Limonin Content of Juice from Marrs and Hamlin Oranges [*Citrus sinensis* (L.) Osbeck]

Roger F. Albach,* George H. Redman, and Bruce J. Lime

Marrs and Hamlin early oranges from five locations in the South Texas citrus belt were harvested and juiced at 2-week intervals during their season of maturity. The juice was heated as in commercial processing so that delayed bitterness due to limonin formation from its tasteless precursor, limonoate A-ring lactone, would develop maximally. Limonin content was analyzed by thin-layer chromatography. By mid-November, the juice of oranges from all five locations contained less limonin than the nominal taste threshold concentration of 6 ppm. Average limonin concentration fell from a high of 6.2 ppm in September to a minimum of 1.8 ppm in January. The two cultivars did not differ significantly in limonin content. Locational differences were most apparent during the early season but persisted in some cases to the last harvest. Linear correlations between limonin and time of harvest was much better than between limonin and °Brix, percent acid, or the °Brix/acid ratio. Results were consistent over two seasons at one location.

"Delayed bitterness" due to limonin in processed orange juice has been the subject of considerable research over the past 40 years (Maier et al., 1977). Remarkable advances in the understanding of its causes and cures have been achieved through chemical, biochemical, and enzymological studies and they are still continuing (Carter et al., 1975; Chandler et al., 1976; Guadagni et al., 1976; Hasegawa, 1976; Levi et al., 1974b). Effects of horticultural factors on juice bitterness, however, have been studied less thoroughly.

The canning industry in California, during early efforts to process single-strength orange juice, found that juice from early-season Washington navel oranges became unpalatably and persistently bitter within a few hours after canning. The problem was not observed there with other cultivars maturing later in the season. It soon became apparent that other varieties grown elsewhere in the world and on different rootstocks also produced juice with delayed bitterness.

Marsh (1953) demonstrated a clear relationship between the type of rootstock upon which the navel orange is grown and the absence or degree of delayed bitterness in the juice. Scott (1970) reported that in Florida citrus juices the component responsible for the bitterness, limonin, is less affected by rootstock than by location and growing conditions.

Scott suggested that in Florida the only orange-type fruit which produces sufficient limonin to pose a possible bitterness problem is Murcott. In Israel the Shamouti produces a very bitter juice at the beginning of its period of maturity in December (greater than 20 ppm of limonin) (Levi et al., 1974a). In Australia the navel oranges especially, and sometimes even Valencia, give a bitter juice (Chandler and Kefford, 1966).

Marrs orange is a relatively new cultivar developed from a mutation of Washington navel (Olsen, 1963). It reaches legal maturity as early as September in the South Texas citrus belt. Because of these factors, it was believed that Marrs juice might exhibit delayed bitterness. Considerable acreages of Marrs have been planted in South Texas as well as in California within the past 15 years. Increases in production, greater diversion of fruit from fresh market to processing channels, and possibly earlier harvest dates will require the industry to learn how to best utilize the juice for maximum consumer appeal and economic gain. An increasing percentage of the world's orange crop continues to be diverted into processed juice products and competition for juice markets is accelerating; hence, greater emphasis must be placed on the organoleptic quality of orange juice. The delayed bitterness problem usually denoted relative unpalatability in the infancy of the citrus-canning industry, whereas it now has a much more subtle connotation of acceptibility.

Guadagni et al. (1973) made a detailed study of individual and group bitterness thresholds for limonin in orange juice as a function of pH, total acidity, and sugar content. Bitterness is just discernible at the taste threshold and not necessarily objectionable or unpalatable. They found that the threshold of limonin bitterness varied over a wide range: the threshold of the least sensitive taste-panel judge was more than 60 times that of the most sensitive judge. A taste-panel threshold is therefore somewhat arbitrary and will exceed the taste thresholds of a certain portion of the population. It is thus not possible to precisely specify the lowest limonin concentration that would constitute a problem with economic proportions for the citrus industry. Such evaluation is best left to the processors and marketing specialist who have information on other pertinent factors. The report of Guadagni et al. (1973) does show clearly that threshold levels of limonin have a significant negative effect on preference for processed orange juice.

This report describes the limonin content of Marrs and Hamlin early oranges from five groves in the Lower Rio Grande Valley of Texas during the course of the season.

MATERIALS AND METHODS

Eight trees each of Marrs and Hamlin sweet oranges on Texas sour orange (*Citrus aurantium* L.) rootstock were selected and marked in each of five groves distributed within the citrus belt of the Lower Rio Grande Valley of Texas (~26° north latitude). Groves were fertilized, irrigated, and treated with pesticides according to the practices of good commercial management.

Seven fruit were randomly harvested from each tree at 2-week intervals beginning the last week of Sept 1972 and continuing into Jan 1973. Fruit of the same cultivar in each grove were combined to give two 56-fruit composite samples for each of the five groves.

Food Crops Utilization Research, Science and Education Administration, U.S. Department of Agriculture, Weslaco, Texas 78596.

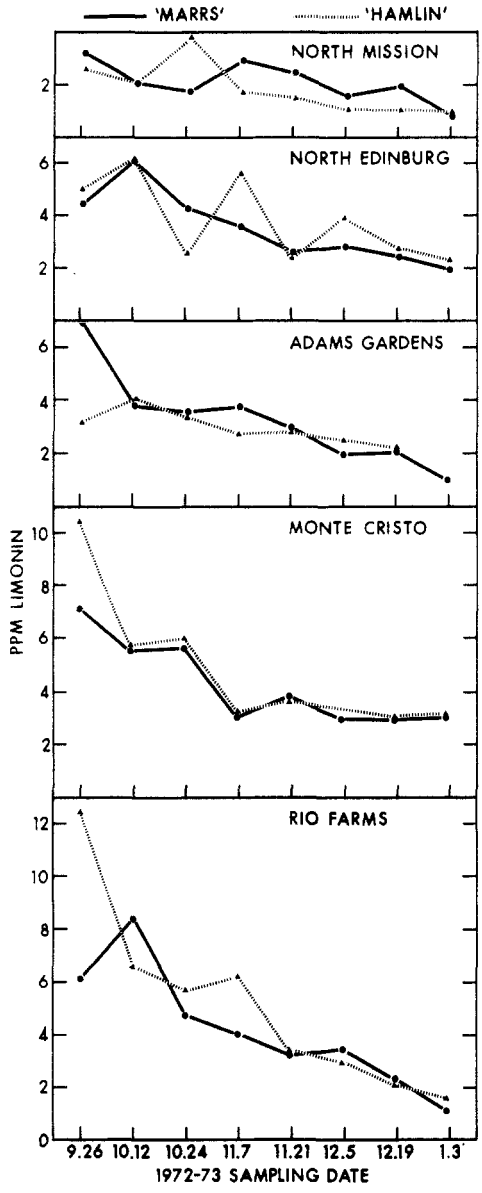

Figure 1. Seasonal trends of limonin content of processed juice from Marrs and Hamlin early oranges grown at five different groves in the South Texas citrus belt.

Each fruit sample was soaked in water, subjected to a water spray and brush rollers, and then drained. Juice was extracted with a Model 091B FMC in-line test extractor (FMC Corp., Citrus Equipment Division, Lakeland, FL), equipped with a No. 1 cup and 0.64-mm (0.025-in.) prefinisher strainer screen and beam pressure of 124 kPa (18 lb/in.^{2}). Juice direct from the extractor was deaerated, heated, refrigerated for 2 days, and frozen until analyzed.

Limonin content of each juice sample was analyzed in triplicate by the thin-layer chromatography (TLC) method of Tatum and Berry (1973), using the solvent system listed as no. 8 (benzene–hexane–acetone–acetic acid, 65:22:10:3 v/v) for a double migration. The visualizing spray was 10% sulfuric acid in ethanol. After heat treatment of the developed and sprayed TLC plate, two of us independently evaluated the amount of limonin present by visual comparisons with limonin standards. The limonin contents of the samples as determined by the two examiners were averaged. Each plate contained duplicate spots for one variety from each of the five groves sampled on a particular date, and a triplicate set of plates was run. Values from the six determinations for each juice sample were averaged. Six parts per million of limonin was selected as a nominal

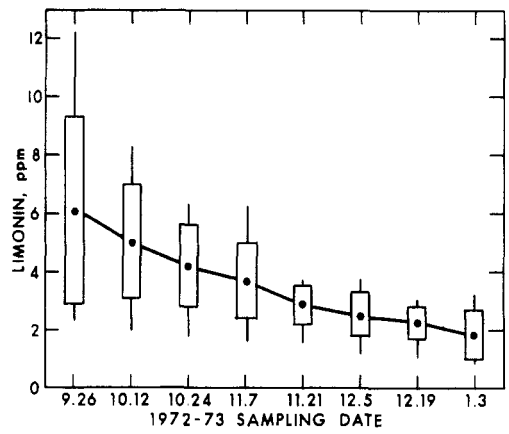

Figure 2. Seasonal trends of mean limonin content of processed juice from Marrs and Hamlin early oranges in the South Texas citrus belt. The line connects the mean limonin values for dates. The rectangular box represents ±one standard deviation. The whiskers represent the maximum and minimum values.

threshold value for bitterness, as in the work of Levi et al. (1974b). Attempts to automate the quantitation of limonin on the TLC plates by scanning reflectance spectrophotometry and fluorescence were unsuccessful.

RESULTS AND DISCUSSION

Only at the earliest four harvests did any of the samples from Marrs and Hamlin oranges grown at the five grove locations yield juice with a limonin content above the nominal threshold level of 6 ppm (Figure 1). Limonin levels were still well below those of juice from oranges grown in some other citrus production areas of the world (Kefford and Chandler, 1970; Levi et al., 1974b); they were, however, sufficiently high for a major portion of consumers to judge the juice as being bitter.

Variations between the two cultivars were mainly temporary and likely reflected short-term influences (e.g., irrigation timing) on a generally similar environment. The magnitude of the variations between the two cultivars was greatest during the early, hotter portion of the season when water stress is more likely to influence crop development. By use of the five groves as replications of the limonin determinations for the two cultivars, no significant difference was found between them. Figure 2 is a box and whisker plot of mean limonin values for the two cultivars at the various harvest dates. The mean, variability, and range of limonin concentration of juice from oranges received at the processing plant are more meaningful to processors than corresponding values for individual groves. The boxes in Figure 2 represent plus and minus one standard deviation from the mean and represent the amount of variation between groves. Variation in limonin concentration is greatest during the early season and becomes less as the season progresses.

Locational influences, although subtle, were indeed real, as indicated by the relatively close parallel between curves for the two cultivars. From other experiments on replicate grove samples, we have found the coefficient of variability for limonin to fall in the range of 0.1–6.0%. The two curves for the Monte Cristo grove (Figure 1) are especially similar.

The Adams Gardens grove has heavier soil and is in a more humid location than the other groves; yet its limonin curves were intermediate among the other four. No single location has a set of environmental conditions sufficiently unique to give a distinctive limonin curve.

The North Mission grove consistently produced fruit of both cultivars with the lowest limonin content among all groves for each sampling date. The fruit of this same grove

were generally the most mature, as indicated by the juice °Brix/acid ratio, which was consistently highest for Hamlin and predominantly so for Marrs relative to these cultivars in all of the other groves at the same harvest dates.

The Hamlin juices with the highest limonin contents (12.2 ppm for Rio Farms and 10.3 ppm for Monte Cristo) at the September harvest also had the two lowest °Brix/acid ratios (11.5 and 8.5, respectively) relative to the Hamlin juice from the other groves.

Marrs juice from the Adams Gardens grove was highest in limonin (7.0 ppm) and second lowest in the °Brix/acid ratio (14.6) at the September harvest. The limonin content was highest (8.1 ppm) and °Brix/acid ratio lowest (13.8) in fruit harvested on Oct 12th at Rio Farms.

The apparent relationship between fruit maturity and limonin content did have exceptions; thus, juice from Marrs harvested in September at the Monte Cristo grove had a limonin content of 7.0 ppm but a °Brix/acid ratio equal to the highest.

Any apparent correlations between limonin and °Brix, percent acid, or °Brix/acid ratio may be strictly fortuitous since all these variables are functions of time. Indeed, when linear regression equations were developed for the relationships between limonin and any of the other three values at a fixed harvest date, the coefficients of linear correlation, r, were relatively low, averaging for all dates 0.48 for limonin and °Brix, 0.56 for limonin and percent acid, and 0.62 for limonin and °Brix/acid ratio. When regression equations were developed for the same relationships over all dates, the r values were considerably higher, but none were as high as that for limonin vs. time: $r = 0.99$ for Marrs ($y = 6.57 - 0.04x$), and $r = 0.95$ for Hamlin ($y = 7.08 - 0.05x$) where y = ppm of limonin and x = days after Sept 1st.

Marsh (1953) found that sour orange rootstock was intermediate in its ability to impart bitterness to the juice of navel oranges in coastal southern California. In the warmer climate of South Texas, sour orange rootstock appears not to impart excessive bitterness to juice from either Marrs or Hamlin oranges.

It is difficult enough to deduce rootstock influences at a single location and dangerous to extend such deductions to different scion varieties and/or different locations. Thus, in California (Marsh, 1953) and Australia (Kefford and Chandler, 1970), the rough lemon rootstock was found to be associated with navel orange juice with an early season delayed bitterness problem, and rootstock trials in Pakistan (Mahmood et al., 1975) showed rough lemon rootstocks with Jaffa or Valencia scions produced juice with a higher limonin content than four other rootstocks tested. In Florida, however, where a large number of plantings are on rough lemon, bitterness does not appear to be a serious problem (Scott, 1970). Clearly, any rootstock trials for climate adaptability should include tests for limonin delayed bitterness as a juice quality factor.

A major problem in the comparison of limonin levels in juices collected at different times and locations thus far has been the failure to standardize on extraction conditions which also approximate commerical operations. Many other factors undoubtedly are involved in determining ultimate juice limonin content, such as precursor content of the tissues, peel thickness, tissue strength, fragment size, pulp–juice contact time, extraction pressure (Carter et al., 1975), etc.

A commercial test extractor such as the one employed here can be utilized in other places and approximates commercial practices. How close the limonin values come to those found in commercial citrus packs will depend upon the degree to which commercial operations differ from test extraction procedures. Levi et al. (1974b) have made proposals for adjusting commerical extractors to minimize juice limonin content.

How limonin content of juice varies from year to year is largely unknown. During the 1973–1974 crop year, samples of Marrs and Hamlin juice obtained from Adams Gardens fruit were analyzed monthly. Between the corresponding dates of the 1972–1973 season, the maximum difference in limonin content was only 1.3 ppm with Marrs and 0.2 ppm with Hamlin.

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