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Residual Carbohydrates from in Vitro Digested Processed Rapeseed (*Brassica napus*) Meal

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ABSTRACT: Rapeseed meal (RSM) was subjected to different physical or chemical pretreatments to decrease residual, hard to degrade carbohydrates and to improve fermentability of RSM polysaccharides. Next, these pretreated samples were in vitro digested and fermented, with or without the addition of commercial pectinolytic enzymes. Remaining carbohydrates were quantified, and two physical characteristics were analyzed: (1) water-binding capacity (WBC) of the insoluble residue and (2) viscosity of the soluble fraction. Mild acid pretreatment in combination with commercial pectinolytic enzyme mixtures showed best digestion of RSM carbohydrates; only 32% of the total carbohydrate content remained. For most pretreatments, addition of commercial pectinolytic enzymes had the strongest effect on lowering the WBC of the in vitro incubated RSM. In the cases that less carbohydrate remained after in vitro digestion, the WBC of the residue decreased, and less gas seems to be produced during fermentation.

KEYWORDS: *Brassica napus, in vitro digestion, fermentability, carbohydrate degradability, water binding capacity, viscosity, processing, animal nutrition*

INTRODUCTION

Rapeseed meal (RSM) is a byproduct from the production of rapeseed oil, which is used as an edible oil as well as for biodiesel. After solvent extraction of the oil, the meal is dried by solvent-toasting and used as animal feed for its high protein content. RSM has an energy value (net energy/8.8) of 0.71 MJ/kg for pigs.[1] The high demands for biofuels led to an increase in the production of biodiesel, and therefore more RSM will become available for the animal feed industry. Apart from protein, RSM is rich in nonstarch polysaccharides (NSP), which potentially could be used as an energy source in animal feed. Monogastric animals lack the necessary digestive enzymes that can degrade NSP. Still, in the large intestine (and already in the small intestine of pigs) these carbohydrates are fermented into short-chain fatty acids, gases, and microbial biomass.[2]

Pectins are the main carbohydrates present in dehulled rapeseed.[3] Pectins are a very diverse group of polysaccharides, consisting of homogalacturonan, rhamnogalacturonans I and II, and xylogalacturonan. Along with pectins, cellulose and hemicelluloses (like xyloglucan) are present. Apart from polysaccharides RSM also contains sucrose and oligosaccharides, such as raffinose and stachyose.[4]

An option to increase the energy value of RSM is to stimulate the degradability of polysaccharides for (monogastric) animals by pretreatment of the RSM before inclusion into the diet. Hereto, different techniques such as fine milling, extrusion, chemical pretreatment, and enzyme addition are good candidates. Milling increases the surface area of a product. Shear is used to increase the extractability of soluble carbohydrates and the accessibility for (digestive) enzymes.[5] During extrusion the product is exposed to a high temperature (90−160 °C) for a short time (30−120 s) at high pressures and at relatively low moisture contents (<30%).[5] Chemical pretreatment with sulfuric acid has been the traditional method to increase extractability of carbohydrates from lignocellulosic material. Recently, also dicarboxylic organic acids at elevated temperatures have been shown to open up the cell wall and to increase carbohydrate solubility.[6] These could be more suitable in animal nutrition, because no additional sulfates will be introduced in the diet.

Apart from the above-described pretreatments, addition of NSP-degrading enzymes is an option to open up the cell wall material and make it more accessible for digestive enzymes. Along with polysaccharide degradation, they can reduce digesta viscosity and detrimental effects on digestion and absorption.[7]

So far, RSM digestion studies have focused on protein digestibility,[8,9] or when focused on polysaccharide digestibility, only neutral and acid detergent fiber content were analyzed.[10] In vitro digestibility studies of other oil seeds, for example, sunflower meal and soybean meal, have been performed. Addition of commercial enzymes was shown to increase total carbohydrate solubilization and decreased viscosity;[11] however, constituent glycosyl residues were not analyzed. The aim of the present study is to improve the in vitro digestibility of RSM and to characterize residual carbohydrates and their constituent glycosyl residues. Especially the combination of pretreatment and the addition of commercial pectinolytic enzymes are hypothesized to have significant effects on carbohydrate digestion. Digestibility is evaluated by an in vitro digestion and fermentation study. The changes in carbohydrate

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composition and molecular weight distribution are analyzed and related to water-binding capacity (WBC) and viscosity, respectively, which are also important aspects for the transit in the gastrointestinal tract.

MATERIALS AND METHODS

Plant Material. Rapeseed meal (90% w/w dry matter) was supplied by Nutreco (Boxmeer, The Netherlands).

Treatments. The effects of technological pretreatment and commercial pectinolytic enzyme addition were studied. Different technologies and accompanying settings, such as temperature, flow, concentration, and residence time, were tested in pre-experiments, with the criterion being the optimal solubilization of NSP from rapeseed meal.

Wet-milling was performed using a laboratory-scale refiner (Sprout-Waldron, Muncy, PA, USA) with a feed rate of 150 kg/h, a water flow rate of 480 L/h at 3000 rpm, and a distance of 0.07 mm between the disks. The temperature of the processed product was around 36 °C. Samples were freeze-dried before analysis.

Extrusion was performed with a double-screw extruder (Baker-Perkins Ltd., Peterborough, UK) at 120 °C (product temperature = 119 °C), 250 rpm screw speed, and die size = 6 mm at 20% (w/w) moisture.

Autoclave pretreatment was performed using a Varioklav 25T tabletop (Thermo Scientific, Waltham, MA, USA) at 120 °C for 30 min at 20% (w/w) moisture.

Mild acid pretreatment was performed after the samples had been soaked in 11 mM (or 1.4% w/w acid based on dry matter of the substrate) maleic acid at a concentration of 100 g/L. Samples were heated in the above-described autoclave for 30 min at 130 °C. The final pH of the solution after heat treatment was 5.2. Samples were freeze-dried before analysis.

Commercial enzyme mixtures Pectinex UltraSP (Novozymes, Bagsvaerd, Denmark) and Multifect Pectinase PE (Genencor, Rochester, NY, USA) were, if appropriate, added (250 μL per 10 g of substrate for each enzyme mixture) in the first step of the in vitro digestion.

In Vitro Digestion and Fermentation. In vitro digestion was performed according to the method of Boisen and Fernandez[12] as modified by Sappok et al.[13] without further milling. Briefly, RSM was incubated in duplicate with pepsin at pH 3.5 at 40 °C for 75 min, followed by the addition of pancreatin and amyloglucosidase and incubation at pH 6.8 at 40 °C for 3.5 h. The final residues were washed with demineralized water, centrifuged (twice 10 min at room temperature and 3030*g*), decanted, freeze-dried, and used for analyses. Also, the corresponding supernatants were collected and kept frozen for further analyses of the soluble fraction.

Residues were used as a substrate for in vitro fermentation according to the method of Williams et al.[14] using fecal inocula from sows receiving commercial diets containing the NSP sources 30% barley, 20% wheat middlings, 10% maize, 7.5% rapeseed meal, 5% wheat, 5% soy hulls, and 1.5% linseed. Besides this, the diet contained synthetic amino acids, salts, and oil. Samples were incubated at pH 6.8 at 40 °C for 72 h, and cumulative gas production was measured in time with the fully automated time-related gas production system.[15]

Analytical Methods. *The geometric mean diameter (GMD)* of untreated RSM was analyzed using the wet sieve method and calculated according to the ASAE method.[16] The GMD of pretreated RSM was analyzed on a CoulterCounter (Beckman Coulter, Brea, CA, USA).

Protein content (N × 5.3) was determined according to the Dumas method of the AOAC[17] on a Thermo Quest NA 2100 Nitrogen and Protein Analyzer (Interscience, Troy, NY, USA). Sample (~10 mg) was weighed into a cup and directly analyzed. D-Methionine was used for calibration.

Total starch content was determined enzymatically using the total starch assay procedure K-TSTA 04/2009 (Megazyme, Bray, Ireland).

Neutral sugar composition was determined by gas chromatography according to the method of Englyst and Cummings.[18] After a pretreatment with 72% (w/w) H_2SO_4 for 1 h at 30 °C, the samples were hydrolyzed with 1 M H_2SO_4 at 100 °C for 3 h. Afterward, the constituent glycosyl residues were derivatized into alditol acetates and analyzed using a GC (Focus-GC, Thermo Scientific, Waltham, MA, USA). Inositol was used as internal standard.

Uronic acid content was determined according to the automated colorimetric *m*-hydroxydiphenyl assay[19] using an autoanalyzer (Skalar Analytical, Breda, The Netherlands). Galacturonic acid was used for calibration.

Lignin content was determined gravimetrically. After a pretreatment with 72% (w/w) H_2SO_4 for 1 h at 30 °C, samples were hydrolyzed with 1 M H_2SO_4 at 100 °C for 3 h. Samples were filtered over a G4 glass filter (Schott Duran). The acid-insoluble residue was dried (105 °C, 18 h) and weighed.

Water-binding capacity (WBC) was determined by soaking 250 mg of raw material or freeze-dried residue in 10 mL of water for 24 h at room temperature. Samples were centrifuged at 3274*g* for 20 min at room temperature and subsequently drained inverted for 15 min. WBC was calculated as grams of water held per gram of dry material.

Viscosity of the soluble fraction was determined with a rheometer (RheoLab QC, Anton Paar GmbH, Graz, Austria) with a double-gap module attached. Prior to measurement, samples were freeze-dried and solubilized in water to $^1/_{10}$ of the original volume. Viscosity was measured at 40 °C and a shear rate of 122 s^{-1}, mimicking the gastrointestinal tract.[20]

High-performance size exclusion chromatography (HPSEC) was performed on an Ultimate 3000 system (Dionex, Sunnyvale, CA, USA) equipped with a set of four TSK-Gel superAW columns (Tosoh Bioscience, Tokyo, Japan) in series: guard column (6 mm i.d. × 40 mm) and separation columns 4000, 3000, and 2500 (6 mm i.d. × 150 mm). Samples (25 μL) were eluted with filtered aqueous 0.2 M sodium nitrate at 40 °C at a flow rate of 0.6 mL/min followed by refractive index detection (Shodex RI 101; Showa Denko K.K., Kawasaki, Japan). Prior to analysis, samples were freeze-dried and solubilized in water to $^1/_{10}$ of the original volume. Calibration was performed using pullulan standards of 180 and 738 Da and 6, 12, 23, 47, and 112 kDa (Sigma, St. Louis, MO, USA).

From the HPSEC elution, fractions were collected using a Gilson FC204 fraction collector (Gilson Inc., Middleton, WI, USA), collecting 0.42 min per well. Of the fractions of interest, sugar composition was determined after drying at 40 °C under a stream of nitrogen gas. Samples were analyzed by methanolic HCl/TFA hydrolysis, by first solvolyzing them in 2 M HCl in dry methanol at 80 °C for 16 h, followed by hydrolysis with 2 M TFA at 121 °C for 1 h.[21] The monomers were analyzed by high-performance anion exchange chromatography (HPAEC), and postcolumn addition was performed on an ICS-3000 unit (Dionex, Sunnyvale, CA, USA) using a CarboPac PA1 column (2 × 250 mm). Samples (25 μL) were eluted isocratically in 30 min with Millipore water. Afterward, the following elution profile was applied: 30−45 min, 0.1 M NaOH−0.4 M NaOAc in 0.1 M NaOH; 45−50 min, 1 M NaOAc in 0.1 M NaOH; 50−58 min, 0.1 M NaOH; 58−73 min, Millipore water (equilibration). A flow of 0.1 mL/min 0.5 M NaOH was added postcolumn allowing pulsed amperiometric detection.

Calculations and Statistical Analysis. *Calculations on in Vitro Fermentability.* Data of cumulative gas production (mL/g DM) for each bottle were modeled according to the monophasic model described by Groot et al.[22]

$$G = \frac{A}{1 + \left(\frac{B}{t}\right)^C} \quad (1)$$

where G is the total gas production (mL/g DM), A the asymptotic gas production (mL/g DM), B the time at which half of the asymptotic gas production has been reached (h), C the switching characteristic of the curve, and t the time (h).

Statistical Analysis. In a 5 × 2 factorial arrangement, the effects of technological pretreatment (T) and commercial pectinolytic enzyme addition (E) were studied. The results from the in vitro experiment were statistically analyzed using the General Linear Models procedure

with a SAS program (SAS Institute, version 9.2). The model used to describe the data was

$$Y_{ij} = \mu + T_i + E_j + T_i \times E_j + \varepsilon_{ij} \quad (2)$$

where Y_{ij} is the response variable, μ the overall mean, T_i the technological pretreatment (i = untreated, wet mill, autoclave, extruder, or mild acid pretreatment), E_j the addition of commercial pectinolytic enzymes (j = yes or no), $T_i \times E_j$ the interaction between technological pretreatment and enzyme addition, and ε_{ij} the error term. Residuals were tested for normality, using the Shapiro–Wilk test.

A posthoc test was performed using multiple comparisons with Tukey adjustment.

RESULTS AND DISCUSSION

Characteristics of RSM. Industrial RSM (Table 1; untreated) is mainly composed of carbohydrates (48%), protein (33%), lignin (13%), fat (3%), and ash (3%). Apart from cellulose, the sugar composition (table 1) indicated the presence of homogalacturonan, arabinan, and minor amounts of xyloglucan and arabinogalactan, which was expected.[3,23] Wet-milling and mild acid pretreatment decreased the total sugar content slightly, from 48% in untreated RSM to 45 and 43% w/w DM, respectively. Only small changes in sugar composition were noted, so all glycosyl residues were assumed to be stable during processing. The starch content was very low (<1% w/w DM) for all samples.

Table 1. Composition, Particle Size, and Water-Binding Capacity (WBC) of Rapeseed Meal (RSM), before and after Treatment

	untreated	acid	autoclave	extruder	wet mill
protein[a]	33	29	29	26	30
total carbohydrates[a]	48	43	48	50	45
(of which starch[a])	(1)	(1)	(1)	(1)	(1)
lignin[b]	13	na[c]	na	na	na
molar composition of carbohydrates[d]					
Rha	0	1	1	0	1
Ara	13	14	13	13	14
Xyl	6	7	6	5	6
Man	6	5	4	12	9
Gal	7	8	7	7	7
Glc	28	29	27	21	24
UA	40	38	43	42	40
particle size (μm)	520	499	520	343	135
WBC (g water/g material)	2.4	4.8	2.4	3.9	3.5

[a]g/100 g of dry matter. [b]Acid-insoluble lignin. [c]Not analyzed. [d]mol %; presented as anhydrosugar moieties; Rha = rhamnose, Ara = arabinose, Xyl = xylose, Man = mannose, Gal = galactose, Glc = glucose, UA = uronic acid.

The particle size distribution was affected by the various types of pretreatment (Table 1). The untreated RSM used in this study had a GMD of 520 μm; autoclave and mild acid pretreatment were similar. After extrusion and wet-milling, the GMD decreased to 343 and 135 μm, respectively. Particle size reduction is known to increase digestibility in pigs of dry matter and NSP in barley[24] and dried distillers grains with solubles (DDGS).[25]

The WBC was affected by processing (Table 1). Without processing, 1 g of untreated RSM could hold 2.4 g of water. After wet-milling, extrusion, and mild acid pretreatment, the WBC increased to 3.5, 3.9, and 4.8 g water/g RSM, respectively. During milling, the surface area of the product increases, potentially increasing the water-holding capacity as reported for, for example, peas.[26] During extrusion, the cell wall is opened up by heat and shear, creating pores in which water can be trapped.[27] During mild acid pretreatment, the lower pH probably degrades acid labile pectin structural elements, thereby increasing the WBC even more.

In Vitro Incubation. For the first time, this paper presents a detailed study on residual carbohydrates and their constituent glycosyl residues after in vitro digestion of RSM. Solubles were separated from the residues after in vitro digestion. Residues were analyzed separately, because the carbohydrate structures that cannot be digested are of interest and it is known that soluble carbohydrates are more easily digested and fermented in vivo than insoluble carbohydrates.[28] However, the in vivo part of the solubilized carbohydrates will still be available for fermentation.

After in vitro digestion, 45–50% of total NSP remained for (un)treated RSM. When looking at the untreated RSM after in vitro incubation, nearly all of the arabinosyl (19% w/w of total sugar) and xylosyl (8% w/w) residues remained in the residue, whereas up to 60% of the glucosyl and uronyl residues are digested. Between pretreatments no large differences were seen (Figure 1). When commercial pectinolytic enzymes were added after wet-milling and extrusion, remaining NSP decreased to 36% and for mild acid pretreatment even to 32%. This indicates that the NSP fraction becomes more accessible for commercial pectinolytic enzymes after these two pretreatments. The interaction between thermal pretreatment and addition of commercial pectinolytic enzymes has previously been found for apparent ileal digestibility of pretreated soybean.[8]

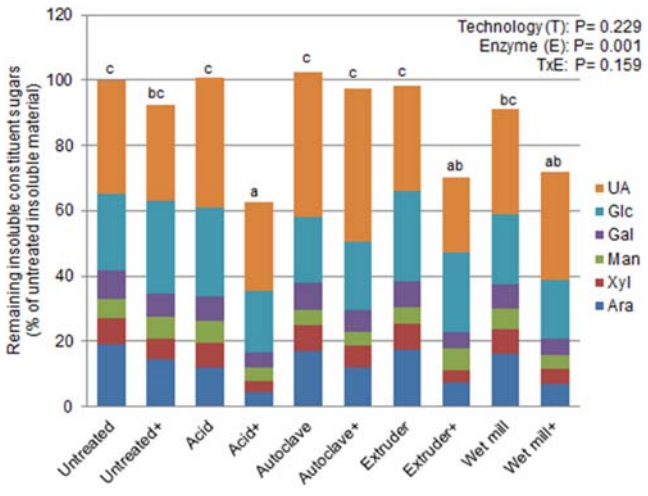

Figure 1. Constituent NSP sugars in insoluble fraction after in vitro incubation of (pretreated) RSM. Ara = arabinose, Xyl = xylose, Man = mannose, Gal = galactose, Glc = glucose, UA = uronic acid, + = with the addition of commercial pectinolytic enzymes. Bars with the same letter on top represent total sugar levels that are not significantly different.

With regard to individual constituent sugars, large differences were seen between pretreatments. An obvious decrease was determined for polymeric arabinosyl (shown in Figure 2) and

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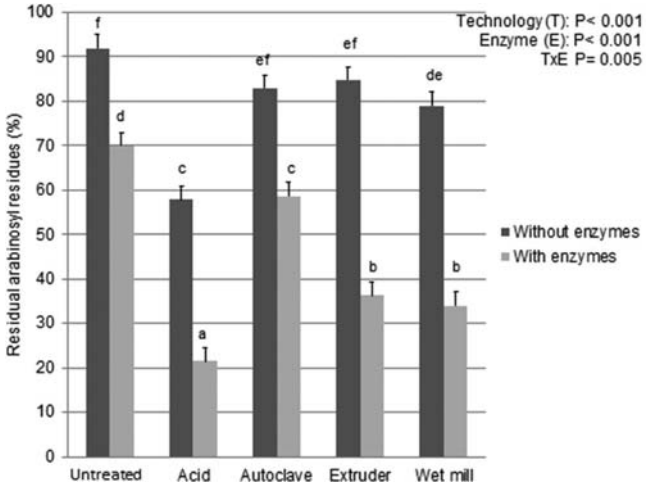

Figure 2. Residual arabinosyl residues (as percent of starting material) after in vitro incubation of (pretreated) RSM either without or with the addition of commercial pectinolytic enzymes. Bars with the same letter on top are not significantly different.

xylosyl (not shown) after the mild acid pretreatment. These five-carbon sugars are known to be more acid-labile than six-carbon sugars.[29] The studied pretreatments, without commercial pectinolytic enzyme addition, hardly affected the contents of mannosyl, glucosyl, and uronyl residues (Figure 1).

After commercial pectinolytic enzyme addition to untreated, wet-milled, extruded, and mild acid pretreated RSM, fewer arabinosyl residues remained than without the addition of commercial pectinolytic enzymes (70, 34, 36, and 21% remaining after in vitro incubation, respectively). Apparently, arabinan in RSM is rather well enzyme degradable when preceded by a pretreatment. After commercial pectinolytic enzyme addition to mild acid pretreated RSM, also fewer uronyl residues remained than without commercial pectinolytic enzyme addition.

The WBC of the incubated residues is shown in Figure 3-I. For untreated RSM it was 9 g water/g material (or 5 g water/g starting material, Figure 3-II), whereas untreated RSM that has not been digested in vitro binds 2.4 g water/g material (Table 1). Expression in grams of water bound per gram of starting material takes into account that less DM remained after in vitro incubation. An increase in WBC during in vitro incubation means that the cell wall structure is affected in such a way that more water can be held per gram of material. Addition of commercial pectinolytic enzymes did not have an effect on the WBC of untreated RSM. After commercial pectinolytic enzyme addition to wet-milled, extruded, and mild acid pretreated RSM, the WBC of the in vitro incubation residue decreased to 5.6, 6.4, and 6.5 g water/g residue, respectively, compared to without commercial pectinolytic enzyme addition. When WBC was expressed as grams of water bound per gram of starting material, there was a decrease compared to the untreated RSM after in vitro incubation (2.4−2.8 and 4.8 g water/g starting material, respectively). As expected, cell wall material is degraded by the addition of commercial pectinolytic enzymes so extensive that the material can hold less water.

Viscosity and Molecular Weight Distribution of Soluble Polysaccharides from in Vitro Incubation. The intestinal viscosity is partly determined by the soluble polysaccharides in the digesta and influences the passage rate in vivo.[28] Therefore, the soluble fraction of the in vitro

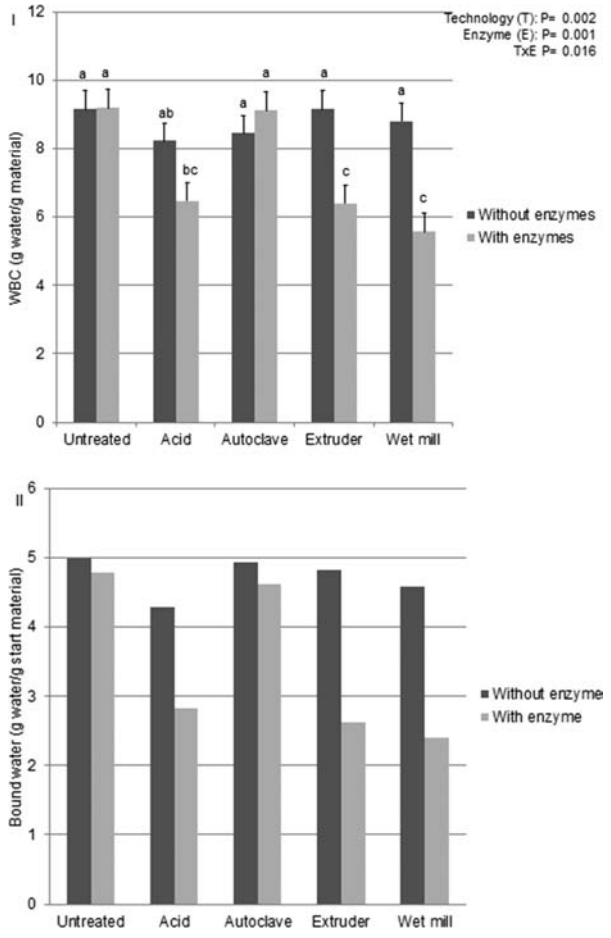

Figure 3. Water-binding capacity (g water/g dry matter) of starting material and residues from in vitro incubation of (pretreated) RSM either without or with the addition of commercial pectinolytic enzymes: (I) WBC represented as g water/g residue from in vitro incubation; (II) WBC represented as g water/g starting material for in vitro incubation. Bars with the same letter on top are not significantly different.

incubation was analyzed for its viscosity (Figure 4) together with the apparent molecular weight of the solubilized polysaccharides (HPSEC; Figure 5). It should be noted that viscosity continuously changes as digestion proceeds,[30] and only the end point has been analyzed in this study. Nevertheless, the values indicate trends in viscosity changes provoked by the various treatments.

The viscosity of the soluble fraction of the incubation of untreated RSM was 2.8 mPa·s. This value is in the same order of magnitude as the viscosity of in vivo digesta at the end of the small intestine for pigs fed a wheat-containing diet.[31] Wet-milling and mild acid pretreatment increased viscosity to 4.6 and 4 mPa·s (Figure 4), respectively, even though similar amounts of sugars were solubilized (assuming no sugars were lost). In Figure 5, it can be seen that high molecular weight material (peak A; >400,000 Da) was solubilized during in vitro incubation of untreated RSM. From wet-milled RSM, the area under peak A increased 1.8 times, meaning that more of this molecular weight population was solubilized during wet-milling compared to untreated RSM. Simultaneously, viscosity increased 1.6 times by wet-milling compared to untreated RSM. With the addition of commercial pectinolytic enzymes to wet-milled RSM, the area under peak A still increased 1.3 times

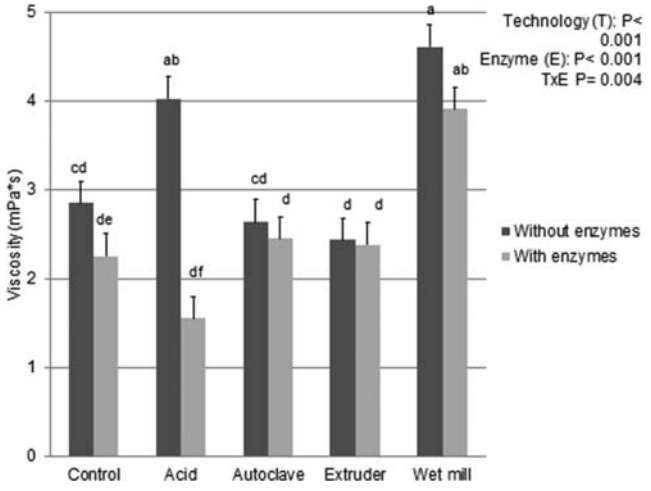

Figure 4. Viscosity (mPa·s) of solubles from in vitro incubation of (pretreated) RSM either without or with the addition of commercial pectinolytic enzymes. Bars with the same letter on top are not significantly different.

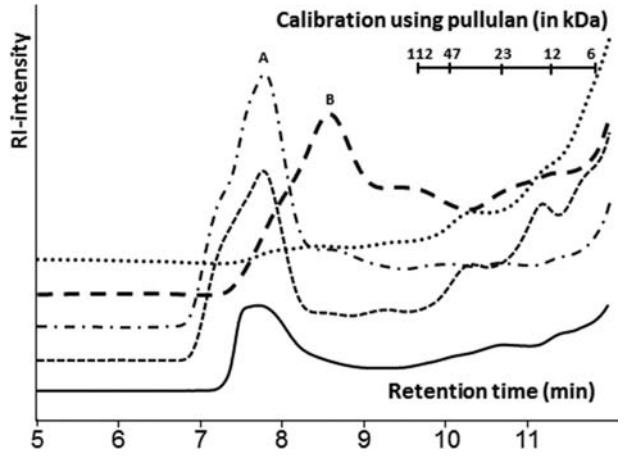

Figure 5. HPSEC elution pattern of supernatants after in vitro incubation of pretreated RSM either without or with the addition of commercial pectinolytic enzymes compared to untreated RSM. From bottom to top: untreated (solid line), wet mill (square dotted line), wet mill with enzymes (dash-dotted line), mild acid (dashed line), and mild acid with enzymes (round dotted line).

compared to wet-milled RSM to which no enzymes have been added. Viscosity decreased slightly by enzyme addition to wet-milled RSM, although not significantly. Apparently, besides increasing solubilization of material under peak A, the pectinolytic enzymes added also broke down interactions between carbohydrates, thereby lowering the viscosity.

After in vitro incubation, in the supernatant of mild acid pretreated RSM, peak A is absent and peak B (around 306,000 Da) appeared (Figure 5). After commercial pectinolytic enzyme addition, this peak B disappeared. Apparently, the mild acid pretreatment made the NSP fraction better accessible for the commercial pectinolytic enzymes. Commercial pectinolytic enzyme addition to mild acid pretreated sample decreased viscosity from 4.0 to 1.6 mPa·s, so peak B was responsible for the increase in viscosity when compared to untreated RSM (2.8 mPa·s). Peaks A (untreated) and B (mild acid pretreated) were collected and analyzed for their glycosyl composition by methanolic HCl/TFA hydrolysis. Peak A was found to consist of 67 mol % glucosyl and 16 mol % xylosyl residues. Peak B was found to consist of 55 mol % glucosyl and 24 mol % xylosyl residues. Carbohydrates represented in peaks A and B were possibly xyloglucans, which are common in dicotyledonous plants.[32] The xyloglucan in peak B has a higher xylose/glucose molar ratio than peak A (0.44 and 0.24, respectively). Arabinosyl, galactosyl, and mannosyl residues were found in equal ratios, which could be present as side chains on the xyloglucan.

In Vitro Fermentation. The washed residues from in vitro digestion were inoculated and fermented. The cumulative volume (DMCV) of gas produced is presented in Figure 6. In

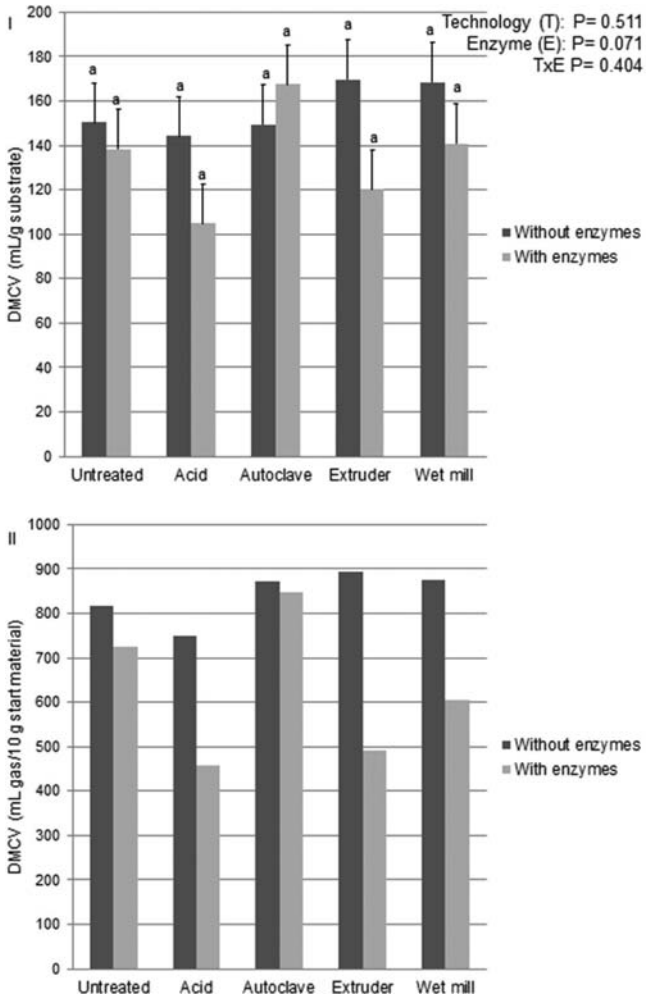

Figure 6. Cumulative gas produced during in vitro fermentation of (pretreated) RSM either without or with the addition of commercial pectinolytic enzymes: (I) DMCV represented as milliliters of gas produced per gram residue from in vitro incubation; (II) DMCV represented as millliters of gas produced per gram starting material for in vitro incubation. Bars with the same letter on top are not significantly different.

Figure 6-I the DMCV is expressed in milliliters of gas produced per gram residue of in vitro digestion, and in Figure 6-II the DMCV is expressed as milliliters of gas produced per gram of starting material before in vitro incubation. Expression per gram starting material represents better the fermentability of the *whole* feed material than the amount of gas per gram residue, which is generally used in the literature (and shown in Figure 6-I). The effect of the in vitro digestion of (un)treated RSM was

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as such that most of the carbohydrates were solubilized and removed before fermentation. In vivo these soluble carbohydrates will also be available for fermentation by the microflora in the large intestine if they are not digested.

From samples with no commercial pectinolytic enzymes added, on average 84 mL of gas was produced per gram of starting substrate. No significant differences were seen between the different technological pretreatments ($P = 0.511$). Although no significant difference was found, a trend is visible that addition of commercial pectinolytic enzymes to mild acid pretreated, extruded, and wet-milled RSM decreases gas production compared to untreated RSM. Commercial pectinolytic enzyme addition to autoclaved RSM did not show this trend in decreasing gas production.

Overall, gas production from RSM was low when compared to other substrates, which were around 240 mL gas/g of organic matter after in vitro digestion of corn silage, rye grass, and Jerusalem artichoke.[13] These raw materials are higher in neutral detergent fiber and lower in protein compared to RSM,[33] which could explain the lower amount of gas produced.

When less DM and less carbohydrate remained in the residue after in vitro digestion, less gas seemed to be produced. This seems to be the trend for mild acid pretreated, extruded, and wet-milled RSM to which commercial pectinolytic enzymes were added. Although differences in the sugar composition of the in vitro digestion residues exist (Figure 1), analysis of the fermentation residues showed very similar sugar compositions (data not shown).

In conclusion, from the different technological pretreatments tested, mild acid pretreatment only when used in combination with commercial pectinolytic enzymes showed to be the best in digesting RSM carbohydrates, because the least residual carbohydrate was found. Especially residual arabinosyl and uronyl contents decreased with mild acid pretreatment and enzyme addition. This treatment also showed to be the best in decreasing the WBC of insoluble material and viscosity of soluble fraction. No significant differences between the treatments were seen in the fermentation of in vitro digestion resistant residues. Subsequent studies will be in vivo digestion trials using pretreated RSM fed to chicken and pigs.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

DM, dry matter; NSP, nonstarch polysaccharides; RSM, rapeseed meal; WBC, water-binding capacity.

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