Fermentation of the endosperm cell walls of monocotyledon and dicotyledon plant species by faecal microbes from pigs

The relationship between cell wall characteristics and fermentability

Harmen van Laar*, Seerp Tamminga, Barbara A. Williams, Martin W.A. Verstegen

Animal Nutrition Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen Agricultural University, PO Box 338, 6700 AH, Wageningen, Netherlands

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Abstract

Cell walls from the endosperm of four monocotyledons (maize, wheat, rye, and rice) and four dicotyledons (soya bean, lupin, faba bean, and pea) seeds were studied to relate cell wall composition and structure with fermentation characteristics. Cell wall material was isolated from the endosperm of the mono- and dicotyledons. The fermentation characteristics of isolated cell walls from mono- and dicotyledons were analysed in two separate in vitro gas production experiments. At 0, 12, 24, 36, 48, and 144 h of fermentation, fermentation was stopped in selected bottles to analyse VFA production (144 h only) and sugar degradation patterns. The relationship between cell wall characteristics (composition, particle size) and fermentation characteristics (half-time of gas production and maximal rate of substrate degradation) was analysed using linear regression. For the monocotyledon cell walls, the rate of substrate degradation was decreased by increasing particle size of the cell walls, a clear effect of cell wall composition on fermentation characteristics could not be determined, though this might have been obscured by the differences in particle size. During fermentation of the monocotyledon cell wall, arabinoxylans (arabinose and xylose) and cellulose (glucose) appeared to be degraded simultaneously. For the dicotyledon cell walls, an increase in total sugar content decreased the half-time of gas production, though total sugar content was probably confounded with the crude protein content. During fermentation of the dicotyledon cell wall, pectins or pectin-related sugars (galactose, arabinose, uronic acids) appeared to be degraded faster than cellulose, whereas for the monocotyledon cell walls, arabinoxylans and

* Corresponding author. Tel.: +31-0317-483281; fax: +31-0317-484260.
E-mail address: harmen.vanlaar@alg.vv.wau.nl (H. van Laar).
cellulose were degraded simultaneously. The differences in cell wall fermentation and sugar degradation pattern between monocotyledon and dicotyledon cell walls are discussed in relation to differences in cell wall architecture. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

In the past, most cell wall research has focused on fermentation of whole plant material. However, whole plant material contains a mixture of primary and secondary cell walls, which clearly differ in their fermentation characteristics (Chesson, 1993). Therefore, to relate the composition of an individual cell wall type to its fermentation characteristics, it is important to study isolated cell walls of a single cell wall type. Also, earlier cell wall research has mainly focused on secondary cell wall fermentation in ruminants, whereas the fermentation of primary (mostly un lignified) cell walls, predominantly present in the endosperm of seeds commonly used in pig nutrition (e.g., maize and soya beans) has received much less attention. Feed evaluation for monogastrics would benefit from a better understanding of the factors influencing the fermentation characteristics of seed endosperm cell walls.

Current feed evaluation for cell walls is mostly based on ‘crude’ fractions such as neutral detergent fibre and acid detergent fibre (Van Soest et al., 1991). These methods provide a crude separation into different polysaccharide fractions, but do not give information on differences in polysaccharide composition within those fractions. Analysis of the composite sugars gives a more detailed view of the composition of the polysaccharides in the cell wall. However, this is complicated by the fact that in different cell wall types the same sugar may be present in totally different polysaccharides, within a completely different cell wall architecture. This is especially the case for arabinose, which in monocotyledonous cell walls is present mainly in the arabinoxylan fraction, whereas in dicotyledonous cell walls it is present mainly in the pectin fraction. This illustrates the importance of knowing the general cell wall architecture of a cell wall when interpreting sugar composition data.

Monocotyledon and dicotyledon cell walls are different in their composition and the structure of their primary cell walls. Generally, monocotyledon cell walls contain mainly hemicellulose and cellulose, with only little pectin, whereas dicotyledon cell walls contain mainly pectin and cellulose and little hemicellulose (Bailey et al., 1976). Furthermore, these polysaccharides have a different arrangement within the cell wall, resulting from differences in cell wall architecture. The monocotyledon cell wall is composed of a tightly packed network of both arabinoxylans and cellulose, whereas the dicotyledon cell wall has a very open hydrated cell wall structure (Hatfield, 1993). These differences in cell wall structure are likely to have an effect on the fermentation of these cell walls. Whereas differences in cell wall structure may influence cell wall fermentation, differences in sugar composition within a certain cell wall type might also be important.
A study of differences in the composition of polysaccharides could be done using structurally similar cell walls, which differ in composition of their polysaccharides. However, surface area to volume ratio (or particle size/cell wall thickness) which influences fermentation rate (Fisher et al., 1989; Weimer, 1996) would have to be similar, to be able to compare differences in cell wall composition. This experiment was designed to study the relationship between the sugar composition of primary (mostly unlignified) cell walls and their fermentation by gastrointestinal microbes of pigs, and to study differences in the fermentation characteristics of mono- and dicotyledon cell walls.

2. Materials and methods

2.1. Feedstuffs

Four species from two plant groups (monocotyledonous and dicotyledonous plants) were chosen to be analysed for fermentation characteristics of their endosperm cell walls. For the monocotyledonous feedstuffs, cell walls were obtained from maize endosperm (Zea mays) (Meneba Meel Weert b.v., the Netherlands), wheat flour (Triticum aestivum) (Meneba), rye flour (Secale cereale) (Vibe Glitsø, Danish Institute of Animal Science) (Glitsø and Bach Knudsen, 1999), and dehulled polished rice (Oryza sativa) (food grade). For the dicotyledonous feedstuffs, cell walls were obtained from the cotyledons of Argentinean soya beans (Glycine max) (Schouten Giessen b.v., the Netherlands), lupins (Lupinus albus), faba beans (Vicia faba) (white hulled variety), and peas (Pisum sativum) (Goelema, 1999).

2.2. Cell wall extraction

The dicotyledonous materials were soaked overnight in iced water. After soaking, they were manually separated into hulls and cotyledons. Cell walls (CWs) were isolated from the cotyledons as described by Huisman et al. (1998). Briefly, cotyledons were ground over a 0.5 mm sieve, and extracted with petroleum ether BP 40–60°C in a Soxhlet extractor to remove fat. Defatted endosperm was sequentially extracted using demineralized water (3 h, room temperature) and a solution of 10 g l\(^{-1}\) sodium dodecyl sulphate (SDS) and 1.5 g l\(^{-1}\) dithiothreitol (3 h, room temperature). After gelatinization (pH 5, 85°C, 1 h), starch was removed using a maleic acid buffer containing 2 mg l\(^{-1}\) porcine \(\alpha\)-amylase (Merck 16312) (pH 6.5 for 16–20 h at 30°C). After each extraction step, the material was centrifuged at 11,000 g for 30 min, the supernatant discarded, and the pellet subjected to the subsequent extraction step. The final residue was freeze-dried and left to air-equilibrate for a minimum of 3 h.

For the monocotyledonous feedstuffs, the endosperm fractions were subjected to the same cell wall extractions procedure as for the dicotyledonous feedstuffs, but without soaking or grinding, except for rice which was ground over a 0.5 mm sieve. Furthermore, the materials were wet-sieved over a 0.45 \(\mu\)m sieve width, to remove most of the starch granules prior to gelatinization. Thus the extraction procedures yielded eight cell wall fractions, four from monocotyledonous feedstuffs (maize, wheat, rye, rice) and four from dicotyledonous feedstuffs (soya beans, lupins, faba beans, peas).
2.3. Experimental procedures

The monocotyledonous and dicotyledonous cell wall substrates were assessed for their fermentability in two separate gas production runs, using the in vitro cumulative gas production technique described by Theodorou et al. (1994). Approximately 0.5 g substrate dry matter (DM) was weighed into 100 ml serum bottles. To these bottles, 82 ml of semi-defined medium, supporting growth of most micro-organisms from the rumen (Lowe et al., 1985) was added. Bottles were inoculated with 5 ml of an inoculum which had been prepared from faeces of pigs by diluting 1:5 with saline (9 g l⁻¹ NaCl), mixing in a blender for 1 min, and straining through a double layer of cheesecloth. The maize based diet, fed to the pigs, contained no added antibiotics or copper. Bottles were incubated in an incubator at 39°C. Sufficient replicates were used so that bottles containing substrate could be removed after 0, 12, 24, 36, 48, and 144 h of incubation. For 144 h of incubation, four bottles were used for every substrate, and four bottles were used as a blank, which contained only medium and inoculum. For all other combinations of fermentation time and substrate, the number of bottles used was determined based on an estimated total residue of approximately 1 g DM (minimum of two, maximum of eight bottles depending on substrate and fermentation time). For each bottle, gas production was measured by recording pressure and volume of gas produced, at regular intervals during fermentation.

Fermentation was stopped by autoclaving the bottles at 110°C for 10 min, after which the bottles were stored at −18°C pending residue collection. After thawing, the contents of each bottle were rinsed into a centrifuge tube using demineralized water and centrifuged at 11,000g for 30 min. The pellets were resuspended in demineralized water and centrifuged again. Subsequently pellets (residues) were transferred to a 50 ml plastic bottle, freeze-dried, and residual DM determined (freeze-drying). Subsequently, samples were pooled by substrate and fermentation time and reground with a porcelain pestle and mortar. The supernatants from the centrifugation procedure were pooled by bottle and brought up to a volume of 250 ml with demineralized water. A sub-sample of 10 ml was taken and 0.5 ml of phosphoric acid (85%) was added, after which the sample was stored at −18°C pending volatile fatty acid (VFA) analysis.

2.4. Chemical analyses, particle size analyses and contamination analyses

Substrates were analysed for DM, ash, nitrogen (N), fat, and sugar composition. Fermentation residues were only analysed for sugar composition. DM was determined by drying to a constant weight at 103°C (ISO, 1983), ash by combustion at 550°C (ISO, 1978), N was determined using the Kjeldahl method with CuSO₄ as the catalyst (ISO, 1979), and fat was determined using a Soxhlet extraction with petroleum ether (ISO, 1996). Neutral sugar composition was analysed by high-pressure liquid chromatography (HPLC; Dionex PA-10 column and PA-10 guard column) with pulsed amperiometric detection, using allose as an internal standard, similar to Lebet et al. (1997). The samples were pre-treated with 12 M sulphuric acid (1 h, 30°C) followed by hydrolysis with 1 M sulphuric acid for 3 h at 100°C. After hydrolysis, uronic acids were determined by a colorimetric m-hydroxydiphenyl assay using a spectrophotometer at 520 nm.
VFA in fermentation liquids were analysed using gas chromatography (Packard 419, CE Instruments, Milan, Italy; glass column filled with chromosorb 101, carrier gas N₂ saturated with methanoic acid, at 190°C with iso-caproic acid as the internal standard).

To analyse the particle size distribution, the cell wall materials were analysed with a Coulter laser LS 130 particle size analyser (Keetels, 1995), using the Fraunhofer optical mode. This method assumes that all particles are spherical, which for cell wall material is not the case. The Coulter counter measures both the number of particles, and the volume and surface area of specific particle size classes. For this experiment only the ratio of volume (μm³) to surface area (μm²), the \(d_{3/2}\) value, was used as a measure of particle size.

Earlier experiments indicated that cell wall extraction using a detergent such as SDS can contaminate the cell wall sample with residuals of the detergent used. This could possibly affect the rate of fermentation. By analysing the CW material with pyrolysis gas chromatography mass spectrometry (P-GC–MS), which is usually used for analyses of the lignin composition (Lapierre, 1993), the relative amount of contamination (relative to other samples) could be quantified. However, the exact chemical structure and the absolute concentration of the contaminant could not be determined.

2.5. Calculations and statistics

2.5.1. Gas production

To smooth the gas production profiles, the gas volume was regressed against the gas pressure yielding a linear volume pressure relationship for each individual bottle. This relationship was used to recalculate the gas volume produced for each pressure, which were added per measurement time to give the cumulative gas production profiles. Gas production profiles were corrected for gas production in the blank bottles.

For each bottle fermented for 144 h, gas production profiles (millilitre per gram of total sugars in material, TS) were fitted to a mono- and di-phasic model as described in model 1 (Groot et al., 1996):

\[
Y = \sum_{i=1}^{n} \frac{A_i}{1 + (C_i/t)^{B_i}}, \quad \text{(Model 1)}
\]

where \(Y\) is the total gas production (ml g⁻¹ TS), \(A_i\) the asymptotic gas production for phase \(i\), \(B_i\) the switching characteristic factor for phase \(i\), \(C_i\) the half-time for asymptotic gas production for phase \(i\), and \(t\) the time (h).

For each phase, the maximal fractional rate of substrate degradation (\(R_M\)) was calculated, using Eq. (1) adapted from Groot et al. (1996):

\[
R_M = \frac{(B - 1)^{B/(B-1)}}{C}
\]

where \(R_M\) is the maximal fractional rate of substrate degradation, \(B\) the switching characteristic of gas production equation (Model 1), and \(C\) the half-time of gas production (Model 1).
The statistical comparison between the mono- and di-phasic models was done with an F-test as described by Motulsky and Ransnas (1987).

Parameter fittings and other results within each separate gas production run of monocotyledons and dicotyledons were analysed with Model 2 using the GLM procedure of SAS (1989). Differences between individual substrates were analysed by a multiple comparison test (Tukey), using the means statement of SAS (1989):

\[ Y = \mu + \text{Substrate}_i + \varepsilon_{ij} \quad \text{(Model 2)} \]

where \( Y \) is the result, \( \mu \) the mean, \( \text{Substrate}_i \) the effect for substrate \( i \), and \( \varepsilon_{ij} \) the error term.

2.5.2. Cell wall characteristics and fermentability

The relationship between particle size, contamination, and sugar composition, with the maximal rate of substrate degradation and the half-time of gas production for the fermentation of the cell wall materials was analysed by linear regression using the REG procedure of SAS (1989) (Model 3). This analysis was performed using the means of the maximal rate of substrate degradation and half-time of gas production for each cell wall fraction, yet separately from mono- and dicotyledons. However, differences in particle size could be confounded with contamination or sugar composition. Therefore, this simple linear regression must be seen as a tentative approach to investigate the factors governing the fermentation of different feedstuff cell walls:

\[ Y_{ij} = \beta_0 + \beta_1 x_i + \varepsilon_{ij} \quad \text{(Model 3)} \]

where \( Y \) is the \( R_M, C \) (maximal rate of substrate degradation, half-time of gas production), \( \beta_0 \) the intercept, \( \beta_1 \) the slope, \( x_i \) the characteristic (e.g. particle size, sugar composition), and \( \varepsilon_{ij} \) the error term.

3. Results

3.1. Cell wall composition and extraction

The composition of the cell walls from the mono- and dicotyledonous feedstuffs is given in Table 1. The protein content of both monocotyledon and dicotyledon cell walls varied from 25 to 109 g kg\(^{-1}\) DM. In terms of sugars, the cell walls of the monocotyledons contained large amounts of arabinose, xylose and glucose, except for rice cell walls, which were mainly glucose (cellulose). For the cell walls of the dicotyledons, lupins contained a lot of galactose, as did soya, whereas faba beans and peas contained large amounts of arabinose.

Table 2 shows the yield of cell wall material (total amount of DM left after isolation compared to the amount of DM in the original material prior to extraction, expressed as a percentage) and the average particle size, expressed as the \( d_{3/2} \) value. For the monocotyledons, the cell wall yield was around 2% or lower. For the dicotyledons the cell wall yield differed from 3.8% for peas to 32% for lupins. For the monocotyledons only maize and rice cell wall had a measurable contamination with extraction residue, and was twice as high for rice compared to maize. For the dicotyledons the relative
contamination (relative to lowest contamination) with extraction residue was 14.2, 10.5, 1.0, and 1.25 for soya, lupin, faba bean and pea cell wall materials, respectively. The analysis with P-GC–MS confirmed that there was only very little lignin present in the endosperm cell walls of the mono- and dicotyledons.

3.2. Gas production results

3.2.1. Gas production profiles

Fig. 1 shows the gas production profiles for all substrates divided into mono- and dicotyledons. For the monocotyledons, there were marked differences in gas production profiles, both in lag time and total gas production. Most prominent is the rapid and extensive degradation of the rice cell wall, and the relatively long lag time for the

Table 1

|                        | DM (g kg⁻¹), organic matter (OM), crude protein (CP), crude fat (CF), and TS content (g kg⁻¹ DM), and sugar composition (g kg⁻¹ TS) for both the dicotyledonous and monocotyledonous endosperm cell wall materials
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>Ara: arabinose; Gal: galactose; Glc: glucose; Xyl: xylose; and UA: uronic acids.</td>
</tr>
<tr>
<td><strong>Monocotyledonous cell walls</strong></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>909.7 989.1 93.7 11.0 742 254 52 281 315 77</td>
</tr>
<tr>
<td>Wheat</td>
<td>890.0 1000 25.0 0 899 244 15 256 408 26</td>
</tr>
<tr>
<td>Rye</td>
<td>891.7 998.2 25.2 0 873 207 19 364 284 21</td>
</tr>
<tr>
<td>Rice</td>
<td>898.8 995.4 70.6 1.6 761 36 7 880 36 32</td>
</tr>
<tr>
<td><strong>Dicotyledonous cell walls</strong></td>
<td></td>
</tr>
<tr>
<td>Soya</td>
<td>921.3 964.0 109.3 0.9 752 184 356 164 65 172</td>
</tr>
<tr>
<td>Lupin</td>
<td>909.1 985.6 53.5 1.7 796 128 646 93 23 86</td>
</tr>
<tr>
<td>Faba (w)</td>
<td>925.0 953.0 28.1 3.7 819 426 56 202 48 220</td>
</tr>
<tr>
<td>Pea</td>
<td>928.4 960.7 30.4 2.2 793 464 59 182 44 201</td>
</tr>
</tbody>
</table>

Table 2

|                        | Yield (%) of cell wall material (total amount of DM left after isolation compared to the amount of DM in the original material prior to extraction) from the original material and particle size expressed as volume over area (d_{3/2} value, μm³ μm⁻²) for both the monocotyledonous and dicotyledonous endosperm cell wall materials
<table>
<thead>
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<tbody>
<tr>
<td></td>
<td>Monocotyledonous cell walls</td>
</tr>
<tr>
<td></td>
<td>d_{3/2} value</td>
</tr>
<tr>
<td>Maize</td>
<td>2.1ᵃ</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.7</td>
</tr>
<tr>
<td>Rye</td>
<td>1.5</td>
</tr>
<tr>
<td>Rice</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Dicotyledonous cell walls</strong></td>
<td></td>
</tr>
<tr>
<td>Soya</td>
<td>10.4ᵇ</td>
</tr>
<tr>
<td>Lupin</td>
<td>32.0ᵇ</td>
</tr>
<tr>
<td>Faba</td>
<td>4.1ᵇ</td>
</tr>
<tr>
<td>Pea</td>
<td>3.8ᵇ</td>
</tr>
</tbody>
</table>

ᵃ Value from earlier experiment (Van Laar, unpublished).
ᵇ Yield calculated on an air DM basis.
degradation of maize cell walls. For the dicotyledons, the asymptotic gas production was similar, though for soya bean cell walls there seemed to be an increased lag phase, compared to the other three cell walls, which had similar gas production profiles. The lag phase of the gas production profiles are rather long (up to 15 h), which is probably an artefact of the in vitro technique, which uses a very diluted inoculum.

3.2.2. Gas production curve fitting

The fitted parameters and the maximal rates of substrate degradation are shown in Table 3. For the mono-phasic curve fit of the monocotyledons, the rice cell walls had the highest asymptotic gas production and highest maximal rate of substrate degradation. Maize cell walls had the lowest maximal rate of substrate degradation and had a very high half-time of gas production. The asymptotic gas production was lower and much more

Fig. 1. Gas production patterns (ml g\(^{-1}\) TS) for the fermentation of: (A) monocotyledon and (B) dicotyledon endosperm cell walls.
variable for the monocotyledons compared with the dicotyledons. For the dicotyledons, the asymptotic gas production was similar for all the cell walls, though there were some differences in maximal rate of substrate degradation. Soya cell walls had a very high half-time of gas production compared to the other three dicotyledons. The half-times of gas production for mono- and dicotyledons were comparable, whereas the maximal rate of substrate degradation was slightly higher for the dicotyledons.

Fitting a di-phasic curve through the gas production profiles significantly improved the curve fit for all bottles. The division of gas production into two phases could be related to fermentation of specific sugars within each substrate. For the monocotyledons, the proportion of gas produced in the first phase for maize (26.8%), wheat (85.5%), rye (89.2%), and rice (64.8%) was not similar to the proportion of arabininoxylan (arabinose and xylose) of the total cell wall sugars (56.9, 65.2, 57.1, and 7.2%, respectively). In the case of the dicotyledons, the proportion of gas produced in the first phase for soya (71.8%), and faba beans (59.5%) was fairly similar to the proportion of pectin-related sugars (arabinose, galactose and uronic acids) (71.2 and 64.8%, for soya and faba beans, respectively). However, the proportion of gas produced in the first phase for lupin (53.6%) and pea (58.9%) did not agree with the proportion of pectin-related sugars (86 and 72.4%, respectively).

### Table 3

Asymptotic gas production ($A; \text{ml g}^{-1} \text{TS}$), switching characteristic ($B$), half-time ($C; \text{h}$), and maximal rate of substrate degradation ($R_M, \% \text{h}^{-1}$) for the fermentation of the monocotyledonous and dicotyledonous endosperm cell wall materials, when fitted with the mono-phasic or the di-phasic model.

<table>
<thead>
<tr>
<th>Mono-phasic</th>
<th>Di-phasic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$A$</td>
</tr>
<tr>
<td><strong>Monocotyledonous cell walls</strong></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>400 b</td>
</tr>
<tr>
<td>Wheat</td>
<td>337 c</td>
</tr>
<tr>
<td>Rye</td>
<td>343 c</td>
</tr>
<tr>
<td>Rice</td>
<td>461 a</td>
</tr>
<tr>
<td>SEM</td>
<td>12.0</td>
</tr>
<tr>
<td><strong>Dicotyledonous cell walls</strong></td>
<td></td>
</tr>
<tr>
<td>Soya</td>
<td>430 a</td>
</tr>
<tr>
<td>Lupin</td>
<td>446 a</td>
</tr>
<tr>
<td>Faba</td>
<td>429 a</td>
</tr>
<tr>
<td>Pea</td>
<td>441 a</td>
</tr>
<tr>
<td>SEM</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*Means with different letters within the groups of monocotyledons and dicotyledons, within one column differ significantly ($P < 0.05$).
Although differences in the amount of VFA produced were not statistically significant, the higher residue was reflected in the lower amount of VFA produced. Fermentation of rice cell wall led to a different pattern of VFA production, with less acetic acid and more propionic acid, as reflected in the lower A/P ratio, compared to the other monocotyledons. For dicotyledons, the percentage of residue was fairly similar for the different feedstuffs. However, the amount of VFA produced was slightly lower for soya and lupin cell walls. For lupins, the production of acetic acid was very low, whereas the propionic acid production was very high, reflected in a low A/P ratio.

### Table 4

Percentage of residue after 144 h fermentation, amount of VFA produced (expressed as acetic acid equivalents: AAE, mmol g\(^{-1}\) TS), VFA production pattern (%), acetic acid to propionic acid ratio (A/P), acetic acid equivalents yield (AAEY, AAE produced per DM fermented, mmol g\(^{-1}\) gas production yield (gas produced per DM fermented, ml g\(^{-1}\)) for the fermentation of the dicotyledonous and monocotyledonous endosperm cell wall materials

<table>
<thead>
<tr>
<th></th>
<th>Residue</th>
<th>AAE</th>
<th>HAc(^{b})</th>
<th>HPr(^{c})</th>
<th>HBu(^{d})</th>
<th>A/P</th>
<th>AAEY</th>
<th>Gas Y</th>
</tr>
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<tbody>
<tr>
<td><strong>Monocotyledonous cell walls</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Maize</td>
<td>19.9 b</td>
<td>10.8 a</td>
<td>59.0 a</td>
<td>28.5 c</td>
<td>12.5 a</td>
<td>2.1 a</td>
<td>10.1 a</td>
<td>375 b</td>
</tr>
<tr>
<td>Wheat</td>
<td>27.8 a</td>
<td>8.3 a</td>
<td>59.8 a</td>
<td>30.2 b</td>
<td>10.0 c</td>
<td>2.0 a</td>
<td>10.4 a</td>
<td>437 a</td>
</tr>
<tr>
<td>Rye</td>
<td>34.0 a</td>
<td>8.5 a</td>
<td>60.0 a</td>
<td>28.9 bc</td>
<td>11.1 bc</td>
<td>2.1 a</td>
<td>11.2 a</td>
<td>464 a</td>
</tr>
<tr>
<td>Rice</td>
<td>14.9 b</td>
<td>10.2 a</td>
<td>55.6 b</td>
<td>33.0 a</td>
<td>11.4 ab</td>
<td>1.7 b</td>
<td>9.1 a</td>
<td>423 ab</td>
</tr>
<tr>
<td>SEM</td>
<td>1.6</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.04</td>
<td>0.7</td>
<td>13.4</td>
</tr>
<tr>
<td><strong>Dicotyledonous cell walls</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Soya</td>
<td>17.1 a</td>
<td>8.0 b</td>
<td>54.3 c</td>
<td>33.6 b</td>
<td>12.1 a</td>
<td>1.6 b</td>
<td>7.2 b</td>
<td>391 b</td>
</tr>
<tr>
<td>Lupin</td>
<td>12.8 b</td>
<td>7.8 b</td>
<td>47.8 d</td>
<td>43.4 a</td>
<td>8.8 b</td>
<td>1.1 c</td>
<td>7.1 b</td>
<td>420 ab</td>
</tr>
<tr>
<td>Faba</td>
<td>14.4 ab</td>
<td>10.4 ab</td>
<td>60.2 a</td>
<td>31.4 b</td>
<td>8.4 b</td>
<td>1.9 a</td>
<td>9.8 ab</td>
<td>428 a</td>
</tr>
<tr>
<td>Pea</td>
<td>15.7 ab</td>
<td>12.1 a</td>
<td>57.8 b</td>
<td>32.8 b</td>
<td>9.4 b</td>
<td>1.8 ab</td>
<td>11.3 a</td>
<td>433 a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.9</td>
<td>0.7</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>0.04</td>
<td>0.7</td>
<td>6.7</td>
</tr>
</tbody>
</table>

\(^{a}\) Means with different letters within the groups of monocotyledons and dicotyledons, within one column differ significantly (\(P < 0.05\)).

\(^{b}\) HAc: acetic acid.

\(^{c}\) HPr: propionic acid.

\(^{d}\) HBu: butyric acid.

\(^{e}\) Standard error of the means.

### 3.4. Sugar degradation patterns

Fig. 2 shows the sugar degradation pattern of arabinose, xylose and glucose, for the four monocotyledonous substrates. The other sugars were present in low amounts, which made the analysis less reliable, and could have introduced large errors to the degradation pattern, and have, therefore, been omitted. For maize and wheat, the degradation of arabinose, xylose and glucose was similar. For rye, the degradation of glucose seemed more rapid, whereas for rice the degradation of glucose was slower than for arabinose and xylose.

Fig. 3 shows the sugar degradation pattern of arabinose, xylose, galactose, glucose, and uronic acids for the four dicotyledonous cell wall substrates. The sugar degradation pattern was fairly similar for the four substrates, with arabinose and galactose having the
most rapid, uronic acids and xylose an intermediate, and glucose the slowest degradation rate.

3.5. Cell wall characteristics and fermentation characteristics

The half-time of gas production (C) and maximal rate of substrate degradation (RM) were regressed on volume to surface area ratio (d_{3/2}, particle size), relative contamination, proportion of arabinoxylans, proportion of pectin, and content of: crude protein, TS, arabinose, xylose, galactose, glucose, and uronic acids. For the monocotyledons, three linear relationships (20 analysed) were significant (P < 0.05), these were:

\[
C \text{ (h)} = 18.9 + 0.31 \times \text{galactose content (g kg}^{-1}\text{TS}) \quad (r^2 = 0.86)
\]

\[
C \text{ (h)} = 16.15 + 0.25 \times \text{uronic acid content (g kg}^{-1}\text{TS}) \quad (r^2 = 0.93)
\]

\[
RM \text{ (% h}^{-1}) = 9.34 - 0.19 \times d_{3/2} \text{ value (µm}^3\text{ µm}^{-2}) \quad (r^2 = 0.91)
\]

The relationship between arabinose content and the maximal rate of substrate degradation was nearly significant (P = 0.1002):

\[
RM \text{ (% h}^{-1}) = 9.32 - 0.016 \times \text{arabinose content (g kg}^{-1}) \quad (r^2 = 0.71)
\]
For the dicotyledons, only one significant ($P < 0.05$) linear relationship was found:

$$C(h) = 146.8 - 0.151 \times \text{total sugar content (g kg}^{-1} \text{TS}) \quad (r^2 = 0.86)$$

The relationship between crude protein content and half-time of gas production was nearly significant ($P = 0.095$):

$$C(h) = 21.64 + 0.105 \times \text{crude protein content (g kg}^{-1}) \quad (r^2 = 0.73)$$

4. Discussion

4.1. Cell wall composition and characteristics

The crude protein content of the monocotyledon cell walls (wheat, rye and rice) was low, as would be expected for primary cell walls. However, maize cell walls had a higher crude protein content, which, however, was lower than reported by Van Dijk (1996). For the dicotyledons, the crude protein content of lupin, faba bean and pea cell walls was around 4% as was expected for the cotyledon cell walls of dicotyledons (Selvendran,
1983). The crude protein content of the soya cotyledon cell walls was higher than expected (Huisman et al., 1998; Van Laar et al., 1999), though similar levels have been found previously (Van Laar et al., 2000). The extraction procedures are not to be considered as absolute, isolating neither pure cell wall material nor all cell wall materials present, but are designed to obtain an isolate approximating the cell walls in the original material.

The sugar composition of the monocotyledon cell walls (rich in arabinose, xylose, and glucose) is characteristic for the monocotyledon primary cell wall, which is mainly composed of arabinoxylans and cellulose (maize: Harris et al., 1997; wheat: Gruppen et al., 1992). However, the rice cell wall is mainly composed of glucose, which is probably present mostly as cellulose and some xyloglucan (Selvendran, 1983). Some loss of β-glucans, which may be present in monocotyledon cell walls, may have occurred as these can be largely water-soluble (e.g. for barley: Viétor, 1992). The dicotyledon cell walls were either rich in arabinose or in galactose, whereas the levels of glucose and uronic acids were comparable (except for lupin cell walls). The results of Huisman et al. (1998) show that in similar cell wall extraction procedures, using endosperm soya bean material, more than 90% of the pectin fraction was recovered in the isolation procedure. Therefore some loss of pectin materials during isolation of the different endosperm cell wall types may have occurred. The arabinose and galactose in the dicotyledon cell wall are believed to be associated mainly with the pectin fraction by covalent linkage of arabinose and galactose containing polymers to a galacturonic acid backbone (for soya beans and lupins: Van de Vis, 1994). However, for lupins the galactose content is very high, which suggests that not all galactose can be covalently linked to a galacturonic acid backbone.

For the monocotyledons, the average particle size ($d_{3/2}$ value) varied considerably. Only the sieve widths for grinding of rice (0.5 mm) and rye flour (0.25 mm; Glitsø and Bach Knudsen, 1999) were known, whereas the wheat flour and maize endosperm were obtained in a ground state and were not reground. Although the sieve width used for rice was larger than for rye endosperm, the cell wall particles were smaller for rice than for rye (Table 2). For the cell walls from the dicotyledons, particle sizes were fairly similar, probably because the intact cotyledons had all been ground over a sieve width of 0.5 mm, and were similar in characteristics that influence particle size during grinding (all cotyledons).

4.2. Cell wall characteristics and fermentation

4.2.1. Linear regression between cell wall and fermentation characteristics

Studying the effects of cell wall characteristics on the maximal rate of substrate degradation ($R_{M, \% h^{-1}}$) and half-time of gas production ($C$, h) using linear regression, is a tentative approach to uncover the principles governing fermentation kinetics of a cell wall. For the monocotyledons, there was an increase in the half-time of gas production with increasing amounts of uronic acids and galactose. Possibly the micro-organisms fermenting the cell wall needed more time to be able to adapt their enzyme systems to uronic acids and galactose (pectin) in the cell wall. The maximal rate of substrate degradation was mainly influenced by the difference in particle size of the different
monocotyledon cell walls. Particle size, more correctly, surface area to volume ratio, has been known to be an important factor in cell wall fermentation (Chesson et al., 1997; Fisher et al., 1989; Weimer, 1996). Other significant relationships between cell wall characteristics and maximal rate of fermentation were not found, though these may have been concealed by the effect of particle size.

For the dicotyledon cell walls, the only significant relationship was between TS content and half-time of gas production. A higher TS content of the cell wall led to a shorter half-time of gas production. However, this relationship could be confounded with the nearly significant positive relationship between crude protein content and half-time of gas production (more crude protein, longer half-time). For the dicotyledons, there was no significant linear relationship between any of the individual sugars, and fermentation characteristics.

For both monocotyledons and dicotyledons, there was no significant linear relationship between contamination of the cell wall material with detergent residue, and the half-time of gas production nor with the maximal rate of substrate degradation. Therefore, the effect of the presence of a contaminant on the cell wall fermentation characteristics is considered to be negligible at the concentrations found in the present experiment.

4.2.2. VFA production profiles

For the monocotyledons, the VFA production profiles were similar for the fermentation of maize, wheat, and rye cell walls (Table 4). Therefore, it would appear that the similar composition (arabinoxylans and glucose) of these three monocotyledons led to similar VFA production profiles. The high propionic acid production for the fermentation of the rice cell walls is most probably a reflection of the rapid fermentation, which can stimulate propionic acid production (Van Houtert, 1993). This is contrary to the high glucose (cellulose) content of the rice cell wall, which would be expected to lead to an acetogenic fermentation (Hungate, 1966; Van Houtert, 1993).

For the dicotyledons, the differences in VFA production profiles between the substrates were much larger than for the monocotyledons. The VFA production profiles for faba bean and pea cell wall fermentation were fairly similar, with the highest relative acetic acid production, which may have reflected the high pectin content (Marounek et al., 1985). This would also be the case for the fermentation of soya bean cell walls, though this fermentation had a slightly lower proportion of acetic acid, and a higher proportion of butyric acid. The faba bean and pea cell walls are rich in arabinose, whereas the soya bean cell wall contains more galactose than arabinose. The high arabinose content of the pectin could possibly stimulate acetic acid production, which could be an explanation for the low proportion of acetic acid for the fermentation of lupin cell walls, which are mainly composed of galactose.

4.2.3. Sugar degradation patterns

The sugar degradation pattern (disappearance from the insoluble cell wall fraction) for the monocotyledons, was different for the four cell walls (Fig. 2). For maize and wheat cell walls, the degradation of arabinose and xylose was similar to the degradation of glucose. This is as would be expected from the degradation of monocotyledon cell walls from previous experiments with maize cell walls (Van Laar, unpublished) and with
grasses (Chesson et al., 1986; Gordon et al., 1983). Probably this is caused by the structure of the monocotyledon cell wall, which is a very tightly associated cellulose and hemicellulose (arabinoxylan) network (Carpita, 1996; Hatfield, 1993). Due to this tight association, arabinoxylans and glucose are degraded at similar rates, and one cannot be degraded without degradation of the other. For rye cell walls the degradation of glucose was more rapid than the degradation of arabinose and xylose. Glitsø et al. (1998) found a more complete faecal digestion of non-cellulosic cell wall glucose than for cellulose and arabinoxylans, when rye endosperm was fed to pigs. This more rapidly fermented non-cellulosic cell wall glucose was believed to be mainly \( \beta \)-glucan. For rice, glucose seemed to disappear more slowly from the cell wall than arabinose and xylose. However, the content of arabinose and xylose in the rice cell wall was very low, and, therefore, hard to determine accurately. For rice cell walls, it is hard to conclude whether the degradation rates of arabinose, xylose and glucose were different.

The sugar degradation patterns for dicotyledons were fairly similar with respect to the sequence with which cell wall sugars disappeared from the cell wall (Fig. 3). The relatively rapid degradation of arabinose and galactose, the slightly slower degradation of uronic acids and xylose, and the slow degradation of glucose, has also been found in previous experiments using soya bean cell walls (Van Laar et al., 1999). The cause of the sequence of degradation is believed to be a combination of the structures of dicotyledon cell walls and pectin. The pectin in most dicotyledons is composed of a galacturonic backbone, with branches of arabinose and galactose (Huisman et al., 1998; Van de Vis, 1994). Because the pectin molecule would be degraded from the outside inward the arabinose- and galactose-containing side-chains would be removed prior to degradation of the backbone. The pectin-rich cell wall of dicotyledons are composed of an relatively ‘open’ structure, in which a cellulose–xyloglucan network forms the backbone of the cell wall, and the space between this network is filled with pectin (Hatfield, 1993; Carpita and Gibeaut, 1993; McCann and Roberts, 1991). Probably because of this ‘open’ structure, pectin can and must be degraded first, before degradation of the cellulose, hemicellulose network, thus causing the differences in degradation of the different sugars within one cell wall.

4.2.4. Di-phasic curve fit

Fitting two phases through the gas production data can be used to obtain more information, when these phases can be correlated with the fermentation of a specific fraction from the substrate (Beuvink and Kogut, 1993). However, for the monocotyledons the division of the gas production into two phases did not agree with a division of the cell wall into arabinoxylans and cellulose. From the sugar degradation pattern it could be concluded that the cell wall of the monocotyledons is degraded as a whole, without preferential degradation of a specific polysaccharide. Rye may have been an exception, where some \( \beta \)-glucans may have been preferentially degraded, though these could not be separated form the degradation of the other glucose (cellulose). Therefore, the size and characteristics of the two phases for a di-phasic fit do not yield extra information on the fermentation of the monocotyledon cell wall.

For the dicotyledon cell wall, a division of the gas production into two phases agreed well with a division of the cell wall into a pectin (arabinose, galactose, uronic acids) and a
hemicellulose/cellulose fraction, for the soya and faba bean cell walls. The lack of agreement between the gas production phases and amount of pectin (arabinose, galactose, uronic acids) for lupin cell walls, might be explained by the assumption that a large proportion of galactose is not structurally related to the pectin molecule, and, therefore, would not be fermented in a single phase. The lack of agreement between the gas production phases and amount of pectin (arabinose, galactose, uronic acids) for pea cell walls remains unclear, although it is also possible that for pea cell walls not all arabinose is part of the pectin molecule.

5. Conclusions

For the monocotyledon cell walls an increase in particle size decreased the maximal rate of substrate degradation, which seemed to conceal possible compositional effects on fermentation rate. For the dicotyledon cell wall the amount of TS and protein seemed to influence the half-time of gas production. No clear effects of differences in sugar composition on cell wall fermentation could be found. This may be because of the small number of feedstuffs used, with too many confounding parameters (unequal particle size for the monocotyledons, unequal protein content for the dicotyledons).

For the monocotyledons, the disappearance of the individual sugars from the cell wall during fermentation was similar for cell walls, which did not contain β-glucans. Therefore, arabinoxylans and cellulose from the monocotyledon primary cell walls were broken down simultaneously. For the primary cell wall of dicotyledons, the sugars associated with the pectin molecule (arabinose, galactose, and uronic acids) were broken down more rapidly than glucose (cellulose). The cause of this discrepancy in the mode of breakdown of monocotyledon and dicotyledon cell walls is probably related to the structural arrangement and functional properties of the polysaccharides within the cell wall.

This research shows that even between the primary cell walls of feedstuffs (monocotyledons and dicotyledons) there are differences in rate and extent of fermentation. Therefore, a better description of the fermentable carbohydrate fraction (e.g. what type of cell walls and polysaccharides) in pig diets may lead to a better control over the fermentation processes in the large intestines of pigs.

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