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ENZYME-PEELING OF VALENCIA ORANGES FOR FRESH-CUT SLICES

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Abstract. **In spite of the booming market for fresh cut fruit, fresh cut citrus products have not been successfully commercialized due to technical difficulties in peeling the fruit and concerns over microbial contamination and juice leakage. The USDA and the Florida Department of Citrus have developed processes using enzyme infiltration under vacuum as well as water infusion to facilitate citrus peeling. However, the enzymes (cellulase and/or pectinase) continue their lytic action on the slices after the peeling process, reportedly degrading the slices during storage, and likely contributing to objectionable softening and juice leakage. There is also a perceived fear of microbial contamination in the process of enzyme infiltration or water infusion. The objective of the present study was to investigate low level enzyme concentrations and use of an acid solution (0.1 N HCl) rinse and cold conditioning (2°C for 24 hours) to slow down enzymatic activity after peeling of 'Valencia' oranges. In addition, the infiltration system was used to compare two commercial enzymes and water infusion for efficacy of peeling and effect on microbial contamination. Quality factors including juice leakage, firmness, pH, titratable acidity, soluble solids, and surface microbial counts were evaluated over three different harvests. Water infusion resulted in less attractive slices and a tougher texture compared to enzyme-treated fruit slices. Microbial counts were highest in water-infused fruit compared to those treated with enzymes or manually peeled, which in both cases were very low. Of the two enzymes tested, Ultrazym resulted in firmer slices and generally less juice leakage, although leakage was minimal for both enzymes within a two-week storage period at the enzyme concentrations used. Juice leakage, however, was not affected by the acid treatment or temperature conditioning.**

The fresh cut industry is driven by affluence, convenience, and desire for healthful alternatives in a ready-to-eat food (Hodge, 2003) making fresh-cut fruits and vegetables the fastest growing segment of the fresh produce industry. In the first quarter of 2006, more than $1.3 billion in sales was reported for fresh-cut produce, indicating a 6.5% increase from last year. Of those, $242 million was for fresh-cut fruit and the rest ($1 billion) was for fresh-cut vegetables, including salad. Overall, the fresh-cut fruit experienced strong growth of 15.7% compared to 2005 (Anonymous, 2006a). However, it is still difficult to buy ready-to-eat oranges, despite that the major factor limiting fresh citrus consumption in the U.S. is the necessity of peeling by hand (Pao et al., 1997). Moreover, unlike melon, which has a near neutral pH, the high acidity and postharvest stability of citrus fruit make oranges suitable for a minimally-processed product (Abeles, 1973; Rocha et al., 1995).

Commercialization of fresh-cut oranges is limited mostly by technical difficulties in peeling, due to the peculiarity of citrus peel (presence of albedo) and pulp (vesicle structure). The use of mechanical peelers with blades is not as efficient as for other fruit (for example, kiwifruits and apples) since it is not possible to completely remove the peel from the citrus segments without damaging the segment surface, losing edible material and generating juice leakage (Senesi et al., 2005).

In the late 1970s, a method to peel citrus was developed by U.S. Department of Agriculture (USDA). Fruit, whose peel was previously scored, could be easily peeled after infusion under vacuum with an enzyme solution that digested the albedo (Bruemmer, 1981). Since then, many studies (Pao et al., 1996a, b; Pao et al., 1997; Rouhana and Mannheim, 1994) have been carried out to peel citrus by pectinase and/or cellulase infiltration or water infusion. The Florida Department of Citrus (FDOC) developed a machine able to remove the peel from enzyme-infused citrus (Ismail and Thomas, 2002). Unfortunately, due to enzyme infiltration and continued action of the enzymes, juice leakage, loss of texture, microbial contamination and off-flavors are a concern (Baker and Bruemmer, 1989; Baker and Hagenmaier, 1995; Ismail et al., 2005; Senesi et al., 2003) and have reportedly prevented this technique from being commercially adapted for the production of fresh-cut citrus on large-scale.

For this reason, further studies compared vacuum and high pressure infusion in presence or absence of enzymes, determining that water infiltration alone could also result in peeling of oranges, but with significantly less juice leakage and firmness loss during storage (Pao et al., 1996b; Pao and Petracek, 1997). However, the resulting citrus segments were not as attractive due to some residual albedo tissue, especially early in the citrus season (less senescent peel).

The aim of this study was to compare fresh segments and slices from oranges infused under vacuum with different solutions of water and enzymes along with post treatment acid dips and temperature conditioning for effect on quality, residual enzyme activity related juice leakage, shelf life and microbial stability.

Materials and Methods

Fruit Material

'Valencia' oranges (*Citrus sinensis* L.) were obtained from a commercial grower in Haines City, Fla. in March (experiment 1), April (experiment 2), and May (experiment 3) of

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2005. Fruit were brought back to the laboratory and stored at 7°C for 2-4 d before processing.

Processing

Before processing each fruit was carefully cleaned with an abrasive pad and hot water. For the third experiment (microbial analysis), oranges were sanitized with peroxyacetic acid (PAA) (StorOx®, BioSafe Systems, Glastonbury, Conn.) just before scoring by dipping fruit in a 100 ppm PAA solution at 35°C for 3 min (Narciso and Plotto, 2005). All handling surfaces and equipment were sanitized with a chlorine solution at 400 ppm or ethanol and, whenever possible, knives and foil sheets were sterilized in an autoclave. Fruit were always handled with sanitized gloved hands.

The peel was scored by hand with a citrus peeler (Sunkist) making six cuts from the stem to the blossom end to permit infusion of solution into the albedo. Scored fruit were kept submerged with weights in three different solutions, prepared with double deionized water at room temperature: 1) water; 2) 0.1% pectinase (Ultrazym 100G, Novozymes, Dittingen, Switzerland); 3) 0.1% pectinase (Rohapect PTE, AB Enzymes, Darmstadt) and placed in a vacuum chamber. These enzymes were chosen since pectinases were reportedly more effective than cellulases (Pretel et al., 1997; Rouhana and Mannheim, 1994) and these pectinases are commonly used in food processing and recommended for peeling of citrus (Anonymous, 2006b). Oranges were infused by evacuating the chamber to ~90 kPa, holding the vacuum for 2 min and then slowly releasing it over a 3 min interval. Water-infused oranges were immediately peeled by hand while enzyme-infused fruit were peeled after 30 min of incubation in air at room temperature. Peeled oranges from enzyme-infused oranges were rinsed individually with deionized water to wash any residual enzyme and gently rubbed to remove excess albedo.

In the first experiment, after water or enzyme infusion, whole oranges were blotted dry with a paper towel, then manually divided into segments. In the second experiment, in order to stop enzymatic activity, peeled oranges from enzymatic infusion (Ultrazym or Rohapect) were dipped in a solution of 0.1 M hydrochloric acid (HCl) or water for 2 min, then washed and blotted dry, or further held at 2°C (conditioning) for 24 h before cutting. Low acidity and low temperature are reported to reduce pectinase activity (Pretel et al., 1997). Since in the first experiment, the enzyme concentration was not high enough to easily facilitate segment separation, fruit were cut radially into 6 sections using a sharp knife (Pao et al., 1997). In the third experiment, oranges were infused in either water or Ultrazym as described above, peeled and cut as in the second experiment. Slices were then air dried and packed. One set of fruit was manually peeled, without prior water or enzyme infusion.

Slices were then placed in 453.6 mL (16 oz) plastic containers with lids (Deli/food containers, Sweetheart cup company, Inc., Owings Mills, Md.) and stored at 5°C for 14 d. Four or five slices were placed in each container.

Orange slices were evaluated weekly for juice leakage, firmness, pH, titratable acidity (TA), and soluble solids content (SSC) in the first and second experiments. In the third experiment, microbial populations were monitored in storage.

Quality Parameters

Firmness. Firmness was tested on orange slices using a XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell and a 5 cm diameter flat probe. Test was carried out with a stroke speed of 2 mm·sec^{-1}, evaluating the maximum force during a 50% strain of the slice. For each treatment 12-15 whole slices were tested, from three replicated containers.

pH, titratable acidity and soluble solids content. pH, titratable acidity (TA) and soluble solids content (SSC) were measured on juice squeezed from the slices used in firmness testing. For pH and TA, a 10 mL sample from each replication, diluted with 50 mL double deionized water, was titrated with 0.1 N NaOH to a pH 8.1 endpoint, using an Orion 950 titrator (Thermo Electron Corporation, Beverly, Mass.). Total SSC was determined with two measurements for each replication with a digital ATAGO PR-101 refractometer (Atago Co, Ltd., Tokyo, Japan). Each replication represents juice from 4-5 slices.

Microbial Assays

For each replication, three representative slices were taken and placed in sterile 950 mL sampling bags (Fisherbrand, Fisher Scientific, Pittsburgh, Pa.). After weighing, 99 mL of sterile phosphate buffer (pH 7.2) was added to the bags and samples were gently massaged by hand for 2 min to disperse all microorganisms present on the fruit slice surface into the buffer. Small aliquots (~5 mL) of buffer were then taken from each bag and plated using a Whitley Automatic Spiral Plater (DW Scientific, Ltd., Shipley, West Yorkshire, UK) onto potato dextrose agar (PDA), orange serum agar (OSA) and plate count agar (PCA) (BD/Difco Brand, Sparks, Md.). The different media were chosen to isolate a broad range of organisms (PCA for bacteria, OSA for microorganisms of citrus products, PDA for yeasts and molds). The plates were incubated at 35°C for 48 h and then left for 2-3 d at room temperature (25°C). The results were read with a ProtoCOL colony counter (Synoptics, Ltd., Cambridge, UK). Colony counts for replicate plates, were averaged and results were reported as cfu/g (cfu = colony forming units).

Statistical Analyses

Data were analyzed by analysis of variance (ANOVA) with the SAS statistical software (SAS System Software Version 9.1, SAS Institute, Cary, N.C., 1999). Separation of means was performed with the Duncan's Multiple Range test, with $\alpha = 0.05$.

Results and Discussion

Juice leakage. In the first experiment, slices from Rohapect enzyme-infused oranges had the most juice leakage, although the variation between replicates was high (Table 1). In the second experiment, juice leakage was generally greater, because segments were cut, instead of being manually separated as in

Table 1. Juice leakage (% juice ± SE) of orange slices infused with double deionized water or commercial enzyme solutions (Ultrazym or Rohapect). Data are means of 3 replicate containers with 4-5 slices per container.

	Days in storage at 5°C	
Treatment	7	14
Water	0.0	0.0
Enzyme (Ultrazym)	0.14 ± 0.14	0.18 ± 0.18
Enzyme (Rohapect)	0.16 ± 0.16	0.37 ± 0.19

the first experiment (Table 2). Indeed, Pao et al. (1997) showed that the higher cut surface area increased juice leakage. The effect of HCl increased juice leakage if the slices were not held at 2°C prior to processing, while holding fruit at 2°C after treatment did not affect juice leakage except in the case of HCl-induced leakage of the Rohapect enzyme-treated fruit. Nevertheless, juice leakage was minimal, probably due to the low concentration of enzymes used compared to most reports (Pretel et al., 1997; Soffer and Mannheim, 1994).

Firmness. In the first experiment, water-infused oranges were firmer than enzyme-infused fruit after 7 and 14 d of storage (Table 3). Increasing firmness for water-infused oranges over storage time was likely due to dehydration of the fruit surface. Decreasing firmness over storage time for Rohapect enzyme-infused oranges was likely due to residual enzyme activity, while Ultrazym enzyme-infused fruit did not change in firmness over the storage period. In the second experiment, slices from HCl-treated fruit were less firm (day 0 for Rohapect, day 7 for both enzymes) if they were not held at 2°C prior to slicing (Table 4). Loss of firmness appeared to be correlated to juice leakage (data not shown), and fruit treated with Rohapect seemed to be more prone to softening than those treated with Ultrazym. In an informal taste panel with 5 laboratory personnel, slices from enzyme-peeled oranges were preferred because they were softer and had a better mouth-feel (data not shown). This was later confirmed by a 20-panelist ranking test (Pinnavaia et al., submitted). In any case, at the enzyme levels used, over-softening of the fruit segments or slices was not a problem according to preliminary sensory data. Therefore, due to results showing less juice leakage and softening compared to the Rohapect enzyme, only Ultazyme was used in the final experiment.

pH, TA and SSC. In the first experiment, pH of water-infused fruit was slightly higher than for enzyme-infused oranges and the TA was lower on day 7 than for the enzyme-treated fruit and the Ultrazym-treated fruit on day 14 (Table 5). Values for SSC were higher in Rohapect enzyme-infused fruit on day 7 and for water-infused fruit on day 14 (Table 5), but differences were slight and were not perceived by an informal taste panel. Also, higher SSC could be due to higher electrolyte leakage, as SSC measures not only soluble sugars, but also any soluble material including acids and soluble pectins. In the second experiment, slices from oranges infused with Ultrazym, treated with HCl and not conditioned at 2°C prior to cutting had the lowest pH (Table 6). For Rohapect enzyme-treated fruit, generally those treated with HCl had a lower pH regardless of conditioning. For TA, slices from oranges infused with Ultrazym, treated with HCl and conditioned had the lowest TA (Table 7). There was no effect of HCl treatment and conditioning for slices from oranges infused with Rohapect, except after 14 days in storage where control slices from Rohapect enzyme-treated fruit (no HCl and no conditioning) had the lowest TA. It appears that the HCl treatment tended to contribute to acid levels, but not always. For SSC, the Ultrazym enzyme-treated fruit treated with HCl generally had slightly higher solids, especially if conditioned at 2°C (Table 8). For Rohapect enzyme-infiltrated fruit solids were higher in conditioned fruit not dipped in HCl.

Microbial analysis. In the third experiment, manual peeling of fruit was compared to water and enzyme (Ultrazym) infusion (Fig. 1). Ultrazym was used since it caused less fruit tissue softening than the Rohapect product in experiment 1. Infusion of any solution into oranges provides an opportunity for infiltration of microorganisms. Results showed that the manual peeling and enzyme-infusion resulted in the lower counts than did water infusion, with the enzyme treatment exhibiting the least counts at the end of storage for all three media representing general microbial populations (OSA), bacteria (PCA), yeast and molds (PDA).

Differences in microbial counts between water and enzyme infused oranges, with enzyme infusion resulting in lower counts, could be due to residual albedo left on water-

Table 3. Firmness (N) of orange slices from oranges infused with double deionized water or commercial enzyme solutions (Ultrazym or Rohapect). Data are means of 12-15 slices from three replicated containers per treatment.[z]

	Days in storage at 5°C		
Treatment	0	7	14
Water	43.06 aB	47.71 aAB	54.00 aA
Ultrazym	40.79 aA	43.24 aA	40.75 bA
Rohapect	38.42 aA	30.83 bB	28.69 cB

[z]Means followed by the same letter within a column (small letters) or row (capital letters) are not significantly different by the Duncan's Multiple Range test ($\alpha = 0.05$).

Table 2. Juice leakage (% juice ± SE) of orange slices infused with commercial enzyme solutions (Ultrazym or Rohapect) followed or not by an HCl dip and held or not at 2°C for 24 h. Data are means of 3 replicate containers with 4-5 slices per container.[z]

			Storage (days at 5°C)	
Enzyme	HCl (M)	Hold at 2°C	7	14
Ultrazym	0.0	no	2.37 b	3.60 b
	0.0	yes	2.70 ab	4.60 ab
	0.1	no	3.93 a	6.40 a
	0.1	yes	3.20 ab	5.23 ab
Rohapect	0.0	no	3.03 b	4.40 b
	0.0	yes	2.63 b	3.67 b
	0.1	no	5.23 a	7.80 a
	0.1	yes	3.10 b	4.53 b

[z]Means followed by the same letter within a column and within an enzyme group are not significantly different by the Duncan's Multiple Range test ($\alpha = 0.05$).

Table 4. Firmness (N) of slices treated or not with 0.1 M HCl to stop enzymatic activity, and conditioned or not at 2°C for 24 h.[z]

			Storage (days at 5°C)		
Enzyme	HCl (M)	Hold at 2°C	0	7	14
Ultrazym	0.0	no	30.75 a	35.17 a	30.66 a
	0.0	yes	33.06 a	32.77 ab	28.18 a
	0.1	no	28.65 a	29.60 b	29.29 a
	0.1	yes	30.31 a	33.78 ab	31.35 a
Rohapect	0.0	no	36.81 a	28.59 ab	28.82 a
	0.0	yes	36.54 a	31.80 a	31.84 a
	0.1	no	22.79 b	25.88 b	27.71 a
	0.1	yes	31.77 a	30.23 ab	31.61 a

[z]Means followed by the same letter within a column and within an enzyme group are not significantly different by the Duncan's Multiple Range test ($\alpha = 0.05$).

Table 5. Values for pH, TA, SSC of orange slices infused with double deionized water or commercial enzyme solutions (Ultrazym or Rohapect). Data are means of three replicate containers with 4-5 slices per container.[z]

	Storage (days at 5°C)		
Treatment	0	7	14
		pH	
Water	4.09 a	4.16 a	4.16 a
Ultrazym	3.98 b	4.04 b	4.15 a
Rohapect	4.05 ab	4.04 b	4.01 b
		TA (% citric acid)	
Water	0.673 a	0.607 b	0.572 b
Ultrazym	0.703 a	0.668 a	0.642 a
Rohapect	0.665 a	0.675 a	0.577 b
		SSC (°Brix)	
Water	10.17 a	10.03 b	10.90 a
Ultrazym	10.37 a	10.32 ab	10.35 b
Rohapect	10.08 a	10.52 a	10.30 b

[z]Means followed by the same letter within a column are not significantly different by the Duncan's Multiple Range test ($\alpha = 0.05$).

infused fruit. After infusion, fruit were rinsed in deionized water and gently brushed to remove residual enzyme and albedo from the fruit surface. Water-infused fruit was treated in the same way. While this action removed albedo from the

Table 6. pH of slices treated or not with 0.1 M HCl to stop enzymatic activity, and conditioned or not at 2°C for 24 h.[z]

			Storage (days at 5°C)		
Enzyme	HCl (M)	Hold at 2°C	0	7	14
Ultrazym	0.0	no	3.78 a	3.76 a	3.75 a
	0.0	yes	3.72 b	3.67 b	3.69 b
	0.1	no	3.61 c	3.61 c	3.60 c
	0.1	yes	3.79 a	3.69 b	3.79 a
Rohapect	0.0	no	3.89 a	3.85 b	3.96 a
	0.0	yes	3.83 ab	3.91 a	3.91 a
	0.1	no	3.83 ab	3.86 b	3.81 b
	0.1	yes	3.80 b	3.88 ab	3.84 b

[z]Means followed by the same letter within a column and within an enzyme group are not significantly different by the Duncan's Multiple Range test ($\alpha = 0.05$).

Table 7. TA (% citric acid) of slices treated or not with 0.1 M HCl to stop enzymatic activity, and conditioned or not at 2°C for 24 h.[z]

			Storage (days at 5°C)		
Enzyme	HCl (M)	Hold at 2°C	0	7	14
Ultrazym	0.0	no	1.025 b	0.978 bc	0.958 b
	0.0	yes	1.095 b	1.013 ab	1.023 a
	0.1	no	1.205 a	1.082 a	1.013 a
	0.1	yes	0.940 c	0.920 c	0.868 c
Rohapect	0.0	no	0.900 a	0.815 a	0.732 b
	0.0	yes	0.960 a	0.800 a	0.802 a
	0.1	no	0.903 a	0.775 a	0.808 a
	0.1	yes	0.947 a	0.817 a	0.845 a

[z]Means followed by the same letter within a column and within an enzyme group are not significantly different by the Duncan's Multiple Range test ($\alpha = 0.05$).

Table 8. SSC (°Brix) of slices treated or not with 0.1 M HCl to stop enzymatic activity, and conditioned or not at 2°C for 24 h.[z]

			Storage (days at 5°C)		
Enzyme	HCl (M)	Hold at 2°C	0	7	14
Ultrazym	0.0	no	11.4 b	11.8 c	11.7 bc
	0.0	yes	11.9 ab	12.0 b	11.6 c
	0.1	no	12.2 a	12.0 b	12.0 b
	0.1	yes	12.3 a	12.6 a	12.5 a
Rohapect	0.0	no	11.5 b	11.6 c	11.5 b
	0.0	yes	12.1 a	12.4 a	12.1 a
	0.1	no	11.1 c	11.1 d	11.6 b
	0.1	yes	11.7 b	12.0 b	12.2 a

[z]Means followed by the same letter within a column and within an enzyme group are not significantly different by the Duncan's Multiple Range test ($\alpha = 0.05$).

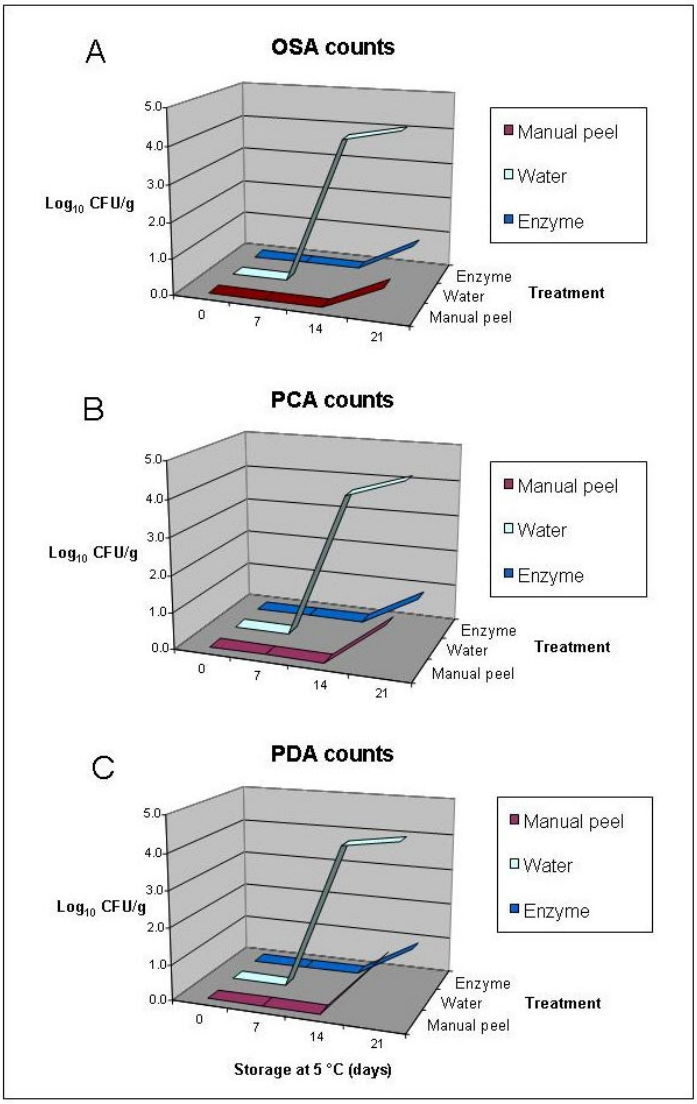

Fig. 1. Orange Serum Agar (OSA) counts (A), Potato Dextrose Agar (PDA) counts (B) and Plate Count Agar (PCA) counts (C) for stored orange segments that were manually peeled or infiltrated with double deionized water or 1% pectinase (Ultrazym) solution.

enzyme-treated fruit, it did not for water-infused fruit. The residual albedo may then have provided safe harbour to any bacteria left. Reduced counts in manually-peeled fruit was expected because of reduced handling. Any added steps in processing of fresh cut fruit increases risk of contamination.

Conclusions

Quality of the enzyme-treated oranges was preferred by the informal sensory panel due to a more attractive appearance and softer texture of the resulting slices. Juice leakage was not that significant at enzyme levels used, and therefore, not considered a great deterrent to commercialization if the fruit are only stored for two weeks, which is within the commercial window for cut fruit products (Del Monte Fresh, personal communication). Previous studies that showed significant juice leakage stored the enzyme-peeled fruit for 3-6 weeks (Baker and Hagenmaier, 1997; Pao et al., 1996b) and generally used higher enzyme levels. Enzyme levels used in this study (1000 ppm) was at the lower range for those reported in the literature (1000 ppm and above) (Ismail et al., 2005; Pretel et al., 1997). Nevertheless, enzyme activity, as reflected by fruit softening, and less by juice leakage, was not greatly affected by either the acid dip or cold conditioning. Water-infusion, in the case of early and mid-season Florida 'Valencia' fruit, resulted in tougher, less attractive slices due to adhering albedo tissue to the peeled fruit as well as higher microbial counts compared to manually peeled or enzyme infiltrated fruit. Of the two commercial enzymes, Ultrazym performed best with minimal juice leakage, softening of slices and microbial contamination despite the infusion process for fruit stored up to two weeks.

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