

Aroma of Muscat Grape Varieties

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The terpenic derivatives that occur in the aroma of Muscat grapes have been studied by gas and thin-layer chromatography and by infrared and mass spectrophotometry. Eight compounds have been identified: linalol, geraniol, nerol, α -terpineol, two furanic, and two piranic oxides of linalol. Furthermore, two other substances that are not identified definitively have been placed in a prominent position. The terpenes of the Muscat juice have been titrated individually by gas chromatog-

raphy, after concentration by salting-out of the aqueous solution into an extractive solvent; the total content varies between 1 and 3 mg/l. The influence of each substance on the whole aroma has been precisely checked by the determination of thresholds, which vary between 100 and more than 6000 $\mu\text{g/l}$. Some transformations of terpenes may explain the losses of aroma that are sometimes observed during the processing and the storage of grape juices and wines.

Chemists have always been particularly interested in the study of the characteristic aroma of Muscat grape varieties. Furthermore, for a long time a synthetic reproduction of this aroma has been attempted in common juices and wines with different plant extracts.

It was in 1946 that Austerweil hypothesized for the first time that this aroma was attributable to terpenic compounds, especially to linalol and to its derivatives. Cordonnier (1956), in a fundamental work about the Muscat wines and the substances used to imitate their aroma, has suggested the presence of linalol, limonen, geraniol, and α -terpineol in the aroma of these wines.

For some years, with the introduction of gas chromatography, many workers have been interested by the study of these aromas. The work of Stevens et al. (1966), Webb et al. (1966), Usseglio-Tomasset (1966, 1969), Usseglio-Tomasset et al. (1966), Wenzel and de Vries (1968), Prillinger and Madner (1969, 1970), Hardy (1970), and Bayonove and Cordonnier (1970) concerns the identification and the role of the different terpenic compounds in the aroma of grapes and wines. But their results are not always in agreement; some authors attributed the characteristic Muscat aroma to the presence only of linalol, while for others linalol is only one compound, surely important, but not the only component of the fraction responsible for the aroma. The contradictions mentioned in the literature prompted us to again attempt the separation, isolation, and identification of the terpenic compounds present naturally in the Muscat grapes. Also, we hoped to titrate the terpenic derivatives previously identified in a great number of samples of grapes, grape juices, and wines. Thus, it was important to study the organoleptic part of these substances in the whole aroma of grape juices; for that, we have searched for the threshold value of each of them, individually and mixed. Finally, we have attempted to specify the transformations that take place in grape juices and wines and which may be accompanied by a loss of aromatic characteristics. Indeed, it is well known that the fruit aroma may disappear during the transformations that follow the processing and storage of grape juices and wines. All the works were the subject of several previous publications (Terrier, 1972; Terrier et al., 1972a,b; Terrier and Boidron, 1972a,b).

IDENTIFICATION

Prior Works. If the presence of linalol and α -terpineol seems definitively proven, many other alcohols and terpenic hydrocarbons have been pointed out by different authors; their formulas are given in Chart I. Also, the intervention of different oxides that are derived from linalol has

been pointed out; their formulas and the preparative methods by chemical reactions are given in Scheme I.

Experimental. The grapes were collected under sanitary conditions in absolutely perfect shape. The berries were separated from the stems. The grapes were crushed in the presence of sodium fluoride (500 mg/kg) to avoid fermentation and in the presence of ascorbic acid (500 mg/kg) to avoid oxidation. The crude mixture so obtained was delicately crushed during 4 min. at 400 rpm at a temperature of 0°. Then the crush was centrifuged at 9000 rpm during 3 min, also at low temperature. The liquid was filtered on a membrane so as to make the medium sterile, in order to be able to keep it until the time of analysis. The extraction of terpenic compounds was effected with methylene chloride; the solvent must be purified very carefully so as to avoid artefacts on the chromatograms, attributable to the presence of these impurities. Grape juice (650 ml) was stirred with 360 ml of solvent during 180 min at 40 rpm; a second extraction was effected with 300 ml of solvent. The organic phases were collected and dried during 24 hr on anhydrous sodium sulfate. The solvent was distilled in a water bath; the residue of the distillation (about 0.2 ml), which contains the substances to be studied, very concentrated, was stored at -20°, until the time of the analysis.

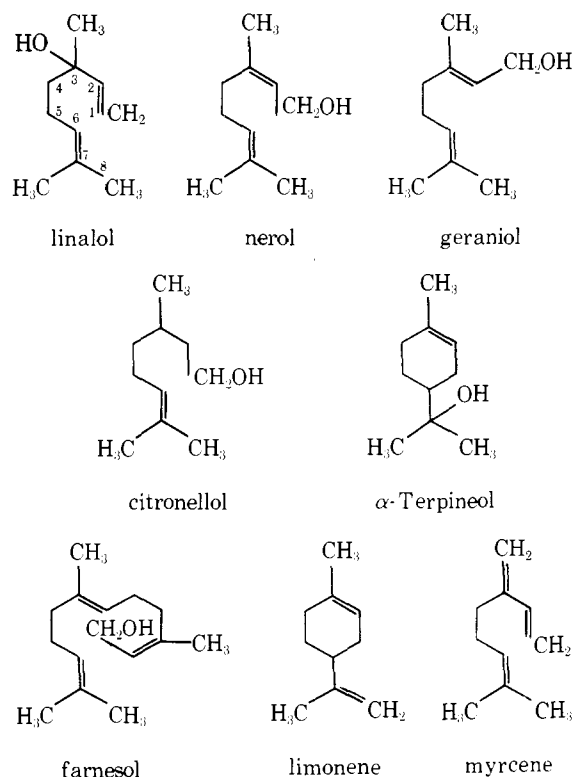
The identification of the components of this extract was achieved with different techniques: (1) gas chromatography with many columns: Carbowax 20M, SF 96, and FFAP; the columns were 10 m long and the temperature was between 150 and 250°; (2) thin-layer chromatography on silica gel with 20 solvents among which the most important were (a) ethyl acetate-acetic acid-cyclohexane (20:1:79) and (b) benzene-methanol (40:2); the revelation is achieved either with sulfuric vaniline or with anisaldehyde solution in methanol; (3) coupling gas chromatography with thin-layer chromatography; (4) coupling thin-layer chromatography with gas chromatography; (5) coupling gas chromatography with mass spectrophotometry. The comparisons are achieved with reference compounds of commercial origin. The oxides of linalol have been synthesized in the laboratory. In all cases, these compounds have been purified by preparative gas chromatography.

Results. Figure 1 shows the separation of terpenic derivatives by gas chromatography. The identifications mentioned in the legend have been achieved by comparison with reference substances; the chromatographic characteristics of all the substances are mentioned in Table I.

Chromatographic couplings might confirm these identifications. Concerning peak 3 of Figure 1, it is in an amount important enough to be able to be collected, in order to achieve its infrared spectrum, which is absolutely identical with that of pure linalol.

Finally, a definitive and complete identification of all the substances was obtained by compiling their mass spectra,

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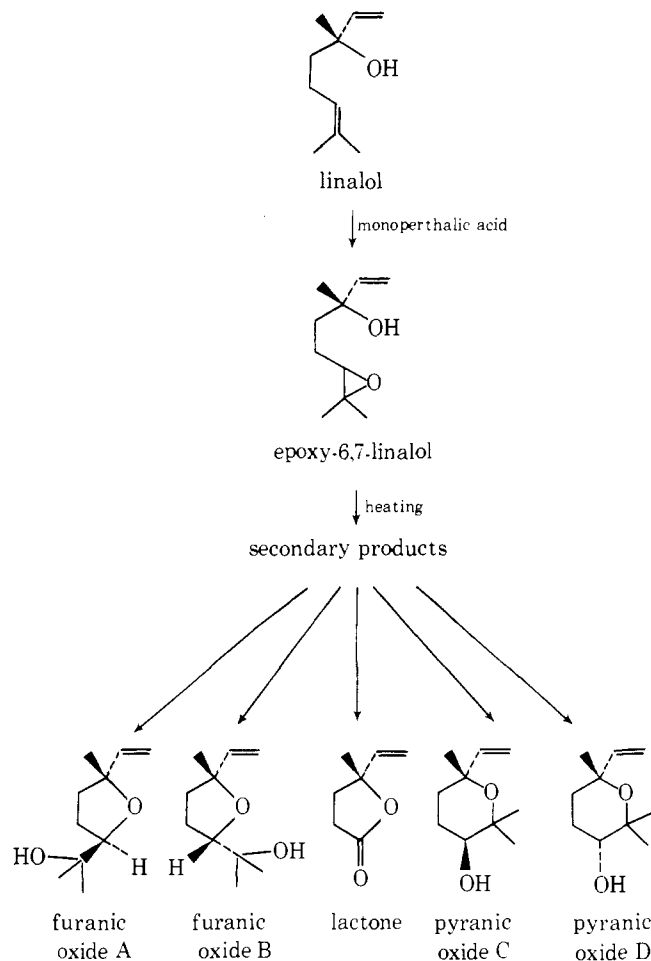
Chart I. Chemical Structures of Terpenic Alcohols and Hydrocarbons Found in Wine

which are identical with those of reference substances. Our work had given us the possibility of obtaining the mass spectra of substances X and Y (peaks 3 and 4 of Figure 1), but this information was not enough to allow identification. The mass spectrum might also identify the ionol (peak 10 of Figure 1) that is probably an impurity of the solvent.

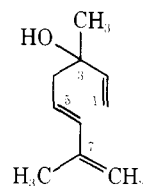
The following conclusions were obtained from the different experiments: (1) linalol and terpineol are present in the Muscat grapes; this identification is now admitted by all the authors; (2) nerol, geraniol, and piranic and furanic oxides, the identification of which is not admitted by all the authors, are also present naturally in these grapes; (3) limonene, myrcene, citronellol, and farnesol, mentioned in some works, are not present in sufficient amounts to be identified in the grapes that we have analyzed; (4) an unknown compound X is also present in these same grapes. This is not an artefact, because it is absent in nonaromatic grapes and in synthetic solutions of terpenes, treated under the same conditions; some facts seem to show that it would come from a transformation of monoterpenic acyclic alcohols. We think it is the "Muscat compound", mentioned by Prillinger and Madner in 1969. We could not identify this substance which had an aroma like the linden tree, but we have given its chromatographic characteristics and the mass spectrum. The results given in Table I show that the chromatographic characteristics of X and linalol are about the same. The results of Table I explain why X has escaped identification and been mistaken for linalol.

Since the publication of our results which we previously summarized, two important works have been published about this subject. Cordonnier and Bayonove (1974) have shown the existence of a monoterpenic bound fraction in the berry of Muscat Alexandria grapes, the hydrolysis of which, by the enzymes of fruit, liberates essentially linalol. It would probably concern a heterosidic form, in which the terpene is associated with a glucose molecule. Nevertheless, these combinations might not interfere with the technology of fruit juice and wine making, as they are probably destroyed immediately after the crushing of the berry.

In addition, Schreier et al. (1974) have identified dimethyl-3,7-octatrien-1,5,7-ol-3 in grapes other than the

Scheme I. Chemical Structure and Synthesis of Linalol Oxides

Muscat varieties, the mass spectrum of which is identical with that of the compound we have called X; there are reasons to suppose that the same terpenic derivative is present in Muscat grapes.



dimethyl-3,7-octatrien-1,5,7-ol-3

QUANTITATIVE DETERMINATION

Experimental. To achieve a preliminary concentration of compounds to be studied, extraction by solvent which gives a good concentration in a large amount is one of the best techniques for identification. However, such a method is difficult and not reproducible; consequently it is used with difficulty for quantitative determination; for this reason, terpenes have been salted-out of the aqueous solution into an extractive solvent; the manipulation is more simple and gives reproducible results. This method consists of forming an aqueous phase and an organic phase which are not miscible, by addition of a large amount of mineral salt to the aqueous phase. In the case of wines, the organic solvent is ethanol that it contains; in the case of grape juices, dimethyl ketone is added. In the experimental method that we used, 1.6 ml of dimethyl ketone and 35 ml of grape juice were mixed; successively, 24.1 g of pure and dry monosodic phosphate and 42.1 g of hydrated magnesium sulfate were added; the operation was done at 25°. The organic compounds, contained in the initial aqueous solution, were di-

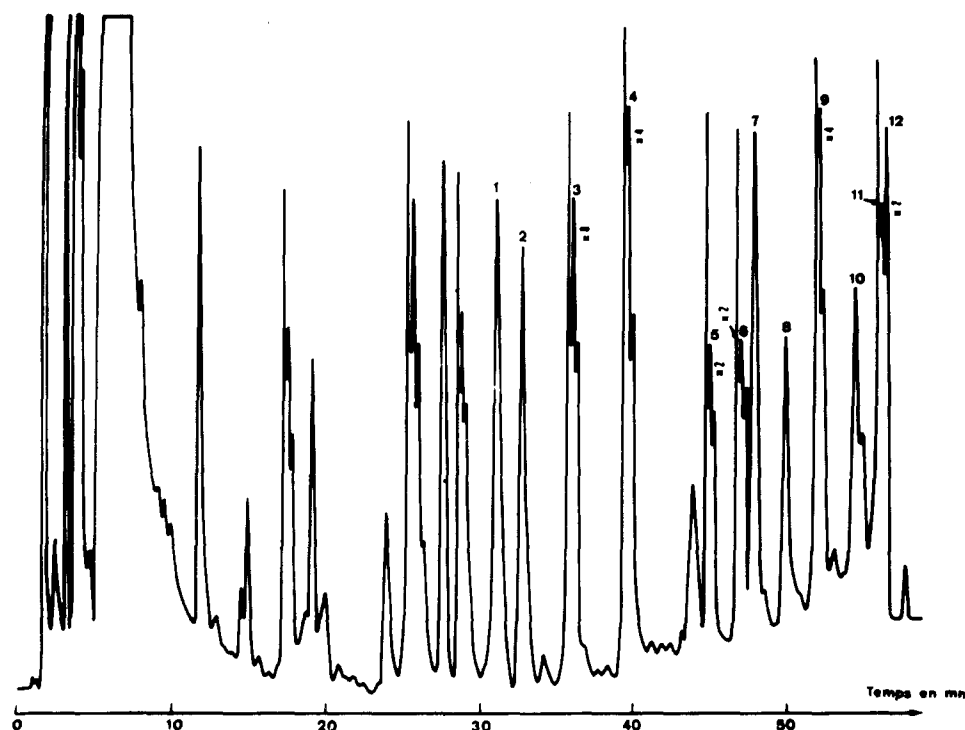


Figure 1. Chromatogram of an extract by CH_2Cl_2 of Muscat of Frontignan grape (column, FFAP; nitrogen, 20 ml/min; programmed temperature, from 65 to 200° with a 2° change for 1 min): (1) oxide A; (2) oxide B; (3) linalol; (4) compound X; (5) α -terpineol; (6) oxide C; (7) oxide D; (8) nerol; (9) geraniol; (10) ionol; (11) phenyl-2-ethanol; (12) compound Y.

Table I. Chromatographic Characteristics of Terpenic Derivatives from Muscat Grapes

Solvent no.	R_f values ^a								
	Linalol (3; 35, 42, 28.5)	α -Terpineol (5; 44, 51, 35)	Nerol (8; 50, 56, 38)	Geraniol (9; 52, 59, 42)	Oxide A (1; 29, 35, 26.7)	Oxide B (2; 31, 37, 28)	Oxide C (6; 47, 53, 33)	Oxide D (7; 48, 54, 33)	Compd X (4; 37, 46, -)
1	100	78	75	75					92
2	100	70	70	66					96
3	100	77	80	73					102
4	100	78	80	76					100
5	100		85	81					97
6	100	100	100	100					100
7	100	50	50	50					93
8	100	53	47	47					88
9	100	66	56	45					90
10	100	79	73	66					92
11	100	96	96	92					102
12	100	78	78	78					95
13	100	75	63	63					93
14	100	69	62	62					93
15	100	70	70	70					100
16	100	90	85	81					107
17	100	77	74	69	81	81	50	50	100
18	100	74	77	77					100
19	100	79	79	79					100
20	100	70	83	73					100

Color after spraying ^b									
Sulfuric vanillin (0.5 hr)	G	Bl	G	G	Gy	Gy	Gy	Gy	B
Sulfuric vanillin (12 hr)	Bl	Br	Gy	Gy	R	R	R	R	B
Anisaldehyde	Yg	Ps	Yg	Yg	Gp	Gp	Gp	Gp	M

^a For all column heads, the first number in parentheses is the peak number (from Figure 1). The other three numbers are retention times by gas chromatography for FFAP (4 m), Carbowax 20M (6 m), and SF96 (6 m) columns, respectively. R_f values refer to linalol by thin-layer chromatography in 20 different solvents (Terrier, 1972). ^b Abbreviations are: G, green; Gy, green-yellow; Gp, pale green; M, mallow; Ps, pink salmon; R, red; Bl, blue; Br, brown; Yg, yellow-green.

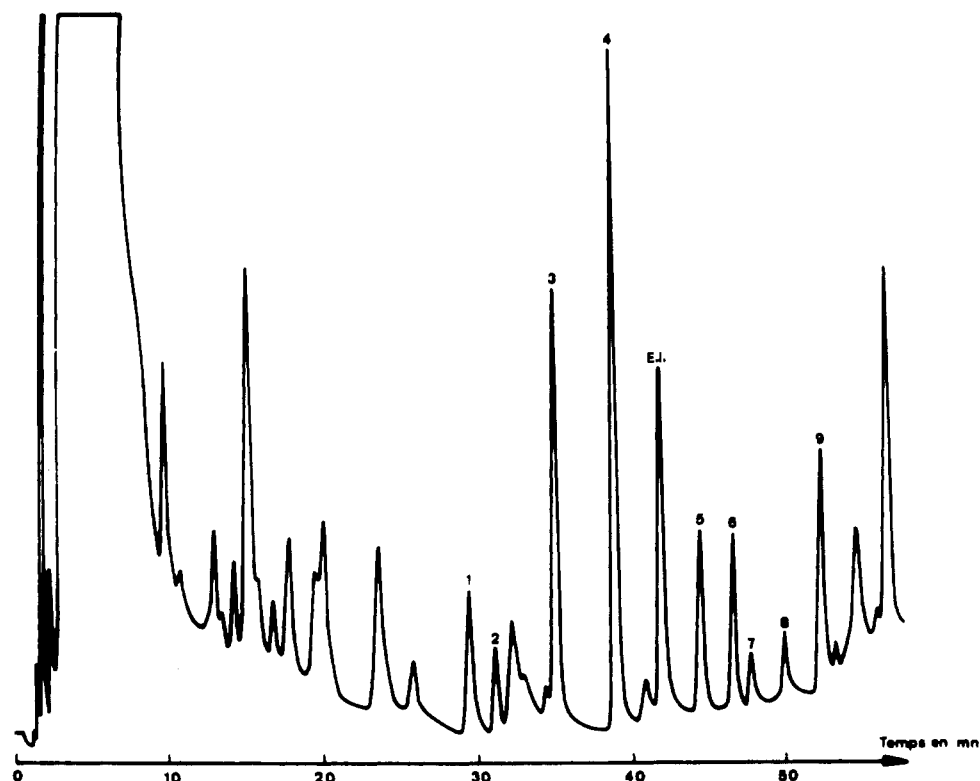


Figure 2. Chromatogram of direct injection of a 2- μ l organic phase salting-out of Muscat of Alexandria juice (column FFAP): (1) oxide A; (2) oxide B; (3) linalol; (4) compound X; (5) α -terpineol; (6) oxide C; (7) oxide D; (8) nerol; (9) geraniol; E.I. internal standard (nonanol-1).

Table II. Terpenic Derivatives Content (Micrograms per Liter) in Different Samples of Muscat Grape Juice

Muscat varieties	Linalol	Geraniol	Nerol	α -Terpineol	Oxide A	Oxide B	Oxide C	Oxide D	Total terpenes
Alexandrie									
Maximum	815	1059	263	117	190	103	194	100	2720
Minimum	170	223	14	23	86	39	65	20	685
Average	455	506	94	78	133	73	141	44	1525
Frontignan									
Maximum	846	702	405	145	400	247	349	232	3326
Minimum	245	225	35	62	81	62	135	55	1020
Average	473	327	135	87	225	157	208	96	1716
Saint Vallier									
Maximum	1506	441	46	140	226	109	201	51	2655
Minimum	240	204	29	44	124	83	89	23	1020
Average	764	311	37	86	167	92	145	37	1640
Italia									
Maximum	638	285	47	53	58	37	75	77	1127
Minimum	160	97	12	23	21	10	20	10	353
Average	359	202	34	36	33	22	52	40	778
Hambourg									
Maximum	489	618	447	114	239	183	176	115	2381
Minimum	62	130	11	20	24	18	30	21	316
Average	308	257	103	39	97	71	91	58	1018
Maximum value	1506	1059	447	145	400	247	349	232	3326
Minimum value	62	89	11	19	21	10	20	10	316
Average value	433	321	84	60	129	83	127	56	1294

vided between both phases, depending on their distribution factor. The volatile compounds, particularly the terpenic derivatives, were concentrated reproducibly in the organic phase, in a very small amount, that can be analyzed by direct gas chromatography (Figure 2). The measurement of each peak is referred to a reference curve that is obtained from standard solutions treated exactly in the same condi-

tions as for the titration itself. The titration is made more accurate by adding an internal standard (nonanol-1).

Results. Many analyses of all the terpenic derivatives have been performed on different Muscat juices; Table II sums up all these results. It is noticed that the differences are evident enough between the varieties. On the other hand, the terpenic derivatives that occur in the aroma of

Table III. Composition of Two Synthetic Solutions of Terpenic Derivatives

	Mixture I		Mixture II	
	$\mu\text{g/l.}$	%	$\mu\text{g/l.}$	%
Linalol	300	24.4	600	33.1
Geraniol	268	21.8	430	23.8
Nerol	84	6.9	84	4.6
α -Terpineol	92	7.5	137	7.5
Oxide A	100	8.1	200	11
Oxide B	100	8.1	100	5.5
Oxide C	190	15.5	189	10.4
Oxide D	92	7.5	74	4
Total amount	1226	100	1814	100

Muscat grapes have a concentration that does not exceed 1–3 mg/l. Recent results that Bayonove and Cordonnier (1971) have obtained about linalol content agree with the values mentioned in this work, but the titrations that these authors have carried out refer only to this substance.

The varieties of vines that produce aromatic grapes but which do not belong to Muscat varieties, particularly the vines cultivated in the Rhine valley (Riesling), have the same terpenic substances, but their whole terpene content is much lower, about 0.1–0.3 mg/l. The varieties known to produce nonaromatic grapes give juices that contain no terpenic derivatives at all.

INTERVENTION OF TERPENIC COMPOUNDS IN THE AROMA OF MUSCAT GRAPE JUICES

Since we now knew the chemical composition of the Muscat aroma, it was interesting to study the role of each compound in the whole Muscat aroma. This was done with authentic samples of terpenic derivatives of commercial origin or synthesized by chemical reactions; in both cases, they were purified by preparative gas chromatography. Then we measured the aromatic thresholds of the terpenes in water and sugar, which is the same medium that they occupy in grape juices. These determinations were made not only with pure terpenes found in Muscat grapes but also with two mixtures of all these substances in the same amount that they are in Muscat juice (Table III); in mixture I the two main compounds (linalol and geraniol) were 46% and in mixture II, 57%.

The determination of the different thresholds has been made by a panel of 18 tasters, using the triangular test; each taster looks for the lowest amount he is able to characterize only by smell. The threshold of a component is expressed by the minimum amount of this compound 50% of the people in the panel are able to appreciate. Table IV gives the results. The two main terpenes in Muscat grapes, linalol and geraniol, have the lowest thresholds; that means they are the most aromatic. For linalol, the amount is 100 $\mu\text{g/l.}$ and for geraniol it is a little less, 132 $\mu\text{g/l.}$; this last amount is found by 59% of the panel. This fact is important; not only, as we showed previously, do Muscat grapes contain about the same amount of geraniol as linalol, but also, as we show now, these two compounds have the same aromatic strength. This confirms that geraniol plays the same role as linalol in the Muscat aroma. Nerol and terpineol have thresholds three or four times higher, between 400 and 500 $\mu\text{g/l.}$ Finally, for the linalol oxides, the values are even higher, about 3000–5000 $\mu\text{g/l.}$ for oxides C and D and more than 6000 $\mu\text{g/l.}$ for oxides A and B. These compounds are 30 to 60 times less aromatic than linalol.

Now if we look at the results for mixtures I and II, we see that the thresholds are very low; this means that their aroma is intensive. For mixture I, 180 $\mu\text{g/l.}$ is found by 72%

Table IV. Determination of Aromatic Thresholds of Terpenic Derivatives in Synthetic Medium (Percentage of Tasters Able to Appreciate the Amounts Checked) (the Amount for Which the Threshold Is Reached or Passed Is in Italics)

Derivative	Thresholds		
Linalol	100 $\mu\text{g/l.}$ 50%	200 $\mu\text{g/l.}$ 69%	300 $\mu\text{g/l.}$ 88%
Geraniol	132 $\mu\text{g/l.}$ 59%	265 $\mu\text{g/l.}$ 82%	530 $\mu\text{g/l.}$ 94%
Nerol	400 $\mu\text{g/l.}$ 47%	600 $\mu\text{g/l.}$ 71%	800 $\mu\text{g/l.}$ 94%
α -Terpineol	230 $\mu\text{g/l.}$ 24%	460 $\mu\text{g/l.}$ 53%	690 $\mu\text{g/l.}$ 77%
Oxide A	4000 $\mu\text{g/l.}$ 6%	5000 $\mu\text{g/l.}$ 17%	6000 $\mu\text{g/l.}$ 34%
Oxide B	4000 $\mu\text{g/l.}$ 6%	5000 $\mu\text{g/l.}$ 17%	6000 $\mu\text{g/l.}$ 45%
Oxide C	1800 $\mu\text{g/l.}$ 28%	3600 $\mu\text{g/l.}$ 72%	5400 $\mu\text{g/l.}$ 78%
Oxide D	1800 $\mu\text{g/l.}$ 17%	3600 $\mu\text{g/l.}$ 45%	5400 $\mu\text{g/l.}$ 56%
Mixture I ^a (total amount)	184 $\mu\text{g/l.}$ 72%	306 $\mu\text{g/l.}$ 89%	430 $\mu\text{g/l.}$ 95%
Mixture II ^a (total amount)	64 $\mu\text{g/l.}$ 29%	91 $\mu\text{g/l.}$ 65%	136 $\mu\text{g/l.}$ 82%

^a See Table III for the composition of these mixtures.

of the tasters; this result gives a threshold value of about 100 $\mu\text{g/l.}$ and, of that amount, only 46% are linalol and geraniol, which have about the same threshold. The remaining amount, that is to say 54%, includes all the other Muscat terpenes, even the linalol oxides, which are much less aromatic. The same result is even more evident with mixture II, containing 57% of linalol and geraniol; its threshold is below 90 $\mu\text{g/l.}$, which means below the corresponding value for linalol, which has the lowest threshold among the pure compounds included in this mixture.

It appears from these results that, concerning their aromatic characteristics, terpenes react with each other; more particularly, one compound can increase the aroma of another and a mixture is more aromatic than the most aromatic of all the compounds which belong to that mixture.

In the discussion of muscat aroma, we have not considered the intervention of compound X, which has been identified very recently. But we hope to be able to make use of a pure sample and to appreciate its aroma in a short time. This compound probably plays a fundamental part in the Muscat aroma.

Finally, it is interesting to consider the chemical transformations of the Muscat aroma during the processing of juices, wine making, and during the storage of juices and wines. It is well known that linalol can be easily oxidized into the four oxides previously described. Also, it is known that geraniol can be transformed into terpineol; nerol, which is an isomer of geraniol, can probably react likewise, but it is less important because nerol is three times less aromatic than geraniol.

If we consider the thresholds of all these compounds it is evident that these chemical transformations involve a loss of aroma for these juices or wines; also, it is possible to hypothesize that these reactions take place in the transformations responsible for losses of aroma in commercial juices or in wines.

CONCLUSION

In conclusion, the characteristic aroma of Muscat grapes can be attributed to the presence of different terpenic de-

rivatives: linalol, nerol, geraniol, α -terpineol, and four oxides of linalol; moreover, two other substances are present: one is probably dimethyl-3,7-octatrien-1,5,7-ol-3. The total amount of these substances represents 1–3 mg/l. in Muscat juices.

Each of these compounds has different organoleptic characteristics and an aroma which is not identical with that of Muscat, that is found correctly in the mixture of all these different substances. The threshold of these different compounds is between 100 and >6000 μ g/l. Geraniol and linalol are, in the whole aroma, the most important substances, not only because they are the most concentrated, but also because they have the lowest thresholds. In the whole aroma, the different terpenes react with each other because the mixtures are more aromatic than each individual compound. The most aromatic of these substances can be involved in chemical transformations which lead to other terpenes less aromatic. These chemical transformations must result in the losses of aroma often observed during the processing or the storage of commercial juices and wines.

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Cheddar Cheese Flavor. A Review of Current Progress

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Research on Cheddar cheese flavor from 1968 to 1974 is reviewed with respect to factors affecting flavor development, flavor volatiles identified, and the divergent views on the volatile components

most significant to the characteristic Cheddar aroma. Recent reexaminations of methods of isolating cheese volatiles are noted.

During the period from 1968 to 1974, work on cheese flavor has been reviewed from several points of view. Schormuller's 1968 review deals with the chemistry and biochemistry of a variety of cheeses. Fryer (1969) published a comprehensive review of the microflora of Cheddar cheese and its influence on flavor. Forss (1969) reviewed the flavor of dairy products, including pertinent current scientific and patent literature on Cheddar flavor. Sandine and Elliker (1970) reviewed flavor in fermented dairy products, including Cheddar cheese. Reiter and Sharpe (1971) provided an update on the work being done at the National Institute for Research in Dairying in England, using aseptic cheese vats. Evans (1972) also reviewed the subject, but with greater emphasis on analytical aspects. Dwivedi (1973), in a review on the role of enzymes in flavor of dairy products, briefly deals with Cheddar cheese. There is also a review of cheese flavor in general, by Panouse et al. (1972), and an extensive review of flavor development in Swiss cheese by Langsrud and Reinhold (1973a–c, 1974).

In this review an attempt is made to summarize and critically assess recent work on factors involved in the development in Cheddar flavor and the flavor significance of components that have been identified.

Early work on Cheddar flavor was based on the hypothesis that there was one compound or one class of compounds that provided the characteristic Cheddar flavor. Since such a compound could not be found, Kosikowski and Mocquot (1958) formalized the component balance theory, the essence of which had been stated earlier by Mulder (1952). This theory proposed that cheese flavor was produced by a blend of compounds, no one of which produced the characteristic flavor. If the proper balance of components was not achieved, then undesirable or defective flavors occurred. The component balance theory, to my knowledge, has not been questioned in recent years.

There are essentially two approaches to the study of Cheddar flavor. One approach is to isolate and identify components which contribute to the flavor. The other approach is to determine the factors or agents which influence or control the development of flavor.

FACTORS INFLUENCING DEVELOPMENT OF FLAVOR

The work being done at NIRD in England by Reiter and Sharpe (1971) is of particular interest. They make cheese in aseptic vats using δ -gluconic acid lactone as the acid-producing agent. No starter culture is used, and the resulting cheese is completely devoid of Cheddar flavor. This of course demonstrates that they have eliminated the previously uncertain influences of chance contamination by bacteria from the cheese plant environment. It also demon-

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