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# Polyphenols in Golden Delicious Apple Juice in Relation to Method of Preparation

J. Van Buren,*[1] L. de Vos, and W. Pilnik

Apple juices prepared by conventional and enzymatic procedures were examined for phenolic materials and color before and after storage. Measurement of both the total phenolic material and the phenolic material precipitated by formaldehyde was supplemented by specific measurement of chlorogenic acid and flavonol glycosides, as well as inspection of chromatograms for flavans. The total phenolics ranged from 25 mg/100 ml in juices from well-oxidized pulps to 150 mg/100 ml in juices made with minimal enzymatic oxidation. Chlorogenic acid and considerable flavonoids were easily extracted into the juice, but they were also lost during the pre-pasteurization period. On the other hand, flavonol glycosides went into the juice slowly, were relatively stable in unpasteurized juices, and were lost during storage, particularly in concentrates. Enzymatic oxidation of apple pulp, presumably by polyphenol oxidase, caused almost complete loss of chlorogenic acid and flavans, but had little effect on flavonol glycosides. Treatment with pectolytic enzymes before pressing led to increased extraction of nonflavonoid Folin-Ciocalteu reactive materials into juice. Reducing materials increased during storage of juice concentrate. Observations of color changes in stored juices indicate that brown pigment development was associated with flavonoids.

The polyphenolic materials of apple juice are of particular interest because the color and turbidity of juices and concentrates are due, in large part, to these compounds. Furthermore, the conditions of juice preparation create opportunities for oxidative reactions involving polyphenols; thus the types of polyphenols present in finished juices are affected by juice-making procedures.

Different types of phenolic compounds react differently under juice making conditions. The catechin-type phenolics and the chlorogenic acid serve as good substrates for apple polyphenol oxidase, but the products from catechin oxidation are darker (Siegelman, 1955) and have a greater tendency to condense. The catechins oxidize more readily than the cinnamic acid derivatives. Flavonol glycosides are not direct substrates for the oxidase (Baruah and Swain, 1959; Walker, 1964b). It might be assumed that the amounts of particular phenolic compounds remaining in a juice would be as important as the total phenolic content in affecting the characteristics of the juice.

In recent years methods have been developed for the enzymatic treatment of apple pulp which improve both the ease of pressing and the yield of juice (Pilnik and de Vos, 1970; Verspuy et al., 1970; de Vos, 1970). The enzymatic treatment, employing pectolytic preparations, was most advantageous when used with apples of poor pressing characteristics, either due to variety, i.e. Golden Delicious, or to deterioration during storage. Since such apples are forming an ever greater part of the apples used for juice production the enzymatic procedure could be of considerable practical importance. Therefore it is desirable to have information on the polyphenol composition of juices derived from enyzme-treated pulp in order to provide a basis for the use of the process by the apple juice industry. Some of the effects of such treatments on the character of apple juice have already been described (Pilnik and de Vos, 1970; de Vos, 1970) and include changes in the phenolic content, the color, and the aroma.

The major cinnamic acid derivative in apples, chlorogenic acid, has been measured in a number of varieties (Kuusi and Pajunen, 1971; Macheix, 1970; Walker, 1963) and amounts in apple flesh are reported as 20 mg to 200 mg per 100 g of fresh tissue. Little or no loss takes place during juice storage or concentration (Tanner and Rentschler, 1956).

Flavonol glycosides have been measured in apple epidermal tissues (Walker, 1964a; Workman, 1963) where they are concentrated. Typical values are between 0.5 and 18 mg per g of fresh peel. These are mainly glycosides of quercetin having galactose, glucose, arabinose, rhamnose, or xylose as the sugar group (Fisher, 1965; Siegelman, 1955).

While a number of the particular phenolic compounds of apples have been identified (Durkee and Poapst, 1965; Fisher, 1965; Van Buren, 1971; Van Buren et al., 1966) many of the studies on the phenolic content of apple juices have involved the determination of classes of compounds, such as total phenols, vanillin–sulfuric acid reactive phenols, and leucoanthocyanidins. None of these is a distinct or an exclusive group of phenolic compounds although studies of these classes have provided considerable insight into changes occurring in apple juice. In particular, the ratio of total phenolic groups to vanillin–sulfuric acid reactive groups has provided a measure of the degree of polymerization of the phenolic compounds indicating that oxidation in a juice or pulp results, initially, in an increase in the degree of polymerization of the phenolic material (Pilnik and de Vos, 1970) accompanied by a rapid loss of vanillin–sulfuric reactive material,

Sprenger Instituut and Agricultural University, Wageningen, Holland.
[1] Permanent address: Department of Food Science and Technology, Cornell University, Geneva, New York 14456.

presumably flavans, and a slower loss of Folin-Denis reagent reactive material representing the total phenolic material.

In the present work further differentiation was possible. Separate measurements of the chlorogenic acid and flavonol glycosides were supplemented by the use of the Folin-Ciocalteu reagent (Singleton and Rossi, 1965) to measure the phenolic materials of the whole juice and of the juice remaining after precipitation of the flavonoids with formaldehyde (Kramling and Singleton, 1969; Hillis and Urbach, 1959). These measurements gave values for the flavonoid phenolics and the nonflavonoid phenolics. A similar analysis has been used on wines by Peri et al. (1971).

This report deals with the effect of several pulp handling techniques, including enzyme treatment, on the concentration of several classes of phenolic material in the juice. Also studied were changes that took place during storage of single strength juice and concentrate. Color comparisons were made on the products.

## EXPERIMENTAL SECTION

The apples used were of the Golden Delicious variety held in cold storage at 1 °C for 1 to 4 months before juice making.

Juices were prepared in the following manner. (1) **Conventional Juice.** Apples were pulped in a rasp mill (Bucher Central III) and pressed without delay on an Ensink band press (de Vos, 1970). After pressing the juices were depectinized with Ultrazym 100 Spezial (Dr. Schubert AG), 0.04 g/l., at room temperature for 2–3 h. Following this the juices were heated briefly to 80 °C, centrifuged, and filtered. Single strength juices (10–12% soluble solids) were pasteurized again at the bottling stage. Concentrates at 65–74% soluble solids, obtained using a Sako low-temperature vacuum evaporator (SMS, Butzbach), were stored without further pasteurization. Normal storage was at 3 °C while accelerated storage testing was carried out at 35 and 50 °C.

(2) **Rapidly Pasteurized Juice.** This was prepared in the same manner as conventional juice but the juice was heated briefly to 80 °C immediately after pressing. The total time between pulping and pasteurization was about 10 min.

(3) **NaCl-Treated Pulp Juice.** This was prepared in the same manner as conventional juice except that 1 g of NaCl/kg was added to the apple at the pulping stage.

(4 and 5) **Oxidized Pulp Juice.** This was prepared in the same manner as conventional juice except that the pulp was stirred in air for the indicated times before pressing.

(6) **Held and Nonoxidized Pulp Juice.** This was prepared in the same manner as conventional juice except that 1 g of NaCl and 10 mg of $SO_2$ per kg were added to the pulp, which was held for 4 h before pressing.

(7) **Oxidized and Enzyme-Treated Pulp Juice.** This was prepared in the same manner as 1 h oxidized pulp juice except that, after oxidation, Ultrazym 100 (0.1 g/kg of pulp) was added and the pulp held a further 2 h before pressing. Ultrazym 100 is a pectolytic enzyme preparation.

(8) **PVP and Enzyme Treated Pulp Juice.** This was prepared in the same manner as conventional juice except that added to the pulp were 0.6 g of PVP (polyvinylpyrrolidone) (Kollidin 25, BASF) and 0.2 g of Ultrazym 100, per kg, and the pulp was held 4 h before pressing.

Chlorogenic acid and flavonol glycoside concentrations were measured by a chromatographic–fluorometric procedure using cellulose thin-layer plates for the separations and a Vitatron densitometer to measure fluorescence (Van Buren et al., 1973). For flavonol glycosides the plates were treated with aluminum sulfate in order to have these compounds fluoresce under ultraviolet light. Total phenolic materials were measured with the Folin-Ciocalteu reagent according to the procedure developed for wine analyses (Singleton and Rossi, 1965). Total nonflavonoid material (including chlorogenic acid and other hydroxycinnamic acids) was determined on the soluble juice components remaining after precipitation of the flavonoids with formaldehyde (Kramling and Singleton, 1969). Subtraction of the nonflavonoid values from the total phenolics yielded a value for the total flavonoids (including flavonol glycosides and flavans) in the juice. Subtraction of chlorogenic acid from the nonflavonoids gave the concentration of "other nonflavonoids" while subtraction of flavonol glycosides from the total flavonoids gave the concentration of "other flavonoids".

The "other nonflavonoids" and "other flavonoids" do not necessarily represent only phenolic materials, but should be considered as the materials indicated by particular analytical procedures and calculations.

Inspection of cellulose thin-layer chromatograms under uv light immediately after exposure to ammonia fumes allowed detection of *p*-coumarylquinic acid while treatment of chromatograms with a solution of Fast Black Salts K permitted observation of flavans, including proanthocyanidins (Van Buren et al., 1966).

**Table I. Flavonoid Phenolics in Apple Juice**

| Juice procedure | Flavanol glycosides,[a] mg/100 ml | Other flavonoids,[b] mg/100 ml |
|---|---|---|
| Conventional (1) | 0.4 | 20 |
| Rapidly pasteurized (2) | 0.2 | 71 |
| NaCl-treated pulp (3) | 0.3 | 73 |
| Oxidized pulp, 1 h (4) | 0.7 | 9 |
| Oxidized pulp, 3 h (5) | 6.0 | 1.0 |
| Held and nonoxidized pulp (6) | 8.0 | 101 |
| Oxidized and enzyme-treated pulp (7) | 6.5 | 12 |
| PVP and enzyme-treated pulp (8) | 8.5 | 12 |

[a] Expressed as rutin. [b] Expressed as catechin.

## RESULTS

**Flavonoids.** The marked influence of juice-making procedure on the polyphenol composition of apple juice can be noted in Tables I and II. The procedures were intended to give a variety of oxidative conditions in connection with various pulp holding times and pectolytic enzyme treatments.

In procedures 1, 2, and 3 pressing followed shortly after the pulping operation. With the conventional process oxidation began at the pulping step and continued in the juice until pasteurization. In the case of the rapidly pasteurized juice the oxidase action took place only in the pulp and during pressing. When the pulp was prepared with NaCl the polyphenol oxidase was inhibited (Sharon and Mayer, 1967); thus oxidation was near a minimum.

With treatments 4 and 5 enzymatic oxidation was promoted by the continuous mixing of the pulp with air, while with treatment 6 the pulp was held for a considerable time during which oxidation was inhibited by the NaCl and a small amount of $SO_2$. In both the pectolytic enzyme treatments, 7 and 8, oxidation took place in the pulp, but it was somewhat less in the PVP and enzymed pulp as

Table II. Nonflavonoid Phenolic Materials in Apple Juice

| Juice type | Chlorogenic acid, mg/100 ml | Other nonflavonoids, mg/100 ml[a] |
|---|---|---|
| Conventional | 14 | 17 |
| Rapidly pasteurized | 23 | 8 |
| NaCl-treated pulp | 26 | 10 |
| Oxidized pulp, 1 h | <1 | 15 |
| Oxidized pulp, 2 h | <1 | 21 |
| Held and nonoxidized pulp | 32 | 20 |
| Oxidized and enzyme-treated pulp | 3 | 32 |
| PVP and enzyme-treated pulp | <1 | 32 |

[a] Expressed as chlorogenic acid.

evidenced by a decrease in brown color of the portion of the pulp not exposed to air.

The amounts of flavonol glycoside found in the finished juices were dependent on the length of time the pulp was held before pressing and independent of the degree of enzymatic oxidation. The flesh of the Golden Delicious apple contains very low amounts of flavonol (Workman, 1963) in contrast to the epidermal tissue where most of it is found. During the pulping operation the epidermal tissues were not broken as much as the flesh cells, although they were certainly bruised by passage through the pulping machine. Other work has shown that very little flavonol glycoside moved out of nonbruised apple peels suspended in water for 24 h. Some tissue injury may be needed to release epidermal cell contents. This may be related to the general experience with grapes where a heat treatment or the presence of alcohol is needed in order to obtain a high extraction of polyphenolic compounds from the skins (Berg and Akiyoshi, 1957).

Oxidative conditions or pectolytic enzymes were not necessary for the extraction of flavonols from the pulp since considerable amounts were extracted from pulp where oxidation during the holding period was inhibited by 0.1% NaCl and 10 ppm of $SO_2$ (treatment 6).

The behavior of "other flavonoids" was somewhat different since they were rather easily extracted into the juice and were quite susceptible to oxidations yielding products no longer soluble or not reacting with the Folin-Ciocalteu reagent. A major component of the "other flavonoids" fraction in juices protected against enzymatic oxidation was the flavans, including the proanthocyanidins. These materials serve as good substrates for polyphenol oxidase and also condense nonenzymatically to form polymers (Weinges, 1969).

Work by Johnson et al. (1969), indicating a large difference between the total phenolics of juices prepared with and without oxidation, gave results similar to those found in the present work. Furthermore, they report an even greater percentage difference in the amounts of proanthocyanidins, in agreement with the findings here of a dramatic decrease in "other flavonoids".

The virtual disappearance of "other flavonoids" from juice derived from oxidized pulps may be compared with the moderate amounts present in juices coming from enzyme-treated pulps. The enzymatic treatments released into the juice some flavonoid-type materials which were not, by chromatographic testing, identifiable as flavans or flavonol glycosides; therefore, the "other flavonoids" from enzyme treated pulps may have had a different composition from the "other flavonoids" from pulps protected against oxidations. The presence of "other flavonoids" was associated with darkening of stored juice since their removal through the use of polyclar AT (Mennett and Nakayama, 1969) greatly decreased color changes during storage at 35 °C.

**Nonflavonoids.** Chlorogenic acid concentrations (Table II) in finished juices were found to be lowered when the pulps or juices had been permitted to undergo enzymatic oxidation, but the degree of loss was less than seen with the "other flavonoids" (Table I). Others (Roberts, 1957) have found that flavans were oxidized in preference to chlorogenic acid by polyphenol oxidase. Holding pulp under nonoxidative conditions led to higher chlorogenic acid levels in the juice. Much of the loss of chlorogenic acid in conventional juice was a result of the juice holding period associated with enzymatic depectinization of the juice.

The "other nonflavonoid" fraction presented a difficult matter for interpretation. Derivatives of coumaric and ferulic acids would be expected to be measured in this fraction, and they could be detected on cellulose chromatograms of juices from the less oxidized samples. But their concentrations can be expected (Macheix, 1974) at $^1/_{10}$ to $^1/_5$ the level of chlorogenic acid. Ascorbic acid would be measured by the Folin-Ciocalteu reagent, but this also disappears in the more oxidized samples. Therefore, most of the material in this fraction might be unidentified nonphenolic compounds capable of reacting with the Folin-Ciocalteu reagent. Treatment of pulp with the pectolytic enzyme was particularly effective in augmenting the amount of this unknown material in the juice.

*p*-Coumarylquinic acid was not measured by a quantitative procedure, but a visual estimate was made on chromatograms regarding the relative amounts of this phenolic in the juice samples. Pulp and juice treatments that resulted in decreases in chlorogenic acid caused similar changes in the apparent amounts of *p*-coumaryl acid. This might be expected in view of reports that *p*-coumaric acid serves as a substrate for apple polyphenol oxidase (Walker, 1964b).

**Juice Storage.** Of the various phenolic fractions measured, only the flavonol glycosides showed an appreciable decrease during 35 °C storage. However, the flavans, except with the juice from $SO_2$ treated pulp, showed greatly decreased mobility on chromatograms, indicating that considerable polymerization had taken place. This polymerization of flavans was accompanied by greatly increased turbidity in these samples.

**Color.** Since polyphenols are believed to influence the color of fruit juice color or darkness comparisons were made on the experimental juices. Observations on stored juice (35 °C) indicated that the principal materials causing darkening in juice were the flavonoids, both flavonol glycosides and "other flavonoids". This was seen most directly in that those samples having the highest flavonoid content developed the darkest color (Table III). For instance, the rapidly pasteurized juice darkened the most during storage while the juice obtained from pulp oxidized 1 h had little darkening. Furthermore, the removal of flavonoids (but not a large percentage of the nonflavonoids) through the use of 2 g/l. of polyclar AT resulted in juice that darkened very little in storage. Addition of specific compounds to polyclar AT treated juice showed that storage darkening did not result from adding 20 mg/100 ml of chlorogenic acid, moderate darkening resulted from adding 5 mg/100 ml of rutin, and severe darkening occurred where 10 mg/100 ml of catechin had been added.

**Concentrates.** When the analytical procedures were applied to juice concentrates stored at 35 or 50 °C there was seen an increase in both the "other flavonoid" and the

Table III. Relative Darkness of Finished Apple Juices

| Freshly bottled | Bottles stored at 35 °C for 90 days |
|---|---|
| Lightest | |
| Conventional plus 2 g/l. of polyclar AT | Conventional plus 2 g/l. of polyclar AT |
| Rapidly pasteurized (2) | Oxidized pulp, 1 h (4) |
| Held and nonoxidized pulp (6) | Oxidized and enzymed pulp plus 2 g/l. of polyclar AT |
| NaCl-treated pulp (3) | Oxidized pulp, 3 h (5) |
| Oxidized pulp, 1 h (4) | PVP and enzyme-treated pulp (8) |
| Oxidized and enzyme-treated pulp plus 2 g/l. of polyclar AT | Oxidized and enzyme-treated pulp (7) |
| Oxidized pulp, 3 h (5) | Conventional (1) |
| PVP and enzyme-treated pulp (8) | Held and nonoxidized pulp (6)[a] |
| Oxidized and enzyme-treated pulp (7) | NaCl-treated pulp (3)[a] |
| Conventional (1) | Rapidly pasteurized (2)[a] |
| Darkest | |

[a] Darkness measured after removal of turbid material by centrifugation.

Table IV. Changes in the Apparent Amounts of "Other Flavonoids" and "Other Nonflavonoids" during Storage of Apple Juice Concentrates at Elevated Temperatures[a]

| | Component at storage | | | |
|---|---|---|---|---|
| | "Other flavonoids"[b] | | "Other nonflavonoids"[c] | |
| Juice type | 35 °C, 90 days | 50 °C, 20 days | 35 °C, 90 days | 50 °C, 20 days |
| Conventional (1) | +13 | +23 | +7.5 | +13.5 |
| Oxidized pulp, 3 h (5) | +0.6 | +4.5 | +11 | +23 |
| Oxidized and enzyme-treated pulp (7) | +3 | +4.5 | +21 | +30 |

[a] Values given as milligrams of phenolic per 100 ml of reconstituted juice containing 12% soluble solids. [c] Expressed as catechin. [c] Expressed as chlorogenic acid.

"other nonflavonoid" fraction (Table IV). A previous report (Van Buren et al., 1972) indicated that, under the same conditions, flavonol glycosides decreased significantly, and chlorogenic acid decreased very little. Flavans were present in rather small amounts similar to those in fresh concentrate.

The types of materials responsible for the increased measurement of phenolics are not known, but the strong probability exists that the amounts measured do not represent phenolics at all, but are reducing substances of other sorts, possibly carbohydrate breakdown products or reductones.

Darkening took place as a result of storage at elevated temperatures with concentrate from conventional juice darkening to about the same extent as that from oxidized and enzyme-treated pulp. The least darkening took place with the juice concentrates from oxidized pulp. Although darkening in apple juice concentrates is caused by carbohydrate as well as polyphenol reactions, the differences in darkening among these concentrates could well be due to their differences in flavonoid content.

From a practical standpoint it is important that enzyme-treated pulps yield juices and concentrates with normal color stability during storage. When one desires an exceptionally light concentrate, pulp oxidation can lead to such a product. The flavor or aroma in such juices is somewhat different, but not disagreeable, and weaker than in juices from the conventional procedure. When flavor is of secondary importance, the higher juice yields and satisfactory color stability indicate an advantage for enzyme treatment of Golden Delicious and other apples with poor pressing characteristics.

The analytical values for the experimental juices clearly show that the absolute and relative concentrations of various types of polyphenols changed considerably with degree of oxidation, and to a lesser extent with pulp holding time. The flavonoids were lost most readily, then the chlorogenic acid, while increased pulp holding time led to higher levels of flavonol glycosides. In protected juices the flavonoids predominate, but exposure to oxidation leaves chlorogenic acid as the predominant identifiable polyphenol. Still further oxidation, accompanied by pulp holding, results in a greatly increased proportion of flavonol glycoside.

The nature of the materials designated as "other flavonoids" and "other nonflavonoids" is somewhat in doubt, especially with the more extensively oxidized samples and those from pulp treated with pectolytic enzyme. There is a strong possibility that some of it is nonphenolic in nature. The same can be said of the increased amounts of Folin-Ciocalteu reactive material found in stored apple juice concentrate. This, in turn, indicates that caution should be used in interpreting the values obtained by the Folin-Ciocalteu procedure.

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