Colour analysis of honey samples produced in Thailand using near-infrared spectroscopy

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Introduction

Honey is one of the most complex mixtures of carbohydrates and other minor components produced in nature.[1] The quality of honey differs because of various factors such as geographical, seasonal and processing conditions, floral source, packaging and storage conditions.[2] The colour of honey is one of the factors determining its price in the world market, and consumer's acceptability.[3] Light-coloured honey is usually milder in flavor and has higher commercial value than dark-coloured honey. To improve honey quality control, it is necessary to develop a rapid, simple, and accurate method for the routine quality assessment of honey. The aim of this study is to investigate near-infrared (NIR) spectroscopy in a transflection mode as a rapid analytical tool for the simultaneous quantitative determination of colour characteristics of unifloral and multifloral honey samples produced in Thailand.

Materials and methods

Honey samples

A total of 127 bottles of honey samples produced between 2007 and 2008 in Thailand were randomly selected and categorised into unifloral and multifloral honey. To identify the botanical source of the honey samples, information regarding seasons and available floral sources were obtained by asking beekeepers. Unifloral honey samples, which include sunflower ($n=30$), longan ($n=30$), litchi ($n=13$), and bitter bush ($n=18$); and multifloral honey ($n=36$) were analysed. All samples were kept in the dark at 25 ± 2°C in plastic jars. Prior to colour and spectroscopic analyses, honey samples were incubated in a water bath at about 40 ± 2°C until all the sugar crystals were melted.

Colour analysis

Colour characteristics in terms of L*, a*, b*, hue angle, and chroma were assessed by the CIE L*a*b* method[4] using a Minolta CM-3500d spectrophotometer. The *L**-values ranging between 0 and 100 were used as a measure of brightness. Positive or negative of a*-value corresponds to the increase in red and green colour properties. The b*-value represents colour ranging from yellow (+) to blue (–). Hue angle, which defines the colour, was calculated as arctangent (b*/a*). Chroma, a measure of colour intensity, was calculated as $\sqrt{a^{*2} + b^{*2}}$. All of the analyses were performed in triplicate, and the mean values of the triplicate determination were used in calibration and validation studies.

NIR spectral acquisition

NIR spectra were recorded using a Büchi NIRFlex Solids spectrometer, which was operated with the NIRLabWare 3.0 software and equipped with a rotating sample holder (Büchi Labortechnik AG, Flawil, Switzerland) to level out effects of sample non-homogeneity. The measurements were performed at room temperature 25 ± 2°C. Ten grams of liquefied honey were poured into a clean glass Petri dish, which was then covered with an aluminium plate. As a result, there would be a 0.75 mm layer of honey between the bottom of the Petri dish and its surface, which would function as a reflection material. Thirty-two scans with a resolution of 8 cm^{-1} were recorded in transflection mode for each spectrum, in the 1000–2500 nm region.

Data analysis

The Unscrambler software (version 7.8: CAMO AS, Trondheim, Norway) was used to build the partial least squares (PLS) regression with five mathematical treatment methods: normalisation, smoothing, multiplicative scatter correction (MSC), first derivative, and second derivative. Seventy samples were randomly selected and formed a calibration set, and the remaining 50 samples formed a validation set. All the data were checked carefully to detect and eliminate outlier samples. A random cross-validation technique was used to develop calibration models. The optimal calibration equations were chosen, based on the highest correlation coefficient (*R*) and the lowest root mean square error of cross-validation (*RMSECV*). The performances of the established calibration equations were further validated using the validation set. The predicted values were correlated with the reference values, and the accuracy of prediction was assessed by the root mean square error of prediction (*RMSEP*) and bias.

Results and discussion

Table 1 shows information regarding sample statistics for the calibration and validation sets, showing the minimum, maximum, mean, and standard deviation values.

Outlier samples were identified by principal component analysis (PCA). Figure 1 shows NIR spectra of 142 honey samples.

A broad feature at around 1450 nm contains contributions from OH, CH, and CH_2 vibrations, a band at 1936 nm is due to OH combinations, and bands at 2100 nm and 2280 nm are assigned CH combinations.[5] Transflectance spectra of aqueous solutions of fructose and glucose have been reported to contain absorbance peaks at almost identical locations.[6]

Table 1. Colour characteristics of calibration and validation sample sets.

Colour characteristics	Calibration set					Validation set				
	n	Min	Max	Mean	*SD*	*n*	Min	Max	Mean	*SD*
L*	77	58.2	88.6	77.0	7.6	40	59.9	86.0	77.2	7.2
a*	67	–2.2	28.9	8.3	7.5	36	0.02	28.7	9.0	7.6
b*	70	31.9	102.9	68.8	16.8	36	39.3	103.4	69.6	17.0
Hue angle (°)	77	32.0	107.3	70.3	18.7	39	37.4	107.0	70.5	18.0
Chroma	70	72.5	93.8	84.1	4.4	38	74.2	91.3	84.2	3.9

The PLS calibration models developed gave satisfying accuracies for the determination of colour characteristics of honey samples. The outline of the calibration and validation results is given in Table 2.

The correlation coefficients (*R*) of calibration for all measured quantities are relatively high (>0.88), which suggests that the calibration models are able to match the NIR data to the values obtained by the reference method. The *RMSEP* for L*, a*, b*, hue angle, and chroma values were 4.272, 4.244, 10.780, 9.940, and 2.629, respectively, and no statistically significant differences (p= 0.05) were found between actual values and NIR predicted values. The regression lines for all measured quantities are shown in Figure 2.

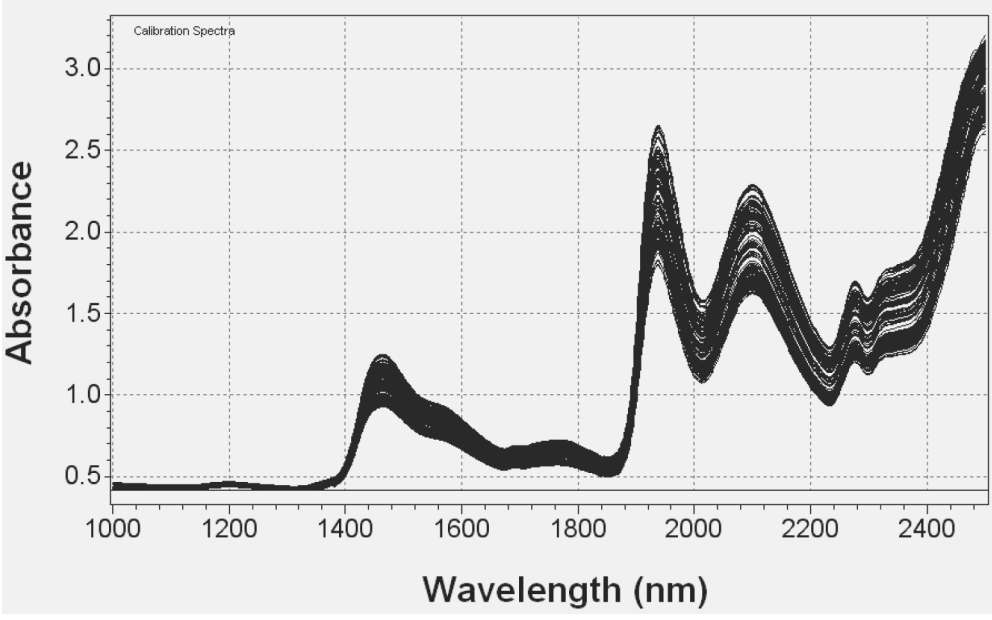

Figure 1. NIR absorbance spectra of 127 Thai commercial honey samples.

Table 2. Calibration and validation results for colour characteristics of honey samples.

Colour characteristics	Mathematical treatment methods	Number of PLS factors	Calibration set			Validation set		
			n	*R*	*RMSECV*	*n*	*RMSEP*	Bias
L*	1st derivative	14	77	0.881	5.578	40	4.272	0.603
a*	MSC	12	67	0.895	5.843	36	4.244	0.571
b*	Smoothing	15	70	0.920	10.867	36	10.780	0.818
Hue angle (°)	1st derivative	14	77	0.908	11.160	39	9.940	–0.828
Chroma	1st derivative	16	70	0.948	2.724	38	2.629	–1.142

PLS-factors: number of partial least squares factors used to build the model in cross-validation and validation
R: Correlation coefficient, *RMSECV*: root mean square error of cross-validation, *RMSEP*: root mean square error of prediction

Conclusions

This study suggests that NIR can be used routinely as a non-destructive measurement for the prediction of colour characteristics of unifloral and multi floral honey samples. Further research

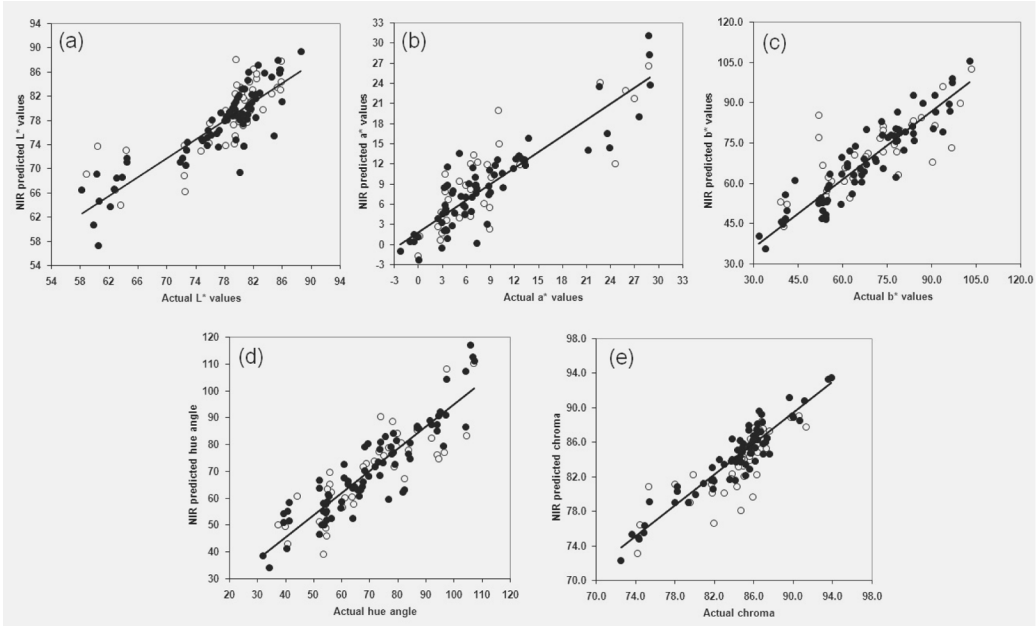

Figure 2. Plots of laboratory measured values against values predicted by NIR spectroscopy for (a) L*, (b) a*, (c) b*, (d) hue angle, and (e)chroma values. ● = calibration set, ○ = validation set.

is still needed to improve the accuracy and precision of prediction for other honey quality properties.

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