

Infiltration of Tomatoes Immersed at Different Temperatures to Different Depths in Suspensions of *Erwinia carotovora* subsp. *carotovora*

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ABSTRACT

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In tests with freshly destemmed tomatoes, infiltration did not occur as soon as fruits were immersed in suspensions of *Erwinia carotovora* subsp. *carotovora* unless depths equaled or exceeded 122 cm (4 ft). The barrier to immediate infiltration at shallower depths was the hydrophobic nature of the stem scar tissues, a conclusion derived from two observations. First, drops of an oil-based fruit wax were quickly absorbed by the stem scar tissues, whereas water had to be forced into those tissues. Second, addition of surfactants to suspensions of *E. carotovora* subsp. *carotovora* increased the amount of infiltration twofold to threefold and decreased the time between immersion and onset of infiltration.

Additional key words: *Lycopersicon esculentum*, postharvest decay

In a report by Bartz and Showalter (3), warm (26–40 C) tomatoes immersed for 10 min or longer in cool (20–22 C) suspensions of bacteria in water were infiltrated by water and bacteria. This activity was predicted by the general gas law. Gases in the intercellular spaces of cooling fruit exert reduced pressure, which allows the combined atmospheric and hydrostatic forces on the immersed fruit to force some of the external environment into pores in the stem scar. For tomatoes immersed in suspensions of *Erwinia carotovora* subsp. *carotovora* (Jones, 1901) Bergey et al, 1923 (*Ecc*), the infiltration resulted in the development of bacterial soft rot (BSR) during storage.

Deductively, hydrostatic or waterhead forces would also cause immersed fruits to be infiltrated. Indeed, in the report by Bartz and Showalter (3), fruits held 8 cm below the surface of a water bath for 30 min increased in weight an average of 0.3 g/fruit. Shorter immersion times were not used. In commercial tomato packing-houses in Florida, most tomatoes are immersed in water for 2–5 min to maximum depths of 91.5 cm (3 ft) (Bartz, unpublished). Some fruits may be immersed for more than 5 min during equipment breakdowns, lunch breaks, etc, but these fruits represent a minority of the total volume being packed.

In the following experiments, short immersion times of 15 min to about 10 sec

were used in both temperature differential and immersion depth experiments to assess the potential for infiltration during normal packinghouse operations.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum* Mill.) fruits, mature green or riper, were hand-harvested from commercial fields and experimental plots in Florida. The same cultivar was not available for all tests; fruits within each test were from the same cultivar. Whenever possible, fruits were harvested by clipping the stem (pedicle) with pruning shears so that part of the stem remained on the fruit. The stems were removed just before immersion treatments so that stem scars were fresh. Older stem scars (more than 2 days old) are less porous than fresh scars (8; Bartz, unpublished). However, even with stems attached, longer storage leads to stem scars that were less porous (Bartz, unpublished). As a result, fruits were used within 3–4 days after harvest.

Bacterial suspensions used were prepared from nutrient broth shake cultures about 24 hr old of *Ecc*, isolate SR-12, as described previously (1,2). The inoculum concentration ranged from 5×10^5 to 1×10^6 cells per milliliter. Temperatures of bacterial suspension, water, and fruit were measured to within ± 0.05 C. In the immersion treatments, fruits were weighed, immersed, removed from the bath, rinsed in a solution of sodium hypochlorite (50 mg/L), wiped dry with a cotton towel, reweighed, and stored. Fruit weights were measured to the nearest 0.01 g. The rinse was omitted in the last three experiments.

Fruits were immersed to different depths either by a direct or simulated method. Tomatoes normally float in water with stem scars oriented upward. This orientation was used in all tests. In

direct immersion tests, fruits were forced downward quickly but gently using a wooden plunger, until the desired depth was reached. Simulated depth immersion tests were conducted with a 22-qt Presto aluminum pressure cooker (National Presto Industries, Inc., Eau Claire, WI 54701). Tomatoes were placed into a suspension in the canner, the slotted bottom plate of the canner was placed on the fruits, a flask of water was placed on the plate to force the fruits below the surface of the liquid, the unit was sealed, compressed air was applied, and the timing for the treatment was begun when the desired pressure was achieved. Air pressure in the chamber was continuously monitored with a manometer. The pressure was expressed as centimeters of water.

In some tests, the wetting agents Tergitol anionic-7 (T-7) and Tergitol nonionic-NPX (T-NPX) (Union Carbide Corp.) were added to bacterial suspensions at rates from 0.2 to 0.01% (v/v) of suspension. The viscosity of these solutions was compared with that of distilled water in a Cannon-Fenske Routine Viscometer (Cannon Instrument Co., P.O. Box 16, State College, PA 16801).

In an attempt to block the pores in the stem scar, drops of an oil-based fruit wax, WT-3 (Decco Div., Pennwalt Corp., Monrovia, CA 91016), were added to stem scars of 15 fruits until the entire scar appeared oil-soaked. Wax-treated and control fruits at 37 C were immersed for 10 min in suspensions of *Ecc* at 20 or 37 C.

After treatment, fruits were stored on plastic dinner trays at greater than 75% relative humidity and 30 C, optimum for development of BSR in tomatoes (Bartz, unpublished). Fruits were observed daily for the development of BSR. To discourage secondary spread, diseased fruits were discarded immediately. Tabular data on the percentage of fruits with BSR did not include those where lesions clearly arose from contaminated wounds or from fruit-to-fruit spread on the trays.

Comparisons of weight increases were made with the one-way analysis of variance and Duncan's new multiple range tests. For these analyses, the data were transformed by the formula $\frac{1}{2} + x$ as suggested by Steel and Torrie (7). Each fruit was treated as a replicate.

RESULTS

As observed in previous tests (3), a

weight increase followed by BSR in storage occurred in tomatoes immersed for 10 min just under the surface of a suspension of *Ecc* that was initially 17 C cooler than the incoming fruits (Table 1). If the time of immersion was reduced to 5 min, only a slight weight increase was recorded. Fruits immersed for only 2 min apparently did not increase in weight and had an incidence of BSR similar to that in the control (no temperature differential) treatments.

Diseased fruits in these tests, as well as in those reported previously (3), usually resulted when fruits increased in weight at least 0.1 g. Healthy fruits in treatments where weight increases averaged more than 0.1 g were those that did not absorb at least 0.1 ml of suspension rather than fruits that were resistant to *Ecc*. Apparently, the stem scars of fruits from a given lot often differ greatly in porosity. For example, in the 10 min, 20 and 37 C treatment in test 1, individual fruits gained 0.02–1.01 g. The first fruit was free of BSR at the end of the storage period, whereas the second had BSR within 48 hr of treatment. In addition, fruits that gained more weight generally developed BSR earlier during storage. In test 1, lesions developed on 25% of the fruits by 48 hr after treatment; the average weight gain in these fruits was 0.7 g. Between 48 and 72 hr, BSR appeared in an additional 55% of the fruits; the weight gain of these fruits was 0.4 g.

Hydrostatic forces should be additive with forces associated with the cooling of the immersed fruits. However, when mature green fruits at 37 C were immersed to a depth of 15 cm for 10 min in a suspension of *Ecc* at 20 C, the average weight increases were not statistically greater for fruits immersed to 15 cm than for fruits immersed to about 1 cm (Table 2). Similar results occurred in a test with pink and red fruits (test 2). Hydrostatic forces from 15 cm of water were not associated with greater infiltration in either test.

The failure of hydrostatic forces to be associated with infiltration in the preceding tests (Table 2) may have been caused, at least in part, by the temperature of the suspension used (20 C). In a different test, 10 red tomatoes at 20 C immersed to 31 cm for 15 min in a 20-C suspension gained little weight—0.02 g—whereas fruits at 30 or 37 C immersed in suspensions at those temperatures gained an average of 0.08 and 0.18 g, respectively. The percentages of BSR for 20, 30, and 37 C were 20, 80, and 70%, respectively. The weight increase differences were significant at $P=0.05$. Because temperature differentials were absent, hydrostatic forces were responsible for infiltration.

Greater intake at higher water-fruit temperatures may be associated with reduced surface tension, decreased viscosity, or increased fruit temperature.

Ten green fruits at 37 C immersed for 10 min in a 20-C suspension containing a surfactant (0.2% v/v of T-7) gained an average of 0.76 g; 10 fruits similarly treated in a suspension without surfactant gained an average of 0.41 g (difference significant at $P = 0.01$). However, the respective disease incidences after 2 days in storage were similar, 90 and 85%. Subsequently, in a similar temperature differential experiment with 10 green tomatoes per treatment, fruits immersed in suspension plus surfactant gained nearly twice the weight and had twice the incidence of BSR in storage as did fruits immersed in the control (surfactant-free) suspension. The control fruits gained an average of 0.49 g and the incidence of BSR was 37% after 48 hr in storage.

Fruits in two surfactant concentrations,

0.01 and 0.1% T-7, gained 1.33 and 1.20 g and had 61 and 69% BSR, respectively. The differences between the weight increase of the control and of the two T-7 treatments were significant ($P = 0.05$). These concentrations of T-7 as well as the 0.1% concentration of another surfactant, T-NPX, did not appreciably reduce the viscosity of water as measured by a Cannon-Fenske viscometer.

In the tests with surfactants in the suspensions, the stem scar tissues appeared hydrophobic. Evidence supporting that conclusion was provided by observations made in a test of the possibility that fruit pox (4) (necrotic ruptures in the epidermis of fruit) could serve as portals for ingress. Fruits with pox were treated with a commercial tomato fruit wax before immersion in an

Table 1. Bacterial soft rot and weight increase associated with immersion^v of mature green tomatoes for different lengths of time in suspension of *Erwinia carotovora* subsp. *carotovora*^w

Temperature (C)		Time (min)	Weight increase (g/fruit) ^x		Disease (%) ^y	
Suspension	Fruit		Test 1	Test 2	Test 1	Test 2
20	37	10	0.41 a ^z	0.17 a	90	90
		5	0.06 b	0.03 b	20	30
		2	0.01 b	0.01 c	15	0
37	37	10	0.00 b	0.00 c	10	0
		5	0.03 b	0.00 c	10	0
		2	0.00 b	0.00 c	5	0

^v Fruit were held about 1 cm under surface of suspension.

^w Florida isolate SR-12 at 1×10^6 cells per milliliter.

^x Average for 20 fruits; weights to nearest 0.01 g.

^y After storage at 30 C for 5 and 3 days for tests 1 and 2, respectively.

^z Values not followed by the same letter were different at $P = 0.05$.

Table 2. Bacterial soft rot and weight increase associated with 10-min immersion of tomatoes at two depths in suspensions of *Erwinia carotovora* subsp. *carotovora*^w

Temperature (C)		Depth (cm)	Weight increase (g/fruit) ^x		Disease (%) ^y	
Suspension	Fruit		Test 1	Test 2	Test 1	Test 2
20	37	15	0.34 a ^z	0.07 a	30	50
		1	0.17 a	0.03 b	30	40
37	37	15	0.08 b	0.01 b	0	10
		1	0.04 b	0.02 b	0	10

^w Florida isolate SR-12 at 1×10^6 cells per milliliter.

^x Average of 10 mature green and 15 riper (10 pink, 5 red) fruits in tests 1 and 2, respectively; weights to nearest 0.01 g.

^y After storage at 30 C for 5 and 4 days for tests 1 and 2, respectively.

^z Values not followed by the same letter were different at $P = 0.05$.

Table 3. Bacterial soft rot and weight increase associated with 10-min immersion^v of mature green tomatoes with or without waxed stem scars^v in suspensions of *Erwinia carotovora* subsp. *carotovora*^w

Treatment	Temperature (C)		Weight increase (g/fruit) ^x	Disease (%) at days ^y	
	Suspension	Fruit		4	8
Waxed stem scar	20	37	0.50 a ^z	60	100
	37	37	0.49 ab	60	100
Control	20	37	0.20 bc	50	60
	37	37	0.02 c	0	0

^v Held about 1 cm surface of suspension.

^v All exposed tissues of stem scar wetted by commercial tomato fruit wax.

^w Florida isolate SR-12 at 1×10^6 cells per milliliter.

^x Average of 10 and 5 fruits per 20–37 and 37–37 C treatment, respectively; weights to nearest 0.01 g.

^y After storage at 30 C.

^z Values not followed by the same letter were different at $P = 0.05$.

effort to block the pores in the stem scars. Drops of the viscous, oily wax were absorbed by the stem scar tissues nearly as rapidly as they were applied; drops of water were never absorbed by those tissues. After several drops of fruit wax were placed in the stem scar, parts of the adjacent fruit wall became translucent, evidence that the intercellular spaces in the fruit wall were also hydrophobic. The wax treatment did not block the pores.

Table 4. Bacterial soft rot and weight increase associated with immersion of mature green tomatoes to depth of 31 cm in suspension of *Erwinia carotovora* subsp. *carotovora*^v

Time (min)	Weight increase (g/fruit) ^w	Disease (%) at days ^x	
		2	3
15	0.67 a ^y	79	100
10	0.24 b	21	50
5	0.01 c	0	0
2	0.01 c	0	0
0 ^z	0.00 c	0	0

^v Florida isolate SR-12 at 5×10^5 cells per milliliter.

^w Average of 14 fruits; each weighed to the nearest 0.01 g. Temperature of fruit and suspension = 37 C.

^x After storage at 30 C.

^y Values not followed by the same letter were different at $P = 0.01$.

^z Time at depth about 1 sec.

Fruits with waxed stem scars gained more weight during a 17-C temperature differential treatment and had a higher incidence of BSR after treatment as compared with control treatments (Table 3). The pox lesions were not readily infiltrated by the suspensions. Few BSR lesions originated at pox lesions; most were associated with the stem scar. Fruits in the waxed 37 and 37 C treatment also gained weight and developed BSR; those in the unwaxed 37 and 37 C treatment did not. The wax treatment increased the porosity of the stem scar tissues to water rather than sealing them.

In the absence of surfactants in the suspensions of *Ecc*, fruits immersed to 31 cm were infiltrated if the exposure time was 10 min or longer (Table 4). Fruits immersed for 0-5 min did not gain weight and were not diseased during storage. However, if a surfactant was added to the suspension and the total pressure on the immersed fruits was equivalent to a 68-cm (2.2-ft) waterhead, ingress of water and *Ecc* occurred within 2 min and was quite extensive by 5 min (Table 5) as compared with previous experiments. But infiltration was still not instantaneous with immersion: fruits immersed to 68 cm and immediately allowed to rise to the surface did not increase in weight and were still free of BSR after 6 days of storage.

Table 5. Bacterial soft rot and weight increase associated with simulated immersion^u of green tomatoes in 0.1% (v/v) surfactant^v in water or surfactant in suspension of *Erwinia carotovora* subsp. *carotovora*^w

Treatment	Time (min)	Depth (cm)	Weight increase (g/fruit) ^x	Disease (%) ^y
Suspension	15	1	0.06 d ^z	20
	15	68	0.68 b	100
	10	68	0.88 ab	100
	5	68	0.39 c	100
	2	68	0.17 d	70
	0	68	0.03 d	0
Water	15	68	1.01 a	0

^u Total force on immersed fruit = immersion depth + air pressure; 0-time treatment = about 1 sec.

^v Tergitol nonionic-NPX at 0.1% (v/v) water or suspension.

^w Florida isolate SR-12 at 1×10^6 cells per milliliter.

^x Average of 10 fruits weighed to the nearest 0.01 g; temperature of fruit and suspension = 26 C.

^y After 24 hr at 30 C.

^z Values not followed by the same letter were different at $P = 0.05$.

Table 6. Bacterial soft rot and weight increase associated with 2- or 10-min simulated immersion^v of green tomatoes to various depths in suspension of *Erwinia carotovora* subsp. *carotovora*^w

Depth (cm)	Weight increase (g/fruit) ^x	2 min		10 min	
		Disease at days ^y		Disease (%) at days ^y	
		1	5	1	5
68	0.20 bc ^z	100	100	0.54 a	90
51	0.09 cd	90	90	0.31 b	100
34	0.01 d	0	30	0.08 cd	70
17	0.02 d	0	0	0.03 cd	30
1	0.00 d	0	0	0.02 d	0

^v Total force on immersed fruit = immersion depth + air pressure.

^w Florida isolate SR-12 at 1×10^6 cells per milliliter and 0.1% (v/v) Tergitol nonionic-NPX in water.

^x Average of 10 fruits per treatment; weights to nearest 0.01 g; temperature of fruit and suspension = 26 C.

^y After storage at 30 C.

^z Values not followed by same letter were different at $P = 0.05$.

Fruits immersed to 68 cm for 15 min in water gained more weight than those similarly treated with the bacterial suspension. The reason for this difference was not clear, because fruits treated in the suspension for only 10 min gained just as much weight, statistically, as did those in the 15-min water treatment. The fruits in the latter treatment gained the most weight in the test but were free of disease. Water ingress per se did not initiate BSR. Furthermore, in this test the chlorine rinse after immersion was eliminated. Fruits of the 0-time treatment were free of either internal or external BSR lesions even after 6 days of storage. This was evidence that most diseased fruits in infiltration experiments were inoculated by ingress of *Ecc*; few were inoculated by contamination of the surface of the fruits with *Ecc*.

Subsequently, in a test with 2- and 10-min immersions, ingress occurred with either exposure time at the 34-cm depth, but only the 10-min immersion resulted in ingress at 17 cm (Table 6). Fruits exposed to less force for shorter periods of time were free of BSR. Once again the postimmersion chlorine rinse was omitted and, as in the preceding test, inoculation was associated with ingress, not with surface contamination.

Evidence that ingress may occur instantaneously with the application of pressure was obtained in a test with a maximum immersion depth of 244 cm (8 ft) and a 5-day storage period (Table 7). Bacterial soft rot developed within 48 hr of treatment in 30 and 70% of fruits immersed to 122 and 244 cm, respectively, in the 0-time treatments. In the 0-time treatment, the elapsed time that the fruits were in contact with the suspension was about 15 sec. The total time at the indicated depth was less than 1 sec and from the application of pressure to release of pressure was less than 6 sec.

Fruits treated with less force or for shorter times had no disease after storage. This again supported the connection between the lack of ingress and lack of BSR. However, when BSR appeared, it did so very quickly, especially among fruits that absorbed at least 0.1 ml of the suspension of *Ecc*; 85 and 98% were diseased by 24 and 48 hr after treatment, respectively.

DISCUSSION

Infiltration must be prevented, not simply limited, in commercial tomato packinghouses. An inadvertent inoculation by infiltration is much more devastating in terms of postharvest losses than inoculation through contamination of the surface of fruits. Several characteristics of inoculation by infiltration lead to that conclusion. First, any or all fruits in a given lot may be infiltrated. With inoculation by surface contamination, only fruits with small wounds, up to about 2 mm³ in volume or

the size of a puncture made by a grain of sand (1,2), would be involved; fruits with larger wounds would be culled out and those without wounds would not be inoculated.

Second, infiltration may involve large, even milliliter, volumes of water, whereas the maximum volume contained by a 2-mm³ wound is 2 μ l. Consequently, with infiltration, the concentration of microbes suspended in water need not be as great to achieve inoculation as with surface contamination.

Third, with inoculation by infiltration, the pathogen is deposited in unwounded tissues in the interior of the fruits; the environment should be ideal for growth. With surface contamination, the pathogen is deposited in an environment that may be acid because of damaged cells, that may become dry, or that may be affected by a wound response of the host (1).

Fourth, with infiltration the inoculum may be dispersed over a relatively large area within the host (3). Colonization of extensive areas inside infiltrated fruits is probable; at disease onset, the initial lesions may involve large portions of the fruits. With inoculation by surface contamination, the pathogens begin colonization in a limited area; the initial lesion will originate from a point.

Finally, infiltrated fruits were frequently visibly diseased within 24 hr after inoculation; bacterial ooze often emanated from these fruits within 24 hr of disease onset. In fact, even daily removal of fruits with BSR did not always prevent fruit-to-fruit spread on the dinner trays containing the fruits (Bartz, unpublished). Previously, with inoculation of small wounds on fruits, the first lesions were observed from 48 hr to more than 4 days after treatment, and removal of diseased fruits every other day prevented fruit-to-fruit spread (1).

When immersed in water, tomatoes may be infiltrated because of two different phenomena. The temperature differential phenomenon, discussed previously (3), involves the cooling of immersed fruits; it requires that the immersed fruits be initially warmer than the water and that fruits be in contact with the water long enough to cool. Consequently, this phenomenon depends on time and temperature. The second phenomenon arises from hydrostatic forces and is theoretically independent of both time and temperature. The forces that cause infiltration are created as the fruits are immersed. However, in the tests reported here, the onset of infiltration lagged behind the creation of force unless depths of 122 cm or more were used.

The two phenomena affect each other. When warm fruits are immersed in cool suspensions, the extent of the infiltration should be governed by the sum of forces due to immersion and those due to cooling. However, because higher temperatures in immersion depth tests led

Table 7. Bacterial soft rot and weight increase associated with simulated immersion^v of mature green tomatoes to various depths for various times in suspension of *Erwinia carotovora* subsp. *carotovora*^w

Depth (cm)	0 min		2 min		10 min	
	Weight increase (g/fruit) ^x	Disease (%) ^y	Weight increase (g/fruit)	Disease (%)	Weight increase (g/fruit)	Disease (%)
244	0.00 e ^z	70	2.52 b	100	3.94 a	100
122	0.00 e	30	0.52 d	90	1.08 c	100
61	0.00 e	0	0.00 e	20	0.12 e	40
31	0.00 e	0	0.00 e	0	0.04 e	20
1	0.00 e	0	0.00 e	0	0.00 e	0

^v Total force on immersed fruit (expressed in centimeters of water) = immersion depth + air pressure; 0-time treatments = about 1 sec at indicated depths.

^w Florida isolate SR-12 at 1×10^6 cells per milliliter with 0.01% (v/v) Tergitol nonionic-NPX in water.

^x Average of 10 fruits per treatment, each weighed to nearest 0.01 g; temperature of fruit and suspension = 26 C.

^y After 3 days at 30 C.

^z Values not followed by the same letter were different at $P = 0.01$.

to increased infiltration, the combined action of cooling and immersion depth on submersed fruit can not be determined through simple calculation. The air-water surface tension for pure water at 40 C is only 4% less than that at 20 C; it appears unlikely that the high-temperature effect on infiltration is related to decreased surface tension. On the other hand, the viscosity of pure water at 40 C is more than 34% lower than that at 20 C, and warmer fruit could be expected to have larger pores through expansion than would cooler fruit.

In addition, as noted by Bartz and Showalter (3), the warming of cool fruits in warm suspensions should result in an expansion of the gases inside the fruits, thereby counteracting the fluid pressures on them; ingress should be blocked. However, this balance of the two phenomena with regard to ingress of water in immersed tomatoes may occur over a relatively narrow range of depths and temperatures. Clearly, in the tests reported here, the release of pressure on tomatoes immersed to various depths did not result in an immediate expulsion of inoculum. Tomatoes that are infiltrated because of depth are not likely to be freed of inoculum because of expansion of warming internal gases.

A complicating factor in making practical and effective recommendations on ways to prevent infiltration is the inherent variation in the porosity of tomato stem scars. Variation was found not only among fruits within a given lot, as discussed here, but also among different cultivars (Bartz, unpublished). Another consideration is that the surface tension of the water in the dump tank may vary because of residues washed from the surfaces of the incoming fruits. As a result of these problems, recommendations must be based on the worst possible case.

Some may argue that the maintenance of adequate hypochlorous acid concentrations in the water (5,6) eliminates the need for prevention of infiltration.

However, because infiltration can be instantaneous with immersion, hypochlorous acid levels must be sufficient to inactivate microbes immediately when fruits enter the water.

When we consider the inactivation of hypochlorous acid by debris accompanying harvested fruits (5), it may be impossible to maintain adequate levels of the germicide under all conditions. Utilizing both chlorination and prevention of infiltration provides a margin of safety for packinghouse operators that either technique, by itself, fails to provide.

Based on observations reported here and those reported previously (3), one may prevent infiltration of tomatoes that are handled in water by keeping exposure times to less than 2 min and immersion depths to less than 17 cm (7 in); with a maximum 2-min exposure, the water temperature need not be controlled. The 17-cm depth would not appear to be unrealistic for commercial handling because that depth would easily allow for a double layer of floating tomatoes in a flume system. In most flume systems I have observed, the fruits were in a single layer by the time they reached the conveyor that took them out of the tank. The dump tank water should be continuously chlorinated as recommended (5,6) to keep the inoculum concentration below the level (about 500 cells or spores per milliliter) that would inoculate small wounds or initiate disease as a result of trace infiltration of exceptionally porous fruits.

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