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Analytical Methods

Discriminating authentic *Nostoc flagelliforme* from its counterfeits by applying alternative ED-XRF and FTIR techniques

Angela Li *, Yun Wei Yat, Wee Kim Yap, Chee Wei Lim, Sheot Harn Chan

Food Safety Laboratory, Applied Sciences Group, Health Sciences Authority, 11 Outram Road, Singapore 169078, Singapore

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ABSTRACT

Nostoc flagelliforme is an edible blue-green algae belonging to the Nostocaceae family. It is recognised as a Chinese delicacy in south-eastern Asia and is widely consumed. Due to its high economic value and diminishing supply, as a result of overharvesting, counterfeits have often been found in the retail markets. Methods involving microscopy and histochemistry were conventionally applied to differentiate the authentic *N. flagelliforme* from its counterfeits. In this paper, we report an alternative analytical approach, using a combination of non-destructive energy dispersive X-ray fluorescence (ED-XRF) and Fourier-transform infrared (FTIR) spectroscopy, to achieve the objective of authentic *N. flagelliforme* verification. In view of the scarcity of this Chinese delicacy, such a non-destructive methodology would be ideal to preserve the integrity of the sample and yet provide a means to discriminate between authentic and counterfeit samples.

1. Introduction

Nostoc flagelliforme is a terrestrial blue-green alga, grown in the arid or semi-arid geographic regions, including Algeria, China, Czechoslovakia, France, Mexico, Mongolia, Morocco, Russia, Somalia and USA. The alga adapts well to extreme environmental conditions, such as dramatic daily and yearly temperature variations and frequent wind, thereby demonstrating its ecological drought-adaptation and physiological heat-resistant capabilities (Gao, 1998). In China, it has been reported to be found in the northern and north-western regions: Qinghai, Xinjiang, Ningxia, Gansu, Shanxi and Shaanxi, Inner Mongolia and Hebei (Diao, 1996).

N. flagelliforme is known to the southern Chinese population as "Facai", due to its black, hair-like appearance. This alga is widely used in Chinese cuisine especially during the festive seasons as its name "Facai" is a homonym with prosperity. The use of *N. flagelliforme* as a delicacy can be dated back to the Jin dynasty (A.D. 265–316). In addition, it has also been recognised to possess herbal properties, as documented in the Compendium of Materia Medica, recorded more than 400 years ago (Gao, 1998).

Rising demand for *N. flagelliforme* during the last century has led to extensive damage to the vegetation residing in northern and north-western China, thereby giving rise to desertification. As a result, the State Council of the People's Republic of China imposed a ban on the collection and trading of *N. flagelliforme* in 2000, in an effort to begin the ecological conservation of northern China. This conservation strategy created a domino effect on the supply and demand food chain for "Facai". Specifically, it triggered a sharp reduction of supplies in countries outside China, causing a resultant price hike. Being a premium foodstuff, it has been transformed into a prime target for adulteration by profit-driven producers.

Conventional methods used to differentiate the authentic alga from its counterfeits include microscopy, iodine staining colour test and elemental analysis involving spectroscopy. Microscopy emphasises on the unique cellular structure characteristics of the alga, to achieve a visual screening only. In iodine staining (a histochemical method), imitation alga sample will turn dark blue or black in the presence of iodine solution, indicating presence of amylase (a natural component of starch) while the authentic alga will remain dull greenish. However, the latter offers no discriminatory advantage for adulterated samples containing both authentic and fake algae. Elemental analysis techniques often involve a sample preparation step, such as acid digestion, before performing analysis. Such techniques include atomic absorption spectroscopy (AAS) and inductively-coupled plasma mass spectrometry (ICP/MS). This paper describes the application of dispersive X-ray fluorescence spectroscopy, which can effectively exclude contamination sources originating from apparatus, reagents and environment, since minimum sample preparation is required. Instead, direct (non-destructive) sample analysis can be performed with the added advantage of shorter analysis time.

In this paper, we applied an alternative combination of microscopy and spectroscopic techniques, aimed at providing qualitative

* Corresponding author. Tel.: +65 6213 0735; fax: +65 6213 0749.
E-mail address: angela_li@hsa.gov.sg (A. Li).

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and quantitative (relative abundance) fingerprints of alga samples obtained from different sources, to establish a systematic workflow tailored for their unambiguous positive identification and classification. For comparison purposes, microscopy was applied, to obtain the morphological features of the algae, operating under transmission mode to distinguish between the alga samples. With the alternative analytical approach, elemental compositions were first obtained by applying energy dispersive X-ray fluorescence spectroscopy, followed by structural elucidation using Fourier-transform infrared (FTIR) spectroscopy.

2. Materials and methods

2.1. N. flagelliforme samples

Due to the rarity of the *N. flagelliforme*, only seven samples could be obtained for this study. The authentic samples were sourced from reliable wholesalers while the remaining five samples were purchased from the retail markets. A portion of the dry samples of *N. flagelliforme* were first left to soak in water overnight and then examined under a polarising microscope the next day. Another portion of the as-received dry sample of *N. flagelliforme* was cut into small pieces and transferred into a disposable sample holder (about 0.5 g each), which was then placed in the analysis chamber for analysis using the ED-XRF. The remaining dry samples of *N. flagelliforme* were analysed directly using the FTIR. The same set of samples was used to perform all three types of analysis throughout this study.

2.2. Polarising microscope analysis

A DMRP cross-polarising microscope (Leica, Wetzlar, Germany) was used to study the cellular characteristics using the authentic sample micrograph as a reference standard. The soaked strands of the *N. flagelliforme* were drained and cut diagonally or ground with mortar and pestle to expose the underlying bead-like filament of prokaryotic cells, visible through the loosening of the gelatinous sheath on the alga before mounting on glass slides for examination.

2.3. ED-XRF analysis

Elemental analysis was performed by using an energy dispersive X-ray spectrometer EDX-720 (Shimadzu, Tokyo, Japan). Quantitative analysis was carried out using the Standard-less Quantitative Analysis Software, by setting the accelerating voltage to 50 kV for elements from titanium to uranium, and 15 kV for elements from sodium to scandium. To achieve higher sensitivity for analysis, primary X-ray filters were used for elements from zinc to arsenic and lead, as well as from sulphur to potassium. Two detection time settings were used for data acquisitions. All scans were set to a detection time of 100 s while scans acquired using the primary filter, zinc to arsenic and lead, were set to a detection time of 200 s. The number of Kα or Lα counts per second (cps/μA) was measured for each of the elements present and quantified using the preset algorithm built into the software. Due to the unavailability of a calibration standard using elements detected in the same sample matrix, the values obtained were further computed to reflect their relative abundance with respect to the iron content present in the individual samples. An average for each sample category (authentic, imitation and adulterated) was then taken. This form of data treatment takes into account variation in the proportions of macromolecular pools among algae samples and their respective nutrient availability.

Fig. 1a. Bead-like cell filaments of the authentic *N. flagelliforme*.

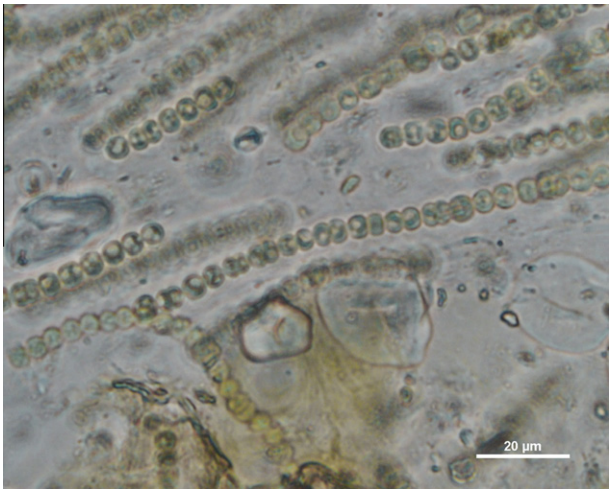

Fig. 1b. Adulterated sample containing some authentic *N. flagelliforme* strands.

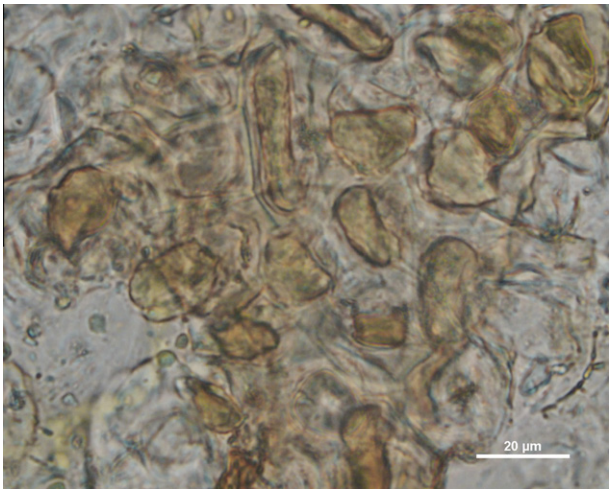

Fig. 1c. Non-cellular material with masses of brown and black pigments.