Characterisation of the Aroma of the Hybrid Ferdinand de Lesseps (*Vitis vinifera x Vitis labrusca*)

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Freon 11-extracted volatiles of Ferdinand de Lesseps grape juice were studied by capillary gas chromatography, capillary gas chromatography-mass spectrometry and gas chromatography-sniffing. The berries were crushed under an inert atmosphere and the juice extracted with Freon 11 for 20 hours. Thirty-one compounds were reported. The juice was quantitatively characterised mostly by esters, particularly hydroxy esters. These esters are most probably responsible for the sweetish pineapple aroma of Ferdinand de Lesseps grapes whereas *o*-aminoacetophenone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone could contribute to its hybrid note.

Ferdinand de Lesseps, an American-French hybrid, was exhibited at the Royal Horticultural Society Show in 1870, where it received a First Class certificate. Royal Muscadine (or Chasselas doré) had been crossed with 'Isabella' (*Vitis vinifera* x *Vitis labrusca*), a prolific American grape, to produce the new variety. It was noted that it had somewhat of Isabella's strawberry-like aroma (Perold, 1927).

In South Africa Ferdinand de Lesseps has a distinctive character and has been known as the "pineapple" or "honey" grape. It was grown on a small scale as an early table grape and was also used for producing highly aromatic sweet wines. Until recently Ferdinand de Lesseps was used in small quantities as a blending partner to enhance the fruity bouquet of some white table wines.

Several compounds have been identified that are considered to play an important role in the aroma of labrusca grapes. Large amounts of esters are found in these grapes, unlike in vinifera grapes. Among these are methyl anthranilate, unsaturated esters and hydroxy esters. Other compounds of interest are *o*-amino-acetophenone, 2,5 dimethyl-4-hydroxy-3(2H)-furanone (Acree & Lavin, 1990), its methoxy derivative (Schreier, 1980), ß-damascenone (Acree, 1981, Braell *et al.*, 1986) and ethyl-2-mercaptopropionate (Kolor, 1983).

This investigation was aimed at the identification of aroma compounds that are pertinent to the fruity character of Ferdinand de Lesseps. This was achieved by extracting the grape aroma compounds with Freon 11 and submitting the concentrated extract for GC-MS identification of the compounds and for subsequent odour characterisation by GC-sniffing.

MATERIALS AND METHODS

Plant material: Ferdinand de Lesseps grapes were harvested at 23°B from a local vineyard in Stellenbosch, South Africa.

Sample preparation: Whole, undamaged berries were selected. These were crushed by hand in plastic bags filled with nitrogen and the juice recovered and centrifuged under a nitrogen atmosphere to limit oxidation and artefact formation as far as possible.

Isolation of free volatiles: Free volatile grape aroma was extracted using a method described by Marais (1986). A 250 mL aliquot of grape juice was subjected to continuous liquid extraction with Freon 11 for 20 hours. The extracts were concentrated to approximately 100 µL and stored at -14°C prior to analysis.

Analysis: The extracts were analysed by combined gas chromatography-mass spectrometry using a Finnegan 4600 Quadrupole mass spectrometer. The volatiles were identified by either comparing mass spectra with published data or spectra obtained from standard compounds analysed under the same conditions. Relative concentrations of each compound were determined using 3-decanol as internal standard without considering the recovery of volatiles and detector response factors.

The sample was then submitted for GC sniffing to identify compounds responsible for the varietal aroma. An interesting Ferdinand de Lesseps-like aroma was later identified using a Finnegan GCQ.

Instrumental operating conditions:

Gas chromatography and gas chromatography-mass spectrometry conditions:

Finnegan 9610 gas chromatograph / 4600 Quadrupole mass spectrometer system: Gas chromatographic parameters: Column Supelcowax 10 (60 m x 0.32 mm ID x 0.5 µm df) fused silica capillary. Operating conditions: Injector temperature, 200°C, oven temperature programme, 60°C (10 min) x 1°C/min to 190°C, carrier gas He, injection volume 1 µL, split ratio 30:1.

Mass spectrometer parameters: Source temperature, 240°C; interface temperature, 210°C; manifold temperature, 105°C; ionisation current, 0.31 amps; acceleration potential, 70 eV, multiplier voltage 850, scanning range 35 to 450 amu, scan time 0.95s with a 0.05s pause between scans.

Finnegan GCQ: Gas chromatographic parameters: Column Restek Stabilowax DA (60 m x 0.32 mm ID x 0.25 µm df) fused silica capillary. Operating conditions: Injector temperature, 225°C, oven temperature programme, 60°C (5 min) x 1.5°C/min to 180°C, carrier gas He, injection volume 1 µL, split ratio 30:1.

Mass spectrometer parameters: Source temperature, 180°C; transfer line temperature, 260°C; emission current 250 micro amps, acceleration potential 70 eV, electron multiplier, 1450 volts with a scanning range of 35 to 450 amu each second.

Gas chromatography-sniffing conditions: A Carlo Erba 4200 Gas Chromatograph equipped with a sniffing port was used for the odour assessment. Two experienced sniffers alternating every 15 minutes to overcome nasal fatigue assessed the GC effluent. Aroma descriptions were assigned to the identified compounds on the basis of matching relative retention times.

Gas chromatographic parameters: Column Supelcowax 10 (60 m x 0.32 mm ID x 0.24 µm df) fused silica capillary. Operating conditions: Injector temperature 200°C, oven temperature programme, 60°C (10 min) x 1°C/min to 190°C, carrier gas He, injection volume 1 µL, split ratio 30:1.

RESULTS AND DISCUSSION

The identities of 31 volatile compounds in the Ferdinand de Lesseps grape extract were confirmed by GC-MS analysis. All of these compounds had been reported in grapes previously. Their relative concentrations and associated aroma as determined by GC and GC-sniffing, respectively, are listed in Table 1.

***o*-Aminoacetophenone:** The identity of a compound responsible for an intense Ferdinand de Lesseps grape-like aroma nosed during GC-sniffing was later verified as *o*-aminoacetophenone. Due to its low concentration, its presence was missed in the initial GC-MS study of the extract. *o*-Aminoacetophenone has been implicated as the component responsible for the "foxy" character of *labrusca* grapes (Acree & Lavin, 1990), muscadine grapes (Baek *et al.*, 1997) and as providing a *labrusca* character in Concord grapes (Shure & Acree, 1995). In spite of its low concentration in muscadine grape juice (10-19 µg/L), *o*-aminoacetophenone gave a high flavour dilution factor, i.e. the highest dilution at which an aroma active compound could be detected. In their implication of *o*-aminoacetophenone as being associated with the "foxy" character of labrusca grapes, Acree & Lavin (1990) measured levels of 130 – 280 ng/L. Rapp, Versini & Ullemeyer (1993) identified *o*-aminoacetophenone as the component responsible for an off-odour in the *Vitis vinifera* cultivars Müller-Thurgau, Riesling and Sylvaner, which was described as an atypical ageing note ("napthalene", "hybrid" or "wet dirty towel"). In the faulty wines analysed by Dollmann *et al.* (1999) amounts of *o*-aminoacetophenone ranged from 0.7 to 12.8 mg/L. The "hybrid" note was recognised sensorially from a concentration of 700 ng/L and greater in fermented model wine solutions (Rapp, Versini & Engel, 1995). Baek *et al.* (1997) used a threshold value of 400 ng/L determined in skin milk to calculate an aroma value (concentration/aroma threshold) for *o*-aminoacetophenone. Acree & Lavin (1990) found 130–280 ng/L of *o*-aminoacetophenone in cultivars of labrusca grapes exhibiting the foxy-like odour. The relative concentration of 500 ng/L of *o*-aminoacetophenone found for Ferdinand de Lesseps grape juice is in the region of the quoted threshold values and hence could be at a level where it contributes to the aroma of this cultivar.

2,5-Dimethyl-4-hydroxy-3(2H)-furanone: The presence of 2,5-dimethyl-4-hydroxy-3(2H)-furanone, (DMHF or furaneol) in Ferdinand de Lesseps aroma is of particular interest as this compound has previously been reported as responsible for a sweet candy-like aroma in cultivars derived from labrusca (Acree & Lavin, 1990) and for the "strawberry" off-flavour in berries and wines of interspecific grapevine breedings (Rapp *et al.*, 1980). Guedes de Pinho & Bertrand (1995) developed an analytical method for determining 2,5-dimethyl-4-hydroxy-3(2H)-furanone, whose presence indicated a non-*Vitis vinifera* element in the wine. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone has also been established to be a major character impact compound of pineapple flavour concentrate (Rodin *et al.*, 1965) and a contributor to pineapple flavour (Takeoka *et al.*, 1989). It exists both in the free and glycosidically bound forms and its glucoside has been identified in strawberries (Mayerl, Näf & Thomas, 1989). Furaneol was most abundant in the free and bound forms identified in Muscadine grape juice (Baek & Cadwallader, 1999). At high concentrations, furaneol has a burnt candy-like aroma and at low concentrations a pineapple- or strawberry-like aroma. The odour threshold of furaneol in water has been reported as 31 µg/L at pH 4.5 (Buttery as quoted by Baek *et al.*, 1997). Rapp *et al.* (1995) reported the taste threshold for the recognition of the strawberry note at 80–150 µg/L in wine. At a relative concentration of 14.2 µg/L furaneol may not seem to be such an important aroma contributor to the Ferdinand de Lesseps grape aroma, but its contribution to wine aroma could be increased with release from its glycosidically bound forms during the wine-making processes.

Esters: Esters featured prominently in the Ferdinand de Lesseps aroma profile. As a group they contributed to 69% of the total relative concentration of the GC-registered volatiles. This may be an indication of the *labrusca* parentage of Ferdinand de Lesseps. *Vinifera* grapes differ distinctly from *labrusca*, with only trace amounts having been detected (Schreier, Drawert & Junker, 1976; Schreier, 1980). The high concentration of volatile esters has been used as an index of the "fruity" character of *labrusca* grapes (Fuleki as quoted by Schreier, 1980). Of particular quantitative interest were the polar hydroxy esters, *i.e.* ethyl-3-hydroxy butanoate, methyl-3-hydroxy butanoate and ethyl-2-butanoate and to a lesser extent ethyl-3-hydroxy hexanoate and ethyl-3-hydroxy propanoate. Most polar esters have low odour detection thresholds and contribute favourably to the fruit and flower notes of wine (Baumes *et al.*, 1986). Schreier (1980) reported that the hydroxy esters contributed to the "fruity" character of labrusca grapes. Methyl-3-hydroxy butanoate and ethyl-3-hydroxy hexanoate have been reported as aroma constituents in pineapple (Schreier, 1980; Rodin *et al.*, 1965). Baek *et al.* (1997) reported an average concentration range of 390-550 µg/L ethyl-3-hydroxy butanoate in muscadine grape juice, which exhibited a burnt marshmallow and muscadine-like aroma note, but it had a relatively low flavour dilution factor. Other esters present in Ferdinand de Lesseps aroma are ethyl butanoate and ethyl hexanoate with odour thresholds of 1 µg/L and 1.8 µg/L, respectively. These compounds have been identified as important contributors to fresh pineapple aroma (Takeoka *et al.*, 1989). The odour of ethyl hexanoate has been described as fruity with pineapple undertone (Fenaroli as quoted by Takeoka *et al.*, 1989). GC-sniffing performed in this investigation showed that the esters, specifically ethyl butanoate, ethyl-3-hydroxy butanoate and methyl-3-hydroxy butanoate,

TABLE 1

Volatile compounds identified by GC-MS in freon extracts of Ferdinand de Lesseps grapes.

No.	Compound	Concentration relative to 3-Decanol µg/L	Aroma Description
1	Ethyl butanoate	57	Sweet
2	Hexanal	53	Grassy
3	*n*-Butanol	Trace	*
4	Ethyl-2-butenoate	20	Sweet, fruity
5	*n*-Pentanol	Trace	*
6	*trans*-2-Hexenal	34	Grassy
7	Ethyl hexanoate	10	Sweet
8	Hexyl acetate	2	*
9	3-Hydroxy-2-butanone	8	Floral, sweet
10	*n*-Hexanol	Trace	*
11	*trans*-3-Hexen-l-ol	5	*
12	*cis*-3-Hexen-1-ol	5	*
13	Nonanal	Trace	*
14	*trans*-2-Hexen-1-ol	60	Green
15	*cis*-2-Hexen-1-ol	<1	*
16	*cis*-furan Linalool oxide	<1	*
17	Ethyl-2-hydroxy propanoate	4	Slightly sweet
18	Methyl-3-hydroxy butanoate	161	Sweet, fruity
19	2-Ethyl-1-hexanol	<1	*
20	Ethyl-3-hydroxy butanoate[1]	320	Sweet, dried fruit
21	Linalool	<1	*
22	∝-Terpineo[1]	2	*
23	Ethyl-3-hydroxy hexanoate	15	Sweet, fruity
24	*trans*-pyran Linalool oxide	2.5	*
25	3-Methyl-3-buten-2-one	2.5	*
26	Hexanoic acid	8	Slight stink
27	Benzyl alcohol	9	*
28	2-Phenyl ethanol[1]	43	Rose
29	Terpene-diol-1	12.5	*
30	2.5-Dimethyl-4-hydroxy-3(2H)-furanone[1]	14.2	Candy-floss
31	*o*-Aminoacetophenone[1]	0.5	Ferdinand de Lesseps-like

* No perceived aroma for compound (concentration ≤ odour threshold).

[1)] Compounds identified using authentic standards.

contributed sweet, fruity odours to the aroma profile of the Ferdinand de Lesseps extract.

Unsaturated compounds: Schreier & Paroschy (1981) identified a number of unsaturated compounds considered as contributing to the sweet-fruity odour of certain aroma fractions of labrusca grapes. Of these, only ethyl-2-butenoate, whose odour was perceived as sweet and fruity on sniffing, was identified in this study.

Alcohols: Of the small amounts of alcohols present in Ferdinand de Lesseps grape juice, quantitatively *trans*-2-hexen-1-ol and 2-phenyl ethanol are of interest. The odour of identity of *trans*-2-hexen-1-o1 was perceived as green. In our study 2-phenyl ethanol

exhibited a strong rose-like note with GC-sniffing. It has been implicated as a major aroma component of muscadine grapes and wine (Lamikanra, Grimm & Inyang, 1996). 2-Phenyl ethanol occurs both in free and glycosidically bound forms and has been described as having a rose-like note at high concentrations and a honey-like note at low concentrations (Baek *et al.*, 1997).

Labrusca compounds not detected in Ferdinand de Lesseps grape juice: We were unable to detect the presence of methyl anthranilate and ß-damascenone in Ferdinand de Lesseps grape aroma when using the GCQ mass spectrometer in the Selected Ion Monitoring mode. These compounds have been reported as contributors to the aroma of labrusca grapes (Acree, 1981). ß-Damascenone, which has a pleasant floral odour and a very low threshold of 2-20 pg/g in water, is thought to contribute to the sweet perfume aroma. It is possible that it required a further fractionation of the extract for its determination.

CONCLUSIONS

The combination of sniffing of GC fractionated volatiles of a Ferdinand de Lesseps grape aroma extract with GC-MS analyses for assessing the identity of individual compounds enabled us to identify the main aroma-contributing compounds of this cultivar. From our aroma description of these compounds, as well as those reported in grapes and other fruits with similar aroma tones, it appears as though the hybrid note of the Ferdinand de Lesseps could most likely be attributed to the presense of *o*-amino-acetophenone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone, whereas the esters, ethyl butanoate and the ethyl and methyl esters of 3-hydroxy butanoic acid, and to a lesser extent, ethyl 3-hydroxy hexanoate and ethyl-3-hydroxy propanoate, are most probably responsible for the sweetish pineapple aroma.

LITERATURE CITED

ACREE, T.E., 1981. The odour quality of *Labrusca* grapes. In: TERANISHI, R. & BERRER-BENITEZ, H. (eds). *Quality of selected fruits and vegetables of North America.* ACS Symposium Series 170. American Chemical Society, Washington, DC. pp. 11-19.

ACREE, T.E. & LAVIN, E.H., 1990. *o*-Aminoacetophenone the 'foxy'-smelling component of *labruscana* grapes. In: BESSIÉRE, Y. & THOMAS, A.F. (eds). *Flavour science and technology.* John Wiley & Sons Ltd, Chichester. pp. 49-52.

BAEK, H.H., CADWALLADER, K.R., MARROQUIN, E. & SILVA, J.L., 1997. Identification of predominant aroma compounds in Muscadine grape juice. *J. Fd. Sci.* **62**, 249-252.

BAEK, H.H. & CADWALLADER, K.R., 1999. Contribution of free and glycosidically bound volatile compounds to the aroma of Muscadine grape juice. *J. Fd. Sci.* **64**, 441-443.

BAUMES, R., CORDONNIER, R., NITZ, S. & DRAWERT, F., 1986. Identification and determination of volatile constituents in wines from different vine cultivars. *J. Sci. Food Agric.* **37**, 927-943.

BRAELL, P.A., ACREE, T.E, BUTTS, R.M. & ZHOU, P.G., 1986. Isolation of nonvolatile precursors of ß-damascenone from grapes using CHARM analysis. In: PARLIAMENT, T.H. & CROTEAU, R. (eds). *Biogeneration of aroma.* American Chemical Society, Washington, DC. pp. 75-84.

DOLLMANN, B., WICHMANN, D., SCHMITT, A., KOEHLER, H. & SCHREIER, P., 1996. Quantitative analysis of 2-aminoacetophenone in off-flavoured wines by stable isotope dilution assay. *J. AOAC Int.* **79**. 583-586.

GUEDES de PINHO, P. & BERTRAND, A., 1995. Analytical determination of furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone). Application to differentiation of white wines from hybrid and various *Vitis vinifera* cultivars. *Am. J. Enol. Vitic.* **46**, 181-186.

LAMIKANRA, O., GRIMM, C.C. & INYANG, I.D., 1996. Formation and occurrence of flavor components in noble Muscadine wine. *Fd. Chem.* **56**, 373-376.

KOLOR, M.G., 1983. Identification of an important new flavour compound in Concord grape: ethyl-3-mercaptopropionate. *J. Agric, Food Chem.* **31,** 1125-1127.

MARAIS, J., 1986. A reproducible capillary gas chromatographic technique for the determination of specific terpenes in grape juice and wine. *S. Afr. J. Enol. Vitic.* **7**, 21-25.

MAYERL, F., NÄF, R. & THOMAS, A.F., 1989. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone glucoside: Isolation from strawberries and synthesis. *Phytochem.* **28**, 631-633.

PEROLD, A.I., 1927. *Treatise on Viticulture.* Macmillan and Co., Ltd., London.

RAPP, A., KNIPSER, W., ENGEL, L., ULLEMEYER, H. & HEIMANN, W., 1980. Off-flavour compounds in the berry and wine aroma of grapevine hybrids. I. The strawberry-like flavour. *Vitis* **19**, 13-23.

RAPP, A., VERSINI, G. & ULLEMEYER, H., 1993. 2-Aminoacetophenone: causal component of 'untypical ageing flavour' ('napathalene' note, 'hybrid'note) of wine. *Vitis* **32**, 61-62.

RAPP, A., VERSINI, G. & ENGEL, L., 1995. Determination of 2-aminoacetophenone in fermented model wine solutions. *Vitis* **34**, 193-194.

RODIN, J.O., HIMEL, C.M., SILVERSTEIN, R.M, LEEPER, R.W. & GORTNER, W.A, 1965. Volatile flavour and aroma components of pineapple. I. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3(2H)-furanone. *J.Fd. Sci.* **30**, 280-285.

SCHREIER, P., 1980. Volatile constituents in different grape species. *Grape and wine centennial symposium proceedings.* University of California, Davis. pp. 317-321.

SCHREIER, P., DRAWERT, F. & JUNKER, A., 1976. Identification of volatile constituents from grapes. *J. Agric. Food Chem.* **24**, 331-336.

SCHREIER, P. & PAROSCHY, J.H., 1981. Volatile constituents from Concord, Niagara (*Vitis labrusca*, L.) and Elvira (*V. labrusca*, L. x *V. riparia*, M.) grapes. *Can. Inst. Food Sci. Technol. J.* **14**, 112-118.

SHURE, K.B. & ACREE, T.E., 1995. *In vivo* and *in vitro* flavour studies of *Vitis labruscana* Cv. Concord. In: ROUSEFF, R.K. & LEAHY, M. (eds). *Fruit flavors – biogenesis, characterization and authentication.* ACS Symposium Series 596, American Chemical Society, Washington. pp. 127-133.

TAKEOKA, G., BUTTERY, R.G., FLATH, R.A., TERANISHI, R., WHEELER, E.L., WIECZOREK, R.L. & GUENTERT, M., 1989. Volatile constituents of pineapple (*Ananas cosmosus* [L.] Merr.). In: BESSIÉRE, Y. & THOMAS, A.F., (eds). *Flavour science and technology,* John Wiley & Sons Ltd, Chichester. pp. 223-237.