Discrimination of Storage Periods for *Macrocybe gigantea* (Massee) Pegler & Lodge using UV Spectral Fingerprints

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Abstract

Li Y., Zhang J., Liu H.-G., Jin H., Wang Y.-Z., Li T. (2015): **Discrimination of storage periods for *Macrocybe gigantea* (Massee) Pegler & Lodge using UV spectral fingerprints**. Czech J. Food Sci., 33: 441–448.

Macrocybe gigantea (Massee) Pegler & Lodge is a species of edible mushrooms which has important nutritional, medicinal, and economic values. Discrimination of *M. gigantea* with different storage periods is helpful to guarantee the authenticity and to promote marketing of this species. We focused on the establishment of the fingerprints based on UV spectroscopy to discriminate the wild-grown *M. gigantea* samples stored for different years and to distinguish the wild-grown and cultured samples which were stored for the same years. The analyses of the data were performed by multivariate analyses such as principal component analysis (PCA) and cluster analysis (CA). UV spectral fingerprints showed that the major composition and content of the main components were significantly variable over time. All the *M. gigantea* samples which were stored for different years could be successfully grouped by PCA and CA. The results demonstrated that this qualitative assessment could be regarded as a rapid and reliable method for discrimination of *M. gigantea* with different storage times.

Keywords: mushrooms; storage time; differentiation; UV spectroscopy; multivariate analyses

In recent years, food quality has become an object of public concern, and emphasis is increasingly being put on the relationship between food and nutritional ingredients (Lairon 2010). It is reported that some factors have effects on food quality, such as marked age, origins, harvesting time, different storage conditions and so on (Riovanto *et al.* 2011; Ferreira-Lima *et al.* 2013; Figueiredo-González *et al.* 2013; Ouyang *et al.* 2013). In addition, the storage time could also influence food quality (Lee & Cho 2012; Marquez *et al.* 2014; Touati *et al.* 2014). Nowadays, some unscrupulous traders sell inferior foods which have been stored for an extended period as the good ones for profiteering, which leads to unfair competition. Therefore, it is necessary to guarantee the authentic food products and to prevent the adulteration.

Up to now, some analytical techniques have been used for food quality control such as UV-Vis, GC-MS, HPLC, HPLC-MS, ICP-MS and so on (Yu *et al.* 2006; Pereira *et al.* 2011a; Gordillo *et al.* 2012; Bandoniene *et al.* 2013; Kuś *et al.* 2014). Kuś *et al.* (2014) used general HPLC fingerprints combined with multivariate analysis to distinguish the honey types. Gordillo *et al.* (2012) developed HPLC-MS and tristimulus colorimetry to assess the ageing aptitude of Syrah wine. However, some deficiencies such

as high time consumption and expenses have existed in chromatographic methods and the standards and calibration are also required (Fernández-González *et al.* 2014). Comparatively, UV spectral fingerprints which could provide the comprehensive fuzz information of specimens have gained wide acceptance in the food field because of their simplicity, cost-effective and rapid tests for detecting samples (Pereira *et al.* 2011b; Roshan *et al.* 2013). For example, UV spectral fingerprints have been used to discriminate the food specimens which were obtained from different years and stored for a period of time (Brudzynski & Kim 2011; Pereira *et al.* 2011a; Prieto *et al.* 2012). Furthermore, they could also analyse the characteristics of the sample chemical compositions because they have the absorption band of their chemical information (Li *et al.* 2013). In addition, it is difficult to discriminate the samples just by visual inspection in the UV spectra because the spectra contain complex data information. To address this concern, the aid from multivariate analysis is needed.

Macrocybe gigantea (Massee) Pegler & Lodge is a macro-fungus which grows under the environment of high temperature and humidity and distributes in hylaea and subtropical rain forests in Africa and Asia (Huang 2001). In China, it distributes in Fujian, Guangdong, Hainan, Yunnan, and Taiwan (Dai *et al.* 2013). *M. gigantea* tastes sweet and is rich in nutrient components such as protein, polysaccharide, fat, amino acid and many mineral elements (Wang *et al.* 2004). It is appreciated primarily for its flavour, but can also be a healthy supplement to the diet. In general, its nutritive value compares favourably to that of most vegetables (Giri *et al.* 2013). In addition to edibleness, many medicinal properties in *M. gigantea*, such as anti-bacterial, anti-oxidation and anti-tumour

effects, have been claimed (Mau *et al.* 2002; Dai *et al.* 2009; Giri *et al.* 2012). It has been reported that the UV spectral technique could be applied for analysis of edible mushrooms (Dobrinas *et al.* 2013; Nagajyothi *et al.* 2014; Yang *et al.* 2014). Yang *et al.* (2014) used UV spectral fingerprints combined with chemometrics to discriminate different parts of edible bolete mushrooms. Dobrinas *et al.* (2013) determined trace element levels of three species of mushrooms by UV/visible spectrometry. However, no reports were found in relation to the utilization of UV spectral fingerprints for discrimination of *M. gigantea* which had different storage periods.

The main objective of this work was to develop an analytical method for the discrimination of wild-grown *M. gigantea* which was stored for different times by UV spectroscopy combined with multivariate analyses. The differentiation between the wild-grown and cultured samples which had the same storage period was concurrently studied by this method.

MATERIAL AND METHODS

Material. The mature wild-grown *M. gigantea* fruiting bodies were collected annually from one population in Pu'er, Yunnan Province during 2010 to 2013 and the cultured *M. gigantea* fruiting bodies were collected in Yuanjiang, Yunnan Province in 2011. The wild-grown *M. gigantea* is constituted of many small fruiting bodies, so do the cultured ones (Figures 1 and 2). All the samples were authenticated by Dr. Yu-Cheng Dai, Institute of Microbiology, Beijing Forestry University. The voucher specimens were preserved in the Herbarium of Beijing Forestry University. All the samples (Table 1) were tested in

Figure 1. The wild-grown *M. gigantea* samples

Figure 2. The cultured *M. gigantea* samples

Table 1. Information on *M. gigantea* fruiting body samples (*n* = 10)

No.	Date of collection	Form	Storage periods (years)
1 2	June, 2010	wild-grown	3
3 4	June, 2011	wild-grown	2
5 6		cultured	2
7 8	June, 2012	wild-grown	1
9 10	June, 2013	wild-grown	0

n – number of specimens in a pool; the two wild-grown samples obtained from the same year belong to different clusters, so do the cultured ones

October, 2013. The mushroom specimens collected in 2010, 2011 and 2012 had the storage times of three, two and one years, respectively, while the samples which were collected in 2013 were tested in the year of collection.

Sample processing. After acquisition, the fresh *M. gigantea* fruiting bodies were cleaned up and air-dried in an oven at 50°C. Then all the samples were ground to fine powder, and passed through a 100-mesh stainless steel sieve. The powders were kept in the Ziploc bags at room temperature.

200 mg of each powdered sample and 6.0 ml petroleum ether (analytical grade) were put into a 25 ml colorimetric tube and extracted by ultrasonication for 30 minutes. The extracts were filtered and then tested.

Data acquisition. Each *M. gigantea* sample extract was analysed by UV-2550 UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan) equipped with a quartz cell with an optical path of 1 cm. The UV spectra were scanned by the spectrophotometer at 0.2 nm sampling interval and 2.0 nm slit width. The spectra were recorded in the working range from 190 nm to 400 nm, and then treated by the three groups of average, two-point smoothing and second derivative processing methods to calibrate and exclude interference, in order to increase the resolution of spectra.

Multivariate analyses. The raw spectra data were interpreted and processed by principal component analysis (PCA) and cluster analysis (CA). The recorded absorption wavelengths were exported to Excel 2007 software (Microsoft, USA), and then analysed by PCA and CA. These two statistical approaches were performed using the SPSS 20.0 (IBM Corp, Armonk, USA). Data were visualised by using PCA and CA. Each point on the PCA two-dimensional diagram represented an individual sample. From the CA dendrogram, the relationships between the fruiting body samples could be recognised.

RESULTS AND DISCUSSION

Method validation. Sample No. 4 was randomly selected to be used for verifying the efficacy of UV method. The repeatability was assessed by testing seven independently prepared sample extracts which were from the same sample using the uniform method. The relative standard deviations (RSD%) of wavelengths of common peaks were arranged from 0.03 to 0.92. The precision was determined by testing the same sample extract in duplicate seven times. The RSD% of wavelengths of common peaks were in the range from 0.00 to 1.39. The stability was measured by testing one sample extract at 1, 5, 10, 20, and 30 h at room temperature. The RSD% of wavelengths of common peaks were arranged from 0.00 to 0.64. It turned out that the developed method was sensitive, precise and accurate.

UV spectral fingerprints of *M. gigantea*. The UV absorption bands of the presented samples are usually associated with the presence of different chromophores exemplified in conjugated systems as well as other UV-absorbing systems (Zeng & Zhang 2010). Because of the detection range of the UV-Vis spectrophotometer, the wavelengths of absorption peaks were arranged from 212 nm to 330 nm for the sake of avoiding the spectral noise. It displayed that the UV spectral fingerprints of the samples had a higher overlap rate from 250 nm to 330 nm than that of other absorption wavelengths. The UV spectra of all the *M. gigantea* samples have shown their characteristics. Every sample had some characteristic absorption peaks and showed their fingerprint features.

The UV spectral fingerprints of wild-grown *M. gigantea* samples are presented in Figure 3. It shows that some chemical components appear to be very similar among the wild-grown mushroom samples because they have some common peaks such as 240, 254, 265, 276, 287, and 299 nm. However, there are obviously differences in the number of absorption peaks and peak heights. The longer the storage time,
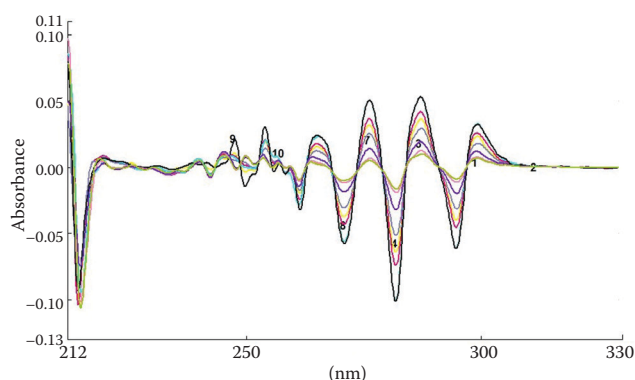
Figure 3. UV spectral fingerprints of the wild-grown *M. gigantea* samples

Figure 4. UV spectral fingerprints of the wild-grown and cultured *M. gigantea* samples stored for the same years

the correspondingly lower absorbance is presented. To a certain degree, when the substance was in high concentration, the corresponding absorbance was also high (Wei *et al.* 2011). It indicated that the content of chemical components of the *M. gigantea* samples may decrease with the increasing storage times. Similar results were reported in previous researches that the content of some chemical compounds of mushrooms can decrease obviously during the storage period (Jaworska & Bernaś 2009; Pennazza *et al.* 2013). It could be inferred that the chemical constituents of *M. gigantea* may be degraded during storage.

The spectral fingerprints of the wild-grown and cultured *M. gigantea* samples which were stored for the same years were compared directly (Figure 4). There are differences in the absorbance of different samples and the absorbance of wild-grown *M. gigantea* is higher than that of cultured ones. It revealed that the content of chemical components of cultured *M. gigantea* was lower than that of wild-grown ones after storing for about two years. It may be related to the stability of the compositions of the wild-grown and cultured samples, and it could also be relative to the growing environment between wild-grown *M. gigantea* and the cultured ones.

Principal component analysis. Principal component analysis (PCA), a well-known unsupervised method, is one of the most commonly used multivariate analysis methods with the aim of reducing th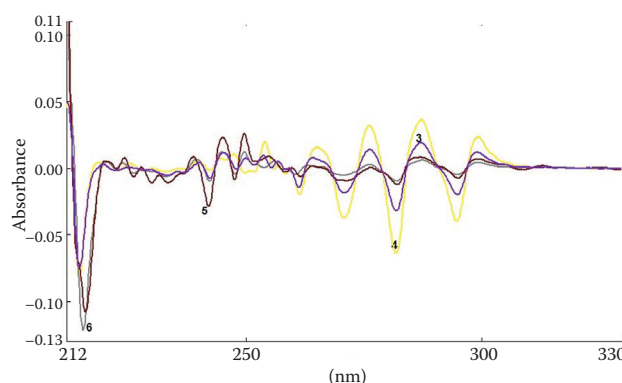e dimensionality of response matrix in principal components (PCs), which changes the multi-index to a few comprehensive indexes (Li *et al.* 2013). This statistical method is a commonly used multivariate tool for discrimination and it could facilitate the subsequent analysis, reduce the risk of incorrect inferences as well as avoid subjective decisions (Pereira *et al.* 2011a; Sârbu *et al.* 2012). The visual distribution patterns generated from the data could be correlated with general characteristics of the samples analysed. It also enabled to explain the qualitative evaluation of the resemblances and differences in the spectra. Moreover, it was employed to distinguish samples according to their storage times.

It was revealed that 89.45% of information regarding the chemical component variability of the fruiting bodies of *M. gigantea* can be described by two principal components (PCs) (Table 2 and Figure 5). It shows that the first two principal components are on a very steep slope, and for the third principal component the slope becomes flat gradually. So the first two principal components were chosen to describe the characteristics of the specimens. It was obtained from Table 2 that the eigenvalue of PC1 is 6.691, 55.762% of the sum of total eigenvalues, and the total eigenvalue of PC1 and PC2 is 10.734, 89.45% of the sum of total eigenvalues (greater than 85%), which are sufficient to show the clustering of different samples. As shown in Figure 6, all samples could be clearly clustered into five groups based on their storage times by a spectrographic method. It

Table 2. Eigenvalues of correlation matrix and related statistics

PC	Eigenvalue	Total variance (%)	Cumulative eigenvalue	Cumulative (%)
1	6.691	55.762	6.691	55.762
2	4.043	33.688	10.734	89.45

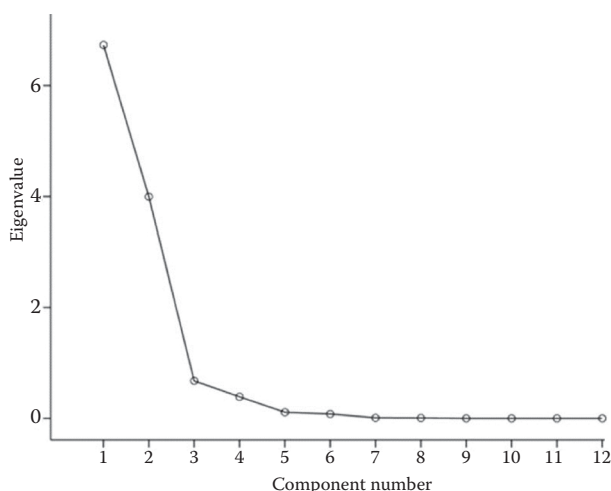Minimum eigenvalue = 1; PC – principal component

Figure 5. Scree plot-plot of eigenvalues of correlation matrix

displayed that the chemical components of cultured *M. gigantea* were obviously different from wild-grown ones, and they could be distinguished by this method in a distinct way. It also indicated that the chemical components of wild-grown *M. gigantea* may change in varying degrees as the variation of storage times. So, PCA could be useful to discriminate
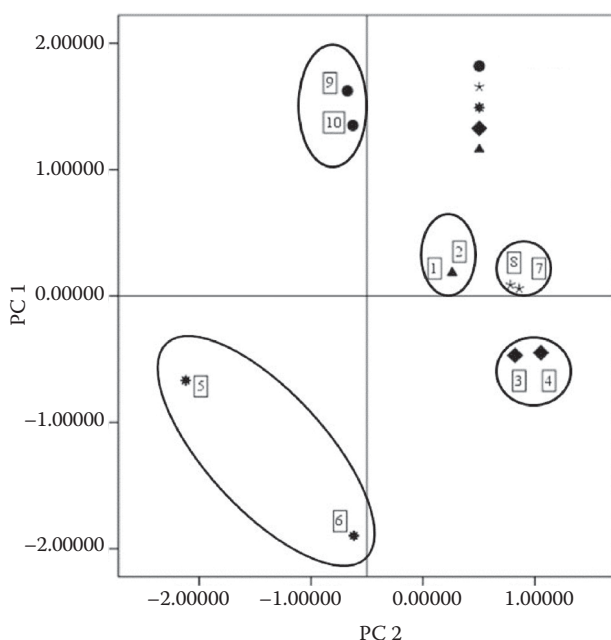
the *M. gigantea* with different storage periods and to distinguish the wild-grown and cultured samples which were stored for the same years.

Cluster analysis. Cluster analysis (CA), another unsupervised technique, was used to evaluate the relationship in terms of similarity or dissimilarity among samples (Sârbu *et al.* 2012; Luo *et al.* 2013). It was utilized to sort the samples into groups using the complete linkage method for cluster building and the distance between clusters was computed by the squared Euclidean method. The main principle of CA is assuming that there are *m* observations, and then the algorithm starts with *m* clusters. With the calculation of the squared Euclidean distance between observations, the closest points are grouped into a single cluster and the process is repeated until all the observations are included in one cluster (Ren *et al.* 2012). CA of the spectra data set obtained did how on some similarity of sample components among the fruiting bodies of *M. gigantea* with different storage periods.

The CA diagram (Figure 7) divided all cases into three main fractions when their distance was fifteen, and which apparently reflected interdependent relationships occurring among them. Wild-grown *M. gigantea* fruiting bodies which were stored for three, two and one years (collected in 2010, 2011 and 2012, respectively) were joined to form the first cluster. This cluster can be divided into two fractions, the

Figure 6. Two-dimensional diagram of *M. gigantea* samples by PCA (PC1-PC2)
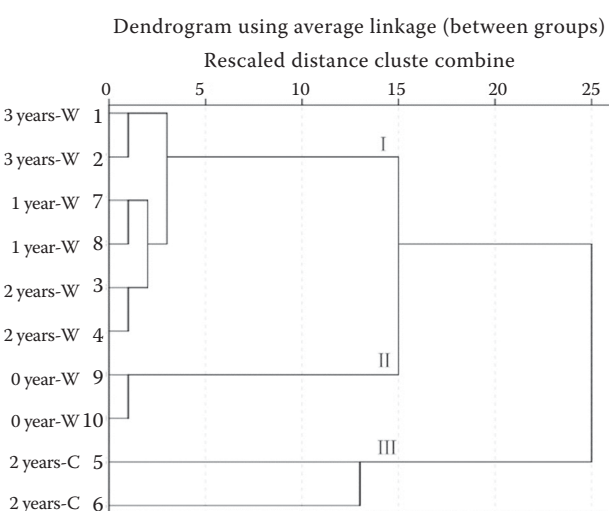
Figure 7. Bundle diagram of similarity of components among the fruiting bodies of *M. gigantea* samples

0 year-W the fruiting bodies of wild-grown *M. gigantea* collected in 2013 and tested in the year of collection; 1 year-W the fruiting bodies of wild-grown *M. gigantea* stored for about one year; 2 years-C the fruiting bodies of cultured *M. gigantea* stored for about two years; 2 years-W the fruiting bodies of wild-grown *M. gigantea* stored for about two years; 3 years-W the fruiting bodies of wild-grown *M. gigantea* stored for about three years

wild-grown samples stored for one and two years were joined to the same group to differentiate that of three-year storage. The distance among them was less than three, which indicated that the chemical components of wild-grown samples which had the storage period of one and two years were the most similar. In other words, after one year and two years of storage, the fluctuation of components could be relatively small. Additionally, during the three-year storage period, the fluctuation of constituents may be relatively large. The second case is the fruiting bodies of wild-grown mushroom samples collected in 2013. In the third case, cultured samples after two years of storage can be recognized. Obviously, the chemical constituents of *M. gigantea* samples could be affected by storage periods and the fluctuation in compositions was different during storage. It can be inferred that the degrees of degradation of chemical components in *M. gigantea* fruiting bodies were different during the storage period. It is similar to the result reported by Jawordka *et al.* (2009) and Patras *et al.* (2011). Moreover, the cultured *M. gigantea* was the most different in all the mushroom samples. It implied that after two years of storage, the chemical components of wild-grown and cultured mushroom samples which were collected in the same year were distinctly different. It is concluded that CA could preferably discriminate the *M. gigantea* with different storage times and measure the similarity among samples.

Mushrooms belong to the most perishable foodstuffs and tend to lose quality after harvest (Fernandes *et al.* 2012). The storage conditions and storage periods may directly affect the chemical composition of mushrooms (Pennazza *et al.* 2013). In this study, all the samples have uniform storage conditions. So, the storage times may play an important role in the change of *M. gigantea* quality. Moreover, all the samples could be discriminated accurately according to the storage period and their relationships have been clearly presented. In contrast to the results of previous studies related to the discrimination of food based on other analytical approaches, such as HPLC-MS (Wiczkowski *et al.* 2015) and GC-MS (Karabagias *et al.* 2014), it can be concluded that the proposed method is a reliable and fast tool for discriminating food quality.

CONCLUSION

This is the first study to present a qualitative method to discriminate *M. gigantea*, and it avoids the need of a quantitative method that would require the use of standards, calibration and time-consuming analysis. A spectrographic fingerprinting analysis of *M. gigantea* which was stored for different years has been performed by employing UV spectroscopy. The results showed that UV spectral fingerprints combined with multivariate analyses could clearly discriminate the relationship between temporal variations and similarities in the compositions of *M. gigantea*. In conclusion, UV spectroscopy with multivariate analysis methods could be considered as a useful tool for discrimination and correlation in *M. gigantea* with different storage periods. Moreover, this qualitative method would provide more reasonable references for utilization and quality evaluation of *M. gigantea.*

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