Detection of endangered species animal parts used in traditional Chinese medicine

Ci Wen Yang[a,b], Suming Chen[b], Chun-Chi Huang[b], Chang En Pu[c], Kuo Chuan Wu[c], Wen-Hsiung Ho[c], Kuang-Yu Hu[d] and Paul J. Brimmer[e]

a Taipei Zoo, Taipei, Taiwan, dwx10@zoo.gov.tw, bDept. of Bio-Industrial Mechatronics Engineering, National Taiwan University, Taipei, Taiwan, schen@ccms.ntu.edu.tw , cInvestigation Bureau, Ministry of Justice, Taipei, Taiwan, dNational Defense Medical Center , Taipei, Taiwan, eFoss-NIRSystems, Australia

Introduction

Tradition Chinese Medicine (TCM) is the quintessence of Chinese Culture with a long history. It has for many years, enriched the quality of people's lives. TCM is mainly obtained from nature. Unfortunately, some of the natural resources are no longer sustainable due to habitat destruction and over-exploitation. Many of the animals and plants that are used in TCM are listed as endangered species. The tiger and the rhino are the best-known examples but a number of many other less well-known species have also been threatened with extinction. To ensure sustainable use of our precious natural resources, international trade in medicines containing these endangered species is regulated under the Convention on Internal Trade in Endangered Species of Wild Fauna and Flora (CITES) through an established licensing system.

The objective of the proposed study is to discuss the methods of identification of those endangered species that are used in TCM. Two main approaches, Near-Infrared spectroscopy (NIR) and DNA studies would be done in tandem - NIR as a pre-screening method and DNA as a definitive proof. This paper contains two studies, one to demonstrate the ability to differentiate different types of horns (from endangered and non-endangered species), as well as counterfeit horn material, and in the second, to demonstrate the ability to differentiate the scales from pangolin (an endangered species) from other animal derived material.

Methods and analysis

Case 1 --- Horn Samples

NIR studies

Horn Samples of rhino (two species), Saitaricae Tataricae (two samples), buffalo, cattle (3 samples) were used to build up the NIR spectral library to test two unknown samples from the Customs Department, whose interest was to obtain evidence of the illegal trade in these materials. Samples were ground and scanned by the spectrometer (Foss NIRSystems, model 6500, Silver Spring MD, USA). Ground samples were respectively placed into a 1 ml mini-sample cup, which was placed onto the sampling stage of the Rapid Content Analyser (RCA) for the NIR Analysis. Thirty-two co-added scans of the samples were rationed to 32 coadded scans of a white ceramic reference to give the NIR spectra for identification. Total scanning time for each sample was less than 60 seconds.

Figure 1 shows the NIR absorbance (Log 1/R) spectra of 8 different horn samples. Figure 2 shows the spectra after conversion to the second derivative, which eliminates baseline differences between spectra, and allows for better comparison of the spectra.

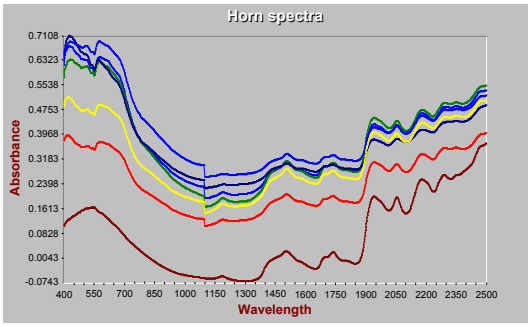

Figure 1. NIR spectra of 8 different horn samples.

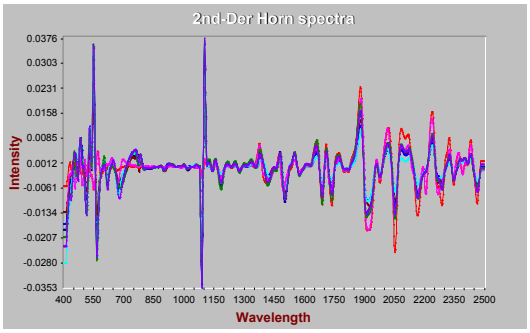

Figure 2. Second derivative spectra of horn samples.

The spectra of the horn samples from animal origin, while not identical, do contain many of the same features. In addition to the comparison by visual inspection, mathematical pattern recognition techniques are used to identify whether the spectral patterns match those from samples previously scanned and stored in a spectral library. In this study, samples of the "real" horn samples were used to create a library that shows they can each be distinctly identified as different kinds of horn samples. Figure 3 shows the results of the library development using only the real horn samples. The pattern recognition method used was residual variance, based on principal component scores from the NIR second derivative spectra, and the spectral regions used were 412-1080 nm, 1120-1350 nm, 1600-1850 nm, and 2000-2480 nm. (This was done to exclude the water band regions, as differences in the moisture in the samples could cause problems with identification of future samples whose moisture contents were not the same as the samples in the library.) None of the samples were incorrectly identified, showing that each horn sample could be conclusively identified as belonging to a specific species.

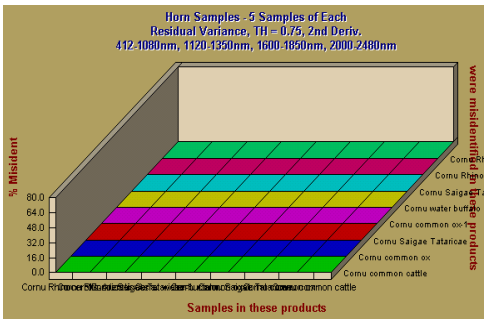

Figure 3. The ID of the horn samples.

Table 1. The identification results of the horn samples showing all of the horn samples have passed the identification method.

Product Name	P/F	Match Index	ID as	True ID
Cornu Rhinocerotis-Afica	Pass	0.0277	Cornu Rhinocerotis-Afica	Cornu Rhinocerotis-Afica
Cornu Rhinocerotis-Asia	Pass	0.3816	Cornu Rhinocerotis-Asia	Cornu Rhinocerotis-Asia
Cornu Saigae Tataricae-1	Pass	0.3284	Cornu Saigae Tataricae-1	Cornu Saigae Tataricae-1
Cornu water buffalo	Pass	0.018	Cornu water buffalo	Cornu water buffalo
Cornu common ox-1	Pass	0.0869	Cornu common ox-1	Cornu common ox-1
Cornu Saigae Tataricae	Pass	0.3806	Cornu Saigae Tataricae	Cornu Saigae Tataricae
Cornu common ox	Pass	0.2917	Cornu common ox	Cornu common ox
Cornu common cattle	Pass	0.1145	Cornu common cattle	Cornu common cattle

As shown in Figures 4 and 5, the spectra of the "unknown" samples do not appear anything like the spectra of the real horn samples, as described below. Using the library made from 8 "real" horn samples, the spectra of the unknown 'plastic' horn samples were tested to see if they would be identified as one of the members of the Horn library. Five scans of 2 unknown samples are used in this example. The results are shown in Table 2. All of the scans of these two samples are reported as "No Match", meaning that by this pattern recognition method, their results are not above a threshold that identifies them as a member of the horn library. In this case, the ID result is the probability of not belonging to a member of this library. The results of the NIR analysis showed that the two unknown samples (scanned 5 times each) were not identified by the horn library.

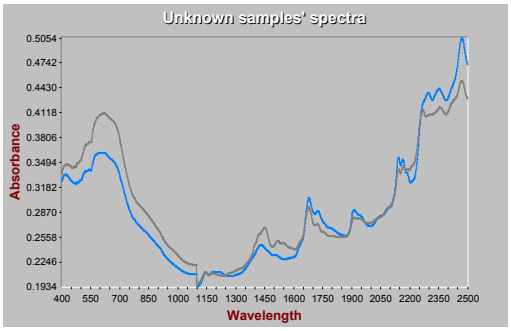

Figure 4. NIR spectra of 2 unknown samples.

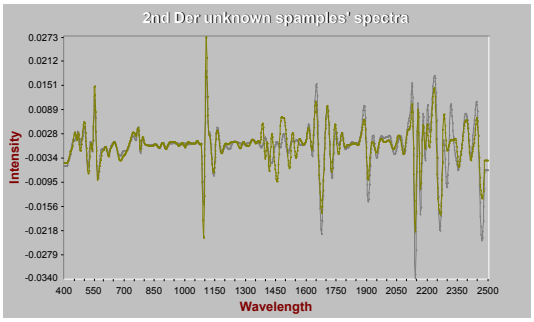

Figure 5. Second derivative spectra of 2 unknown samples.

Table 2. The ID report for unknown samples

Library:						
Output Project:		20020830				
Date	Time	Sample ID	Selected	ID as	ID Result	P/F
27/08/02	9:16:31	2	unknown	No Match	0.971	**Fail**
27/08/02	9:17:29	3	unknown	No Match	0.970	**Fail**
27/08/02	9:18:58	4	unknown	No Match	0.970	**Fail**
27/08/02	9:19:43	5	unknown	No Match	0.970	**Fail**
27/08/02	9:15:17	1	unknown	No Match	0.971	**Fail**
27/08/02	9:29:04	1	unknown	No Match	0.966	**Fail**
27/08/02	9:30:05	2	unknown	No Match	0.966	**Fail**
27/08/02	9:30:42	3	unknown	No Match	0.966	**Fail**
27/08/02	9:31:21	4	unknown	No Match	0.966	**Fail**
27/08/02	9:32:00	5	unknown	No Match	0.966	**Fail**

DNA studies

DNA analysis was used to confirm the result of the NIRS analysis. The purified DNA was subjected to polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and DNA sequencing analysis. The cytochrome *b* gene sequences of rhino horn samples matched the published sequences of the rhino cytochrome *b* gene in GCG database, with identities from 98% up to 100%.

Case 1 --- Pangolin Scales

NIR studies

There were 3000~4000 kg of pangolin scales from one Indonesian ship were investigated from Customs Department in 1999. In order to identify these products, 23 pangolin samples were collected from Taiwan, Thailand, and China from 2000-2003 to set up the database for this purpose. Figure 6 shows the NIR absorbance (Log 1/R) spectra of 7 different pangolin scale samples. Figure 7 shows the spectra after conversion to the second derivative.

The identification results of the library pangolin samples showing all of the samples have passed the identification method. In order to check that other animal derived products do not mis-identify as pangolin scales (false negative result) the spectra of the horns from Case 1 were checked against the pangolin library. The results are shown in Table 4. The identification results of the horns tested versus Pangolin library confirms they do not misidentify as pangolin samples showing all of the samples have failed the identification method. As can be seen from this table, none of the animal derived materials was mis-identified as pangolin scales, indicating that a reasonable stable library for identifying pangolin scales has been developed.

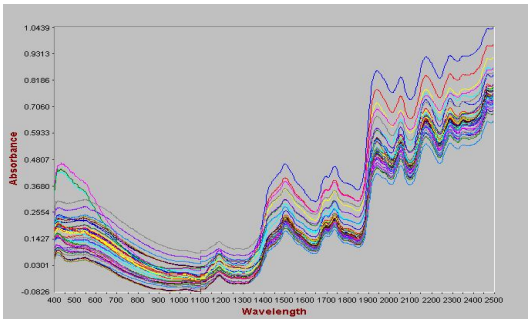

Figure 6. NIR spectra of 8 different horn samples.

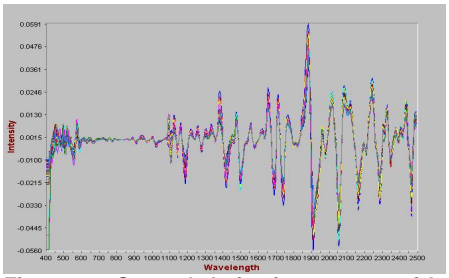

Figure 7. Second derivative spectra of horn samples

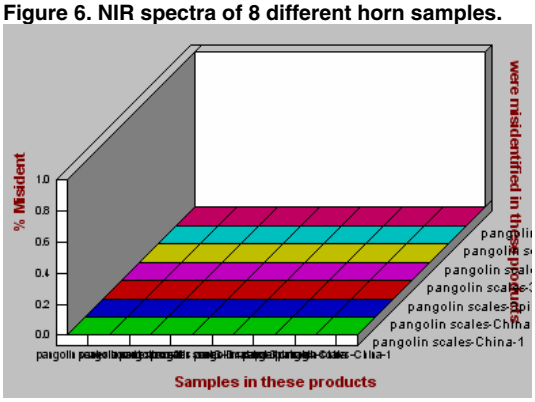

Figure 8. The ID of the pangolin scale samples.

Table 3. The identification results of the library pangolin samples.

Sample ID	Selected	ID as	ID Result	P/F
sample1	pangolin	pangolin	0.997	Pass
Sample2	pangolin	pangolin	0.996	Pass
Sample3	pangolin	pangolin	0.997	Pass
Sample4	pangolin	pangolin	0.997	Pass
Sample5	pangolin	pangolin	0.998	Pass
Sample6	pangolin	pangolin	0.998	Pass

Table 4. The identification results of the horns tested versus Pangolin library.

Sample ID	Selected	ID as	P/F
Cornu water buffalo	pangolin	No Match	Fail
Cornu Rhinocerotis-Afica	pangolin	No Match	Fail
Cornu Rhinocerotis-Asia	pangolin	No Match	Fail
Cornu Saigae Tataricae-1	pangolin	No Match	Fail
Cornu Saigae Tataricae	pangolin	No Match	Fail
Cornu common ox-1	pangolin	No Match	Fail
Cornu common ox	pangolin	No Match	Fail
Cornu common cattle	pangolin	No Match	Fail

DNA studies

The cytochrome *b* gene sequences of investigated pangolin scale samples matched the sequences of the other Asian pangolin cytochrome *b* gene in the pangolin gene database of sample collection.

Conclusion

The NIR study shows the possibility using the NIRS to identify the species of the endangered species and differentiate between them and non-endangered species samples. Spectra library of endangered species versus other horns was successfully developed. In addition, this "horn" library was able to be used to identify "fake" horn material. Pangolin versus "other" animal material results were shown to conclusively be able to differentiate scales from the endangered pangolin from other animal derived material.

With the use of NIR reflectance spectra, the rapid analysis was performed for the identification of the evidence samples. While DNA is the confirmation test for species identification, NIR spectra can be used successfully to pre-screen animal derived material to determine if they have come from endangered species. The NIR analysis can be performed in a matter of seconds, which can be used to pre-screen samples before a longer, more labourious DNA test (which is the definitive identification) needs to be performed.

References

1. Agriculture, Fisheries and Conservation Department (AFCD), Hong Kong. Protect endangered species. The first International conference and exhibition of the modernization of Chinese medicine (2002).
2. J. Jarcho, Restriction fragment length polymorphism analysis. In Current Protocols in Human Genetics (N.C. Dracopoli, J.L. Haines, B.R. Korf, D.T Moir, C.C. Morton, C.E. Seidman, J.G. Seidman, and D.R. Smith, eds.) pp. 2.7.1-2.7.15. John Wiley. Sons, New York (1994).
3. I.Kheterpal and R.A. Mathies,. Capillary array electrophoresis DNA sequencing. Anal. Chem. 71:31A-37A (1999).

4. R.K.Saiki, D.H.Gelfand, S.Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, and H.A.Erlich, Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491(1988).
5. C. W. Yang, and P. J. Brimmer. Identification of Herbal Medicines by Near-Infrared Spectroscopy, 26th Eastern Analytical Symposium. USA (2001).
6. C. C. Huang, S. Chen and C. W. Yang. Determination of constituents in Chinese medicine using near infrared spectroscopy. In "Proceedings of International Symposium on Automation and Mechatronics of Agricultural and Bioproduction Systems", 375-381. Chiayi, Taiwan : National Chiayi University. (2002)
7. C. W. Yang, S. Chen, C. C. Huang, C. E. Pu, K. C. Wu, W. H. Ho, K. Y. Hu and P. J. Brimmer. Detection of endangered species used in the traditional Chinese medicine. In " Proceedings of International Symposium on Automation and Mechatronics of Agricultural and Bioproduction Systems", 285-292. Chiayi, Taiwan：National Chiayi University. (2002)