

Spectroscopic Analysis of Polyphenols in White Wines

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Spectroscopic analysis was used to study the effect of wine processing on phenolic composition. Various classes of phenolic compounds were detected and characterized by ultraviolet (UV) and infrared (IR) spectroscopy in white grapes of Sauvignon Blanc and French Colombard, as well as in wines prepared from these grapes. Combined treatment with bentonite, egg albumin and Polyclar AT decreased the amounts of catechols, flavonols, anthocyanins and leucoanthocyanins. Polyphenols (32-17%), anthocyanogens (64-48%) and proteins (62-77%) were removed by this technological process. The best results were received when not only wines, but also musts were pretreated with bentonite. Comparisons of the polyphenol compositions of wines made from the same grape variety grown in different locations of the same vintage and between two vintages are reported.

Phenolic compounds contribute in an important manner to the taste, bitterness, and bacteriological effects of wines. These compounds include catechins, leucoanthocyanidins, flavonols, flavonol glycosides, high-molecular-weight tannins, hydrocinnamic acid-tartaric esters and their glucose esters, proanthocyanidins and anthocyanidins, phenolic benzoic and phenolic cinnamic acids (1, 2).

Turbidity formation caused by the interaction between must proteins and phenols has been studied by several authors (3-8).

The concentration of phenolic compounds in white wines depends on the methods of grape processing, grape crushing and must preparation (9-12).

High levels of polyphenols increase susceptibility to oxidation, leading to decreased visual and organoleptic qualities (13). Sauvignon Blanc is the least susceptible to browning (9, 14). The levels of polyphenols and proteins are reduced in wine material treated with different adsorbents, such as bentonite, polyclar, gelatin, egg albumin and others (8, 15-18). There is a lack of data corresponding to the quality of final products and the fractions of polyphenols and proteins in Israeli white wines. Therefore, two varieties of white grapes (Sauvignon Blanc and French Colombard of 1988 vintage), musts and the corresponding wines were subjected to spectroscopic analysis using variables selected by correlation to the quality of the final product.

MATERIALS AND METHODS

Two types of Israeli grapes Sauvignon Blanc from three growing areas (Shaalabim, Gshor and Hulda) and French Colombard (M. Tut). These two varieties of grapes were selected as the basic grapes for different technological treatments. All technological processes (crushing, fermentation, racking, and fining) were carried out under industrial conditions for grapes for Sauvignon Blanc at Carmel Wine Corporation at Rishon-Le-Zion and for French Colombard at the same Company at Zichron-Yakov.

Analysis of wines Samples of wines were treated in the laboratory with either bentonite (=B), egg albumin (=E) and Polyclar AT (=P)-PVPP-(polyvinylpyrrolidone), or with a mixture of these three additives (B, E and P) in different variations in order to determine if this treatment affects the stability of the final product with emphasis on the content of polyphenols.

Bentonite was mixed with water at ratios of 1:10 and 1:7 w/v. All details of such fining are summarized in Tables 1 and 2. The time of fining with different adsorbents was the same as for the industrial conditions (7-10 d). Juice of French Colombard grapes was treated with bentonite (0.5 g/l) at 10°C during 24 h. Control 1 was the wine sample before treatment and Control 2 represented the same wine sample after treatment at the winery (Table 1). Samples of musts and wines were subsampled during different stages of wine preparation, such as fresh grape juice, complete juice fermentation, last racking, before fining, after fining with different adsorbents and also samples after filtration.

Standard wine and must analysis was carried out using conventional methods (19). The wine samples were dialyzed against water for 72 h at 4°C and then freeze-dried. Concentrates of wines were diluted to their original degrees Brix before analysis.

Analysis of phenolic acids

UV spectroscopy Twenty one phenols were chosen as standards for UV analysis: (catechin; rutin; quercetin; fisetin; cinnamic acid; caffeic acid gallic acid; salicylic acid; *p*-hydroxybenzoic acid; 2,3-dihydroxybenzoic acid; 2,4-dihydroxybenzoic acid; 2,5-dihydroxybenzoic acid; 3,4-dihydroxybenzoic acid, 2,4,6-trihydroxybenzoic acid; 2,3,4-trihydroxybenzoic acid; 4-hydroxyphenylacetic acid; resorcinol; tannic acid; catechol; caffeine and vanillin).

Sample preparation All determinations were carried out in triplicate. Samples were prepared according to Tryon *et al.* (20), with some of our modifications, based on the solubility of phenols and origin of wine. 5 ml of wine were diluted to 100 ml with a solution of 12% (v/v) ethanol and 5% (v/v) dextrose.

Wine samples which were previously freeze-dried were

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TABLE 1. Results* of fining with different adsorbents in white wine (Sauvignon Blanc) from grapes collected at Gshor

Indices	Control 1	Control 2	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8
Bentonite (1 : 10), g/l	—	+	+				+		+	
Bentonite (1 : 7), g/l	—			+				+		+
Egg albumin, mg/l	—				+		+	+		+
Polyclar AT, mg/l	—					+			+	+
Proteins, mg/l	34.8±1.0	18.2±0.7	16.5±0.5	14.8±0.3	14.5±0.4	30.5±1.1	13.7±0.2	12.5±0.3	11.2±0.2	10.2±0.1
Polyphenols, mg/l	420±9.0	390±7.3	390±7.1	385±6.5	383±7.0	365±8.8	370±6.9	370±6.8	360±8.1	350±7.3
Leucoanthocyanin, mg/l	180±3.1	165±2.4	160±1.9	155±1.8	153±2.0	112±2.3	150±1.5	148±1.7	104±1.1	95±0.9
Cu, mg/l	0.15±0.09	0.13±0.08	0.12±0.10	0.11±0.10	0.10±0.09	0.10±0.09	0.10±0.09	0.09±0.01	0.08±0.03	0.07±0.01
Fe, mg/l	6.0±0.2	5.7±0.1	5.6±0.2	5.2±0.1	5.1±0.2	5.0±0.2	4.9±0.3	4.7±0.4	4.6±0.2	4.4±0.2
Stability -5°C	Unstab.	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.
+55°C										

* Each value is the mean of three determinations±SD.

TABLE 2. Results* of fining with different adsorbents in white wine (French Colombard) from grapes collected at M. Tut

Indices	Control 1	Control 2	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8
Bentonite (1 : 10), g/l	—	+	+				+		+	
Bentonite (1 : 7), g/l	—			+				+		+
Egg albumin, mg/l	—				+		+	+		+
Polyclar AT, mg/l	—					+			+	+
Proteins, mg/l	10.3±0.3	5.5±0.2	5.3±0.4	5.1±0.2	4.9±0.1	9.4±0.4	5.0±0.3	4.8±0.2	4.5±0.4	4.0±0.3
Polyphenols, mg/l	450±9.8	380±8.4	380±8.4	375±8.0	375±5.7	330±6.9	375±7.9	370±7.6	320±6.8	310±5.9
Leucoanthocyanin, mg/l	210±3.6	160±3.7	150±3.2	148±2.8	146±3.1	100±1.6	145±2.9	140±3.0	90±1.8	72±1.2
Cu, mg/l	0.15±0.04	0.12±0.05	0.10±0.06	0.08±0.08	0.08±0.02	0.08±0.03	0.09±0.01	0.07±0.03	0.06±0.01	0.05±0.01
Fe, mg/l	5.6±0.4	5.3±0.4	5.1±0.3	5.0±0.4	4.9±0.2	4.9±0.1	4.9±0.5	4.7±0.3	4.2±0.4	4.0±0.2
Stability -5°C	Unstab.	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.	Stab.
+55°C										

* Each value is the mean of three determinations±SD.

dissolved in water according to the lyophilized weight in order to add 5 ml of original wine, and were then diluted to 100 ml with an ethanol-dextrose solution. Phenols (quercetin, rutin and fisetin) as well as phenolic fractions of lyophilized wine samples were first dissolved in ethanol and then diluted with the ethanol-dextrose solution. Stock solutions (100 mg/l) of each phenol were prepared by dissolving the sample in ethanol-dextrose solution. The absorption of standard solutions (10 mg/l), as well as wines were measured with a Wicon 930 Kautron UV spectrophotometer at a scan range of 250–350 nm and a scan speed of 200 nm/min. All ultraviolet spectra were recorded using ethanol-dextrose solution as a blank.

The amount of total polyphenols extracted with methanol was determined spectrophotometrically at 275 nm. Resorcinol was used as a standard (21). Phenolic compounds were also determined by the Folin-Ciocalteu method (22).

IR analysis The IR spectra of lyophilized wine samples were measured by Fourier Transformation Infrared Spectroscopy (FTIR) as a film between two KBr plates with a Fourier Transformation (FT) IR Analect instrument. The recording was done from 4,000 to 2,500 cm^{-1} wave number. Standards for total polyphenol content were purchased from Sigma Chemical Co. (St. Louis, USA).

Protein analysis Total proteins in musts and wines were determined in 1 ml of the sample which was treated with 4 ml Brilliant Blue G-250 for 10 min, followed by colorimetry at 595 nm (23, 24).

100 ml of juice or wine at different stages of vinification were lyophilized and the weight of dry substance was determined. Dry precipitate was dissolved in acetone 1 : 5 (w/v). The acetone extractable fractions, containing proteins, were dissolved in varying amounts of 2-mercapto-

ethanol (2-ME) from 2–20% for maximum extraction.

Total proteins were precipitated also from 1 l of wine by saturating the solution with ammonium sulfate (540 g), leaving it stand overnight at 4°C, centrifuging at 9,000 rpm, and then dialyzation followed by lyophilization.

Acetone extractable and ammonium sulphate precipitable proteins were investigated in 10–20% gradients by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (25). Standards obtained from Sigma Chemical Co. of 8.1; 14.2; 20.0; 24.0; 29.0; 36.0; 45.0 and 66.0 kDa were used for the molecular weight estimation of protein subunits.

Other procedures Stability to heat/cold testing was done at 55°C and -5°C for 48 h. The PVPP-treated wines were also subjected to a forced browning test at +50°C for 72 h.

Cu and Fe were determined by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Treatment with bentonite suspension under different concentrations in water together with the addition of egg albumin and Polyclar AT depressed the amount of polyphenols up to 17–32% and the leucoanthocyanin content of the wines was diminished to 48–64% compared to initial control samples (Tables 1 and 2).

Our data show similar amounts of polyphenols and leucoanthocyanins in Sauvignon Blanc white wines made from grapes collected in Shaalabim, Gshor and Hulda. Therefore original reports of Shaalabim and Hulda have been omitted in this report. Differences in the phenolic make-up of the 2 cultivars (Tables 1 and 2) were small.

The phenol and tannin contents were identical with ones

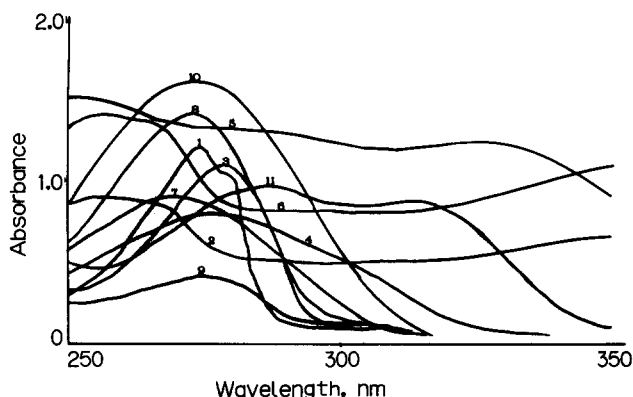


FIG. 1. UV spectra of some phenolics. 1: Resorcinol; 2: catechol; 3: catechin; 4: rutin; 5: quercetin; 6: fisetin; 7: cinnamic acid; 8: caffein; 9: 4-hydroxyphenyl acetic acid; 10: tannic acid; 11: caffeic acid.

reported in the literature (26). However, some authors have found very high amounts of leucoanthocyanin content in white wines (11, 27).

Mean values of phenolic substances were lower in this season than in the 1978 vintage (6, 15). French Colombard investigated in this study was treated with bentonite (Table 2) during must fermentation. The amount of total polyphenols, as well as proteins decreased more abruptly (Table 2) than in samples not treated with adsorbents during must fermentation (Table 1). Similar results have been reported by Amati (3) and Yokotsuka *et al.* (8).

Total polyphenols and leucoanthocyanins were measured in all wine samples treated with Polyclar AT and after aging during four months. The amounts of polyphenols and leucoanthocyanins were similar for Control 2 and Tests 7 and 8 of wine samples, prepared from grapes collected in four different locations (Tables 1 and 2). Brown color test was negative in all samples treated with Polyclar. These data are similar to previous reports (3, 28). There is no literature data available on the phenolic content of Israeli Sauvignon Blanc and French Colombard wines to which a comparison can be made.

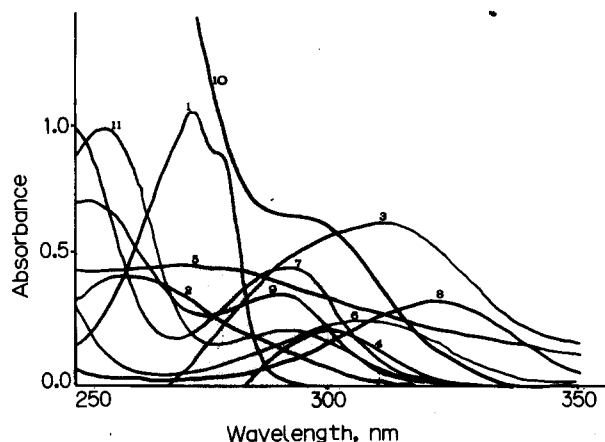


FIG. 2. UV spectra of benzoic acid derivatives and nonflavonoids. 1: Resorcinol; 2: gallic acid; 3: vanillin; 4: salicylic acid; 5: *p*-hydroxybenzoic acid; 6: 2,3-dihydroxybenzoic acid; 7: 2,4-dihydroxybenzoic acid; 8: 2,5-dihydroxybenzoic acid; 9: 3,4-dihydroxybenzoic acid; 10: 2,3,4-trihydroxybenzoic acid; 11: 2,4,6-trihydroxybenzoic acid.

TABLE 3. Absorption maxima of some phenols

Phenolic compounds	Absorption peaks, nm					
	1	2	3	4	5	6
Flavonoids						
Catechin	328	279				
Rutin	299	295	256			
Quercetin	326	282	274	253		
Fisetin	301	256				
Nonflavonoids						
Cinnamic acid	340	333	328	270		
Caffeic acid	312	287	285			
Benzoic acid derivatives						
Gallic acid	344	340	333	261		
Salicylic acid	299	295	256			
<i>p</i> -Hydroxybenzoic acid	271					
2,3-Dihydroxybenzoic acid	306					
2,4-Dihydroxybenzoic acid	342	292				
2,5-Dihydroxybenzoic acid	321					
3,4-Dihydroxybenzoic acid	341	290	253			
2,4,6-Trihydroxybenzoic acid	341	293	255			
2,3,4-Trihydroxybenzoic acid	347	295	260			
Others						
Caffeine	340	333	328	324	314	272
Catechol	346	335	328	273		
4-Hydroxyphenylacetic acid	343	325	317	306	275	
Resorcinol	335	328	314	309	302	273
Tannic acid	348	340	338	277		
Vanillin	311					

The UV and IR spectra of several white Israeli wines were used to determine their phenolic content, as a result of PVPP treatment with and without other adsorbents (Tables 1 and 2). Figures 1 and 2 show the UV spectra for all phenols investigated. The characteristic absorption data of Figs. 1 and 2 are shown in Table 3. As can be seen from Table 3, the absorption maxima for all standards were between 350 and 250 nm.

Figure 3 presents two wine samples in comparison with catechin, caffeic, gallic and tannic acids, the most representative acids in the phenolic composition of wines.

The wine spectrum (Fig. 3) was similar to one reported by Tryon *et al.* (1988), where the percentages of phenols adsorbed by PVPP were the following: quercetin (69–73); rutin (14); catechin (36); caffeic acid (70–78); gallic acid (57) and salicylic acid (45). The absorbance of wine fining

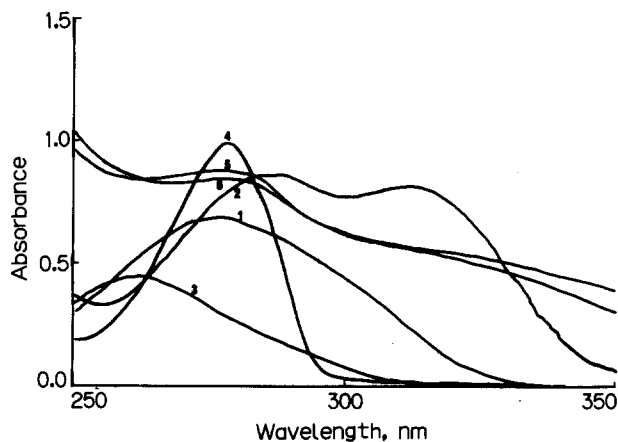


FIG. 3. UV spectra of 1, Tannic acid; 2, caffeic acid; 3, gallic acid; 4, catechin; 5, wine treated with bentonite and egg albumin (corresponding to Test 6, Table 1); 6, wine treated with bentonite, egg albumin and Polyclar (corresponding to Test 8, Table 1).

with PVPP was lower than in untreated samples, indicating the removal of phenols had occurred (Fig. 3).

Table 4 illustrates that wine samples revealed absorbance peaks in the range of all 21 phenolic standards (Figs. 1 and 2, Table 3). The number of peaks in wine samples was smaller than in pure standards, suggesting that the extracted compounds were a mixture of phenols.

The phenolic O-H stretch absorbs at $3705\text{--}3125\text{ cm}^{-1}$ for phenols, catechols and resorcinols. Aromatic carboxylic acids as cinnamic, gallic and others O-H stretch absorbs at $3,335\text{--}2,500\text{ cm}^{-1}$.

IR spectra recorded the following peaks (in cm^{-1}): for catechin (2643; 2746, 2849; 2937; 3106); caffeic acid (2541; 3210); gallic acid (2875; 3132; 3544) and tannic acid (3232; 3441).

In wine samples shown in Fig. 4 peaks were recorded nearly in the same range (cm^{-1}): in samples of wine treated with bentonite and egg albumin (corresponding to Test 6, Table 1): 2515; 3081; 3389; 3492; 3544 and for samples of wine treated with bentonite, egg albumin and polyclar (corresponding to Test 8, Table 1): 2566; 2721; 2926; 3184; 3284; 3441; 3698. Therefore the FTIR spectra of standards and wine samples were totally consistent with one another in the O-H-stretch region.

Three tested fining agents decreased the amount of polyphenols and leucoanthocyanins in the order Polyclar AT > Bentonite > Egg albumin. Leucoanthocyanin content is an indicator of wine stability. A wine sample from Shaalabim (Test 8) was the most stable and its amount of leucoanthocyanin was the lowest. Most likely proteins produce turbidity only with tannin fractions. This has been shown in a model system (8).

All wine samples had $<400\text{ mg/l}$ polyphenols and showed resistance to oxidation, findings which correspond to those of Vacca *et al.* (13). The decreases in the amounts

TABLE 4. Absorption maxima in white wine (Sauvignon Blanc) from grapes collected at Gshor

Investigated samples	Treatment	Absorption peaks, nm		
		1	2	3
Fresh juice (L) ^a	—	282		
Fresh juice (L+D) ^b	—	278		
Ext. phenols from fresh juice ^c (L)	—	281	279	277
Must (L+D)	after fermentation	278	263	
Wine (L+D)	before fining	277	266	
Ext. phenols from wine ^a (L)	before fining	272		
Ext. phenols from wine ^c (L+D)	before fining	275	273	271
Wine (L)	B (1 : 7) ^g	282	279	
Wine (L+D)	B (1 : 7)+E ^h	277	263	
Wine (original) ^f	B (1 : 7)+E+P ^h	266		
Wine (L)	B (1 : 10) ^g	281		
Wine (original)	B (1 : 10)+E	268		
Wine (L+D)	B (1 : 10)+E+D	281	276	

^a Lyophilized.

^b Dialyzed.

^c Phenolic compounds extracted with methanol from lyophilized fresh juice.

^d Phenolic compounds extracted with methanol from lyophilized wine.

^e Phenolic compounds extracted with methanol from lyophilized and dialyzed wine.

^f Fresh original wine.

^g Fining with bentonite (1 : 7), g/l.

^h Fining with egg albumin, mg/l; fining with Polyclar AT, mg/l.

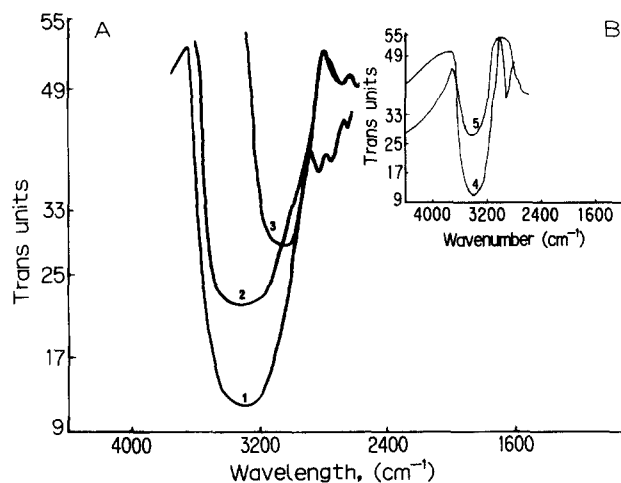


FIG. 4. FTIR spectra of A: 1, tannic acid; 2, catechin; 3, gallic acid; B: 4, wine treated with bentonite and egg albumin (corresponding to Test 6, Table 1); 5, wine treated with bentonite, egg albumin and Polyclar (corresponding to Test 8, Table 1).

(mg/l) of Fe and Cu were around 5.6–3.0 and 0.15–0.04, respectively (Tables 1 and 2). These values were lower than for vintage 1978 (6, 15).

The amount of protein decreased in the range of 62 to 72% compared to initial amounts. According to the results of isoelectric focusing of proteins in musts of Sauvignon Blanc and French Colombard, only two main bands were found at pH 4.0. The wines fermented from the same musts showed 2 protein bands at about pH 3.8. The differences in the compositions of proteins in wines and musts were very small. The total number of isoelectric focusing bands was about 8 between pH 3.8 and 7.0. These results are similar to those of other authors (29, 30).

SDS-PAGE demonstrated that the amounts of must and wine proteins were about 8; 10; 15; 22; 29 kDa, with minor bands of 45 and 66 kDa. It was observed that fermentation as well as the fining process reduced the total amount and number of fractions. Using chromatography on Superose

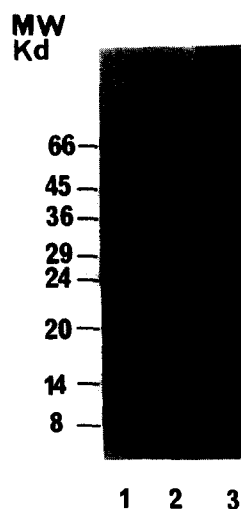


FIG. 5. SDS-PAGE of wine proteins in 10–20% gradient PAAG. 1, Standard (10 μl); 2, wine (20 μl) treated with bentonite, egg albumin and Polyclar (corresponding to Test 8, Table 1); 3, wine (20 μl) treated with bentonite, egg albumin and Polyclar (corresponding to Test 8, wine from grapes collected in Hulda).

12 with different solvent systems, 9 protein fractions were found between 190 and 1 kDa (31). However, Mesrob *et al.* (32) reported that wine proteins were separated into 4 fractions, 28; 128; 144; and 160.

Wine proteins extracted by ammonium sulfate and separated by SDS-PAGE showed diffused and unseparated bands in the range between 8 and 20 kDa, while acetone extractable proteins demonstrated sharp bands in the same region. Acetone extraction was investigated as a method of avoiding possible interference from polyphenolic compounds which have been encountered in the fractionation of plant proteins. Two wine samples (Fig. 5) from grapes collected in Gshor (line 2) and Hulda (line 3) were similar for bands of 8, 20 and 29 kDa. Test 8 (wine from grapes collected in Hulda) showed some additional bands between 8 and 14 kDa and very weak ones at 45 and 66 kDa. Slight differences were found between 24 and 29 kDa. This sample showed more protein content between 24 and 29 kDa than in the Gshor sample (line 2, Fig. 5). These data correspond exactly to the amount of protein (test 8 in Table 1 and in wine from grapes collected in Hulda), as well as to the stability of wine samples. Some separated samples are not shown in this paper because the bands were similar to ones which have been presented. The order of adsorbents used in this study for protein treatment is the following; Polyclar < Bentonite < Egg Albumin.

These studies make it possible to establish optimizing profiles of Israeli white wines with regards to phenolic substances and proteins which can then be used as indicators of wine quality.

Protein haze seems to be caused by the removal of low molecular weight proteins by bentonite treatment. These results are comparable with those of different authors for various types of wine which found that the values obtained are within a reasonable range (8, 17, 18, 29, 33).

Differences in the three Sauvignon Blanc grape samples were small and could generally be attributed to climatic viticultural conditions (different times and areas of grapes collection) and some slight differences during fining. Composition was similar within an individual grape variety (different areas of collection) and differed between varieties (Sauvignon Blanc and French Colombard). During the ten years between vintages 1978 and 1988 there has been an improvement in irrigation, as well as variations in climate. However, these factors had very little influence on the quality of the final product. Treatment with different adsorbents influenced the quality and stability of white wines. UV and IR spectroscopy can be applied to grapes and wines with satisfactory results. Fining with different adsorbents was effective at decreasing the amount of phenolics.

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