

## DETERMINATION OF CAROTENOIDS AND DOBI CONTENT IN CRUDE PALM OIL BY SPECTROSCOPY TECHNIQUES: COMPARISON OF RAMAN AND FT-NIR SPECTROSCOPY

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**ABSTRACT:** Crude palm oil (CPO) is a rich source of carotenoids which are a precursor of vitamin A as an important antioxidant. Carotenoids and Deterioration Of Bleachability Index (DOBI) are important factors to indicated quality of CPO. The conventional method for DOBI and carotenoids determination use UV-Vis spectrophotometry and HPLC, respectively which methods use solvents and spend time for analysis. This research interests Raman and FT-NIR spectroscopy for carotenoids and DOBI determination due to those techniques are an alternative technique for rapid measurement, non-destruction of material, and environmental suitability. The results indicated that Raman is better for determination than FT-NIR spectrometry. In Raman analysis, multiplicative scatter correction (MSC) and standard normal variate (SNV) treated methods were the best models for carotenoids and DOBI, respectively. The bands of carotenoids were at 1,100 and 1,500  $\text{cm}^{-1}$  wavenumber. Validation on carotenoids produced 0.94 of correlation coefficient (R), 0.88 of coefficient of determination ( $R^2$ ), 40.65 ppm of root mean square error of prediction (RMSEP) and 3.25 of the ratio of standard error of prediction to standard deviation (RPD). The validation of DOBI produced 0.76 of R, 0.57 of  $R^2$ , 0.31 of RMSEP, 1.92 of RPD of 1.92. The limitation of carotenoids and DOBI determination using FT-NIR is having sufficient concentration of carotenoids for analysis. In addition, data for various samples regarding location, season, and oil palm species are important to build models for precise prediction.

**Keywords:** DOBI, Carotenoids, Crude palm oil, Rapid analysis, Raman, FT-NIR

### 1. INTRODUCTION

Thailand is an agricultural country where the major crops include rice, para rubber, cassava, oil palm, sugarcane, and corn as top exports [1]. However several years ago, oil palm became important as an economic crop because it has many advantages for consumption (such as in food, cosmetics, pharmaceuticals, etc.) and alternative energy (such as lubrication oil, biodiesel, biofuel, etc.). Furthermore, the Thai government has promoted oil palm plantations to farmers by launching several policies to facilitate and create stakeholder activity. Consequently, oil palm cultivation and the crude palm oil (CPO) yield have increased every year.

At present, commercial palm oil is a highly competitive commodity with importance placed on CPO properties such as free fatty acid, iodine value, peroxide value, oil content and Deterioration of Bleachability Index (DOBI). The CPO quality is a trade barrier and the DOBI value is of interest because it is an important factor for CPO classification of crude palm mills Thailand and the carotenoids content is depended on the DOBI value. The price of the CPO in the commercial is decided by the CPO quality. There are a premium quality (The  $\text{DOBI} \geq 3$ ), a good quality (grade A;

$2.3 < \text{DOBI} < 3$ ) and a poor quality (grade B;  $\text{DOBI} < 2.3$ ). The high quality gives the high price for purchase. The DOBI is an index for determining the bleachability of palm oil [2] and carotenoids in CPO. However, mills cannot detect it themselves because they lack the knowhow, laboratory skills, and instruments for analysis. Good CPO quality requires not only high carotene but also should contain low secondary oxidation products (peroxide, aldehyde, ketone, acid, etc.) [3], [4]. Other characteristics of the DOBI could indicate the ripeness of palm fruits, the amounts of carotenoids, and the freshness of palm fruits.

The conventional methods for DOBI and carotenoids determination involve UV-Vis spectrophotometry or high performance liquid chromatography [2], [3], [5]. However, the sample preparation uses chemical solvent (introducing a waste chemical problem) and the analysis is time-consuming. Many researchers have studied spectroscopy techniques for carotenoids analysis in various vegetables and fruits. Spectroscopy offers a powerful, nondestructive, and intensively applied method for rapid analysis such as; (1) Fourier Transform Raman (FT-Raman) and Fourier Transform near Infrared (FT-NIR) techniques were applied to determine carotenoid in tomato fruits. The concentration range of carotenoids was 28.5-631.83 ppm and the major component of

carotenoids was lutein. The results indicated that FT-Raman produced better analysis than FT-NIR with the coefficient of determination ( $R^2$ ) and standard error of cross-validation (SECV) using FT-Raman and FT-NIR being 0.91, 74.31 and 0.85, 91.19, respectively [6]. (2) FTIR spectroscopy was determine the commercial  $\beta$ -carotene in refined-bleached and deodorized (RBD) palm olein. The commercial carotene was spiked into RBD at 200-2,000 ppm. A partial least squares (PLS) calibration model indicated this was a good method that was efficient and accurate for quality control analysis [7]. (3) Raman was used to determine carotenoids in various vegetables and fruits (such as apricot, carrot, saffron, Broccoli, chamomile, nautilus, periwinkle, etc.). They found two strong bands in the 1,100-1,200  $\text{cm}^{-1}$  and 1,400-1,600  $\text{cm}^{-1}$  region, of the Raman spectrum. These bands are due to C-C stretching vibrations of the polyene chain, C=C stretching of conjugate carotenoids, with the shift of bands depending on the length of the chain and the number of double bonds of carotenoids [8]. (4) The application of NIR for carotenoids determination in CPO, a calibration curve range from 200 to 800 ppm was prepared by spiking a known concentration of carotenoid into a normal CPO. PLS calibration was developed from 18,315-12,210  $\text{cm}^{-1}$  of the spectral region. The  $R^2$  and standard error calibration (SEP) values were 0.95 and 23.6, respectively, while validation produced an  $R^2$  of 0.98 and an SEP of 19.96 [9]. (5) Using NIR spectroscopy to study the chemical properties of oils (a mixture of soybean, rapeseed and palm oil) such as the iodine value, moisture content, acid value, etc. [10]. Although many researchers have studied carotenoids determination using spectroscopy techniques, there has been no report on the study of the DOBI or carotenoids content in extracted CPO from under-ripe palm fruits, with the reported work involving extracted CPO from ripe-palm fruits, whereas the palm oil mills extract CPO from under-ripe and ripe palm fruits. The under-ripe palm fruits (inner section of the spikelet from the oil palm bunch) have a very low carotenoids content which may affect measurement using spectrometry.

This research investigated the application of Raman and FT-NIR for rapid and direct measurement of carotenoids and DOBI in extracted CPO from ripe and under-ripe palm fruits. The results were compared and the best calibration model was determined using the Unscrambler program to confirm possible implementation in a palm oil mill. The Raman and the NIR techniques are the novelty of determination of the DOBI and carotene content in the crude palm oil. They are destructure of crude palm oil which the conventional method has to sample preparation with n-hexane solvent and the both techniques can reduce the operated time of analysis from 5 mins to

less than 1 min. One advantage of the both technique is friendly environment. The one objective of my research was determination the DOBI and carotene from unripe-palm fruit which is not found in any research.

## 2. MATERIAL AND METHOD

### 2.1 Sample Preparation

This research focused on the *Tenera* species of oil palm obtained from the Oil Palm Technology Development for Local Commercial Biodiesel Industry in Newly Planted Area Project, in the Faculty of Agriculture, Kasetsart University, Thailand. For sample preparation, the fresh palm bunches (FPBs) were divided into three zones (apical, equatorial and basal zones). Each zone was divided into two categories of palm fruit sample—ripe and under-ripe palm fruit. The ripe palm fruits were the outer section of the spikelet having dark-orange or dark-red palm fruits while the under-ripe palm fruits were from the inner section of the spikelet and were light-yellow to light-orange whole fruit. For each sample the CPO was obtained using solvent extraction with hexane solvent and solvent evaporation in a vacuum evaporator. Oil palm fruit samples were collect every day for a week, resulting in 126 CPO samples.

### 2.2 Measurement Using Spectroscopy Techniques

#### 2.2.1 Raman spectra measurement

The all samples were warmed at 60°C using a hot-air oven before measurement of Raman. Then, Raman spectra were recorded using a Serstech Indicator Kit (model Serstech 100 Indicator; Serstech Chemical Intelligence Solutions, Sweden). Spectra data of CPO were accumulated at 20.50 seconds of scan time in the range 400-2,300  $\text{cm}^{-1}$  with a laser power of 300 mW. For each sample, two spectra were collected and subsequently averaged.

#### 2.2.2 NIR spectra measurement

The all samples were analyzed using a Bruker spectrometer (model MPA, Bruker, GmbH, Ettlingen, Germany). This FT-NIR spectrometer is designed for transmittance analysis of a liquid sample. The oil sample was dropped in a quartz with 1mm path length (S10-SQ-1; GLSciences Inc. Japan) and all samples were warmed in a dry thermo bath (model MG-2000, Eyela thermo bath, Germany) at 60°C before analysis for homogeneous CPO samples. NIR spectra were measured directly on the sample and acquired between 4,000 to 12,500  $\text{cm}^{-1}$ , setting the resolution to 8  $\text{cm}^{-1}$  at 60°C, and 64 scans of the average reading. For each sample, two

spectra were collected and subsequently averaged.

### 2.2.3 DOBI and carotenoids content analysis (reference analysis)

DOBI was determined according to the Palm Oil Research Institute of Malaysia (PORIM) test method which was developed by Swoboda (1982) and the carotenoids content was determined according to the PORIM test method (1995). About 0.1 g of CPO was weighed and dissolved in 25 ml of hexane solvent. The solution sample was placed in a 1 cm wide of cuvette and absorbance measured at 446 nm (measurement of carotenoids) and at 269 nm using a spectrophotometer (T70+UV/VIS spectrometer, PG Instruments, USA). The DOBI and carotenoids content were calculated using eq. 1 and eq. 2, respectively.

$$\text{DOBI} = \frac{A_{446}}{A_{269}} \quad (1)$$

$$\text{Carotenoids (ppm)} = \frac{383 \times A_{446} \times V}{W \times 100} \quad (2)$$

Which: A446 = absorbance at 446 nm, A269 = absorbance at 269 nm, 383 = diffusion coefficient, V = value of hexane (ml) and W = weight of CPO sample (g).

### 2.2.4 Calibration parameters and prediction model

PLS regressions were fitted to describe the relationship between dependent variables (DOBI and carotenoids in CPO samples) and Raman or NIR spectra using a multivariate statistic program (the Unscrambler 9.7 version). The calibration models were developed from the 126 spectra of all CPO samples. PLS calibrations were fitted using the PLS-1 method. Model validation was performed using the full cross validation method. The number of latent variables (factor) adopted for each model was suggested by the statistical program.

The statistical results of PLS models built using original and pretreated spectra were compared using MSC (multiplicative correction), SNV (standard normal variate), 1D (first derivative), and 2D (second derivative). The criteria for selection of the prediction model included: factor-number of latent variables; correlation coefficient of calibration ( $R_c$ ); coefficient of determination of calibration ( $R_c^2$ ); root mean square error of calibration (RMSEC); correlation coefficient of validation ( $R_v$ ); coefficient of determination of validation ( $R_v^2$ ); root mean square error of prediction (RMSEP); and the ratio of standard error of prediction to standard deviation (RPD), being the ratio of the standard deviation of data (SD) to RMSEC. Models with a high  $R$ ,  $R^2$ , and RPD and a low RMSEC and RMSEP are considered to be optimum.

## 3. RESULT AND DISCUSSION

The DOBI and carotenoids content of the extracted CPO from ripe palm fruits were greater than in the extracted CPO from under-ripe palm fruits. With increased palm fruit ripening (observed from the color change from light yellow to dark red), the carotenoids content in the CPO increased but the DOBI only increased to a maximum value at 3 days of storage time and after that the DOBI decreased due to the autoxidation reaction of oil over longer storage. The fatty acid or triglyceride was in radical forms. The hydrogen atom from the fatty acid or triglyceride in oils is removed and lipid alkyl radicals are converted into lipid peroxide for which oxygen, UV, and heat are the catalysts for autoxidation. The lipid peroxide decomposed to alkoxy radicals and then formed aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons (secondary products of oxidation). Table 1 shows the distribution of the DOBI and carotenoids content in all samples. The maximum carotenoids content was 539.79 ppm in CPO extracted from ripe palm fruit while the minimum carotenoids content was 2.79 ppm in CPO extracted from under-ripe palm fruit. In general, commercial CPO has a carotenoids content between 400 and 700 ppm [3–6] but the current experiment resulted in a low carotenoids content because of the drought in Thailand at the time the samples were collected and this affected the production of oil palm products (less weight of oil palm bunch, less palm oil yield, slow growth of oil palm, slow ripeness of oil palm bunch, etc.).

Table 1 Distribution of DOBI and carotenoids content in crude palm oil samples.

Parameters	Min.	Max.	X	SD
DOBI	0.07	2.09	0.87	0.50
Carotenoids	2.79	539.79	191.02	121.96

### 3.1 Spectra Analysis

Carotenoids are a group of tetraterpenoids consisting of isoprenoid units. Double bonds in carotenoids are conjugated forms and usually are all trans forms. CPO is a source of carotenoids, having 60%  $\beta$ -carotene and 40%  $\alpha$ -carotene [6]. Fig. 1 and Fig. 2 show Raman spectra and NIR spectra for the DOBI and carotenoids content determination, respectively.

#### 3.1.1 Raman spectra

Figure 1 (a) compares spectra between the extracted CPO from ripe and under-ripe oil palm fruits. The spectra show the C=C stretching vibration of carotenoids with a dominant signal observed in the range 1,500-1,550  $\text{cm}^{-1}$ ; the position

of this band depends on the ripeness of the oil palm fruit. At  $1,168\text{ cm}^{-1}$  the C-C group with the C-CH<sub>3</sub> of carotenoids is presented. The two wavenumber positions of the carotenoids are characteristic of the spectrum of the  $\beta$ -carotene standard (Fig. 1 (b)). In the spectrum of the  $\beta$ -carotene standard, the characteristic bands have three main peaks which are located at  $1,512$ ,  $1,154$ , and  $1,004\text{ cm}^{-1}$ . These wavenumber positions are correlated with the wavenumbers of the polyene chain (C=C), C-C coupled with C-CH<sub>3</sub>, and C-C stretching of carotenoids, respectively [9–14]. At other positions in the CPO,  $1,442$  and  $1,656\text{ cm}^{-1}$  are correlated with the wavenumbers of CH<sub>2</sub> or CH<sub>3</sub> deformation of fatty acid or triglyceride, and C=C stretching of unsaturated fatty acid or triglyceride, respectively.

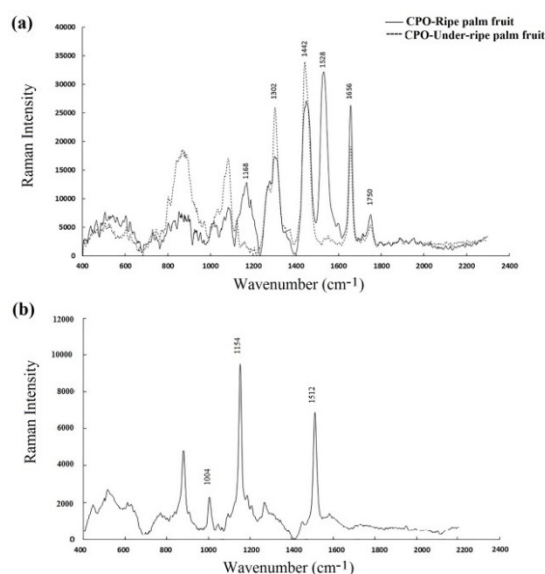


Fig.1 Comparison between Raman spectra of CPO from ripe and under-ripe palm fruit (a), and  $\beta$ -carotene spectrum (b).

### 3.1.2 NIR spectra

The NIR region was more difficult to explain mainly because of the extensive band overlapping due to overtones and combinations of fundamental vibrations involving hydrogen stretching modes. However, it was possible to predict the DOBI and carotenoids content in CPO. Fig. 2 (a) shows the comparison of NIR spectra between the extracted CPO from ripe and under-ripe palm fruits. The results indicated that the spectra had the same patterns, with the bands at around  $5,789$  and  $5,681\text{ cm}^{-1}$  due to the combination of bands and the first overtone of the C-H of methylene of the aliphatic group of oil and the second overtone being observed at  $8,211\text{ cm}^{-1}$  [15]. The bands at around  $4,655$  and  $4,535\text{ cm}^{-1}$  may be attributed to combination bands of C-C and C-H stretching vibrations of cis-unsaturated fatty acids, and at  $7,058$  and  $7,139\text{ cm}^{-1}$  are attributed to a C-H combination band of

methylene [11]. Quantitative analysis of carotenoids by FT-Raman was based on the trans-CH=CH-functional group but this characteristic band did not exist in the NIR region [9]. In the current research, the best conditions for NIR spectra pretreatment were in the wavenumber range between  $5,500$  and  $4,600\text{ cm}^{-1}$  (Fig. 2 (b)) where there were strong correlations.

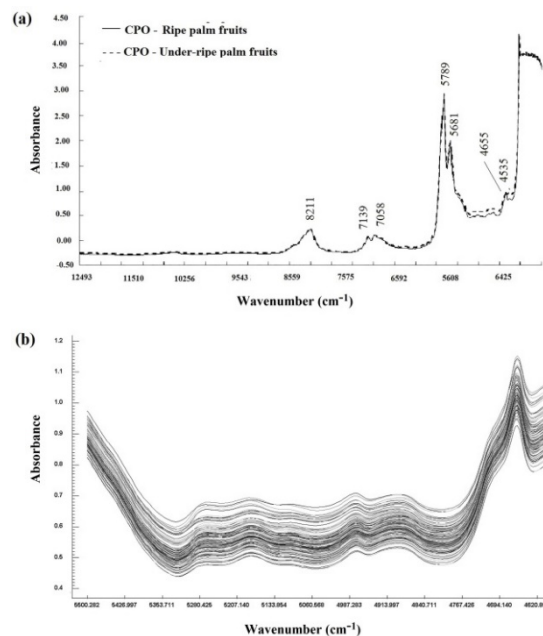


Fig.2 Comparison between NIR spectra of CPO from ripe and under-ripe palm fruits,  $12,500$ – $4,000\text{ cm}^{-1}$  wavenumber (a), and  $5,500$ – $4,600\text{ cm}^{-1}$  wavenumber (b).

## 3.2 Calibration and Cross-Validation Results

### 3.2.1 Raman analysis

The precision calibration models can be checked using the cross-validation method. The model was calibrated using different mathematical treatments of spectra, selected by the variable uncertainty test used for the validation method. The criteria for best model selection were the highest  $R^2$  and RPD, and the lowest RMSEP. MSC pretreatment was the best method for all spectral improvement and this model can predict the carotenoids content in CPO. The statistical results of the prediction model were  $0.94$  for  $R_v$ ,  $0.88$  for  $R_v^2$ ,  $40.65\text{ ppm}$  for RMSEC, and  $3.25$  for RPD. RPD is calculated from the ratio of the standard deviation of the data to RMSEC thus providing a ratio of the performance to the deviation. Hence, the highest RPD value indicates the best method too. In the DOBI prediction model, the SNV pretreatment was the best. The statistical results of the prediction model were  $0.76$  for  $R_v$ ,  $0.57$  for  $R_v^2$ ,  $0.31$  for RMSEP, and  $1.92$  for RPD using the cross-validation method.

Figure 3 (a) and Fig. 3 (b) show the MSC-

Raman spectra for carotenoids and the SNV - Raman spectra for DOBI, respectively. In the 400-2,300  $\text{cm}^{-1}$  wavelength range of the Raman spectra, the results showed broad bands around 1,100 and 1,500  $\text{cm}^{-1}$  due to the C-C group with C-CH<sub>3</sub> and C=C stretching of carotenoids [7–9], [12–13], [15]. Both wavenumbers were significant for carotenoids determination and they were clearly confirmed with the regression coefficients in Fig. 5 (a). The other positions, around 1,400 and 1,600-1,700  $\text{cm}^{-1}$  have been assigned to the fatty acid or triglyceride.

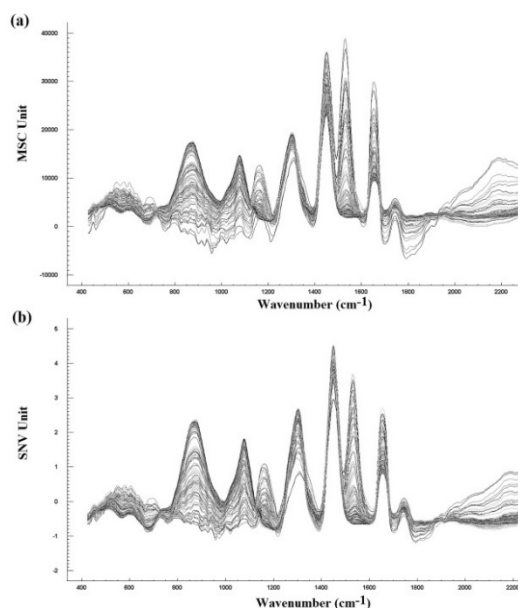


Fig.3 Raman spectra pretreatment of crude palm oil on carotenoids and DOBI, carotenoids- MSC (a), and DOBI-SNV (b).

### 3.2.2 FT-NIR analysis

The calibration models using cross validation for carotenoids and DOBI determination in CPO where all samples were measured using FT-NIR. The 1D pretreatment using the Gap-Segment method was the best method for improvement of CPO spectral base for carotenoids and DOBI. The statistical results for carotenoids were 0.71 for  $R_v$ , 0.50 for  $R_v^2$ , 86.70 ppm for RMSEP, and 1.63 for RPD. In the DOBI prediction, the 1D pretreatment by the Gap-Segment method was the best method. The statistical results were 0.80 for  $R_v$ , 0.64 for  $R_v^2$ , 0.29 for RMSEP, and 1.72 for RPD.

Figure 4 shows the NIR spectra in the 5,500-4,600  $\text{cm}^{-1}$  range for spectral samples pretreatment. The results indicated that the PLS model built using the 1D method was the best model for carotenoids and DOBI determination. The data on the carotenoids content and DOBI following analysis using the Unscramble program showed very low contents (2.79-537.79 ppm for carotenoids and 0.07-2.09 for DOBI) and the regression of

carotenoids showed a wavelength of 5,276  $\text{cm}^{-1}$  (Fig. 5 (b)) which is near the 5,263  $\text{cm}^{-1}$  of H-O-H stretching and bending vibrations of water [13]. Therefore, the amount of carotenoids, the DOBI, and the amount of water affected measurement using FT-NIR.

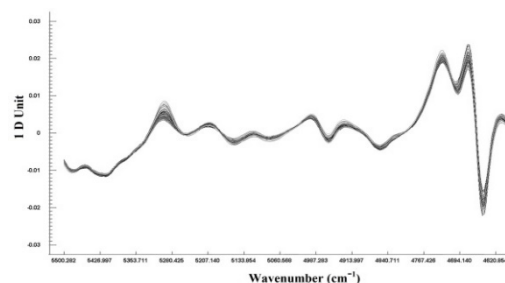


Fig.4 1D NIR spectra pretreatment of crude palm oil on DOBI and carotenoids.

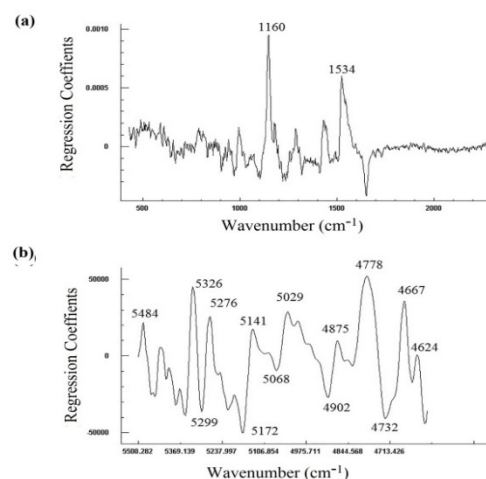


Fig.5 Regression coefficients of the MSC-pretreated PLS model measured using Raman (a) and the 1D-pretreated PLS model measured using FT-NIR (b) on carotenoids content in the extracted crude palm oil from palm fruits.

## 4. CONCLUSIONS

Raman and FT-NIR techniques are environmentally friendly, use no solvent, and provide rapid measurement which is required for industrial application. The results showed that Raman spectrometry was better than the conventional method and FT-NIR spectrometry. Raman provided good DOBI and carotenoids determination in CPO and provided clear carotenoids determination at around 1,100 and 1,500  $\text{cm}^{-1}$  in CPO. Raman was better at measuring both parameters than FT-NIR, although the concentration of carotenoids was very low. In addition, model development has to be extended with calibrations using more samples of palm fruits obtained from many locations, in different seasons,

and for different species to ensure maximum accuracy of analysis.

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