JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Effects of Cultivar and Processing Method on the Contents of Catechins and Procyanidins in Grape Juice

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The aim of the presented work was to study the effect of pressing method, pasteurization, cultivar, and vintage on the content of (+)-catechin, (−)-epicatechin, and nine procyanidins in grape juice. The results showed that the concentration of these flavan-3-ols in the juice was influenced, in decreasing order of importance, by pressing method, cultivar, pasteurization, and vintage. Cold pressing without maceration was the least and hot pressing after maceration at 60 °C for 60 min the most effective method for extracting the flavan-3-ols. Pasteurization increased the concentration of catechins in cold-pressed juices, but it decreased concentrations in hot-pressed juices. The concentration of most procyanidins was increased by pasteurization. Among the white cultivars, Seyval and Niagara were highest in procyanidins and Elvira and Chardonnay were highest in catechins. Vincent, Foch, and Baco were the red cultivars highest in catechins, and Vincent also had the highest content of procyanidins.

**KEYWORDS: Flavan-3-ols; catechins; procyanidins; nutraceutical; grape juice; cultivar; extraction**

## INTRODUCTION

Grapes and their products are rich sources for flavan-3-ols. In addition to the monomers (+)-catechin, (−)-epicatechin, and (−)-epicatechin 3-*O*-gallate, 14 dimeric, 11 trimeric, and 1 tetrameric procyanidin have been identified in grape seeds. Nine of these procyanidins are esterified with one or two gallic acid molecules attached to an (−)-epicatechin unit (*1*). There are larger, still unidentified oligomeric and polymeric procyanidins in grapes that constitute a greater proportion of the total flavan-3-ols (*2*−*6*). Recently, 18 additional galloylated and nongalloylated procyanidin oligomers, up to octamers, were identified in grape seed extract (*7*). Only procyanidin-type proanthocyanidins were detected in the seeds, whereas the skin of the grapes contained prodelphinidin as well as procyanidin (*8*).

Flavan-3-ols influence the sensory characteristics of grape products and play important roles in the technology of juice- and wine-making. They affect bitterness and astringency (*9*−*11*), haze formation and interactions with proteins (*10*, *12*−*14*), and color and its stability (*10*, *13*, *15*−*17*). Furthermore, flavan-3-ols are nutraceuticals playing important roles in the prevention of disease, particularly those of the cardiovascular system (*1*, *18*−*22*). There are numerous publications on the flavan-3-ols content of wine (*2*, *3*, *5*, *16*, *23*−*30*), but very little information is available on their concentration in grape juice and on the effects of cultivar and juice processing methods on these concentrations.

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An early publication on the presence of procyanidins in grape juice reported the separation of (+)-catechin, (−)-epicatechin, and four unidentified procyanidins from purple grape juice (*31*). Jaworski and Lee (*32*) identified (in order of their quantity) (−)-epicatechin, (+)-catechin, and procyanidins B3 [(+)-catechin-(4α→8)-(+)-catechin] and B2 [(−)-epicatechin-(4β→8)-(−)-epicatechin] in juice prepared in their laboratory from cv. Niagara grapes. Using the same methods these authors determined, in addition to the above flavan-3-ols, procyanidin B1 [(−)-epicatechin-(4β→8)-(+)-catechin] in 21 white grape cultivars grown in New York state (*33*). Large differences in the concentration of individual flavan-3-ols among cultivars were observed. In most cultivars (−)-epicatechin > (+)-catechin and procyanidin B3 were the most abundant. Spanos and Wrolstad (*34*) produced cold-pressed juice (CPJ) and concentrate from the white *vinifera* cv. Thompson Seedless to study the influence of processing and storage variables on phenolic composition. The juices and concentrates from various stages of processing were analyzed for flavan-3-ols. The juices contained (in order of their quantity) (+)-catechin, (−)-epicatechin, procyanidins B1, B2, B3, and B4 [(+)-catechin-(4α→8)-(−)-epicatechin], a trimer, and a tetramer as well as eight unidentified procyanidins. The effects of processing on the (+)-catechin, (−)-epicatechin, and procyanidin B3 contents of Cabernet Sauvignon, Chambourcin, and Noble (*Vitis rotundifolia* Mich.) juices were studied by Auw and co-workers (*35*). They found in the first two cultivars (+)-catechin > procyanidin B3 > (−)-epicatechin, whereas cv. Noble exhibited a different pattern [(−)-epicatechin > (+)-catechin > B3]. Hot-pressed juice (HPJ) had a significantly higher flavan-3-ol content than the CPJ. Frankel and his

10.1021/jf020689m CCC: $25.00 
Published on Web 12/24/2002

group (*22*) analyzed two of each white and purple grape juices from a major producer for their phenolic contents including total flavan-3-ols and their antioxidant activity. They found the purple Concord juice had antioxidant activity comparable to that of red wines, whereas the white juices were less active. Lazarus and co-workers analyzed two brands of purple grape juice, one from a major producer and the other a local retailer's private label (S. A. Lazarus, personal communication). They detected the presence of catechins and procyanidins up to tetramers in the first one. The primary components were (+)-catechin, (−)-epicatechin, and dimeric procyanidins. There were no detectable quantities of flavan-3-ols in the local brand (*7*).

Juice is an important grape product. In North America purple commercial grape juice is primarily made from cv. Concord grapes. Cv. Niagara, another *labrusca*-type cultivar, is responsible for the typical flavor of commercial white grape juice. Both of these cultivars are extensively grown for juice production in the Niagara region of the province of Ontario, Canada. This study was undertaken to extend the database on the (+)-catechin, (−)-epicatechin, and individual procyanidin contents of juices produced from various grape cultivars and to study the effects of juice processing methods on these concentrations.

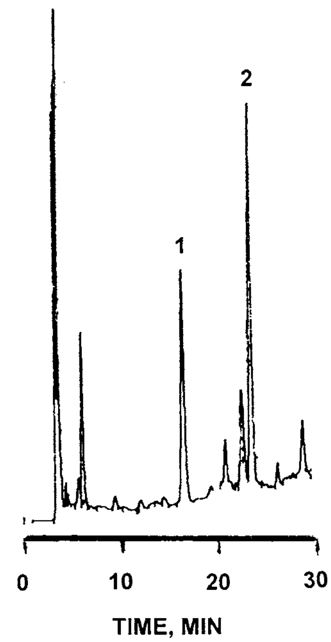


**Figure 1.** Chromatogram of the catechin fraction [acetonitrile/water (30:70) eluate from the polyamide column] of hot-pressed (30 min) pasteurized Concord grape juice monitored at 280 nm. Peak identification ($t_R$, min): 1, (+)-catechin (16.1); 2, (–)-epicatechin (23.3).

## MATERIALS AND METHODS

**Grapes.** Hand-harvested mature clusters of grapes were obtained from the Rittenhouse Grape Research Station, Beamsville, part of the Department of Plant Agriculture, University of Guelph at Vineland Station, or from the vineyards of Wiley Brothers Ltd., St. Catharines, ON, Canada, in the 1993 and 1994 growing seasons. To randomize the fruit within each cultivar, clusters were picked from every 25 kg container of harvested fruit to make up ∼45 kg batches, which were processed in duplicate. The grapes were kept in cold storage (∼4 °C) for 2−3 days prior to processing.

**Production of Juice.** To study the flavan-3-ol content of grape juices and the effects of processing variables, bottled pasteurized juices were produced from cvs. Concord, Niagara, and Elvira in 1993 and 1994. For comparison, freshly pressed juice samples were also collected from these and three wine grape cultivars (Gamay, Riesling, and Seyval) in the 1993 season. In 1994, the analyses of unpasteurized juices were expanded by four cultivars (Baco noir, Chardonnay, Maréchal Foch, and Vincent). The unpasteurized juice samples were frozen and held at −30 °C until analyzed. Each sample was coded with a random number. The juice samples, including the frozen ones that were packed in ice in insulated containers, were shipped by air to Portugal for analyses of flavan-3-ols.

*Cold-Pressed Juice.* In the cold-pressing (CP) treatment, the clusters were passed through the roller-crusher attached to the press and pressed without delay on a small rack and frame type hydraulic press (model TPZ 7, Bucher-Guyer, Niederweningen, Switzerland) at 6895 kPa for 5 min. When maceration on the skin was required prior to pressing, the grapes were destemmed and crushed in a model DPV-25 stainless steel stemmer-crusher (Mearelli Fraz., Cinquemiglia Cite di Castello, Italy). The crushed grapes were allowed 18 or 30 h of skin contact in stainless steel containers prior to pressing. The juice was sulfited (50 mg of $SO_2$/L), cold settled at 0 °C for 3 days, decanted, and cold detartrated at 0 °C for ∼8 weeks. Juice was passed through C5 and SL "Steinmat" filter pads using a PG.14 all-glass filter press (Carlson-Ford Ltd., Ashton-under-Lyne, U.K.). The filtered juice was flash pasteurized at 85 °C in a laboratory scale pasteurizer (*36*) and hot filled into 375 or 340 mL green glass bottles, which were sealed with rolled-on, pilferproof plastic-lined aluminum screwcaps. The bottles were inverted, air-cooled, and stored in the dark at 0 °C until analyzed.

*Hot-Pressed Juice.* The grapes were destemmed and crushed as described above. For hot pressing (HP), the crushed grapes were heated in a 45 L stainless steel, steam-jacketed kettle equipped with stirring paddles. When the temperature reached 60 °C, 50 mg/L DL5 pectolitic enzyme (Rhöm, Malden, MA) was added, and this temperature was maintained for 30 (standard), 60, or 90 min. The pulp was stirred continuously to prevent scorching and ensure uniform heating. The grapes were pressed and the juice was processed as described above for CPJ.

**Chemical Standards.** (+)-Catechin and (−)-epicatechin standards were purchased from Sarsyntex (Merignac, France). Procyanidin dimers B1, B2, B3, B4, B1-3-*O*-gallate [(−)-epicatechin-3-*O*-gallate-(4*β*→8)-(+)-catechin], B2-3-*O*-gallate [(−)-epicatechin-3-*O*-gallate-(4*β*→8)-(−)-epicatechin], and B2-3′-*O*-gallate [(−)-epicatechin-(4*β*→8)-(−)-epicatechin-3-*O*-gallate] and trimers C1 [(−)-epicatechin-(4*β*→8)-(−)-epicatechin-(4*β*→8)-(−)-epicatechin] and T2 [(−)-epicatechin-(4*β*→8)-(−)-epicatechin-(4*β*→8)-(+)-catechin] were isolated from grape seed as described by Ricardo da Silva et al. (*37*).

**Analytical Procedures.** The procyanidins and catechins were purified and fractionated by placing 5 mL of juice on a polyamide (TLC6, Macherey-Nagel, Düren, Germany) column and washing it with $H_2O$ adjusted to pH 7.0. The catechins were eluted with acetonitrile/water (30:70) followed by acetone/water (75:25) to desorb the procyanidins (*38*).

The individual catechins and procyanidins were separated by HPLC on a reversed-phase C18 Supersphere 100 (Merck, Darmstadt, Germany) column and detected at 280 nm (*1*). The mobile phases for the separation of monomers (catechins) were (A) acetic acid/water (2.5:97.5) and (B) acetonitrile/A (80:20). The procyanidins were chromatographed separately, and the mobile phases for them were (A) acetic acid/water (10:90) and (B) water. Linear gradients were used for the separation of both groups of compounds. Representative chromatograms of the catechin and procyanidin fractions of the 30 min hot macerated, pasteurized Concord juice are shown in **Figures 1** and **2**. The monomers were quantified as (+)-catechin, dimers and trimers as procyanidin B2, and gallates as B2-3′-*O*-gallate.

Total phenolics content was measured using Singleton and Rossi's (*39*) modification of the Folin−Ciocalteu colorimetric method. Total anthocyanin was determined by the single-pH method (*40*). A Zeiss DMR 21 spectrophotometer (C. Zeiss, Oberkochen, Germany) was used for colorimetric analyses. Total soluble solids content of juices was determined using a model A Abbé refractometer equipped with a flow-through cell (C. Zeiss). The temperature of the cell was controlled with a model 2000 Masterline constant-temperature water bath and circulator (Forma Scientific Inc., Marietta, OH). Titratable acidity and pH were determined with a model 28 pH meter (Radiometer A/S, Copenhagen, Denmark). All analyses were performed at least in duplicate.

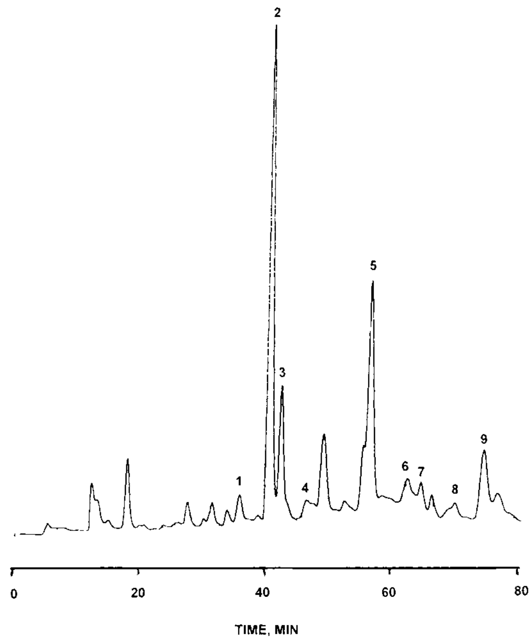


**Figure 2.** Chromatogram of the procyanidin fraction [acetone/water (75:25) eluate from the polyamide column] of hot-pressed (30 min) pasteurized Concord grape juice monitored at 280 nm. Peak identification ($t_R$, min): 1, B3 (35.7); 2, B1 (40.4); 3, T2 (42.3); 4, B4 (46.7); 5, B2 (57.0); 6, B2-3-*O*-gallate (62.9); 7, B2-3′-*O*-gallate (65.1); 8, B1-3-*O*-gallate (70.5); 9, C1 (74.9).

**Statistics.** Analyses of variance and Duncan's multiple-range test were carried out on the log transformed data using the General Linear Model procedure of the Statistical Analysis System program package (SAS Institute, Raleigh, NC).

## RESULTS AND DISCUSSION

**Effects of Pressing Method.** *Cold Pressing versus Hot Pressing.* CP without maceration was compared to HP with 30 min of hot maceration for Concord, Gamay, and Baco noir cultivars. The results presented in **Table 1** and Supplement 2 (Supporting Information) show significantly higher quantities of catechins and procyanidins in the HPJ. Because CP recovers mainly the juice from the flesh, which has very low flavan-3-ol content (*24*, *26*), CPJ contained very low concentrations of catechins and procyanidins. Hot maceration of the crushed and destemmed grapes permitted good skin extraction and presumably some extraction of the seed, which resulted in juice rich in catechins and procyanidins. Auw and co-workers (*35*) also found significantly higher concentration of total phenolics, catechins, and procyanidins B3 and B4 in HPJ as compared to CPJ produced from three red grape cultivars. Frankel et al. (*22*) compared two samples each of HP Concord juice and CP white grape juice (a blend of Niagara, Thompson Seedless, and possibly other white grape cultivars) produced by a major manufacturer. The flavan-3-ol concentrations were 10.7 and 40.3 for the two whites and 33.1 and 51.6 mg of catechin equivalents/L for the Concords. Processing generally reduces the catechin and procyanidin contents of fruit juices (*34*) and the production of commercial grape juice frequently involves the use of concentrates, so the low Concord value possibly occurred as the result of processing variables. The high value for one of the whites suggests excessive skin contact prior to pressing. This can occur unintentionally when mechanically harvested, partly crushed grapes are kept too long in large 1, 2, or 4 tonne collection/transportation bins.

**Effects of Skin Contact.** *Cold Maceration.* The catechins and procyanidins contents of CPJ pressed after 18 h of cold maceration were compared to those of CPJ produced without maceration, and results for Concord, Niagara, Elvira, and Chardonnay are presented in **Tables 1** and **2**. As expected cold maceration resulted in greater extraction of skin components and resulted in significantly higher catechin and procyanidin contents. Maceration extended for 30 h did not extract more flavan-3-ol than 18 h of treatment. It is noteworthy that 18 h of cold maceration of Concord increased the concentration of

**Table 1.** Effects of Pressing Method on the Catechin and Procyanidin Composition (Milligrams per Liter) of Pasteurized Concord Grape Juice in 1993 and 1994[a]

| | | catechins | | procyanidins[b] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pressing method | *n* | (+)-catechin | (−)-epicatechin | B1 | B2 | B3 | B4 | B1-3-*O*-g | B2-3-*O*-g | B2-3′-*O*-g | C1 | T2 | TSS, % | TA,[c] g/L | pH | Tacy,[d] mg/L | TPh,[e] mg/L |
| 1993 | | | | | | | | | | | | | | | | | |
| cold press | 2 | 0.15b | 0.21b | 0.21b | 0.68b | 0.08 | 0.22 | 0.02 | 0.11 | 0.11b | 0.02 | 0.47 | 13.00 | 0.71c | 3.14bc | tr[f]c | 23.06d |
| cold press, 18 h | 2 | 6.30a | 9.29a | 17.78a | 16.67a | 0.42 | 0.83 | 0.06 | 1.92 | 1.45a | 0.08 | 2.98 | 12.70 | 0.98b | 3.10c | 10.64b | 77.46c |
| hot press, 30 min | 2 | 4.68a | 5.97a | 18.15a | 13.12a | 0.12 | 0.08 | 0.05 | 1.25 | 0.55ab | 0.11 | 4.84 | 12.64 | 0.95b | 3.15ab | 24.86a | 148.05b |
| hot press, 60 min | 2 | 5.53a | 6.89a | 18.03a | 11.61a | 1.53 | 1.01 | 0.44 | 0.09 | 0.82a | 0.88 | 4.83 | 13.00 | 1.13a | 3.17ab | 20.01a | 145.81b |
| hot press, 90 min | 2 | 4.04a | 5.98a | 9.95a | 6.87a | 0.29 | tr | tr | 1.10 | 0.51ab | tr | 2.93 | 13.60 | 1.09a | 3.20a | 28.50a | 195.34a |
| 1994 | | | | | | | | | | | | | | | | | |
| cold press | 2 | 0.85c | 0.86c | 0.75b | 0.38 | 0.01b | 0.01 | 0.08b | tr | 0.05b | 0.04b | 0.04b | 16.24ab | 0.90b | 3.12c | nd[g] | nd |
| cold press, 18 h | 2 | 10.88ab | 13.96ab | 20.42a | 0.19 | 1.20a | tr | 3.08a | tr | 8.28a | 3.48a | 6.65a | 15.60b | 0.95b | 3.12c | nd | nd |
| hot press, 30 min | 2 | 7.29b | 5.90b | 25.35a | 11.09 | 0.67a | tr | 2.22a | tr | 14.56a | 5.20a | 6.15a | 16.89a | 1.07a | 3.19b | nd | nd |
| hot press, 60 min | 2 | 13.15a | 16.48a | 32.14a | 21.52 | 1.80a | tr | 3.04a | tr | 9.80a | 3.51a | 8.51a | 15.95ab | 1.12a | 3.23a | nd | nd |
| hot press, 90 min | 2 | 8.36ab | 9.46ab | 34.75a | 21.23 | 0.85a | tr | 2.84a | tr | 10.28a | 2.94a | 7.98a | 16.65ab | 1.11a | 3.19b | nd | nd |
| 1993 and 1994 | | | | | | | | | | | | | | | | | |
| cold press | 4 | 0.36b | 0.43b | 0.40b | 0.51b | 0.03b | 0.06 | 0.03 | 0.02 | 0.07b | 0.03 | 0.13b | 14.53 | 7.94c | 3.13b | nd | nd |
| cold press, 18 h | 4 | 7.06a | 9.68a | 17.34a | 12.65a | 0.65a | 0.15 | 0.20 | 0.26 | 1.64a | 0.23 | 3.67a | 13.60 | 9.70b | 3.10b | nd | nd |
| hot press, 30 min | 4 | 5.83a | 5.93a | 21.48a | 12.08a | 0.28ab | 0.02 | 0.34 | 0.02 | 2.79a | 0.75 | 5.46a | 14.61 | 10.07ab | 3.17a | nd | nd |
| hot press, 60 min | 4 | 8.53a | 10.67a | 24.10a | 15.81a | 1.66a | 0.07 | 1.16 | 0.10 | 2.83a | 1.75 | 6.41a | 14.40 | 11.22a | 3.20a | nd | nd |
| hot press, 90 min | 4 | 5.81a | 7.52a | 18.58a | 12.08a | 0.50ab | tr | 0.12 | 0.07 | 2.29a | 0.12 | 4.84a | 15.05 | 10.97ab | 3.20a | nd | nd |

[a] Means within each column within each year followed by the same or no letter are not significantly different using Duncan's multiple range test ($p = 0.05$). [b] B1, B2, B3, B4 = dimers; B1-3-*O*-g, B2-3-*O*-g, B2-3′-*O*-g = dimer-gallates; C1, T2 = trimers. [c] Titratable acidity (TA) expressed as tartaric acid. [d] Total antocyanins (Tacy) determined by the single pH method and expressed as cyanidin 3-galactoside. [e] Total phenolics (TPh) expressed as gallic acid. [f] tr = trace. For statistical calculations they were assumed to be 0.005 mg/L. [g] nd = not determined.

**Table 2.** Effects of Skin Contact on the Catechin and Procyanidin Composition (Milligrams per Liter) of Cold-Pressed Juice from Three White Grape Cultivars

| | | catechins | | procyanidins[a] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| treatment | *n* | (+)-catechin | (−)-epicatechin | B1 | B2 | B3 | B4 | B1-3-*O*-g | B2-3-*O*-g | B2-3′-*O*-g | C1 | T2 | TSS, % | TA,[b] g/L | pH | TPh,[c] mg/L |
| | | | | | | | | 1993 | | | | | | | | |
| Niagara | | | | | | | | | | | | | | | | |
| 0 h | 2 | 0.98 | 1.07 | na[d] | 0.35 | 0.04 | 0.16 | tr[e] | 0.07 | 0.15 | tr | 0.20 | 13.25 | 7.09 | 2.93 | 32.81 |
| 18 h | 2 | 4.13 | 4.26 | na | 7.60 | 1.68 | 1.12 | 0.43 | 0.21 | 0.60 | 0.25 | 1.41 | 13.30 | 7.74 | 2.95 | 80.13 |
| signif[f] | | ns | ns | na | ** | ** | * | ** | ns | * | *** | * | ns | ns | ns | ns |
| | | | | | | | | 1994 | | | | | | | | |
| 0 h | 2 | 1.13 | 1.38 | 0.21 | 0.07 | tr | tr | 0.09 | 0.02 | tr | 0.07 | 0.24 | 14.80 | 7.65 | 3.01 | nd[d] |
| 18 h | 2 | 6.26 | 6.65 | 9.77 | 3.68 | 0.80 | 0.36 | 0.20 | 0.08 | 0.09 | 0.37 | 0.71 | 14.70 | 7.20 | 3.06 | nd |
| signif[f] | | ** | * | ns | * | *** | *** | * | ns | ** | * | ns | ns | ns | ns | nd |
| | | | | | | | | 1993 and 1994 | | | | | | | | |
| 0 h | 4 | 1.05 | 1.21 | 0.21 | 0.12 | 0.04 | 0.02 | 0.02 | 0.03 | 0.02 | 0.02 | 0.44 | 14.00 | 7.36 | 2.97 | nd |
| 18 h | 4 | 5.09 | 5.32 | 1.22 | 5.28 | 1.16 | 0.63 | 0.29 | 0.13 | 0.23 | 0.30 | 1.00 | 13.98 | 7.47 | 3.00 | nd |
| signif[f] | | *** | *** | ns | ** | *** | * | * | ns | ns | * | * | ns | ns | ns | nd |
| | | | | | | | | 1993 | | | | | | | | |
| Elvira | | | | | | | | | | | | | | | | |
| 0 h | 2 | 0.33 | 0.52 | 1.48 | 0.14 | tr | 0.05 | tr | tr | 0.27 | tr | 0.74 | 11.85 | 8.79 | 2.98 | 22.35 |
| 18 h | 2 | 3.20 | 4.51 | 11.32 | 5.24 | 0.61 | 0.92 | 0.20 | 0.14 | 1.00 | 0.35 | 2.88 | 12.55 | 10.05 | 3.03 | 46.52 |
| signif[f] | | * | ** | ns | ** | ** | * | * | * | * | ** | * | ns | ns | ns | * |
| | | | | | | | | 1994 | | | | | | | | |
| 0 h | 2 | 0.91 | 0.97 | 0.26 | tr | tr | tr | 0.09 | tr | tr | 0.09 | 0.23 | 16.40 | 12.54 | 3.08 | nd |
| 18 h | 2 | 6.21 | 7.19 | 3.74 | 1.81 | 0.12 | 0.19 | 0.16 | tr | 0.03 | 0.06 | 0.71 | 15.15 | 13.25 | 3.10 | nd |
| signif[f] | | * | ** | ns | ** | ns | ** | ns | ns | ns | ns | * | ns | ns | ns | nd |
| | | | | | | | | 1993 and 1994 | | | | | | | | |
| 0 h | 4 | 0.55 | 0.71 | 0.62 | 0.03 | tr | 0.02 | 0.02 | tr | 0.04 | 0.02 | 0.41 | 13.94 | 10.50 | 3.03 | nd |
| 18 h | 4 | 4.46 | 5.69 | 6.50 | 3.08 | 0.27 | 0.42 | 0.18 | 0.03 | 0.18 | 0.14 | 1.43 | 13.79 | 11.54 | 3.07 | nd |
| signif[f] | | *** | *** | * | ** | *** | ** | ns | ns | ns | ns | ns | ns | ns | ns | nd |
| | | | | | | | | 1994 | | | | | | | | |
| Chardonnay | | | | | | | | | | | | | | | | |
| 0 h | 2 | 0.12 | 0.05 | tr | 0.02 | tr | tr | tr | 0.02 | tr | tr | tr | nd | nd | nd | nd |
| 18 h | 2 | 4.12 | 4.83 | 4.06 | 0.98 | 0.08 | 0.25 | 0.24 | 0.31 | 0.04 | 0.04 | 1.13 | nd | nd | nd | nd |
| signif[f] | | * | * | * | ns | ns | * | ** | ns | ns | ns | * | nd | nd | nd | nd |

[a] B1, B2, B3, B4 = dimers; B1-3-*O*-g, B2-3-*O*-g, B2-3′-*O*-g = dimer-gallates; C1, T2 = trimers. [b] Titratable acidity (TA) expressed as tartaric acid. [c] Total phenolics expressed as gallic acid. [d] na = data not available; nd = not determined. [e] tr = traces. For statistical calculation, they were assumed to be 0.005 mg/L. [f] *, **, *** = significant at the $p \leq 0.05$, 0.01, and 0.001 confidence levels, respectively, by analysis of variance; ns = nonsignificant.

catechins and procyanidins above that of standard HP, but the total phenolics and anthocyanin content of this CPJ, the latter critical to consumer acceptance, was less than half of the HPJ (**Table 1**). The oligomers above trimers and the polymeric procyanidins, which represent on a weight basis the major part of the procyanidins (*2*−*6*), were not analyzed in this study. However, total phenolics correlate closely with the total procyanidin content, including the larger oligomers and polymers (*6*); hence, the total phenolics data presented in **Table 1** suggest that whereas the 18 h of cold maceration is more effective than HP extracting the catechins, procyanidin dimers, and trimers, the opposite is true for the higher oligomers and polymers. It should be also noted that although the total phenolics content of the 18 h macerated CPJ was triple that of the direct CPJ, the increases in the analyzed catechins and procyanidins were up to 44- and 85-fold, respectively.

*Hot Maceration*. The effects of hot maceration for 30, 60, and 90 min at 60 °C on the catechin and procyanidin contents of HPJ produced from Concord are shown in **Table 1**. Although most differences were statistically nonsignificant, there was a tendency for the catechins and procyanidins to increase in concentration as the heating period increased from 30 to 60 min, but declined at 90 min. It appears that excessive heating (90 min treatment) resulted in thermal degradation of the catechins and procyanidins to derived products [e.g., (+)-catechin to catequinic acid]. Because these reactions are oxidative (*41*, *42*), constant stirring applied to prevent scorching aided the reaction by aerating the crushed heated grapes. Spanos and Wrolstad (*34*) studied the effects of processing on the catechin and procyanidin contents of CPJ produced from Thompson Seedless grapes. They found that operations involving heating (e.g., bottling and concentrating) reduced the flavon-3-ol content, particularly in the absence of $SO_2$.

**Effects of Pasteurization.** Commercial grape juice production includes pasteurization, so it was of interest to see whether this process affected the catechins and procyanidins. The compositions of direct CP, 18 h cold macerated, and 30, 60, and 90 min hot macerated unpasteurized Concord juices were compared to that of pasteurized juices. A comparison of the results (**Table 3**) indicates that pasteurization increased the content of catechins in the two CP juices and decreased them in the three HP treatments. In contrast, the concentrations of most analyzed procyanidins were increased by pasteurization in all but the direct CP treatment. Reactions such as thermal degradation (*29*, *41*−*43*) and depolymerization and polymerization (*41*, *42*) of flavan-3-ols occur during pasteurization. The net outcome of these reactions is reflected in the reported results. Depolymerization of higher oligomeric and polymeric procyanidins, which constitute a much larger proportion of total flavan-3-ols than those analyzed here (*2*−*7*), into dimers and trimers appears to be the dominant reaction, resulting in an increase of most analyzed procyanidins in all but the CPJ. Thermal degradation

**Table 3.** Effects of Pasteurization on the Catechin and Procyanidin Composition (Milligrams per Liter) of Concord Grape Juice Produced by Different Pressing Methods in 1994[a]

| | | catechins | | procyanidins[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| treatment | *n* | (+)-catechin | (–)-epicatechin | B1 | B2 | B3 | B4 | B1-3-*O*-g | B2-3-*O*-g | B2-3′-*O*-g | C1 | T2 |
| cold press | | | | | | | | | | | | |
| unpasteurized | 2 | 0.42 | 0.37 | 1.24 | 0.58 | 0.20 | 0.04 | 0.05 | 0.29 | tr[c] | 0.15 | 0.57 |
| pasteurized | 2 | 0.86 | 0.87 | 0.91 | 0.47 | 0.03 | 0.02 | 0.09 | tr | 0.07 | 0.06 | 0.23 |
| cold press, 18 h | | | | | | | | | | | | |
| unpasteurized | 2 | 1.66 | 2.51 | 2.98 | 3.03 | 0.50 | 0.45 | 0.20 | 0.43 | tr | 0.24 | 1.38 |
| pasteurized | 2 | 11.10 | 14.60 | 20.90 | 3.70 | 1.30 | tr | 3.20 | tr | 9.60 | 4.20 | 6.80 |
| hot press, 30 min | | | | | | | | | | | | |
| unpasteurized | 2 | 8.70 | 13.68 | 9.95 | 10.46 | tr | 0.40 | tr | tr | tr | 1.28 | 3.48 |
| pasteurized | 2 | 7.30 | 6.00 | 25.40 | 11.20 | 0.70 | tr | 2.30 | tr | 14.80 | 5.20 | 6.20 |
| hot press, 60 min | | | | | | | | | | | | |
| unpasteurized | 2 | 16.33 | 29.28 | 18.13 | 18.21 | 0.90 | 0.67 | 0.33 | tr | tr | 4.18 | 5.69 |
| pasteurized | 2 | 13.20 | 16.70 | 32.20 | 21.60 | 2.30 | tr | 3.10 | tr | 11.00 | 4.90 | 8.70 |
| hot press, 90 min | | | | | | | | | | | | |
| unpasteurized | 2 | 12.71 | 28.38 | 26.31 | 13.64 | 3.24 | 8.15 | 2.65 | 3.50 | tr | 2.42 | 10.33 |
| pasteurized | 2 | 8.40 | 9.60 | 34.80 | 21.30 | 0.90 | tr | 2.90 | tr | 10.70 | 3.40 | 8.00 |

[a] Non log transformed means. [b] B1, B2, B3, B4 = dimers; B1-3-*O*-g, B2-3-*O*-g, B2-3′-*O*-g = dimer-gallates; C1, T2 = trimers. [c] tr = trace.

**Table 4.** Effects of Cultivar on the Catechin and Procyanidin Composition (Milligrams per Liter) of 18 h Cold Macerated and Pressed Unpasteurized Grape Juice in 1994[a]

| | | catechins | | procyanidins[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cultivar | *n* | (+)-catechin | (–)-epicatechin | B1 | B2 | B3 | B4 | B1-3-*O*-g | B2-3-*O*-g | B2-3′-*O*-g | C1 | T2 |
| Chardonnay | 2 | 4.12abc | 4.83ab | 4.06 | 0.98 | 0.08 | 0.25 | 0.24 | 0.31 | 0.04 | 0.04 | 1.13 |
| Riesling | 2 | 2.61bc | 1.62c | 5.09 | 1.92 | 0.66 | 0.91 | 0.36 | 0.38 | 0.04 | 0.63 | 1.95 |
| Seyval | 2 | 9.06a | 9.38a | 13.03 | 4.38 | 2.34 | 0.69 | 0.50 | 0.36 | 0.19 | 1.58 | 4.79 |
| Elvira | 2 | 8.32ab | 10.14a | 8.28 | 4.20 | 1.24 | 0.50 | 0.90 | 0.34 | 0.18 | 1.10 | 2.32 |
| Niagara | 2 | 4.72abc | 5.70ab | 4.42 | 1.60 | 0.08 | 0.08 | 0.16 | 0.14 | 0.02 | 0.02 | 1.33 |
| Concord | 2 | 1.64c | 2.51bc | 0.17 | 2.04 | 0.07 | 0.07 | 0.05 | 0.07 | tr[c] | 0.05 | 0.12 |

[a] Means within each column followed by the same or no letter are not significantly different using Duncan's multiple range test ($p = 0.05$). [b] B1, B2, B3, B4 = dimers; B1-3-*O*-g, B2-3-*O*-g, B2-3′-*O*-g = dimer-gallates; C1, T2 = trimers. [c] tr = trace. For statistical calculations they were assumed to be 0.005 mg/L.

**Table 5.** Effects of Cultivar on the Catechin and Procyanidin Composition (Milligrams per Liter) of 30 h Cold Macerated and Pressed Unpasteurized White Grape Juice in 1994[a]

| | | catechins | | procyanidins[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cultivar | *n* | (+)-catechin | (–)-epicatechin | B1 | B2 | B3 | B4 | B1-3-*O*-g | B2-3-*O*-g | B2-3′-*O*-g | C1 | T2 |
| Chardonnay | 2 | 11.22 | 8.95 | 16.84a | 3.70bc | 1.69b | 0.34 | 0.37 | 0.47a | 0.36a | 0.88b | 6.37ab |
| Seyval | 2 | 7.15 | 4.47 | 6.43b | 1.85c | 1.14b | 0.16 | 0.21 | 0.14b | 0.09ab | 0.25c | 2.04b |
| Elvira | 2 | 10.62 | 11.59 | 14.82ab | 6.70ab | 1.33b | 1.27 | 1.52 | 0.49a | tr[c]b | 3.61a | 3.67ab |
| Niagara | 2 | 2.78 | 3.44 | 26.05a | 10.27a | 5.72a | 2.07 | 0.96 | 0.51a | 1.29a | 1.19b | 8.95a |

[a] Means within each column followed by the same or no letter are not significantly different using Duncan's multiple range test ($p = 0.05$). [b] B1, B2, B3, B4 = dimers; B1-3-*O*-g, B2-3-*O*-g, B2-3′-*O*-g = dimer-gallates; C1, T2 = trimers. [c] tr = trace. For statistical calculations they were assumed to be 0.005 mg/L.

and/or polymerization of the monomers was the dominant reaction affecting the concentration of catechins in HPJ, resulting in reduction of their content upon pasteurization.

**Effects of Cultivar.** Because catechins and procyanidins are poorly extracted in CP without maceration, the concentration of these phenolics is very low, making comparison among cultivars difficult.

*Cold Pressed after 18 h of Maceration Juices.* Cold maceration of the crushed, destemmed grapes prior to pressing extracted more catechins and procyanidins than CP without maceration; hence, this gave a better base for comparison among cultivars. The results presented in **Table 4** show that in 1994 the highest concentrations of (+)-catechin and procyanidins B1, B2, B3, B2-3′-*O*-gallate, C1, and T2 were in Seyval; the highest concentrations of (−)-epicatechin and B1-3-*O*-gallate were found in Elvira, and the highest concentrations of B4 and B2-3-*O*-gallate were found in Riesling. In 1993 Seyval contained a significantly greater amount of B3 than Riesling (Supplement 3, Supporting Information).

*Cold Pressed after 30 h of Maceration Juices.* In the 1994 season four white cultivars were cold macerated for 30 h prior to pressing (**Table 5**). Catechin was in highest concentration in Chardonnay and (−)-epicatechin in Elvira. The highest concentration of the analyzed procyanidins was in Niagara, with the exception of B1-3-*O*-gallate and C1, which were highest in Elvira. In contrast, Niagara was lowest in both catechins. The lowest concentration of individual procyanidins was in Seyval except B2-3′-*O*-gallate, which was the least abundant in Elvira. Catechin was present in higher concentration than (−)-epicatechin in Chardonnay and Seyval, whereas the reverse was the case for Elvira and Niagara. The most abundant procyanidin was B1 in every cultivar. Significant quantities of procyanidins T2, B2, and B3 were also present in every cultivar. The three galloylated procyanidins were detected in the lowest concentration in most cases. Lee and Jaworski (*33*) analyzed the catechins and procyanidins in the berries of 21 white grape cultivars. Their survey covered Niagara and three other cultivars (Chardonnay, Riesling, and Seyval) used here. They found Niagara to be the

**Table 6.** Effects of Cultivar on the Catechin and Procyanidin Composition (Milligrams per Liter) of Hot-Pressed Unpasteurized Red Grape Juice in 1994[a]

| | | catechins | | procyanidins[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cultivar | *n* | (+)-catechin | (–)-epicatechin | B1 | B2 | B3 | B4 | B1-3-*O*-g | B2-3-*O*-g | B2-3′-*O*-g | C1 | T2 |
| Gamay | 2 | 9.00ab | 7.36b | 5.64b | 5.27ab | 1.19ab | 0.66 | 0.88b | 0.01a | 0.10 | 0.99 | 3.73b |
| Baco noir | 2 | 6.64b | 17.26ab | 1.81c | 3.23b | 0.08bc | 0.13 | 0.01c | 0.01a | 0.07 | 0.18 | 0.01c |
| Foch | 2 | 30.62a | 16.18ab | 11.19b | 5.22ab | 3.30ab | 1.03 | 0.60b | tr[c]b | 0.06 | 1.00 | 5.01b |
| Vincent | 2 | 18.41ab | 33.11a | 32.14a | 17.99a | 6.55a | 11.12 | 6.46a | tr b | 0.19 | 10.86 | 13.68a |
| Concord | 2 | 6.81b | 10.47ab | 9.82b | 10.45ab | tr c | 0.06 | tr c | tr b | tr | 0.11 | 3.48b |

[a] Means within each column followed by the same or no letter are not significantly different using Duncan's multiple range test ($p = 0.05$). [b] B1, B2, B3, B4 = dimers; B1-3-*O*-g, B2-3-*O*-g, B2-3′-*O*-g = dimer-gallates; C1, T2 = trimers. [c] tr = trace. For statistical calculations they were assumed to be 0.005 mg/L.

highest in procyanidins B1, B2, and B3 and second highest in (+)-catechin among these four cultivars.

*Hot-Pressed Juices*. The standard HP (30 min of maceration) was employed with five cultivars in 1994. The results presented in **Table 6** show that the highest concentrations of most catechins and procyanidins were found in the highly pigmented Vineland hybrid Vincent (*44*). Only Foch had a higher (+)-catechin content, and the procyanidin B2-3-*O*-gallate, which was detected only in traces in Vincent, was present in measurable quantities in Gamay and Baco noir. Catechin was present in a greater amount than (−)-epicatechin in Gamay and Foch, whereas the reverse was true for Baco noir, Vincent, and Concord. The procyanidin found in highest concentration was B1 in Gamay, Foch, and Vincent, whereas B2 was dominant in Baco noir and Concord. A relatively high quantity of T2 was detected in all cultivars with the exception of Baco noir. The procyanidin in lowest concentration was B2-3-*O*-gallate in all five cultivars.

**Effects of Vintage.** The growing season in 1994 was slightly warmer (1356 vs 1315 heat units by the end of October) and wetter (764 vs 650 mm) than that in 1993 (K. H. Fisher, personal communication). Concord was harvested about the same time and extensively studied in both years. The results in **Table 1** show that (+)-catechin, (−)-epicatechin, and procyanidins B1, B3, B1-3-*O*-gallate, B2-3′-*O*-gallate, C1, and T2 were consistently higher and B4 and B2-3-*O*-gallate were lower in 1994. One of the major procyanidins, B2, had higher values in 1994 for the 60 and 90 min HP but not for the 30 min HP and the two CP treatments. Gamay was the other red cultivar which was analyzed in both years (Supplement 2, Supporting Information). The HPJ had higher catechins and procyanidins B1, B4, B2-3′-*O*-gallate, and T2 contents in 1993. Procyanidins B2, B3, B1-3-*O*-gallate, B2-3-*O*-gallate, and C1 concentrations were slightly higher in 1994. Because Gamay was harvested 10 days earlier in 1993, a difference in ripeness could be responsible for the higher values in that year.

The CPJ produced after 18 h of cold maceration was used for the whites to compare the vintages. Niagara and Elvira (**Table 2**) responded similarly, producing juice with higher (+)-catechin and (−)-epicatechin contents in 1994. However, every procyanidin except B1 and C1 for Niagara had lower values in that year. Riesling and Seyval (**Table 4** and Supplement 3, Supporting Information) responded differently, the Riesling having lower catechin contents, whereas the Seyval was higher in these compounds in 1994. In both cultivars almost every procyanidin had higher values in 1994. These results for both red and white grape cultivars demonstrate that vintage influences the flavan-3-ol contents of grape juice.

These results showed that pressing method had the greatest influence, but cultivar and, to a lesser degree, pasteurization and vintage also played a role in determining the flavan-3-ol contents of grape juice. Analyses on the *labrusca*-type juice grape cultivars used in commercial juice production in North America demonstrated that HPJ from Concord is a good source for the flavan-3-ol nutraceuticals. The white juice produced by CP without maceration from Niagara was a poor source for these compounds, but their concentration could be increased by cold maceration prior to pressing.

## ABBREVIATIONS USED

CP, cold pressing; CPJ, cold-pressed juice; HP, hot pressing; HPJ, hot-pressed juice.

## ACKNOWLEDGMENT

We thank Dr. K. H. Fisher for critically reading the manuscript and supplying the grapes; Dr. C. L. Chu for advice on statistical analyses; R.B. Palabay and P. Hipolito-Reis for their able technical assistance.

**Supporting Information Available:** Tables containing the experimental design showing the juice treatments from 11 grape cultivars (1), effects of cold and hot pressing (2) and comparing two white cultivars for their flavon-3-ol content (3). This material is available free of charge via the Internet at http://pubs.acs.org.

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**Received for review June 20, 2002. Revised manuscript received October 28, 2002. Accepted October 31, 2002. We gratefully acknowledge the financial support of the Ontario Food Processing Research Fund of the Ontario Ministry of Agriculture, Food and Rural Affairs; the Ontario Grape Growers' Marketing Board, and Wiley Brothers Ltd.**

JF020689M