STUDY ON EGYPTIAN FRESH AND AGED WINES. II. TOTAL NITROGEN AND AMINO ACIDS CONTENT OF WINES OF THREE VINTAGES

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ABSTRACT

Total nitrogen, amino nitrogen, nonamino nitrogen, and amino acids content was determined in 10 brands of Egyptian wine of three vintages. The brands represented three dry white wines, three semi-sweet white wines, and four dry red wines. Most of the total nitrogen content was in the form of amino nitrogen rather than nonamino nitrogen. The amino acids content of wine confirms the same trend observed with the amino nitrogen content. No defined trend could be detected for the quantitative sequence of amino acids in wines of different brands and vintages. Proline constituted 43.6-64.3 and 30.8-53.4% of the amino nitrogen and total nitrogen, respectively.

Very little is known about the chemical composition of Egyptian wines (12). Accordingly, the present study aims to cover the changes in the total nitrogen, amino nitrogen, nonamino nitrogen, and amino acids content, particularly proline, in the most important Egyptian wines produced by the main winery, Gianaclis, in Alexandria.

MATERIALS AND METHODS

Samples source: Samples of white and red wine (10 brands) were obtained from the Egyptian Vineyards and Distilleries Company Gianaclis, in Alexandria. The samples taken in 1975 represented three consecutive seasons according to the year of production, and are as follows: 1) fresh wines produced in 1975 without further storage; 2) one-year-old wines, produced in 1974 and stored in tanks for one year, and 3) two-year-old wines produced in 1973 and stored the first year in tanks and the second year in bottles.

Samples of the fresh and aged wines of 1974 and 1975 vintages were bottled and tightly closed in the Gianaclis winery. The samples were kept in the refrigerator for chemical analysis.

Total nitrogen: Total nitrogen was determined by micro-Kjeldahl according to the A.O.A.C. (2).

Amino acids extraction: The amino acids were extracted from the wine samples by the method used by Kliewer (4).

Amino acids nitrogen: The amino acids nitrogen was determined by micro-Kjeldahl as described by the A.O.A.C. (2).

Nonamino acids nitrogen: Nonamino acids nitrogen is the difference between total nitrogen and amino acids nitrogen.

Chromatographic separation of amino acids: The amino acids were separated by paper partition chromatography, using a solvent system of n-butanol:acetic acid: water (144:13:34), according to Mikes (7).

Elution and colorimetric estimation of amino acids: The air-dried amino acids chromatograms were dipped in a solution of 0.25% ninhydrin in acetone containing 1% acetic acid, as followed by Roland and Gross (11). The chromatograms were then dried at room temperature and placed in the dark for 18 hours over sulfuric acid, for maximum color development. The amino acid spots of the unknown were unidentified by comparison with those of standard amino acids.

For the colorimetric determination, the individual spots were cut out and eluted separately in test tubes for 30 minutes, using 5 ml of 75% ethyl alcohol containing 0.5 mg copper sulfate, as suggested by Majunders et al. (6). The color intensities of the different amino acids were measured using the Spekol Spectrophotometer at a wavelength of 515 mμ. Standard curves were constructed with standard amino acids.

Proline: The acidic ninhydrin photometric method of Chinard (3), as modified later by Ough (9), was used for proline content determination.

RESULTS AND DISCUSSION

Total amino and nonamino nitrogen: The results shown in Table 1 indicate that the total nitrogen content as mg nitrogen per 100 ml wine ranged as follows: fresh wines 19.0-26.5 and 16.7-24.6; for one-year-old wines 30.5-39.2 and 27.1-30.5; for two-year-old wines 30.0-33.4 and 24.8-26.5 in white and red wines, respectively.

The amino nitrogen content, as mg nitrogen per 100 ml of white and red wines, is shown in Table 2 and

ranges, respectively, as follows: fresh wine 14.1-18.1 and 12.8-17.6; one-year-old samples 22.7-29.1 and 21.0-32.0; two-year-old samples 20.0-24.5 and 19.6-21.2.

The nonamino nitrogen content as mg nitrogen per 100 ml of white and red wines ranged, respectively, as follows: fresh samples 4.9-8.4 and 3.9-7.0; one-year-old samples 7.3-11.1 and 6.1-8.7; two-year-old samples 7.2-11.4 and 3.8-6.9, as shown in Table 3.

According to Poux et al. (10) and Amerine et al. (1), the increase in the total nitrogen content during wine aging in contact with yeast is related to the autolysis of yeast, and hence most of the total nitrogen was in the form of amino nitrogen. The results obtained in the present work agreed with those reported by the aforementioned investigators, as the total nitrogen content of the wine brands was mostly amino nitrogen rather than nonamino nitrogen.

The results obtained in the present work generally indicate that white wines show higher total nitrogen content than red wines. This may be attributed to the higher content of phenolic compounds present in red wines than in white wines. These phenolic compounds form insoluble complexes with the nitrogenous compounds and then they are easily precipitated during wine clarification, according to Singleton (13).

Table 1. Total nitrogen content of wine of different ages.

Brand		Total nitrogen (mg/100 ml of wine)		
		Year of production		
		1975 (fresh)	1974 (1-year-old)	1973 (2-year-old)
White wines				
Clo Mariote	Dry	19.8	35.7	31.7
Gianaclis	Dry	—	35.2	31.7
Natural Abarka	Dry	19.0	35.2	30.0
Cleopatra	Semisweet	26.5	37.8	33.4
Nefertiti	Semisweet	21.3	30.5	30.0
Potlemo	Semisweet	21.6	39.2	32.3
Red wines				
Clo Matamir	Dry	—	36.6	24.8
Gianaclis	Dry	16.7	—	25.9
Kaser Gianaclis	Dry	24.6	27.1	26.5
Pharaons	Dry	21.3	30.5	24.8

Table 2. Amino nitrogen content of wine of different ages.

Brand		Amino nitrogen (mg/100 ml of wine)		
		Year of production		
		1975 (fresh)	1974 (1-year-old)	1973 (2-year-old)
White wines				
Clo Mariote	Dry	14.2	28.4	24.5
Gianaclis	Dry	—	26.2	24.3
Natural Abarka	Dry	14.1	26.4	20.5
Cleopatra	Semisweet	18.1	26.6	23.8
Nefertiti	Semisweet	14.8	22.7	21.2
Potlemo	Semisweet	16.3	29.1	20.9
Red wines				
Clo Matamir	Dry	—	27.9	20.9
Gianaclis	Dry	12.8	—	21.2
Kaser Gianaclis	Dry	17.6	21.0	19.6
Pharaons	Dry	15.8	23.2	20.2

Table 3. Nonamino nitrogen content of wine of different ages.

Brand		Nonamino nitrogen (mg/100 ml of wine)		
		Year of production		
		1975 (fresh)	1974 (1-year-old)	1973 (2-year-old)
White wines				
Clo Mariote	Dry	5.4	7.3	7.2
Gianaclis	Dry	—	9.0	7.4
Natural Abarka	Dry	4.9	8.8	10.0
Cleopatra	Semisweet	8.4	11.2	9.6
Nefertiti	Semisweet	6.5	7.8	8.8
Potlemo	Semisweet	5.3	10.1	11.4
Red wines				
Clo Matamir	Dry	—	8.7	3.8
Gianaclis	Dry	3.9	—	4.7
Kaser Gianaclis	Dry	7.0	6.1	6.9
Pharaons	Dry	5.5	7.3	4.6

Amino acids content: Seventeen amino acids were identified and estimated quantitatively in both white and red wine brands. The amino acids content of wine was tabulated according to their separation sequence in the chromatograms, as shown in Tables 4, 5 and 6. The total amounts of amino acids in white and red wines of the three vintages as mg per 100 ml ranged, respectively, as follows: fresh samples 115-148 and 98-140; one-year-old 176-217 and 169-223; two-year-old 152-185 and 147-173. From the results presented in the three tables mentioned above, it seems that no defined trend can be detected for the quantitative sequence of amino acids in all wine brands of different ages. This may be due to the seasonal variation, which depends mainly on grape variety, climate, soil and other vinification practices in the winery, according to Amerine et al. (1).

Table 4. Amino acids content of fresh wines.

Amino acids	Amino acids, mg per 100 ml wine							
	White brands							Red brands
	Dry		Semisweet					Dry
	Clo Mariote	Natural Abarka	Cleopatra	Nefertiti	Potlemo	Gianaclis	Kaser Gianaclis	Pharaons
Cystine	5.00	7.50	3.75	2.50	6.25	4.38	0.00	0.00
Lysine	5.60	4.08	2.80	5.13	3.03	6.77	8.87	6.07
Histidine	3.08	3.08	3.85	3.85	3.46	3.08	6.35	5.77
Arginine	4.33	2.52	6.73	3.37	2.16	5.53	4.81	3.37
Aspartic acid	5.52	3.92	5.23	3.20	5.67	3.49	11.63	4.94
Serine	7.70	3.85	6.11	5.81	2.69	3.78	6.11	3.92
Glycine	2.27	2.78	3.54	4.55	3.54	1.77	3.79	3.54
Glutamic acid	8.00	5.67	8.00	4.44	3.89	4.44	6.11	3.89
Threonine	1.37	1.27	2.13	2.54	4.30	1.17	3.91	1.95
Alanine	3.20	2.67	5.60	2.80	5.13	2.53	3.67	2.87
Tyrosine	4.48	4.04	8.97	10.76	6.73	2.69	5.83	5.38
Proline	58.71	69.07	77.71	62.17	70.80	51.80	65.62	70.80
Methionine	0.00	0.00	0.00	0.00	7.50	0.00	0.00	0.00
Valine	7.11	2.22	5.78	5.00	5.89	3.78	6.89	3.78
Phenylalanine	1.24	0.62	2.78	2.78	3.40	0.93	4.48	3.40
Isoleucine and leucine	1.29	1.29	5.16	1.80	2.19	2.06	1.93	1.55
Total	119	115	148	121	137	98	140	121

Table 5. Amino acids content of one-year-old wine.

Amino acids	Amino acids, mg per 100 ml wine								
	White brands						Red brands		
	Dry			Semisweet			Dry		
	Clo Mariote	Gianaclis	Natural Abarka	Cleopatra	Nefertiti	Potlemo	Clo Matamir	Kaser Gianaclis	Pharaons
Cystine	8.75	0.00	9.38	5.63	6.25	0.00	4.38	0.00	0.00
Lysine	5.13	11.67	5.83	5.02	6.30	9.22	3.38	6.30	6.30
Histidine	12.31	3.85	13.65	5.77	7.69	9.39	11.15	7.31	6.92
Arginine	3.97	5.05	3.61	8.53	6.01	5.79	5.05	2.40	3.85
Aspartic acid	10.17	9.30	14.24	11.34	5.38	7.56	11.34	5.23	4.07
Serine	4.80	5.52	5.81	6.18	5.81	5.74	5.96	3.63	3.42
Glycine	4.70	5.18	7.45	4.92	5.05	8.84	7.96	6.57	6.82
Glutamic acid	11.33	12.67	11.00	12.79	14.22	11.33	10.56	5.33	5.66
Threonine	0.98	2.34	0.98	0.78	0.96	2.54	1.86	1.56	2.54
Alanine	6.67	7.20	7.87	5.33	6.40	6.53	7.73	4.53	3.87
Tyrosine	2.69	2.22	3.14	3.14	2.92	1.79	4.93	3.81	4.26
Proline	120.9	113.9	107.0	112.2	86.3	124.3	127.8	108.8	117.4
Methionine	7.50	4.38	2.50	2.50	5.63	9.38	6.25	3.13	3.13
Valine	4.11	5.33	3.67	6.22	5.67	4.00	3.67	3.22	4.11
Phenylalanine	3.09	2.47	3.55	2.01	3.09	5.09	3.40	3.09	2.32
Isoleucine and leucine	7.08	6.17	8.51	5.41	7.99	5.67	7.22	3.87	3.48
Total	217	197	208	198	176	217	223	169	178

Table 6. Amino acid content of two-year-old wine.

Amino acids	Amino acids, mg per 100 ml wine									
	White brands						Red brands			
	Dry			Semisweet			Dry			
	Clo Mariote	Gianaclis	Natural Abarka	Cleopatra	Nefertiti	Potlemo	Clo Matamir	Gianaclis	Kaser Gianaclis	Pharaons
Cystine	3.75	3.75	3.13	2.50	3.75	2.50	5.00	4.38	0.00	4.38
Lysine	5.02	6.65	4.99	6.65	7.58	3.99	2.92	5.02	6.65	5.02
Histidine	7.12	5.77	5.19	6.35	5.39	5.58	4.04	10.96	2.50	3.08
Arginine	4.33	3.97	3.13	5.05	3.61	3.85	2.52	3.85	1.68	2.28
Aspartic acid	7.41	8.14	6.69	11.34	6.98	7.99	4.07	9.88	2.47	3.33
Serine	6.83	5.02	3.49	5.38	4.65	4.65	2.11	5.02	3.05	3.56
Glycine	6.57	5.57	4.42	5.93	4.92	5.18	4.55	6.95	4.04	5.56
Glutamic acid	5.56	6.00	3.78	4.89	9.33	4.22	3.33	9.33	4.22	3.56
Threonine	2.93	2.64	1.95	2.54	3.61	2.15	2.25	1.56	1.66	3.90
Alanine	5.40	6.00	5.47	6.47	5.87	6.93	2.47	6.87	3.20	4.20
Tyrosine	2.92	3.59	2.24	2.69	3.37	3.14	4.04	4.04	4.48	4.04
Proline	110.5	103.6	96.70	103.6	75.9	94.9	108.8	89.8	103.6	100.1
Methionine	3.75	5.00	2.50	3.13	5.00	3.13	3.13	2.50	0.00	6.25
Valine	3.33	4.78	2.67	4.00	3.33	2.67	2.56	3.67	2.56	3.22
Phenylalanine	2.16	2.16	1.70	1.85	1.85	2.16	1.85	2.78	1.54	2.47
Isoleucine and leucine	7.47	3.87	5.41	8.25	5.80	5.41	2.71	6.83	2.06	2.32
Total	185	176	152	181	151	158	156	173	147	156

Proline: As noted from Tables 4, 5 and 6, proline is the most predominant amino acid in wine and is present in higher amounts than the other amino acids. The proline nitrogen, calculated as percentage of the amino nitrogen, is shown in Table 7 and ranges as follows: fresh wine 49.5-59.5 and 49.1-54.3; one-year-old wine 46.1-52.8 and 55.6-62.9; and two-year-old wine 43.6-58.7 and 51.5-64.3 in white and red wines, respectively. The percentage of proline as nitrogen within the total nitrogen in the Egyptian wine brands ranged in the fresh, one-year-old, and two-year-old wine as follows: 32.5-44.2; 34.4-48.8; 30.8-53.4. Ough (8) reported that the amount of proline in wine varied from 0 to about 90% of the total nitrogen, and the amount detected in California wines ranged from 0 to 3400 mg per liter. The proline content of Egyptian wine was less than that found in California wine. Many investigators used the proline content of wine as an index of its origin and genuineness. Lachkhi and Tsiskarichvili (5) stated that dry wines containing more than 200 mg proline per liter were known to have been produced by fermentation of grape juice.

Table 7. Proline content of wine of different ages.

		Proline nitrogen % of the amino nitrogen			Proline nitrogen % of the total nitrogen		
		Year of production			Year of production		
		1975 (fresh)	1974 (1-year-old)	1973 (2-year-old)	1975 (fresh)	1974 (1-year-old)	1973 (2-year-old)
White wines							
Clo Mariote	Dry	49.5	51.8	54.9	36.1	41.1	42.4
Gianaclis	Dry	—	52.8	51.7	—	39.4	39.7
Natural Abarka	Dry	59.5	49.4	58.7	44.2	37.0	39.2
Cleopatra	Semisweet	52.0	51.2	53.0	35.6	36.2	37.7
Nefertiti	Semisweet	50.9	46.1	43.6	35.4	34.4	30.8
Potlemo	Semisweet	52.9	51.9	55.3	39.8	38.6	35.8
Red wines							
Clo Matamir	Dry	—	55.6	63.3	—	42.5	53.4
Gianaclis	Dry	49.1	—	51.5	37.7	—	42.1
Kaser Gianaclis	Dry	45.4	62.9	64.3	32.5	48.8	47.5
Pharaons	Dry	54.3	61.5	60.4	40.4	46.7	49.1

LITERATURE CITED

1. Amerine, M. A., H. W. Berg, and W. V. Cruess. The Technology of Wine Making. 3rd ed. p. 802. Avi Publishing Co., Westport, Ct. (1972).

2. Assoc. Off. Anal. Chem. Official Methods of Analysis. A.O.A.C., Washington, D.C. (1975).

3. Chinard, F. P. Phostometric estimation of proline and ornithine. J. Biol. Chem. 199:91-5 (1952).

4. Kliewer, W. M. Free amino acids and other nitrogenous substances of table grape varieties. J. Food Sci. 34:274-8 (1969).

5. Lachkhi, A. D., and T. P. Tsiskarichvilli. Proving the natural origin of wine obtained by fermentation of grape juice. Vignes et Vin 211:5-7 (1972).

6. Majunders, S., D. R. Glosh, and N. C. Gunguli. Amino acid composition of some Indian vegetables as determined by paper chromatography. Food Res. 21:447 (1956).

7. Mikes, O. Laboratory Handbook of Chromatographic Methods. De Van Norstrand Co. Ltd., London (1966).

8. Ough, C. S. Proline content of grapes and wines. Vitis 7:321-31 (1968).

9. Ough, C. S. Rapid determination of proline in grapes and wines. J. Food Sci. 34:228-30 (1969).

10. Poux, C., C. Flanzy, and M. Flanzy. Alcohol yeasts in wines: Proteolysis and proteogenesis. Ann. Technol. Agric. 13:5-18 (1964).

11. Roland, J. F., and A. M. Gross. Quantitative determination of amino acids using mono-dimensional paper chromatography. Anal. Chem. 26:502 (1954).

12. Sachde, A. G., E. M. El-Zalaki, A. M. El-Tabey, and S. A. Abo-Donia. Study on Egyptian fresh and aged wines: I. pH, acidity, esters, and SO_2 content. Am. J. Enol. Vitic. 30:205-7 (1979).

13. Singleton, V. L. Role of polyphenolics in aging of wine and a few contrasts with beer. MBAA Technical Quarterly 11:135-8 (1974).