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Research article

Identification of ginseng root using quantitative X-ray microtomography

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ABSTRACT

Background: The use of X-ray phase-contrast microtomography for the investigation of Chinese medicinal materials is advantageous for its nondestructive, *in situ*, and three-dimensional quantitative imaging properties.

Methods: The X-ray phase-contrast microtomography quantitative imaging method was used to investigate the microstructure of ginseng, and the phase-retrieval method is also employed to process the experimental data. Four different ginseng samples were collected and investigated; these were classified according to their species, production area, and sample growth pattern.

Results: The quantitative internal characteristic microstructures of ginseng were extracted successfully. The size and position distributions of the calcium oxalate cluster crystals (COCCs), important secondary metabolites that accumulate in ginseng, are revealed by the three-dimensional quantitative imaging method. The volume and amount of the COCCs in different species of the ginseng are obtained by a quantitative analysis of the three-dimensional microstructures, which shows obvious difference among the four species of ginseng.

Conclusion: This study is the first to provide evidence of the distribution characteristics of COCCs to identify four types of ginseng, with regard to species authentication and age identification, by X-ray phase-contrast microtomography quantitative imaging. This method is also expected to reveal important relationships between COCCs and the occurrence of the effective medicinal components of ginseng.

1. Introduction

As a result of the spatial coherence improvement of X-rays from synchrotron radiation facilities [1,2], the application of X-ray phase-contrast microtomography (XPCMT) as a useful scientific research method has been expanded to the comprehensive study of Chinese medicinal materials (CMMs) to nondestructively investigate the characteristic microstructures of ginseng and evaluate ginseng quality [3,4]. XPCMT has many advantages; for instance, it provides nondestructive, *in situ*, and three-dimensional (3D) quantitative imaging. The current study applied a phase-retrieval process to generate a quantitative image using XPCMT and specifically to identify ginseng.

Ginseng is a slow-growing perennial herbaceous plant as well as a CMM that is well known worldwide. *Panax quinquefolius* L. (American ginseng) and *Panax ginseng* Meyer (Asian ginseng) are the two predominant *Panax* species grown commercially in North America and Asia, respectively [5]. Ginseng is grown for its highly valued root, which plays an important role as a medicinal resource and in nutrition. The major attraction of ginseng is its wide range of pharmacological effects [6–8]. The medicinal value of ginseng is attributed mostly to ginsenosides. The content of ginsenosides varies depending on the light level [9], fertility level [10], plant part, age [11], harvest date [12], and drying condition [13]. In the marketplace, the quality of ginseng root is graded based on several factors, such as species, production area, growth pattern, age grade, and external shape, and these characteristics are closely related to its medicinal value. Currently, ginseng is primarily identified with the naked eye. It is particularly important to judge the growing conditions nondestructively during the quality grading process of ginseng.

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With the development of new technologies, both physicochemical and microscopic identification methods have been used in CMM research. Physicochemical methods are well adapted for quantitative research, whereas microscopic identification methods are appropriate for qualitative research. In general, both quantitative and qualitative research methods have been adapted for the authentication, quality assessment, and age assessment of ginseng species. However, only biological and chemical methods can provide accurate data to quantitatively identify and evaluate ginseng [14–17]. Qualitative identifications of CMMs have been performed using imaging techniques by employing light and scanning electron microscopy [18,19]. Sample preparations rely on traditional paraffin sectioning; thus, this technique provides information only about static cross sections, and samples are greatly damaged during this process. Although the traditional slice accumulation method can yield 3D imaging information, the disadvantages of this method are the unavoidable deviations introduced during the process of slicing. Moreover, reconstruction of the 3D appearance of the microstructure from dissection or a sectional series requires particular scientific drawing skills, which are always a matter of the artist's interpretation. This approach also causes wastage of materials, and much time is required for sample preparation. Magnetic resonance imaging leads to low image resolution, and temperature easily influences sample moisture. This irregular moisture distribution makes it more difficult to produce an acceptable magnetic resonance image [20]. Therefore, these methods are not completely suitable for the study of precious samples.

Currently, all the methods discussed above have their new applications; however, these methods are unable to satisfy the demand for nondestructive and *in situ* research. Practical application requires that wild ginseng is left intact; therefore, destructive methods are not suitable for research on highly valuable wild ginseng. Nondestructive research methods for *in situ* ginseng identification continue to rely on the rich experience of prestigious experts who judge its quality based on appearance and other characteristics. Owing to a lack of scientific data, identification by experience occasionally results in errors. Recently, a kind of computed tomography (CT) scanner was used to evaluate ginseng quality via nondestructive inspection of the internal tissue density of ginseng [21]. However, the CT scanner neither achieves independent or effective detection, nor provides high-resolution imaging results. Thus, the CT scanner is unable to provide reliable identification information or to lead to a quantitative analysis. The current study addresses these problems.

Calcium oxalate cluster crystals (COCCs) are a kind of secondary metabolite found in medicinal plants; the shape, distribution, and amount of crystals are important characteristics for microscopic identification of medicinal plants. Accumulation of secondary metabolites in medicinal plant roots is also correlated with plant age [13]. We undertook this study to obtain quantitative information on ginseng microstructures and identify different types of ginseng using XPCMT. The objectives of this study were (1) to report the efficiency of XPCMT with regard to the quantitative identification of ginseng root and (2) to correlate the accumulation characteristics of ginseng COCCs with the identification results of the different types of ginseng.

2. Materials and methods

2.1. Materials

Many investigations on American and Asian ginseng have been published over the past decade [22]. Asian ginseng can be either wild or cultivated. Wild ginseng is classified as a national protected plant of the first class in China. Therefore, not only is wild ginseng rare in CMMs, but its specimens also have an extremely high value to collectors. Samples of Asian and American ginseng were used in this investigation. Cultivated and wild ginseng of the same species, but with different ages and different growth patterns, were selected from the same cultivated area (Changbai Mountain, China). Korean ginseng is cultivated in South Korea, and American ginseng is cultivated in Canada. All these ginsengs were purchased from Beijing Tong Ren Tang. The age of wild ginseng is 26 yr and cultivated ginseng is 6 yr; the Korean ginseng and American ginseng are several years old, but there is no exact age recorded for them. Based on the characteristics of the four samples of raw ginseng root, three different groups were established to distinguish them by their different growing conditions. The major differences were between species (Korean ginseng and American ginseng), ignoring the influence of the different growing areas. In addition to species, the major difference between the samples was the location where they were grown. The main differences between cultivated ginseng and wild ginseng were their age grades and their growing conditions.

2.2. Sample preparation

In contrast with techniques such as scanning electron microscopy and transmission electron microscopy, XPCMT does not require samples to be sliced. Other features of XPCMT are of major interest, such as avoiding time-consuming preparation, reducing chemicals, eliminating the artifacts introduced by different sample preparation procedures, and avoiding internal structural damage to the whole ginseng. However, we must also consider the problem of deformation due to sample shrinkage during XPCMT. Sample deformation directly destabilizes imaging quality and can even prevent the acquisition of experimental data. Sample deformation is caused by the high moisture content that is then heated by X-ray irradiation. Therefore, most of the water must be removed during sample preparation to ensure that the internal structure of the ginseng is not damaged or deformed. The allowed deformation value is $\leq$3.7 μm in every direction of the overall sample tissue. Sample drying should be conducted at a constant temperature and constant humidity. In the current study, all the ginseng samples were dried at a constant temperature (25°C) for 1 month. Studies were conducted on the lower portion of taproot in wild ginseng and on the upper part of the lateral root in the other samples. All sample diameters ranged between 4 mm and 7 mm.

2.3. Methods and experimental setup

As ginseng weakly absorbs X-rays, X-ray absorption imaging is not suitable for such low-*Z* materials. X-ray phase-contrast microscopy is advantageous given its high sensitivity for low-*Z* materials [1,2,23,24]. XPCMT is a highly promising method because of its nondestructiveness, suitability for *in situ* and 3D measurements, and sensitivity for low-*Z* materials [25]. To date, XPCMT has been used successfully for plant research [26–28]. X-ray phase-contrast microscopy has already provided preliminary exploratory results [29,30], and XPCMT has been adapted to study CMM raw materials [3,4]. In this study, XPCMT data processing using the phase-retrieval method was employed to obtain quantitative information about the microstructure of ginseng. We found that XPCMT can be used to identify ginseng.

XPCMT experiments on ginseng were performed at the X-ray imaging and biomedical application beamline (BL13W1) of the Shanghai Synchrotron Radiation Facility in China. The third-generation synchrotron radiation facility provides an excellent X-ray imaging resource with the advantages of monochromaticity, high spatial coherence, and high flux density. The white beam that results from wiggler radiation is monochromatized via a Si(111) and