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Physicochemical differences between malanga (*Xanthosoma sagittifolium*) and potato (*Solanum tuberosum*) tubers are associated with differential effects on the gut microbiome

Brittany L. Graf^{1,*}, Li Zhang¹, Maria G. Corradini^{2,†}, Peter Kuhn¹, Susan S. Newman³, J. Michael Salbaum³, and Ilya Raskin¹

¹Department of Plant Biology, Rutgers University, New Brunswick, NJ 08901, USA

²Department of Food Science, Rutgers University, New Brunswick, NJ 08901, USA

³Pennington Biomedical Research Center, 6400 Perkins Rd, Baton Rouge, LA 70808, USA

Abstract

Malanga (*Xanthosoma sagittifolium*) is used as a medicinal food for infant development and gastritis. We compared the physicochemical properties and gut microbial effects of malanga versus potato (*Solanum tuberosum*) using nutritional analysis, rheometry, *in vitro* TNO Intestinal Model, and C57Bl/6J mouse models. Malanga was characterized by higher starch (70.7% v. 66.3%), lower amylose:amylopectin (0.33 v. 0.59), higher free sugar (5.44% v. 3.23%), lower viscosity (271.0 v. 863.0 mPa.s), and higher bioaccessible and bioavailable sugar (0.89 v. 0.11 g bioaccessible sucrose per 20 g load *in vitro*; blood glucose levels of 129.1 v. 95.2 and 133.8 v. 104.3 mg/dL after 20 and 60 min *in vivo*). Gut microbiota of mice fed a high fat diet containing 20% malanga for 14 d exhibited significantly higher α diversity than those fed 20% potato, indicating that minor physicochemical differences between similar tuber crops are associated with significantly different effects on the gut microbiome.

Keywords

carbohydrate; digestion; microbiome; starch

*Corresponding author: 59 Dudley Rd, New Brunswick, NJ 08901; Phone: (848) 932-6336; britgraf@gmail.com.

†Current affiliation: Department of Food Science, University of Massachusetts, Amherst, MA, 01003, USA

DATA DEPOSITION

The microbial DNA sequences encoding bacterial 16S rRNA V4 region reported in this paper have been deposited in the Sequence Read Archive (SRA) under the accession number SRP114686.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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1. INTRODUCTION

Known as “malanga” in Cuba, “yautía” in the Dominican Republic, “taioba” in Brazil, and “tannia” or “new cocoyam” in other regions, *Xanthosoma sagittifolium* (L.) Schott (Araceae) is an important food crop for over 400 million people throughout the tropics. High in starch (77.1% of flour) (Hoyos-Leyva, et al., 2017b), *X. sagittifolium* tubers are commonly consumed as a boiled vegetable similarly to potatoes (*Solanum tuberosum* L., Solanaceae), but also used to produce flour, soup thickener, porridge, and beverages (Owusu-Darko, et al., 2014). In Cuba and other countries, malanga tubers are widely claimed to be easily digestible, and are used as a functional food to promote infant development during weaning and to reduce symptoms of gastritis in adults (Owusu-Darko, et al., 2014). However, limited studies have assessed malanga’s nutritional properties and food applications, and, to our knowledge, no processed malanga products are currently available on the market.

Comparatively, potatoes are the world’s third largest food crop, the most extensively consumed root vegetable, the source of a wide variety of processed food products (ie. instant mashed potatoes), and one of the most well studied sources of starch (Ek, et al., 2012). Potato starch functionality and digestibility are known to be determined by granule size, structure, amylose:amylopectin ratio, and mineral content (Ek, et al., 2012; Singh, et al., 2003; Srichuwong, et al., 2005). Food processing techniques, such as the heating, cooling, and dehydrating processes used to produce instant mashed potatoes (Lamberti, et al., 2004), have also been shown to affect potato digestibility compared to cooked tubers by resulting in higher levels of resistant starch (Mishra, et al., 2008). Rehydration of dehydrated samples then re-crystallizes the starch to return it to a state that mimics its condition after boiling (Mishra, et al., 2008), though retrograded starches with higher amylose content are more difficult to re-gelatinize (Ek, et al., 2012).

Few studies have directly compared the morphological and physicochemical properties of starches of potato to those of malanga. Using electron scanning microscopy and X-ray diffraction, one study showed that malanga starch granules were smaller in diameter ($20.7 \pm 8.1 \mu\text{m}$) and tended to display spherical-polygonal shapes distinct from those of potato ($42.3 \pm 38.1 \mu\text{m}$ in diameter, oval-spherical in shape) (Srichuwong, et al., 2005). Generally, small starch granules are more rapidly digested than large granules due to larger surface area:volume ratio that facilitates access of digestive enzymes (Ek, et al., 2012). Another study found that malanga starches were characterized by an A-type X-ray diffraction pattern, whereas potato starches were B-type (McPherson & Jane, 1999; Srichuwong, et al., 2005). A-type starches generally have shorter average amylopectin branch chain lengths than B-type, which comparatively reduces the structural stability of their amylopectin helices and makes them more prone to enzymatic digestion (Ek, et al., 2012; McPherson & Jane, 1999; Singh, et al., 2003; Srichuwong, et al., 2005). Hoyos, et al. (2017b) confirmed the A-type crystalline structure and small granule size of malanga starches ($21.2 \pm 3.2 \mu\text{m}$ in diameter, with a large population of granules $1 - 10 \mu\text{m}$), and also found that malanga contained lower amylose content (11.3% of isolated starch) than has been reported in potato starch (18.4–26.2% amylose) (Noda, et al., 2007). Amylose:amylopectin content is an important factor in determining starch digestibility because linear amylose chains form stabilized amorphous

regions whereas the branch points of amylopectin forms double helices that arrange in external crystalline structures which are more susceptible to hydrolysis (Srichuwong, et al., 2005).

Studies on the biological implications of different botanical sources of starch have predominantly focused upon differing starch structures and compositions, and their effects upon rate of hydrolysis *in vitro* and glycemic response *in vivo*. Very few studies have measured effects of different botanical sources of starches on the lower gastrointestinal tract or gut microbial communities. However, it is estimated that 10–60 g/d dietary carbohydrates reach the colon in human adults, including 8–40 g/d resistant starch, 8–18 g/d non-starch polysaccharides, 2–10 g/d unabsorbable sugars, and 2–8 g/d oligosaccharides (Gibson & Roberfroid, 1995). These residual carbohydrates are fermented by gut microbes that are differentially equipped to produce specific carbohydrate-degrading enzymes (Flint, et al., 2012a; Martens, et al., 2014), and play a major role in animal health and disease. Gut microbial community composition has been shown to impact child growth and development (Arrieta, et al., 2014; Edwards & Parrett, 2002; Gibson & Roberfroid, 1995; Koleva, et al., 2015; Marques, et al., 2010), intestinal pathogen invasion (Martens, et al., 2014), energy intake and expenditure, bone mineralization, nervous system development, and immunomodulation (Flint, et al., 2012a; Marques, et al., 2010).

Two studies have assessed the effect of potato starch consumption on the colon/hindgut. Han, et al. (2008) showed that potato flake consumption increased total anaerobes and short-chain fatty acids (SCFAs) in the cecum of rats compared to a corn starch diet. Panasevich, et al. (2015) demonstrated that potato fiber consumption increased the abundance of key fermenting bacteria and SCFA levels in the feces of dogs. Based upon evidence that distinct physicochemical properties of carbohydrates differentially affect the composition and function of gut microbial communities, malanga consumption may uniquely impact the gut compared to potato.

Inspired by the traditional uses and alleged digestive health benefits associated with malanga, we aimed to assess the composition, digestibility and gut microbial effects of processed malanga tubers in comparison to the most commonly consumed starchy root vegetable, potato. We chose to investigate the effect of whole foods, rather than isolated chemical constituents, to most closely mimic the effects of human consumption of the processed food products. Malanga and potato are both comprised mainly of starch, but also contain low levels of non-starch polysaccharides, cell wall fibers, proteins, vitamins and minerals that may impact gut communities (Flint, et al., 2012a; Le Tourneau, 1956). Understanding the interactions between gut bacterial communities and complex food matrices that represent true dietary choices may provide a better depiction of outcomes following human dietary interventions.

2. MATERIALS AND METHODS

2.1 Preparation of botanical material

White malanga tubers and white Idaho potatoes (*Solanum tuberosum* L., Solanaceae) were purchased from New Jersey food stores in November 2015. A malanga voucher specimen

was deposited in the Chrysler Herbarium at Rutgers University (accession no. 146337). Tubers were processed according to common cooking methods. Briefly, tubers were peeled, cut into 2.5 cm slices, and boiled in Millipore water in 2 kg batches (1:1 tubers:water by weight) for 20 min. Fork-tender tuber slices were mashed, cooled to room temperature for 1 h, stored at -20°C for at least 24 h, and freeze-dried. Freeze-drying was selected as the dehydration method for this study because it is considered the best method to preserve starch properties compared to other drying techniques (Pelletier, et al., 2010). Dry malanga powder and dry potato powder were weighed to calculate yield (Supplemental Table 1).

2.2 Nutritional analysis

Nutritional analysis of malanga and potato powders was performed by Medallion Labs (Minneapolis, MN) in accordance with Association of Analytical Communities (AOAC) methods for protein (Dumas assay, AOAC 968.06), fat (acid hydrolysis, AOAC 996.06), ash (AOAC 923.03), free sugars [high performance liquid chromatography (HPLC) assay, AOAC 977.20 with modifications], and dietary fiber (soluble, insoluble, resistant starch types RS2 and RS3, and resistant oligosaccharides) by enzymatic digestion followed by HPLC quantification of low molecular weight soluble dietary fiber (AOAC 991.43/2001.03). Moisture was determined by vacuum oven at 70°C for 16 h. Total carbohydrates were determined by difference and calories by calculation. Total starch content was determined using hydrolysis, and amylose:amylopectin composition was determined using reported methods (Matheson & Welsh, 1988; Yun & Matheson, 1990). Briefly, samples were heat-dispersed in dimethyl sulfoxide (DMSO), and starch was precipitated in ethanol and dissolved in acetate/salt solution. Amylopectin was precipitated by the addition of concanavalin A and removed by centrifugation. Amylose and total starch were measured from aliquots of the supernatant via enzymatic hydrolysis to D-glucose, which was analyzed using glucose oxidase/peroxidase reagent. The concentration of amylose in the starch sample was estimated as the ratio of glucose oxidase and peroxidase (GOPOD) absorbance at 510 nm of the supernatant of the concanavalin A precipitated sample to that of the total starch sample.

2.3 *In vivo* glycemic response

All animal protocols used in this study were approved by the Rutgers University Institutional Care and Use Committee and followed federal and state laws. Five-week-old C57Bl/6J male mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and fed a standard chow diet (10% fat) *ad libitum* (cat. No. 5015; Purina) during their 1-wk acclimatization period and for the next 5 wk. Animals were housed 4 per cage with free access to water in a room with a temperature of $24 \pm 1^{\circ}\text{C}$ and 12:12-h light:dark cycle (7:00 AM – 7:00 PM). At 6 wk of age, postprandial glycemic response tests were performed on 24 mice (12 mice each for malanga and potato treatment groups). Mice were fasted overnight (14 h) with *ad libitum* access to water. Baseline (0 min) fasting blood glucose levels were determined via tail-nick using a handheld glucometer (AlphaTRAK® 32004-02; Abbott Animal Health, Randor, PA) (Graf, et al., 2014). Mice were then administered the highest concentration of malanga or potato suspension that could pass through a gavage needle (1.67 g/kg body weight) by dosing 0.2 mL/20 g mouse of a suspension of 3.34 g powder/20 mL water). Suspensions were prepared immediately before the experiment by rehydrating powders in water for 20

min at 95 °C, and were mixed by inversion between dosing each animal to maintain homogeneity. Blood glucose levels were measured again at 20, 40, 60, 90 and 120 min after gavage. Area under the curve (AUC_{0–120 min}) was calculated using the trapezoidal method (Muruganandan, et al., 2005).

2.4 *In vitro* digestion in TIM-1

The TNO Intestinal Model 1 (TIM-1) (Ribnický, et al., 2014) was used to simulate human gastrointestinal digestion (stomach, duodenum, jejunum, and ileum) of 20 g malanga or potato powder. Powders were each rehydrated for 20 min at 95 °C in 300 mL gastric electrolyte solution containing a final concentration of sodium chloride (6.2 g/L), potassium chloride (2.2 g/L), and calcium chloride dihydrate (0.3 g/L); then the suspensions were cooled for 20 min and fed to the TIM-1 warm. Experiments were conducted as described previously (Fassler, et al., 2006). Briefly, amylase, pepsin, lipase, trypsin, fresh porcine bile, and pancreatin (containing amylase, trypsin, lipase, and protease) were introduced into various compartments of the system. The pH of the entire system was monitored and auto-adjusted by injections of HCl (1 M) or NaHCO₃ (1 M). Hollow fiber filtration devices, composed of semi-permeable membranes, were connected to jejunal and ileal compartments to simulate human intestinal absorption (Fassler, et al., 2006). Jejunal and ileal absorption samples, as well as the ileal excretion, were collected every 30 min for 300 min, weighed to determine volume, diluted 1:1 with water, and stored at –20 °C until further analysis. Three replicates were performed for each malanga and potato.

2.5 HPLC-ELSD analysis

Jejunal/ileal filtrates and ileal efflux collected from TIM-1 experiments were analyzed using Waters Alliance e2695 Separation Module High Performance Liquid Chromatography with Waters 2424 Evaporative Light Scattering Detector (HPLC-ELSD) according to previously described methods (Shanmugavelan, et al., 2013) with modifications. Identification and quantification of the individual sugars was performed using external standards of glucose, sucrose, and maltose (Sigma-Aldrich, Bellefonte, PA) (0.5 – 5.0 mg/mL). A SUPELCOSIL™ LC-NH₂, 5 µm (25 cm × 4.6 mm) column (Sigma-Aldrich) was used in combination with a Supelguard™ LC-NH₂ guard column 2 cm cartridge. The stroke volume for the system was 50 µL, and an isocratic pump mode was used with a solvent mixture of 20% HPLC-grade water and 80% HPLC-grade acetonitrile (EMD Chemicals, Inc., Gibbstown, NJ). Column temperature was set at 30 °C.

2.6 Rheological properties

Steady state rheological properties of malanga and potato suspensions (1 g powder heated for 20 min at 95 °C in 9 mL water each) were evaluated using a Discovery Hybrid Rheometer HR-2 (TA Instruments, DE) equipped with a 60 mm cone plate geometry. Flow curve was recorded at 25 °C at a shear rate from 0.25–150 s^{–1}. Herschel-Bulkley model was selected to fit the flow curves using nonlinear regression (Moelants, et al., 2013). This model is expressed as: $\tau = \tau_0 + k \dot{\gamma}^n$, where τ is shear stress, $\dot{\gamma}$ is shear rate, τ_0 is yield stress, k is consistency coefficient and n is flow index. Samples were compared in triplicate based on the parameters of the model and their apparent viscosity at 30 s^{–1}.

2.7 Chronic feeding of C57Bl/6J mice

Sixteen-week-old C57Bl/6J male mice were purchased from Jackson Laboratories (Bar Harbor, ME). These mice had been fed a basal high fat diet (HFD; D12492, Research Diets, NJ) containing 62% kcal from fat, 18% from protein, and 20% from carbohydrate (Supplemental Table 2) for 11 wk to induce obesity, hyperglycemia, and gut microbial dysbiosis (Murphy, et al., 2015). High-fat-diet mice were chosen as a model of intestinal dysbiosis because alterations in the gut microbiota have been well characterized in this model, and this model has been commonly used to explore the effect of dietary interventions on gut microbial community structure (Hildebrandt, et al., 2009; Murphy, et al., 2015; Turnbaugh, et al., 2008). Upon arrival at Rutgers University animal facility, mice were fed the same high fat diet *ad libitum* during their 1-wk acclimatization period. Animals were housed 2 per cage under the same conditions described for the glycemic response study. On the day that the mice turned 17 wk of age, mice were placed in individual cages until 7 fecal pellets were collected from each mouse using sterilized forceps. Fecal pellets were immediately placed on dry ice and stored at -80°C . Then, mice were randomly fed high fat diets containing 20% malanga or potato powder (12 mice per group) for 14 d (Experiment Diet #1). Using nutritional composition data, all diets were formulated by Research Diets to be comparable in the percentage of kcal contributed by protein, fat, carbohydrates, and fiber (Supplemental Table 2). On day 14, mice were placed in individual cages until 7 fecal pellets were collected from each mouse. Mice were then switched back to the standard high-fat diet (D12492) to begin a 14 d washout period. On day 28, fecal pellets were collected a third time. Using a crossover design, groups were then switched so that mice that had consumed malanga during Experiment Diet #1 were now fed potato for 14 d, and vice versa. Fecal pellets were collected the fourth time at the end of the second intervention (Experiment Diet #2). Over the course of the study, body weights and food intake were recorded 3 times per wk. Food intake per mouse per day was calculated as follows: [total food intake per cage]/[mice per cage]/[days of food consumption].

2.8 16S rRNA gene sequencing

Genomic DNA was extracted from 100 mg of each fecal sample using the PowerLyzer PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA). The V4 region of bacterial 16S rRNA gene was amplified using primers (Integrated DNA Technologies) adopted from the MiSeq Wet Lab standard operating procedure (SOP) (Kozich, et al., 2013): forward 5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC<8-nt index>TAT GGT AAT TGT GTG CCA GCM GCC GCG GTA A-3' and reverse 5'-CAA GCA GAA GAC GGC ATA CGA GAT<8-nt index>AGT CAG TCA GCC GGA CTA CHV GGG TWT CTA AT-3'. PCR reactions were conducted with 20 ng of template DNA, 1 μL of each paired set of indexed primers (10 μM), 25 μL of NEBNext High-Fidelity 2X PCR Master Mix (New England BioLabs) in a total reaction volume of 50 μL in 96-well plates. PCR conditions were 2 min at 95°C ; 30 cycles of 20 s at 95°C , 15 s at 55°C , 5 min at 72°C ; followed by 10 min at 72°C . Success of PCR was confirmed by running random samples in a 1% agarose gel at 100 v for 30 min. PCR products were cleaned using Agencourt AMPure XP system (Beckman Coulter), and quantified using Spectramax Quant Accuclear Nano dsDNA Assay Kit (Molecular Devices). An equal amount of PCR product from each sample was pooled to construct a library pool, which was then checked using Agilent Bioanalyzer with

Agilent High Sensitivity DNA Kit, and quantified using KAPA HiFi HotStart ReadyMix (Kapa Biosystems). Sequencing was performed using Illumina MiSeq with MiSeq Reagent Kit v3.

2.9 Sequencing data analysis

Sequences were de-multiplexed and trimmed to eliminate barcodes and primers using MiSeq Reporter v2.2. Contigs were generated using Mothur v1.36.1 (Schloss, 2009), and those with a length of 252–253 bp, no ambiguous bases, and homopolymers shorter than 8 bp were kept. The contigs were further de-replicated with VSearch 1.10.2 (Rognes, et al., 2016).

Operational taxonomic units (OTUs) were generated using USEARCH v8.1.1861 (Edgar, 2013). Unique sequences except singletons were clustered at 97% sequence homology. Chimeras were firstly filtered by the UPARSE-OTU algorithm and then by the UCHIME algorithm (Edgar, et al., 2011) using the rdp_gold database and the default threshold. The depths of the resulting OTU table ranged from 19,015 to 116,227 with a median of 64,914. Taxonomies of OTU representative sequences were assigned using the Ribosomal Database Project (RDP) Classifier v2.12 (Wang, et al., 2007) with a confidence threshold of 50%. OTUs were then aggregated at different phylogenetic levels.

Microbiota α diversity (Shannon index and OTU richness) and β diversity were analyzed based on 19,000 sequences per sample using Qiime 1.9.1 (Caporaso & et.al., 2010). A phylogenetic tree was built from OTU representative sequences as previously described (Zhang, et al., 2017). Principle coordinate analyses (PCoA) were performed on unweighted UniFrac distances (Lozupone & Knight, 2005) between bacterial communities. ADONIS tests were performed to assess the differential clustering of bacterial communities using the vegan R package v2.4-1 (Oksanen, et al., 2015).

To discover OTUs/genera/phyla that were differently altered by malanga and potato diets, features that were present in less than 50% of samples in both groups and features with average relative abundances lower than 0.02% in both groups were filtered out. Zero values in the relative abundance table were replaced with the detection limit $0.5/60000 = 0.00083\%$ for calculating fold-changes. The matrices of fold-changes/relative abundances were permuted 10,000 times, and p values represent fraction of times that permuted differences assessed by Welch's t test were greater than or equal to real differences. P values from multiple testing were adjusted (q values) using the Benjamini-Hochberg false discovery rate (FDR) with a significance level of 0.05 (Benjamini & Hochberg, 1995).

2.10 Statistics

ANOVA analyses were performed using GraphPad Prism 6.0 software (La Jolla, CA, USA). T-test comparisons were performed in Excel. Statistical analysis of 16S rRNA DNA sequencing metagenomics was performed as described above. $P/q < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties and digestibility of malanga versus potato

Malanga and potato powders displayed differences in nutritional composition (Table 1). Malanga powder had higher total carbohydrate and lower protein content than potato. Higher carbohydrate content in malanga resulted from higher total starch, as well as slightly higher total fiber and free sugars, especially sucrose. The fiber profile for malanga and potato differed in that malanga contained lower soluble fiber and resistant starch, but higher levels of insoluble fiber and resistant oligosaccharides. Both malanga and potato powders contained low levels of fat and similar levels of ash. As shown in Supplemental Table 1, malanga tubers also contained lower water content than potato tubers, indicated by higher yield in dry weight.

Malanga starch was characterized by a lower amylose:amylopectin ratio than potato starch. These results corroborate previous findings that malanga contains low amylose levels (Hoyos-Leyva, et al., 2017b). Combined with the nutritional profile showing higher carbohydrate level in malanga, these results would lead us to expect higher bioaccessible and bioavailable sugar levels following malanga digestion compared to potato. Starches higher in amylopectin are generally more susceptible to enzymatic hydrolysis due to easier enzymatic access of the external crystalline helices formed by amylopectin (Srichuwong, et al., 2005). Furthermore, retrograded starches lower in amylose content undergo more successful re-crystallization during rehydration (Ek, et al., 2012).

In agreement with the expectation that malanga starch would be more rapidly digested than potato, we found significantly higher bioavailability of sugars in healthy mice treated with malanga compared to potato, as measured by a glycemic response test (Fig. 1). Bioavailability of malanga and potato carbohydrates were estimated by quantifying blood glucose levels in 5-wk-old C57Bl/6J mice at 20, 40, 60, 80, 100, and 120 min post-gavage of rehydrated powders. Malanga resulted in significantly higher blood glucose levels at 20 and 60 min time points (129.1 v. 95.2 mg/dL at 20 min; 133.8 v. 104.3 mg/dL at 60 mins; Fig. 1A), as well as a trend for higher overall glycemic response, as calculated by the AUC from 0 – 120 min ($P = 0.0691$, t-test) (Fig. 1B). The test used in our mouse study resembles the postprandial glycemic response test used in humans to determine the glycemic index of carbohydrate-rich foods, which is directly related to the enzymatic digestibility of the carbohydrates and the subsequent bioavailability of their sugar monomers (Tahvonon, et al., 2006). Though more bioavailable sugar levels may not be desirable for adults with diabetes, rapid digestion of carbohydrates could be beneficial for young infants with developing digestive systems whose amylase enzymatic activity is lower than that of adults (Sevenhuysen, et al., 1984).

To further explore the digestibility of malanga versus potato, we utilized the TIM-1 simulated digestive model and quantified the level of bioaccessible free sugars (glucose, maltose, and sucrose) over a 6 h digestion time (Fig. 2, Table 2). Malanga powder yielded significantly higher bioaccessible sucrose than potato powder at 3 time points: 280.9 ± 229.3 v. 16.9 ± 15.0 mg at 90 min, 201.2 ± 73.1 v. 21.9 ± 19.5 mg at 120 min, and 194.4 ± 83.9 v. 19.1 ± 18.2 mg at 150 min (Fig. 2C), and significantly higher total bioaccessible sucrose

over the 6 h digestion time (Table 2). Supplemental Figure 1 shows representative HPLC-ELSD chromatograms for the TIM-1 jejunal filtrates of malanga and potato powder digestion at time point 120 min (the point of generally highest bioaccessible sugar levels), in which a higher sucrose level in the malanga powder sample is apparent by the larger area of peak 3. Total amounts of bioaccessible sucrose following malanga and potato feeding are equivalent to the initial amounts of free sucrose delivered to the TIM-1 by each of these tubers, respectively (Table 2).

Maltose levels also tended to be higher at 120 and 150 min for malanga (Fig. 2B), but this trend was insignificant. Glucose and maltose are shown as peaks 1 and 2 in Supplemental Figure 1, respectively. Peaks eluted after t_R 16.0 min indicate the presence of other starch degradation products (ie. maltotriose, other oligosaccharides) in the jejunal and ileal filtrates that could not be measured by our HPLC-ELSD method. The total combined weights of glucose and maltose recovered in the jejunal and ileal filtrates following malanga and potato feeding were 59.5% and 62.2% of the initial weights of digestible starches delivered to the TIM-1 by a 20 g dose of each of these tuber powders, respectively (calculated after subtracting free glucose and maltose levels in the initial meal) (Table 2). This indicates that about 37.8% – 40.5% of the total digestible starches in malanga and potato were hydrolyzed to maltotriose and other oligosaccharides. Though we expected to observe significantly higher levels of bioaccessible glucose and maltose following malanga feeding compared to potato, it is possible that malanga starches were more quickly hydrolyzed to oligosaccharide units than potato, and that the resulting oligosaccharides would be further hydrolyzed to mono- and disaccharides at different rates in the human gut by brush border enzymes compared to potato. Digestibility of malanga starches may also be related to their viscosity, molecular weight and other physicochemical properties (Dos Santos, et al., 2016; Ek, et al., 2012; Hoyos-Leyva, et al., 2017a; Singh, et al., 2003). We examined the rheological properties of the suspensions to gain insights into the relationship between viscosity, size, structure, and digestibility of malanga and potato starches (Table 3, Supplemental Fig. 2). The apparent viscosity, yield stress (τ_0), and consistency coefficient (k) of the malanga suspension were lower than those of the potato suspension, while the flow index (n) value for malanga was closer to Newtonian behavior (closer to 1) than potato (Table 3). Lower apparent viscosity of starchy crops has previously been correlated with higher amylopectin content (Xie, et al., 2009), as well as higher digestibility due to enhanced availability for enzymatic hydrolysis (Norton, et al., 2011), thereby corroborating our previous observations (Table 1, Figure 1). Furthermore, flow index values closer to Newtonian behavior (closer to 1), as observed for malanga compared to potato (Table 3), have been attributed to smaller molecular size (Kang, et al., 2016), aligning with previous reports that malanga starch granules are smaller in size than those of potato (Srichuwong, et al., 2005). Smaller sized starch granules tend to result in less entanglements and consequently lower viscosity, as well as more rapid hydrolysis (Ek, et al., 2012).

Rheological properties and digestibility of starch suspensions, however, can also be determined by starch phosphate content and amylopectin branch chain lengths, which were not investigated in this study. Potato starch is known to contain high phosphate content, which may have increased the viscosity and lowered the digestibility of our potato starch suspension (Dos Santos, et al., 2016; Kanazawa, et al., 2008; Singh, et al., 2003). Recently,

Hoyos et al. (2017a) found malanga starch to be comprised of a higher proportion (42%) of shorter chains (degree of polymerization less than 13), than has been reported in potato starch (34% shorter chains) (Srichuwong, et al., 2005). Longer amylopectin branch chain lengths stabilize starch, thereby reducing its hydrolysis (Ek, et al., 2012; Srichuwong, et al., 2005). It is also important to note that agricultural crops are subject to variation in chemical composition across cultivars due to genetic variety and environmental factors (Hoyos-Leyva, et al., 2017b; Kaur, et al., 2007; Noda, et al., 2007); our results are indicative of a single sample of malanga and potato tuber cultivars. Future studies that directly compare the phosphate content, branch chain length, digestibility, and other properties of a variety of malanga and potato cultivars is needed.

3.2 Effect of malanga versus potato consumption on gut bacterial communities

In a digestive system, molecules that are not absorbed into the bloodstream in the upper gastrointestinal tract become available for bacterial fermentation throughout the digestive process (Hammer & Hammer, 2012; Zhou, et al., 2010). As noted in our physicochemical analysis of malanga and potato powders, they each contained 11.2%–11.9% total fiber (Table 1). We also observed small, partially digested food particles, possibly resistant starches, in the ileal efflux of the TIM-1 experiments, that would be delivered to the colon. Previous studies have shown that, following high-starch feeding, not all starch granules are fully hydrolyzed to mono- and disaccharide units. Rather, many starch molecules are only partially digested and escape to the colon (Gibson & Roberfroid, 1995; Hammer & Hammer, 2012; Zhou, et al., 2010). Therefore, it is plausible that differences in the physicochemical properties of malanga and potato powders could lead to distinct impacts on the gut microbial communities.

We measured the effect of chronic feeding of malanga and potato powders on the bacterial composition of the gut microbiota in C57Bl/6J high-fat-diet mice supplemented with 20% malanga or potato powders (Supplemental Table 2) in a cross-over study using 16S rRNA gene sequencing of fecal specimens. High-fat-diet C57Bl/6J mice were used for this study because their gut microbiota have been well characterized and serve as a model of intestinal dysbiosis (Mosca, et al., 2016; Murphy, et al., 2015), which influences susceptibility to chronic and systemic disease (Brown, et al., 2012). We hypothesized that malanga would mitigate intestinal dysbiosis by improving the overall diversity of the gut microbiome and promoting the abundance of bacterial groups associated with health benefits.

Though both malanga and potato diet groups consumed similar amounts of food over the 6 wk experiment (Fig. 3A) and no differences in weight gain were observed between groups (Fig. 3B), malanga consumption significantly improved overall gut bacterial diversity in high-fat-diet mice compared to potato (Fig. 4A and B). Malanga treatment lead to significantly higher α diversity than potato after 14 d of Experiment Diet #1, as demonstrated by higher Shannon index (mean $\pm$ SD: 4.48 ± 0.40 vs. 3.90 ± 0.41 , $p = 0.002$) and higher OTU richness (mean $\pm$ SD: 152.82 ± 10.46 vs. 124.01 ± 9.09 OTUs/19,000 reads; $p < 0.0001$). PCoA of unweighted UniFrac distance matrices showed a clear separation between microbiotas from the malanga and potato-treated mice after Experiment Diet #1 (Fig. 4C). Furthermore, the effect of malanga treatment on gut microbiota was

durable; α and β diversity was not completely reversed by the end of the 14 d wash out (Fig. 4A, B, and C). Due to this finding, we focused our statistical analysis of alterations in bacterial composition during Experiment Diet #1 only.

Higher gut bacterial diversity is generally associated with health, as higher diversity has been found in healthy human subjects compared to individuals with obesity (Le Chatelier, et al., 2013; Turnbaugh, et al., 2009), metabolic syndrome (Le Chatelier, et al., 2013), inflammatory bowel disease (Walters, et al., 2014), chronic fatigue syndrome (Giloteaux, et al., 2016), allergies, and intestinal cancers (Doré & Blottiere, 2015). Furthermore, ecological theory links species richness to stability, fitness (Tuohy & Del Rio, 2015), and resistance to intestinal pathogens, which may result from reduced gut permeability or occupation of all available ecological niches (Lozupone, et al., 2012; Martens, et al., 2014).

In terms of bacterial composition, our results suggest that malanga and potato diets differentially affected OTUs involved in carbohydrate metabolism (Flint, et al., 2012a) within the Lachnospiraceae and Ruminococcaceae families (phylum Firmicutes, class Clostridia) (Fig. 4D). The relative abundances of eight OTUs within the Firmicutes phylum (class Clostridia) were found to be significantly different ($q < 0.05$) between groups after Experiment Diet #1. Malanga promoted the increase of the relative abundance of two OTUs belonging to Ruminococcaceae, including one OTU within *Clostridium* cluster IV, and three OTUs belonging to Lachnospiraceae, including two OTUs assigned as *Ruminococcus2* and *Acetatifactor*, respectively. Moreover, malanga intake led to the decrease of the relative abundance of another two OTUs belonging to Lachnospiraceae, including one OTU within the *Clostridium* cluster XIVa.

Interestingly, all bacterial genera/clusters which were significantly affected by the malanga or potato treatments are potential butyrate-producing bacteria (Flint, et al., 2012a). Butyrate, a breakdown product of carbohydrate fermentation in the gut by bacteria, is the primary energy source for colonocyte health and function, and regulates systemic metabolism as an important signaling molecule (Flint, et al., 2012b). This is in line with the fact that malanga and potato are comprised of high amounts of different types of carbohydrates. Gut microbial genomes are differentially equipped to digest the diversity of carbohydrates potentially available in the diet based on substrate preferences and competitive abilities to anchor to and enzymatically metabolize specific sugar linkages (Flint, et al., 2008; Flint, et al., 2012a; Martens, et al., 2014). Furthermore, the physicochemical attributes of carbohydrates (i.e. amylose:amylopectin ratio of starches, fiber type) can stimulate gut microbial gene signaling to produce enzymes that degrade specific carbohydrate structures (Martens, et al., 2014). Through these mechanisms, intestinal microbial communities are known to be strongly influenced by the carbohydrate content and quality (Flint, et al., 2012a). Our findings that malanga differentially affect the growth of bacterial genera associated with gut health indicate that further studies are warranted to test the role of malanga constituents as potential prebiotics (Gibson, et al., 2017).

Bacterial compositions at phylum level clearly show the effects of dietary switch between experimental diets and wash-out diet (Supplemental Fig. 3A). The relative abundances of all major phyla did not show any difference between groups at d 0, but mice fed the malanga

diet for 14 d had lower relative abundances of Actinobacteria (mean $\pm$ SD: 0.09% $\pm$ 0.09% vs. 0.26% $\pm$ 0.22%, $q = 0.047$) and Verrucomicrobia (mean $\pm$ SD: 0.24% $\pm$ 0.35% vs. 1.92% $\pm$ 2.83%, $q = 0.04$), as well as higher relative abundances of Proteobacteria (mean $\pm$ SD: 0.95% $\pm$ 1.37% vs. 0.02% $\pm$ 0.01%, $q = 0.04$), than mice fed the potato diet. Of these phyla, only the fold-change of relative abundances of Proteobacteria was outstandingly different between the two groups (mean $\pm$ SD: 13.21 $\pm$ 23.70 vs. 0.21 $\pm$ 0.20, $q = 0.003$), and the increase of genus *Desulfovibrio* contributed the most to this difference (fold change, mean $\pm$ SD: 831.16 $\pm$ 1304.56 vs. 0.94 $\pm$ 0.30, $p = 0.0006$, Supplemental Fig. 3B). No difference in abundances or fold-changes of other genera belonging to Proteobacteria, i.e. *Enterobacter* and *Escherichia/Shigella* was detected. Though *Desulfovibrio* has been associated with negative gut health outcomes in previous studies (Heberling, et al., 2013), the relative abundance of this bacteria remained below 1% in our study, and the bloom is likely the result of higher abundance of hydrogen gas as a byproduct of polysaccharide fermentation (Heberling, et al., 2013). Further evaluation of the effect of malanga consumption upon particular classes of gut bacteria, including the butyrate-producers and Proteobacteria, is needed in additional animal and human models.

CONCLUSION

This study was the first to compare the biochemical, physiochemical and physiological differences between shelf-stable food products prepared from boiled-and-freeze-dried malanga and potato tubers. We found that malanga carbohydrates occurred in higher abundance, were characterized by higher amylopectin content and lower viscosity, and were more bioaccessible and bioavailable than those of potato on a w/w basis of the whole food. We also found that malanga consumption significantly improved overall diversity of the gut microbiome in mice, whereas potato did not. It is plausible that the folkloric benefits of malanga for infant development and the management of adult gastritis may be due to both facilitated digestion of malanga starches in the upper gastrointestinal tract, and positive changes to the diversity of the gut microbial community. This research supports the traditional uses of malanga and opens opportunities for the development of malanga-derived functional foods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

BLG and IR designed the research. LZ conducted bioinformatic analysis of microbiome data. MGC conducted rheological measurements and supported HPLC-ELSD method development. PK supported animal experiments. SN and MS conducted library construction and sequencing of fecal DNA. BLG conducted and analyzed data from all other experiments, wrote the manuscript, and had primary responsibility for final content. All authors have read and approved the final manuscript.

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Malanga displays higher starch, amylopectin, and free sugars with lower viscosity than potato

Malanga carbohydrates are more bioaccessible and bioavailable than those of potato

Malanga consumption leads to higher alpha diversity of the gut microbiome than potato

Figure 1. Mice fed malanga powder show a significantly higher glycemic response compared to those fed potato powder

(A) C57Bl/6J mice (6-wk old) were fed a standard chow diet for 6 wk, then fasted for 14 h. Blood glucose levels (mg/dL) expressed as mean $\pm$ SD ($n = 12$) were measured at baseline and again 20, 40, 60, 90, and 120 min after gavage of 1.67 g malanga or potato powder/kg body weight. * $P < 0.05$, Fisher's LSD. (B) Area under the curve (AUC), shown as the mean $\pm$ SD ($n = 12$), $P = 0.0691$, unpaired, two-tailed t -test.

Figure 2. *In vitro* digestion of malanga powder using the TNO Intestinal Model 1 (TIM-1) yields significantly higher levels of bioaccessible sucrose at 90, 120, and 150 mins compared to digestion of potato powder, while yielding similar levels of bioaccessible glucose and maltose. Sugar levels (mg), expressed as mean $\pm$ SD ($n = 3$), were measured via HPLC-ELSD from TIM-1 jejunal and ileal absorption samples every 30 min for 300 min after feeding 20 g malanga or potato powder. (A) Bioaccessible glucose (mg). (B) Bioaccessible maltose (mg). (C) Bioaccessible sucrose (mg). **** $P < 0.0001$, * $P < 0.05$, Sidak's multiple comparison 2-way ANOVA.

Figure 3. Food intake and body weight gain were not significantly affected by replacement of sucrose and maltodextrin with malanga or potato powder in high fat diets
 (A) Food intake, (B) Weight gain. Results are expressed as the mean $\pm$ SD ($n = 12$). There were no significant differences in food intake or weight gain between malanga and potato-fed groups ($P > 0.05$, one-way ANOVA).

Figure 4. Gut microbial communities were significantly affected by replacement of sucrose and maltodextrin with malanga or potato powder in the high fat diets

(A) Shannon index and (B) OTU richness as indexes for microbiota alpha diversity. Data are the mean $\pm$ SD ($n = 12$). Asterisks represent significant differences between the two groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, unpaired t test or Mann-Whitney test). (C) Principle coordinate analysis (PCoA) on unweighted UniFrac distances. One PCoA model was applied on each timepoint. p values are listed for differential clustering (ADONIS test) and R^2 values represent the percentages of variation explained by diet. In A, B, and C, analyses were performed on 19,000 sequences per fecal sample. (D) Heatmaps showing the fold-change of OTUs differing between the potato-treated and malanga-treated mice after 14 d of Experiment diet #1. Taxonomy is reported at the lowest identifiable level. OTUs showed higher fold-changes in the malanga group are indicated in red and the opposite in blue. Statistical comparison of the two groups was done by 10,000 times of permutation; p values represent fraction of times that permuted differences assessed by Welch's t-test were greater than or equal to real differences, and were adjusted by FDR correction (* $q < 0.05$, ** $q < 0.01$).

Table 1
Nutritional profiles of malanga and potato powders

Malanga and potato tubers were each peeled, boiled, mashed, frozen, and freeze-dried. Analysis was performed by Medallion Labs (Minneapolis, MN, USA).

Nutritional component	Malanga powder (% w/w)	Potato powder (% w/w)
Carbohydrates	85.01	77.20
Protein	4.35	8.82
Fat	0.20	0.00
Ash	3.60	3.38
Moisture	6.84	10.60
Total fiber	11.9	11.2
Soluble fiber	4.1	4.6
Insoluble fiber	7.8	6.6
Resistant oligosaccharides	0.5	0.2
Total starch	70.70	66.30
Resistant starch	4.21	4.71
Amylose: amylopectin	24.55: 75.45 (ratio 0.33)	36.94: 63.06 (ratio 0.59)
Total free sugars	5.440	3.230
Fructose	0.540	0.802
Glucose	0.551	0.865
Maltose	0.156	0.274
Sucrose	4.190	1.290

Table 2

Cumulative amount of bioaccessible sugars in jejunal and ileal compartments after 300 min digestion of malanga versus potato powder in the TNO Intestinal Model 1 (TIM-1) as compared to the initial levels of digestible starch and free sugars fed to TIM-1

Results for glucose, maltose, and sucrose are the mean $\pm$ SD ($n = 3$).

Cumulative amount of bioaccessible sugars after 300 min TIM-1 digestion (g ± SD)			Initial digestible starch and free sugars delivered to TIM-1 in 20 g powder (g)			Combined bioaccessible glucose and maltose derived from digestible starch			
	Glucose	Maltose	Sucrose	Digestible starch	Free glucose	Free maltose	Free sucrose	Combined amount subtracted from free sugars (g)	% of digestible starch
Malanga	2.27 ± 0.90	5.79 ± 1.67	0.89 ± 0.40 *	13.30	0.11	0.03	0.84	7.92	59.5
Potato	2.52 ± 1.45	5.37 ± 0.70	0.11 ± 0.13 *	12.32	0.17	0.06	0.26	7.66	62.2

* $P < 0.05$ (t-test).

Subsequent results were determined via calculation using the mean values for glucose, maltose, and sucrose, and the values for digestible starch, free glucose, free maltose, and free sucrose determined by nutritional analysis (Table 1).

Table 3
Rheological parameters of malanga and potato suspensions

τ_0 = yield stress, k = consistency coefficient, n = flow index. Data are the mean $\pm$ SD ($n = 3$).

Rheological parameters (mean $\pm$ SD)				
	Apparent viscosity @ 30 s ⁻¹ (mPa.s)	τ_0 (Pa)	k (Pa.s ^{<i>n</i>})	n (-)
Malanga	271.0 $\pm$ 22.4 **	1.7 $\pm$ 0.3 **	0.6 $\pm$ 0.0 *	0.7 $\pm$ 0.0 *
Potato	863.0 $\pm$ 34.5	7.3 $\pm$ 0.7	2.6 $\pm$ 0.3	0.6 $\pm$ 0.0

* $P < 0.05$,

** $P < 0.01$,

t-test comparing malanga vs. potato for each parameter.