Rapid detection of cAMP content in red jujube using near-infrared spectroscopy*

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(Received 25 July 2018; Revised 6 August 2018)

In this paper, a new method for the rapid, economical and convenient detection of cyclic adenosine monophosphate (cAMP) in jujube is proposed and verified. Based on near-infrared (NIR) fiber spectroscopy combined with stoichiometric analysis, the cAMP content in red jujube can be quickly detected. 68 red jujube samples were used for the NIR spectroscopy data acquisition and the corresponding chemical values were determined. The sample set was adjusted based on the joint *XY* distance (SPXY) to select the correction sample set. After different preprocessing on the spectra, the partial least squares (PLS) method was used to establish the model, and the smoothed and normalized PLS model result was obtained better. The model's correction correlation coefficient (R_c), correction set mean square error (R_{MSEC}), prediction correlation coefficient (R_p), and prediction and mean square error (R_{MSEP}) are 0.951 5, 25.793 7, 0.910 8 and 28.228 0, respectively. The results show that NIR combined with specific chemometric methods can achieve rapid detection of cAMP in red jujube.

Document code: A **Article ID:** 1673-1905(2018)05-0380-4

DOI https://doi.org/10.1007/s11801-018-8120-z

Cyclic adenosine monophosphate (cAMP) is one of the most important functional ingredients in red jujube, and its content is thousands to tens of thousands times of that in common plants and animals. Exogenous cAMP can inhibit the growth of cancer cells, dilate the blood vessels, improve the liver function, promote the regeneration of nerves, regulate the metabolism of substances, and reduce the division of blood sugar[1-4]. And, the application of cAMP-based red jujube quality identification, functional food deep processing, health care and medical high-purity extraction has high requirements for real-time and rapid quantitative detection of cAMP. Therefore, it is necessary to develop a method for detecting cAMP content in time.

At present, the traditional cAMP detection methods are mainly chemical reagent damage detection methods, such as paper chromatography, thin layer chromatography, ultraviolet spectrophotometry, high performance liquid chromatography (HPLC), etc. However, these methods all have some defects such as long inspection time, complicated operation procedure, expensive reagents, tedious experimental data processing and so on[5, 6].

Spectral analysis is an important method for rapid qualitative and quantitative analysis. The near-infrared (NIR) spectroscopy has been widely used in the fields of agriculture, food, medicine and petroleum because of its advantages of fast, nondestructive and easy operation, low cost, simple data processing and green environmental protection[7,8]. However at present, there are few reports on the detection of red jujube cAMP by NIR. In this paper, the feasibility of rapid quantitative determination of red jujube camp by using NIR combined with stoichiometric analysis is demonstrated. The results are satisfactory.

The spectrum acquisition instrument is the VERTEX 70 infrared spectrometer from BRUKER company of Germany, with the air as the background, the scanning range of 4 000—10 000 cm^{-1}, the resolution of 8 cm^{-1}, and the number of scanning times of 32.

Drying box, pulverizer, ultrasonic cleaner and centrifuge were used for red jujube samples processing. The HPLC was used for chemical value measurement. NIR data processing and statistical analysis software was MATLAB R2016b.

The 17 kinds of red jujube samples used in experiment were from Xinjiang Quality Supervision Bureau, which

* This work has been supported by the Science and Technology Project on aid to Xinjiang Uygur Autonomous Region (No.2018E02058).

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were numbered in turn and dried and crushed through a 90-mesh sieve. Two of the 17 kinds of red jujube samples were mixed randomly with equal mass to get 68 kinds of samples in total, which were put into self-sealing bags for subsequent use. The reagents for HPLC were methanol, potassium dihydrogen phosphate and adenosine monophosphate. The experimental water was distilled water.

The 68 samples of red jujube powder without any chemical treatment were put into the sample tube of 4 mL and the spectra were measured directly by the optical fiber spectrometer. Each sample was measured for three times and the average value was used as spectral data for subsequent modeling. The original average NIR spectra of the red jujube powder were measured, which are shown in Fig.1. The resulting spectral data were stored in the computer in the form of text.

At the same time, the cAMP contents of the corresponding samples were determined by HPLC. In the experiment, 1 g red jujube powder and 10 mL distilled water were added to the conical bottle, and the ultrasonic treatment was performed for 10 min. After that, the solution was centrifuged at a rate of 3 000 r/min. The supernatant was collected into the 25 mL capacity bottle, then the red jujube residue was added to the 10 mL distilled water. The supernatant was extracted repeatedly, and combined with the supernatant before. The volume of the supernatant was fixed with distilled water. An appropriate amount of the supernatant was added onto the 0.45-μm-thick filter membrane, and the chemical value was calculated through HPLC[9].

50 of 68 samples were selected as the correction set by the SPXY method[10,11], while the remaining 18 samples were used as prediction set. The sample division is shown in Tab.1. It can be seen that the cAMP content in the prediction set is within the range of the correction set, so the division of the sample set is reasonable, and the correction model will be more representative.

Tab.1 Sample division

Sample set	Sample number	Mean value ($mg \cdot kg^{-1}$)	Maximum ($mg \cdot kg^{-1}$)	Minimum ($mg \cdot kg^{-1}$)	Standard deviation ($mg \cdot kg^{-1}$)
Full sample	68	236.541 4	501.710 0	63.592 5	77.018 0
Calibration set	50	234.085 6	501.710 0	63.592 5	83.897 2
Prediction set	18	243.546 0	376.552 5	153.648 8	53.746 6

In order to reduce the external interference information and the random error caused by noise, this experiment used the classical spectral preprocessing method, such as smoothing, normalization, multi-scattering correction and their combinations, etc[12,13]. The optimal preprocessing scheme can be obtained according to the treatment results. Tab.2 lists the results of several well-prepared preprocessing methods.

Tab.2 Results of PLS models with different preprocessing methods

PLS main factor number	Preprocessing method	Calibration set		Prediction set	
		R_C	R_{MSEC}	R_P	R_{MSEP}
15	Nothing	0.973 7	19.101 5	0.830 8	39.248 2
15	Smooth (window7)	0.959 6	23.607 5	0.884 1	33.268 9
14	Smooth (17)+MSC	0.924 2	32.031 7	0.882 0	29.676 1
15	Smooth (7)+ normalization	0.953 8	25.207 3	0.910 8	28.386 2

It can be concluded that the preprocessing effect is the best when the number of main factors in PLS is 15, and the smoothing (window width of 7)+ normalization is selected, namely, the model can get a higher prediction set correlation coefficient and a smaller prediction set mean squared variance. It can also be seen from Fig.2 that the processed spectra become smoother and the alignment is more tidy and tight than that before processing. This indicates that the preprocessing removes some of the noise in the original spectrum while preserving the main information. Therefore, when the final model is established, the smoothing (7) + normalization is selected as the pretreatment method in this experiment.

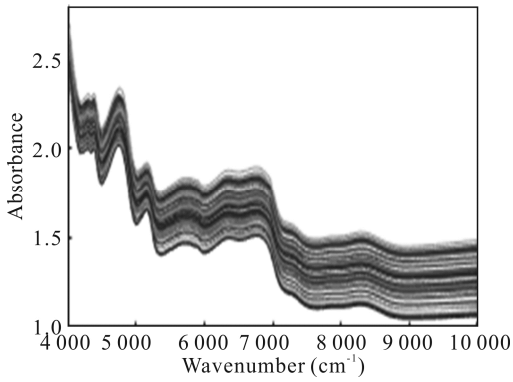

Fig.1 Original mean spectra

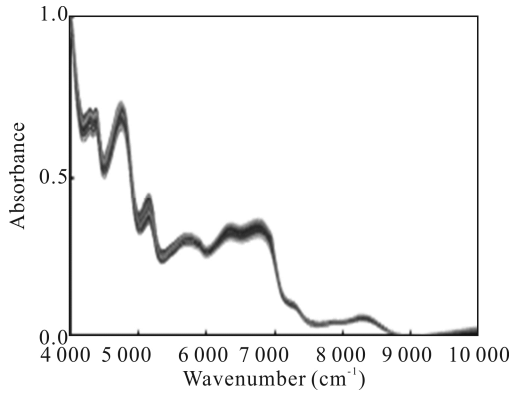

Fig.2 Smooth+normalization pre-processed spectra

The full-band spectra of the 50 pre-processed correction samples were subjected to PLS statistical regression to establish a cAMP quantitative correction model, and then the model was validated with 18 prediction set samples. The quality of the model is evaluated by the correction set correlation coefficient (R_c), correction set mean square error (R_{MSEC}), prediction set correlation coefficient (R_p), and prediction set mean square error (R_{MSEP}). For the same batch of samples, the smaller the R_{MSEC} and R_{MSEP} values, the higher the accuracy of the model, and the closer the two values, the better the stability of the model[14].

The size of the principal factor of the PLS model is related to the actual prediction ability of the model. If the number of principal factors is too small, the model will have insufficient fitness; if the number of principal factors is too large, the model will be over-fitting[15]. The experimental results show that when the number of principal factors is 15, the correlation coefficient between the correction set and the prediction set of the finally established cAMP quantitative correction model is above 0.9, and the values of R_{MSEC} and R_{MSEP} are very close, that is, the obtained model has better predictive ability and stability. The scatter plot diagrams of the chemical value contents for the correction set and the predicted set of the cAMP quantitative model along with predictive values are shown in Fig.3 and Fig.4.

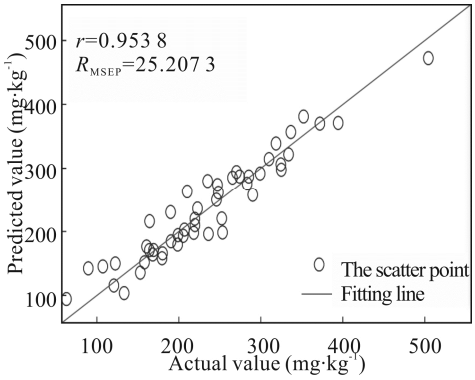

Fig.3 Correction result of the PLS quantitative model

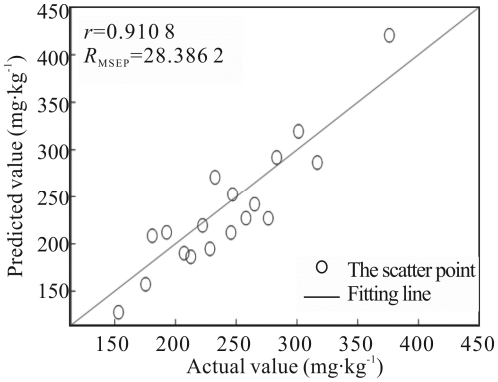

Fig.4 Prediction result of the PLS quantitative model

It can be seen that there is a good linear relationship between the predicted value and the chemical value of cAMP content in red jujube, which proves that the method can quantitatively determine cAMP content in red jujube.

The experimental results show that the R_c and R_p of the cAMP quantitative model are 0.951 5 and 0.910 8, respectively. The R_{MSEC} and R_{MSEP} are 25.793 7 and 28.228 0, respectively. The prediction ability and stability are better, which verifies the feasibility of rapid detection of cAMP content in red jujube by NIR spectroscopy combined with stoichiometric analysis. This method is more environmental, economical and faster than the widely used HPLC, without the need for complex preprocessing of the sample. It is of great significance to purify cAMP from red jujube and develop the market of its processed product.

Although the PLS modeling method used in this paper has a better prediction correlation coefficient, R_{MSEP} still has room for improvement. In the next study, it is necessary to find a better preprocessing method and a more accurate chemometric analysis method to get a cAMP quantitative measurement model with less prediction error. At the same time, it is necessary to further use more samples from different sources to improve the NIR quantitative model, and then it will be widely used in the actual detection of red jujube cAMP content.

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