Research Article

Mango (*Mangifera indica* L.) polyphenols ameliorate functional constipation symptoms in humans beyond equivalent amount of fiber

Vinicius P. Venancio[1], Hyemee Kim[1], Maritza A. Sirven[1], Carmen D. Tekwe[2], Gilson Honvoh[2], Stephen T. Talcott[1], Susanne U. Mertens-Talcott[1]

1 Department of Nutrition and Food Science, Texas A&M University, College Station, TX, USA

[2] Department of Epidemiology and Biostatistics, Texas A&M University, College Station, TX, USA

Correspondence:

Susanne U. Mertens-Talcott

Associate Professor

Department of Nutrition and Food Science, Texas A&M University

1500 Research Parkway Room 220K College Station/TX 77843 Phone: (979) 458-1819

(smtalcott@tamu.edu).

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Abbreviations: GIP, gastrin inhibitory polypeptide; **GLP-1,** glucagon-like peptide 1; **IL-1β,** interleukin 1 beta; **IL-6,** interleukin 6; **IL-10,** interleukin 10; **MCP-1,** monocyte chemotactic protein 1; **PAI-1,** plasminogen activator inhibitor 1; **PYY,** peptide YY; **SCFA,** short-chain fatty acids; **TNF-α,** tumor necrosis factor alpha.

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Abstract

Scope: Chronic constipation is a common gastrointestinal condition associated with intestinal inflammation and considerably impaired quality of life, affecting about 20% of Americans. Dietary fiber and laxatives aid in its treatment but do not fully address all symptoms, such as intestinal inflammation. Mango (*Mangifera indica* L.), a fiber- and polyphenol-rich fruit may provide anti-inflammatory effects in constipation.

Methods and Results: The 4-week consumption of mango fruit (300g) or the equivalent amount of fiber was investigated in otherwise healthy human volunteers with chronic constipation that were randomly assigned to either group. Blood and fecal samples and digestive wellness questionnaires were collected at the beginning and end of the study. Results show that mango consumption significantly improved constipation status (stool frequency, consistency, and shape) and increased gastrin levels and fecal concentrations of short chain fatty acid (valeric acid) while lowering endotoxin and interleukin 6 concentrations in plasma.

Conclusion: In this pilot study, the consumption of mango improves symptoms and associated biomarkers of constipation beyond an equivalent amount of fiber. Larger follow-up studies would need to investigate biomarkers for intestinal inflammation in more detail.

1 Introduction

Functional constipation is a symptom-based disorder, characterized by a low frequency of bowel movements [1, 2]. Although studies on the prevalence of chronic constipation differ in the selection of the included criteria, estimates suggest that up to 20% of the North American population suffer from this disease [1, 3-5]. The perception of constipation by patients is based on straining or hard stools [6], and may also be diagnosed by the duration of the episodes, the number of bowel movements, and the occurrence of abdominal pain [7]. According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK-NIH), complications of long-lasting constipation include hemorrhoids, anal fissures, rectal prolapse, and fecal impaction.

Recent studies show that fiber supplements are among the first-line of choice in treating chronic idiopathic constipation (CIC) and constipation-predominant irritable bowel syndrome (IBS-C) [8], where most subjects reported considering fiber supplements as a satisfying treatment for their symptoms. Dietary guidelines from the Office of Disease Prevention and Health Promotion recommend 22-37 g of fiber per day. Fiber is particularly useful in promoting intestinal regularity through increasing stool volume and improving stool consistency [9]. Dietary sources of fiber include whole fruits, vegetables, and whole grains [10]. Non-digestible carbohydrates cannot be metabolized by digestive enzymes and therefore are not considered a source of energy to humans. However, these compounds can be fermented by certain commensal intestinal microbes into short-chain fatty acids (SCFA) [11-14]. The anti-inflammatory effects of SCFA such as butyrate and propionate have been previously described [12, 15].

Polyphenols and polyphenol-rich foods (fruits, teas, spices) also increase the concentration of SCFA and consequently protect intestinal cells from damage [12, 16, 17]. Mango (*Mangifera indica* L.) is not only a fiber-rich (1.3 – 3.8 g/100 g) but also a polyphenol-rich fruit [18-20]. A previous animal study [21] showed that mango supplementation restored the *Bifidobacteria* and *Akkermansia* population and increased fermentation rates in the gut of C57BL/6 mice previously exposed to a 60% fat kcal diet. While fiber may ameliorate symptoms of chronic constipation, polyphenols in mango may additionally decrease inflammation and further decrease symptoms. Although a few in vitro and animal studies have been performed in this aspect, no research has investigated the additive effects of the mango polyphenols in gut health. The objective of this study was to evaluate the effects of mango intake (compared to the same amount of fiber supplementation) in subjects with functional constipation.

2 Materials and Methods

2.1 Study treatments: mango polyphenol composition and fiber powder

Chemical Analysis of mango pulp: 6.4 ± 0.8 g of frozen mango was transferred to a 50-mL falcon tube and homogenized five times with 1:1 acetone and methanol (10 mL/extraction). Homogenized mango was then centrifuged at room temperature for 10 minutes at 3,500 rpm and the supernatant aliquoted to a round bottom flask. Pooled supernatant was evaporated under reduced pressure at 60 °C until only endogenous water from the mango pulp remained in the round bottom flask. The resulting mango extract was frozen at -20°C until analysis via liquid chromatography-mass spectrometry (LC-MS). Extraction was performed in triplicate; 3 different samples from 2 different portions of mango was analyzed.

LC-MS characterization and quantification were performed utilizing a Thermo Finnigan LCQ Deca XP Max MSn ion trap mass spectrometer equipped with an ESI source. Separations were carried out with a Phenomenex (Torrance, CA, USA) Kinetix C18 column (150 x 4.6 mm, 2.6 um) with 0.1% formic acid (A) and 0.1% formic acid in methanol (B) as mobile phases. Gradient elution began with 100% A at a flow rate of 4.5 mL/min. 100% A was maintained for 2 minutes before decreasing to 90% after 4 minutes and held at these conditions for 10 minutes. Mobile phase A continued to decrease to 60% after 25 minutes, 35% after 35 minutes, and finally 15% after 41 minutes. 15% A was held for 5 minutes before equilibrating at 100% A for 4 minutes prior to the next run. The electrospray interface ran in the negative ionization mode and optimized with methyl gallate. Sheath and auxiliary gas were set to 10 and 5 units/minute, respectively. Capillary temperature was 325˚C, source voltage was 4 kV, source current was 80 uA, and capillary voltage was -43 V. Gallic acid, mono-galloyl-glucosides, and penta-galloyl-glucoside were quantified using their perspective standards. Gallotannins that contained 6 or more gallic acid moeities were quantified in Penta-galloyl-glucose equivalents, due to the lack of available standards for each individually sized polymer (Luo, 2014). Results were expressed as mg/kg mango.

The fiber treatment used in this study consisted of a commercially-available Psyllium husk powder containing 5 g of total fiber per teaspoon (5.8 g). Minor inert ingredients included maltodextrin, citric acid, natural and artificial flavoring, and artificial coloring.

2.2 Ethics

The study design was submitted and approved by Texas A&M University Institutional Review Board (IRB) before its execution, under IRB# 2014-0585. A written informed consent form, with all details of the study, was signed by each subject.

2.3 Subjects

A total of 48 male and female adults (18-65 years old) self-reportedly suffering from chronic constipation were screened for eligibility, and 36 subjects completed the study. The study was advertised at healthcare providers in the Bryan-College Station area using an IRB-approved flyer. Emails were also sent to the Texas A&M University community. Bowel constipation was diagnosed using the ROME III criteria [7]. All participants attended a familiarization session before beginning the study. Exclusion criteria included: history of acute cardiac event, stroke, or cancer; recurrent hospitalization or drug treatment within the last six months; history of alcohol or substance abuse; smoking (> 1 pack/week); excessive exercise (> 60 minutes; >5 times/week); allergy against mango or fiber; hepatitis B or C; HIV; drug treatment against constipation, including steroids.

2.4 Nutritional intervention treatments

Subjects were randomly assigned to either the mango group or the fiber group. Patients in the mango group were asked to include one daily serving of approximately 300 g of mango fruit in their diet for 4 weeks, while subjects in the fiber group included the equivalent amount of fiber powder (one teaspoon of fiber powder, equivalent to 5 grams of fiber) into their diet for the same duration.

2.5 Subject monitoring

Participants attended the first sample collection session and donated a blood and a stool sample before beginning the mango or fiber treatment. Subjects were contacted weekly by a research nurse, when they were asked to provide information about any adverse events, as well as symptoms of

constipation. At the end of the 4-week treatment period, blood and stool samples were collected additionally.

The overall dietary intake of each subject was evaluated using a 72-hour food record, collected during the first and last week of the study. Dietary intake data were analyzed using iProfile 3.0 (http://iprofile.wiley.com) and calculated as calories, fat, cholesterol, carbohydrates, dietary fiber, and protein.

2.6 Evaluation of constipation symptoms

Severity of constipation symptoms was evaluated weekly throughout the study duration. The evacuation categorization was based on stool consistency and shape according to the scale of Bristol [22]. There were seven categories: (1): nut-like; (2): lumpy sausage; (3): sausage with cracks; (4): smooth snake; (5): soft blobs; (6): fluffy pieces; (7): watery. Evacuation categorization was determined by the difference from category 4 (ideal stool form and consistency).

Constipation intensity was assessed following the constipation scoring system proposed by Agachan [23]. The AGACHAN score was calculated weekly throughout the study duration and assessed frequency of bowel movements, difficulty/straining to evacuate, pain on evacuation, sensation of incomplete evacuation, abdominal pain, time taken to start the evacuation, type of assistance (digital assistance or enema) for evacuation, attempts per day and duration of constipation [23, 24].

2.7 Plasma preparation and analysis

One 10-mL blood sample was collected using Vacutainer® system and K_2EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA) on day 1 and 28 of the study. Blood tubes were centrifuged at 1,500 × g for 10 minutes at 4 ºC. Plasma was then stored at -80 ºC until analysis. Inflammatory biomarkers, hormones, and adipokines were assessed in plasma samples from the subjects as described below.

2.8 Determination of inflammatory biomarkers, metabolic hormones, and gastrin

Plasmatic levels of inflammatory biomarkers, adipokines, and metabolic hormones were quantified by xMAP Multiplex Assay (Luminex 200, Luminex Corporation, Austin, TX, USA) using magnetic beads acquired from EMD Millipore (Billerica, MA, USA) and following the manufacturer's protocol. The analytes included: Interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor alpha (TNF-α), adiponectin, resistin, plasminogen activator inhibitor 1 (PAI-1), C-peptide, gastrin inhibitory polypeptide (GIP), glucagon-like peptide 1(GLP-1), glucagon, insulin, leptin, monocyte chemotactic protein 1(MCP-1), and peptide YY(PYY). All determinations were performed in duplicate.

Gastrin concentration was analyzed by ELISA kit acquired from Abcam (Cambridge, UK). All samples were analyzed in duplicate.

2.9 Stool short-chain fatty acids (SCFA) analysis

SCFA analysis was performed by gas chromatography (HP 5890, Hewlett-Packard, Palo Alto, CA, USA) coupled to a quadrupole mass spectrometer (HP-5989A). Ground feces (0.5g) were vortexed in 2N HCl for 30 minutes followed by centrifugation at 3,000 rpm for 20 min. The upper phase was transferred to C18 cartridge after adding 200 mM internal standard (d7-butyric acid) and then eluted with diethyl ether. Diethyl ether was added again to the sample and the tube vortexed for 15 minutes. The top layer (supernatant) was again removed, MTBSTFA (N-tert-butyldimethylsilyl-N ethytrifluoracetamide) was added to the tubes, and samples was transferred to vials for the gas chromatography-mass spectrometry injection. Dry matter weights of fecal samples were used to normalize the concentration of SCFA. SCFA results were expressed as μmol/mL [25].

2.10 EndoLISA test

Endotoxin levels in the stool samples were measured by EndoLISA (Hyglos, Germany), according to the manufacturer's instructions. In brief, frozen, ground feces (0.2g) was vortexed and diluted in binding buffer. The samples were incubated at 37 °C for 90 minutes with shaking. The plate was

washed with washing buffer, and assay reagent was added. The signal was detected in a FLUOstar Omega plate reader at 380 nm excitation and 445 nm emission (BMG Labtech, Durhan, NC).

2.11 Statistical analysis

Summary statistics of the demographic variables were obtained and expressed as means ± SD by treatment group, see Table 1. All the outcome variables included in our study were determined to have distributions that deviated from the normal distribution based on the Shapiro-Wilk test [26]. Therefore, nonparametric tests were used to determine differences by treatment group and time. To test for the effects of time within each treatment group, we first obtained paired differences by subtracting measures obtained at baseline from those obtained at the end of the study. Wilcoxon signed rank tests were subsequently used to assess the effects of time within each group [27]. Differences in the treatments groups were tested by comparing the medians using the exact Wilcoxon Mann-Whitney test [28]. All tests were considered statistically significant for p-values ≤ 0.05. The analyses were performed using PROC UNIVARIATE and PROC NPAR1WAY of SAS 9.4 (SAS Institute, Cary NC).

3 Results and Discussion

The polyphenol composition of mango provided to subjects (Table 1) was in accordance with others [29-32]. Mango pulp polyphenols are predominantly comprised of gallotannins, which are glucose esterified with 5 or more gallic acid moieties [31]. Gallic acid and mono-galloyl-glucose are also commonly found in mango pulp, and the concentrations found here are similar to another reported analysis of the same mango cultivar, Keitt [32]. Flavonoids have also been commonly reported in mango, but they were not found in significant concentrations in this analysis (data not shown) [31].

A total of 36 subjects completed the study. Twelve subjects withdrew from the study for various reasons, such as scheduling. The number of participants in each group (17 in the fiber and 19 in the mango group) is higher than previous studies with similar outcomes [33-35]. No adverse events occurred during this study.

Subjects were randomly assigned to either the fiber or mango group. Overall, the compliance in the mango group was higher compared to the fiber group. The distribution of subjects in the fiber and mango groups was not significantly different for gender, age, height, weight, and body (Table 2). The distribution of gender in the study population (i.e., higher number of female participants) is consistent with previous studies [33, 36].

The Rome III criteria were applied to this study as a reliable tool for the diagnostic of functional constipation [37-39]. Other nutritional intervention studies have used the same tool in their interventions, such as Wojtyniak et al. (*Lactobacillus casei rhamnosus Lcr35*) [40], Mirghafourvand et al. (probiotic yogurt) [41], and Waitzberg et al. (symbiotic) [24]. The Rome III criteria characterize functional constipation as the occurrence of three or fewer bowel movements over the period of one week [7].

The food intake (calories, fat, cholesterol, carbohydrates, dietary fiber and protein) data (Table 3), assessed by food questionnaires (72-h recall) shows no significant differences in food intake profile of subjects between the fiber or mango group (including dietary fiber consumption), indicating that the subjects did not change their food intake over the course of the study and both groups consumed equivalent amounts of macronutrients.

The overall outcome of this intervention study was to evaluate how mango and fiber may modulate the severity of constipation and the symptoms of the condition where evacuation category was selected as primary outcome and AGACHAN score data as secondary outcome, both obtained from weekly surveys. Data indicate that both mango and fiber supplementation improved the AGACHAN score over the course of the study (Figure 1). This scoring system was developed as a tool to assess constipation based on the patient's self-evaluated symptoms that include both subjective symptomatic complaints and physiologic findings. The AGACHAN score considers the frequency of bowel movements, difficulty, completeness, pain, time, assistance, failure, and history, for an accurate assessment of the severity of constipation [23].

Evacuation categorization is a 7-point scale that categorizes bowel transit time based on the stool form. A score of 4 is attributed to a normal stool shape, and evacuation categorization is often expressed as the average difference from a normal shape [22]. In this study, subjects in the mango group reported increased evacuation categorization throughout the treatment phase. Subjects in the fiber group did not report any changes in this parameter.

Biomarkers for inflammation, gastrin, adipokines, and metabolic hormones were assessed at baseline and the end of this study (Table 4). The mango treatment significantly decreased the expression of IL-6 over the 4-week treatment while the fiber treatment did not show any improvement. In vivo and in vitro anti-inflammatory activity of mango polyphenols have been described previously [42, 43]. IL-6 is relevant to intestinal health because it triggers cytokine production in response to inflammation [44], and plays a role in the mucosal protection against enteric bacterial pathogens [45].

Both mango and fiber treatment increased the level of gastrin throughout the 4-week study treatment phase ($p = 0.0002$ and $p = 0.0001$, respectively – Table 4). However, statistical analysis indicates that the increase in participants treated with mango was higher compared to those who received the fiber supplementation ($p = 0.0288$, Table 4). Gastrin has been associated with low intestinal motility [46, 47].Therefore, increased gastrin levels are associated with improved intestinal health, as previously discussed [46, 48, 49].

SCFA (total, acetic acid, propionic acid, butyric acid, isovaleric acid and valeric acid) were analyzed in stool samples from this 4-week nutritional intervention, as well as endotoxin concentrations (Figure 2). Participants in the mango group exhibited increased levels of valeric acid after four weeks of treatment compared to the baseline levels of this fatty acid in stool. SCFAs play an important role in the maintenance of gut and immune homeostasis [50]. Increased levels of valeric acid were reported in constipated patients treated with *Lactobacillus parasei*-enriched artichokes [51]. Other nutritional intervention studies have reported increased concentrations of SCFA and improvement of constipation symptoms after treatment with rye bread [52] and wheat bran [53] in humans. A nutritional

intervention with mango in obese C57BL/6 mice described increased levels of SCFA acetic and n-butyric acids [21].

Compared to the fiber group, mango consumption decreased the concentration of fecal endotoxins. Endotoxins are relevant in intestinal health because these compounds may be absorbed and contribute to increased systemic inflammation, relevant to chronic inflammatory diseases (including cardiovascular diseases, diabetes, insulin resistance, and obesity) [54, 55].

4 Concluding remarks

Taken together, our results indicate mango supplementation was more effective in mitigating the symptoms of functional constipation in human subjects than fiber alone. Our investigation showed that the consumption of mango fruit decreased IL-6 in plasma and increased fecal valeric acid after a four-week intervention. Both mango and fiber increased gastrin levels in plasma, but the intervention with mango fruit caused a significantly greater increase of this biomarker. Subjects reported improvements in their intestinal health, measured by their stool consistency and the severity of their symptoms, but once again the supplementation with mango fruit proved to be more effective in modulating these parameters. Molecular mechanistic studies are necessary to elucidate the mechanism of action involved in the mango protective effect in functional constipation and which role mango polyphenols may play in supporting the beneficial effects of fiber.

V.P.V. has conducted the clinical trial, analyzed plasma samples and questionnaire entries, and written the manuscript. H.K. has analyzed samples for short chain fatty acids and endotoxin. M.A.S. performed the polyphenol composition of mango fruit. C.D.T. and G.H. performed the statistical analyses. S. T. T. and S. U. M. T. have supervised the all steps of the clinical trial and written the manuscript.

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The authors have declared no conflicts of interest.

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Figure legends

Fig. 1 – Evacuation categorization (A-C) and AGACHAN score (D-F) of subjects from fiber and mango groups.

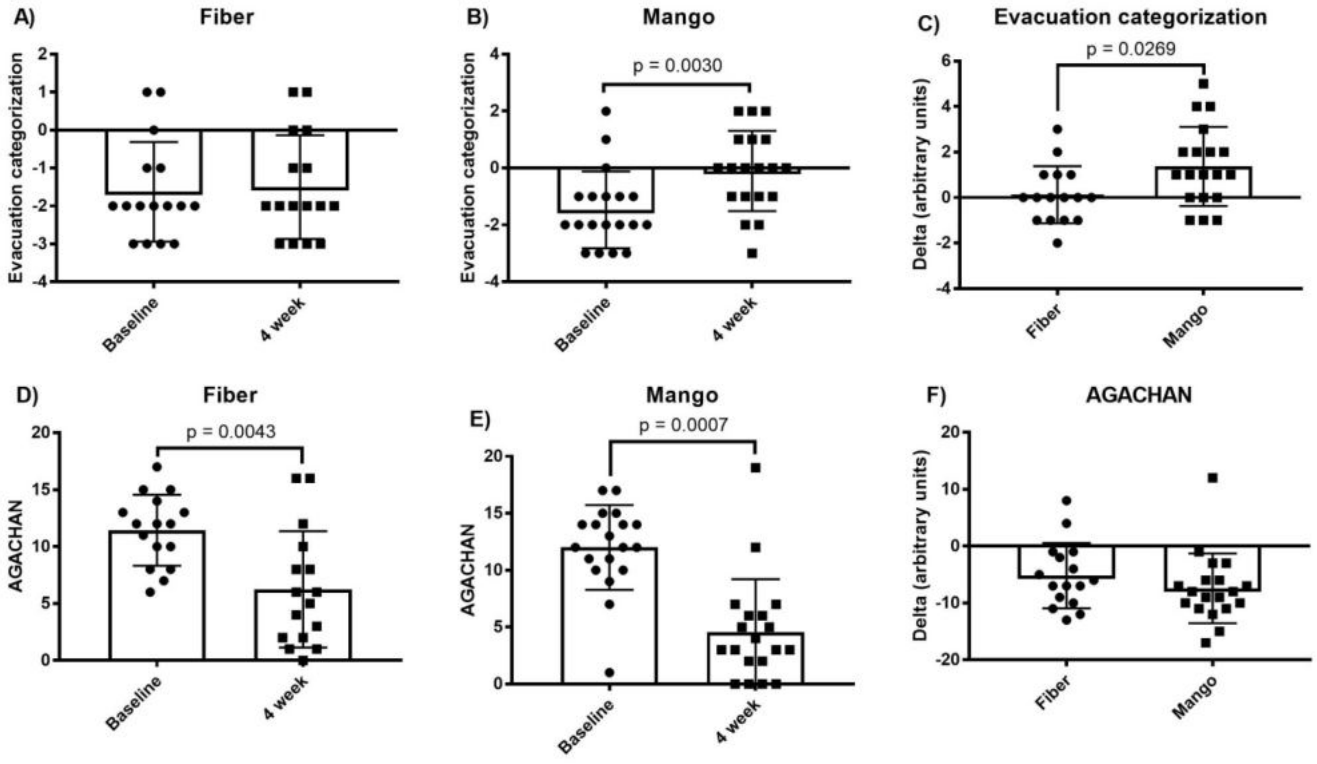
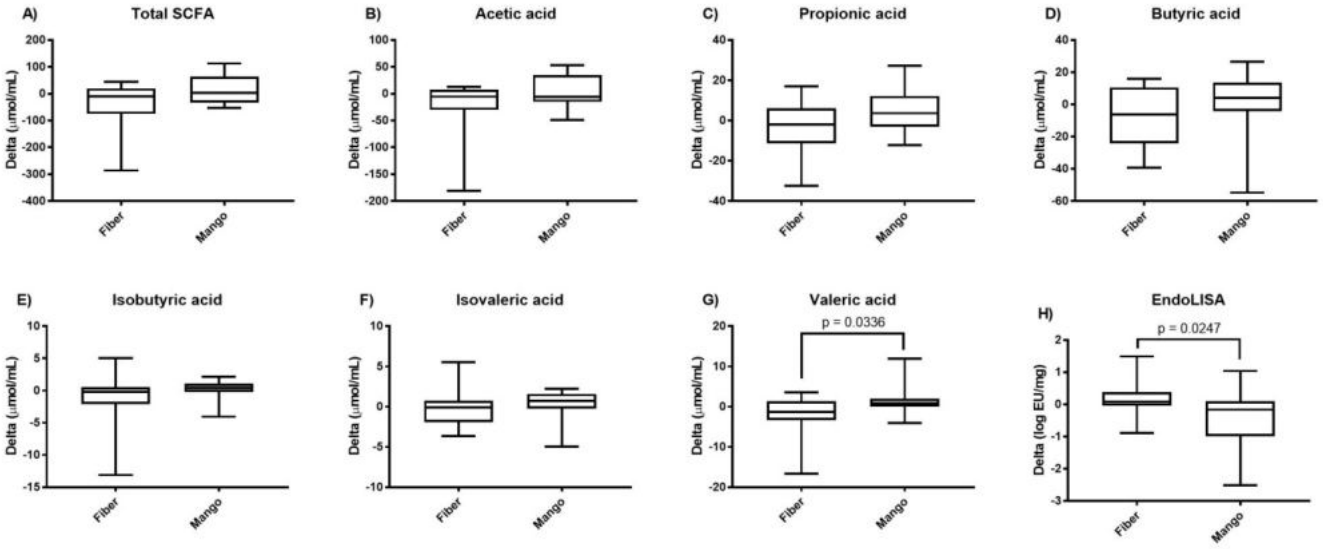


Fig 2 – Short chain fatty acids (A-G) and endotoxins (H) in fecal samples of participants in the fiber and mango groups.



Graphical Abstract – Text

Mango or the equivalent amount of fiber powder were offered to subjects with constipation for four weeks. Significant changes include decreased AGACHAN score and evacuation categorization, increased gastrin and valeric acid levels, and reduced concentrations of interleukin 6 and endotoxins.

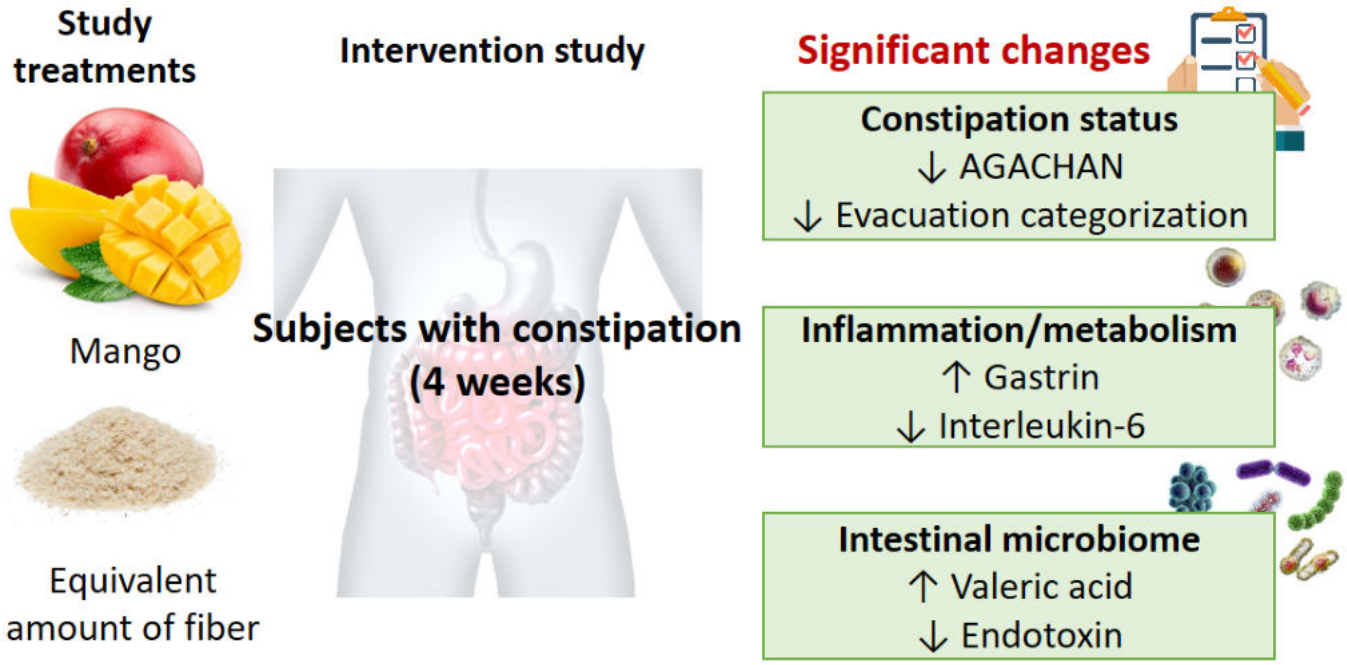


Table 1. Characterization and quantification of predominant polyphenols in mango cv. 'Keitt' that was provided for subjects during the 4-week period.

Compound	Concentration (mg/kg mango)
Gallic Acid	6.43 ± 0.31
Monogalloyl glucoside (ester)	34.09 ± 11. 12
Monogalloyl glucoside (ether)	4.30 ± 0.92
Penta-*O*-galloylglucose	87.33 ± 17.56
Hexa-*O*-galloylglucose	63.19 ± 17.60
Hepta-*O*-galloylglucose	66.02 ± 21.79
Octa-*O*-galloylglucose	29.08 ± 15.89
Nona-*O*-galloylglucose	27.09 ± 15.54
Total Gallotannins	272.71 ± 48.69

Values are mean ± SD. n = 3.

Table 2. Distribution of subjects: gender, age, height, weight and body mass index.

Group	Fiber	Mango
n	17 (11 female, 6 male)	19 (14 female, 5 male)
Age (years)	28.9 ± 8.9	23.5 ± 4.4
Weight (kg)	70.6 ± 21.3	65.6 ± 9.0
Height (m)	1.7 ± 0.1	1.6 ± 0.1
BMI	24.2 ± 7.1	24.5 ± 3.4

No differences were found between groups ($p > 0.05$).

Table 3. Daily intake obtained from 72-hour food questionnaire.

Variable	Group	Fiber			Mango			Delta		
		Baseline	4 week	p value	Baseline	4 week	p value	Fiber	Mango	p value
Calories (kcal)	Mean	2566	2273	0.2783	2319	1939	0.1055	-292	-379	0.8094
	SD	1028	761		640	345				
Fat(g)	Mean	106	96	0.8311	97	86	0.6523	-10	-11	0.8094
	SD	51	37		47	18				
Cholesterol (mg)	Mean	297	312	0.9658	371	316	0.5566	15	-55	0.4262
	SD	169	154		204	196				
Carbohydrates (g)	Mean	319	279	0.2061	266	222	0.0840	-40	-44	0.9177
	SD	134	109		94	55				
Dietary fiber (g)	Mean	29	28	0.8828	23	18	0.0508	-1	-4	0.3190
	SD	13	10		10	8				
Protein (g)	Mean	93	81	0.2783	106	81	0.0840	-12	-25	0.8506
	SD	16	34		43	23				

Table 4. Changes in the level of inflammatory biomarkers, gastrin, adipokines and metabolic hormones at baseline and 4 weeks of the program participation for the fiber and mango groups.

Variable	Group	Fiber			Mango			Delta		
		Baseline	4 week	p value	Baseline	4 week	p value	Fiber	Mango	p value
Inflammatory biomarkers										
IL-1β (pg/mL)	Mean	10.14	9.51	0.8702	9.23	8.70	0.5217	-0.63	-0.53	0.6294
	SD	5.66	4.00		4.86	2.53				
IL-6 (pg/mL)	Mean	14.24	15.12	0.2312	15.17	11.67	0.0141	0.88	-3.50	0.0120
	SD	8.76	6.60		7.25	4.64				
IL-10 (pg/mL)	Mean	48.69	47.25	0.1796	46.06	38.95	0.1019	-1.44	-7.11	0.0307
	SD	46.55	32.28		23.76	16.08				
TNF-α (pg/mL)	Mean	5.13	4.71	0.2261	6.38	4.81	0.9924	-0.42	-1.56	0.4561
	SD	5.21	1.92		7.75	2.87				
Gastrin, adipokines and metabolic hormones										
Gastrin (pg/mL)	Mean	1.40	1.51	0.0002	1.70	1.92	0.0001	0.11	0.22	0.0288
	SD	0.16	0.18		0.24	0.24				
Adiponectin (μg/mL)	Mean	22.15	25.54	0.3225	22.03	20.78	0.4653	3.39	-1.25	0.2432
	SD	13.21	13.72		12.63	12.05				
Resistin (ng/mL)	Mean	52.34	51.67	0.8999	54.97	45.33	0.0728	-0.67	-9.64	0.2569
	SD	34.87	23.57		28.46	21.28				

PAI-1 (ng/mL)	Mean	45.35	50.14	0.4637	56.97	48.93	0.0546	4.79	-8.03	0.1247
	SD	21.55	27.03		34.06	28.34				
C-Peptide (pg/mL)	Mean	1461.3	1513.36	0.9399	1317.74	1384.25	0.5412	52.05	66.51	0.5669
	SD	586.38	539.58		908.34	878.02				
GIP (pg/mL)	Mean	95.31	102.29	0.8999	72.21	86.17	0.2935	6.98	13.96	0.7314
	SD	66.44	56.13		51.43	49.24				
GLP-1 (pg/mL)	Mean	211.51	240.31	0.4332	214.54	243.70	0.3899	28.81	29.16	0.9025
	SD	108.37	137.78		91.72	135.87				
Glucagon (pg/mL)	Mean	17.16	17.51	0.4332	17.71	18.29	0.4180	0.35	0.58	0.7561
	SD	3.72	3.40		3.98	4.24				
Insulin (pg/mL)	Mean	217.32	236.54	0.3484	195.52	241.80	0.2101	19.22	46.28	0.5448
	SD	130.15	130.02		112.10	163.39				
Leptin (ng/mL)	Mean	3.84	4.42	0.2744	5.30	5.05	0.8596	0.58	-0.25	0.3498
	SD	2.21	2.61		3.64	2.43				
MCP-1 (pg/mL)	Mean	60.07	64.54	0.3225	60.56	59.91	0.9530	4.48	-0.25	0.4814
	SD	16.21	18.42		19.97	28.59				
PYY (pg/mL)	Mean	60.51	58.79	0.9399	67.57	73.99	0.3124	-1.72	6.42	0.2432
SD	SD	19.83	16.06		33.95	21.04				

IL-1β, interleukin 1 beta; IL-6, interleukin 6; IL-10, interleukin 10; TNF-α, tumor necrosis factor alpha; PAI-1, plasminogen activator inhibitor 1; GIP, gastrin inhibitory polypeptide; GLP-1, glucagon-like peptide; MCP-1, monocyte chemotactic protein 1; PYY, peptide YY.