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2-Acetylfuran-3-Glucopyranoside as a Novel Marker for the Detection of Honey Adulterated with Rice Syrup

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ABSTRACT: The determination of honey authenticity is of importance to ensure its quality and safety. There is an urgent need of effective methods to detect adulterated honey. A simple, rapid, and effective HPLC−DAD method was developed to detect honey adulteration by rice syrup, using a characteristic compound from rice syrup, which is presently difficult to detect by current analytical methods. The characteristic compound was identified as 2-acetylfuran-3-glucopyranoside (AFGP) by MS and NMR. Based on HPLC analyses, the average concentration of AFGP was 92 ± 60 mg/kg in rice syrup. However, AFGP was not detected in any of the natural honey samples, so it could be used as a marker for the detection of honey adulteration by rice syrup. The developed method enabled a rapid detection of honey samples adulterated with 10% rice syrup. Using the developed method, 16 out of 186 honey samples from some markets were found to be adulterated with rice syrup.

KEYWORDS: *2-acetylfuran-3-glucopyranoside, rice syrup, HPLC-DAD, adulterated honey*

INTRODUCTION

Honey is a natural sweet substance produced by honey bees from flower nectar or plant secretions. Honey bees combine these plant compounds with specific substances and store them in honeycombs.[1] Honey is widely consumed throughout the world due to its nutritional and therapeutic properties. In comparison with other sweeteners, honey is highly expensive which makes it more prone to adulteration. Authentication of honey is of utmost importance for both consumers and the food industry. Efficient quality control and assurance of honey authentication is required to ensure its quality and safety.

Generally, honey is adulterated with inexpensive sweeteners such as corn syrup (CS), invert sugar syrup (IS), and high fructose corn syrup (HFCS). This type of adulteration is difficult to detect due to normal natural variations in honey carbohydrates and also similarities in the sugar composition between these syrups and natural honey.[2,3] Despite these limitations, several analytical methods are available for the detection of honey adulteration including thin-layer chromatography (TLC),[2,4] gas chromatography (GC), or gas chromatography−mass spectrometry (GC−MS),[5−7] high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC−PAD),[8−12] stable carbon isotopic ratio analysis (SCIRA),[13−17] infrared spectroscopy (IR),[18−20] and nuclear magnetic resonance (NMR).[21] These analytical methods are both reliable and valid for the detection of honey adulteration by sugar syrups but they have some limitations. TLC, GC, GC−MS, and HPAEC−PAD are very effective for the detection of honey adulterated with HFCS and use oligosaccharides, polysaccharides, and difructose anhydrides as adulteration markers. HPAEC−PAD, which was developed by Mehdi,[12] have been used in many testing laboratories in China. However, HPAEC−PAD is not valid for honey adulterated with syrups that contain low levels of oligosaccharides, difructose anhydrides, and polysaccharides. In many countries, SCIRA has been used as an official analytical method to detect honey adulteration by HFCS. However, this analytical method is limited for the detection of syrups from C4 plants (e.g., corn and sugar cane). It is difficult to detect syrups from C3 plants because of their similarities in the isotope composition of natural honey.[8] IR and NMR combined with chemometrics constitute the simplest and most rapid screening methods for the detection of adulterated honey and also they rely on simple extraction and sample preparation methods. But, IR and NMR do have certain limitations: they require a large amount of samples and arduous data analyses.

Honey adulterated with rice syrup has recently emerged in the honey market. Rice syrup, which is obtained from rice, is very difficult to detect by the current analytical methods. It is impossible to detect this syrup by common SCIRA, as it is from C3 plants and thus follows a similar Calvin cycle of photosynthesis as natural honey. Additionally, the production of rice syrup involves the hydrolysis of polysaccharides and oligosaccharides, making it difficult to detect the presence of rice syrup by TLC and HPAEC−PAD. Currently, honey adulterated with rice syrup has become a serious problem that affects its quality and safety. Therefore, there is an urgent need for rapid, accurate, and reliable methods for the detection of honey adulterated with rice syrup. To the best of our knowledge, no studies have been conducted about this type of adulteration.

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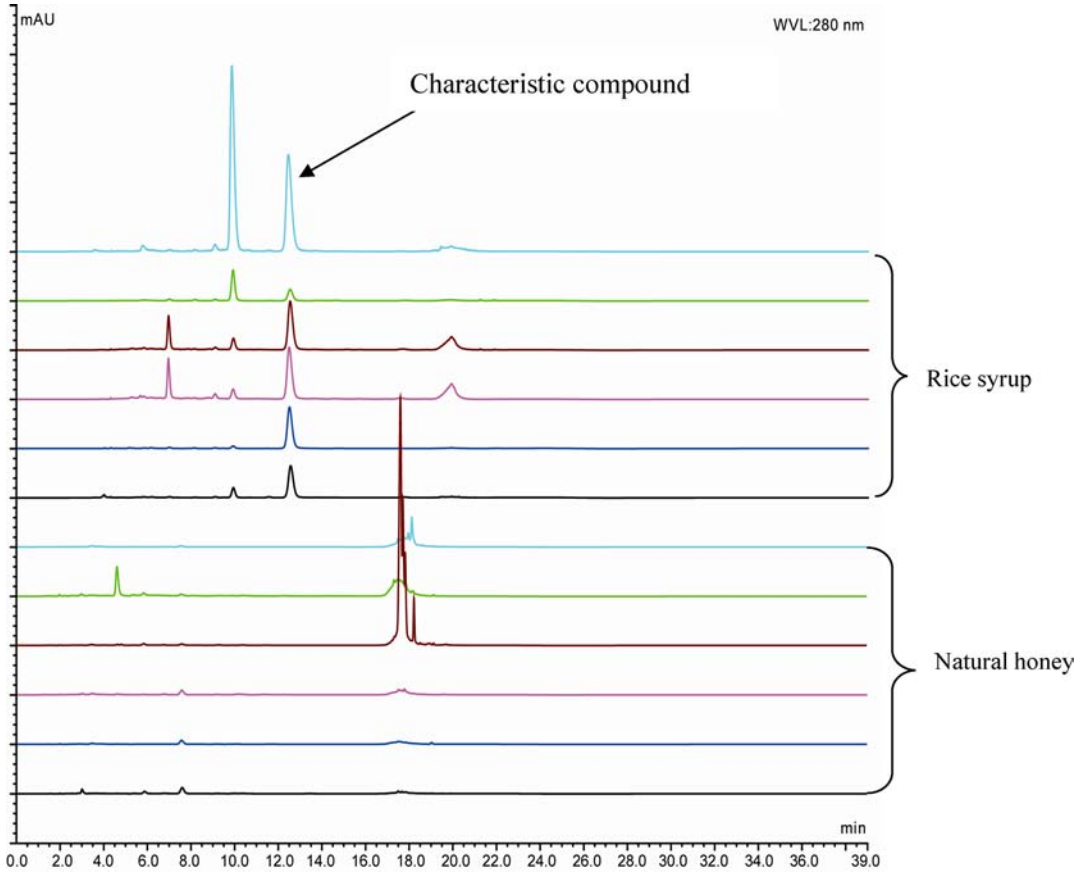

Figure 1. Chromatograms of rice syrup and natural honey. Arrow indicates the characteristic compound of rice syrup.

The aims of this study were (i) to find a specific compound in rice syrup that can be used as a marker for the detection of honey adulteration, and to use this marker in the development of a simple, rapid, and effective analytical method based on high performance liquid chromatography with diode array detection (HPLC–DAD); and (ii) to apply the developed analytical method to investigate the presence of adulterated honey in the market with rice syrup.

MATERIALS AND METHODS

Sample Collection. One hundred and sixty honey samples were as follows: Acacia honey (25, labeled A1–A25), jujube honey (20, labeled J1–J20), rape honey (30, labeled R1–R30), linden honey (15, labeled L1–L15), litchi honey (20, labeled LZ1–LZ20), clover honey (25, labeled C1–C25), and multifloral honey (25, labeled M1–M25) were obtained from 34 beekeepers located in Beijing, Zhejiang province; Hubei, Sichuan, Yunnan, Shannxi, Liaoning, Xinjiang and Shandong Province, China, respectively. The honey samples were selected according to strict criteria with the quality charter ensuring their authenticity.

Thirty two representative rice syrup samples (labeled RS1–RS32) were purchased from some markets in China. Syrup RS26 was added in different proportions (10, 20, or 50%, w/w) to an authentic honey sample to intentionally simulate honey adulteration.

One hundred and eighty six commercial honey samples (labeled sam1–sam186) were randomly purchased from some markets in Beijing, Hebei, Henan, Jiangsu, Zhejiang, and Shandong Province, China.

Reagents and Standards. The compound 2-acetylfuran-3-glucopyranoside (≥95% purity determined by HPLC) was obtained by preparative high performance liquid chromatography (PHPLC) and lyophilization. Acetonitrile of HPLC grade was purchased from Fisher Chemicals (FairLawn, NJ). Deionized water was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA).

Sample Preparation. For sample preparation, 5 g of sample and 5 mL of deionized water were added to a 30 mL centrifuge tube, mixed in a vortex for 3 min, and centrifuged at 10 000 rpm for 10 min. The resulting supernatant was filtered through a 0.45 μm PVDF membrane. The filtrate was taken up for further analysis.

HPLC Analysis. HPLC analysis was performed using a Dionex Ultimate 3000 HPLC system (Blaine, MN) equipped with a P680 pump, an ASI-100 auto injector, a TCC-100 column oven, a DAD 100 detector, and an Agilent ZORBAX AqC$_{18}$ (100 × 2.1 mm, 3.5 μm) column. Instrument control and data acquisition were performed using Chromeleon software. The mobile phase gradient consisted of 99% water (solvent A) and 1% acetonitrile (solvent B) for the first 10 min. Solvent B increased to 10% acetonitrile over 5 min and held for 1 min. Solvent B was then adjusted to 90% and held for 6 min. Finally, solvent B was reduced to 1% over 1 min and held for 10 min. The flow rate was 0.2 mL/min and the injection volume was 20 μL. The column temperature was maintained at 30 °C. The detection wavelength was set to 280 nm, the maximum absorption of the standard compound.

PHPLC. PHPLC (Agilent, Waldbronn, Germany) consisted of a 1362A preparative pump equipped with a G1365D multiple wavelength detector (MWD) and a preparative column (Prep C$_{18}$, 150 × 21.2 mm, 5 μm, Agilent, Santa Clara, CA). The flow rate was set to 15 mL/min, the injection volume was 5.0 mL, and the column temperature was maintained at 30 °C. The mobile phase, elution conditions, and detection wavelength were same to those used in HPLC. The sample was added to the column and the elute containing the desired compound was added several times to the column until the compound was purified. Purified compounds were freeze-dried and analyzed by both liquid chromatography–quadrupole–time of flight mass spectrometry (LC–Q–TOF MS) and NMR.

LC–Q–TOF MS. LC was performed in an Agilent 1100 Series HPLC (Agilent, Palo Alto, CA, USA) equipped with an autoinjector

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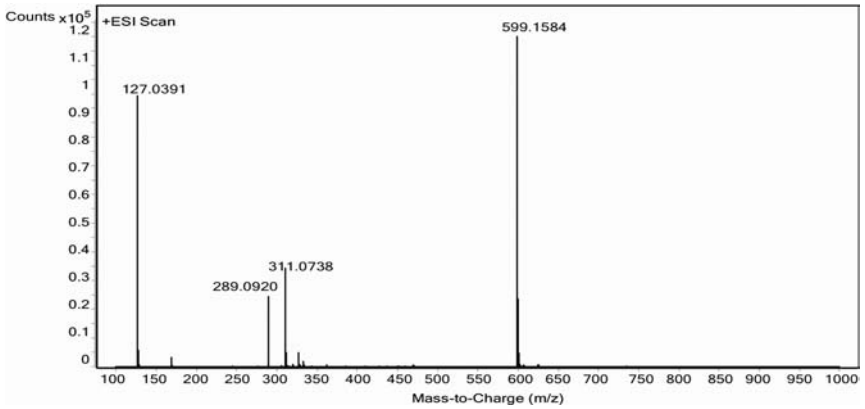

Figure 2. Mass spectrum of the marker compound. Peak assignment is highlighted.

and a quaternary HPLC pump. The separation was performed on an Agilent ZORBAX AqC_{18} (100 × 2.1 mm, 3.5 μm) column. The injection volume was 5 μL. The mobile phase consisted of acetonitrile (solvent A) and water (solvent B) in a 99:1 (v/v) ratio. Total run time was 15 min with flow rate of 0.2 mL/min.

MS was performed in an Agilent 6510 ESI−Q−TOF. The optimized conditions consisted of a capillary voltage of 4.0 kV in positive ionization mode, a fragmentor voltage of 125 V, and a skimmer voltage of 65 V. Gas temperature was 350 °C, drying gas flow rate was 9 L/min, and nebulizer pressure was 45 psi. Nitrogen was used as the collision gas. MS spectra were acquired within the range of 100−1,000 m/z using an extended dynamic range and a scan rate of 1.4 spectra/s by varying collision energy with mass. The Mass Hunter Workstation software (Version B.01.03) was used. A reference mass solution containing reference ions (121.0508 and 922.0097) was used to maintain mass accuracy during the run time.

NMR Spectroscopy. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), and ^{1}H−^{1}H correlation spectroscopy (COSY) were recorded at 25 °C using a Varian 600 MHz spectrometer in DMSO-d_6 and DMSO-d_6+D_2O. Tetramethylsilane (TMS) was used as an internal standard for the determination of chemical shifts.

RESULTS AND DISCUSSION

Selection of a Marker Compound in Rice Syrup. Selection of an adulteration marker is crucial for the detection of adulterated honey. Suitable markers can be selected from the adulterants (i.e., foreign additives) or from natural honey. In addition to glucose and fructose, natural honey contains organic acids, proteins, amino acids, phenolic acids, and flavonoids. These compounds could be used as markers for the detection of adulterated honey. For example, honey amino acids and proteins have been used to detect adulterated honey.[22,23] However, the concentrations of amino acids and proteins vary considerably depending on the type of honey, making it difficult to detect adulterated honey when low levels of adulterants have been added.

Ideally, markers for the detection of adulterated honey should be selected from the adulterants. Oligosaccharides, polysaccharides, and difructose anhydrides are suitable markers for the detection of honey adulteration by corn-based syrups and sugar cane syrup. Researchers have used SCIRA, TLC, HPLC−PAD, and GC to detect honey adulteration using these markers. In this study, a suitable marker was found from rice syrup to detect honey adulteration.

In this study, 32 rice syrup samples and 160 natural honey samples were analyzed by HPLC−DAD. A representative HPLC−DAD chromatogram is shown in Figure 1. By comparing the HPLC−DAD chromatograms obtained from rice syrup and natural honey, an unknown compound at about 12.5 retention time was detected in rice syrup. This compound was present in all rice syrup samples, was not seen in the natural honey samples. Therefore this compound was selected as a suitable marker.

Isolation and Purification of the Marker Compound. To obtain more information on the marker compound, we followed a thorough analytical procedure. The rice syrup sample labeled RS6 was selected as the preparative material due to its relatively higher content of marker. RS6 (200 g) was dissolved in 200 mL of hot water (at approximately 50 °C) and filtered through cotton wool to remove solid particles. The filtrate was passed through a chromatography column (400 mm ×12 mm, C_{18}, 10 μm), which was washed with 50 mL of water to remove sugars and other polar compounds present in honey. The adsorbed compounds were eluted with acetonitrile (50 mL). The eluted solution was concentrated at 40 °C under reduced pressure using a rotary evaporator. The residue was dissolved in 10 mL of water. The final solution was filtered through a 0.45 μm membrane filter and injected into PHPLC. PHPLC analyses were performed as previously described. The purified compounds were lyophilized, yielding approximately 20 mg of marker compound. Based on HPLC analysis, the purity of the marker compound was >95%. The purity and amount of the marker compound met NMR requirements.

Chemical Identification of the Marker Compound. Mass determination was performed using LC−Q−TOF MS. Background noise and irrelevant ions were excluded from the results using molecular feature extraction (MFE) data files, a function of the Mass Hunter Workstation software. Mass values were obtained within an error of <5 ppm, which allowed us to rapidly generate possible molecular formulas. The mass spectrum of the marker compound is shown in Figure 2. The mass spectrum had an m/z of 289.0920 $[M+H]^+$, calculated for $C_{12}H_{17}O_8$ (deviation: +0.7 ppm); an m/z of 311.0738 $[M+Na]^+$, calculated for $C_{12}H_{16}O_8Na$ (deviation: −0.3 ppm); an m/z of 599.1584 $[2M+Na]^+$, calculated for $C_{24}H_{32}O_{16}Na$ (deviation: +0.2 ppm) and an fragmentation m/z 127.0391 calculated for $C_6H_7O_3$ (deviation +0.8 ppm). Based on these mass data, the Mass Hunter Workstation software revealed that a possible molecular formula for the marker compound was $C_{12}H_{16}O_8$.

^{1}H NMR (Figure 3) and ^{13}C NMR of the marker compound, with an arbitrary numbering of the carbon atoms, refer to the structure shown in Figure 4. ^{1}H NMR results (600 MHz, DMSO-d_6, δ, ppm, J/Hz) were 7.81 (1H, d, J = 2.1, H-6); 6.78

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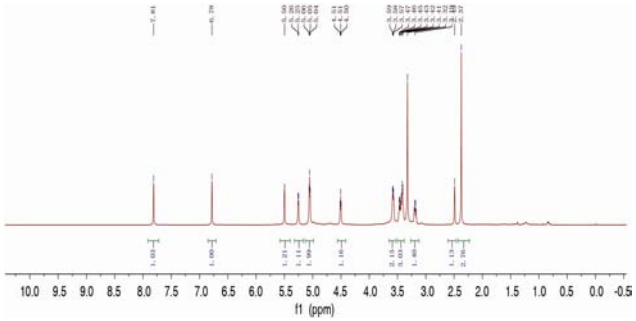

Figure 3. ^{1}H NMR spectrum of the marker compound.

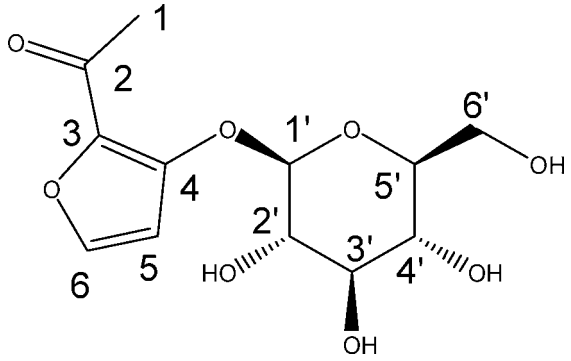

Figure 4. Chemical structure of the marker compound.

(1H, d, J = 2.1, H-4); 5.50 (1H, d, J = 2.4, H-1′); 5.25 (1H, d, J = 5.4, OH); 5.06 (1H, d, J = 4.8, OH); 5.05 (1H, d, J = 6.0, OH); 4.51 (1H, t, J = 6.0, OH); 3.59−3.17 (6H, m, H2′−H6′); and 2.37 (3H, s, H-1). ^{13}C NMR results (150 MHz, DMSO-d6, δ) were 183.2 (C-2); 152.4 (C-4); 149.2 (C-6); 137.4 (C-3); 104.8 (C-5); 99.3 (C-1′); 74.6 (C-5′); 73.0 (C-3′); 71.1 (C-2′); 69.6 (C-4′); 60.6 (C-6′); and 27.3 (C-1). These results were confirmed by distortionless enhancement polarization transfer (DEPT) and ^{1}H−^{1}H COSY.

Combining the data obtained from NMR and MS analyses, the marker compound was identified as 1-[3-(3,4,5-trihydroxy-6-methoxy-tetrahydro-2*H*-pyran-2-yloxy) furan-2-yl]-ethanone. Using ChemDraw Ultra 7.0(CambridgeSoft Corporation), the structure of the marker compound is shown in Figure 4. The marker compound was abbreviated as 2-acetylfuran-3-glucopyranoside (i.e., AFGP).

Determination of AFGP by HPLC−DAD. A simple and fast HPLC−DAD method was developed for the determination of AFGP. The optimum chromatography conditions were described in the HPLC Analysis section. Six AFGP standard solutions (0.5, 1, 10, 50, 100, and 200 mg/L) were prepared by serial dilution with water and analyzed by HPLC. The AFGP standard curve resulted in a linear relationship described by $y = 0.6354x - 0.0364$, where y and x represent the peak area and concentration of the standard solution, respectively. The AFGP standard curve had a good linearity in the range of 0.5−200 mg/L (r = 0.9997). Accuracy, determined at three concentrations (1, 5, and 10 mg/kg) was satisfactory (recovery rates 99.2−101.4% with RSD <2.7%). Based on signal-to-noise rate (S/N) of 3 and 10, the limits of detection (LOD) and quantification (LOQ) were determined by HPLC−DAD using the AFGP standard solutions analyzed by HPLC. The resulting LOD and LOQ were 0.15 mg/kg and 0.35 mg/kg, respectively.

Analyses of AFGP in Rice Syrup and Natural Honey Samples. The presence of AFGP in all rice syrup and natural honey samples was analyzed using the developed method. Representative HPLC chromatograms of AFGP standard, rice syrup, natural honey and positive sample are shown in Figure 5a−d, respectively. A total of 32 rice syrup samples and 160

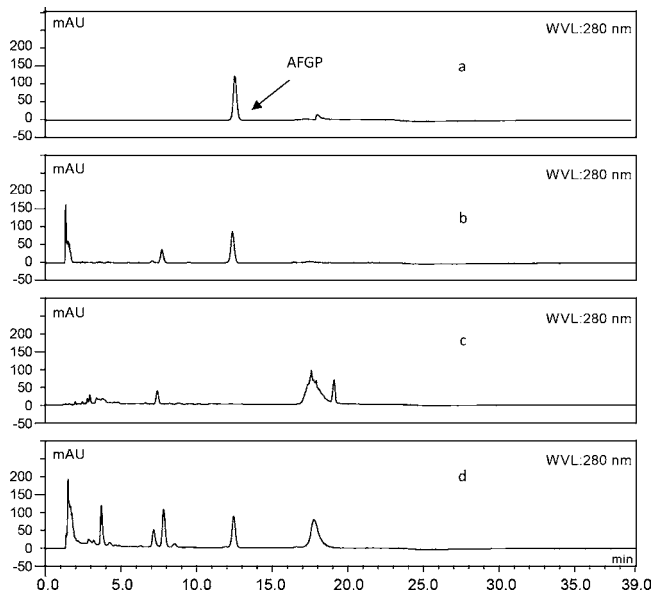

Figure 5. Representative chromatograms of AFGP standard (a), rice syrup (b), natural honey(c), and a positive sample (d).

natural honey samples were analyzed. As expected, AFGP was detected in all rice syrup samples; its concentration ranged from 32 to 152 mg/kg. On the other hand, AFGP was not detected in any of the natural honey samples.

Analyses of AFGP in Adulterated Honey with Rice Syrup and in Honey Market Samples. Honey samples, adulterated with 10%, 20%, and 50% rice syrup, were analyzed using the developed method; the resulting AFGP concentrations were 3.3, 6.4, and 15.9 mg/kg, respectively. As shown in Figure 6, AFGP was easily detected and quantified at the 10% adulteration level. Below the 10% adulteration level, AFGP was easily detected due to good S/N rates present at the 10% adulteration level (26:1). However, this low level of adulteration was not a concern in this study because there is little profit to earn when adulteration is at a lower level from economics/commerce perspective.

Using the developed HPLC−DAD method, 186 honey samples from different origins were analyzed. The results revealed that AFGP was detected in 16 samples with its concentration ranged from 21.5 to 145.6 mg/kg. The results are summarized in Table 1. By comparing the results to the AFGP concentration present in rice syrup, it was concluded that the 16 honey market samples almost consisted of 100% rice syrup.

Comparison of Results with Traditional Analytical Methods. To validate the developed method for the detection of adulterated honey with rice syrup, the 16 positive samples were simultaneously analyzed by two traditional analytical methods: TLC[2] and SCIRA[14]. The results are summarized in Table 1. According to the results, SCIRA was not suitable for the detection of honey adulteration by rice syrup because it failed to detect any positive samples. Only four adulterated samples were detected using TLC method. Thus, TLC was

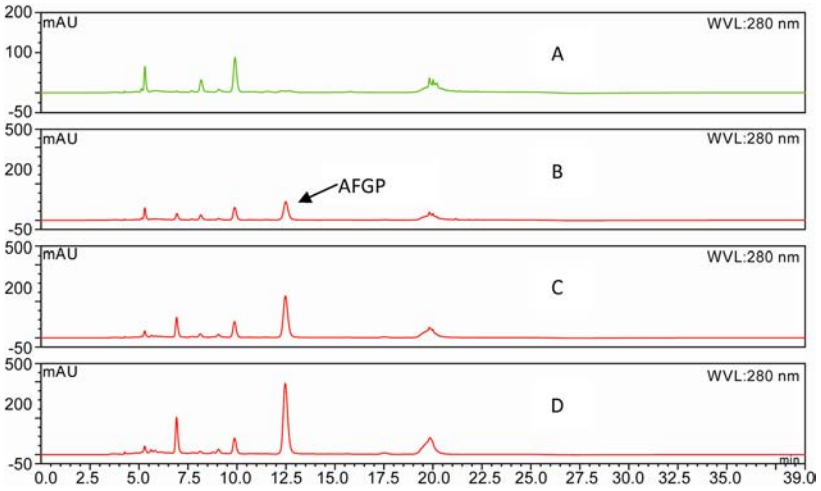

Figure 6. HPLC analysis of natural honey (A), honey adulterated with 10% rice syrup (B), honey adulterated with 20% rice syrup (C), and honey adulterated 50% rice syrup (D).

Table 1. Detection of 16 Positive Honey Samples by AFGP, TLC, and SCIRA[a]

sample no.	AFGP content (mg/kg)	TLC	SCIRA
sam9	132.2	−	<7%
sam21	125.4	−	<7%
sam22	121.0	−	<7%
sam55	90.4	+	<7%
sam73	130.2	−	<7%
sam74	128.4	−	<7%
sam75	132.2	−	<7%
sam97	21.5	+	<7%
sam112	60.6	−	<7%
sam116	70.4	−	<7%
sam119	90.6	+	<7%
sam126	145.6	−	<7%
sam143	140.7	−	<7%
sam149	139.2	−	<7%
sam163	120.8	+	<7%
sam177	86.4	−	<7%

[a]"+" detected, "−" not detected. "< 7%" addition of syrup is less than 7%, which is a negative sample (SCIRA method).

partly effective for detecting adulterated honey with rice syrup. Compared with the two traditional methods, the developed method was very effective in detecting adulterated honey with rice syrup.

In this study, a characteristic compound was detected in rice syrup and used as a marker of honey adulteration by rice syrup. Newly adulterated honey samples in the market are difficult to detect using the available and traditional methods. The characteristic compound in rice syrup was 1-[3-(3,4,5-trihydroxy-6-methoxy-tetrahydro-2*H*-pyran-2-yloxy)furan-2-yl]-ethanone, which was abbreviated as 2-acetylfuran-3-glucopyranoside or AFGP. The results reveal that AFGP meets the requirements of an adulteration marker: (a) it is not present in natural honey, (b) it is easily detected by HPLC, (c) it is specifically present in rice syrup, and (d) it is present at a certain concentration in rice syrup. The presence of AFGP in honey sample is a clear indication of the addition of rice syrup.

Using AFGP as a marker, a simple, fast, and effective HPLC method was developed for detecting adulterated honey with rice syrup. By analyzing intentionally adulterated honey samples with different levels of rice syrup, the developed method enabled a rapid detection of honey adulterated with 10% rice syrup. In comparison with two traditional methods, the developed method was more accurate and effective for the detection of rice syrup as an adulterant. Using this new method, 16 out of 186 honey market samples were found to be adulterated with rice syrup. This result indicates that adulteration by rice syrup is currently very serious in the honey market. According to the AFGP structure, this compound is a furan derivative, thus it may have some toxicity effects. Therefore, future study is needed to evaluate the risks associated with AFGP consumption.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

HPLC, high performance liquid chromatography; PHPLC, preparative high performance liquid chromatography; LC-Q-TOF MS, liquid chromatography-Quadrupole-time of flight mass spectrometry; NMR, nuclear magnetic resonance; COSY, correlation spectroscopy; DEPT, distortionless enhancement polarization transfer; AFGP, 2-acetylfuran-3-glucopyranoside

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