Determination of oak lactones in barrel-aged wines and in oak extracts by stable isotope dilution analysis

Alan P. Pollnitz[a,*], Graham P. Jones[b], Mark A. Sefton[a]

[a]*The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, SA 5064, Australia*
[b]*Department of Horticulture, Viticulture and Oenology, The University of Adelaide, PMB 1, Glen Osmond, SA, 5064, Australia*

Abstract

The *cis*- and *trans*-isomers of 5-butyl-4-methyl-4,5-dihydro-2(3H)-furanone, the so-called oak lactones, are derived from oakwood, and the *cis*-isomer is an important contributor to wine flavour. Their deuterium-labelled forms, [^{2}H$_4$]*cis*-oak lactone and [^{2}H$_4$]*trans*-oak lactone, were synthesised from the unlabelled analogues, and were utilised in a new method employing gas chromatography–mass spectrometry to determine the concentration of these compounds in wine or extracts of oak shavings in a single analysis. The method can employ either liquid–liquid extraction or solid-phase microextraction, and is both rapid and accurate. There was some artefactual generation of *cis*-oak lactone during the analysis of model wine extracts of unheated oak shavings when diethyl ether extraction and injector block temperatures at or above 225°C were employed. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Wood; Wine; Food analysis; Butylmethyldihydrofuranones; Oak lactones

1. Introduction

Of the volatile components of oakwood that are extracted into wine during barrel-ageing, the *cis*-isomer of the so-called oak lactones [viz. 4S,5S-5-butyl-4-methyl-4,5-dihydro-2(3H)-furanone], is regarded as among the most important to the sensory characteristics of wine (for a comprehensive review of the literature on *cis*- and *trans*-oak lactones, see Maga [1]).

In a recent sensory study in our laboratory [2], the concentration of the *cis*-isomer was positively correlated with the aroma intensity of the "coconut" descriptor in a Chardonnay wine, and with the aroma intensity of "coconut", "vanilla", "berry" and "dark chocolate" descriptors in a Cabernet Sauvignon wine. The tasting panel also preferred wines with higher concentrations of *cis*-oak lactone. Chatonnet et al. [3] found that additions of a 1:1 mixture of *cis*- and *trans*-isomers (as a racemate) to a red wine enhanced "woody", "coconut" and "varnish" characters, but resulted in a decrease in "preference" at a concentration above 235 µg/l. Oak lactones have also been directly linked to the quality of oak-aged spirits [4,5]. Thus, the measurement of the oak lactones in wines and wood-lots is likely to be important to quality determination and to understanding the sensory characteristics of wine.

Quantification of volatiles in foodstuffs and beverages by stable isotope dilution analysis is finding increasing use [6–11]. We have developed stable

*Corresponding author. Fax: +61-8-8303-6601.
E-mail address: apollnitz@awri.adelaide.edu.au (A.P. Pollnitz)

isotope dilution assays for 2,4,6-trichloroanisole [12], vanillin [13], vanillyl ethyl ether [14] and vanillyl alcohol [14] in wines, and the technique has been used for over a decade to determine the concentration of methoxypyrazines in grapes and in wine [15]. Kotseridis and co-workers [16,17] have reported similar methods for quantitative analysis of the important grape-derived flavour compounds β-damascenone, α- and β-ionone and 2-isobutyl-3-methoxypyrazine. Recently, Hayasaka and Bartowsky [18] combined isotope dilution assays and solid-phase microextraction (SPME) to measure diacetyl in wine.

Previous analyses of oak volatiles in our laboratory [19] used large sample volumes (200 ml), exhaustive liquid–liquid extractions (1–3 days), required a concentration step and lacked precision. The method of Marsal and Sarre [20] is also time consuming and requires adjustment of the pH to 8.5, and uses diethyl ether followed by diethyl ether–hexane (1:1) as extraction solvents, factors which are conducive to artefact formation (see below and Ref. [13]). The relative standard deviations (RSDs) obtained by Marsal and Sarre [20] were 8.0% for the *cis* isomer and 9.6% for the *trans* isomer (after reassigning the isomers correctly [19]).

This paper reports a method of determination of oak lactones in wines or oak extracts by stable isotope dilution analysis. Sample preparation takes just a few minutes, requires only a small sample volume and gives high precision and accuracy.

2. Experimental

2.1. Synthesis of deuterium-labelled oak lactone

2.1.1. 4-Hydroxy-3-methyloctanoic acid, sodium salt

A racemic mixture of *cis*- plus *trans*-oak lactone (Allied Flavours, Allied Mills Industries, 20 g) was added to aqueous sodium hydroxide (18 ml, 6.5 *M*, 0.9 molar equiv.) and stirred at 75°C for 6.5 h under nitrogen with the addition of water (80 ml) after the first hour. The reaction mixture was then washed with diethyl ether (2×50 ml) to remove unreacted oak lactone, concentrated, and dried to constant mass

in vacuo to yield the sodium salt of 4-hydroxy-3-methyloctanoic acid as a white powder (22.6 g).

2.1.2. [²H₄]cis plus [²H₄]trans-5-butyl-4-methyl-4,5-dihydro-2(3H)-furanone (i.e., [²H₄]cis plus [²H₄]trans-oak lactone)

Chromium (VI) trioxide (13.4 g, 99.9%, Aldrich) was added to a solution of the sodium salt (5 g) of 4-hydroxy-3-methyloctanoic acid in anhydrous pyridine (ca. 125 ml) and stirred overnight at 25°C. The reaction mixture was cooled to 0°C and acidified to pH<1 with dilute mineral acid. The sodium hydrogencarbonate-soluble organic fraction of the crude product was isolated with ethyl acetate by liquid–liquid extraction, dried over magnesium sulphate, filtered and the filtrate distilled in vacuo with a Kugelrohr distillation apparatus to yield 3-methyl-4-oxooctanoic acid (2.1 g) as a clear liquid, m/z 172 (M$^+$, 2%), 130 (38%), 115 (17%), 112 (17%), 103 (13%), 87 (13%), 85 (100%), 57 (74%). Several batches of the keto acid were prepared in this way.

Sodium hydride (0.6 g, dry, 95%, Aldrich), then a solution of 3-methyl-4-oxooctanoic acid (2.73 g) in deuterium oxide (10 ml), was added to deuterium oxide (30 ml) and stirred at room temperature under nitrogen overnight. Sodium borodeuteride (3.0 g, 98 atom% ^{2}H, Aldrich) and deuterium oxide (20 ml) were then added and the reaction mixture was stirred at room temperature under nitrogen for a further 24 h before quenching with aqueous hydrogen chloride (32%, 10 *M*) and stirring for an additional 24 h. The crude product was isolated with diethyl ether and purified by column chromatography on silica gel 60 using hexane–diethyl ether (3:1) as eluent, yielding pure [²H₄]*trans*-oak lactone (**1**, 85 mg), a mixture of [²H₄]*cis* and [²H₄]*trans*-oak lactone (**1**+**2**, 1.31 g), and pure [²H₄]*cis*-oak lactone (**2**, 53 mg). Structures are shown in Fig. 1. All fractions were redistilled in vacuo to give clear liquids. [²H₄]*trans*-Oak lactone (**1**), m/z 160 (M$^+$, 3%), 132 (4%), 118 (6%), 102 (6.5%), 101 (100%), 90 (13%), 72 (13%), 71 (6%). [²H₄]*cis*-Oak lactone (**2**), m/z 160 (M$^+$, 3%), 132 (4%), 118 (6%), 102 (6.5%), 101 (100%), 90 (21%), 72 (21%), 71 (10%). No m/z 159 or 157 ions were detected in the mass spectra of the lactone isomers. A small m/z 158 ion in the spectra was attributed to loss of deuterium from the molecular ion, associated
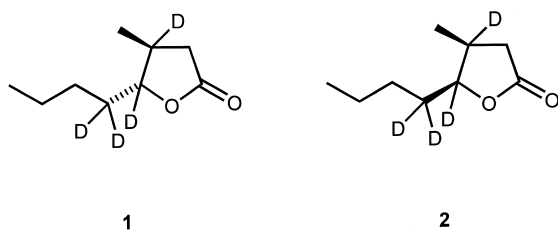
Fig. 1. Chemical structures of [²H₄]*trans*-oak lactone (**1**) and [²H₄]*cis*-oak lactone (**2**) (compounds are racemates).

with fragmentation of the C_5–H bond (furanone numbering). Analysis of a mixture of weighed aliquots of the pure isomers was used to determine the composition of the major (mixed) fraction obtained from the synthesis. This major fraction was used for all analyses of oak lactones in the wine samples.

2.2. Preparation of samples for analysis

For analysis of liquid–liquid extracts of wines or oak extracts, a solution of [²H₄]*trans*-oak lactone (3.37 μg) and [²H₄]*cis*-oak lactone (1.90 μg) in ethanol (100 μl) was added to the sample (5 ml) in a screw cap vial using a glass syringe (100 μl, Hamilton). The organic solvent (ca. 2 ml) was added and the mixture was shaken briefly. A portion of the organic layer was then placed in a vial ready for instrumental analysis. For analysis by SPME, a solution of [²H₄]*trans*-oak lactone (3.37 μg) and [²H₄]*cis*-oak lactone (1.90 μg) in ethanol (100 μl) using a glass syringe (100 μl, Hamilton), and sodium chloride (ca. 1 g) were added to the wine (5 ml) in a screw cap vial with a PTFE seal. A SPME portable field sampler (Supelco, Bellefonte, PA, USA) fitted with a 65 μm Carbowax–divinylbenzene (DVB) fibre assembly (Supelco) was used to sample the headspace above the stirred wine sample for 20 min at room temperature, immediately prior to instrumental analysis.

2.3. Instrumental analyses

Samples were analysed with either a Hewlett-Packard (HP) 5890A Series II gas chromatograph coupled to a HP 5971 mass spectrometer or with a HP 6890 gas chromatograph coupled to a HP 5973

mass spectrometer. The analytical method was the same for both gas chromatography–mass spectrometry (GC–MS) systems, except that the 6890 gas chromatograph was run in the purge splitless mode. The gas chromatograph was fitted with a 30 m×0.25 mm J&W fused-silica capillary column DB-1701, 0.25 μm film thickness. The carrier gas was helium (Air Liquide High Purity), linear velocity 31 cm/s, flow-rate 0.72 ml/min. The oven temperature was started at 50°C, held at this temperature for 1 min then increased to 250°C at 10°C/min and held at this temperature for 20 min. The injector was held at 200°C and the transfer line at 280°C. For liquid injections, the sample volume injected was 2 μl and the splitter, at 30:1, was opened after 36 s. The residence time for the needle in the injector block was 100 ms. Positive ion electron impact spectra at 70 eV were recorded in the range m/z 50–350 for scan runs.

For quantification of the oak lactones, mass spectra were recorded in the selective ion monitoring (SIM) mode. The ions monitored in SIM runs were: m/z 90, 101, 118, 132 and 160 for [²H₄]*cis* and [²H₄]*trans*-oak lactone (internal standards) and m/z 99, 114, 128 and 156 for unlabelled *cis*- and *trans*-oak lactone. Selected fragment ions were monitored for 25 ms each. The analysis by SPME was done in the same manner, except that the splitter was opened after 5 min after which the fibre was baked for at least another 10 min before retraction. The oven temperature was started at 50°C, held at this temperature for 1 min then increased to 250°C at 10°C/min and held at this temperature for 10 min. The injector was held at 220°C and the transfer line at 280°C.

2.4. Validation

The method was validated by a series of duplicate standard additions to a model wine and two red wines. The calibration curves obtained were linear, with coefficient of determination, $r^2 = 1.000$ (0, and 10 to 1000 μg/l total oak lactone, $N = 8 \times 2$) for analysis of both the *cis* ($y = 1.12x + 0.12$ for m/z 99 vs. m/z 101 and $y = 5.15x + 0.51$ for m/z 99 vs. m/z 90) and *trans* isomers ($y = 1.03x + 0.048$ for m/z 99 vs. m/z 101) in pentane extracts of a red wine, $r^2 = 1.000$ (0, and 1 to 1000 μg/l for each oak

lactone isomer, $N = 11 \times 2$) for analysis of both the isomers in pentane–diethyl ether extracts for the model wine [*cis* ($y = 1.12x$ for m/z 99 vs. m/z 101, $y = 4.99x$ for m/z 99 vs. m/z 90) and *trans* ($y = 1.07x$) for m/z 99 vs. m/z 101] and a bag in box red wine [*cis* ($y = 1.12x + 0.119$ for m/z 99 vs. m/z 101 and $y = 5.15x + 0.514$ for m/z 99 vs. m/z 90, and *trans* ($y = 1.03x + 0.0476$) for m/z 99 vs. m/z 101] and a bottled red wine [*cis* ($y = 1.10x + 0.720$ for m/z 99 vs. m/z 101 and $y = 5.11x + 3.22$ for m/z 99 vs. m/z 90), and *trans* ($y = 1.03x + 0.0804$) for m/z 99 vs. m/z 101] and $r^2 = 0.998$ (0, and 10 to 1000 µg/l total oak lactone, $N = 8 \times 2$) for SPME analysis of both the *cis* ($y = 1.12x + 0.092$ for m/z 99 vs. m/z 101 and $y = 5.21x + 0.397$ for m/z 99 vs. m/z 90) and *trans* ($y = 1.08x + 0.005$ for m/z 99 vs. m/z 101) isomers in another bottled red wine. The repeatability of the analysis at 25 µg/l and 500 µg/l was determined by spiking model, white and red wines in septuplicate. At the lower level the RSDs in model, white and red wines, respectively, were 2.9%, 2.6% and 2.3% for *trans*-oak lactone and 2.8%, 3.2% and 4.9% for *cis*-oak lactone. At the higher level the RSDs in model, white and red wines, respectively, were 1.9%, 1.9% and 2.1% for *trans*-oak lactone and 2.1%, 2.4% and 1.0% for *cis*-oak lactone.

2.5. Investigation into acid catalysed trans/cis isomerisation of oak lactone

Solutions of oak lactone (*trans/cis* = 4:1, and *trans/cis* = 1:2, each approx. 10 mg total), obtained from column chromatography, were each made up in 12% aqueous ethanol (10 ml) acidified to pH 1 with hydrochloric acid (32%, 10 *M*). The solutions were sealed in glass ampoules, and stored at 55°C for 53 days. The ampoules were then opened, and the contents analysed by GC-MS.

2.6. Studies on the artefactual generation of oak lactones during analysis

Fine shavings (1 mm thickness) were taken from six oak wood samples, two staves of *Quercus alba*, fine grain, two of *Q. alba*, medium grain, and two of chestnut oak (*Q. prinus*) all supplied by Southcorp Wines in South Australia. The species of the oak samples was determined by the suppliers, but has not

been independently confirmed by us. Half of the shavings from each oak sample was heated in a constant temperature oven at $175 \pm 1°C$ for 2 h and then allowed to cool. All 12 samples of shavings (ca. 100 g) were soaked in 1 l of model wine (10% ethanol, adjusted to pH 3.4 with potassium hydrogentartrate and tartaric acid) for one week at room temperature, after which time the shavings were removed by filtration through glass wool. Separate 5-ml aliquots from these 12 solutions were extracted and analysed in triplicate by SPME and also by liquid–liquid extraction with pentane (2 ml), pentane–diethyl ether (2:1) (2 ml) or diethyl ether (2 ml). The liquid–liquid extracts were each prepared in triplicate, and each replicate was analysed at three different injector block temperatures; 200°C, 225°C and 250°C, giving a total of 30 determinations for each model wine solution.

3. Results and discussion

3.1. The analytical method

Using [²H₄]*trans*- and [²H₄]*cis*-oak lactones (**1** and **2**, Fig. 1) as internal standards, *trans*- and *cis*-oak lactones in wines could be quantified in pentane, pentane–diethyl ether or diethyl ether extracts at concentrations down to 1 µg/l and sometimes even lower. The greatest sensitivity was obtained by monitoring the base peak fragments of the labelled and unlabelled compounds, m/z 101 and 99, respectively. These odd numbered ions are, however, common to the spectra of a number of wine components, and caution is required if they are to be used in quantification. Although smaller in comparison to the base peak, the even-number ion m/z 90 of the internal standards proved more reliable than m/z 101 for quantification. The ions 118, 132 and 160 (M⁺) in the internal standard, and 114, 128 and 156 (M⁺) in the analyte were monitored to ensure peak homogeneity. The relative ratios of these qualifier ions were used to ensure the authenticity of the analytes and standards. The stability of the deuterium label at wine pH can be assumed, as no loss of deuterium took place under the conditions of the analysis.

The oak lactones in the wines could be determined

with equal facility by SPME of the headspace above the wine. In practice, this technique gave cleaner chromatograms, but with lower sensitivity than those using the organic solvent extracts. PMDS (100 μm polydimethylsiloxane), as the bonded phase, proved to be a more efficient adsorbent of the lactones than did 65 μm Carbowax–DVB (partially crosslinked). The PMDS fibre also gave more efficient desorption in the gas chromatograph injector, because it was stable at 270°C whereas the Carbowax–DVB was stable only to 220°C. The PMDS fibre extracted an extra 10% *trans*-oak lactone relative to *cis*-oak lactone when compared to the Carbowax–DVB fibre. However, the Carbowax–DVB fibre was, in general, preferred for the analysis of oak lactones as the chromatograms were cleaner than those obtained with the PMDS fibre when real wine and oak extract samples were analysed.

Efficiency of adsorption by the SPME fibre appeared to be controlled mainly by kinetic factors, especially for the less volatile *cis*-isomer, as demonstrated by the time study shown in Fig. 2. Long adsorption times gave greater sensitivity.

Salting the wine extracts prior to SPME gave recoveries 2.5 times better than unsalted wine. The more volatile *trans* isomer was salted out 1.14-times more readily than the *cis* isomer. Stirring the wine extracts during SPME gave approximately 10% higher recoveries for extractions up to 45 min
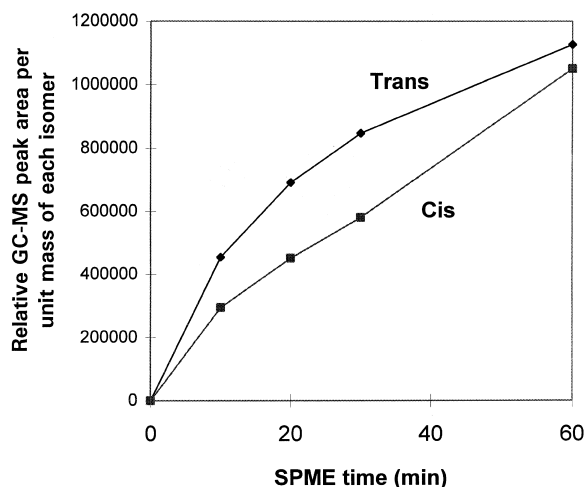
duration, but made no detectable difference after 60 min.

In practice, an absorption time of 20 min allowed for a level of detection below 1 μg/l and accuracy of the analysis was not compromised as the internal standards were absorbed at the same rate as their non-isotopically labelled analogues.

Analysis of a mixture of *cis*- and *trans*-oak lactones made from the pure isomers showed that the relationship between the amount of each isomer present, and the peak area in both the total and single ion current chromatograms varied according to the operating conditions of the GC–MS system. Similarly, the ratio of the intensity of common fragments for labelled and unlabelled analogues of either the *cis*- or *trans*-isomer also varied. It is therefore important to know the proportion of *cis*- and *trans*-isomers for both labelled and unlabelled lactones in the standards used to calibrate the analytical method by determining the molar ion response factors under the same instrumental conditions as the analyses of each set of samples.

3.2. Investigation into acid catalysed trans/cis isomerisation of oak lactone

Two model wine samples of the oak lactones, enriched in the *cis*- and *trans*-isomers, respectively, were heated to 55°C at pH 1 for 53 days. No change in the isomer ratios was observed during this time and no other products were formed. Significant acid-catalysed *cis/trans* isomerization does not, therefore, take place in the weakly acidic medium of wines and spirits during preparation of samples for analysis. Indeed, such acid catalysed isomerisation will also not take place during normal storage, even over prolonged periods, as has been suggested elsewhere [21,22].

3.3. Artefactual formation of oak lactones during analysis

To test the possibility that oak lactone was released from bound forms by thermal degradation in the gas chromatograph injector block during the analysis, model wine extracts of oak shavings were analysed under a variety of conditions. Each model wine extract was analysed in triplicate via SPME. In

Fig. 2. Solid-phase microextraction of oak lactone isomers from model wine solution over time.

addition, triplicate extracts of the model wine solutions with each of three organic solvent systems, namely pentane, pentane–diethyl ether (2:1) and diethyl ether were prepared, and each of these nine extracts was, in turn, injected into the gas chromatograph at three different injector block temperatures; 200°C, 225°C and 250°C. There was no significant difference, for any of the model wine oak extracts, in the concentration of oak lactones determined by SPME or by liquid–liquid extraction with pentane or pentane–diethyl ether (2:1) at an injector temperature of 200°C. There were slight but statistically significant increases ($P < 0.05$) in the *cis*-oak lactone concentration determined when pentane–diethyl ether (2:1) or diethyl ether was employed as an extracting solvent and the gas chromatograph injector temperature was 225°C or 250°C, with the largest increases (12%) ($P < 0.001$) occurring for diethyl ether solutions injected at 250°C (Fig. 3). These results indicate that the more polar solvent, diethyl ether, may be extracting one or more conjugates of *cis*-oak lactone from the model wine oak extracts, and these conjugates can generate *cis*-oak lactone at high injector block temperatures. Thus, regardless of the internal standard used, care should be taken to avoid the use of polar organic solvents for extraction

of samples for analysis of oak lactones by GC, and high injector temperatures should also be avoided.

3.4. Determination of trans- and cis-oak lactones in wood samples

Mean results are shown in Table 1 for the determinations of *trans*- and *cis*-oak lactones in wood samples by SPME and by liquid–liquid extraction [pentane–diethyl ether (2:1), injection at 200°C]. All concentrations are in μg of oak lactone per gram of unheated oak wood. RSDs for these determinations ranged from 0.36% up to 2.34%.

There was approximately 10-times more *trans*-oak lactone found in the chestnut oak samples than in the *Q. alba* samples. Similar amounts of *cis*-oak lactone were found in all the samples, except for *Q. alba* fine grain B which had about four-times more *cis*-oak lactone than the other oak wood samples.

Heating the wood slightly decreased the amounts of *cis* and *trans* oak lactone found in most samples. This might have been a result of driving off a small percentage of the oak lactone from the finely shaved oak samples during heating.

The ratios of *cis/trans* isomers found in the *Q. alba* samples are in agreement with Waterhouse and
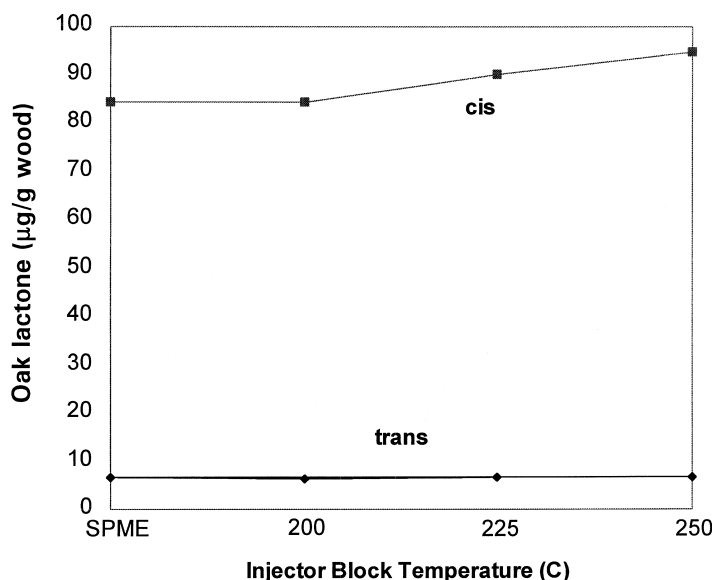

Fig. 3. Diethyl ether extracts of unheated *Q. alba*, fine grain stave B (each data point is a mean of three determinations).

Table 1
Determination of *trans*- and *cis*-oak lactones in wood samples[a]

	trans-Oak lactone (μg/g oak)	*cis*-Oak lactone (μg/g oak)	Ratio *cis/trans*
Q. alba fine grain stave A, unheated	1.98	14.0	7.07
Q. alba fine grain stave B, unheated	6.65	86.0	12.9
Q. alba medium grain stave A, unheated	3.94	15.2	3.86
Q. alba medium grain stave B, unheated	1.69	22.6	13.4
Chestnut oak stave A, unheated	36.6	19.4	0.53
Chestnut oak stave B, unheated	40.6	19.1	0.47
Q. alba fine grain stave A, heated	1.65	11.0	6.66
Q. alba fine grain stave B, heated	5.33	73.4	13.8
Q. alba medium grain stave A, heated	3.93	14.9	3.79
Q. alba medium grain stave B, heated	1.47	14.9	10.1
Chestnut oak stave A, heated	42.2	21.6	0.51
Chestnut oak stave B, heated	35.5	16.0	0.45

[a] Mean results (of six determinations) are shown in Table 1 for the determinations by SPME and by liquid–liquid extraction [pentane–diethyl ether (2:1), injection at 200°C]. Concentrations are expressed as μg oak lactone per gram of unheated oak shavings. RSDs for these determinations ranged from 0.36% up to 2.34%.

Towey [23], Guichard et al. [24], Towey and Waterhouse [25], Swan [26], Maga [1] and within the typical range of samples analysed in our laboratory in conjunction with industry trials.

In the chestnut oak samples, however, there was about twice as much *trans*-oak lactone as *cis*-oak lactone. This finding has not been previously reported and is in contrast with the findings of Swan [26] who reports ratios of *cis/trans* from 2 to 1 up to 4 to 1 in chestnut oaks. Furthermore, in none of the oak wood species analysed by Waterhouse and Towey [23] was the ratio of *cis/trans* less than or equal to one, and for all their American wood samples (except the Oregon oak *Q. garryana*) the ratios were within the range of 5 to 8 *cis* to 1 *trans*. Similarly, Maga [1] does not report more *trans* than *cis* in any example, except when citing earlier literature where the isomers were incorrectly assigned.

4. Conclusions

The analytical method described here is fast, precise, accurate and reliable. Combined with automated instrumental analysis, the method has enabled our laboratory to measure oak lactones in numbers that were hitherto impossible. The method is now widely used to support oak-barrel quality trials throughout the Australian wine industry and could prove equally useful in research and quality management for barrel-aged spirits.

References

[1] J.A. Maga, Food Rev. Int. 12 (1996) 105.
[2] P.J. Spillman, K.F. Pocock, R. Gawel, M.A. Sefton, in: C.S. Stockley, R.S. Johnson, A. Sas, T.H. Lee (Eds.), Proceedings of the 9th Australian Wine Industry Technical Conference, Winetitles, Hyde Park Press, Adelaide, July 1995, p. 66.
[3] P. Chatonnet, D. Dubourdieu, J.N. Boidron, Sci. Aliments 10 (1990) 565.
[4] K. Otsuka, Y. Zenibayashi, M. Itoh, A. Totsuka, Agric. Biol. Chem. 38 (1974) 485.
[5] G.H. Reazin, Am. J. Enol. Vitic. 32 (1981) 283.

[6] H. Guth, W. Grosch, Lebensm. Wiss. Technol. 23 (1990) 513.

[7] C. Masanetz, I. Blank, W. Grosch, Flavour Fragr. J. 10 (1995) 9.

[8] P. Schieberle, W. Grosch, J. Agric. Food Chem. 35 (1987) 252.

[9] P. Semmelroch, G. Laskawy, I. Blank, W. Grosch, Flavour Fragr. J. 10 (1995) 1.

[10] A. Sen, G. Laskawy, P. Schieberle, W. Grosch, J. Agric. Food Chem. 39 (1991) 757.

[11] A. Sen, W. Grosch, Z. Lebensm. Unters. Forsch. 192 (1991) 541.

[12] A.P. Pollnitz, K.H. Pardon, D. Liacopoulos, G.K. Skouroumounis, M.A. Sefton, Aust. J. Grape Wine Res. 2 (1996) 184.

[13] P.J. Spillman, A.P. Pollnitz, D. Liacopoulos, G.K. Skouroumounis, M.A. Sefton, J. Agric. Food Chem. 45 (1997) 2584.

[14] P.J. Spillman, A.P. Pollnitz, K.H. Pardon, D. Liacopoulos, M.A. Sefton, J. Agric. Food Chem. 46 (1998) 663.

[15] R.L.N. Harris, M.J. Lacey, W.V. Brown, M.S. Allen, Vitis 26 (1987) 201.

[16] Y. Kotseridis, R. Baumes, G.K. Skouroumounis, J. Chromatogr. A 824 (1998) 71.

[17] Y. Kotseridis, A. Anocibar Beloqui, A. Bertrand, J.P. Doazan, Am. J. Enol. Vitic. 49 (1998) 44.

[18] Y. Hayasaka, E. Bartowsky, J. Agric. Food. Chem. 47 (1999) 612.

[19] M.A. Sefton, I.L. Francis, K.F. Pocock, P.J. Williams, Sci. Aliments 13 (1993) 629.

[20] F. Marsal, Ch. Sarre, Connaissance Vigne Vin 21 (1987) 71.

[21] P. Chatonnet, Ph.D. Dissertation, Université de Bordeaux II, Bordeaux, 1991.

[22] J.R. Piggott, J.M. Conner, J.L. Melvin, in: G. Charalambous (Ed.), Food Flavours – Generation, Flavours and Food Process Influence, Elsevier, Amsterdam, 1995, p. 1695.

[23] A.L. Waterhouse, J.P. Towey, J. Agric. Food Chem. 42 (1994) 1971.

[24] E. Guichard, N. Fournier, G. Masson, J.-L. Puech, Am. J. Enol. Vitic. 46 (1995) 419.

[25] J.P. Towey, A.L. Waterhouse, Am. J. Enol. Vitic. 47 (1996) 163.

[26] J. Swan, Wines Vines June (1997) 26.