

Determination of Basic Components in White Wines by HPLC, FT-IR Spectroscopy, and Electrophoretic Techniques

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Received December 13, 1991, and in revised form May 18, 1992

Analysis of the carbohydrates of a selection of 11 different Israeli white wines of the Sauvignon Blanc type revealed five sugars: fructose, glucose, sucrose, maltose, and maltotriose. Fructose (0.2–2.0 g/liter) and glucose (0.4–0.8 g/liter) were the major components, followed by sucrose, maltose, and maltotriose. Glycerol (6.3–8.3 g/liter) was found in all samples. The presence of aromatic C=C bands in the infrared (1600 cm⁻¹) suggests the presence of polyphenols. The intense bands in the infrared O—H and C=O regions were attributed mainly to the presence of acetic acid. The major protein fractions were similar in all wines. Some minor differences in peptides were noted in the low molecular weight range. © 1992 Academic Press, Inc.

INTRODUCTION

Many compounds affect the appearance, taste, flavor, and nutritional quality of wines. The presence of carbohydrate contents including glycerol in a particular sample can be useful indicators of aroma, taste, sweetness, and quality (Ciolfi *et al.*, 1983; Datunashvili *et al.*, 1987; Noble and Bursick, 1984).

The ratios of different carbohydrates, glycerol, and acetic acid are attributed to the fermentation process (Ciolfi *et al.*, 1983; Datunashvili *et al.*, 1987), and the fining process of wines (Alexandre Benavent, 1987; Ogorodnik *et al.*, 1984). Polyphenol contents play an important role in the organoleptic characterization of wines, their susceptibility to oxidation, and their stability during storage (Amati, 1986; Khristyuk *et al.*, 1981; Yokotsuka *et al.*, 1983). White wines contain high molecular weight proteins and neutral polysaccharides, both of which can interact with polyphenols to form a colloidal turbidity during storage (Avakyants and Vladimirova, 1983; Flores *et al.*, 1990; Hsu and Heatherbell, 1987; Manrikyan and Ezhov, 1985; Yokotsuka *et al.*, 1983). In our previous studies some of the components of wines were investigated (Gorinstein *et al.*, 1980, 1984a,b), but an in depth study of basic components of Israeli white wines was not done.

The main objective of the present study is to determine the amounts of carbohydrates, glycerol, polyphenols, and proteins in white wines, to compare their content in the same grape variety during different vintages, and to determine the optimal profiles for protein stability of selected white wines.

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MATERIALS AND METHODS

Sample Preparation

Wines used in this study were produced by different Israeli wine companies from white grapes of the Sauvignon Blanc variety. Investigation was conducted during 1989–1991. Eighty-eight samples of eleven typical white wines were investigated. (A) Carmel Wine Company: Rishon Le Zion-Premier Cru (P. Cru, 1985), Premier Cru (P. Cru, 1986), Carmel (C., 1986), Selected (S., 1986), Selected (S., 1987), and Baron Edmond Rothschild (B.E.R., 1987). (B) Galilee Wine Company: Yarden (Yar., 1986) and Gamla (Gamla, 1987). (C) Eliaz, Beniyamina: Chateau Golan (Ch. Golan, 1986). (D) Stock, Ltd.: Nathanya-Monfort (Monf., 1982) and Monfort (Monf., 1986). Years of samples collection are shown in parenthesis. All wineries used the standard technology of wine making. For wine making of Premier Cru, Baron Edmond Rothschild, Yarden, and Gamla first running juice was used. Selected, Ch. Golan, Carmel, and Monfort were prepared by mixing of first and second pressure running juices. Differences in microclimates of Israel influence the grapes. For wines Baron Edmond Rothschild, Yarden, and Gamla, grapes growing in Galilee (600 m elevation) were used. In wines produced by the same company, the same variety of grapes was used but from different locations. All grape musts were fermented with *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. Samples of wines were treated at the wineries with bentonite in water (1:10), $K_4[Fe(CH)_6]$, heating and cooling.

The percentage of alcohol by volume was 11.5. For carbohydrate determination by high-pressure liquid chromatography (HPLC) the original wine samples were used. Lyophilized wine samples were prepared for Fourier Transform–Infrared Spectroscopy. Ethanol was removed from the wine sample with a rotary evaporator and the sample was freeze-dried. Stability to heat/cold testing was done at 55 and $-5^{\circ}C$ for 48 h.

HPLC Analysis

A high-pressure liquid chromatography apparatus (LKB 2150 HPLC pump connected to an LKB 2152 HPLC controller, LKB Producter AB, Bromma, Sweden) was equipped with a refraction index (RI) detector (EPC-7510, ERMA Inc. Instrument, Tokyo, Japan). Separation of glycerol and saccharides was carried out on a 4.5-mm \times 25-cm CN column at room temperature ($20^{\circ}C$). Glycerol as well as carbohydrates were eluted with an isocratic solvent system of 75% acetonitrile in water, at a flow rate of 2 ml/min (sample 20 μ l) (Duarte-Coelho *et al.*, 1985; Israelian *et al.*, 1978). Standards of glycerol, fructose, glucose, sucrose, maltose, and maltotriose were purchased from Sigma Chemical Co.

IR Analysis

The lyophilized wine samples were measured by Fourier Transform–Infrared Spectroscopy (FT–IR) as a film between two KBr plates on a FT–IR spectrometer (Analect Instruments fx-6160). The recording was done from 4000 to 400 cm^{-1} wave number. Standards for total polyphenol content such as resorcinol and tannic acid catechin (flavonoid), hydroxycinnamic acid (nonflavonoid), and gallic acid (benzoic acid derivative) were purchased from Sigma Chemical Co.

Protein Preparation

Preparation of high molecular weight fractions of wine samples was done according to Dale and Young (1988). Samples of wines were placed in dialysis tubing (Medicell International Ltd; size 12-1½ in.) and dialyzed against several portions of distilled water for 72 h. The material retained by the dialysis membrane was freeze-dried. In addition 100 ml of the wine sample was placed into a 1-liter round-bottom flask followed by 500 ml acetone. The mixture was shaken, then allowed to stand. The precipitate which formed was washed twice with fresh acetone, then freeze-dried. Proteins were precipitated by ammonium sulphate and then dialyzed and lyophilized. The protein content in each of the precipitates was determined by the method of Bradford (1976). Serva blue G from Serva (Terochem. Laboratories Ltd.) was used instead of Coomassie brilliant blue G-250. Freeze-drying was done first by freezing the sample in Cryobath (CB-60, NesLab, Bioinstrument) at -10°C in liquid alcohol. Then, freeze-drying was continued at room temperature, at 100 milliTorr for 24 h, using freeze mobile 12 (Virtis Co., Inc., Gardiner, No. 4).

Polyacrylamide Gel Isoelectric Focusing (PAGIF)

PAGIF was done using Pharmacia Phase System with ready gel PhastGel IEF (isoelectric focusing) media 3-9, containing Pharmalyte carrier ampholytes, pH range 3 to 9. All stages of PAGIF were performed according to the Owners Manual (1987). *pI* calibration kit broad *pI* range (pH 3-10) was provided by Pharmacia. Protein concentration of the sample was about 200 $\mu\text{g}/\text{ml}$. One microliter of the sample volume per lane was applied to the gel.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Preliminary separation of proteins was done using the Pharmacia Phast System with ready PhastGel gradient 10-15% and PhastGel buffer strips. All procedures were carried out according to the Owners Manual (1987). Protein concentration was 2.2 mg/ml. Ten microliters of sample was dissolved in 10 μl of sample buffer and the applicator picked up 0.5 μl per lane. All wine protein samples (dialyzed, lyophilized, acetone and ammonium sulphate precipitable) were applied to SDS-PAGE for molecular weight (MW) determination using slab gel according to Laemmli (1970).

The equipment used included: SE 200 mighty small I slab unit, Hoefer Scientific Instruments; voltage current regulated power supply, Brinkmann Instruments; gradient former, Buchler Instruments; peristaltic pump, Buchler Instruments. The gel dimensions were 83 mm \times 73 mm \times 1.5 mm. Chemicals were purchased from Bio-Rad Laboratories. M_r values for protein subunits were obtained from a standard graph prepared for the following standard subunits (Sigma Chemical Co.) analyzed by SDS-PAGE (subunit M_r in parentheses): 1. (MW-SDS-70 L kit): albumin bovine (60,000); albumin egg (45,000); glyceraldehyde-3-phosphate dehydrogenase (36,000); carbonic anhydrase (29,000); trypsinogen bovine pancreas (24,000); trypsin inhibitor soybean (20,000) and α -lactalbumin bovine milk (14,000); 2. (MW-SDS-200 kit): myosin rabbit muscle (205,000); β -galactosidase prestained from *Escherichia coli* (116,000); phosphorylase B rabbit muscle (97,400); albumin bovine plasma (66,000); albumin egg (45,000) and carbonic anhydrase (29,000). The separation gel contained 10-20% gradient. The running Tris-glycine buffer was at pH 6.8. The running time was 130 min,

using 18 mA per gel. The diluted concentration of lyophilized sample buffer was about 432 $\mu\text{g/ml}$ and 20 μl were applied per lane.

Molecular weight determinations of lyophilized wine protein samples were performed by gel filtration. A column of Superose 12 Pharmacia HP 10/30 (flow rate 0.4 ml/min, chart speed 0.5 cm/ml) equilibrated with 0.1 M Tris-HCl buffer, pH 7.4, containing 1 mM EDTA was calibrated with standard proteins (M_r in parenthesis) β -amylase (200,000); alcohol dehydrogenase (150,000); albumin bovine serum (66,000); carbonic anhydrase (29,000); and cytochrome C (12,400) to obtain a standard graph of V_e/V_o against $\log M_r$. Lyophilized wine samples were subsequently chromatographed on the column and their molecular weights calculated from the corresponding V_e/V_o values. For protein separation two systems of buffers were used: (1) 0.1 M Tris-HCl pH 7.4, containing 0.25 M NaCl, and (2) 25% formic acid (Croy *et al.*, 1984).

Mean values of three determinations were reported in this study. Analysis of variance was done on the original data. In this study all statistical data were determined by Duncan's (1955) multiple range test.

RESULTS

A typical chromatogram is shown in Fig. 1. Each compound was identified by its retention time and/or spiking with standards under the same conditions. Glycerol content as well as the carbohydrate composition are shown in Table 1.

The carbohydrate contents of the wines in g/liter were: glycerol (6.3–8.3); fructose (0.2–2.0); glucose (0.4–0.8); sucrose (0.3–1.0); maltose (0.3–0.6), and maltotriose (0–0.3).

The Carmel, Galilee, Eliaz, and Stock, Ltd. wines showed the following ratios of glycerol to total saccharides: Premier Cru, 1985 (3.7:1.0); Premier Cru, 1986 (1.7:1.0);

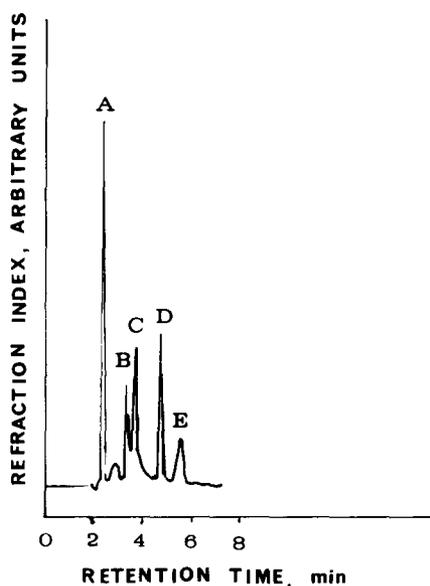


FIG. 1. High-performance liquid chromatogram of carbohydrate compounds in fermentation standard. (A–E) Peaks of glycerol, fructose, glucose, sucrose, and maltose, respectively.

TABLE I
GLYCEROL AND CARBOHYDRATES IN ISRAELI WHITE WINES***

| Type of Wine* | Glycerol | Fructose | Glucose | Sucrose | Maltose | Maltotriose |
|----------------|---------------|-----------|-----------|-----------|-----------|-------------|
| | Amount, g/L** | | | | | |
| P. Cru, 1985 | 7.9 ±0.07 | 0.2 ±0.04 | 0.7 ±0.07 | 0.6 ±0.07 | 0.6 ±0.04 | - |
| P. Cru, 1986 | 6.3 ±0.04 | 2.0 ±0.03 | 0.4 ±0.01 | 0.8 ±0.03 | 0.4 ±0.01 | - |
| C., 1986 | 6.7 ±0.08 | 1.4 ±0.04 | 0.8 ±0.04 | 0.6 ±0.01 | 0.3 ±0.02 | 0.2 ±0.03 |
| S., 1986 | 7.3 ±0.07 | 0.6 ±0.01 | 0.6 ±0.01 | 0.9 ±0.03 | 0.4 ±0.03 | 0.3 ±0.01 |
| S., 1987 | 7.0 ±0.07 | 1.3 ±0.06 | 0.5 ±0.03 | 0.5 ±0.01 | 0.4 ±0.01 | 0.3 ±0.02 |
| B.E.R., 1987 | 7.3 ±0.08 | 1.3 ±0.03 | 0.8 ±0.05 | 0.3 ±0.01 | 0.5 ±0.02 | - |
| Yar., 1986 | 7.9 ±0.09 | 0.8 ±0.02 | 0.3 ±0.01 | 0.5 ±0.03 | 0.5 ±0.03 | 0.2 ±0.01 |
| Gamla, 1987 | 7.8 ±0.07 | 1.0 ±0.04 | 0.2 ±0.01 | 0.6 ±0.06 | 0.6 ±0.04 | - |
| Ch.Golan, 1986 | 8.1 ±0.09 | 0.7 ±0.07 | 0.2 ±0.01 | 0.5 ±0.02 | 0.5 ±0.03 | - |
| Monf., 1982 | 8.3 ±0.10 | 0.2 ±0.01 | 0.6 ±0.03 | 0.6 ±0.03 | 0.3 ±0.01 | - |
| Monf., 1986 | 8.0 ±0.11 | 0.8 ±0.04 | 0.3 ±0.02 | 0.5 ±0.01 | 0.3 ±0.03 | - |

* - Investigated wine samples:

A. Carmel Wine Company, Rishon Le Zion - Premier Cru (P. Cru, 1985); Premier Cru (P. Cru, 1986); Carmel (C., 1986); Selected (S., 1986); Selected (S., 1987); and Baron Edmond Rothschild (B.E.R., 1987). B. Galilee Wine Company -Yarden (Yar., 1986) and Gamla (Gamla, 87). C. Eliaz, Beniyamina - Chateau Golan (Ch. Golan, 1986). D. Stock, Ltd., Nathanya - Monfort (Monf., 1982) and Monfort (Monf., 1986).

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

*** -Each wine group contained eight samples.

Carmel, 1986 (2.1:1.0); Selected, 1986 (3.7:1.0); Selected, 1987 (2.3:1.0); Baron Edmond Rothschild (2.6:1.0); Yarden, 1986 (3.7:1.0); Gamla, 1987 (3.3:1.0); Ch. Golan, 1986 (4.2:1.0); Monf., 1982 (5.0:1.0); and Monf., 1986 (4.1:1.0).

The IR spectra of lyophilized wine sample Premier Cru, 1986, and Gamla, 1987 are shown in Figs. 2 and 3. The aromatic C=C bands (1600 cm^{-1}) might be characteristic of the presence of simple polyphenols, such as gallic acid, catechin, and hydroxycinnamic acid (Roggero and Archier, 1989). Premier Cru, 1985 showed more polyphenols than Premier Cru, 1986 in this region. These 2 IR spectra were chosen from 88 investigated ones as the most typical, showing the difference in these two types of wines. The relative amount of polyphenols was in the following order: Premier Cru, 1985 > P. Cru, 1986 > Monf., 1982 > Monf., 1986 > Carmel, 1986 > Selected, 1987 > Ch. Golan, 1986 > Yar, 1986 > Baron Edmond Rothschild, 1987 > Gamla, 1987.

Proteins in different wine samples were characterized in terms of isoelectric points. pI values of the proteins were between 3.75 and 5.85. The lyophilized wine samples contained all or most of the major protein subunits of 29,000 and 14,000. The minor proteins showed subunits in the region of 60,000, 45,000, 36,000, 18,000, and lower than 10,000 (Fig. 4).

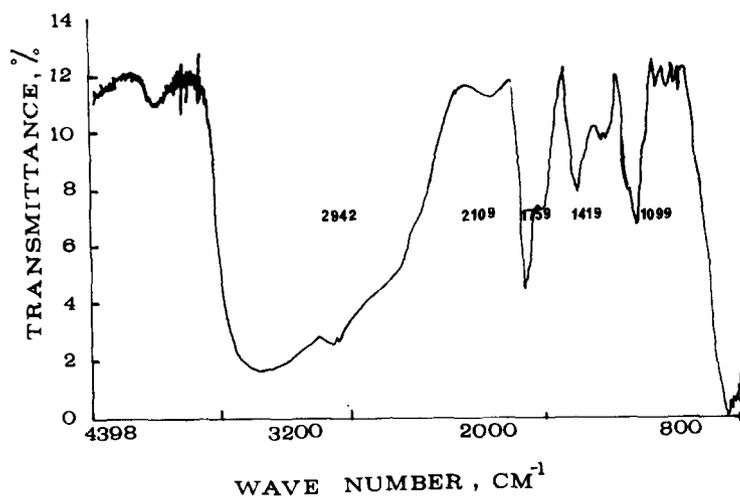


FIG. 2. IR-spectrum of lyophilized wine sample Premier Cru, 1986.

Chromatography of the lyophilized wine samples on Superose 12 using 0.1 M Tris-HCl, pH 7.4, containing 0.25 M NaCl and comparison of V_e/V_o values with those for standard proteins gave values of $190,000 \pm 10\%$, $160,000 \pm 10\%$, $12,400 \pm 10\%$, $10,000 \pm 10\%$, $2,000 \pm 10\%$, and lower. Chromatography of the same lyophilized wine samples on Superose 12, using 25% formic acid, gave better separation for the lower molecular weights (36,000, 11,000, 10,000, 2,000, and 1,000) (Fig. 5).

DISCUSSION

Glycerol, which is normally found in wine, contributes to sweetness and fullness of taste in the amount of 1–11 g/liter (Curvelo Garcia *et al.*, 1990; Lozano Chaves

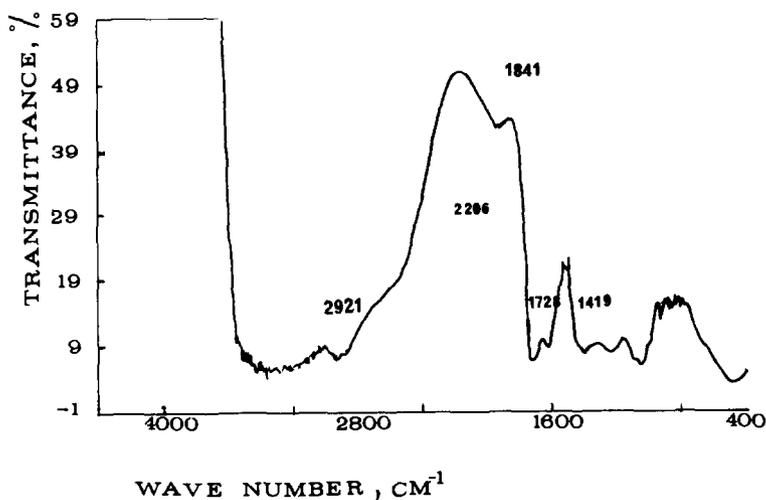


FIG. 3. IR-spectrum of lyophilized wine sample Gamla, 1987.

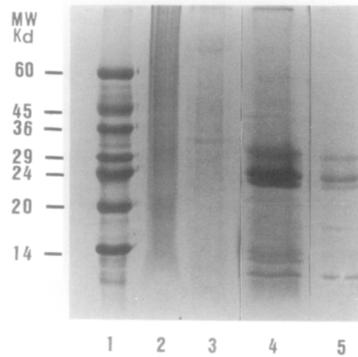


FIG. 4. Electrophoretic patterns of wine samples. 1, Standard (10 μ l); 2, 3, 4, 5, respectively, 20 μ l of lyophilized samples from Sauvignon Blanc grapes of fresh juice, final product, fermented must, and fermented must after acetone precipitation.

and Munoz Leyva, 1988; Noble and Bursick, 1984). The amount of glycerol could be used to detect the degree of enzymatic hydrolysis of fatty compounds and to predict the taste and viscosity (Gorinstein *et al.*, 1980). The amount of glycerol as well as the ratios of glycerol to total saccharides were similar among various wine samples obtained during vintages with the highest one for Monf., 1982. The glucose: fructose relation in the wine samples appeared in the following order: Premier Cru, 1985 (2.9:1.0); Premier Cru, 1986 (1:5); Carmel, 1986 (1.0:1.7); Selected, 1986 (1:1); Selected, 1987 (1.0:2.5); Baron Edmond Rothschild (1.0:1.7); Yarden, 1986 (1.0:2.5); Gamla, 1987 (1:5); Ch. Golan, 1986 (1.0:3.9); Monf., 1982 (3:1); and Monf., 1986 (1.0:2.5). Samples of Carmel, 1986 and Baron Edmond Rothschild, 1987 were equal in the ratios of glucose:fructose, but the same types of wines, Premier Cru and Selected ones, differed during two vintages.

The glucose:fructose relationship depends on the fermentation process and yeast strains of various species. Concurrently with ripening of grapes the ratio of glucose to fructose decreases from values >1 to values <1 . During the fermentation of grape musts glucose concentrations decrease faster than those of fructose (Lemperle *et al.*, 1988).

Saccharomyces species (*cerevisiae* and *bayanus*) fermented fructose about half as fast as they did glucose in Selected, 1986, Baron Edmond Rothschild, 1987, Yar., 1986, and Monf., 1986. In Premier Cru, 1986 and Gamla, 1987 only one-fifth of the fructose was fermented, while in Premier Cru, 1985 and Monf., 1982 nearly all fructose was fermented. Only one sample, Selected, 1986, had a ratio of about 1:1. These data correspond well with other communications (Ciolfi *et al.*, 1983). The glucose-fructose ratio in wines is employed in the evaluation of sample quality and gives an indication of the origin of the residual sugar (Bailer, 1990).

The largest band at O—H and C=O regions was attributed mainly to the presence of acetic acid. For example, Selected, 1986 had a larger band for acetic acid than Selected, 1987. According to Kholiddinov and Gribov (1985), phenols, esters, acetals, and ketals showed absorption maxima at 900–750 cm^{-1} . All wine samples showed absorption peaks in this region. The highest wine stability in turbidity was in samples Selected, 1986, Selected, 1987, Baron Edmond Rothschild, 1987, Yar., 1986, and Gamla, 1987. The observed differences between wines and years can be explained by

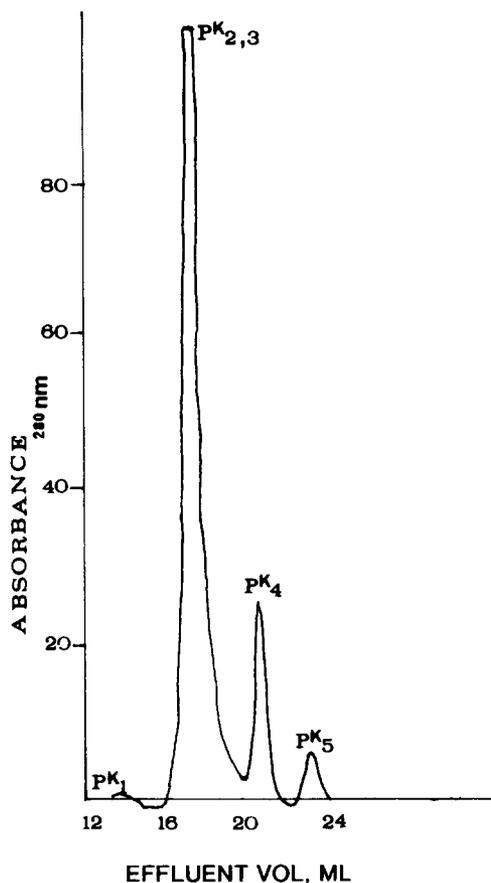


FIG. 5. UV-absorbance (280 nm) elution profiles of lyophilized wine sample from Superose 12.

oxidation during aging. Separation on Superose 12 proved that wine samples have full spectrum from lower to high molecular weight proteins, and this is in agreement with others (Flores *et al.*, 1990; Goerg *et al.*, 1981). According to Mesrob *et al.* (1983) only four fractions were separated in wines with molecular weights of 28,000, 128,000, 144,000, and 160,000. Differences in low molecular weight proteins in some wine samples was a reflection of treatment. Heating, cooling, bentonite, and $K_4[Fe(CN)_6]$, which were used during wine technology at the wineries, caused qualitative and quantitative modifications of protein fractions. Protein haze seems to be caused by the low molecular weight proteins which are removed by bentonite treatment (Flores *et al.*, 1990; Hsu *et al.*, 1987; Mesrob *et al.*, 1983). Therefore the difference in samples produced by different companies reflect this process. These results confirm those of different authors for various types of wine fining, since the values obtained are within a reasonable range (Goerg *et al.*, 1981; Mesrob *et al.*, 1983; Molnar, 1975).

The results obtained from this study show a relationship between the total ratios of saccharides and glycerol, glucose and fructose, and the fermentation process. Acetic acid, glycerol, and polyphenols are the factors which affect the taste and stability of white wines. These indices have to be used together for a complete wine spectrum. Premier Cru, 1985 and Yarden, 1986 had equal amounts of glycerol (7.86–7.88), but

the ratios of glucose/fructose differed, at 2.9:1 and 1:2.5, respectively; Selected, 1986 and Baron Edmond Rothschild, 1987 had the same amount of glycerol (7.25) and different ratios of glucose/fructose as 1:1 and 1:1.7; Chateau Golan, 1986 and Monfort, 1986 had the same amount of glycerol (8.03–8.06) and the difference was in glucose/fructose ratios of 1:3.9 and 1:2.5.

The same samples have to be characterized also in protein composition, stability, and polyphenols. Techniques used in this study indicate the possibility of protein separation in wine samples without pretreatment and isolation. The combination of gel filtration and electrophoresis was efficient in revealing detectable amounts of low molecular weight proteins, which are the cause of instability of wines. All the above parameters, which characterize taste and protein stability, were present in all of the vintages, but in different portions in each individual wine.

HPLC, FT-IR, and electrophoresis can be used together as a rapid and quantitative method for the determination of the main constituents in wine solids.

ACKNOWLEDGMENT

The authors are thankful to Miss S. Dubetz, University of Alberta, for her excellent technical assistance.

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