Citrus Tissue Extracts Affect Juice Cloud Stability

RANDALL G. CAMERON, ROBERT A. BAKER, and KAREL GROHMANN

ABSTRACT

Salt extractable proteins were isolated from hand expressed juice, rag and peel of Valencia oranges. Each tissue extract was divided into dialysis supernatant (DS) and precipitate (DP) (forms during dialysis). DP, DS and DS heated for 2 min at 80°C (HDS) were added to pasteurized, reconstituted frozen concentrated orange juice at 5 U · mL^{-1} of pectinmethylesterase. Samples were incubated either at 25°C for 14 days or 4°C for 28 days and periodically sampled to determine the effects of tissue extracts on juice cloud stability. Tissue specific differences were observed for the rate of juice cloud precipitation and among the HDS, DS, and DP fractions of a given tissue. HDS fractions destabilized juice cloud more rapidly than DS or DP at both 25 and 4°C.

Key Words: citrus, juice cloud, cloud stability, pectinmethylesterase, pectin

INTRODUCTION

SINCE THE INTRODUCTION of frozen concentrated orange juice (FCOJ) in the 1940s, a major quality defect has been juice cloud precipitation. Early studies indicated that pectinmethylesterase (PME; EC 3.1.1.11) initiates a sequence of events that leads to juice cloud destabilization (Joslyn and Sedky, 1940; Stevens et al., 1950; Guyer et al., 1956). Hydrolysis of methyl esters attached to C6 of galacturonic acid moieties in the main pectin chain produces free acid groups. When sufficient divalent cations such as calcium are present, these cations crosslink acid functions on adjacent pectin chains, reducing their solubility leading to juice cloud precipitation (Stevens et al., 1950; Joslyn and Pilnik, 1961; Baker and Bruemmer, 1969).

Multiple forms of PME have been identified in various citrus fruits (Versteeg et al., 1980; Seymour et al., 1991; Macdonald et al., 1993, 1994; Cameron et al., 1994; Cameron and Grohmann, 1995, 1996). One form, purified from a commercial 'Fresh Frozen Orange Juice' was relatively thermally stable, retaining ~50% activity after 60 sec incubation in a 90°C water bath (Cameron and Grohmann, 1996). Thermally tolerant forms of PME (TT-PME) also have been identified from navel oranges (Versteeg et al., 1980), white grapefruit (Seymour et al., 1991), citrus tissue culture cells (Cameron et al., 1994), lemon fruit (Macdonald et al., 1994), and red grapefruit (Cameron and Grohmann, 1995). Versteeg et al. (1980) reported that at 5°C only a TT-PME was capable of destabilizing juice cloud in <4 wk (see Guyer et al., 1956, Bissett et al., 1957 and Carroll et al., 1957 for discussion and definition of juice cloud clarification). Rouse (1953) demonstrated that PME activity was present in all fruit tissues of Valencia oranges and that juice sacs contained ~75% of total PME activity. Rombouts et al. (1982), using nonequilibrium pH gradient electrophoresis and activity prints, reported a PME with the same Rf as TT-PME studied by Versteeg et al. (1980) in all tissues of Navel oranges. However, Rombouts et al. (1982) did not determine the levels of TT-PME activity in the various tissues.

Snir (1996) reported data suggesting that the method of juice extraction may affect the level of TT-PME in the juice. No data are available on the amounts of TT-PME present in various citrus fruit tissues or whether any TT-PME's that might occur would be equally effective at destabilizing citrus juice cloud. Therefore our objective was to detemine the effects of fruit tissue extracts on juice cloud stability.

The authors are with the USDA, ARS, South Atlantic Area, Citrus and Subtropical Products Laboratory, 600 Avenue S, N.W. (P.O. Box 1909), Winter Haven, FL 33883-1909.

MATERIALS & METHODS

Fruit tissue extracts

Valencia oranges (1.5 bushels) were purchased from a local supplier on 21 Feb 1995, the beginning of the harvesting season for that cultivar. The oranges were halved and the juice was hand expressed using a kitchen-type juicer. A total of 17.8 L of juice was collected. Rag, composed of intersegmental septa, squeezed juice sacs and fruit core tissue, was then separated (4.2 kg) from peel (flavedo and albedo, 5.9 kg). The rag was rinsed with deionized water to remove residual juice and frozen at −20°C until needed. Peel was ground with a meat grinder and frozen at −20°C until needed.

Juice was brought to 0.1M Tris and 1M NaCl, the pH adjusted to 8.0 with solid NaOH and stirred overnight at 5°C. Juice (8L) was brought to 75% ammonium sulfate saturation and stirred overnight at 4°C. After centrifugation in 250 mL bottles at 12,100 × *g* for 30 min at 4°C, the supernatant was discarded (no PME activity) and 50 mL of 10 mM Tris, pH 7.5 (at 31°C), 20 mM NaCl (TBS) was added to each bottle to solubilize precipitated proteins. Insoluble material was still present, so the suspension was re-centrifuged (as above) to remove this material. The supernatant (containing PME activity) was decanted and frozen at −75°C in 50 mL aliquots. The remaining juice (9.8L) was centrifuged at 12,100 × *g* for 15 min at 4°C to remove insoluble materials. Ammonium sulfate was added to the resulting serum to 75% saturation, stirred overnight at 4°C, and then centrifuged as described. The supernatant was discarded and protein pellets solubilized as described. The solubilized protein was dialyzed (6000 dalton molecular weight cut-off dialysis tubing) exhaustively at 6°C against 3L of solubilization buffer, with a total of 4 buffer changes. During dialysis a precipitate (DP) formed (Cameron and Grohmann, 1996) which was separated from the dialysis supernatant (DS) by centrifugation at 12,100 × *g* for 30 min at 4°C. The juice DS was then pooled and concentrated to a final volume of 90 mL using a 10,000 dalton molecular weight cut-off hollow fiber cartridge. The juice DP was solubilized in a total volume of 200 mL of 10 mM Tris, 1M NaCl, pH 7.5. The solubilized juice DP was centrifuged at 12,100 × *g* for 15 min at 4°C to remove any remaining insoluble material. The supernatant was decanted (contained PME activity), pooled and concentrated to 70 mL with a hollow fiber cartridge as described. The concentrated juice DP was frozen at −75°C in 50 mL aliquots.

Rag was mixed with 2 volumes of 0.1M Tris, pH 8.0 at 27°C, 1M NaCl (w/v; 1 kg rag plus 2L buffer) and homogenized (~5 min) in a pre-chilled blender (in a 4°C room). The rag homogenate was stirred overnight at 4°C. It was then filtered through four layers of cheesecloth. The filtrate was centrifuged at 12,100 × *g* for 45 min at 4°C to remove any remaining insoluble material. The supernatant was stored overnight at 4°C. The rag material retained by the cheesecloth was mixed with extraction buffer as described and stirred overnight at 4°C. After filtering through cheesecloth and centrifugation both rag extracts were pooled and brought to 75% ammonium sulfate saturation. After stirring overnight at 4°C the precipitated proteins were removed by centrifugation at 12,100g for 30 min at 4°C. Proteins in the pellet were solubilized in TBS and dialyzed vs 4L TBS overnight at 6°C with a total of four buffer changes. The precipitate formed during dialysis was removed by centrifugation at 27,200 × *g* for 30 min at 4°C. The supernatant (rag DS) was pooled and frozen at −75°C. The pellet (rag DP) was solubilized as described for juice DP, pooled and frozen at −75°C. Both rag DS and rag DP were then concentrated with a hollow fiber cartridge as described.

Ground peel was mixed with 3 volumes (w/v) of extraction buffer and homogenized (~5 min) in a blender at 4°C. After stirring overnight at 4°C it was filtered through two layers of cheesecloth. The filtrate was pooled and stored at 4°C. Recovered peel tissue was mixed with 3 volumes of extraction buffer and further homogenized (~3 min) with a

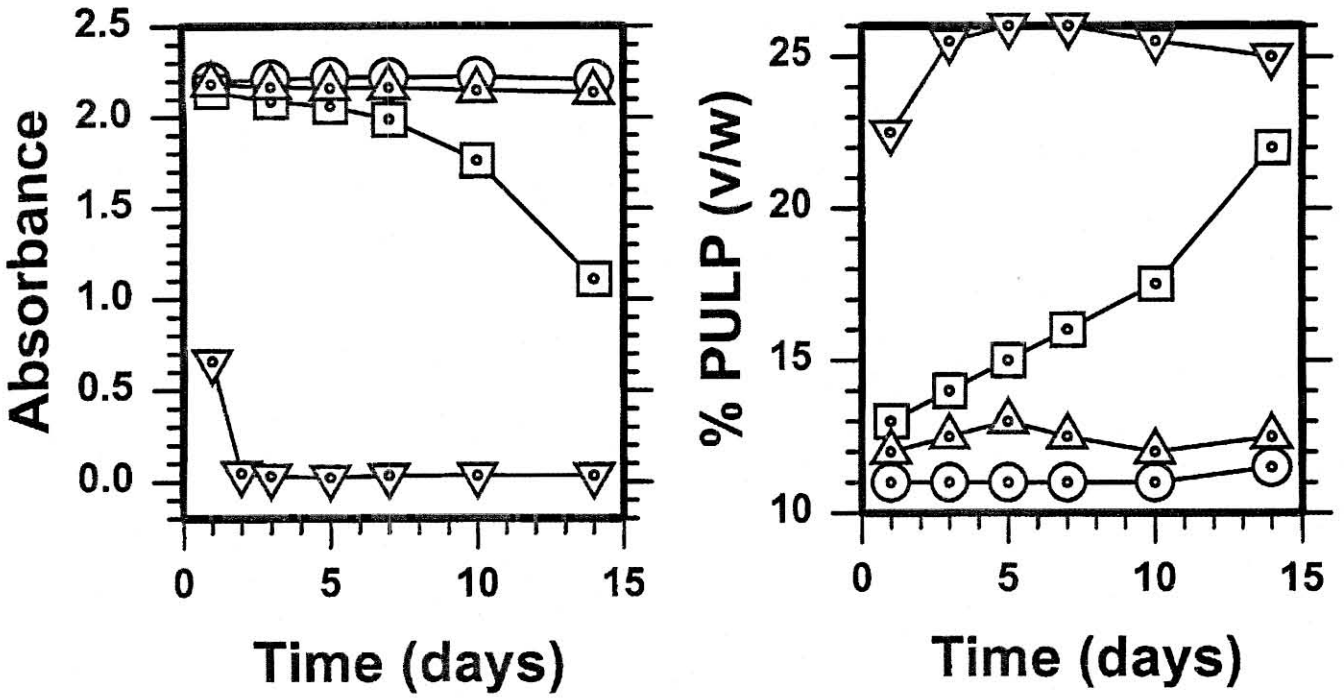

Fig. 1—Effects of peel extracts on juice cloud stability at room temperature. Absorbance was measured at 660 nm and % pulp = settling pulp. ○ = control (reconstituted FCOJ only), □ = DS, △ = DP, ▽ = HDS. Absence of error bars indicated the S.E. was contained within the symbol.

Polytron. Homogenized peel tissue was again stirred overnight at 4°C and filtered through cheesecloth. Filtrates from the first and second extraction were pooled and centrifuged at 12,100 × *g* for 30 min at 4°C. Peel oil was aspirated from the top of the centrifuge bottles and discarded. Extracted peel proteins were precipitated with 75% ammonium sulfate saturation and overnight stirring at 4°C. Precipitated proteins were removed by centrifugation at 12,100 × *g* for 30 min at 4°C. The proteins were solubilized in TBS and dialyzed overnight vs 4L TBS with four buffer changes. The peel DS was pooled and frozen at −75°C in 50 mL aliquots. The peel DP was removed and solubilized as described and frozen at −75°C in 50 mL aliquots. Both peel DS and peel DP were concentrated with hollow fiber cartridges as described.

Enzyme and protein assays

Quantitative assays were performed according to Cameron et al. (1992) utilizing a kinetic microplate reader. Activity (one Unit = 1 μEq acid · min^{-1} · μL^{-1}) was estimated for each concentrated tissue extract before and after 2 min incubation in an 80°C water bath, which inactivated all but TT-PME (=HDS, activity which remained after 2 min incubation), (Cameron and Grohmann, 1995). The microplates were read every 15 sec for 20 min. Reported values are the mean ± standard error (P = 0.05) for a minimum of three replicates.

Protein amounts were estimated by the method of Bradford (1976) with BSA as standard.

Juice cloud stability

To determine the effects of tissue extracts on juice cloud stability, pasteurized FCOJ was obtained from a local processor (Citrus World, Lake Wales, FL). The concentrate was reconstituted to 11 °Brix with deionized water plus the appropriate amount of tissue extract to provide 5 U · mL^{-1} of PME activity (final volume = 200 mL). The reconstituted juice also contained 0.02% sodium azide (to prevent microbial contamination) and 2.18*g* · L^{-1} potassium metabisulphite.

To determine the effects of only the TT-PME on cloud stability the appropriate volume of each extract, to give a final activity of 5 U · mL^{-1}, was incubated, in 150 μL aliquots, for 2 min in a 80°C water bath (Cameron and Grohmann, 1995) and then mixed with the reconstituted FCOJ (final volume = 200 mL). The samples with PME added were placed in glass bottles and stored on the laboratory bench (room temperature ~25°C) or in a refrigerator (4°C). Three samples per treatment were taken from each bottle per time interval by inverting the bottle three times, pipetting 10 mL into a 15 mL graduated, conical centrifuge tube, and then centrifuging for 10 min at 360 × *g*. One mL of supernatant from each tube was transferred to a cuvette and the absorption at 660_{nm} was recorded (Krop, 1974). The amount of settling pulp was estimated by reading the volume occupied by the pelleted solids. Reported means ± standard errors (P = 0.05) are for three replicates.

RESULTS

BASED ON SPECIFIC ACTIVITY, the data indicated (Table 1) that rag DS contained the greatest amount of total and TT-PME activity (54.6% and 47.3% respectively). Juice DP and rag DP contained nearly equal amounts of total PME activity (15.3% and 14.1% respectively) although juice DP had 10 times more TT-PME specific activity than rag DP (34.8% vs 2.5%). Juice extracts contained the greatest proportion of total specific activity as TT-PME activity.

Tissue specific differences for juice cloud destabilization were observed at room temperature (Fig. 1 to 3) and at 4°C (Fig. 4 to 6). The most rapid destabilization at room temperature occurred when peel HDS (Fig. 1) or juice HDS (Fig. 3) was added to pasteurized juice. The TL-PME's comprised 96.7% and 95.8% of the total PME activity for peel DS and juice DS respectively (Table 1). Although juice treatments were clarified by peel HDS and juice HDS after 2 days, peel HDS destabilized juice cloud faster than juice HDS as indicated by the one day sampling points (Figs. 1, 3). Peel DP had no effect on juice cloud stability and peel DS had only a marginal effect through the 14 day sampling (Fig. 1). Juice DS also had no effect but juice DP (Table 1, 94.4% TL-PME) destabilized the cloud nearly as fast as juice HDS (Fig. 3).

All treatments using rag extracts destabilized juice cloud (Fig. 2). As with peel and juice extracts, the most rapid destabilization was produced by rag HDS (TT-PME). However, its action was slower than peel HDS or juice HDS (Fig. 1 to 3). The rag DS (97.9% TL-PME) and rag DP (99.4% TL-PME) both destabilized cloud slightly slower than rag HDS. In all cases the increase in settling pulp occurred with, or slightly before, the decrease in absorbance of the supernatant (data not shown).

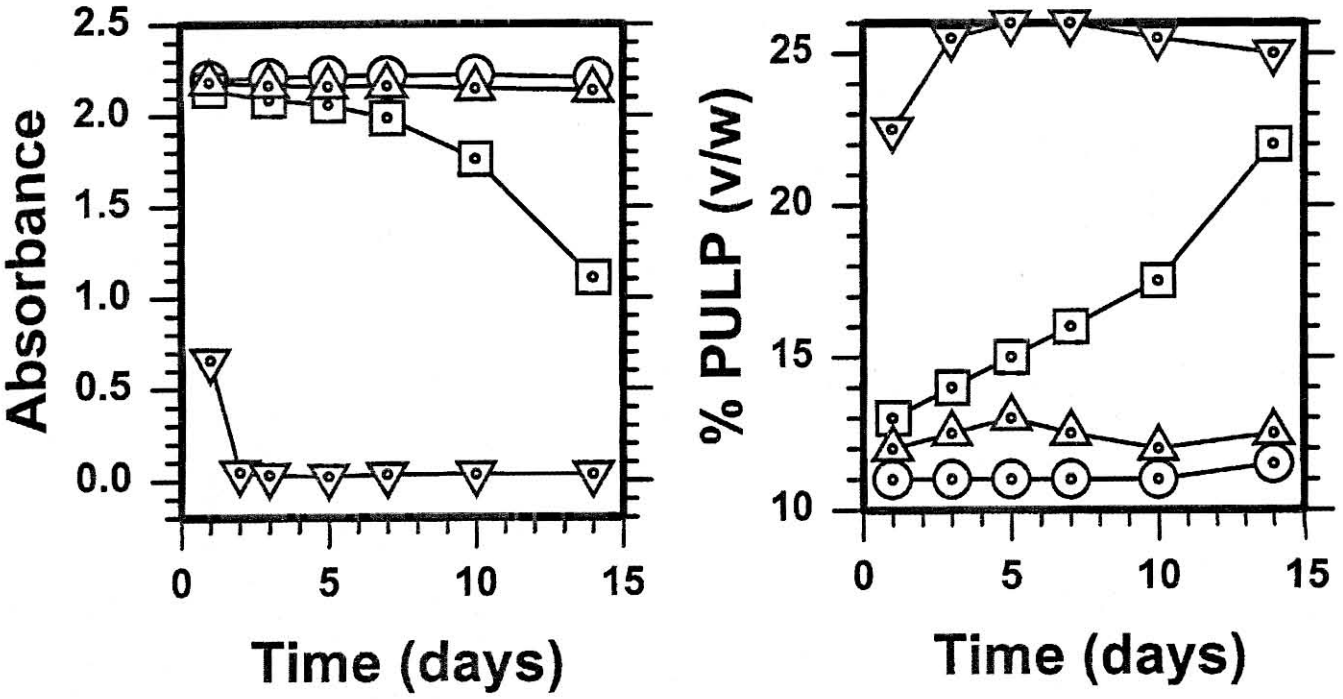

Fig. 1—Effects of peel extracts on juice cloud stability at room temperature. Absorbance was measured at 660 nm and % pulp = settling pulp. ○ = control (reconstituted FCOJ only), □ = DS, △ = DP, ▽ = HDS. Absence of error bars indicated the S.E. was contained within the symbol.

Polytron. Homogenized peel tissue was again stirred overnight at 4°C and filtered through cheesecloth. Filtrates from the first and second extraction were pooled and centrifuged at 12,100 × *g* for 30 min at 4°C. Peel oil was aspirated from the top of the centrifuge bottles and discarded. Extracted peel proteins were precipitated with 75% ammonium sulfate saturation and overnight stirring at 4°C. Precipitated proteins were removed by centrifugation at 12,100 × *g* for 30 min at 4°C. The proteins were solubilized in TBS and dialyzed overnight vs 4L TBS with four buffer changes. The peel DS was pooled and frozen at −75°C in 50 mL aliquots. The peel DP was removed and solubilized as described and frozen at −75°C in 50 mL aliquots. Both peel DS and peel DP were concentrated with hollow fiber cartridges as described.

Enzyme and protein assays

Quantitative assays were performed according to Cameron et al. (1992) utilizing a kinetic microplate reader. Activity (one Unit = 1 μEq acid · min^{-1} · μL^{-1}) was estimated for each concentrated tissue extract before and after 2 min incubation in an 80°C water bath, which inactivated all but TT-PME (=HDS, activity which remained after 2 min incubation), (Cameron and Grohmann, 1995). The microplates were read every 15 sec for 20 min. Reported values are the mean ± standard error (P = 0.05) for a minimum of three replicates.

Protein amounts were estimated by the method of Bradford (1976) with BSA as standard.

Juice cloud stability

To determine the effects of tissue extracts on juice cloud stability, pasteurized FCOJ was obtained from a local processor (Citrus World, Lake Wales, FL). The concentrate was reconstituted to 11 °Brix with deionized water plus the appropriate amount of tissue extract to provide 5 U · mL^{-1} of PME activity (final volume = 200 mL). The reconstituted juice also contained 0.02% sodium azide (to prevent microbial contamination) and 2.18*g* · L^{-1} potassium metabisulphite.

To determine the effects of only the TT-PME on cloud stability the appropriate volume of each extract, to give a final activity of 5 U · mL^{-1}, was incubated, in 150 μL aliquots, for 2 min in a 80°C water bath (Cameron and Grohmann, 1995) and then mixed with the reconstituted FCOJ (final volume = 200 mL). The samples with PME added were placed in glass bottles and stored on the laboratory bench (room temperature ~25°C) or in a refrigerator (4°C). Three samples per treatment were taken from each bottle per time interval by inverting the bottle three times, pipetting 10 mL into a 15 mL graduated, conical centrifuge tube, and then centrifuging for 10 min at 360 × *g*. One mL of supernatant from each tube was transferred to a cuvette and the absorption at 660_{nm} was recorded (Krop, 1974). The amount of settling pulp was estimated by reading the volume occupied by the pelleted solids. Reported means ± standard errors (P = 0.05) are for three replicates.

RESULTS

BASED ON SPECIFIC ACTIVITY, the data indicated (Table 1) that rag DS contained the greatest amount of total and TT-PME activity (54.6% and 47.3% respectively). Juice DP and rag DP contained nearly equal amounts of total PME activity (15.3% and 14.1% respectively) although juice DP had 10 times more TT-PME specific activity than rag DP (34.8% vs 2.5%). Juice extracts contained the greatest proportion of total specific activity as TT-PME activity.

Tissue specific differences for juice cloud destabilization were observed at room temperature (Fig. 1 to 3) and at 4°C (Fig. 4 to 6). The most rapid destabilization at room temperature occurred when peel HDS (Fig. 1) or juice HDS (Fig. 3) was added to pasteurized juice. The TL-PME's comprised 96.7% and 95.8% of the total PME activity for peel DS and juice DS respectively (Table 1). Although juice treatments were clarified by peel HDS and juice HDS after 2 days, peel HDS destabilized juice cloud faster than juice HDS as indicated by the one day sampling points (Figs. 1, 3). Peel DP had no effect on juice cloud stability and peel DS had only a marginal effect through the 14 day sampling (Fig. 1). Juice DS also had no effect but juice DP (Table 1, 94.4% TL-PME) destabilized the cloud nearly as fast as juice HDS (Fig. 3).

All treatments using rag extracts destabilized juice cloud (Fig. 2). As with peel and juice extracts, the most rapid destabilization was produced by rag HDS (TT-PME). However, its action was slower than peel HDS or juice HDS (Fig. 1 to 3). The rag DS (97.9% TL-PME) and rag DP (99.4% TL-PME) both destabilized cloud slightly slower than rag HDS. In all cases the increase in settling pulp occurred with, or slightly before, the decrease in absorbance of the supernatant (data not shown).

Citrus Tissue Extracts Affect Juice Cloud Stability

RANDALL G. CAMERON, ROBERT A. BAKER, and KAREL GROHMANN

ABSTRACT

Salt extractable proteins were isolated from hand expressed juice, rag and peel of Valencia oranges. Each tissue extract was divided into dialysis supernatant (DS) and precipitate (DP) (forms during dialysis). DP, DS and DS heated for 2 min at 80°C (HDS) were added to pasteurized, reconstituted frozen concentrated orange juice at 5 U · mL^{-1} of pectinmethylesterase. Samples were incubated either at 25°C for 14 days or 4°C for 28 days and periodically sampled to determine the effects of tissue extracts on juice cloud stability. Tissue specific differences were observed for the rate of juice cloud precipitation and among the HDS, DS, and DP fractions of a given tissue. HDS fractions destabilized juice cloud more rapidly than DS or DP at both 25 and 4°C.

Key Words: citrus, juice cloud, cloud stability, pectinmethylesterase, pectin

INTRODUCTION

SINCE THE INTRODUCTION of frozen concentrated orange juice (FCOJ) in the 1940s, a major quality defect has been juice cloud precipitation. Early studies indicated that pectinmethylesterase (PME; EC 3.1.1.11) initiates a sequence of events that leads to juice cloud destabilization (Joslyn and Sedky, 1940; Stevens et al., 1950; Guyer et al., 1956). Hydrolysis of methyl esters attached to C6 of galacturonic acid moieties in the main pectin chain produces free acid groups. When sufficient divalent cations such as calcium are present, these cations crosslink acid functions on adjacent pectin chains, reducing their solubility leading to juice cloud precipitation (Stevens et al., 1950; Joslyn and Pilnik, 1961; Baker and Bruemmer, 1969).

Multiple forms of PME have been identified in various citrus fruits (Versteeg et al., 1980; Seymour et al., 1991; Macdonald et al., 1993, 1994; Cameron et al., 1994; Cameron and Grohmann, 1995, 1996). One form, purified from a commercial 'Fresh Frozen Orange Juice' was relatively thermally stable, retaining ~50% activity after 60 sec incubation in a 90°C water bath (Cameron and Grohmann, 1996). Thermally tolerant forms of PME (TT-PME) also have been identified from navel oranges (Versteeg et al., 1980), white grapefruit (Seymour et al., 1991), citrus tissue culture cells (Cameron et al., 1994), lemon fruit (Macdonald et al., 1994), and red grapefruit (Cameron and Grohmann, 1995). Versteeg et al. (1980) reported that at 5°C only a TT-PME was capable of destabilizing juice cloud in <4 wk (see Guyer et al., 1956, Bissett et al., 1957 and Carroll et al., 1957 for discussion and definition of juice cloud clarification). Rouse (1953) demonstrated that PME activity was present in all fruit tissues of Valencia oranges and that juice sacs contained ~75% of total PME activity. Rombouts et al. (1982), using nonequilibrium pH gradient electrophoresis and activity prints, reported a PME with the same Rf as TT-PME studied by Versteeg et al. (1980) in all tissues of Navel oranges. However, Rombouts et al. (1982) did not determine the levels of TT-PME activity in the various tissues.

Snir (1996) reported data suggesting that the method of juice extraction may affect the level of TT-PME in the juice. No data are available on the amounts of TT-PME present in various citrus fruit tissues or whether any TT-PME's that might occur would be equally effective at destabilizing citrus juice cloud. Therefore our objective was to detemine the effects of fruit tissue extracts on juice cloud stability.

The authors are with the USDA, ARS, South Atlantic Area, Citrus and Subtropical Products Laboratory, 600 Avenue S, N.W. (P.O. Box 1909), Winter Haven, FL 33883-1909.

MATERIALS & METHODS

Fruit tissue extracts

Valencia oranges (1.5 bushels) were purchased from a local supplier on 21 Feb 1995, the beginning of the harvesting season for that cultivar. The oranges were halved and the juice was hand expressed using a kitchen-type juicer. A total of 17.8 L of juice was collected. Rag, composed of intersegmental septa, squeezed juice sacs and fruit core tissue, was then separated (4.2 kg) from peel (flavedo and albedo, 5.9 kg). The rag was rinsed with deionized water to remove residual juice and frozen at −20°C until needed. Peel was ground with a meat grinder and frozen at −20°C until needed.

Juice was brought to 0.1M Tris and 1M NaCl, the pH adjusted to 8.0 with solid NaOH and stirred overnight at 5°C. Juice (8L) was brought to 75% ammonium sulfate saturation and stirred overnight at 4°C. After centrifugation in 250 mL bottles at 12,100 × *g* for 30 min at 4°C, the supernatant was discarded (no PME activity) and 50 mL of 10 mM Tris, pH 7.5 (at 31°C), 20 mM NaCl (TBS) was added to each bottle to solubilize precipitated proteins. Insoluble material was still present, so the suspension was re-centrifuged (as above) to remove this material. The supernatant (containing PME activity) was decanted and frozen at −75°C in 50 mL aliquots. The remaining juice (9.8L) was centrifuged at 12,100 × *g* for 15 min at 4°C to remove insoluble materials. Ammonium sulfate was added to the resulting serum to 75% saturation, stirred overnight at 4°C, and then centrifuged as described. The supernatant was discarded and protein pellets solubilized as described. The solubilized protein was dialyzed (6000 dalton molecular weight cut-off dialysis tubing) exhaustively at 6°C against 3L of solubilization buffer, with a total of 4 buffer changes. During dialysis a precipitate (DP) formed (Cameron and Grohmann, 1996) which was separated from the dialysis supernatant (DS) by centrifugation at 12,100 × *g* for 30 min at 4°C. The juice DS was then pooled and concentrated to a final volume of 90 mL using a 10,000 dalton molecular weight cut-off hollow fiber cartridge. The juice DP was solubilized in a total volume of 200 mL of 10 mM Tris, 1M NaCl, pH 7.5. The solubilized juice DP was centrifuged at 12,100 × *g* for 15 min at 4°C to remove any remaining insoluble material. The supernatant was decanted (contained PME activity), pooled and concentrated to 70 mL with a hollow fiber cartridge as described. The concentrated juice DP was frozen at −75°C in 50 mL aliquots.

Rag was mixed with 2 volumes of 0.1M Tris, pH 8.0 at 27°C, 1M NaCl (w/v; 1 kg rag plus 2L buffer) and homogenized (~5 min) in a pre-chilled blender (in a 4°C room). The rag homogenate was stirred overnight at 4°C. It was then filtered through four layers of cheesecloth. The filtrate was centrifuged at 12,100 × *g* for 45 min at 4°C to remove any remaining insoluble material. The supernatant was stored overnight at 4°C. The rag material retained by the cheesecloth was mixed with extraction buffer as described and stirred overnight at 4°C. After filtering through cheesecloth and centrifugation both rag extracts were pooled and brought to 75% ammonium sulfate saturation. After stirring overnight at 4°C the precipitated proteins were removed by centrifugation at 12,100g for 30 min at 4°C. Proteins in the pellet were solubilized in TBS and dialyzed vs 4L TBS overnight at 6°C with a total of four buffer changes. The precipitate formed during dialysis was removed by centrifugation at 27,200 × *g* for 30 min at 4°C. The supernatant (rag DS) was pooled and frozen at −75°C. The pellet (rag DP) was solubilized as described for juice DP, pooled and frozen at −75°C. Both rag DS and rag DP were then concentrated with a hollow fiber cartridge as described.

Ground peel was mixed with 3 volumes (w/v) of extraction buffer and homogenized (~5 min) in a blender at 4°C. After stirring overnight at 4°C it was filtered through two layers of cheesecloth. The filtrate was pooled and stored at 4°C. Recovered peel tissue was mixed with 3 volumes of extraction buffer and further homogenized (~3 min) with a

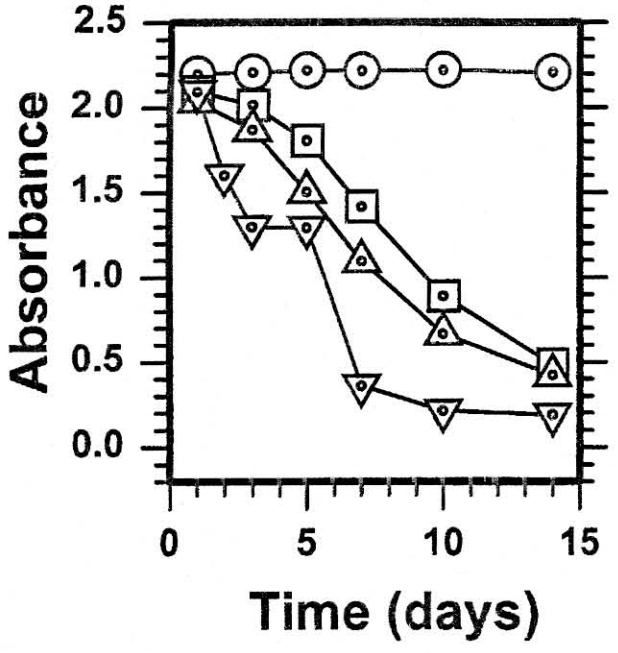

Fig. 2—Effects of rag extracts on juice cloud stability at room temperature. Measurements, symbols and error bars as in Fig. 1.

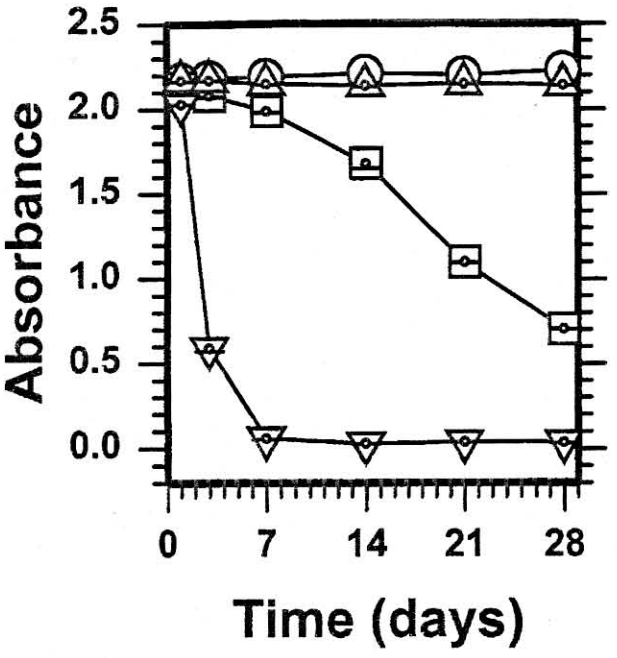

Fig. 4—Effects of peel extracts on juice cloud stability at 4°C. Measurements, symbols and error bars as in Fig. 1.

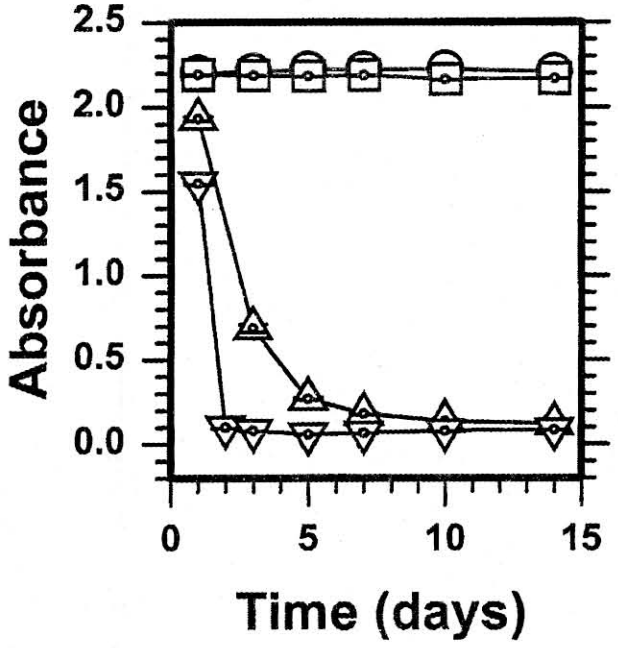

Fig. 3—Effects of juice extracts on juice cloud stability at room temperature. Measurements, symbols and error bars as in Fig. 1.

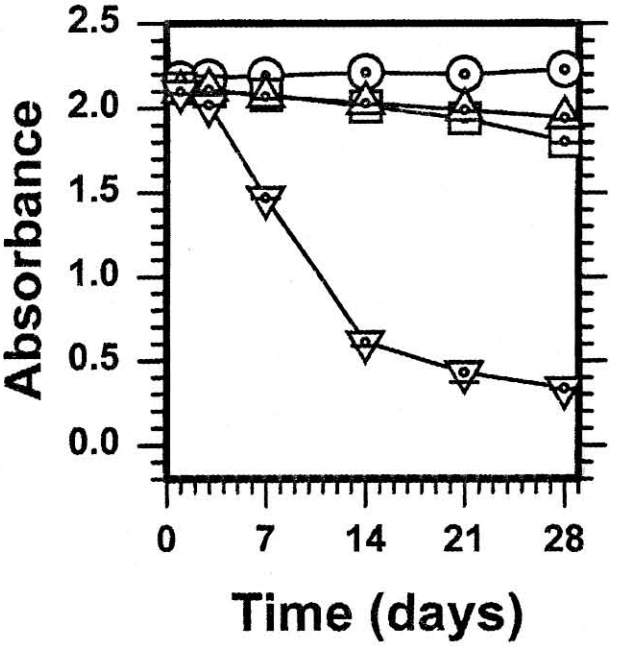

Fig. 5—Effects of rag extracts on juice cloud stability at 4°C. Measurements, symbols and error bars as in Fig. 1.

At 4°C peel HDS (100% TT-PME) clarified the juice before other extracts (Fig. 4). At 3 days the peel HDS reduced the A_{660} to ~0.6 and to < 0.1 at 7 days. The only other treatment that totally clarified the juice during the 28 day study was juice HDS (100% TT-PME) which took ~14 days (Fig. 6). At 28 days rag HDS (100% TT-PME, Fig. 5) decreased the A_{660} to ~0.3, while the juice DP sample (Fig. 6) had an A_{660} of ~0.5 and the peel DS sample, an A_{660} (Fig. 4) near 0.7. The other treatments had negligible effects on juice cloud stability (Fig. 4 to 6). Again, increases in settling pulp corresponded to decreases in A_{660} (Fig. 4 to 6).

DISCUSSION

FRUIT TISSUE EXTRACTS from Valencia oranges, used to add to pasteurized, reconstituted FCOJ destabilized the juice cloud. Since heat treatment inactivates all but the TT-PME, the TT-PME in those tissues destabilized the juice cloud faster than a mixture of thermally labile PME's (TL-PME) and TT-PME. All tissues contained TT-PME although its relative proportion in tissue extracts varied from 0.2% (peel DP) to 5.6% (juice DP). Data from Rouse et al. (1962) suggest that tissues from these fruit contained near the seasonal average for PME activity. At room temperature and 4°C, peel HDS (100% TT-PME) destabilized the juice cloud more rapidly than other extracts. Versteeg et al. (1980) also reported that a purified TT-PME destabilized cloud faster than two TL-PMEs. At room temperature one extract, juice DP, which contained only 5.6% TT-PME, destabilized the juice cloud more rapidly than the rag HDS (100% TT-PME). At 4°C the effects of juice DP and rag HDS were

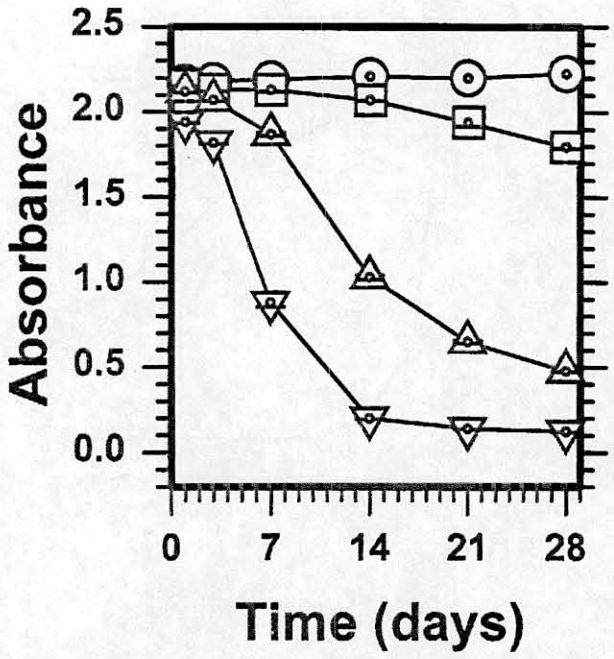

Fig. 6—Effects of juice extracts on juice cloud stability at 4°C. Measurements, symbols and error bars as in Fig. 1.

Table 1—Estimation of total PME and TT-PME specific activity (μEq · min^{-1} · μg^{-1} protein) in concentrated fruit tissue extracts[a]

Sample	μ protein · μL^{-1}	RT activity[b]	80°C activity[c]	% TT-PME[d]
Peel DS	13.39 ± 0.27	0.11 ± 0.004	0.004 ± 1.9^{-5}	3.3
Peel DP	2.33 ± 0.34	0.11 ± 0.006	2.15^{-4} ± 0	0.2
Rag DS	12.18 ± 0.98	0.89 ± 0.07	0.019 ± 0.002	2.1
Rag DP	2.40 ± 0.49	0.23 ± 0.01	0.001 ± 1.1^{-5}	0.6
Juice DS	41.07 ± 2.08	0.04 ± 0.001	0.002 ± 3.9^{-5}	4.2
Juice DP	1.22 ± 0.27	0.25 ± 0.01	0.014 ± 4.6^{-4}	5.6

[a] All estimates are means ± standard error for three replicates.
[b] Specific activity in unheated extracts.
[c] Specific activity remaining after 2 min incubation in 80°C water bath.
[d] % of total (RT activity + 80°C activity) PME specific activity that is TT-PME specific activity.

nearly equivalent. Tissue specific differences also were evident for unheated DS and DP treatments.

These results suggest that the different tissues may contain different forms of TL- and TT-PMEs. As indicated, many different forms of PME have been reported in citrus. Tissue specific forms of PME have also been reported from tomato (Gaffe et al., 1994; Warrilow and Jones, 1995). Multiple forms of PME from tomato exhibited differences in salt effects, kinetics pI and pH optima (Warrilow and Jones, 1995). Consequently the more rapid destabilization of juice cloud with TT-PME, compared to TL-PME's, may be related to the retention of higher levels of activity at the pH of juice.

Peel HDS extract (100% TT-PME) had a more rapid effect on cloud stability than unheated DS or DP extracts (~95% TL-PME) but the amount of TT-PME did not appear to be the only factor affecting cloud stability. Juice DP extract (5.6% TT-PME) precipitated cloud more rapidly than rag HDS (100% TT-PME) at room temperature and was nearly equivalent at 4°C. Additionally, the heated peel DS clarified the juice almost two times faster than heated juice DS.

Since peel extracts caused the most rapid cloud destabilization, the cloud stability of lightly or nonpasteurized orange juice may be extended if care is taken to prevent peel tissue or peel juice from entering the juice.

REFERENCES

Baker, R.A. and Bruemmer, J.H. 1969. Cloud stability in the absence of various orange juice soluble components. Proc. Fla. Hort. Soc. 82: 215–220.

Bissett, O.W., Veldhuis, M.K., Guyer, R.B., and Miller, W.M. 1957. Stability of frozen concentrated orange. IV. Effect of heat treatment on Hamlin, Pineapple, and Valencia juices at different stages of maturity. Food Technol. 11: 512–515.

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Cameron, R.G., Buslig, B.S., and Shaw, P.E. 1992. Adaptation of a spectrophotometric assay for pectinmethylesterase to a kinetic microplate reader. J. Food Sci. 57: 1006–1008.

Cameron, R.G. and Grohmann, K. 1995. Partial purification and thermal characterization of pectinmethylesterase from red grapefruit finisher pulp. J. Food Sci. 60: 821–825.

Cameron, R.G. and Grohmann, K. 1996. Purification and characterization of a thermally tolerant pectinmethylesterase from a commercial Valencia 'Fresh Frozen Orange Juice'. J. Agric. Food Chem. 44: 458–462.

Cameron, R.G., Niedz, R.P., and Grohmann, K. 1994. Variable heat stability for multiple forms of pectinmethylesterase from citrus tissue culture cells. J. Agric. Food Chem. 42: 903–908.

Carroll, E.A., Guyer, R.B., Bissett, O.W., and Veldhuis, M.K. 1957. Stability of frozen concentrated orange juice. V. The effect of heat treatment at intermediate stages of concentration on juices prepared from Valencia oranges. Food Technol. 11: 516–519

Gaffe, J., Tieman, D.M., and Handa, A.K. 1994. Pectin methylesterase isoforms in tomato (*Lycopersicon esculentum*) tissues. Plant Physiol. 105: 199–203.

Guyer, R.B., Miller, W.M., Bissett, O.W., and Veldhuis, M.K. 1956. Stability of frozen concentrated orange juice. I. The effect of heat treatment on enzyme inactivation and cloud stability of frozen concentrates made from pineapple and valencia oranges. Food Technol. 10: 10–16.

Joslyn, M.A. and Pilnik, W. 1961. Enzymes and enzyme activity. Ch. 12 in *The Orange: Its Biochemistry and Physiology*, W.B. Sinclair (Ed.), p. 373–435. Univ. of California.

Joslyn, M.A. and Sedky, A. 1940. Effect of heating on the clearing of citrus juices. Food Research 5: 223.

Krop, J. 1974. The mechanism of cloud loss phenomena in orange juice. Ph.D. thesis, Agricultural Univ., Wageningen, The Netherlands.

Macdonald, H.M., Evans, R., and Spencer, W.J. 1993. Purification and properties of the major pectinesterases in lemon fruits (*Citrus limon*). J. Sci. Food Agric. 62: 163–168.

Macdonald, H.M., Evans, R., and Spencer, W.J. 1994. The use of continuous-flow electrophoresis to remove pectin in the purification of the minor pectinesterase in lemon fruits (*Citrus limon*). J. Sci. Food Agric. 64: 129–134.

Rombouts, F.M., Versteeg, C., Karman, A.M., and Pilnik, W. 1982. Pectinesterases in component parts of citrus fruits related to problems of cloud loss and gelation in citrus products. In *Use of Enzymes in Food Technology*, P. Dupay (Ed.), p. 483–487. Technique et Documentation Lavoisier, Paris, France.

Rouse, A.H. 1953. Distribution of pectinesterase and total pectin in component parts of citrus fruits. Food Technol. 7: 360–362.

Rouse, A.H., Atkins, C.D., and Moore, E.L. 1962. Seasonal changes occurring in the pectinesterase activity and pectic constituents of the component parts of citrus fruits. I. Valencia oranges. J. Food Sci. 27: 419–425.

Seymour, T.A., Preston, J.F., Wicker, L., Lindsay, J.A., and Marshall, M.R. 1991. Purification and properties of pectinesterases of Marsh White grapefruit pulp. J. Agric. Food Chem. 39: 1075–1079.

Snir, R., Koehler, P.E., Sims, K.A., and Wicker, L. 1996. Total and thermostable pectinesterases in citrus juices. J. Food Sci. 61: 379–382.

Stevens, J.W., Pritchett, D.E., and Baier, W.E. 1950. Control of flocculation of cloud in citrus juices. Food Technol. 4: 469–473.

Versteeg, C., Rombouts, F.M., Spaansen, C.H., and Pilnik, W. 1980. Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. J. Food Sci. 45: 969–971, 998.

Warrilow, A.G.S. and Jones, M.G. 1995. Different forms of tomato pectinesterase have different kinetic properties. Phytochemistry 39: 277–282.

Ms received 5/14/96; revised 8/27;96; accepted 9/4/96.

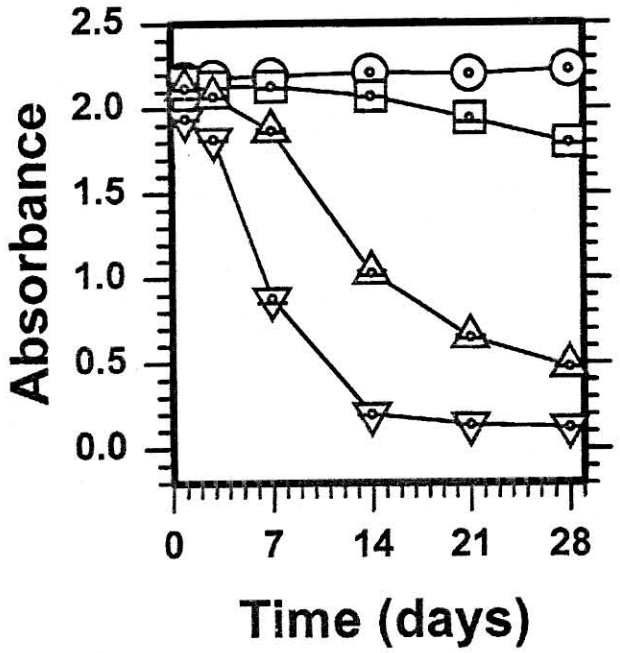

Fig. 6—Effects of juice extracts on juice cloud stability at 4°C. Measurements, symbols and error bars as in Fig. 1.

Table 1—Estimation of total PME and TT-PME specific activity (μEq · min^{-1} · μg^{-1} protein) in concentrated fruit tissue extracts[a]

Sample	μ protein · μL^{-1}	RT activity[b]	80°C activity[c]	% TT-PME[d]
Peel DS	13.39 ± 0.27	0.11 ± 0.004	0.004 ± 1.9^{-5}	3.3
Peel DP	2.33 ± 0.34	0.11 ± 0.006	2.15^{-4} ± 0	0.2
Rag DS	12.18 ± 0.98	0.89 ± 0.07	0.019 ± 0.002	2.1
Rag DP	2.40 ± 0.49	0.23 ± 0.01	0.001 ± 1.1^{-5}	0.6
Juice DS	41.07 ± 2.08	0.04 ± 0.001	0.002 ± 3.9^{-5}	4.2
Juice DP	1.22 ± 0.27	0.25 ± 0.01	0.014 ± 4.6^{-4}	5.6

[a] All estimates are means ± standard error for three replicates.
[b] Specific activity in unheated extracts.
[c] Specific activity remaining after 2 min incubation in 80°C water bath.
[d] % of total (RT activity + 80°C activity) PME specific activity that is TT-PME specific activity.

nearly equivalent. Tissue specific differences also were evident for unheated DS and DP treatments.

These results suggest that the different tissues may contain different forms of TL- and TT-PMEs. As indicated, many different forms of PME have been reported in citrus. Tissue specific forms of PME have also been reported from tomato (Gaffe et al., 1994; Warrilow and Jones, 1995). Multiple forms of PME from tomato exhibited differences in salt effects, kinetics pI and pH optima (Warrilow and Jones, 1995). Consequently the more rapid destabilization of juice cloud with TT-PME, compared to TL-PME's, may be related to the retention of higher levels of activity at the pH of juice.

Peel HDS extract (100% TT-PME) had a more rapid effect on cloud stability than unheated DS or DP extracts (~95% TL-PME) but the amount of TT-PME did not appear to be the only factor affecting cloud stability. Juice DP extract (5.6% TT-PME) precipitated cloud more rapidly than rag HDS (100% TT-PME) at room temperature and was nearly equivalent at 4°C. Additionally, the heated peel DS clarified the juice almost two times faster than heated juice DS.

Since peel extracts caused the most rapid cloud destabilization, the cloud stability of lightly or nonpasteurized orange juice may be extended if care is taken to prevent peel tissue or peel juice from entering the juice.

REFERENCES

Baker, R.A. and Bruemmer, J.H. 1969. Cloud stability in the absence of various orange juice soluble components. Proc. Fla. Hort. Soc. 82: 215–220.

Bissett, O.W., Veldhuis, M.K., Guyer, R.B., and Miller, W.M. 1957. Stability of frozen concentrated orange. IV. Effect of heat treatment on Hamlin, Pineapple, and Valencia juices at different stages of maturity. Food Technol. 11: 512–515.

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Cameron, R.G., Buslig, B.S., and Shaw, P.E. 1992. Adaptation of a spectrophotometric assay for pectinmethylesterase to a kinetic microplate reader. J. Food Sci. 57: 1006–1008.

Cameron, R.G. and Grohmann, K. 1995. Partial purification and thermal characterization of pectinmethylesterase from red grapefruit finisher pulp. J. Food Sci. 60: 821–825.

Cameron, R.G. and Grohmann, K. 1996. Purification and characterization of a thermally tolerant pectinmethylesterase from a commercial Valencia 'Fresh Frozen Orange Juice'. J. Agric. Food Chem. 44: 458–462.

Cameron, R.G., Niedz, R.P., and Grohmann, K. 1994. Variable heat stability for multiple forms of pectinmethylesterase from citrus tissue culture cells. J. Agric. Food Chem. 42: 903–908.

Carroll, E.A., Guyer, R.B., Bissett, O.W., and Veldhuis, M.K. 1957. Stability of frozen concentrated orange juice. V. The effect of heat treatment at intermediate stages of concentration on juices prepared from Valencia oranges. Food Technol. 11: 516–519

Gaffe, J., Tieman, D.M., and Handa, A.K. 1994. Pectin methylesterase isoforms in tomato (*Lycopersicon esculentum*) tissues. Plant Physiol. 105: 199–203.

Guyer, R.B., Miller, W.M., Bissett, O.W., and Veldhuis, M.K. 1956. Stability of frozen concentrated orange juice. I. The effect of heat treatment on enzyme inactivation and cloud stability of frozen concentrates made from pineapple and valencia oranges. Food Technol. 10: 10–16.

Joslyn, M.A. and Pilnik, W. 1961. Enzymes and enzyme activity. Ch. 12 in *The Orange: Its Biochemistry and Physiology*, W.B. Sinclair (Ed.), p. 373–435. Univ. of California.

Joslyn, M.A. and Sedky, A. 1940. Effect of heating on the clearing of citrus juices. Food Research 5: 223.

Krop, J. 1974. The mechanism of cloud loss phenomena in orange juice. Ph.D. thesis, Agricultural Univ., Wageningen, The Netherlands.

Macdonald, H.M., Evans, R., and Spencer, W.J. 1993. Purification and properties of the major pectinesterases in lemon fruits (*Citrus limon*). J. Sci. Food Agric. 62: 163–168.

Macdonald, H.M., Evans, R., and Spencer, W.J. 1994. The use of continuous-flow electrophoresis to remove pectin in the purification of the minor pectinesterase in lemon fruits (*Citrus limon*). J. Sci. Food Agric. 64: 129–134.

Rombouts, F.M., Versteeg, C., Karman, A.M., and Pilnik, W. 1982. Pectinesterases in component parts of citrus fruits related to problems of cloud loss and gelation in citrus products. In *Use of Enzymes in Food Technology*, P. Dupay (Ed.), p. 483–487. Technique et Documentation Lavoisier, Paris, France.

Rouse, A.H. 1953. Distribution of pectinesterase and total pectin in component parts of citrus fruits. Food Technol. 7: 360–362.

Rouse, A.H., Atkins, C.D., and Moore, E.L. 1962. Seasonal changes occurring in the pectinesterase activity and pectic constituents of the component parts of citrus fruits. I. Valencia oranges. J. Food Sci. 27: 419–425.

Seymour, T.A., Preston, J.F., Wicker, L., Lindsay, J.A., and Marshall, M.R. 1991. Purification and properties of pectinesterases of Marsh White grapefruit pulp. J. Agric. Food Chem. 39: 1075–1079.

Snir, R., Koehler, P.E., Sims, K.A., and Wicker, L. 1996. Total and thermostable pectinesterases in citrus juices. J. Food Sci. 61: 379–382.

Stevens, J.W., Pritchett, D.E., and Baier, W.E. 1950. Control of flocculation of cloud in citrus juices. Food Technol. 4: 469–473.

Versteeg, C., Rombouts, F.M., Spaansen, C.H., and Pilnik, W. 1980. Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. J. Food Sci. 45: 969–971, 998.

Warrilow, A.G.S. and Jones, M.G. 1995. Different forms of tomato pectinesterase have different kinetic properties. Phytochemistry 39: 277–282.

Ms received 5/14/96; revised 8/27;96; accepted 9/4/96.

Mention of a trademark or proprietary product is for identification only and does not imply a guarantee or warranty of the product by the U.S. Department of Agriculture. All programs and services of the U.S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

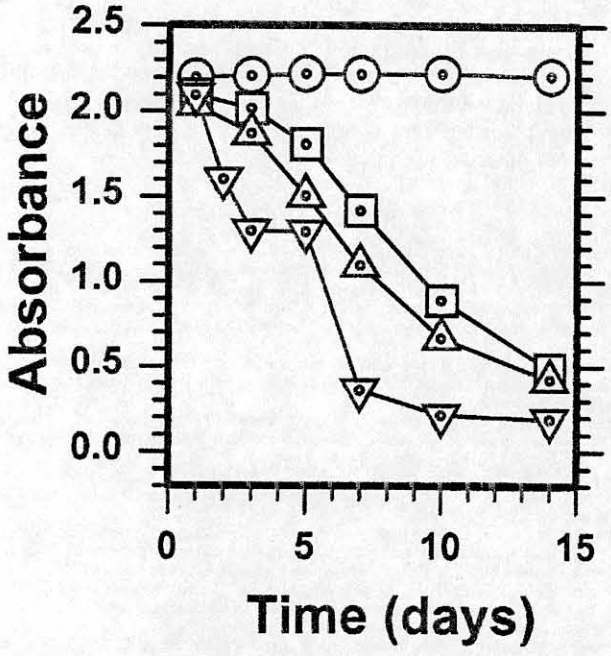

Fig. 2—Effects of rag extracts on juice cloud stability at room temperature. Measurements, symbols and error bars as in Fig. 1.

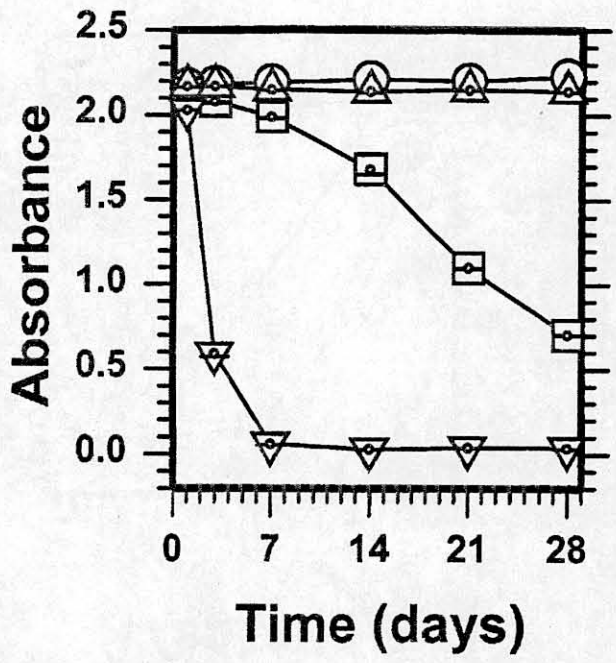

Fig. 4—Effects of peel extracts on juice cloud stability at 4°C. Measurements, symbols and error bars as in Fig. 1.

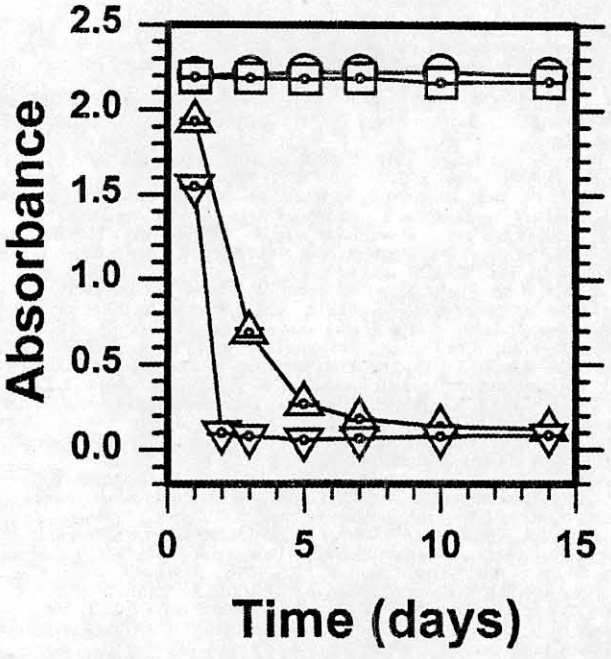

Fig. 3—Effects of juice extracts on juice cloud stability at room temperature. Measurements, symbols and error bars as in Fig. 1.

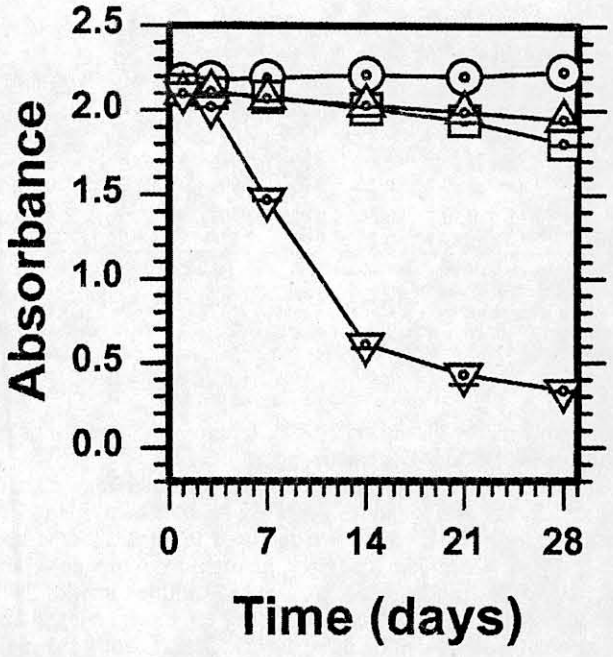

Fig. 5—Effects of rag extracts on juice cloud stability at 4°C. Measurements, symbols and error bars as in Fig. 1.

At 4°C peel HDS (100% TT-PME) clarified the juice before other extracts (Fig. 4). At 3 days the peel HDS reduced the A_{660} to ~0.6 and to < 0.1 at 7 days. The only other treatment that totally clarified the juice during the 28 day study was juice HDS (100% TT-PME) which took ~14 days (Fig. 6). At 28 days rag HDS (100% TT-PME, Fig. 5) decreased the A_{660} to ~0.3, while the juice DP sample (Fig. 6) had an A_{660} of ~0.5 and the peel DS sample, an A_{660} (Fig. 4) near 0.7. The other treatments had negligible effects on juice cloud stability (Fig. 4 to 6). Again, increases in settling pulp corresponded to decreases in A_{660} (Fig. 4 to 6).

DISCUSSION

FRUIT TISSUE EXTRACTS from Valencia oranges, used to add to pasteurized, reconstituted FCOJ destabilized the juice cloud. Since heat treatment inactivates all but the TT-PME, the TT-PME in those tissues destabilized the juice cloud faster than a mixture of thermally labile PME's (TL-PME) and TT-PME. All tissues contained TT-PME although its relative proportion in tissue extracts varied from 0.2% (peel DP) to 5.6% (juice DP). Data from Rouse et al. (1962) suggest that tissues from these fruit contained near the seasonal average for PME activity. At room temperature and 4°C, peel HDS (100% TT-PME) destabilized the juice cloud more rapidly than other extracts. Versteeg et al. (1980) also reported that a purified TT-PME destabilized cloud faster than two TL-PMEs. At room temperature one extract, juice DP, which contained only 5.6% TT-PME, destabilized the juice cloud more rapidly than the rag HDS (100% TT-PME). At 4°C the effects of juice DP and rag HDS were