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# Rapid extraction of aroma compounds in must and wine by means of ultrasound

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The aromatic compounds of must and wine were extracted with a new rapid method using ultrasound. The new technique, showing good recovery, linearity and reproducibility for most of the compounds, together with rapidity and simplicity, has proven itself to be better than resin ($C_{18}$) extraction. Suitable operating procedures (sample volume, temperature and extraction conditions) gave very good results which, coupled with shorter operating times, allows the method to be applied routinely, particularly for rapid screening in wine yeast selection.

## INTRODUCTION

Identification of aroma compounds in grape musts and/or in fermented samples is one of the most important steps in the evaluation of aromatics in musts and quality of wines as well as in the selection of wine yeast strains. Laboratory screening experiments with several yeast strains during the selection phase usually require high analysis costs and long operating times (Suomalainen & Matti, 1978; Di Stefano *et al.*, 1981), the reduction of which needs an extraction method that is as rapid and simple as possible.

Taking into consideration all the sample preparation techniques in use so far Amati *et al.*, 1973; Di Stefano *et al.*, 1981; Williams *et al.*, 1982; Simpson & Miller, 1983; Di Stefano 1985; Gunata *et al.*, 1985; Versini, 1985; Baumes *et al.*, 1986; Etievant, 1987; Herraiz *et al.*, 1989; Edwards & Beelman, 1990; Gianotti & Di Stefano,1991; Herraiz *et al.*, 1991; Gerbi *et al.*, 1992; Abbot *et al.*, 1993; Dirninger *et al.*, 1993; Gerbi & Zeppa, 1993; Moio *et al.*, 1993; Razungles *et al.*, 1993), sonication has proven very useful in giving a rapid extraction of the aromatics from musts and wines.

## MATERIALS AND METHODS

### Reagents and chemicals

Methanol, dichloromethane and pentane were obtained from Merck, Darmstadt, Germany; bidistilled water was used.

Two standard mixtures were used: A in absolute ethanol and B in dichloromethane (Merck, Darmstadt, Germany; Fluka, Buchs, Switzerland; Carlo Erba, Milan, Italy).

### Grape musts and wines

- Musts: Moscato di Canelli.
- Wines: Moscato di Chambave, Blanc de Morgex-La Salle, fermented by different yeast strains (*Saccharomyces cerevisiae* ABM15, ABM47, ABM135, S189c (before malolactic fermentation), S189c M (after malolactic fermentation), AM 18, AM 46, AM 48, AM 49, AM 81, AM 211, AM 218). Table 1 reports the composition.
- Synthetic Nutrient Medium (MNS) according to Delfini (Delfini, 1982).

### Ultrasound (US) apparatus

A US bath Bransonic Mod 220, US fixed-frequency 48 kHz ± 10%, equipped with a 2-litre vessel, was used.

### Solid phase extraction (SPE) columns

J. T. Baker Inc. extraction columns 'Bakerbond' spe Octadecyl ($C_{18}$), packed with reversed phase octadecylsilane ($C_{18}$) bonded to silica gel (40 μm APD, 6 nm), were used.

### Apparatus and chromatographic conditions

The gas chromatographic analysis was performed with a Carlo Erba HRGC 5300 Mega series gas chromatograph equipped with a flame ionisation detector (FID).

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**Table 1. Composition of musts and wines used in the experiments**

| | d 20°C | pH | Total acidity (meq) | Volatile acidity (meq) | Ethanol (%) |
|---|---|---|---|---|---|
| *Must* | | | | | |
| Moscato di Canelli: | 1·0752 | 3·36 | 95 | 0·00 | 0·60 |
| *Wines* | | | | | |
| Blanc de Morgex-La Salle: | | | | | |
| ABM 15 | | 3·30 | 113 | 3·3 | 9·58 |
| ABM 47 | | 3·30 | 111 | 2·5 | 10·40 |
| ABM 135 | | 3·31 | 113 | 5·0 | 9·58 |
| ABM 138 | | 3·39 | 108 | 5·0 | 9·66 |
| S189c | | 3·35 | 97 | 8·3 | 9·58 |
| S189c (with malolactic bacteria) | | 3·60 | 79 | 11·7 | 9·58 |
| Moscato di Chambave: | | | | | |
| AM 48 | | 3·34 | 101 | 6·8 | 11·49 |
| AM 49 | | 3·32 | 114 | 10·4 | 11·61 |
| AM 81 | | 3·25 | 122 | 8·7 | 11·41 |
| AM 218 | | 3·28 | 124 | 8·2 | 11·61 |

The column was a Supelcowax capillary column (Supelco) (30 m × 0·25 mm i.d., film thickness 0·25 $\mu$m). Operating conditions were as follows: injector and detector temperature 250°C; split ratio 1 : 20; carrier gas, $H_2$, 1 ml min$^{-1}$; oven temperature programme, 10 min at 40°C then 3·5°C min$^{-1}$ up to 210°C and finally 10 min at 210°C; volume injected, between 1·0 and 1·5 $\mu$l.

The chromatographic data were analysed on a Varian STAR 2000 GC Star Workstation.

## Ultrasound extraction

The absorption coefficients, and the velocity of mechanical waves, depend on liquid composition, the frequency, the temperature and probably the sample volume. The extraction solvent, sample volume and temperature for the optimum extraction efficiency (grape juice, wine and MNS), were verified in wines at two different temperatures (10 and 20°C) by extracting a volume of 50, 100 and 200 ml, respectively. In this test, 20 ml of standard solution A were added to 2 litres of Blanc de Morgex-La Salle wine. The procedure is as follows:

*Experiment at 20°C*: 50, 100 and 200 ml of the sample, put respectively into a 200, 300 and 500 ml spherical flask were extracted by means of ultrasound during 10 min with, respectively, 15, 30 and 60 ml of dichloromethane. After separation, the organic layers were dried on anhydrous sodium sulphate and transferred to a vacuum flask. The second and third extractions were performed with 5, 10 and 20 ml of dichloromethane, respectively; the organic layers were collected in the same flask. The extracts, concentrated to a final volume of about 100 $\mu$l in a rotary evaporator (at 40°C without vacuum) were ready for gas chromatographic (GC) analyses.

*Experiment at 10°C*: the above experiment was repeated in a refrigerator at 10°C with precooled ultrasound equipment and sample.

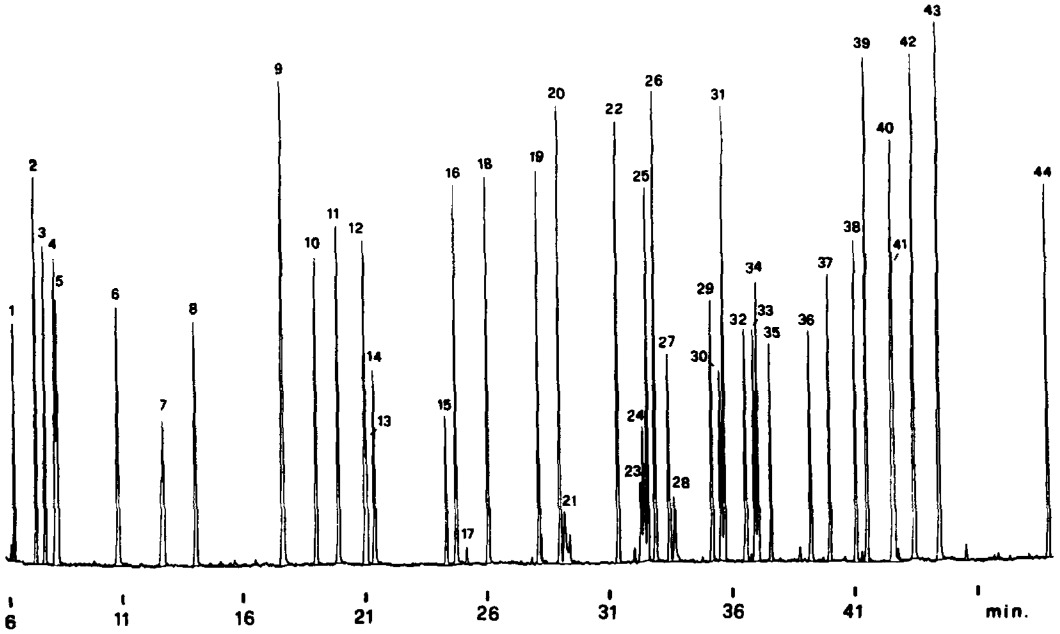

**Fig. 1.** Chromatogram of standard mixture A (1) 2,3-butanedione; (2) isobutyl acetate; (3) ethyl butyrate; (4) l-propanol; (5) 2-butanol; (6) 2-methyl-l-propanol; (7) isoamyl acetate; (8) l-butanol; (9) 3-methyl-1-butanol and 2-methyl-l-butanol; (10) ethyl hexanoate; (11) 1-pentanol; (12) hexyl acetate; (13) ethyl pyruvate; (14) 3-hydroxy-2-butanone; (15) ethyl lactate; (16) 1-hexanol; (17) trans-3-hexen-l-ol; (18) cis-3-hexen-1-ol; (19) ethyl octanoate; (20) 1-heptanol; (21) acetic acid; (22) benzaldehyde; (23) propionic acid; (24) (-) -butan-2,3-diol; (25) linalool; (26) 1-octanol; (27) isobutyric acid; (28) (R,S)-butan-2,3-diol; (29) $\gamma$-butyrolactone; (30) butyric acid; (31) ethyl decanoate; (32) furfuryl alcohol; (33) isovaleric acid; (34) diethyl succinate, (35) $\alpha$-terpineol, (36) valeric acid; (37) citronellol; (38) nerol; (39) phenethyl acetate; (40) hexanoic acid; (41) geraniol; (42) benzyl alcohol; (43) 2-phenyl ethanol; (44) octanoic acid.

*Comparison of the methods:* 50 ml of a wine, 50 ml of a grape must and 50 ml of the MNS were extracted by means of sonication a 20°C in comparison with the extraction resin ($C_{18}$) method (Gianotti & Di Stefano, 1991).

## Extraction with resin ($C_{18}$)

A reversed phase octadecylsilane ($C_{18}$, particle size distribution <64 $\mu$m) column was conditioned with 10 ml $CH_3OH$, 10 ml $H_2O$, 20 ml $CH_2Cl_2$, 10 ml $CH_3OH$ and finally with 5 ml water. A sample of 20 ml of wine, diluted 1 : 1 with water to which 200 $\mu$l of 0·360 mg ml$^{-1}$ solution of 1-octanol in ethanol as internal standard had been added, was allowed to percolate through the resin to a complete absorption. Sugars and other water-soluble compounds were removed by rinsing the column with 20 ml of water; the organics were then eluted by gravity with 40 ml of dichloromethane. The dichloromethane extract was dried on anhydrous sodium sulphate and concentrated to a final volume of 100 $\mu$l in a rotary evaporator at 40°C without vacuum. The column, washed with 20 ml methanol and 5 ml water, was ready for re-utilisation.

## Statistical analysis of data

The wines obtained by different yeast strains were extracted with both methods and data were treated with the multifactor analysis of variance using the STATGRAPHICS 5-PLUS (STSC, Inc.) software.

## Evaluation of aromatic compounds

Identification was made from both retention data and peak enhancement with authentic materials by spiking the samples with standard solution B.

To verify the reproducibility of the ultrasound method at 20°C, the test was repeated five times on a sample of

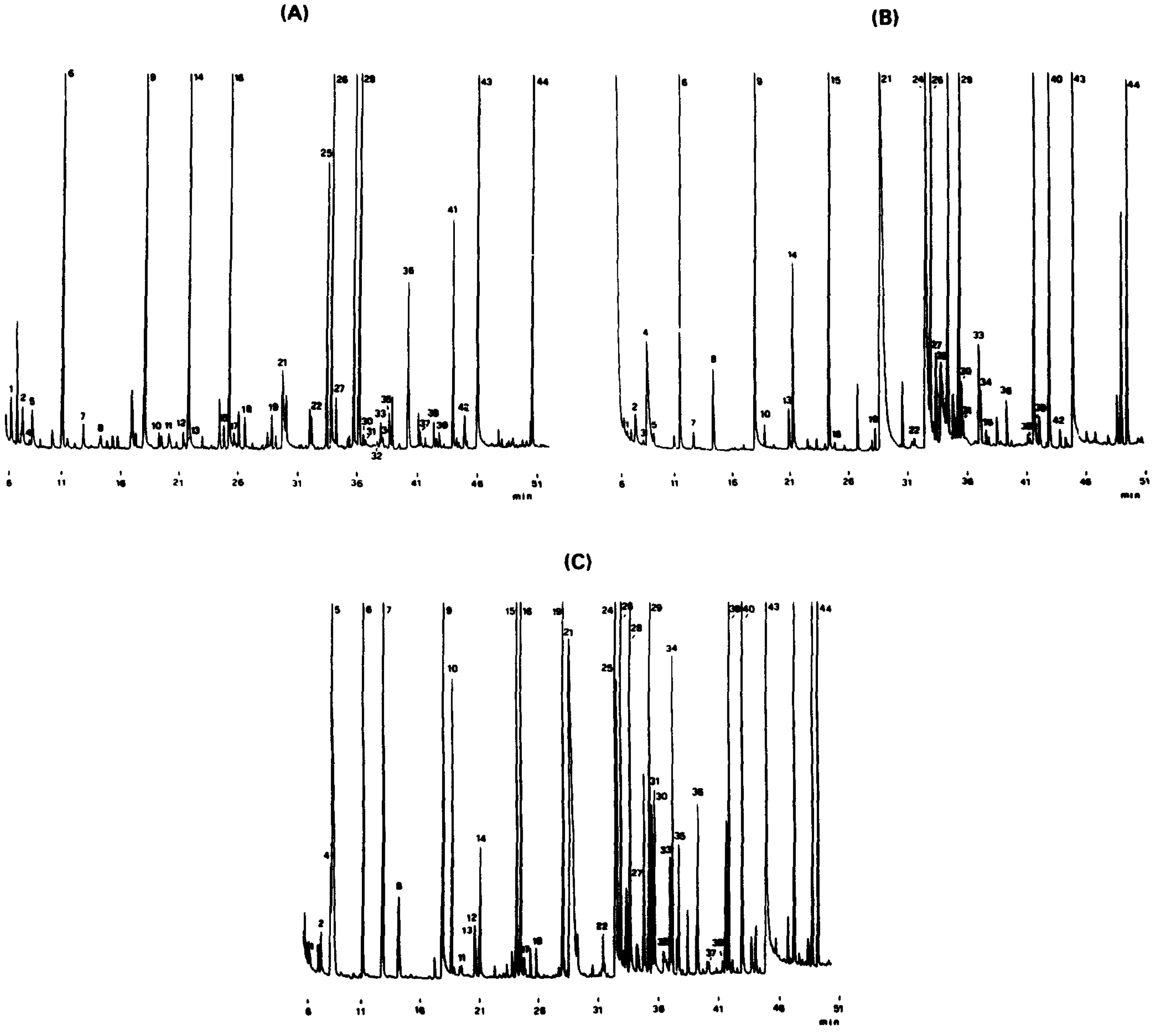

Fig. 2. Example of an aroma chromatogram in a sample must (A), in a sample MNS (B), in a sample wine (C) (to identify peaks, see Fig. 1).

Blanc de Morgex-La Salle wine, IS solution being added to give a final concentration of 2·88 mg litre$^{-1}$.

In order to verify the repeatability, linearity and the percentage recovery, 0·5, 1, 1·5 and 2 ml of standard solution A were added to four samples of the above wine. All the samples were extracted at 20°C using the ultrasound method, repeating the gas chromatographic determination for each extraction fraction twice.

## RESULTS AND DISCUSSION

The standard solution (B) chromatogram appears in Fig. 1. The gas chromatographic conditions chosen allow the separation of most compounds. Ethyl pyruvate, propionic acid and hexanoic acid were not completely separated from hexyl acetate, (-)-butan-2,3-diol and geraniol, respectively, due to their very close retention times.

Chromatograms of must, MNS and different wines are reported in Figs 2(a)–(c).

The reproducibility of the chromatographic method can be seen in Table 2, in which the mean value, the range of eight estimations, the standard deviation and the coefficient of variation of each response factor are listed: the highest coefficients of variation were those of trans-3-hexen-1-ol, l-heptanol, (-)-butan-2,3-diol, furfuryl alcohol, nerol and hexanoic acid (from 10% to 14%).

### Extraction efficiency

The method proved rapid. Repeatability and linearity of the results are shown in Tables 3 and 4.

The precision of the ultrasound technique, as measured by the coefficients of variation of the main volatiles, is illustrated in Table 3. Generally, the coefficient of variation for any compound was less than 9%, the major exceptions being isobutyl acetate which approached 25%, 1-propanol (16%), 2-methyl-1-propanol and l-butanol (about 11%).

Concerning the linearity, the method generally gave

**Table 2. Reproducibility of the chromatographic method**

| Peak table | Mean (mg litre$^{-1}$) | Range of eight estimations | Standard deviation | Coefficient of variance |
|---|---|---|---|---|
| 2,3-butanedione | 3·28 | 2·89–3·49 | 0·175 | 5·35 |
| isobutyl acetate | 1·20 | 1·17–1·22 | 0·016 | 1·32 |
| ethyl butyrate | 1·07 | 1·04–1·09 | 0·015 | 1·41 |
| 1-propanol | 1·14 | 1·09–1·18 | 0·035 | 3·04 |
| 2-butanol | 1·27 | 1·26–1·29 | 0·013 | 1·03 |
| 2-methyl-1-propanol | 0·89 | 0·86–0·91 | 0·014 | 1·61 |
| isoamyl acetate | 0·92 | 0·90–0·95 | 0·019 | 2·07 |
| 1-butanol | 1·11 | 1·08–1·13 | 0·019 | 1·65 |
| 3+2-methyl-1-butanol | 0·96 | 0·94–0·99 | 0·016 | 1·71 |
| ethyl haxanoate | 1·16 | 1·13–1·17 | 0·016 | 1·36 |
| 1-pentanol | 1·14 | 1·09–1·18 | 0·028 | 2·44 |
| ethyl pyruvate | 1·07 | 1·03–1·11 | 0·026 | 2·45 |
| 3-hydroxy-2-butanone | 1·00 | 0·97–1·02 | 0·017 | 1·65 |
| ethyl lactate | 2·85 | 2·74–2·91 | 0·053 | 1·85 |
| 1-hexanol | 0·99 | 0·98–1·01 | 0·013 | 1·29 |
| *trans*-3-hexen-1-ol | 1·03 | 0·85–1·18 | 0·105 | 10·26 |
| *cis*-3-hexen-1-ol | 0·98 | 0·97–0·99 | 0·008 | 0·77 |
| ethyl octanoate | 1·05 | 1·01–1·07 | 0·021 | 2·03 |
| 1-heptanol | 0·92 | 0·74–1·00 | 0·105 | 11·47 |
| acetic acid | 1·96 | 1·77–2·20 | 0·166 | 8·47 |
| benzaldehyde | 1·16 | 1·13–1·19 | 0·020 | 1·76 |
| (-)-butan-2,3-diol | 1·69 | 1·40–1·99 | 0·214 | 12·64 |
| linalool | 1·41 | 1·35–1·49 | 0·042 | 2·98 |
| (R,S)-butan-2,3-diol | 1·70 | 1·52–1·88 | 0·130 | 7·66 |
| isobutyric acid | 1·63 | 1·60–1·68 | 0·031 | 1·89 |
| τ-butyrolactone | 1·79 | 1·76–1·82 | 0·020 | 1·10 |
| butyric acid | 1·59 | 1·55–1·68 | 0·051 | 3·20 |
| ethyl decanoate | 1·06 | 1·01–1·08 | 0·020 | 1·89 |
| furfuryl alcohol | 2·86 | 2·36–3·36 | 0·315 | 11·01 |
| isovaleric acid | 1·33 | 1·29–1·40 | 0·037 | 2·80 |
| diethyl succinate | 1·81 | 1·78–1·84 | 0·017 | 0·94 |
| *α*-terpineol | 0·78 | 0·74–0·81 | 0·024 | 3·09 |
| valeric acid | 1·34 | 1·30–1·40 | 0·033 | 2·46 |
| citronellol | 1·50 | 1·40–1·61 | 0·067 | 4·48 |
| nerol | 1·40 | 1·21–1·66 | 0·158 | 11·28 |
| phenethyl acetate | 1·18 | 1·17–1·20 | 0·010 | 0·85 |
| hexanoic acid | 0·82 | 0·72–0·98 | 0·113 | 13·75 |
| geraniol | 1·79 | 1·66–1·91 | 0·122 | 6·79 |
| benzyl alcohol | 1·08 | 1·07–1·12 | 0·015 | 1·36 |
| 2-phenyl ethanol | 1·02 | 0·93–1·08 | 0·045 | 4·41 |
| octanoic acid | 1·26 | 1·15–1·39 | 0·083 | 6·61 |

**Table 3. Repeatability of the ultrasound extraction method**

| Peak table | Mean (mg litre$^{-1}$) | Range of four estimations | Standard deviation | Coefficient of variance |
|---|---|---|---|---|
| 2,3-butanedione | 0·32 | 0·30– 0·34 | 0·020 | 6·23 |
| isobutyl acetate | 0·50 | 0·38– 0·67 | 0·129 | 25·46 |
| 1-propanol | 3·49 | 2·84– 4·24 | 0·576 | 16·47 |
| 2-methyl-1-propanol | 9·59 | 8·10–10·46 | 1·087 | 11·34 |
| isoamyl acetate | 3·45 | 3·09– 3·76 | 0·296 | 8·56 |
| 1-butanol | 0·42 | 0·36– 0·46 | 0·048 | 11·45 |
| 3+2-methyl-1-butanol | 85·08 | 77·11–91·67 | 6·508 | 7·65 |
| ethyl hexanoate | 0·52 | 0·47– 0·56 | 0·044 | 8·40 |
| ethyl pyruvate | 1·37 | 1·26– 1·44 | 0·080 | 5·83 |
| 3-hydroxy-2-butanone | 9·74 | 8·95–10·54 | 0·725 | 7·44 |
| ethyl lactate | 41·26 | 38·43–44·45 | 2·673 | 6·48 |
| 1-hexanol | 1·37 | 1·30– 1·41 | 0·051 | 3·74 |
| *cis*-3-hexen-1-ol | 0·29 | 0·26– 0·31 | 0·021 | 7·14 |
| ethyl octanoate | 0·95 | 0·87– 1·03 | 0·073 | 7·68 |
| 1-heptanol | 5·94 | 5·54– 6·54 | 0·472 | 7·95 |
| (-)-butan-2,3-diol | 33·39 | 30·93–34·92 | 1·717 | 5·14 |
| (R,S)-butan-2,3-diol | 13·41 | 12·62–14·77 | 0·934 | 6·96 |
| isobutyric acid | 0·62 | 0·60– 0·64 | 0·018 | 2·97 |
| $\tau$-butyrolactone | 3·51 | 3·31– 3·75 | 0·195 | 5·55 |
| butyric acid | 0·70 | 0·68– 0·75 | 0·033 | 4·68 |
| ethyl decanoate | 0·43 | 0·38– 0·46 | 0·039 | 9·01 |
| isovaleric acid | 0·44 | 0·42– 0·48 | 0·028 | 6·29 |
| diethyl succinate | 0·72 | 0·69– 0·78 | 0·046 | 6·35 |
| valeric acid | 1·33 | 1·28– 1·36 | 0·037 | 2·79 |
| phenethyl acetate | 2·44 | 2·36– 2·57 | 0·095 | 3·88 |
| hexanoic acid | 1·20 | 1·14– 1·32 | 0·087 | 7·27 |
| benzyl alcohol | 0·28 | 0·27– 0·30 | 0·013 | 4·60 |
| 2-phenyl ethanol | 74·36 | 71·31–77·53 | 2·864 | 3·85 |
| octanoic acid | 11·71 | 11·30–12·06 | 0·335 | 2·86 |

**Table 4. Linearity of the ultrasound extraction method**

| Peak table | Correlation coefficient | Standard error of estimate | *R*-squared % |
|---|---|---|---|
| 2,3-butanedione | 0·990 | 0·108 | 98·03 |
| isobutyl acetate | 0·998 | 0·115 | 99·57 |
| ethyl butyrate | 0·998 | 0·021 | 99·51 |
| 1-propanol | 0·891 | 0·107 | 79·37 |
| 2-butanol | 0·985 | 0·277 | 96·96 |
| 2-methyl-1-propanol | 0·968 | 0·250 | 93·64 |
| isoamyl acetate | 0·986 | 0·256 | 97·22 |
| 1-butanol | 0·999 | 0·042 | 99·71 |
| 3+2-methyl-1-butanol | 0·947 | 1·981 | 89·63 |
| ethyl hexanoate | 0·998 | 0·075 | 99·69 |
| 1-pentanol | 0·999 | 0·040 | 99·92 |
| ethyl pyruvate | 0·999 | 0·050 | 99·94 |
| 3-hydroxy-2-butanone | 0·974 | 0·274 | 94·95 |
| ethyl lactate | 0·963 | 0·690 | 92·75 |
| 1-hexanol | 0·999 | 0·067 | 99·82 |
| *trans*-3-hexen-1-ol | 0·974 | 0·007 | 94·89 |
| *cis*-3-hexen-1-ol | 0·999 | 0·019 | 99·92 |
| ethyl octanoate | 0·977 | 0·345 | 95·39 |
| acetic acid | 0·997 | 0·049 | 99·46 |
| benzaldehyde | 0·991 | 0·488 | 98·17 |
| linalool | 0·999 | 0·045 | 99·96 |
| isobutyric acid | 0·999 | 0·031 | 99·93 |
| $\tau$-butyrolactone | 0·998 | 0·159 | 99·61 |
| butyric acid | 0·999 | 0·045 | 99·81 |
| ethyl decanoate | 0·986 | 0·079 | 97·25 |
| furfuryl alcohol | 0·999 | 0·636 | 99·89 |
| isovaleric acid | 0·998 | 0·085 | 99·58 |
| diethyl succinate | 0·999 | 0·061 | 99·91 |
| $\alpha$-terpineol | 0·999 | 0·039 | 99·97 |
| valeric acid | 0·999 | 0·054 | 99·90 |
| citronellol | 0·996 | 0·168 | 99·30 |
| nerol | 0·998 | 0·164 | 99·53 |
| phenethyl acetate | 0·979 | 0·682 | 95·81 |
| geraniol | 0·986 | 0·570 | 97·26 |
| benzyl alcohol | 0·998 | 0·176 | 99·56 |
| 2-phenyl ethanol | 0·942 | 1·448 | 88·81 |
| octanoic acid | 0·994 | 0·302 | 98·77 |

good results (Table 4). Correlation coefficients were relatively high, over 97–99%; only in the case of 1-propanol, 3- and 2-methyl-1-butanol, ethyl lactate and 2-phenyl ethanol were they 89%, 95%, 96% and 94%, respectively. The highest standard errors of estimate were those of 3- and 2-methyl-1-butanol and 2-phenyl ethanol, even though their values were always acceptable.

### Influence of the extraction solvent, temperature and sample volume

Different solvent extraction mixtures were tested (pentane, dichloromethane, pentane: dichloromethane 60: 40 v/v, pentane and dichloromethane in sequence). Only dichloromethane (up to 30% of the sample volume) allowed the extraction without formation of stable emulsions. As regards the other mixtures, absence of emulsion formation strongly depended on the wine and must composition.

The statistical analysis of variance shows no significant difference between the data (Fig. 3 and Table 5) obtained with different sample volumes. Only for the higher alcohols 2-butanol, 2-methyl-1-propanol, 3- and 2-methyl-1-butanol and benzaldehyde did there seem to be a decrease of the extracted amounts when the sample volume is greater. Thus a sample of 50 ml proved optimal.

Regarding the effect of temperature on the efficiency and repeatability of the extraction, the analysis of variance (Fig. 3 and Table 5) does not show a significant difference between the two temperatures assayed, except for (-)-butan-2,3-diol, (R,S)-butan-2,3-diol, isobutyric acid and $\gamma$-butyrolactone for which a different extraction efficiency seems to exist. In fact, for

(-)-butan-2,3-diol, (R,S)-butan-2,3-diol and γ-butyrolactone there seems to be a decrease of the extracted amounts at 20°C while for isobutyric acid there is an increase. However, the results of these three compounds do not affect the generally good response of the method both at 10 and 20°C. Obviously 20°C appears to be the best analytical temperature.

### Comparison between the ultrasound method and $C_{18}$ column extraction

A typical chromatogram of a wine ultrasound extract is shown in Fig. 2(c). While most compounds occurring in wine were identified, the identity of a few peaks remains unknown.

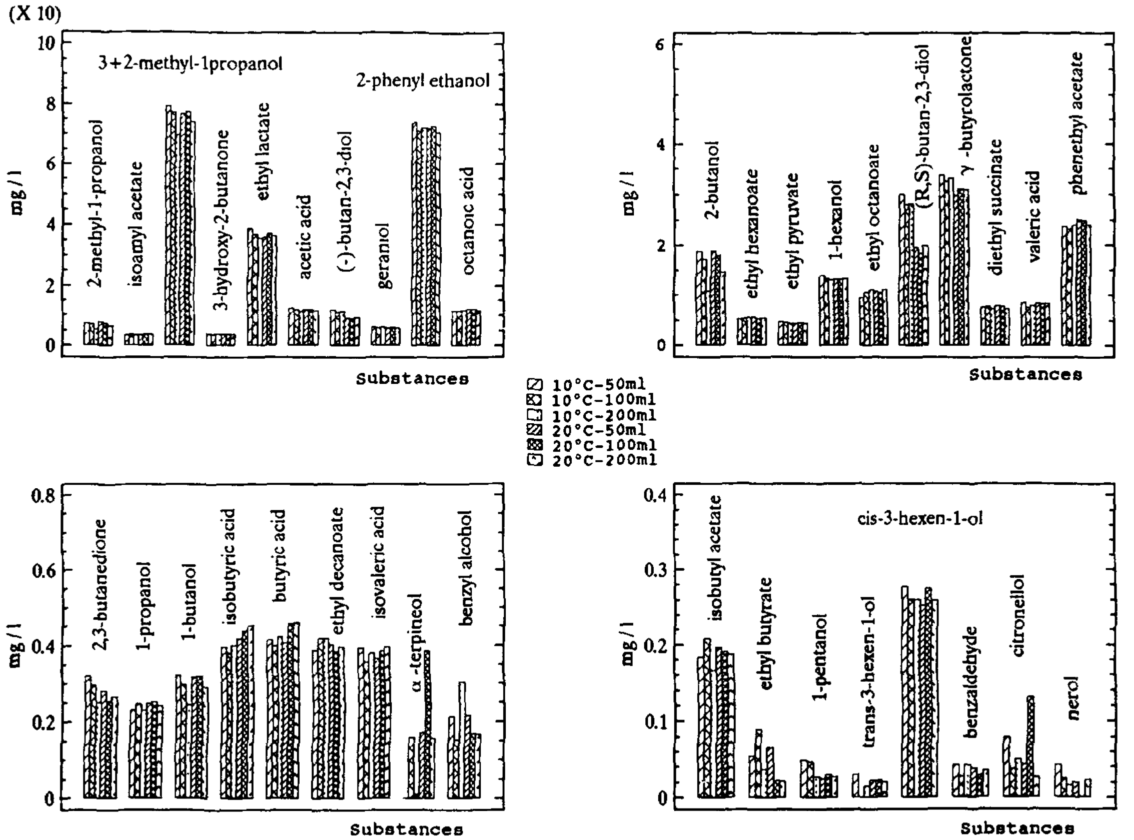

**Fig. 3.** Comparison between the two different temperatures (10 and 20°C) by extracting with three different sample volumes (50, 100 and 200 ml, respectively).

**Table 5. Variance analysis of aromatic compound concentrations (see Fig. 3) found at the two different temperatures (10–20°C) and the three sample volumes (50, 100 and 200 ml)**

| | % Significance level | | | % Sigificance level | |
|---|---|---|---|---|---|
| Substances | Temp. | Vol. | Substances | Temp. | Vol. |
| 2,3-butanedione | 34·7 | 36·5 | benzaldehyde | 62·9 | 6·9 |
| isobutyl acetate | 68·4 | 33·0 | (-)-butan-2,3-diol | 0·1 | 95·8 |
| ethyl butyrate | 32·8 | 56·1 | (R,S)-butan-2,3-diol | 0·0 | 97·3 |
| 1-propanol | 9·9 | 45·2 | isobutyric acid | 1·7 | 84·5 |
| 2-butanol | 59·9 | 6·0 | τ-butyrolactone | 0·8 | 99·1 |
| 2-methyl-1-propanol | 53·8 | 7·5 | butyric acid | 18·8 | 62·0 |
| isoamyl acetate | 27·2 | 25·1 | ethyl decanoate | 29·4 | 82·4 |
| 1-butanol | 42·3 | 17·6 | isovaleric acid | 72·9 | 68·9 |
| 3+2-methyl-1-butanol | 85·5 | 10·8 | diethyl succinate | 52·1 | 15·3 |
| ethyl hexanoate | 22·2 | 73·4 | α-terpineol | 11·5 | 37·4 |
| 1-pentanol | 13·0 | 62·9 | valeric acid | 34·1 | 38·3 |
| ethyl pyruvate | 47·6 | 73·8 | citronellol | 75·5 | 59·9 |
| 3-hydroxyl-2-butanone | 59·2 | 86·1 | nerol | 29·1 | 49·3 |
| ethyl lactate | 54·8 | 53·3 | phenethyl acetate | 5·6 | 87·8 |
| 1-hexanol | 51·5 | 62·3 | geraniol | 68·1 | 64·4 |
| *trans*-3-hexen-1-ol | 47·7 | 42·3 | benzyl alcohol | 47·6 | 48·3 |
| *cis*-3-hexen-1-ol | 66·6 | 79·2 | 2-phenyl ethanol | 66·1 | 49·2 |
| ethyl octanoate | 46·6 | 40·0 | octanoic acid | 10·1 | 89·8 |
| acetic acid | 81·1 | 18·0 | | | |

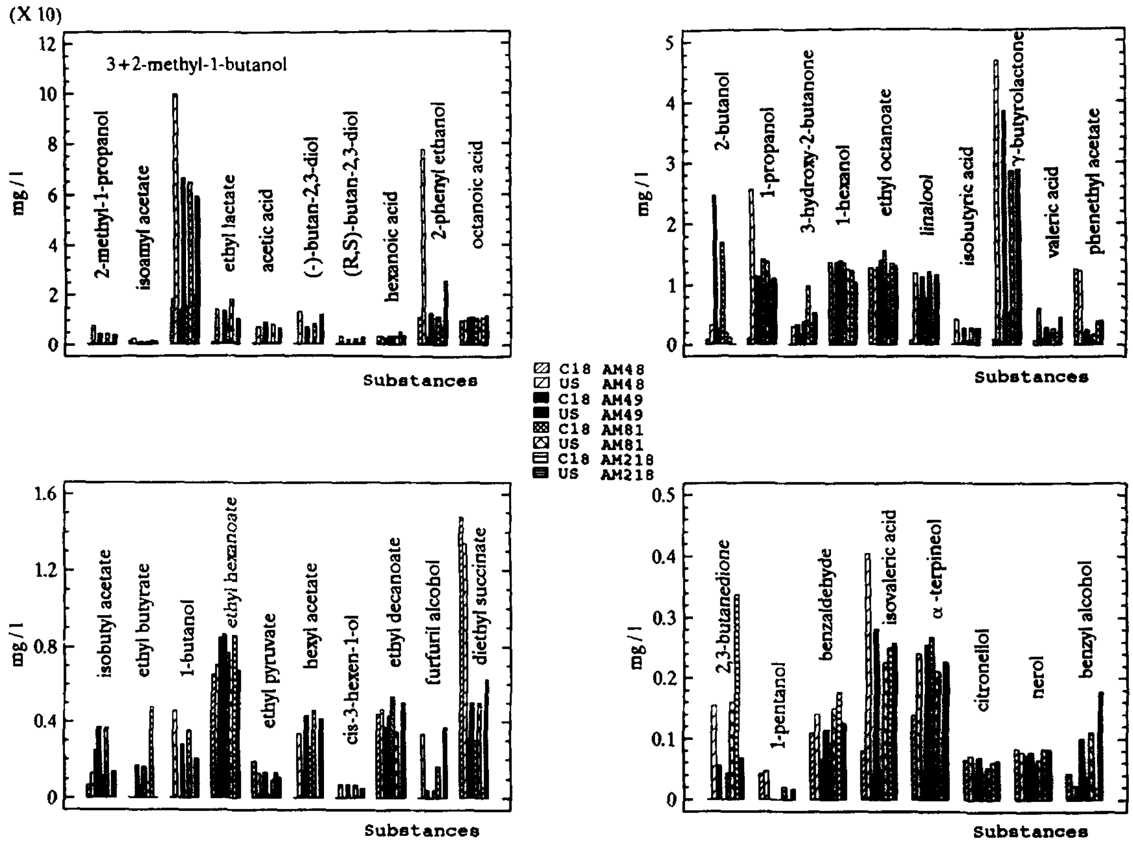

**Fig. 4.** Comparison between aromatic compound concentrations (as mg litre$^{-1}$) found with the $C_{18}$ and ultrasound extraction methods from Chambave (C) wines, fermented by different yeast strains (AM 48, AM 49, AM 81, AM 218).

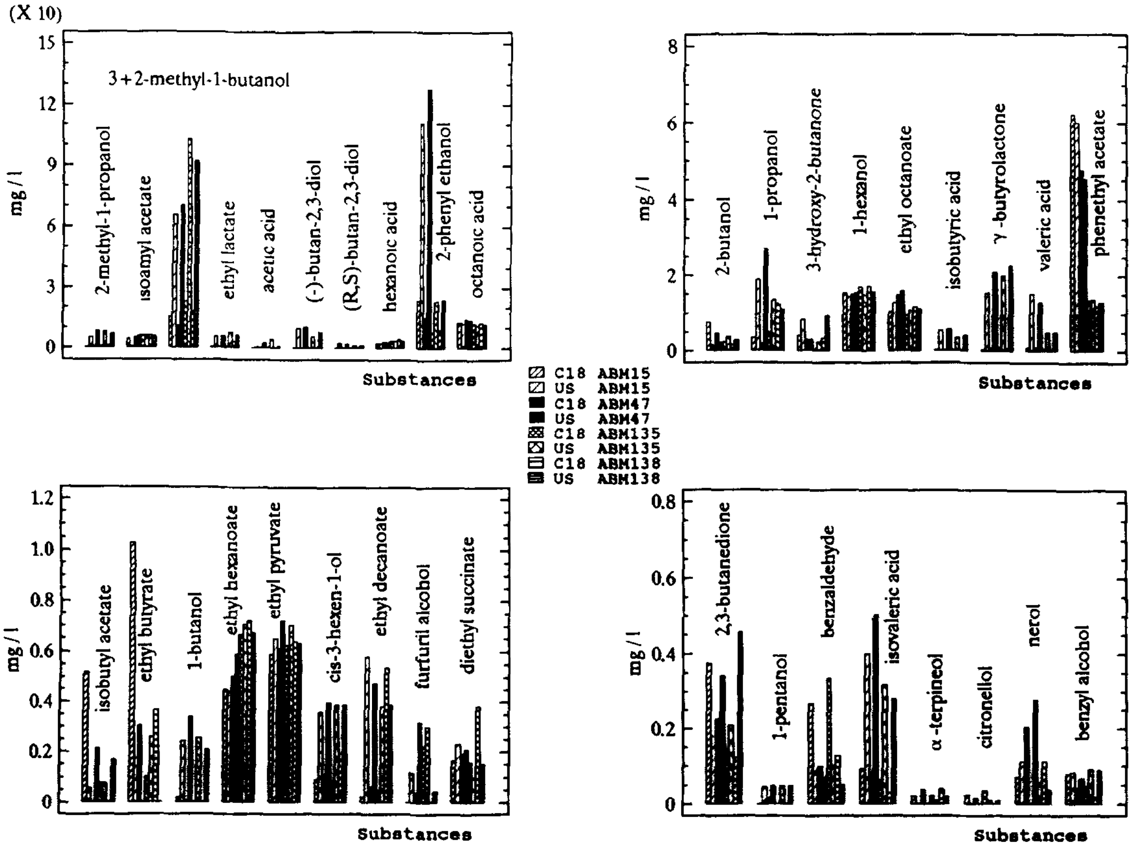

**Fig. 5.** Comparison between aromatic compound concentrations (as mg litre$^{-1}$) found with the $C_{18}$ and ultrasound extraction methods from Blanc de Morgex-La Salle (B) wines, fermented by different yeast strains (Abm15, Abm47, Abm135, Abm138).

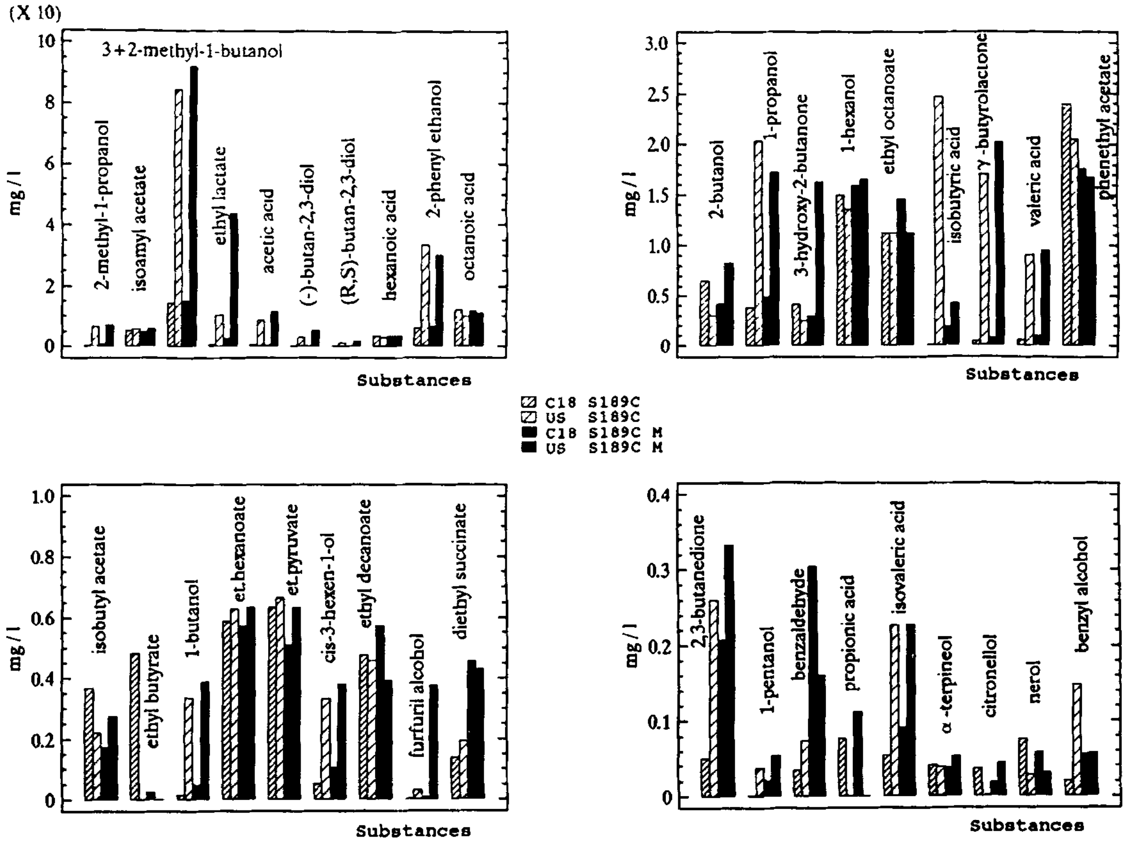

**Fig. 6.** Comparison between aromatic compound concentrations (as mg litre$^{-1}$) found with the $C_{18}$ and ultrasound extraction methods from Blanc de Morgex-La Salle before (B) and after malolactic fermentation (M) wines, fermented by S189c.

Gas chromatographic data from two wines (Blanc de Morgex before (B) and after (M) malolactic fermentation and Chambave (C) that were fermented by different yeast strains and analysed with both methods) appear in Figs 4–6; the variance analysis of which are listed in Table 6.

The results of the compound concentrations found in three different wines with the ultrasound extraction method (particularly those of 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol and 2-methyl-1-butanol, 1-pentanol, hexyl acetate, ethyl lactate, cis-3-hexen-1-ol, acetic acid, (-)-butan-2,3-diol, linalool, isobutyric acid, (R,S)-butan-2,3-diol, γ-butyrolactone, isovaleric acid, valeric acid, 2-phenyl ethanol) were higher than those obtained by the $C_{18}$ extraction method (Figs 4–6). However, from the analysis of some single data of Figs 5 and 6, it turns out that for some compounds (e.g. propionic acid, hexanoic acid and octanoic acid) this trend is emphasised in the $C_{18}$ method; on the other hand, this fact suggests the existence of variability factors for both methods which appear to be less important in the case of sonication.

The extraction efficiency for almost the totality of the must and wine aromatic compounds is higher than that achieved with the $C_{18}$ method. In fact, the extraction of complex matrices with resin columns depends on both the column loading capacity and the concentration of substances competing for the adsorbing sites. If this concentration is unknown and extremely variable, as in musts and wines, a resin method can be uncontrollably affected.

Furthermore, the wine ethanol concentration (from 10% to 15%) can partially affect the resin adsorbing capacity for both polar and non-polar substances. On the other hand, sonication always gives a quantitative extraction of all substances. Thus, considering the simplicity and quantitative accuracy of the ultrasound extraction, this method can be considered a valid alternative to long, laborious extractions with solvent and columns, and this is particularly true for rapid screening, as in the case of wine yeast selection.

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**Table 6. Variance analysis of data summarized in Figs 4–6. Significance level of variance between yeast strains and between methods**

| | | % Significance level | | | | % Significance level | |
|---|---|---|---|---|---|---|---|
| | | Yeast | Method | | | Yeast | Method |
| 2,3-Butanedione | B | 77·4 | 29·2 | Propionic acid | B | 50·0 | 19·3 |
| | C | 67·0 | 89·9 | | C | — | — |
| | M | 22·5 | 15·7 | | M | 49·2 | 11·9 |
| Isobutyl acetate | B | 65·0 | 73·0 | (-)-Butan-2,3-diol | B | 50·4 | 0·7 |
| | C | 6·4 | 3·9 | | C | 49·8 | 0·6 |
| | M | 67·6 | 88·1 | | M | 48·3 | 17·9 |
| Ethyl butyrate | B | 48·1 | 8·5 | Linalool | B | 50·0 | 37·4 |
| | C | 55·3 | 21·3 | | C | 51·2 | 3·0 |
| | M | 49·2 | 46·2 | | M | 49·2 | 49·2 |
| 2-Butanol | B | 77·8 | 28·0 | Isobutyric acid | B | 50·4 | 10·2 |
| | C | 45·8 | 22·0 | | C | 66·0 | 0·5 |
| | M | 77·2 | 95·6 | | M | 56·6 | 43·3 |
| 1-Propanol | B | 95·7 | 4·4 | (R,S)-Butan-2,3-diol | B | 50·0 | 3·1 |
| | C | 97·0 | 38·1 | | C | 21·4 | 0·1 |
| | M | 71·6 | 9·3 | | M | 51·0 | 51·6 |
| 2-Methyl-1-propanol | B | 41·3 | 0·0 | τ-Butyrolactone | B | 46·1 | 0·0 |
| | C | 53·0 | 1·4 | | C | 67·0 | 0·7 |
| | M | 31·8 | 2·4 | | M | 42·5 | 4·6 |
| Isoamyl acetate | B | 25·0 | 54·4 | Butyric acid | B | 72·7 | 90·3 |
| | C | 28·0 | 59·4 | | C | 47·3 | 0·4 |
| | M | 54·7 | 20·5 | | M | 25·6 | 6·0 |
| 1-Butanol | B | 47·6 | 0·0 | Ethyl decanoate | B | 68·2 | 16·2 |
| | C | 46·8 | 1·0 | | C | 75·3 | 54·4 |
| | M | 15·7 | 2·1 | | M | 85·2 | 43·2 |
| 3+2-Methyl-1-butanol | B | 18·4 | 0·0 | Furfuryl alcohol | B | 8·9 | 7·6 |
| | C | 45·1 | 0·8 | | C | 65·3 | 13·1 |
| | M | 44·3 | 3·0 | | M | 48·1 | 44·6 |
| Ethyl hexanoate | B | 1·0 | 41·2 | Isovaleric acid | B | 25·9 | 0·2 |
| | C | 29·6 | 50·1 | | C | 58·8 | 6·8 |
| | M | 73·2 | 13·3 | | M | 47·5 | 7·3 |
| 1-Pentanol | B | 31·2 | 0·0 | Diethyl succinate | B | 62·7 | 60·2 |
| | C | 2·6 | 11·8 | | C | 3·3 | 25·4 |
| | M | 6·6 | 3·6 | | M | 8·9 | 77·9 |
| Ethyl pyruvate | B | 63·2 | 5·1 | α-Terpineol | B | 12·0 | 2·8 |
| | C | 38·4 | 63·2 | | C | 65·5 | 16·7 |
| | M | 33·3 | 33·0 | | M | 63·1 | 58·6 |
| Hexyl acetate | B | 22·9 | 37·4 | Valeric acid | B | 42·9 | 1·2 |
| | C | 15·8 | 0·6 | | C | 54·2 | 2·9 |
| | M | — | — | | M | 6·8 | 0·3 |
| 3-Hydroxy-2-butanone | B | 27·8 | 20·1 | Citronellol | B | 65·0 | 7·6 |
| | C | 28·2 | 24·9 | | C | 20·5 | 11·8 |
| | M | 56·0 | 58·3 | | M | 76·8 | 90·0 |
| Ethyl lactate | B | 60·4 | 0·5 | Nerol | B | 68·2 | 10·9 |
| | C | 51·1 | 0·4 | | C | 4·1 | 49·4 |
| | M | 45·0 | 34·2 | | M | 58·1 | 15·6 |
| 1-Hexanol | B | 13·9 | 4·2 | Phenethyl acetate | B | 0·0 | 19·3 |
| | C | 14·2 | 12·3 | | C | 0·0 | 46·3 |
| | M | 30·6 | 78·1 | | M | 15·6 | 34·2 |
| *Trans*-3-hexen-1-ol | B | 32·0 | 4·8 | Hexanoic acid | B | 0·1 | 1·0 |
| | C | 45·3 | 18·6 | | C | 98·1 | 24·3 |
| | M | 5·7 | 3·7 | | M | 41·3 | 28·8 |
| *Cis*-3-hexen-1-ol | B | 18·2 | 0·0 | Geraniol | B | 50·0 | 18·9 |
| | C | 50·0 | 0·1 | | C | 50·0 | 18·4 |
| | M | 1·8 | 0·3 | | M | 49·2 | 49·2 |
| Ethyl octanoate | B | 1·3 | 17·5 | Benzyl alcohol | B | 80·4 | 6·1 |
| | C | 89·6 | 49·6 | | C | 68·8 | 14·2 |
| | M | 49·0 | 49·2 | | M | 74·9 | 48·7 |
| Acetic acid | B | 44·4 | 9·6 | 2-Phenyl ethanol | B | 32·8 | 6·3 |
| | C | 46·4 | 0·1 | | C | 36·0 | 16·2 |
| | M | 53·9 | 9·4 | | M | 59·4 | 4·9 |
| Benzaldehyde | B | 32·9 | 12·1 | Octanoic acid | B | 2·2 | 5·8 |
| | C | 50·7 | 46·2 | | C | 14·4 | 95·2 |
| | M | 29·6 | 67·4 | | M | 72·7 | 28·7 |

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