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Postharvest Biology and Technology 14 (1998) 317–324

Postharvest Biology and Technology

Effects of freezing rate on astringency reduction in persimmon during and after thawing

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Received 24 April 1998; accepted 12 August 1998

Abstract

We compared the reduction of astringency in the flesh of persimmon fruit (*Diospyros kaki* Thunb. cv. 'Hiratanenashi') either slowly frozen in a conventional freezer or rapidly frozen with liquid nitrogen, during and after thawing. Astringency and concentration of soluble tannins in flesh frozen by either method had decreased little by the start of thawing. In slowly frozen flesh, soluble tannins decreased greatly during thawing, and the thawed flesh was almost non-astringent. However, rapidly frozen flesh was still astringent after being thawed. Astringency reduction in frozen and thawed persimmon flesh is probably not related to changes in pectic substances, which changed little during and after thawing, whatever the mode of freezing. Microscopic observations suggested that these differences may be related to the degree of damage that occurred in flesh cell walls and plasma membranes during freezing. Soluble tannins, released from tannin cells during thawing, became insoluble on contact with fragments of cell walls and plasma membranes. The process of tannin insolubilization was greatest for slowly frozen flesh. Such adhesion of soluble tannins to fragments of cell walls and plasma membranes may be a factor that reduce astringency in persimmon fruit frozen slowly during and after thawing.

Keywords: Persimmon; *Diospyros kaki*; Astringency; Freezing; Thawing

1. Introduction

The astringency of astringent persimmon (*Diospyros kaki* Thunb.) fruits can be removed by insolublizing the soluble tannins that give rise to the astringent taste. These tannins are polymerized by acetaldehyde that can accumulate in the flesh during exposure to either ethanol vapour or high levels of carbon dioxide gas (Taira, 1996). In addition, it is known that astringency of persimmon fruits can also decrease during freezing and thawing even though little acetaldehyde accumulation occurs (Nakamura, 1961). Taira and Watanabe (1995) recently found that there is only a slight loss of astringency in 'Hiratanenashi'

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0925-5214/98/$ - see front matter
PII S0925-5214(98)00057-X

fruits during the freezing phase, but it decreases markedly during and after thawing. Therefore, we believe that some unknown factors other than acetaldehyde may be involved in the reduction of astringency during and after the thawing of frozen persimmon fruits.

We have previously reported that the formation of a complex between soluble pectins released from the flesh cell walls and tannins released from the vacuoles in tannin cells can result in reduced persimmon astringency during postharvest fruit softening, with no relationship to acetaldehyde accumulation (Taira et al., 1997). It is also possible, although not yet shown, that this complex formation may be involved in astringency reduction during and after the thawing of frozen persimmon fruits.

The objective of this study was to identify the factors that may cause astringency reduction during and after thawing of frozen persimmon fruit tissue. Although the effects of different rates of freezing on astringency reduction in persimmon fruit flesh were examined, we used freezing as a treatment to help identify the factors related to astringency reduction, rather than as a postharvest technology for removing astringency from persimmon fruits.

2. Materials and methods

2.1. Fruit materials

Mature and fully coloured fruits of the astringent persimmon (*D. kaki* Thunb. cv. 'Hiratanenashi') were harvested in late October at the orchard of Yamagata University at Tsuruoka City. After harvest, fruits were held at 20°C for about 48 h prior to experimentation, either in air or carbon dioxide gas ($>90\%$) in order to produce astringent or non-astringent fruit, respectively.

2.2. Methods of freezing and thawing

Astringent and non-astringent fruit flesh was cut into $1 \times 1 \times 4$ cm sticks each weighing about 5 g. Individual flesh sticks obtained from different fruit, wrapped in a piece of aluminum foil, were frozen in one of two ways: slow freezing in a conventional freezer (Medical Freezer, Sanyo Denki Co., Tokyo) at -20°C (it took ≈ 1 h to freeze), or rapid freezing by immersion in liquid nitrogen for 1 min before being placed in the same freezer. The sticks were kept frozen for about 30 days and then thawed in their wrapping at room temperature (20°C).

2.3. Assays of astringency and tannins

Three sensory panelists evaluated the intensity of astringency for small cubes of flesh ($5 \times 5 \times 5$ mm) both when initially placed in the mouth and after chewing, as described in our previous paper (Taira et al., 1997). Scores used are defined as follows: 0, not astringent; 1, almost not astringent; 2, slightly astringent; 3, rather astringent; 4, strongly astringent. Several minutes were allowed between evaluations. Statistical analysis was performed using a paired comparison test, by counting samples which had more astringency.

The soluble tannins of whole sticks and thawed drips, prepared from astringent fruit frozen in either treatment, were extracted with 80% MeOH and measured by the Folin–Denis method (Taira, 1996). Insoluble tannins in the frozen sticks were assayed in the pelleted residue of the above extract. The residue was re-suspended and sequentially extracted with 0.02% HCl in MeOH, 0.1% HCl in MeOH, and 1% HCl in MeOH, at room temperature, then with 1% HCl in MeOH at 40°C for 30 min, and finally at 60°C for 30 min, by a modification of the method described by Taira and Ono (1997). The tannin concentration in the supernatant after each extraction was assayed by the Folin–Denis method. Measurements were performed on non-frozen sticks, on sticks at removal from the freezer, and after 6 and 24 h during thawing. Measurements were replicated using three different flesh sticks from different fruit.

2.4. Assay of pectin and hemicellulose

The pectins and hemicellulose of alcohol-insoluble solids in both astringent and non-astringent flesh sticks were measured by the method de-

scribed previously (Taira et al., 1997). Measurements were performed in parallel with tannin measurement, and replicated using three different flesh sticks.

2.5. Acetaldehyde assay

The acetaldehyde in the flesh sticks during and after thawing was measured by gas chromatography as described by Taira et al. (1989).

2.6. Microscopy

Samples of flesh sticks from astringent fruit and sticks at the beginning of thawing and after being thawed were cut into $5 \times 5 \times 5$ mm blocks for scanning electron microscopy (SEM). The blocks were fixed with FAA and dehydrated through a graded EtOH series. After plating with gold, the surface of each block was observed by SEM (ABT-32, ABT Co., Tokyo). Samples were cut out from thawed flesh sticks with a stainless steel razor blade, stained with 5% $FeCl_3$, and pressed with a cover glass for observation by light microscopy (LM).

3. Results and discussion

3.1. Effects of freezing rate on astringency and tannin concentration

Astringency of the flesh sticks at the start of thawing was the same as that prior to freezing by either of the freezing methods (Table 1). However, 6 h after the start of thawing, the sticks that had been frozen slowly showed only slight astringency, even after the cube had been chewed. In contrast, the rapidly frozen sticks were still very astringent. Twenty-four hours after the start of thawing, the astringent taste had disappeared from the sticks that had been frozen slowly, but those that had been frozen rapidly were still astringent.

The soluble tannin concentrations of the flesh sticks during and after thawing paralleled the degree of astringency (Fig. 1). Changes in soluble tannin concentration were negligible during the freezing phase for both treatments. During thawing, the decline in tannin concentration was greatest for slowly frozen sticks.

Little or no acetaldehyde accumulation was detected in the flesh sticks during freezing or after thawing with either freezing method (data not shown). Some drips appeared during thawing from sticks frozen at either rate, and the amounts of these drips varied little. Drips from slowly frozen sticks were almost completely non-astringent, while those from rapidly frozen sticks were strongly astringent.

We previously reported that there was little or no reduction of astringency during the freezing of intact persimmon fruit, when frozen slowly in a conventional freezer, and that the concentration of soluble tannins decreased during and after thawing with little acetaldehyde accumulation in the flesh (Taira and Watanabe, 1995). The results of the present study with flesh sticks frozen slowly were almost the same, but this was not the case in those frozen rapidly. The degree of reduction in astringency and the change in the concentrations of soluble tannins during and after thawing, how-

Table 1
Effects of freezing rate on flesh astringency of frozen persimmon during and after thawing

	Astringency score	
	After being put into mouth	After being chewed
Before freezing	4.0	4.0
Just after the start of thawing		
Slow freezing	4.0	4.0
Rapid freezing	4.0	4.0
Significance	NS	NS
6 h after the start of thawing		
Slow freezing	0.0	1.0
Rapid freezing	3.0	4.0
Significance[a]	5%	5%
24 h after the start of thawing		
Slow freezing	0.0	0.0
Rapid freezing	2.5	3.5
Significance[a]	5%	5%

NS, not significantly different.
[a] Significantly different at 5% level.

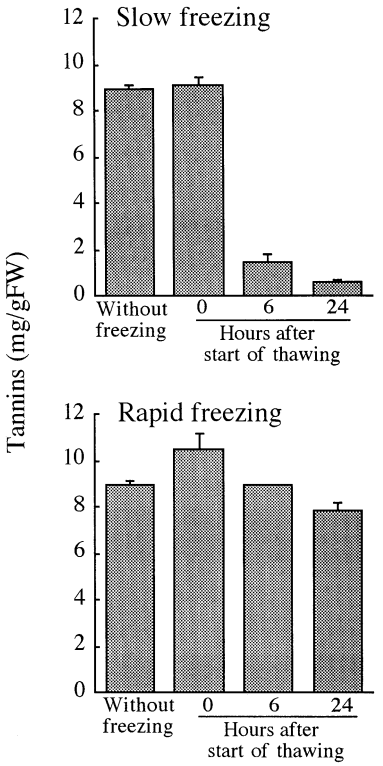

Fig. 1. Effects of freezing rate on soluble tannin concentration in frozen persimmon flesh during and after thawing. Bars indicate S.E. ($n = 3$).

ever, may depend almost entirely on the rate of freezing of the tissues.

3.2. Characteristics of insoluble tannins in frozen and thawed flesh sticks

Insoluble tannins can be resolubilized by extraction with acid in methanol. Ben-Arie and Sonego (1993) found that the tannins in 'Triumph' persimmon fruit made insoluble by carbon dioxide gas treatment could be made soluble again with 1% HCl in MeOH at room temperature. Oshida et al. (1996) showed that 80% of the tannins made insoluble by on-tree ethanol vapour treatment of 'Hiratanenashi' fruit were made soluble by extraction with 1% HCl in MeOH at room temperature, but the percentage decreased gradually during the browning reaction of the insoluble tannin cells after treatment, while fruit was still on the tree. When softened mature 'Hiratanenashi' fruits after harvest were massaged gently by hand, causing a proportion of the tannins to become insoluble, some of the insoluble tannins were made soluble again only by heat extraction with 1% HCl in MeOH (Taira and Ono, 1997). The results of these studies suggested that the proportion of insoluble tannins that became soluble again was affected by treatment for removal of astringency and probably also varied with the cultivar.

In our study, much greater amounts of soluble tannins became insoluble during and after thawing in the sticks frozen slowly than in those frozen rapidly. Most of the insoluble tannins in flesh sticks frozen slowly and then thawed were made soluble with a low concentration (0.02 and 0.1%) of HCl in MeOH at room temperature (Fig. 2). This finding suggested that the lost astringency of persimmon flesh slowly frozen and thawed might reappear quite easily.

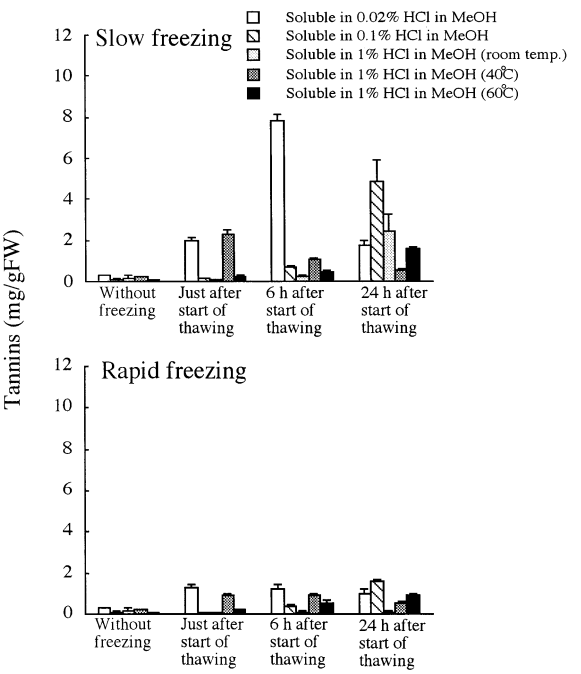

Fig. 2. Effects of freezing rate on resolubilization of insoluble tannins from frozen persimmon flesh during and after thawing. Bars indicate S.E. ($n = 3$).

3.3. Changes in pectins and hemicellulose during and after thawing

Taira et al. (1997) reported that soluble pectins which rise during flesh softening may form a complex with soluble tannins, resulting in a reduction of astringency in intact persimmon fruit. Thus, if pectic substances in the flesh depolymerize during freezing and after thawing, increasing the amount of water-soluble pectin, complex formation between soluble pectin and soluble tannins may reduce astringency.

The concentrations of total pectin, WSP, ESP, HSP, and hemicellulose changed little in astringent flesh sticks frozen at either freezing rate during and after thawing (Fig. 3). This tendency was the same in non-astringent flesh sticks that had been treated with carbon dioxide gas to make tannins insoluble, except for the higher concentrations of WSP and hemicellulose than in astringent flesh sticks (Fig. 4). In either case, there were no differences in the changes in pectic substances between flesh sticks frozen slowly and those frozen rapidly. These findings suggest that reduction in astringency or decrease in levels of soluble tannins in flesh sticks frozen slowly may have no relationship to water-soluble pectins in the sticks.

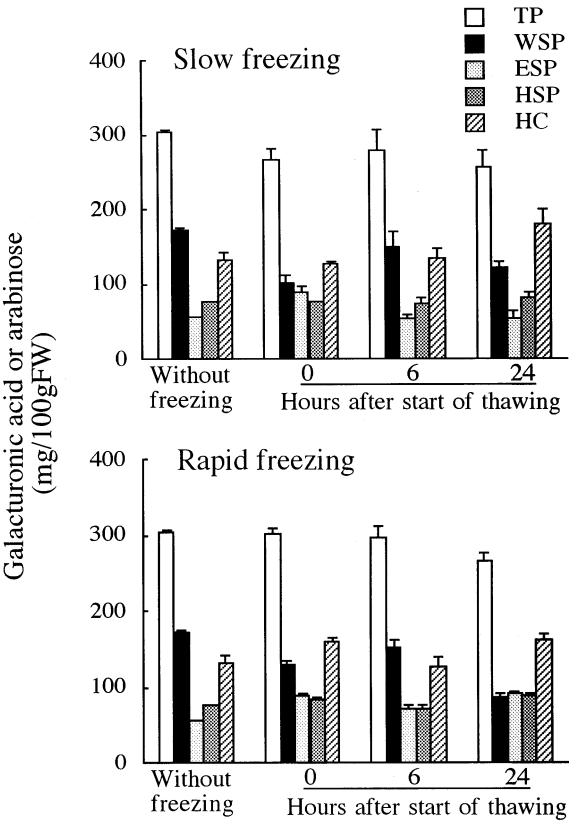

Fig. 3. Mean changes in concentrations of pectins and hemicellulose of slowly and rapidly frozen astringent persimmon flesh during and after thawing. Pectic substances and hemicellulose are expressed as equivalents of galacturonic acid and arabinose, respectively. TP, total pectin; WSP, water-soluble pectin; ESP, EDTA-soluble pectin; HSP, HCl-soluble pectin; HC, hemicellulose. Bars indicate S.E. ($n = 3$).

3.4. Microscopy

The differences in the changes in astringency depending on the rate of freezing may be due to the different effects of these treatment on the structure of flesh tissues. Fuchigami et al. (1994) studied the effects of freezing rate on the texture and histological structure of frozen and thawed carrot disks. They showed that cell damage increases with slower rates of freezing. The larger ice crystals in plant tissue frozen slowly may account for this difference (Ashworth, 1992). Our results showing that sticks frozen rapidly have little cell damage appear consistent with the observations by Ashworth (1992) and Fuchigami et al. (1994) in other plant tissues.

SEM observation showed that the damage in cells was greater in flesh sticks frozen slowly than in those frozen rapidly (Fig. 5). The shapes of non-frozen flesh cells were regular (Fig. 5A), but damage, and a large gap between the cell wall and cell contents caused by slow freezing, were seen in cells just after and at 6 and 24 h after the start of thawing (Fig. 5B–D). Rapid freezing resulted in much less cell damage, except at 24 h after the start of thawing, at which time point cells in the sticks were constricted (Fig. 5E–G).

Under LM, the fragments of cell walls and plasma membranes of flesh samples slowly frozen and then thawed turned more black when stained with $FeCl_3$ than when flesh was rapidly frozen and then thawed (micrographs not shown). This suggested that tannins in the flesh frozen slowly, which have become insoluble during and after thawing, bind with these fragments.

These observations suggested that flesh cells, including tannin cells, were damaged or destroyed during slow freezing, and soluble tannins were ready to flow out from the tannin cells but were frozen. Soluble tannins that had been frozen were released from the collapsed tannin cells during and after thawing; they probably adhered loosely to the fragments of the cell walls or plasma membrane, becoming insoluble and resulting in lower astringency as we suggested previously (Taira and Ono, 1997). The tannin cells of flesh sticks frozen rapidly remained almost intact during and after thawing, so soluble tannins could be released from the thawed tannin cells directly into the $FeCl_3$ solution when the flesh samples were pressed with a cover glass for observation by LM.

Fuchigami et al. (1995) postulated that slow freezing caused more pectin to be released from the cell walls of frozen and thawed carrot disks than rapid freezing. The present results showed that pectic substances varied little between the flesh sticks frozen slowly and those frozen rapidly. Thus, soluble pectin may not contribute to reduction of astringency during and after thawing in persimmon flesh frozen slowly.

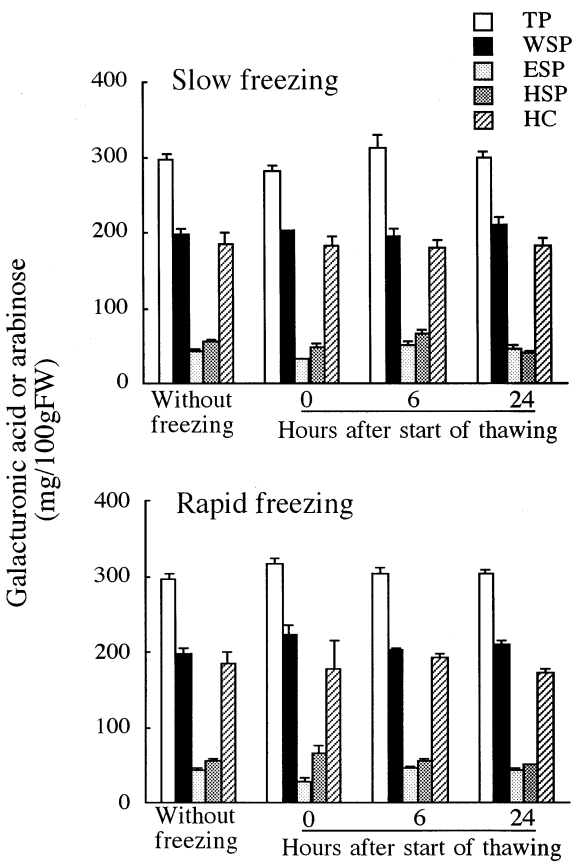

Fig. 4. Mean changes in concentrations of pectins and hemicellulose of slowly and rapidly frozen non-astringent persimmon flesh during and after thawing. Pectic substances and hemicellulose are expressed as equivalents of galacturonic acid and arabinose, respectively. TP, total pectin; WSP, water-soluble pectin; ESP, EDTA-soluble pectin; HSP, HCl-soluble pectin; HC, hemicellulose. Bars indicate S.E. ($n = 3$).

3.5. Mechanisms of reduction of astringency in frozen and thawed persimmon flesh

Gottreich and Blumenfeld (1991) compared the structural characteristics of cell walls of astringent and non-astringent persimmon cultivars. They demonstrated that the cell walls of astringent cultivars were weaker than those of non-astringent cultivars. Such relatively weak cell walls could be severely damaged during slow freezing. Soluble tannins in damaged tannin cells would be readily released from their vacuoles, but would not yet bind or adhere to the fragments of cell components due to being frozen. During thawing of the flesh, thawed soluble tannins may bind loosely to the sections of walls and membranes of the cell, becoming insoluble. Complex formation between apoplastic soluble pectins and tannins, which can reduce astringency (Taira et al., 1997), may not play an important role in reduction of astringency in frozen and thawed persimmon flesh. In the flesh frozen rapidly, the cells remained almost intact and the whole population of tannin cells that contain soluble tannins can be frozen. On thawing, these tannins would remain within the vacuoles and would not come into contact with apoplastic cell components. Thus, the thawed flesh was still strongly astringent.

Taira and Watanabe (1995) reported that fruit maturity affected the degree of reduction of astringency during and after thawing when intact

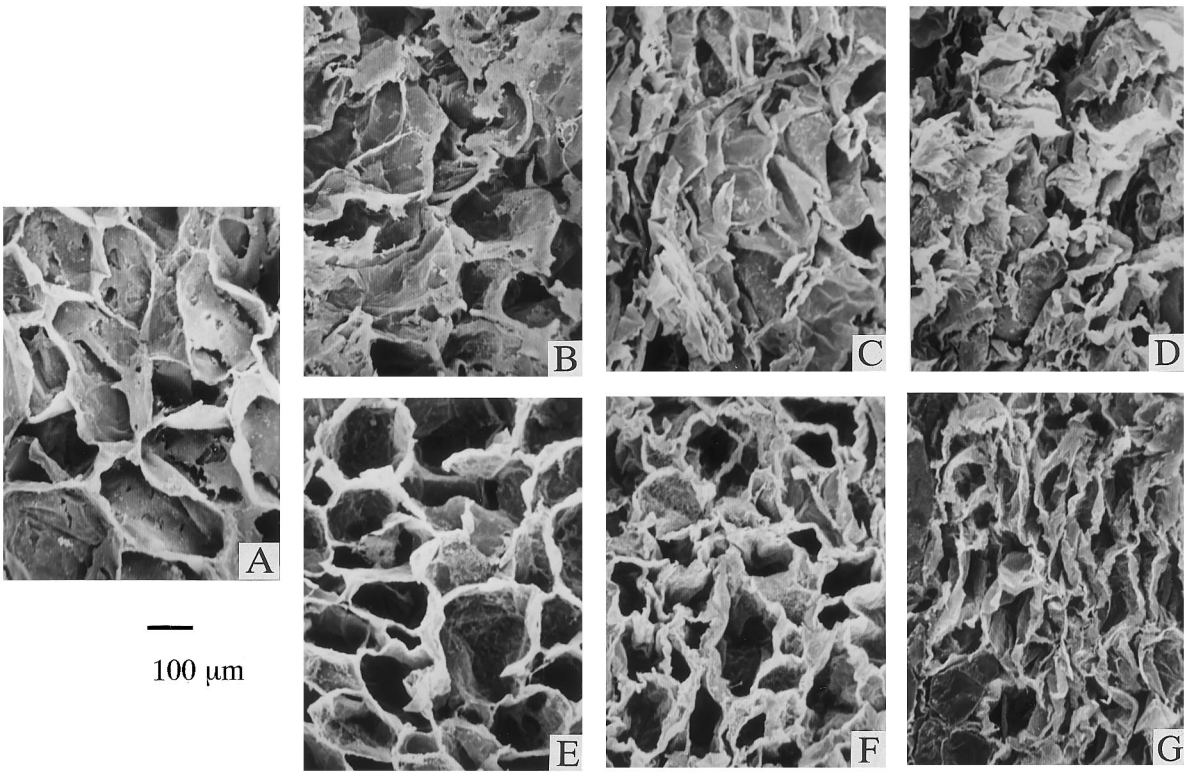

Fig. 5. Scanning electron micrographs of slowly and rapidly frozen persimmon flesh during and after thawing. (A) Before freezing; (B–D) slowly frozen flesh; (E–G) rapidly frozen flesh; (B) and (E) at the start of thawing; (C) and (F) 6 h after the start of thawing; (D) and (G) 24 h after the start of thawing.

fruits were frozen slowly. This may have some correlation with the concentration of soluble tannins in the flesh and the qualitative differences in characteristics of cell walls, plasma membranes, and other cell components during fruit development. The effects of fruit maturity on astringency reduction in frozen persimmon flesh should be studied further.

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