Note

Effects of Fermentation Conditions and Aging Temperature on Volatile Ester Contents in Wine*

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In a previous study¹⁾ we developed a rapid and accurate method and determined the amounts of volatile esters in wine by gas chromatography. The analysis of esters in various wines has led to the suggestion that the differences of wine-making processes participate in the formation of volatile esters. In the present work the effects of yeast strains, grape varieties, clarification of must, sugaring, sulfurous acid, anaerobic fermentation, temperature and aging on the formation of volatile esters in wines were examined. The fermentation process was the same as described previously.²⁾

The effective conditions to produce a large amount of volatile esters were provided by *Sacch. cerevisiae* var. *ellipsoideus* BIG 65, sugaring, anaerobic fermentation and clarification of must (Tables I and II), while the other

conditions examined (Tables III and IV) were less effective for ester formation. The results indicate that ester content in wines is increased during fermentation under the following conditions: (1) use of a yeast strain with high ability to produce the esters, (2) anaerobic environment to suppress oxygen uptake of yeasts, since synthesis of fatty acid esters in yeast cells increased in anaerobic cultures,^{3,9,10)} and (3) appropriate sugar content in grape must, as the more ethyl alcohol the more esters seem to be produced (Table I).⁷⁾

The changes of volatile ester contents in wine during aging are shown in Fig. 1. The contents of acetate esters rapidly decreased at higher temperatures (above 20°C), while fatty acid esters gradually decreased and at lower temperatures (below 10°C) the volatile esters decreased very slowly. However, diethyl succinate (DES) increased with time and the formation is promoted by temperature elevation.⁸⁾ The influence of temperature is also shown in Table V. The results show that higher temperatures (above 20°C) promote the decrease of the esters except DES in wine during aging, and this well agreed with the observations in Fig. 1 and the previous results.^{5~7)}

In conclusion, significant improvement to give a fruity and good flavor to wine was attained by the use of a selected yeast strain like *Sacch. cerevisiae* var. *ellipsoideus* and well matured grapes, fermentation under anaerobic conditions of clarified must, and aging of wines at lower temperatures (below 20°C).

Acknowledgments. We wish to express our sincere

FIG. 1. Changes of Volatile Ester Contents in Wines during Storage at Different Temperatures.

White wines were stored in bottles (720 ml size) closed with corks.

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Abbreviations: EtC6, ethyl caproate; EtC8, ethyl caprylate; EtC10, ethyl caprate; AmOAc, iso-amyl acetate; HxOAc, *n*-hexyl acetate; PhEtOAc, 2-Phenylethyl acetate; DES, diethyl succinate.

TABLE I. FORMATION OF VOLATILE ESTERS UNDER VARIOUS FERMENTATION CONDITIONS-(1)

		EtC6	EtC8	EtC10	Sum-A ^b	AmOAc	HxOAc	PhEtOAc	Sum-B ^c	Sum A+B ^b
		(milligrams per liter)								
1. Yeast strains ^a										
No.										
1	<i>Sacch. cerevisiae</i> IAM4274	0.7	0.8	0.4	1.9	1.1	t ^c	0.3	1.4	: 3.3
2	<i>Sacch. cerevisiae</i> IAM4195	0.6	1.3	0.4	2.3	0.8	t	0.2	1.0	: 3.3
3	<i>Sacch. cerevisiae</i> NJKW304	0.7	0.8	0.4	1.9	1.1	t	0.5	1.5	: 3.4
4	<i>Sacch. cerevisiae</i> var. <i>ellipsoideus</i> IAM4268	0.8	1.0	0.4	2.2	1.6	0	0.2	1.8	: 4.0
5	<i>Sacch. cerevisiae</i> var. <i>ellipsoideus</i> BIG65	0.4	0.5	0.3	1.2	3.7	t	1.2	4.9	: 6.1
6	<i>Sacch. oviformis</i> IAM4351	0.7	0.9	0.4	2.0	1.6	t	0.4	2.0	: 4.0
7	<i>Sacch. delbrueckii</i> IFO316	0.8	1.1	0.5	2.4	2.2	t	0.3	2.5	: 4.9
8	<i>Sacch. bayanus</i> IFO0206	0.4	0.8	0.4	1.6	0.5	0	0.2	0.7	: 2.3
9	<i>Schizo. pombe</i> BIG228	0.3	0.5	t ^d	0.8	t ^c	0	0	t ^c	: 0.8
10	<i>Kl. apiculata</i> IFO0865	0.9	0.7	0.5	2.1	1.1	t	0.2	1.2	: 3.3
2. Sugaring ^e										
Sugarcane content in must										
16% (Control)		0.5	0.5	t	1.0	3.0	t	0.4	3.4	: 4.4
22%		0.7	0.8	t	1.5	5.3	0.1	0.5	5.9	: 7.4
25%		0.5	0.7	t	1.2	4.1	t	0.6	4.7	: 5.9
30%		0.4	0.8	t	1.2	2.1	t	0	2.1	: 3.3

^{a,e} Grape musts (*Koshu*, sterile filtered) were fermented at 20°C in hermetic vessels equipped with fermentation bungs.^b Sum-A, EtC6+EtC8+EtC10; Sum-B, AmOAc+PhEtOAc+HxOAc; Sum A+B, Sum-A+Sum-B.^c t, traces, less than 0.1 mg/liter.^d t, traces, less than 0.3 mg/liter.^e Saccharose was used for amelioration and the strain, *Sacch. cerevisiae* IAM4274, was employed for fermentation. Ethyl alcohol (v/v%) formed was 9.1, 12.0, 11.3 or 10.4 at 16, 22, 25 or 30% sugar, respectively.

TABLE II. FORMATION OF VOLATILE ESTERS UNDER VARIOUS FERMENTATION CONDITIONS-(2)

	EtC6	EtC8	EtCl0	Sum-A:	AmOAc	HxOAc	PhEtOAc	Sum-B:	Sum A + B
	(milligrams per liter)								
3. Anaerobic fermentation <i>Sacch. cerevisiae</i> IAM4274									
Anaerobic ^a	0.6	0.8	0.4	1.8	1.1	t	0.3	1.4	: 3.2
Control ^b	0.2	0.2	0	0.4	0.2	0	0.1	0.3	: 0.7
<i>Sacch. cerevisiae</i> NJKW304									
Anaerobic	0.7	0.8	0.4	1.9	1.1	t	0.5	1.6	: 3.5
Control	0.2	0.3	0	0.5	0.2	0	0.3	0.5	: 1.0
4. Clarification of must ^c									
No. 1 Clear must	0.9	0.7	0.3	1.9	7.5	0.1	1.3	8.9	:10.8
2 Clouded must	0.5	0.4	t	0.9	5.2	0	0.4	5.6	: 6.5
3 Clouded must with skins	0.2	0.1	0	0.3	1.3	0	0.2	1.5	: 1.8

^{a,b} Grape musts (*Koshu*, sterile filtered) were fermented at 20°C in hermetic vessels equipped (a) with fermentation bungs or (b) with cotton plugs.^c *Cabernet-Sauvignon* grapes were used. Clear must: suspended materials were removed from must after settling at 5°C for 15 hr. Clouded must: must with suspended materials. Clouded must with skins: crushed and stemmed grapes. The strain, *Sacch. cerevisiae* IAM4274, was used for fermentation.

TABLE III. FORMATION OF VOLATILE ESTERS UNDER VARIOUS FERMENTATION CONDITIONS-(3)

	EtC6	EtC8	EtCl0	Sum-A:	AmOAc	HxOAc	PhEtOAc	Sum-B:	Sum A+B
	(milligrams per liter)								
5. Grape varieties ^a									
<i>Koshu</i>	0.8	1.2	0.6	2.6	1.8	0.1	0.2	2.1	: 4.7
<i>Zenkoji</i>	0.4	0.5	0.3	1.2	2.8	0.3	0.2	3.3	: 4.5
<i>Chardonnay</i>	0.4	0.3	0.4	1.1	1.9	0.2	0.3	2.4	: 3.5
<i>Riesling</i>	0.6	0.6	0.3	1.5	2.0	0.2	0.4	2.6	: 4.1
<i>Delaware</i>	0.5	0.8	t	1.3	3.0	t	0.4	3.4	: 4.7
6. Sulfurous acid ^a									
<i>Sacch. cerevisiae</i> IAM4274									
SO ₂ 0 mg/liter	0.9	1.2	0.4	2.5	2.6	0.1	0.5	3.2	: 5.7
SO ₂ 50 mg/liter	0.7	1.2	0.4	2.3	2.9	0.1	0.7	3.7	: 6.0
SO ₂ 100 mg/liter	0.7	1.2	0.4	2.3	1.6	t	0.5	2.1	: 4.4
<i>Sacch. cerevisiae</i> NJKW304									
SO ₂ 0 mg/liter	0.5	0.7	0	1.2	1.8	0.1	0.8	2.7	: 3.9
SO ₂ 50 mg/liter	0.4	0.7	t	1.1	1.3	t	0.5	1.8	: 2.9
SO ₂ 100 mg/liter	0.8	0.9	0.3	2.0	2.0	t	0.6	2.6	: 4.6
<i>Sacch. cerevisiae</i> var. <i>ellipsoideus</i> IAM4268									
SO ₂ 0 mg/liter	0.8	0.7	0	1.5	4.0	t	0.7	4.7	: 6.2
SO ₂ 50 mg/liter	0.5	0.8	t	1.3	3.0	t	0.4	3.4	: 4.7
SO ₂ 100 mg/liter ^b	—	—	—	—	—	—	—	—	: —

^a Grape musts were fermented as described in the footnote to Table I.^b This lot was not fermented due to the high concentration of SO₂.

TABLE IV. FORMATION OF VOLATILE ESTERS UNDER VARIOUS FERMENTATION CONDITIONS-(4)

	EtC6	EtC8	EtC10	Sum-A:	AmOAc	HxOAc	PhEtOAc	Sum-B:	Sum A + B
(milligrams per liter)									
7. Fermentation temperature ^a									
<i>Sacch. cerevisiae</i> IAM4274									
15°C	0.7	0.9	t	1.6	1.2	t	0.2	1.4	: 3.0
20	0.7	0.8	0.4	1.9	1.1	t	0.3	1.4	: 3.3
25	0.5	0.7	0.4	1.6	1.0	0	0.4	1.4	: 3.0
<i>Sacch. cerevisiae</i> NJKW304									
15°C	0.7	0.8	0.3	1.8	0.7	t	0.2	0.9	: 2.7
20	0.7	0.8	0.4	1.9	1.1	t	0.5	1.6	: 3.5
25	0.5	0.8	0.4	1.7	1.1	0	0.2	1.3	: 3.0
<i>Sacch. cerevisiae</i> var. <i>ellipsoideus</i> IAM4268									
15°C	0.6	1.1	0.4	2.1	2.1	t	0.2	2.3	: 4.4
20	0.8	1.0	0.4	2.2	1.6	0	0.2	1.8	: 4.0
25	0.4	0.7	0.4	1.5	1.2	0	0.2	1.4	: 2.9

^a Grape musts were fermented as described in the footnote to Table I.

TABLE V. CONTENTS OF VOLATILE ESTERS IN WHITE WINES STORED FOR 12 MONTHS AT DIFFERENT TEMPERATURES^a

	EtC6	EtC8	EtC10	Sum-A:	AmOAc	HxOAc	PhEtOAc	Sum-B:	Sum A+B:	DES ^b
(milligrams per liter)										
<i>Zenkoji 1977</i>										
5°C	0.4	0.3	0	0.7	1.3	0	0	1.3	: 2.0	3
10	0.3	0.4	0	0.7	1.1	0	0	1.1	: 1.8	5
20	0.3	0.2	0	0.5	0.8	0	0	0.8	: 1.3	8
30	0.2	0.2	0	0.4	0.3	0	0	0.3	: 0.7	20
37	0.2	0.1	0	0.7	t	0	0	t	: 0.3	33
<i>Delaware 1975</i>										
5°C	0.6	0.4	0	1.0	1.1	0	0	1.1	: 2.1	8
20±5	0.4	0.3	0	0.7	0.1	0	0	0.1	: 0.8	18
30	0.3	0.4	0	0.7	t	0	0	t	: 0.7	23

^a Bottled wines (720 ml size, closed with corks) were used for storage.

^b DES, diethyl succinate.

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