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Micellar electrokinetic chromatography method for the simultaneous determination of furanic compounds in honey and vegetable oils

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ABSTRACT

A simple micellar electrokinetic chromatography (MEKC) method for the simultaneous determination of 2-furfural (2-F), 3-furfural (3-F), 5-methylfurfural (5-MF), 5-hydroxymethylfurfural (5-HMF), 2-furoic acid (2-FA) and 3-furoic acid (3-FA) in honey and vegetable oils is described. Parameters affecting the separation such as pH, buffer and surfactant concentrations, applied voltage, capillary temperature, injection time and capillary length were studied and optimized. The separation was carried out in normal polarity mode at 20 °C, 22 kV and using hydrodynamic injection (17 s). The separation was achieved in a bare fused-silica capillary (46 cm × 50 μm i.d.) with a background electrolyte of 75 mM phosphoric acid (pH 7.3), containing 200 mM of sodium dodecyl sulphate (SDS). The detection wavelengths were at 200 nm (2-FA and 3-FA) and 280 nm (2-F, 3-F, 5-MF, 5-HMF). The furfurals were well separated in less than 20 min. The method was validated in terms of linearity, limit of detection and quantitation, precision and recoveries. Calibration curves of the six furfurals were well correlated ($r^2 > 0.991$) within the range 1–25 μg mL^{-1}. Relative standard deviations of intra- and inter-day migration times and corrected peak areas ≤9.96% were achieved. The limit of detection (signal:noise, 3) was 0.33–0.70 μg mL^{-1} whereas the limit of quantitation (signal:noise, 10) was 1.00–2.12 μg mL^{-1}. The method was applied to the determination of furanic compounds in honeys and vegetable oils (palm, walnut, grape seed and rapeseed). The effects of thermal treatment and gamma irradiation on the formation of the furanic compounds in honey were also investigated.

1. Introduction

Furfurals are organic compounds that are formed through dehydration of sugars when foods or drinks that are rich in carbohydrate are heated [1,2]. For years, the study of furfurals especially 5-hydroxymethylfurfural (5-HMF) in foodstuffs has attracted much attention as these compounds have been reported to exhibit cytotoxic [3], mutagenic [4], carcinogenic [5] and genotoxic [6] effects. The toxicological concerns on furfurals have prompted investigations on various processed foods and beverages. However, the toxicity of these compounds is still under debate as conflicting reports have been made [7].

5-HMF and furfurals such as 2-furaldehyde (2-F) and 5-methylfurfural (5-MF) (Fig. 1) are parameters that are related to the freshness and quality of certain foods. Normally, 5-HMF is nearly absent in fresh and untreated food but its concentration tend to rise during heating or long term storage [8]. Thus, it has been employed in food surveillance to assess both the quality of the processing method and the organoleptic characteristics of the final products. Recent reports have shown that furfurals, especially 5-HMF can serve as a useful chemical marker to evaluate browning reactions in different foodstuffs [8,9].

Honey is one of the saccharide-rich products that have the potential to form furfurals as it offers favorable conditions (high concentration of carbohydrates, acidic environments, high water content, etc) for the formation of furanic compounds (e.g. 2-F, 5-HMF) [10]. The presence of high concentration of 5-HMF in honey are indication of quality deterioration [11], adulteration [11,12], overheating [11,13] and stress during storage. The Codex Alimentarius of the WHO and EU have established a maximum 5-HMF quality level in honey (40 mg kg^{-1}), with the exception of honey of tropical origin (80 mg kg^{-1}) [14,15]. No limits have yet been established for the other furfurals. Of much interest to us are the local honey (known as Tualang honey) which is produced by the Asian rock bees (*Apis dorsata*), which build their hives up in the Tualang tree (*Kompassia excels*) in the tropical forests. The honey has been found to be effective in treating of wound infection [16] and has significant anticancer activity against

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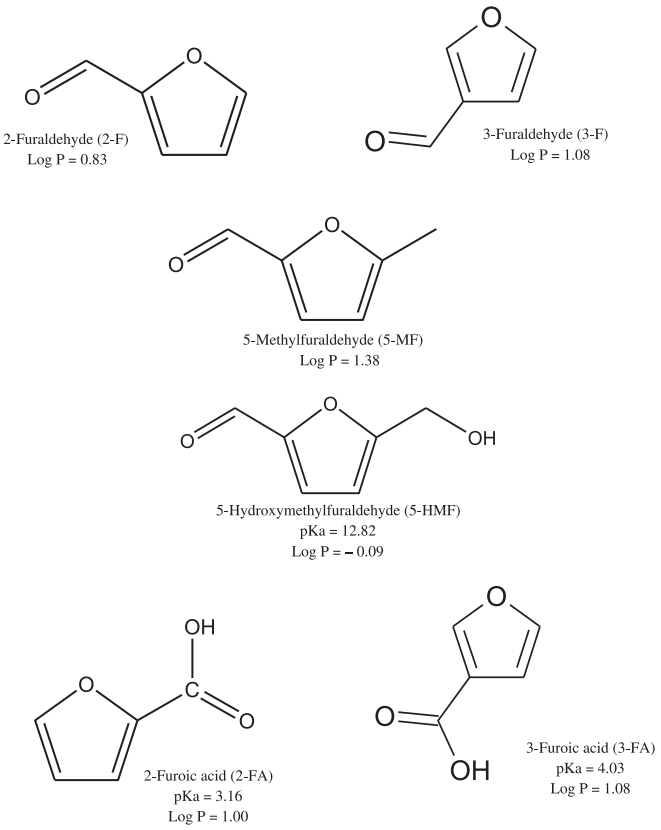

Fig. 1. Chemical structures of the furanic compounds studied.

human breast and cervical cancer cell lines [17]. Contrary to moderate climate honeys, tropical honeys have a high water contents (~40%). For traditional medical applications, the water content needs to be reduced (usually by heat treatment). The samples are then sterilized. The effects of heat treatment and sterilization of tropical honeys on the composition of furfurals are unknown.

Various analytical methods have been reported for the determination of 2-F, 3-furaldehyde (3-F), 5-MF, 5-HMF, 2-furoic acid (2-FA) and 3-furoic acid (3-FA) either separately or their combinations, in fruit juices [9,18,19], alcoholic beverages [20], honey [9,10,21–24], carbonated soft drinks [25], jam [1,9,21], biscuits [9,21], milk based products [26–28], baby foods [29] and oil samples [30,31]. Classical methods for the quantitative determination of furfurals in food product are based on colorimetric measurement which are plagued by the lack of selectivity, time consuming and consumes hazardous or toxic chemicals. The Association of Official Analytical Chemists (AOAC method 980.23, 1996) had prescribed liquid chromatography (LC) with ultraviolet (UV) detection as a reference method for the determination of 5-HMF [32]. However, homogentisic was conclusively found to interfere in the determination of 5-HMF in honey [33]. Other methods described include gas chromatography (GC) – flame ionization detector [22,25] or mass spectrometer (MS) [9,22], high performance liquid chromatography (HPLC) – UV detector [10,19,23,25,26] or MS [21,25] and capillary electrophoresis (CE) with UV detector[24,27,28,34]. In general, LC and GC methods provide satisfactory quantification together with acceptable sensitivity. The LC methods, however, require long analysis time and consume large amounts of solvent. A derivatization procedure is mandatory in GC analysis to increase the volatility and overcome adsorption of the polar functional groups to the GC column. Moreover, the majority of the reported methods usually do not allow direct analysis of the sample as a clean-up step is needed in order to remove interferences. The clean-up steps commonly used include liquid–liquid extraction [25], clarifying agents (Carrez I and II) [2,21,26], solid phase extraction [9,35], direct immersion solid phase microextraction (DI-SPME) [22], etc.

Capillary electrophoresis has developed into an attractive separation technique, including for food analysis, due to the short analysis time, small injection volumes (a few nanoliters), high resolution power and low consumption of solvents [36]. It can be applied in numerous modes of separation. So far, CE methods for the determination of 5-HMF in food [34], milk [27,28], and honey [24] have been reported.

Even though many analytical methods have been developed for the determination of furfurals, the simultaneous determination of all furanic compounds have not been reported. The aim of our work is to develop a suitable and rapid MEKC method for the separation and simultaneous determination of these compounds. The method will be validated and applied to the determination of these furanic compounds in honey and some vegetable oils.

2. Experimental

2.1. Chemicals and reagents

2-furaldehyde (2-F, 99%), 3-furaldehyde (3-F, 97%), 5-methylfurfural (5-MF, 99%), 5-hydroxymethylfurfural (5-HMF, 99%), 2-furoic acid (2-FA, 98%) and 3-furoic acid (3-FA, 98%), sodium dodecyl sulphate (SDS) and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical reagent grade n-hexane was purchased from Merck (Darmstadt, Germany). Phosphoric acid (85%) was supplied by Univar (Ingleburn, Australia). Ultra pure water (18.2 MΩ cm^{-1}) was produced by a Milli-Q system (Millipore, MA, USA).

2.2. Honey samples

Eighteen honey samples were purchased from the local markets. Most of the samples were of Malaysian origin from different states (Penang, Kedah, Terengganu and Johor), while the rest were from New Zealand (1), Saudi Arabia (1) and Vietnam (1). Some of the samples were subjected to gamma irradiation and heat treatment. Prior to the analysis, samples were vortexed for 5 min with a LMS Mixer UZUSIO (Bunkyo-Ku, Tokyo, Japan).

2.3. Vegetable oil samples

Grape seed, rapeseed and walnut oil samples were purchased from local supermarkets. Crude palm oil (cold and hot press), crude palm olein and crude palm stearin samples were kindly donated by Carotino Sdn. Bhd. (Johor Bahru, Malaysia).

2.4. Instrumentation and electrophoretic conditions

Separations were conducted on a $HP^{3D}CE$ capillary zone electrophoresis system, model G 1600 A (Agilent Technologies, Waldbronn, Germany). The CE was equipped with photo diode array (PDA) detector. The separations were performed using a bare fused-silica capillary 46 cm × 50 μm i.d.; (detection length, 8.5 cm from the outlet end of the capillary) supplied by Agilent Technologies. Data acquisition was performed with ChemStation software. The new capillary was equilibrated by flushing for 15 min with NaOH (1.0 M), 10 min with water and 10 min with running buffer. The capillary was regenerated every day before the analysis by flushing for 5 min with NaOH (0.1 M), 5 min with ultra pure water and 5 min with running buffer.

Between injections, the capillary was preconditioned with NaOH (0.1 M), water and followed by running buffer, each for 3 min between the runs. Samples and standards were introduced hydrodynamically (50 mbar) for 17 s under the following conditions: background electrolyte (BGE) was a mixture of 75 mM H_3PO_4 and 200 mM SDS, pH 7.3; capillary temperature, 20 °C; voltage, 22 kV and detection wavelengths were at 200 nm (2-FA and 3-FA) and 280 nm (2-F, 3-F, 5-MF and 5-HMF). After the last analysis, the capillary was flushed for 3 min with NaOH (0.1 M) and 3 min with water. All standards, sample solutions, BGE, and NaOH solutions were filtered through 0.45 μm nylon filter membranes (Agilent Technologies, Waldbronn, Germany).

The identity of the peaks was confirmed by scanning from 190 to 400 nm and comparing to that of the standard spectra. For peaks of low concentrations, further confirmation was done by comparing the peak area ratios of the respective peak measured at two different wavelengths (260 and 280 nm for 2-F, 3-F, 5-MF and 5-HMF; 200 and 220 nm for 2-FA and 3-FA) to that of the standards peaks.

2.5. Stock and standard solutions

Stock solutions (100 μg mL^{-1}) of each furfural were prepared in water by sonication for 3 min and then diluted to the desired concentrations. Calibration standards were prepared using serial dilution of the stock solution in water. All the solutions were stored refrigerated (4 °C) in the dark when not in use.

2.6. Preparation of honey samples

The degree Brix was measured using HI 96801 Digital Refractometer (Hanna Instruments, Woonsocket, RI, USA). Accurately weighed amounts of the honeys were diluted to 10° Brix using water, followed by sonication for 1 min. Further dilution to 2.5° or 0.5° Brix was performed when the concentration exceed the linear ranges. The diluted honey samples were filtered through double layer of 0.45 μm nylon filter membranes (Agilent Technologies, Waldbronn, Germany) before introducing to the CE system.

2.6.1. Heat and irradiation treatment

Twenty one honey samples (20 g) were filled into thermal treatment tubes and tightly sealed. The tubes were placed in a thermostated oven either at 60, 80 or 100 °C for different duration (0.25, 0.50, 1, 3, 6, 12 and 24 h). The sample was then cooled and analyzed using the proposed method (Section 2.6). Six bottles (230 g) of Tualang honey (AgroMas; Malaysia) from the same batch were randomly subjected for gamma irradiation using a cobalt-60 irradiator at 5, 15, 20, 25 and 30 kGy and the last bottle of honey was used as control (i.e., not irradiated). The gamma irradiation was performed at Sterilgamma (M) Sdn Bhd (Selangor, Malaysia).

2.7. Preparation of vegetable oil samples

A miniaturized liquid–liquid extraction procedure with a slight modification of the reported method by Durmaz et al. was employed [31]. Prior to the analysis, samples were vortexed for 3 min. Oil sample (0.5 g) was transferred to a test tube (15 mL), followed by the addition of n-hexane (1 mL) and vortexed for 30 s. It was then extracted with water (1 mL) by vortexing the mixture for 1 min. The mixture was centrifuged (4000 rpm) for 10 min. After the centrifugation, the aqueous layer was isolated. The extraction was repeated three times under the same conditions. The combined aqueous layer was immediately filtered and subjected to the CE analysis.

3. Results and discussion

MEKC is an extension of the basic CE technique that allows the separation of neutral analytes as well as charged ones. A surfactant (anionic, cationic or neutral) is added to the BGE at a concentration above its critical micelle concentration. Advantages of the MEKC technique include the high separation efficiency, low chemical consumption and the user-friendly operation [37].

3.1. Optimization of separation conditions

The initial MEKC conditions (adopted from the work of Teixidó, et al., for the determination of 5-HMF in foodstuffs [34]) were used: 75 mM of phosphate buffer and 100 mM SDS (pH 8.0) as BGE; capillary temperature, 25 °C; voltage 25 kV, using a 50 cm × 50 μm i.d fused-silica capillary. Under these conditions, the separation of all the furanic compounds was not possible. Hence, experimental conditions such as pH, buffer and surfactant concentration, temperature, voltage, capillary dimensions that influence the separation were studied. To evaluate the effects of these parameters, a mixture that contained 25 μg mL^{-1} of each furfural was used. The SDS concentration and voltage were adjusted to 200 mM and 20 kV in order to improve the separation of the six furanic compounds and at the same time avoid Joule heating. As the compounds exhibited different sensitivities, detection wavelengths that produced the more intense peaks were used. Thus, 200 nm was used for the detection of 2-FA and 3-FA while the others were detected at 280 nm (Fig. 3).

3.1.1. Optimization of BGE

The charge on the analytes (2-FA and 3-FA) is pH-dependent, and thus, the selectivity of the separation is affected substantially by pH. It also affects the magnitude of the electroosmotic flow (EOF) as negative charge on the silanol groups increase with pH. Hence, under alkaline conditions, the EOF may be too strong that incomplete separations may occur. In this study, the effect of pH over the range 6.7–7.9 on resolution, migration time and the peak shape was investigated. Fig. 2(A) depicted the electrophoretic mobility (μ_{ep}) of the analytes at different pH. Priority was given to the last two peaks (2-FA and 3-FA) which possess almost similar chemical structure but different pKa (3.16 and 4.03 for 2-FA and 3-FA, respectively). At pH > 4.03, both 2-FA and 3-FA exist predominantly as anionic moiety and migrate towards the anode. The negative charge will cause some degree of repulsion between the analytes and the negatively charged micelles. The degree of repulsion affects the mobility of these analytes as the pH changed. From Fig. 2(A), it can be seen that the μ_{ep} of the analytes decreased as the pH increase. The change in EOF allows the analytes to achieve different mobility which eventually causes the separation to occur. At pH above 7.1, separation of the six furanic compounds can be observed. However, as the pH increased (above 7.3), peaks shape become worse. Therefore, BGE with pH 7.3 was selected.

The concentrations of phosphate buffer ranging from 25 to 100 mM were studied (Fig. 2(B)). It is known that increasing the ionic strength (concentration) of the buffer enhances the thickness of the ionic double layer which eventually lowers the EOF. The increase or decrease in EOF will affect the analyte mobility. From Fig. 2(B), both 75 and 100 mM of phosphate resulted in satisfactory separation ($R_s \geq 3.64$) of the analytes. However, high buffer concentration (100 mM) promotes the generation of Joule heating Therefore, 75 mM phosphate was chosen.

At low concentration (< 1 mM), SDS exists as a single entity. Therefore, to facilitate formation of micelles, concentration higher than its critical micelle concentration (CMC) was used

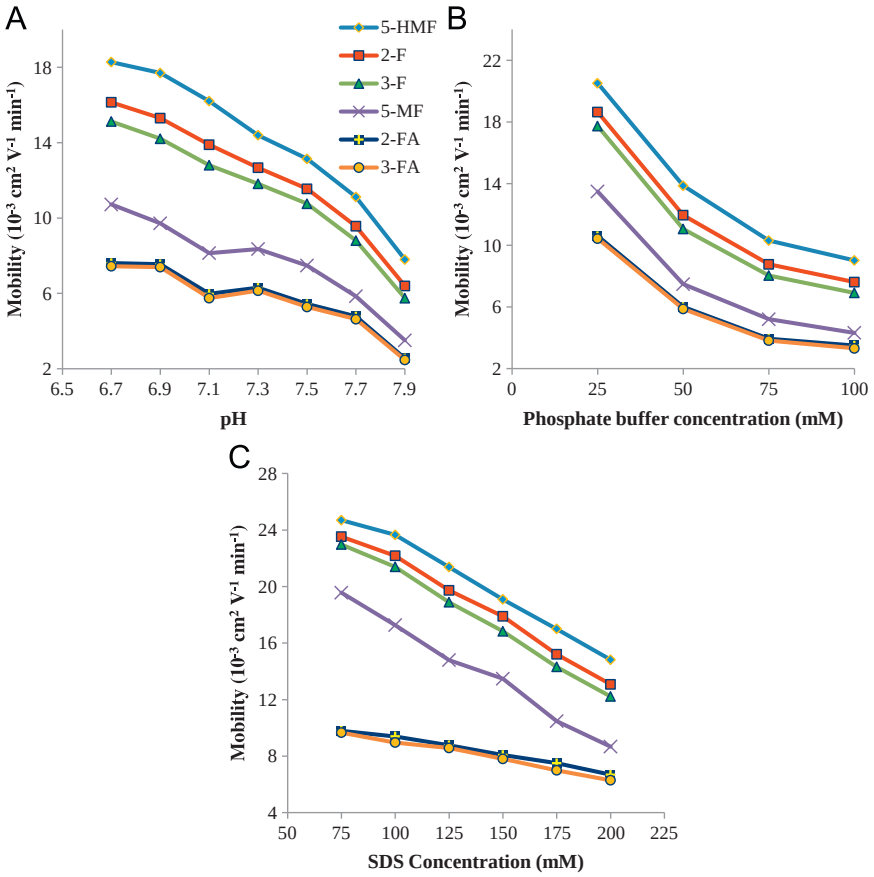

Fig. 2. Effect of: (A) pH, (B) buffer concentration, and (C) SDS concentration, on the analytes mobility.

(CMC of SDS, 8 mM) [38]. The micelles formed have the ability to organize analytes at the molecular level based on hydrophobic and electrostatic interactions. Moreover, SDS micellar systems are reported to work similarly to an octadecylsilane stationary phase in HPLC for moderately water-soluble compounds [39].

The effects of different concentration of SDS (75–200 mM) on the analyte electrophoretic mobility was investigated (Fig. 2(C)). Since the analytes are different in their chemical and geometrical structures, the micelles can interact with them through both hydrophobic and electrostatic interactions. The presence of SDS in the running buffer slows down the migration of these compounds, depending on how strongly they partition in and out of the micelles. The micelles move towards the anode while the EOF towards the cathode. Hence, the more the solutes interact with the micelle the longer is its migration time. In most cases, increasing the SDS concentration results in a more efficient separation but at the same time raises the current in the capillary and increase the migration time of the analytes [40]. From our results, incomplete separation of the analytes occurred when the surfactant concentration is lower than 200 mM. Further attempts to employ higher concentration of SDS ($>$ 200 mM) were halted due to the tremendous increase in the generated current that reduces the sharpness of the peaks and the separation. Therefore, 200 mM SDS was maintained for the subsequent analysis.

3.1.2. Optimization of instrumental conditions

The influence of temperature (15–30 °C) on the migration times and separation efficiency were studied. It is imperative to control Joule heating since this parameter is directly linked to analyte mobility, stability, as well as the system reproducibility.

Table 1
Adopted CE operating conditions.

Background electrolyte	75 mM H_3PO_4; pH 7.3; 200 mM SDS
Applied voltage	22 kV (normal polarity)
Injection time	17 s hydrodynamically
Capillary temperature	20 °C
Bare fused silica capillary	50 μm i.d × 46 cm, (detection length, 8.5 cm from the outlet end of the capillary)
Detection wavelengths	200 nm and 280 nm

Acceptable speed of analysis and improvement in the separation efficiency were obtained when separated at 20 °C.

The effect of applied voltage (18–28 kV) was also investigated, when 22 kV was used, sharper peaks were obtained with good resolution. However, voltage above 22 kV was avoided due to Joule heating. In view of the sensitivity and peak shape, the injection time for the analytes was varied from 5 to 20 s at 50 mbar. As the injection time was increased, peak area will also increase accordingly. Peak broadening was observed with injection time above 17 s. Hence, injection time of 17 s was chosen.

In order to reduce the analysis time, the effect of effective capillary lengths (40–56 cm) on the migration time and resolution were also investigated. The use of 46 cm capillary resulted in acceptable resolution and analysis time. However, further reduction in capillary length resulted in poor resolution between the last two peaks (2-FA and 3-FA).

The adopted conditions are summarized in Table 1 while Fig. 3 shows typical electropherograms of the standards. Faster separation and stable baseline of the proposed MEKC method (~20 min) compared to the reported DI-SPME coupled with GC–MS method

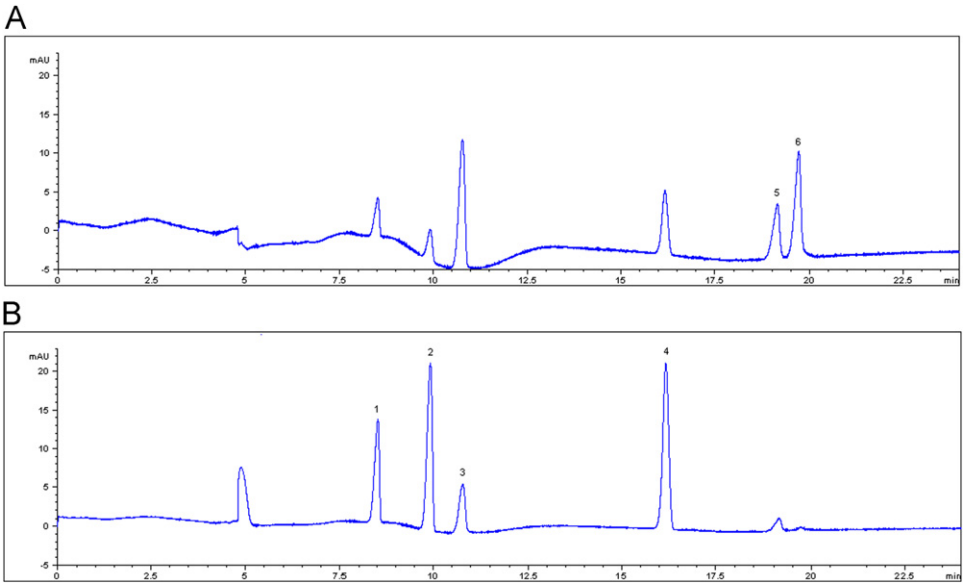

Fig. 3. Electropherograms obtained from the introduction of standard mixture containing 2-F, 3-F, 5-MF, 5-HMF, 2-FA and 3-FA (25 μg mL^{-1}) measured at 200 nm (A) and 280 nm (B), operated under the optimum electrophoretic conditions (Table 1). Peaks: **1**, 5-HMF; **2**, 2-F; **3**, 3-F; **4**, 5-MF; **5**, 2-FA; and **6**, 3-FA.

Table 2
Intra-day and inter-day repeatability for mixtures of 2-F, 3-F, 5-MF, 5-HMF, 2-FA and 3-FA standard solutions.

Analyte concentration (μg mL^{-1})	Relative standard deviation, RSD (%)											
	Migration time						Corrected peak area					
	2-F	3-F	5-MF	5-HMF	2-FA	3-FA	2-F	3-F	5-MF	5-HMF	2-FA	3-FA
Intra-day precision (n=9)												
3	4.43	2.47	4.03	4.05	2.84	2.85	7.89	6.67	3.41	6.89	7.35	7.72
13	0.89	0.97	1.18	0.79	1.21	1.25	2.61	3.53	2.51	1.89	4.05	4.18
25	0.68	0.72	0.96	0.61	0.92	0.97	6.69	4.56	6.38	7.04	8.87	9.78
Inter-day precision (n=54)												
3	3.61	3.71	5.18	3.19	5.70	5.88	8.80	9.45	9.32	7.07	7.05	9.95
13	3.00	3.20	5.95	2.69	5.02	5.55	6.45	5.83	6.83	5.11	7.96	6.51
25	1.45	1.56	4.08	1.29	1.47	1.53	4.91	3.39	5.33	4.77	6.40	5.70

(~24 min) for the separation of 2-F, 5-MF and 5-HMF was observed [22]. Moreover, the present method enables the separation of six furanic compounds compared to the reported reversed phase (RP) HPLC method (5 furanic compounds) [10]. Furthermore, the RP-HPLC method employed acidic mobile phase (0.1 M H_2SO_4), this is detrimental to the column lifetime and the internal parts of the system.

3.2. Validation of analytical method

3.2.1. Linearity

Linearity of the calibration plots was studied using standard mixtures of 2-F, 3-F, 5-MF, 5-HMF, 2-FA and 3-FA at eight concentration levels (1, 2, 3, 4, 5, 10, 15 and 25 μg mL^{-1}). Calibration curve was constructed by plotting the corrected peak area (y) as a function of analyte concentration (x) in μg mL^{-1}. Good linearity was observed over the range studied with regression equations and correlation coefficients (R^2) values of: 2-F (y=0.786x-0.005, R^2=0.999), 3-F (y=0.371x+0.113, R^2=0.994), 5-MF (y=0.615x, R^2=0.999), 5-HMF (y=0.599x-0.032, R^2=0.999), 2-FA (y=0.382x+0.276, R^2=0.991) and 3-FA (y=0.502x+0.106, R^2=0.993).

3.2.2. Limits of detection and quantitation

The limits of detection (LOD), with signal-to-noise ratio of 3 for 2-F, 3-F, 5-MF, 5-HMF, 2-FA and 3-FA were 0.33, 0.70, 0.42, 0.43, 0.68 and 0.52 μg mL^{-1}, respectively. Limits of quantitation (LOQ) were calculated at signal to noise ratio of 10. Values obtained for LOQ were 1.00, 2.12, 1.28, 1.31, 2.07 and 1.56 μg mL^{-1}, respectively. The sensitivity achieved meets the requirements for quality control purpose of honey, with maximum permitted 5-HMF concentration of 40 and 80 mg kg^{-1} (tropical origin) by the European Union (EU) and Codex Alimentarius of World Health Organization (WHO).

3.2.3. Precision

Intra-day precision was assessed by introducing standard mixtures of 2-F, 3-F, 5-MF, 5-HMF, 2-FA and 3-FA at three concentration levels (3, 13 and 25 μg mL^{-1}) on the same day (n=9). The relative standard deviation (RSD) for migration times and corrected peak area were less than 4.43 and 9.79%, respectively (Table 2). The inter-day precision was assessed by introducing three concentration levels (3, 13, 25 μg mL^{-1}) for six consecutive days (n=54). RSD for migration times and corrected peak areas were $<$5.96% and $<$9.96%, respectively (Table 2).

3.2.4. Accuracy studies for honey

Accuracy studies were performed in three replicates, by spiking the honey (10° Brix) with known amounts of standard and each sample was analyzed thrice. The results are summarized in

Table 3. Recoveries obtained ranged from 75.8 ± 9% to 108 ± 9% which is comparable to the method reported by Spano, et al. (≥ 81%) for the determination of 2-F, 3-F, 5-HMF, 2-FA and 3-FA [10]. The good accuracy values obtained indicate the potential of this method for the determination of the furanic compounds in honey. Fig. 4(A) shows the electropherograms obtained for spiked honey samples.

3.2.5. Accuracy studies for oil

Rapeseed oil (0.5 g) was spiked with different amounts of standards and analyzed thrice. The obtained recoveries ranged between 70.1 ± 8 and 120 ± 10 (Table 3) The recoveries for 5-MF (≤ 76.82%) were rather low compare to other furfurals (≥ 80.5%) as 5-MF is less polar (log P=1.38) compare to the other furfurals. Hence, the extraction using water was less efficient for 5-MF. The obtained recoveries for 2-F and 5-HMF (≥ 85.0% and ≥ 103%) are comparable with the method reported by Durmaz, et al. (≥ 98.1% and ≥ 94.3%) [31].

3.3. Analysis of honey samples

The validated method was applied for the determination of 2-F, 3-F, 5-MF, 5-HMF, 2-FA and 3-FA. Fig. 4(B) depicts typical electropherogram obtained for a honey sample. Results are shown in Table 4. 5-HMF ranging from 11 ± 0.2 to 1145 ± 19 mg kg^{-1} was found. These values are much higher than those observed by Spano et al. and Nozal et al. [10,35]. The results suggest that heat treatment have been applied on some of these samples. Another possibility is probably due to the relatively high hexose and 3-deoxyosone contents in these honey samples, as 5-HMF originates from reactions such as acid degradation of hexose and decomposition of 3-deoxyosone during the Maillard reaction [41,42]. It is also interesting to note that 5-HMF content is different according to the botanical origin, higher values are found in Tualang honey compared to the Acacia honey. 2-F was found in seven samples, ranging from 1.1 ± 0.1 (Acacia 2) to 6.5 ± 0.3 mg kg^{-1} (Tualang 3). The oxidative degradation reactions of ascorbic acid under anaerobic conditions can account for the formation of 2-F in the honey [43]. 5-MF was also found in one of the samples with concentration of 5.6 ± 0.6 mg kg^{-1} (Tualang 6). These findings suggests that 3-F, 5-MF, 2-FA and 3-FA are either minor constituents of honey or degradation products obtained through a complex, intermolecular rearrangement reactions from compounds which are not always present in the honey. Data also shows that most of the tropical honeys violate the Codex Alimentarius and EU limits established for 5-HMF.

3.3.1. Effect of heating

3-F, 5-MF, 2-FA and 3-FA were not detected during the whole heating treatment. 2-F levels remained almost constant in all samples regardless of heating temperature and duration. However, a small increase after 24 h of heating was found: 60 °C (4.44 to 7.17 mg kg^{-1}); 80 °C (4.44 to 11.2 mg kg^{-1}) and 100 °C (4.44 to 20.0 mg kg^{-1}). Fig. 5 (A, B and C) shows the changes in 5-HMF concentration when heated at different temperatures (60, 80 and 100 °C). Higher heating temperature and duration favours formation of 5-HMF, i.e., 100 °C (2374 mg kg^{-1}); 80 °C (508 mg kg^{-1}); and 60 °C (314 mg kg^{-1}) was found after 24 h of heating. The results clearly suggests that 5-HMF is a more sensitive chemical indicator for heat treatment compare to 2-F.

3.3.2. Gamma irradiation

Fig. 4(C) shows typical electropherograms obtained for non-irradiated and irradiated samples. No new peaks were observed from the irradiation effects. A steady reduction in 5-HMF levels was found when the irradiation dosage was increased (Fig. 6). The changes in 5-HMF levels were many orders of magnitude lesser compared to variations in temperature.

3.4. Analysis of vegetable oils

The recent reports on the presence of 2-F and 5-HMF in crude palm oil (CPO) [30], roasted nut and seed oils [31] are very interesting. The presence of 5-HMF in the oil is particularly intriguing as it is the most polar (log P = −0.09) than the other furanic compounds (2-F (log P=0.83), 3-F (log P=1.08), 5-MF (log P=1.38), 2-FA (log P=1.00) and 3-FA (log P=1.24)) and thus is expected to have low solubility in the oil. The roasting process applied to the nuts and seeds before the oil extraction have indirectly induced MR through the interaction of amino acids with reducing sugars or lipid oxidation products that eventually contribute to the formation of furfurals [8]. The formation of the furfurals in CPO has been attributed to the chemical breakdown of hemicelluloses to 5-carbon xylose that further dehydrated to form furfurals during the sterilization processing of fresh fruit bunches (FFBs) [30].

In the present studies, four palm oil samples were analyzed, i.e., cold press CPO, hot press CPO, crude palm olein (CPL) and crude palm stearin (CPS) (which are fractions of the hot press CPO). Low levels of 2-F and 3-F, below the limit of detection of the proposed method in three samples (hot and cold press CPO and CPS) were found. These findings suggest that furfurals are minor compounds in the samples, formed from the sterilization process. Post dehydration of the hydrolyzed hemicelluloses product

Table 3
Accuracy of the MEKC method (n=9).

Level spiked (μg mL^{-1})	Mean recovery (% ± SD)					
	2-F	3-F	5-MF	5-HMF	2-FA	3-FA
Honey						
2	94.9 ± 3	96.9 ± 6	95.4 ± 5	85.2 ± 5	75.8 ± 9	82.4 ± 8
5	108 ± 9	100 ± 8	104 ± 8	101 ± 6	85.9 ± 7	82.0 ± 7
13	106 ± 1	97.1 ± 5	107 ± 2	106 ± 2	98.1 ± 6	83.7 ± 6
20	98.1 ± 3	99.4 ± 5	94.8 ± 5	101 ± 3	89.3 ± 7	95.7 ± 7
Oil						
2	85.8 ± 7	80.5 ± 5	76.8 ± 9	106 ± 5	120 ± 10	93.9 ± 9
5	92.5 ± 4	86.2 ± 9	74.1 ± 5	110 ± 4	93.6 ± 10	114 ± 8
13	92.2 ± 3	82.8 ± 4	76.1 ± 3	103 ± 4	103 ± 8	101 ± 10
20	85.0 ± 3	83.4 ± 8	70.1 ± 8	107 ± 8	95.5 ± 10	94.8 ± 9

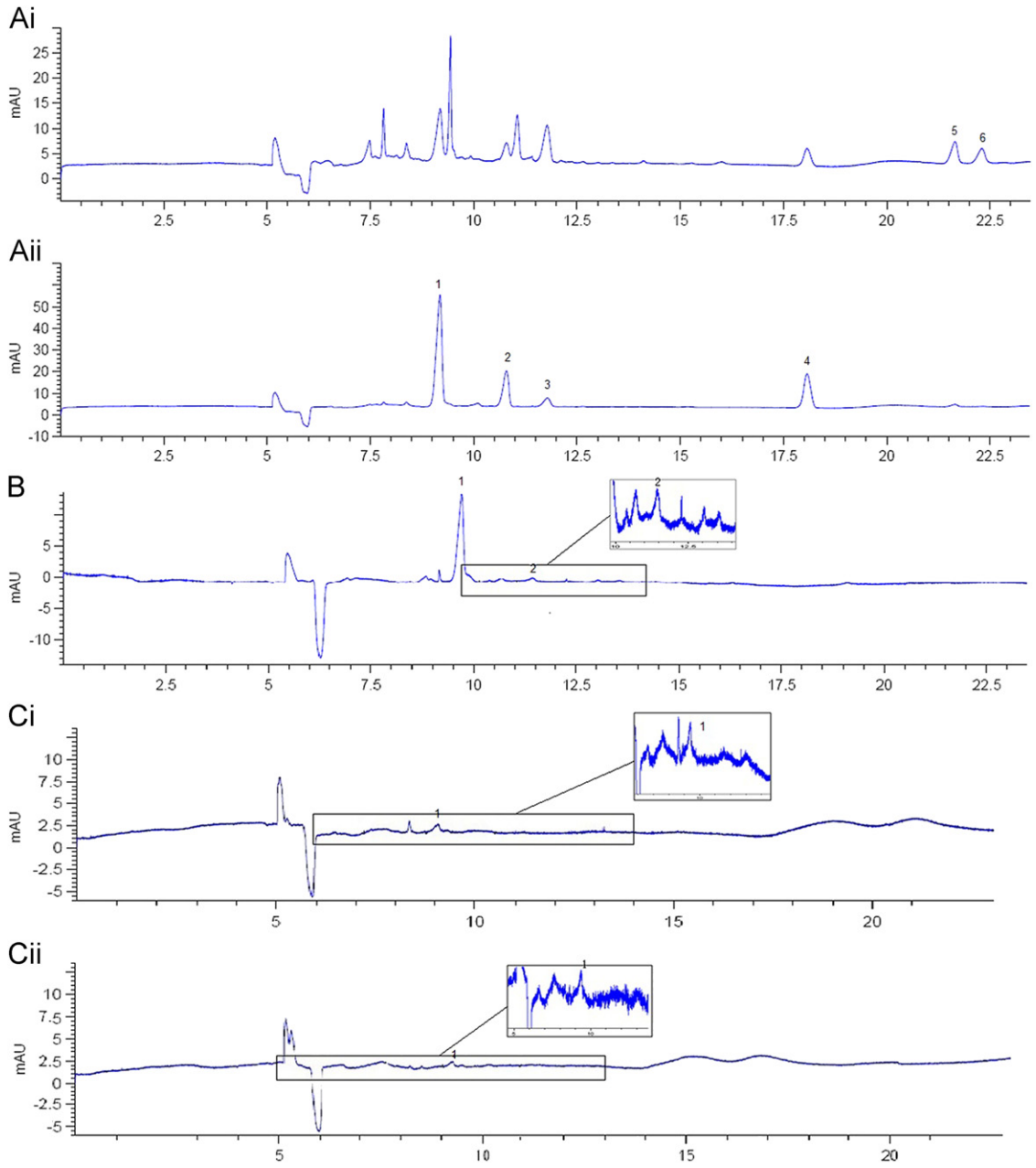

Fig. 4. Electropherograms of (A) (i) spiked honey measured at 200 nm, (ii) spiked honey; (B) honey sample (Tualang 7); (C) (i) non-irradiated honey (ii) irradiated (30 Gy) honey. Measured at 280 nm with the exception of A(i) (200 nm). Peaks: **1**, 5-HMF; **2**, 2-F; **3**, 3-F; **4**, 5-MF; **5**, 2-FA; and **6**, 3-FA.

Table 4
Details of honey samples studied and the levels of furanic compounds found.

Sample no.	Origin	Degree Brix (%) at 26.5 °C	Average concentration ± SD (mg kg^{-1})					
			2-F	3-F	5-MF	5-HMF	2-FA	3-FA
1	Malaysia (Penang)	75.9	< LoD	< LoD	< LoD	11 ± 0.2	< LoD	< LoD
2	Vietnam	74.5	2.3 ± 0.2	< LoD	< LoD	64 ± 4	< LoD	< LoD
3	Saudi Arabia	74.1	< LoD	< LoD	< LoD	208 ± 6	< LoD	< LoD
4 (Tualang 1)	Malaysia (Johor)	73.8	4.4 ± 0.1	< LoD	< LoD	168 ± 6	< LoD	< LoD
5 (Tualang 2)	Malaysia (Johor)	81.0	< LoD	< LoD	< LoD	112 ± 4	< LoD	< LoD
6 (Tualang 3)	Malaysia (Penang)	76.4	6.5 ± 0.3	< LoD	< LoD	468 ± 11	< LoD	< LoD
7 (Tualang 4)	Malaysia (Kedah)	74.4	< LoD	< LoD	< LoD	1145 ± 19	< LoD	< LoD
8 (Tualang 5)	Malaysia (Kedah)	73.4	3.0 ± 0.5	< LoD	< LoD	107 ± 2	< LoD	< LoD
9 (Tualang 6)	Malaysia (Terengganu)	70.8	< LoD	< LoD	5.6 ± 0.6	19 ± 1	< LoD	< LoD
10 (Tualang 7)	Malaysia	73.2	3.3 ± 0.4	< LoD	< LoD	149 ± 1	< LoD	< LoD
11(Tualang 8)	Malaysia	71.5	< LoD	< LoD	< LoD	16 ± 0.8	< LoD	< LoD
12 (Tani)	Malaysia	77.0	< LoD	< LoD	< LoD	75 ± 2	< LoD	< LoD
13 (Borneo)	Malaysia	76.7	< LoD	< LoD	< LoD	108 ± 3	< LoD	< LoD
14 (Borneo)	Malaysia	74.5	< LoD	< LoD	< LoD	175 ± 3	< LoD	< LoD
15 (Manuka)	New Zealand	78.4	< LoD	< LoD	< LoD	104 ± 3	< LoD	< LoD
16 (Gelam)	Malaysia (Terengganu)	74.1	< LoD	< LoD	< LoD	249 ± 3	< LoD	< LoD
17 (Acacia 1)	Malaysia (Johor)	75.5	< LoD	< LoD	< LoD	53 ± 0.5	< LoD	< LoD
18 (Acacia 2)	Malaysia (Johor)	74.2	1.1 ± 0.1	< LoD	< LoD	56 ± 2	< LoD	< LoD

[a] < LoD, below limit of detection.

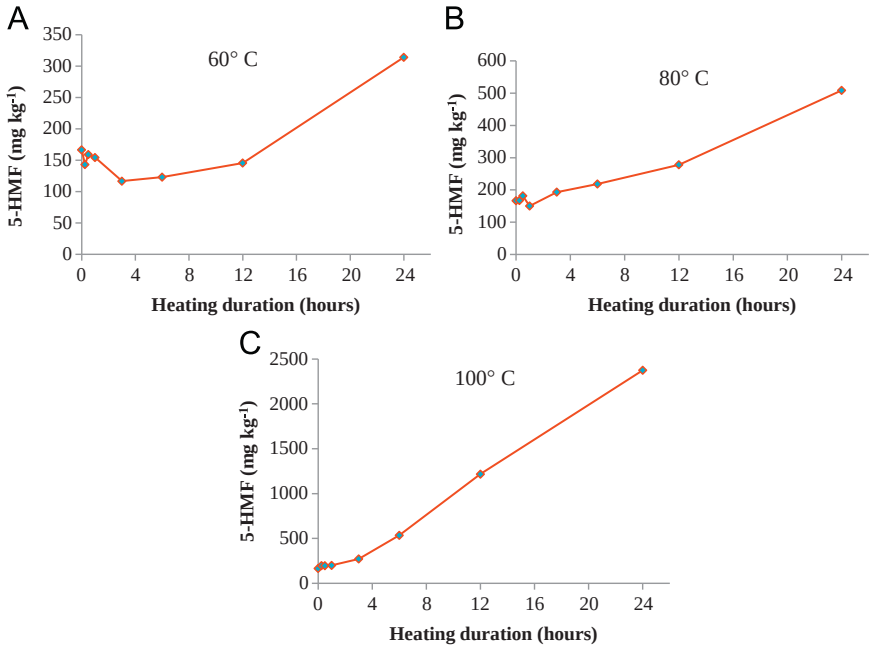

Fig. 5. Effect of heating (60 (A), 80 (B) and 100 °C (C)) on 5-HMF concentration in Tualang honey samples ($n=7$).

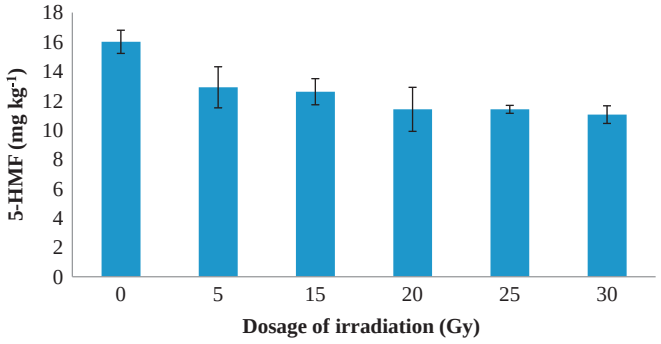

Fig. 6. Effect of irradiation on 5-HMF concentration in Tualang honey samples ($n=6$).

(xyloses) possibly attributed to the formation of furfurals in the palm oil samples. No furfurals were detected in nut and seed oils, which is due to the refining process after the extraction.

4. Conclusion

An analytical method for the simultaneous determination of 2-F, 3-F, 5-MF, 5-HMF, 2-FA and 3-FA was for the first time reported. The developed MEKC method was validated and applied for the determination of these furanic compounds in honey and vegetable oil samples. Heating resulted in significant formation of 5-HMF while irradiation causes a slight reduction in 5-HMF levels. It is also clear that 5-HMF is the better indicator for the assessment of the history of the honey sample e.g., heat and irradiation treatment. The formation of some of the furfurals in palm oil samples warrant a more comprehensive studies as both hot and cold press methods resulted in low levels of furfural formation. The formation of furfurals is probably due to the sterilization processes. The developed method is useful for routine quality control of honey that focuses on the analysis of chemical markers and adulteration of honeys. The satisfactory separation of the six furanic compounds will also be valuable in nutrition studies [44,45].

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