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Carbohydrate Polymers 59 (2005) 247–255
Carbohydrate Polymers
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# Influence of structure on in vitro fermentability of commercial pectins and partially hydrolysed pectin preparations

Marianna Gulfi, Eva Arrigoni[1], Renato Amadò[2,*]

*Swiss Federal Institute of Technology (ETH), Institute of Food Science and Nutrition, ETH-Zentrum, Schmelzbergstrasse 9, CH-8092 Zurich, Switzerland*

Received 12 May 2004; accepted 28 September 2004
Available online 2 November 2004

**Abstract**

Pectins are of interest for nutritionists as dietary fibres, as they have been shown to exhibit a hypocholesterolemic effect and to be protective against cancer. The aim of the present study was to investigate the physiological behaviour of pectin preparations differing in composition and degree of polymerisation by a well established in vitro fermentation method and to correlate their fermentation pattern to chemical and physical features. The analysed samples exhibited some chemical and physical differences. The in vitro fermentation experiments indicated a very good fermentability for all substrates. Small differences in the degradability by colonic microorganisms were only found depending on the degree of methoxylation. Partially hydrolysed pectins showed a similar behaviour as the corresponding starting materials, therefore higher amounts could be added to foods without causing gelling, and health effects due to colonic fermentation would be enhanced. Further experiments will be carried out to identify other factors influencing the fermentability of pectins.


*Keywords:* Pectin; Chemical composition; Degree of methoxylation; Partial hydrolysis; In vitro fermentability

## 1. Introduction

Pectin is an important component of the plant cell wall. It is present in the highest concentration in the middle lamella, where it acts as a cementing substance between adjacent cells. Pectin is a complex polysaccharide being polymolecular, polydisperse and heterogenic with respect to chemical composition and structure. It consists of smooth regions of α-D-(1,4)-galacturonic acid and of hairy regions, where neutral sugar side chains are attached to a backbone of rhamnogalacturonan (RG) (Schols & Voragen, 1996). Of the two types of RG existing, RG I contains arabinan, galactan and arabinogalactan side chains (Carpita & Gibeaut, 1993), whereas in RG II side chains different characteristic monosaccharides are present, e.g. apiose (Vidal et al., 2000).

Pectin is used in the food industry as a natural ingredient due to its ability to form gels at low concentrations and to increase the viscosity of liquid foods. It is also applied as a stabilizer in acid milk products and as a fat mimetic (Voragen, Plinik, Thibault, Axelos, & Renard, 1995). Although pectin is commonly present in most plant tissues, for the commercial manufacture only limited sources are available. Apples and citrus fruits are the main sources of commercial pectin at present (Thakur, Rakesh, & Handa, 1997).

Beside its technological function, pectin is also of interest for nutritionists as dietary fibre. Several experiments on rats as well as on humans have clearly demonstrated a reduction of serum cholesterol after oral intake of pectin (Anderson, Jones, & Riddel-Mason, 1994; Arjamandi, Craig, Nathani, & Reeves, 1992; Cerda, Robbins, Burgin, Baumgartner, & Rice, 1988; Judd & Truswell, 1982). Different mechanisms have been proposed for the cholesterol lowering effect. The gel forming capacity of pectin has been found to be of importance to prevent the absorption of cholesterol and fat, and possibly to slow down lipolytic activity (Judd & Truswell, 1985).

* Corresponding author. Tel.: +41 1 632 32 91; fax: +41 1 632 1123.
*E-mail addresses:* marianna.gulfi@ilw.agrl.ethz.ch (M. Gulfi), eva.arrigoni@ilw.agrl.ethz.ch (E. Arrigoni), renato.amado@ilw.agrl.ethz.ch (R. Amadò).
[1] Tel.: +41 1 632 3374; fax: +41 1 632 1123.
[2] Tel.: +41 1 632 3291; fax: +41 1 632 1123.

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doi:10.1016/j.carbpol.2004.09.018

Ahrens, Hagemeister, Pfeuffer, and Barth (1986) concluded that the hypocholesterolemic effect was mainly due to the sequestration of bile acids and cholesterol in the small intestine. Komai and Kimura (1987) found an increase in the daily bile acid excretion and a change in their profile in mice. These effects were more marked in conventional than in germ free rats, suggesting that colonic fermentation of pectin may also play a role in the balance of the bile acids.

Different kinds of pectin polysaccharides, e.g. low and high methoxyl (LM resp. HM), low and high molecular weight (MW) pectins, have been investigated on their influence on cholesterol and fat in blood and liver. A dose-dependent hypocholesterolemic effect in rats for LM and HM pectin was observed, where HM pectin seemed to be more efficient at lower dose (Ahrens et al., 1986). In man, however, HM and LM pectins were found to influence blood and faecal lipids to similar extent (Judd & Truswell, 1982). Yamaguchi, Shimizu, and Hatanaka (1994) fed rats with a cholesterol diet enriched with a HM citrus pectin and with a corresponding enzymatically degraded polysaccharide. A significant and similar decrease in liver cholesterol was measured with both preparations, but no changes in the total serum cholesterol were assessed compared to the control group.

Pectin is also thought to be protective against colon cancer. A main role is probably played by the short-chain fatty acids (SCFA) released by the fermentation of the polysaccharide in the colon. Acetate, propionate and butyrate favour the proliferation of normal crypt cells (Scheppach, Bartram, & Richter, 1995), but only propionate and butyrate seem to induce terminal differentiation or apoptosis of cancer cells (Gamet, Daviaud, Denis-Pouxviel, Remesy, & Murat, 1992; Hague et al., 1993). Olano-Martin, Rimbach, Gibson, and Rastall (2003) demonstrated that HM and LM pectins, as well as oligosaccharide preparations obtained from these, induce apoptosis of human colonic adenocarcinoma HT29 in vitro. The largest effect was observed for the oligosaccharide preparation from LM pectin.

Another positive aspect is that fermentable fibres can bind bile acids, and the pH reduction due to their fermentation also causes a precipitation of bile acids, which can be cytotoxic (Rafter, Haza-Duaso, & Glinghammer, 1988).

The aim of this study was to investigate the influence of physical and chemical features of commercial pectins on their behaviour in the colon. For that purpose, preparations of different origin and varying in degree of methoxylation and viscosity were characterised and subjected to a well established in vitro fermentation procedure. Pectin preparations of low viscosity are particularly of interest as they could be added to food in higher amounts to increase its dietary fibre content, without causing gelling or changing the food texture in a critical way.

## 2. Materials

All samples were obtained from Obipektin AG, Bischofszell, CH. They were prepared from apple pomace and citrus peel, respectively, with hot acidic extraction, a commonly used method for commercial pectins. The ULV (ultra low viscosity) preparations were obtained by partial enzymatic hydrolysis. LM and HM pectins were available, as well as pectic acid from citrus fruits (non-methoxylated). For every pectin type a ULV sample was obtained, for the HM citrus pectin two of them differing in viscosity.

## 3. Methods

### 3.1. Chemical characterisation

The degree of methoxylation (DM) and the degree of acetylation (DA) were determined by HPLC according to a method published by Voragen, Schols, and Pilnik (1986), with some modifications. The saponification was performed with sodium hydroxide 2 M and isopropanol 5:1 (v/v) and the solutions were neutralised with hydrochloric acid prior to injection. The peaks were quantified by the external standard method.

The uronic acid (UA) content was determined photometrically according to the method of Blumenkrantz and Asboe-Hansen (1973) and expressed as polygalacturonic acid content. A calibration curve with galacturonic acid was used.

Neutral sugars (NS) were determined quantitatively as alditol acetates by GLC–FID after hydrolysis in sulphuric acid 1 M and derivatisation according to the method described by Blakeney, Harris, Henry, and Stone (1983) and performed as proposed by Strasser (1998).

The substitution pattern of neutral sugar and uronic acid residues was determined by methylation analysis after carbodiimide-activated reduction of the uronic acids. The carbodiimide-activated reduction was performed according to the method of Kim and Carpita (1992) as modified by Wechsler (1997). An additional modification was applied to the dialysis step, in order to limit the loss of small pectin fractions: dialysis tubes with cut off 4–6 kDa (Carl Roth GmbH, Karlsruhe, D) were used. Methylation analysis was performed as described by Lutz, Oechslin, and Amadò (2003). Identification of the peaks by GC–MS was done using the database recorded by Wechsler (1997) and relative retention times. Quantification was achieved by GLC–FID with consideration of calculated effective carbon response-factors and expressed as mol%-distribution of the total of identified sugar derivatives.

To model the structure, the following calculations were done. The amount of polygalacturonic acid (PG) in mol% comprises the PG present in homogalacturonan as well as in rhamnogalacturonan regions. The size of rhamnogalacturonan (RG) type I regions is calculated as twice the amount of total rhamnose (mol%), considering the fact that rhamnose alternates to galacturonic acid residues in RG I. The degree of branching (DB) is calculated as the ratio of ramified rhamnose to total rhamnose. For galactan side

chains (GalSC) terminal, 1,4- and 1,4,6-β-D-galactose were considered as constituent monomers. For arabinogalactans type II (AGSC) terminal and 1,3-α-L-arabinose and terminal, 1,3-, 1,3,6- and 1,6-β-D-galactose were taken into account (Dey & Brinson, 1984).

### 3.2. Physical characterisation

Molecular weight (MW) and intrinsic viscosity (IV) were assessed with high performance size exclusion chromatography (HPSEC) combined with viscometer, light scattering and refractometer (Viscotek Triple Detection System TDA 302, Viscotek, Houston, USA). Pectin powder was dissolved in sodium nitrate 0.05 M and heated to 70 °C for 10 min. After filtration through a 0.2 μm membrane of regenerated cellulose (Titan Syringe Filters, Scientific Resources, Inc., Eatonwon, USA), the solutions were injected on a Ultrahydrogel Linear column (Waters Corp., Milford, USA) and eluted at 40 °C with sodium nitrate 0.05 M at 0.7 ml/min. Data was evaluated by the provided software (OmniSEC, Viscotek) with the method of the universal calibration, thus using only the data of refractometer and viscometer (Haney, 1985). Calibration was done with pullulan standards (Shodex Standard P-82, Showa Denko K.K., Kawasaki, Japan) with known MW.

### 3.3. In vitro fermentation

A batch technique as described by Lebet, Arrigoni, and Amadò (1998) was used. Minor modifications were applied. The substrates of about 100 mg (pectin powder or lactulose as control substrate) were hydrated over night at 4 °C under continuous shaking with 8 ml fermentation buffer under reducing and anaerobic conditions.

Fresh human faeces from three healthy volunteers were collected, mixed with three parts of buffer (w/w), homogenised, filtered through a 0.5 mm sieve and added as inoculum (2 ml) to the hydrated substrates. All samples were incubated at 37 °C in a shaking water bath. Duplicates were taken after 0, 1, 2, 3, 4, 6, 8 and 24 h. Sampling of lactulose and of blank samples (inoculum and buffer only) was done after 0, 2, 4, 6, 8, 24 h ($n=1$). Selected parameters were measured to follow fermentation kinetics. Overpressure was measured to calculate total gas production. Hydrogen concentration was assessed in a headspace aliquot. After stopping bacterial fermentation by adding 0.1 ml of saturated $HgCl_2$ solution, pH was measured and fermentation samples were centrifugated. An aliquot of the supernatant was taken to quantify SCFA and the remainder was stored at −28 °C. The centrifugation residue was freeze-dried. To follow substrate disappearance uronic acid and total neutral sugars were measured using an automated segment flow analyser (Skalar Analytical B.V., Breda, NL), in both supernatants and dried residues. A pre-hydrolysis according to Ahmed and Labavitch (1977) was performed only for the solid residues. Uronic acids were determined by the *m*-hydroxydiphenyl assay (Thibault, 1979) and neutral sugars by the anthrone assay (Bailey, 1958). Calibration curves were obtained with galacturonic acid and glucose. Results are given as the sum of anhydro uronic acids and anhydro neutral sugars.

## 4. Results

### 4.1. Chemical characterisation

The pectin samples were characterised for their composition (Table 1). All samples had a high uronic acid content of about 70 g/100 g, except for both apple HM pectins which contained 50–60 g/100 g uronic acid. The neutral sugar content was higher in these two samples than in the others, reaching total values of 18–20 g/100 g. HM pectins had DM higher than 50%, LM pectins lower than 40% and pectic acid samples lower than 1%. The partial hydrolysis to ULV pectins seemed to elevate the DM slightly. This may be due to the loss of unmethylated uronic acid oligomers. DA were low in general (<2%). Galactose was found to be the most abundant neutral sugar, followed by arabinose. Rhamnose, xylose, and very small amounts of fucose were found as well (not shown). A slight decrease in the arabinose and galactose content was observed from starting materials to ULV samples, except for apple HM

Table 1
Uronic acid content (UA), degree of methoxylation (DM) and of acetylation (DA), and neutral sugar (NS) content of pectin samples ($n=2;3$)

| | UA (g/100 g) | DM (%) | DA (%) | Galactose (g/100 g) | Arabinose (g/100 g) | Total NS (g/100 g) |
|---|---|---|---|---|---|---|
| Apple HM | 51 | 56 | 1.5 | 7.3 | 1.6 | 20.0 |
| Apple HM ULV | 59 | 63 | 1.9 | 7.8 | 1.2 | 17.6 |
| Apple LM | 70 | 36 | 0.1 | 4.6 | 0.2 | 10.0[a] |
| Apple LM ULV | 68[a] | 40 | 0.1 | 3.8 | 0.1 | 8.6 |
| Pectic acid | 71 | 1 | 0 | 3.9 | 0.5 | 6.3 |
| Pectic acid ULV | 72 | 0 | 0.1 | 2.9 | 0.4 | 5.0 |
| Citrus HM | 76 | 58 | 0.7 | 5.3 | 0.7 | 8.4 |
| Citrus HM ULV 1 | 74 | 64 | 0.6 | 3.1 | 0.4 | 5.1 |
| Citrus HM ULV 2 | 72 | 69 | 0.7 | 3.6 | 0.5 | 6.1 |

SD<7%.
[a] SD<10%.

Table 2
Degree of branching (DB), proportion of polygalacturonic acid (PG) and rhamnogalacturonan (RG) and average size of galactan (GalSC) and arabinogalactan (AGSC) side chains (for calculations, see methods) of pectin samples ($n=2$)

| | DB (–) | PG (mol%) | RG (mol%) | Average size[a] | |
|---|---|---|---|---|---|
| | | | | GalSC | AGSC |
| Apple HM | 0.37 | 72 | 7 | 10 | 7 |
| Apple HM ULV | 0.39 | 63 | 10 | 12 | 5 |
| Apple LM | 0.30 | 78 | 6 | 10–11 | 6 |
| Apple LM ULV | 0.34 | 78 | 7–8 | 5 | 6 |
| Pectic acid | 0.33 | 76 | 8 | 9 | 8 |
| Pectic acid ULV | 0.30 | 78 | 6 | 11 | 6–7 |
| Citrus HM | 0.28 | 84 | 6 | 8–10 | 7 |
| Citrus HM ULV 1 | 0.30 | 80 | 5 | 6–7 | 5 |
| Citrus HM ULV 2 | 0.29 | 83 | 6 | 4–6 | 3–4 |

[a] Number of monomeric units.

Table 3
Molecular weight ($M_w$, $M_p$) and intrinsic viscosity ($IV_w$) of pectin samples ($n=3;4$)

| | $M_w$ (kDa) | $M_p$ (kDa) | $IV_w$ (dl/g) |
|---|---|---|---|
| Apple HM | 123 | 57 | 3.6 |
| Apple HM ULV | 83 | 39 | 1.5 |
| Apple LM | 190[a] | 73 | 2.2[a] |
| Apple LM ULV | 64 | 20[a] | 0.4[b] |
| Pectic acid | 65 | 34 | 1.1 |
| Pectic acid ULV | 23 | 35 | 0.3 |
| Citrus HM | 107 | 52 | 3.7 |
| Citrus HM ULV 1 | 68 | 45 | 2.8 |
| Citrus HM ULV 2 | 41 | 29 | 1.7 |

SD < 10%.
[a] SD < 18%.
[b] SD = 29%.

samples which were produced from different batches. This may be a consequence of the enzymatic hydrolysis.

With methylation analysis sugar monomers and their linkages were identified. Characteristic values which may lead to a better understanding of the molecular structure were calculated (Table 2). RG type I as well as galactan and arabinogalactan type II side chains of different sizes were identified. The higher neutral sugar content of apple HM and apple HM ULV was the result of a higher DB, larger RG I regions and longer side chains, and was confirmed by lower values for PG. In general, there were only small differences in the structural features of the samples. A tendential shortening of the neutral side chains from starting material to ULV samples was observed for apple LM and citrus HM, but not for pectic acid and apple HM.

### 4.2. Physical characterisation with HPSEC combined with triple detector

In Table 3 weight average MW ($M_w$), peak MW ($M_p$, a MW calculated for the peak maximum) and weight average IV ($IV_w$) are given.

The highest $IV_w$ were measured for apple HM and citrus HM. The enzymatic treatment led to a 2–3-fold decrease of the $M_w$ compared to the starting materials. Pectic acid ULV was the furthest degraded sample. There was no correlation between the decrease of $M_w$ or $M_p$ and the one of $IV_w$ observed from starting materials to ULV samples. The partial hydrolysis seemed to have had a stronger effect on $IV_w$ than on the MW.

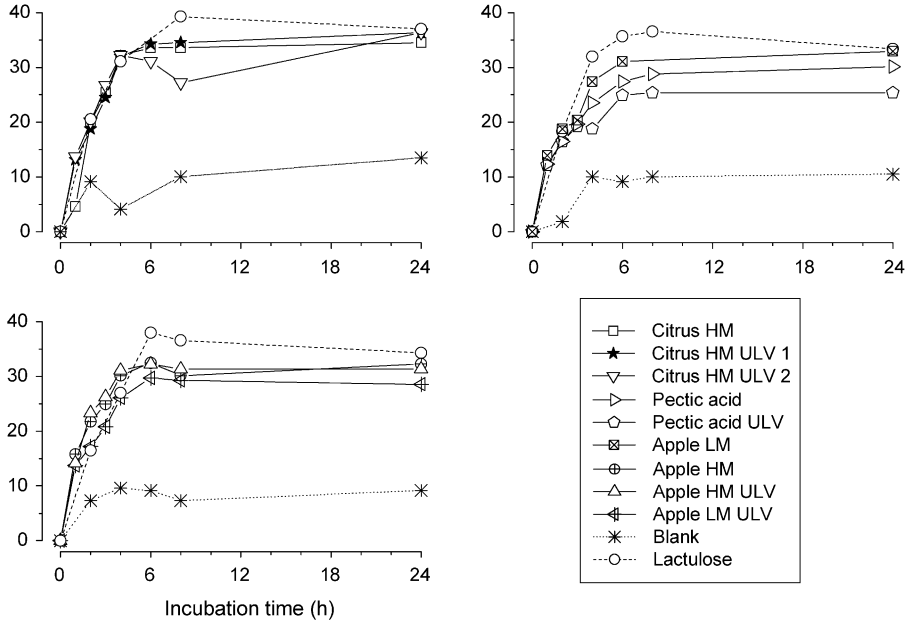


Fig. 1. Total gas production (ml/100 mg substrate) during in vitro fermentation of pectin samples.

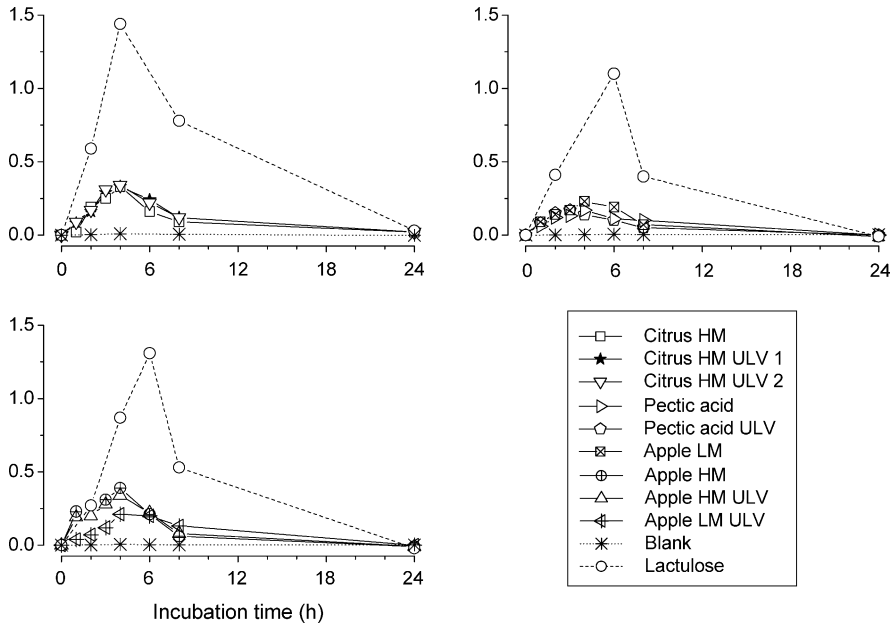


Fig. 2. Hydrogen accumulation (ml/100 mg substrate) during in vitro fermentation of pectin samples.

### 4.3. In vitro fermentability

Three fermentation experiments with three pectin samples each were carried out. During in vitro fermentation almost as much total gas was produced from pectin samples as from the control substrate (lactulose) (Fig. 1). No relevant differences in the rate of production over time were found. Lactulose accumulated higher amounts of hydrogen compared to the pectin samples (Fig. 2). Hydrogen accumulation was higher during the fermentation of HM pectins (0.3–0.4 ml/100 mg substrate after 4 h) compared to LM pectins ($\leq$0.2 ml/100 mg). Fig. 3 shows the pH reached after 24 h in vitro fermentation. Pectins induced a less pronounced decrease in pH compared to lactulose, with citrus HM samples reaching the lowest values among the pectins. The considerably higher pH end value of pectic acid ULV was due to the high mineral content of this sample, which exerted a buffering effect. Pectic acid ULV contained about 14% minerals, the other samples only 2–3% (personal communication of the supplier).

The production of SCFA from pectins was almost as high as from lactulose (Fig. 4). It reached for all pectins values between 8 and 10 mmol/g substrate. Table 4 shows the proportion of acetic acid, propionic acid and butyric acid after 24 h fermentation. Iso-butyric, valeric and iso-valeric acid were also quantified and accounted for 1–2% of

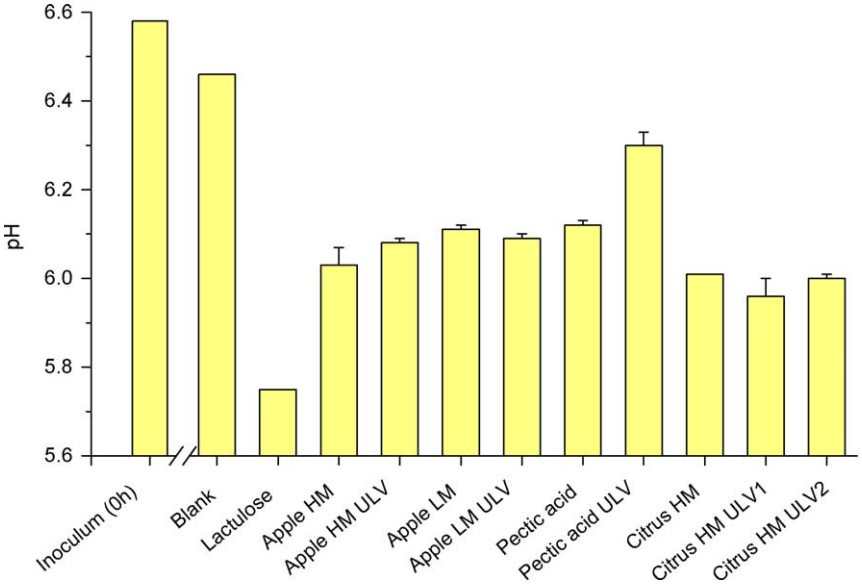


Fig. 3. pH values after 24 h of in vitro fermentation of pectin samples compared to inoculum.

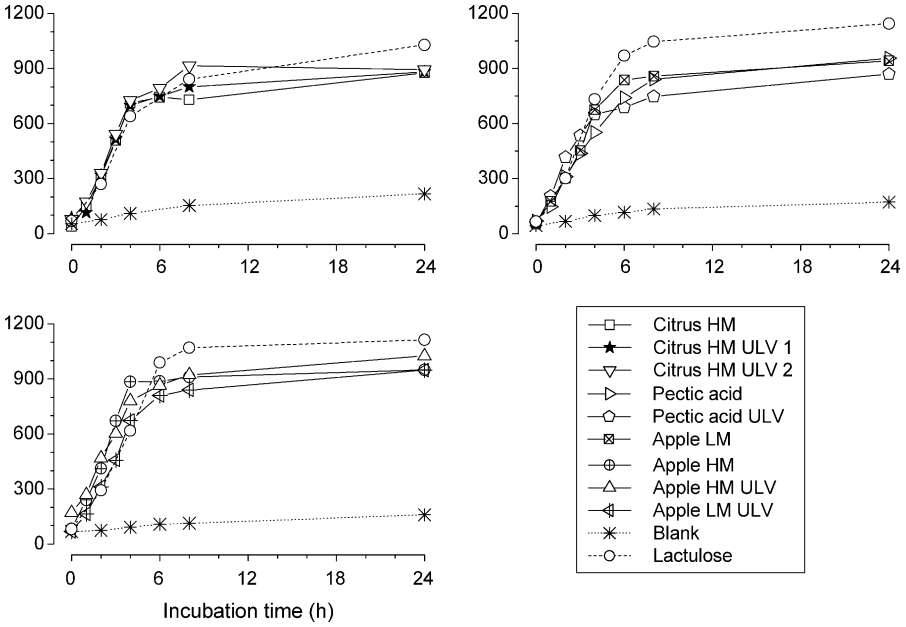


Fig. 4. SCFA production (μmol/100 mg substrate) during in vitro fermentation of pectin samples.

the total SCFA produced from pectins. Pectins confirmed to be good acetic acid producers. Only small differences could be observed in the SCFA proportions among different samples. HM apple pectins produced tendentially more propionic acid than the other samples.

Substrate disappearance as sum of UA and NS is shown in Fig. 5. All pectin samples and lactulose were quickly and completely fermented. HM pectins disappeared within 4 h of fermentation. LM pectins needed 6 h to be completely fermented, whereas for pectic acid and pectic acid ULV 8 h were necessary for their complete disappearance. This may suggest a correlation between the rate of substrate disappearance and the degree of methoxylation.

Table 4
Proportions of the three main SCFA measured in pectin samples after 24 h of in vitro fermentation ($n=2$)

| | Acetic acid (mol%) | Propionic acid (mol%) | Butyric acid (mol%) |
|---|---|---|---|
| Apple HM | 74 | 11 | 13 |
| Apple HM ULV | 74 | 12 | 13 |
| Apple LM | 75 | 11 | 12 |
| Apple LM ULV | 77 | 10 | 12 |
| Pectic acid | 79 | 9 | 10 |
| Pectic acid ULV | 79 | 9[a] | 10[a] |
| Citrus HM | 76 | 9 | 13 |
| Citrus HM ULV 1 | 77 | 9 | 12 |
| Citrus HM ULV 2 | 78 | 9 | 12 |
| Lactulose[b] | 73 | 10 | 15 |
| Blank[b] | 49 | 17 | 14 |

SD < 6%.
[a] SD < 10%.
[b] $n=1$.

## 5. Discussion

### 5.1. Composition and structural features

Pectins which are used for nutritional studies with animals or humans are usually scarcely described (the fruit source is hardly mentioned). In this work, a great importance has been given to the chemical and physical characterisation of the pectin samples, which were then investigated and correlated with their physiological behaviour in vitro.

Apple pomace and citrus peel, being the main sources for commercial pectin preparations, contain slightly different pectins, which make them suitable for specific applications (May, 1990). Possible differences in composition and structure among the nine available samples (four apple and five citrus pectins) were identified. The investigated apple pectins showed a higher neutral sugar content (ranging from 8 to 20 g/100 g) compared to citrus pectins, which contained 5–8 g neutral sugars/100 g (Table 1). Commercial apple pectins are expected to have higher amounts of neutral sugars than citrus pectins (Kravtchenko, Voragen, & Pilnik, 1992). The lower neutral sugar content of LM compared to HM apple pectin, or of pectic acid compared to LM citrus pectins, may be due to the acidic treatment for the de-esterification, which is concomitant with the splitting off of some neutral sugar side chains (Voragen et al., 1995).

Low amounts of arabinose were found in general, and no arabinan side chains were modelled after methylation analysis (only arabinogalactans). It is well known that the treatment with hot acid induces breakdown of arabinosyl linkages, and with increasing extraction time also galactose is expected to decrease (Guillon & Thibault, 1990). With

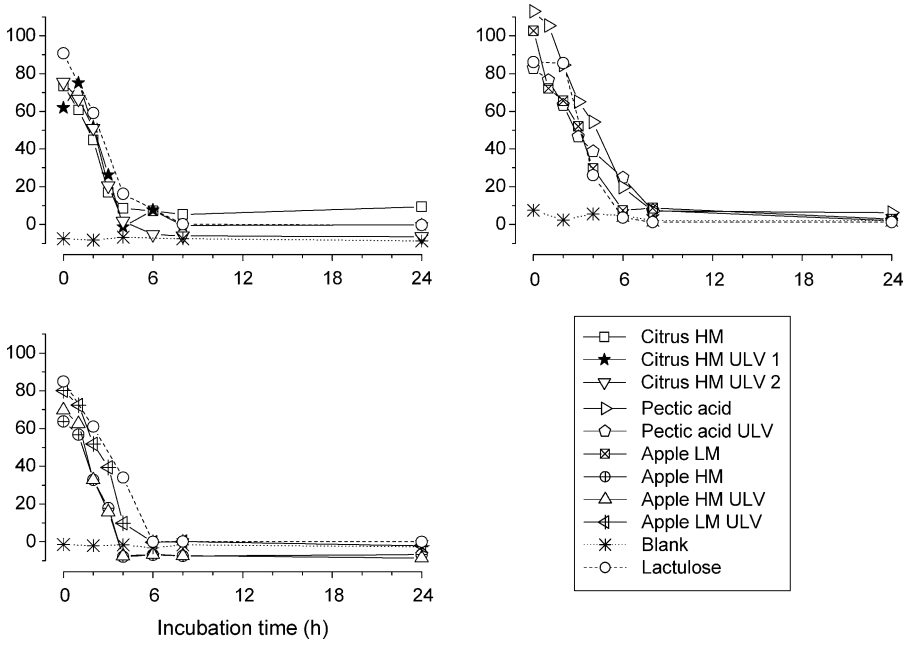


Fig. 5. Substrate disappearance (sum of uronic acids and neutral sugars in mg/100 mg substrate) during in vitro fermentation of pectin samples.

other extraction methods (enzymes, chelating agents), it would be possible to produce arabinan rich pectins (Renard & Thibault, 1993; Thibault & De Dreu, 1988).

Concerning chemical and structural differences between commercial pectins and their partially hydrolysed preparations, a tendential loss of neutral sugars was observed for ULV samples. This fact was only partially confirmed by methylation analysis. It should be taken into account that polysaccharide fractions "smaller than 4–6 kDa are not included" in the modelled molecules, since a dialysis step is necessary after the carbodiimide-activated reduction of the uronic acid residues. Information about smaller fractions is then lost, and ULV samples are more affected by these losses. However, methylation analysis allowed the modelling of pectic structures which were similar among the samples, but differences in their size and in the frequency of side chains were observed (Table 2). The extraction procedure at low pH is known to act as a 'homogenising' factor for the pectin structures, but some special features of the samples were still observable.

### 5.2. Physical characterisation

With HPSEC combined with RI-detector and viscometer the decrease of the molecular weight for the ULV samples vs. their starting materials could be easily observed. There was no direct correlation between $M_w$ and $IV_w$. Indeed, viscosity is better correlated with the length of the polysaccharide backbone than with MW, and the presence of oligomers which may act as diluting agent is of influence as well (Kravtchenko, 1992). Both MW and IV shall be considered for a proper characterisation of the samples. The extent of degradation and the MW distribution may be of importance when tailoring ULV samples: alcohol soluble oligomers are expected to be lost to some extent during alcohol precipitation, if this method is chosen for the production of ULV pectins. However, viscosity is a feature of main importance with respect to industrial applications and physiological behaviour. Depending on the viscosity of the produced ULV pectins, different amounts of the polysaccharide can be added to foods like fruit juices without causing a critical change of the consistency, which would make the product unacceptable for the consumer. In this regard, apple LM ULV and pectic acid ULV would be the most adequate samples for food enrichment of the investigated ones, having $IV_w < 0.4$ dl/g. Though, pectin viscosity is important in several mechanisms taking place in the small intestine, which may lead to a decrease of serum cholesterol (Ahrens et al., 1986; Judd & Truswell, 1985). If the ability of building gels is still desirable for ULV pectins, it may be compensated by adding higher amounts of polysaccharides and by limiting the hydrolysis extent during pectin production.

### 5.3. In vitro fermentability

The degree of methoxylation revealed to be the only factor of influence for the physiological behaviour in the colon. Neither pectin source nor viscosity appeared to be of relevance for the degradability of the preparations by colonic microorganisms. The rate of substrate disappearance during in vitro fermentation appeared to be slower for LM pectins and pectic acid. Also hydrogen accumulation and, partially, the pH decrease, suggested that HM pectins are faster and more extensively fermented than lowly esterified pectins. This is not in accordance with

the results of Dongowski and Lorenz (1998), who reported a slower fermentation of a very highly esterified citrus pectin (DM = 95%) compared to a LM one (DM = 34%). The substrate disappearance of the two samples was in general much slower than in the present experiment, with a certain amount of very highly esterified pectin being still detectable after 24 h. This may be partially explained with the higher faeces and substrate concentrations of the present experiment. Olano-Martin, Gibson, and Rastall (2002) also concluded that LM pectins and LM pectin oligosaccharides (POS, $M_w \sim 4$ kDa) with a DM of 8% were faster fermented than HM preparations (DM = 66%), since a higher growth rate of pure cultures of bacteria isolated from faeces was observed in a LM pectin and a LM POS medium, respectively. In a more complex system, using a faecal slurry prepared from fresh faeces of four donors, the same authors reported a significant enhancement of the growth rate of *Bifidobacteria* for all preparations, where the highest values were assessed for the LM preparations in general and for LM POS in particular. Other genera (*Bacteroides*, Lactic acid bacteria and *Clostridium*) were counted as well, and did not seem to be influenced by the pectin preparations. The different conclusions about the rate of fermentability of the pectins between these two works (Dongowski & Lorenz, 1998; Olano-Martin et al., 2002) and the present one may be explained on the one hand with a different composition of the complex gut microflora and of the pectin substrates. On the other hand, the two methods of evaluation (bacterial growth vs. substrate disappearance) which were applied consider the process from different points of view. Further investigations, comprehensive of both aspects, would be needed on this subject.

Whether a faster or slower fermentability is preferable for health is still not clear. Olano-Martin et al. (2002) showed LM POS to be the most rapidly fermented substrate and at the same time the best prebiotic candidate, since in the first 8 h of fermentation it seemed to favour *Bifidobacteria* more than the other pectin types. From another point of view, a longer persistence of the soluble fibres in the gut might be beneficial as a diluting and binding agent for carcinogens and heavy metal cations.

Since viscosity did not appear to be of relevance for the in vitro fermentability of the pectin preparations, ULV pectins from either citrus or apple could be added to foods to increase their content of dietary fibres without losing their positive effects on colonic fermentation, such as the production of SCFA. Some studies about the health effects of pectin hydrolysates have been already carried out. Compared to a control group, a decrease in liver weight and in liver cholesterol of rats was assessed after adding a partial pectin hydrolysate of $M_w = 66$ kDa to a high cholesterol diet, but no changes in serum cholesterol were observed (Yamaguchi et al., 1994). Moreover, anti-proliferative effects of human colonic adenocarcinoma HT29 were demonstrated for both pectin and POS ($M_w \sim 4$ kDa; Olano-Martin, Rimbach et al., 2003). POS have also been shown to inhibit Shiga-like toxins from *Escherichia coli* (Olano-Martin, Williams, Gibson, & Rastall, 2003) and to block adherence of *E. coli* on uroepithelial cells (Guggenbichler, De Bettignies-Dutz, Meissner, Schellmoser, & Jurenitsch, 1997). Olano-Martin, Rimbach et al. (2003) reported the largest decreasing effect on the yield of HT29 cells (after 2 days treatment) for LM POS, when HM and LM pectins, HM and LM POS preparations were compared. All samples induced a significant decrease from 100 to 58–70% cell growth using a pectin concentration of 10 mg/ml.

In conclusion, it can be stated that neither pectin source nor intrinsic viscosity seemed to be of influence on in vitro fermentability. However, other pectin sources which may give preparations with larger differences in the molecular structure and composition might be of interest. As the degree of methoxylation was shown to influence fermentability, pectins with very high DM or with other functional groups (e.g. amidated or acetylated pectins) may have a particular physiological behaviour.

Since ULV pectins do not behave differently from native pectins, they may be adequate for dietary fibre enrichment. Their low viscosity would allow the addition of higher amounts to food and therefore a higher intake of soluble fibres by the consumers.

## Acknowledgements

This work was partially supported by Obipektin AG, Bischofszell (CH), which also provided the pectin samples. The authors wish to thank Barbara Eder, Dorothea Kemmler and Sandro Janett for technical assistance.

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