Research Article

Ultraviolet Spectroscopy Used to Fingerprint Five Wild-Grown Edible Mushrooms (Boletaceae) Collected from Yunnan, China

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Nowadays, wild-grown edible mushrooms which are natural, nutritious, and healthy get more and more popular by large consumers. In this paper, UV spectra of different Boletaceae mushrooms with the aid of partial least squares discriminant analysis (PLS-DA) and hierarchical cluster analysis (HCA) were shown to be a practical and rapid method for discrimination purpose. The specimens of *Boletus edulis*, *Boletus ferrugineus*, *Boletus tomentipes*, *Leccinum rugosiceps*, and *Xerocomus* sp. were described based on the UV spectra. From the results, all the specimens were characterized by strong absorption at the wavelengths of 274 and 284 nm and showed the shoulder at 296 nm. However, changes could be seen in the peak heights at the same wavelength for different samples. After analyzing by chemometrics, visual discrimination among samples was presented and the relationships among them were also obtained. This study showed that UV spectroscopy combined with chemometrics methods could be used successfully as a simple and effective approach for characterization of these five wild-grown edible mushrooms at species and genus levels. Meanwhile, this rapid and simple methodology could also provide reference for the discrimination of edible mushrooms.

1. Introduction

Wild-grown edible mushrooms are considered as healthy food sources and have long been attracting a great deal of interest by humankind for the natural and nutritional effects [1]. Some species of them are consumed as a delicacy and constitute an increasing share in the world diet [2]. The consumption of wild-grown edible mushrooms is 5.6 kg of fresh product per household yearly in Czech Republic while higher value is found to be 20–24 kg in China [3, 4]. Moreover, these mushrooms are not only an important source of revenue for rural economies but also a substantial economic resource in several regions of the world [5].

In the daily life, many edible mushrooms are usually sliced and dried after collecting for better storage and sales.

However, since the market demands for wild-grown edible mushrooms increased, some unscrupulous traders sell inferior mushrooms for profiteering such as mixing different species of dried mushroom slices even toxic ones which lead to unfair competition [6]. In a previous study, Dentinger and Suz [7] analyzed 15 pieces of dried Chinese porcini from a single commercial packet purchased in London and identified three species of mushrooms that have never been formally described by science until now. It suggested that insufficient knowledge of the porcini species contained within food products may pose a health concern. Therefore, for economical, biodiversity-related reasons and so forth, it is often important to discriminate the species of wild-grown edible mushrooms accurately.

Classical mushroom discrimination is according to careful observation of microscopic and macroscopic morphological characters. However, these methods require trained and experienced people. At present, some analytical techniques for discrimination of edible mushrooms have been published including high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and DNA sequence analyses [8–12]. Since these techniques provide a nonselective signal, the aid of appropriate chemometrics methods is also necessary for interpreting them [13, 14]. Nevertheless, some imperfections about these methods have been reported. For example, IR needed the experienced technicians and it was hard to model. GC-MS which was expensive could be used to analyze the substance which had the low boiling point and good thermal stability, merely. The ideal chemical technique for the discrimination of mushroom species would provide rapid and accurate analysis. Recently, ultraviolet (UV) spectrometry that reflect the comprehensive fuzz information of samples should be accessible as discrimination tool in diverse research fields, such as the analysis of foods [15], herb medicines [16], automotive window tints [17], for the advantages like rapidity, simplicity, and low cost. Additionally, Li et al. [18] distinguished wild and cultured *Macrocybe gigantea* with different storage times based on UV spectroscopy combined with multivariate analyses. As reported by Yang et al. [19] UV spectra fingerprints in combination with chemometrics methods could be used to discriminate different parts of edible mushrooms. The rapid and reliable method of UV spectroscopy has shown huge potential for the analysis of edible mushrooms.

Mushrooms in the family Boletaceae with 50 genera and 800 species, which are mainly characterized by soft fleshy context, are important groups in the macrofungi of basidiomycete [20, 21]. They are wildly collected and consumed in the main production areas such as eastern Asia, Europe, and North America [22, 23]. In southwestern China, Yunnan Province which is mild and rainy in summer and autumn providing ideal conditions for fungal growth is one of the most important centers for producing, consuming, and trading Boletaceae mushrooms [24]. *Boletus edulis* which is one of the most famous delicious edible mushrooms in the world is widely liked by people [25]. Other species such as *Boletus tomentipes* are generally trading on the market during summer and autumn [26]. Apart from flavor and taste, the fruiting bodies of the wild-grown edible mushrooms in this family are considered sources of proteins, amino acids, vitamins, and carbohydrates, as well as minerals and antioxidants [1, 26]. In addition to edibility, many medicinal properties in these mushrooms, such as antioxidation, antitumor, and antibiotic effect, have been claimed [27, 28].

In this study, an analytical method for the discrimination of five wild-grown edible mushrooms (Boletaceae) by UV spectroscopy was established and verified. All the spectroscopic data were analyzed by partial least squares discriminant analysis (PLS-DA) and hierarchical cluster analysis (HCA) that aimed to distinguish different specimens and find correlations among these species. The results represented

a detailed report on the differentiation of tested Boletaceae species which may provide a utility of methodology for discriminating wild-grown edible mushrooms rapidly and accurately.

2. Experiment

2.1. Materials. For the real samples used in this study, the fruiting bodies of wild Boletaceae including five species were collected from Yunnan Province during the collection season (June to September) in 2011. All the samples were authenticated by Dr. Honggao Liu, College of Agronomy and Biotechnology, Yunnan Agricultural University, and preserved in the specimen room of this university. The sample information is listed in Table 1.

2.2. Apparatus. Apparatus were as follows: UV-2550 UV-Vis Spectrophotometer (Shimadzu, Japan); DFT-100 type grinder (Zhejiang Wenling City Linda Machinery Company, China); SY3200-T type ultrasonic washer (Shanghai Shengyuan Ultrasonic Equipment Company, China); 100-mesh stainless steel sieve (Beijing Zhongxi Tai'an Technology Service Company, China); and AR1140 Electronic Analytical Balance (NJ, USA).

2.3. Sample Preparations. All the samples were cleaned up and dried at the temperature of 50°C before analysis. Then all of them were ground to fine powder and passed through a 100-mesh stainless steel sieve. The sieved powders were stored in the labeled Ziploc bags at room temperature until further analysis. In each mushroom species, the mixed mushroom samples ($n = 7$) of the same collection site were used to perform the experiment. 0.1 g of each mixed powdered sample and 10.0 mL chloroform (analytical grade) were put into a 25 mL colorimetric tube and extracted by ultrasonication for 30 min. The extracts were filtered and kept as stock solutions for testing.

2.4. Data Acquisition and Chemometrics Methods. Each stock solution was analyzed by UV-2550 UV-Vis spectrophotometer at 0.2 nm sampling interval and 1.0 nm slit width. Scans were collected over a range of 190–400 nm and each sample was measured in triplicate. The UV spectra were treated by the three groups of average and second derivative, in order to eliminate the solvent interference and increase accuracy of spectra. The number of points for derivative was five and the spectral data were mean centered during the analyzed.

The absorption readings obtained over the spectral points of all the samples were converted into a data matrix using Excel 2007 (Microsoft, USA) with the spectral points as variables represented by columns and the corresponding spectral absorption measurements represented by rows. Then the raw spectral data of all the samples were analyzed by partial least squares discriminant analysis (PLS-DA) and hierarchical cluster analysis (HCA) to evaluate the relationships in terms of similarity or dissimilarity among groups of multivariate data. The two statistical analyses were performed by using SIMCA-P⁺ 11.5 (Umetrics, Umeå, Sweden) and SPSS

TABLE 1: Information of all the samples.

Number	Species	Site of collection
1	<i>Leccinum rugosiceps</i> (Peck) Singer	Wuhua District, Kunming, Yunnan
2	<i>Leccinum rugosiceps</i> (Peck) Singer	Qujing, Yunnan
3	<i>Leccinum rugosiceps</i> (Peck) Singer	Pubei, Yimen, Yunnan
4	<i>Boletus ferrugineus</i> Schaeff.	Wuding, Yunnan
5	<i>Boletus ferrugineus</i> Schaeff.	Qujing, Yunnan
6	<i>Boletus ferrugineus</i> Schaeff.	Wuhua District, Kunming, Yunnan
7	<i>Xerocomus</i> sp.	Nanhua, Chuxiong, Yunnan
8	<i>Xerocomus</i> sp.	Yao'an, Chuxiong, Yunnan
9	<i>Xerocomus</i> sp.	Qujing, Yunnan
10	<i>Boletus tomentipes</i> Earle	Fuliangpeng, Eshan, Yunnan
11	<i>Boletus tomentipes</i> Earle	Pu'er, Yunnan
12	<i>Boletus tomentipes</i> Earle	Xiaojie, Eshan, Yunnan
13	<i>Boletus tomentipes</i> Earle	Qianchang, Yao'an, Yunnan
14	<i>Boletus tomentipes</i> Earle	Tianshentang, Nanhua, Yunnan
15	<i>Boletus tomentipes</i> Earle	Tongchang, Yimen, Yunnan
16	<i>Boletus tomentipes</i> Earle	Shaqiao, Nanhua, Yunnan
17	<i>Boletus tomentipes</i> Earle	Pu'er, Yunnan
18	<i>Boletus edulis</i> Bull.	Baofeng, Jinning, Yunnan
19	<i>Boletus edulis</i> Bull.	Yulu, Nanhua, Yunnan
20	<i>Boletus edulis</i> Bull.	Pubei, Yimen, Yunnan
21	<i>Boletus edulis</i> Bull.	Longchuan, Nanhua, Yunnan
22	<i>Boletus edulis</i> Bull.	Qianchang, Yao'an, Yunnan
23	<i>Boletus edulis</i> Bull.	Qianchang, Yao'an, Yunnan
24	<i>Boletus edulis</i> Bull.	Shaqiao, Nanhua, Yunnan

20.0 (IBM Corp., Armonk, USA), respectively. Data were visualized by using the two statistical approaches.

3. Results and Discussion

3.1. Selection of Extraction Solvent. Every powdered mushroom sample was taken out to form the mixed sample in order to select the extraction solvent. A total of four different extraction solvents (petroleum ether, chloroform, absolute ethanol, and 0.5 mol/L NaOH) were used and all reagents were of analytical grade. The number of the absorption peaks was used to validate the most appropriate extraction solvent. As shown in the result in Figure 1, the number of the absorption peaks about the chloroform extract is the highest of all the extracts while others have only one or two absorption peaks. It suggested that chloroform extract may include more constituent information about the mushroom samples to reflect their characteristics and chloroform could be the most appropriate extraction solvent.

3.2. UV Spectra of the Wild Edible Boletaceae Mushrooms. The UV spectra of all the specimens are shown in Figure 2. On account of the detection range of the UV-Vis spectrophotometer, the wavelengths of absorption peaks are arranged from 235 to 400 nm for the sake of avoiding the spectral noise. It shows that all the specimens have higher overlap rate

from 235 to 335 nm than that of other wavelengths. Every sample has some characteristic absorption peaks to reveal its fingerprint feature. In Figure 2, it indicated that some chemical components appeared to be very similar among these five species of Boletaceae mushrooms because all the specimens are characterized by strong absorption at the wavelengths of 274 and 284 nm and show the shoulder at 296 nm. However, changes can be seen in the peak heights at the same wavelength for different samples and in the ratios between absorbance values at different wavelengths for the same sample. The UV absorption bands of the presented specimens were usually associated with the presence of different chromophores exemplified in conjugated systems as well as other UV-absorbing systems [29]. To a certain degree, when the substance was in high concentration, the corresponding absorbance was high, too [30]. It suggested that the contents of chemical compositions in different species of mushrooms were variable. This result was in agreement with the reports in previous literatures that the accumulation of chemical composition may be affected by the mushroom species [31, 32]. These differences may be used to discriminate the Boletaceae specimens.

3.3. Partial Least Squares Discriminant Analysis. PLS-DA is a well-established chemometric approach for supervised analyses based on a PLS model in which the dependent variable

FIGURE 1: UV spectra of different extraction solvents.

FIGURE 2: UV spectra fingerprints of the wild edible mushrooms (Boletaceae).

(Y block) represents class membership [33]. This method was used as a representative technique to discriminate the mushroom samples according to their species in this study. The first four principal components (PCs) of PLS-DA could explain 85% of the total variance. The score plot of the sample data with 95% confidence ellipses is shown in Figure 3. Clear separation of the five species of wild-grown Boletaceae mushrooms is observed in the two-dimensional diagram. The mushroom samples which belong to the same species could form cluster and be distinct with other species. PC 1 is determined mainly by negative scores for the samples of

FIGURE 3: PLS-DA score plot based on UV spectra of five species of wild-grown Boletaceae mushrooms.

B. tomentipes while the strongest positive scores on PC 2 are found for samples which belong to *Xerocomus* sp. All the samples could be distinguished into five classes. Additionally, the individual differences among samples of *B. edulis* seem to be relatively obvious because these samples are distributed dispersedly. Similarly, clear differences are also visible in the individuals of *Xerocomus* sp. What is more, the species in the same genus tend to cluster together. As a result, 24 tested samples were classified entirely as their groups by PLS-DA.

According to the spectrographic PLS-DA analysis, a series of scores (variable importance for the projections, or VIPs) were computed to assess the contribution of absorbance to these dimensions. A variable which has the VIP score greater than 1.0 is usually considered important for the discrimination whereas variables with VIPs smaller than 1.0 are less important [34]. Figure 4 shows the contribution of information from each individual variable to the overall samples separation by PLS-DA. As shown in Figure 4, a total of 159 variables have the VIPs greater than 1.0 and about 50% of them are in the region of 270–300 nm. It showed that the absorption of the wavelength from 270 to 300 nm was likely to be considered as main factor for discrimination of all the mushrooms. Moreover, in this region, the absorption in the wavelength of 272.4, 274.6, 278, 279.4, 280.2, 280.4, 282.2, 283.2, 284, 285, 285.2, 289.8, 291, 292.2, 296, 296.2, 296.4, 297.8, and 298 nm makes a relatively great contribution because the VIPs of these variables are greater than 2.0 (Table 2).

From the corresponding loading plot (Figure 5), the contribution of different selected variables (VIP > 1.0 and marked by hollow red square) on the PCs has been recognized. The loadings of variables which made a relatively great contribution (VIPs > 2.0) are shown in Table 2. PC 1 plays a significant role in discriminating *B. tomentipes* and *Xerocomus* sp. from other samples. Variables in the wavelength of 280.2, 289.8, and 291 nm have contributions to PC 1, which separate samples number 10 to 17 from the others, with *B. tomentipes* having a negative loading value. The most relevant variables for discriminating *Xerocomus* sp. from other mushrooms are in the wavelength of 278, 279.4, 280.4,

TABLE 2: VIPs and loadings of selected variables of PLS-DA.

Var ID/nm	VIP	Loading 1	Loading 2
272.4	2.27903	0.10182	−0.034039
274.6	2.13471	0.0953719	−0.048547
278	2.48458	−0.111003	0.0341494
279.4	2.05394	−0.091763	0.0367178
280.2	2.2159	−0.098999	−0.024235
280.4	2.22486	−0.099399	0.0190163
282.2	2.24673	0.100376	0.0255896
283.2	2.17515	0.0971786	−0.015926
283.8	2.02639	0.0905325	−0.002524
284	2.07926	0.0928945	−0.02318
285	2.28531	0.1021	−0.017045
285.2	2.1474	0.0959388	−0.005978
289.8	2.46534	−0.110143	−0.039376
291	2.15336	−0.096205	−0.006975
292.2	2.45586	−0.10972	0.0165164
296	2.18898	0.0977962	−0.083971
296.2	2.08193	0.0930139	−0.03437
296.4	2.26702	0.101283	0.0613701
297.8	2.1033	0.0939687	−0.005287
298	2.18613	0.097669	0.0402933

FIGURE 4: Variable Importance for the Projection (VIP) plot of absorbance for the contribution to sample separation from PLS-DA.

and 292.2 nm. In addition, the absorption of wavelength of 282.2, 296.4, and 298 nm plays the discriminating roles for *B. ferrugineus* from other species while *L. rugosiceps* and *B. edulis* are in the same quadrant where the absorbance of wavelength of 272.4, 274.6, 283.2, 283.8, 284, 285, 285.2, 296, 296.2, and 297.8 nm contributes to separating these two species from the other samples.

3.4. Hierarchical Cluster Analysis. HCA is an unsupervised pattern recognition method for clustering samples according to the similarities among them [35]. It was used based on the first four PCs of PLS-DA to classify the samples into groups using the single linkage method for cluster building

and the distance between clusters was computed by the squared Euclidean method. As shown in the dendrogram (Figure 6), all the samples have the correct cluster at species level. Overall, all the five species are divided into three main fractions when the distance is 19 and that apparently did reflect interdependent relationships occurring among them. In the first case up to three subfractions can be recognized that relate to *B. ferrugineus* (samples number 4, 5, and 6), *B. tomentipes* (samples number 10 to 17), and *B. edulis* (samples number 18 to 24), respectively, which belonged to genus *Boletus*, family Boletaceae [36, 37]. As a consequence, these three species mushrooms had the correct cluster at genus and family levels and corresponded with the fungal classification. Three samples—samples number 7 to 9—are similar and join to form the second cluster. They are all classified as *Xerocomus* sp. [20]. The last one also contains three mushroom samples. Combined with the information in Table 1, these samples were *L. rugosiceps* and pertained to genus *Leccinum*, family Boletaceae [20, 37].

Generally, the different species of edible mushrooms were discriminated based on the morphological characteristics and macroscopic color reactions. However some literatures suggested that these two methods relied on experience and subjective factors [8, 38]. In addition, the color reactions of the mushrooms may be affected by the environment, climate, agrototype, growing season, and physiological status of the fruiting bodies and this may cause the deviation of subjective judgment [38]. In this study, discrimination of five wild-grown edible mushrooms (Boletaceae) profiled by UV spectroscopy analysis combined with chemometrics allowed for the digitalization of these sample properties providing a novel approach for objective annotation of different edible mushroom (Boletaceae) attributes such as species. In contrast, this analysis was efficient, rapid, and reliable, as based on the chloroform extracts.

PLS-DA was a good tool that could provide an overall look at the initial differences in UV spectra and it was possible to show clear differences in chemical components among the five species of wild-grown edible Boletaceae mushrooms. It suggested that this method could be used to differentiate mushroom specimens according to their species. However, although PLS-DA showed clear separation of the mushrooms, better clustering could be observed in the HCA dendrogram (Figure 6). All the specimens could be distinguished accurately at species level and the relationship among them has been also presented.

Obviously, although there were differences among the five species of wild-grown edible mushrooms, some similar constituents between *B. edulis* and *B. tomentipes* could be reflected on account of these two species samples joined together at first. More interestingly, according to the results of dendrogram, the interspecific differences between *B. edulis* and *B. tomentipes* could be the smallest in *B. edulis*, *B. ferrugineus*, and *B. tomentipes* even though these three species belonged to the same genus. This may be related to the genetic stability and variability of different species of Boletaceae mushrooms during the long-term evolution process. With regard to the level of genus, *Boletus* may have similar relationship with *Xerocomus* based on the chemical analysis

FIGURE 5: Loading plot generated from the PLS-DA model of the mushroom samples.

FIGURE 6: Dendrogram resulting from hierarchical cluster analysis.

because these two genera could cluster together firstly among the three genera studied in this study. This was consistent with the previous study that some mycologists have incorporated *Xerocomus* into *Boletus* [39]. Additionally, *Boletus* was clearly different from *Leccinum* that the samples which belonged to the same genus could be clustered to differentiate with the other one. Indeed, a recent paper has demonstrated this result by molecular analyses that explained this phenomenon in a genetic way [40]. On the other hand, this method for chemical analysis verified the consequence of previous study. It could provide a new way to classify the edible mushrooms.

4. Conclusions

This paper described the combination of UV spectroscopy and chemometrics as a rapid discrimination approach of wild-grown Boletaceae mushrooms. The results demonstrated that obvious differences could be found in the whole chemical components based on the chloroform extracts of *B. edulis*, *B. ferrugineus*, *B. tomentipes*, *L. rugosiceps*, and *Xerocomus* sp. All the samples could be distinguished accurately at species and genus levels and the relationships among them have been presented. On the other hand, this study could offer an economical, effective, and useful discrimination approach of wild-grown edible mushrooms.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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