Molecular Weight Distribution, Measured by HPSEC, and Viscosity of Water-Soluble Dietary Fiber in Carrots following Different Types of Processing

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The molecular weight distribution, measured by HPSEC, and the viscosity of the water-soluble dietary fiber isolated from variously processed/cooked carrots were investigated. The carrots were studied raw, frozen, blanched, soured, microwaved, and boiled. The fibers were isolated after enzymatic digestion of protein and starch to simulate the situation in the gastrointestinal tract. The degree of polymerization (DP) and the viscosity were highly dependent on the type of processing/cooking and in general followed the degree of heat treatment. Thus, the DP values were similar with raw and frozen carrots, whereas blanched carrots had a lower DP. Further, only minor differences in DP could be observed among blanched, soured, and microwaved material, and the most pronounced degradation was obtained in boiled material. The viscosity was in accordance with the DP measurements and decreased in the order raw > blanched > boiled.

Keywords: *Processing; soluble fiber; viscosity; molecular weight distribution; carrots; HPSEC; freezing; boiling; microwave treatment; souring; blanching*

INTRODUCTION

Vegetables are quantitatively the most important source of soluble dietary fiber. In Sweden one of the most commonly eaten vegetables is carrot, at an annual consumption of about 6.5 kg per person. Carrots are available throughout the year and are consumed either fresh or following various kinds of processing and cooking.

In processing/cooking, glycosidic linkages in the dietary fiber polysaccharides may be broken (Björck et al., 1984; Nyman et al., 1987a,b; Plat et al., 1988). At a moderate degradation the amount of soluble fiber will increase (Nyman et al., 1987a,b), whereas there will be an apparent decrease in the total fiber content if the decomposition is extensive enough to yield alcohol-soluble fragments (Nyman et al., 1987a,b). Such effects are easy to determine by using one of the current analyses of dietary fiber (Asp et al., 1983; Englyst and Cummings, 1984; Prosky et al., 1985; Theander and Westerlund, 1986). However, to gain insight into the extent of depolymerization, the molecular weight distribution has to be studied.

The depolymerization of fiber polysaccharides may be of interest from the point of view of nutrition. For instance, a breakage of glycosidic linkages may result in an increased fermentability of the fiber in the large intestine and consequently a lower bulking capacity (Björck et al., 1984; Nyman et al., 1987a). Further, as the viscosity is reduced already with the cleavage of a few glycosidic linkages (Albersheim et al., 1960), processing/cooking may also reduce beneficial effects on carbohydrate and lipid metabolism. In glucose tolerance tests, with the addition of different dietary fibers, the decrease in blood glucose responses may be correlated to the viscosities of the dietary fibers (Jenkins et al., 1978). It was also shown that the glucose and insulin reducing effects of guar gum were abolished by hydrolysis and loss of viscosity (Jenkins et al., 1978). Furthermore, raw carrots in a mixed meal elicited a lower glucose response than boiled or microwaved ones (Gustafsson et al., 1995).

The molecular weight distribution of soluble dietary fiber in vegetables has been investigated earlier by using conventional gel filtration (Nyman et al., 1993, 1994). In those studies a Sephadex (G-75) column with a fractionation range between 1000 and 50 000 was used, with which the separation of polysaccharides with an approximate degree of polymerization between 5 and 250 is possible. A depolymerization of the soluble fiber in carrots was indicated following microwave treatment, boiling, or canning but not after blanching (Nyman et al., 1993). Further, there was evidence of a depolymerization of the soluble fiber in the canning process of green beans, green peas, and Brussels sprouts compared to blanching (Nyman et al., 1994). However, analysis by conventional gel filtration is very time-consuming, and it would therefore be an advantage if more modern methodologies, such as high-performance size exclusion chromatography (HPSEC), could be used. HPSEC is now more or less routinely applied to starch and its degradation products (Kennedy et al., 1992). There are also reports that this technique can be applied to β-glucans, and the molecular weight of β-glucans in oats has been studied after passage through the stomach and the duodenum in pigs by using this technique (Johansen et al., 1993). However, vegetables that contain large

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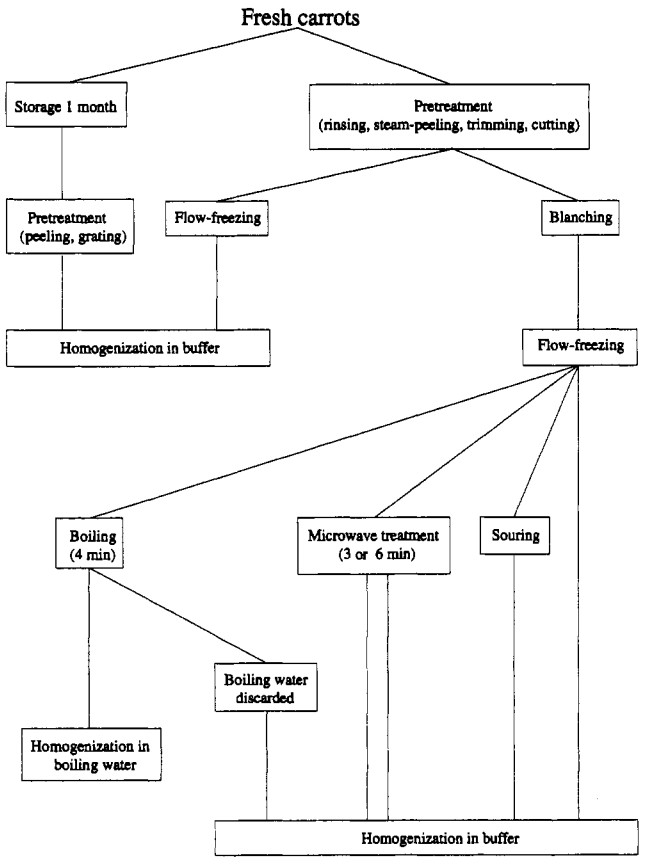

Figure 1. Flow chart: processing of raw carrots.

amounts of charged and highly viscous pectin may be more difficult to analyze.

The purpose of the present investigation was to study the molecular weight distribution, by using HPSEC, and the viscosity of the soluble fiber both in raw carrots and in carrots following various processing and cooking methods. The fiber was isolated by simulating the process in the gastrointestinal tract. The effect of pH and concentration of the soluble fiber was evaluated and discussed in relation to the processing effects. The carrots used were of the same variety as in earlier experiments in man (Gustafsson et al., 1994, 1995) and from the same harvest and batch in which dose–response effects on postprandial glucose and hormonal responses were evaluated (Gustafsson et al., 1994). Pure pectins and guar gum were used as reference materials.

MATERIALS AND METHODS

Materials. Carrots (*Daucus carota* sp. *sativus*) were investigated raw and following different types of processing/cooking, i.e. freezing, blanching, boiling, microwaving, and souring, and on the whole eight samples were studied (Figure 1).

In the viscosity measurements low methoxylated (LM) pectin (degree of esterification 37%), high methoxylated (HM) pectin (degree of esterification 74%), and guar gum were also included (Copenhagen Pectin Factory Ltd., Skensved, Denmark).

Processing. Raw carrots were rinsed, steam-peeled, trimmed, and cut into cubes (10 × 10 × 10 mm). These were either frozen directly or blanched before freezing (Figure 1). The blanched/frozen carrots (200 g) were then poured into boiling, lightly salted water (1 g of NaCl in 150 mL of H_2O, 4 min), microwaved (700 W, 30 mL of H_2O), or soured (25 mL of 10% lactic acid, overnight, 4 °C). The microwaved carrots were cooked either for 3 min as recommended by the producer or for 6 min. Raw carrots were stored at 5 °C for about 1 month until analyzed and then peeled by hand and roughly grated by using a food processor (the holes of the grater were 5 × 5 mm).

Isolation of Water-Soluble Dietary Fiber. To simulate the situation in the gastrointestinal tract, water-soluble dietary fiber was isolated after enzymatic digestion of protein and starch. The method is based on that of Asp et al. (1983) with some modifications. The initial gelatinization step at 100 °C was excluded since the effects of heat treatment were to be studied, and the amount of enzymes (pepsin and pancreatin) and buffer was reduced for practical reasons. Raw and variously processed/cooked carrots (150 g on an "as is" basis) were homogenized in sodium phosphate buffer (150 mL, 0.1 M, pH 6.0). Boiled carrots were also homogenized in the water in which they were boiled instead of buffer (Figure 1). After the homogenization, the suspension was adjusted to pH 1.5 (5 M HCl) and incubated with pepsin (0.5 g, 2000 FIP-U/g; Merck, Darmstadt, Germany) at 40 °C with agitation for 60 min. The pH was then adjusted to 6.8 (5 M NaOH), and the addition of pancreatin (0.5 g, 100 U/mg; BioChemica, Fluka Chemie, Buchs, Switzerland) was followed by a second incubation at 40 °C with agitation for 60 min. The pH was adjusted to 4.5, and the solution was centrifuged at 5000 rpm for 30 min. The supernatant and washings (2 × 100 mL) were evaporated to approximately 100 mL and then dialyzed (Spectra/Por, cutoff molecular weight = 1000, Spectrum, Houston, TX) and freeze-dried. The freeze-dried samples were used for viscometry measurements and studies of molecular weight distribution.

HPSEC. The instrument employed consisted of an M-590 pump, an M-715 automatic injector, and an M-411 refractive index detector. The system was controlled and the data were handled with a Maxima workstation (all equipment was from Millipore/Waters, Milford, MA).

The chromatography was carried out on μHydrogel 2000, 250, and 120 (7.8 × 300 mm) columns at 70 °C. The eluent was 50 mM ammonium acetate (pH 6.0) at a flow rate of 0.5 mL/min. Samples were dissolved in the eluent (2000 mg/L) with gentle stirring overnight and injected (100 μL) into the HPLC instrument without further manipulation.

Pullulans of various molecular weight (M_w)—12 200, 48 000, 186 000, and 853 000 (Showa Denko, Japan)—were used for calibration. However, it has been shown that pullulan molecules are very flexible, and their use as standards has been shown to give molecular weights too high for rigid molecules such as pentosans and β-glucans (Vårum et al., 1991) and therefore probably also for the carrot fibers used in this study. Thus, all reported molecular weights are apparent weights. The variation in retention times of the standard component was less than 0.3% and the variation in areas less than 2% between different injections (Suortti, 1995).

Viscometry Measurements. The freeze-dried preparations were redissolved in 0.1 M sodium phosphate buffer (pH 6.0) giving a final concentration of 4% (w/w). With raw carrots 2.5 and 5% (w/w) solutions were also prepared. The solutions were allowed to equilibrate overnight before measurement. Four percent (w/w) solutions of LM pectin and HM pectin as well as 3% (w/w) solutions of LM pectin, HM pectin, and guar gum were included as references. Further, to get an indication of the effect of pH, a 5% (w/w) solution of LM pectin at different pH values (2.4, 2.9, and 3.4) was tested. The viscosity was measured in a Haake viscotester (VT 501, Tillquist, Kista, Sweden). The carrot samples and the pectins were measured with sensor system NV and the guar gum with sensor system SV1. The shear rate was swept both up and down in intervals of 10–1000 s^{-1} for carrot samples and pectins and 2–200 s^{-1} for guar gum. The analyses were performed in duplicate for most materials.

The viscosity of "random coil" polysaccharides was characterized by two parameters, the maximum "zero-shear" viscosity (η_0) and the shear rate ($\gamma_{1/2}$) at which viscosity is reduced to $\eta_0/2$ (Morris, 1990). These two parameters, η_0 and $\gamma_{1/2}$, were calculated for all materials and statistically evaluated with a one-sided analysis of variance by Duncan's procedure for multiple comparison.

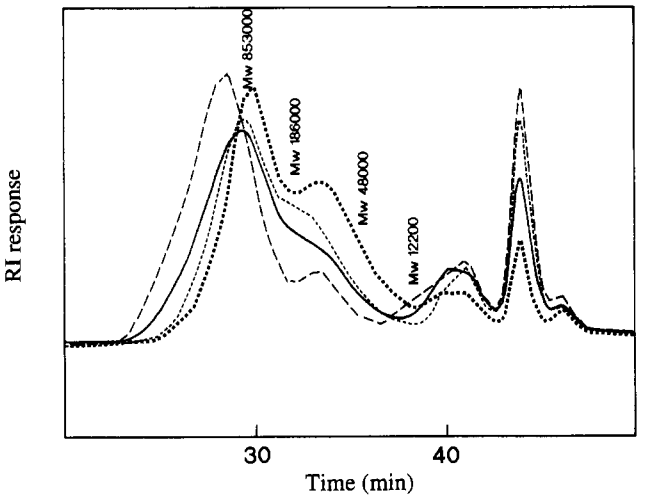

Figure 2. Molecular weight distribution of the water-soluble fiber isolated from raw carrots (— —), blanched carrots (—), carrots microwaved for 6 min (- - -), and boiled carrots homogenized in buffer (• • •).

RESULTS

HPSEC. The molecular weight distribution of the soluble fiber isolated from the variously processed carrots is shown in Figures 2 and 3. All molecular weights reported are apparent and obtained by calibration with pullulans. The pullulans exhibited a linear relationship between retention times and the logarithm of molecular weights. The HPSEC chromatograms showed four peaks, one main peak of high molecular weight ($M_w \geq 850\,000$), one peak at a molecular weight of about 100 000; and two peaks of lower molecular weight ($M_w < 12\,000$). The elution time of the main peak increased with increasing heat treatment, indicating a decreased degree of polymerization (DP) (Figure 2). The differently processed/cooked carrots could be divided into three groups according to their DP, with raw and frozen carrots having the highest DP (Figure 3a), blanched, microwaved, and soured carrots intermediate DP (Figure 3b), and boiled carrots the lowest DP (Figure 3c). Further, the second peak ($M_w \sim 100\,000$) was considerably higher in the case of boiled carrots (Figure 3c) than the other products (Figure 3a,b).

Some differences were also obtained with the material of lower molecular weight ($M_w < 12\,000$). The height of the low molecular weight peaks was lower for boiled carrots homogenized in buffer than for those homogenized in the boiling water (Figure 3c). Similarly, frozen carrots had lower amounts of low DP than raw carrots (Figure 3a). On the other hand only minor differences were obtained between blanched, soured, and microwaved material (Figure 3b).

Viscometry Measurements. The viscosity of the water-soluble polysaccharides isolated from carrots was highly dependent on the type of processing and in general followed the degree of heat treatment (Figure 4a). The highest viscosity was obtained with frozen carrots, followed by raw, microwaved for 3 min, blanched, soured, microwaved for 6 min, boiled, and boiled homogenized in water (Figure 4a). The curves from the upsweep and the downsweep of the shear rate coincide, which indicates that no time-dependent structural rearrangements occur during the shear process. All material showed a pseudoplastic behavior, i.e. the viscosity decreased with increasing shear rate.

The maximum viscosity, η_0, and the shear rate, $\gamma_{1/2}$, are listed in Table 1. For frozen and raw material the

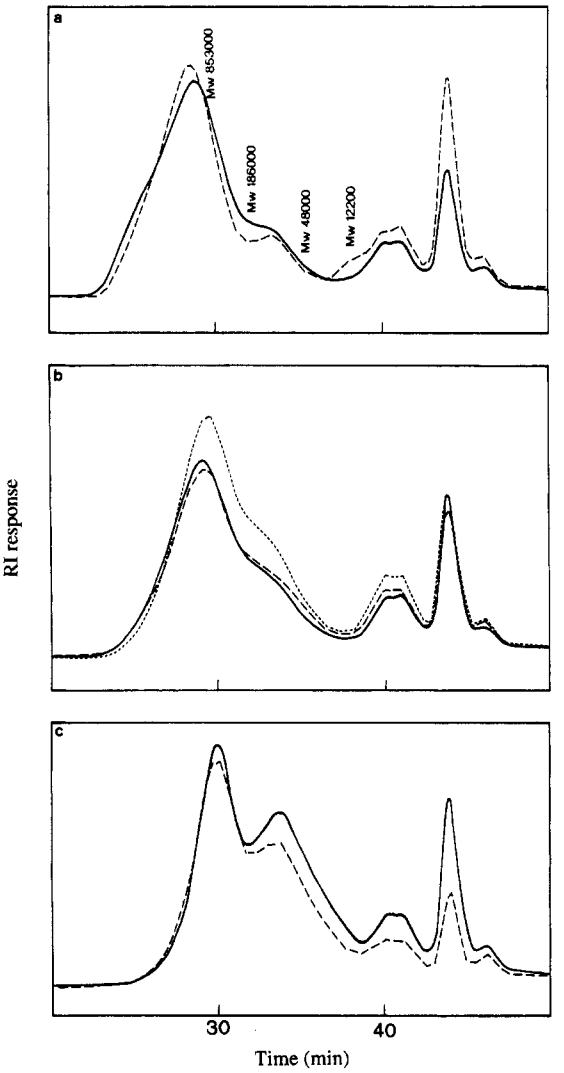

Figure 3. Molecular weight distribution of the water-soluble fiber isolated from (a) raw carrots (- - -) and frozen carrots (—); (b) blanched carrots (- - -), carrots microwaved for 3 min (—), and soured carrots (• • •); and (c) boiled carrots homogenized in buffer (- - -) and boiled carrots homogenized in boiling water (—).

maximum viscosities, η_0, were 2200 and 1 700 mPa s, respectively, while blanching decreased η_0 to approximately half of that found in the frozen samples. Soluble fiber in microwaved (6 min) carrots had a viscosity which was about one-fourth of that in the frozen material (500 mPa s). The variously boiled carrots showed the lowest η_0 and, compared with the frozen ones, η_0 was about 10 times lower (200 mPa s) for those homogenized in buffer and 20 times lower for those homogenized in boiling water (100 mPa s).

The effect of different concentrations of soluble fiber from raw carrots is shown in Figure 4b. The maximum viscosities, η_0, were 200, 1700, and 7000 mPa s and the shear rates, $\gamma_{1/2}$, 230, 23, and 8 s^{-1} for the 2.5, 4, and 5% (w/w) solutions, respectively.

A 4% (w/w) solution of LM pectin had a viscosity of the same magnitude as the soluble fiber isolated from boiled carrots (Table 1). The viscosity of guar gum (3% w/w) was very high, especially at low shear rates (Figure 5a). The maximum viscosity, η_0, and the shear rate, $\gamma_{1/2}$, could not be calculated, and consequently viscosities at different shear rates are reported for this material. At shear rates of 1.78, 17.8, and 178.1 s^{-1} the viscosities were 80 000, 9000, and 900 mPa s, respectively, for the guar gum (3% w/w) solution. Corresponding viscosities

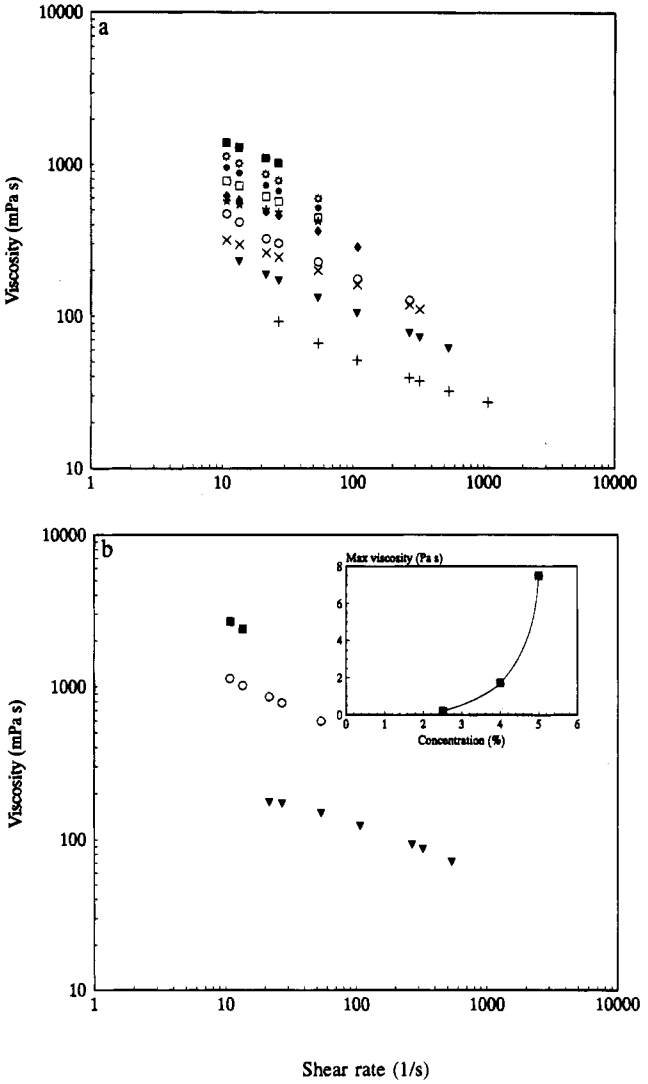

Figure 4. (a) Viscosity versus shear rate of the water-soluble fiber 4% (w/w), isolated from carrots following different types of processing: (■) frozen carrots; (starburst) raw carrots; (●) carrots microwaved for 3 min; (□) blanched carrots; (◆) soured carrots; (○) carrots microwaved for 6 min; (▼) boiled carrots homogenized in buffer; and (+) boiled carrots homogenized in boiling water. Reference material: (*) HM pectin and (×) LM pectin. (b) Viscosity versus shear rate of the water-soluble fiber at different concentrations isolated from raw carrots: (■) 5% (w/w); (○) 4% (w/w); and (▼) 2.5% (w/w).

Table 1. Maximum Viscosity, η_0, and Shear Rate, $\gamma_{1/2}$, for Soluble Fiber in Carrots (4% w/w) Processed in Various Ways and Pectins (4% w/w)[a]

process	maximum viscosity η_0 (Pa s)	shear rate $\gamma_{1/2}$ (s^{-1})
frozen	2.2[a]	21[a]
raw	1.7[b]	23[a]
microwaved for 3 min[b]	1.4[b]	25[a]
blanched	1.0[c]	38[a]
soured[b]	0.8[cd]	48[a]
microwaved for 6 min[b]	0.5[de]	51[a]
boiled[b]	0.2[ef]	120[b]
boiled, with boiling water[b]	0.1[f]	318[c]
LM pectin	0.3[ef]	113[b]
HM pectin	0.6[d]	118[b]

[a] Mean values with different superscript letters in the same column are significantly different ($P < 0.05$) according to Duncan's method. [b] Carrots were blanched and frozen before further processing.

at shear rates of 11, 27, and 108 s^{-1} were 200, 180, and 135 mPa s, respectively, for HM pectin and 100, 90, and 70 mPa s, respectively, for LM pectin (Figure 5a).

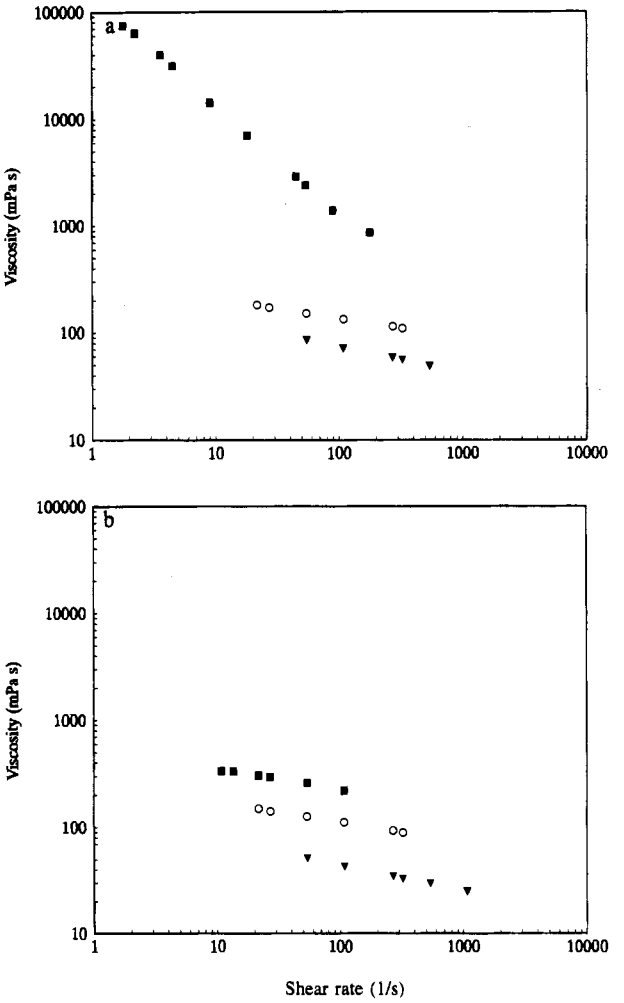

Figure 5. (a) Viscosity versus shear rate of different reference materials 3% (w/w): (■) guar gum; (○) HM pectin; and (▼) LM pectin. (b) Viscosity versus shear rate of LM pectins, 5% (w/w) at different pH values: (▼) pH 2.4; (■) pH 2.9; and (○) pH 3.6.

The effect of three different pH values on the viscosity of LM pectin (5% w/w) is shown in Figure 5b. The viscosities were all rather low and highest at pH 2.9 (η_0 = 400 mPa s). Thus, it decreased both with higher and lower pH.

DISCUSSION

The viscosity and the molecular weight distribution of the soluble fiber in carrots essentially followed the degree of processing/cooking, indicating that the time and intensity of heat treatment are crucial for the splitting of glycosidic linkages. Thus, the viscosity and the molecular weight were higher in frozen carrots than in blanched ones, which in turn were higher than in boiled carrots. However, the viscosity of raw carrots was unexpectedly lower than that of frozen ones. One contributing factor could be that raw carrots were stored for about 1 month before analysis, whereas frozen carrots were frozen directly after harvest. Endogenous pectinases may degrade the dietary fiber during storage, and a 6 month storage of carrots has been shown to decrease the viscosity to a small extent (Nyman and Nilsson, 1994). Another factor of importance may be the activation, by freezing, of the enzyme pectin methylesterase (PME), which catalyzes demethoxylation of the pectic substances and hence the formation of free carboxyl groups (Van Buren, 1979). Calcium or other divalent ions present in carrots may then be cross-linked

to the free carboxyl groups by salt bridges, thus giving rise to a firmer cell structure. PME has been reported to be relatively inactive in the raw plant but to be activated by freezing (Van Buren, 1979), and this activation may explain the higher viscosity of the soluble dietary fiber in frozen carrots.

The lowest viscosity and molecular weight were found for boiled carrots. The viscosity was about 7 times lower and the M_w of the main peak was about half of that found for carrots microwaved for 3 min (Table 1; Figure 3). The considerable difference between these two processes may be due to the heat treatment as such, boiling being more intense, keeping the carrots at a higher temperature during a longer time, than microwave treatment, causing more breakage of glycosidic linkages. Another explanation may be that salt was added to the boiling water but not in the microwave treatment. It has been reported that the β-elimination of pectin in carrots is enhanced with extensive heat treatment (100 °C, 1 h) if cations are added (Sajjaanantakul et al., 1993). The degradation increased with salt concentration, probably due to interaction between cations and the carboxylic groups on the uronic acids. It has also been shown that the addition of salt per se may decrease the viscosity when added to solutions of flax seed mucilage (Mazza and Biliaderis, 1989). This was explained by increasing association between the counterions and the fiber molecules, leading to decreased repulsion of charged groups and a more compact structure of the fiber molecules (Mazza and Biliaderis, 1989).

Boiled carrots homogenized in boiling water had a lower viscosity than boiled carrots homogenized in buffer, most probably due to the comparatively higher concentration of lower molecular weight polysaccharides (Figure 3c). This may be explained by a higher extent of low molecular weight polysaccharides leaking into the boiling water.

The decreased viscosity of the soluble fiber in carrots in vitro may also have nutritional implications in vivo. Experiments in man, with the same genotype of carrots but another harvest as in this study, showed an effect of processing. Thus, raw carrots elicited lower glucose and hormonal responses than blanched and microwaved ones (Gustafsson et al., 1995).

The viscosity was dependent not only on the type of processing but also on the concentration. If the concentration of the soluble fiber isolated from raw carrots increased from 2.5 to 4%, the maximum viscosity increased by more than 8 times—from 200 to 1700 mPa s—i.e. the difference was of the same magnitude as between boiled and raw carrots. Further, by increasing the concentration of soluble fiber from 4 to 5% the zero shear viscosity increased from 1700 to 7000 mPa s. Thus, the viscosity increased exponentially with concentration (Figure 4b), and small variations in the intake of carrots at certain concentrations may therefore be of importance for metabolic effects. Similar observations have been made in vivo in man. Thus, it was shown that the larger the amount of carrots added to a meal the lower were the glucose and hormonal responses (Gustafsson et al., 1994).

It may be questioned whether the viscosity of the soluble fiber in carrots is sufficient to elicit any metabolic effects, without increasing the intake of carrots to an unrealistic level. The soluble fiber in boiled carrots had a viscosity similar to that of the purified LM pectin. In different studies it has been reported that about 10–20 g of pectin may improve the glucose metabolism when added to a meal (Jenkins et al., 1977; Vaaler et al., 1980; Schwartz et al., 1988). Supposing the viscosity of various pectins to be approximately in the same magnitude, an intake of about 600 g of boiled or about half that of raw carrots is necessary. However, when studies in man were performed with the same batch of carrots as in this study, a significant decrease in glucose response was obtained only with 200 g of the boiled carrots when added to a carbohydrate-rich test meal (Gustafsson et al., 1994).

The viscosity of pectin was shown to be dependent on pH, having a maximum viscosity at pH 2.9 (Figure 5b). Similar observations have been made on HM pectin by Michel et al. (1985). Augmented effects on carbohydrate metabolism with fermented and soured products obtained in different studies (Torsdottir et al., 1992; Gustafsson et al., 1994) are sometimes thought to be mediated by a higher viscosity of the fiber (Torsdottir et al., 1992). However, soluble fiber in blanched or soured carrots isolated by simulating the passage through the gastrointestinal tract had very similar maximum viscosities, indicating that the effect of pH is reversible. This has also been found by others (Crean, 1969). With the body being a very effective regulator of pH in the stomach, a similar in vitro viscosity of blanched and soured products most probably also will be valid in vivo. Consequently, the lower glucose and insulin responses after a meal containing soured carrots instead of boiled ones, using the same carrots as in this study, seemed not to be explained in terms of an increased viscosity (Gustafsson et al., 1994).

The viscosity was characterized by two parameters according to the guidelines of Morris (1990). Reforming the viscosity in this way makes it easier to compare the viscosities of various polysaccharides. However, the connection is not true for all polysaccharides and cannot be generally used. An exception was the 3% "solution" of guar gum, which in fact can be characterized more as a gel than a solution, while other exceptions are starch and xanthan (Morris, 1990). The viscosity of various soluble fibers is important in relation to carbohydrate metabolism. However, as the viscosity varies considerably and is different at various shear rates, the question arises as to what the actual shear rate of the food is in the stomach. This is, of course, very difficult to ascertain but is presumably fairly low, and comparisons at zero shear rate are probably quite relevant.

The HPSEC method has several advantages over gel filtration (Sephadex G-75). The fractionation range is much broader (M_w = 1000–1 000 000) compared with the Sephadex G-75 column ($M_{w\,dextran}$ = 1000–50 000) in earlier studies (Nyman et al., 1993, 1994). This makes it possible to observe a degradation and a breakage of only a few glycosidic linkages of the fiber polysaccharides. The HPSEC chromatograms showed four peaks, two in the high and two in the low molecular weight area. In contrast with the gel filtration (Nyman et al., 1993), a degradation of the high molecular weight fraction could be observed. Another advantage is the much shorter time needed to complete an analysis, less than 1 h as compared to a couple of days.

In conclusion, the viscosity and the DP of the soluble fiber in carrots differ considerably with various types of heat treatment. Therefore, variously processed/cooked carrots can also be expected to differ considerably in their physiological effects. However, with larger amounts, entailing a higher concentration and a higher viscosity, the influence of heat treatment may be reduced.

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