Effect of Juice Extractor Settings on Juice Cloud Stability

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Juice was extracted from Valencia oranges using three different extractor settings. Differential juice cloud stability was observed. Soft-extracted juice was the most stable, and hard-extracted juice was the least stable. The medium-extracted juice had intermediate cloud stability. Yearly (1997 versus 1998) differences were observed, but the relationship among the juices did not change. Addition of protein extracts, obtained from each juice, to pasteurized juice also resulted in differential cloud stability. Using pectinmethylesterase (PME) activity estimated at pH 4.5, the effects of the protein extract mirrored results from raw juice. Estimating PME activity at pH 7.5 produced contradictory results, indicating that predicting consequences of PME activity estimated at pH 7.5 is unreliable.

Keywords: *Citrus; juice processing; orange juice; juice cloud; cloud stability*

INTRODUCTION

The majority of citrus fruit harvested in the United States is used by processors for juice extraction (Florida Citrus Mutual, 1998). Processors can manipulate commercial juice extractors in numerous ways to alter the yield and quality of the expressed juice. One juice characteristic influenced by extractor settings is the amount of fruit peel material incorporated into the juice (Swift, 1951; Swift and Veldhuis, 1957). Properties of one juice constituent, the enzyme pectinmethylesterase (PME), influence several aspects of juice processing. Unstabilized juice contains significant PME activity, which can lead to clarification [see Guyer et al. (1956), Bissett et al. (1957), and Carroll et al. (1957) for a discussion and definition of juice cloud clarification]. Precipitation of juice cloud results in an unattractive sediment and a clear serum, which is essentially flavorless. PME creates a blockwise distribution of free galacturonic acid (GA) residues in the homogalacturonic acid region of soluble pectin by its sequential hydrolysis of C-6 methyl esters of GA. This action, if allowed to proceed in juice, yields low-methoxyl pectins with sequences of free acid groups. When the proportion of free acid groups reaches a critical level, such pectins become susceptible to cross-linking with divalent cations such as calcium (Baker and Bruemmer, 1969; Baker, 1979). Cross-linking increases the pectin apparent molecular weight, which reduces solubility, thereby leading to flocculation. Precipitation of pectins in this manner was presumed to occlude cloud particulates and remove them from suspension (Stevens et al., 1950; Joslyn and Pilnik, 1961). However, there is some evidence that PME may also act on insoluble pectins at the surface of cloud particulates (Yufera et al., 1965) and that an active binding of cloud particulates to flocculating pectins may occur (Baker and Bruemmer, 1972).

When low levels of low-methoxyl pectins are present in juice, calcium cross-linking results in flocculation; at higher pectin concentrations it can lead to gelation of juice concentrate (Olsen, 1951). Depending on pectin concentration and demethylation level, this gelation can range from simple curdiness to complete, firm gel formation. Such gels constitute a product defect by hindering reconstitution and may interfere with concentrate pumping and blending. To prevent these serious problems, citrus juices are pasteurized at temperatures ranging from 90 to 95 °C and for holding times of 15−60 s (Chen et al., 1993).

In previous studies we have demonstrated that forms of PME present in Valencia orange fruit peel tissue destabilized juice cloud of pasteurized, reconstituted orange juice more rapidly than PME extracts from rag (intersegmental septa, squeezed juice sacs, and fruit core tissue) or hand expressed juice (Cameron et al., 1997). Additionally, we were able to demonstrate that at least four forms of PME were present in peel tissue and that two of them, one thermally labile and one thermally tolerant, were able to rapidly destabilize juice cloud at 4 °C (Cameron et al., 1998). Results obtained from these studies suggested that PME forms present in fruit peel tissue would be especially detrimental to juice stability and quality. Therefore, it may be advantageous to the juice processor to set extractor conditions so that a minimum of fruit peel tissue is incorporated into the juice.

The purpose of this study was to determine (1) if extractor settings affected cloud stability of raw, unpasteurized juice and (2) if the total, salt extractable PME present in juices obtained from different extraction conditions (i.e., soft, medium, and hard juice extraction) caused differential effects on juice cloud of pasteurized, reconstituted orange juice.

MATERIALS AND METHODS

Juice Extraction. Field-run Valencia oranges (late season, May 27, 1997, and May 12, 1998) were purchased from a local growers' association (Haines City Citrus Growers Association, Haines City, FL). The fruit was washed and juice was extracted with an FMC model 291 juice extractor and an FMC model 35 finisher (0.40 in.). A $^5/_8$ in. long bore orifice tube and $^1/_8$ in. beam was used for the soft extraction (soft-extracted juice = SEJ), a $^7/_{16}$ in. long bore orifice tube and $^3/_4$ in. beam was used for the medium extraction (medium-extracted juice =

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MEJ), and a $^{7}/_{16}$ in. long window tube with $^{1}/_{8}$ in. beam was used for the hard extraction (hard extracted juice = HEJ).

PME Extraction. Total salt extractable proteins were obtained from 16 L of each juice sample (Cameron et al., 1997). Briefly, juice was brought to 0.1 M Tris Base/1.0 M NaCl and then adjusted to pH 8.0 with solid NaOH. This solution was stirred overnight at 5 °C. The following morning the juice was filtered through four layers of cheesecloth to remove large particulates and then centrifuged at 12000*g* for 30 min at 4 °C. The supernatant was decanted, pooled, and then brought to 75% saturation with solid ammonium sulfate, stirred overnight at 5 °C, and then centrifuged as described above. The resulting pellets were solubilized in 10 mM Tris, pH 7.5 (at 31 °C), 20 mM NaCl, and 0.02% sodium azide (w/v). The solubilized protein was dialyzed (6000 Da molecular weight cutoff dialysis tubing) exhaustively at 5 °C against 4 L of solubilization buffer, with a total of four buffer changes. A precipitate that formed during dialysis was removed by centrifugation as previously described.

Enzyme Activity Assays. PME activity in the protein extracts was estimated according to the method of Cameron and Grohmann (1996) using a kinetic microplate method (Cameron et al., 1992) at pH 7.5 ($n = 3$) and 0.05% (w/v) citrus pectin (degree of methylation = 72%, Sigma). Activity of PME also was estimated at pH 4.5 ($n = 9$) by adapting the colorimetric method of Vilarino et al. (1993) to a kinetic microplate reader. All solutions were adjusted to pH 4.5 just prior to use. The final reaction mixture contained 0.05% (w/v) citrus pectin, 61.8 μg·mL^{-1} bromocresol green, and 0.2 M NaCl.

Juice Cloud Stability. Raw juice was brought to 0.02% (w/v) sodium azide, and 1.74 g·L^{-1} potassium metabisulfite was added. Juice samples were stored in glass bottles at both 4 and 30 °C. At selected times the juice was sampled and juice cloud stability was determined as previously described (Cameron et al., 1997) by monitoring absorbance at 660 nm.

Pasteurized frozen concentrated orange juice (FCOJ) was obtained from a local processor (Citrus World, Lake Wales, FL) and reconstituted to 11 °Brix with deionized water plus one of the PME-containing extracts from either the SEJ (soft PME), MEJ (medium PME), or HEJ (hard PME). For PME activity estimated at pH 7.5, concentrations of 1, 0.5, and 0.25 unit·mL^{-1} PME (1 unit is the volume required to release 1 μequiv·min^{-1}) were added to the juice. Only 0.5 and 0.25 unit·mL^{-1} were added when using the pH 4.5 PME activity estimate. Preliminary experiments indicated that when using pH 4.5 activity estimates of 1 unit·mL^{-1} and pH 7.5 estimates of 2.5 unit·mL^{-1} destabilized the juice cloud too rapidly to be able to ascertain differences between the treatments. In addition to the PME-containing extract the reconstituted FCOJ was made to contain 0.02% (w/v) sodium azide and 1.74 g·L^{-1} potassium metabisulfite. At selected times juice was sampled as previously described (Cameron et al., 1997).

RESULTS

Cloud loss in raw juice was most rapid in the HEJ and MEJ (Figures 1 and 2). Cloud was most stable in the SEJ, whereas MEJ had a cloud stability intermediate between those of HEJ and SEJ in 1997 but was very similar to that of HEJ in 1998. These results were more pronounced when samples were incubated at 30 versus 4 °C (Figures 1 and 2). In fact, during the 1998 season no cloud destabilization occurred at 4 °C in any of the juices. PME activities in these juices, after homogenization and adjustment of the pH to 7.5, were very similar (Table 1). PME activity in juice adjusted to pH 4.5 was qualitatively present but at levels too low for quantitation.

Estimation of PME activity in salt extractable proteins obtained from hard, medium, and soft juice extraction demonstrated differential pH effects on PME activity (Table 1). The ratio of pH 4.5 to pH 7.5 enzyme activity was nearly identical for the hard and medium

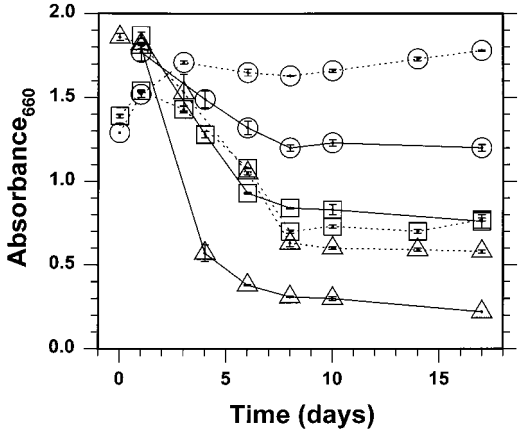

Figure 1. Cloud stability of raw juices incubated at 30 °C: (—) 1997; (- - -) 1998; (○) soft; (□) medium; (△) hard.

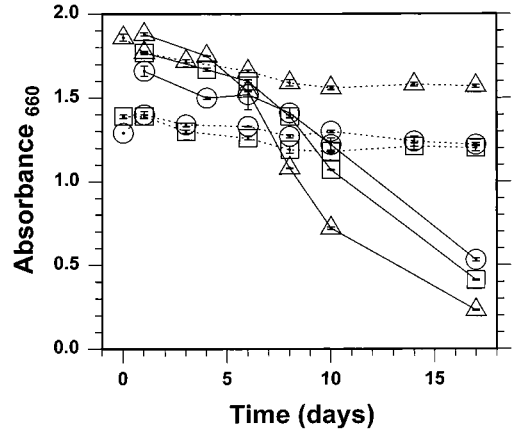

Figure 2. Cloud stability of raw juices incubated at 4 °C. Lines and symbols are as in Figure 1.

Table 1. PME Activity in Raw Juice and Protein Extracts from Soft, Medium, and Hard Juice Extraction of Valencia Oranges

		activity (μequiv·min^{-1}·mL^{-1})		
sample	juice extraction	pH 4.5	pH 7.5	4.5/7.5 × 100 (%)
raw	soft	NM[a]	1.1 ± 0.01	
	medium	NM	1.4 ± 0.01	
	hard	NM	1.2 ± 0.01	
protein extract	soft	23.9 ± 0.6	49.1 ± 3.6	48.7
	medium	27.9 ± 0.6	89.7 ± 2.5	31.1
	hard	26.9 ± 0.6	91.9 ± 3.7	29.3

[a] NM, not measurable.

PMEs (0.29 and 0.31, respectively). However, the soft PME ratio was 0.49, nearly 40% greater than the hard or medium PME.

The addition of PME-containing extract to pasteurized, reconstituted FCOJ at 0.25 and 0.5 unit·mL^{-1} (activity estimated at pH 4.5) destabilized the juice cloud (Figures 3 and 4). At both 0.25 and 0.5 unit·mL^{-1} the most rapid destablization was with addition of PME from the HEJ (hard PME). PME from the SEJ (soft PME) had the least effect on cloud stability. Differences among the three PME extracts were most evident at 4 °C with 0.5 unit·mL^{-1} (Figure 3). At 0.25 unit·mL^{-1} and 4 °C the cloud of all samples remained stable over 23 days (Figure 4). Settling pulp in these samples increased concomitantly with the decrease in juice cloud (Figure 5).

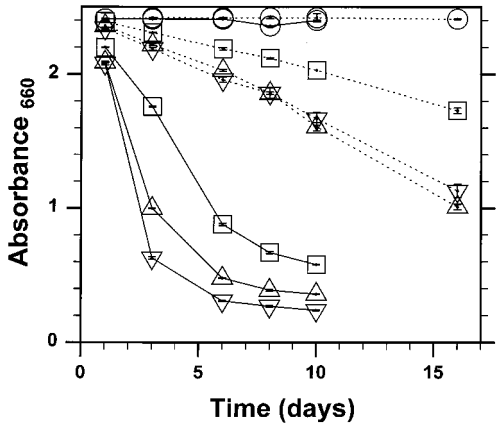

Figure 3. Cloud stability at 30 °C of pasteurized, reconstituted frozen concentrated orange juice to which PME-containing protein extracts from soft, medium, or hard juice extraction had been added: (- - -) 0.25 unit·mL^{-1}; (—) 0.5 unit·mL^{-1}; (○) control; (□) soft; (△) medium; (▽) hard. PME activity was estimated at pH 4.5.

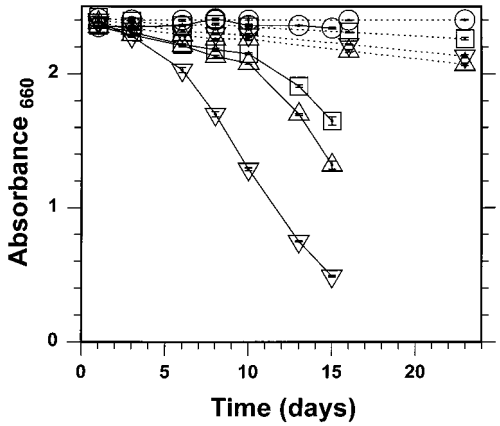

Figure 4. Cloud stability at 4 °C of pasteurized, reconstituted frozen concentrated orange juice to which PME-containing protein extracts from soft, medium, or hard juice extraction had been added. PME activity was estimated at pH 4.5. Lines and symbols are as in Figure 3.

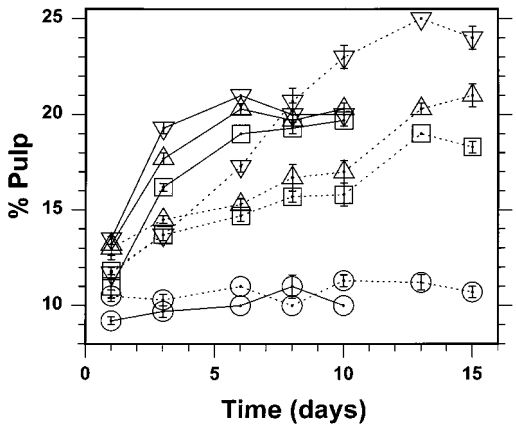

Figure 5. Settling pulp in pasteurized, reconstituted frozen concentrated orange juice to which PME-containing protein extracts from soft, medium, or hard juice extraction had been added at 0.5 unit·mL^{-1}: (—) 30 °C; (- - -) 4 °C; symbols are as in Figure 3. PME activity was estimated at pH 4.5.

Adding 1.0 or 0.5 unit·mL^{-1} PME activity estimated at pH 7.5 to pasteurized, reconstituted FCOJ produced different results than when using pH 4.5 activity estimates (Figures 6 and 7). At 1.0 unit·mL^{-1} the soft

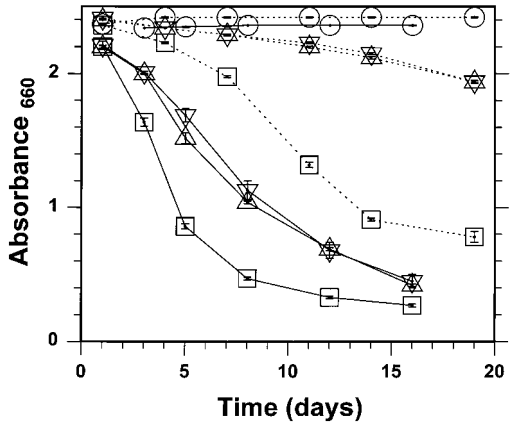

Figure 6. Cloud stability at 30 °C of pasteurized, reconstituted frozen concentrated orange juice to which PME-containing protein extracts from soft, medium, or hard juice extraction had been added: (—) 1.0 unit·mL^{-1}; (- - -) 0.5 unit·mL $^{-1}$; symbols are as in Figure 3. PME activity was estimated at pH 7.5.

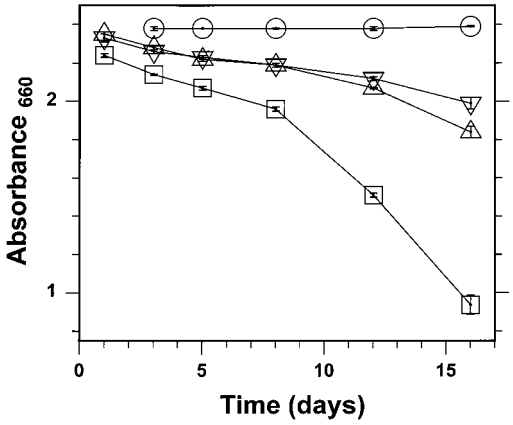

Figure 7. Cloud stability at 4 °C of pasteurized, reconstituted frozen concentrated orange juice to which PME-containing protein extracts from soft, medium, or hard juice extraction had been added at 1.0 unit·mL^{-1}. PME activity was estimated at pH 7.5. Symbols are as in Figure 3.

PME destabilized the juice cloud more rapidly than the hard or medium PME (Figure 6). Decreasing the activity level to 0.5 unit·mL^{-1} slowed the cloud destabilization, but the soft PME still produced the most rapid effect (Figure 6).

DISCUSSION

Data presented here indicate cloud stability in raw juice is dependent on juice extractor settings. Juice (both 1997 and 1998 seasons) extracted under mild conditions (SEJ) had a more stable cloud than juice extracted under harsher conditions (HEJ or MEJ). Juice extracted under intermediate conditions (MEJ) had an intermediate cloud stability (more evident during the 1997 season). Cloud stability differences in raw juice are not due to total PME activity present. MEJ contained greater amounts of PME activity (estimated at pH 7.5), but the cloud was more or as stable as HEJ. SEJ contained either the same amount (1998 season) or 92% (1997 season) of the activity (estimated at pH 7.5) present in HEJ, but cloud in the SEJ never destabilized at 30 °C. At 4 °C different results were obtained. During the 1997 season cloud in all treatments was destabilized, whereas juice cloud from the 1998 season remained stable throughout the study period.

Estimating PME activity in salt-extracted protein preparations at pH 4.5 and using them to provide reconstituted orange juice with a known activity level demonstrated that cloud stability is dependent on the PME source. These results parallel those obtained with raw juice. The minimal effect on juice cloud (most stable cloud) was observed with PME from the SEJ. PME from HEJ destabilized the juice cloud most rapidly.

The dramatic difference in effect on cloud stability comparing PME activity estimated at pH 7.5 versus 4.5 was unexpected. However, on inspection of Table 1 it is clear that PME activity estimated at pH 7.5 is not equivalent to activity estimated at pH 4.5. Published pH curves for citrus PME demonstrate pH optima at near neutral pH values, with dramatic decreases below pH 5 (Cameron and Grohmann, 1995). Differences in pH optima also have been reported for multiple forms of PME from tomato (Warrilow and Jones, 1995). More recently, Catoire et al. (1998) have suggested that pH may affect the mode of action and demethylation patterns of different isoforms of PME. In addition, the reduction in activity at pH 4.5 compared to 7.5 is not equal in the PMEs extracted from soft-, medium-, and hard-extracted juice. Whereas only 31 and 29% of the pH 7.5 activity was present at pH 4.5 for the hard and medium PMEs, respectively, the soft PME retained 49% of its pH 7.5 activity. Consequently, addition of the PME extracts, based on activity estimated at pH 7.5, to reconstituted orange juice (pH 3.9) would result in only a fraction of the intended activity remaining. Juice containing the soft PME would retain a greater proportion of the added PME activity than juice to which medium or hard PME had been added. This may account for the observed results in which the soft PME caused the most rapid cloud loss. One other possible factor may be related to the nature of raw juice versus reconstituted FCOJ. Settling pulp in raw juice ranged from 14 to 15% (SEJ and MEJ) to 19% (HEJ, unpublished data); the reconstituted FCOJ had only 9−11% settling pulp (Figure 5).

Results from this study suggest that extracting juice under harsh conditions, which increases the amount of peel content in juice, may precondition the juice for subsequent quality defects related to cloud stability and gelation, especially with improper handling. Additionally, PME activity estimates at neutral pH will not provide accurate or reliable indications of what will occur in juice at acidic pH.

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