TITLE: APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY TO IDENTIFY POTATO CULTIVARS

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INTRODUCTION

Recent advances in technology connected with the potato seed industry and with breeding and plant biotechnology programs have heightened the need for an accurate, repeatable, rapid technique to identify potato cultivars. There is a need to characterize and archive new cultivars to support visual descriptions. In particular, potatoes are often propagated in a controlled, disease-free environment by means of a mini-tuber technique, but various cultivars increased by this technique are often extremely difficult to identify visually.

Previous research targeted at these needs has centered on either electrophoretic analysis of isoenzymes or on direct analysis of DNA, using polymerized chain reactions (PCR). Although both are valuable techniques, especially PCR which can be used to identify lines that differ by only a few genes, the procedures are involved are complex and costly.

We suggest that potato cultivars can be identified by means of IR spectroscopy, using a fine powder prepared from freeze-dried tubers and a specially designed sampling accessory. Use of a freeze dried powder would provide a simple, inexpensive method of shelf storage of archival material, as well as a simple, quick, inexpensive means of analyzing samples based on a computer library of spectra. The technique may also be useful for characterization or identification of other plant and animal tissue.

MATERIALS AND METHODS

(i) Summer studies - 1994

Two sets of duplicate tubers from each of six cultivars grown in 1993 in Weld county and stored at 2-3 C since September 1993 (Russett Burbank, Russett Norkotah, Bintje, Yukon Gold, Norland, Norchip) were sliced, freeze-dried, pulverized into a fine powder using a Wiley Mill and a mortar and pestle, then sifted through a 400 mesh screen (38 micron openings) to produce an extremely fine powder which can be stored indefinitely. The two sample sets were labelled "A" and "B".

Infrared absorbance spectra of each of the powders were obtained with a Biorad FTS 7 spectrometer equipped with a MTS detector and a specially designed horizontal attenuated total reflectance accessory. The principles of operation are described by Coltrup (1990) and the features of the accessory by Remmele and Stushnoff (1994) and Remmele et al. (1994). The ATR technique uses a specially designed device in which mirrors are placed in the ordinary light pathway of an IR spectrometer. A laser light beam is reflected into a Germanium crystal, and refracted onto the surface of the crystal, creating an evanescent wave about one micron thick on the crystal surface. The sample is placed onto the crystal surface where it absorbs a tiny amount of the IR energy contained in the wave (Fig. 1). Interference from H_2O or CO_2 in either sample

chamber or the light pathway is eliminated by use of a specially designed device attached to the spectrometer which supplies air that has been dried and scrubbed of carbon dioxide and which allows both the light pathway and sample chamber to be placed under a vacuum (Fig. 2).

Although absorbance is quite weak, the signal to noise ratio is improved by means of Fourier Transform Spectroscopy (Coltrup, 1990). Numerous scans in the form of interferograms are taken, signal averaged and converted to absorbance spectra by means of Fourier transforms, resulting in reduction of noise by the square root of the number of scans taken. Although the mathematical computations would have been prohibitive by hand, "Lab Calc" software allows conversions by computer in seconds. By taking a total of 1024 scans of each sample, the noise, in relation to the signal, was reduced to insignificant levels.

Spectra of all six cultivars of each "A" and "B" samples were assembled, baseline corrected, uniformly scaled and stored in respective libraries on disk.

A second scan, obtained from a different tuber of the same cultivar , was then called into computer memory and the appropriate, corresponding library was searched. Lab-Calc software includes a mathematical means of comparing spectra which produces a hit quality index (HQI) of 0.0 for a perfect match and a 1.414 for the worst possible match. Search results are listed in order of hit quality from best to worst.

Additional sample preparation techniques were tested , including division of tubers into several sub-fractions - soluble peel, soluble centers, insoluble peel, and insoluble centers to optimize sample preparation and spectral analyses.

(ii) Fall and winter studies - 1994-95

Tubers and minitubers of twenty four cultivars and selections were obtained from SLV in September 1994 and stored at 3-4 C. Freeze-dried samples of tubers were prepared as in (i) in October and in January to search for changes which may have occurred in storage. Minituber samples were limited in number and samples were prepared only in October.
Samples will again be prepared in May to test the impact of season-long storage.

Additional studies were conducted to determine the optimum wavenumber for computer library comparisons by systematically studying selected absorbance spectra.

RESULTS AND DISCUSSION

(i) Summer studies

When samples were first prepared by sifting through a coarser screen (150 microns), results were inconclusive, indicating poor ability to distinguish between cultivars. These unsatisfactory results may be due to voids between sample particles or ineffective contact with the crystal surface, in either case very fine samples (38 microns) are essential and resulted in an improved ability to distinguish between cultivars and more consistent results in searching unknowns.

Also, better results were obtained by improving crystal cleaning procedures, washing and swabbing twice with water followed by acetone compared to vacuum cleaning.

Spectra were obtained in the wavenumber range from 600 cm^{-1} to 4000 cm^{-1} , but only

the region 850 cm^{-1} and 1800 cm^{-1} , which includes the Amide I and Amide II peaks from proteins, as well as peaks due to carbonyl groups and starch branching patterns, proved to be of interest (Fig. 3). An example of a successful search of unknown Bintje B when compared to the A library is shown in Fig. 4. When similar searches were conducted for all cultivars using 850 cm^{-1} to 1800 cm^{-1} wavenumbers, four of the six samples were correctly identified and in the two mismatches the correct cultivar was picked as the second best hit. When spectra from B samples were searched against A library using 1200 cm^{-1} to 1800 cm^{-1} the correct cultivar was identified in five of the six cases and was the second best hit in the remaining case (Fig. 5).

Spectral comparisons of soluble and insoluble fractions were inferior to the whole tuber powders. The soluble fractions were very difficult to handle because of their extremely hydrophilic nature.

The tubers used in these studies had been in storage for about ten months - near the end of their useful storage life - and some of the less than ideal results may be attributable to storage senescence. Under the circumstances it is encouraging that accuracy was still quite good.

(ii) Fall and winter studies - 1994-95

Narrowing of the spectral bands, and use of two regions in combination, for search routines revealed that the overtone region for protein and starch improved the hit quality compared to the summer studies. Very high quality HQIs were obtained (0.1 - 0.2) .

Frequency of obtaining correct hits is being studied with October and January stored samples using the improved protocol. Preliminary evidence suggests that minituber identity can be detected using minituber libraries (Table 1). Regular tubers can also be identified when searched against a minituber library (Table 2), but minituber unknowns could not be identified when searched against a library of 10-month old regular tuber spectra (Table 3). Loss of sensitivity was particularly apparent in the protein fingerprint region, suggesting a loss of specific proteins in senescing tubers. These comparisons are still underway and not all cultivars have been characterized, nor have all libraries been completed for the tubers still in storage.

CONCLUSIONS

Results obtained in 1994-95 strongly suggest that the technique can be used to fingerprint and identify potato cultivars. Best results were obtained when the following procedures were used.

1. Freeze-dried whole tubers are better than either soluble or insoluble peel or tuber fractions.

2. Sifting through a 400 mesh (38 micron) screen is essential. Indefinite storage of powders in sample vials sealed to prevent uptake of moisture should be possible. Refrigerated storage may or may not enhance shelf life because moisture levels are 1% (fwb) or less.

3. Comparisons made on small, but different segments of the infrared spectrum, especially in the amide I and amide II overtone regions, have markedly improved sensitivity and accuracy of identification. Some modifications to the software program will improve ease of analysis using this approach.

4. Minituber and whole tuber unknowns have been correctly identified from minituber libraries, but not from libraries of 10-month old tuber powders.

Work yet to be done

1. Complete preparation and analyses of stored tubers.
2. Determine if identification can be made at the onset of tuber initiation from field samples.
3. Determine if leaf samples can be used for identification purposes.
4. Modify the software program to simplify analyses.
5. Obtain samples of similar cultivars from different regions in the US to test impact of environment on identification parameters.
6. Disclose procedure for possible patent application.
7. Improve precision to enable identification of somatic mutant clones differing by only a few genes.
8. Test applicability to distinguish transgenic lines with genes for increased starch content, BT insect resistance, protein coat virus resistance and herbicide resistance. This would need to be explored with Monsanto Company.
9. Explore obtaining funding to test the procedure with other storage organs containing starch and protein such as cereal grains.

REFERENCES

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Remmele Jr LR, Stushnoff C, Carpenter JF (1994) Real-time infrared spectroscopic analysis of lysozyme during lyophilization: structure/hydration behavior and influence of sucrose. In JL Cleland and CB Langer (Eds) Protein Formulation and Delivery, Amer Chem Soc. Pub., NY

Table 1. Yukon Gold minituber search from minituber library. Data are Hit Quality Indices; 0=perfect match, 1.141=perfect mismatch. ( ) indicates rank position for best match based on HQI .

	Starch region wavenumber			Protein region wavenumber	
	650-1100	650-950	950-1100	1400-2000	1400-1700
All Blue	0.68	0.70	0.30	0.43	0.24
Centennial Russet	0.68	0.67	0.35	0.74	0.57
Ranger Russet	0.71	0.73	0.31	0.43	0.27
Red Lasoda	0.72	0.73	0.29	0.43	0.23
R. Burbank	1.01	1.01	0.40	0.60	0.48
Yukon Gold	0.54 (1)	0.48 (1)	0.25 (1)	0.28 (1)	0.14 (1)

Table 2. Yukon Gold whole tuber search from minituber library. Data are Hit Quality Indices; 0=perfect match, 1.141=perfect mismatch. ( ) indicates rank position for best match based on HQI .

	Starch region wavenumber			Protein region wavenumber	
	650-1100	650-950	950-1100	1400-2000	1400-1700
All Blue	0.69	0.73	0.33	0.48	0.27
Centennial Russet	0.69	0.69	0.40	0.75	0.58
Ranger Russet	0.72	0.76	0.36	0.48	0.28
Red Lasoda	0.73	0.77	0.33	0.49	0.26
R. Burbank	1.03	1.04	0.44	0.64	0.49
Yukon Gold	0.55 (1)	0.51 (1)	0.30 (1)	0.31 (1)	0.17 (1)

Table 3. Yukon Gold minituber search from 10 month-old whole tuber library. Data are Hit Quality Indices; 0=perfect match, 1.141=perfect mismatch. ( ) indicates rank position for best match based on HQI .

	Starch region wavenumber			Protein region wavenumber	
	650-1100	650-950	950-1100	1400-2000	1400-1700
Bintje	1.06	0.95	0.27	0.30	0.18
Norchip	0.78	0.73	0.31	0.53	0.23
Norland	0.75	0.73	0.34	0.54	0.13
R. Norkotah	0.64	0.65	0.34	0.48	0.17
R. Burbank	0.84	0.75	0.37	0.49	0.19
Yukon Gold	0.82 (3)	0.74 (4)	0.38 (6)	0.53 (5)	0.26 (6)

Fig. 1

Attenuated Total Reflectance (ATR) Spectroscopy

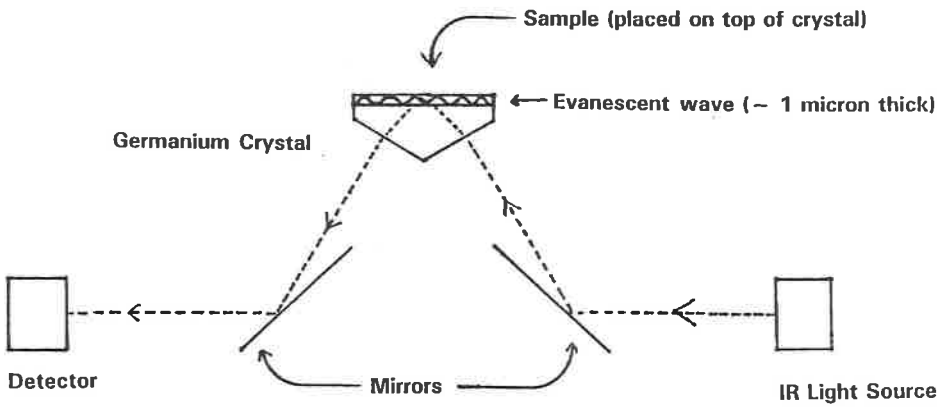
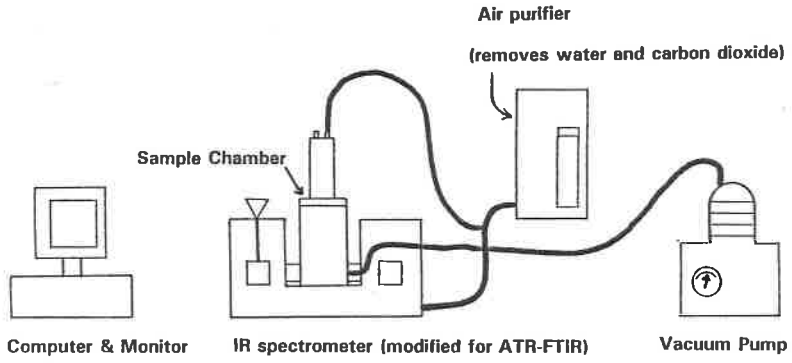


Fig. 2

Infrared Spectrometer Modified for ATR/FTIR



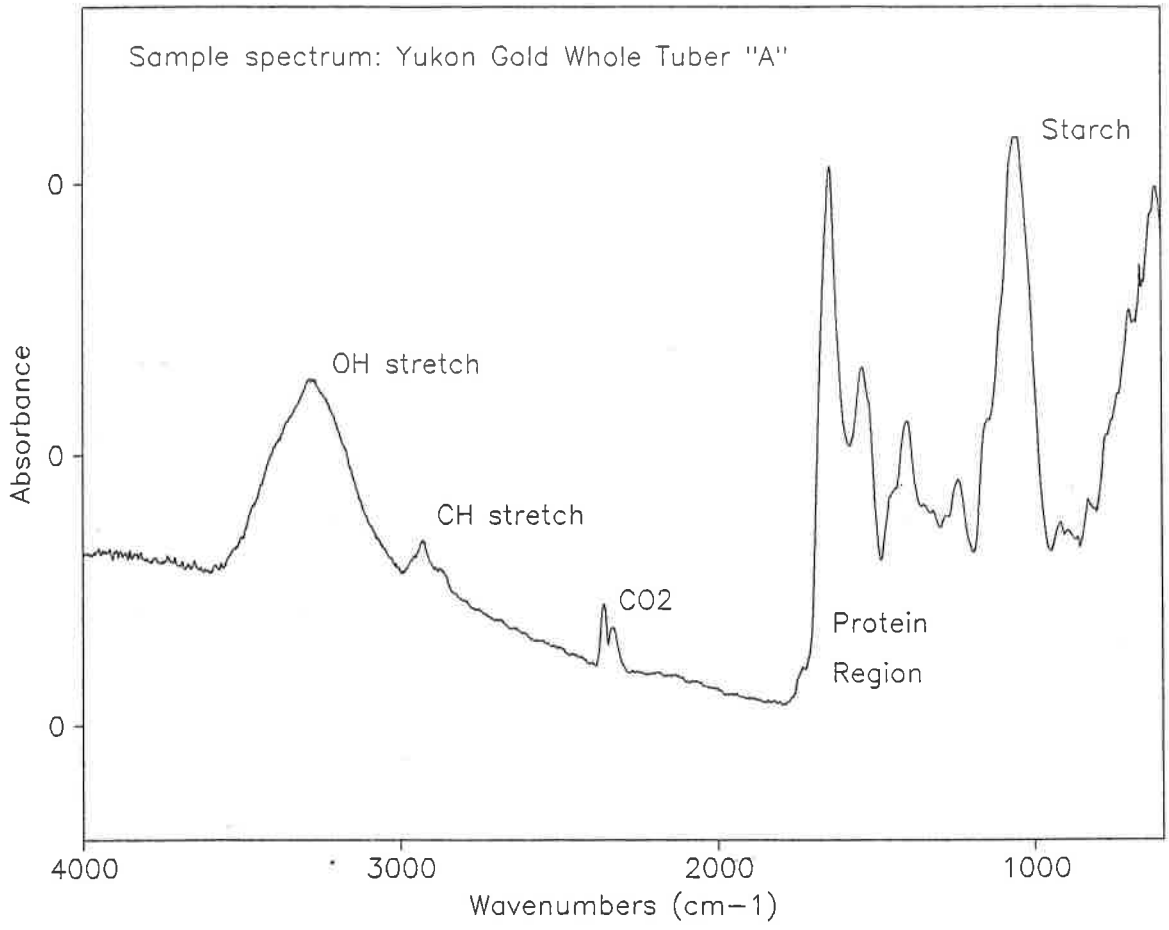


Fig. 3 A scan of a whole potato tuber, showing the entire 600 cm^{-1} to 4000 cm^{-1} absorbance spectrum. Key information areas are labeled on the spectrum, illustrating that the sector of the scan containing information of interest is the 850 cm^{-1} to 1800 cm^{-1} range. That range contains absorbance peaks attributable to proteins and starch branching patterns, which vary from one cultivar to another.

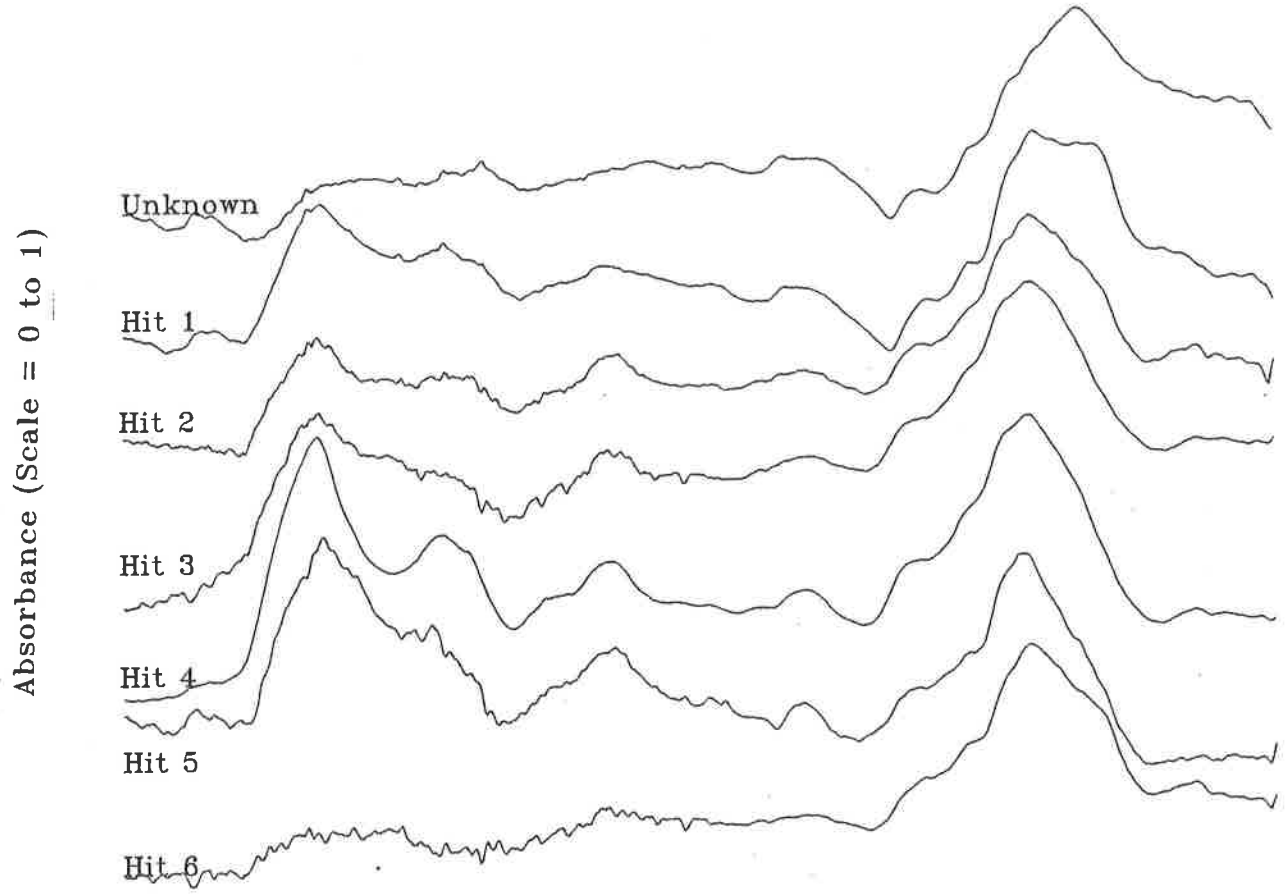


Fig. 4 **Results of a library search with spectra trimmed to the 850 cm^{-1} to 1800 cm^{-1} range, baseline corrected, and adjusted to a standardized (0 to 1) absorbance scale. Mathematic results of the search are listed below.**

Unknown: Bintje "B"

Hit #1: Bintje	**--- HQI = 0.255**
Hit #2: Norland "A"	**--- HQI = 0.465**
Hit #3: Russett Burbank "	**--- HQI = 0.532**
Hit #4: Yukon Gold "A"	**--- HQI = 0.554**
Hit #5: Russett Norkota "A"	**--- HQI = 0.565**
Hit #6: Norchip "A"	**--- HQI = 0.582**

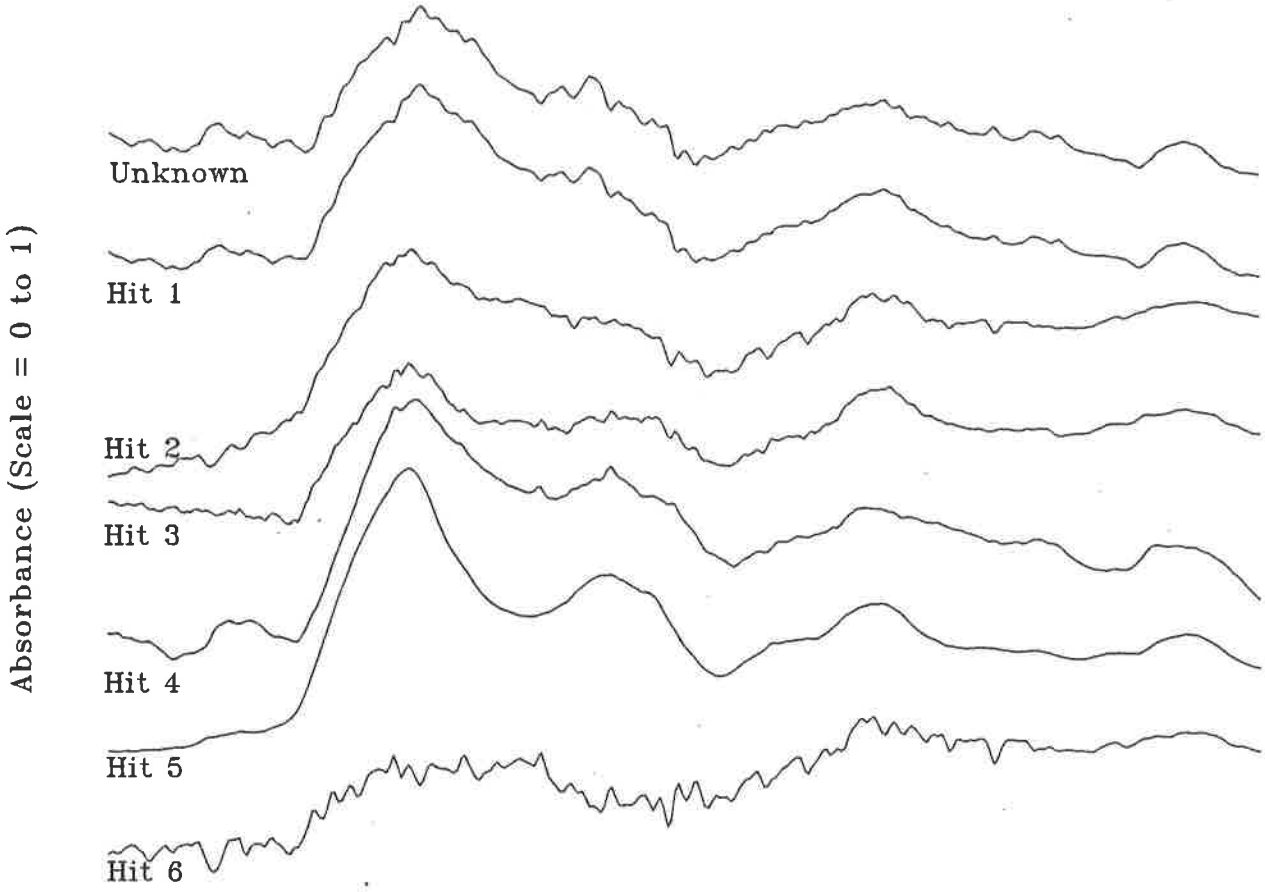


Fig. 5 Results of a library search with spectra trimmed to the 1200 cm^{-1} to 1800 cm^{-1} range, baseline corrected, and adjusted to a standardized (0 to 1) absorbance scale. Mathematic results of the search are listed below.

Unknown: Russett Norkota "B"

Hit #1: Russett Norkota "A"	**--- HQI = 0.105**
Hit #2: Russett Burbank "A"	**--- HQI = 0.232**
Hit #3: Norland "A"	**--- HQI = 0.244**
Hit #4: Bintje "A"	**--- HQI = 0.244**
Hit #5: Yukon Gold "A"	**--- HQI = 0.260**
Hit #6: Norchip "A"	**--- HQI = 0.404**