FT-RAMAN SPECTROSCOPIC STUDY OF YAM PROTEIN CONFORMATION

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Abstract: We reported Raman spectroscopic study on the molecular structure of yam proteins isolated from three popularly consumed cultivars including *D. alata* L., *D. purpurea*, and *D. Japonica*. Each yam species expressed its specialty in conformation which is highly consistent with the result of compositional analysis by amino acid analysis.

Yam, the tuber of Dioscorea *spp*., is an important staple food in many tropical countries [1]. It also receives much attention on its functional properties and pharmaceutical potential. In Chinese society, yam has been traditionally used as a health food and Chinese herbal medicine. Several beneficial properties of yams have been reported [2-4]. Yam extracts shows antioxidative activity and modifies serum lipid levels in human [3]. More than 600 species of yam has been planted in the world [3] and various yam cultivars would definitely behave of difference in their biological and functional properties. To unveil the distinct biological properties of yam proteins, a number of studies have been focused on their sequence determination and functional properties exploration [5]. However, very little is known about the structural relevance of yam proteins to date. It is of great importance and worthwhile to determine the molecular structure of yam protein because protein structure plays key role in biological functions. For example, protein folding–unfolding process is crucial to enzymatic activity. Generally, x-Ray crystallography is used to determine the three-dimensional structure of a target protein and is able to figure out the spatial location of certain important amino acid residues. However, the difficulty and complexity of crystallization often limits x-ray's application. Circular dichroism (CD) maybe serves a powerful tool in search of protein secondary structures; however, the requirement for clear samples in CD analysis limits its application to dilute protein solutions or transparent gels. Interference due to absorbance of various salts and buffer substances in the far-UV region also limits the use of CD spectroscopy in studying the effects of environmental conditions on protein conformation [6]. FT-Raman with multiplex and high throughput property is able to obtain high quality structural information at a molecular level of proteins. Structural characterization of food proteins by FT-Raman has been successfully reported recently [6]. This further proved that FT-Raman spectroscopy is a powerful technique for elucidating the molecular structure of plant proteins. In the current study, we report the structural characterization of yam proteins isolated from three locally popular yam cultivars including *D. alata*, *D. alata* var. *purpurea*, and *D. japonica*.

Fig.1 displayed the FT-Raman spectra of yam proteins, 1a for *D. alata* L., 1b for *D. alata* L. var *purpurea*, and 1c for *D. Japonica.* The secondary structure of major yam proteins can be easily determined by examining the vibrational stretching of amide I and amide III. For the *D. alata* L., the secondary structure of dioscorin was major in α-helix as proved by the vibrational mode of amide I at 1662 cm^{-1} and amide III at 1263 cm^{-1}. The secondary structure in *D. alata L.* var *purpurea* was major in random coil as evidenced by the amide I at 1669 cm^{-1} and amide III at 1247 cm^{-1}. The locations of amide I at 1667 cm^{-1} and amide III at 1257cm^{-1} indicated that the secondary structures of Dioscorea *D. Japonica* was in a mixed form of α-helix and random coil. The microenvironmental property of major amino acids in yam proteins was also easily illustrated by the Raman profile in the low wavenumber range, such as 621 cm^{-1} for phenylalanine, 643 cm^{-1} for tyrosine, and 759 cm^{-1} for tryptophan. Different Raman ratio of $I_{643/621}$ (1.46 for *D. alata*, 0.90 for *D. alata* var. *purpurea*, and 1.00 for *D. japonica*,) among various yam cultivars reflected to their

variation in the relative content of tyrosine to phenylalanine. Raman spectral fluctuation in the range from 800 cm^{-1} to 1000 cm^{-1} among various yam proteins revealed their significant differences in the microenvironment of tyrosine and tryptophan. This is in agreement with the result obtained via amino acid analysis.

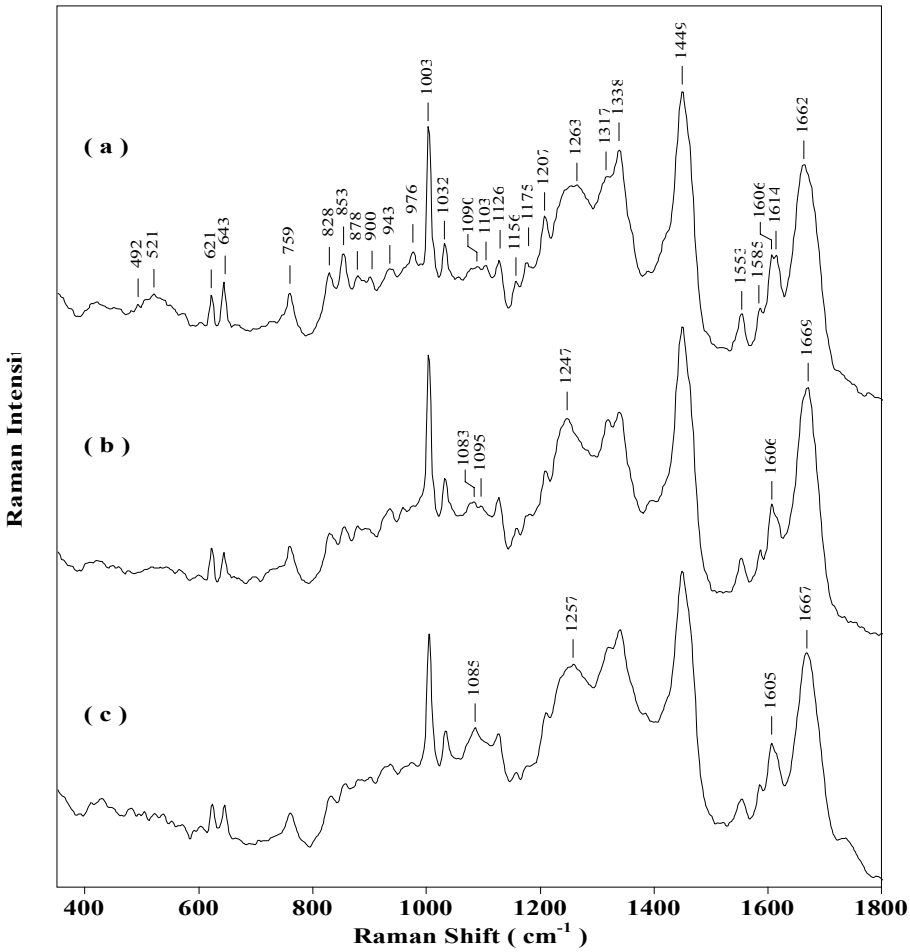

Figure 1: Raman spectra in the 350~1800 cm^{-1} region of yam proteins isolated from various yams (a) *D. alata* L. (b) *D. alata* L. var *purpurea* (c) *D. Japonica*. Data acquisition conditions: excitation wavelength = 1064nm; laser power = 150mW; spectral resolution = 4.0 cm^{-1}, and coadded scan = 500 (~15 min).

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