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Changes in molecular weight of water-soluble and EDTA-soluble pectin fractions from carrot after heat treatments

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The molecular weight of water-soluble and EDTA-soluble pectin from carrot following blanching, blanching + dehydration, and dehydration without blanching was determined by the viscometric measurement and gel permeation methods.

The viscometric measurement indicated that after blanching + dehydration, the molecular weight of the water-soluble pectin increased 2·5-fold and that of EDTA-soluble pectin 2·3-fold as compared with the untreated tissue. Dehydration without blanching drastically decreased the molecular weight of the pectin in both fractions.

INTRODUCTION

Molecular weights (MW) of pectin have been reported to be in the range 60,000–200,000 daltons (Knee, 1978). However, elution from Sephadex G-200 (Barrett & Northcote, 1965) indicates the aggregation of pectin molecules to form a higher MW. The changes in the relative MW distribution of pectin using gel permeation chromatography and intrinsic viscosity were studied by Anger and Berth (1985). Studies of the size distribution of high MW species in the pectin fraction from apples have been made by O'Beirne and Van Buren (1983). The thermal degradation of pectin in various solutions was analyzed by calibration and application of high performance size exclusion columns for MW distribution of pectin (Deckers & Olieman, 1986).

In a previous paper (Plat *et al.*, 1991), the authors demonstrated the changes in water-soluble pectin (WSP) and EDTA-soluble pectin (EDTA-SP) after blanching, dehydration and blanching plus dehydration. Both 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-SP with the appearance of a new large fraction which had not been there previously.

The aim of this work was to study the changes in the MW in the WSP and in the EDTA-SP during three different heat treatments: blanching, blanching + dehydration, and dehydration without blanching.

MATERIALS AND METHODS

Carrots var. 'Chantenay Red Core' were obtained from Deco-Dehydration Plant in Brur-Chael, Israel. The carrots were hand-peeled (control), cut into 8mm × 8mm × 8mm cubes and divided into samples of 0·5 kg. One sample was steam-heated (blanching) for 4 min at 85°C (the time found to inactivate pectin esterase), one was blanched and dehydrated (Levi *et al.*, 1988; Plat *et al.*, 1988), and one was dehydrated without blanching.

Alcohol-insoluble solids (AIS) were prepared from all four samples by repeated extractions with 70% ethanol, followed by a fifth extraction with 100 ml of 100% acetone at room temperature, according to Levi *et al.* (1988). The WSP and EDTA-SP fractions were prepared according to Levi *et al.* (1988) and Plat *et al.* (1988).

WSP was prepared by sequential extraction of the AIS with water at room temperature until no galacturonic acid appeared in the extract. EDTA-SP was extracted from the washed pellet of the WSP with 0·2% EDTA and tris-HCl (0·02 M, pH 6·2), dialyzed against water, and freeze-dried.

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The WSP and EDTA-SP (20 mg of galacturonic acid) were solubilized, dialyzed with sodium phosphate buffer (1 mM, pH 6·2) and applied to a column of DEAE-cellulose (Whatman, Maidstone, UK) (1·6 cm × 10 cm) that had been equilibrated previously with the same buffer (Plat *et al.*, 1991).

Elution was done initially with 1 mM sodium phosphate (150 ml) and then with the same buffer, in a linear gradient of 0–0·4M (300 ml). Fractions (3–4 ml) were collected and monitored for galacturonic acid by the *m*-hydroxyphenol method (Blumenkrantz & Asboe-Hansen, 1973). Appropriate fractions eluted from the column were combined, dialyzed and freeze-dried. The composition and amount of individual neutral sugars were obtained by hydrolysis in trifluoroacetic acid (Plat *et al.*, 1991). The respective alditol acetates were analyzed by gas chromatography as described by Albersheim *et al.* (1967). Pectic substances (PS) were calculated, after DEAE separation, as the sum of galacturonic acid and neutral sugars which eluted with them. The MWs of the pectic fractions were determined by viscometric measurements according to Christensen (1954) and by gel permeation using Sephacryl S-300 (Pharmacia Corp., Uppsala, Sweden). PS (10 mg), which was received from the DEAE column, was dissolved in 2 ml of phosphate buffer and loaded on to a 1·6 cm × 90 cm column. The buffer consisted of tris, 20 mM, pH 6·2, EDTA, 0·2% and NaCl, 0·1 N. The PS were dialyzed before they were loaded on to the column, at a rate of 20 ml/h. Samples of 3 ml were collected and the amount of galacturonic acid was measured in each fraction. Calibration of the column was done by Dextran 2000 and glucose.

RESULTS AND DISCUSSION

In order to compare the influence of different heat processes on pectic polymers, the WSP and EDTA-SP fractions were separated and partially characterized.

Blanching the carrot tissue or dehydration of the tissue after blanching slightly decreased the amount of the WSP as compared with the untreated tissue (Table 1). A pronounced decrease in the amount of the WSP was observed only after dehydration without blanching. The amount of the PS in the EDTA-SP increased after blanching, and blanching + dehydration.

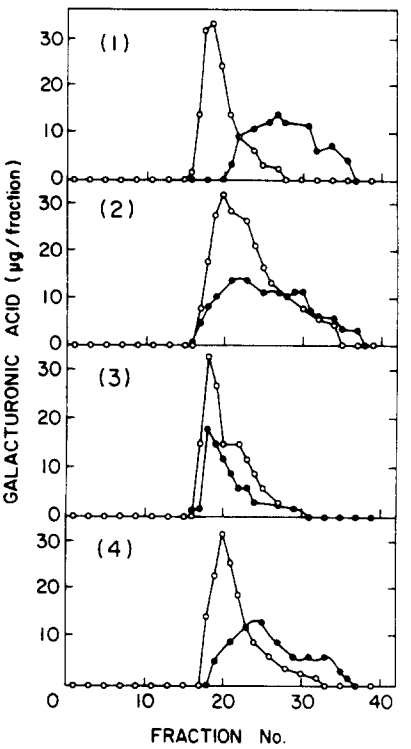

Fig. 1. Separation of soluble pectin (●) from carrot and of EDTA-SP (○) on a Sephacryl S-300 column after different treatments: (1) not treated; (2) blanching; (3) blanching + dehydration; (4) dehydration.

Changes in the PS after heat treatment were reported in other publications (Doesberg, 1965; Thibault, 1983; Levi *et al.*, 1988; Sajjaanantakul *et al.*, 1989). In most cases WSP was measured and found to increase as a result of the process (Keijbets *et al.*, 1976; Lee *et al.*, 1979; Knee & Bartley, 1981). The observation that the amount of the EDTA-SP increased after blanching and blanching + dehydration seems to be very interesting, and is already documented in the authors' previous publications (Levi *et al.*, 1988; Plat *et al.*, 1988). From the literature it was expected that heat processing in near-neutral pH (like the carrot) would degrade the pectin and decrease its amount through the eliminative cleavage of the chains (Thibault, 1983; Sajjaanantakul *et al.*, 1989). One of the possibilities to explain the increase in EDTA-SP is the assumption that interchanges of pectic fractions occurred in the cell wall and new de-

Table 1. The amount and molecular weight of carrot pectic substances (galacturonic acid plus neutral sugars) in WSP and EDTA-SP after separation on a DEAE-cellulose column

Treatment	Pectic substances (mg/g dry matter)		Molecular weight (× 10^{-3})	
	WSP	EDTA-SP	WSP	EDTA-SP
Untreated tissue	8·8 + 0·4	11·3 + 0·7	21·2 + 1·0	61·3 + 2·8
Blanching	8·7 + 0·3	19·3 + 0·8	21·7 + 0·8	64·4 + 3·3
Blanching + dehydration	8·5 + 0·5	18·0 + 0·9	53·5 + 3·3	138·1 + 2·7
Dehydration	7·4 + 0·3	4·0 + 0·3	11·3 + 0·6	36·5 + 1·7

graded pectin appeared in the EDTA-SP during this process. This observation was supported by the changes in MW of the WSP and the EDTA-SP fractions after the process of blanching + dehydration (Table 1). High MW pectin appeared in both fractions. The MW of the WSP and the EDTA-SP increased by more than 100% after this process.

In an attempt to gain further support for the MW measurements, the authors ran the pectic fractions through Sephacryl S-300 (Fig. 1). In general, the WSP was distributed all over the column, compared with a sharp peak of elution of the EDTA-SP. From the K_{av} of the different peaks it seems that the peaks of EDTA-SP and the WSP after the blanching + dehydration process were eluted before the control, which means higher MW.

Dehydration of the tissue without blanching resulted in a marked decrease in the amount of the PS in the WSP, in the EDTA-SP and in their MWs (Table 1). Endogenous pectolytic enzyme activity in the carrot tissue during the dehydration process seems to be the reason for the increase in the degradation and the decrease in the MW.

CONCLUSIONS

The increase in the MW of the WSP and EDTA-SP and in the amount of the PS of the EDTA-SP during the blanching + dehydration treatment led the authors to assume that interchange of pectic fractions occurred during the heating process and a new pectin moiety was formed.

The significant decrease in the total percentage of all the PS after dehydration alone, compared with the decrease caused by blanching + dehydration, led to the conclusion that controlled blanching contributes to the reduction in the changes in PS and can improve the texture of the final product.

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