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Prebiotic effects of cassava bagasse in TNO's *in vitro* model of the colon in lean *versus* obese microbiota

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ABSTRACT

Obesity is currently a worldwide epidemic that has serious consequences for health. It has been suggested that the gut microbiota can influence body weight, e.g., by producing short-chain fatty acids (SCFA), which are substrates for the host and induce the release of satiety hormones (e.g., PYY). To test the potential prebiotic effect of cassava bagasse (a by-product from cassava flour and starch production) *in vitro* fermentation experiments were performed using fecal microbiota originating from lean and obese subjects in a validated model of the colon (TIM-2). The microbial composition and production of microbial metabolites were analysed. Cassava was found to modulate the microbiota composition, since it stimulated the growth of the genera Bifidobacterium and Roseburia. The production of SCFA was similar to that for inulin (positive control). Although supplementary *in vivo* studies with cassava bagasse are needed, this study shows for the first time the prebiotic potential of cassava bagasse.

1. Introduction

"Globesity" is the term used to describe the global epidemic of overweight and obesity (Elli, Colombo, & Tagliabue, 2010), including occurrence in inhabitants from developing nations like Brazil. Currently, more than 1 billion adults are overweight, of which one third is clinically obese (WHO, 2013).

In addition to the already known factors that can contribute to obesity, such as genetics and unsuitable dietary habits, new evidence is showing that the gut microbiota can also affect the nutritional metabolism of the host (Fleissner et al., 2010).

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Abbreviations: TNO, Netherlands Organization for Applied Scientific Research; TIM-2, TNO *in vitro* model of the large intestine; SCFA, short-chain fatty acids; PYY, peptide YY; LPS, lipopolysaccharide; SIEM, simulated ileum effluent medium; BCFA, branched-chain fatty acids; GLP-1, glucagon-like peptide 1; FOS, fructooligosaccharides; GOS, galactoligosaccharides

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Several studies indicate the role of the gut microbiota in obesity-linked diseases (Backhed et al., 2004; Venema, 2010), although the mechanism is not yet entirely clear.

Short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate, are the major metabolites produced by the microbiota in the large intestine and these deliver additional energy to the host, contributing to about 5–15% of the total caloric requirements of humans (Blaut & Klaus, 2012). Since even a small daily excess of energy ingestion – as low as 1% of the recommended daily intake can contribute to increased corporal weight in the long term, all mechanisms modifying food-derived energy availability are important for the balance of body weight (Cani, Delzenne, Amar, & Burcelin, 2008). This means that the colonic microbiota has a very important role in energy supply.

Besides the extra energy provided by SCFA, it has been hypothesized that these can also influence upper gut motility, satiety and, through this, obesity. Endocrine L-cells present in the colonic mucosa secrete peptides such as peptide YY (PYY), which is involved in appetite regulation (Hamer et al., 2008). PYY release is stimulated directly by nutrients and in response to lipids in distal ileum and colon (Voortman, Hendriks, Witkamp, & Wortelboer, 2012). An *in vitro* study showed that butyrate increased the expression and release of PYY in epithelial cells (Hamer et al., 2008). Hence, there is some evidence that the effect of fermentable dietary fibre on satiety and body weight could be mediated through the increased colonic production of SCFA (Delzenne, Neyrinck, & Cani, 2013).

Another mechanism that links obesity and gut microbiota is the higher amounts of plasma lipopolysaccharide (LPS), called metabolic endotoxemia (Cani et al., 2007), common in obese and type II diabetic people. LPS is involved in the release of several cytokines that are key factors in activating insulin resistance, and is also being considered a factor for the triggering of obesity (Cani et al., 2007).

Current recommendations for the management of obesity and diabetes propose an increase in consumption of dietary fibre, which may contribute to the control of several metabolic disorders. Among the dietary fibres, prebiotics can be used as a tool to modulate the gut microbiota. A prebiotic is defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" (Gibson & Roberfroid, 1995).

Cassava (*Manihot esculenta* Crantz) is a tuber, and an original species from Brazil, where the consumption *per capita* is around 50 kg/year (Cardoso, Hühn, & Junior, 1999). It can be consumed as natural tuber, nevertheless, as it spoils easily, the production of flour or starch is a manner to conserve it. During the flour or starch processing, a solid residue – called bagasse – is produced, which contains the fibre material from the root and the remaining starch that was not extracted during the manufacturing (Rodrigues, Caliari, & Asquieri, 2011).

To make one ton of starch, around 930 kg of cassava bagasse is generated and in Brazil, per year, around 97.000 tons of bagasse are produced (Leonel, Cereda, & Roau, 1999). This residue is considered an industrial waste and it is generally used in animal feed or even disposed of in the environment and thus considered an ecological hazard (Ferreira, Oliveira, Cardoso, Magalhães, & Brito, 2007). Since currently it does not have a great value in the market, the main limitation of the commercialization and use of this residue is the drying process, which is considered much too expensive (Rodrigues et al., 2011).

Cassava bagasse is a good source of fibre and some investigations have demonstrated the beneficial aspects of cassava bagasse intake both in rat and human studies (Costa, Bramorski, Silva, Teixeira, & Amboni, 2005; Osundahunsi, Williams, & Oluwalana, 2012). These benefits are due to the high insoluble fibre content (~86%), and its associated physiological effects, such as the increase in volume and frequency of defaecations – and therefore relief of constipation – and the possibility to be incorporated into food products specially designed for celiac disease patients. However, little is known about the possible prebiotic property of this by-product. A study using human faecal inoculum revealed that *in vitro* batch fermentation of the isolated fibre showed an increased amount of SCFA (Mallillin, Trinidad, Raterta, Dagbay, & Loyola, 2008), placing cassava bagasse in a promising position that deserves to be further investigated.

The aim of the present study was to investigate the potential prebiotic effect of cassava bagasse. This was performed assessing the stimulation of growth and/or activity of beneficial colonic bacteria using TNO's dynamic *in vitro* model of the large intestine (TIM-2) in response to the administration of 7.5 g/day cassava bagasse over a period of 3 days. The microbial composition, levels and type of microbial metabolites as well as the levels of LPS were analysed before and after the fermentation experiments. All analyses compared faecal homogenates from lean with those from obese subjects. As a preliminary study, the influence of cassava bagasse on the release of the satiety hormone PYY was determined after incubating TIM-2 samples with pig intestinal segments.

2. Material and methods

2.1. *Study design*

Faecal material was collected from lean (body mass index – BMI – 18.5–25 kg/m^2, mean 23.6 ± 1.4 kg/m^2, age range 23–31 years, two females (F) and two male (M)), and obese (BMI >30 kg/m^2, mean 33.5 ± 2.6 kg/m^2, age range 23–61 years, 3 F and 1 M) volunteers recruited from TNO and Maastricht University Medical Centre, respectively.

The exclusion criteria for both groups included the use of antibiotics or any other medical treatment influencing gut transit or intestinal microbiota during the preceding 3 months, severe chronic disease, gastrointestinal disease, severe food allergy and intake of probiotics and prebiotics.

In vitro experiments were performed in duplicate with mixed faecal material from either obese or lean individuals in the presence of a) dried and milled cassava bagasse (Grazimara, Biguaçu, Brazil), b) chicory inulin as the "gold standard prebiotic" with purity ≥ 90% and degree of polymerization > 10 (Orafti, Amsterdam, the Netherlands), and c) the Simulated Ileum Effluent Medium (SIEM) simulating the material reaching the colon (further on referred to as "standard") – described in detail below.

2.2. *Faeces collection and standardization*

Faecal samples were collected from each subject. Volunteers were instructed to put the samples in a gastight bag and to place it immediately into a plastic jar containing an anaerocult strip (AnaeroGen™, Cambridge, UK) to create anaerobic conditions during transport to the anaerobic cabinet and/or freezer (–80 °C). This transport never lasted longer than 3 hours.

The standardized lean or obese microbiota used to inoculate the TIM-2 system was prepared in an anaerobic cabinet (Sheldon Lab – Bactron IV, Cornelius, OR, USA) where the faecal samples (separate for lean and obese individuals) were mixed for standardization (Venema, van Nuenen, Smeets-Peeters, Minekus, & Havennar, 2000) and yielded a total of 500 grams of faecal material. Then, 450 mL of 10 × concentrated dialysis liquid, 2490 mL of demi-water and 560 g of glycerol was added.

The faecal material was aliquoted, snap-frozen in liquid nitrogen and stored at –80 °C.

This method was validated previously and showed that standardized stool samples are similar in composition and activity to fresh faecal samples (Venema et al., 2000). Besides that, since every person has a different microbiota composition, by pooling faeces from different people it was possible to: a) have sufficient amounts of faecal slurry for all experiments and therefore be able to compare the results from the different runs without being cautious about the use of different microbiota (Venema et al., 2000), and b) to create a standardized microbiota that encompasses species from different individuals and therefore can be considered to represent a larger population.

2.3. *TNO in vitro model of the colon – TIM-2*

TIM-2 (Fig. S1) simulates the conditions in the lumen of the human proximal colon and all parameters are computer-controlled (Minekus et al., 1999). The model has been described extensively in recent publications (Kovatcheva-Datchary et al., 2009; Maathuis, Hoffman, Evans, Sanders, & Venema, 2009). In short, it simulates body temperature, pH in the lumen, composition and rate of secretion, absorption of water and microbial metabolites through a semipermeable membrane inside the model, mixing and transport of the intestinal contents through the simulation of peristaltic movements, and the presence of a complex, high density, metabolically active, anaerobic microbiota of human origin (Reimer et al., 2014).

2.3.1. *Experimental setup*

To start the experiment, TIM-2 was inoculated with 70 mL of the standardized microbiota (described above) plus 50 mL of dialysis liquid (described below) yielding a total of 120 mL (total volume of the system). The microbiota adapted to the model conditions with the standard medium (SIEM) for 16 hours (Fig. 1) and after that, a period of 4 hours of starvation allowed the bacteria to ferment all available carbohydrates in the system. Next, samples were collected at time zero (t0) after which the test compound was added. From this point onwards, a 3-day experimental period started, where the microbiota received the standard feeding (that yields 7.5 g/day of carbohydrates) or standard feeding without carbohydrate, in which the test compounds were mixed in (7.5 g/day of cassava bagasse or inulin), added through the feeding syringe (Fig. S1g; 2.5 mL/hour). Samples of the lumen and dialysate were collected at time 0 h, 24 h, 48 h and 72 h (Fig. 1).

2.3.2. *Simulated ileal effluent medium and dialysate*

The standard feeding, simulated ileal effluent medium (SIEM), simulates the material reaching the colon and was slightly modified for experiments in TIM-2 compared to the medium which was described by Gibson, Cummings, and Macfarlane (1988), mainly concerning a lower amount of water. SIEM contained the following components (g/L): 9.0 pectin, 9.0 xylan, 9.0 arabinogalactan, 9.0 amylopectin, 43.7 casein, 74.6 starch, 31.5 Tween 80, 43.7 bactopepton, 0.7 ox-bile, 4.7 $K_2HPO_4.3H_2O$, 8.4 NaCl, 0.009 $FeSO_4.7H_2O$, 0.7 $MgSO_4.7H_2O$, 0.8 $CaCl_2.2H_2O$, 0.05 bile, 0.02 haemin and 0.3 cysteine·HCl, plus 1.5 mL of a vitamin mixture containing (per litre): 1 mg menadione, 2 mg D-biotin, 0.5 mg vitamin B12, 10 mg pantothenate, 5 mg nicotinamide, 5 mg *p*-aminobenzoic acid and 4 mg thiamine. The pH was adjusted to 5.8. Dialysis liquid contained (per litre): 2.5 g $K_2HPO_4.3H_2O$, 4.5 g NaCl, 0.005 g $FeSO_4.7H_2O$, 0.5 g $MgSO_4.7H_2O$, 0.45 g $CaCl_2.2H_2O$, 0.05 g bile and 0.4 g cysteine·HCl, plus 1 mL of the vitamin mixture. All medium components were purchased at Tritium Microbiology (Eindhoven, the Netherlands).

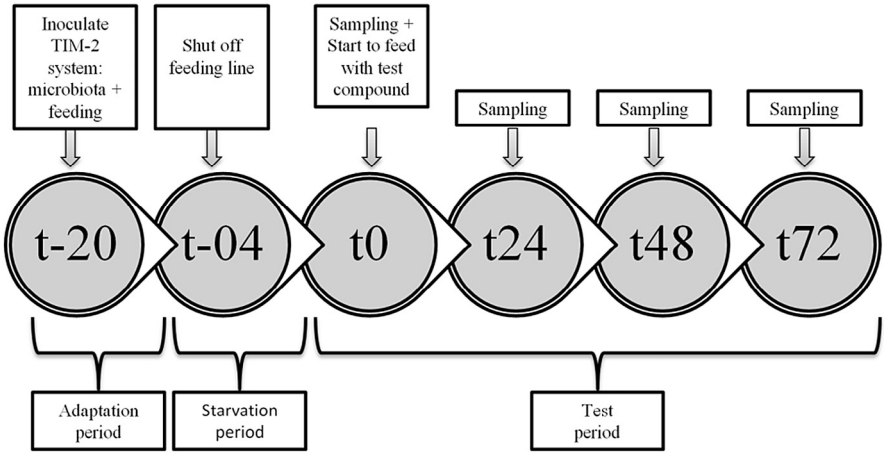

Fig. 1 – Experimental set-up with timeline for TIM-2 experiments.

SIEM only contains indigestible carbohydrates and hence did not require pre-digestion. SIEM was administered at 60 mL/d (corresponding to ~7.5 g carbohydrate/d).

2.4. *Cassava bagasse*

Cassava bagasse was generated during flour production as described earlier (Pandey et al., 2000). The bagasse was dried by the supplier (Grazimara, Biguaçu, Brazil) in an industrial pan at 250–300 °C for 20 minutes. Carbohydrate and protein composition was analysed at the Food Chemistry Department of Wageningen University. Samples were analysed in duplicate, and data are represented as average (±range). Samples contained 0.25% of nitrogen (N) and 1.7% of protein. The assay used for protein and nitrogen analyses was the "Dumas" method (Pustjens et al., 2012).

Sugar composition was determined by derivatizing the sugars into alditol acetates (Pustjens et al., 2012). Briefly, after a pre-hydrolysis step using 72% (w/w) sulphuric acid at 30 °C for 1 hour, samples were hydrolysed with 1 M sulphuric acid at 100 °C for 3 hours in a heating block. Afterwards the sugars were reduced with $NaBH_4$. Acetylation was performed and samples were analysed with gas chromatography (GC) and a flame ionization detector (FID) (Focus-GC, Thermo Scientific, Waltham, MA, USA) using inositol as an internal standard. The total uronic acid content was determined with the automated *m*-hydroxy-diphenyl assay (Pustjens et al., 2012). Galacturonic acid was used for calibration. Results are given in w/w (%; percentage by weight) and mol % (molar percentage). The determined sugar composition is shown in Table 1. The nature of the fibre (soluble and insoluble portions) as prepared for the fermentation experiments was analysed (n = 4), and on average cassava bagasse was determined to be composed of 86% of insoluble fibre.

2.5. *Microbiota composition*

To determine, among others, whether cassava bagasse would have a prebiotic effect, the composition of the microbiota was evaluated using pyrosequencing of the small subunit ribosomal RNA (16S rRNA) gene. DNA was isolated from 200 mg of "faecal" material at the start and at the end of the TIM-2 experiments (t0h and t72h), using a commercial DNA isolation kit (Agowa, Berlin, Germany) following the instructions of the manufacturer. Generation of PCR amplicon library was performed by amplification of the 16S rRNA gene V5-V7 hypervariable region. Amplification was performed using the forward primer 785F (5'GGATTAGATACCCBRGTAGTC-3') and reverse primer 1175R ('5- ACGTCRTCCCCDCCTTCCTC-3). The primers were fitted with the 454 Life Sciences Adapter A (forward primer) and B (reverse primer). The forward primer also included a unique titanium decemnucleotide sample identification key (barcode). The amplification mix contained two units of Pfu Ultra II Fusion HS DNA polymerase (Stratagene, La Jolla, CA, USA) and 1x Pfu Ultra II reaction buffer (Stratagene), 200 μM dNTP PurePeak DNA polymerase Mix (Pierce Nucleic Acid Technologies, Milwaukee, WI, USA), and 0.2 μM of each primer. After an initial denaturation (94 °C; 2 min), 30 PCR cycles were performed (denaturation (94 °C; 30 sec), annealing (50 °C; 40 sec), extension (72 °C; 80 sec)). Samples with DNA recovery of equal or less than 10 pg/μL of DNA were cycled 35 times using the same protocol. Amplicons were size checked and quantified by gel electrophoresis and Quant-iT Picogreen dsDNA Assay (Invitrogen, Carlsbad, CA, USA) on the Tecan Infinite M200 (Tecan Group Ltd, Männedorf, Switzerland). Amplicons of the individual samples were equimolar pooled and purified from agarose gel by means of QIAquick Gel Extraction Kit Protocol (Qiagen, Hilden, Germany).

The library was sequenced unidirectionally in the reverse direction (B-adaptor) in one run in the 454 GS-FLX-Titanium Sequencer (Life Sciences (Roche), Branford, USA) by Keygene (Wageningen, the Netherlands).

2.5.1. *Sequence processing*

FASTA-formatted sequences and corresponding quality scores were extracted from the .sff data file generated by the GS-FLX-Titatium sequencer using the GS Amplicon software package (Roche, Branford, CT, USA). Sequencing data were processed using modules implemented in the Mothur v. 1.20.0 software platform module (Schloss et al., 2009). Due to the unique barcodes, sequences were binned by sample of origin. For further downstream analyses, barcodes and primer sequences were trimmed, and low quality reads (containing ambiguous base calls (N) in the sequence, > 1 error in the primer, and ≥ 1 error in the barcode, more than 8 homopolymers anywhere in the sequence, and the occurrence of a 50 nucleotide window with a window average below 35, or a length >500 bp or <200 bp and sequences that were too short or too long (automated function)) were removed from the analyses. The data set was simplified by using the "unique.seqs" command to generate a non-redundant (unique) set of sequences. Unique sequences were aligned using the "align.seqs" command and an adaptation of the Bacterial SILVA SEED database as a template (available at: http://www.mothur.org/wiki/Alignment_database). Alignment was performed using the ribosome database project (RDP) template in order to ensure that comparable regions of the 16S rRNA gene across all reads were analysed. Sequences that started before the 2.5-percentile or ended after the 97.5-percentile in the alignment were filtered. Sequences were denoised using the "pre.cluster" command. This command applies a pseudo-single linkage algorithm with the goal of

Table 1 – Sugar composition of cassava bagasse.

	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid
mol %	0.5	0.5	0	1	96	2
w/w % [a]	0.4	0.4	0.2	0.7	85	2

[a] w/w % does not sum up to 100% due to the presence of moisture, ash and proteins.

removing sequences that are likely due to pyrosequencing errors (Huse, Welch, Morrison, & Sogin, 2010).

A total of 149.220 potentially chimeric sequences were detected and removed using the "chimera.uchime" command (Edgar, Haas, Clemente, Quince, & Knight, 2011). High quality aligned sequences were classified by using the RDP-II naïve Bayesian Classifier database. Aligned sequences clustered into Operational Taxonomic Units (OTUs) using OTU's defined by 97% similarity, were calculated by the average linkage clustering command method. For each sample rarefraction curves were plotted and community diversity parameters (Shannon diversity index, CAO1 and Simpson's) calculated. Unweighted and weighted Unifrac dendrograms/trees were generated using the Unifrac module implemented in the Mothur software manual and FastTree2 (Schloss et al., 2009) to plot the distance matrices in trees.

Identification to the species level was performed essentially as described before (de Goffau et al., 2013).

2.6. *Short-chain fatty acids, branched-chain fatty acids, lactate and ammonia*

Lumen and spent dialysis liquid from TIM-2 were analysed for microbial metabolites essentially as described before (Maathuis et al., 2009). Concentrations were calculated based on a calibration curve that was measured together with each series of samples.

2.6.1. *Calculations*

Cumulative amounts of SCFA, BCFA, lactate and ammonia were calculated considering the measurements in spent dialysis liquid and luminal content and the volume of each sample.

2.6.2. *Energy production*

Energy production in the form of SCFA was calculated using the following values for acetate, propionate, butyrate and lactate, respectively: 874, 1536, 2192 and 1364 kJ mol^{-1} (van der Kamp, Jones, Mccleary, & Topping, 2010).

2.7. *Lipopolysaccharide*

Lumen samples from TIM-2 (0 h and 72 h) were analysed for LPS as follows. Samples were centrifuged for 10 minutes at 14.800 g at room temperature. The supernatant – 100 µL – was diluted eightfold with endotoxin-free phosphate buffered saline (PBS – Gibco by Life Technologies, Bleiswijk, the Netherlands) and passed through a disposable 0.20 µm sterile, pyrogen-free filter (Minisart, Sartorius Stedim Biotech, Göttingen, Germany). Samples were heated at 100 °C for 30 minutes and centrifuged for 2 minutes at 10.000 rpm at room temperature (Gozho, Krause, & Plaizier, 2006). Afterwards they were further diluted 1.000 fold using PBS.

The assay was performed using a Limulus Amebocyte Lysate (LAL) kit (Thermo Scientific, Rockfort, IL, USA) in a 96-well microplate (Sarstedt Inc., Newton, NC, USA) according to the instruction of the manufacturer. The absorbance was read at 405 nm using a microplate reader (Biotek Synergy HT, Winooski, VT, USA) (Gozho et al., 2006).

2.8. *Statistical analysis*

Data are represented as average (±range). Since the system yields highly reproducible results, the experiments were performed in duplicate. Due to this it was not possible to perform statistical analyses.

3. Results

3.1. *Microbiota composition*

All the baseline (time zero) samples clustered together (Fig. S2), showing that all fermentations started with the same microbiota composition, although those of the lean microbiota clustered separately from those of the obese microbiota (Fig. S2).

Fig. 2 shows the relative abundance (in percentages) of each phylum at the start and after 72 hours of fermentation with the different substrates using faecal microbiota derived from lean (A) and obese (B) subjects.

3.1.1. *Lean microbiota*

Firmicutes increased 16 and 13% for standard and inulin, respectively, and decreased 4.6% for cassava (Fig. 2A). The final abundance was 92, 89 and 67% for standard, inulin and cassava, respectively.

The phylum *Bacteroidetes* decreased in all groups after 72 hours. It decreased by 15, 16 and 9.5% in standard, inulin and cassava, respectively. The final abundance was 5.3 for standard, 6.1 for inulin and 16% in cassava.

Actinobacteria (including the genus *Bifidobacterium*) decreased 0.70% in standard, resulting in a final abundance of 0.04%. Inulin caused a subtle increase (0.74%), while cassava showed the highest increase of *Actinobacteria* (14%), with a final abundance of 15%.

3.1.2. *Obese microbiota*

The obese microbiota (Fig. 2B) showed a higher percentage of *Firmicutes* at time zero when compared with the lean microbiota. However, it decreased in all groups – by 71, 6.1 and 29% to a final abundance of 22, 87 and 62% for standard, inulin and cassava.

Bacteroidetes showed a major increase in abundance under standard condition. The initial percentage was 3.6% while at the end of the fermentation period this number increased to 34%. A similar tendency was seen for cassava, where the initial amount of *Bacteroidetes* was 4.8% before and 28% after the fermentation period. The abundance of this phylum on inulin did not change much over the fermentation period.

Actinobacteria showed a higher increase in the obese microbiota when fed with inulin compared with standard and cassava. The initial abundance of this phylum was 0.74% and it increased with 6.2%. Cassava also showed a slight increase in this phylum, where the initial proportion was 0.43% and the final was 2.6%. In the standard, *Actinobacteria* did not differ after 72 hours of experiment. There were very few changes in the other phyla.

3.2. *Short-chain fatty acids and lactate*

Fig. 3 shows the average of total cumulative production of the beneficial microbial metabolites acetate, propionate,

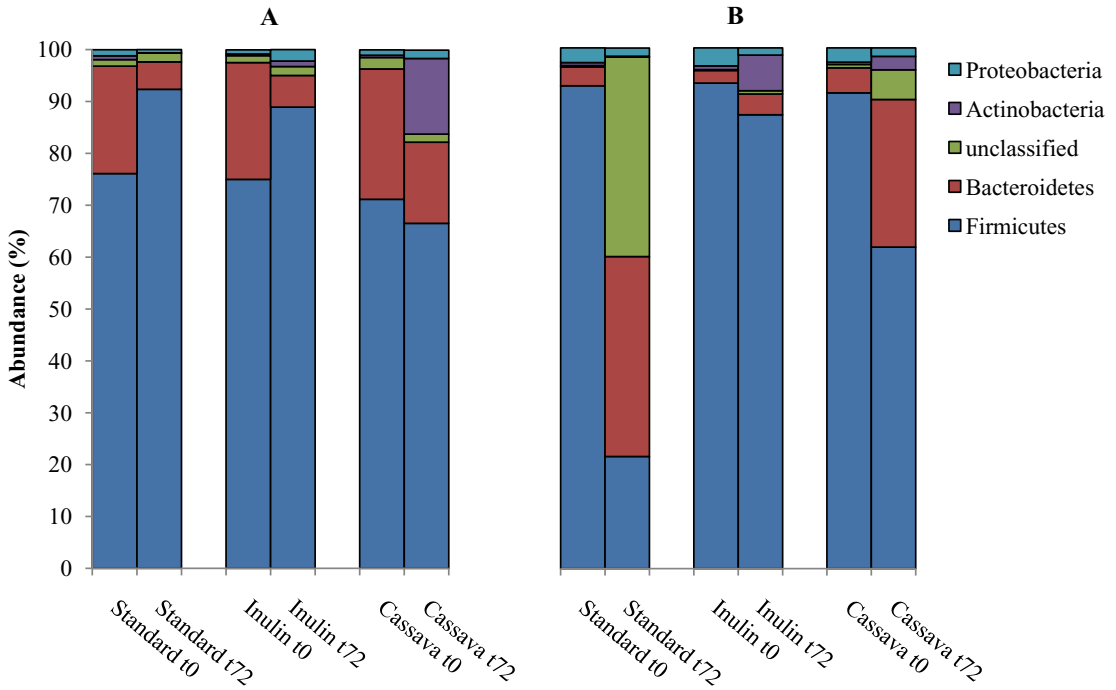

Fig. 2 – Percentage of each phylum at time 0 h and 72 h with standard, inulin and cassava in lean (A) and obese (B) microbiotas.

butyrate and lactate during the TIM-2 experiments by the lean microbiota (A) and obese microbiota (B).

In lean microbiota, acetate production was comparable for the three substrates. Standard showed higher amounts of butyrate, and propionate production was lower than that of other SCFA. Lactate production was higher in the standard, followed by cassava and inulin.

In obese microbiota, acetate production was also comparable for the three substrates, but with higher values than observed for the lean microbiota. Fermentation of cassava showed higher production of propionate and the lowest production of butyrate. Lactate production in obese microbiota was higher for inulin and cassava when compared with lean microbiota.

Fig. S4 shows the kinetics of the cumulative average production of SCFA on the cassava substrate during the entire test period for both microbiotas.

3.2.1. *Production of energy*

In lean microbiota, standard showed the highest production of energy in kJ in the form of SCFA and lactate – 176 kJ. Fermentation of inulin by the lean microbiota produced less energy compared with the obese microbiota (153 *versus* 180 kJ). Lean and obese microbiota extracted similar amounts of energy from cassava (148 *versus* 151 kJ), which was overall lower than for the other substrates.

3.3. *Branched-chain fatty acids and ammonia*

Table 2 shows the cumulative production (mmol) of BCFA – iso-butyrate and iso-valerate – and ammonia at t72 by lean and obese microbiota.

In the lean microbiota, the production of BCFA was slightly higher after fermentation with cassava. With regard to ammonia production, microbiota fed with standard produced the highest amount.

In the obese microbiota, fermentation of cassava resulted in the highest production either of *i*-butyrate, *i*-valerate and ammonia when compared with the standard and inulin.

3.4. *Lipopolysaccharide*

Fig. 4 shows that in the lean microbiota, after a fermentation period of 72 hours, the LPS concentration in all substrates increased in similar amounts.

Table 2 – Cumulative production of BCFA (iso-butyrate and iso-valerate – mmol) and ammonia (mmol) by lean and obese microbiotas after 3 days of addition of the test compounds at 7.5 g/day (n = 2).

	Lean			Obese		
Substance	*i*-butyrate	*i*-valerate	Ammonia	*i*-butyrate	*i*-valerate	Ammonia
Standard	1.3 ± 0.04	2.6 ± 0.05	65.2 ± 3.08	0.13 ± 0.18	0.53 ± 0.31	41.8 ± 2.07
Inulin	1.0 ± 0.04	2.1 ± 0.01	51.2 ± 1.27	0.13 ± 0.13	0.42 ± 0.33	39.1 ± 0.20
Cassava	1.5 ± 0.09	2.9 ± 0.22	60.5 ± 9.23	0.52 ± 0.33	1.3 ± 0.49	55.8 ± 4.9

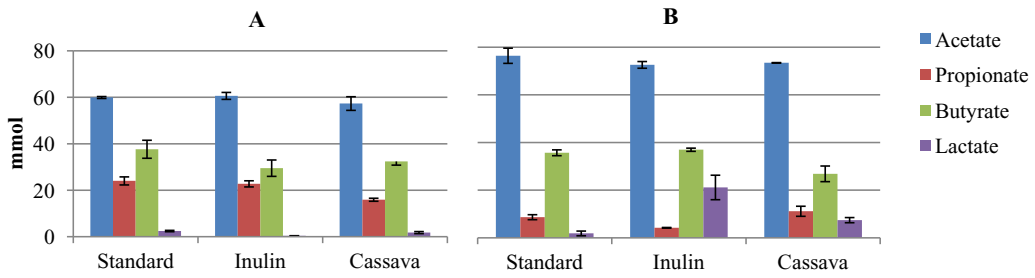

Fig. 3 – Average cumulative SCFA and lactate production (mmol) by the lean (A) and obese (B) microbiotas after 72 hours (n = 2 ± range).

In obese microbiota the initial amount of LPS on cassava was lower compared with the other substrates; however, it presented the highest increase.

4. Discussion

4.1. *Cassava bagasse composition*

As can be observed in Table 1, cassava bagasse is predominantly composed of glucose, probably due the fact that the manufacturing process is not able to extract all the starch present. Resistant starch is indigestible in the small intestine, and it enters the large intestine. It has been proposed that resistant starch has properties similar to dietary fibre and thus could also affect satiety and have a beneficial role in weight regulation (Topping & Clifton, 2001). Initial experiments to determine the digestibility of the starch have been carried out and the results show that the starch in cassava bagasse is partly digestible. Therefore, to reach the colon delivery strategies (such as encapsulation, etc.) may have to be devised.

4.2. *Microbiota composition*

The microbiota composition in the beginning of the TIM-2 experiments (Fig. 2) is in accordance with the finding of Gordon and colleagues (Ley et al., 2005), with an increased abundance of the phylum *Bacteroidetes* and decreased abundance of *Firmicutes* in lean microbiota when compared with obese microbiota. It has been hypothesized that an increased ratio of *Firmicutes* to *Bacteroidetes* may have a significant contribution to the pathophysiology of obesity through the promotion of adiposity or could even represent a host-mediated adaptive response to limit energy uptake/storage through, for instance, reducing the capacity to ferment polysaccharides (Ley et al., 2005). On the one hand is not logical to link this ratio with obesity because it is known that *Bacteroidetes*, especially members of the genus *Bacteroides,* have a great capacity to metabolize an enormous variety of glycans from plants and animals (Martens et al., 2011) and therefore could provide more energy in the form of SCFA and also monosaccharides that can be absorbed by the human gut. On the other hand, it has been proposed that SCFA have satiety-enhancing properties (Darzi, Frost, & Robertson, 2011) and therefore it could be hypothesized that the higher the amounts of SCFA produced, the more satiety effects would occur (and thus the leaner one could become). Additionally, research showed an increase in *Bacteroidetes* in overweight and obese individuals (Schwiertz et al., 2010), which is the opposite of the results published by Gordon and colleagues (Ley et al., 2005). Moreover, these phyla are composed of several dozens of species, and the role in obesity of the species that represent these phyla in the gut ecosystem has not been examined in these early studies.

Cassava had some unique effects regarding the abundance of some species. In lean microbiota abundance of *Ruminococcus obeum* and *Bacteroides uniformis* increased, and *Eubacterium rectale* and *Prevotella copri* decreased upon cassava bagasse feeding.

Inulin also had some unique effects, which in lean microbiota included the increase of *Catenibacterium mitsuokai* and decrease of *Eubacterium rectale* and *Ruminococcus bromii*. In obese microbiota it increased the abundance of *Eubacterium hadrum*, *Lactococcus garvieae* and *Eubacterium biforme*.

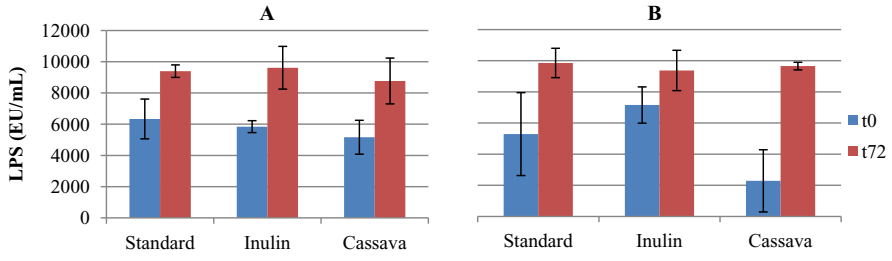

Fig. 4 – LPS concentration (endotoxin units (EU)) in the TIM-2 lumen samples at time 0 h and 72 h of fermentation with different substrates with lean (A) and obese (B) microbiotas.

In lean microbiota, both inulin and cassava stimulated the increase in abundance of the genus *Bifidobacterium*. However, while inulin increased the relative proportion by a factor of 169-fold compared with the standard (Fig. S3), cassava showed a greater response with an overall 1738 fold increase. Interestingly, not entirely the same was observed in obese microbiota. After 72 hours of fermentation with inulin and cassava, inulin showed a stronger effect on *Bifidobacterium* than cassava, with an 80-fold increase relative to the standard. Cassava, although also stimulating the increase of this genus by 42-fold, had a clearly less strong effect when compared with lean microbiota. Obese samples become rather similar to lean samples after 72 hours (Fig. S1), indicating the microbiota becomes healthier.

Bifidobacterium and *Lactobacillus* are genera usually used to characterize the prebiotic effect of substrates, since these organisms have been reported to have health-promoting properties such as inhibition of opportunistic pathogens (Rycroft, Jones, Gibson, & Rastall, 2001). Cassava showed a greater increase in the genus *Bifidobacterium* in the microbiota obtained from lean individuals than that of the well-known bifidogenic prebiotic inulin. Cassava also induced the relative contribution of bifidobacteria in microbiota obtained from obese individuals, but to a lesser extent when compared with lean individuals. Specifically, either in lean or obese microbiota at t72h, *Bifidobacterium dentium* was the most dominant *Bifidobacterium* present. *B. adolescentis*, *B. breve* and *B. pseudocatenulatum* increased in lean and obese microbiota. The effects of inulin and cassava on the genus *Lactobacillus* did not differ from the standard, either in lean or obese microbiota.

The genus *Roseburia* is related to butyrate production and is thus thought to play a key role in colonic health (Venema, 2010). In comparison with the standard, cassava was able to increase the relative proportion of *Roseburia* sevenfold after 72 hours of fermentation in lean microbiota (Fig. S3), especially *Roseburia faecis*. Interestingly, inulin decreased the amount of *Roseburia* in lean microbiota sevenfold. There were no changes in this phylum in microbiota obtained from obese individuals after 72 hours of fermentation.

Cassava bagasse had the ability to increase the amount of *Bacteroides* in lean microbiota compared with the standard, whereas the amount in obese microbiota was not influenced compared with the standard. Known propionate producers belong to the genus *Bacteroides* (Schwiertz et al., 2010) and besides that, large polysaccharides, such as resistant starch, are more readily broken down by *Bacteroides*, while bifidobacteria prefer to ferment shorter-chain oligosaccharides (Maathuis et al., 2009). Interestingly, bifidobacteria and *Bacteroides* were higher in lean microbiota after feeding cassava, indicating that cross-feeding between these two genera might have occurred (Maathuis et al., 2009). Bifidobacteria have been shown to be stimulated upon starch feeding before (Kovatcheva-Datchary et al., 2009).

The genera *Dorea* and *Coprococcus* were stimulated 44- and 14-fold, respectively, by cassava (Fig. S3). Unfortunately these genera have only recently been described as being part of the microbiota, and their importance to host physiology is not yet known. *Dorea* has been found to be stimulated by inulin (Kovatcheva-Datchary, 2010). In our study, this is only observed in the faecal material of obese individuals. In the faecal material from lean subjects, *Dorea* actually decreased upon inulin feeding. There were very few changes in the other phyla. As expected the *Verrucomicrobia*, containing *Akkermansia* (*Akkermansia muciniphila*, is inversely correlated with weight gain; Delzenne et al., 2013), decreased, as there was no mucin added to TIM-2, which is the major substrate of the genus.

4.3. *Short-chain fatty acids and lactate*

It is clear that acetate production by the obese microbiota, for all substrates, was much higher than production of the other two SCFA (Fig. 3 and Fig. S4). Acetate stimulates lipid synthesis, but at the same time propionate may counteract *de novo* lipogenesis from acetate (Delzenne & Cani, 2011). Therefore, it can be speculated that the ratio of SCFA produced by the obese microbiota is in a sense not "protective" against excess lipid production, since acetate concentrations are higher than those of propionate. Indeed SCFA have different metabolic effects in the host and therefore changing the proportional abundance of these metabolites might result in significant changes in responses of host cells (Yadav, Lee, Lloyd, Walter, & Rane, 2013).

It is noteworthy that lactate production by the obese microbiota fed with inulin was higher than on all the other substrates, as well as compared with lean microbiota. Lactate is an intermediate metabolite in intestinal fermentation and it only accumulates when there is a fast fermentation of the substrate (Maathuis et al., 2009). Besides that, it is the major end product of bifidobacteria metabolism, which can explain the amount of lactate production in obese microbiota fed with inulin since it primarily stimulated the increase of abundance of this genus. However, cassava bagasse in lean microbiota increased the abundance of bifidobacteria even more, but was not followed by an increase in either acetate or lactate. Here, lactate may have been converted into the other SCFA.

4.4. *Branched-chain fatty acids and ammonia*

The amounts of BCFA produced during cassava fermentation by the lean microbiota were higher when comparing with the standard and inulin. However, these concentrations were lower when compared with the obese microbiota. What could be hypothesized here is that the difference in microbiota composition between lean and obese may have had an effect on the production of these different metabolites. Certainly further studies are required in order to confirm this hypothesis and find out exactly which bacterial species are related with protein fermentation that in turn yields BCFA and ammonia as metabolites.

4.5. *Lipopolysaccharide*

Given that obesity is considered to be a low-grade inflammatory disease and that previous study demonstrated that obese subjects have higher concentration of plasma LPS (Cani et al., 2007) than lean subjects, it was hypothesized that the luminal concentration of LPS in TIM-2 could also be higher for the microbiota from obese subjects compared with the microbiota from lean subjects. Our first results shown here are not in

agreement with this. Further experiments are required to confirm differences in luminal LPS concentration.

4.6. *Peptide YY*

In an *ex vivo* experiment, pig intestinal segments were incubated with TIM-2 luminal samples and preliminary results indicated a higher release of PYY induced by the obese microbiota metabolites (not shown). Since PYY induces satiety, this was unexpected. However, it may also point to the beneficial effects of dietary interventions with inulin and/or cassava on PYY expression. It remains to be seen which (combination of) metabolites in the obese samples are responsible for the higher release of PYY from colonic tissue.

4.7. *Production of energy*

It was expected that the results of this study would show a higher amount of SCFA produced by the obese microbiota when compared with the lean microbiota, since SCFA provide extra energy to the host. However, the present study did not show this. Inulin was the only substrate that after fermentation by the obese microbiota showed a higher amount of energy extraction when compared with the lean microbiota. It should be noted that equal amounts of carbohydrates were provided to these microbiotas, which is likely to be different *in vivo*, where obese individuals overeat and hence more carbohydrates may reach the colon.

An explanation to this unexpected result could be the fact that it has been suggested that SCFA have satiety-enhancing properties, where for instance, the expression of proglucagon, the precursor of glucagon-like peptide 1 (GLP-1), can be upregulated by SCFA (Parnell & Reimer, 2012). Besides this, it has been reported that butyrate can increase energy expenditure and fatty acid oxidation in rats (Gao et al., 2009). Thus, one can argue that higher amounts of SCFA are responsible to keep the lean individuals lean due to the satiety effects of the gut microbiota metabolites. The results clearly show that SCFA production depends on the substrate provided and on the origin of the microbiota. Accordingly, this study shows that microbiota fermentation of substrates and their consequent productions of SCFA (and therefore the production of extra energy to the host) are not the only factor that can affect body weight. It seems that it involves different and complex mechanisms (not investigated here). It is definitely not only a question of extra calories yielded, but the intricate role that each SCFA has on host metabolism.

5. Conclusions

Health professionals know the difficulties that people experience in changing eating habits and lifestyle necessary for weight management. Therefore, there is an urgent need for alternative solutions that can assist people in their daily battle with overweight and obesity and by this improve the quality of life of a great portion of the world population.

Here we showed for the first time the potential prebiotic properties of cassava bagasse, an industrial residue that currently does not have commercial value. It induced different effects in microbiotas originating from lean and obese individuals, and seemed to drive the obese microbiota composition closer to that of lean individuals. It is thought that particularly the starch fraction was responsible for the increase growth of beneficial bacteria, including the bifidogenic effect. Thus, it shows great promise in becoming a functional food in the future, although further experiments in order to confirm the results obtained in this study are required. Additionally, to follow European Food Safety Authority (EFSA) recommendation it is necessary to show the prebiotic effect of cassava bagasse in supplementary *in vivo* studies. Nevertheless, the methodology used here shows great promise as a rapid screening tool for evaluation of prebiotic activity of indigestible substrates.

Conflict of interest

The authors declare that there is no conflict of interest. Part of the results was presented at the European Network for Gastrointestinal Health Research (ENGIRH) 2013, 18–20 August – Valencia, Spain, at the 15th Gut Day Symposium (2013), 7 November – Groningen, the Netherlands and at the 13th European Training Course on Carbohydrates (2014), 13–17 April – Wageningen, the Netherlands.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2014.09.019.

REFERENCES

Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., Semenkovich, C. F., & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage.

Proceedings of the National Academy of Sciences of the United States of America, *101*, 15718–15723.
Blaut, M., & Klaus, S. (2012). Intestinal microbiota and obesity. *Handbook of Experimental Pharmacology*, *209*, 251–273.
Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A. M., Fava, F., Tuohy, K. M., Chabo, C., Waget, A., Delmee, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrieres, J., Tanti, J. F., Gibson, G. R., Casteilla, L., Delzenne, N. M., Alessi, M. C., & Burcelin, R. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, *56*, 1761–1772.
Cani, P. D., Delzenne, N. M., Amar, J., & Burcelin, R. (2008). Role of gut microflora in the development of obesity and insulin resistance following high-fat diet feeding. *Pathologie-Biologie*, *56*, 305–309.
Cardoso, E. M. R., Hühn, S., & Junior, J. D. D. N. (1999). Processo industrial para beneficiamento da macaxeira. *Novos Cadernos NAEA*, *2*, 177–184.
Costa, L. A. D., Bramorski, A., Silva, M. C., Teixeira, E., & Amboni, R. D. D. M. C. (2005). Desenvolvimento de alimento em barra a base de resíduo da fabricação de farinha de mandioca. *Alimentos e Nutrição*, *16*, 8.
de Goffau, M. C., Luopajarvi, K., Knip, M., Ilonen, J., Ruohtula, T., Harkonen, T., Orivuori, L., Hakala, S., Welling, G. W., Harmsen, H. J., & Vaarala, O. (2013). Fecal microbiota composition differs between children with beta-cell autoimmunity and those without. *Diabetes*, *62*, 1238–1244.
Darzi, J., Frost, G. S., & Robertson, M. D. (2011). Do SCFA have a role in appetite regulation? *The Proceedings of the Nutrition Society*, *70*, 119–128.
Delzenne, N. M., & Cani, P. D. (2011). Interaction between obesity and the gut microbiota: Relevance in nutrition. *Annual Review of Nutrition*, *31*, 15–31.
Delzenne, N. M., Neyrinck, A. M., & Cani, P. D. (2013). Gut microbiota and metabolic disorders: How prebiotic can work? *The British Journal of Nutrition*, *109*.
Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics (Oxford, England)*, *27*, 2194–2200.
Elli, M., Colombo, O., & Tagliabue, A. (2010). A common core microbiota between obese individuals and their lean relatives? Evaluation of the predisposition to obesity on the basis of the fecal microflora profile. *Medical Hypotheses*, *75*, 350–352.
Ferreira, G. D. G., Oliveira, R. L., Cardoso, E. D. C., Magalhães, A. L. R., & Brito, E. L. (2007). Valor nutritivo de co-produtos da mandioca. *Revista Brasileira de Saúde e Produção Animal*, *8*, 364–374.
Fleissner, C. K., Huebel, N., Abd El-Bary, M. M., Loh, G., Klaus, S., & Blaut, M. (2010). Absence of intestinal microbiota does not protect mice from diet-induced obesity. *The British Journal of Nutrition*, *104*, 919–929.
Gao, Z., Yin, J., Zhang, J., Ward, R. E., Martin, R. J., Lefevre, M., Cefalu, W. T., & Ye, J. (2009). Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes*, *58*, 1509–1517.
Gibson, G. R., Cummings, J. H., & Macfarlane, G. T. (1988). Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Applied and Environmental Microbiology*, *54*, 2750–2755.
Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *The Journal of Nutrition*, *125*, 1401–1412.
Gozho, G. N., Krause, D. O., & Plaizier, J. C. (2006). Rumen lipopolysaccharide and inflammation during grain adaptation and subacute ruminal acidosis in steers. *Journal of Dairy Science*, *89*, 4404–4413.
Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., & Brummer, R. J. (2008). Review article: The role of butyrate on colonic function. *Alimentary Pharmacology & Therapeutics*, *27*, 104–119.
Huse, S. M., Welch, D. M., Morrison, H. G., & Sogin, M. L. (2010). Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environmental Microbiology*, *12*, 1889–1898.
Kovatcheva-Datchary, P. (2010). *Analyzing the functionality of the human intestinal microbiota by stable isotope probing* (PhD thesis). Wageningen, The Netherlands: Wageningen University and Research Centre.
Kovatcheva-Datchary, P., Egert, M., Maathuis, A., Rajilic-Stojanovic, M., de Graaf, A. A., Smidt, H., de Vos, W. M., & Venema, K. (2009). Linking phylogenetic identities of bacteria to starch fermentation in an in vitro model of the large intestine by RNA-based stable isotope probing. *Environmental Microbiology*, *11*, 914–926.
Leonel, M., Cereda, M., & Roau, X. (1999). Aproveitamento do resíduo da produção de etanol a partir de farelo de mandioca, como fonte de fibras dietéticas. *Ciência e Tecnologia de Alimentos*, *19*, 241–245.
Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 11070–11075.
Maathuis, A., Hoffman, A., Evans, A., Sanders, L., & Venema, K. (2009). The effect of the undigested fraction of maize products on the activity and composition of the microbiota determined in a dynamic in vitro model of the human proximal large intestine. *Journal of the American College of Nutrition*, *28*, 657–666.
Mallillin, A. C., Trinidad, T. P., Raterta, R., Dagbay, K., & Loyola, A. S. (2008). Dietary fibre and fermentability characteristics of root crops and legumes. *The British Journal of Nutrition*, *100*, 485–488.
Martens, E. C., Lowe, E. C., Chiang, H., Pudlo, N. A., Wu, M., McNulty, N. P., Abbott, D. W., Henrissat, B., Gilbert, H. J., Bolam, D. N., & Gordon, J. I. (2011). Recognition and degradation of plant cell wall polysaccharides by two human gut symbionts. *PLoS Biology*, *9*, e1001221.
Minekus, M., Smeets-Peeters, M., Bernalier, A., Marol-Bonnin, S., Havenaar, R., Marteau, P., Alric, M., Fonty, G., & Huis in't Veld, J. H. (1999). A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. *Applied Microbiology and Biotechnology*, *53*, 108–114.
Osundahunsi, O. F., Williams, A. O., & Oluwalana, I. B. (2012). Prebiotic effects of cassava fibre as an ingredient in cracker-like products. *Food & Function*, *3*, 159–163.
Pandey, A., Soccol, C. R., Nigam, P., Soccol, V. T., Vandenberghe, L. P. S., & Mohan, R. (2000). Biotechnological potential of agro-industrial residues. II: Cassava bagasse. *Bioresource Technology*, *74*, 81–87.
Parnell, J. A., & Reimer, R. A. (2012). Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. *The British Journal of Nutrition*, *107*, 601–613.
Pustjens, A. M., De Vries, S., Gerrits, W. J., Kabel, M. A., Schols, H. A., & Gruppen, H. (2012). Residual Carbohydrates from In vitro Digested Processed Rapeseed (Brassica napus) Meal. *Journal of Agricultural and Food Chemistry*, *60*, 8257–8263.
Reimer, R. A., Maathuis, A. J., Venema, K., Lyon, M. R., Gahler, R. J., & Wood, S. (2014). Effect of the novel polysaccharide PolyGlycopleX(R) on short-chain fatty acid production in a computer-controlled in vitro model of the human large intestine. *Nutrients*, *6*, 1115–1127.

Rodrigues, J. P. M., Caliari, M., & Asquieri, E. R. (2011). Caracterização e análise sensorial de biscoitos de polvilho elaborados com diferentes níveis de farelo de mandioca. *Ciência Rural : Publicação de Universidade Federal de Santa Maria*, *41*, 2196–2202.

Rycroft, C. E., Jones, M. R., Gibson, G. R., & Rastall, R. A. (2001). A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *Journal of Applied Microbiology*, *91*, 878–887.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., & Weber, C. F. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*, 7537–7541.

Schwiertz, A., Taras, D., Schafer, K., Beijer, S., Bos, N. A., Donus, C., & Hardt, P. D. (2010). Microbiota and SCFA in lean and overweight healthy subjects. *Obesity*, *18*, 190–195.

Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews*, *81*, 1031–1064.

van der Kamp, J. W., Jones, J., Mccleary, B., & Topping, D. J. (2010). *Dietary fibre: New frontiers for food and health*. Wageningen Academic Publishers.

Venema, K. (2010). Role of gut microbiota in the control of energy and carbohydrate metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care*, *13*, 432–438.

Venema, K., van Nuenen, M., Smeets-Peeters, M., Minekus, M., & Havenaar, R. (2000). TNO's in vitro large intestinal model: An excellent screening tool for functional food and pharmaceutical research. *Ernährung Nutrition*, *24*, 559–564.

Voortman, T., Hendriks, H. F., Witkamp, R. F., & Wortelboer, H. M. (2012). Effects of long and short chain fatty acids on the release of gastrointestinal hormones using an ex vivo porcine intestinal tissue model. *Journal of Agricultural and Food Chemistry*, *60*, 9035–9042.

WHO. (2013). Obesity and Overweight – Fact sheet n. 311. In (Vol. 2014).

Yadav, H., Lee, J. H., Lloyd, J., Walter, P., & Rane, S. G. (2013). Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. *The Journal of Biological Chemistry*, *288*, 25088–25097.