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Determination of storage stability of the crude extracts of *Ganoderma lucidum* using FTIR and 2D-IR spectroscopy

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ABSTRACT

In this paper, the feasibility and advantage of employing FTIR spectroscopy and the corresponding second-derivative spectroscopy combined with 2D-IR spectroscopy for the analysis of water extracts of *Ganoderma lucidum* with different storage durations were investigated and demonstrated for the first time. In order to compare the second-derivative microscopic fingerprint spectra from the four periods of storage, the range from 1200 to 500 cm^{-1} has to be ignored due to the detection of starch in the samples and the standard as well. This is crucial because the main characteristic band of polysaccharide was assigned within this range. The addition of starch as outer constituent was considered spoilage of polysaccharide content investigation. In fact, polysaccharide content in *G. lucidum* plays a main role as anti-cancer properties. The ranges from 1480 to 1200 cm^{-1} and from 1700 to 1480 cm^{-1} were interpreted and directly compared. For the range (from 1800 to 400 cm^{-1}), the 22 months sample was closest to the control, followed by 38 months, 13 months and 6 months product. The matching of the extract spectra with the control was not consistent and depended on which range of spectra had been chosen. Many factors can be considered which may have possibly affected inferences of the composition of the extract from different storage durations.

1. Introduction

Research on *Ganoderma* spp. has drawn the attention of many scientists on account of it being described as one of the most important of traditional Chinese medicines [1]. This mushroom and its related products have been extensively studied for both its contents or efficacies. Nevertheless, work is still being actively carried on account of its wide and still un-tapped pharmaceutical potential.

Ganoderma spp. is a large polyporacea [2], the fruiting body of which is able to grow up to 30–50 cm in diameter depending on its geographical origin and their growing conditions. It has a glossy exterior and a woody texture [3]. In China, at least 80 species of *Ganoderma* spp. have been authenticated. Among them, the most famous is *Ganoderma lucidum* (Leyss. ex Fr.) Karst on which a majority of the pioneering scientific reports have focused.

The main bio-active components of *Ganoderma* spp. range from β-D-glucan [4], ganoderic acid (triterpenoid) [5], lingzhi-8 [6], sterols, nucleosides, fatty acids, alkaloids and inorganic elements [7]. *G. lucidum* is highly regarded in Chinese traditional medicine. It is believed to promote health and longevity and it lowers the risk of cancer and heart disease [8]. Presently, many commercial *G. lucidum* products include freeze-dried crude water extract of *G. lucidum* the so-called "Lingzhi quintessence". There are also modified methods of application where it seems some communities prefer to consume *G. lucidum* extract by boiling the pulverized mushroom in water. The pharmacological action of water extract of *G. lucidum* includes increasing the function of hyperoxide mutase, the scavenging of free radicals [9], improving the fluidity and sealing of cell membranes, promoting cell distortion ability and reducing the accumulation of blood platelet [10]. The extract is able to promote the synthesis of DNA, RNA and protein and most importantly, is effective in modulating immune functions, preventing oxidative damage [11], protecting the liver [12], reducing serum glucose levels as well as anti-human immunodeficiency virus-1 protease activities [13] while producing no toxic effects.

This mushroom is being commercially applied as a medicine and a supplement not only in China but in other countries including Malaysia. As far as we know, *G. lucidum* is quite scarce in nature and can only be found in particular locations such as virgin forests. In addition, the amount of wild *G. lucidum* cannot support commercial exploitation [14]. In tandem with the increasing demands of the international markets, more and more studies relating to the artificial cultivation of *G. lucidum* have been reported [15].

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Table 1
Types of *G. lucidum* crude water extract from Mushroom Farm in Changchun, China.

No.	Product storage since	Product number	Period storage till October 2008
1	May 2008	A2008	6 months
2	September 2007	B2007	13 months
3	December 2006	C2006	22 months
4	May 2005	D2005	38 months

There are many internal and external criteria from which we can infer the quality of *G. lucidum* extract. The quality of the extract obtained can be interpreted to mean that the sensitivity of the chemical constituents could significantly vary from the original source material due to some factors such as temperature changes; the humidity and brightness of the store room; the method of storage; the packaging material used in storage; the place where the package is kept in the store; the method or process of extraction and freeze-drying. We also must consider the intra and inter chemical reactions of the extracts chemical constituents and the duration of storage could also be a factor that influences the chemical changes and the quality of the original material. Generally, the variations are not significant in terms of texture, color or appearance. At the same time, it is very difficult to identify variations in chemical constituents by routine analytical methods.

Prof. Isao Noda has developed the 2D-IR correlation technique [16–18] while Prof. Sun Su-Qin has been applying related theories successfully in the quality control of traditional Chinese medicine [19,20]. As a non-separate, rapid and non-destructive method, 2D-IR spectroscopy combined with IR (*also referred to as the "IR macro-fingerprint method"*) has the advantage of applicability from simple compound through to very complex systems. This is very crucial in view of the different concepts in the field of traditional Chinese medicine as compared to western medicine. The IR-macro-fingerprint method integrates principles of traditional Chinese medicine without loss of the original natural 'instinct' and compatibility of traditional Chinese medicine [21].

The objective of this study is to reveal the content of raw *G. lucidum* material and its crude water extract under FTIR spectroscopy, second derivative spectroscopy and 2D-IR spectroscopy. It will also look at the differences in crude water extracts of *G. lucidum* in term of their chemicals changes after certain periods of storage. Besides, the other objective is to differentiate the product based on raw material from the extract, to use as a reference for future works and to enhance public awareness on the changes of chemical constituents due to the effectiveness of storage.

2. Experimental

2.1. Samples

The six types of *G. lucidum* products that were purchased from a Mushroom Farm in Changchun, China are:

(a) *G. lucidum* raw material in powder form.
(b) Powder form of *G. lucidum* crude water extract (Lingzhi 'essence' as Control).
(c) Four types (different periods) of the powder form of *G. lucidum* crude water extracts (Table 1).

2.2. Sample preparation

The finely textured *G. lucidum* powder is prepared by two different approaches. In the first, the whole fruiting body that may include the portions of the stalk is pulverized. In the second approach, the mushroom is cut into small pieces or pulverized and followed by boiling in water for a few hours. While the first approach produced powdered *G. lucidum* raw material, the second approach produced a powdered crude extract of *G. lucidum*.

2.3. Apparatus

Spectrum GX Fourier-transform infrared (FTIR) spectrometer (Perkin-Elmer) with an attached DTGS detector has been used as main equipment for the whole experiment. The dynamic FTIR spectra were recorded with the above spectrometer combined with a portable, programmable temperature controller (Model 50-886, Love Control), with a controllable range from room temperature up to 120 °C. The Spectrum software V3.02 (Perkin Elmer) was tested and set up for the most ideal conditions. The first FTIR spectra were obtained after 16 scans at room temperature from the range 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. The second derivative IR spectra were acquired after 13-point smoothing of the original IR spectra.

Dynamic FTIR spectra are obtained after 32 scans and are collected by varying the temperature from 50 °C to 120 °C by steps of 10 °C. The thermal dependent 2D-IR spectra were obtained from the dynamic spectra series using the 2D-IR correlation analysis software, developed by the Analysis Center of Tsinghua University.

2.4. Procedure

The experiment started with a tablet of KBr as blank. There was about 0.01 g of *G. lucidum* product mixed evenly with 0.02 g of KBr crystal. The mixture was ground and pressed into a tablet with a pressure of not more than 10 psi. FTIR spectrum was generally acceptable when a transmission of 60–70% was achieved. Otherwise, the test had to be repeated with either the sample or with KBr added. Later on, the sample tablet was put into the temperature-controlled pool and the FTIR spectra were recorded *in situ*. These results were interpreted for 2D-IR correlation spectroscopy.

3. Results and discussions

3.1. FTIR spectra analysis of Ganoderma raw material and its crude water extract

It is practically impossible to determine the real components of *Ganoderma* powder forms just on the basis of their appearance, texture and smell. The IR spectroscopy used in this experiment has detected subtle differences in the intrinsic composition of these two types of powders.

Figs. 1 and 2 show the FTIR spectra of *G. lucidum* raw material and crude *G. lucidum* water extract. There are almost no similar-

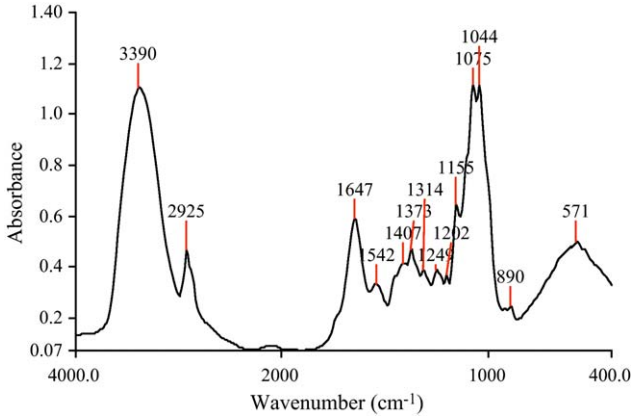

Fig. 1. FTIR spectrum of *G. lucidum* raw material.