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Loss of aroma compounds in carbon dioxide effluent during white wine fermentation

AP-42 Section 9.12.2
Reference 5
Report Sect.
Reference

G.C. MILLER, J.M. AMON and R.F. SIMPSON

The concentration of aroma compounds entrained in the carbon dioxide escaping from the fermentation tank during white winemaking was analysed by gas chromatography. The loss of higher alcohols and fermentation esters was determined for five wines; expressed as a proportion of the amount of compound retained in the newly made wine, 2–24% of the acetate esters, 0–25% of the ethyl esters of the C_4–C_{12} fatty acids and no more than 1% of the higher alcohols were removed by the carbon dioxide.

Several hundred volatile compounds have been identified in wine, e.g. alcohols, carboxylic acids, esters, aldehydes and ketones. Some are original components of the grape; but the majority, especially in young wine, are yeast metabolites formed during alcoholic fermentation (Webb & Muller 1972, Schreier 1979). The formation of yeast metabolites is influenced by juice properties such as composition, clarity and pH, as well as fermentation parameters such as yeast strain inoculated, starter culture preparation and degree of aeration (Daudt & Ough 1973, Groat & Ough 1978, Killian & Ough 1979, Houtman, Marais & du Plessis 1980).

The net production of volatile yeast metabolites does not alone determine the concentration of each in the finished wine. The esters and higher alcohols are adsorbed onto the yeast lees, filters and bentonite (Simpson 1983, Simpson & Miller 1984, Miller *et al.* 1985). The concentration of esters is likely to be affected by chemical equilibrium reactions, although the effect is more pronounced during long-term storage or at elevated temperature (Ramey & Ough 1980). Furthermore, volatile compounds are carried in the carbon dioxide produced during fermentation. The extent of loss of aroma compounds through entrainment in the fermentation gases depends on temperature but is also influenced by the rate of gas evolution, size and shape of the fermentation tank (Simpson 1983). In physicochemical terms, two factors determine the quantity of a compound removed from the fermentation liquid: the extent to which a dynamic equilibrium between the gas (headspace carbon dioxide) and liquid phase (fermenting juice or wine) is approached and the position of the equilibrium, which depends on the concentration of compounds in the liquid and the temperature. The relationship between the quantity of compound present in the gas phase and the temperature is a power function so that progressively larger losses occur at higher fermentation temperatures.

Considerable change has occurred in the production of white table wine in Australia over the past 10–20 years; the advent of must cooling and the trend towards fermentation at low temperature has led to an overall improvement in wine quality and the production of more delicate and flavourful wine (Rankine 1977). Whilst fermentation at low temperature can be expected to reduce the loss of aroma compounds during fermentation, the benefits have not been evaluated by instrumental analysis until recent times. The present study reports the aroma loss of the higher alcohols and esters by entrainment in the carbon dioxide formed during the production of five commercial wines.

Materials and methods

Grape juice and wine

Muscat Gordo Blanco grapes for the preparation of wines 1 and 2 and Riesling grapes for the preparation of wines 3–5 were harvested in the Riverland district of South Australia during the 1985 vintage; 100 g/t of potassium metabisulphite was added. The grapes were crushed in a Miller roller crusher and destemmer; sulphur dioxide was added to the free run juice at a concentration of 30 mg/L total sulphur dioxide.

The juice was centrifuged into tanks from the drainer. Volclay WG bentonite at 0.8 g/L to achieve protein stability was added to the juice from which wine 3 was made. No further addition of bentonite was made. Actively fermenting Chandon yeast culture at ca. 30 mL/L was added to each juice and fermentations were conducted at 15° ± 2 C deg. Bentonite at 1.1, 1.1 and 0.8 g/L was added to the actively fermenting juices from which wines 1, 2 and 4, respectively, were made. No bentonite was added to juice 5 before or during fermentation. The volume of juice, the initial and final reducing sugar content, the dissolved carbon dioxide content and the ethanol content of the new wines are shown in Table 1.

Analytical methods

The composition of the major aroma compounds entrained in the carbon dioxide escaping from the fermentation tank was determined as follows. A 3.2 mm OD teflon tube located ca 800 mm below the top of the 20 000 L stainless steel tank was connected to a Neuberger model N06 ST diaphragm compressor/vacuum pump (Kurt Neuberger KG, Freiburg–Munzingen, West Germany) and in turn to the gas inlet of a liquid–liquid extraction apparatus similiar to that described by Rapp and Knipser (1980). The gas flow was controlled by a Nupro SS - 2SG fine metering valve (Nupro Co., Willoughby, OH, USA) fitted to the inlet of the pump and was set at 50 mL/min using a bubble flowmeter. For convenience, Swagelok Quick-Connects (Crawford Fitting Company, Solon, OH, USA) were attached to teflon tubing located at the top of several fermentation tanks and to tubing leading to the pump.

The quantity of individual aroma compounds in 60 L of carbon dioxide, i.e. collected over 20 h, was monitored throughout active fermentation (14–19 d). The freon extract containing the aroma compounds was concentrated to ca 2 mL by distillation with a Vigreux column; 1-octanol (20 μg) in freon solution was added as internal standard and the extract was further concentrated to ca 50 μL (Simpson & Miller 1983). The quantity of each of the major esters and alcohols in the freon

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Table 1. Grape juice and wine characteristics

Juice and wine composition	Muscat Gordo Blanco		Riesling		
	1*	2	3	4	5
Juice					
Volume (L)	19 000	19 200	19 000	17 900	18 000
Reducing sugar (g/L)	198	176	190	188	192
Wine					
Reducing sugar (g/L)	1.2	0.8	1.3	7.9	2.1
Dissolved carbon dioxide (g/L)	2.0	2.2	2.3	1.9	2.4
Ethanol (mL/L)	126	106	116	111	113

* *Wine number*

Table 2. Loss of individual aroma compounds through entrainment in the carbon dioxide evolved during fermentation

Compound	Quantity*				
	1†	2	3	4	5
Isobutyl acetate	7	13	15	18	22
Isoamyl acetate	5	7	2	7	12
Hexyl acetate	10	21	8	17	24
Ethyl *n*–butanoate	4	7	2	4	12
Ethyl *n*–hexanoate	9	16	7	15	25
Ethyl *n*–octanoate	3	6	3	11	15
Ethyl *n*–decanoate	1	2	8	4	15
Ethyl *n*–dodecanoate	0	1	4	1	1
Isobutanol	0	0	0	0	0
Isoamyl alcohol	0	0	0	0	0
1–Hexanol	0	1	0	0	1
Linalool	0	0	n.d.#	n.d.	n.d.
2–Phenethanol	0	0	0	0	0

* *Expressed as a proportion of that remaining in the new wine (%)*
† *Wine number*
\# *Not determined (minor quantity)*

concentrate was determined by gas chromatography, based on FID response factors (McNair & Bonelli 1967). A correction was made for the retention of a small amount of each compound in the liquid – liquid extractor and the incomplete extraction of the lower molecular weight and more water soluble compounds; the proportion of compound retained in the extractor was: isobutyl acetate 5.5%, isoamyl acetate 3.8%, ethyl *n*-butanoate 4.1%, isobutanol 38%, and isoamyl alcohol 13% (unpublished data).

The volume of water-saturated carbon dioxide at 15°C and 101 kPa evolved during the sampling period was estimated from the expected conversion of glucose and fructose to carbon dioxide and ethanol (Amerine *et al.* 1980) and the sugar attenuation of the must from the commencement to the completion of sampling. Reducing sugar content was determined by the Rebelein method (Amerine & Ough 1980).

The quantity of each compound in the total volume of carbon dioxide produced during sampling was calculated. The quantity of each compound carried away in the fermentation gas between samplings was similarly determined from the volume of carbon dioxide and the mean of the concentrations in adjacent samplings. The overall loss of each compound through entrainment was determined and expressed as a proportion of that remaining in the new wine (Table 2); the aroma composition of the new wine was determined by headspace analysis (Simpson & Miller 1983).

GC–sniff assessment of wine

The freon extracts of a Muscat Gordo Blanco wine (wine 1) and a Riesling wine (wine 5) were chromatographed on a 70 m × 0.5 mm ID SP1000 SCOT glass capillary column (SGE, Melbourne), which was fitted into a Perkin-Elmer Sigma 3B gas chromatograph. Operating conditions were: 2.5 mL/min helium carrier gas; 27.5 mL/min nitrogen make-up gas; oven temperature programmed at 2 C. deg/min from 50 to 180°C; FID. The column effluent was split 1:1 to the FID and to a teflon sniffing port via a heated 0.3 mm ID glass-lined metal tube (Dravnieks & O'Donnell 1971).

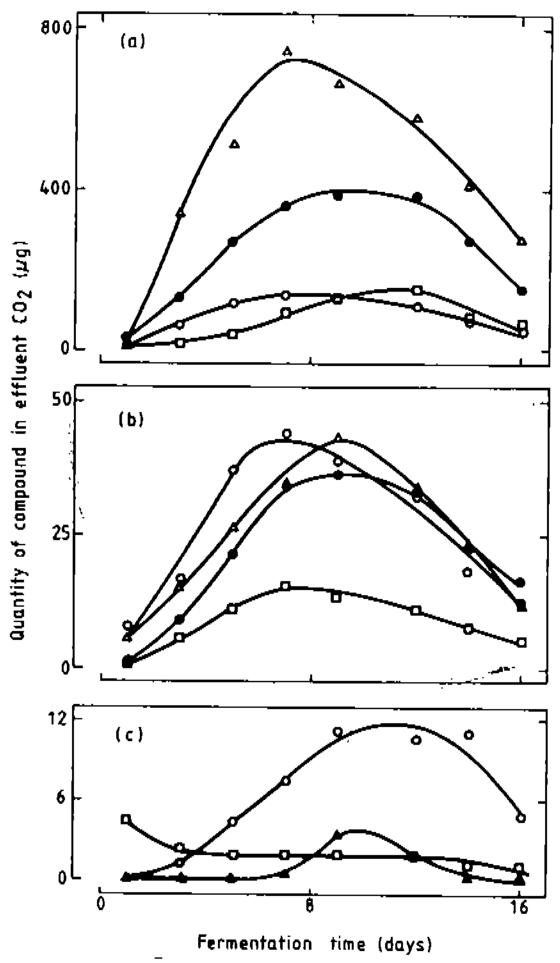

Fig.1. Quantity of compound isolated from 60 L of carbon dioxide effluent during the fermentation of wine
1: (a)△, isoamyl acetate; ○, ethyl n*–hexanoate; ■, ethyl* n*–octanoate; ●, isoamyl alcohol; (b) □, isobutyl acetate; ○, hexyl acetate; ●, ethyl* n*–butanoate; △, isobutanol; (c) ○, ethyl* n*–decanoate; □, 1-hexanol; ▲, 2-phenethanol*

The identity of the compounds contributing most to wine aroma was based on GC retention and results from previous investigations (Simpson & Miller 1983, 1984). The identity of each compound, a description of its perceived aroma and its relative intensity are shown in Table 5.

Results

The concentration of the higher alcohols and esters in carbon dioxide changed throughout the course of the fermentation; except for 1–hexanol, which is derived from lipids in the grape material and therefore is not a primary fermentation product (Cordonnier & Bayonove 1981), the concentration of the compounds in carbon dioxide initially increased, then decreased during the later part of the fermentation. The maximum concentration of each compound was reached at different times during the course of the fermentation; but individual compounds showed the same pattern of evolution for the five wines, which were made at different times, commencing in late February (wines 4, 5) and late March 1985 (wines 1–3). The quantity of the major aroma compounds present in 60 L samples of fermentation gas analysed during the fermentation of wine 1 is shown in Figure 1; the reducing sugar content of the must and the ethanol content, calculated from sugar attenuation, are shown in

Table 3. Flavour threshold, concentration and aroma values of some aroma compounds in wine

Compound	Flavour threshold (mg/L)*	Concentration (mg/L) 1†	2	3	4	5	Range	Aroma value (AU)#
Isobutyl acetate	0.9	0.12	0.10	0.16	0.06	0.07	0.06–0.16	0.1–0.2
Isomayl acetate	1.0	7.76	9.36	3.04	6.56	6.44	3.04–9.36	3.0–9.4
Hexyl acetate	2.4	0.26	0.36	0.41	0.59	0.54	0.26–0.59	0.1–0.2
Ethyl *n*–butanoate	0.4	0.42	0.33	0.32	0.40	0.30	0.30–0.42	0.7–1.1
Ethyl *n*–hexanoate	0.3	0.94	0.97	1.03	1.11	1.17	0.94–1.17	3.1–3.9
Ethyl *n*–octanoate	0.8	1.84	1.61	1.66	1.64	2.10	1.61–2.10	2.0–2.6
Ethyl *n*–decanoate	1.5	0.58	0.38	0.38	0.41	0.49	0.38–0.58	0.2–0.4
Ethyl *n*–dodecanoate	3.5	0.16	0.10	0.08	0.09	0:11	0.08–0.16	0.02–0.05
Isobutanol	200	22.7	18.9	27.7	8.73	9.42	8.73–26.7	0.04–0.13
Isoamyl alcohol	60	146	145	69.9	97.4	96.2	69.9–146	1.2–2.4
1–Hexanol	4.0	0.94	1.04	0.92	0.86	0.86	0.86–1.04	0.2–0.3
Linalool	0.10	0.50	0.52	n.d.	n.d.	n.d.	n.d.–0.52	n.d–5.2
2–Phenethanol	100	23.5	42.8	7.9	23.6	29.8	7.9–42.8	0.1–0.4

* *Values selected from the literature; see de Wet (1978), Simpson (1979) and reference cited therein*
† *Wine number*
\# *Aroma units*

Table 4. Loss of some compounds contributing strongly to wine aroma through entrainment in the carbon dioxide evolved during fermentation

Compound	Loss (AU)* 1†	2	3	4	5
Isoamyl acetate	0.40	0.69	0.06	0.69	0.80
Ethyl *n*–butanoate	0.04	0.06	0.02	0.07	0.09
Ethyl *n*–hexanoate	0.29	0.52	0.25	0.84	0.97
Ethyl *n*–octanoate	0.07	0.12	0.06	0.34	0.26

* *Aroma units*
† *Wine number*

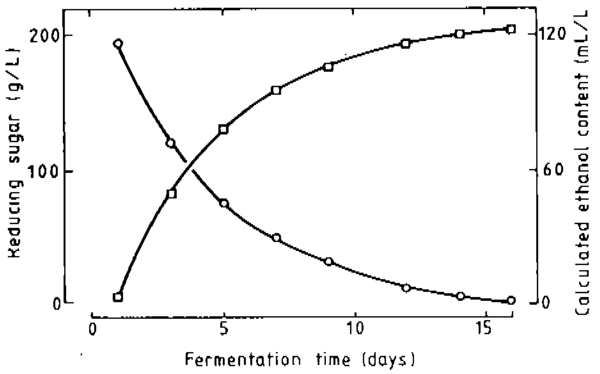

Figure 2. Reducing sugar content (○) and calculated ethanol content (□) during the fermentation of wine 1

Table 5. Aroma assessment of the headspace volatiles isolated from Muscat Gordo Blanco wine and Riesling wine

Compound*	Aroma description	Intensity† 1#	5
Ethyl acetate	Solvent, ethyl acetate	m	m
Ethyl *n*–propionate	Estery, fruity	—	m
Propyl acetate	Estery	—	m
Isobutyl acetate	Estery, banana	m	m
Ethyl *n*–butanoate	Estery, fruity	—	m
Isoamyl acetate	Estery, banana	s	s
Isoamyl alcohol	Sweet, fusel oil	s	s
Ethyl *n*–hexanoate	Estery, fruity	s	m
Hexyl acetate	Estery, fruity	m	s
Unknown. I=1314‡	Amine	m	—
Hexanol	Green	m	m
cis–3–Hexen–1–ol	Green, banana	m	—
Ethyl *n*–octanoate	Estery, pineapple	m	s
Linalool	Floral	m	m
Ethyl *n*–decanoate	Estery, fatty	m	m

**Listed in order of elution on an SP1000 glass capillary column*
† *s, strong; m, moderate; —, weak or not evident*
\# *Wine number*
‡ *Linear retention index (Ettre 1973)*

Figure 2. The quantity of each of the aroma compounds present in the total volume of CO_2 produced per day followed a similar pattern to that contained in the 60 L samples as shown in Figure 1. There was, however, a sharper rise in the quantity of compound present in the CO_2 during the early part of the fermentation and, because of the decrease in both the concentration of compound in the CO_2 and the volume of CO_2 evolved, a more pronounced fall in the quantity towards the end of the fermentation. Combined losses of the individual esters through entrainment in carbon dioxide expressed as a proportion of the quantity in the new wine were: isobutyl acetate 7–22%, isoamyl acetate 2–12%, hexyl acetate 8–24%, ethyl *n*–butanoate 2–12%, ethyl *n*–hexanoate 7–25%, ethyl *n*–octanoate 3–15%, ethyl *n*–decanoate 1–15%, and ethyl *n*–dodecanoate 0–4% (Table 2). Loss of the individual alcohols, isobutanol, isoamyl alcohol, 1–hexanol, linalool and 2–phenethanol was no greater than 1% of the quantity in the new wine.

The flavour threshold in wine of the esters and alcohols, their concentration in the five wines and the corresponding aroma values, i.e. the concentration in wine divided by the flavour threshold, are shown in Table 3. The loss, expressed in aroma units (AU), of compounds having a more marked influence on wine aroma is shown in Table 4.

The GC–sniff assessment of the freon extract of headspace volatiles from Muscat Gordo Blanco and Riesling wine is shown in Table 5; only compounds exhibiting moderate to strong aromas are listed.

Discussion

Large volumes of carbon dioxide are produced during winemaking (Amerine *et al.* 1980); for example, one million litres of water-saturated carbon dioxide at 15°C and 101 kPa would have formed during the fermentation of juice to produce wine 1 (Table 1), based on the conversion of grape sugar to carbon dioxide and on physicochemical gas laws (Liptrot, Thompson & Walker 1982). Consequently, the concentration of each of the major alcohols and esters in the fermentation effluent was monitored during the production of wines 1–5 and the loss of each determined by calculating the volume of carbon dioxide produced. The assumptions made in these calculations are now considered.

Soon after the onset of fermentation the must becomes saturated with carbon dioxide; therefore, carbon dioxide produced subsequently will be converted to effluent.

- In practical winemaking, the conversion of reducing sugar is only ca 90–95% (Amerine *et al.* 1980) due to the production of yeast biomass and metabolites in addition to carbon dioxide and ethanol; 93% conversion was assumed in the present studies.
- The strong convection currents during active fermentation in the 20 000 L tanks produced thorough mixing of the must and made samples drawn for reducing sugar estimations representative of the must in a tank.

In the analysis of aroma volatiles in the fermentation effluent, losses of material occur during collection and concentration of the freon solution. A proportion of the aroma volatiles is retained in the liquid–liquid extractor: corrections were made for the loss of the individual compounds (refer to experimental section) which were minor except for isobutanol and isoamyl alcohol that are more strongly retained in the aqueous layer. A small proportion (ca 3%) of volatiles is not trapped by the extractor, according to Rapp and Knipser (1980); no correction was made for this loss. Loss of material through evaporation can be expected during concentration of the freon extract; however, negligible loss should occur during the initial concentration, and the minor loss during the second concentration should be compensated for by the presence of an internal standard (Bemelmans 1981).

The change in concentration of the aroma volatiles in carbon dioxide during the production of wine 1 (Figure 1) is representative of all five wines and is attributable to variation in the concentration and solubility of each in the must. The higher alcohols and esters are formed during the growth phase of the yeast (Kunkee & Goswell 1977), accounting for their increasing concentration in carbon dioxide until ca day 9. The decrease in concentration of aroma volatiles in carbon dioxide from day 12 to day 16 is probably influenced by their higher solubility in the must as the ethanol content increases (Williams & Rosser 1981); but hydrolysis of the esters may also be occurring due to the presence of yeast esterases (Suomalainen 1981). The concentration of 2–phenethanol in the carbon dioxide effluent did not increase until later than other components, presumably due to its different genesis; Williams *et al.* (1983) have shown that glycosides of 2–phenethanol are present in *Vitis vinifera* grapes and that to a large extent the free alcohol is formed by enzymic hydrolysis.

Significant loss of the acetate esters and of ethyl esters of the $- C_8$ fatty acids occurred during fermentation (Table 2), in agreement with earlier reports (Simpson 1983, Simpson & Miller 1984). The loss of isoamyl acetate and the ethyl esters of the $C_4 - C_8$ fatty acids is shown in Table 4 as a loss in wine aroma, i.e. the total quantity of each compound entrained in the carbon dioxide was divided by the volume of new wine to give an effective loss in concentration; this value was divided by its flavour threshold to convert to aroma units.

The loss in the aroma of wines 1–5 was appreciably less than that previously reported during the production of wine from 35 000 L of Riesling juice (Simpson & Miller 1984); in this instance, loss of the major esters was: ethyl *n*–butanoate 0.1, ethyl *n*–hexanoate 1.6, ethyl *n*–octanoate 0.9, ethyl *n*–decanoate 0.2, isoamyl acetate 1.4 and hexyl acetate 0.1 AU. Although the temperature during fermentation was similar for this wine and wines 1–5 presently described, the conditions of fermentation favoured better retention of the esters during the production of wines 1–5.

A negligible proportion of the higher alcohols — also in agreement with previous findings — and of linalool was entrained in the evolving carbon dioxide (Table 2). These alcohols are more strongly retained in the liquid in contrast to the more hydrophobic esters. Linalool and related monoterpene alcohols are important contributors to the aroma of wine from muscat grapes (Ribéreau-Gayon, Boidron & Terrier 1975) and to a lesser extent to varieties such as Traminer and Riesling. No reduction in grape flavour should occur, therefore, through entrainment of volatile compounds in the fermentation gases during the fermentation of muscat-type wines.

The perceptible loss of desirable wine aroma during fermentation has always been a concern of the winemaker. But the task of trapping and recovering these flavour materials so that they might be added back to part or all of the new wine is made difficult by their low concentration in the huge volume of carbon dioxide gas. Consequently, it is necessary to assess if the recovery of aroma compounds is practicable and economically worthwhile. One of the more promising approaches to recovery of wine aroma is that in which the volatile compounds from the fermentation gases are absorbed by a carbon filter, reclaimed by washing with a portion of the new wine and added back to the wine (Izumi Shokuhin 1982). Furtsov, Kostadinov and Genov (1983) developed equipment for the cryogenic trapping of aroma volatiles from fermentation gases during red wine making; these authors indicated that addition of the condensate to wine markedly improved the flavour. The higher fermentation temperatures in red wine production, which may be over 30°C according to Furtsov *et al.* (1983), may give more scope for aroma recovery because of the greater loss of aroma compounds. The cost effectiveness of aroma recovery of volatiles from red or white wine fermentation, however, depends on the realisable improvement in wine quality and increased value of the wine.

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Continued on p. 253

A summary of the current status of fat-soluble vitamin analysis at Lynfield Agricultural Centre in Auckland has been presented. The target of the New Zealand scientists has been to simplify the [illegible]s as much as possible without sacrificing accuracy, so that [illegible]r numbers of samples can be analysed. This is necessary to allow meaningful quality control to be implemented. High technology in the form of HPLC has been the back-bone of the years of study. Without this technology, the quality of dairy products and other fortified food items produced in New Zealand could not have achieved such a high standard.

Acknowledgements

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CHANGES IN THE APPARENT CONSUMPTION OF FOODS AND NUTRIENTS IN AUSTRALIA: 1975–76 to 1983–84 — *Continued from p.245*

Australians. This analysis indicates that the changes that have occurred in the consumption of foodstuffs and nutrients over 1975–84 are consistent with the intent of the Australian Dietary Guidelines. However, while these changes provide support for these dietary guidelines, there is still a need for considerable improvement until the overall % contribution of specific macronutrients (e.g. fat, sugar and carbohydrates) to total energy is comparable to that recommended by nutrition experts.

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