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BRIEF COMMUNICATION

Quality-by-Design: Multivariate Model for Multicomponent Quantification in Refining Process of Honey

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ABSTRACT

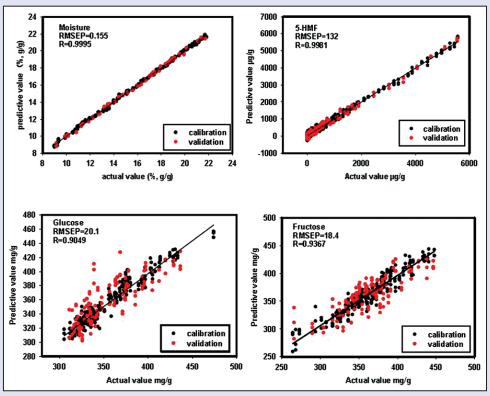
Objective: A method for rapid analysis of the refining process of honey was developed based on near-infrared (NIR) spectroscopy. **Methods:** Partial least square calibration models were built for the four components after the selection of the optimal spectral pretreatment method and latent factors. **Results:** The models covered the samples of different temperatures and time points, therefore the models were robust and universal. **Conclusions:** These results highlighted that the NIR technology could extract the information of critical process and provide essential process knowledge of the honey refining process.

Key words: Honey, near-infrared, partial least squares, rapid analysis, refining process

SUMMARY

- A method for rapid analysis of the refi ning process of honey was developed based on near-infrared (NIR) spectroscopy.

Abbreviations used: NIR: Near-infrared; 5-HMF: 5-hydroxymethylfurfural; RMSEP: Root mean square error of prediction; R: correlation coefficients; PRESS: prediction residual error-sum squares; TCM: Traditional Chinese medicine; HPLC: High-performance liquid chromatography; HPLC-DAD: HPLC-diode array detector; PLS: Partial least square; MSC: multiplicative scatter correction; RMSECV: Root mean square error of cross validation; RPD: Residual predictive deviation; 1D: 1st order derivative; SG: Savitzky-Golay smooth; 2D: 2nd order derivative.



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INTRODUCTION

Honey has been used as food as well as traditional Chinese medicine (TCM). It has the effect of strengthening the middle warmer, moistening the lung to suppress cough, and moistening dryness of intestine and detoxification.[1] Besides, honey has been used as auxiliary material during the process of Chinese medicinal herbs and the preparation of Chinese patent drug.[2]

Honey has been used as adhesive and corrective after being processed in the honey pills. Honey pill is widely approved in China. There are lots of famous and effective prescriptions appeared in the form of honey pill, such as Angong Niuhuang Pill.[3,4] Though the refined honey plays an important role in the preparation of honey pill, once it is not well refined, honey pills will be easily wrinkled, dried, and cracked.

Refined honey, which has been used in Chinese herbal medicine processing, was called honey-fried method. Raw drugs decocted by refining honey are widely used in clinical settings. Thousands of honey-fried crude drugs can be found in Chinese pharmacopeia and local standards.[5-8] The quality of the refined honey influences the quality of herbal medicine.

In the refining process, the quality of honey is affected by heating time, temperature, pressure, evaporation intensity, gas flow, etc., Moisture has long been regarded as the test item of refined honey, and its content can indicate the rank of the refined honey. A previous study has explored the above-mentioned factors according to the index of moisture content.[9,10] However, more components should be explored. 5-hydroxymethylfurfural (5-HMF) is bad for human health. Reports indicate that 5-HMF can cause the paralysis of nonstriated muscle and organ injury. Newly collected honey does not include 5-HMF. 5-HMF is produced during the storage and processing of honey and it is the degeneration product of monosaccharide such as glucose. Therefore, it must be strictly controlled.[11-13] Fructose and glucose are the main contents of the honey.[14] They are easily destroyed during the refining process, and it is necessary to monitor both of them.

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Near-infrared (NIR) spectroscopy is a newly emerging process analytical technology, which is fast, environmental friendly, free of pretreatment, and able to detect different components at the same time. NIR spectroscopy is widely used in the analysis of TCM's manufacturing process, such as the extraction process, the drying process, and the blend process.[15-19] Currently, the application of the technique in honey is focused on the content determination, distinction of different producing areas and floral resources, and adulteration. There is no literature reporting the application of NIR in the process of refining honey.

Since the quality of the refining honey has profound effects, the control of the refining process of the refining honey can not only guarantee the quality of the refining honey, but also make a meaningful exploration of the nature of the process. In this study, we studied the role of the temperature and time played in the refining process of honey according to the contents of moisture, 5-HMF, fructose, and glucose. NIR technique was applied in the refining process of honey.

MATERIALS AND METHODS

Materials

Honey was purchased from Tong Ren Tang Technologies Co., Ltd. (Beijing, China). 5-hydromethylfurfural (5-HMF) (lot number: 110777–201005), fructose (lot number: 100231–200904), and glucose (reference standards: 110833–200904) were supplied by the National Institute for Food and Drug control (Beijing, China). Methanol and acetonitrile of high-performance liquid chromatography (HPLC) grade were purchased from Fisher Scientific Co., Ltd. (New Jersey, USA). Distilled water was purchased from Watsons Co., Ltd. (Hong Kong, China).

Sample preparation

Amounts of honey in the two-necked round-bottomed flask were refined for 10 h in the oil-bathing of constant temperature of 100°C, 110°C, and 120°C, respectively. Samples were collected every 15 min from 0 min to 600 min, and 123 kinds of samples were obtained.

Near-infrared equipment

The NIR spectra were collected by the transflective mode using the Antaris Ⅱ NIR spectrophotometer (Thermo Electron Co., USA). Each spectrum was scanned 32 times with a resolution of 8/cm. The spectra range was from 4000/cm to 10,000/cm. Spectra of each sample were collected 3 times and the average result of three spectra was used for future analysis. Data analysis was performed with the TQ Analyst V8.0 software (Thermo Electron Co., USA).

Determination of water content

The moisture in the honey was measured by Abbe refractometer. The detail of the method can be found in the industry standard of Import and Export Inspection and Quarantine of China (SN/T0852–2000). The temperature of the water flowed through the refractometer was 40°C.

Determination of 5-hydroxymethylfurfural content

A 2.5 g sample of refining honey was dissolved by 10% methanol (v/v), and then transferred into a 25 mL volumetric flask, 10% methanol was added to the scale. After the sample solution was filtrated through a 0.45 μm filter, the filtrate was transferred into an HPLC vial before HPLC-diode array detector (HPLC-DAD) analysis.

A Shimadzu LC-20AT system consisting of two pumps, DAD detector, a thermostat maintained at 30°C, and an auto sampler was adopted. The sample filtrate was separated and analyzed by an Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm). The mobile phase consisted of 94% solvent A (water) and 6% solvent B (methanol). The flow rate was 0.8 mL/min. The absorbance was measured at a wavelength of 283 nm. The chromatographic peaks were identified by comparing their retention time against standards.

Determination of fructose and glucose contents

A 0.2 g sample of refining honey was dissolved by 10% methanol (v/v), and then transferred into a 100 mL volumetric flask, 10% methanol was added to the scale. After the sample solution was filtrated through a 0.45 μm filter, the filtrate was transferred into an HPLC vial before HPLC-DAD analysis.

An Agilent 1100 system equipped with Alltech 3300 ELSD detector was used in the determination of fructose and glucose. The samples were separated and analyzed by an APS-2 Hypersil NH2-column (250 × 4.6 mm, 5 μm), which was used as a reversed-phase column. The mobile phase was 25% solvent A (water) and 75% solvent B (acetonitrile), and its flow rate was 0.8 mL/min. The gas flow rate was 3.0 mL/min. The temperature of the drift tube was set at 80°C. The gain of the instrument was 1. The injection volume was 5 μL.

Software and basic theory

TQ Analyst V8.0 software related to the equipment was applied to process data. Partial least square (PLS) analysis can consider both variable matrix Y (data or property collected by traditional method) and matrix X (the spectra) at the same time, thus PLS can solve problems, which cannot be solved by common multiple regression. Therefore, PLS was chosen to establish the regression model. Stratified sampling method was used to divide the calibration set, and validation set for the data of 5-HMF showed skewed distributions. In other words, one of every three samples is divided into the validation set. Finally, 82 samples were chosen as calibration set and 41 samples were chosen as validation set.

To evaluate the result of the models established by PLS, 5 indicators were introduced. They are root mean square error of prediction (RMSEP), root mean square error of cross validation, prediction residual error-sum squares (PRESS), correlation coefficient (*R*), and standard deviation/SEP.

$$\text{RMSEP}=\sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i-y_i)^2}{n}}$$

$$\text{RMSECV}=\sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_{\backslash i}-y_i)^2}{n}}$$

$$\text{R}=\sqrt{1-\frac{\sum_{i=1}^{n}(\hat{y}_i-y_i)^2}{\sum_{i=1}^{n}(\hat{y}_i-\overline{y})^2}}$$

RESULTS AND DISCUSSION

Result of moisture content by reference method

Moisture loss is an important characteristic of the refining process of honey. Figure 1 shows how the content of moisture changed during the refining process. As we can see from the figure, as the refining time goes on, moisture content declines apparently. The loss rate of the moisture exaggerates with the increasing temperature. That is, the loss rate was higher when the temperature was 120°C than 110°C and 100°C. The linear regression equations were calculated, and the slopes were − 0.15, −0.17, and − 0.22, respectively.

The refining honey was divided into three grades according to traditional Chinese pharmaceutical theory for different purposes. In Grade A, the water content varies between 17% and 20%, and after 105, 90, and 120 min heating at 100, 110, and 120°C, the refining honey began to be Grade A. In Grade B, the water content varies between 14% and 16%, and after 435, 360, and 195 min heating at 100, 110, and 120°C, the refining honey began to be

Grade B. In Grade C, the water content was < 10%, and after 525 min heating at 120°C, the refining honey began to be Grade C. After 10 h heating, the final moisture content was 11.88%, 11.26%, and 8.99%, respectively.

Quantitative analysis of 5-hydroxymethylfurfural by high-performance liquid chromatography method

HPLC method was explored to determine 5-HMF. Figure 2 shows the chromatograms of 5-HMF reference standard and the honey sample. 5-HMF in honey sample has the same retention time with the reference standard. The methodology study was investigated. The calibration curve exhibited good linearity ($r = 0.9999$), within the quantitative range from 3.76 to 188.00 μg. The methodology parameters were investigated before the realistic sample analyses such as the precision, stability, the average recovery, and repeatability test. It comes to the conclusion that HPLC method can satisfy all the demands of quantitative analysis, and can provide accurate data for NIR calibration.

Figure 3 reveals that the refining temperature and time had a great effect on the content of 5-HMF. 5-HMF content increases along with the time extension and temperature rise. The growth rate of 5-HMF also increases along with the temperature rise, in other words, the growth rate of 5-HMF was higher in 120°C than in 100°C and 110°C. After 120, 75, and 60 min heating at 100, 110, and 120°C, the content of 5-HMF was higher than 0.04 mg/g, which was the highest limit of European standard. The suitable curves were calculated, and the slopes were 0.001, 0.003, and 0.011, when the temperature was 100, 110, and 120°C. The final content of 5-HMF were 0.7408, 1.9039, and 5.5721 mg/g, respectively

Quantitative analysis of fructose and glucose by high-performance liquid chromatography method

Figure 4 shows the chromatograms of fructose reference standard, glucose reference standard, and the honey sample. Fructose and glucose in honey sample have the same retention time with the reference standards. The methodology studies were also investigated. The calibration curve of fructose and glucose exhibited good linearity ($r = 0.9993$ and $r = 0.9996$, respectively), within the quantitative ranges from 1.3720 to 13.7200 μg and 1.2048 to 12.0480 μg, respectively. What is more, other tests such as the precision, stability, the average recovery ratio, and repeatability test perform well. Hence, it comes to the conclusion that HPLC method can satisfy all the demands of quantitative analysis, and can provide accurate data for NIR calibration.

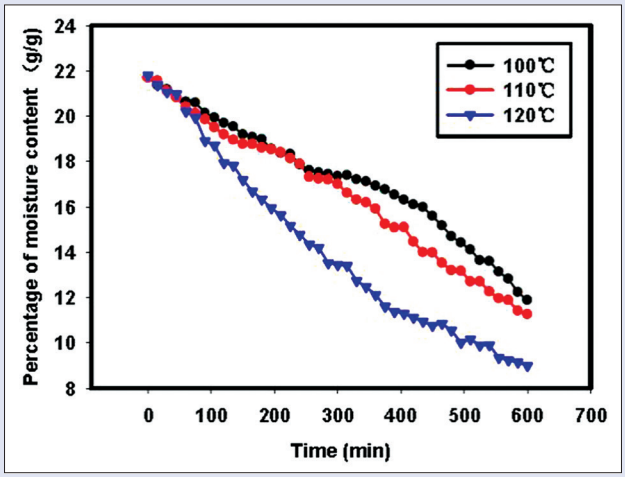

Figure 1: The change in the content of water during the refining process

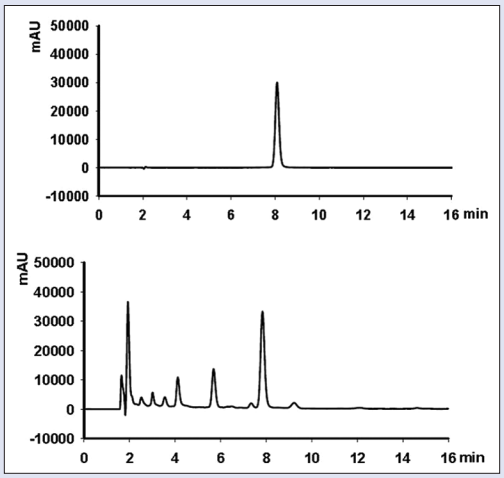

Figure 2: The chromatograms of 5-hydroxymethylfurfural reference standard and honey sample

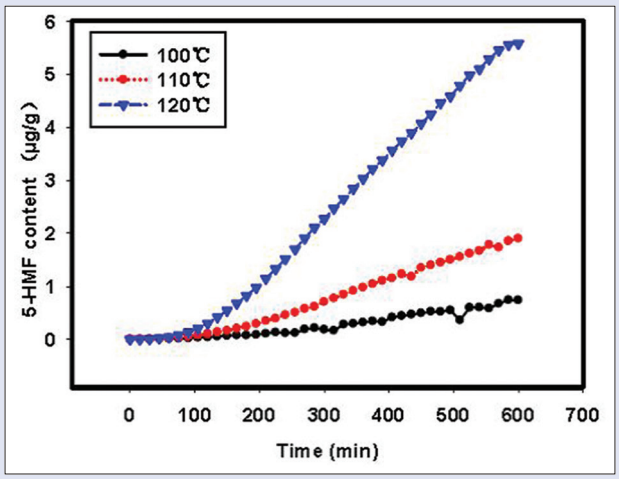

Figure 3: The content change of 5-hydroxymethylfurfural during the refining process

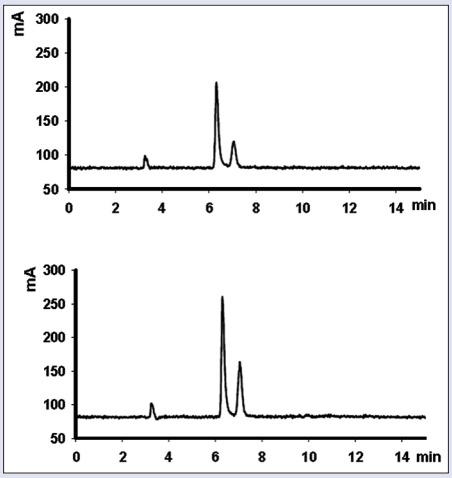

Figure 4: The chromatograms of reference standards and honey sample. Peak 1 represents fructose and Peak 2 represents glucose

Figure 5 shows the influence of the refining temperature and time on the content of fructose and glucose. The refining temperature and refining time do affect the content of fructose and glucose. However, the change rule or trend is not clear. The content of fructose varied from 315.8933 to 374.6548, 349.8052 to 447.8220, and 264.1140 to 418.3368 mg/g in 100, 110, and 120°C, respectively, whereas the content of glucose varied from 312.1638 to 350.9069, 305.4088 to 474.0710, and 311.2195 to 405.1648 mg/g. Additional research should be conducted to thoroughly evaluate the change rules of fructose and glucose.

Spectral pretreatment

The original NIR spectra may be affected by the physical properties of the samples and other environmental factors. Therefore, the pretreatment of the spectra is of great importance. Reducing the systematic noise, removing the drift of the baseline, and eliminating the effect of the lighting scattering will make it easier to get effective information. Preprocessing techniques such as 1st order derivative (1d), 2nd order derivative, Savitzky–Golay smooth (SG, 9, 2), multiplicative scatter correction (MSC), and their combinations are used to seek the optimal models. Table 1 shows the result of different pretreatments. From Table 1, it is obvious that for moisture model, 5-HMF model, and fructose model, 1d combined with SG smooth and MSC (1d-sg-MSC) method is superior to the other methods; for glucose model, 1d is the best method.

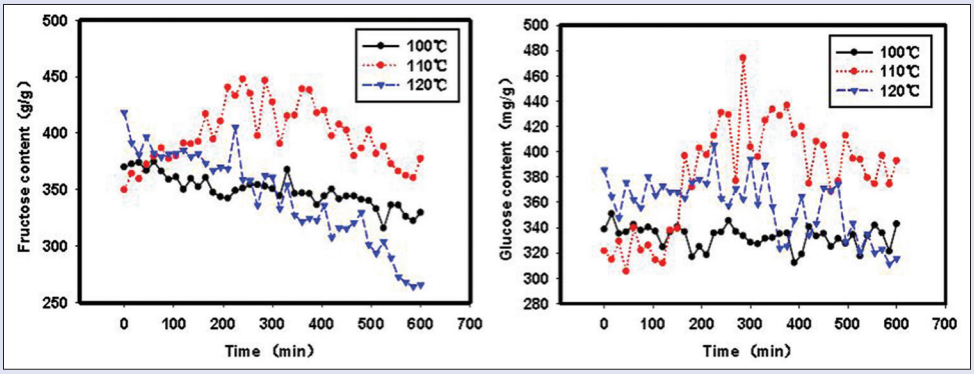

Figure 5: The changes in the content of fructose and glucose during the refining process

Table 1: Comparison of different spectral pretreatment methods

	Pretreatment	RMSEP	R^2	RMSECV	RMSEP/RMSECV	RPD	Latent factor	PRESS
Moisture	RAW	0.210	0.9984	0.268	0.7836	16.2687	5	18.05
	1D	0.225	0.9980	0.243	0.9259	15.1841	4	14.83
	1D-SG	0.225	0.9981	0.242	0.9298	15.1841	4	14.70
	1D-SG-MSC	0.155	0.9990	0.174	0.8908	22.0415	7	7.65
	2D	0.378	0.9938	0.336	1.1250	9.0382	3	28.49
	2D-SG	0.216	0.9980	0.264	0.8182	15.8168	5	17.52
	2D-SG-MSC	0.183	0.9986	0.198	0.9242	18.6690	5	9.92
5-HMF	RAW	158	0.9950	213	0.7418	9.5126	18	11,558,367.00
	1D	323	0.9766	338	0.9556	4.6532	14	29,211,018.00
	1D-SG	132	0.9963	177	0.7458	11.3862	15	7,988,959.00
	1D-SG-MSC	132	0.9963	168	0.7857	11.3862	14	7,234,764.50
	2D	458	0.9526	500	0.9160	3.2816	5	63,715,060.00
	2D-SG	364	0.9712	385	0.9455	4.1291	10	37,864,720.00
	2D-SG-MSC	374	0.9700	379	0.9868	4.0187	13	36,622,896.00
Fructose	RAW	22.9	0.8206	18.5	1.2378	1.6559	12	84,165.76
	1D	19.9	0.8593	19.3	1.0311	1.9055	7	91,949.96
	1D-SG	19.1	0.8752	17.5	1.0914	1.9853	11	75,314.90
	1D-SG-MSC	18.4	0.8777	17.9	1.0279	2.0608	10	79,138.22
	2D	21.4	0.8259	20.6	1.0388	1.7719	4	104,230.77
	2D-SG	20.0	0.8641	18.9	1.0582	1.8960	8	87,424.38
	2D-SG-MSC	20.2	0.8504	18.6	1.0860	1.8772	7	85,051.42
Glucose	RAW	20.4	0.8482	24.7	0.8259	1.6506	11	150,038.14
	1D	20.1	0.8188	23.4	0.8590	1.6752	8	134,453.86
	1D-SG	22.1	0.7809	23.2	0.9526	1.5236	11	132,787.98
	1D-SG-MSC	22.9	0.7540	23.8	0.9622	1.4704	10	139,159.59
	2D	21.5	0.7813	24.6	0.8740	1.5661	3	149,027.06
	2D-SG	21.2	0.7913	25.2	0.8413	1.5883	5	156,538.98
	2D-SG-MSC	22.6	0.7531	25.9	0.8726	1.4899	6	165,440.25

RMSEP: Root mean square error of prediction; RMSECV: Root mean square error of cross validation; RPD: Residual predictive deviation; PRESS: Prediction residual error-sum squares; 5-HMF: 5-hydroxymethylfurfural; 1D: 1st order derivative; SG: Savitzky–Golay smooth; MSC: Multiplicative scatter correction; 2D: 2nd order derivative

The selection of principal component number

Confirming the principal component number is another effective way to eliminate the noise and make the best use of the spectral data. If fewer principal component numbers are considered in the model, the predictive ability of the model is not tenable, and this is called under fitting. However, if too many principle component numbers are considered; for example, the principal component number which represents the noise may be considered in the model, the predictive ability of the model is not tenable, either, and it is called over fitting. The optimal latent factors were chosen based on the lowest PRESS. Figure 6 shows how the numbers of latent factors affect the values of PRESS when determining all the components with different spectral pretreatments. The optimal numbers of latent factors were 7, 14, 10, and 8, for moisture, 5-HMF, fructose, and glucose, respectively.

Result of the near-infrared method

After the spectral preprocessing method and the number of latent factors were selected, the PLS models were built to the determination of moisture, 5-HMF, fructose, glucose, and the decrease of sugars, according

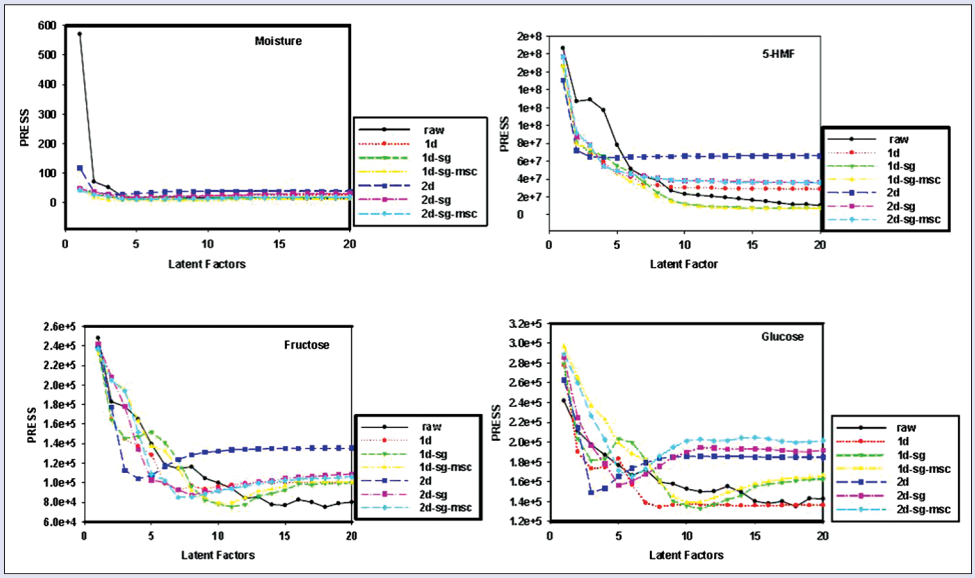

Figure 6: Effects of numbers of partial least squares latent factors on prediction residual error-sum squares values for moisture, 5-hydroxymethylfurfural, fructose, and glucose

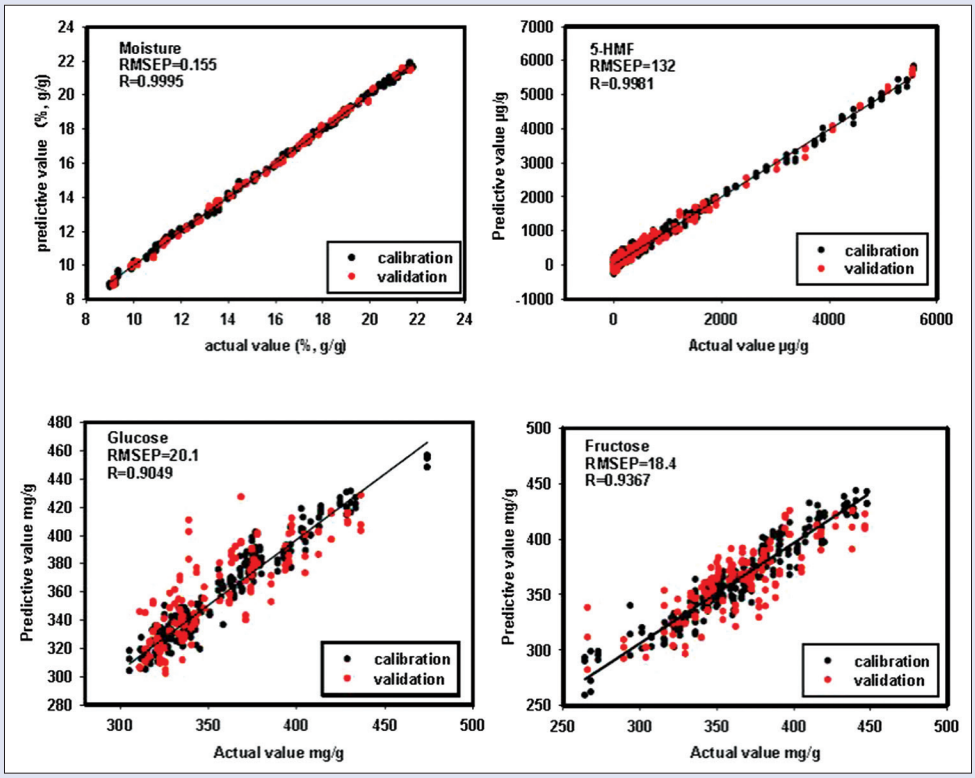

Figure 7: The correlation of predictive value of near-infrared and actual value of reference method for moisture, 5-hydroxymethylfurfural, fructose, and glucose

to all the evaluation parameters. The RMSE*P* values in the established models for moisture, 5-HMF, fructose, and glucose were 0.155% (g/g), 132 μg/ml, 18.4 mg/g, and 20.1 mg/g, respectively. The R values were 0.9995, 0.9981, 0.9367, and 0.9049, respectively. Figure 7 shows the correlation of predictive value of NIR and actual value of reference method for moisture, 5-HMF, fructose, and glucose. The calibration sets of the correction models covered samples that were got from different refining temperature and refining time. Thus, the models allowed the determination of the content of moisture, 5-HMF, fructose, and glucose to be applied at a wide range rapidly and conveniently

CONCLUSIONS

Honey refining is a typical case for process analysis. The technologies such as NIR spectroscopy used in process analysis can be applied in the refining process of honey. Our study explored the change of content of moisture, 5-HMF, fructose, and glucose in the refining process of honey, and investigated the influence of the refining time and temperature. Moreover, PLS models were built to analyze the refining process rapidly. The results exhibited that NIR spectroscopy is a good solution for the quality control of the refining process of honey. NIR spectroscopy avoids the time-consuming, costly, and destructive chemical analysis, and guarantees the quality of the refining honey at the same time. Once there was homogeneous and stable refining honey, the quality and effect of the drugs or crude drugs were ensured. Thus, it will bring tremendous economic and social benefits. This research is meaningful in illuminating the essence of honey refining project.

This research was carried out in the laboratory and had limitations, but more work should be done in the manufacturing process to make sure that NIR technology can be used in monitoring the refining process of honey.

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Conflicts of interest

There are no conflicts of interest.

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