The chemical characteristics of apple pectin influence its fermentability in vitro

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Abstract

The aim of the present study was to characterize the fermentability of different apple pectins, as soluble dietary fibres, in vitro. High- and low-methoxyl pectins were fermented completely and very fast by human colonic bacteria, whereas pectic acid and amidated pectin were degraded more slowly. The main fermentation metabolites, i.e. short-chain fatty acids (SCFA) and gases, were produced in lower amounts from pectic acid and amidated pectin. Based on the higher production of SCFA, pectins with a higher degree of methoxylation might be the best candidates for food enrichment due to a beneficial effect on colonic health. On the other hand, the longer persistence of the slowly degraded pectins in the gut may be of advantage as binding and diluting agents for carcinogens.

Keywords: Degree of methoxylation; Degree of amidation; In vitro fermentability; Short-chain fatty acids

1. Introduction

Pectins are complex polysaccharides present in the cell wall of higher plants, where they act as cementing materials in the cellulosic network. Pectin is important in the food industry as an additive enhancing the texture of different food products. It is mainly extracted from citrus peel, apple pomace, and sugar beet pulp. After extraction, pectin is suitable for further modification, e.g. saponification or amidation. Products with different gelling behaviour at different conditions are thus obtained (Thakur, Singh, & Handa, 1997; Thibault & Ralet, 2001).

Pectin is not metabolized in the human upper digestive tract and is therefore considered a dietary fibre. Pectin is recognized to have a preventing effect on different Western diseases such as coronary heart diseases and diabetes type II (Reiser, 1987). Different mechanisms in both upper and lower intestine have been proposed to explain its hypocholesterolemic effect. Bile acid absorption and the production of short-chain fatty acids (SCFA) probably contribute to the positive health effects on the level of the lower intestinal tract (Anderson, Deakins, & Bridges, 1990). SCFA are also supposed to prevent carcinogenesis in the large bowel (Scheppach, Bartram, & Richter, 1995).

Several experiments have been performed in vitro and in vivo in order to characterize the colonic fermentation patterns of pectins with different structural properties and their possible effect on metabolism and health. Judd and Truswell (1982) compared the influence of high- and low-methoxyl (HM and LM, respectively) pectins on lipid metabolism in humans. Olano-Martin, Gibson, and Rastall (2002) compared the bifidogenic properties of HM and LM pectins and pectic oligosaccharides. Rats studies (Dongowski, Lorenz, & Proll, 2002) as well as in vitro experiments with human faecal flora (Dongowski & Lorenz, 1998) showed an influence of the degree of methoxylations (DM) of pectin on fermentability. In a previous work, we have shown that partially hydrolysed pectins behave similarly to their starting material when fermented in vitro with human faecal bacteria (Gulfi, Arrigoni, & Amadò, 2005). Only the DM was found to be of influence on the fermentation pattern, but in a different way than observed by Dongowski et al. (2002) and Dongowski and Lorenz (1998).

The aim of the present work was to confirm the influence of DM and to investigate the effect of the degree of
amidation (DA) of pectins on in vitro fermentability. Apple pomace was the source for all samples, which were commercially produced, but not standardized for application in food.

2. Material and methods

2.1. Materials

HM, LM and amidated non-standardized apple pectins were produced from different apple pomace batches by Obipektin AG (CH-Bischofszell), pectic acid from apple pomace was a gift of Herbstreith & Fox KG (D-Neuenbürg/Württ).

2.2. Methods

Uronic acids (UA) were quantified spectrophotometrically, neutral sugars (NS) by GLC-FID after derivatization and DM by HPLC as described by Gulfi et al. (2005). The DA was assessed by the producer according to the method of Kjeldahl. The quantification of weight average molecular weight (M_w) and of the intrinsic viscosity ([η]) was done with high performance size exclusion chromatography combined with refractometer, right and low angle light scattering (LS) detectors and viscometer (Gulfi et al., 2005) with following modifications: as eluent sodium nitrate 0.1 mol/l was used, and the LS detector was calibrated with a pullulan standard with M_w = 112 kDa (Shodex Standard P-82, Showa Denko KK, J-Kawasaki). Pectin solutions were prepared in concentrations between 0.4 and 2 mg/ml and filtered (cut-off 0.45 μm) for the calculation of the refractive index increment (dn/dc). M_w was assessed using the LS signal, the calculated dn/dc and the detector constant.

In vitro fermentation was performed according to the method described earlier (Gulfi et al., 2005). It was a batch system where fresh human faeces were added to pectin solutions (hydrated over night). Fermented samples were separated in a liquid and a solid phase by centrifugation and filtered (cut-off 0.45 μm) for analysis. SCFA analysis was performed by GLC-FID. For the substrate disappearance NS and UA were measured spectrophotometrically after a color reaction in both liquid and solid phase. This assessment was not specific for pectin, sugars present in the inoculum could also react.

3. Results and discussion

3.1. Characterization of the pectins

Samples were characterized by their chemical composition, degrees of methoxylolation and amidation, and physical features (Table 1). As already indicated in a previous work (Gulfi et al., 2005), pectins which underwent a further treatment (saponification) had decreased NS contents. This was also the case for the amidated pectin presently analysed. Amidated, LM pectin and pectic acid had similar NS contents. LM and amidated pectin had a similar DM, whereas the DM of pectic acid was significantly lower. HM pectin had slightly more than 50% of its carboxyl groups methylated. 21% of the methoxyl groups of the amidated pectin were substituted with ammonia; indicatory values for the DA are ≤25% for commercial pectins.

M_w between 200 and 260 kDa were measured for three out of four samples. Thibault, Renard, Axelos, Roger, and Crépeau (1993) characterized a commercial apple sample for its M_w by multi-angle laser LS and [η] with an Ubbelohde capillary. Their M_w value, 89 kDa, was rather low compared to those presented in this work, whereas their [η], 2.7 dl/g, was well in agreement with the ones of Table 1 (except for pectic acid).

Dn/dc values between 0.130 and 0.136 ml/g had been assessed for the amidated, HM and LM pectins, and were used for the calculation of M_w. These values were near to the one given by Theisen, Johann, Deacon, and Harding (2000) for pectin and for many other polysaccharides (0.146 ml/g).

Pectic acid was found to have a lower M_w than the other samples. A dn/dc value of 0.121 ml/g only was obtained for this sample. The low dn/dc might be due to the low solubility of pectic acid, because losses during sample preparation influence the accuracy of the concentration given. Approx. 20% of poorly soluble material was estimated to be lost during filtration. Both M_w and [η] probably represented only the best soluble part of this sample.

For pectins with M_w below 200 kDa and [η] below 3.7 dl/g, respectively, differences in M_w and [η] of magnitude 4–6 times were not expected to have an effect on in vitro fermentability (Gulfi et al., 2005).

3.2. In vitro fermentability

Pectins, a blank sample and lactulose, a highly fermentable substrate, underwent an in vitro fermentation with fresh human faeces for 24h. Table 2 shows different parameters that were monitored in order to characterize the fermentation behaviour of each sample. All parameters indicated a complete fermentability for lactulose (n = 1): low end pH, high gas and SCFA production and high accumulation of the intermediate product hydrogen. In the blank samples (n = 1), SCFA and gases were hardly produced, and the pH did not change during the course of the experiment (starting pH 6.7). As expected, pectin samples (n = 2) were fermented in a different way. The ranking of substrates based on fermentability rate was HM pectin > LM pectin > pectic acid > amidated pectin. Gas and SCFA production as well as pH decrease were lower for amidated pectin and pectic acid compared to HM and LM apple pectin. Dongowski and Lorenz (1998) observed a SCFA production which was inversely proportional to the DM, whereas in the present study the trend was opposite.
Substrate disappearance was assessed in both liquid and solid phases (Fig. 1). Lactulose completely disappeared within 6 h. The curves of apple HM and apple LM pectins differed only slightly, both of them disappearing also completely within 6 h of fermentation. Apple pectic acid was fermented more slowly, particularly between 4 and 8 h. The degradation of the amidated pectin was much slower and small amounts of UA and NS were still found after 24 h in the fermentation suspensions.

By separating the liquid and the solid phase of the fermentation samples after centrifugation, it was possible to monitor how much pectin was properly dissolved and how much was only suspended or adhering to dispersed particles such as bacteria or residual insoluble fibres of the inoculum. All pectins except for pectic acid were completely dissolved in the liquid phase and only bacterial mass contributed to the solid residue after freeze-drying. On the contrary, parts of pectic acid were found in the solid phase until \( t = 8 \) h. This may be due to its poor solubility or its interacting with insoluble particles, which could also influence its rate of fermentability. Amidated pectin was, on the contrary, well soluble, therefore the amide groups are probably the only cause for the slower and less efficient fermentation.

The present results obtained for Apple HM, LM and pectic acid support those described previously (Gulfi et al., 2005) but are in contrast with the conclusions of another research group (Dongowski et al., 2002; Dongowski & Lorenz, 1998; Dongowski, Lorenz, & Anger, 2000), who stated that LM pectins are a preferred substrate for faecal bacteria (isolated cultures or fresh faeces) compared to HM pectins. This may suggest that the conditions in which an in vitro fermentation experiment with pectins is carried out may be determinant for the results. Bacterial composition, which is strongly influenced by the donors, is a key factor influencing fermentation patterns. Moreover, the treatment of the flora, the composition of the fermentation buffer and the ratio in which substrates, buffer and colonic flora are mixed, can influence the course of the fermentation. It would be interesting to compare the two methods further in
detail and to perform a comparative study using the same samples and colonic microbiota.

4. Conclusions

Nowadays, partially degraded pectin is used by the food industry to increase the dietary fibre content in products like fruit juices. Usually low viscosity pectin preparations are added to liquid foods because of their good solubility and their low tendency to form gels even when added in high amounts. Size and viscosity of the polymers were shown to have no influence on their fermentability (Gulfi et al., 2005), higher amounts of partially hydrolysed pectins can therefore be added to food thus increasing its dietary fibre content. Others features may be considered in order to improve the nutritional value of this soluble fibre. On the basis of the results presented here, HM pectin could be regarded as the best candidate for dietary fibre enrichment of food, since it is fermented more thoroughly and produces slightly higher amounts of SCFA. But if it is considered that a longer persistence of soluble fibres in the gut contributes to dilute and to bind carcinogens and heavy metal cations, pectic acid or amidated pectin would be the preferred additives. However, it would be necessary to elucidate the mechanism of degradation of amidated pectin. Ammonia is considered a toxic metabolite indeed, commonly originating from the metabolism of proteins. If high amounts of ammonia are released from amidated pectin in the gut, food enrichment with this polysaccharide could hardly be taken into consideration. The measurement of ammonia would be feasible in an in vitro batch system.

References


