Near Infrared Spectroscopy as a rapid method for sandalwood oil determination

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Abstract

The remaining chips from 295 sandalwood (S. austrocaledonicum) cores previously analysed using GC-MS were scanned using Near infrared Spectroscopy (NIRS). The correlation between the NIR spectral data and the a-santalol content from the GC-MS analysis was very high (R^2 = 0.9258). Such a high correspondence between these two techniques indicates that it is possible to use NIRS to predict a-santalol content in sandalwood chip samples. The relative advantages of using NIRS for quantifying a-santalol content in raw sandalwood is discussed in terms of its rapid and potentially inexpensive application to quality control for processing and breeding new cultivars.

Introduction

It is evident that sandalwood oil composition may vary depending on its geographical and taxonomic origin, which may reflect current international demand (Howes et al., 2004). Despite several studies on sandalwood in Vanuatu and elsewhere in the region, there is a lack of quantitative information about the sources of variation in oil yield and quality and prediction of this variation from morphological characteristics. In many parts of the world, essential oil and associated product markets are becoming increasingly competitive and complex. At present, no standard method is readily available to determine oil quality or aid the identification of the species from which the oil was obtained. Not only is there the need for the rapid, inexpensive and reliable identification of sandalwood oil quality in its final form, but also the quantity and quality of the oil in its raw form prior to processing. Taking this one step further would be the real-time evaluation of the plant in-situ saving valuable time, effort and dollars on grow-out, harvesting and subsequent processing. Research into technologies for measuring agricultural products quality attributes is progressing in a number of areas with varying levels of success.

The adoption of Near Infrared Spectroscopy (NIRS) to rapidly evaluate products such as Sandalwood oil has the potential to give Australian producers and manufacturers a competitive advantage in their markets. Such technologies may be utilised as rapid assessment tools for quality control within a manufacturing plant or in the production environment. Links also could be established with breeding programs, and these technologies used as part of the selection process.

In view of the current issues associated with sandalwood (quality and sustainability), a feasibility study was conducted to assess the ability of NIR spectroscopy as a low-cost analytical alternative to gas chromatography-mass spectrometry (GC-MS) for santalol determination, in particular α-santalol and *b*-santalol. If successful this will have direct commercial application in quality control of marketed sandalwood oil.

Background Information on Near Infrared Spectroscopy

NIRS is a non-destructive method of using optical light rather than wet chemistry methods to determine chemical composition of various liquid and solid biological materials. Of the current non-invasive techniques (e.g., NMR, acoustics, etc) NIRS is probably the most advanced technology with regard to instrumentation, applications, accessories and statistical software packages. NIR is a small part of the electromagnetic spectrum of radiation between the visible and the infra-red regions of the spectrum. Wavelengths in the near infra-red spectrum (700 - 2500 nm) are absorbed by certain electronic bonds at specific wavelengths (i.e., carbon-hydrogen, oxygen-hydrogen, nitrogen-hydrogen bonds that form the basis of all biological material). Although these wavelengths are not visible to the unaided human eye, they can be used to obtain information on the chemical make-up of a material.

The technique involves beaming NIR into a product; electronic bonds absorb some NIR light whilst the rest is reflected/transmitted and their corresponding wavelengths are picked up by detectors giving a spectrum or finger print of absorbance over wavelength. NIRS assessment offers the advantage of being non-destructive and taking only a fraction of a second per test, with the potential to test every piece of product in an in-line application for various internal attributes. However, it must be remembered that NIRS is a secondary method of measurement and therefore must be calibrated against a primary

Figure 1. Sandalwood core samples prepared as sliced cores (left) medium chips (centre) and milled powder (right)

Figure 2. Near infrared bench-top research spectrometer (left) and hand-held solids probe (right) used to evaluate the sandalwood samples.

reference method. That is, we must train the NIRS instrument using a known reference method, for example, in our analysis of santalol oil content gas chromatography-mass spectrometry (GC-MS) method was used. Once trained, we then have a calibration model (a mathematical equation) for that reference method.

The advantage of NIRS over wet chemistry analyses lies in the fact that it can be non-destructive and in-situ, allowing determination of the chemical composition of the sample in its environment. The technique requires no or minimal sample preparation and avoids the need for reagents as well as wastage. Furthermore, the technique is multi-analytic allowing several determinations simultaneously.

NIRS has been in routine use for the assessment of a range of components in dry materials, for example: protein in grains, in the pharmaceutical industry (primarily for product identification), the petrochemical industry (for octane analysis), the food and fodder industries (quality assessment of flour, baked products, dairy products, and forage), and the horticultural industry (whole fruit and vegetables). With regards to the present application, NIRS has had a demonstrated success for various plant essential oils, including oils from basil, chamomile, thyme, and oregano (Schulz et al., 2004; Steur et al., 2001). In addition, NIR spectroscopy has also been applied as both a qualitative and quantitative analytical tool in the forestry industry (Schimleck et al., 2003; Steur et al., 2001; Brunner et al., 1996).

Methods

The sample set of Sandalwood cores used in the current study were collected from various sampling sites in different regions of Vanuatu and Cape York. Samples were analysed for *a*-santalol using the destructive GC-MS technique. The remainder of each of the sandalwood cores following GC-MS analysis were individually presented as fine chips (2-5 mm diameter). A total of 295 samples of Sandalwood chips were then scanned using a commercially available, research grade NIRS instruments (MPA, Bruker Optics, Ettlingen, Germany) (Figure 3). Statistical modelling techniques such as partial least squares (PLS) were utilised to establish a correlation between the NIR spectral data and the *a*-santalol content (from the GC-MS analysis) for the sample set.

Results and discussion

The results of this feasibility study are summarised and graphically depicted in Figure 3. The results of the trial were very encouraging and indicate that it is possible to use NIRS to predict *a*-santalol content in sandalwood chip samples. An R^2 (coefficient of determination) of 0.9258 was obtained, meaning that 92.58% of the variance in the reference samples (GC-MS results) can be explained. An RPD (**R**atio of (standard error of) **P**rediction (Validation) to (standard) **D**eviation) of 3.67 indicated that the model prediction would be suitable for process control purposes . The fairly large error (root mean standard error of cross validation RMSECV) of 3.74 % may have resulted from the combined effects of (i) the NIR spectrum was not obtained on the exact sandalwood sample that was tested by the GC-MS reference method and (ii) there was a 12 to 19 month time lapse between the GC-MS and NIR sampling. This time lapse may have resulted in a decrease in santalol content due to the volatility of the oil in the samples.

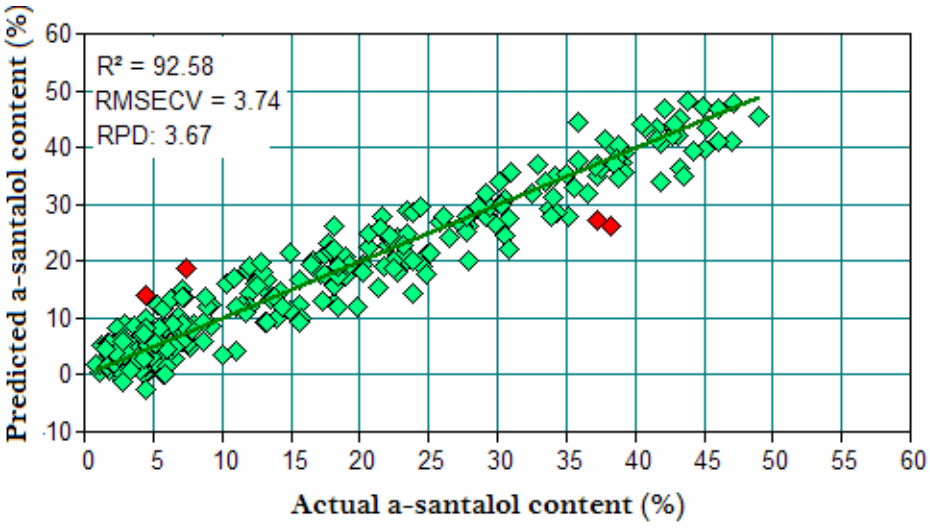

Figure 3. Predicted vs. actual *a*-santalol content for sandalwood chips using 295 samples (13 outliers removed from this dataset).

Summary
NIRS shows tremendous potential to be used as a rapid tool to assess Sandalwood oil (*a*-santalol) content in chipped sandalwood samples. The technique of utilising NIRS technology for sandalwood quality and quantity determination needs to be further developed to be utilised as a tool for commercial applications. The technology offers the potential of being a rapid, non-invasive tool for assessing not only oil sample purity and quality of liquid oil samples, but also core wood samples in a processing plant situation, seedlings and trees in a field environment. This has enormous possibilities for field selection of plants for processing and may be linked to selective breeding programs. This would enable genetic improvement programs to not only focus on quantity but also on the quality of the raw material, thus targeting the raw material to specific processes and products.

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About the Rapid Assessment Unit
The Rapid Assessment Unit (RAU) is a collaborative initiative between the Queensland Department of Primary Industries (QDPI&F) and James Cook University (JCU), and is located within the JCU Centre for Tropical Agri-Tech Research on their Cairns campus. The RAU has an ongoing program of research encompassing non-invasive rapid assessment technologies such as near infrared spectroscopy (NIRS) to rapidly and non-destructively evaluate food and agricultural biological products.

Short Communication.
An effective technique for performing *in-vitro* pollination experiments with flowers of *Santalum spicatum* and *Santalum album*.

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Experimental investigations of plant breeding systems frequently employ *in-situ* bagging of inflorescences to exclude natural pollinators so that hand pollinations can be carried out (Kearns and Inouye 1993). In a similar fashion, pollination experiments were performed for the Western Australian sandalwood *S. spicatum*, the Indian *S. album* and the Quandong, *S. acuminatum* (Sedgley 1982; Sindhuveerendra and Sujatha 1989; Rugkhla et al. 1997; Ma et al. 2006). The flowers and fruits of *S. spicatum* and *S. album* are, however often loosely held on the branches of the tree and abscise easily on contact (Fox and Reeve 1992). The aim of the present project was therefore to establish an effective technique for performing in-vitro pollination experiments for these two species.

OASIS® Floral foam has been successfully used to maintain viability of flowers and shoots (Jefferies et al. 1982; van Tuyl et al. 1991; Salom and Broeckling 2003; Wise et al. 2006). OASIS® Floral foam is an open-celled phenolic foam that resembles the biological make-up of plant stem cell structure. The foam draws water through capillary action and retains it in the cells. OASIS® Floral foam was soaked in a diluted solution of the fungicide Previcur (Bayer, active ingredient: 600 g/l propamocarb) (1.5 ml previcur/ 1l water) and then immersed in a container filled with tap water. Flowers were cut before anthesis and placed immediately with the pedicel first, into the foam. The flowers of *Santalum spicatum* and *S. album* remained viable and healthy for up to two weeks (the approximate lifetime of the flowers in-situ) (see also Barrett 1987; Ma et al. 2006) using OASIS® Floral foam. The porous nature of the foam drained nectar from the sandalwood flowers. Pollen viability and stigma receptivity were evaluated and hand pollinations were successfully performed using flowers stored in Oasis foam. Sandalwood flowers (of both species) stored in the foam experienced a change in colour (from green to pink to dark red), and related to it, in stigma receptivity in a similar fashion as those maturing on the tree (for *S. spicatum*: Muir 2004; for *S. album*: Bhaskar 1992). Further research is required to refine this in-vitro technique to enable

Figure 1. Excised flower buds from *S. album* positioned in OASIS® Floral foam (image is black & white) .