The Relationship Between Metals, Polyphenols, Nitogenous Substances and Treatment of Red and White Wines

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The effect of treatment of red and white wines was studied according to the following criteria: stability (non-biological and non-crystalline), presence of soluble nitrogen-containing compounds (total, amine and amino acid nitrogen), metals and polyphenols with respect to their changes. Among the findings is the decrease of protein and amino acid content during wine treatment, and this is noted especially when using bentonite.

It has been found that by treating red wines with 70 mg/L of gelatin, and white wines with 0.4 g/L of bentonite during the cooling process, stability and overall quality of wines was improved.

It has been shown that the stability of wines with regard to taste, color, clarity and stability is dependent on the relationships between several components, which include metals, proteins and phenolic substances (1,5,6,7). However, non-biological and non-crystalline hazes are dependent on the protein-polyphenol reaction and interaction between the metals, sulfurous and protein compounds which tend to form complexes with iron and copper (12,14,20).

Potassium and calcium tartrates have also been shown to be insoluble in wines (4,10).

In order to control the stability of wine during the winemaking and storage stages, accurate monitoring of all of the metal concentrations, the total content of nitrogenous and polyphenol compounds, and proteins (14) is required. Among the precipitates formed in this medium of high protein content are iron and copper (16,20).

Therefore, the causes for wine turbidity are due to metals, crystallization, and various colloids. The haze in white wines is caused by proteins and in red wines mostly by large tannins (19). As a means of reducing this turbidity, filtration and subsequent treatment with bentonite, gelatin, silicagel, perlite, tannin-gelatin, gallotannin, vinylpolypyrolidone complexes, casein and polyclar are effective in reducing the levels of metals and can stabilize the proteins (3,15,21). Regarding taste, amino acids are known to play an important role, especially their interactions with sugars (13,23).

The storage of wine in Israel has to be done with extra care due to the climate, which is often much hotter than in other wine-producing countries, and ultimately affects the taste. Until recently, there has been very little data available on the stability of Israeli wines with regard to their chemical contents and the various treatments during production. The stability of wines can be related to the presence of nitrogenous and polyphenol compounds, and the changes occurring in them during the treatment of the wine.

The purpose of this research was to establish the relationships between different methods of wine treatment, such as cooling and absorption periods, and the stability of the resulting wine. During this study, some important indices (such as amino acids, proteins, polyphenols and metals) have also been investigated in order to give indications of the quality and stability of the final product.

Materials and Methods

In this study, two wines were selected: one a semi-dry, red wine, whose trade name is Lod (Stock, Ltd.); the other a white wine, Advat (Rishon-Le-Zion). These were selected as basic types of wine for a closer investigation of the different types of treatments. The work was comprised of two series of experiments. The initial set was carried out in order to determine if variation in the absorbent content for varying time periods during treatment affected the stability of the final product and in which way. In the second set of experiments, the effects on stability were related to the optimal times allowed for the wine to cool during the second half of its treatment. This test used the same samples of wine at various temperatures, for a series of time periods. Samples 1 to 10 of the red wine were treated in the laboratory with either gelatin (G) or bentonite (B) or with a mixture of these two additives (G & B) for varying time periods, and this is summarized in Tables 1a and 1b (Rows 1 to 3). On completion of fining, all of the samples were cooled at −5°C for six days. The sample Control I is wine taken before fining and Control II, a sample taken after treatment. Sample 6 and Control I[2] were treated under similar laboratory conditions while Control II at the winery was used in the second series of experiments in which Samples 11 to 17 were kept at temperatures of −3°C and −5°C for three, four, five and six day periods. (Table 2).

Tables 3a and 3b depict the treatment of white wine Samples 100 to 114 under laboratory conditions, while Table 4 shows the way in which Samples 115 to 124 were cooled for differing time durations.

Standard wine and must analysis was carried out using conventional methods (1,2).

Nitrogen was determined by the methods of Dumas

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Table. 1a. Result data[a] of gelatin fining before refrigeration in red wine Lod[b] (semidry) Stock.

Indices	Control I	Samples of Wine 1	2	5	6	7	8	Control II
Time of fining, days	—	7	10	7	10	7	10	10
Bentonite, g/L	—	—	—	1.1	1.1	1.4	1.4	1.1
Gelatin, mg/L	—	70	70	70	70	70	70	70
Total nitrogen, mg/L	185.4	152.0	151.3	140.7	138.6	132.4	124.3	143.1
Amine nitrogen, mg/L	93.4	91.4	90.7	83.0	82.6	83.5	84.7	89.4
C, %	36.67	36.25	36.47	37.01	37.38	38.18	39.03	36.73
H, %	6.32	6.27	6.29	6.29	6.27	6.38	6.40	6.32
Na, mg/L	50.0	58.0	58.0	66.0	68.00	73.0	76.0	68.0
K, mg/L	1520	1505	1510	1504	1500	1490	1498	1500
Ca, mg/L	145	142	140	140	138	135	133	138
Fe, mg/L	8.0	8.0	8.0	7.0	7.0	7.0	6.0	7.5
Stability +55°C	—	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.

[a] Each value is the mean of two replicates. [b] Eight field replications.

Table 1b. Result data[a] of bentonite fining before refrigeration in red wine Lod[b] (semidry) Stock.

Indices	Samples of wine Control I	3	4	9	10	Control II
Time of fining, days	—	7	10	7	10	10
Bentonite, g/L	—	0.8	0.8	1.1	1.1	1.1
Gelatin, mg/L	—	—	—	—	—	70
Total nitrogen, mg/L	185.4	150.1	148.4	147.3	144.8	143.1
Amine nitrogen, mg/L	93.4	87.0	87.4	86.3	85.4	89.4
C, %	36.67	37.00	37.15	37.65	38.01	36.73
H, %	6.32	6.34	6.34	6.38	6.40	6.32
Na, mg/L	50.0	62.0	62.5	66.0	68.0	68.0
K, mg/L	1520	1510	1512	1480	1485	1500
Ca, mg/L	145	142	140	142	141	138
Fe, mg/L	8.0	7.0	7.0	7.0	6.5	7.5
Stability +55°C	—	Stab.	Stab.	Stab.	Stab.	Stab.

[a] Each value is the mean of two replicates. [b] Eight field replications.

Table 2. Result data[a] of cooling in red wine Lod[b] (semidry) Stock.

Indices	Control I[2]	Control II[2]	Samples of wine 11	12	13	14	15	16	17
Time of cooling, days	—	12	4	5	6	3	4	5	6
Temperature (−)°C	—	3	3	3	3	5	5	5	5
Na, mg/L	68.00	65.0	77.5	65.0	65.0	65.0	62.5	60.0	70.0
K, mg/L	1500	1500	1200	1200	1200	1380	1140	1160	1140
Ca, mg/L	138	125	135	130	115	125	125	120	120
Total nitrogen, mg/L	138.6	127.5	130.7	130.0	130.0	120.4	118.7	116.5	116.4
Protein, mg/L	0.39	0.30	0.34	0.34	0.34	0.28	0.26	0.26	0.26
Stability −5°C	—	stab.	unstab.	unstab.	unstab.	stab.	stab.	stab.	stab.
Stability +55°C	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.

[a] Each value is the mean of two replicates. [b] Eight field replications.

Table 3a. Result Data[a] of bentonite fining in white wine Avdat[b] Rishon-le-Zion.

Indices	Control I[3]	Control II[3]	Samples of Wine 100	101	102	103	104	105	106	107	108
Time of fining, days	—	8	4	4	4	6	6	6	8	8	8
Bentonite, g/L	—	0.6	0.4	0.6	0.9	0.4	0.6	0.9	0.4	0.6	0.9
6 days refrigeration at −5°C	—	12(−3°C)	—	—	—	—	—	—	—	—	—
Total nitrogen, mg/L	190.4	158.9	174.5	169.2	170.2	172.3	167.3	163.2	170.3	165.2	152.4
Amine nitrogen, mg/L	108.7	88.4	97.8	93.2	92.3	96.4	92.4	90.3	95.1	91.4	88.5
C, %	34.65	37.35	39.03	38.61	38.23	38.92	36.10	38.15	38.72	38.35	37.92
H, %	7.11	6.92	6.67	7.23	6.70	6.93	6.86	6.79	7.13	6.60	6.86
Fe, mg/L	6.5	4.0	4.0	4.0	4.0	4.5	4.0	4.0	4.5	4.5	4.0
Cu, mg/L	0.20	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Na, mg/L	50.0	52.8	50.0	52.5	55.0	50.0	52.5	55.0	50.0	55.0	55.0
K, mg/L	920	600	880	880	880	880	880	880	900	900	880
Ca, mg/L	224	120	216	120	119	152	118	115	134	122	102
Stability −5°C	—	stab.	—	—	—	—	—	—	—	—	—
Stability +55°C	unstab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.

[a] Each value is the mean of two replicates. [b] Eight field replications.

Table 3b. Results Data[a] of bentonite fining in white wine Avdat[b] Rishon-le-Zion.

Indices	Control I[3]	Control II[3]	Samples of wine 109	110	111	112	113	114
Time of fining, days	—	8	6	8	6	8	6	8
Bentonite, g/L	—	0.6	0.4	0.4	0.6	0.6	0.9	0.9
6 days refrigeration at −5°C	—	12(−3°C)	+	+	+	+	+	+
Total nitrogen, mg/L	190.4	158.9	162.3	159.3	158.5	153.4	144.7	138.4
Amine nitrogen, mg/L	108.7	88.4	89.9	88.0	87.1	86.7	85.8	84.5
C, %	34.65	37.35	37.65	37.34	36.00	37.25	37.15	35.93
H, %	7.11	6.92	6.97	6.94	6.88	6.85	7.00	6.91
Fe, mg/L	6.5	4.0	4.0	5.0	4.0	4.0	4.0	3.5
Cu, mg/L	0.20	0.10	Traces	0.10	0.10	0.10	0.10	0.10
Na, mg/L	50.0	52.8	55.0	55.0	55.0	55.0	55.0	52.5
K, mg/L	920	600	640	700	620	700	620	700
Ca, mg/L	224	120	136	118	107	112	108	72
Stability −5°C	—	stab.	stab.	stab.	stab.	stab.	stab.	stab.
Stability +55°C	Unstab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.

[a] Each value is the mean of two replicates. [b] Eight field replications.

Table 4. Result Data[a] of cooling in white wine Avdat[b] Rishon-le-Zion.

Indices	Control I[4]	Control II[4]	Samples of wine 115	116	117	118	119	120	121	122	123	124
Time of cooling, days	—	12	4	6	8	10	12	4	6	8	10	12
Temperature (−)°C	—	3	3	3	3	3	3	5	5	5	5	5
Na, mg/L	55.0	52.8	52.5	52.0	52.0	52.5	50.0	48.0	48.0	48.0	48.0	48.0
K, mg/L	900	600	720	720	660	640	640	720	700	680	680	580
Ca, mg/L	122	120	102	96	96	88	88	104	100	80	80	80
Total nitrogen, mg/L	165.2	158.9	162.0	160.3	157.4	156.5	154.7	157.0	156.3	155.0	152.4	150.3
Protein, mg/L	0.81	0.78	0.81	0.80	0.76	0.74	0.70	0.63	0.63	0.62	0.60	0.58
Stability −5°C	—	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.
Stability +55°C	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.	stab.

[a] Each value is the mean of two replicates. [b] Eight field replications.

and Kjeldahl, the latter using a Büchi 425 Nitrogen Determination System. Protein was precipitated by a 20% TCA solution, and nitrogen content was determined both before and after precipitation.

Carbon, hydrogen and chloride were found by microanalysis. Sodium, potassium, calcium, iron and copper were determined by absorption spectrophotometry.

The stability of the wines was investigated at temperatures of under +55°C and at −5°C after 48 hours. In order to determine the glycerol content, IR spectroscopy was used (8), and for amino acids, a Beckman model 120C automatic amino acid analyzer was used after hydrolysis of the samples for 24 hours at 110°C with a 6*M* solution of HCl in evacuated tubes. Also determined were the free amino acids (9).

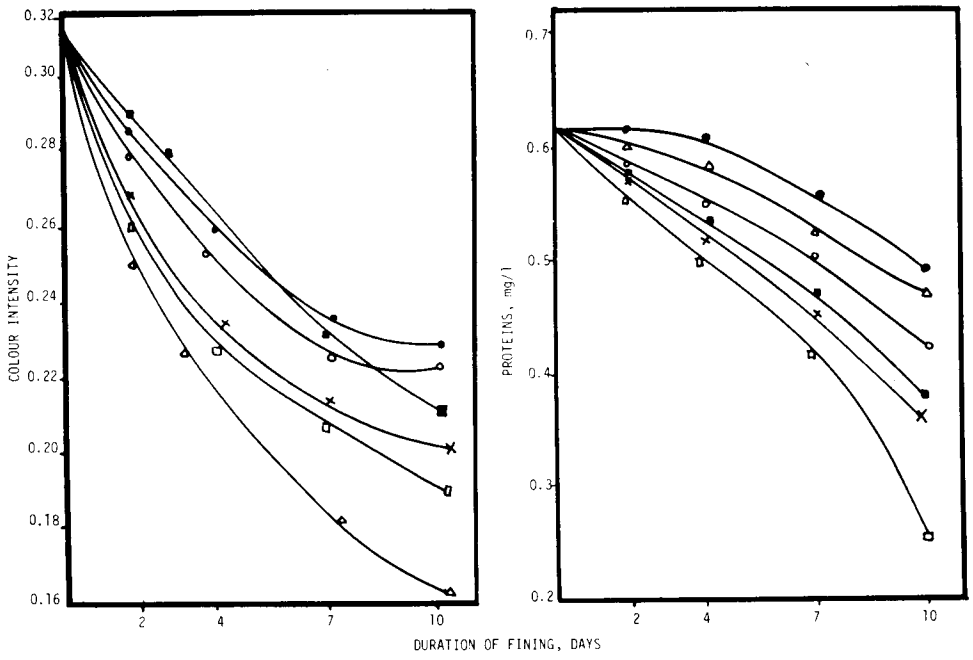

Fig. 1. Effect of gelatin (G) and bentonite (B) treatment of red wine, Lod, on color intensity (a) and protein content (b).
(o) 0.8 g/L B
(●) 70 mg/L G
(□) 1.4 g/L B + 70 mg/L G
(x) 1.1 g/L B + 70 mg/L G
(■) 1.1 g/L B
(▽) finished product.

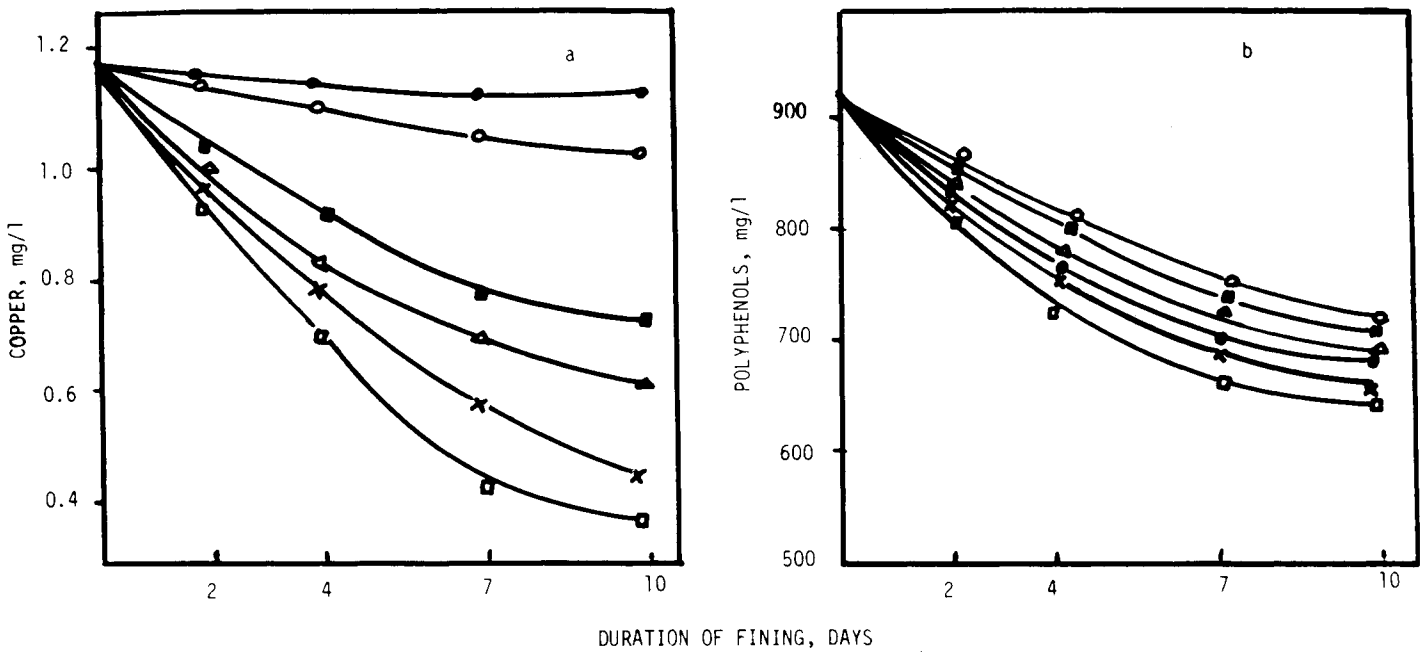

Fig. 2. Effect of bentonite (B) and gelatin (G) treatment of red wine, Lod on copper (a) and polyphenols (b) contents. (o) 0.8 g/L B; (●) 70 mg/L G; (□) 1.4 g/L B + 70 mg/L G; (x) 1.1 g/L B + 70 mg/L G; (■) 1.1 g/L B; (▽) finished product.

Results and Discussion

Table 1a shows the main variations of indices occurring during the treatment of the wine, Lod, with gelatin of 70 mg/L modified by two levels of bentonite at 1.1 and 1.4 g/L. Table 1b supplies effect of bentonite.

From Tables 1a and 1b it can be seen that a reduction occurred in the iron, calcium and potassium content. The increase in sodium content can be accounted for by the use of bentonite, which also contains this metal as a slight contaminent. (Table 1b). Total and amine nitrogen changed during gelatin and bentonite fining. As a measure of stability after treatment with bentonite and gelatin, the samples were kept at +55°C, and a lack of turbidity showed protein stabilization.

From Figure 1a, the relationships between color intensity and fining with bentonite and gelatin treatments are shown. This shows that the bentonite reduced the wine color by a higher degree than the gelatin. In Figure 1b, the relationship between protein content and the same treatment is shown. The decrease in copper and polyphenols by gelatin and bentonite treatment can be

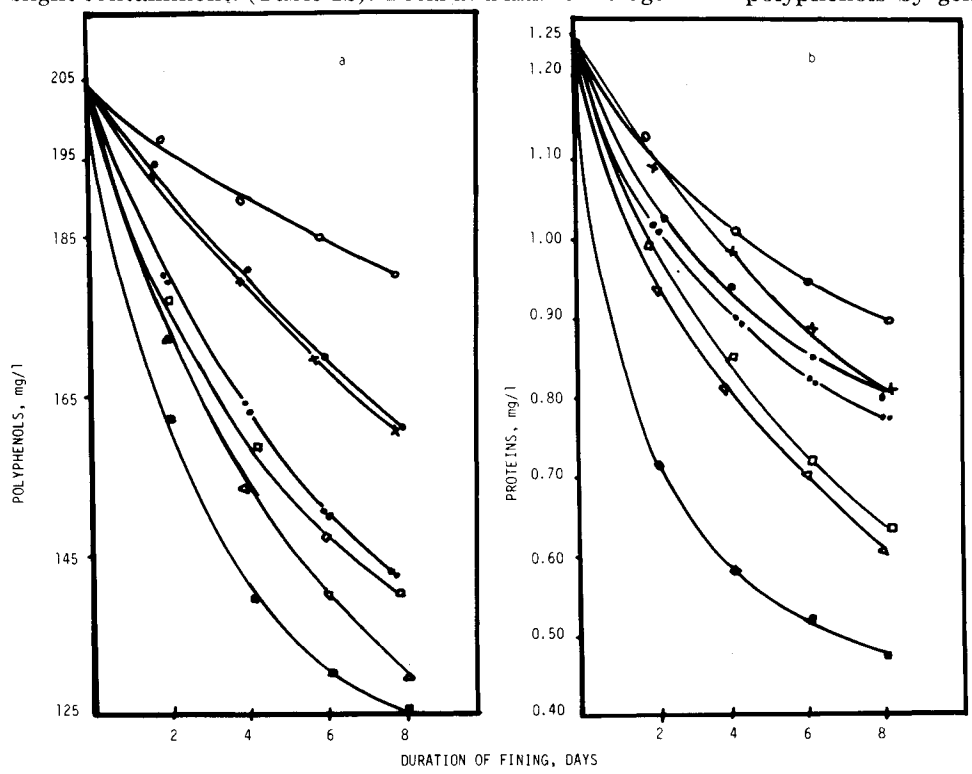

Fig. 3. Effect on bentonite (B) and cooling (C) treatment of white wine, Avdat, on polyphenols (a) and proteins (b) contents.
(o) 0.4 g/L B
(●) 0.6 g/L B
(□) 0.9 g/L B
(x) 0.4 g/L B + C
(▽) 0.6 g/L B + C
(■) 0.9 g/L B + C
(●●) finished product.

clearly seen in Figures 2a and 2b. It was observed that gelatin alone was as efficient as a gelatin-bentonite mixture in absorbing the polyphenols. It can thus be concluded that bentonite is more efficient in the absorption of protein, while gelatin has more emphasis on polyphenol absorption. The results of cooling treatments are shown in Table 2.

The effects of cooling at −3°C and at −5°C are a reduction in calcium content. After four and five days at −3°C, the samples were seen to be unstable, using protein stability as a factor. However, when wines were kept for similar periods at −5°C, the samples were stable.

Tables 3a and 3b depict the effect of bentonite fining in all the samples of white wine with details of the indices found after treatment with bentonite.

Contrary to the results with the red wine samples, copper did not show any variation throughout the treatment of white wine samples. The samples can be divided into three groups: a) 100-102; b) 103-105; and c) 106-108 (Table 3a). Each of the groups was treated with differing amounts of bentonite, but for the same periods of time. A slight change was noted in amine nitrogen, in the polyphenol, and protein contents. The maximum stability was attained in Samples 109 to 114, which were treated with bentonite and cooled at −5°C simultaneously (Table 3b). This last treatment caused a great reduction in all of

Table 5. Range of indices measured after wine treatment.

Parameters	Red wine Lod	White wine Avdat
Density, 20/20	1.0009 - 1.0016	0.9929 - 0.9936
Alcohol, 20°C	10.8 - 11.3	10.7 - 10.9
Reducing sugars, g/L	13.5 - 14.1	1.1 - 1.2
Total extract, g/L	39.8 - 42.9	19.0 - 20.9
Reducing extract, g/L	27.3 - 30.0	18.9 - 20.8
Total acidity (tart. acid, g/L)	4.82 - 6.17	4.83 - 4.86
Volatile acidity (acetic acid, g/L)	0.49 - 0.84	0.34 - 0.38
Fixing acidity (tart, acid, g/L)	4.21 - 5.30	4.36 - 4.41
pH	3.61 - 3.75	3.45
SO_2 free, mg/L	10 - 22	12 - 20
SO_2 total, mg/L	102 - 131	104 - 110
Ash, g/L	3 450 - 4 540	1925 - 2300
Alkalinity of ash, meq/L	31.0 - 40.5	18.5 - 22.5
Malic acid, g/L	0.5 - 1.0	0
Tartaric acid, g/L	1.5 - 3.0	2
Citric acid, g/L	0.5	0 - traces
Lactic & succinic acid, g/L	1.5 - 2.0	2.5
Glycerol, g/L	18.1 - 25.3	14.0 - 17.5
Cl %	1.12 - 1.30	0.85 - 1.34

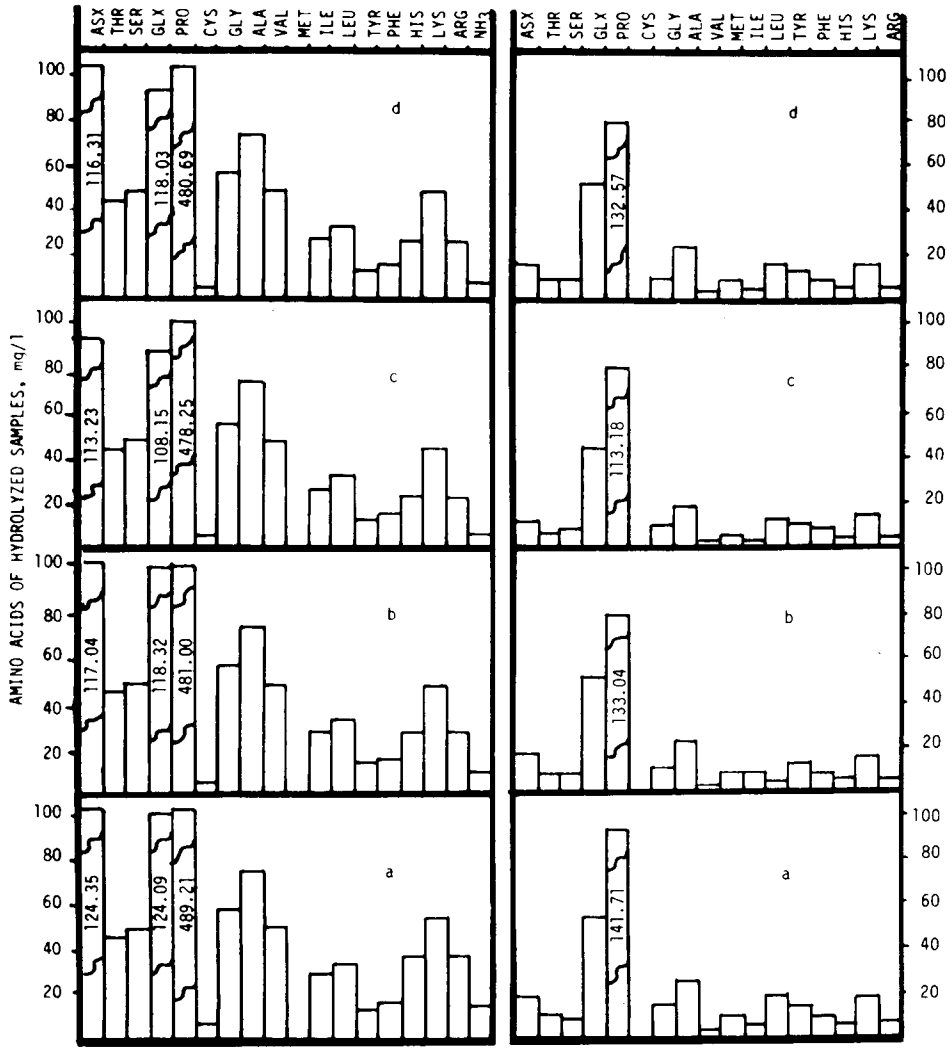

Fig. 4. Amino acid content (mg/L) of red wine, Lod at differnet stages of gelatin (G) and bentonite (B) treatment.
(a) untreated wine
(b) with 70 mg/L G/7 days
(c) with (1.4 g/L B + 70 mg/L G)/10 days
(d) finished product.

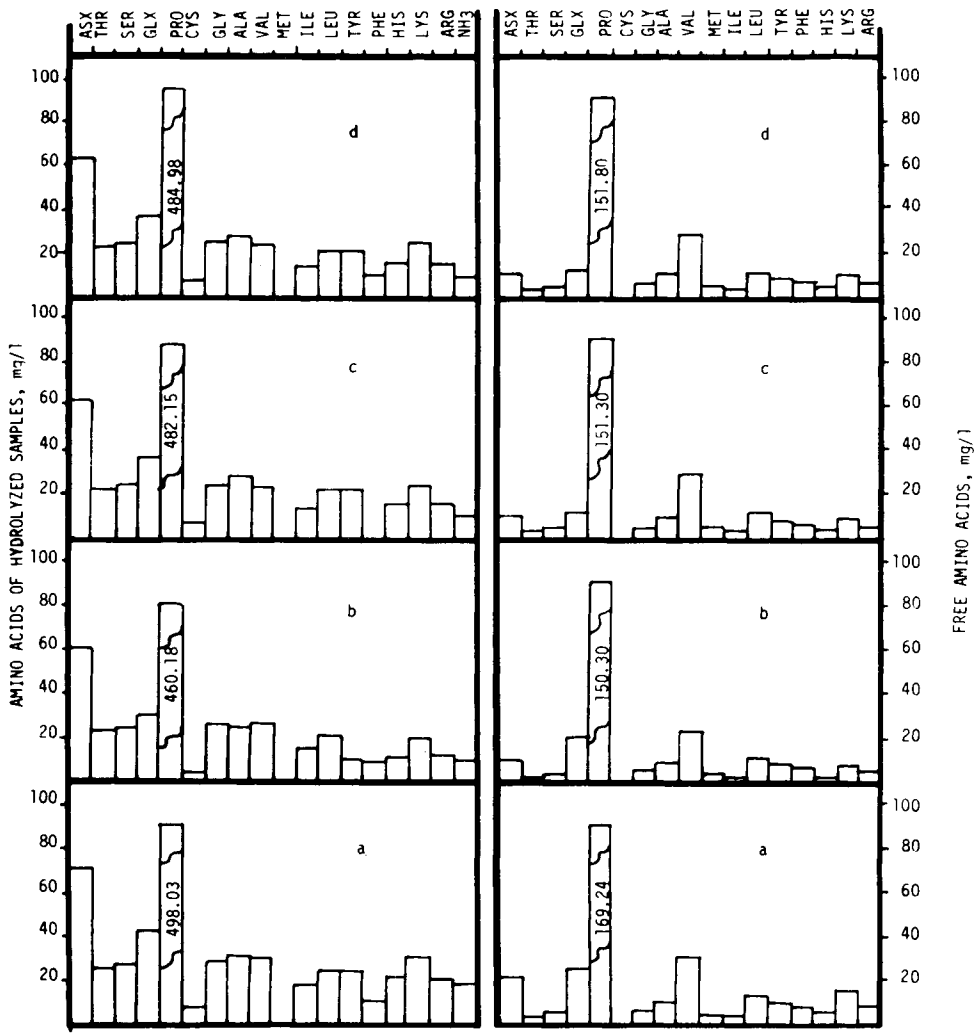

Fig. 5. Amino acid content (mg/L) of white wine Avdat at different stages of bentonite (B) and cooling (C) treatment.
(a) untreated wine
(b) with 0.6 g/L B/8 days + C
(c) with 0.9 g/L B/8 days + C
(d) finished product.

the indices, especially in nitrogenous compounds, polyphenols and calcium. Table 4, like Table 2, shows the effects of cooling after treatment with bentonite for white wine. The only notable reductions were in calcium and potassium. The total polyphenols were higher in comparative terms in the red wine, Lod, than in the white wine, Avdat. Therefore, it would appear that the bentonite and gelatin treatments were successful in allowing precipitation of the true proteins in the red wine (Fig. 1b). From Figure 3, it can be seen that the protein content in Avdat is in excess of the polyphenols (tannins) and large enough to form an insoluble complex, which can be removed as a protein-tannin precipitate. In general, it can be stated that when proteins are excessive, bentonite treatment aids protein stabilization, and a clear reduction in protein after bentonite fining has been observed. The data found on this aspect of wine treatment are in agreement with earlier work (19).

Table 5 shows that the changes in standard wine analysis were in normal ranges according to the literature (1,9,17,18).

Figures 4 and 5 show the relationships between different types of wine treatments and the amino acid composition. During the stages of treatment of the red wine, Lod, no large changes in amino acid composition were found, so Figure 4 only shows four samples; Control I, Samples 1 and 8, and Control II. The changes which did occur are also shown in Tables 1a and 1b; and from these, the reductions in the total nitrogen and protein concentrations are the most significant factors (9).

The order, from highest to lowest, in which the amino acids were decreased during the treatment according to sample number is shown: $7 > 8$; Control II > 1; $1, 2 > 3, 4$; and $5, 6 \approx 8, 9$. The smallest amino acid concentration was noted in Sample 8.

Bentonite was more efficient in the absorption of lysine, arginine, histidine, aspartic and glutamic acids, but less so with glycine, alanine, valine, leucine, isoleucine, serine, threonine. The effect on other amino acids was insignificant during the treatment of red wine.

In the case of white wine, similar results were obtained and are shown for Avdat in Figure 5, which represents four samples; Control I[3], Samples 112 and 114, and Control II[3]. The sample with the least amino acid content, after fining was 114. The content of Sample 112 was approximately equal to that of Control II[3] and 111.

Another point of interest was that the amino acid content after fining and cooling was lower than in fining only for the same samples. Therefore, Samples 109 and 114 were lower in amino acid content than 103, 106, 104,

107, 105 and 108. The relationships may be summed up thus: 102 < 107; 107 > 105. In assessing the relationships when using greater amounts of bentonite in the fining process and for a shorter period, we found the following: 101 > 104; 103 > 106; 100 > 103; 103, 104, 105 < 109, 111, 112. (Series, 100 > 106; 101 > 107; 103 > 110.)

In all, the same degrees of change were noted in the amino acids of both red and white wines.

Conclusion

From this research, it was found that the quantities of additives in the fining process, bentonite and gelatin, which are used at present in Israeli wineries, can be reduced by up to 20%, thus saving manufacturing costs. Qualitative improvement was also attained in color intensity and stability.

The dynamics of change in wine amino acid composition during fining were displayed graphically as an aid for relating the protein complex formation to its initial amino acid content. The proteins in wines after treatment were rich in proline, aspartic and glutamic acid, glycine, alanine, threonine and serine in their free and bound forms, but poor in basic, aromatic, S-containing, and heterocyclic amino acids.

In general, the free and bound amino acids in wines were found in concentrations similar to those reported in the literature (11,13,15,22,23). The best treatment is by cooling and fining simultaneously. The use of bentonite at the same time as cooling improved the utilization of the tanks, allowing for a greater savings and shortening treatment by two weeks. Cooling can be more effective at lower temperatures and for shorter periods of time.

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