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Modification of physicochemical properties of dietary fibre in carrots by mono- and divalent cations

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Abstract

Physicochemical changes of dietary fibre in the presence of NaCl or $CaCl_2$ (0, 100 or 400 mM) during boiling (4 or 25 min) of blanched/frozen carrots were investigated. Addition of NaCl (100 mM) to the boiling water reduced total dietary fibre (TDF) content, due to a reduction of insoluble polymers. Higher concentrations had no further effects. NaCl showed minor effects on molecular weight distribution as well as on viscosity of water-soluble polysaccharides (WSP) isolated. The low concentration of $CaCl_2$ did not affect TDF content, but there was a redistribution of soluble (SDF) to insoluble dietary fibre (IDF), mainly uronic acids but to some extent also arabinose and galactose. The higher concentration of $CaCl_2$ caused a significant loss of TDF (~18%), both soluble (uronic acids ~9%, galactose ~2% and arabinose ~2%) and insoluble polymers (glucose ~4%). Reduced amounts of the various WSP fractions were isolated (60–70%) in the presence of $CaCl_2$. The viscosities of these fractions were comparatively lower, which may be explained by the proportionally lower amounts of arabinose and galactose in the high molecular weight fraction. All low molecular weight fractions isolated (not quantified in the dietary fibre analyses) contained high proportions of non-starch glucose. It is concluded that $CaCl_2$ and NaCl modify physicochemical properties of dietary fibre to a great extent and, as a consequence, also nutritional effects.

Keywords: Carrots; *Daucus Carota*; Boiling; Blanching; Heat-treatment; Salt addition; $CaCl_2$; NaCl; Physicochemical properties; Dietary fibre; Dietary fibre composition; Viscosity; Molecular weight distribution

1. Introduction

Physiological effects of dietary fibre are greatly dependent on the physicochemical properties of the ingested material, e.g. the water-binding capacity, the molecular weight distribution and the viscosity (Guillon et al., 1998). Another factor of great nutritional importance is the plant cellular structure, which may also play a role in storage stability and sensory characteristics. To be able to produce foods with a high nutritional quality, there is a need to gain more knowledge on how physicochemical properties of dietary fibre polysaccharides are affected by processing and cooking.

During processing, physicochemical properties of dietary fibre may be modified (Kunzek, Kabbert, & Gloyna, 1999) and, as a consequence, physiological effects may be changed. Glycosidic linkages in the dietary fibre polysaccharides and associations between the polysaccharide chains can be broken (Albersheim, Neukom & Deuel, 1960; Reinders & Thier, 1999; Selvendran & Robertson, 1994), resulting in a solubilization or loss of dietary fibre (Björck, Nyman, & Asp, 1984; Nyman, Björck, Håkansson, & Asp, 1987; Phillips & Palmer, 1991). The degradation may be elevated in the presence of cations and it has been shown that the β-eliminative degradation of pure pectin increased when cations were present at heat-treatment (Keijbets & Pilnik, 1974; Krall & McFeeters, 1998; Sajjaanantakul, Van Buren & Downing, 1993). Divalent cations promoted a higher degradation than monovalent cations. In contrast to these results, others have found (Ng, Parker, Smith, & Waldron, 1999) that the occurrence of β-elimination and depolymerization of pectin, isolated from onion, decreased on heat-treatment with Ca^{2+} and Sr^{2+}. The solubility of the cell wall polymers also decreased.

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The purpose of the present investigation was to study changes of physicochemical properties of dietary fibre in carrots following addition of mono- (NaCl) and divalent ($CaCl_2$) cations, at different concentrations, to the boiling water. Content and composition of the dietary fibre and of various molecular weight fractions of water-soluble polysaccharides were studied. Also the viscosities of these polysaccharides were investigated.

2. Materials and methods

2.1. Materials

Blanched/frozen carrots (*Daucus carota* sp. *sativus*) were boiled (4 or 25 min) with different concentrations (0, 100 and 400 mM) of cations (Na^+ or Ca^{2+}). The carrots (18 kg) were obtained from Nestlé R&D Center Bjuv AB (Sweden). Before delivery, raw carrots had been washed, steam-peeled, trimmed, cut into cubes (10×10×10 mm), water-blanched (100 °C, 90 s) and water-cooled (10 °C, 2 min) before freezing and packaging in batches of 200 g.

In the viscosity measurements LM- (low methoxylated) and HM- (high methoxylated) pectin were used as reference materials.

2.2. Processing

Blanched/frozen carrots (3 batches of 200 g) were poured into boiling water (150 ml for 4 or 25 min) containing different concentrations (0, 100 and 400 mM) of NaCl or $CaCl_2$. The boiled carrots were drained for 15 min before further treatment. The various carrot samples were freeze-dried and analysed for dietary fibre. Water-soluble polysaccharides (WSP; Mw > 1000) were also isolated for further studies of viscosity and composition of different molecular weight fractions.

2.3. Analytical methods

2.3.1. Dietary fibre

Total dietary fibre (TDF)—separated into a soluble and an insoluble fraction—in the carrots was isolated with a procedure based on the enzymatic method of Asp, Johansson, Hallmer, and Siljeström (1983). As effects of boiling were to be studied, the initial gelatinization step at 100 °C was excluded but, as carrots only contain minor (< 0.1 g/100 g DW) amounts of starch, such an exclusion had little influence on TDF content (Svanberg, Nyman, Andersson, & Nilsson, 1997). Further, due to the high viscosity of the carrot fibre the amount of sample for analyses was reduced.

The compositions of the insoluble (IDF) and soluble (SDF) dietary fibre residues were analysed by gas–liquid chromatography (GLC) for the neutral sugars, as their alditol acetates, after hydrolysis in 0.4 M H_2SO_4, and by a spectrophotometric method for the uronic acids (Theander, Åman, Westerlund, Andersson, & Petterson, 1995). The contents of SDF and IDF were then calculated as the sum of the neutral polysaccharide and the uronic acid residues.

2.3.2. Isolation of water-soluble polysaccharides

To be able to study the viscosity and composition of various molecular weight fractions of the soluble dietary fibre, these were also isolated by centrifugation and dialysis and referred to as water-soluble polysaccharides. The procedure used simulated conditions of the upper part of the gastrointestinal tract (Svanberg, Gustafsson, Suortti, & Nyman, 1995). Carrots boiled for 25 min with $CaCl_2$ or NaCl (0, 100 or 400 mM) were then homogenized (200 g) in phosphate buffer (250 ml, 0.1 M, pH 6.0).

2.3.3. Fractionation of WSP

The isolated WSP was separated with high-performance size-exclusion chromatography (HPSEC). The chromatographic system used consisted of a GP40 gradient pump, an AS3500 auto-sampler and a pulse amperometric detector (PAD). Carbohydrate detection was carried out with the following pulse potentials and durations: $E_1 = 0.05$ V ($t_1 = 480$ ms); $E_2 = 0.60$ V ($t_2 = 120$ ms); $E_3 = -0.60$ V ($t_3 = 60$ ms), and chromatography was carried out on μHydrogel 2 000, 250 and 120 (7.8×300 mm) columns (Millipore/Waters, Milford, MA) in series at 70 °C. The mobile phase was phosphate buffer (50 mM, pH 6.5) at a flow-rate of 0.5 ml/min. The freeze-dried samples from the isolated carrots were dissolved in eluent (10 000 mg/l) with gentle stirring overnight, and injected (100 μl) into the HPLC instrument without further manipulation.

The samples were reinjected 10 times to obtain enough material for analysing composition of the WSP. Three different molecular weight fractions were collected, one of high molecular weight (35–43 min; apparent molecular weight $MW_{app} > 200\,000$), one of intermediate molecular weight (43–54 min; MW_{app} 200 000 − 1 500) and one of low molecular weight (54–65 min; $MW_{app} < 1500$). The various fractions were then hydrolysed, derivatized and analysed by GLC (Theander et al., 1995).

As standards, pullulans of various molecular weights (MW)—5800, 12 200, 23 700, 48 000, 100 000, 212 000, 380 000 and 1 600 000 (Showa Denko, Japan)—were used. These were separated by weight, but high molecular weight polysaccharides gave a lower response than those of low molecular weight.

2.3.4. Viscosity

The freeze-dried WSP-fraction was redissolved in phosphate buffer (0.1 M, pH 6.0), giving a final concentration

of 4% (w/w). The solutions were allowed to equilibrate at 4 °C overnight before measurements. The viscosity was measured in a Haake viscotester (VT 501, Tillquist, Kista, Sweden) in the shear rate interval 10–1000 s^{-1} (Svanberg et al., 1995). The maximum "zero-shear" viscosity (η_0) and the shear rate ($\gamma_{1/2}$) at which the viscosity is reduced to $\eta_0/2$ were calculated for all materials (Morris, 1990).

2.4. Calculations and statistical analysis

All dietary fibre analyses were performed at least in duplicate and the maximum error was less than 5%. The dietary fibre constituents were expressed as polymers (0.9×monomer weight). Statistical evaluation was performed by one-way analysis of variance, followed by the Tukey's procedure for multiple comparison with the software Minitab for Windows version 12 (Minitab Inc., USA).

3. Results and discussion

3.1. Dietary fibre content and composition

The TDF content in blanched frozen carrots was 28.2×100 g^{-1} of dry weight (DW) and of that 31% was soluble (Table 1). The SDF mostly consisted of uronic acids (61%), galactose (23%) and arabinose (11%), while the IDF consisted of glucose (48%), uronic acids (27%) and galactose (13%).

There was a significant increase ($P<0.05$) in TDF from 28.2 g/100 DW to 30.7 g/100 g DW following 4 min of boiling (Table 1). The increase was mainly due to an increase ($P<0.05$) in SDF content, while IDF content was unaffected. However, there is generally a considerable loss of dry substance into the water with boiling and, in a previous study using the same type of carrots and similar process-conditions, the loss of dry substance was 25% (Nyman, Pålsson, & Asp, 1987; Svanberg et al., 1997). Thus, the increase of SDF was most probably apparent. This was further indicated by the very similar distribution of monomers in the SDF before and after boiling. Instead there was a change in the composition of IDF. Thus, the proportion of uronic acids was lower in boiled carrots than those, which had only been blanched (20% versus 27%). These results, together with the knowledge that low molecular weight components leak into the boiling water during cooking, instead indicate a loss of insoluble uronic acid-containing polymers. This could be due to the pectic substances being lost into the boiling water, e.g. by breakage of weak bonds between polysaccharide chains of intermediate or low molecular weight (Selvendran & Robertson, 1994).

The TDF content was not affected by 100 mM $CaCl_2$, either with 4 min of boiling or with 25 min of boiling (Fig. 1). However, there was a redistribution from SDF (uronic acids) to IDF (uronic acids) at both boiling-

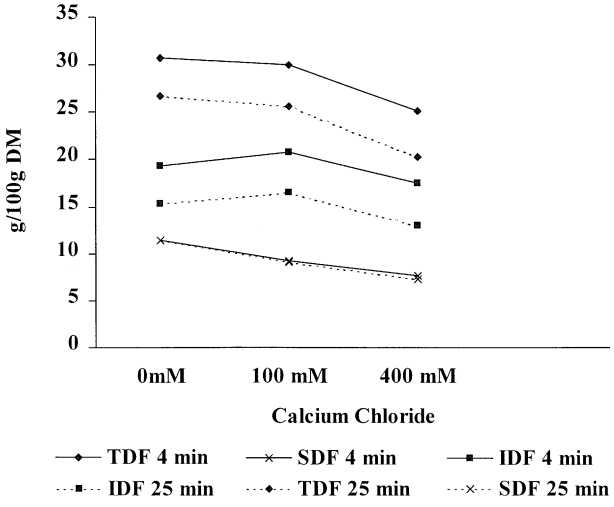

Fig. 1. Effect of $CaCl_2$ (0, 100, 400 mM) on dietary fibre content following boiling.—=4 min of boiling, ...= 25 min of boiling, ◆=total dietary fibre (TDF), ■=insoluble dietary fibre (IDF) and ×=souble dietary fibre (SDF).

Table 1
Dietary fibre content (g/100 g DW) and monomeric composition (%) in carrots boiled for 0, 4 and 25 min in water[a]

	TDF			IDF			SDF		
	0 min	4 min	25 min	0 min	4 min	25 min	0 min	4 min	25 min
Rhamnose	2 a	2 a	3 a	1 a	2 a	2 a	4 a	3 a	4 a
Arabinose	7 a	8 a	8 a	6 a	5 a	5 a	11 a	11 b	12 c
Xylose	2 a	2 a	2 a	2 a	3 a	3 a	0 a	0 a	0 a
Mannose	2 a	2 a	2 a	3 a	4 a	4 a	0 a	0 a	0 a
Galactose	16 a	16 a	16 a	13 a	12 a	10 b	23 a	23 b	24 b
Glucose	34 a	35 b	35 a	48 a	54 b	60 a	1 a	2 a	1 a
Uronic acids	37 a	35 a	34 b	27 a	20 b	16 c	61 a	61 b	59 b
Total	28.2 a	30.7 b	26.7 a	19.3 a	19.3 a	15.3 b	8.8 a	11.4 b	11.4 b

TDF, total dietary fibre; IDF; insoluble dietary fibre; SDF, soluble dietary fibre.

[a] Mean values within the same row with different letters are significantly ($P<0.05$) different from each other according to Tukey's test

times, suggesting an increased cross-linking between calcium and the carboxyl groups in the pectin-molecule. A lower solubility of the cell wall polymers in the presence of Ca^{2+} has also been reported to occur in onion waste (Ng et al., 1999). With the higher concentration of $CaCl_2$ there was instead a decrease in TDF. With 4 min of boiling, TDF content was only 25.2 g/100 g DW versus 30.7 g/100 g DW ($P<0.05$) without any cations, which corresponds to a loss of about 18%. The decrease was due to a loss of both soluble polymers, containing uronic acids (~9%, $P<0.05$), galactose (~2%), and arbinose (~2%), and insoluble polymers, containing glucose (~4%). The same phenomena occurred for both boiling temperatures. This is in agreement with Sajjaanantakul et al. (1993), who found an enhanced degradation of pectin, through the β-elimination reaction, when cations were added at heat-treatment. A low concentration of $CaCl_2$ may thus be of importance when producing vegetables and fruits with a more stable plant structure, whereas a higher concentration causes degradation of the plant structure.

NaCl caused a reduction of TDF, even at the low concentration, i.e. about 15% with 4 min of boiling ($P<0.05$) and 11% with 25 min of boiling ($P<0.05$; Fig. 2). The lower value could be explained by a decreased content of insoluble polymers containing uronic acids (~9% and ~6% with 4 min and 25 min of boiling, respectively; $P<0.05$), glucose (~3%) galactose (~1%, $P<0.05$) and arabinose (~1%), while SDF content was approximately the same. No further decrease in TDF could be seen with 400 mM NaCl, either with 4 min of boiling or with 25 min.

Thus, only Ca^{2+} could cross-link the pectin molecules, giving a more stable plant structure. Both $CaCl_2$ and NaCl catalyse the degradation of dietary fibre, Na^+ already at the low concentration and Ca^{2+} at the high concentration. Ca^{2+} mediated its effects via the SDF, first by cross-linking and then by degradation, whereas Na^+ only had an influence on IDF.

3.2. Composition of WSP of various molecular weight fractions

The composition of the various molecular weight fractions isolated is shown in Fig. 3a. The high and the intermediate fraction isolated from blanched carrots contained similar amounts of polymers, and both consisted mainly of uronic acids (64 and 71%, respectively), galactose (22 and 15%, respectively) and arabinose (on average 10%). The low molecular weight fraction contained mainly glucose (72%), but also appreciable amounts of mannose (13%) and uronic acids (7%). The low molecular weight fraction was not quantified in the dietary fibre analyses, as judged from the low amount of glucose found in the SDF fractions (Table 1). Callose, a β-(1,3) linked glucan, has been reported to be synthesised when plant materials are wounded, as a protection against leakage of water and nutrients through the vessel elements of the plant (Shea, Gibeaut, & Carpita, 1990). Possibly, callose can be formed when carrots are trimmed, peeled and cut into cubes before blanching, a procedure causing an area which is highly wounded. Some of the glucose found in the low-molecular fraction might also be starch-remnants, but carrots only contain small amounts of starch (<0.1 g/100 g DW) and, of this, minor amounts will be soluble (Svanberg et al., 1997).

During boiling, there was an enrichment of polymers in the intermediate fraction, while the fraction containing high molecular weight polymers decreased (Fig. 3a). The increase was mainly due to a higher uronic acid content, which was nearly trebled, but to some extent also to galactose and arabinose. Also, polymers (uronic acids and glucose) of low molecular weight increased somewhat, demonstrating a depolymerization of polysaccharides during boiling. A depolymerization of WSP in carrots and beans following other types of heat-treatments has also been seen in previous studies (Svanberg et al., 1995; Svanberg, Suortti & Nyman, 1997).

With $CaCl_2$ there was a considerable reduction (60 to 70%) in all the various molecular weight fractions isolated, suggesting formation of insoluble complexes. This is in agreement with the dietary fibre analyses, where a lower content of SDF was also obtained. The proportion of high molecular material also decreased with $CaCl_2$ (from 18 to 14%), due to an increase of low molecular weight material, indicating a further degradation of

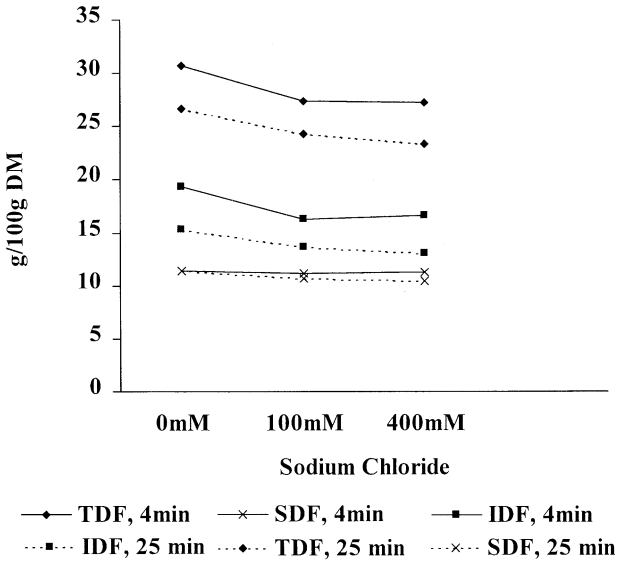

Fig. 2. Effect of NaCl (0, 100, 400 mM) on dietary fibre content following boiling.— = 4 min of boiling, ... = 25 min of boiling, ◆ = total dietary fibre (TDF), ■ = insoluble dietary fibre (IDF) and × = souble dietary fibre (SDF).

high molecular weight material when $CaCl_2$ was present in the boiling water.

Addition of NaCl (400 mM) to the boiling water had minor effects on the various molecular weight fractions isolated.

3.3. Viscosity

All materials showed a pseudoplastic behaviour, i.e. the viscosity decreased with increasing shear rate. The curves from the upsweep and the downsweep of the shear rate coincided in all samples, which indicates that no time-dependent structural rearrangements occurred during the process.

The viscosity of WSP isolated from carrots was highly dependent on time of boiling. The highest viscosity was obtained with blanched/frozen carrots and decreased with increased boiling time (Fig. 4). WSP in blanched/frozen carrots showed a similar viscosity profile to HM-pectin at most of the shear rates investigated, while WSP in carrots boiled for 25 min had a similar profile to LM-pectin. The maximum viscosity (η_0) was about 1000 mPa/s, for WSP isolated from blanched/frozen carrots, while 4 min of boiling decreased η_0 to approximately a fourth (256 mPas) and 25 min of boiling further four times (64 mPas; Table 2). A decrease in viscosity of SDF, following heat-treatment of vegetables has been observed in previous studies (Nyman, Svanberg, & Asp, 1994; Svanberg et al., 1995).

Addition of $CaCl_2$, at the higher concentration (400 mM), reduced the viscosity (Fig. 5a), while the low concentration (100 mM) had minor effects compared

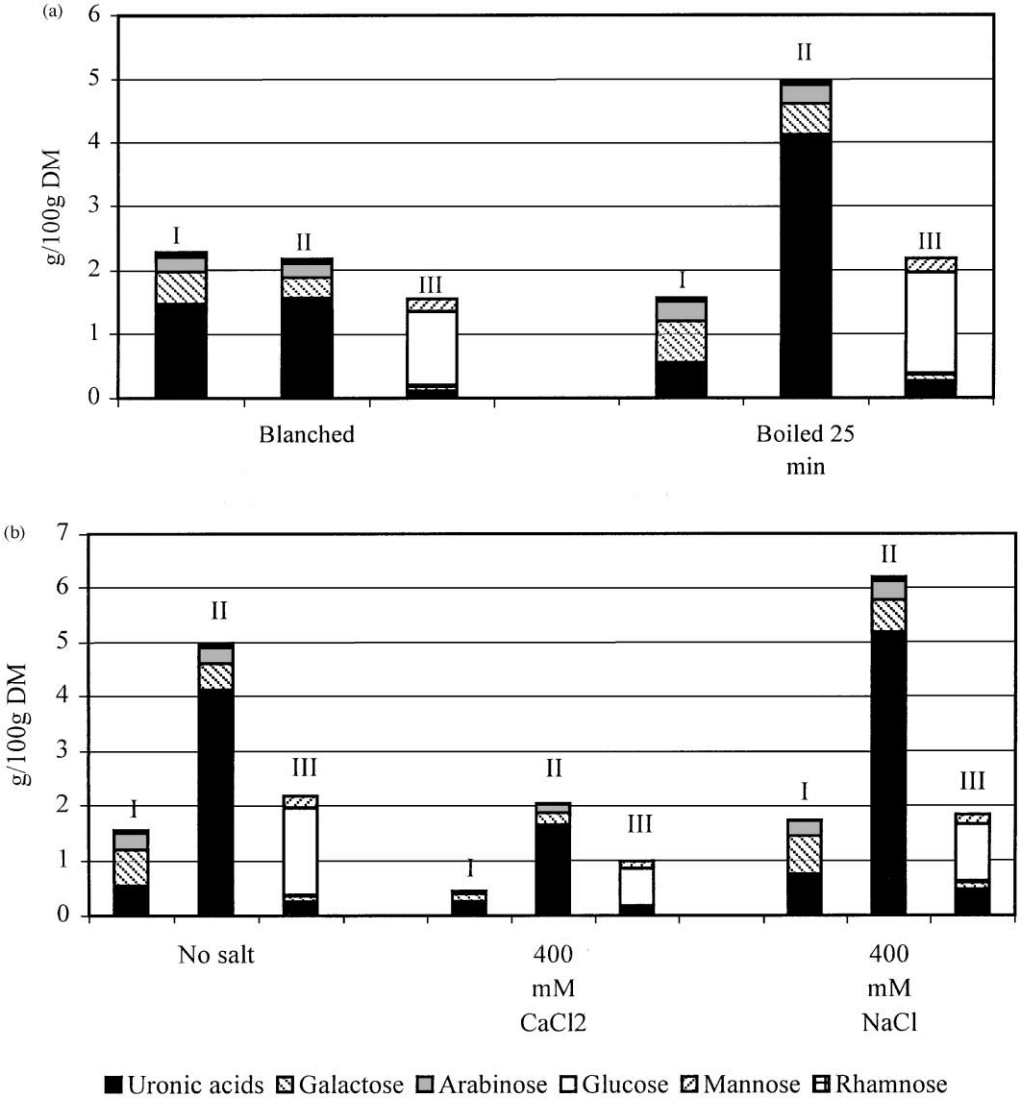

Fig. 3. Composition (g/100 g DW) of the different molecular weight fractions of the water-souble polysaccharides (WSP) isolated from (a) blanched and boiled (25 min) carrots and (b) boiled (25 min) carrots with or without the addition of 400 mM $CaCl_2$ or NaCl to the boiling water. Fraction I $MW_{app} > 200\,000$, fraction II MW_{app} 200 000 − 1 500, fraction III $MW_{app} < 1500$.

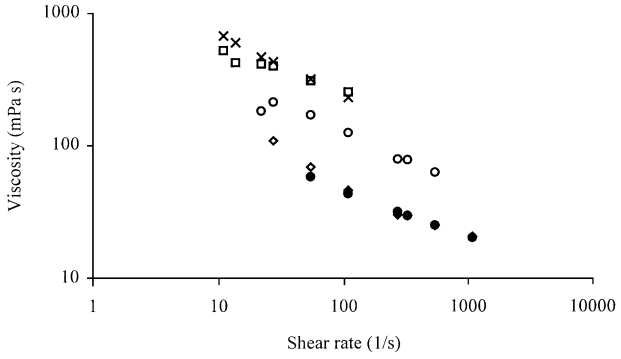

Fig. 4. Viscosity versus shear rate of water-souble polysaccharides (WSP; 4% w/w) isolated from blanched carrots (×) and carrots boiled for 4 (○) or 25 min (●). Reference material: high methoxylated-pectin (□) and low methoxylated-pectin (◇).

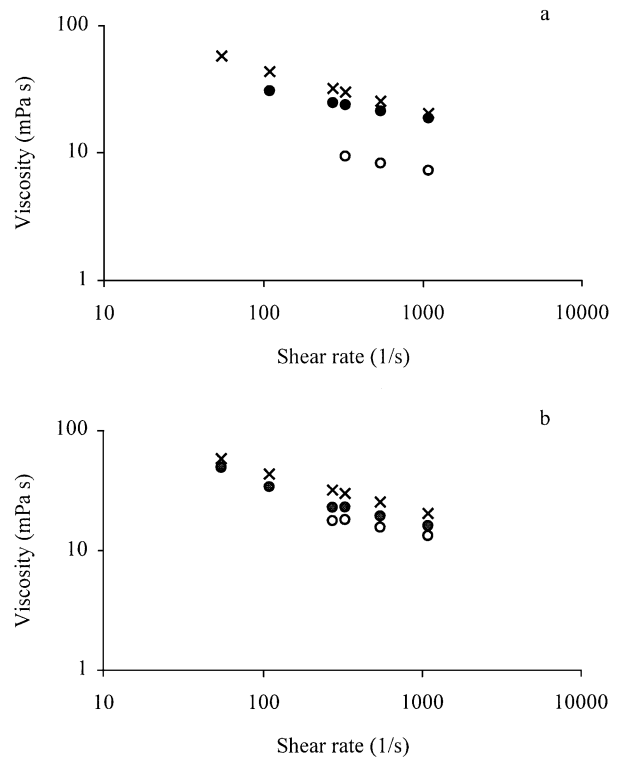

Fig. 5. Viscosity versus shear rate of water-soluble polysaccharides (4% w/w) isolated from carrots boiled for 25 min with (a) $CaCl_2$ or (b) NaCl, (×) 0 mM, (●) 100 mM and (○) 400 mM.

Table 2
Maximum viscosity (η_o) and shear rate ($\gamma_{1/2}$) for WSP in carrots boiled for 25 min[a]

	η_o	$\gamma_{1/2}$
0 mM	64 e	346 a
100 mM $CaCl_2$	33 c	1370 b
400 mM $CaCl_2$	11 a	2374 c
100 mM NaCl	50 d	329 a
400 mM NaCl	22 b	1826 bc

WSP, water-souble polysaccharides

[a] Mean values within the same column with different letters are significantly ($P<0.05$) different from each other according to Tukey's test.

with no cations. Calculation of η_0 gave similar information (Table 2). This is in accordance with results on molecular weight distribution, where the proportion of the high molecular weight fraction was comparatively lower when $CaCl_2$ (400 mM) was added (14:57:29 versus 18:57:25).

The effect of NaCl on the viscosity was small (Fig. 5b). The maximum viscosity, η_0, decreased (from 64 to 22 mPa/s) when the concentration of NaCl increased (Table 2).

4. General discussion

The present study shows that both NaCl and $CaCl_2$ may affect physico-chemical properties of dietary fibre in carrots. However, the cations seem to have different effects, where NaCl generally was of less importance than $CaCl_2$. Nevertheless the IDF content decreased considerably when carrots were boiled with NaCl. Possibly weak bonds between polysaccharide chains are broken during heat-treatment. This has been shown for raw potatoes, where the strong associations between pectic and cellulosic polysaccharides were lost after cooking (Ryden & Selvendran, 1990). This seems to be consistent also for carrots, and this reaction seems to be catalysed by NaCl, as judged by the more pronounced reduction in IDF when NaCl was present. A lower content of IDF may also have nutritional implications, by reducing the bulking capacity and increasing the formation of short-chain fatty acids (Schneeman, 2001).

With low concentrations of $CaCl_2$ there was a redistribution of soluble to insoluble dietary fibre, due to an increased cross-linking between polysaccharide chains (pectins) when divalent cations were added, resulting in a more stable plant structure. This may also be of nutritional significance. The release of digestible carbohydrates may be slower in a stable cellular structure, thus decreasing glycaemic response. Whole apples have been shown to give a lower blood glucose response than apple puree (Haber, Heaton, & Murphy, 1977). Similarly, blanched/microwaved carrots gave a higher blood glucose response than raw carrots with an intact cellular structure (Gustafsson, Asp, Hagander, Nyman, & Schweizer, 1995). Insoluble dietary fibre may also increase the faecal bulking effect, due to their resistance against fermentation in the large intestine (Schneeman, 2001). Thus, this type of fibre may decrease the risk of constipation and also possibly colonic cancer.

It has been suggested that an increased concentration of cations might result in a more compact structure of cell wall polymers (Mazza & Biliaderis, 1989). However, simultaneously, there is also a degradation of pectin by the β-elimination reaction, which is enhanced with heat-treatment and added cations (Sajjaanantakul et al.,

1993). In the present study, there was a complex-binding between pectin molecules with low concentrations of $CaCl_2$ but with a higher concentration, a considerable degradation of the polysaccharides occured. At the higher concentration there was also a loss in viscosity, a factor that has been considered to be of great importance for effects on glucose and lipid metabolism. On the other hand, degraded dietary fibre polysaccharides may be more easily fermented to short-chain fatty acids. Some of these acids have increasingly been considered as nutritionally important, and formation of a high proportion of propionic acid is suggested to affect glucose and lipid metabolism beneficially.

The production of an indigestible glucan with peeling and cutting is interesting. High proportions of butyric acid have been shown to be formed by β-glucans from barley (Berggren, Björck, & Nyman, 1993). This acid, which is one of the most important substrates for the colonic mucosa, is thought to protect against colonic diseases, such as ulcerative colitis and colonic cancer (Cummings, Rombeau, & Sakata, 1995).

In conclusion, by adding cations to the boiling water, the physico-chemical properties of the dietary fibre can be changed in different ways. Various concentrations have different effects. Changes of physico-chemical properties are also of importance from a nutritional point of view.

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