Determination of Geographical Origin and Protein Content of *Acacia* Gums Using Infrared Spectroscopy and Chemometrics

ZOU Xiaobo[1], Alaa Kamal Mohmmed KHAIR KHOGLY[1], SHI Jiyong[1], Mel HOLMES[2]
(1. School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China;
2. School of Food Science and Nutrition, the University of Leeds, Leeds LS1 3AB, United Kingdom)

Abstract: The feasibility of using Fourier transform infrared (FTIR) spectroscopy and chemometrics as a rapid and non-invasive technique to determine the geographical origin and protein content of Sudanese *Acacia* gums was investigated. Seventy-two samples of *Acacia* gums were collected from six different regions (12 samples from each region). Linear discriminant analysis (LDA) was used to discriminate the geographical origin of *Acacia* gums, and backward interval partial least squares (Bi-PLS) was applied to build a prediction model for the protein content of *Acacia* gums. The results showed that the recognition rates of LDA for calibration set (48 samples) and prediction set (24 samples) were both 100% when the first 6 principal components were used. In addition, Bi-PLS yielded a good prediction model (R_P = 0.937 3 and RMSEP = 0.173%) for protein content by using the optimal combination of 4 out of 20 spectral intervals. Hence, FTIR spectroscopy coupled with chemometrics can be considered as a valid approach for the determination of the geographical origin and protein content of *Acacia* gums.

Key words: *Acacia* gums; Fourier transform infrared (FTIR) spectroscopy; geographical origin; protein content; linear discriminant analysis (LDA); backward interval partial least squares (Bi-PLS)

应用红外光谱结合化学计量学方法检测阿拉伯胶产地和蛋白质含量

邹小波[1]，Alaa Kamal Mohmmed KHAIR KHOGLY[1]，石吉勇[1]，Mel HOLMES[2]
（1.江苏大学食品与生物工程学院，江苏 镇江 212013；
2.利兹大学食品科学与营养学院，英国 利兹 LS1 3AB）

摘 要：研究利用傅里叶红外光谱结合化学计量学方法来实现对苏丹阿拉伯胶的产地和蛋白质含量的快速无损检测的可行性。采集自6 个不同的产地，每个产地12 个，总计72 个阿拉伯胶样本，作为研究对象，运用线性判别分析（linear discriminant analysis，LDA）和反向区间偏最小二乘（backward interval partial least squares，Bi-PLS）法分别实现对苏丹阿拉伯胶的产地区分和蛋白质含量检测。结果表明，当主成分数为6时，LDA对样本的训练集（48 个样本）和预测集（24 个样本）的识别率都为100%。Bi-PLS法回归联合20 个光谱子区间中的4 个子区间得到最佳的蛋白质预测模型，其预测集相关系数为0.937 3，均方根误差为0.173%。因此，利用傅里叶红外光谱结合化学计量学方法可实现对苏丹阿拉伯胶的产地以及蛋白质的含量的快速无损检测。

关键词：阿拉伯胶；傅里叶红外光谱；产地；蛋白质含量；线性判别分析；反向区间偏最小二乘法
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作者简介：邹小波（1974—），男，教授，博士，研究方向为食品与农产品无损检测。E-mail：zou_xiaobo@ujs.edu.cn

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Acacia gums, also called gums Arabic, are the dried exudates extracted from the stem and branches of *Acacia* trees. They are often used as non-digestible food ingredients in the foodstuffs, such as an emulsifier in the manufacture of soft drinks and a stabilizer in fermentation milk[1]. The chemical composition of gum Arabic is complex and consists of a group of macromolecules characterized by a high proportion of carbohydrates (−97%), which are predominantly composed of *D*-galactose and *L*-arabinose units and a low proportion of proteins (<3%)[2]. *Acacia* gums from different *Acacia* species exhibited different characteristics, and even gums in the same species bear some difference because of their geographical origin. For example, *Acacia senegal* (*A. senegal*) has a higher degree of branching and better emulsifying properties than *Acacia seyal* (*A. seyal*), gum does[3]. This is because their biochemical composition and molecular characteristics can vary, depending on internal and external factors (*Acacia* specie, tree location, or weather conditions) and postharvesting processes (storage conditions, filtration, spray drying, irradiation, or heat treatments)[4]. Hence, recognition of these *Acacia* gums is of great significance for the market and the end use.

Traditionally, the identification of the speices or quality of *Acacia* gums can be conducted by sensory analysis, such as observing the color and smelling, which are unfortunately impeded the subjectivity of human. Other physicochemical methods, such as detection of the sugar and nitrogen content[5], and component analysis by using combined gel permeation chromatography and multi-angle laser light scattering (GPC-MALLS)[6], are either destructive to samples or time-consuming. Nuclear magnetic resonance (NMR) was also regarded as an effective technology to identify *A. senegal* and *A. seyal*, but it may be inaccessible for industrial high throughput screening purposes[7]. As a result, a rapid, non-invasive and economical method for distinguishing the speices or the quality of *Acacia* gums is highly desired.

Fourier transform infrared (FTIR) spectroscopy, which keeps the advantages of being robust, cost-effective and non-destructive to sample, has been widely used as an effective identification and quantitative analysis technique[8]. In recent years, many studies have shown the feasibility of using FTIR spectroscopy in discriminating similar foodstuffs and medicines, such as walnut oil[9], *Cortex Eucommiae*[10], minced beef[11], and detecting specific components, such as free acids, invertase, moisture, hydroxymethyl furfural, polyphenol oxidase and electrical conductivity[12-14]. Quantification of gums is another interesting issue for determining the quality of the gums. For *A. senegal* and *A. seyal* gums, one of the most significant difference is their protein content (−2.5% for *A. senegal*, −1% for *A. seyal* gums)[15]. Accordingly, the protein content can be used as an imporatant quality index of these *Acacia* gums.

Sudan is a dominant leader in *Acacia* gum production in the world[16]. The aim of the present work is discriminating the *A. senegal* and *A. seyal* gums collected from Sudan, and predicting their total protein content by using FTIR spectroscopy and chemometrics.

1 Materials and Methods

1.1 Materials

Two categories of *Acacia* gum samples (*n*=72) were collected from different regions in Sudan, namely *A. senegal* (*n*=36) (Group (A), *n*=12: West Bara Locality in North Kordofan State; Group (B), *n*=12: Ennuhud Locality in West Kordofan State; Group (D), *n*=12: *Acacia* gum Ltd., Sudan) and *Acacia sayal* (*n*=24) (Group (C), *n*=12: South Kordofan State; Group (E), *n*=12: *Acacia* gum Ltd., Sudan). One other *Acacia* gum sample was obtained from China (Group (F), *n*=12: Siyuan Biology Ltd., Henan, China). All other reagents are of analytical grade purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.

1.2 Methods

1.2.1 Determination of total protein content

Firstly, all raw *Acacia* gum samples were dried in

a forced draught oven (Shanghai Yi-Heng Machine Co., Shanghai, China) at 50 ℃ for 24 h and then mashed into powder by using a cyclone mill. The total protein contents of the gums were measured by using the Kjeldahl method[17].

1.2.2 FTIR spectroscopy

FTIR spectra of gum powders were collected by using a FTIR spectrometer (Thermo Scientific Nicolet iS50, Thermo Fisher, USA). The spectra were obtained at reflectance mode from 650–4 000 cm^{-1} at a resolution of 4 cm^{-1} and the total number of scans was 32. Three spectra were recorded for each sample and the average spectrum was used for subsequent data analysis.

1.2.3 Chemometrics

Principle component analysis (PCA) is a statistical procedure which is able to reduce the dimensions of the data set by using an orthogonal transformation[18]. After PCA, the useful information can be extracted from the original spectral data by eliminating overlapping information and the remained dimensions are defined by principal components (PCs). It is able to provide the visual graphical information for determining differences within and between cluster trends[19].

Linear discriminant analysis (LDA) is a classical statistical approach for feature extraction and dimension reduction[20]. LDA can classify the objects into groups by estimating the distance between each observation form all group centers[21]. It manages to find out the optimal transformation (projection) that minimizes the intra-class distance and maximizes the inter-class distance simultaneously in order to achieve the maximum discrimination.

Partial least squares (PLS) regression is one of the multiple linear regression methods[22]. PLS is useful when number of predictors (i.e. spectral peaks) is much higher than number of samples in data set[23]. Backward interval partial least squares (Bi-PLS) regression is an extension of PLS. The basic principle of Bi-PLS is as follows[24]: The full-spectrum region is split into a number of equidistant spectral subintervals. Then, PLS models are calculated with each subinterval left out. The first left out interval is the one that when it gives the poorest performing model with respect to the root mean square error of cross-validation (RMSECV).

A cross-validation process was used in model validation with leave-one-out method. The performance of the regression models was evaluated according to the correlation coefficient of calibration set (R_c) and prediction set (R_P), RMSECV and root mean standard error of prediction set (RMSEP). Generally, a good model should have high correlation coefficients along with low RMSECV and RMSEP[25].

1.3 Data analysis

Matlab V7.0 (MathWorks, USA) was used for data processing under Windows 7.

2 Results and Analysis

2.1 The total protein content of *Acacia* gums

All 72 samples were randomly separated into two subsets, including the calibration set (48 samples) used to build models and the prediction set (24 samples) used to test the robustness of models. The total protein content in *Acacia* gums is shown in Table 1. It can be seen that the total protein contents of *A. senegal* (Groups (A), (B), and (D)) and *A. sayal* (Groups (C) and (E)) were in the range of 1.53%–2.22% and 0.56%–1.87%, respectively. These results are close to the Sudanese Standards and Metrology Organization (SSMO) in which the protein contents for *A. senegal* and *A. sayal* gums are recorded to be in the range of 1.50%–2.70% and 0.7%–1.0%, respectively. In comparison, the *Acacia* gums obtained from Group (D) had the highest protein content, while the *Acacia* gums obtained from China had the lowest protein content. These difference were closely associated with the species of the *Acacia* gums and also with the gum origin, age, storage conditions, and so forth[26].

Table 1 Protein content of *Acacia* gums

Classification	Protein content/%			
	Min	Max	Mean	SD
Group (A)	1.77	2.10	1.93	0.09
Group (B)	1.75	2.20	1.98	0.14
Group (C)	1.53	1.87	1.71	0.11
Group (D)	1.91	2.22	2.06	0.08
Group (E)	0.56	1.00	0.74	0.13
Group (F)	0.52	0.72	0.63	0.06
Calibration set	0.51	2.22	1.25	0.51
Prediction set	0.52	2.17	1.16	0.50

Note: SD. Standard deviation.

2.2 Spectra investigation

The FTIR spectra for the *Acacia* gum samples acquired in the range of 650–4 000 cm^{-1} are shown in Fig. 1. In order to avoid the strong absorption of water, especially in the amide I band region (1 720–1 580 cm^{-1})[17], *Acacia* gums were fully dried before spectra collection. For all of the *Acacia* gums, there are four main characteristic spectral bands. No significant difference can be observed between the FTIR

spectra of different groups. This is because all of them consist of similar kinds of chemical components. Table 2 shows the wavenumbers and attribution of the characteristic spectral bands of these *Acacia* gums.

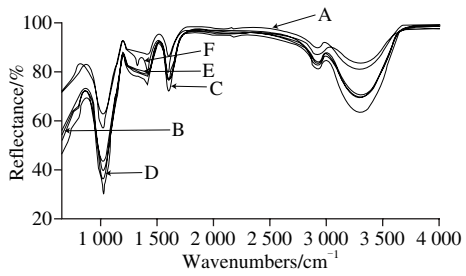


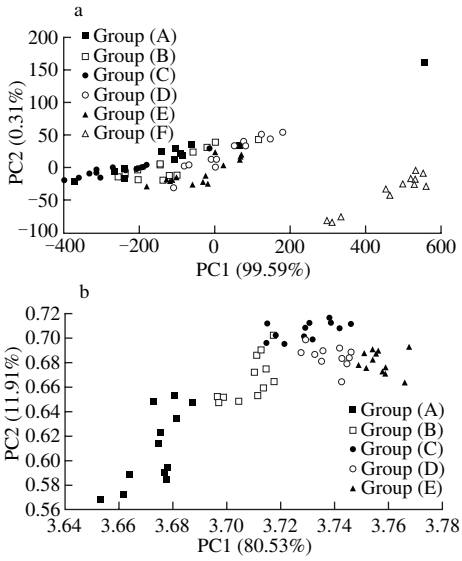
Fig. 1 FTIR spectra of *Acacia* gums

Table 2 Wavenumbers and ttribution of the characteristic spectral bands in FTIR spectra of *Acacia* gums

Wavenumbers/cm^{-1}	Attribution
3 000–2 839	C–H stretching (carbohydrates)[27] O–H stretching (carboxylic acids) NH_3 stretching (free amino acids)[28]
1 550–1 650	Band Amide Ⅰ and Ⅱ from proteins Deprotonated carboxylic function (COO–) from uronic acids
1 309–1 456	O–H stretching/bending C–O stretching (carbohydrates) C–H stretching (carbohydrates) C=O stretching of ketones
977–1 107	C–O & C–C stretching (carbohydrates) Ring vibrations (mainly from carbohydrates)

2.3 Principle component analysis of FTIR spectra of *Acacia* gum

Prior to regression analysis, an exploratory analysis was performed in order to investigate any trend of discrimination among the *Acacia* gums from different regions. PCA is the most widely applied linear projection method for unsupervised exploratory multivariate data analysis to visualize the similarities and differences between the spectra[29]. Before PCA, the raw mean spectra were preprocessed by using standard normal variate (SNV). Fig. 2a shows the score plot of the two-dimensional component space of six categories of *Acacia* gum samples. The accumulated variance contribution rates were 99.33% for the top two PCs. The classification trend of these six categories of *Acacia* gum samples can be also observed from the score plot. The *Acacia* gum samples obtained from China were clearly separated from that obtained from Sudan, indicating the obvious difference of the chemical constituents between the *Acacia* gum from different geographical origins. In order to furtherly visualize the difference between *Acacia* gum collected from Sudan, PCA was conducted for the *Acacia* gum regardless of Group (F) and the score plot of the two-dimensional was shown in Fig. 2b. It can be seen that the five categories of *Acacia* gum from Sudan are clearly distinguished from each other, demonstrating their intrinsic difference. Nevertheless, PCA is not able to define the boundaries of the six categories to discriminate the *Acacia* gum samples so that further analysis was carried out in the next sections.



a. including Group (F); b. excluding Group (F).

Fig. 2 Score cluster plot of top two principal components (PCs) for *Acacia* gum samples

2.4 Determination of geographical of *Acacia* gum by LDA

LDA focus on finding the optimal boundaries between the classes[30]. The number of PCs is crucial to the performance of the LDA discrimination model. The discrimination rates by cross-validation were used to optimize the number of PCs. Fig. 3 shows the discrimination rates of LDA model according to different PCs by cross-validation. The optimal LDA models were achieved when the number of PCs was 6, and meanwhile the discrimination rate was up to 100% for both calibration set and prediction set. These results indicated that an ideal separation can be achieved for these six categories of *Acacia* gum. On the basis of LDA, a cluster analysis was conducted. Samples were grouped in clusters based on their nearness or similarity. Fig. 4 shows the dendrogram of *Acacia* gum samples. All the *Acacia* gums in the same group gathered together and were distinguished from other groups. In addition, when all the *Acacia* gum were clustered into two big groups, Group (E) and Group (F) were defined into the same group. This may be due to the reason that Group (E) and (F) had similar protein contents, which were much lower than those of other groups.

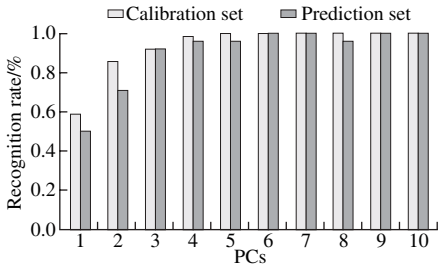


Fig. 3 Recognition rates of LDA models

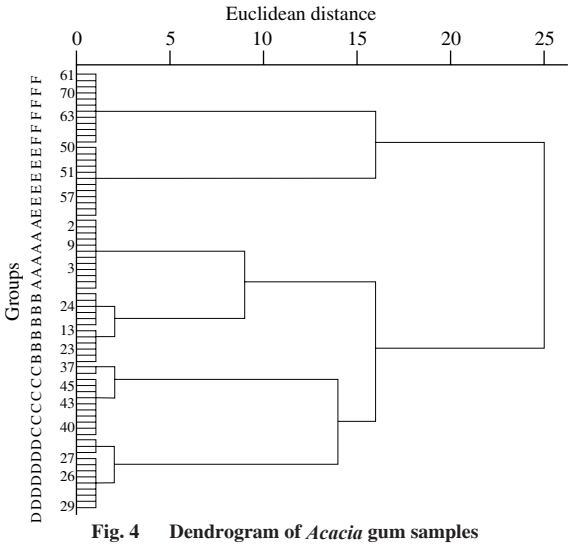


Fig. 4 Dendrogram of *Acacia* gum samples

2.5 Prediction of the total protein content in *Acacia* gum by Bi-PLS

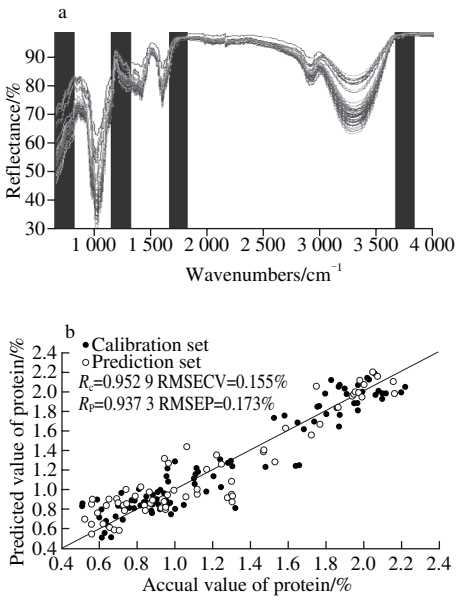


Fig. 5 Spectral interval selected by Bi-PLS (a) and actual versus predicted protein contents (b) in calibration set and prediction set

In this study, the Bi-PLS approach was applied for the quantification of the total protein contents in *Acacia* gums. Bi-PLS aimed to select the most important subintervals in the whole data matrix. The spectra data set was split into some subintervals, and then PLS models were calculated when each subinterval left out. When the whole spectrum region was split into 20 subintervals, the optimal Bi-PLS model for the total protein content was obtained with the combination of four subintervals, resulting in the lowest RMSECV of 0.155%. As shown in Fig. 5a, the optimal subintervals are [1 4 7 19], which correspond to 650–817, 1 152–1 319, 1 652–1 819 and 3 666–3 833 cm^{-1}, respectively. Fig. 5b presents the performance of the Bi-PLS model on the calibration and prediction set. For the prediction set, R_P was 0.937 3 and RMSEP was 0.173%, demonstrating that the Bi-PLS model can be used to predict the total protein content of *Acacia* gums.

3 Conclusion

FTIR spectroscopy technique combined with chemometrics was successfully established and employed to distinguish the geographical origins and predict the total protein contents of *Acacia* gums. The results showed that the optimal LDA model was achieved in determining the geographic origins with 100% of discrimination rate for both the calibration and prediction set when the number of PCs was 6. The Bi-PLS model showed a good performance in predicting the total protein content (R_P = 0.937 3 and RMSEP = 0.173%) by using the optimal combination of 4 spectral subintervals among 20 subintervals. FTIR spectroscopy coupled with chemometrics can be considered as a valid approach for determination of the geographical origin and protein content of *Acacia* gums.

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