Journal of Functional Foods 64 (2020) 103685

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Prebiotic effects of a mixture of agavins and green banana flour in a mouse model of obesity

Guadalupe Monserrat Alvarado-Jasso[a], Brenda Hildeliza Camacho-Díaz[a], Martha Lucía Arenas Ocampo[a], Jesús Enrique Jiménez-Ferrer[b], Rosalva Mora-Escobedo[c], Perla Osorio-Díaz[a,*]

[a] Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, Km 8.5 Carr. Yautepec-Jojutla, Col. San Isidro, Yautepec, Morelos C.P. 62731, Mexico
[b] Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social (IMSS), Argentina No. 1, Xochitepec, Morelos 62790, Mexico
[c] Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional. Campus Zacatenco, Unidad Profesional “Adolfo López Mateos”, Calle Wilfrido Massieu esquina Cda. Manuel Stampa. C.P, Ciudad de Mexico 07738, Mexico

ARTICLE INFO

Keywords:
Dietary fiber
Obesity
Short-chain fatty acids

ABSTRACT

Dietary fibers from agavins and green banana flour are resistant to digestion but promote beneficial fermentation by gut microbiota. Therefore, the main of this work was evaluate potential synergistic effects of AV and BF on energy intake; body weight gain; metabolic markers, and gut short-chain fatty acid (SCFA) levels in ad libitum-fed, obese mice. C57BL/6 male mice were fed a standard (ST) or high-fat (HF) diet, supplemented with agavins (AV), green banana flour (BF), or with the mixture (MX). As relevant results HF diet-fed mouse showed a significant increase in body weight, and decrease in SCFA (*t*-test, $p < 0.05$). Mice fed with BF diet significantly decreased body weight and metabolic markers ($p < 0.05$). The use of a combination of AV and BF offer synergistic protection against the metabolic sequelae of obesity and warrant consideration as interventions against obesity-related disorders.

1. Introduction

Obesity is a global problem and is a risk factor for several chronic degenerative diseases (Sanghera, Bejar, Sharma, Gupta, & Blackett, 2019). The current obesity epidemic has many clear causes, including impacts of modern diets and lifestyles and reduced physical activity. Although many interventions have been recommended, the prevalence of obesity continues to rise and has prompted a re-evaluation of potential interventions. In recent years, altered gastrointestinal microbiota have been recognized in obese individuals (Escobar et al., 2018). The Mexican population is no an exception, and according to data from the National Health and Nutrition, 73% of the adults were overweight or obese in 2016.

Diet is currently recognized as a controllable parameter through which microbiota can be influenced to positively affect metabolism (Bretin, Gewirtz, & Chassaing, 2018). Recent data show benefits of dietary fiber fermentation. When hydrolyzed by digestive enzymes, the resulting fermentation products in the colon are mainly short-chain fatty acids (SCFAs) (Lee, Yacyshyn, & Yacyshyn, 2019). The three most abundant SCFAs acetate, propionate, and butyrate, each of which exert favorable and unique physiological effects (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004).

Several types of prebiotic fibers can be distinguished by considering their fermentation products (Castillo Andrade et al., 2018). Fructans have been recognized as prebiotic ingredients because they stimulate the growth of bacteria in the colon, which benefits gastrointestinal health and has various beneficial metabolic effects (Huazano-García, Shin, & López, 2017). Recent research has found that cecal SCFAs contents in rats fed with branched fructans (agavins) were significantly greater than in high-fat (HF) diet-fed rats (Huazano-García & López, 2015). Moreover, other evidence suggests that agavins from *Agave tequilana* and *A. angustifolia* have complementary effects against obesity-related metabolic disorders (Rendón-Huerta, Juárez-Flores, Pinos-Rodríguez, Aguirre-Rivera, & Delgado-Portales, 2012; Urías-Silvas et al., 2008).

Dietary fiber is generally obtained from cereals. However, unripe fruits, such as green bananas (*Musa paradisiaca* L.), are rich in resistant starch granules and can be considered an unconventional source of dietary fiber because their structures are indigestible. Specifically, these fruits contain 73.6–79.4% starch, of which 47.3–54.2% is considered

---

* Corresponding author at: Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos, Mexico.
E-mail address: posorio@ipn.mx (P. Osorio-Díaz).

https://doi.org/10.1016/j.jff.2019.103685
Received 1 May 2019; Received in revised form 12 November 2019; Accepted 15 November 2019

resistant starch (Almeida-Junior, Curimbaba, Chagas, Quaglio, & Di Stasi, 2017; da Mota, Lajolo, Cordenunsi, & Ciacco, 2002; Faisant et al., 1995). Resistant starches are non-digestible polysaccharides that act as dietary fiber in the small intestine and are used by colonic microbiota to produce SCFAs through anaerobic fermentation (Batista et al., 2017; Sánchez-Zapata, Viuda-Martos, Fernández-López, & Pérez-Alvarez, 2015).

SCFAs, such as acetate, propionate, and butyrate, are the end products of intestinal microbial fermentation of dietary fibers and resistant starches (Fernández et al., 2016). Plasma and colonic SCFAs are associated with metabolic syndrome (Hu, Lin, Zheng, & Cheung, 2018). Recently, the roles of SCFAs in the regulation of energy homeostasis have been extensively studied. The importance of SCFAs in energy metabolism indicates the potential of modulating SCFAs as nutritional targets to prevent and counteract obesity-related metabolic disorders and their associated diseases, such as type 2 diabetes, hypertension (Pingitore et al., 2019).

Few studies of health benefits have been performed with mixes of dietary fibers with different chemical structures. Two fibers with different chemical structures, and showed that they were fermentable by intestinal microbiota. Fermentation types and parts of the colon in which ferment occur vary with chemical structures of fiber substrates (Le Blay, Michel, Blottière, & Cherbut, 2003). These observations confirm that fibers have particular properties that are worthy of consideration. The synergistic effects of fibers with different fermentation rates and chemical structures. Their mixtures of the fibers exerted intestinal anti-inflammatory effects that were associated with increased numbers of beneficial bacteria (lactobacilli and bifidobacteria) and through positive regulation of epithelial defense mechanisms (Rodríguez-Cabezas et al., 2010).

Green bananas (*Musa paradisiaca* L.) are an important source of resistant starch. Moreover, flour from these fruits has been widely studied as a potential nutraceutical ingredient to increase fiber and reduce starch digestibility (García-Solís, Bello-Pérez, Agama-Acevedo, & Flores-Silva, 2018). Various components of bread (Juarez-Garcia, Agama-Acevedo, Sáyago-Ayerdi, Rodríguez-Ambriz, & Bello-Pérez, 2006), spaghetti, (Hernández-Nava, Berrios, Pan, Osorio-Díaz, & Bello-Pérez, 2009) cereal bars, (Utrilla-Coello, Agama-Acevedo, Osorio-Díaz, Reynoso-Camacho, & Bello-Pérez, 2013) and snacks (Flores-Silva, Tovar, Reynoso-Camacho, & Bello-Pérez, 2017) have been related to reductions in postprandial glycemic and insulinemic responses, with potential benefits for the management of obesity. The objective of the present study was to evaluate these metabolic impacts of native banana flour. We initially determined whether dietary supplementation with green banana flour or branched fructans (agavins) produces protective effects relating to the prebiotic capacity of these dietary fibers to counteract the metabolic disorders associated with an HF diet in a mouse model of obesity. Subsequently, we determined whether the combined supplementation with green banana flour and agavins produces synergistic effects on SCFAs contents in the same animal model.

2. Materials and methods

2.1. Extraction of agavins fructans

Powder containing AV from *Agave angustifolia* Haw was obtained through a patented process (no. MX/a/2015/016512, under the title “Modular system and process to obtain different agave fructan products” using spray drying). The reducing sugar concentration was 0.46% and the AV content was 99.22% g dry weight.

2.2. Banana flour production

Green bananas were purchased from a local market in Cuautla, Morelos, Mexico, and flour was produced from the pulp of the fruit using the procedure described by Ovando-Martinez, Sáyago-Ayerdi, Agama-Acevedo, Goñi, and Bello-Pérez (2008). Here, the term “green banana” refers to unripe bananas of the species *Musa paradisiaca* L. Green bananas have a moisture content of 9% with 1.5% lipids, 3% ash, 3.5% proteins, and 62% RS.

2.3. Experimental animals

C57BL/6 male mice (5-week old) were housed in a temperature-controlled room with a 12-h light/12-h dark cycle. During the experimental period, food intake was measured daily, body weights were determined weekly, and feces were collected weekly to evaluate 24-h fecal volumes and to analyze SCFAs contents. Mean daily energy intake (kJ d^{-1}) was calculated as follows: intake of food (g) × energetic value of diet (kJ g^{-1}). Maintenance of the animals, studies, and development of the experiments were carried out accordance with the Official Mexican Norm 062 ZOO-1999 and the international ethical guidelines for the care and use of laboratory animals.

2.4. Diets and diet-induced obesity

Initially, animals were fed an HF diet *ad libitum* to generate overweight mice, and the effects of dietary fiber supplements were then compared. Mice were fed with standard (ST, n = 10, 5008 LabDiet) or an HF diet (n = 40) containing 40% carbohydrate (fructose, sucrose), 21% protein (caseinate), and 40% lipid (lard) for 12 weeks. Subsequently, healthy control mice were maintained on the standard diet 5008 LabDiet (ST, n = 10) and overweight mice were divided into four groups and continued to receive the HF diet for 6 weeks. Mice of the control HF diet group (n = 10) received no fiber supplements, whereas mice of the other diet groups received agavins (HF + AV, n = 10), banana flour (HF + BF, n = 10), or the mixture of agavins and banana flour (HF + MX, n = 10). Diets were administrated in pellet form. Food and water were provided *ad libitum*.

2.5. Biochemical parameters

Biochemical measurements were made at baseline and after six weeks. Glucose (GLU), triglyceride (TG), and total cholesterol (TC) contents were measurement in blood samples after 5 h without food consumption. Blood samples of approximately 25 µl were then taken from tail incisions. Samples were quantified in mg/dL using an Accutrend® plus system (enzymatic colorimetry).

2.6. Blood pressure measurements

After the experimental diet-feeding period, mice were anesthetized with intraperitoneal (i.p.) injections of 50-mg/kg pentobarbital (Pfizer) and were placed in a LETICA Storage Pressure Meter, LE 5002, (Biopac System MP150, Goleta, CA, USA). Ten measurements were recorded for each mouse and average values for blood pressure (BP), systolic (SBP) and diastolic (DBP) were calculated for each treatment.

2.7. Detection of fecal SCFAs

Stool samples were collected in polypropylene vials using metabolic cages and were immediately stored at −70 °C until the day of evaluation. SCFAs contents in 150-mg stool samples were quantified using gas chromatography (GC) as described by Tangerman and Nagengast (1996). Briefly, stool samples were homogenized in Mili-Q water for 3 min, were kept at room temperature for 10 min, and were then centrifuged at 21,475*g* for 10 min at 4 °C. Supernatants were then filtered through the 0.45-µm cellulose acetate filters and 400-µl filtrates were mixed with 100 µl of 50-µmol/mL standard internal solution, 10 µl of formic acid, and 490 µl of Mili-Q water in polypropylene vials. After centrifugation at 12,000*g* for 15 min at 4 °C, 700-µl aliquots of supernatant were collected. SCFAs content was the determined using GC

(Clarus 580 Gas Chromatograph, PerkinElmer, Inc. USA) equipped with a capillary column (CARBOWAX 25 m, capillary and 25 m × 320 µm × 0.30 µm nominal) coupled with a flame detector. Helium was used as the mobile phase at a flow rate of 1 mL/min. The starting temperature was 90 °C and was raised at 10 °C/min to 220 °C and then maintained for 5 min.

2.8. Statistical analysis

Results are presented as the mean ± standard error of the mean (SEM). Differences between the ST and HF groups were assessed using the Student's *t*-test. All percentage data were subject to angular transformation ($\arcsin\sqrt{X}$) and SEM data were square root transformed prior to analysis. Differences between the HF and DF-supplemented HF groups were identified using analysis of variance, followed by the Tukey's multiple comparisons test with SigmaPlot version 11 software (Systat Software, Inc., San Jose, CA, USA). A probability (*p*) value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Food intake and body weight gains

All mice fed with supplemented diets (HF + AV, HF + BF, and HF + MX) had reduced body weight gains compared with the control HF group (Fig. 1A). Moreover, daily food and/or energy intake throughout the experimental period was significantly lower ($P \leq 0.05$) in HF + AV, HF + BF, and HF + MX mice than in HF mice (Fig. 1B). Mice of the HF + AV, HF + MX, and HF + BF groups ate 25%, 27%, and 48% less food, respectively, than the mice of the control HF diet group. Average energy values were 18.24 kJ g^{-1} for the ST diet, 23.26 kJ g^{-1} for HF diet, and 20.93 kJ g^{-1} for the supplemented diets.

3.2. Blood pressure evaluations

All fiber-supplemented mice had reduced BP compared with mice of the control HF group, in which values were elevated by 75 mm Hg. Specifically, compared with 137.36 mm Hg in the HF group, mice in the HF + AV, HF + BF, and HF + MX groups showed BP values of 115.34–64.15, 120–72, and 124.56–66.8 mm Hg, respectively, and these levels were similar to those in the ST group (Fig. 2).

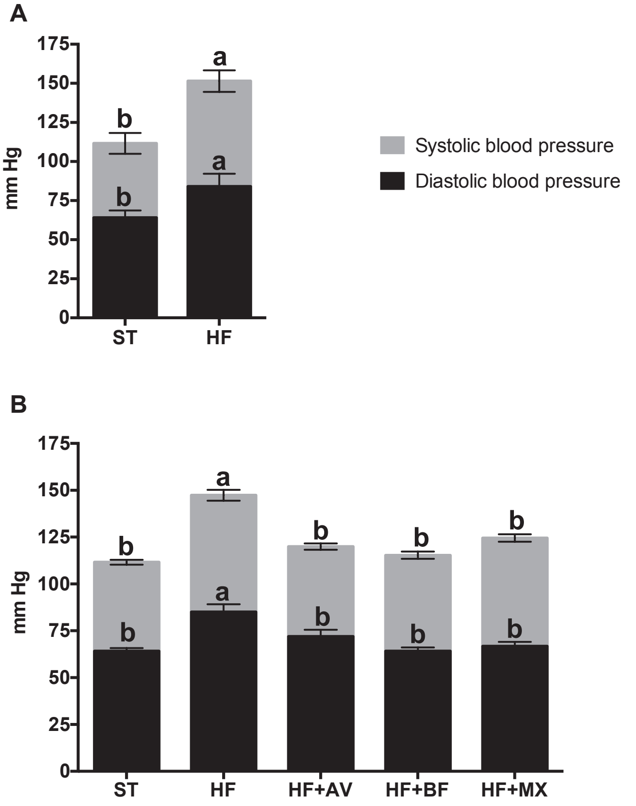

Fig. 2. Systolic and diastolic blood pressure of mice fed (A) a standard diet or (B) a high-fat diet supplemented with agavins (HF + AV), banana flour (HF + BF), or a mixture of both (HF + MX). Values are presented as the means ± standard deviation (vertical bars). Ten mice per group. Mean values with different letters indicate significantly differences ($p \leq 0.05$).

3.3. Evaluations of blood biochemistry

To investigate the metabolic impacts of the present interventions, serum GLU, TC, and TG concentrations were evaluated as summarized in Table 1. Serum GLU concentrations were significantly lower in mice of the HF + AV and HF + BF groups than in mice of the HF group.

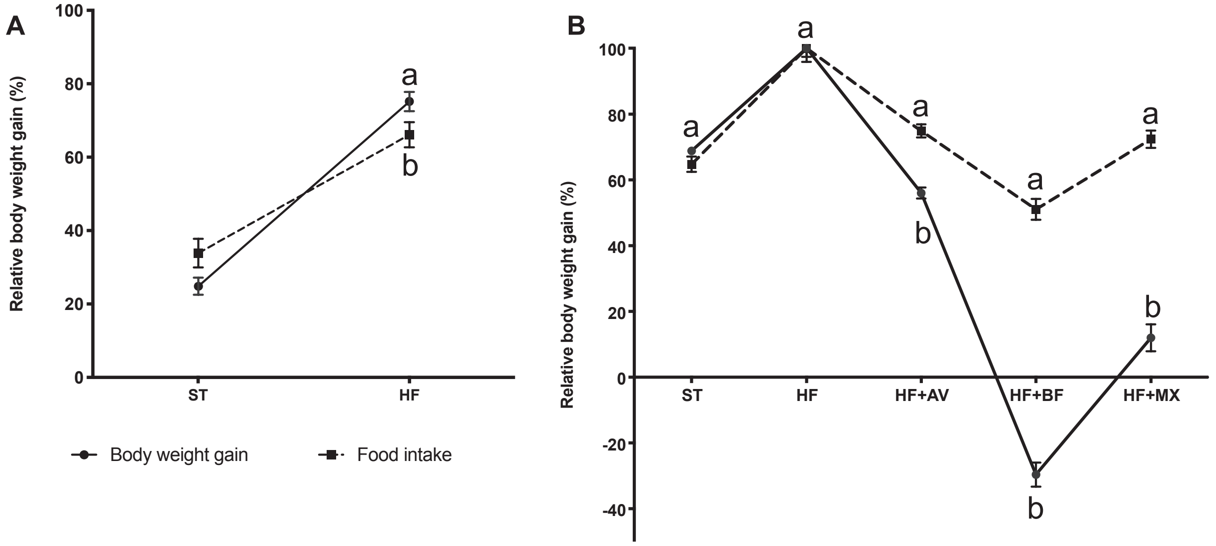

Fig. 1. Relative body weight gain of mice fed a standard diet (ST) or a high-fat diet supplemented with agavins (HF + AV), banana flour (HF + BF), or a mixture of both (HF + MX). (A) First phase: induction of obesity. (B) Second phase: treatment. Values are presented as the means ± standard errors (vertical bars). Ten mice per group. Mean values with different letters indicate significant differences ($p \leq 0.05$).

Table 1
Plasma concentrations of glucose and lipid profile (triglycerides (TG) total and cholesterol (COL)) of mice fed with a standard diet (ST), or High-fat diet (HF) or diets supplemented with Agavins (HF + AV) or Banana flour (HF + BF) and Mixture (HF + MX).

	Glucose (mM)		Triglyceride (mM)		Cholesterol (mM)	
Diet	Mean	SEM	Mean	SEM	Mean	SEM
ST	7.31^{c}	0.52	0.87^{d}	0.04	40.98^{ab}	1.34
HF	11.12^{a}	0.78	1.99^{a}	0.28	43.29^{a}	2.29
HF + AV	7.88^{c}	0.85	1.31^{bc}	0.09	40.07^{b}	0.94
HF + BF	7.19^{c}	0.33	1.19^{cd}	0.43	40.07^{b}	0.83
HF + MX	9.21^{b}	0.96	1.56^{b}	0.09	42.18^{ab}	2.54

Values are means with the standard errors of the mean (SEM). Means values with different letters are statistically different, ($P \leq 0.05$).

Serum TG concentrations in HF + AV, HF + BF, and HF + MX groups were also significantly lower than in HF mice. TC concentrations did not differ significantly between HF, HF + AV, HF + BF, and HF + MX groups and were within the normal range (García Sevillano et al., 2014).

3.4. Fecal short-chain fatty acid contents

Fecal concentrations of the SCFAs acetate, propionate, and butyrate are presented in Fig. 3. Acetate was the most abundant SCFA and the concentration was significantly higher in the HF + MX group ($p > 0.05$) (Fig. 3A), while propionate concentrations were significantly increased in the HF + AV and HF + MX groups compared to those of the HF group ($p > 0.05$) (Fig. 3A). Moreover, butyrate concentrations were significantly higher in the HF + AV and HF + MX groups than in the HF group ($p > 0.05$) (Fig. 3A). The HF + MX group had the highest content of total SCFAs ($p > 0.05$) (Fig. 3A). Total SCFA production (acetate:propionate:butyrate) was 77:13:10 in the HF + AV group, 90:7:3 in the HF + BF group, and 81:11:8 in the HF + MX group (Fig. 3B), demonstrating notable differences among the groups.

4. Discussion

Dietary fiber supplementation has previously been considered as a nutraceutical aid that facilitates weight loss (Howarth, Saltzman, & Roberts, 2009), and multiple mechanisms of fiber supplementation have been proposed. Among them, increased of satiety and satiation may occur through the formation of gastric masses and the regulation of signaling hormones of cephalic and peripheral satiety (Wanders et al., 2013), such as ghrelin and peptide YY (PYY). Although we did not evaluate these changes in our experiments, they likely contributed to decreases in blood GLU in the present mouse groups. Moreover, reduced energy absorption in the presence of non-digestible fiber could interfere with the digestion or absorption of macronutrients, and we confirmed this by measuring energetic intake, which was reduced by fiber supplements (Chater, Wilcox, Pearson, & Brownlee, 2015). The increased energy expenditure (diet-induced thermogenesis) during the digestion process could be attributed to increased gastrointestinal mobility (Pereira & Ludwig, 2001). Fiber also acts as a substrate that influences gut microbiota favorably and consequently enhances the production of fermentation products with positive metabolic effects, such as SCFAs (Cani, 2019). The chemical structures and physical forms of DFs determine the fermentation rate and subsequently the rate of SCFA production (Bae, Jun, Lee, & Lee, 2016). Chemical characteristics include the proportion of monosaccharides and linkage compositions, molecular size, and the arrangements of sugars at the molecular level (Hamaker & Tuncil, 2014; Wong, De Souza, Kendall, Emam, & Jenkins, 2006). In this study, consumption of HF diets supplemented with agavins, banana flour, and the mixture of both reduced GLU and TG contents in the blood of mice. However, no changes in blood TC contents were observed. This may reflect the longer time frames in which cellular mechanisms regulate cholesterol in response to excess caloric intake (Basurto, Hernández-Valencia, Zárate, Saucedo, & Manuel-Apolinar, 2017). Previous studies with agavins show positive effects on weight loss in obese mice (Castillo Andrade et al., 2018), and these effects were accompanied by reductions in GLU and TG (Huazano-García et al., 2017), as shown in Table 1.

DF intake regulates the production of GLU and TG through various mechanisms. The first is related to the physical effects of DFs. Eating foods high in DFs increases the chewing time, which increases the secretion of saliva and gastric juices. As the stomach expands, the sensation of satiety increases. Moreover, DFs shift the availability of dietary calories and nutrients (Slavin & Green, 2007). The second mechanism involves the physiochemical effects of soluble DFs, which form viscous solutions that partially prevent contact between digestive enzymes and nutrients, such as bile acids and fat. This mechanism decreases the digestion and absorption of nutrients in the small intestine, thereby forcing the use of glycogen and fats as energy sources to maintain adequate blood GLU and TG levels (Mudgil & Barak, 2013). These mechanisms had a more significant influence on mice fed the supplemented diets. Analyses of green banana flour showed that this ingredient is rich in dietary fiber and resistant starch (RS), which promotes colonic fermentation and influences glycemic control in mice, in turn resulting in improved insulin sensitivity (Dan et al., 2015). These contributions to reductions of blood GLU contents can be attributed to high contents of fiber and resistant starch. The RS in GBF are classified as type 2, which are compact native granules without pores that display B- or C-type polymorph crystallites and are highly resistant to enzymatic hydrolysis (Espinosa-Solis, Jane, & Bello-Pérez, 2009; Jane et al., 2003). All these attributes of granules limit enzyme activities during digestion. However, release of intestinal peptides such as glucagon 1 (GLP-1) and PYY through intestinal L cells could promote sensations of satiety following stimulation by SCFAs (Guyenet & Schwartz, 2012). In particular, GLP-1 stimulates insulin secretion and inhibits glucagon secretion. Moreover, the PYY can inhibit appetite, reduce energy intake and improve insulin secretion, thereby influencing body weight gains (Murphy & Bloom, 2006). Although we did not evaluate these hormones, these mechanisms may all contribute to the beneficial effects of increased intake of dietary fiber.

BP measurements of mice fed the supplemented diets (HF + AV, HF + BF, and HF + MX) were reduced to levels similar to those of mice fed the standard diet. Although the mechanisms underlying the regulation of BP remain unclear, several potential mechanisms involving DF reduce BP and hypertension risk have been proposed. For example, reductions in food/dietary energy density and increased satiety and satiation can reduce the risk of BW gain and obesity (Wanders et al., 2013). In addition, enhanced insulin sensitivity has been shown to improve vascular and endothelial function (Dahl & Stewart, 2015). The consumption of DF promotes healthier high- and low-density lipoprotein profiles for improved endothelial health and a slower rate of arterial plaque buildup and can attenuate elevated systemic inflammation and oxidation of low-density lipoproteins (Santisteban et al., 2017). Furthermore, it promotes a healthier microbiota and increased fermentation to SCFAs, leading to potential improved cardiometabolic health (Hu et al., 2018). Dysfunctional sympathetic gut communication is associated with gut pathology, dysbiosis, and inflammation, and plays crucial roles in hypertension (Santisteban et al., 2017). Our observations demonstrate that high BP is associated with dysbiosis of gut microbiota. In addition, a recent report describes the influence of the vagal anti-inflammatory pathway on intestinal muscle layers and resident macrophages. These data suggest that shifts in the balance of sympathetic and parasympathetic activities in the gut contribute to hypertension-associated gut pathophysiology, dysbiosis, and immune system activation (Yang et al., 2015). The present data suggest that dietary interventions that correct gut microbiota offer an innovative

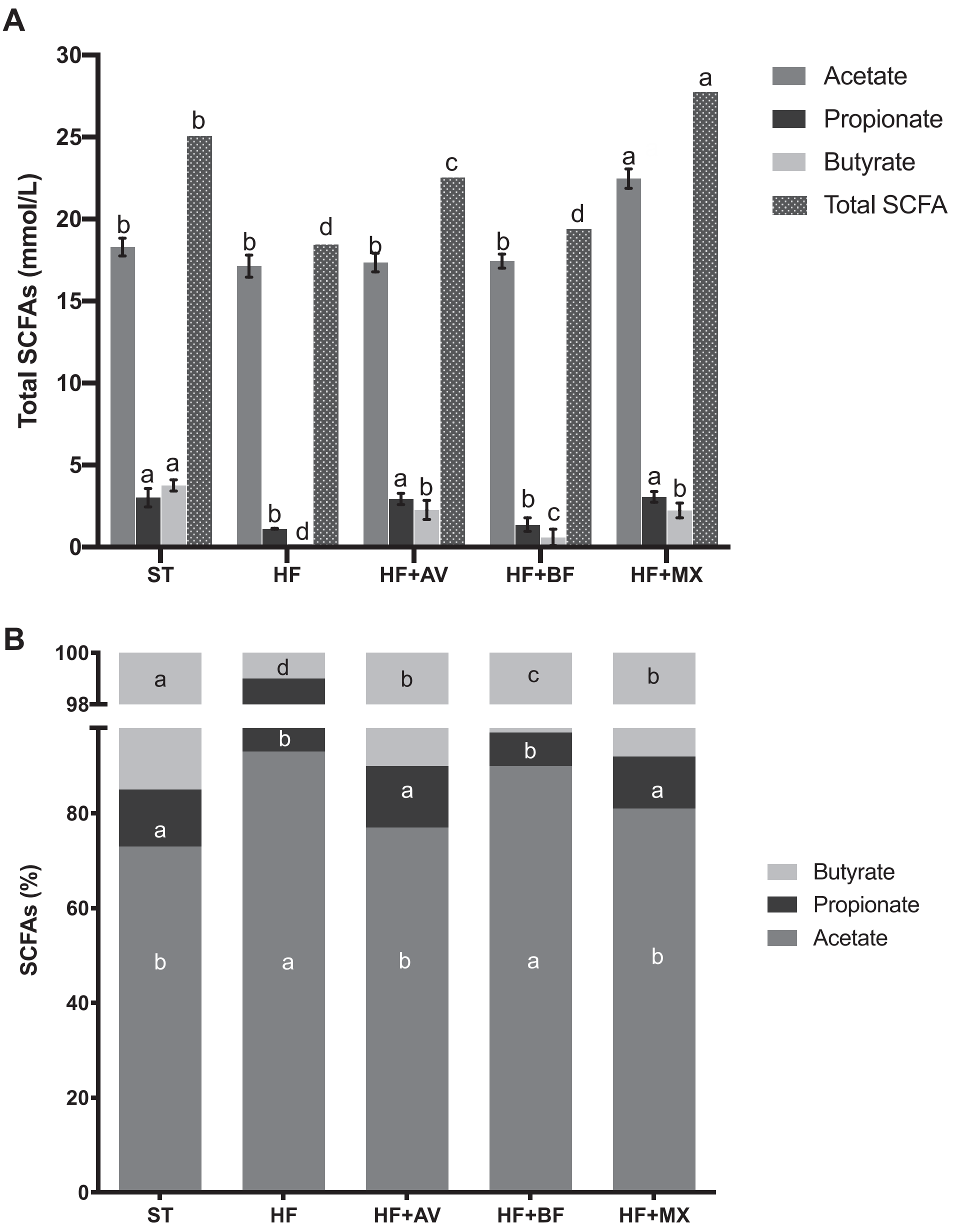

Fig. 3. Short-chain fatty acid (SCFA) concentrations (mM wet content) of mice fed a standard diet or a high-fat diet supplemented with agavins (HF + AV), banana flour (HF + BF), or a mixture of both (HF + MX). A) Total SCFA production. B) Proportions of acetate, propionate, and butyrate to total SCFA production. Mean values with different letters indicate significant differences ($p \leq 0.05$).

nutritional therapeutic strategy for the treatment of hypertension (Santisteban et al., 2017). Although several mechanisms influence BP independently of obesity (García Casilimas et al., 2017), the relationship between dietary fiber consumption and reduced hypertension is of significant importance.

In our analyses of SCFAs, mice of the HF + MX group had higher production than those of HF + AV and HF + BF groups. These observations were also associated with decreased daily energy intake and body weight gains (Zaibi et al., 2010). Among SCFAs, acetate has been associated with appetite regulation through the stimulation of leptin secretion from adipocytes (Smith et al., 2010). Moreover, dietary agavins from various species of agave have demonstrated beneficial effects (Padilla-Camberos, Barragán-Álvarez, Diaz-Martinez, Rathod, & Flores-Fernández, 2018).

Previous comparisons of SCFA production showed that supplementation with HF + AV increased fecal SCFA contents, which was related to the structures (β bonds) of the AV (Huazano-García et al., 2017) but was reversed with a banana mixture. DFs can regulate the yield and molar ratios of SCFAs. Generally, acetate and butyrate are produced by fermentation of aldehydes (e.g., GLU, galactose, mannose, and xylose), whereas propionate is produced mainly by ketone fermentation (e.g., fructose, arabinose, and tagatose) (Plongbunjong, Graidist, Knudsen, & Wichienchot, 2017; Yang, Martínez, Walter, Keshavarzian, & Rose, 2013). Hence, it is likely that digestive enzymes of the large intestine contribute fermentative substrates for microbiota. Moreover, agavins have a rapid fermentation pattern that is similar to that of fructo-oligosaccharides, which are fermented mainly in the proximal colon (Rodríguez-Cabezas et al., 2010; Santiago-García & López, 2014). Accordingly, fecal SCFAs contents were higher in mice fed HF + AV (22%) compared with those fed HF + BF (16%). These observations may reflect RS2 contents of fiber from banana flour, which are fermented slowly and predominantly in the distal colon (Dan et al., 2015; Rodríguez-Cabezas et al., 2010). SCFAs contents were also increased in feces from mice of the HF + MX (24%) and were greater than those of HF and standard diet (20%) groups. Moreover, mice fed the HF diet had lower contents of SCFAs (17%). Therefore, supplementing the HF diet with 10% agavins or green banana flour promotes the production of SCFAs at differing degrees, and synergetic effects were observed after supplementation with both fiber sources.

Agavins increase the quantity and diversity of the microbiome (Huazano-García et al., 2017), potentially through a two-step fermentation mechanism for RS granules. Initially, fermentation of external parts of the granules exposes the internal parts for extensive fermentation, which may be important in the entire length of the colon. Therefore, AV and BF supplements may be complementary, because although AV is particularly active in the proximal colon, BF acts in the distal colon.

In conclusion, the results of the present study demonstrate that co-administration of DFs, AV, and BF promoted health following fermentation throughout the colon, suggesting that this process mitigates the adverse metabolic effects of HF diets. The combination of two DFs, with different rates of fermentability along the large intestine, resulted in a synergistic effect by SCFA production and therefore conveyed a more evident prebiotic effect that may confer a more significant health benefit to the host.

Authorship

G.M.A-J., P.O-D., B.H.C-D, and J.E.J-.F designed the study. G.M.A-J performed the experiments. G.M.A-J, P.O-D, and B.H.C.D wrote the manuscript. All authors read the manuscript and provided comments. G.M.A-J and P.O-D. reviewed and edited the manuscript.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgments

The authors appreciate the support from CONACYT, Mexico; SIP-IPN; COFAA-IPN; and EDI-IPN as well as the scholarships from CONACYT and Centro de Investigación Biomédica del Sur of the Instituto Mexicano del Seguro Social (IMSS), Mexico.

References

Almeida-Junior, L. D., Curimbaba, T. F. S., Chagas, A. S., Quaglio, A. E. V., & Di Stasi, L. C. (2017). Dietary intervention with green dwarf banana flour (Musa sp. AAA) modulates oxidative stress and colonic SCFAs production in the TNBS model of intestinal inflammation. *Journal of Functional Foods, 38*, 497–504. https://doi.org/10.1016/j.jff.2017.09.038.

Bae, I. Y., Jun, Y., Lee, S., & Lee, H. G. (2016). Characterization of apple dietary fibers influencing the in vitro starch digestibility of wheat flour gel. *LWT – Food Science and Technology, 65*, 158–163. https://doi.org/10.1016/j.lwt.2015.07.071.

Basurto, L., Hernández-Valencia, M., Zárate, A., Saucedo, R., & Manuel-Apolinar, L. (2017). Hypercholesterolemia as a risk factor for cardiovascular disease: Current controversial therapeutic management. *Archives of Medical Research, 47*(7), 491–495. https://doi.org/10.1016/j.arcmed.2016.11.009.

Batista, A. L. D., Silva, R., Cappato, L. P., Ferreira, M. V. S., Nascimento, K. O., Schmiele, M., ... Cruz, A. G. (2017). Developing a synbiotic fermented milk using probiotic bacteria and organic green banana flour. *Journal of Functional Foods, 38*, 242–250. https://doi.org/10.1016/j.jff.2017.09.037.

Bretin, A., Gewirtz, A. T., & Chassaing, B. (2018). Microbiota and metabolism: What's new in 2018? *American Journal of Physiology-Endocrinology and Metabolism, 315*(1), E1–E6. https://doi.org/10.1152/ajpendo.00014.2018.

Cani, P. D. (2019). Targeting gut microbiota with a complex mix of dietary fibers improves metabolic diseases. *Kidney International, 95*(1), 14–16. https://doi.org/10.1016/j.kint.2018.11.012.

Castillo Andrade, A. I., Rivera Bautista, C., Godínez Hernández, C., Ruiz Cabrera, M. A., Fuentes Ahumada, C., García Chávez, E., & Grajales Lagunes, A. (2018). Physiometabolic effects of Agave salmiana fructans evaluated in Wistar rats. *International Journal of Biological Macromolecules, 108*, 1300–1309. https://doi.org/10.1016/j.ijbiomac.2017.11.043.

Chater, P. I., Wilcox, M. D., Pearson, J. P., & Brownlee, I. A. (2015). The impact of dietary fibres on the physiological processes governing small intestinal digestive processes. *Bioactive Carbohydrates and Dietary Fibre, 6*(2), 117–132. https://doi.org/10.1016/j.bcdf.2015.09.002.

da Mota, R. V., Lajolo, F. M., Cordenunsi, B. R., & Ciacco, C. (2002). Composition and functional properties of banana flour from different varieties. *Starch/Stärke, 52*(2–3), 63–68. https://doi.org/10.1002/(sici)1521-379x(200004)52:2/3<63::aid-star63>3.3.co;2-m.

Dahl, W. J., & Stewart, M. L. (2015). Position of the academy of nutrition and dietetics: Health implications of dietary fiber. *Journal of the Academy of Nutrition and Dietetics, 115*(11), 1861–1870. https://doi.org/10.1016/j.jand.2015.09.003.

Dan, M. C. T., Cardenette, G. H. L., Sardá, F. A. H., Giuntini, E. B., Bello-Pérez, L. A., Carpinelli, Â. R., ... Menezes, E. W. (2015). Colonic fermentation of unavailable carbohydrates from unripe banana and its influence over glycemic control. *Plant Foods for Human Nutrition, 70*(3), 297–303. https://doi.org/10.1007/s11130-015-0493-6.

Escobar, J. S., Abad, J. M., Velásquez-Mejía, E. P., Corrales-Agudelo, V., de la Cuesta-Zuluaga, J., & Carmona, J. A. (2018). Gut microbiota is associated with obesity and cardiometabolic disease in a population in the midst of Westernization. *Scientific Reports, 8*(1), https://doi.org/10.1038/s41598-018-29687-x.

Espinosa-Solis, V., Jane, J. L., & Bello-Pérez, L. A. (2009). Physicochemical characteristics of starches from unripe fruits of mango and banana. *Starch/Staerke, 61*(5), 291–299. https://doi.org/10.1002/star.200800103.

Faisant, N., Buleon, A., Colonna, P., Molis, C., Lartigue, S., Galmiche, J. P. P., ... Champ, M. (1995). Digestion of raw banana starch in the small intestine of healthy humans: Structural features of resistant starch. *British Journal of Nutrition, 73*(01), 111–123. https://doi.org/10.1079/BJN19950013.

Fernández, J., Redondo-Blanco, S., Gutiérrez-del-Río, I., Miguélez, E. M., Villar, C. J., & Lombó, F. (2016). Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: A review. *Journal of Functional Foods, 25*, 511–522. https://doi.org/10.1016/j.jff.2016.06.032.

Flores-Silva, P. C., Tovar, J., Reynoso-Camacho, R., & Bello-Pérez, L. A. (2017). Impact of chickpea- and raw plantain-based gluten-free snacks on weight gain, serum lipid profile, and insulin resistance of rats fed with a high-fructose diet. *Cereal Chemistry*. https://doi.org/10.1094/CCHEM-03-16-0065-FI.

García Casilimas, G. A., Martin, D. A., Martínez, M. A., Merchán, C. R., Mayorga, C. A., & Barragán, A. F. (2017). Pathophysiology of hypertension secondary to obesity. *Archivos de Cardiologia de Mexico, 87*(4), https://doi.org/10.1016/j.acmx.2017.02.001.

García Sevillano, L., Pellón, M., Treceño, C., Sáinz, M., Salado, I., Velasco, V., ... Carvajal, A. (2014). Knowledge upon the emergency contraceptive pill in Spain. *Atencion Farmaceutica, 16*(3), 224–228. https://doi.org/10.1248/cpb.37.3229.

García-Solís, S. E., Bello-Pérez, L. A., Agama-Acevedo, E., & Flores-Silva, P. C. (2018). Plantain flour: A potential nutraceutical ingredient to increase fiber and reduce starch digestibility of gluten-free cookies. *Starch/Staerke, 70*(1–2), 1–5. https://doi.org/10.1002/star.201700107.

Gibson, G. R., Probert, H. M., Loo, J. Van, Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews, 17*(02), 259. https://doi.org/10.1079/NRR200479.

Guyenet, S. J., & Schwartz, M. W. (2012). Regulation of food intake, energy balance, and body fat mass: Implications for the pathogenesis and treatment of obesity. *Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jc.2011-2525.

Hamaker, B. R., & Tuncil, Y. E. (2014). A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *Journal of Molecular Biology*. https://doi.org/10.1016/j.jmb.2014.07.028.

Hernández-Nava, R. G., Berrios, J. D. J., Pan, J., Osorio-Díaz, P., & Bello-Pérez, L. A. (2009). Development and characterization of Spaghetti with high resistant starch content supplemented with banana starch. *Food Science and Technology International, 15*(1), 73–78. https://doi.org/10.1177/1082013208102379.

Howarth, N. C., Saltzman, E., & Roberts, S. B. (2009). Dietary fiber and weight regulation. *Nutrition Reviews, 59*(5), 129–139. https://doi.org/10.1111/j.1753-4887.2001.tb07001.x.

Hu, J., Lin, S., Zheng, B., & Cheung, P. C. K. (2018). Short-chain fatty acids in control of energy metabolism. *Critical Reviews in Food Science and Nutrition, 58*(8), 1243–1249. https://doi.org/10.1080/10408398.2016.1245650.

Huazano-García, A., & López, M. G. (2015). Agavins reverse the metabolic disorders in overweight mice through the increment of short chain fatty acids and hormones. *Food and Function, 6*(12), 3720–3727. https://doi.org/10.1039/c5fo00830a.

Huazano-García, A., Shin, H., & López, M. G. (2017). Modulation of gut microbiota of overweight mice by agavins and their association with body weight loss. *Nutrients, 9*(9), 821. https://doi.org/10.3390/nu9090821.

Jane, J., Ao, Z., Duvick, S. A., Wiklund, M., Yoo, S.-H., Wong, K.-S., & Gardner, C. (2003). Structures of amylopectin and starch granules: How are they synthesized? *Journal of Applied Glycoscience, 50*(2), 167–172. https://doi.org/10.5458/jag.50.167.

Juarez-Garcia, E., Agama-Acevedo, E., Sáyago-Ayerdi, S. G., Rodríguez-Ambriz, S. L., & Bello-Pérez, L. A. (2006). Composition, digestibility and application in breadmaking of banana flour. *Plant Foods for Human Nutrition, 61*(3), 131–137. https://doi.org/10.1007/s11130-006-0020-x.

Le Blay, G. M., Michel, C. D., Blottière, H. M., & Cherbut, C. J. (2003). Raw potato starch and short-chain fructo-oligosaccharides affect the composition and metabolic activity of rat intestinal microbiota differently depending on the caecocolonic segment involved. *Journal of Applied Microbiology, 94*(2), 312–320. https://doi.org/10.1046/j.1365-2672.2003.01836.x.

Lee, P., Yacyshyn, B. R., & Yacyshyn, M. B. (2019). Gut microbiota and obesity: An opportunity to alter obesity through faecal microbiota transplant (FMT). *Diabetes, Obesity and Metabolism, 21*(3), 479–490. https://doi.org/10.1111/dom.13561.

Mudgil, D., & Barak, S. (2013). Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: A review. *International Journal of Biological*

Macromolecules, 61, 1–6. https://doi.org/10.1016/j.ijbiomac.2013.06.044.

Murphy, K. G., & Bloom, S. R. (2006). Gut hormones and the regulation of energy homeostasis. *Nature*. https://doi.org/10.1038/nature05484.

Ovando-Martinez, M., Sáyago-Ayerdi, S. G., Agama-Acevedo, E., Goñi, I., & Bello-Pérez, L. A. (2008). Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta. *Food Chemistry, 113*(1), 121–126. https://doi.org/10.1016/j.foodchem.2008.07.035.

Padilla-Camberos, E., Barragán-Álvarez, C. P., Diaz-Martinez, N. E., Rathod, V., & Flores-Fernández, J. M. (2018). Effects of Agave fructans (Agave tequilana Weber var. azul) on body fat and serum lipids in obesity. *Plant Foods for Human Nutrition, 73*(1), 34–39. https://doi.org/10.1007/s11130-018-0654-5.

Pereira, M. A., & Ludwig, D. S. (2001). Dietary fiber and body-weight regulation: Observations and mechanisms. *Pediatric Clinics of North America, 48*(4), 969–980. https://doi.org/10.1016/S0031-3955(05)70351-5.

Pingitore, A., Gonzalez-Abuin, N., Ruz-Maldonado, I., Huang, G. C., Frost, G., & Persaud, S. J. (2019). Short chain fatty acids stimulate insulin secretion and reduce apoptosis in mouse and human islets in vitro: Role of free fatty acid receptor 2. *Diabetes, Obesity and Metabolism, 21*(2), 330–339. https://doi.org/10.1111/dom.13529.

Plongbunjong, V., Graidist, P., Knudsen, K. E. B., & Wichienchot, S. (2017). Starch-based carbohydrates display the bifidogenic and butyrogenic properties in pH-controlled faecal fermentation. *International Journal of Food Science and Technology, 52*(12), 2647–2653. https://doi.org/10.1111/ijfs.13553.

Rendón-Huerta, J. A., Juárez-Flores, B., Pinos-Rodríguez, J. M., Aguirre-Rivera, J. R., & Delgado-Portales, R. E. (2012). Effects of different sources of fructans on body weight, blood metabolites and fecal bacteria in normal and obese non-diabetic and diabetic rats. *Plant Foods for Human Nutrition, 67*(1), 64–70. https://doi.org/10.1007/s11130-011-0266-9.

Rodríguez-Cabezas, M. E., Camuesco, D., Arribas, B., Garrido-Mesa, N., Comalada, M., Bailón, E., ... Zarzuelo, A. (2010). The combination of fructooligosaccharides and resistant starch shows prebiotic additive effects in rats. *Clinical Nutrition, 29*(6), 832–839. https://doi.org/10.1016/j.clnu.2010.05.005.

Sánchez-Zapata, E., Viuda-Martos, M., Fernández-López, J., & Pérez-Alvarez, J. A. (2015). Resistant starch as functional ingredient. In K. G. Ramawat, & J. M. Mérillon (Eds.). *Polysaccharides* (pp. 1911–1931). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-16298-0_34.

Sanghera, D. K., Bejar, C., Sharma, S., Gupta, R., & Blackett, P. R. (2019). Obesity genetics and cardiometabolic health: Potential for risk prediction. *Diabetes, Obesity and Metabolism, 21*(5), 1088–1100. https://doi.org/10.1111/dom.13641.

Santiago-García, P. A., & López, M. G. (2014). Agavins from Agave angustifolia and Agave potatorum affect food intake, body weight gain and satiety-related hormones (GLP-1 and ghrelin) in mice. *Food and Function, 5*(12), 3311–3319. https://doi.org/10.1039/c4fo00561a.

Santisteban, M. M., Qi, Y., Zubcevic, J., Kim, S., Yang, T., Shenoy, V., ... Raizada, M. K. (2017). Hypertension-linked pathophysiological alterations in the gut. *Circulation Research, 120*(2), 312–323. https://doi.org/10.1161/CIRCRESAHA.116.309006.

Slavin, J., & Green, H. (2007). Dietary fibre and satiety. *Nutrition Bulletin, 32*(SUPPL.1), 32–42. https://doi.org/10.1111/j.1467-3010.2007.00603.x.

Smith, D. M., Cawthorne, M. A., Stocker, C. J., Davies, A., O'Dowd, J., Bellahcene, M., ... Zaibi, M. S. (2010). Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. *FEBS Letters, 584*(11), 2381–2386. https://doi.org/10.1016/j.febslet.2010.04.027.

Tangerman, A., & Nagengast, F. M. (1996). A gas chromatographic analysis of fecal short-chain fatty acids, using the direct injection method. *Analytical Biochemistry, 236*(1), 1–8. https://doi.org/10.1006/abio.1996.0123.

Urías-Silvas, J. E., Cani, P. D., Delmée, E., Neyrinck, A., López, M. G., & Delzenne, N. M. (2008). Physiological effects of dietary fructans extracted from Agave tequilana Gto. and Dasylirion spp. *British Journal of Nutrition, 99*(2), 254–261. https://doi.org/10.1017/S0007114507795338.

Utrilla-Coello, R. G., Agama-Acevedo, E., Osorio-Díaz, P., Reynoso-Camacho, R., & Bello-Pérez, L. A. (2013). Glycemic response in healthy rats fed with composite cereal bars. *Starch/Staerke, 65*(3–4), 354–359. https://doi.org/10.1002/star.201200129.

Wanders, A. J., Jonathan, M. C., Van Den Borne, J. J. G. C., Mars, M., Schols, H. A., Feskens, E. J. M., & De Graaf, C. (2013). The effects of bulking, viscous and gel-forming dietary fibres on satiation. *British Journal of Nutrition, 109*(7), 1330–1337. https://doi.org/10.1017/S0007114512003145.

Wong, J., De Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J. (2006). Colonic health: Fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology, 40*(3), 235–243.

Yang, J., Martínez, I., Walter, J., Keshavarzian, A., & Rose, D. J. (2013). Invitro characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe, 23*, 74–81. https://doi.org/10.1016/j.anaerobe.2013.06.012.

Yang, T., Santisteban, M. M., Rodriguez, V., Li, E., Ahmari, N., Carvajal, J. M., ... Mohamadzadeh, M. (2015). Gut dysbiosis is linked to hypertension. *Hypertension, 65*(6), 1331–1340. https://doi.org/10.1161/HYPERTENSIONAHA.115.05315.

Zaibi, M. S., Stocker, C. J., O'Dowd, J., Davies, A., Bellahcene, M., Cawthorne, M. A., ... Arch, J. (2010). Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. *FEBS Letters, 584*(11), 2381–2386. https://doi.org/10.1016/j.febslet.2010.04.027.