Food Chemistry **39** (1991) 1–12

Changes in Pectic Substances in Carrots during Dehydration with and without Blanching

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(Received 14 August 1989; revised version received and accepted 2 March 1990)

ABSTRACT

Changes in the fractions of the pectic substances in carrots were studied after blanching, dehydration and blanching plus dehydration. The water-soluble pectin and EDTA-soluble pectin fractions were studied after ion-exchange chromatography. A different pattern of uronic acids from the column and a different degree of esterification and neutral sugar composition indicated significant changes in both pectic fractions, especially in the EDTA-soluble pectin with the appearance of a new large fraction which had not been there previously. The significant decrease in the total percentage of all the pectic substances after dehydration alone, compared with the decrease caused by blanching plus dehydration, led to the conclusion that controlled blanching contributes to reducing the changes in pectic substances.

INTRODUCTION

It is well known that unblanched carrots deteriorate in quality when stored for a long time in a dehydrated state. Enzymatic reactions can produce many changes in the tissue but blanching inactivates the enzymes, reducing changes such as the development of unpalatable flavors and other undesirable reactions. However, changes in texture resulting from blanching may also be undesirable. Pectin is a polysaccharide which is widely believed to contribute to the texture of fruit and vegetables (Jarvis, 1982). In addition,

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Food Chemistry 0308-8146/90/$03·50

the cohesion of pectin gel is probably the most critical factor in determining fruit texture (Williams & Knee, 1980). The changes in the pectic polysaccharides in carrot tissue were investigated by Asamizu *et al.* (1983), in suspension culture during growth by Stevens and Selvendran (1984), and in whole tissue by Plat *et al.* (1988) and Massiot *et al.* (1987). The effect of blanching and drying on the pectic substances in peaches was studied by Levi *et al.* (1988). According to Albersheim *et al.* (1960) and Doesberg (1965), the pectic polymer can be broken down by the beta-elimination reaction after heating pectin at neutral or weakly acidic pH. The reaction is catalyzed by several cations and anions (Keijbets & Pilnik, 1974; Ben-Shalom *et al.*, 1982). In addition, heat-induced degradation by beta-elimination was found in the potato (Keijbets *et al.*, 1976) and cherry (Thibault, 1983).

In order to assess the effect of heat-pretreatment on the composition of pectic polymers in the water-soluble pectin and EDTA-soluble pectin, partial purification and characterization of pectic substances in the carrot cell wall were studied after blanching and after dehydration with and without blanching.

MATERIALS AND METHODS

Blanching and dehydration of carrots

Carrots (cv 'Chantenay Red Core') were obtained from Deco—a dehydration plant in Israel. For each experiment 10 kg of carrots were hand-peeled, cut into 8 mm cubes and divided into four batches: one was left untreated; one was steam-heated (blanched) for 4 min at atmospheric pressure (until no pectin esterase activity was detected); one was blanched and dehydrated; and one was dehydrated without blanching. The carrots to be dehydrated were spread on perforated aluminum trays, the trays weighed and the carrots dried to 90–95% dry matter (DM) in a cabinet dryer under a constant flow of air, for 3 h at 70 ± 5°C followed by 7–10 h at 50 ± 5°C. The experiments were repeated three times with carrots of the same variety and the results are representative of the trends observed in all the experiments.

Preparation of alcohol-insoluble solids

The alcohol-insoluble solids (AIS) were prepared according to Levi *et al.* (1988) and Plat *et al.* (1988), by repeated extractions with 70% alcohol from the untreated (0·1 g/30 ml) and the blanched tissue and from the dehydrated carrot cubes. Water-soluble pectin (WSP) was prepared by sequential extraction of the AIS (0·2 g/50 ml) with water at room temperature until no

galacturonic acid appeared in the extract. EDTA-soluble pectin (EDTA-SP) was extracted from the washed pellet of soluble pectin with 0·2% EDTA and Tris/HCl (0·02M; pH 6·2) and then dialyzed against water.

Separation of the WSP and EDTA-SP on DEAE-cellulose

The WSP pectin and the EDTA-SP calcium pectate (20 mg galacturonic acid) were dissolved in and dialyzed with a sodium phosphate buffer (1 mM, pH 6·2), then applied to a DEAE-cellulose column (Whatman 52, 160 × 20 mm) which had been brought to equilibrium with the same buffer. In order to remove the starch, prior to application on the DEAE column the soluble pectin and calcium pectate fractions were treated with 40 ppm alpha amylase at 37°C for 2 h. Elution was initially performed with 1 mM sodium phosphate (150 ml) and then with the same buffer over a linear gradient of 0–4·0M (300 ml). Fractions (3–4 ml) were collected and monitored for galacturonic acid (GA) using the *m*-hydroxyphenol method developed by Blumenkrantz and Asboe-Hansen (1973). Appropriate fractions eluted from the column were combined, dialyzed and freeze-dried.

Neutral sugar composition

The presence of six particular neutral sugars and their relative amounts were determined by hydrolysis in trifluoracetic acid. The respective alditol acetates were analyzed by gas chromatography as described by Albersheim *et al.* (1967).

Degree of esterification

Methanol derived after demethylation was converted to methyl nitrite and quantitatively evaluated by gas chromatography using the method developed by Litchman and Upton (1972) as modified by Versteeg (1979).

RESULTS AND DISCUSSION

The influence of the different treatments on the content of the WSP as separated on the DEAE column is depicted in Fig. 1. The chromatogram of WSP showed three main fractions: nonabsorbed material, which was washed with 1 mM phosphate buffer, pH 6·2 (A); absorbed material, which was eluted with a linear gradient of sodium phosphate (B); and residual pectin (still bound to the column), which was eluted with 0·05M NaOH (C).

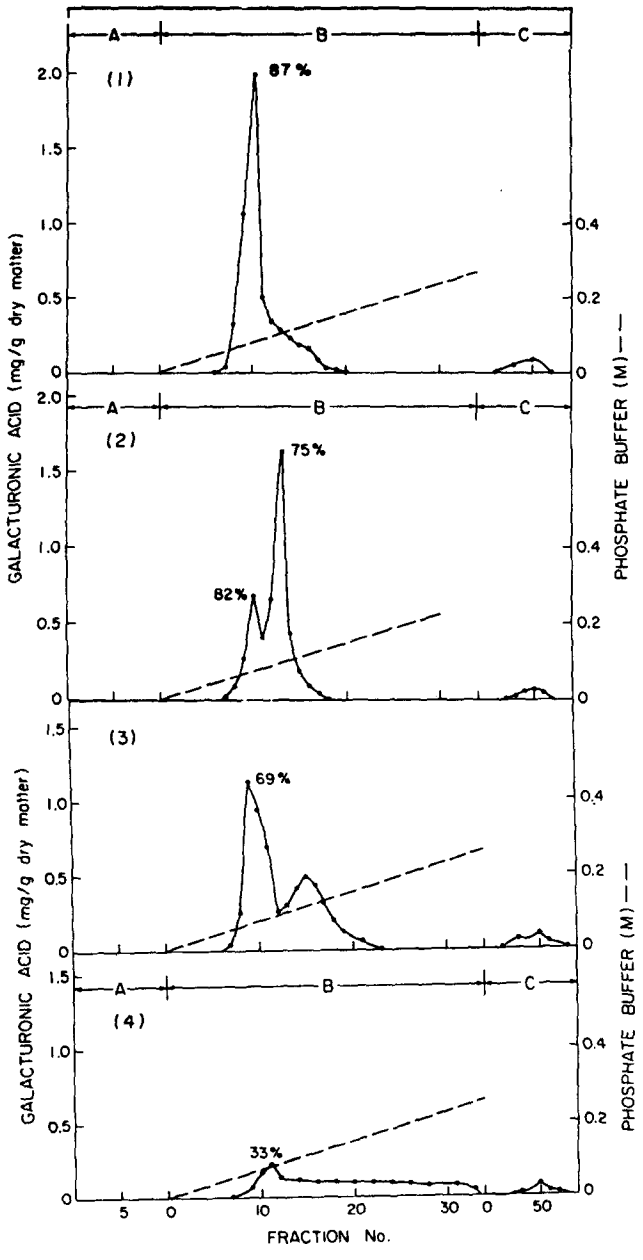

Fig. 1. Separation of soluble pectin on a DEAE-cellulose column of untreated (1); blanched (2); blanched plus dehydrated (3); dehydrated (4) carrot tissue. The two main fractions shown in the chromatogram are: [B] the absorbed material, eluted with a linear gradient of sodium phosphate, and [C] the residual pectin (still bound to the column), eluted with 0·05N NaOH. The percentage given in the figure represents the degree of esterification of the pectin.

Large differences were found between the fingerprints of the WSP fractions after the different treatments (Fig. 1). Only one peak appeared in the non-blanched tissue instead of two peaks in the blanched or blanched + dehydrated. The DE decreased, reaching a lower level after blanching and an even lower one after dehydration of the blanched tissue. The lowest DE was observed in the dehydrated non-blanched carrots probably because of pectin esterase activity de-esterifying the WSP during the dehydration. Separation of the WSP of the non-blanched carrot on the DEAE column showed that it contained two fractions (Table 1). The main one is fraction B, with 78% of the GA and 93% of the total pectic substances. Peak C contained an unusual type of pectin which bound strongly to the DEAE column. It consists mainly of neutral sugars (NS) (88%), GA and phenol groups (data are not shown). The unusual composition of this pectin enabled it to form hydrogen bonds with high affinity to the DEAE column.

TABLE 1

The Content of Total Galacturonic Acid and Neutral Sugars in the Soluble Pectin Fraction of Untreated, Blanched, Blanched Plus Dehydrated, and Dehydrated Carrot Tissues After their Separation on a DEAE Column. (The sugar content is expressed in mg/g dry matter.)

Treatment	*Sugar content (mg/g DM)*[a] *fraction*		*Total sugars*
	B	*C*	
Untreated tissue			
Neutral sugars	1·35 (15·3)	0·55 (6·2)	1·90 (21·5)
Galacturonic acid	6·84 (77·5)	0·08 (1·0)	6·92 (78·5)
Pectic substances	8·19 (92·8)	0·63 (7·2)	8·82 (100·0)
Blanched tissue			
Neutral sugars	1·07 (12·3)	1·27 (14·7)	2·34 (27·0)
Galacturonic acid	6·28 (72·6)	0·03 (0·4)	6·31 (73·0)
Pectic substances	7·35 (84·9)	1·30 (15·1)	8·65 (100·0)
Blanched plus dehydrated tissue			
Neutral sugars	1·46 (17·1)	0·54 (6·4)	2·00 (23·5)
Galacturonic acid	6·40 (75·4)	0·10 (1·1)	6·50 (76·5)
Pectic substances	7·86 (92·5)	0·64 (7·5)	8·50 (100·0)
Dehydrated tissue			
Neutral sugars	0·75 (10·1)	0·74 (10·1)	1·49 (20·2)
Galacturonic acid	5·82 (79·2)	0·05 (0·6)	5·87 (79·8)
Pectic substances	6·57 (89·3)	0·79 (10·7)	7·36 (100·0)

[a] Mean of three samples. Percentage of soluble pectin fraction in parentheses.

The WSP contained approximately 20% NS which probably is bound covalently to the GA and was separated with it from the anion-exchange column. In this case the pectic substances are the sum of GA and NS, as shown in Table 1.

Blanching the carrot tissue slightly decreased the amount of GA and WSP (Table 1), but mainly affected the NS in fractions B and C. Dehydration of the blanched tissue hardly changed the amount of the pectic substances and the ratio between GA and NS. Dehydration of the carrot with-

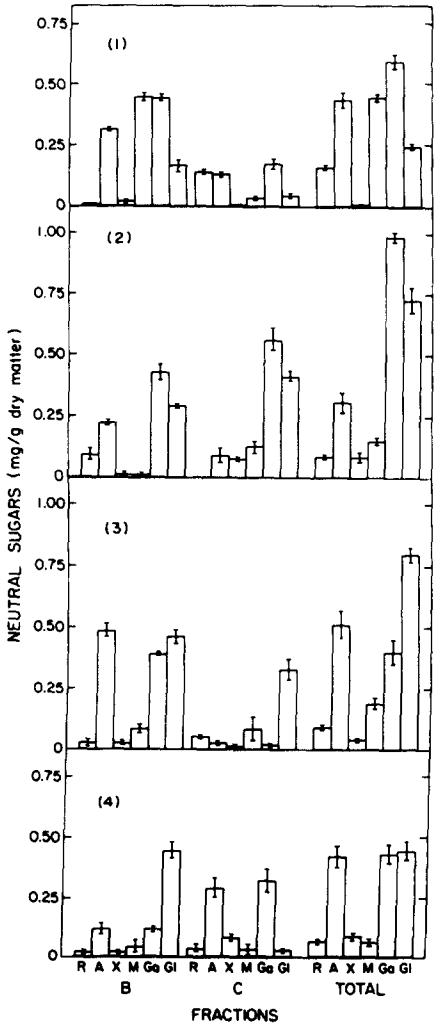

Fig. 2. Composition of neutral sugars in the soluble pectin fraction of (1) untreated, (2) blanched, (3) blanched plus dehydrated, and (4) dehydrated carrot tissue after their separation on a DEAE-cellulose column. R, rhamnose: A, arabinose: X, xylose: M, mannose: Ga, galactose; Gl, glucose. Bars represent SE.

out blanching caused large changes in the pectic substances probably because of enzymatic activity. In fraction B the GA decreased by 15% compared with the control, and the NS by 45%, as the total WSP decreased by *c.* 17%. This phenomenon probably occurred as a result of acceleration of the activity of the endogenous pectolytic enzymes during this process (Bateman & Miller, 1966). The degradation of the soluble pectin after dehydration without blanching was found to be more drastic than blanching plus dehydration or blanching alone.

The composition of the NS in the WSP of the fresh carrot showed (Fig. 2(1)) that they are composed mainly of galactose, mannose and arabinose, and less of glucose and rhamnose; no xylose was found. Rhamnose appeared in fraction C but not in B. Blanching the tissue completely changed the composition and the ratios between the various NS (Fig. 2(2)). Rhamnose appeared in fractions B and C and xylose in fraction C. In the total NS, galactose and glucose increased as mannose, arabinose and rhamnose decreased. Dehydration of the blanched tissue (Fig. 1(3)) showed that, although the amounts of the NS barely changed (Table 1) as compared with

TABLE 2

The Content of Total Galacturonic Acid and Neutral Sugars in the Calcium Pectate Fraction of Untreated, Blanched, Blanched Plus Dehydrated, and Dehydrated Carrot Tissues after Their Separation on a DEAE Column. (The sugar content is expressed in mg g dry matter)

Treatment	*Sugar content (mg/g DM)*[a] *fraction*			*Total sugars*
	A	*B*	*C*	
Untreated tissue				
Neutral sugars	— (—)	1·09 (9·6)	0·27 (2·4)	1·36 (12·0)
Galacturonic acid	— (—)	6·17 (54·7)	3·75 (33·3)	9·92 (88·0)
Pectic substances	— (—)	7·26 (64·3)	4·02 (35·7)	11·3 (100·0)
Blanched tissue				
Neutral sugars	1·34 (6·9)	0·95 (4·9)	0·41 (2·1)	2·70 (13·9)
Galacturonic acid	10·3 (53·0)	6·21 (32·1)	0·18 (1·0)	16·6 (86·1)
Pectic substances	11·6 (59·9)	7·16 (37·0)	0·59 (3·1)	19·3 (100·0)
Blanched plus dehydrated tissue				
Neutral sugars	0·92 (5·1)	1·20 (6·6)	0·05 (0·3)	2·21 (12·2)
Galacturonic acid	8·31 (46·3)	7·18 (39·9)	0·30 (1·6)	15·8 (87·8)
Pectic substances	9·23 (51·4)	8·38 (46·5)	0·35 (1·9)	18·0 (100·0)
Dehydrated tissue				
Neutral sugars	0·05 (1·2)	0·373 (9·2)	0·036 (0·9)	0·46 (11·3)
Galacturonic acid	0·34 (8·6)	3·14 (78·8)	0·05 (1·3)	3·53 (88·7)
Pectic substances	0·39 (9·8)	3·51 (88·0)	0·086 (2·2)	3·99 (100.0)

[a] Mean of three samples. Percentage of soluble pectin fraction in parentheses.

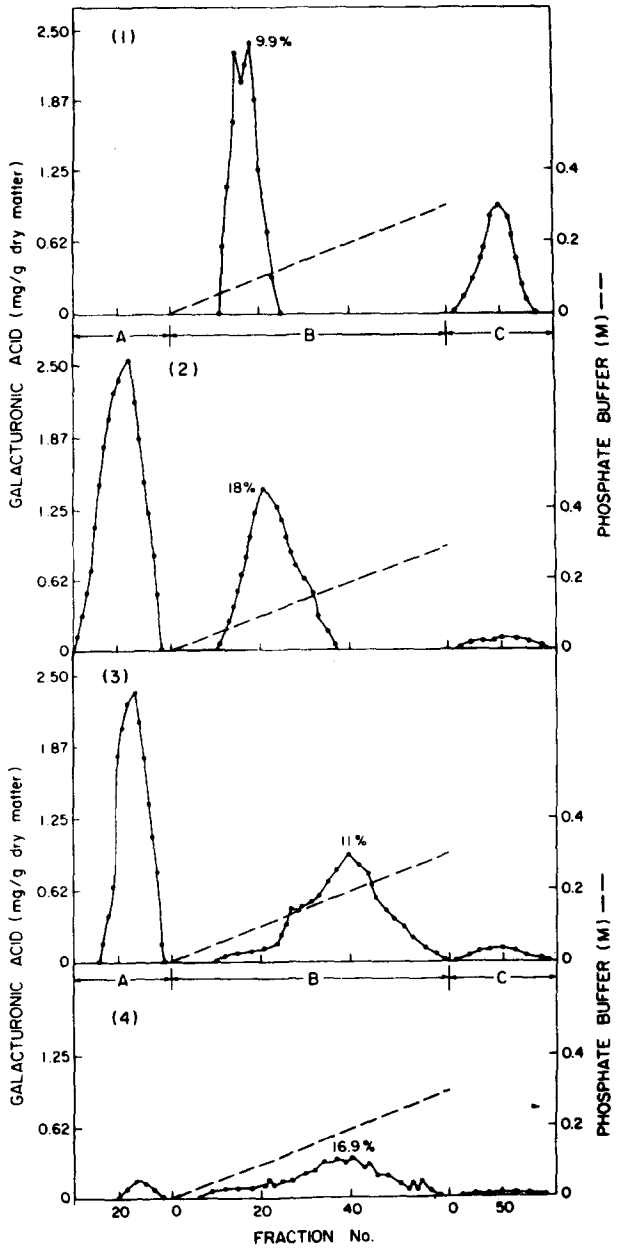

Fig. 3. Separation of carrot calcium pectate on a DEAE-cellulose column. (1) Untreated; (2) blanched; (3) blanched plus dehydrated; (4) dehydrated carrot tissue. Three main fractions shown in each chromatogram are: [A] nonabsorbed material washed with 1 mM phosphate buffer at pH 6·2; [B] absorbed material, eluted with a linear gradient of sodium phosphate; [C] residual pectin (still bound to the column), eluted with 0·05N NaOH. The percentage given in the figure represents the degree of esterification.

the untreated tissue, the ratios between the NS changed completely. Dehydration of the tissue without blanching (Fig. 2(4)) caused a decrease in the contents of the mannose, rhamnose and galactose as the amount of glucose increased.

Major changes have been found in the chromatogram of the EDTA-SP fraction after blanching (Fig. 3(2)). A new large peak of GA appeared in fraction A of the EDTA-SP (Fig. 3(2)). The DE in fraction B increased from 9·9% in the untreated tissue to 18% in the blanched one. The changes in the fingerprint of the EDTA-SP chromatogram after blanching plus dehydration have shown the same tendency as found after blanching alone. A large new peak of GA appeared in fraction A (Fig. 3(3)) and the DE increased in comparison with the control. A small amount of GA appeared in fractions A, B and C and spread all over the column (Fig. 3(4)) after dehydration of the tissue, without blanching. The EDTA-SP of the carrot tissue contained 88% GA (Table 2), compared with 79% in the WSP, and its fraction C had a low content of NS. The new, large peak of pectin, which appeared in fraction A of the EDTA-SP after blanching, consisted of 60% of all the pectic substances which were extracted by the EDTA (Table 2). The total amount of pectic substances in the EDTA-SP fraction increased after blanching by 70% as compared with the control. Dehydration of the blanched tissue also showed that a new peak of pectin which appeared in fraction A consisted of 51% of the total pectic substances.

Tissue dehydration without blanching drastically degraded the GA—by 64% and the NS—by 66% (Table 2). It was interesting to find that the endogenous pectolytic enzymes broke down the EDTA-SP fraction, which seems to be resistant to degradation as a result of crosslinking through calcium bonds. The changes in the composition of the NS in the EDTA-SP fraction after the various heat processings (Fig. 4) occurred mainly in arabinose, galactose and glucose.

The question which may be asked is, why did the amount of the EDTA-SP fraction increase after heat was applied to the carrot tissue—by blanching or by blanching plus dehydration? Pectic substances, cross-linked inter- and intramolecularly by calcium, are thought to be largely responsible for tissue rigidity.

Most studies have focused on the factor responsible for the augmented levels of calcium (Grant *et al.*, 1973; Clarkson & Hanson, 1980), and relatively little is known about the degradation and the interaction of calcium and pectic polymers during heat-treatment *in vivo*.

Williams and Knee (1980) and Stevens and Selvendran (1984) showed that polysaccharides from carrot tissue contain polyuronides over the whole cell wall. Exposure of the carrot tissue to heat seems to stimulate changes in the WSP and EDTA-SP fractions, probably within and between the pectic

fractions and the whole cell wall, as was shown also in our previous study on baby carrots (Plat *et al.*, 1988) and in peaches (Levi *et al.*, 1988). In the present work, applying heat to the carrot tissue significantly changed the composition of the pectic polymers in the WSP and especially in the EDTA-SP, with the appearance of a new large fraction. On the basis of work by Albersheim *et al.* (1960), Doesberg (1965), Thibault (1983) and Sajjaanantakul *et al.* (1989), who have shown that the pectins are degraded by beta-

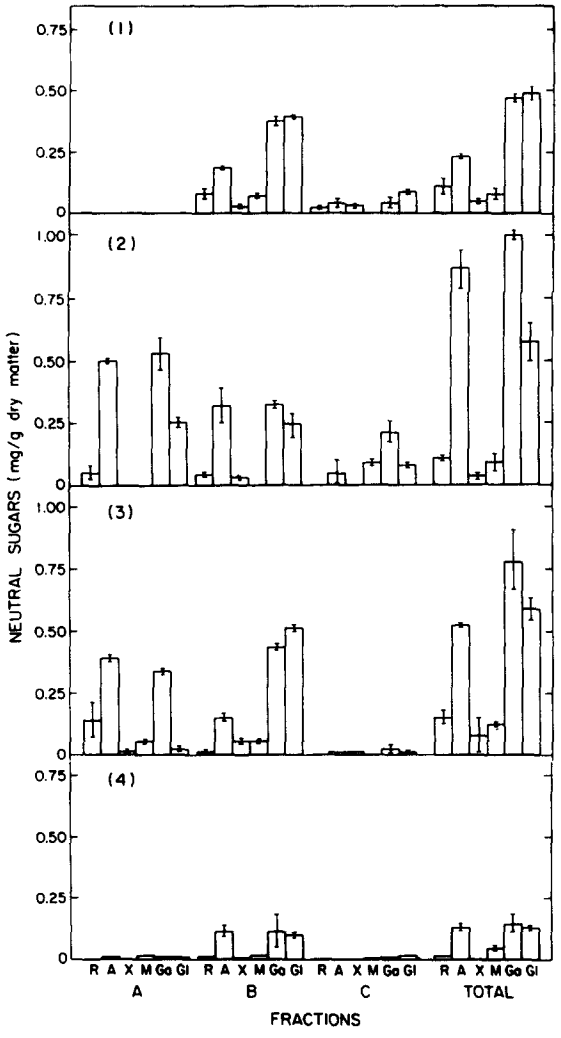

Fig. 4. Composition of neutral sugars in the calcium pectate fraction of (1) untreated, (2) blanched, (3) blanched plus dehydrated and (4) dehydrated carrot tissues after their separation on a DEAE-cellulose column. R, rhamnose; A, arabinose; X, xylose; M, mannose; Ga, galactose; Gl, glucose. Bars represent SE.

elimination, we propose that in the carrot tissue, the WSP and EDTA-SP are changed and there appear fractions which differ fron the original ones in the untreated carrot tissue.

CONCLUSIONS

The significant decrease in the amount of PS after dehydration without blanching, as compared with blanching plus dehydration, led to the conclusion that careful blanching may be used as a means for reducing the changes in the PS in carrot tissue.

As a result of controlling the blanching during the dehydration process, the texture, water uptake, and reconstitution rate of the final product could be improved.

The changes in the PS after non-enzymatic and enzymatic dehydration seem to be a very complicated process. The dynamic systems were identified by the various changes which occurred simultaneously in the soluble pectin, calcium pectate and probably other pectic substance fractions in the carrot cell wall. The degradation process could be influenced by the structure of pectic polysaccharides, and by the recreation of calcium bonds. The main changes in the EDTA-SP fraction have shown that more research on the structure of the pectic polysaccharides is needed in order to determine which regions and side chains of pectic polymers are more susceptible to enzymatic and heat-induced changes.

ACKNOWLEDGEMENTS

This contribution is from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, No. 2893-E 1990 series. The work was supported by the US–Israel Binational Agricultural Research and Development Fund (BARD), Project No. US-452-81. The authors wish to thank Ms Rivka Pinto, ARO, The Volcani Center, Bet Dagan, Israel, for her excellent assistance.

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