLC-Grade) and 5–(Hydroxymethyl) furfural standard used in the study were purchased from commercial suppliers and used directly without any purification process.

2.2. Samples

95 honey samples including 12 kinds of monofloral honey (MH) samples [*n=55honeys; thyme (MH_TV1 to TV6), acacia (MH_A1 to A2), carob (MH_C1 to C5), chestnut (MH_CH1 to CH6), black cumin (MH_BC1 to BC4), milk vetch (MH_MV1 to MV7), sunflower (MH_S1 to S3), citrus (MH_CF1 to CF8), rhododendron (MH_R1 to R3), thistle (MH_T1), linden (MH_L1 to L5), lavender (MH_FL1 to FL5)*],20 polyfloral honey (PH) samples obtained from 2019–2020 seasons(n=20; PH_1 to 20) and 2 kinds of honeydew honeys (HH) [*n=20honeys; honeydew (HH_H1 to H14) and oak (HH_O1 to O6)*] were used. All samples were purchased from the local markets, factory outlets and local beekeepers, and they were stored at +4°C in a cabinet away from daylight.

2.3. HMF Analysis by HPLC

An Agilent 1260 Series HPLC equipped with a DAD system was used for the recognition of HMF content. The method was optimized and validated by us in our laboratory. 5 mL of ultrapure water was added to1 g sample and this solution was passed through 0.45 µm-membrane filter, and 5 µL of sample was injected into the HPLC system. A Novapak C_{18} column (30×0.39 cm×4 µm) was used.

The method comprised of a mobile phase of methanol:water (10:90, v/v) with a flow rate of 1.0 $mL.min^{-1}$. The temperature of column was set as 25 °C. The peak of HMF was recorded at λ_{Abs}=285 nm. The standard HMF solutions in different concentrations (0.1, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0ppm) was used and method validation studies were carried out based on the parameters of specificity, recovery, linearity, limits of detection (LOD) and quantification (LOQ). The findings of HMF content in honeys were reported as mg $HMF.kg^{-1}$ honey.

3. RESULTS

The chromatogram of HMF standards used for the calibration graphics shown in Figure 1. As depicted in Figure 1, the peak of HMF was eluted in 11.32 ±0.01 min and the linearity of method was assessed from the calibration curve at seven concentration levels. The method performance was validated based on the statistical parameters of linearity, sensitivity, precision and accuracy (Table 1).

The findings in this table clearly show the method to be sensitive and accurate for the recognition of HMF content. The linear calibration graph was constructed by the peak areas versus the concentrations of HMF. The correlation coefficient (R^2) was found as 0.99, and the accuracy of method was 96.90%. The %RSD for HMF was estimated about 0.12%, and the sensitivity of HPLC method (LOD and LOQ) was also computed as 0.86 and 2.61$mg.kg^{-1}$

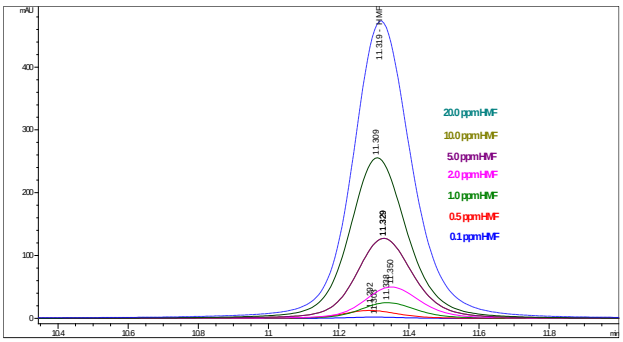
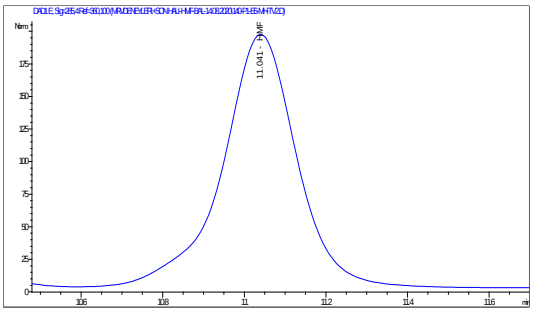


Figure 1. HMF chromatogram of the HMF standards in different concentrations

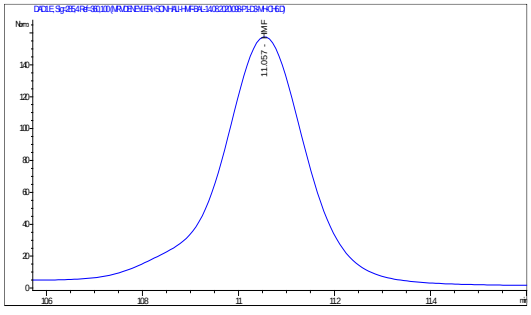
Table 1. Method validation results for HMF detection

Method validation study			
linearity	**Calibration**	**t_R±SD (min)**	11.32 ±0.01
		working range (mg.kg^{-1})	0–20
		equality	y=290.24+26.922
		R^2	0.99
		RSD, %	0.12
accuracy		**regain, %**	96.90
sensibility		**LOD (mg.kg^{-1})**	0.86
		LOQ (mg.kg^{-1})	2.61

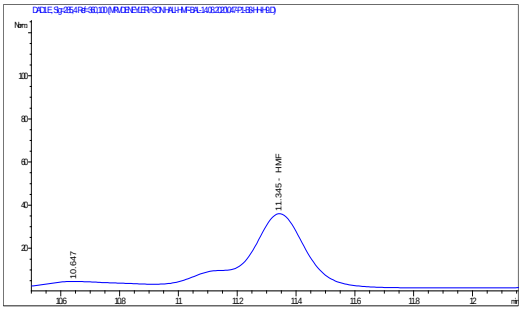
The chromatograms of some honey samples are illustrated in Figure 2, and the HMF contents of all samples are given in Table 2.



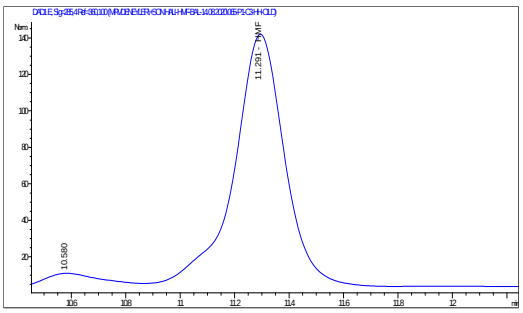
(a)



(b)



(c)



(d)

Figure 2. HMF chromatogram of honey samples **(a)** MH_TV2, **(b)** MH_CH6, **(c)** HH_H9 and **(d)** HH_O1

Table 2. HMF contents of honey samples, mg.kg^{-1}

Monofloral Honey (MH) Samples	**HMF content (mg.kg^{-1})**
MH_A1	nd*
MH_A2	nd*
MH_BC1	nd*
MH_BC2	nd*
MH_BC3	nd*
MH_BC4	nd*
MH_C1	0.76 ±0.00
MH_C2	nd*
MH_C3	1.52 ±0.00
MH_C4	5.70 ±0.01
MH_C5	0.89 ±0.06
MH_CF1	nd*
MH_CF2	nd*
MH_CF3	nd*
MH_CF4	nd*
MH_CF5	nd*
MH_CF6	nd*
MH_CF7	nd*
MH_CF8	nd*
MH_CH1	nd*
MH_CH2	nd*
MH_CH3	nd*
MH_CH4	3.00 ±0.02
MH_CH5	0.78 ±0.03
MH_CH6	6.70 ±0.02
MH_FL1	nd*
MH_FL2	nd*
MH_FL3	nd*
MH_FL4	nd*
MH_FL5	nd*
MH_L1	nd*
MH_L2	nd*
MH_L3	nd*
MH_L4	nd*
MH_L5	nd*
MH_MV1	5.20 ±0.03
MH_MV2	2.53 ±0.00
MH_MV3	**16.65 ±0.03**
MH_MV4	**15.77 ±0.00**
MH_MV5	5.09 ±0.10
MH_MV6	nd*
MH_MV7	nd*
MH_R1	nd*
MH_R2	nd*
MH_R3	nd*
MH_S1	nd*
MH_S2	nd*
MH_S3	nd*
MH_T1	nd*
MH_TV1	1.84 ±0.01
MH_TV2	8.56 ±0.03

MH_TV3	nd*
MH_TV4	nd*
MH_TV5	nd*
MH_TV6	nd*
Polyfloral Honey (PH) Samples	
PH1	nd*
PH2	nd*
PH3	nd*
PH4	nd*
PH5	nd*
PH6	nd*
PH7	nd*
PH8	nd*
PH9	nd*
PH10	nd*
PH11	nd*
PH12	nd*
PH13	nd*
PH14	nd*
PH15	nd*
PH16	nd*
PH17	nd*
PH18	nd*
PH19	nd*
PH20	nd*
Honeydew Honey (HH) Samples	
HH_H1	0.83 ±0.02
HH_H2	1.20 ±0.24
HH_H3	1.21 ±0.01
HH_H4	1.17 ±0.04
HH_H5	0.36 ±0.01
HH_H6	2.69 ±0.02
HH_H7	2.00 ±0.03
HH_H8	1.78 ±0.01
HH_H9	1.65 ±0.03
HH_H10	0.40 ±0.04
HH_H11	1.72 ±0.04
HH_H12	0.40 ±0.04
HH_H13	1.85 ±0.01
HH_H14	0.67 ±0.00
HH_O1	6.19 ±0.03
HH_O2	1.28 ±0.03
HH_O3	nd*
HH_O4	1.28 ±1.81
HH_O5	nd*
HH_O6	nd*

nd*: not detected.

As is well known that, HMF is a product of non-enzymatic browning reactions and is formed in foods as a result of the condensation reaction of sugars and proteins. It is one of the products of Maillard reactions that occur between sugars and biomolecules that contain amine groups, such as amino acids. Its low level in honey is an indicator of the freshness of honey. As can be seen from Table 2, the HMF values in honey samples under study were detected as 0.00-16.65mg.kg^{-1}. The maximum HMF contents were determined in the samples of MH_MV3 and MH_MV4. In the study, HMF formation was detected in honey types coded MH_C, MH_CH, MH_MV and MH_TV from monofloral honey samples from different botanical origins, while HMF content was not found in polyfloral honey samples. Low amounts of HMF formation were also detected in two different honeydew honey samples analyzed. It is possible that the detected HMF contents in these samples provided from markets is due to different process parameters like time, temperature, pH and humidity applied in their production and storage. For instance, the HMF content in samples of fresh honey is typically zero, but with long-term storage, depending on pH and storage temperature, it increases (Ghramh et al. 2020). Even at low temperatures and in an acidic environment, HMF could easily develop (Shapla et al. 2018). When honey sample is subjected to thermal treatment for reducing viscosity, delaying or preventing crystallization and eliminating microorganisms that contaminate it and the HMF content could increase (Cozmuta et al. 2011). The amount of HMF in honey also depends on sugar ratio, protein content and botanical characteristics. Considering these reasons stated in literature; it is predicted that the HMF contents in the honey samples detected in the study are mainly due to factors such as sugar and protein contents originating from botanical differences, temperature, time, pH and humidity applied during production and storage.

4. DISCUSSION AND CONCLUSION

Herein, the recognition of HMF in honeys was successfully performed by the HPLC-DAD method under laboratory conditions by validating certain conditions. The HMF contents of 95 different honey samples from different botanical origins were vary between 0.00-16.65 mg.kg^{-1}, and all of them were well below the Honey Communiqué limit (40mg.kg^{-1}). The HMF could not be detected in 64 of 95 pure honeys. Thus, it was concluded that all of the honey samples were compatible with the Honey Communiqué report in terms of HMF and had appropriate honey quality values. It could be concluded that the analyzed honey samples met the desired quality standards for HMF content.

CONFLICT OF INTEREST

All authors declare that they have no further conflict of interest.

THANKS

The research was financially supported by the project with a number of 220O089 (TUBITAK–1002).

REFERENCES

Bastos DM, Monaro, Siguemoto E, Sefora M (2012). Maillard reaction products in processed food: pros and cons. In: Food industrial processes - methods and equipment. InTech.

Chua LS, Abdul-Rahaman N, Sarmidi MR, Aziz R (2012). Multi-elemental composition and physical properties of honey samples from Malaysia. Food Chemistry, 135: 880-887.

Cozmuta AM, Cozmuta LM, Varga C, Marian M, Peter A (2011). Effect of thermal processing on quality of polyfloral honey. Romanian Journal of Food Science, 1: 45-52.

Das S, Uddin MN, Haque MS, Chakraborty D, Mostafa M, Hasnaine A, Das SK, Uddin M (2022). Hydroxymethylfurfural Content and Sugar Profile of Honey Available in Bangladesh Using Validated HPLC-PDA and HPLC-RID. Journal of Food Quality and Hazards Control, 9: 160-168.

De Almeida AMM, Oliveira MBS, Da Costa JG, Valentim IB, Goulart MOF (2016). Antioxidant capacity, physicochemical and floral characterization of honeys from the northeast of Brazil. Revista Virtual de Quimica, 8: 57-77.

El Sohaimy SA, Masry SHD, Shehata MG (2015). Physico-chemical characteristics of honey from different origins. Annals of Agricultural Science, 60: 279-287.

Elhamdaoui O, El Orche A, Cheikh A, Mojemmi B, Nejjari, Bouatia M (2020). Development of fast analytical method for the detection and quantification of honey adulteration using vibrational spectroscopy and chemometrics tools. Journal of Analytical Methods in Chemistry, 2020(1): 8816249.

Ghramh HA, Khan KA, Zubair A, Ansari MJ (2020). Qualityevaluation of Saudi honey harvested from the Asir province by using high-performance liquid chromatography (HPLC). Saudi Journal of Biological Sciences, 27: 2097-2105.

Glatt H, Schneider, H, Murkovic M, Monien BH, Meinl W (2012). Hydroxymethyl-substituted furans: mutagenicity in salmonella typhimurium strains engineered for expression of various human and rodent sulphotransferases. Mutagenesis, 27(1): 41-48.

Harmonised methods of the international honey commission (2009). Harmonised methods of the international honey commission.

Islam A, Khalil I, Islam N, Moniruzzaman M, Mottalib A, Sulaiman SA,d Gan SH (2012). Physicochemical and antiox-idant properties of Bangladeshi honeys stored for more than one year. BMC Complementary and Alternative Medicine, 12: 177.

Jandrić Z, Frew R, Fernandez-Cedi LN, Cannavan A (2017). An investigative study on discrimination of honey of various floral and geographical origins using UPLC QToF MS and multivariate data analysis. Food Control, 72: 189-197.

Kurtagić H, Sarić E, Abdelfattah N, Murtabegović N (2021). Determination of Hydroxymethylfurfural Content (HMF) in Fresh Bee Honey Produced in Bosnia and Herzegovina (B&H) by HPLC DAD Method. International Journal of Environmental Sciences and Natural Resources, 26(5): 153-159.

Lewkowski J (2001). Synthesis, chemistry and applications of 5-hydroxymethylfurfural and its derivatives. Arkivoc, (1): 17-54.

Makawi SZA, Taha MI, Zakaria BA, Siddig B, Mahmod H (2009). Identification and quantification of 5-hydroxymethyl furfural HMF in some sugar-containing food products by HPLC. Pakistan Journal of Nutrition, 8(9): 1391-1396.

Mehryar L, Esmaiili M, Hassanzadeh A (2013). Evaluation of some physicochemical and rheological properties of Iranian honeys and the effect of temperature on its viscosity. American-Eurasian Journal of Agricultural and Environmental Sciences, 13: 807-819.

Pastoriza de la Cueva S, Álvarez J, Végvári A, Montilla-Gómez J, Cruz-López O, Delgado-Andrade C, Rufián-Henares JA (2017). Relationship between HMF intake and SMF formation in vivo: An animal and human study. Molecular Nutrition & Food Research, 61(3).

PM da Silva PM, Gauche C, Gonzaga LV, Costa ACO, Fett R (2016). Honey: Chemical composition, stability and authenticity. Food Chemistry, 196: 309-323.

Ruiz Matute AI, Weiss M, Sammataro D, Finely J, Sanz ML (2010) Carbohydrate composition of high-fructose corn syrups (HFCS) used for bee feeding: effect on honey composition. Journal of Agricultural and Food Chemistry, 58(12): 7317-7322.

Sabireen SN, Sulaiman SA, Salleh RM, Fuad WEM (2020). Correlation between 5-Hydroxymethylfurfural (HMF) Concentration and Physicochemical Properties in Fresh and Aged Tualang Honey. Asian Journal of Medicine and Biomedicine, 4(2): 10–16.

Samarghandian S, Farkhondeh T, Samini F (2017). Honey and health: a review of recent clinical research. Pharmacognosy Research, 9: 121-127.

Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH (2018). 5-Hydroxymethylfurfural (HMF) levels in honey and other food products. Effects on bees and human health. Chemistry Central Journal, 12(1): 35.

Vorlová L, Borkovcová I, Kalábová K, Večerek V (2006). Hydroxymethylfurfural contents in foodstuffs determined by HPLC method. Journal of Food and Nutrition Research, 45(1): 34-38.

Zappala M, Fallico B, Arena E, Verzera A (2005). Methods for the determination of HMF in honey: a comparison. Food Control, 16(3): 273-277.