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Sugar content changes in persimmon fruits (*Diospyros kaki* L.) during artificial ripening with CO_2: a possible connection to deastringency mechanisms*

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The existence of a very active invertase was demonstrated in persimmon fruits (*Diospyros kaki* L., var. Triumph). However, this high activity becomes manifest only after crushing the fruit. On analysis of the soluble sugars, under conditions causing protein denaturation, persimmon was shown to contain mainly sucrose and much less glucose and fructose, these two monosaccharides being at a ratio larger than 1 : 1. The soluble sugar content decreased significantly during the CO_2 treatment of the fruits for deastringency, without, however, loss of sweetness. Also, the total soluble solids content remained as high as before the treatment. Upon acid treatment of the soluble fraction, its soluble sugar content increased, remaining constant during the deastringency process. A possible interaction between tannins and soluble sugars is proposed to explain the loss of astringency in persimmon fruits upon treatment with CO_2.

INTRODUCTION

Persimmon fruits (*Diospyros kaki* L.) may be classified into two groups: astringent and non-astringent 9 (Itoo, 1980). 'Triumph' represents an Israeli astringent variety (Oppenheimer, 1942) that is consumable only after post-harvest treatments: ethanol vapours, immersion in warm water and, most generally, CO_2 (Matsuo *et al.*, 1976). These treatments dramatically reduce the astringency level. Nevertheless, the fruits remain sweet and tasty, and retain their excellent appearance and succulent flavour. Moreover, contrary to the on-tree fully ripening varieties that become very soft, the 'Triumph' variety persimmon fruits remain hard with a 'crispy' texture that appeals to the consumer. This hardness confers to the fruit a longer shelf-life and a better durability in transportation.

Persimmon fruits (*Diospyros kaki* L.) are rich in soluble and nonsoluble condensed tannins (proanthocyanidins). Low molecular weight soluble tannins are believed to be responsible for the astringency of the fruits (Matsuo & Ito, 1978). Their main feature is to bind very strongly to proteins (Hagerman & Butler, 1980; Hagerman, 1992). In the mouth, they precipitate the proteins present in the saliva—mostly amylases—and bind to the taste receptors, causing a feeling of dryness and puckeriness throughout the entire palate, characteristic of astringent foods (Joslyn & Goldstein, 1964). During the treatments mentioned above, the level of soluble tannins decreased considerably, reaching levels imperceptible by humans.

Since the discovery of the deastringency method by means of CO_2 (Gore & Fairchild, 1911), many studies have been performed to explain the mechanism of action of this gas and the whole process of astringency removal.

The ripening of persimmon fruit and the loss of its astringency were initially attributed to the association of soluble tannins with colloidal substances (Lloyd, 1911). This process was believed to render the tannins insoluble. A similar mechanism was proposed (Kitagawa, 1969) to explain the removal of astringency from persimmon fruits during warm water treatment. Later, two processes in the disappearance of astringency were distinctly recognized (Gazit & Adato, 1972) and carefully studied (Matsuo & Ito, 1977), i.e., an induction process in the presence of CO_2 and a deastringency process without the gas. Acetaldehyde, a product of anaerobic respiration, accumulates during the deastringency process of persimmon fruits (Pesis & Ben-Arie, 1984) and is considered to play an important role in the removal of astringency of these fruits (Pesis *et al.*, 1984; Matsuo *et al.*, 1991) through a condensation process with the soluble tannins.

The purpose of this study is to investigate the possible

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involvement of other cell metabolites, namely, soluble sugars, in the removal of astringency of persimmon fruits.

MATERIALS AND METHODS

Plant materials and deastringency process

Sample fruits (*Diospyros kaki* L., var 'Triumph'), an astringent cultivar, were picked from the orchard of a local grower in November 1990 and 1991. The harvested fruits were kept at 0°C until their use. Uniformly shaped and coloured fruits were chosen for the experiments. About 100 fruits were inserted into a 20 litre container, and CO_2 (100%) was allowed to flow into it for 30 min in order to ensure complete displacement of air by the gas; thereafter, the container was closed hermetically. A sample of 12 fruits was taken every 4 h and the container refilled with CO_2 for 20 min before closing. The fruits were analysed for their soluble tannins, soluble sugars and total soluble solids (TSS) contents at three different times: immediately after the sampling, and 24 and 48 h later.

Sample preparation

Method 1
One gram of each peeled fruit was placed directly into 25 ml of 80% methanol solution in water, cut in this solution into small pieces with fine scissors and homogenized for 1 to 2 min using a Polytron (Luzern, Switzerland). One ml of the extract was evaporated overnight to dryness at room temperature (RT). The semisolid syrupy residue was taken into 1 ml of the mobile phase (see below), centrifuged at 10 000 rpm for 20 min at RT, filtered through a 0·45 μm membrane, and 25 μl were injected into the chromatograph.

Method 2
Four peeled and cut fruits were crushed in a blender for 5 min at normal speed, and samples (tetraplicates) of 1 g each were homogenized for 30 s in 25 ml of 80% methanol solution in water, using a Polytron. This extract was used directly for the tannin analysis. Injection samples were prepared as above.

Method 3
One gram of peeled fruit was treated as in Method 1 up to the syrupy residue stage, then 200 μl of 2N HCl was added, and hydrolysis was performed for 30 min at 80°C. After cooling to RT, 800 μl of the mobile phase were added and the acid was neutralized carefully with solid calcium carbonate. After centrifugation and filtration, 25 μl were injected into the chromatograph.

Analytical methods

Soluble tannins were determined from the methanolic extract (Method 2) by a new method (Ittah, 1991), using a 96-well polystyrene plate, and were expressed as mg of purified persimmon tannin (Matsuo & Ito, 1980).

High performance liquid chromatography (HPLC) was used to estimate the glucose, fructose and sucrose content using a Hewlett-Packard (Avondale, PA, USA) HP 1090 instrument equipped with a refractive index detector (Erma Optical Works, ERC-7510, Tokyo, Japan). The separation was performed on an Aminex Carbohydrate HPX-87C column (Richmond, CA) at 85°C using a 0·02 mM $CaSO_4$ aqueous solution as mobile phase. The flow rate was reduced from 1 to 0·5 ml/min over a 6 min period and then kept constant until the end of the chromatography process. The three peaks were separated fully and the retention times for sucrose, glucose and fructose were 8·98, 11·59 and 15·06 min, respectively.

RESULTS AND DISCUSSION

The activity of invertase has been reported to increase during the on-tree ripening of persimmon fruits (Hirai *et al.*, 1986; Zheng & Sugiura, 1990). Consequently, the sugar composition was thought to vary along with the ripening, leading to the almost complete disappearance of sucrose (Daood *et al.*, 1992). Our first aim in studying the sugar composition during the CO_2 treatment was thus to establish whether or not such an increase in invertase activity would take place during the much shorter artificial deastringency process (24–48 h). The findings, as shown in Table 1, indicate the values obtained from fruits treated according to Method 1. They demonstrate clearly the presence of sucrose in very significant amounts. In fact, it forms the major component of the soluble sugar fraction. The highest values for sucrose were obtained for the untreated fruits. While the decrease in sucrose content starts immediately upon commencement of the treatment with CO_2, the changes in glucose and fructose content seem to be very slow. This behaviour appears to be related to some moderate activity of invertase in the fruit. Following the deastringency treatment, the fruits were kept at RT for 24 and 48 h. Although no firmness determinations were conducted (e.g. with a Hunter Spring pressure tester, Hatfield, PA), it appears, from manual observation that, during this period, the fruits softened slightly, and that the degree of softening increased with the duration of treatment with CO_2. Nevertheless, this softening seems not to have altered the activity of invertase as the content of the three sugars was approximately the same after 24 or 48 h. It is interesting to note that the ratio between glucose and fructose was invariably higher than 1 (between 1·2 and 1·9), even after 24 or 48 h on the shelf at RT.

Such a high ratio between the two reducing sugars is probably the result of slow respiratory processes, combined with possibly high activity of enzymatic systems responsible for glucose production in the persimmon fruit, such as gluconeogenesis pathway enzymes, fructose isomerase and cellulase (Flohr, 1992). However, despite

Table 1. Changes in sugar content of persimmon fruits during the CO_2 treatment for deastringency[a] (Method 1)

Duration of treatment with CO_2	0 h after treatment				24 h after treatment				48 h after treatment			
	Sucrose (mg/g, FW)	Glucose (mg/g, FW)	Fructose (mg/g, FW)	Gluc./fruc. ratio	Sucrose (mg/g, FW)	Glucose (mg/g, FW)	Fructose (mg/g, FW)	Gluc./fruc. ratio	Sucrose (mg/g, FW)	Glucose (mg/g, FW)	Fructose (mg/g, FW)	Gluc./fruc. ratio
0·0	100·8	22·5	14·9	1·51	97·0	30·7	20·7	1·48	113·1	20·6	14·0	1·47
4·0	91·1	26·9	19·6	1·37	88·6	22·9	18·3	1·25	105·0	29·5	22·9	1·29
8·0	66·9	40·5	26·9	1·51	NM	NM	NM	NM	39·3	50·1	37·4	1·34
16·0	68·2	30·0	20·9	1·44	94·7	17·1	14·2	1·20	86·5	16·1	13·6	1·18
20·0	64·3	26·9	16·9	1·59	NM	NM	NM	NM	62·6	23·9	17·7	1·35
24·0	45·5	39·2	26·7	1·47	71·4	26·6	17·8	1·49	NM	NM	NM	NM
32·0	68·7	19·2	10·0	1·92	42·7	29·8	19·2	1·55	NM	NM	NM	NM
48·0	54·2	16·4	12·4	1·32	52·5	16·4	12·4	1·32	54·2	15·8	10·3	1·53

[a] All the values were calculated from the area of each peak by comparison with standard solutions of the three sugars.
Each value represents the mean of four determinations.
NM: not measured.

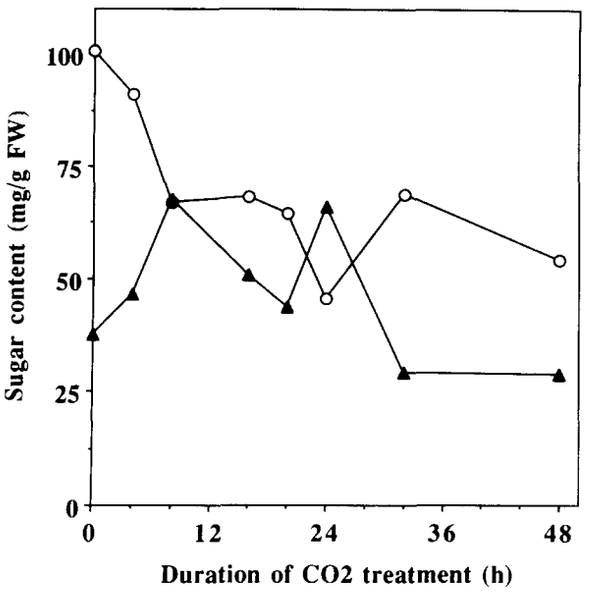

Fig. 1. Sugar content of persimmon fruits vs. duration of CO_2 treatment. The sugars (glucose, fructose and sucrose) were analysed by HPLC as described in 'Materials and Methods': (▲) glucose + fructose; (○) sucrose.

the high ratio, the changes in sucrose content, on the one hand, and those in glucose combined with fructose on the other, are closely correlated, as shown in Fig. 1: when the sucrose content was high, the glucose–fructose fraction was low, and vice versa. Therefore, it appears that sucrose remains the main source of both glucose and fructose overall.

Remarkably in 'Fuyu', a nonastringent variety of persimmon fruits, when treated according to Method 1, the sugar fraction consisted also mainly of sucrose (63·5 mg/g fresh weight (FW)), albeit with a relatively high content of glucose (45·6 mg/g, FW) and fructose (32·9 mg/g, FW). However, in this case too, the glucose/fructose ratio was much greater than 1:1.

When persimmon fruits were treated according to Method 2, the sugars profile was different from that obtained with Method 1, as shown in Table 2. Complete inversion took place, and only glucose and fructose could be detected by HPLC, with a ratio around 1:1. It appears that, upon fine crushing, invertase was activated substantially and, within few minutes, all the sucrose was hydrolysed to glucose and fructose. Such an increase in invertase activity might result from the release of the enzyme from specific vacuoles during the fine crushing. Interestingly, the sugar content, as detected by HPLC analysis, was found to be constant. This finding suggests that other enzymes, e.g. glycosidases were also released upon the crushing process. It seems, therefore, that when these releases occur in methanol solution (Method 1), the enzymes are instantly inhibited or denaturated and most (if not all) of the sucrose and other sugar-containing species remain intact.

Hence, it appears that, in persimmon fruits, invertase is indeed very active, as found by Daood *et al.* (1992). However, this exceptionally high activity does not become manifest inside the fruit; a crucial, external step would be needed for its expression. Under proper conditions and cautious handling, one could show easily that persimmon fruits contain large amounts of sucrose that apparently contribute substantially to their sweetness.

Table 2. Changes in sugar content of persimmon fruits during the CO_2 treatment for deastringency[a] (Method 2)

Duration of treatment with CO_2	Glucose (mg/g, FW)	Fructose (mg/g, FW)	Gluc./fruc. ratio	Total sugars (mg/g, FW)
0·0	79·2	75·0	1·06	154
3·0	81·2	77·7	1·05	159
20·0	84·3	79·1	1·07	163
24·0	83·0	82·5	1·01	166
48·0	81·5	78·2	1·04	160

[a] All the values were calculated from the area of each peak by comparison with standard solutions of the two sugars. Each value represents the mean of four determinations.

As shown in Fig. 2, the content of the total soluble sugars of the fruits decreases gradually during the treatment with CO_2. After completion of the process (48 h), their level dropped to about half the original value. Such a large and rapid decrease of the sugar level might not be attributed to an exceptionally high combustion rate (the artificial ripening taking place in CO_2), therefore, no aerobic respiration process could be possible under these conditions and the anaerobic processes are known to be much less energy-consuming. The sugars appear to have reacted with other species present in the cell during the deastringency process.

Moreover, the sugar content of the fruits, left in the air at RT for 24 or 48 h after the same duration of treatment, also had a similar profile. After the deastringency process, and despite this substantial reduction in sugar, the fruits still possessed a level of sweetness much higher than would be expected from such a sugar content. This finding seems to indicate that the sugars reacted with low molecular weight (MW) cell components, thereby retaining their sweetening properties.

Furthermore, the products of such interactions apparently have low MWs as the TSS value of the fruits remained quite constant throughout the entire deastringency process. As the level of soluble tannins has obviously decreased to undetectable values, we postulate that these two species, sugars and tannins, might have interacted during the treatment with CO_2 to form a nonastringent adduct or astringency-removing factor (Gazit & Adato, 1972), whose nature has yet to be elucidated. On the other hand, when the residue from the methanolic extraction (Method 3) was subjected to acid hydrolysis conditions, the sugar content (consisting of glucose and fructose only) was shown to remain constant during the deastringency process (Fig. 2).

We therefore conclude that the methanolic extract contains, besides soluble sugars and soluble tannins, species which release sugar upon acid hydrolysis, such

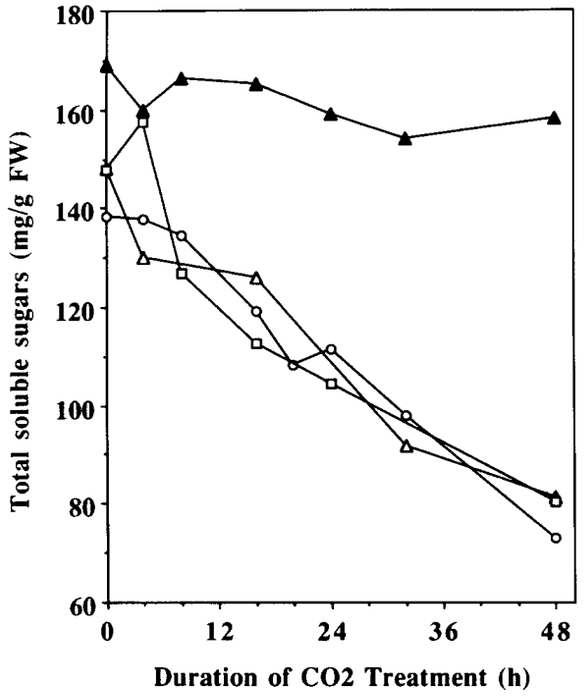

Fig. 2. Total soluble sugars (glucose + fructose + sucrose) vs. duration of CO_2 treatment. The three sugars were analysed by HPLC as described in 'Materials and Methods': (○) 0 h*; (□) 24 h*; (Δ) 48 h*; (▲) after acid hydrolysis. (* After the deastringency process, without acid hydrolysis.)

as glycosides. As glycosylated flavonoids are ubiquitous in plants (Chopin & Bouillant, 1975),we hereby propose a new hypothesis to explain the removal of astringency from persimmon fruits, namely, the formation of a glycoside between the soluble tannins and the soluble sugars during the treatment with CO_2. Such a glycosidation of the tannins would probably decrease their hydrophobicity, thereby reducing their ability to precipitate proteins, and the tannin-rich fruits (or foods) would cease to be astringent.

Currently, we are focusing our research on the isolation, mainly by HPLC, of these sugar addicts in order to elucidate their structure. We also intend to study the kinetics of the glycosyl transferases during the treatment of the persimmon fruits with CO_2. A significant enhancement of their activity might explain the reduction in the soluble sugar content in these fruits.

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