Sandalwood Research Newsletter

September 2008 | Issue 23 | ISSN 1321-022X

EDITOR'S NOTE

The quality and value of sandalwood oil is typically determined by the level of α- and β-santalol. There are various methods of extraction currently used such as solvent extraction, super critical fluid extraction, water distillation and steam distillation. Making direct comparisons between studies using different means of oil extraction can be problematic given the variable results between them. Since steam distillation is the primary method used by industry using bulk wood samples, there is a need for a standardised 'desk top' method that gives equivalent results. In this issue Hettiarachichi compares the relative merits of two widley used methods of extraction and proposes a standardised method that may be developed for use across industry to allow for more accurate comparison between studies.

With interest in improving product quality across all sandalwood markets there is also a need for rapid determination of α- and β-santalol in both harvested logs and standing trees. This will assist sandalwood merchants in stratifying their harvest into those suitable for oil extraction and powdering for agarbatti. There is also a need for determining the levels of santalol in living trees that may contribute to future improvement programmes and cultivar development. To meet this challenge Wedding et. al. has developed a rapid method using Near Infra-Red Spectroscopy to assess oil quality in heartwood across a large number of samples. There are many potential applications of this technology for both sandalwood science and industry alike.

Developing a sound understanding of the breeding systems for sandalwood often involves meticulous work with controlled pollination and isolation of very small and fragile flowers. Given that only a small percentage of flowers pollinated this way are useful in studies of breeding systems Shepherd has developed a laboratory-based protocol that is less painstaking to undertake, which could have implications for reproductive biologists working with sandalwood.

Tony Page

Volatile oil content determination in the Australian sandalwood industry: Towards a standardised method

Dhanushka S. Hettiarachichi
Wescorp Sandalwood Pty Ltd. Bibra Lake, Western Australia.

Abstract

Quality assurance of Western Australian Sandalwood has been identified as being important for industry. The main parameters of sandalwood timber quality are yield and quality of its oil. Sandalwood oil already has an Australian standard, but there is no specified method to determine yield. A hydro-distillation method was developed along with a simple solvent extraction method to represent the currently contemporary methods. Despite the significant time and resource consumption of hydro-distillation it was found to be a superior method of analysis since it more accurately reflects the results obtained during industrial processing. More economical and rapid methods are demanded by different sectors involved in sandalwood industry. This article is an early step in seeking the most appropriate method suited for volatile oil determination.

Introduction

Sandalwood plays an important role in the Western Australian economy as an export commodity and involves many organisations for its large scale processing. Western Australian Sandalwood (*Santalum spicatum*) is placed fifth in the quality scale (Erligmann 2001) relating to its relatively lower percentage of santalol when compared with its Indian and Pacific cousins. While different species have different chemical composition quality also varies within species which may be influenced by factors such as tree age, soil, climatic conditions, host vegetation, pests or other idiosyncratic causes.

Most of the sandalwood harvested in Western Australia is distilled for its oil since it has a very high perfumery value. The balance of the annual harvest is used in the incense industry and for certain other cultural uses in different parts of the world. Australian (AS 2112:2003) and international (ISO 3518:2002) standards have been drawn for the quality parameters of the oil with reference to santalol levels and physical parameters. Gas chromatography is the preferred method of analysis to determine the α- and β-santalol and the other sesquiterpene concentrations (Howes *et al.* 2004).

The two primary parameters determining the quality of timber will be the yield of volatile oil and the santalol content of the obtained oil. While a standard exists for oil quality, a standard method for the determination of volatile oil yield accepted by the Sandalwood industry in Australia is still required.

To study the volatile oil content of plant material there are few methods

inside...

published in 'standards' documents. The ISO method (ISO 6571: 2008) of determining volatile content of spices and condiments shows much similarity with other widely accepted methods stated on British Pharmacopeia (Appendix XI E 2008) and AOAC international (962.17-2006). All these methods follow a similar procedure of hydro distillation with minor variations. Other methods which are commonly used to isolate the volatile oil content are solvent extraction, microwave extraction, super critical fluid extraction, dense liquid carbon dioxide extraction, solid phase micro extraction and headspace gas analysis (Min (2007); Lucchesi *et al.* 2004; Moretta *et al.* 2001).

The current study has focused on contemporary analytical methods used in the Western Australian sandalwood industry, namely hydro distillation and solvent extraction (Brand *et al.* 1999). Optimal experimental conditions and possible errors of both these methods are discussed as an initial step to develop a standard method for the industry.

Methods

Plant material

A root sample of *Santalum spicatum* was selected out of 54 specimens kept at Wescorp Sandalwood. Particle size was reduced with a hammer mill and separated into two samples (i) as coarse (420-4760µm / 4-40 mesh size) and (ii) fine(<420µm / 40 mesh).

Hydro-distillation

Samples of 50g and 25g were each placed in a 1000mL round bottom flask, 600mL distilled water was added to each, and kept on an electric heating mantel set to boil (Isopad LG2ER,Tyco Thermal controls GmbH) . Dean-Stalk arm (12.5mL /0.1 mL, LAB GLASS) was fixed to the flask with a Liebig condenser (Figure 1). Temperature in the condenser was monitored by a thermocouple (HI935005, K type,Hanna) (within bottom 10cm) and water flow was controlled to maintain <80^{o}C. Distillation operated for 18 hours, oil was collected in the Dean-Stalk arm and volume was recorded every 3hrs. Samples of same timber with different weights 15g, 25g, 40g, 50g, 75g and 100g were distilled for 9hrs to obtain their percentage yields.

Particle size	Time of Extraction	% Extract in wood	% Volatiles in wood	% Volatiles in extract	% Non-volatiles in extract
> 40 mesh	1 hr.	7.24	3.83	53	47
> 40 mesh	2 hrs.	8.08	3.39	42	58
> 40 mesh	4 hrs.	7.02	2.93	41.5	58.5
> 40 mesh	8 hrs.	7.21	2.96	41	59
> 40 mesh	24 hrs.	7.31	2.92	40	60
4 – 40 mesh	1 hr.	5.58	1.89	34	66
4 – 40 mesh	2 hrs.	5.56	1.97	35.5	64.5
4 – 40 mesh	4 hrs.	5.63	2.47	44	56
4 – 40 mesh	8 hrs.	5.80	2.38	41	59
4 – 40 mesh	24 hrs.	5.77	2.36	41	59

Table 1.Variations in solvent extraction yield with change to time and particle size

Oil recovery

A similar distillation unit was employed as mentioned above, sample of wood was replaced by a Sandalwood oil samples (oil of *S.austrocaledonicum* standardised for total santalols of 33.87%). Distillation was carried out for 18 hrs, volume of oil collected in the Dean-Stalk arm was noted every three hours. This was employed to determine the recovery of volatile oil.

Figure 1.Dean-Stalk apparatus used for hydro-distillation of the sandalwood oil from milled heartwood.

Solvent extraction

Five sub-samples (1g) were taken from each of the coarse and fine samples and placed in a sintered glass vial and 10mL of *n*-hexane (HPLC grade, Lab Scan, Ireland) was added and shaken intermittently. Solven was withdrawn from one sample from each particle size after 1, 2, 4, 8 and 24 hours. Extract was filtered through a Whatman filter paper and collected to a pre-weighed vial. Hexane was evaporated under air flow for 24 hours and observed for constant weight. Dry extract obtained was weighed and dissolved in 2mL of hexane, 100µL was further diluted to 1000µL with hexane. Final concentration of the sample was measured by gas chromatography using a calibration curve.

Gas chromatography

Shimadzu GC2010 Ver.2 instrument (Shimadzu scientific, Japan) attached to a Shimadzu AOC-20i auto sampler was used. Column used was Rtx-WAX (Restek, PA, USA) 60m X 0.25mm with 1µm film thickness. 1µL of the sample was injected as a split injection with a ratio of 10. The injector port and flame ionisation detector was kept a constant temperature of 220^{o}C. Column was held at 100^{o}C for 5 min and increased by 3^{o}C/min until it attained 220^{o}C where it maintained for 25 min. The carrier gas was helium (ultra high purity grade, BOC gas, Australia) with a linear velocity of 3mL/min.

Parameter	Hydro distillation	Solvent extraction
Sample size	40g or above	~1g
Electricity consumption	significant	nil
Water consumption	significant	nil
Time	9 hrs	4 hrs
Solvent consumption	nil	modest
Non-volatile composition	nil	~50%
Auxiliary methods	nil	separation of volatiles
Actual interpretation	appropriate	non appropriate
General experimental error	very modest	significant
Human error	modest	significant
Standard methods	many	nil
Effect to oil quality	very modest	quite significant

Table 2.Comparison between the hydro-distillation an solvent extraction over 12 different parameters.

Calibration Curve

Oil obtained from hydro distillation was prepared in dilution sequence from 0.5% to 10% in 0.5% increments. Sample was analysed by gas chromatography to produce a calibration curve considering α-santalol as the internal standard. This calibration curve was used to find the concentration of volatile composition of the solvent extracts. Auto generated calibration curve (GC Lab Solutions, Shimadzu Scientific, Japan) was manually verified using Microsoft Excel©.

Results

Distillation time

An progressive increase in oil yield was observed in all timber and oil distillation experiments from 3 to 9 hours. No increase in oil yield was measured after distillation from 9 to 18 hours (Table 1). These results were reproduced in a second replicated experiment. Using the described method it is therefore proposed that hydro-distillation for 9hrs is sufficient to separate the volatile oils from timber. All the experiments hereafter were performed only for 9hrs. Shorter distillation time is beneficial to the industry, as it not only consumes less resources but it also manages to provide a result within a quicker time frame.

Sample weight and particle size for distillation

Different sample weights of the same timber were distilled for 9 hours and the oil yield estimate was highest in the 15g followed by the 25g sample. All three oil yield estimates for the 40, 50 and 75g samples were the same (Figure 2). The 100g samples and samples of fine particle size (<420 µm) samples exhibited irregular particle movement and colliding, hence the experiments were terminated to avoid any accident. With the above results 50g coarse particle sample was identified as an appropriate standard for a 1000mL capacity distillation vessel to perform hydro distillation.

Solvent extraction

Solvent extraction with hexane has shown greater variation with the time and particle size (Table 1). Solvent extracts consist of both volatile oil and non-volatile hydrocarbon soluble compounds such as waxes and lignans. The non-volatile component of solvent extracts can comprise more than 50% of the extract, which contrasts with the hydro distillation method where very little non-volatile components. Calculated percentage yield for solvent extraction varies nearly 2% v/w, which is inadequate for estimating yield of an industrial sample. Solvent extraction estimate is closest to steam distillation is when a coarse sample of 4760-420µm (4-40 mesh) used and extracted with hexane for 4 hrs in a solvent to sample ratio of 1:10. Future research can find a better suitable particle size, solvent, ratio and an extraction time.

Gas chromatography

An increase in the oil percentage was observed with solvent extraction compared with hydro distillation, since more compounds were extracted thus reducing the percentage of total santalol content. Chromatogram of the solvent extract showed higher concentration of non-polar minor compounds due to higher extraction capacity and is substantially different the chromatagram of the hydro distilled sample (Figure 3). The misinterpretation of quality using solvent extraction is disadvantageous to the industry. These results support the findings of Piggott *et al.* (1997) which revealed that steam distillation yields fewer

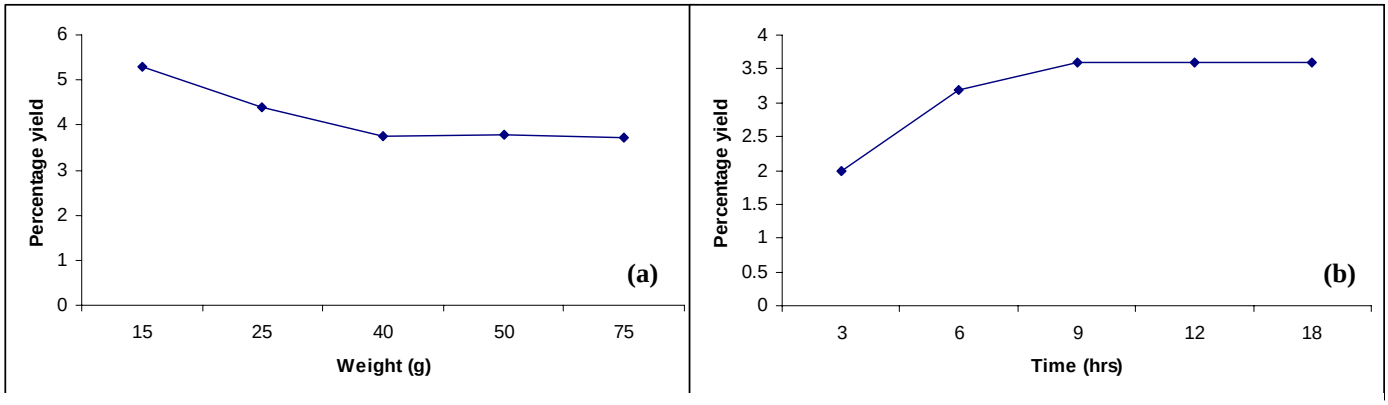

Figure 2 .Hydro-distillation volatile oil yield with (a) increasing time and (b) sample weight

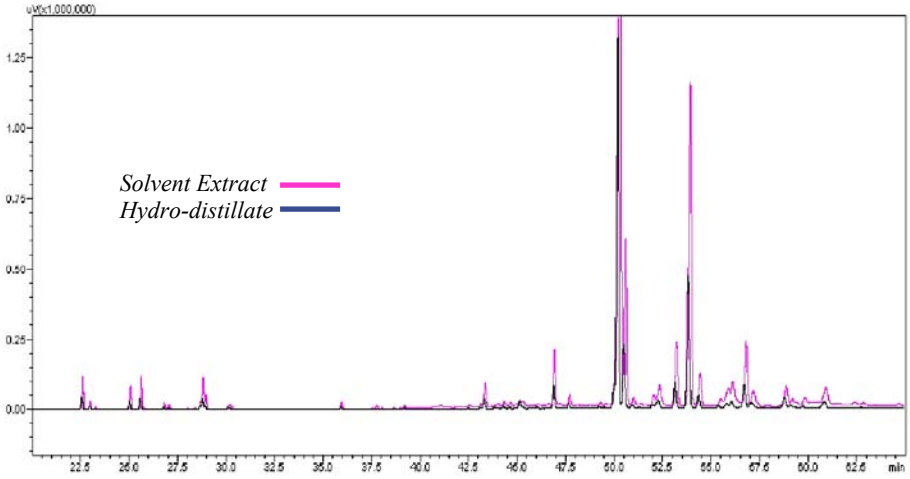

Figure 3 Gas chromatogram of the extracts obtained from the two methods.

extracts but consists of a higher percentage (>90%) of volatile oils compared with solvent extraction. Sesquiterpene analysis of the above study revealed that the percentages of major compounds in solvent extracts are nearly half of those found in the distilled oil.

Discussion

Hydro distillation is a time and resource consuming method, it is also necessary to have a much larger sample for analysis than solvent extraction. For more accurate measurement of oil yield a v/w% can be replaced by w/w% where the yielded oil will be dried and weighed. The ISO method can also be practiced followed by drying and evaporation of solvent used in oil recovery. Possible experimental error or the likelihood of human error is very high in solvent extraction method when compared to hydro distillation, a minor fault can cause a significant difference as many factors will affect the final results such as temperature, sample size, solvent ratio and frequency of particle movement where as in hydro distillation there is a broader window for experimental error (Table 2).

In this study total volatile composition of the solvent extract was determined using a calibration curve, in a routine experiment an external standard can be used instead of the internal standard method used in this study, which again is subject to human error. A more accurate method is needed to separate the volatile from the non-volatile compounds in an extract, such as headspace analysis, liquid chromatography or partial distillation analysis. These methods can verify the volatile composition accurately, but a possible drawback will be the cost effectiveness of the instrument.

Conclusion

Steam distillation is the most widely accepted commercial method for sandalwood oil extraction. Any 'bench top' analysis should therefore closely match the commercial yield rather than reporting total volatiles or extractable matter. Analysis of smaller samples from living trees provided by the plantation and forestry sector cannot practically use steam distillation method. Researchers and process operators need to continue to develop cost effective and rapid new methods for utilising small test samples to accurately estimate commercial yield and quality. Even though such a method may be developed in future, proper quality management must be enforced to minimise the experimental error. Until a generally accepted method is developed for sandalwood industry the findings of this study suggest the continued use of the standard method of hydro distillation. This conclusion can be justified, as the hydro distillation is the closest bench top method to the industrial process and less affected by experimental error when compared to solvent extraction. Hydro distillation has already been used in different standard documents in the pharmaceutical, flavour and fragrance industries, and is inarguably the most accepted method despite its high consumption of resources and time.

Acknowledgement

The author wishes to render his sincere acknowledgement to Mr. Norman Butler and
Mr. Tim Coakley of Wescorp Sandalwood Pty Ltd for there extended support and guidance given throughout this study.

References

AOAC International (2006), Volatile Oil in Spices, Official Method 962.17.

Brand JE (1999) Sandalwood (*Santalum spicatum* (R.Br.) A.DC.) excavation study *Sandalwood Research Newsletter,* 8:1-3.

British Pharmacopeia (2008), Volatile oil in Drugs, IV: Appendix XI E, The Stationary Office, London,

Erligmann A (2001) Sandalwood oils *The International Journal of Aromatherapy*, 11:4

Howes M-J.R, Simmonds MSJ and Kite GC (2004) Evaluation of sandalwood essesntial oils by gas chromatography-mass spectroscopy *Journal of Chromatography A*, 1028:307-312.

International Standards Organisation (ISO) (1984), Spices, condiments and herbs – Determination of volatile oil content, ISO 6571:2008, Geneva.

International Standards Organisation (2002), Oil of Sandalwood (*Santalum album* L.) ISO 3518:2002(E), Geneva.

Lucchesi ME, Chemat F, and Samdja J (2004) Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro distillation, *Journal of Chromatography A,* 1043:323-327

Min DB (on line resource) Flavour compounds isolation, Flavour Chemistry, Ohio State University, Ohio. http://class.fst.ohio-state.edu/fst820/820-2.pdf, Accessed 09/08/2007

Moretta P, Ghisalberti E and Trengove R (2001) Longitudinal variation in the Yield and Composition of Sandalwood Oil from *Santalum spicatum. Sandalwood Research Newsletter*, 14: 5-7.

Piggott MJ, Ghisalberti EL and Trengove RD (1997) Western Australian Sandalwood Oil: Extraction by different Techniques and Variations of the Major Components in Different Sections of a Single Tree, *Flavour and Fragrance Journal,* 12:43-46.

Standards Australia International (2003), Oil of Sandalwood [*Santalum spicatum* (R.Br) A.DC.] AS 2112-2003, Sydney.

Near Infrared Spectroscopy as a rapid method for sandalwood oil determination

B.B. Wedding[1], R.D. White[2], S. Grauf[1], B. Tilse[3], and P.A. Gadek[3]

[1] Rapid Assessment Unit, Centre for Tropical Agri-tech Research, and Innovative Food Technologies, Queensland Department of Primary Industries & Fisheries, Cairns 4870 QLD Australia
[2] Rapid Assessment Unit, Centre for Tropical Agri-tech Research, and School of Mathematics, Physics and IT, James Cook University, Townsville, 4811 QLD Australia
[3] Rapid Assessment Unit, Centre for Tropical Agri-tech Research, and School of Marine and Tropical Biology, James Cook University, Cairns QLD 4870 Australia

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.

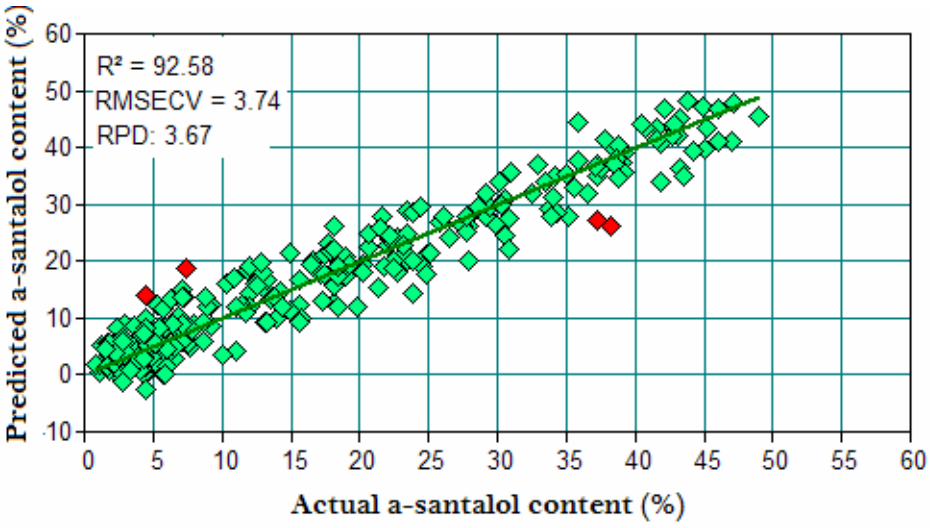

Figure 3. Predicted vs. actual *a*-santalol content for sandalwood chips using 295 samples (13 outliers removed from this dataset).

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.

References
Brunner M, Eugster R and Trenka E (1996) FT-NIR spectroscopy and wood identification. *Holzforschung*. 50: 130-134.
Howes M-J.R., Simmonds MSJ and Kite GC (2004) Evaluation of sandalwood essential oils by gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1028: 307-312 .
Schimleck, lP. Doran JC and Rimbawanto A (2003) Near infrared spectroscopy for cost effective screening of foliar oil characteristics in a *Melaleuca cajuputi* breeding population. *Journal of Agricultural and Food Chemistry*, 51: 2433-2437.
Schulz H, Baranska B, Belz HH, Rosch P, Strehle MA and Popp J (2004) Chemotaxonomic characterisation of essential oil plants by vibrational spectroscopy measurements. *Vibrational Spectroscopy,* 35: 81-86.
Steur B, Schulz H and Lager E (2001) Classification and analysis of citrus oils by NIR spectroscopy. *Food Chemistry*, 72: 113-117.

Acknowledgements
We acknowledge the financial support of James Cook University and the research group colleagues in the Rapid Assessment Unit at Cairns. The authors also wish to thank Tony Page of James Cook University, the officers at the Vanuatu Department of Forests and indigenous communities in Cape York for the organisation, collection and supply of sandalwood samples. Recognition also for Mike Russell of Wollongbar Agricultural Institute for GC analysis.

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*.

Shepherd, D.P., Fox, J. E. D., Duff, H. and Schatral, A.
Mulga Research Centre Curtin University of Technology, GPO Box U1987, Perth WA 6845, Australia. Tel: +61 8 9266 7872, Email: A.Schatral@curtin.edu.au

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

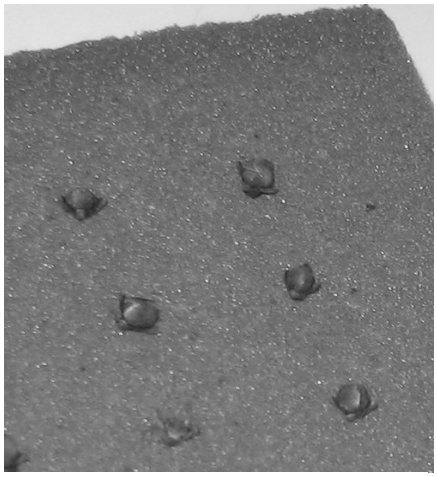

Figure 1. Excised flower buds from *S. album* positioned in OASIS® Floral foam (image is black & white) .

detailed observations on pollen viability and morphological and physiological changes in individual flowers with maturity and under different environmental conditions.

Acknowledgments
Many thanks to Mrs. Jan Barrington and Mr. William Parkinson for helping with the laboratory work. We are also very grateful to Dr. Tony Page for his critical and valuable comments on this manuscript.

References

Barrett DR (1987) Initial Observations on Flowering and Fruiting in *Santalum spicatum* (R. Br.) A.DC. The Western Australian Sandalwood. *Mulga Research Centre Journal*, 9**:** 33-37.
Bhaskar V (1992) Pollination biology and fertilization in *Santalum album* L. (Santalaceae). *Flora*, 187: 73-78.
Fox JED and Reeve PE 1992. Fruit Production on sandalwood (*Santalum spicatum*) trees. A report to the Sandalwood Research Institute, 37 pp.
Jefferies CJ, Brain P, Stott KG and Belcher AR (1982) Experimental systems and a mathematical model for studying temperature effects on pollen-tube growth and fertilization in plum. *Plant, Cell and Environment*, **5**: 231-236.
Kearns CA and Inouye DW (1993) 'Techniques for Pollination Biologists.' (University Press of Colorado: Nivot, Colorado).
Ma GH, Brunn E, Zhang JF and Wu GJ (2006) Evidence of Dichogamy in *Santalum album* L. *Journal of Integrative Plant Biology*, **48:** 300–306.
Muir K (2004) Floral Biology and Mating system in native sandalwood, *Santalum spicatum*. Honours thesis, Curtin Uni.
Rugkhla A, McComb J, Jones MGK (1997) Intra- and Inter-specific pollination of *Santalum spicatum* and *S. album*. *Australian Journal of Botany*, 45: 1083-195.
Salom SM, Broeckling CD (2003) Volatile emissions of eastern hemlock, *Tsuga Canadensis*, and the influence of hemlock wooly adelgig. *Phytochemistry* 62: 175–180.
Sedgley M (1982) Floral anatomy and pollen tube growth in the Quandong (*Santalum acuminatum*) (R. Br.) A. DC. *Australian Journal of Botany*, 30: 601–609.
Sindhuveerendra HC and Sujatha M (1989). Pollination Studies in *Santalum album* L. *Current Science*, **58:** 629-630.
Van Tuyl JM, van Dien MP, van Creij MGM, van Klienwee, TCM, Franken J and Bino RJ (1991) Application of *in vitro* pollination, ovary culture, ovule culture and embryo rescue for overcoming incongruity barriers in interspecific *Lilium* crosses. *Plant Science*, 74: 115 –126.
Wise JC, Coombs AB, Vandervoort C, Gut LJ, Hoffmann EJ and Whalen ME (2006) Use of Residue profile analysis to identify modes of insecticide activity contributing to control plum curculio in apples. *Journal of Economic Entomology*, 99: 2055–2064.

Corresponding Authors:

Dhanushka S. Hettiarachchi
Wescorp Sandalwood Pty Ltd.
PO Box 1033 Bibra Lake, Western Australia.
Tel: +61 8 9418 2888
Email: danny@wescorp.com.au

Andrea Schatral
Mulga Research Centre Curtin University of Technology,
GPO Box U1987, Perth WA 6845, Australia.
Tel: +61 8 9266 7872
Email: A.Schatral@curtin.edu.au

Brett B. Wedding
Rapid Assessment Unit, Centre for Tropical Agri-tech Research, and Innovative Food Technologies
Queensland Department of Primary Industries & Fisheries
PO Box 652 Cairns 4870 QLD Australia
Tel: +61 7 4057 3604
Email: brett.wedding@dpi.qld.gov.au

If you would like to contribute an article to the SRN or wish to be included on the SRN email alerts, please contact the editor .

Editor: Tony Page
James Cook University, PO Box 6811,Cairns, 4870 Australia
Telephone: + 61 7 4042 1673, Facsimile: + 61 7 4042 1319
Email:tony.page@jcu.edu.au

Back issues of the SRN and individual articles can be downloaded (pdf) from the SRN website: http://www.jcu.edu.au/mbil/srn/index.html

The Sandalwood Research Newsletter is produced by James Cook University, Queensland Australia.