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FTIR Study of White and Green Broad Beans Based on Curve-fitting

Xiaohua WANG, Gang LIU*, Quanhong OU, Xingxiang ZHAO, Jianming HAO, Xiangping ZHOU

School of Physics and Electronic Information, Yunnan Normal University, Kunming 650500, China

Abstract Fourier transform infrared (FTIR) spectroscopy was used to study two kinds of broad beans with white and green cotyledons respectively. The results show that the infrared spectra of the two kinds of broad beans are similar and mainly made up of the absorption bands of protein, and polysaccharides. The second derivative infrared spectra amplified the differences and revealed that there were some obvious differences in the range of $1\,800 - 700\text{ cm}^{-1}$ and $1\,200 - 700\text{ cm}^{-1}$. Hierarchical cluster analysis (HCA) were used for the discrimination of the two kinds broad beans based on the second derivative spectral data in the region of $1\,611 - 1\,100\text{ cm}^{-1}$, and yielded 88.9 % accuracy. The spectra in the range from $1\,700$ to $1\,600\text{ cm}^{-1}$ were used to perform Fourier self-deconvolution and curve fitting, which obtained nine peaks. The ratios of relative areas of the bands at α -helix, β -sheet, β -turn and the unordered structure of protein in white beans were 67.71%, 35.6%, 35.6% and 21.09% respectively, while the ratios in green beans were 8.02%, 31.59%, 37.12% and 23.27% respectively. The results indicate that the secondary structure of protein was different in the two kinds of broad beans.

Key words Fourier transform infrared (FTIR) spectroscopy, Hierarchical cluster analysis, Second derivative infrared spectra, Curve fitting, Broad bean

Broad bean is a world-wide crop. Broad bean seeds contain eight essential amino-acids, vitamins, and no cholesterol, which are of benefit to human. The seeds as well as the stem and leaves can be feedstuff for pig and other domestic animals. There are two kinds of broad beans with white and green cotyledons. Green cotyledons broad bean is a special rare and precious species, which grows in Baoshan city of Yunnan province. The nutrient contents of green bean are higher than normal white beans, and have higher price. So, there are counterfeit green beans which were processed with chemicals in local market. It is not easy to discriminate which is true for customers. The conventional chemical analysis and instrumental methods such as ICP-MS for beans have a lot of limitations. These methods involve tedious procedures and trouble pretreatments. Therefore, it is necessary needing a quick and efficiency method to evaluate these samples. Over the last decades, FTIR has been proven to be a simple, efficient, non-destructive method, and needs minimum sample preparation. FTIR spectroscopy is a useful tool for elucidating structures and composition of chemical compounds^[1]. This technique has been widely used in plants and medicine investigations recently^[2-5]. In this paper, FT-IR spectroscopy combine with hierarchical cluster analysis (HCA) and curve fitting was utilized to study broad beans. The purpose is to differentiate two different kinds of beans by spectroscopy.

1 Materials and methods

Broad bean seeds were collected in Baoshan city of Yunnan province in the year of 2011. All samples were dried and stored in dry

condition for use. IR spectra were recorded at a resolution of 4 cm^{-1} on a Frontier FT-IR spectrometer (PerkinElmer, UK), equipped with a DTGS detector. The KBr sample pellets were prepared by mixing 1 mg of a sample with approximately 100 mg of KBr using a pestle and mortar. The IR spectra of the sample pellets were recorded with a total of 16 scans, and transmission spectra thus obtained. A sample-free KBr pellets was used to obtain the spectral background, as opposed to the KBr background used in previous transmission measurements. Each spectrum was collected in triplicate and the average spectrum was used for analysis. The infrared spectra and the second derivative infrared spectra were processed with 9-point smoothing, baseline correction and normalization by OMNIC 6.0. Hierarchical cluster analysis was carried out using SPSS and curve fitting was carried out by Origin 8.5.

2 Result and discussion

2.1 Analysis FTIR spectra of broad beans Fig. 1 shows the FT-IR spectra of white bean (a) and green bean (b). The peak at about $3\,298\text{ cm}^{-1}$ was due to O-H and N-H stretching vibration^[6], while the band at $2\,929\text{ cm}^{-1}$ was due to asymmetric stretching vibration of C-H in CH_2 groups^[7], which mainly come from protein, lipids and carbohydrates; the strong peak near $1\,654\text{ cm}^{-1}$ was attributed to the C=O stretching vibration of amide I of protein; the peak of N-H bending vibration and the C-N stretching vibration of amide II is observed at near $1\,538\text{ cm}^{-1}$ ^[8]; the peaks at $1\,451$ and $1\,391\text{ cm}^{-1}$ were mainly attributed to the bending vibration of CH_2 and CH_3 respectively^[9]; the peak at about $1\,245\text{ cm}^{-1}$ was due to the vibration of amide III^[10]; the peak at $1\,156$ and $1\,077\text{ cm}^{-1}$ were assigned to C-OH stretching vibration of carbohydrate. The weak peak at 855 cm^{-1} was attributed to α -glycosidic bond^[11]. The results showed that the spectra of broad beans were mainly made up of

the absorption bands of protein and polysaccharides.

The second derivative spectrum can amplify the tiny differences and improve the resolution. Fig. 2 shows the second derivative infrared spectra of white bean (a) and green bean (b) in the range of $1800 - 1300 \text{ cm}^{-1}$ and $1200 - 700 \text{ cm}^{-1}$. The obvious differences between them are the peaks at 986, 792, 771 and 756 cm^{-1} in green bean (b) are stronger than those in white bean (a), while the peaks at 1 743, 1 725, 1 692, 1 514, 1 451 and 861 cm^{-1} in green bean (b) are weaker than those in white bean (a). There are also some tiny differences between them. White bean (a) has three peaks at 1 481, 833 and 811 cm^{-1} , while these peaks disappear in green bean (b). Green bean (b) has four peaks at 1 423, 1 392, 1 033 and 933 cm^{-1} , while white bean does not have these peaks. There are two peaks at 1 608 and $1 585 \text{ cm}^{-1}$ in white bean (a) while those peaks shift to 1 614 and $1 593 \text{ cm}^{-1}$ in green bean (b). According to Fig. 2, white bean

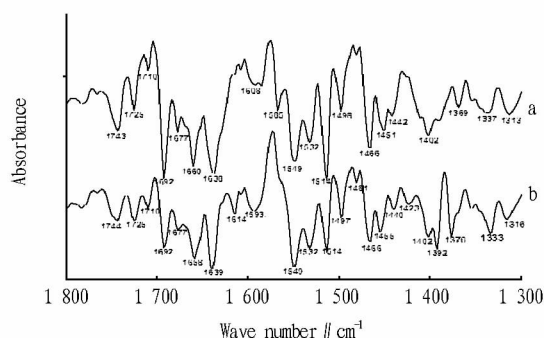


Fig. 2 Second derivative infrared spectra of white bean (a) and green bean (b)

2.2 Hierarchical cluster analysis (HCA) Hierarchical cluster analysis was applied to observe dissimilarities between white beans and green beans. The dissimilarity of different clusters was utilized by a Euclidean distance and calculated by Ward's method^[12]. The second derivative infrared spectra in the range of $1600 - 1100 \text{ cm}^{-1}$ were chosen to perform HCA. The dendrogram was shown in Fig. 3. 54 samples were analyzed, with approximately 88.89% classification accuracy. Samples were clearly divided into two main branches in Fig. 3: one carried the spectra of all white beans except 110, 111, 113 and 124. The other major branch carried the spectra of all green beans except b21 and b23.

2.3 Curve fitting Curve fitting, also known as band-fitting, is a mathematical tool for modeling the experimental data. Fourier deconvoluted spectra are usually curve-fitted using Lorentzian or Gaussian bands. The intensity of the absorption peak was replaced by the peak area after the procedure of the Fourier self-deconvolution and curve fitting^[13, 14].

The original spectra in amide I band ($1700 - 1600 \text{ cm}^{-1}$) was used for curve fitting. The decomposition of amide I band, presented in Fig. 4, was performed in order to extract the information about the secondary structure of proteins.

From Fig. 4, it can be seen that there are nine peaks at 1 692, 1 681, 1 673, 1 666, 1 657, 1 648, 1 639, 1 631 and 1619 cm^{-1} in green beans (B), and at 1 691, 1 680, 1 673,

and green bean can be distinguished by the second derivative infrared spectra.

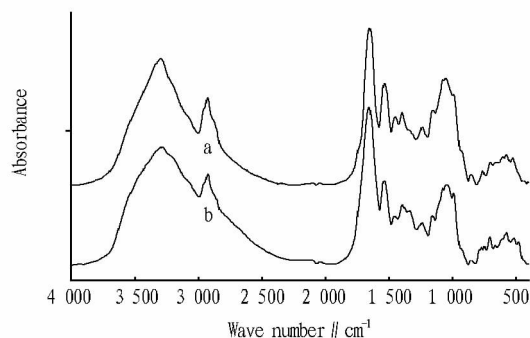
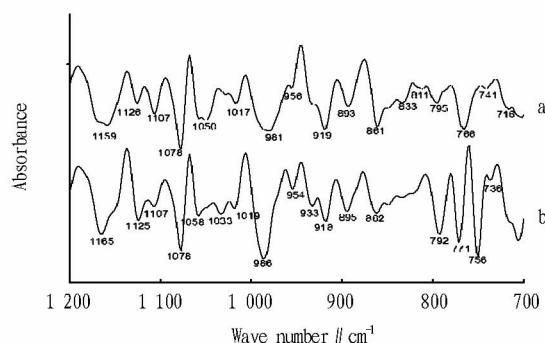


Fig. 1 FTIR of white bean (a) and green bean (b)



1 666, 1 657, 1 648, 1 639, 1 631 and 1 619 cm^{-1} in white beans (A). The α -helix absorption was in the range of 1 650 – 1 660 cm^{-1} , the bands at 1 610 – 1 640 cm^{-1} and 1 670 – 1 680 cm^{-1} were assigned to β -sheet cm^{-1} , β -turn structure absorption was in the region of 1 660 – 1 670 cm^{-1} , the band at about 1 645 cm^{-1} was assigned to the unordered structure^[15–17].

Table 1 The composition of amide I bands of broad beans

Peak no.	White bean		Peak no.	Green bean	
	Wave number	Peak area percentage//%		Wave number	Peak area percentage//%
1	1 619	9.66	1	1 619	11.09
2	1 631	14.91	2	1 631	11.75
3	1 639	7.12	3	1 639	6.61
4	1 648	21.08	4	1 648	23.27
5	1 657	7.82	5	1 657	8.02
6	1 666	18.84	6	1 666	18.52
7	1 673	3.91	7	1 673	2.14
8	1 680	13.68	8	1 681	15.83
9	1 691	2.97	9	1 692	2.77

The α -helix is supposed to contribute to the stabilization of the protein structure^[17]. Therefore, a decrease of α -helix may cause proteins in white beans unstabilization compared to green

beans. The results showed a significant increase of α -helix, β -turn and unordered structure and decrease of β -sheet in green beans compared to white beans. These findings indicate that the different secondary structure of proteins might cause the nutrient differences between green beans and white beans.

Table 2 Content of secondary structure of broad beans proteins by FT-IR analysis

Samples	Secondary structure//%			
	α -helix	β -sheet	β -turn	unordered
White bean	7.71	35.6	35.6	21.09
Green bean	8.02	31.59	37.12	23.27

3 Conclusions

FTIR spectroscopy was adopted to study and compare the differences between white beans and green beans. Hierarchical cluster analysis (HCA) on the second derivative infrared spectra could distinguish green beans from white beans. The results of curve fitting indicated that the secondary structures of protein were dissimilar between green beans and white beans. It was demonstrated that FTIR spectroscopy was a simple, efficient and non-destructive method for distinguish white beans and green beans.

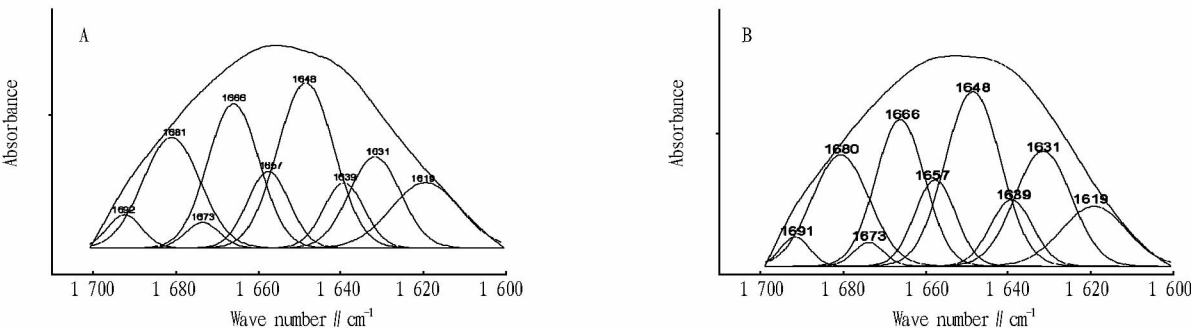


Fig.4 Original (top)and curve-fitting (bottom) spectra of amide I band (1 700 – 1 600 cm^{-1}) of broad beans (A: white bean, B: green bean)

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to physical and chemical properties should be separated from other materials. Agricultural scientific research institutions will generally use some harmful and toxic reagents in some scientific research experiments. For example, molecular marker experiment needs EB coloring, while EB is a strong carcinogen; in tissue culture, HgCl_2 is toxic chemical agent; in molecular biology, phenol is a toxic agent; formamide may lead to monster. Thus, these agents should be stored in special cabinets. Some open laboratories have

Table 3 Registration form for use of special materials

Name of material purchased	Purchasing date	Purchased amount	Date of requisition	Quantity of requisition	Handled by	Application	Remarks
			_____Date				
			_____Month				
			_____Year				

5 Establishing general ledger of special materials, to realize accounts agree with physical inventory

It is recommended to establish auxiliary account for accounting of special materials, and reinforce financial accounting management of special materials. Material safekeeping and use departments (research teams) should establish and improve general ledger for purchasing, selling and storage of materials, strengthen physical management of special materials, and do well in registration of account books, to ensure accurate and complete data of special materials.

6 Conclusions

From the above analysis and discussion, we can reach following conclusions. Firstly, it is required to take effective management of special materials, enhance organization leadership, reinforce procurement procedure of special materials, establish and perfect procurement system, strengthen physical management, and establish general ledger of special materials, to make accounts agree with physical inventory. In addition, it is required to strengthen training of personnel in procurement management department, fully un-

derstand market situation and price trend, find out strength of suppliers, know whether quotation of suppliers is reasonable, and visit suppliers to check their supplying capacity^[6], and improve professional quality of purchasers and strengthen professional ethics and sense of duty.

4.2 Warehouse exit should be registered Before using special materials, each research team should handle warehouse exit formality, and make registration of use of special materials (see Table 3), to ensure tracing of all materials.

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