




Hydroxymethylfurfural Content and Sugar Profile of Honey Available in Bangladesh Using Validated HPLC-PDA and HPLC-RID

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HIGHLIGHTS

- Hydroxymethylfurfural (HMF) values were ranging from 1.41 mg/kg to 2,063.90 mg/kg.
- The Limit of Detection and Limit of Quantification were 0.10 and 0.33 mg/kg, respectively with $R^2=0.9994$ for HMF.
- Fructose, glucose, and sucrose ranged 14.75-52.44%, 8.19-42.63%, and 0.10-21.12% respectively.

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Acronyms and abbreviations

F/G=Fructose/Glucose Ratio
HFCS=High Fructose Corn Syrup
HMF=Hydroxymethylfurfural
HPLC=High Performance Liquid
Chromatography
LOD=Limit of Detection
LOQ=Limit of Quantification

ABSTRACT

Background: Honey has a lot of reputation because of its supposed medicinal properties. In this study, Hydroxymethylfurfural (HMF), sugars, and Fructose/Glucose ratio of honey in Bangladesh were assessed for adulteration and authenticity evaluation.

Methods: Seventy honey samples collected from different districts of Bangladesh were analyzed by High Performance Liquid Chromatography (HPLC) for HMF content and sugar profile. The samples were prepared by using Carrez I and Carrez II prior to injecting into HPLC. The samples were then filtered through syringe filter and taken in 1.5 ml vial for injecting into the HPLC system.

Results: HMF values were ranging from 1.41 mg/kg to 2,063.90 mg/kg. The Limit of Detection (LOD) and Limit of Quantification (LOQ) was found 0.10 mg/kg and 0.33 mg/kg with $R^2=0.9994$. The average values of fructose, glucose, and sucrose were in the range of 14.75-52.44%, 8.19-42.63%, and 0.10-21.12%, respectively. From validation parameters, LOD values for fructose, glucose, and sucrose were 0.003, 0.008, and 0.004%, respectively; and LOQ values were 0.01, 0.028, and 0.015%, respectively with an excellent linearity with R^2 for fructose=1.0, glucose=0.9999, and sucrose=1.0.

Conclusion: Some samples had higher HMF content which may be due to the storage time was increased and improper processing with high temperature or adulteration by High Fructose Corn Syrup (HFCS), sugar cane syrup, rice syrups or rice molasses. The sugar profiles showed that the most of honey samples were nectar honeys.

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Introduction

Honey bees collect nectar from flowers and generate honey in honeycombs (Codex Alimentarius Commission,

2001). Honey is one of the most valuable and well-liked therapeutic substances due to the presence of minor but

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essential organic acids, amino acids, minerals, vitamins, lipids, phenolic compounds, pigments, pollen, and other phytochemicals (Amiry et al., 2017; De Almeida-Muradian et al., 2013; Uran et al., 2017). Honey is a sweetening agent and it can be used by a human without processing. It is one of the most complex foods, which is produced by honeybees from the nectar of different plants and honeydew (Elhamdaoui et al., 2020).

Sucrose may be present in honey samples at concentrations of less than 1%, but during the spring, if beekeepers over feed sugar solutions to the bees, the concentration of sucrose in honey may significantly increase (Ghramh et al., 2020). According to British and German honey rules, a honey sample may contain up to 5% sucrose at the most. The monitoring of honey composition is crucial to keep its quality because it has a very complex composition containing more than 180 substances; mostly sugars, including 33.3-43.0% (w/w) of fructose, 25.2-35.3% (w/w) of glucose, and 0-2% (w/w) sucrose; and water (Aljohar et al., 2018).

Honey has a lot of attention due to its medicinal and therapeutic properties and widespread consumption (Samarghandian et al., 2017). Climate, meteorological, floral, and entomological factors all affect the composition and characteristics of honey (De Almeida et al., 2016; El Sohaimy et al., 2015). Additionally, the composition of honey is significantly influenced by processing temperature, storage interval, and storage circumstances (Islam et al., 2012; Mehryar et al., 2013). Due to its potential prebiotic properties, honey is a widely used substance. It considerably contributes its high nutritional value, which aids in human gut microbiota growth and balance (Meo et al., 2017).

Because honey is a value-added food and rising demand, it always is alluring to adulterate by blending with inexpensive High Fructose Corn Syrup (HFCS), sugar syrups, and molasses for illegal purposes which hurts the quality of honey and the health of consumers (Cengiz et al., 2014; Jandrić et al., 2017; Karabagias et al., 2018). The majority of the honey shipped from several Asian nations to Europe, the United States, and Japan is adulterated with rice syrups or rice molasses (Sobrino-Gregorio et al., 2017). Physical and chemical characteristics of the honey samples such as the amount of HMF, sugars, water, minerals, vitamins, acidity, organic acids, amino acids, proline, proteins, enzyme activity, electrical conductivity, and organoleptic characteristics are established by the European Union regulation as common quality standards but fructose to glucose ratio, sucrose content, and HMF draw more concern as markers of good quality honey (Jandrić et al., 2017).

HMF a furan ring skeleton heteroaromatic compound is found in honey which is derived from carbohydrates (sucrose, glucose, fructose, etc.) through the Maillard

reaction where acid catalytic hydrolysis and dehydration steps are mainly occurred. Freshly harvested authentic honey contains very little amount HMF and according to Codex Alimentarius Commission guideline HMF limit should be 40 mg/kg whereas should not exceed 80 mg/kg for tropical countries (Bastos et al., 2012). The quality is not getting affected by processing honey at the temperature range of 32-40 °C, but heating above 60 °C HMF tends to increase (Shapla et al., 2018). Upper HMF content indicates the deterioration of honey quality due to processing defects mainly heating above 60 °C to consolidate viscosity and eliminate solidification or fermentation, inappropriate storage conditions, the addition of adulterants such as sugar solution, HFCS, ageing, etc. (Shapla et al., 2018).

Numerous investigations have focused on physicochemical qualities such as heavy metals, flowers, pigments, mineral contents, and antibacterial, antioxidant, and other capabilities (Alghamdi et al., 2020; Aljohar et al., 2018). Some analysis were done on honey available in Bangladesh such as physiochemical and antioxidant properties (Islam et al., 2012), phenolic acids and flavonoids in monofloral honey by High Performance Liquid Chromatography (HPLC) (Linkon et al., 2015), comparative analysis of physicochemical and antioxidant properties (Islam et al., 2014), antioxidant and physicochemical properties of Lichi honey (Ali et al., 2018), and qualitative evaluation of some Bangladeshi honey (Ali et al., 2018). From the above review, based on our knowledge, it seems that there is no research done on the quantification of HMF content and sugar content (fructose, glucose, and sucrose) of honey available in Bangladesh using HPLC. To see the adulteration, the fresh or bad stored honey, the condition, temperature effect, and the aging of honey were evaluated in this study by assaying HMF and three sugars (fructose, glucose, and sucrose) in honey samples of Bangladesh by HPLC a modern analytical technique which could give a new dimension for the concern of national authority.

Materials and methods

Chemicals and reagents

HPLC-Grade ACN, HMF, fructose, glucose, and sucrose standards were supplied by Sigma-Aldrich®. Water was used from Milli-Q water purification system from Millipore (Millipore, Bedford, MA, USA).

Honey samples

Seventy Bangladeshi honey samples were collected from different districts with 19 different flowers, a few unknown flowers and mixed flowers. Different honey

samples were collected with different production dates (2017-2020) but one of them was from the year 2000 (HY-47). Those all were tested within three months, of their arrival.

Sample preparation

For HMF content analysis and sugar profiling, 5 g of honey sample was taken in a 50 ml volumetric flask and added 10 ml ultra-pure water then sonicated for 5 min to dissolve honey in water. A 0.5 ml of Carrez I reagent (0.25 M solution of potassium hexacyanoferrate(II) ($K_4Fe(CN)_6 \cdot 3H_2O$) and 0.5 ml of Carrez II reagent (1.0 M solution of zinc acetate ($Zn(CH_3COO)_2 \cdot 2H_2O$) were added after making up to the mark the sample solution was centrifuged for 5 min at 3,000 rpm (Eslamizad et al., 2020). The solution was then filtered through a 0.22 μ m Polytetrafluoroethylene (PTFE) syringe filter and taken in a 1.5 ml HPLC vial for injecting into the HPLC system. For sugar profiling, the prepared sample was diluted further two times as required.

HPLC

Fructose, glucose, and sucrose were analyzed using a quaternary low-pressure gradient HPLC system (LC-2030C, 3D Prominence-i plus) assembled with a Refractive Index Detector-20A (RID-20A) (Shimadzu Corporation, Japan). An isocratic mobile phase of acetonitrile: water (80:20, v/v), with a flow rate 1.2 ml/min pass through the Shim-pack GIST Amino (NH₂), (5 μ m, 250 \times 4.6 mm) column for 10 min and recorded the chromatogram. HMF was analyzed in HPLC-PDA using a Shim-pack GIST C18 column (5 μ m, 250 \times 4.6 mm), with an isocratic mobile phase of water: acetonitrile (90:10, v/v), retained at a flow rate 1.5 ml/min and the peak detected at λ =285 nm and run time 5.5 min. In both case, the sample injection volume was 20 μ l.

Statistical analysis

The statistical analysis was conducted using Microsoft excel version 10.0 for calibration curve, standard deviation, relative standard deviation, Limit of Detection (LOD), and Limit of Quantification (LOQ) determination.

Results

Validation parameters

The described HPLC methods were validated in terms of the International Council for Harmonisation (ICH) of technical requirements for pharmaceuticals for human use analytical performance parameters; linearity, recovery,

accuracy, precision, selectivity, specificity, sensitivity, stability, column efficiency, system suitability, and robustness.

-Linearity

Peak areas of a mixture of standards (fructose, glucose, and sucrose) and a single HMF standard were used to create calibration curves and were plotted against nominal concentrations of the analytic. Calibration equations were for fructose, $y=1,207,487x+618$, for glucose, $y=972,268x-1,990$, for sucrose, $y=1,396,210x-816$, for HMF, $y=91,926x-6,061$. The calibration curves were linear the range of 0.05-2% for mixtures of fructose, glucose, and sucrose standard and the range of 0.5-10 mg/ml for HMF standard. The correlation coefficients (r) were 1.0, 0.9999, 1.0, and 0.9994 for fructose, glucose, sucrose, and HMF, respectively as indicated in Table 2.

-Sensitivity

The LOD was calculated from the calibration graph by the formula; $LOD=3 \cdot S_{xy}/a$, and the $LOQ=10 \cdot S_{xy}/a$. The LOD and LOQ were shown in Table 2 for fructose, glucose, sucrose, and HMF. These results indicated that method was sensitive enough for the analytic of interest.

-Recovery/accuracy

The results of recovery studies obtained from the intraday assay at 6 concentrations (n=6) by the proposed method were fructose 99-101.20%, glucose 98-100.80%, sucrose 98-101%, and HMF 96.73-104%. Inter-day assay at 5 different days was for fructose 95-100%, for glucose 98-100%, for sucrose 98-100.05%, and for HMF 95-102.60% indicated high accuracy of the mixture of standards. Intraday and inter-day recovery data for the proposed method are presented in Table 2.

-Precision

The Relative Standard Deviations (RSD) obtained for the intraday assay in the range for fructose 0.10-1.98%, for glucose 0.50-1.58%, for sucrose 0.30-1.22%, and for HMF 0.08-2.00% and for inter-day assay the corresponding values in the range for fructose 0.10-1.22%, for glucose 0.05-1.02%, for sucrose 0.03-1.02%, and for HMF 0.57-3.31% indicating the high precision of the method. Intraday and inter-day precision data for proposed method are presented in Table 2.

-Specificity/selectivity

The specificity was demonstrated showing that the standards of fructose, glucose, and sucrose were

determined to be free of interference from potential impurities and degradation products by the absence of any peak in the same retention times. The selectivity of the method was checked by injecting fructose, glucose, and sucrose standard solution, background control sample. There was no interference at a retention time of fructose, glucose, and sucrose standards due to back ground control sample. From the chromatogram shown in Figure 1, it is evident that under the chosen chromatographic conditions, fructose 5.79 min, glucose 6.40 min, sucrose 8.53 min, and HMF 4.17 min (Table 3), the HPLC method did not suffer interference since there was no another peak on the retention times of fructose, glucose, sucrose, and HMF. Results indicated the high specificity of the method and could be used in the routine analysis for the investigation of concentrations of fructose, glucose, sucrose, and HMF in honey samples.

-Robustness

Under most circumstances, it was discovered that the percent recoveries were excellent and remained unaffected by small deliberate adjustments to experimental parameters such as the flow rate and isocratic program, even when retention duration and resolution were reduced as was expected.

-System suitability

A system suitability test was an integral part of the method development to verify that the system was adequate for the analysis of fructose, glucose, sucrose, and HMF to be performed. The system suitability was assessed by replicate injections ($n=5$) of the sample at 0.5% and 5 mg/ml concentration levels including intraday and inter-day assessments. To assess the system's appropriateness, the precision of the retention time and peak area was looked at. The RSD of fructose, glucose, sucrose, and HMF for peak area and retention time indicated excellent suitability of the system as shown in Table 3.

-Column Efficiency

The column efficiency parameters were calculated for a representative chromatogram. To make sure a chromatographic system was operating efficiently, this test was required. The calculated values of the theoretical plate number, tailing factor, and capacity factor as shown in Table 4 revealed the excellent performance of the analytical column.

Sugars profile

A typical chromatogram obtained for 3 sugars (fructose, glucose, and sucrose) and HMF is shown in Figure 1. Fructose and glucose were present in all types of

honey (Table 4). Fructose and glucose were found to be the major sugars in all of the tested samples and sucrose were found in 18 samples. Twenty four out of 70 honey samples contained fructose less than expectable limit (Table 4). Fructose was quantitatively the main sugar found in 52 samples of honey but in 18 samples glucose was found to be predominant (Table 4). Sucrose was detected in 18 samples out of 70, which 16 honeys satisfied the minimum amount ($<5\%$), but two sample (HY-72, HY-83) exceeded the maximum level 7.08% and 21.12%, respectively (Table 5). The Fructose/Glucose (F/G) ratio in all types of honey ranged from 3.49 to 0.83 where expected ratios should be near about 1.0.

HMF content

The HMF content in 18 samples out of 70 (25.71%) was higher than acceptable limit. Honey sample (HY-47, Production date April, 2000) had the highest amount of HMF (2,063.90 mg/kg). Others were found in the limit of the Codex Alimentarius and European Union (EU) which was 40-80 mg/kg and some were found less than 40 mg/kg, those could be considered as fresh and good conditioned honey.

Discussion

Honey is predominantly constituted of carbohydrates which among these, monosaccharides (fructose and glucose) are significant constituents, with fructose always being the primary sugar after glucose (Habib et al., 2014; Rodríguez Flores et al., 2014). We observed that the monosaccharide was the main sugars and the fructose contents overrun quantitatively glucose in 52 samples (74.29%). The nectar sources (flowers or plant secretions) that the bees use to make honey, the regional and climatic circumstances, and the storage conditions all affect the sugar content of the honey (Bastos et al., 2012; Dobre et al., 2012; Sobrino-Gregorio et al., 2017). Our findings were consistent with information gathered from earlier studies by researchers who examined samples of honey from various regions of Saudi Arabia, Morocco, Pakistan, Romania, and the United Arab Emirates (Aazza et al., 2014; Abdallah and Hamed, 2019; Dobre et al., 2012; Habib et al., 2014; Khan et al., 2016; Mohammed et al., 2017; Rodríguez Flores et al., 2014).

We found that in 18 samples (25.71%), glucose exceeded very marginally fructose. Since fructose and glucose are the two main sugars in honey, fructose often has a little advantage, but there are some remarkable honeys that have more glucose than fructose for instance, rape, and dandelion honeys (Kirs et al., 2011). In this study, honey samples were obtained from 19 different flower sources (Table 1); perhaps as a result, glucose

outperformed fructose in 18 samples, supporting the study of Kirs et al. (2011). Fructose, with a mean concentration of 34.15 g/100 g, was the most prevalent sugar in all of the honey samples evaluated (Table 5). Lower glucose readings were observed, with a mean of 28.97 g/100 g. The average combined content of glucose and fructose was determined to be 63.10 g/100 g comply with European Legislation (European Union, 2014). One of the quality indicators used to spot adulteration in honey samples is sucrose. Some popular methods of adulterating

honey include the addition of sucrose, overfeeding bees with sucrose solution, or premature honey harvesting. Sucrose should not be more than 1% of the dried mass of natural honey (Alghamdi et al., 2020). In the current study, sucrose was discovered in 18 samples, 2 of which had concentrations that were higher than the allowed maximum (>5%). The results demonstrated that the majority of honey samples were superiorly ripened and free of sugar adulteration.

Table 1: Sample Information of honey collected from different district of Bangladesh

Sample ID	Collection area and manufacturing date	Name of flowers	Sample ID	Collection area and manufacturing date	Name of flowers
HY-1	Dinajpur (March, 2019)	Lychee	HY-36	Shundarban (April, 2020)	Khalisha
HY-2	Dinajpur (March, 2019)	Lychee	HY-37	Shundarban (May, 2020)	Khalisha
HY-3	Dinajpur (March, 2019)	Lychee	HY-38	Natore (March, 2020)	Lychee
HY-4	Madaripur (February, 2019)	Coriander	HY-39	Pabna (March, 2020)	Lychee
HY-5	Shundarban (October, 2019)	Plum	HY-40	Chattogram (April, 2019)	Unknown
HY-6	Shundarban (October, 2018)	Plum	HY-41	Chattogram (April, 2020)	Unknown
HY-7	Bogra (January, 2019)	Drumstick	HY-42	Tangail (April, 2020)	Mixed
HY-8	Shundarban (April, 2019)	Khalisha	HY-43	Shundarban (December, 2019)	Mustard
HY-9	Shundarban (May, 2019)	Khalisha	HY-44	Chattogram (December, 2019)	Mustard
HY-10	Shundarban (April, 2019)	Goran	HY-45	Faridpur (March, 2020)	Fennel
HY-11	Gopalganj (February, 2019)	Grass pea	HY-46	Sylhet (May, 2019)	Mixed
HY-12	Shundarban (July, 2018)	Gewa	HY-47	Shundarban (April, 2000)	Khalisha
HY-13	Jamalpur (January, 2020)	Mustard	HY-48	Faridpur (March, 2020)	Fennel
HY-14	Shirajganj (December, 2019)	Mustard	HY-49	Faridpur (March, 2017)	Fennel
HY-15	Shirajganj (December, 2019)	Mustard	HY-50	Jhinaidah (May, 2020)	Sesame
HY-16	Chattogram (ShajibModhu) (2019)	Plum	HY-51	Chattogram (April, 2020)	Mixed
HY-17	Chattogram (MiyarModhu) (2019)	Mustard	HY-52	Chattogram (November, 2019)	Mixed
HY-18	Chattogram (April, 2020)	Mixed	HY-53	Shundarban (November, 2019)	Mixed
HY-19	Nilphamari (August, 2020)	Olive	HY-54	Chattogram (April, 2017)	Unknown
HY-20	Tangail (August, 2019)	Mimosa	HY-55	Chattogram (March, 2020)	Lychee
HY-21	Shundarban (April, 2020)	Khalisha	HY-56	Sylhet (July, 2019)	Mixed
HY-22	Tangail (April, 2020)	Lemon	HY-57	Pabna, Ishwardi (April, 2020)	Lychee
HY-23	Chattogram (April, 2020)	Mixed	HY-58	Pabna, Ishwardi (March, 2020)	Lychee
HY-24	Shatkira (October, 2017)	Plum	HY-59	Chattogram (June, 2020)	Mixed
HY-25	Khulna (April, 2020)	Sesame	HY-60	Rajshahi (August, 2020)	Mixed
HY-26	Faridpur (May, 2020)	Fennel	HY-61	Chattogram (August, 2020)	Mixed
HY-27	Tangail (April, 2020)	Rabar	HY-62	Chattogram (June, 2020)	Unknown
HY-28	Faridpur (April, 2018)	Mahogany	HY-63	Chattogram (March, 2020)	Lychee
HY-29	Pabna (March, 2020)	Lychee	HY-64	Shundarban (June, 2020)	Mixed
HY-30	Shirajganj (December, 2019)	Mustard	HY-65	Mymensingh (August, 2019)	Mixed
HY-31	Tangail (April, 2020)	Radhuni	HY-66	Jessore (September, 2020)	Mixed
HY-32	Sherpur (November, 2018)	German Iota	HY-67	Shirajganj (March, 2020)	Rosy Rain lily
HY-33	Shundarban (April, 2020)	Khalisha	HY-68	Gazipur (March, 2020)	Unknown
HY-34	Shundarban (April, 2017)	Khalisha	HY-69	Chattogram (September, 2020)	Unknown
HY-35	Shundarban (April, 2020)	Khalisha	HY-70	Brammonbaria (August, 2020)	Mixed

HY=Honey Sample

Table 2: Validation parameters for Hydroxymethylfurfural (HMF) and three sugar (fructose, glucose, and sucrose)

Validation parameters	HMF	Fructose	Glucose	Sucrose
Linear range	0.5-10 (ppm)	0.05-2 (%)	0.05-2 (%)	0.05-2 (%)
Linearity equation	$y=91.926x-6061$	$y=1,207.487x+618$	$y=972,268x-1990$	$y=1,396,210x-816$
RSD of the slope	0.13	0.33	0.99	0.04
Correlation coefficient (r)	0.9994	1.00	0.9999	1.00
RSD (%)				
Intraday	0.08-2.00	0.10-1.98	0.50-1.58	0.30-1.22
Interday	0.57-3.31	0.10-1.22	0.05-1.02	0.03-1.02
Recovery (%)				
Intraday	96.73-104.00	99.00-101.20	98.00-100.80	98.00-101.00
Interday	95.00-102.60	95.00-100.00	98.00-100.00	98.00-100.05
LOD	0.10	0.003	0.008	0.004
LOQ	0.33	0.01	0.028	0.015

RSD= Relative Standard Deviation; LOD=Limit of Detection; LOQ=Limit of Quantification

Table 3: Validation parameters in terms of system suitability (concentration 0.5% for three sugar and 5 mg/l for Hydroxymethylfurfural) for the analysis of sugar content (fructose, glucose, and sucrose) and Hydroxymethylfurfural

Validation parameters	Retention Time (n = 5)		Area (n=5)	
	Average	RSD (%)	Average	RSD (%)
HMF	4.17	0.06	443,281.4	0.04
Fructose	5.79	0.16	612,273.4	1.71
Glucose	6.40	0.24	485,160.4	1.32
Sucrose	8.53	0.32	696,994	0.63

RSD=Relative Standard Deviation; HMF=Hydroxymethylfurfural

Table 4: Validation parameters in terms of column sufficiency (concentration 0.5% for three sugar and 5 mg/l for Hydroxymethylfurfural) for the analysis of sugar content (fructose, glucose, and sucrose) and Hydroxymethylfurfural

Validation parameters	NTP (n=5)		HETP (n=5)		T.F (n=5)	
	Average	RSD (%)	Average	RSD (%)	Average	RSD (%)
HMF	8,772	0.91	17.10	0.90	1.16	0.11
Fructose	2,770	1.56	54.14	1.55	0.92	2.98
Glucose	2,753	0.84	54.48	0.84	0.96	2.44
Sucrose	2,496	1.44	60.10	1.43	0.84	1.81

NTP=Number of Theoretical Plate; HETP=Height Equivalent to Theoretical Plate; T.F=Tailing Factor; HMF=Hydroxymethylfurfural

Table 5: Three sugar (fructose, glucose, and sucrose) and Hydroxymethylfurfural (HMF) content in 70 samples collected from different district of Bangladesh, only mean is given excluding standard deviation.

Sample ID	Fru (%)	Glu (%)	Suc (%)	F/G Ratio	HMF (mg/kg)	Sample ID	Fru (%)	Glu (%)	Suc (%)	F/G Ratio	HMF (mg/kg)
HY-1	31.95	26.98	2.88	1.18	21.13	HY-36	42.57	28.62	ND	1.49	4.61
HY-2	34.68	29.67	ND	1.17	28.43	HY-37	42.67	32.28	ND	1.28	7.66
HY-3	41.72	33.17	ND	1.26	23.28	HY-38	41.14	33.09	ND	1.24	3.68
HY-4	35.58	25.87	ND	1.71	58.67	HY-39	37.80	34.15	ND	1.11	10.22
HY-5	32.42	26.67	ND	1.22	12.87	HY-40	12.07	27.89	2.12	0.90	164.50
HY-6	31.30	25.70	ND	1.22	12.17	HY-41	36.09	42.63	ND	0.85	525.01
HY-7	39.10	25.42	ND	1.54	60.98	HY-42	35.46	29.94	ND	1.18	3.96
HY-8	35.58	25.97	ND	1.37	36.46	HY-43	37.60	28.56	ND	1.32	7.18
HY-9	31.58	22.75	ND	1.39	90.38	HY-44	35.46	36.25	ND	0.98	7.22
HY-10	33.87	24.12	ND	1.40	35.55	HY-45	38.14	28.35	ND	1.35	15.99
HY-11	36.08	28.87	ND	1.25	43.50	HY-46	35.88	34.03	ND	1.05	36.94
HY-12	32.02	23.70	ND	1.35	343.85	HY-47	27.53	25.27	ND	1.09	2,063.90
HY-13	33.68	30.82	0.64	1.09	35.94	HY-48	40.76	26.66	ND	1.53	20.32
HY-14	41.67	23.42	0.96	1.78	123.02	HY-49	34.09	34.89	ND	0.98	323.76
HY-15	42.83	28.84	ND	1.49	4.07	HY-50	32.18	34.03	ND	0.95	28.71
HY-16	35.60	29.30	ND	1.21	20.03	HY-51	32.78	34.63	ND	0.95	7.72
HY-17	36.63	31.07	ND	1.18	110.22	HY-52	26.05	28.97	ND	0.90	42.54
HY-18	34.09	29.36	ND	1.16	18.31	HY-53	37.67	32.73	ND	1.15	74.28
HY-19	33.08	38.00	ND	0.87	1,210.72	HY-54	22.84	26.16	0.53	0.87	760.62
HY-20	37.82	32.84	0.54	1.15	46.44	HY-55	37.63	34.52	ND	1.09	24.78
HY-21	40.43	29.60	0.10	1.37	5.74	HY-56	35.23	35.75	0.14	0.99	109.06
HY-22	35.05	33.33	ND	1.05	2.13	HY-57	40.86	33.64	ND	1.22	6.65
HY-23	52.44	21.80	ND	2.40	2.21	HY-58	39.18	31.96	ND	1.23	8.20
HY-24	37.13	35.43	ND	1.05	950.06	HY-59	14.75	8.19	ND	1.80	111.5
HY-25	33.94	32.71	ND	1.04	16.33	HY-60	24.47	27.71	7.08	0.88	343.25
HY-26	33.21	27.76	ND	1.20	5.72	HY-61	24.89	29.90	4.73	0.83	639.96
HY-27	32.60	29.17	ND	1.12	3.25	HY-62	28.59	30.50	0.38	0.94	1.41
HY-28	24.22	26.76	ND	0.90	62.66	HY-63	35.88	32.49	ND	1.10	174.34
HY-29	32.17	32.38	0.32	0.99	3.66	HY-64	30.08	35.02	3.23	0.86	327.08
HY-30	37.82	27.94	ND	1.35	4.97	HY-65	37.73	10.80	ND	3.49	57.78
HY-31	31.50	34.44	0.32	0.92	3.21	HY-66	33.03	31.25	0.18	1.06	22.94
HY-32	38.44	14.36	ND	2.68	9.21	HY-67	25.68	28.88	4.88	0.89	13.55
HY-33	40.60	27.80	ND	1.46	2.37	HY-68	26.80	24.10	ND	1.12	9.35
HY-34	36.60	27.42	ND	1.30	333.12	HY-69	19.18	13.57	21.12	1.41	3.45
HY-35	39.28	30.17	ND	1.30	31.41	HY-70	29.38	27.21	1.42	1.08	2.42

ND=Not Detected; Fru=Fructose; Glu=Glucose; Suc=Sucrose; F/G ratio=Fructose/Glucose ratio

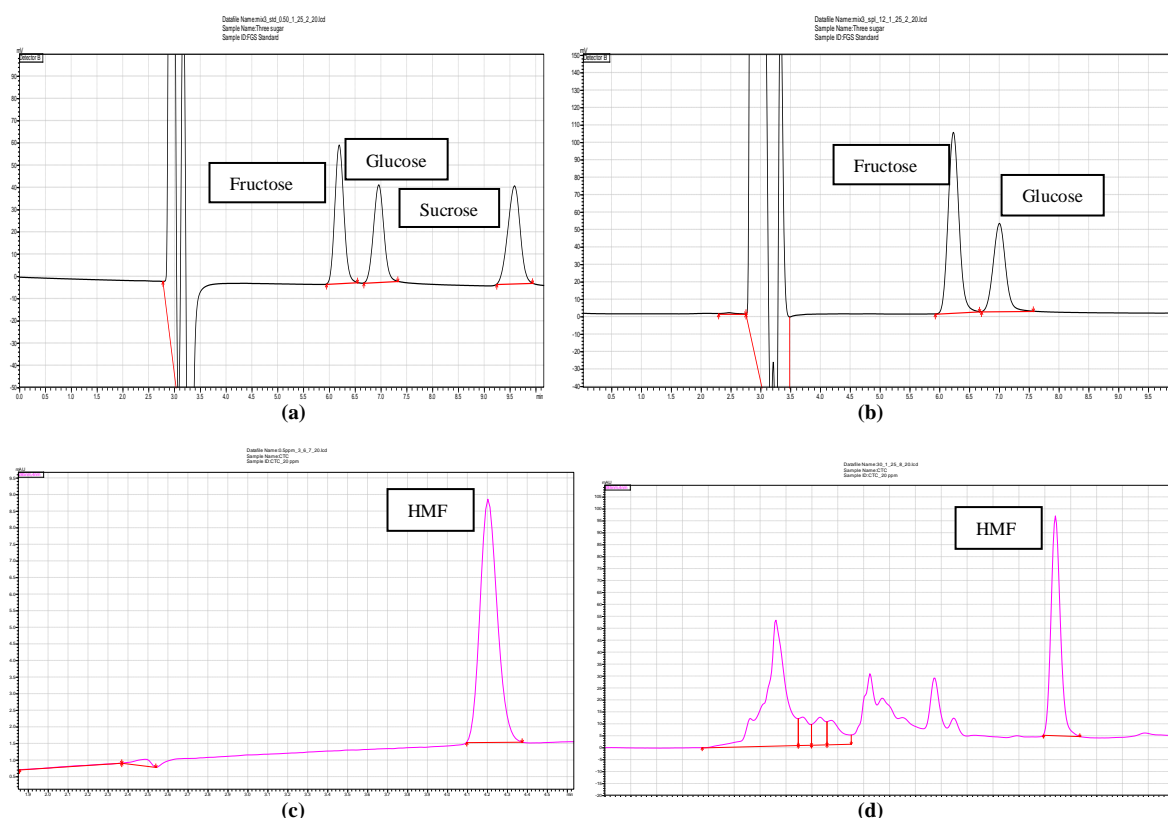


Figure 1: High Performance Liquid Chromatography (HPLC) chromatograms (a) for sugars (fructose, glucose, and sucrose) standard (0.5%); (b) for honey sample; (c) for standard Hydroxymethylfurfural (HMF) (5 mg/l); and (d) for HMF in honey samples

Since fructose is more soluble in water than glucose, the F/G ratio can probably be used to gauge how well honey crystallizes (Ma et al., 2017). Several indexes based on sugar content have also been linked to the potential to crystallize, with a F/G ratio of 1.14 or less in honey being related with quick crystallization in European honeys and a ratio value of more than 1.58 being associated with no tendency to crystallize. Generally, the F/G ratio in honey can be significantly influenced by the honey varieties and place of origin, indicating to their, the origin of floral sources as flower honeys show a F/G ratio of about 1 and honeydew honeys of about 1.5-2.0 (Kirs et al., 2011). The F/G ratio was calculated for all honey samples and it showed values 0.83 to 3.49. Among 70 honey samples, 71.42% samples had the F/G ratio near about 1-1.20 indicating honey samples originated from flower sources, 14.29% having F/G ratio ≤ 0.90 which means these honey samples tends to crystallization, and another 14.29% carrying F/G ratio ≥ 1.50 indicated no tendency to crystallize. So, it is confirmed that the crystallization of honey is a natural process, not due

to any adulteration. If the glucose content is greater than the fructose then the honey samples could be solidified (Ma et al., 2017).

HMF is a crucial component of quality that is used to determine if honey is overheated or too fresh. HMF content in samples of fresh honey is typically zero, but with long-term storage, depending on pH and storage temperature, it increases (Ghranh et al., 2020). Even at low temperatures and in an acidic environment, HMF can develop (Shapla et al., 2018). The amounts of HMF are influenced by a number of variables, including temperature, heating intervals, storage conditions, pH, and the nectar source of a honey (Uran et al., 2017). In this study, we quantified the HMF content from the year of 2017, 2018, 2019, 2020, and one honey from 2000. From the HMF values of the samples, it was found that 52 (74.29%) out of 70 honey samples, the HMF contents were within acceptable limit (Table 5). In 34 samples from 2020, the most recent honey samples had much lower HMF content. Honey is subjected to thermal treatment for reducing viscosity, delaying or preventing crystallization, and

eliminating microorganisms that contaminate the honey (Cozmuta et al., 2011). Bangladesh is a tropical country, thus in the summer it gets quite hot and humid, and in the winter it gets very cold. Since higher water content (humidity) and temperature ($>30^{\circ}\text{C}$) can be the causes which may promote the production of HMF, thus Bangladeshi honey samples are particularly susceptible to HMF formation. Vendors may use thermal treatment to liquefy honey throughout the winter when honey tends to crystallize. In our nation, honey is made from various floral sources and typically crystallizes because it sometimes contains more glucose than fructose. Consumer do not like solid honey that why honey has been heated and HMF content is increased. May be these were the reasons behind the HMF content surpass acceptable limit in 18 samples out of 70 (25.71%).

Conclusion

It was found that the above developed HPLC methods are rapid, valid, and suitable for HMF and sugar profile from the honey sample as well as applicable for other processed foods such as juice, soft drinks, confectionery, etc. From the HMF values of the samples, it was evidenced that some samples had higher HMF content in honey which may be due to improper processing with high temperature or adulteration by HFCS, sugar cane syrup, rice syrups or rice molasses. From sugar profiling, it is seen that the composition of sugars in honey is affected by contributions of the plant floral and environmental conditions. The sugar profiles show that most of honey samples were nectar honeys and may be 2 samples were adulterated by sucrose. Future studies are advised to validate the results that can be made from the study by taking into account more physicochemical and qualitative factors of honey.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: S.D., M.N.U., M.M.; experimental work: S.D., M.S.H., A.H.; data analysis and interpretation of results: S.D., M.N.U.; draft manuscript preparation: M.S.H., D.C., S.K.D., M.U. All authors reviewed the results and approved the final version of the manuscript.

Conflicts of interest

All authors declare that they have no conflicts of interest to disclose.

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