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Volatile amines in *Vitis vinifera* varieties and changes during maturation

by

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Flüchtige Amine in *Vitis-vinifera*-Sorten und ihre Veränderungen während der Beerenreife

Zusammenfassung. — Bei den *Vitis-vinifera*-Sorten Cabernet Sauvignon und Chenin blanc wurden die Konzentrationsänderungen der flüchtigen Amine vom Weichwerden der Beeren bis zur Beerenreife in wöchentlichen Intervallen bestimmt. Diese Veränderungen wiesen bei beiden Sorten grundsätzlich die gleiche Tendenz auf. Zu Beginn der Beerenreife waren die Methylamin- und 2-Phenäthylaminkonzentrationen hoch; während der Reife fielen sie auf erheblich niedrigere Werte ab. Die Äthylaminkonzentration stieg beträchtlich an. Dimethylamin und Isoamylamin zeigten keine klare Tendenz. Die Diäthylkonzentration war bei Cabernet Sauvignon in der Mitte der Reifeperiode am höchsten, bei Chenin blanc dagegen über den ganzen Untersuchungszeitraum recht konstant. Bei der Beerenreife lagen im nichtgepreßten Most von Cabernet Sauvignon, Chenin blanc, Weißem Riesling und Pinot noir folgende Aminkonzentrationen (in µg/l) vor: Methylamin — 500, 180, 850 und 145; Dimethylamin — 45, 10, 25, 35; Äthylamin — 610, 150, 1900, 4900; Diäthylamin — 30, 25, < 1, 30; Isoamylamin — 2, 5, 700, 160; 2-Phenäthylamin — 4, < 1, 200, 25; 2-Methyl-1-butylamin (a-Amylamin) — < 1, < 1, < 1, 3; n-Propylamin — < 1, < 1 (für Riesling und Pinot noir nicht bestimmt).

Introduction

Very little work has been done in amine estimations in wines and none, to our knowledge, has been reported for grapes. Several authors (3, 9) reported in beer that amines are not formed during fermentation, but come from malt, hops, etc. In wines there is no such study with reference to grapes.

According to SMITH (12) the lower aliphatic monoamines are widely distributed in the plant kingdom. They are often produced in flowers at anthesis or by fruiting bodies of fungi. Insects which carry pollen or spores are attracted by the smell of these amines, which may simulate rotting meat. In the Araceae, several aliphatic and aromatic amines are produced as part of a complex mechanism to attract flies which effect pollination.

Among the plants already studied for the presence of volatile amines, no reference is made by SMITH (12) to grapes of *Vitis* spp. In the present study, amine content of four cultivars of grape (*V. vinifera*) var. Cabernet Sauvignon, Chenin blanc, White Riesling and Pinot noir were examined at maturity, and changes occurring during the maturation of the first two varieties were also monitored.

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Materials and methods

Two *V. vinifera* varieties, Chenin blanc and Cabernet Sauvignon (both from University of California vineyards at Davis), were harvested weekly from early July till the 1st week of October, 1980. Two other *V. vinifera* varieties (Pinot noir and White Riesling) were selected at full maturity for analysis.

About 20—24 pounds (9—11 kg) of grapes were stemmed, then crushed in a small electrical screw press at standard conditions. The juice (free of skins) was allowed to settle at 0 °C. Only the clean, particle-free juice was used for analysis. One exception was the Pinot noir sample where the whole crushed grapes, juice and seeds were used for analysis.

The extraction method, derivation and GC method for qualitative analysis were those of DAUDT and OUGH (2). Recovery, standard deviation of measurements and retention times of the TFA derivatives were given. Variations from that report for these grape juice measurements were: 1. The amount of TFA was increased (because of large amounts of ammonia in grapes) to 10—12 ml, first as a 6 ml increment, than as 2 or 3 ml increments; 2. 10—15 ml of 8 % sodim bicarbonate solution were added to neutralize the great amount of acid formed and then dry sodium bicarbonate added until the solutions were completely neutralized.

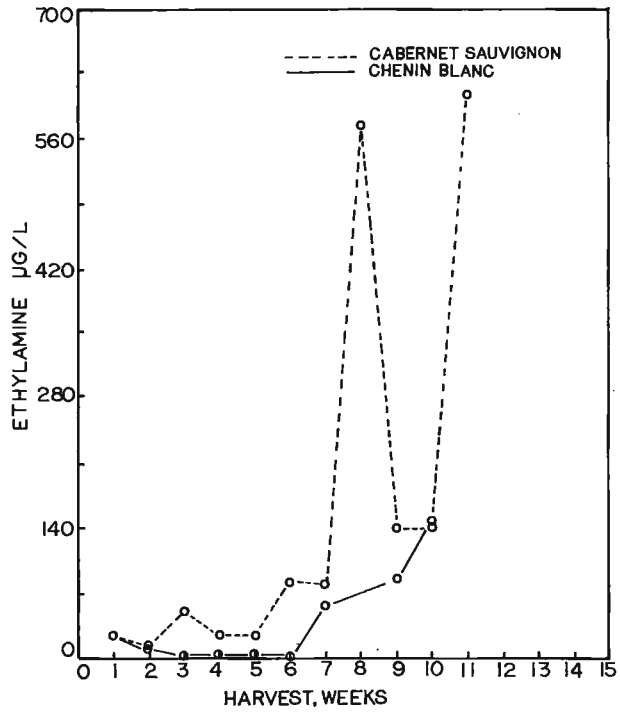

Fig. 1: Ethylamine changes during grape maturation with two varieties of *V. vinifera*. Starting date was 18 July, 1980, represented by 1 on the harvest scale.

Die Veränderungen der Äthylaminkonzentration im Verlaufe der Beerenreife bei zwei *V.-vinifera*-Sorten. Versuchsbeginn am 18. Juli 1980 (= 1 auf der Ernteachse).

The trifluoroacetamides were analyzed by GC with capillary column (25M, fused silica, 0.20 mm I.D., coated with Carbowax 20M) separation and N/P detector determination. Three internal standards were used for the quantification (7).

The sample amines were verified by comparing the retention times and mass spectra on two fused silica capillary columns (Carbowax 20M and SE-54) to those of known synthesized amine TFA derivatives (8).

Results and Discussion

There is a great variation in the amount of amines with degree of maturity (the ripening period from the time the berries reach full size until the sugar accumulation reaches the desired level). Ethylamine (Fig. 1) increased slowly during maturation of Chenin blanc and Cabernet Sauvignon. In the latter variety, however, ethylamine increased and then dropped in the 9th week, remaining at that level for 1 week and increasing towards the end of maturation. Free-run juice of Cabernet Sauvignon was higher in this amine, at maturation, then in the Chenin blanc sample.

For Chenin blanc the methylamine was present in high amounts at the start of the picking scheme. Although methylamine increased in the 4th week, dropping between

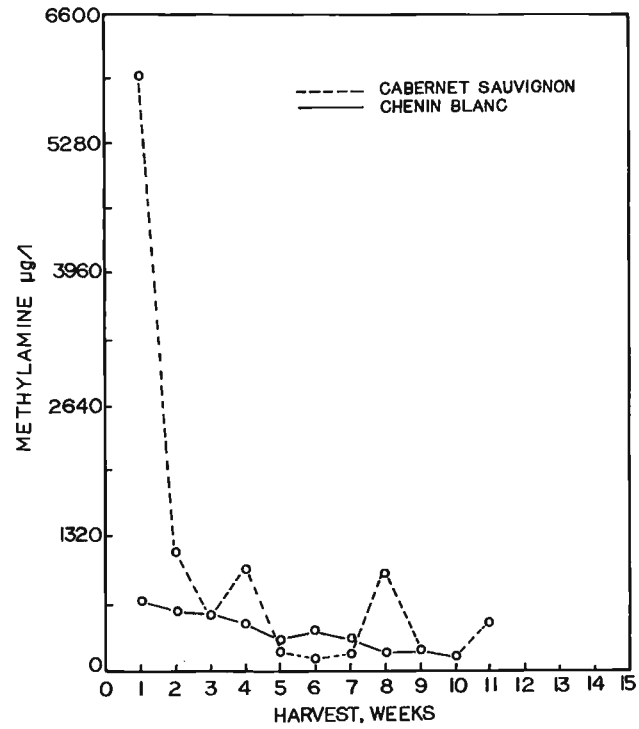

Fig. 2. Methylamine changes during grape muturation for two varieties of *V. vinifera*. Starting date was 18 July 1980, represented by 1 on the harvest scale.

Die Veränderungen der Methylaminkonzentration im Verlaufe der Beerenreife bei zwei *V.-vinifera*-Sorten. Versuchsbeginn am 18. Juli 1980 (= 1 auf der Ernteachse).

Maturity levels and volatile amines determined during and at the end of the maturation period in four *V. vinifera* vars.

Reifezustand sowie Konzentration der flüchtigen Amine während und am Ende der Reifeperiode bei vier *V.-vinifera*-Sorten

Variety	Date of harvest	°Brix sucrose/ 100 g	Total acidity g tart. acid/ 100 ml	pH	Amines — µg/l Methyl	Dimethyl	Ethyl	Diethyl	Isoamyl	2-Phenethyl	n-Propyl	a-Amyl[1])	Isobutyl
Chenin blanc	18 July 1980	4.7	2.70	2.65	700	20	25	35	Traces	195	10	Traces	Traces
	24 July 1980	5.0	3.44	2.65	590	<1	5	15	Traces	200	—	—	—
	31 July 1980	5.4	3.38	2.65	580	35	<1	40	Traces	150	—	Traces	—
	14 Aug 1980	8.5	3.20	2.80	480	5	2	5	Traces	100	—	—	—
	21 Aug 1980	11.7	2.52	2.80	320	4	2	5	Traces	70	—	—	—
	28 Aug 1980	12.9	2.46	2.90	400	10	<1	40	Traces	20	—	—	—
	4 Sept 1980	15.3	2.01	3.00	310	<1	57	15	Traces	5	—	—	—
	11 Sept 1980	18.2	1.45	3.00	180	10	70	5	5	4	Traces	—	Traces
	18 Sept 1980	19.0	1.39	3.05	200	<1	87	25	40	4	Traces	Traces	—
	25 Sept 1980	20.0	1.02	3.10	180	10	150	25	5	<1	—	Traces	—
Cabernet Sauvignon	18 July 1980	4.5	2.65	2.60	6 000	75	25	—[2])	175	580	—	—	—
	24 July 1980	5.1	3.48	2.65	1 200	30	12	5	—	140	—	—	—
	31 July 1980	5.9	3.54	2.65	540	25	50	<1	12	80	—	—	—
	14 Aug 1980	10.5	3.10	2.90	1 050	24	25	<1	—	81	—	—	—
	21 Aug 1980	14.4	2.36	2.90	200	40	25	170	—	110	—	—	—
	28 Aug 1980	16.5	1.80	3.00	120	35	83	150	—	20	—	—	—
	4 Sept 1980	18.7	1.14	3.30	170	75	80	205	12	5	—	—	—
	11 Sept 1980	19.4	0.96	3.30	1 000	25	580	205	30	4	—	—	—
	18 Sept 1980	20.0	0.90	3.30	210	15	140	3	—	5	—	—	—
	25 Sept 1980	21.8	0.79	3.31	140	10	142	10	—	4	—	—	—
	2 Oct 1980	22.6	0.73	3.46	500	45	610	30	2	4	Traces	Traces	—
Pinot noir[3])	13 Sept 1980	27.3	0.78	3.23	145	35	4 900	30	160	25	[4])	3	[4])
White Riesling	27 Sept 1979	22.0	0.68	3.55	850	25	1 900	<1	700	200	[4])	<1	[4])

[1]) 2-Methyl-1-butylamine. — [2]) Not detected. — [3]) In this case the whole grapes were destemmed then crushed and the skins, juice and seeds all distilled. — [4]) Not measured.

the 1st and 2nd week of harvest, it never attained the high levels of the 1st week. The shape of the methylamine curves for both varieties, with the exception of the first three samples is very similar. The extreme variations in both figures may be partially due to sampling or analytical variation, or possibly changes in pool concentrations of the amines in the grapes. From the table, it can be seen that the concentration of the other amines also varied during maturation. This non-uniform concentration might be tentatively explained by a reaction that may happen between an amine and an amino acid with subsequent amide formation. For example, SUZUKI (13) and TAKEO (14) showed that 4-glutamylethylamide (theanine) and 4-glutamylmethylamide were formed in the roots of the tea plant by condensation of ethylamine or methylamine, respectively with glutamic acid.

Two other *V. vinifera* varieties measured at maturity were White Riesling and Pinot noir. The table also lists the amine concentration found for these juice samples.

In the grapes, the amines are probably either formed by the decarboxylation of the respective amino acid as in some plants (1) or by aldehyde amination through a transaminase enzyme system as in other plants (4). In higher plants, HARTMANN *et al.* (5, 6) and PREUSSER (10) reported that, although some similar amines are known to be produced by amino acid decarboxylation, aldehyde amination appears to be a more common biosynthetic pathway. The amounts of these and other amines found in the raw material will vary with the variety of the grapes. As with beer (3, 9), the amines found in wine are present in the raw product before fermentation. Comparably, barley, malt and hop varieties used in brewing also greatly determine the kind of amine and general concentration in beer.

Summary

Changes in the volatile amines of *Vitis vinifera* var. Cabernet Sauvignon and var. Chenin blanc during maturation were measured at weekly intervals from veraison to maturity. The amine concentration changes during maturation for both varieties followed the same general trends. Methylamine and 2-phenethylamine were high early in the season and dropped to much lower levels during maturation. Ethylamine concentration showed substantial increases. The dimethylamine and isoamylamine showed no significant trends in concentration during the sampling period. The diethylamine concentration increased midseason for Cabernet Sauvignon samples and then decreased, but remained fairly constant for Chenin blanc samples. At maturity, free-run juice of Cabernet Sauvignon, Chenin blanc, White Riesling and Pinot noir had 500, 180, 850, and 145 µg/l of methylamine, 45, 10, 25 and 35 µg/l of dimethylamine, 610, 150, 1900 and 4900 µg/l of ethylamine, 30, 25, <1 and 30 µg/l of diethylamine, 2, 5, 700 and 160 µg/l of isoamylamine, 4, <1, 200 and 25 µg/l of 2-phenethylamine, <1, <1, <1, and 3 µg/l of 2-methyl-1-butylamine (a-amyl) and <1, <1 µg/l n-propylamine (Cabernet Sauvignon and Chenin blanc only), respectively.

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