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The influence of variety on the enzymatic degradation of carrots and on functional and physiological properties of the cell wall materials

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Abstract

In order to investigate the influence of variety on the structure and properties of enzymatically degraded cell wall materials (CWM), carrots of the varieties Major (direct consumption) and Karotan (cultivated for industrial processing) were used to prepare cold water extracted reference materials (RM) as well as materials treated with macerating enzymes (MC) and with liquefying enzymes (LF). Rehydrated samples RM and MC showed a good maintenance of the grown biological structure (tissue particles for RM; single cells for MC), a high porosity, excellent hydration properties as well as remarkable visco-elastic and structural viscous properties, independent of the carrot variety used. However, the cell wall of the variety Major was more strongly degraded by enzymatic treatment than that of Karotan. Thus, the strongly degraded LF of Major showed the lowest values for porosity, hydration properties and rheological parameters of their suspensions. In vitro, the CWM from Karotan also gave in higher concentrations of short-chain fatty acids during its fermentation with human faeces flora and had a better binding capacity of glycochenodeoxycholic acid than the CWM from Major. Such CWM from carrots may be promising as ingredients for the production of functional foods.

Keywords: Carrot cell wall materials; Enzymatic degradation; Functional properties; Physiological effects

1. Introduction

Cell wall materials (CWM) are suitable model systems for the dispersed phase of crushed fruit or vegetable products (Kunzek, Kabbert, & Gloyna, 1999; Kunzek, Müller, Vetter, & Godeck, 2002; Müller & Kunzek, 1998). During processing, CWM undergo changes of their composition and structure, leading to several structure-dependent changes in their physicochemical, and physiological properties (Guillon & Champ, 2000; Kunzek et al., 1999). The treatment of apple tissue with commercial pectolytic enzyme preparations resulted in the formation of single cell materials and/or cell fragments which aggregated during drying. The suitability of such degraded CWM as thickening agents is limited due to their functional properties (e.g., porosity, water binding capacity) (Kabbert, Goworek, & Kunzek, 1997). Interactions between process-dependent structural changes and the state transitions of the CWM are still not yet completely understood (Kunzek et al., 1999). Recently, it was shown that an increasing degradation of apple parenchyma tissue by macerating, mash fermenting or liquefying enzymes resulted in a continuous loss of the pectin matrix, a decrease in porosity, an increasing aggregation as well as alterations in water binding and

Abbreviations: BA, bile acid; CWM, cell wall material; DF, dietary fibre; GCDCA, glycochenodeoxycholic acid; LF, liquefied material; MC, macerated material; RM, cold-water-extracted reference material; SCFA, short-chain fatty acid; SEM, scanning electron microscopy; SW, swelling; WR, water retention capacity; WU, water uptake.

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visco-elastic properties of CWM–water suspensions and thermoanalytical behaviour (Förster, Dongowski, & Kunzek, 2002).

Most reports about the effects of pectolytic and cellulolytic enzymes on carrots or carrot cell wall preparations are related to liquid carrot products (Dongowski & Bock, 1977; Grampp, 1969; Massiot, Guiller, Baron, & Drilleau, 1992; Zetelaki-Horvát & Gátai, 1977). Anastasakis, Lindamood, Chism, and Hansen (1987) found that an enzymatic treatment of carrot pulp resulted in alterations of the cell wall structure, viscosity and extracted juice volume. The loss of birefringence was closely related to the pectinase activity.

Besides their functional properties, CWM are of physiological importance as dietary fibre (DF)-rich materials. In most developed Western countries the intake of DF is generally too low (Cummings & Frølich, 1993). Most DF components are to the cell wall polysaccharides, which are resistant to digestion by alimentary enzymes of humans. DF are able to interact with steroids and drugs, as well as to affect glycaemic response or absorption of nutrients in the upper parts of the intestinal tract (Dongowski, 1995; Dongowski, Schnorrenberger, Plätzer, Schwarz, & Neubert, 1997; Kritchevsky & Bonfield, 1995; McCleary & Prosky, 2001). In the large intestine, DF regulates water binding and transit time and is the major substrate for the intestinal microflora. Foremost end-products of the microbial fermentation in the gut are the short-chain fatty acids (SCFA). The SCFA butyrate plays a physiologically outstanding role due to regulating effects in cell cycle and for protection against colon cancer (Jacobasch & Dongowski, 2000; Smith, Yokoyama, & German, 1998). The processes involved in fermentation of DF can be simulated by in vitro experiments with fresh faeces flora (Barry et al., 1995; Casterline, Oles, & Ku, 1997; Dongowski & Lorenz, 1998; Guillon, Renard, Hospers, Thibault, & Barry, 1995).

Carrots are a good source of DF (Bao & Chang, 1994; Horváth-Mosonyi, Rigó, & Hegedüs-Völgyesi, 1983). There is scant information on the influence of carrot variety on its DF contents (Robertson, Eastwood, & Yeoman, 1979) or its functional and physiological properties (Robertson, Eastwood, & Yeoman, 1980). Brunsgaard, Kidmose, Sorensen, Kaack, and Eggum (1994) determined the chemical composition and the nutritive value of 35 carrot samples (different in variety, nitrogen supply, harvest time and cultivation time) in rats and found a connection between the energy digestibility and the proportion of the soluble DF fraction. The fermentation of carrot preparations was investigated only in some studies in vitro and in vivo (McBurney & Thompson, 1990; Roland, Nugonbaudon, Andrieux, & Szylit, 1995; Ryden & Robertson, 1995; Swanson et al., 2001; Wisker, Schweizer, Daniel, & Feldheim, 1994).

Altogether, CWM are DF-rich materials with outstanding functional properties for the production of low-caloric functional foods. Therefore, possible effects of such preparations in nutrition and physiology have to be investigated. In this study, carrot CWM was modified by a treatment with commercial pectolytic and cellulolytic enzymes. The increasing enzymatic degradation of the CWM was examined with respect to changes in (i) structure, composition and physical state, (ii) physicochemical properties, including water binding and rheological properties of the CWM–water suspensions and (iii) physiological properties via binding of bile acids (BA) and in vitro fermentation. A major objective was to determine the influence of carrot variety on the properties of the CWM. In particular, we have investigated whether the cell wall of a carrot variety, determined for direct consumption, has other properties than that cultivated for industrial processing. For this purpose, carrot CWM of the varieties Major (consumption) and Karotan (industrial processing) were chosen and treated with industrial cell wall-degrading enzymes. The structure and properties of the obtained materials were characterized, and essential interrelations or other dependencies between the structural, physicochemical and physiological parameters were tested.

2. Material and methods

2.1. Raw materials

Carrots of the variety Major (mainly cultivated for direct consumption) as well as of the variety Karotan (cultivated for industrial processing) grown near Trebbin, State Brandenburg, Germany, were harvested in September 2001 and stored at 8 °C for some days before the preparation of the CWM. The CWM were isolated from a uniform sample of raw material in both cases within two weeks.

2.2. Preparation of the CWM

The preparation scheme of the CWM is given in Fig. 1. Reference materials (RM) were prepared according to Müller and Kunzek (1998) for cold-water-extracted materials from apples. Carrot mash (1.5 kg) was pressed. The residue was washed with 2.5 l of distilled water and pressed again. The residue was homogenised by an IKA-Ultra-Turrax T 25 (Janke & Kunkel, Germany), using a big dispersing tool (S 25 FN) for 5 min and a small dispersing tool (S 25 GM N) for 2 min. The suspension was fractionated within the range 71–500 μm, washed with distilled water until the conductivity of the drain was below 50 μS/cm, and

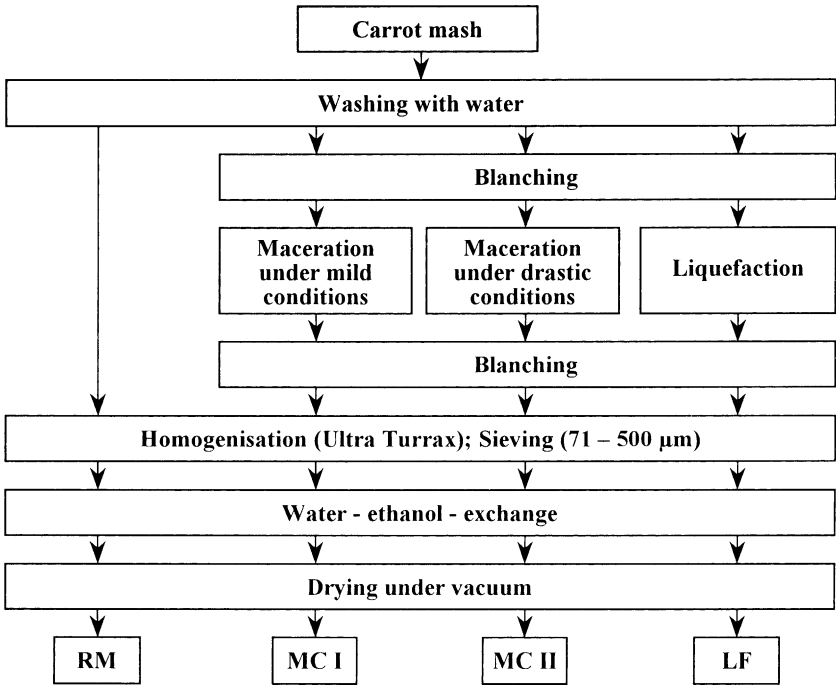


Fig. 1. Preparation of the cell wall materials (CWM). RM, cold water extracted reference material; MC I, CWM treated with macerating enzymes under mild conditions; MC II, CWM treated with macerating enzymes under drastic conditions; LF, CWM treated with liquefaction enzymes.

pressed. Then, the residue was washed with 70%, 90% and 95% ethanol (v/v), successively. The last step was repeated until the ethanol concentration of the discarded solution was greater than 92%. The material was dried in a rotary evaporator (water bath temperature 90 °C) until the dry matter content was above 95%.

The other materials were additionally treated with pectolytic enzymes, as described by Förster et al. (2002). For the preparation of these materials, blanching steps were carried out before and after the enzymatic treatment as follows: The first step with diluted citric acid (5 ml of 5% citric acid per 1 l of water) and the second step with water, always for 5 min at 85 °C (see Fig. 1) (Kabbert et al., 1997; Vetter & Kunzek, 2002; Vetter & Kunzek, 2001). The macerated materials (MC) were obtained using 300 mg/l (MC I) and 500 mg/l (MC II) Rohament PL (polygalacturonan-rich preparation for maceration of fruits or vegetables; Röhm Enzyme GmbH, Darmstadt, Germany) at 45 °C (pH 4.5–5.0) for 1 and 1.5 h, respectively. Liquefied materials (LF) were prepared using a mixture of 200 mg/l Rohapect AP 1 (pectolytic preparation with arabanase activities; Röhm), 100 mg/l Rohapect B 1 L (pectolytic and hemicellulolytic preparations for degradation of pectins and hemicelluloses, Röhm) and 100 mg/l Rohalase 7069 (cellulolytic enzyme preparation for hydrolysis of cellulose, glucans and xylans, Röhm) at pH 4.5–5.0 and 50 °C for 1.5 h. The dosage of the enzyme preparations was as recommended by Röhm.

2.3. Particle analysis and structural properties

The particle size distribution was analysed with a Galai *cis*-1 particle size analyser (L.O.T.-Oriel GmbH, Germany) using a GMCS-7 flow cell. Sample preparation was carried out as described by Kabbert and Kunzek (1995). For the scanning electron microscopy (SEM), the samples were freeze-dried and then fixed on a plate and converted with gold dust. The pictures were obtained using an REM Hitachi S 2700 (Japan) on the Zentraleinrichtung Elektronenmikroskopie, Technische Universität Berlin (see Kabbert, Kotte, Cech, & Kunzek, 1996). The solid densities of the CWM were determined with a multivolume pycnometer 1305 (Micrometries Instruments Corporations, USA) according to Müller and Kunzek (1998). The bulk densities were measured and the porosity was calculated as described by Kabbert et al. (1996).

2.4. Analytical methods

The galacturonan content and the degree of methoxylation were analysed photometrically by the *m*-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973) after hydrolysis (Förster et al., 2002) and by the chromotropic acid method (Bäuerle, Otterbach, Gierschner, & Baumann, 1977), respectively. The protein content was determined according to the Kjeldahl method ($N \times 6.25$). The ash contents were analysed in a muffle furnace at 525 °C for 2 h. Total, insoluble, and

soluble dietary fibre were analysed by the enzymatic–gravimetric AOAC method (Prosky, Asp, Furda, de Vries, & Schweizer, 1988).

2.5. Hydration properties

For characterisation of water binding properties of the CWM, three methods were applied. The swelling (SW) of the dried CWM in water was measured in terms of the bed volume technique (Kuniak & Marchessault, 1972) and calculated in ml hydrated sample per g dry matter. Prior to measurements, 0.1 g of sample in 25 ml water at 37 °C was evaporated (32 mbar for 2 min) for sedimenting the particles (Vetter et al., 2002). The water retention capacity (WR) was determined using the filtration method (Kunzek, Loewe, & Renger, 1993). The WR of samples was measured as the amount of water retained after 2 h incubation in excess water at 37 °C and subsequent removal the surplus water by gravity. Results were expressed as g water per g dry matter of original substrate. The water uptake (WU) was analysed using the capillary suction method (Baumann, 1967) at 20 °C in the modification of Heinevetter and Kroll (1982). Approximately 10 mg of sample were placed on a glass filter G2 (diameter 4 cm, pores 40–90 μm) on top of a closed chamber filled with water and connected to a graduated capillary (filled with water). The water uptake of the sample was measured gravimetrically.

2.6. Rheological properties

Rheological measurements of the re-hydrated samples were carried out by the oscillation method and by shear flow (concentration: 50 g dry matter/l of distilled water) using a Universal Dynamic Spectrometer UDS 200 (Paar Physica, Germany) according to Kunzek, Opel, and Senge (1997).

2.7. Determination of bile acid binding

CWM (100 mg) was incubated for 2 h at 37 °C with 4 ml of a solution of 0.5 mM glycochenodeoxycholic acid (GCDCA) as sodium salt (Sigma Chemical Co., St. Louis, MO, USA) in Sörensen buffer (pH 5.0). In the control experiment, no CWM was used. Then the aqueous phase was separated by filtration. Filter paper used was not able to interact with BA. The BA-containing filtrates were purified by solid phase extraction on Bakerbond spe C_{18} columns in the BAKER spe-12G system (J.T. Baker, Gross Gerau, Germany).

GCDCA was analysed by HPLC on a non-polar stationary phase (Nucleosil 100 Å; C_{18}; 5 μm; 250×4.6 mm) at 40 °C (Gynkotek, Germering, Germany) after a pre-column derivatization with 4-bromomethyl-7-methoxycoumarin (Sigma) and fluorescence detection (excitation λ 320 nm; emission λ 385 nm) (Wang, Stacey, & Earl, 1990). Linear gradients, consisting of acetonitrile (30–100%), methanol (40–0%) and water (30–0%), were applied.

2.8. Fermentation of CWM in vitro

For simulating fermentation in the large intestine, 10 mg of the CWM were incubated an-aerobically (under N_2) with a 1 ml suspension of fresh human faeces flora (20 g/100 ml 0.1 M phosphate buffer; pH 6.5) with shaking for up to 24 h at 37 °C. Samples were taken under sterile conditions at different times and were immediately frozen. Before analysis of SCFA, samples were stored at −20 °C.

2.9. Determination of short-chain fatty acids

SCFA were determined by a modified method of Brighenti (1997) according to Scheppach, Sachs, Bartram, and Kasper (1989). Thawed sample suspensions were diluted with a fourfold amount of water and homogenised. After centrifugation (5 min at 4 °C and 20,000*g*), *iso*-butyrate (internal standard), perchloric acid solution and sodium hydroxide solution were added to the supernatant. Freeze-dried samples were homogenised in a mixture of 5 M formic acid and water (volume ratio 1:4). One μl of the liquid phase was injected on a HP-FFAP capillary column (30 m × 0.53 mm; 1 μm) for gas-chromatographic analysis, using a temperature programme. The Hewlett–Packard 5890A Series II gas chromatograph was equipped with an HP 7673 GC/SFC Injector, HP GC Auto Sampler Controller, flame ionisation detector and HP Chemstation Software. Helium was used as a carrier. All samples were analysed in duplicate and quantified by external and internal calibration with standards (SCFA standard mix from Sigma–Aldrich, Deisenhofen, Germany). Concentrations were calculated in mmol/g substrate.

2.10. Statistical analysis

All measurements were made at least in duplicate. Results are expressed as mean values and standard deviations (SD). Statistical significance was determined using one-way analysis of variance, followed by Student's *t* test. $P < 0.05$ was taken to indicate a statistically significant difference.

3. Results and discussion

3.1. Particle size, structure and composition

With increasing degree of cell wall degradation, yields of cell wall materials decreased in the order: RM > MC

I > MC II > LF (Table 1). Additionally, yields were dependent on the variety of carrots used: Karotan > Major ($P < 0.025$). The SEM (Fig. 2) and the laser particle analysis (Fig. 3) allowed the characterisation of type and shape of the particles obtained. The RM particles consisted mainly of cell clusters of two and more cells, often with destroyed edges (Vetter et al., 2002; Vetter & Kunzek, 2001). In contrast to RM, the particle size distributions of the other samples were shifted to smaller particles (Fig. 3). Before drying, MC I, MC II and LF had a similar distribution pattern of particles especially in the range 50–200 μm. As shown by SEM, MC I and MC II consisted mainly of single cells, whereas LF contained mainly aggregated cell fragments (Fig. 2). Related results were found by Vetter et al. (2002) and Förster et al. (2002) with CWM prepared from apples. Drying and re-hydration of samples had no influence on the results of laser particle analysis (not shown) indicating reversible state transitions occurring during drying and re-hydration. Only during drying of the LF samples, irreversible aggregation was found, to a certain extent.

In contrast to the other samples, the bulk density of LF was relatively high (e.g., LF Major: 0.25 g/cm^3) (Table 1). The solid density increased with increasing cell wall degradation: RM < MC < LF (higher share of crystalline cellulose network; Förster et al., 2002). The highest porosity was found for MC and the least values for LF. Related results were found for CWM prepared from apples (Förster et al., 2002). In any case, bulk densities of Major samples were higher than those of Karotan samples, and the porosity of the Karotan exceeded that of Major preparations (Table 1).

As expected, the galacturonan content decreased with increasing cell wall degradation: RM > MC I > MC II > LF ($P < 0.001$) (Vetter et al., 2002). It is remarkable that the galacturonan content of RM was higher for Major than for Karotan materials (RM-Major > RM-Karotan). However for MC and LF preparations, the opposite relation was found: Karotan > Major ($P < 0.05$) (Table 1). Thus, with increasing cell wall degradation the loss of pectin was lower in Karotan than in Major samples, indicating higher cell wall stability of Karotan materials. In agreement with this finding, higher yields and higher porosities (see above) were obtained for the Karotan cell wall materials.

Presumably, the stability of the Karotan in comparison to the Major cell wall was favoured by a reduced degree of methoxylation ($P < 0.05$) and an increase in non-soluble ash content (Table 1). In particular, the ash content of LF-Karotan was highest.

3.2. Dietary fibre

The CWM were rich in DF. The total DF content was between 80% and 90% (Table 2). There was no distinct difference between total DF of the reference materials (RM) and the two carrot varieties. In principle, the total DF content increased with increasing enzymatic degradation. It is notable that the RM and MC I preparations from Karotan had relatively low amounts of soluble DF. In CWM from both carrot varieties, the

Table 1
Yield, structural parameters and composition of the dried cell wall materials prepared from carrots of the varieties Karotan and Major

Parameter	Variety	RM	MC I	MC II	LF
Yield (g/100 g mash)	Karotan	2.67	1.84	1.61	0.8
	Major	2.06	1.22	1.41	0.65
Dry matter (%)	Karotan	95.97	96.35	94.97	98.07
	Major	95.45	96.02	95.77	95.81
Solid density (g/cm^3)	Karotan	1.57 ± 0.02	1.63 ± 0.01	1.83 ± 0.07	1.75 ± 0.01
	Major	1.48 ± 0.01	1.69 ± 0.00	1.79 ± 0.01	1.87 ± 0.02
Bulk density (g/cm^3)	Karotan	0.10 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.14 ± 0.00
	Major	0.11 ± 0.00	0.11 ± 0.00	0.10 ± 0.00	0.25 ± 0.00
Porosity (%)	Karotan	93.37	94.89	95.45	92.02
	Major	92.80	93.69	94.39	86.43
Galacturonan content (%)	Karotan	37.3 ± 0.32	25.1 ± 0.01	24.5 ± 0.05	22.6 ± 0.08
	Major	39.9 ± 1.21	23.6 ± 0.47	22.5 ± 0.51	14.3 ± 0.05
Degree of methoxylation (%)	Karotan	45.1 ± 0.28	40.3 ± 0.03	41.0 ± 0.25	40.4 ± 0.29
	Major	48.1 ± 0.13	44.2 ± 0.48	50.3 ± 0.02	37.8 ± 0.10
Protein content (%)	Karotan	2.64 ± 0.00	3.56 ± 0.01	3.49 ± 0.00	3.38 ± 0.00
	Major	2.36 ± 0.00	3.30 ± 0.05	3.51 ± 0.01	4.44 ± 0.04
Ash content (%)	Karotan	3.99 ± 0.02	6.53 ± 0.14	4.46 ± 0.02	11.18 ± 0.05
	Major	4.29 ± 0.01	2.22 ± 0.00	2.26 ± 0.07	6.00 ± 0.05

For abbreviations see Fig. 1.

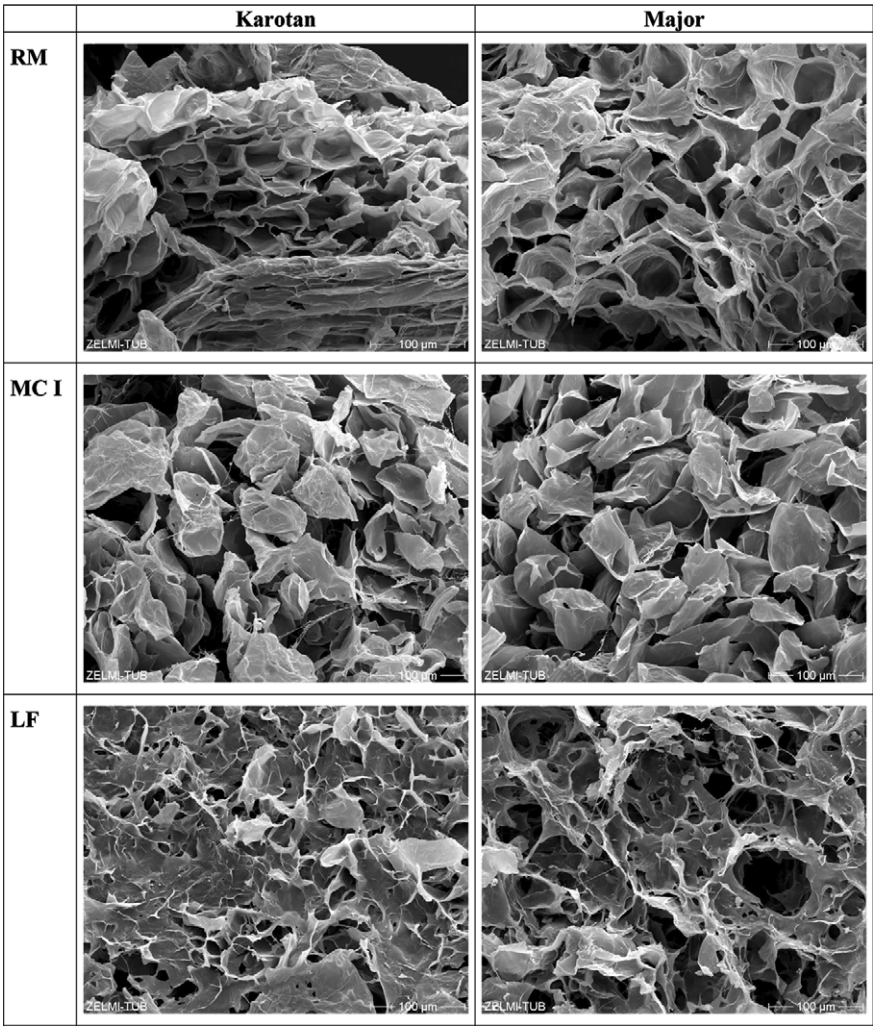


Fig. 2. Scanning electron microscopy of the wet cell wall materials prepared from carrots of the varieties Karotan and Major (before drying). For abbreviations see Fig. 1.

yield in insoluble DF fraction increased from RM to LF. In the case of preparations from Major, the contents of the soluble DF fraction decreased in the same order.

3.3. Hydration properties

For the proper description of the hydration properties, various methods were applied: swelling (Kuniak & Marchessault, 1972; Robertson et al., 2000), water retention capacity (Robertson et al., 2000) determined by filtration (Kunzek et al., 1993), and water uptake using the capillary suction method (Baumann, 1967; Heinevetter & Kroll, 1982; Robertson et al., 2000).

Results of the hydration properties were dependent on the method used. The amount of bound or held water decreased in the following order: $SW > WR > WU$ (Fig. 4) which is in accordance with Vetter and Kunzek (2001) and Robertson et al. (2000). Our values, measured for carrot fibre materials were generally higher than those of other research groups (Bao & Chang, 1994; Bourquin, Titgemeyer, & Fahey, 1993; Robertson et al., 1979). This is probably due to the water–ethanol exchange used during preparation of the materials (Fig. 1) (Kabbert et al., 1996; Kabbert & Kunzek, 1998; Kunzek et al., 2002).

Furthermore, the hydration properties of the CWM were dependent on the degree of the cell wall degradation and on the variety of the carrots used. The cold water-extracted reference material and the macerated materials showed good hydration properties, but LF had strongly reduced water-binding properties ($P < 0.005$; Fig. 4). Excellent hydration properties of the RM were characterised by a partly intact structure of tissue particles with a good firmness and rigidity, a high galacturonan content and a good porosity (Table 1). In contrast to RM, the macerated materials had a dominating single cell structure, reduced galacturonan

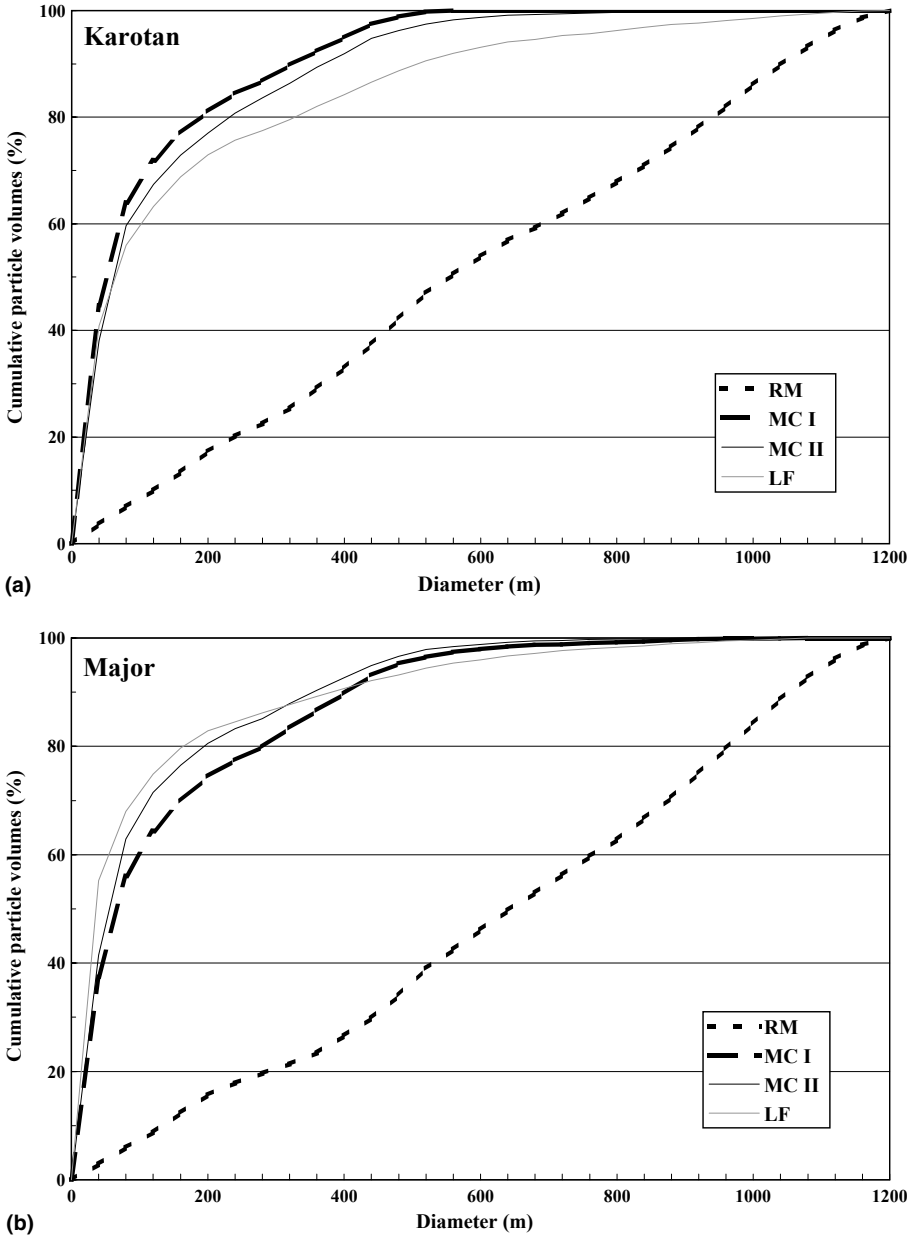


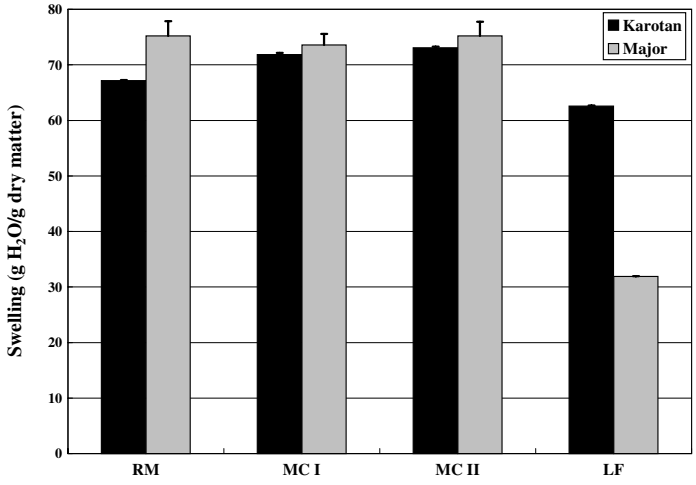
Fig. 3. Curves of the cumulative particle volumes of the cell wall materials prepared from carrots of the varieties Karotan and Major (before drying). For abbreviations see Fig. 1.

Table 2
Dietary fibre (DF) in the cell wall materials (CWM) prepared from carrots

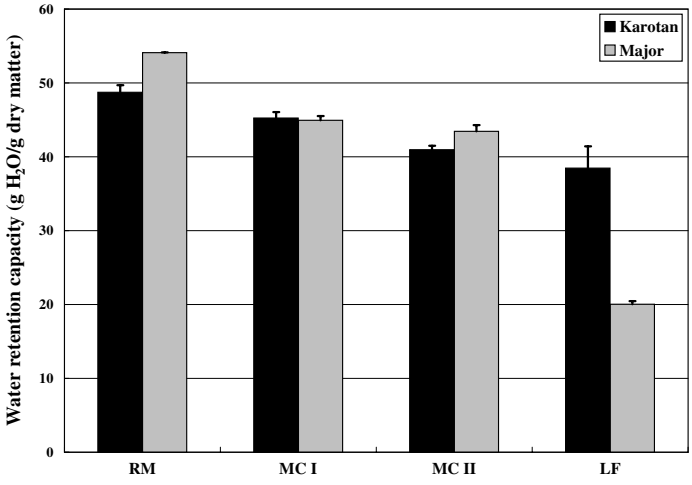
Variety	CWM	Soluble DF (g/100 g)	Insoluble DF (g/100 g)	Total DF (g/100 g)
Karotan	RM	27.4	58.8	82.6
	MC I	27.0	57.6	84.6
	MC II	29.8	59.0	88.8
	LF	24.2	64.4	88.6
Major	RM	32.7	48.0	80.7
	MC I	31.4	53.8	85.2
	MC II	29.8	58.0	87.8
	LF	16.1	70.9	87.0

For abbreviations see Fig. 1.

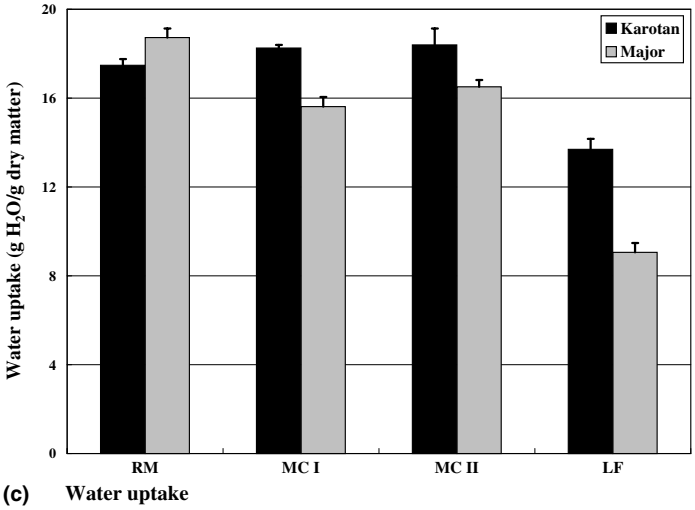
contents, an improved porosity and a softening of the cell wall. These structural features can favour the SW of MC in comparison to RM (in particular for the variety Karotan). The WR of MC was slightly lower than WC of RM but the WU of both materials were comparable (between 16 and 19 g H_2O/g dry matter). Hydration properties of LF were strongly reduced and independent of the method used. In particular, lowest values were found for materials prepared from the variety Major (Fig. 4). Obviously, the hydration properties of the LF were inhibited by shrinkage (reduced porosity) and aggregation of the materials during the drying process.



(a) Swelling



(b) Water retention capacity



(c) Water uptake

Fig. 4. Hydration properties of the dried cell wall materials determined by (a) swelling, (b) water retention capacity and (c) water uptake. For abbreviations see Fig. 1.

The hydration properties of the materials were also markedly influenced by the variety of the carrots used. In all cases, and independent of the hydration method applied, hydration values of RM materials were higher for Major than for Karotan samples. This is probably due to higher galacturonan content and higher degree

of methoxylation of the former samples. With respect to variety, MC materials showed slightly increased SW and partly decreased WU for MC-Major. However, the hydration properties of the LF materials were low, especially for the variety Major as compared to Karotan ($P < 0.05$), independent on the method used. Therefore, it can be assumed that the cell wall of samples of the variety Major was enzymatically degraded to a greater extent than that of Karotan. In agreement with this conclusion, the enzymatic treatment caused a stronger decrease in porosity and galacturonan content for Major than for Karotan materials (Table 1).

3.4. Rheological properties of the re-hydrated materials

The shear flow measurements of the re-hydrated samples gave a decrease of the yield values τ_0 for the variety Karotan in the order RM > (MC I ≈ LF ≈ MC II) and for the variety Major in the order RM > (MC II ≈ MC I) > LF. The oscillation measurements, within the linear viscoelastic region showed that the storage modulus G' of all samples examined was above the loss modulus G'' and increased only slightly with rising frequency (Fig. 5). Thus, all samples showed dominant elastic properties. In dependence on the degree of cell wall degradation and on the variety of carrot used, the

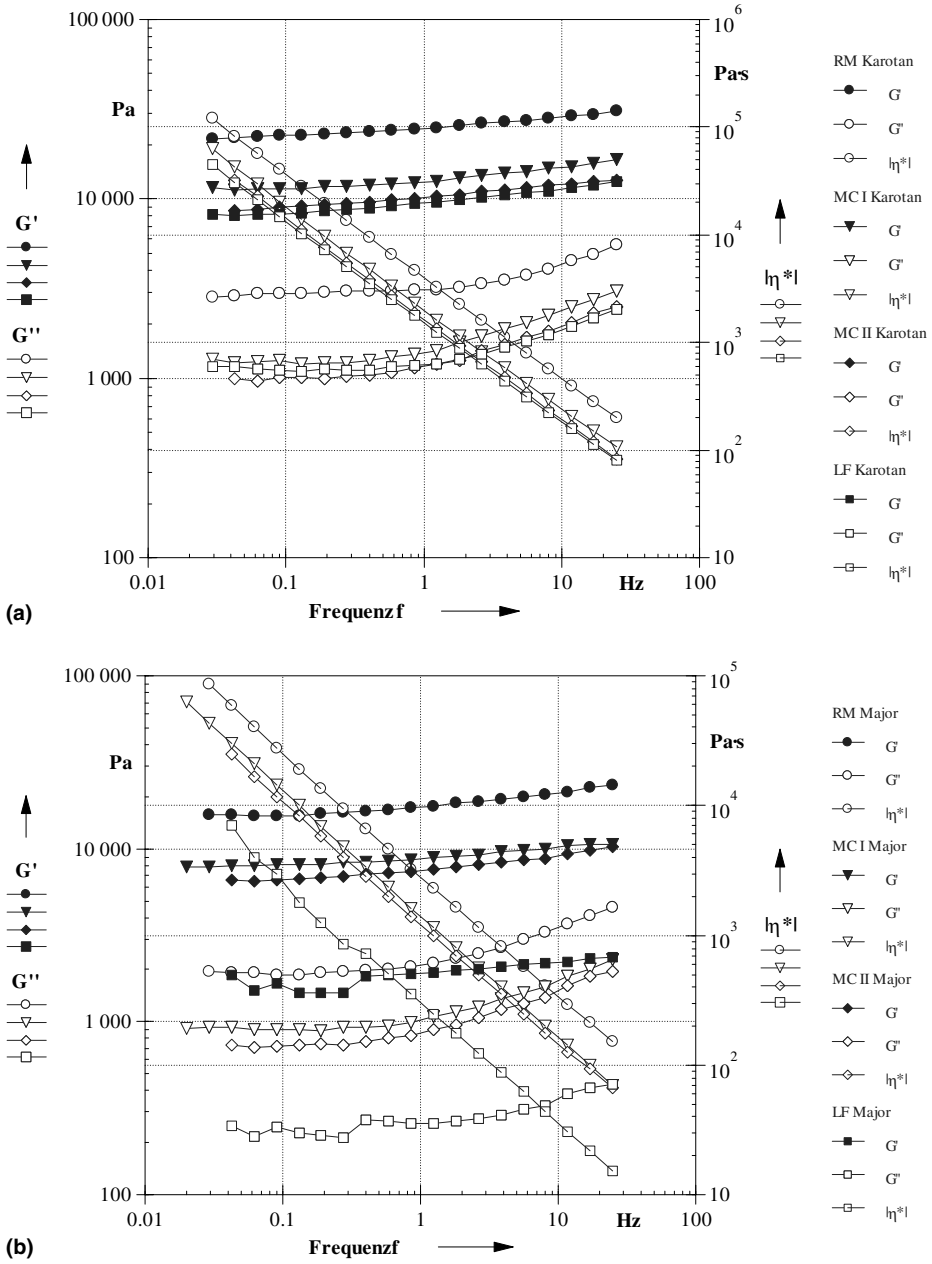


Fig. 5. Frequency sweep of the rehydrated cell wall materials prepared from carrots of the varieties (a) Karotan and (b) Major. Storage modulus G', loss modulus G'' and complex viscosity $|\eta^*|$ versus frequency f. For abbreviations see Fig. 1.

order of the G' values and of the complex viscosity $|\eta^*|$ were comparable with those of the yield values τ_0 (see above): RM > (MC I ⩾ MC II ⩾ LF) (for Karotan) and RM > (MC I ⩾ MC II) > LF (for Major) (Fig. 5). Therefore, it can be assumed that, in any case, the RM suspensions had the best solid properties and the strongest interactions between the swollen particles. In contrast, MC and LF suspensions showed lower structure-viscous and visco-elastic properties. For the Karotan variety, differences between the rheological properties of the MC and LF suspensions were minimal but not for Major suspensions. The lowest values of τ_0, G' and $|\eta^*|$ were found for the LF-Major suspension (Fig. 5), representing no stable dispersed system at the concentration of 50 g/l applied. So, water was separated from the LF-Major suspension very easily under external stress.

For the suspensions investigated, different values for the damping factor $\tan\delta$ were found (Fig. 6). The damping factor is defined as the quotient of G'' and G' and characterises the portion of dissipated energy due to viscous flowing under external stress. In the range 0.1–1 Hz the $\tan\delta$ values were higher for the LF and RM than for the MC suspensions (Fig. 6). The highest $\tan\delta$ value of all samples investigated was measured for the LF-Major suspension (with least structural stability under external stress; see above). Decreasing $\tan\delta$ values were found for Major suspensions in the order LF > RM > MC and for Karotan suspensions in the order (LF ≈ RM) > MC. It is notable that, in every case, the MC suspensions had the best structural stability under external stress (least portion of dissipated energy), although they did not have the highest values for τ_0, G' and $|\eta^*|$ (see above).

The influence of carrot varieties on the rheological properties of CWM suspensions could be clearly proved. In all cases the τ_0 and the G' values were higher for the Karotan than for the Major suspensions. In particular,

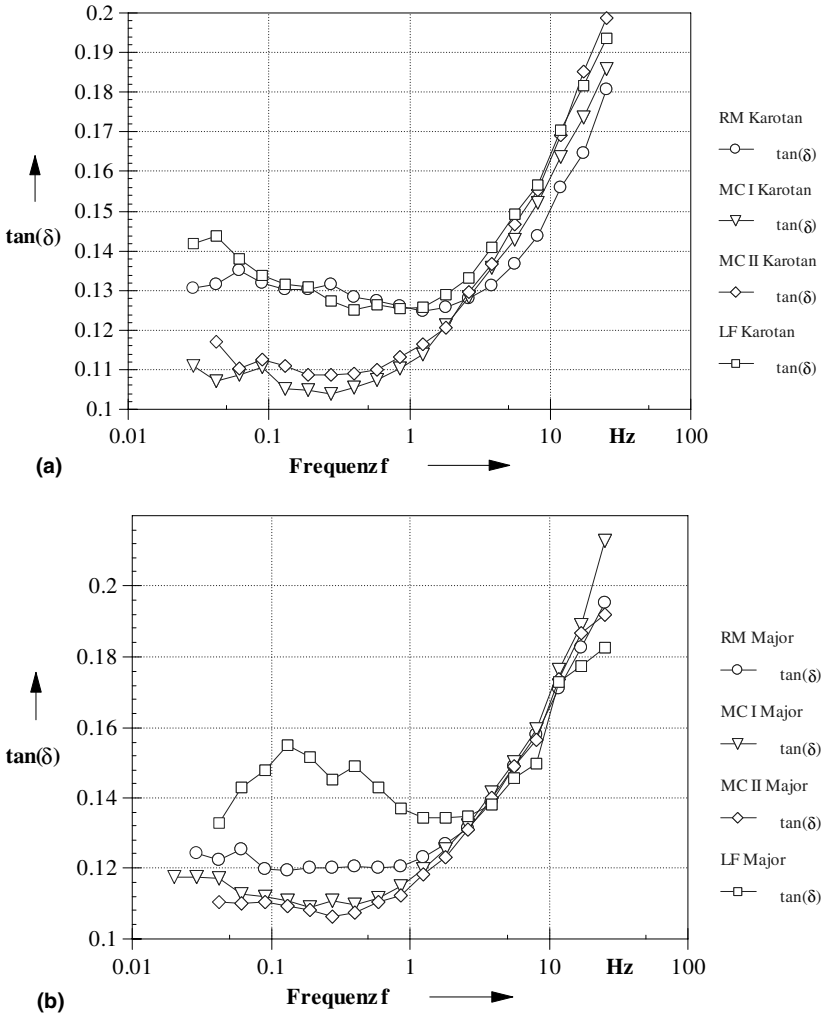


Fig. 6. Frequency sweep of the rehydrated cell wall material prepared from carrots of the varieties (a) Karotan and (b) Major. Damping factor $\tan\delta$ versus frequency f. For abbreviations see Fig. 1.

this finding is valid for the LF suspensions. The structure formation within the suspensions is favoured for Karotan in comparison to Major cell wall materials. This fact results from the high stability of the Karotan cell wall and partly also from the good hydration properties of the Karotan materials (in particular, the LF preparation). The damping factor, $\tan\delta$, of the RM suspensions is higher for the Karotan than for the Major suspension. Thus under external stress, a lower portion of energy is dissipated in the Major suspension, having a higher structural stability. This can be explained by the highest pectin content and the best hydration properties of RM-Major in comparison to all other CWM investigated. In contrast, the damping factor of the LF suspension showed higher values for the Major than for the Karotan sample: LF-Major > LF-Karotan. In this case also, the higher structural stability (LF-Karotan) could result from the higher pectin content and the improved water hydration properties of the used CWM (LF-Karotan > LF-Major).

3.5. Binding of bile acids

As an example of physiological effects in the upper intestinal tract (small intestine), interactions between BA and the prepared CWM were investigated in vitro. We used the bile acid GCDCA, one of the major BA in human bile, for the binding experiments. This primary BA contains hydroxyl groups at the C-atoms 3 and 7 of the steroid nucleus and is conjugated with the amino acid glycine. The experiments were done at pH 5.0, which is typical for gut in the higher part of the small intestine. BA are secreted into the small intestine where they facilitate fat digestion.

All CWM were able to interact with GCDCA under the conditions used (Fig. 7). First, the CWM prepared from Karotan bound the BA to greatest degree than did those made from the variety Major. On the other hand, the intensity of interactions increased with increasing (enzymatic) degradation of carrot material. The least interaction between the CWM and GCDCA occurred with the RM. It is interesting to note that the MC II were able to bind more BA than the MC I preparations. Highest interactions were measured when the liquefied cell wall materials LF were tested. The differences in BA binding between the CWM were significant ($P \geqslant 0.05$). In contrast to our results, Robertson et al. (1980) found no influence of carrot variety on the adsorption of deoxycholic and glycocholic acid to DF, but an effect of development age of the carrots.

As a result of the enzymatic action, cell walls or their fragments were obviously better accessible to absorption of detergent-like molecules, e.g., BA. The binding mechanisms for the interaction between cell wall materials or isolated DF and BA are yet unknown. Model experiments with isolated β-glucan and BA pointed to binding forms other than chemical ones (Bowles, Morgan, Furneau, & Coles, 1996). In the case of isolated pectins, interactions with BA increased with their degree of methylation and were additionally influenced by the arrangement of free and methoxylated carboxyl groups, the mode and degree of derivation, the source of pectin, the fine structure of the BA and the pH value in the system (Dongowski, 1995, 1997). In summary, hydrophobic effects seem to play the major role for the

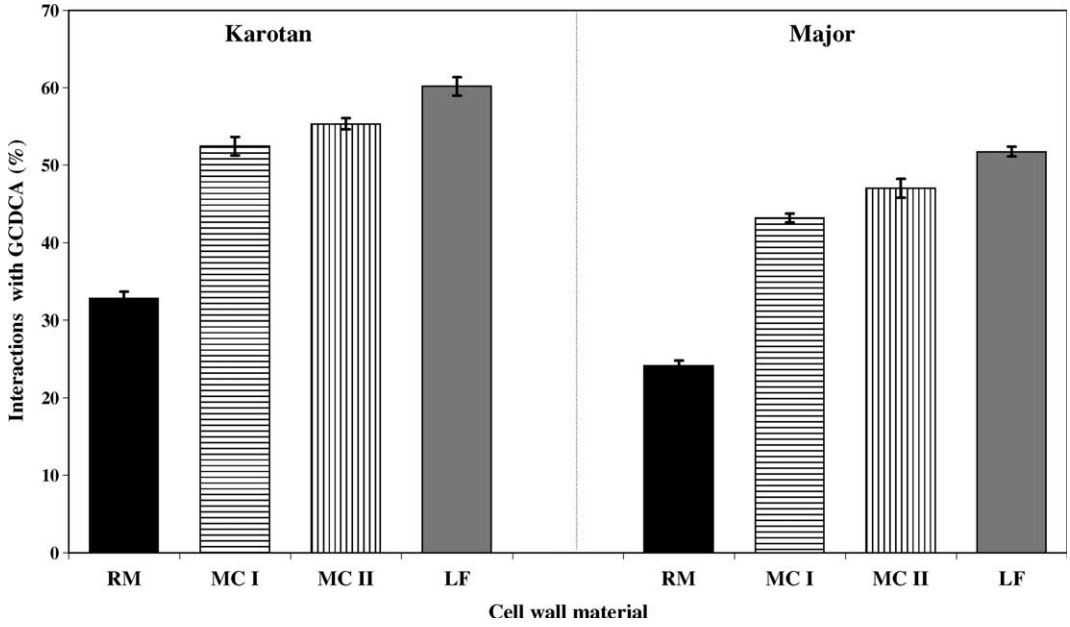


Fig. 7. Interactions between the cell wall materials and glycochenodeoxycholic acid (GCDCA). For abbreviations see Fig. 1.

intensity of the interactions – besides effects due to particle size or surface properties of cell wall preparations, macromolecular properties and structure of isolated DF or conditions in the medium (e.g., viscosity). Mongeau and Brassard (1982) found a correlation between the surface or particle size of cereal DF and BA binding. The interactions were stronger either at lower pH and temperatures or at higher hydrophobicity of the BA (Huang & Dural, 1995). Furthermore, interactions with BA were found with extrudates from whole-grain barley in vitro. This binding was higher with dihydroxy-BA than with trihydroxy-BA (Huth, Dongowski, Gebhardt, & Flamme, 2000). Cell wall preparations (''cellans'') from different sources were also able to bind BA and emulsions, depending on their (over-) structure, the pH in medium and the BA structure (Dongowski & Ehwald, 1999). As a result of binding or interactions between DF and BA in the small intestine, more BA were transported toward the large intestine, where the BA are deconjugated and dehydroxylated by enzymes of the microflora and finally excreted. Higher excretion rate of BA led to higher synthesis of BA from blood cholesterol in the liver. This effect is important in regard to lowering blood cholesterol in hypercholesterolemic individuals or animals (Garcia-Diez, Garcia-Mediavilla, Bayon, & Gonzalez-Gallego, 1996). Whereas Wisker et al. (1994) found no effect of carrot consumption on BA excretion, Robertson et al. (1979) reported an increased BA excretion and reduced serum cholesterol after administering carrots to humans.

3.6. Fermentation of CWM in vitro

To simulate fermentation of DF in the large intestine, in vitro experiments were done with fresh faeces flora and carrot CWM. During fermentation of DF, a spectrum of SCFA is formed. The main fermentation products are acetate, propionate and butyrate.

We used fresh faecal flora samples from two subjects and the following cell wall materials: RM, MA II and LF. Samples were taken after 0, 4, 8 and 24 h, respectively. The total fermentation of the same CWM by the microflora of the two subjects were relatively similar but the composition of the SCFA differed distinctly. The amount of SCFA formed increased with the time of incubation. The enzymatically treated CWM from the variety Major were fermented to a smaller extent than its counterparts (Fig. 8). A reason for this effect might be the lower pectin (and probably hemicellulose) content present in these preparations. It is well known that pectin is fermented faster than the hemicelluloses and especially cellulose (Stevens, Selvendran, Bayliss, & Turner, 1988). Before fermentation takes place, polysaccharides must be split enzymatically by bacterial enzymes to their monosaccharides. These will then be further decomposed by the bacteria via the Emden–Meyerhof–Parnas and pentose phosphate pathways (Macfarlane & Gibson, 1996). The physiologically most important SCFA is butyrate. It is the major energy source for the colonic epithelium, regulates cell growth and differentiation and also plays an important role in

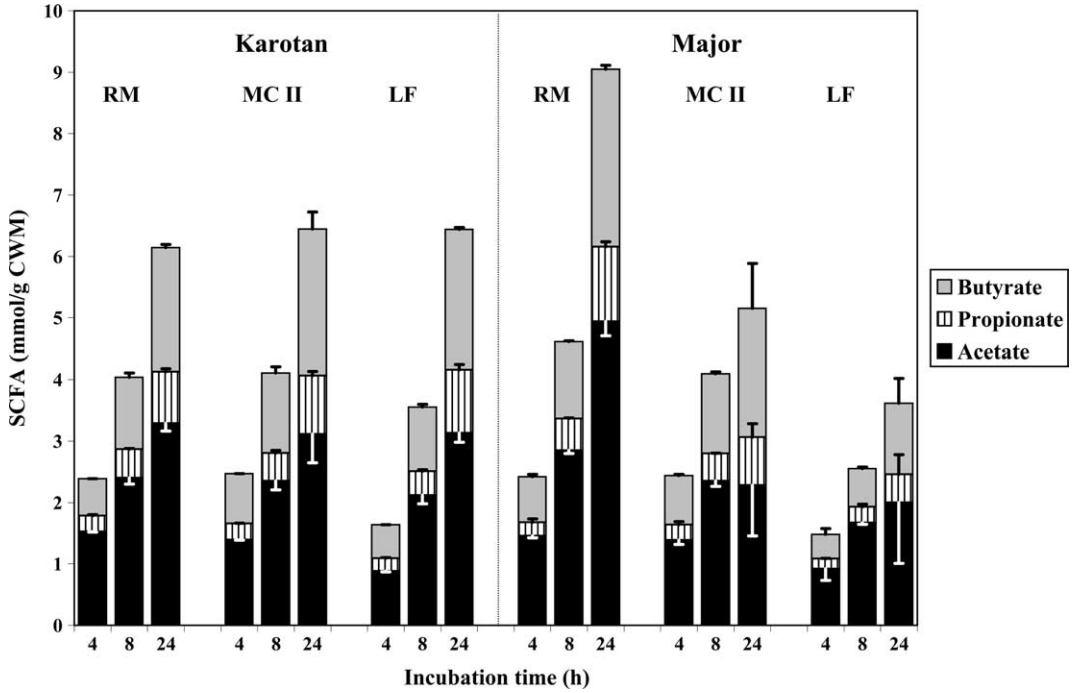


Fig. 8. Molar contents of short-chain fatty acids (SCFA) formed during in vitro fermentation of the cell wall materials. For abbreviations see Fig. 1.

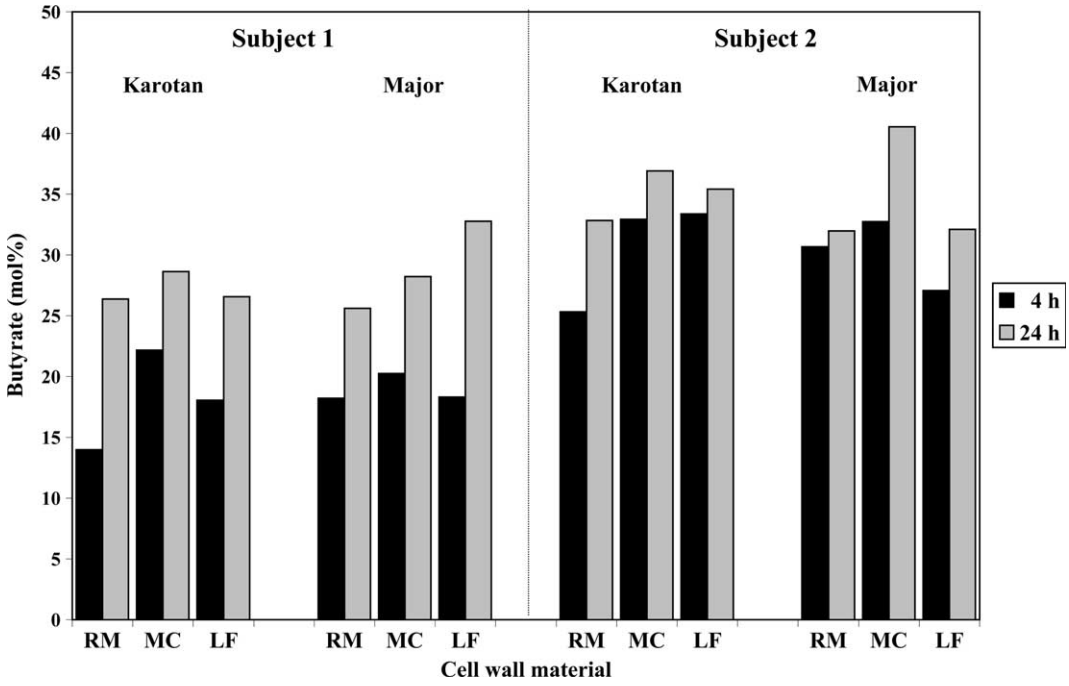


Fig. 9. Molar percentage of butyrate formed during in vitro fermentation of the cell wall materials with faeces flora from two subjects. For abbreviations see Fig. 1.

protection against colon cancer (Jacobasch & Dongowski, 2000; Roediger, 1982; Smith et al., 1998; Topping & Clifton, 2001). Therefore, it is important that the molar proportion of butyrate is increased with the fermentation time (Fig. 9). Additionally, higher butyrate proportions were found when MA II and LF were fermented. The microflora of subject 2 were able to produce higher concentrations and molar proportions of butyrate. In similar experiments with CWM from apples, the liquefied material appeared to be the best source of butyrate during in vitro fermentation (Förster et al., 2002). Bourquin et al. (1993) found a molar ratio of 76:13:11 between acetate, propionate and butyrate during in vitro fermentation of carrot DF. That means lower butyrate concentrations than in our experiments. Fermentability of carrot fibre was high (>90%) in humans (Wisker et al., 1994). Nyman, Asp, Cummings, and Wiggins (1986) found in vivo a faecal recovery of carrot DF of 25% in humans and 29% in rats.

4. Conclusions

It was found that the structure and the properties of the prepared CWM were dependent on the degree of the enzymatic cell wall degradation as well as on the variety of the carrots used. The enzymatic treatments resulted in decreasing yields, reduced galacturonan contents, increasing solid densities as well as reduced values, for the hydration properties and for the structure formation of the re-hydrated samples. Especially our LF materials showed these alterations, to a great extent. However, there are no strongly linear correlations between rising enzymatic cell wall degradation and alterations of structure and properties of the materials. For instance, the MC, consisted mainly of single cells and had a higher porosity, and the re-hydrated materials showed a greater structure stability than the corresponding RM samples.

The enzymatic cell wall degradation was more pronounced in the CWM prepared from carrots of the variety Major than from the Karotan materials. The Karotan cell wall is especially stable against enzymatic degradation due to an enhanced firmness and rigidity. This was demonstrated by a high porosity (reduced shrinkage during drying) and improved hydration properties, as well as structure formation within suspensions of the re-hydrated Karotan, in comparison to the Major samples. Furthermore, the enzymatically treated Karotan materials gave greater amounts of SCFA during in vitro fermentation with fresh human faeces flora, and the interaction with bile acids (GCDCA) was higher than that for the corresponding Major samples.

Altogether, CWM prepared from carrots of the variety Karotan, which was cultivated for industrial processing, showed better functional properties and physiological effects than the CWM from carrots of the variety Major. Therefore, we can say that they can be recommended as health ingredients for the production of functional foods.

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References

Anastasakis, M., Lindamood, J. B., Chism, G. W., & Hansen, P. M. T. (1987). Enzymatic hydrolysis of carrot for extraction of a cloud-stable juice. *Food Hydrocolloids, 1*, 247–261.

Bao, B., & Chang, K. C. (1994). Carrot pulp chemical composition, color, and water-holding capacity as affected by blanching. *Journal of Food Science, 59*, 1159–1161.

Barry, J. L., Hoebler, C., Macfarlane, G. T., Macfarlane, S., Mathers, J. C., Reed, K. A., Mortensen, P. B., Nordgaard, I., Rowland, I. R., & Rumney, C. J. (1995). Estimation of the fermentability of dietary fiber in vitro: A European interlaboratory study. *British Journal of Nutrition, 74*, 303–322.

Bäuerle, D., Otterbach, G., Gierschner, K., & Baumann, G. (1977). Bestimmung des Polyuronidgehaltes und des Veresterungsgrades des Pektinanteiles in Handelspektinpräparaten, Apfelsäften und Apfelmaceraten. *Deutsche Lebensmittel-Rundschau, 73*, 281–286.

Baumann, H. (1967). Apparatur nach Baumann zur Bestimmung der Flüssigkeitsaufnahme von pulverförmigen Substanzen. *Glastechnik und Instrumententechnik - Fachzeitschrift Labor, 11*, 540–542.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*, 484–489.

Bourquin, L. D., Titgemeyer, E. C., & Fahey, G. C. (1993). Vegetable fiber fermentation by human faecal bacteria – cell-wall polysaccharide disappearance and short-chain fatty acid production during in vitro fermentation and water-holding capacity of unfermented residues. *Journal of Nutrition, 123*, 860–869.

Bowles, R. K., Morgan, K. R., Furneau, R. H., & Coles, G. D. (1996). ^{13}C CP/MAS NMR study of the interactions of bile acids with barley β-glucan. *Carbohydrate Polymers, 29*, 7–10.

Brighenti, F. (1997). Simple method for quantitative analysis of short chain fatty acids in serum by gas–liquid chromatography. In F. Guillon, G. Abraham, R. Amadò, H. Andersson, N.-G. Asp, K. E. Bach Knudsen, M. Champ, & J. Robertson (Eds.), *Plant polysaccharides in human nutrition: Structure, function, digestive fate and metabolic effects* (pp. 114–119). Nantes: Institute National de la Recherches Agronomique.

Brunsgaard, G., Kidmose, U., Sorensen, L., Kaack, K., & Eggum, B. (1994). The influence of variety and growth-conditions on the nutritive value of carrots. *Journal of the Science of Food and Agriculture, 65*, 163–170.

Casterline, J. L., Oles, C. J., & Ku, Y. (1997). In vitro fermentation of various food fiber fractions. *Journal of Agricultural and Food Chemistry, 45*, 2463–2467.

Cummings, J. H. & Frølich, W. (1993). *Dietary fibre intake in Europe. A survey conducted by the members of the management committee of COST 92*. Luxemburg: Commission of the European Communities.

Dongowski, G. (1995). Influence of pectin structure on the interaction with bile acids under in vitro conditions. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 201*, 390–398.

Dongowski, G. (1997). Effect of pH on the in vitro interactions between bile acids and pectin. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 205*, 185–192.

Dongowski, G., & Bock, W. (1977). Enzymatische Verflüssigung von Speisemöhren. *Lebensmittel-Industrie, 24*, 33–39.

Dongowski, G., & Ehwald, R. (1999). Binding of water, oil and bile acids to dietary fibers of the cellan type. *Biotechnology Progress, 15*, 250–258.

Dongowski, G., & Lorenz, A. (1998). Unsaturated oligogalacturonic acids are generated by in vitro fermentation of pectin with human faeces flora. *Carbohydrate Research, 314*, 237–244.

Dongowski, G., Schnorrenberger, B., Plätzer, M., Schwarz, M., & Neubert, R. (1997). Interactions between food components and drugs. Part 5: Effect of acetylation and amidation of pectin on the interaction with drugs. *International Journal of Pharmacy, 158*, 99–107.

Förster, S., Dongowski, G., & Kunzek, H. (2002). Structure, physicochemical properties and in vitro fermentation of enzymatically degraded cell wall materials from apples. *Nahrung/Food, 46*, 158–166.

Garcia-Diez, F., Garcia-Mediavilla, V., Bayon, J. E., & Gonzalez-Gallego, J. (1996). Pectin feeding influences fecal bile acid excretion, hepatic bile acid synthesis and serum cholesterol in rats. *Journal of Nutrition, 126*, 1766–1771.

Grampp, E. (1969). Die Verwendung von Enzymen in der Lebensmittelindustrie. *Deutsche Lebensmittel-Rundschau, 65*, 343–347.

Guillon, F., & Champ, M. (2000). Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International, 33*, 233–245.

Guillon, F., Renard, C. M. G. C., Hospers, J., Thibault, J.-F., & Barry, J.-L. (1995). Characterisation of residual fibres from fermentation of pea and apple fibres by human faecal bacteria. *Journal of the Science of Food and Agriculture, 68*, 521–525.

Heinevetter, L., & Kroll, J. (1982). Zur Bestimmung der Wasserbindung pulverförmiger quellfähiger Substanzen mittels einer Kapillarsaugmethode. *Nahrung, 26*, K17–K18.

Horváth-Mosonyi, M., Rigó, J., & Hegedüs-Völgyesi, E. (1983). Study of dietary fibre content and fibre components of carrots. *Acta Alimentaria, 12*, 199–210.

Huang, C.-M., & Dural, N. H. (1995). Adsorption of bile acids to several type food fiber. *Journal of Food Process Engineering, 18*, 243–266.

Huth, M., Dongowski, G., Gebhardt, E., & Flamme, W. (2000). Functional properties of dietary fibre enriched extrudates from barley. *Journal of Cereal Science, 32*, 115–128.

Jacobasch, G., & Dongowski, G. (2000). Ballaststoffe mit spezifischen Wirkungen. In H. F. Erbersdobler & A. H. Meyer (Eds.), *Praxishandbuch functional foods* (pp. 1–34). Hamburg: Behr's Verlag.

Kabbert, R., Goworek, S., & Kunzek, H. (1997). Preparation of single-cell material from enzymatically disintegrated apple tissue - changes in structure and functional properties. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 205*, 380–387.

Kabbert, R., Kotte, K., Cech, B., & Kunzek, H. (1996). Einfluß von Ethanol auf den Trocknungsverlauf und die Makrostruktur eines Einzel-Zellmaterials aus Apfelparenchym (Sorte Granny Smith). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 203*, 512–516.

Kabbert, R., & Kunzek, H. (1995). Charakterisierung der Größenverteilung von Einzel-Zellmaterial mit der Laser-Partikel-Analyse. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 201*, 387–389.

Kabbert, R., & Kunzek, H. (1998). Effects of processing on structure and physico-chemical properties of cell wall material (material with cellular structure). *Polish Journal of Food and Nutrition Sciences, 7/48*(25), 43(S)–48(S).

Kritchevsky, D., & Bonfield, C. (1995). *Dietary fiber in health and disease*. St. Paul: Eagan Press.

Kuniak, L., & Marchessault, R. (1972). Study of cross-linking reaction between epichlorhydrine and starch. *Starch/Stärke, 24*, 110–116.

Kunzek, H., Kabbert, R., & Gloyna, D. (1999). Aspects of material science in food processing: Changes in plant cell walls of fruits and vegetables. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 208*, 233–250.

Kunzek, H., Loewe, B., & Renger, S. (1993). Herstellung und Charakterisierung von zellstrukturiertem Apfelmaterial mit unterschiedlichem Pectingehalt. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 197*, 353–357.

Kunzek, H., Müller, S., Vetter, S., & Godeck, R. (2002). The significance of physico-chemical properties of plant cell wall materials for the development of innovative food products. *European Food Research and Technology, 214*, 361–376.

Kunzek, H., Opel, H., & Senge, B. (1997). Rheological examination of material with cellular structure. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 205*, 193–203.

Macfarlane, G. T., & Gibson, G. R. (1996). Carbohydrate fermentation, energy transduction and gas metabolism in the human large intestine. In R. I. M. Mackie & B. A. White (Eds.), *Gastrointestinal ecosystems and fermentations: Vol. 1. Gastrointestinal microbiology* (pp. 269–318). New York: Chapman and Hall.

Massiot, P., Guiller, I., Baron, A., & Drilleau, J.-F. (1992). Cell wall polysaccharides modification during heat treatment and enzymatic degradation of carrot tissue. *Lebensmittel-Wissenschaft und -Technologie, 25*, 559–563.

McCleary, B. V., & Prosky, L. (2001). *Advanced dietary fibre technology*. Oxford: Blackwell Science.

McBurney, M. I., & Thompson, L. U. (1990). Fermentative characteristics of cereal brans and vegetable fibers. *Nutrition and Cancer, 13*, 271–280.

Mongeau, R., & Brassard, R. (1982). Insoluble dietary fiber from breakfast cereals and brans: Bile salt binding and water-holding capacity in relation to particle size. *Cereal Chemistry, 59*, 413–417.

Müller, S., & Kunzek, H. (1998). Material properties of processed fruit and vegetables. I. Effect of extraction and thermal treatment on apple parenchyma. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 206*, 264–272.

Nyman, M., Asp, N.-G., Cummings, J., & Wiggins, H. (1986). Fermentability of dietary fibre in the intestinal tract: Comparison between man and rat. *British Journal of Nutrition, 55*, 487–496.

Prosky, L., Asp, N.-G., Furda, I., de Vries, J. W., & Schweizer, T. F. (1988). Determination of insoluble, soluble and total dietary fiber in foods, and foods products: Interlaboratory study. *Journal of the Association of Official Analytical Chemists, 71*, 1017–1023.

Robertson, J., Brydon, W. G., Tadesse, K., Wenham, P., Walls, A., & Eastwood, M. A. (1979). The effect of raw carrot on serum lipids and colon function. *American Journal of Clinical Nutrition, 32*, 1889–1892.

Robertson, J. A., De Monredon, F. D., Dysseler, P., Guillon, F., Amadó, R., & Thibault, J.-F. (2000). Hydration properties of dietary fibre and resistant starch: A European collaborative study. *Lebensmittel-Wissenschaft und -Technologie, 33*, 72–79.

Robertson, J. A., Eastwood, M. A., & Yeoman, M. M. (1979). An investigation into the dietary fibre content of named varieties of carrot at different development ages. *Journal of the Science of Food and Agriculture, 30*, 388–394.

Robertson, J. A., Eastwood, M. A., & Yeoman, M. M. (1980). Bile salt adsorption of dietary fiber from named varieties of carrot at different development ages. *Journal of Nutrition, 110*, 1130–1137.

Roediger, W. E. W. (1982). Utilization of nutrients by isolated cells of the rat colon. *Gastroenterology, 83*, 424–429.

Roland, N., Nugonbaudon, L., Andrieux, C., & Szylit, O. (1995). Comparative study of the fermentative characteristics of inulin and different types of fiber in rats inoculated with a whole fecal flora. *British Journal of Nutrition, 74*, 239–249.

Ryden, P., & Robertson, J. A. (1995). The consequences of fruit and vegetable fiber fermentation on their binding capacity for MeIQx and the effects of soluble fiber sources on the binding affinity of wheat bran preparations. *Carcinogenesis, 16*, 1711–1716.

Scheppach, W., Sachs, M., Bartram, P., & Kasper, H. (1989). Faecal short-chain fatty acids after colonic surgery. *European Journal of Clinical Nutrition, 43*, 21–25.

Smith, J. G., Yokoyama, W. H., & German, J. B. (1998). Butyric acid from the diet: Actions at the level of gene expression. *Critical Reviews in Food Science, 38*, 259–297.

Stevens, B. J. H., Selvendran, R. R., Bayliss, C. E., & Turner, T. (1988). Degradation of cell wall material of apple and wheat bran by human faecal bacteria in vitro. *Journal of the Science of Food and Agriculture, 44*, 155–166.

Swanson, K. S., Grieshop, C. M., Clapper, G. M., Shields, R. G., Belay, T., Merchen, N. R., & Fahey, G. C. (2001). Fruit and vegetable fiber fermentation by gut microflora from canines. *Journal of Animal Science, 79*, 919–926.

Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic functions: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews, 81*, 1031–1064.

Vetter, S., & Kunzek, H. (2002). Material properties of processed fruit and vegetables. II. water hydration properties of cell wall materials from apples. *European Food Research and Technology, 214*, 43–51.

Vetter, S., Kunzek, H., & Senge, G. (2001). The influence of the pretreatment of apple cell wall samples on their functional properties. *European Food Research and Technology, 212*, 630–635.

Wang, G., Stacey, N. H., & Earl, J. (1990). Determination of individual bile acids in serum by high performance liquid chromatography. *Biomedical Chromatography, 4*, 136–140.

Wisker, E., Schweizer, T. F., Daniel, M., & Feldheim, W. (1994). Fiber-mediated physiological effects of raw and processed carrots in humans. *British Journal of Nutrition, 72*, 579–599.

Zetelaki-Horvát, K., & Gátai, K. (1977). Disintegration of vegetable tissues by endo-polygalacturonase. *Acta Alimentaria, 6*, 227–240.