

## **Factors Influencing the Clarification of Apple Juice with Honey**

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### *ABSTRACT*

*Six apple cultivars were studied to determine the effect of pH, degree of browning and polyphenol composition on clarification of apple juice by addition of honey. The optimum pH for all apple cultivars was about 3.5. Only browned apple juices, those prepared with no ascorbic acid, flocculated with the honey and consequently clarified. The flocculation time was inversely related to the degree of browning of all apple cultivars except Golden Delicious. The clarification rate did not correlate well with the different phenolic groups present in the juices.*

### INTRODUCTION

Polyphenolic compounds in the apple are known to be the primary substances that interact with protein to form haze in juices (Kieser *et al.*, 1957; Johnson *et al.*, 1968). Several factors influence this interaction including pH and certain chemical properties of polyphenols. Van Buren & Robinson (1969) showed that the pH optimum for the tannic acid–gelatin precipitation was 4.7–4.8, while Gustavson (1954) indicated there was no difference in the precipitation of condensed tannins with gelatin between pH

3.5 and 8.0. Johnson *et al.* (1968) reported that the sediment formed in clarified apple juice consisted of polymeric polyphenols, a form of tannin. Bate-Smith (1973) showed that, as the molecular weight of tannins increased, their ability to precipitate protein increased.

It has been shown that honey has the ability to remove hazy materials from fresh apple juices (Kime, 1982; 1983). Recently, Lee & Kime (1984) reported that the component of honey responsible for clarification of apple juice was protein which was present in relatively low concentrations, ranging from 0.05% to 0.79% (White & Rudyi, 1978). The pH of apple juice ranges from 3.0 to 4.0 and the concentration of polyphenols ranges from 0.05% to 1.10%, the latter based on fresh weight of various cultivars (Van Buren, 1970). In this study the effects of pH, degree of browning and polyphenol composition on the clarification by honey of juices from six apple cultivars were studied.

## MATERIALS AND METHODS

### Apples and juice preparation

The apples used in this study were obtained during the 1983 season from orchards of the New York State Agricultural Experiment Station. Six cultivars; Red Delicious, Jonathan, Golden Delicious, Rhode Island Greening, Rome and McIntosh, were harvested at commercial maturity and held at 1°C prior to processing for juice. Commercial honey (floral type: Locust) was obtained from a local producer. Juices from each cultivar were prepared according to standard commercial practices in our pilot plant. Unoxidized juices were prepared by adding 0.5% ascorbic acid to the fruit prior to grinding in a Fitz mill.

### Analyses

To extract color, apple juices were treated with ethanol to give a final concentration of 60% by volume and then centrifuged at  $500 \times g$  for 15 min in a IEC HN-SII centrifuge. The degree of browning in the supernatant was determined by measuring the absorbance at 440 nm. For the analysis of phenolics, juice containing 60 vol% ethanol was allowed to stand for 1 h at room temperature and then filtered through Whatman No. 4 filter paper under vacuum to remove alcohol-insoluble materials. The ethanol was removed from the filtrate by evaporation under reduced pressure at 35°C. The polyphenols were fractionated into simple phenols, non-tannic flavans, hydrolyzable tannins, and condensed tannins according to the method of

Peri & Ponpei (1971). Each fraction was quantitated by the colorimetric method of Singleton & Rossi (1965) and the results were expressed as mg catechin/100 ml juice.

For the clarification study, honey solution (50% honey in water by weight) was added to each of the following samples to give a final concentration of 4% honey solution (w/w) in (1) regular juice, (2) unoxidized juice, and (3) regular juice adjusted to pH values from 3.0–4.5 with 1N HCl or NaOH. Each sample was thoroughly mixed and observed for 12 h at room temperature to determine the time required to reach initial flocculation (stage 1) according to the method of Lee & Kime (1984).

## RESULTS AND DISCUSSION

### Effect of pH on clarification

The time to reach the stage 1 flocculation of each juice cultivar at different pH values is given in Fig. 1. Flocculation was most rapid within the range of

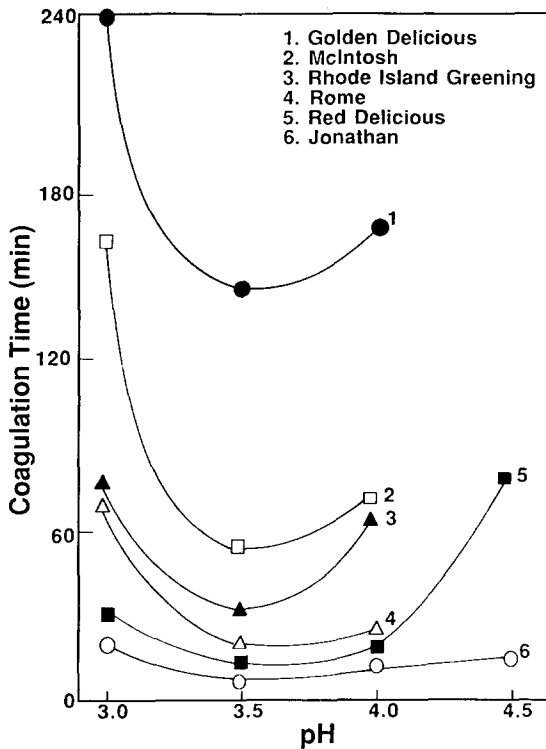


Fig. 1. Time required to reach the initial coagulation stage of apple juices at pH 3.0–4.5 with honey added at 4% level.

pH 3.5–4.0 for all juices. This was consistent with other studies which reported optimal values of pH 3.0–4.0 (Lee & Kime, 1984). Van Buren & Robinson (1969) and Hagerman & Butler (1978) reported that the maximum precipitation of tannin–protein complexes was obtained near the isoelectric point of protein. In agreement with this, the pH optima for the clarification of all cultivars in this study was found to be near, but slightly below, the isoelectric point of honey protein (Paine *et al.*, 1934). Within the pH range tested, Red Delicious, Jonathan and Rome cultivars reached the initial flocculation stage most quickly, Rhode Island Greening and McIntosh were intermediate, and the Golden Delicious cultivar took the longest time. In general, lowering the pH to 3.0 increased the flocculation time significantly for all cultivars except Red Delicious and Jonathan; conversely, at pH 4.5, only Red Delicious and Jonathan reached the initial flocculation stage within the time limits of the experiment.

### Effect of browning on clarification

A plot of the time required to reach the initial flocculation stage prepared against the degree of browning (Fig. 2) shows that, with the exception of Golden Delicious, the flocculation time of juices correlated with the degree of browning on each apple cultivar. The flocculation mechanism in Golden Delicious apple juice is apparently different from the other cultivars. A negative linear correlation ( $r = -0.83$ ) was established for the other five

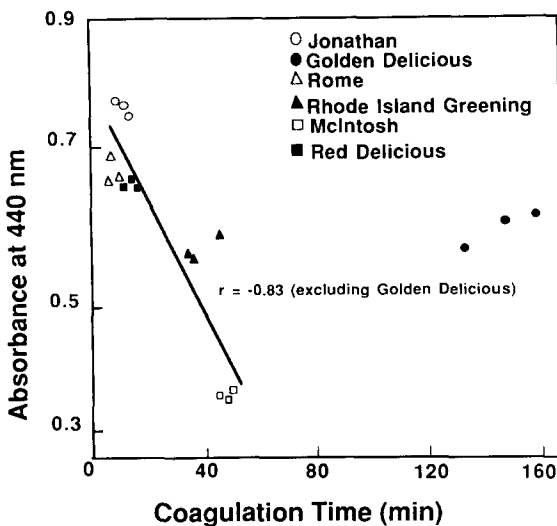


Fig. 2. Effect of the degree of browning on the time required to reach the initial coagulation stage of apple juice with honey added at 4% level.

apple cultivars. In this clarification study, the unoxidized (non-browned) apple juice did not form any detectable flocculates with honey within the time limits of the experiment. Loomis & Battaile (1966) reported that polyphenols are readily oxidized to their corresponding quinones, compounds which bind proteins and peptides to form brown pigments. Negoro (1972) reported also that an oxidized product from chlorogenic acid had great ability to bind protein. In agreement with these observations, our results suggest that the oxidative browning of phenolics to produce quinones might be an essential reaction for the rapid clarification of apple juice using honey. Consequently, the degree of browning may be a good index for determining the time required for honey to clarify apple juice.

### Polyphenol composition of apples and clarification rate

The concentrations of different phenolic groups in the juices of the six cultivars are given in Table 1. The time required to reach the initial flocculation of apple juices did not correlate well with the levels of any one group of phenolics.

Kieser *et al.* (1957) and Johnson *et al.* (1968) reported that the sediments formed in clarified apple juice consisted mainly of polymeric phenolics and protein. Lee (1984) suggested that tannic materials in apple juice might account for the formation of insoluble complexes with honey protein.

TABLE 1

Concentration of Different Phenolic Groups in the Apple Juice of Six Cultivars (as mg catechin/100 ml juice)

	<i>Red Delicious</i>	<i>Jonathan</i>	<i>Golden Delicious</i>	<i>Rhode Island Greening</i>	<i>Rome</i>	<i>McIntosh</i>
Total phenolics	21.8	44.4	30.9	31.1	33.9	16.4
Tannic phenolics	11.7	26.9	14.3	17.8	18.3	6.45
Non-tannic phenolics	10.1	17.4	16.5	13.3	15.6	9.92
Condensed tannins	10.7	25.1	11.6	15.9	15.1	5.42
Hydrolyzable tannins	1.01	1.76	2.68	1.93	3.14	1.04
Non-tannin flavans	0.41	3.24	1.60	2.54	1.75	0.82
Simple phenolics	9.52	13.5	14.9	10.8	13.9	9.10

However, the results obtained in this study indicate that the amount of tannin phenolics in apple juices did not directly correlate with the clarification rate.

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