Vitis 7, 321—331 (1968)

Department of Viticulture and Enology, University of California, Davis, USA

Proline content of grapes and wines

by

C. S. OUGH

The presence of the amino acid proline was noted in grapes and wine by LÜTHI and VETSCH (18) and by SISAKIAN and BEZINGER (32). The latter found it to be unchanged in amount during or after fermentation. In 1956 CASTOR and ARCHER (6) reported on this amino acid in California grapes. They noted it was present in larger amounts in the juices of certain varieties. They showed in a single fermentation that the proline did not change greatly during fermentation. COLAGRANDE (7) found proline was an abundant acid in Italian must. LAFON-LAFOURCADE and PEYNAUD (14) measured the amino acid content of Bordeaux wines and found the white varieties to average higher in proline than the red varieties. Cabernet Sauvignon and Merlot were noted to be particularily high. Seasonal changes were described. Later LAFON-LAFORCADE and GUIMBERTEAU (16) followed the maturity changes in amino acids for several varieties and noted essentially same results as the prior experiments (14). DRAWERT (9) found also the increases in proline with maturity. The proline in the German musts was low compared to other amino acids, however the relatively low final level of maturity may be the reason. MARTINEZ BURGES (19) identified proline in Spanish musts. Reports of the amino content of must (11) of the Dao river area of standard white and red varieties indicated proline was not of major importance. VAN WYK (34), reporting on the amino acids in South African juices, found the predominant amino acid to vary with variety with proline being the most predominant in some. NASSAR and KLIEWER (27) also followed changes in amino acids with grape maturity in Thompson Seedless (Sultana) in various parts of the vine and show proline is only present in large amounts in the berries. MARUTJAN (20) stated that early varieties had higher amounts of amino acids than later varieties and that no pattern in types of amino acids relating to variety could be distinguished. FEUILLAT and BERGERET (10) indicated that in the wines of Burgandy the proline was significantly decreased during fermentation. LASHKHI and TSISKARISHVILI (17) measured the proline content of different Georgian wines and of parts of the grape. They showed a decrease in proline during fermentation then rather spectacular rises to two fold increases over the original amount in the musts.

LAFON-LAFOURCADE and PEYNAUD (15) investigated some effects of fermentation techniques on the amino acid content. They found that time on the skin, up to 8 days, or short term storage on the yeast lees did not alter the proline content significantly. Likewise a malo-lactic fermentation had little effect on the free proline. The effect of years and of variety were very important to the proline concentration in the wine. The degree of pressing of musts appears to increase mainly the arginine and alanine content by freeing these amino acids from the stems, according to GENDRON (12), and that other amino acids are relatively unaffected. MATSUDA and MARAKI (22) found only proline present in large amounts in a freshly made wine and the concentration did not change with various aging treatments. Even extreme treatments of cell rupture and then storage the proline did not increase (21).

THORNE (33) has shown that proline could be assimilated by yeast but to a relatively lesser degree than most other amino acids when the media was relatively rich in nitrogen sources. BARTON-WRIGHT (3) also found proline to be slowly assimulated in beer fermentation. Approximately 20% was reported as normally assimulated. Proline was indicated to be a fair to good source of nitrogen by Schultz and Pamper (31). They checked some 40 strains of *Saccharomyces*. Abadie (1) found that proline was a poor source of nitrogen for a single strain of *S. cerevisiae*. Peynaud and Lafon-Lafourcade (28, 29) show the interrelations of the various nutrient factors in wine yeast growth and assimilation of amino acids.

The analysis of champagne by various Russian workers (13, 30) has shown proline to be a major constituent. It is not easily released by autolyses, as has been demonstrated by Bourdet and Hérard (5), nor does the yeast use appreciable amounts of the proline. In the champagne fermentation experiments of Bergner and Wagner (4), if other favored nitrogen sources for yeast metabolism were available, the proline was spared.

Extensive series of analysis were made on California grapes and wines to verify some of the above work and to determine the expected ranges of proline concentrations. The effects of several other factors not previously investigated were determined.

Materials and Methods

Vitis vinifera grapes used for these measurements were grown under carefully controlled conditions at the University of California at Davis, or various University owned or controlled vineyards throughout the state. The various grape growing areas of the state are classified by Amerine and Winkler (2). The hotter areas being regions V and the coolest region I. The Oakville vineyard is in region II, Davis in region IV, Lodi in warm region III and Kearney (Fresno) in region V. Wines were all made at the Department of Viticulture and Enology using accepted techniques.

The analysis of total nitrogen was done by the macro Kjeldahl procedure. Ammonia was determined by the method of Dimotaki-Kourakou (8) and the proline directly by a colormetric method developed by Ough (23). Total amino nitrogen was measured by a modification of the usual ion exchange-ninhydrin procedure to include proline in the total (Ough, unpublished data).

The measurement of the cluster parts was made on portions of the cluster separated carefully by hand. Then parts were weighed then homogenized with a blender, diluted to given volumes with water, and extracted for several hours at room temperature; then the whole stored at 0° C for several months prior to proline analysis. A part of each was fermented and total nitrogen determined.

The fermentation samples were saved under toluene in a 0° C room until the main fermentations were complete then the desired analyses were made.

Regular juice samples were taken and total nitrogen and ammonia determined immediately and samples saved as above for proline determination later.

Some wine samples were stored at 0° C and others at 11° C until total nitrogen and proline determinations could be made.

Results and Discussion

The analyses of cluster parts for total nitrogen and proline are shown in Table 1 and the percent distribution of each in Table 2. Of particular note is the relatively low percent of the total nitrogen in the juice and the high percent of proline in the juice.

Lashkhi and Tsiskarishvili (17) report, for one variety of grape, proline concentrations of 106, 41 and 11 mg/kg of grapes for the juice, pulp and skins respectively. These are in the same general relative proportions as reported here. Despite this

Table 1

Total nitrogen and proline values for grape cluster parts

Variety	Total N (mg/g component)					Proline (as N mg/g component)				
	Juice	Skins + Seeds	Stems	Pulp	Total	Juice	Skins + Seeds	Stems	Pulp	Total
Cabernet franc	0.35	3.98	1.70	1.36	1.205	0.104	0.050	0.043	0.082	0.089
Petite Bouschet	0.57	3.25	3.13	0.50	1.425	0.061	0.025	0.043	0.009	0.039
Petite Sirah	0.52	5.22	2.49	1.43	1.940	0.084	0.081	0.025	0.053	0.074
Grillo	0.98	4.69	3.90	1.60	1.900	0.150	0.109	0.047	0.122	0.123
Sauvignon blanc	1.73	5.46	4.45	3.16	3.185	0.134	0.089	0.202	0.162	0.135

Table 2

The percentage distribution of the total nitrogen and the proline between the cluster parts

Variety	% of total N in each component					% of proline in each component				
	Juice	Seeds	Skins	Stems	Pulp	Juice	Seeds	Skins	Stems	Pulp
Cabernet franc	18.8	24.7	36.3	6.5	13.6	76.1	8.3	2.1	2.2	11.0
Petite Bouschet	18.1	65.3		9.4	7.4	71.7	18.5		4.7	4.9
Petite Sirah	14.0	64.7		8.8	12.4	59.2	26.3		2.3	12.0
Grillo	26.2	51.2		7.5	15.1	62.3	18.4		1.4	17.8
Sauvignon blanc	28.9	35.6		11.8	17.7	52.5	13.5		12.5	21.3

agreement the possibility that free proline exists inside the intact cells is almost a certainty. However, other experiments tend to confirm the results to a degree in that at most increases in proline by heating the must or by alcohol extraction increase proline by only a relatively small extent compared to the increases in total nitrogen. The biggest gains in proline content in the wine due to fermentation on the skin and seeds versus juice fermentations are about 30% increases. This is close to the amount available as indicated in the pulp, seed and skin measurements. The bulk of the total nitrogen appears to be in the skins and seeds. (Unpublished data show that 2 to 4 or 5 times the amount of total nitrogen present in the juice is present in the whole grape.) These grapes were picked late in the season. (Table 3 gives the juice analyses.) The percent of the total nitrogen accounted for in the juice varies four fold in these few samples.

Table 3

Juice analysis and location of vines of samples used for cluster analyses

Variety	Location	° Brix	Total acid g H_2ta/ 100 ml	pH	NH_3 mg/l	Total N mg/l	Proline (as N) mg/l	
Cabernet franc	Oakville	21.6	0.57	3.60	36	388	116	(29.9)[1]
Petite Bouschet	Davis	22.5	0.62	3.67	65	541	59	(10.8)
Petite Sirah	Oakville	18.4	0.53	3.73	46	559	91	(16.2)
Grillo	Davis	21.8	0.67	3.72	67	984	151	(15.3)
Sauvignon blanc	Davis	25.1	0.65	4.16	126	1924	149	(7.7)

[1]) Values in paranthesis are % of total nitrogen.

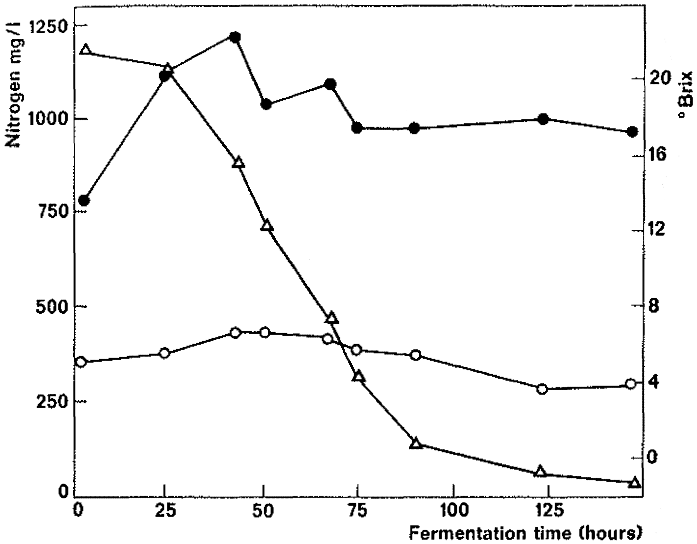

Fig. 1: A must (juice, skins and seeds) fermentation of Oakville Cabernet Sauvignon. Δ (change in °Brix), ● (total nitrogen) and o (proline as nitrogen).

Fig. 1 shows the fermentation changes in °Brix, total acid, total nitrogen and proline occuring in the fermenting juice of a sample of Cabernet Sauvignon grapes from Oakville. The sample was stirred two times daily to keep the skins wet. The decrease in °Brix is accompanied by small changes in the proline. The proline concentration changes are very similar to those reported by Castor and Archer (6) for a white grape juice fermentation. The increases in the total nitrogen are caused by the nitrogen compounds being extracted from the pulp, skins and seeds and the drop occurs because of yeast metabolism and some settling of the yeast prior to sampling. (The samples were mixed and a skin- and seed-free sample drawn off.) Samples were taken at the start and finish of the fermentation to determine the total nitrogen of the whole. These values were about twice as large as those for the nitrogen of the juice plus yeast in the samples. The yeast contained about 1/3 the nitrogen in the seed- and skin-free samples. Adding alcohol above 5% by volume to up to 20% did not cause measurable increase in the proline extracted. The addition of alcohol or heating the sample increased proline in the juice from 350 to 430 mg/l as nitrogen. The total nitrogen was increased by the same treatments from about 790 to 930 mg/l. The higher concentration of alcohol caused measurable nitrogen increases compared to the 5 percent fortifications. The heating and alcohol additions were not additive indicating the source of nitrogen being released was the same for both treatments.

The effect of variety is quite noticeable on the proline content of the must. Table 4 gives the amounts of nitrogen in the juice of samples from several areas. The variations in the proline/total nitrogen ratios are striking between certain varieties. As reported by Lafon-Lafourcade and Peynaud (14) the Cabernet Sauvignon and Merlot were highest in proline. Cabernet franc (see Table 3) had about as high a proline/total nitrogen ratio as did the Merlot. Malbec (four samples from Oakville) was lower, averaging 7 to 10% of the total nitrogen. Ruby Cabernet proline content (four samples from Oakville and one from Lodi) ranged from 15.6 to 19.0% of the juice total nitrogen. With Cabernet Sauvignon, in extreme cases of maturity, the proline (as nitrogen) can be in excess of 40% of the total nitrogen of the juice. Minimum percentages of proline (to total nitrogen) are found in both white and red varieties. The lowest values seen were for St. Macaire and Refosco (4.3 and 4.8% respectively) for the red varieties and Aligote and Grey Riesling (5.8 and 5.5% respectively) for the white varieties. The highest white variety noted was French Clombard (four samples 13.2—20.2% range).

The must and wine nitrogen analyses are presented for several wines in Table 5. The proline content of the wine accounts for a very large percentage of the amino nitrogen. Fermentations on the skins usually show significant proline increases for the red varieties. Some juice fermentations also show increases in the proline. Juices

Table 4

Juice nitrogen analyses from several locations for several varieties and treatments

Variety	Location	Treatment	°Brix	NH_3 mg/l	Proline (as N) mg/l	Total N mg/l
Thompson Seedless	Kearney (Fresno)	Maturity	19.8	146	37 (5.8)[1]	631
		Maturity	21.2	141	53 (7.7)	680
		Maturity	22.0	121	69 (9.4)	732
		Maturity	22.8	89	72 (11.1)	646
		Maturity	24.0	85	95 (12.2)	780
		Rootstock				
Petite Sirah	Lodi	Dogridge	21.4	108	175 (9.7)	1811
		Saltcreek	22.9	91	182 (12.1)	1507
		St. George	23.7	105	192 (13.6)	1451
		1613	24.8	71	160 (14.7)	1087
Barbera	Lodi	Dogridge	23.7	108	125 (7.6)	1654
		Saltcreek	24.3	88	92 (8.7)	1063
		St. George	21.2	50	87 (8.8)	993
		1613	22.0	61	64 (6.5)	986
French Colombard	Lodi	Dogridge	23.3	123	458 (20.2)	2270
		Saltcreek	21.8	108	216 (16.3)	1323
		St. George	21.7	158	357 (18.2)	1954
		1613	19.1	140	154 (13.2)	1164
Ruby Cabernet	Lodi	St. George	18.7	47	132 (16.5)	800
Merlot	Lodi	Own	21.4	—	296 (32.2)	918
Refosco	Lodi	—	21.0	—	71 (4.8)	1490
Peverella	Lodi	Dogridge	22.0	—	171 (13.9)	1227
Peverella	Lodi	St. George	22.8	—	157 (14.2)	1102
Aligote	Lodi	Dogridge	21.2	—	91 (5.8)	1560
Grey Riesling	Lodi	Dogridge	21.5	—	67 (5.5)	1210
St. Macaire	Lodi	Dogridge	22.6	179	77 (4.3)	1790
White Riesling	Lodi	Own	20.2	—	47 (6.3)	728
Groz Manzenc	Lodi	St. George	22.3	224	155 (7.8)	1985
White Riesling	Davis	Own	21.5	51	55 (7.4)	739
White Riesling	Oakville	St. George	25.5	88	48 (7.2)	664
Sauvignon blanc	Oakville	Mixture	22.7	85	62 (7.6)	819
Pinot blanc	Davis	Own	22.5	56	100 (14.1)	706

[1]) Values in paranthesis are % of total nitrogen.

Table 5

Juice and wine nitrogen analyses for some 1967 samples with various treatments

Varieties[1]	⁰ Brix	NH_3 mg/l	Juice analyses: Proline as N mg/l		Total nitrogen mg/l	Type of fermentation	Wine analyses: Proline as N mg/l		Amino nitrogen as N mg/l	Total nitrogen mg/l
Chardonnay	21.3	163	130	(10.6)[2]	1223	Juice	140		221	457
Pinot Gris	22.6	82	67	(8.7)	768	Skin	108	(73)[3]	154	187
Gamay Beaujolais	21.8	128	94	(8.3)	1127	Skin	133	(120)	220	280
Sylvaner	23.1	78	60	(6.0)	998	Juice	79		140	205
Malbec	24.0	163	202	(9.3)	2163	Skin	222	(225)	452	1080
Petite Sirah	21.5	58	95	(11.2)	848	Skin	128	(85)	175	229
Semillon	23.4	92	53	(5.6)	948	Juice	90		136	202
Tinta Madiera	20.4	105	57	(5.7)	998	Skin	151	(104)	210	367
Ruby Cabernet	22.1	77	145	(17.9)	810	Skin	124	(100)	159	195
Emerald Riesling	24.1	80	53	(6.4)	826	Juice	68		154	16[illegible]
Sauvignon blanc	22.4	51	55	(7.4)	739	Juice	86		77	208
						12 hours skins	104		112	237
						24 hours skins	124		178	253
						48 hours skins	125		14[illegible]	234
Pinot blanc	21.0	51	56	(13.0)	432	Juice	1[illegible]7		165	191
						12 hours skins	110		182	184
						24 hours skins	114		21[illegible]	217
						48 hours skins	131		175	217

[1]) Location: Oakville (Sauvignon blanc: Davis).
[2]) Values in paranthesis are % of total nitrogen.
[3]) Values in paranthesis are those for comparison fermentations done with juice only.

Table 6
The effect of rootstock and fertilizer level on the nitrogen composition of the juice and wines[1])

Treatment		Juice analyses				Wine analyses			
Rootstock[2])	Fertilizer[3]) level	°Brix	NH_3 mg/l	Proline as N mg/l	Total nitrogen mg/l	Alcohol Vol.-%	Proline as N mg/l	Total amino nitrogen as N mg/l	Total nitrogen mg/l
St. George	None	22.4	103	96	1074	12.4	157	203	336
	High	22.7	107	108	1157	12.3	147	210	384
99-R	None	22.2	66	58	583	12.4	48	95	121
	High	21.9	73	62	721	12.2	74	129	162

[1]) Average of 10 different varieties from Oakville, 1967 (see Table 5). Cited from Ough *et al.* (24, 25) in part.

[2]) St. George is Rupestris du Lot and 99-R is a *V. rupestris* × *V. berlandieri* cross.

[3]) High fertilizer level was two applications of 150 lb. of N as NH_4NO_3 per acre/year.

with high amounts of pulp would expect to have extra proline released into the juice during fermentation. The free amino acid accounts for a large proportion of the total nitrogen in the wine at low total nitrogen but lesser amounts with high total nitrogens. The further effect of extraction of proline from the skins and seeds is seen in the two sets of data reported in the lower portion of Table 5. About 30% increase in proline resulted from leaving fermenting juice on the skins up to 48 hours. The effect of the pulp is also noted by the increase in the juice fermentation proline.

Some rootstock treatments were shown in Table 4. These were not closely controlled to time of picking so maturity and rootstock had significant interaction on the

Table 7
Oakville maturity study[1]) — Effect of maturity and temperature on nitrogen composition of juice and wine. Juice fermentations

Date of harvest	Must				Wine					
					Total N mg/l			Proline as % of N		
					Fermentation temperature					
	° Brix	Total N mg/l	NH_3 as % of total N	Proline as % of total N	10° C	21° C	33° C	10° C	21° C	33° C
				White Riesling						
13 Sept.	21.2	451	14.6	7.7	62	61	91	6.9	2.1	19.6
20 Sept.	21.9	515	16.4	7.9	80	69	101	29.2	10.7	40.8
27 Sept.	22.7	546	14.0	5.3	108	91	161	27.3	21.6	27.6
5 Oct.	22.8	586	15.7	9.8	255	130	168	20.5	31.8	37.5
11 Oct.	23.3	613	13.7	11.7	152	138	171	51.5	37.9	46.4
				Cabernet Sauvignon						
13 Sept.	19.9	629	17.9	29.5	138	113	163	75.3	86.1	81.5
20 Sept.	20.4	658	16.1	34.3	183	193	224	84.2	79.7	81.6
27 Oct.	22.4	762	12.1	35.4	248	264	245	83.0	87.8	93.4
5 Oct.	22.6	706	13.8	40.2	259	279	323	79.9	66.3	60.9
11 Oct.	23.5	877	10.8	43.4	375	352	444	85.0	93.7	70.7

[1]) Wines stored at 0° C after fermentation. See Ough and Singleton (26).

amount of nitrogen components found. Table 6 reflects the effect of rootstocks in a closely controlled experiment •n the juice and wine nitrogen composition. These data, in part, and orther data in analytical detail has been reported by Ough *et al.* (24, 25). The proline contents, as effected by different rootstocks have not been previously reported. The effect of the vigorous rootstock is to increase nitrogen components at about the same relative degree in the juice. The proline is lowered to a greater extent in the 99-R, no fertilizer, treatment because of the use of some of the proline by the yeast as a growth substrate in several instances. The proline increases, due to St. George rootstock treatments, were caused by releases from pulp and skins into the juice during fermentation and the natural sufficiency of substrate so little if any proline was used for yeast metabolism.

The effect of maturity and fermentation temperature are shown in Table 7 for two varieties from Oakville. The proline increases with maturity both in amount and

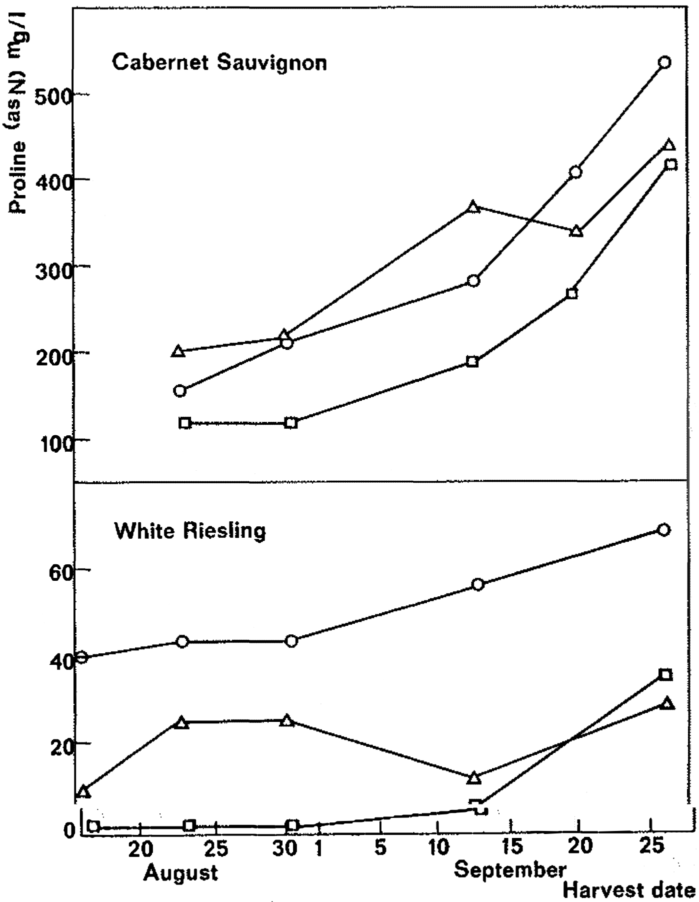

Fig. 2: The proline concentration of a series of maturity samples of Cabernet Sauvignon and White Riesling from Davis. o (juice), □ (wine after juice fermentation at 21° C — small sample with storage at 0° C) and Δ (wine after juice, skins and seeds fermentation for the Cabernet Sauvignon and juice fermentation for White Riesling — standard winery sample with storage at 11° C).

in percentage of the total nitrogen. A similar result for Thompson Seedless maturity can be seen in Table 4. In the extreme case the Cabernet Sauvignon proline increased to over 40% of the total nitrogen. The nitrogen analysis of the wine shows that at 70° F fermentation less total nitrogen and a smaller percentage of proline result than at the other two fermentation temperatures with the White Riesling fermentations. The Cabernet Sauvignon fails to show this temperature effect. This temperature effect appearently is caused by the larger crop of yeast grown at 21° C which uses more nitrogen and hence depletes the sources. Less yeast are grown at 10° C and still less at 33° C. In the case of the Cabernet Sauvignon there is an excess of nutrients, hence, a sparing of the proline. The proline left in the wine accounts for a very large percentage of the wine total nitrogen. This temperature effect was verified by many fermentations. The high percentage of proline in the wine total nitrogen was verified for the Cabernet Sauvignon at another location.

The effect of maturity on the proline content of White Riesling and Cabernet Sauvignon is further demonstrated in Fig. 2. These data show the extreme differences in the two varieties and also indicate the relative changes that occur in the wine proline content when fermentation are with the skins and seeds and with the juice only.

In all these tables and figures the proline was reported as mg/l of nitrogen. To report as mg/l of proline the values must be multiplied by 115/14 or 8.2. Doing this shows the range of proline (mg/l) in the juices to range from 304 to 4600 and in the wine 0—3400. The highest value occuring in the Cabernet Sauvignon from Davis. Castor and Archer (6) found 3490 mg/l proline in a French Colombard juice with a wine value of 3450 mg/l. Drawert (9) reported values as low as 120 mg/l proline in Riesling juice and as high as 535 mg/l proline in another juice. Lafon-Lafourcade and Peynaud (14) and Lafon-Lafourcade and Guimberteau (16) give juice values from 260 to 780 mg/ proline. The higher values of these investigators seem rather small if compared to the data reported in this paper since they are for Merlot and Cabernet Sauvignon. However, at least two factors are causing these to be lower. The grapes did not attain the same degree of maturity. Their latest measurements were taken 21 September which is early for these varieties in Bordeaux. Secondly the levels of total nitrogen are much lower than those found in California grapes and wines. The calculation of the Lafon-Lafourcade and Guimberteau (16) proline data as nitrogen and relating this to the total nitrogen gives percentages of 17.7 to 19.4% for the 4 samples reported. This is not exceedingly far out of range considering the maturity effects due to the cooler climate. Lafon-Lafourcade and Peynaud (14) reported average variations between 2 years of 395 to 700 mg/l of proline. The average of some 78 determinations for proline in the juice gave a value of 742 mg/l of proline. The average of 42 wine samples gave a value 869 mg/l of proline. About one half of these wines were fermented on the skins and half done as juice fermentations. All but two were fermented from Oakville grapes.

Summary

The proline, ammonia and total nitrogen content of some California grapes and the proline, total free amino acids and total nitrogen of the wines have been measured. The juice contains the predominant amount of the proline (50 to 80%) in the grape cluster; while the skins and seeds contain in excess of 50% of the total nitrogen. Very little proline is found in the stems. A moderate portion is in the pulp (5 to 20%). The proline can make up from 5 to 43% of the total nitrogen in the juice

depending on variety and on grape maturity. The Cabernet family of grapes appears to have highest percentages of proline. A large percentage of the total amino acid left in the wine is in the form of proline. The amount of proline in the wine can vary from 0 to about 90% of the total nitrogen. The wines with larger amounts of total nitrogen have higher percentages of residual proline. Rootstock affects the amount of total nitrogen but does not appear to affect the ratio of proline to total nitrogen. The ranges found in California grape juices range from 304 to 4600 mg/l of proline and in the wines from 0 to 3400 mg/l of proline. The average proline value for 78 juices was calculated as 742 mg/l and for the tabulated data of 42 wines (from mainly cool climate grapes) — one half fermented on the skins the other half not — an average value of 869 mg/l of proline was found.

Literature Cited

1. Abadie, F.: Utilisation par levures de quelques acides aminés comme source d'azote et comme source d'azote et de carbone. Ann. Inst. Pasteur 113, 81—95 (1967).
2. Amerine, M. A. and Winkler, A. J.: California wine grapes: Composition and quality of their musts and wines. Univ. Calif., Exp. Sta. Bul. 794, 1—83 (1963).
3. Barton-Wright, E. C.: An analytical approach to some problems in nitrogen relations of yeast. Wallerstein Lab. Comm. 15, 115—129 (1952).
4. Bergner, K. G. und Wagner, H.: Die freien Aminosäuren während der Flaschen- und Tankgärung von Sekt. Mitt. Klosterneuburg A 15, 181—198 (1965).
5. Bourdet, A. et Hérard, J.: Influence de l'autolyse des levures sur la composition phosphorée et azotée des vins. Ann. Technol. Agric. (Paris) 7, 177—202 (1958).
6. Castor, J. G. B. and Archer, T. E.: Amino acids in must and wines, proline, serine and threonine. Amer. J. Enol. 7, 19—25 (1956).
7. Colagrande, O.: Ricerca chromatografica degli aminoacidi nei mosti e nei vini. Ann. Fac. Agraria, Ser. 5 (Perugia) 53, 84—94 (1957).
8. Dimotaki-Kourakou, V.: Dosage de l'azote ammoniacal et des substances azotées macromoleculaires par la methode exchangeurs de cations. Ann. Fals. Expert. Chim. (Paris) 53, 337—348 (1960).
9. Drawert, F.: Biochemisch-physiologische Untersuchungen an Traubenbeeren. Das Verhalten der Aminosäuren während der Reifung und der Zucker nach Einfrieren der Beeren. Vitis 4, 49—56 (1963).
10. Feuillat, M. et Bergeret, J.: Identification et dosage des aminoacides dans les moûts et les vins de Bourgogne. C. R. Hebd. Séances Acad. Sci. (Paris) 264, 1752—1759 (1967).
11. Figueiredo e Silva, F. P. y Sales Pitinga, O.: Identificacão de aminoácidos em mostos de algumas castas dos regiões do Dão e do Oeste. Agros. (Lisboa) 45, 195—199 (1962).
12. Gendron, C.: Influence du pressurage sur le taux d'azote organique dans les moûts. Rev. Ferment. Ind. Aliment. (Bruxelles) 20, 201—205 (1965).
13. Golonina, N. N. and Avakyants, S. P.: Quantitative amino acid analysis by chromatography with ion exchange resins (transl.) Vinodelie Vinogradar. SSSR (Moskau) 26 (4), 4—7 (1966) [Chem. Abstr. 65, 9692h (1966)].
14. Lafon-Lafourcade, S. et Peynaud, E.: Dosage microbiologique des acides aminés des moûts de raisin et des vins. Vitis 2, 45—56 (1959).
15. — — et — — : Composition azotée des vins en fonction des conditions de vinification. Ann. Technol. Agric. (Paris) 10, 143—160 (1961).
16. — — et Guimberteau, G.: Evalution des aminoacides au cours de la maturation des raisins. Vitis 3, 130—135 (1962).
17. Lashkhi, A. D. and Tsiskarishvili, T. P.: Proline in grape products (transl.). Vinodelie Vinogradar. SSSR (Moskau) 27 (1), 19—21 (1967) [Chem. Abstr. 66, 93977z (1967)].
18. Lüthi, H. und Vetsch, U.: Papierchromatographische Bestimmung von Aminosäuren in Weinen. Schweiz. Z. Obst- u. Weinbau 61, 390—394, 405—408 (1952).
19. Martinez Burges, L.: Cromatografia de algunos vinos navarros (amino-acidos libes). Anal. Bromatol. (Madrid) 12, 19—26 (1960).
20. Marutyan, S. A.: Quantitative changes in the amino acids of grapes ripening at different times (transl.). Dokl. Akad. Nauk SSSR (Moskau) 171, 1217—1220 (1966).
21. Matsuda, H. and Muraki, H.: Utilization of yeast cells in wines lees with decompression rupture for improving quality of tables wines. Bull. Res. Inst. Ferment. (Yamanashi University). 39 (11), 37—43 (1964).

22. — — and — — : Utilization of yeast cells in wine making. Bull. Res. Inst. Ferment. (Yamanashi Univ.). 41 (13), 1—9 (1966).
23. Ough, C. S.: Rapid determination of proline in grapes and wine. J. Food. Sci. Submitted (1968).
24. , Lider, L. A. and Cook, J. A.: Rootstock-scion interactions concerning wine making. I. Juice composition changes and effects on fermentation rate with St. George and 99-R rootstocks at two nitrogen fertilizer levels. Amer. J. Enol. Viticult. Submitted (1968).
25. — — , Cook, J. A. and Lider, L. A.: Rootstock-scion interactions concerning wine making. II. Wine compositional and sensory changes attributed to rootstock and fertilizer differences. Amer. J. Enol. Viticult. Submitted (1968).
26. — — and Singleton, V. L.: Wine quality prediction from juice Brix/acid and associated compositional changes for White Riesling and Cabernet Sauvignon from two regions. Amer. J. Enol. Viticult. Submitted (1968).
27. Nassar, A. R. and Kliewer, W. M.: Free amino acids in various parts of *Vitis vinifera* at different stages of development. Proc. Amer. Soc. Hort. Sci. 89, 281—294 (1966).
28. Peynaud, E. et Lafon-Lafourcade, S.: Sur la nutrition azotée des levures de vin. Rev. Ferment. Ind. Aliment. (Bruxelles) 17, 11—21 (1962).
29. — — et — — : Constitution azotée des levures en fonction des conditions de nutrition. Qualit. Plant. Mat. Veg. 9, 365—380 (1963).
30. Rodopulo, A. K. and Pisarnitskii, A. F.: Quantitative determination of amino acids in champagnes by use of an amino acid analyser (transl.). Vinodelie Vinogradar. SSSR (Moskau) 28 (1), 5—7 (1968) [Chem. Abstr. 68, 86118h (1968)].
31. Schultz, A. S. and Pamper, S.: Amino acids as nitrogen source for the growth of yeasts. Arch. Biochem. 19, 184—192 (1948).
32. Sisakian, H. M. and Bezinger, E. N.: Izmenenie aminokeslotnago sostava vina pri ego pervichnoi tekhnologii. Biokhimia 18, 412—422 (1953).
33. Thorne, R. S. W.: Mechanism of nitrogen assimilation by yeasts and their relation to the problem of yeast growth in wort. Wallerstein Lab. Comm. 13, 319—338 (1950).
34. van Wyk, C. J. and Venter, P. J.: The determination of free amino acids in musts and wines by means of high volatage paper electrophoresis and paper chromatography. S. Afr. J. Agricult. Sci. (Pretoria) 8, 57—67, 69—71 (1965).

Eingegangen am 23. 7. 1968

C. S. Ough
Dept. of Viticulture and Enology
Univ. of California
Davis, Calif., 95616
USA