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# **Differential fermentation** of glucose-based carbohydrates in vitro by human faecal bacteria

A study of pyrodextrinised starches from different sources\*

■ **Summary** *Background* Pyrodextrins, modified starches produced by heat/acid treatment, have been used extensively in the paper industry. Recently, pyrodextrinisation has been recognised as a way of producing a "resistant starch" that is watersoluble and has non-starch link-

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ages. However, a full characterisation of the fermentation properties of pyrodextrins has not been reported. Aim of the study To evaluate the effect of pyrodextrinisation on the fermentation characteristics of starches, prepared from Venezuelan crops, in a simple in vitro model of the human colon. *Methods* Potato, lentil and cocoyam pyrodextrins were produced using heat (140 °C for 3 h) and hydrochloric acid as catalyst (1.82 g/kg starch). Then, both native and modified starches were pre-digested with pepsin and pancreatic enzymes and their resistant components fermented anaerobically using human faeces as inocula for 24 h. Short-chain fatty acids (SCFA), pH, residual starch and carbohydrate in the cultures were measured. Results More than 69% of initial carbohydrate disappeared from both pre-digested native and pyroconverted starch cultures. More

than 6.8 and 10.0 mmol net SCFA per gram carbohydrate were produced from pre-digested native and pyrodextrinised starches, respectively. In cultures of predigested pyrodextrins, the molar ratio for propionate doubled, whereas the ratio of acetate decreased by 25 % when compared with pre-digested native starches. The ratio of butyrate did not change. Conclusions The mechanism for the change in SCFA profile is unclear, but may be related to solubility and/or presence of nonstarch linkages. The presence of these bonds may modify the accessibility/affinity of bacterial enzymes to the modified starch structure.

■ **Key words** pyrodextrin – shortchain fatty acids – modified starch pyroconversion – colonic fermentation

# Introduction

Starch modification techniques have been developed for industrial processing to produce a wide range of potential food ingredients, including pyrodextrinised starches. However, interest in modified starches has been restricted mainly to technological aspects with little concern about the possible impact of the modification on the digestibility and fermentability of the product [1, 2].

Pyrodextrins are produced by reactions that take place under the influence of heat, often in the presence of catalytic amounts of ions. These modified starches were first reported nearly 200 years ago, as a water soluble and gummy material from the roasting of starch [3]. Native starch is composed of glucose units linked by  $\alpha(1\rightarrow 4)$  and  $\alpha(1\rightarrow 6)$  glycosidic bonds, which can be broken down by the digestive enzymes present in the small intestine. Starch pyrodextrinisation occurs because of

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hydrolysis, transglucosidation and, in some cases, repolymerisation reactions of glucans. After hydrolysis, the new reducing end group of the glucose chain becomes a glucosyl cation, which can undergo either intermolecular bond formation between two chains or intramolecular dehydration, leading to the formation of 1,6-anhydro-β-D-glucose [4, 5]. Therefore, pyroconverted starches are branched, low molecular weight products showing new glycosidic linkages. It is claimed in the European patent EP 0 540 421 A1 that the non-digestible fraction of a potato pyrodextrin had one-third of its glucose residues at the non-reducing end of a chain, and one-sixth of glucose residues presented atypical linkages, such as  $(1\rightarrow 3)$  and  $(1\rightarrow 2)$ . In addition, four main groups of molecular size for pyrodextrinised maize starch with apparent Mw at 5,000; 10,000; 19,000 and 40,000 have been described [6].

Although it has been recognised for some time that pyrodextrins prepared without any catalyst added were resistant to digestion [7], data about the digestibility and fermentability of pyrodextrins (with or without catalyst) are not readily available. Recently, other authors have found not only that acid catalysed pyroconversion promoted the generation of a significant proportion of non-digestible fractions (around 60%), but also these changes differed in magnitude depending on the starch source [2, 6]. A decrease in rat caecal content pH and an increase in butyrate was reported when rats were fed with corn pyrodextrin enriched diet in comparison with a corn fibre diet [8]. However, full characterisation of the fermentation of pyrodextrins from other plant sources and in comparison to their native starches has not been reported.

In recent years, resistant starches have been recognised for the contribution they can make to human health throughout their interaction with the gut. In particular, the increased butyrate production as this shortchain fatty acid (SCFA) may have anticancer and anti-inflammatory effects on the colo-rectal mucosa [9, 10]. Pyrodextrinised starches share some of the properties of resistant starch. They are partially fermented, producing more butyrate than non-starch polysaccharides and a low colonic pH [8]. On the other hand, pyrodextrinised starch, but not resistant starch [11], decreased gut transit time in rats [12] and may lower serum cholesterol and neutral fat in humans [13]. As pyrodextrinised starches have different glycosidic bonds from resistant starch, they may be fermented by different bacterial species and both the process and end result of fermentation may differ.

The aim of this work, therefore, was to evaluate the effect of pyrodextrinisation on the fermentation characteristics of starches, prepared from potato, lentil and cocoyam, as a substrate in a simple *in vitro* model of the human colon.

### Materials and methods

# Starch sources

Lentil (*Lens esculenta* Medic.) seeds and cocoyam (*Xanthosoma sagittifolium* (L.) Schott.) roots were purchased from a local market in Caracas (Venezuela). Commercially isolated potato starch was purchased from Lyckeby Stärkelsen (Kristianstad, Sweden).

### Starch isolation

Starch was isolated as described elsewhere [14]. Briefly, decorticated lentil grains or peeled and diced cocoyam roots were homogenised in a liquidiser using one volume of water. The suspension was filtered through a 280mesh cloth several times (usually 7 times), each time adding one volume of water, until no material passed through the cloth. The filtered material (essentially starch) was washed out with an equal volume of water, three or four times by centrifugation (1,500 g for 15 min), removing the lipid layer out of the surface in each centrifugation step until none appeared. Before the last washing, the pH was adjusted to neutrality. Then, the starch was dried in an oven at 45 °C for 24 h, sieved out (250 µm pore), and stored at room temperature. Additionally, lentil grains were steeped in 62.5 mmol/l NaOH solution for 24 h at room temperature and washed out extensively afterwards, as a pretreatment to facilitate the peeling of the grains.

Yield was 24% and 45% (d. b.) for lentil and cocoyam starches, respectively. It was low as the method used [14] was developed for the isolation of amaranth starch and no attempt to improve the yield for other sources was made because the emphasis was put on a high starch purity. The estimated starch content of the preparations used in this study was 94.7% and 96.8% for lentil and cocoyam, respectively [6]. Additionally, the protein content was 0.24% for lentil [15] and 0.56% for cocoyam [16] starches. Therefore, the starting materials for the pyrodextrinisation step were mainly starch and the impact of other components, such as antinutritional factors, was most likely minor.

# Starch pyrodextrinisation

Isolated starches (22 g) were sprayed with 0.5 ml HCl (1.82 g acid/kg starch), mixed thoroughly and left overnight at room temperature. Then, they were heated at 140 °C for 3 h, milled and sieved through a 250  $\mu$ m pore size mesh [17].

# Starch pre-digestion

To remove the digestible starch, both native and pyrodextrinised starches were pre-digested using an Englyst starch kit (Englyst Carbohydrate Services Ltd, Cambridge, UK) with some protocol modifications. Briefly, 0.8 g starch and 50 mg guar gum were weighed in a centrifuge tube, then 10 ml of 5 g/l pepsin suspension in 50 mmol/l HCl were added, vortex mixed and incubated for 30 min at 37 °C. To simulate small intestinal digestion, five glass balls and 10 ml 0.25 mol/l sodium acetate solution were added to each tube and they were shaken well by hand. After equilibrating at 37 °C, an enzyme mixture (pancreatin from porcine pancreas, amyloglucosidase from Aspergillus niger and invertase from yeast; 5 ml) was added and incubated for 2 h in a shaking water bath (70 strokes/min). Then, the glass balls were taken out and the tubes were spun at 1,500 g for 5 min. The non-digestible fraction from the pre-digested native starches was recovered from the pellets, freeze-dried, pooled, milled in a mortar, and used for the fermentation experiments. This fraction contained the resistant starch, as defined by Englyst et al. [18], and guar gum.

In contrast, the non-digestible fraction from the predigested pyrodextrinised starches remained in the supernatant after centrifugation, due to their solubility in water. However, as the products of the pre-digestion step were also present in the supernatant, these were removed by dialysis (overnight dialysis against water at 4°C, followed by five 1-hour repeat dialysis steps with stirring) using dialysis bags with small Mw cut off (2,000). Retentates were pooled, freeze-dried, milled in a mortar, and used for fermentation. This fraction contained the non-digestible component from the pre-digested pyrodextrin and the enzymes from the starch kit.

As the SCFA profile was one of the variables studied, it was important to consider the effect of other fermentable substrates present in the system. Therefore, potato native and pyroconverted starches were fermented as described below, but without the pre-digestion step to look at the impact of the presence of either guar gum or the enzymes (from the starch kit) in the non-digestible fractions obtained from native or pyrodextrinised starches, respectively. There were no discernible effects of either component.

### Fermentation of non-digestible fractions

Non-digestible fractions (and glucose, as a readily fermented control) were fermented according to Edwards et al. [19] in four to six independent determinations (actual sample size is given in Table 1). Faecal slurry was made using freshly voided faeces (within 45 min from evacuation), donated by healthy adults (34–36 years, one female), homogenised in 0.1 mol/l Na,K-phosphate

buffer, pH 6.5 (pre-boiled, cooled and kept in an oxygen free nitrogen (OFN) atmosphere until used) using a liquidiser. Starch samples (100 mg for test cultures or none in case of control cultures) were suspended in 10 ml 160 g/l faecal slurry (previously filtered through a nylon stocking) in McCartney bottles (28 ml capacity). Each bottle was fitted with a holed, screw cap with a rubber lining to allow flushing of the culture with OFN before incubation. The bottles were incubated horizontally in a shaking water bath (50 strokes/min) at 37 °C for 24 h.

After incubation, produced gas was released and measured using a calibrated syringe. A culture aliquot (2 ml) was used to measure pH and frozen at -20 °C for later SCFA analysis. The remaining slurry was boiled for 30 min and frozen to assess residual starch and carbohydrate. Time zero cultures were immediately boiled for 30 min and frozen to estimate total starch and carbohydrate

# Short-chain fatty acid assay

SCFA were estimated by gas liquid chromatography using a TRACE™ 2000 gas chromatograph (ThermoQuest Ltd, Manchester, UK) equipped with a flame ionisation detector (250 °C) and using a Zebron ZB-Wax capillary column (15 m x 0.53 mm id x 1 μm film thickness), made of polyethylene glycol (catalogue No. 7EK-G007-22, Phenomenex, Cheshire, UK). Nitrogen (30 ml/min) was used as the carrier gas. Internal standard solution (86.1 mmol/l 3-methyl-n-valeric acid, 0.1 ml) and concentrated orthophosphoric acid (0.1 ml) were added to 0.8 ml culture aliquots. The mixture was extracted three times with 3 ml diethyl ether each time, centrifuged and the ether layers pooled. One microlitre of ether extract was automatically injected (230 °C, splitless) into the column. Then, the column temperature was held at 80 °C for 1 min, increased at 15 °C/min until 210 °C and held for 1 min. The peak integrals were analysed using Chrom-Card 32-bit software version 1.07β5 (2000) by ThermoQuest (Milan, Italy) using an averaged (n=5)each external response factor for standard (166.5 mmol/l 135.0 mmol/l acetic, propionic, 113.5 mmol/l isobutyric, 113.5 mmol/l n-butyric, 97.9 mmol/l isovaleric, 97.9 mmol/l n-valeric, 86.1 mmol/l n-hexanoic, 76.8 mmol/l heptanoic and 69.3 mmol/l n-octanoic acid solutions, pH 8) as calibration method. All the standards were from Sigma-Aldrich Company Ltd. (Dorset, UK), except acetic acid glacial, which was from Fisher Scientific (Loughborough, UK).

# Starch and carbohydrate analyses

Starch and carbohydrate were estimated both before (total) and after (residual) 24 h anaerobic incubation to

quantify the degree of fermentation. Total starch was measured using the enzymatic procedure described by Englyst et al. [18]. Residual starch was estimated according to Edwards et al. [19] based upon Englyst et al. [18]. These two methods are essentially the same and quantify the starch content as glucose released after an *in vitro* simulation of both gastric and small intestine enzymatic digestion. The specificity of the Englyst method, however, underestimates the "starch" content in modified starches such as pyrodextrins because of the presence of atypical bonds that cannot be hydrolysed by the enzymes used in the method. Therefore, measurement of carbohydrate by a more general, chemical assay was necessary to estimate the degree of fermentation of the pre-digested pyrodextrins.

Total and residual carbohydrates were estimated, by sampling from the same preparations made for total and residual starch analyses, using the anthrone-sulphuric acid method, which is a suitable method for the estimation of glucose-based carbohydrates like starches [20, 21]. A modification was made to perform the assay into 96-well microtitration plates. The reaction was carried out mixing 40 μl sample, standard or blank with 100 μl 10.3 mmol/l anthrone in concentrated sulphuric acid and incubated at 92 °C for 3 min. It has been shown that these assay conditions are able to quantify high molecular weight polymers like commercial soluble starch [21]. Absorbance at 630 nm was read using a Dynatech MR5000 microplate reader and analysed with Dynatech Reader software version 1.1 (Dynatech Laboratories Inc., Chantilly, VA, USA). An appropriate calibration curve was made with each plate using glucose as standard [21]. Standard solutions for both starch and carbohydrate determinations were prepared dissolving glucose in slurry supernatants (1,500 g for 5 min) from control cultures at time zero.

## Statistical analysis

Variables were described as mean ± standard deviation. Statistical analysis was made using Minitab® for Windows software, release 10.51 Xtra (Minitab Inc., State College, PA, USA). One- or two-sided unpaired t-test was used to compare means of pre-digested pyrodextrinised against pre-digested native starches where appropriate. A probability level less than 0.05 was used to indicate a significant difference between means.

## Results

# Fermentation of native and pyrodextrinised starches

The fermentation properties of both pre-digested native and pyrodextrinised starches from several sources after 24 h *in vitro* anaerobic incubation with human faeces are shown in Table 1. In cultures containing the pre-digested native starches, 99, 98, and 95 % of potato, lentil, and cocoyam starches, respectively, were fermented. Although a similar trend was found for the pre-digested pyrodextrinised samples, the modified starch was measured as carbohydrate content by a chemical method [21] to overcome the specificity of the enzymatic starch assay [18]. With this approach, 77, 75, and 81 % of the carbohydrate in potato, lentil, and cocoyam pre-digested pyrodextrins were fermented, respectively.

# Total SCFA

The net total SCFA, estimated as mmol/l of fermented culture, was similar for all starches (Table 1). Comparable amounts were produced using glucose as substrate  $(58.8 \pm 4.4 \, \text{mmol/l}, \, \text{n} = 6)$ . However, when net total SCFA were corrected for initial culture carbohydrate content, SCFA production remained similar for all the pre-digested native starch sources, but the total SCFA for the pre-digested pyrodextrinised starches were significantly higher (between 43% and 75%) than their corresponding predigested native starches (p < 0.023, one-sided t-test). Control cultures (n = 6), i. e. those fermented without starch samples, produced  $32.4 \pm 9.1 \,\text{mmol/l}$  of total SCFA, achieved pH  $6.5 \pm 0.1$ and produced  $7 \pm 3$  ml gas.

# Molar proportions of SCFA

All pre-digested pyrodextrins showed a significantly higher (p < 0.017, one-sided t-test) molar ratio of propionate (around twofold) when fermented compared with their pre-digested native starch. In addition, the acetate molar ratio decreased (p < 0.04, one-sided t-test) by about 24% (Table 1). There was no difference in the n-butyrate molar proportion (p > 0.82).

# Fall in pH and gas produced

The culture pH was  $6.6 \pm 0.1$  (n=6) at time zero. After fermentation, all the cultures showed a decrease in pH, but the fall in pH was significantly less for the pre-digested pyroconverted samples (p<0.03, one-sided t-test). The fall in pH observed for glucose (1.7  $\pm$  0.3, n=6) was similar to all the pre-digested native starches (Table 1). The volume of gas produced was the same for all samples, including glucose (17  $\pm$  5 ml, n=6).

**Table 1** Short-chain fatty acid, fall in pH, gas produced, starch and carbohydrate contents in fermented cultures of pre-digested native and pyrodextrinised starches from potato, lentil and cocoyam<sup>b</sup>

	Starch source (pre-digested starches)								
	Potato		Lentil		Cocoyam				
	Native (n = 5)	Pyrodextrin (n = 4)	Native (n = 6)	Pyrodextrin (n = 6)	Native (n = 4)	Pyrodextrin (n = 6)			
Net total SCFA <sup>c</sup> (mmol/l)	55.4±5.8	61.0±5.8	58.2±5.0	56.6±4.4	60.1±14.5	59.1±5.4			
Net total SCFA <sup>a, c</sup> (mol/kg carbohydrate)	$6.9 \pm 0.7$	10.4±1.9*	$7.0 \pm 0.4$	10.0±0.7***	$6.8 \pm 2.3$	11.9±1.8*			
Individual SCFA molar ratio Acetate <sup>a</sup> Propionate <sup>a</sup> n-Butyrate iC4—C8 <sup>a</sup>	649±126 147±34 154±83 50±32	511±35* 254±59* 151±51 85±41	688±121 120±32 148±79 44±34	514±30** 243±56** 156±41 87±32*	660±70 111±17 161±29 69±48	513±31* 244±52*** 161±41 82±37			
Fall in pH $^{\rm a}$ from 6.6 $\pm$ 0.1	$1.5 \pm 0.3$	1.0±0.1**	$1.6 \pm 0.3$	0.9±0.1***	$1.4 \pm 0.2$	1.0±0.1*			
Gas produced (ml)	16±3	20±4	14±8	17±3	10±5	15±6			
Total starch <sup>a, d</sup> (mg SE)	$78.8 \pm 2.4$	5.6±1.8***	85.7 ± 11.0	7.0±6.5***	$90.5 \pm 3.2$	5.6±3.3***			
Residual starch <sup>a, e</sup> (mg SE)	$1.1 \pm 0.7$	$0.8 \pm 0.9$	1.6±1.1	$0.7 \pm 0.6$	$4.3 \pm 2.4$	0.6±0.6*			
Total carbohydrate $^{a, d}$ (mg SE, n = 4)	77.7±8.1	59.8±8.3*	$80.5 \pm 2.3$	55.7±3.9***	87.3±2.9	50.2±4.3***			
Residual carbohydrate $^{e}$ (mg SE, n = 4)	$7.4 \pm 6.0$	13.5±4.4	4.1±3.6	13.9±2.2*	27.1±13.3	9.6±2.2			

Values are means ± standard deviations

SCFA, short-chain fatty acids; iC4–C8, sum of iso-butyrate, iso-valerate, valerate, hexanoate, heptanoate and octanoate; SE, starch equivalents (0.9 x glucose concentration) Mean values were significantly different from their corresponding predigested native starch: \*p < 0.05, \*\*p < 0.01 and \*\*\*p < 0.001 (unpaired t-test, and a sum of iso-butyrate, iso-valerate, hexanoate, heptanoate and octanoate; SE, starch equivalents (0.9 x glucose concentration)

# Discussion

Maize, potato and cassava starches account for almost 90% of the starch produced in the world [22]. To widen the utilisation of underexploited crops such as lentil and cocoyam, produced in Latin American countries like Venezuela, as starch sources, it is important to study their characteristics as potential food ingredients. Previous work has shown that pyrodextrinised starches produced by the acid/heat treatment used here have a 55–65% decrease in the enzymatically available starch. Therefore, pyrodextrins are an excellent source of non-digestible carbohydrates [6]. Moreover, these pyrodextrins differ from other short-chain saccharides because they are composed of a complex mixture of starch derivatives, all with Mw below 105,000 as estimated by gel filtration chromatography [6].

However, the water solubility of the pyrodextrins caused an analytical problem. During the pre-digestion stage, the nondigestible fraction from pre-digested native starches is normally obtained as a pellet after centrifugation at the end of pre-digestion [18]. In contrast, the pyrodextrinised starches remain in the supernatant along with their products of digestion (mainly glucose) and with the enzymes used for the pre-digestion itself. A

dialysis step with small Mw cut-off (2,000) was used to remove the digestion products before fermentation. However, low Mw pyrodextrins, which might be non-digestible and fermentable, were also lost during this step. Consequently, pyrodextrins used in this work had Mw in the range of 2,000 to 105,000.

Pyrodextrins do not belong to any of the three categories of resistant starch originally proposed [18]. Some authors have suggested new categories of resistant starch to include not only chemically modified starches [23], but also physically modified starches (like extruded starch) and amylo-lipid complex [11]. Since pyrodextrins have different glycosidic bonds than those present in native starches, they may belong to the category that includes chemically modified starches.

There was an almost complete fermentation of all starch samples under the fermentation conditions used [19] based on net total SCFA production, fall in pH, starch and carbohydrate content before and after 24h anaerobic incubation (Table 1). Net total SCFA and pH for pre-digested native starches were very similar to those reported elsewhere for raw potato starch [19]. Although raw and native starches are not part of the western diet, they were used here for comparison purposes. The heat/acid treatment did not change the production of gases in the faecal cultures.

<sup>&</sup>lt;sup>b</sup> Fermentation was carried out in anaerobic incubations with human faeces for 24 h

Net total SCFA was calculated by subtracting total SCFA values in control cultures (no carbohydrate) from total SCFA values in test cultures. Total SCFA in faecal slurry (at time zero) was 11.4 ± 4.8 mmol/l (n = 6)

d Before fermentation

<sup>&</sup>lt;sup>e</sup> After fermentation

Two approaches were used to assess the amount of substrate consumed during fermentation. An enzymatic, highly specific approach using Englyst method, as described by Edwards et al. [19], was used for the pre-digested native starches. On the other hand, a more general, chemical approach using a modification of the anthrone-sulphuric acid assay [21] was used for the pre-digested pyrodextrins. These methods differ in their specificity and they were used because the Englyst method underestimated the starch content of pyrodextrins, as shown under 'Total starch' in Table 1. This was possibly due to the presence of non-starch linkages (i. e. not  $\alpha(1\rightarrow 4)$  or  $\alpha(1\rightarrow 6)$  bonds) in such modified starches, which cannot be hydrolysed by the enzymes used in the assay.

Regarding the SCFA profile, pre-digested pyrodextrin fermentation showed the high proportion of butyrate that characterises native starch fermentation [24], yet there was an increase in the molar ratio of propionate with a parallel decrease in acetate. Another study [25] has shown a lower propionate ratio in raw potato and banana starches (resistant starch type 2) when compared with both wheat starch (digestible starch) and retrograded wheat and maize starches (resistant starch type 3). However, differences seen in the present study were 2- to 6.5-fold larger than those previously reported [25]. The drop in pH was higher for the pre-digested native starches than for pyrodextrins (Table 1). This may be due to the higher proportion of acetate, which has a lower pKa (4.74) than the other SCFA, present in the native sample cultures.

It is interesting to note that the only carbohydrate moiety present in pyrodextrins is glucose. The changes during pyrodextrinisation of native starches yield oligoor polysaccharides with lower Mw than native starches,

**Table 2** Short-chain fatty acid molar ratios for several glucose-based carbohydrates in *in vitro* cultures of human faeces or in human faecal associated rats in vivo

along with the new glycosidic bonds. The extent of these changes depends on the condition employed [4], but usually render highly branched [13], water soluble, resistant-to-digest pyrodextrins [6]. It is not clear why pre-digested pyrodextrins had a higher propionate production than their pre-digested native starches. However, it could be due to an increased solubility and/or presence of non-starch bonds caused by transglucosidation reactions. The presence of these new, atypical bonds may change the starch structure in a way that modifies the accessibility and/or affinity of the bacterial enzymes to the bonds.

High solubility, on the other hand, may also be important.  $\alpha$ -Glucooligosaccharide (branched pentasaccharide with  $1\rightarrow 2$ ,  $1\rightarrow 4$  and  $1\rightarrow 6$   $\alpha$ -bonds) and maltodextrin-like oligosaccharides (branched oligosaccharides with 2,000 Mw and  $1\rightarrow 4$ ,  $1\rightarrow 6$ ,  $1\rightarrow 2$  and  $1\rightarrow 3$   $\alpha$ -and  $\beta$ -bonds), both highly soluble and resistant-to-digest carbohydrates yielded similar SCFA molar ratio to the pre-digested pyrodextrins (Table 2) when fermented with human faecal microflora *in vitro* [26].

However, insoluble  $\beta$ -bonded fibres also show a SCFA profile similar to the pre-digested pyrodextrins. A molar ratio of 653:257:90 for acetate, propionate and butyrate, respectively, was reported when oat husk (44% cellulose, 50% hemicellulose+pectin) was used as fibre source in a diet for rats inoculated with human faecal flora [27]. Oat bran (mainly  $\beta$ -glucan) [28] and cellulose [29] fermentation *in vitro* also yielded a high propionate molar ratio (Table 2). Acid catalysed pyrodextrins, as prepared in this study, are thought to contain a significant proportion of  $\beta$ -bonded glycosidic linkages, as shown for wheat starch heated during 4 h at 180°C without addition of any catalyst [7]. However, the presence of such  $\beta$ -bonds in acid catalysed pyrodextrins has yet to be confirmed.

Carbohydrate	SCFA molar ratio*			Soluble - in water	Glycosidic bonda	Reference
	Ac	Pr	Bu	- III watei	DOIIG	
Glucose	640	224	136	yes	none	This work
$\alpha\text{-}Glucooligosaccharide$	598	239	164	yes	α	[26]
Maltodextrin-like oligosaccharide	619	301	80	yes	$\alpha$ and $\beta$	[26]
Pre-digested pyrodextrin <sup>b</sup>	560	270	170	yes	$\alpha$ and $\beta$	This work
Pre-digested native starch <sup>b</sup>	704	133	163	no	α	This work
Cellulose <sup>c</sup>	409	519	72	no	β	[29]
Oat husk <sup>d</sup>	653	257	90	no	β	[27]
Oat brane	560	220	220	no	β	[28]

SCFA short-chain fatty acids; Ac acetate; Pr propionate; Bu butyrate

<sup>\*</sup> Mean values in molar ratio based on net total SCFA (Ac + Pr + Bu)

<sup>&</sup>lt;sup>a</sup> Conformation of glycosidic bonds

<sup>&</sup>lt;sup>b</sup> Mean data from the present study

<sup>&</sup>lt;sup>c</sup> From Sigma (St. Louis, MO, USA)

<sup>&</sup>lt;sup>d</sup> Mainly cellulose and hemicellulose + pectin; in human faecal flora associated rats

e Mainly β-glucar

In conclusion, pyrodextrinisation of starches isolated from potato, lentil and cocoyam resulted in the production of a soluble material that escapes digestion, but was extensively fermented in vitro by the colonic bacteria of healthy adults. This fermentation is characterised by higher proportions of propionate and lower proportions of acetate than the native starch, although the high pro-

portion of butyrate characteristic of starch fermentation was maintained.

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