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Effect of Infusion Method on Peel Removal and Storage Quality of Citrus

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SUMMARY. Peeling and storage characteristics of citrus fruit infused with water or enzyme solution were compared. Fruit were vacuum- or pressure-infused with water or water-containing pectinase. The enzyme treatment did not affect peeling times of white or red grapefruit, oranges, or tangelos. Pressure and vacuum infusion methods produced similar results. Grapefruit and oranges infused with water had significantly less juice leakage and were firmer than fruit infused with enzyme. Microbial levels and respiration rates and ethylene emanation during storage were the same for enzyme- and water-treated fruit.

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Currently, an enzymatic peeling process is used to produce peeled citrus fruit that are convenient for consumption (Adams and Kirk, 1991; Bruemmer, 1981; Bruemmer et al., 1978; McArdle and Culver, 1994). By this process, fruit are scored or abraded, infused with either pectinase or pectinase and cellulase solution by vacuum (Bruemmer, 1981) or pressure (Adams and Kirk, 1991), and incubated at 20 to 45 °C for 0.5 to 2 h. During the incubation period, the enzyme digest the pectin and cellulose of the albedo and regions of the segment membranes, thus, facilitating peel removal and segmentation.

Enzymatically peeled citrus exhibit extensive juice leakage and softening during storage (Baker and Hagenmeier, 1995) despite apparent integrity of segment membranes and juice vesicles. Low-temperature controlled-atmosphere storage (Baker and Bruemmer, 1989; Berry et al., 1988) and edible coatings (Baker and Hagenmeier, 1995) may be used to improve storage quality of peeled citrus. Additional benefits also may be obtained if the enzyme used to peel the fruit was targeted to the peel, inactivated following peeling, or eliminated from the process. The following studies were performed to evaluate the effects of infusion solution (enzyme vs. water), infusion temperature (25 and 45 °C), and infusion method (pressure vs. vacuum) on peeling time and storage quality of peeled citrus.

Materials and methods

Mature 'Marsh' white and 'Ruby Red' grapefruit (*Citrus paradisi* Macf.), 'Hamlin' oranges (*Citrus sinensis* L.), and 'Orlando' tangelos

(*Citrus reticulata* Blanco x *Citrus paradisi* Macf.) were harvested at the Citrus Research and Education Center (CREC) in Lake Alfred, Fla., or were purchased from local packing-houses before washing and waxing. Fruit were harvested in Dec. 1994 and Jan., Feb., and Nov. 1995. Fruit either were processed immediately after acquisition or were stored at 4.4 ± 1.0 °C and $95\% \pm 2\%$ relative humidity for ≤ 2 weeks.

Fruit were washed at CREC on a commercial packingline with fruit cleaner (FMC 395; FMC Corp., Lakeland, Fla.) on the day of peeling. No attempt was made to surface-sterilize the fruit. Fruit were scored to penetrate the cuticle and permit infusion of the solution into the inner part of the peel (albedo). Six radial cuts were made into the peel of each fruit from the stem to the blossom end (Baker and Bruemmer, 1989). Food grade fungal pectic enzyme Spark-L HPG and Clarex-L (Solvey Enzymes, Elkhart, Ind.) were selected based on availability and efficacy (Berry et al., 1988; Bruemmer, 1981). Enzyme solutions (1000 ppm) were prepared in distilled and deionized water. Solutions were poured into vacuum (7.5-L desiccator) or pressure (pressure cooker with 15-L container insert) chambers, and five to 10 fruit were immersed in the solutions. Fruit were submerged with weights to provide complete coverage by solution.

Fruit were vacuum-infused according to Bruemmer (1981) by evacuating the desiccator to ≈ 3 kPa. The vacuum was held for 3 min and gradually released over a 3-min interval. Fruit were pressure-infused according to Adams and Kirk (1991) by filling the pressure chamber with compressed air to ≈ 203 kPa. The pressure was held for 15 s and quickly released. Pressure was applied and released 10 times in succession for each batch of fruit. Fruit infused with enzyme solution were incubated for 30 or 60 min and were peeled. Fruit infused with water were peeled immediately after infusion unless otherwise noted. Infusion and incubation temperatures were maintained by holding the fruit in water at the treatment temperature for 30 min before scoring and infusion, adjusting the infusion solutions to the desired temperature, and holding the fruit in infusion solution during the incubation period.

EFFECT OF INFUSION SOLUTION AND INFUSION TEMPERATURE ON PEEL REMOVAL. The effect of enzyme or water infusion solutions was determined by the time required to remove the peel. Peelers were instructed on processing criteria before peeling. Peeling was judged to be complete when the peel, albedo, and loosened fibers were removed from the segment membrane. Removal of fibers that were separated <2 mm from the segment membrane was judged to be nonessential.

Three independent studies were conducted. In the first study, the effect of infusion solution (water or 1000 ppm enzyme solutions of Spark-L HPG or Clarex-L) and infusion temperature (25 or 45 °C) on peeling time was investigated. Peeling time was calculated as the time required for each peeler (n = four manual peelers) to peel five white grapefruit, red grapefruit, oranges, or tangelos. All fruit were vacuum-infused. Enzyme-treated fruit were incubated for 30 min after infusion and then were peeled. Water-treated fruit were peeled immediately after infusion. Data were organized in a completely randomized factorial design and were analyzed by analysis of variance (ANOVA).

In the second study, the effect of infusion solution (water or 1000 ppm enzyme solution of Spark-L HPG) and infiltration method (vacuum or pressure) on peeling time was investigated. Peeling time was based on the time required for each peeler (n = four manual peelers) to peel five white grapefruit or oranges. Enzyme-treated fruit were infused by vacuum or pressure techniques and incubated at 25 °C for 1 h or 30 min, respectively, and then

were peeled. Water-treated fruit were infused at 25 °C and were peeled immediately after infusion. Data were organized in a completely randomized factorial design and were analyzed by ANOVA.

In the third study, the numbers of peelers, fruit peeled, and peeling time trials were increased to confirm the treatment effect of infusion solution (water or 1000 ppm enzyme solution of Clarex-L). Enzyme- and water-treated fruit were incubated for 30 min at 45 °C after infusion and then were peeled. The time required for each peeler (n = six manual peelers) to peel 20 white grapefruit or oranges was determined. Two time trials were run for each infusion solution and variety combination. Data were organized in a completely randomized design with a split-plot arrangement in which number of time trial was treated as a subplot of a repeated observation.

EFFECT OF INFUSION SOLUTION AND INFILTRATION METHOD ON STORAGE QUALITY. The effect of infusion solution and infiltration method on percent juice loss, fruit firmness, microbial stability, fruit respiration, and ethylene production was evaluated in a series of independent studies.

Juice loss was determined in two studies. In the first study, white grapefruit and oranges were vacuum-infused with either water or enzyme and incubated at 25 °C for 30 min. Fruit were peeled manually (Jan. 1995) using latex gloves and were rinsed individually after peeling with distilled and deionized water for 10 s to remove superficial peeling solution. Individual whole fruit were placed in 0.60- and 0.70-L plastic clamshell containers (UltraPac, Rogers, Minn.) for oranges and grape-

fruit, respectively, and were held at 5, 10, or 15 °C (n = 10 fruit). Four 1-cm slits were cut into the sides of the plastic containers to prevent anaerobic respiration (container atmosphere consisted of 16% to 20% O₂ and 1.5% to 3% CO₂). No additional attempt was made to control or modify the atmosphere. Juice loss was determined by the difference in the weight of container and juice in the presence or absence of the fruit. Juice loss was measured after 1, 2, 3, 7, 14, 21, and 28 d in cold storage. In the second study, white grapefruit and oranges were pressure-infused with either water or enzyme and were incubated at 25 °C for 1 h. Fruit were peeled manually (Feb. 1995), rinsed, placed in containers, and held at 5 °C (n = 10 fruit). Juice loss was determined after 1, 2, 3, 7, 14, and 21 d in cold storage. Data were organized in a completely randomized factorial design and analyzed by ANOVA.

Firmness was evaluated subjectively and objectively. In the first firmness study, multiple comparisons tests (Larmond, 1977) were performed to evaluate main treatment effects of infusion solution (water or 1000 ppm enzyme solutions of Spark-L HPG) and infiltration method (vacuum or pressure) on fruit firmness. White grapefruit and oranges were infused and manually peeled (Feb. 1995) at 25 °C and stored at 5 °C. Reference sample fruit, which were vacuum-infused with water and manually peeled on the day of evaluation, were chilled to 5 °C before evaluation. Fruit peeled and stored for 1 or 7 d and reference samples were halved and placed in containers coded with random numbers. Fruit temperature during evaluation was ≈10 °C. Sensory evaluation of firmness was determined under fluorescent light. A sensory panel (n = 9 judges) experienced in evaluating the quality of fresh citrus was asked to compare the touch and mouth feel of the test fruit to that of the reference sample. Firmness was rated from 1 (extremely softer than reference sample) to 5 (same as reference sample) to 9 (extremely harder than reference sample). Data were organized in a completely randomized factorial design and were analyzed by ANOVA.

In the second firmness study, fruit firmness was measured by an Instron Universal Pressure Tester (Instron Corp., Canton, Mass.). The compres-

Table 1. Effect of infusion solution and infusion and incubation temperature on peeling time.

Infusion solution	Infusion and incubation temp (°C)	Peeling time (s/fruit) ^z			
		'Marsh' white grapefruit	'Ruby Red' grapefruit	'Hamlin' orange	'Orlando' tangelo
Enzyme 1 ^y	25	44 ± 7 ^x	66 ± 9	26 ± 4	27 ± 5
Enzyme 2	25	47 ± 6	56 ± 4	25 ± 2	22 ± 2
Water	25	54 ± 6	67 ± 7	26 ± 5	25 ± 4
Enzyme 1	45	42 ± 2	48 ± 3	24 ± 2	18 ± 2
Enzyme 2	45	42 ± 8	47 ± 4	22 ± 2	19 ± 1
Water	45	48 ± 5	61 ± 3	22 ± 3	20 ± 1

^zPeeling date: 14 Dec. 1994. Average time per fruit for each of four peelers to peel five fruit (±SE).

^yFruit were vacuum- (3 kPa) infused. Enzyme solutions were 1000 ppm Spark-L HPG (enzyme 1) or Clarex-L (enzyme 2).

^xNo significant differences were found among the means for infusion solution or infusion and incubation temperature by analysis of variance at $\alpha = 0.05$.

sion test described by Jackman et al. (1990) was used. White grapefruit and oranges were vacuum-infused with water or enzyme solution (1000 ppm Spark-L), incubated for 30 min, and peeled manually (Nov. 1995). Sections consisting of one-sixth of a whole fruit stored for 7 d at

5 °C were divided by a knife parallel to the segment wall. Each section was placed on a V-shaped sample holder so that the surface of the middle section was compressed by a flat-ended cylindrical probe (5 mm in diameter) at a deformation speed of 50 mm·min⁻¹. Three sections were measured per fruit (n = eight fruit). Firmness values were calculated as the maximum force applied divided by the displacement distance at the point of fracture (in newtons per millimeter).

Microbial stability of peeled citrus was evaluated by procedures described by Redd et al. (1986). White grapefruit and oranges were vacuum-infused with water or enzyme solution (1000 ppm Spark-L), incubated for 30 min, and peeled manually (Jan. 1995). Fruit were stored at 5, 10, or 15 ± 0.5 °C at 0, 1, 2, 3, 7, 14, 21, and 28 d after peeling. Samples (n = 3) of each treatment were macerated by a Stomacher Lab Blender (Stomacher Co., London) at normal speed for 60 s at each evaluation time. Appropriate dilutions of each sample were plated in duplicate on orange serum agar (OSA) and acidified potato dextrose agar (PDA). Plates were incubated for 48 h at 35 °C (OSA) or 5 d at 25 °C (PDA), and plate counts were determined. Numbers of colonies on OSA and PDA represented total aerobic plate counts and yeasts and molds counts, respectively.

Respiration rates of peeled citrus were determined according to a method described by Beaudry et al. (1992). White grapefruit and oranges were vacuum-infused with water or enzyme solution (1000 ppm Spark-L), incubated for 30 min, and peeled manually (Feb. 1995). Two peeled

fruit were weighed (≈400 and 600 g for orange and white grapefruit, respectively), sealed in 30 × 30-cm packages made of 38.3-μm (1.5 mil), low-density polyethylene (Associated Bag Co., Milwaukee, Wis.), and held at 5 ± 0.5 °C throughout analysis. Five replications were prepared for each treatment, and the study was performed twice. Gas samples (0.5 ml) of each package were taken by syringe through a silicone septum 1, 3, 5, and 7 d after packaging. Samples were injected into a flow-through system consisting of O₂ (model 3600, Orbisphere Laboratories, Emerson, N.J.), and CO₂ analyzers (model 6251; Li-Cor, Lincoln, Neb.) connected in series with N₂ used as a carrier gas. Steady-state gas levels measured 7 d after packaging were used to calculate rates of O₂ uptake and CO₂ production. Ethylene emanation was measured by sampling the package headspace and analyzing by GC (HP 5780; Hewlett Packard, Danbury, Conn.) 1 and 7 d after packaging.

Results and discussion

EFFECT OF INFUSION SOLUTION AND INFUSION TEMPERATURE ON PEEL REMOVAL. The processing temperature (25 or 45 °C) did not significantly change the peeling times for fruit vacuum-infused with either enzyme or water solution (Table 1). The effects of temperature were small, as peeling times typically decreased only ≈5% to 15% for 20 °C temperature increases for enzyme- and water-treated fruit. Neither infiltration technique (pressure or vacuum) nor infusion solution (enzyme or water) significantly changed peeling times for grapefruit or oranges (Table 2). In our study, 5%

to 25% of the total area of the albedo of pressure-treated fruit was dry, as also noted in Adams and Kirk's (1991) study. The dry regions in pressure-infused fruit were often noncontiguous and did not affect processing characteristics noticeably.

In the third study, in which the number of peelers and number of fruit peeled was increased, the peels of enzyme-treated fruit were noticeably softer than of water-treated fruit, but this softening did not reduce peeling time significantly (white grapefruit, 35 ± 4 and 38 ± 2 s/fruit; orange, 13 ± 1 and 16 ± 2 s/fruit for fruit infused with enzyme or water, respectively).

EFFECT OF INFUSION SOLUTION AND INFILTRATION METHOD ON STORAGE QUALITY. Juice leakage of vacuum-infused fruit was greater in enzyme-treated fruit than in water-treated fruit at all storage temperatures (Fig. 1). For example, oranges and grapefruit vacuum-infused with enzyme, peeled, and stored for 7 d at 5 °C lost ≈5% of their total weight due to juice leakage compared to <1% for water-infused fruit. Juice leakage of pressure-infused oranges and grapefruit also was greater with enzyme solution compared to water-infusion (Fig. 1).

The sensory panel rated grapefruit and oranges infused with enzyme solution as significantly less firm (Table 2). A reduction in the firmness of grapefruit infused with enzyme solution was perceivable after 1 d of storage at 5 °C, whereas the reduction in firmness of oranges infused with enzyme solution was first detected after 7 d of storage. Enzyme effects on firmness were similar for both infiltration methods. Objective analyses of firm-

Table 2. Effects of infusion solution and infiltration method on firmness of peeled grapefruit and oranges.

Treatment ^y		Peeling time (s/fruit) ^x		Firmness ^z			
				Day 1		Day 7	
Infusion solution	Infiltration method	'Marsh' white grapefruit	'Hamlin' orange	'Marsh' white grapefruit	'Hamlin' orange	'Marsh' white grapefruit	'Hamlin' orange
Enzyme	Vacuum	41 ± 3	16 ± 2	3.7 ± 0.2 ^x	4.3 ± 0.4	2.6 ± 0.3	2.4 ± 0.3
Enzyme	Pressure	48 ± 4	17 ± 3	3.9 ± 0.3	4.8 ± 0.5	2.3 ± 0.3	3.1 ± 0.4
Water	Vacuum	60 ± 7	20 ± 3	5.3 ± 0.2	4.6 ± 0.5	4.1 ± 0.3	4.0 ± 0.3
Water	Pressure	53 ± 7	20 ± 3	5.0 ± 0.0	5.0 ± 0.0	4.6 ± 0.3	4.7 ± 0.2

^zFirmness was rated from 1 (soft) to 5 (firm) to 9 (hard) by sensory panel in reference to a freshly peeled fruit. Fruit were stored 1 and 7 d at 5 °C. Fruit temperature during assessment was between 10 and 15 °C. Mean (± SE) of firmness scores by nine judges. Significant differences were found among the means of infusion solution by analysis of variance at α = 0.05 for grapefruit at day 1 and 7 and oranges at day 7.

^yFruit were vacuum- (3 kPa) or pressure- (207 kPa) infused with 1000 ppm Spark-L HPG enzyme solution and were incubated at 25 °C for 1 h or 30 min, respectively. Water-infused fruit were peeled immediately after infusion.

^xPeeling date: 13 Feb. 1995. Average time per fruit for each of four peelers to peel five fruit (± SE).

ness by an Instron analyzer confirmed that enzyme-treated fruit were significantly less ($\alpha = 0.05$) firm than water-peeled fruit after 7 d of storage at 5 °C. Firmness of white grapefruit was 1.50 ± 0.07 and 1.85 ± 0.10 N·mm⁻¹ and that of orange was 1.52 ± 0.08 and 1.75 ± 0.06 N·mm⁻¹ for enzyme and water treatments, respectively. The enhancement of juice leakage and reduction in firmness in whole fruit infused with an enzyme solution corresponds with that reported on peeled grapefruit segments (Baker and Bruemmer, 1989).

Infusion solution had no significant effect on microbial stability (Fig. 2). Although there is no established standard for peeled citrus, a range of limits of 50,000 to 500,000 colony-forming unites (cfu)/g for total viable aerobes in fresh vegetables has been suggested (National Academy of Sciences, 1964). Assuming that the limit of total viable aerobes is $\approx 100,000$ cfu/g for peeled fruit, the maximum microbiological shelf life of peeled oranges is estimated to be ≈ 10 , 2, and 1 d for fruit stored at 5, 10, and 15 °C, respectively. In comparison, microbiological shelf life of white grapefruit was greater than that of orange (≈ 14 , 3, and 2 d for fruit stored at 5, 10, and 15 °C, respectively). Fruit stored at 25 °C exceeded this level within 1 d after peeling (data not shown), thus, show-

ing the importance of strict control of storage temperature following peel removal.

Differences between total aerobic plate counts (Fig. 2, OSA) and yeast and mold counts (Fig. 2, PDA) indicate that rapid growth of bacteria is responsible for the early stage of spoilage. Spoilage of peeled citrus by bacteria often preceded the emergence of visible colonies of pink yeast and white mold by 1 to 7 d. To ensure quality and microbial safety, industrial scale production of peeled citrus requires proper sanitation and suitable postpeeling treatments to minimize the initial microbial population and to retard microbial growth during storage.

Respiration rate of peeled citrus fruit infused with enzyme or water solutions and stored for 9 d at 5 °C was not significantly different ($\alpha = 0.05$). Oxygen uptake was 0.38 ± 0.05 and 0.39 ± 0.06 mmol·kg⁻¹·h⁻¹ for water- and enzyme-infused white grapefruit, respectively, and 1.09 ± 0.10 and 0.90 ± 0.07 mmol·kg⁻¹·h⁻¹ for water- and enzyme-infused oranges, respectively (package O₂ range: 9% to 13%). Rates of CO₂ production were 0.49 ± 0.07 and 0.53 ± 0.09 mmol·kg⁻¹·h⁻¹ for water- and enzyme-infused white grapefruit, respectively, and 1.30 ± 0.15 and 1.10 ± 0.12 mmol·kg⁻¹·h⁻¹ for water- and enzyme-infused oranges, respectively (package CO₂ range: 4% to 9%).

Ethylene levels in the package headspace were 0.1 ppm for up to 7 d after peeling for enzyme- and water-infused fruit, which suggests that while an enzyme infusion promotes juice loss, it does not stimulate an ethylene wound response during cold storage. While the prolonged effect of enzyme on the fruit is not known, it appears that changes are slow during refrigeration. We observed ethylene wound responses during storage at 5 °C 1 d after wounding nonpeeled grapefruit (data unpublished). Refinement of enzymatic peeling techniques may benefit from determining the precise mechanisms of fruit degradation.

Pectin consists of long chains of polygalacturonic acid that comprise water-soluble and water-insoluble fractions (Van Buren, 1991). In the process of pressure- or vacuum-infusion, we suggest that water dissolves water-soluble pectins in the albedo and that enzyme depolymerizes the polygalacturonic acid backbone of the albedo pectin chain. The lack of substantial effect of enzyme on peeling time indicates that water-solubilization of albedo pectin plays an important role in the peeling process.

Water- and enzyme solution-infusion techniques offer advantages for commercial production of lightly processed citrus. Water-infusion simplifies the peeling process by eliminat-

Fig. 1. Juice leakage of (A and C) vacuum- or (B and D) pressure-infused citrus. (A and B) Oranges and (C and D) white grapefruit with (closed symbol) enzyme solution or (open symbol) water after storage at 5 (●, ○), 10 (■, □), or 15 °C (▲, △). Bars represent $\pm$ SE (n = 10 fruit). Bars are not shown where symbols are larger than bar.

Fig. 2. Microbial level of peeled citrus (A and C) 'Hamlin' orange and (B and D) 'Marsh' white grapefruit vacuum-infused with enzyme solution (closed symbol) or water (open symbol), peeled manually, and stored at 5 (●, ○), 10 (■, □), or 15 °C (▲, △). Colony counts for (A and B) orange serum agar represent total aerobic plate counts. Colony counts for (C and D) acidified potato dextrose agar represent total yeasts and molds counts. Bars represent *se* (*n* = three fruit). Bars are not shown where symbols are larger than bar.

ing the need for enzyme, incubation periods, and high-temperature digestion and produces firmer fruit that lose less juice during storage. Enzyme-infusion enhances peel-softening and fruit-segmenting (Bruemmer et al., 1978). Another alternative could be acidic (pH 2.2) or alkaline (pH 12.2) infusion solutions. Altering pH could depolymerize polygalacturonic acid similar to pectinase and could promote peel softening, segment separation, and fruit softening. Since selected organic acids inhibit some microbes (Banwart 1989), an additional benefit could be to reduce microbial activity.

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