AgriLIFE RESEARCH & EXTENSION
Texas A&M System

TEXAS A&M UNIVERSITY

COLLEGE OF AGRICULTURE AND LIFE SCIENCES

Department of Nutrition and Food Science

Mango in the Promotion of Intestinal Regularity in Subjects with Constipation

FINAL Report

Dr. Susanne Talcott (smtalcott@tamu.edu) and
Dr. Stephen Talcott (stalcott@tamu.edu)
Texas A&M University, Department of Nutrition and Food Science
1500 Research Parkway A
Centeq Research Plaza, Room 220F
College Station, TX 77843-2253

220K Centeq A
1500 Research Parkway
MS 2254
College Station, TX 77843-2253

Email: smtalcott@tamu.edu
Phone: 979-458-1819
Fax: 979-862-7944
Web: http://nfsc.tamu.edu

Introduction and Background

Our ultimate goal is to support the National Mango Board identifying and demonstrating chemical and biochemical properties of mangos that allows the organization to better market fresh mangos. This research presents vital groundwork towards obtaining legal and marketing claims relating to the composition and health benefits of mangos. We realize that this may be a time-consuming process, but recognize that these foundations are critical to build for a strong working case for these ultimate goals.

Introduction

Constipation in the US: Constipation is the number one chronic gastrointestinal complaint in the United States, affecting approximately one in every fifty people, or about 2% of the population.[1] While most cases are minor and short-lived, constipation can also present as a system of more serious conditions. Approximately 92,000 hospitalizations and 900 deaths are reported annually showing constipation as one of the related symptoms[2]. For as prevalent as constipation is, surprisingly little is known about the exact causes behind it[3]. Due to its commonality, varied severity, and minimal systematic research the treatments for constipation are nearly as varied as the patients themselves, even amongst the medical communities in developed countries [4] .Chronic constipation is commonly associated with an insufficient intake of dietary fiber, and many home remedies focus around increased consumption of fruits, vegetables, and other high-fiber foods. However, there are also strong observable correlations between age, race/ethnicity, socioeconomic status, and even gender which are harder to explain by a simple disparity in dietary fiber consumption. Women are almost three times as likely as men to suffer from constipation, whites suffer less than any other race or ethnic group, and persons of lower socioeconomic status are more likely to suffer than their wealthier counterparts. The frequency of constipation shows a sharp increase after age 65 for all demographics[5]. Some studies have suggested a connection between chronic constipation and impaired neural regulation of colonic motility. A neurological link could establish a plausible explanation for the link between socioeconomic status, race, and gender by way of potential non-dietary environmental factors which could play a significant role in overall colonic health [6].

Nutritional Treatment of Constipation

Constituents in mango promoting regular bowel movements

Fiber: Dietary fiber is defined by the IOM[5] as nondigestible carbohydrates and lignin that are intrinsic and intact in plants, including the “plant nonstarch polysaccharides (e.g., cellulose, pectin, gums, hemicelluloses, β-glucans, and fibers contained in oat and wheat bran), plant carbohydrates that are not recovered by alcohol precipitation (e.g., inulin, oligosaccharides, and fructans), lignin, and some resistant starch.” Functional fibers, on the other hand, include fibers that are added to foods (or provided as supplements) and that have been shown to have health benefits. They include, but are not limited to, “isolated, nondigestible plant (e.g., resistant starch, pectin, and gums), animal (e.g., chitin and chitosan), or commercially produced (e.g., resistant starch, polydextrose, inulin, and indigestible dextrins) carbohydrates” (2). The variety of fibers used in the food supply, especially the consistently increasing number of foods with added fibers, renders the examination of total fiber consumption in the U.S. population difficult.

In addition to fiber, mango contains sorbitol, a laxative sugar alcohol that has been found to be laxative when consumed in higher quantities [7]. Potentially, when consumed together with fiber, lower concentrations of sorbitol may contribute to the laxative effects [8].

The effects of polyphenolics on gastro-intestinal motility is not well investigated however, polyphenolics have been shown to reduced intestinal inflammation in our preliminary studies in

chemical-induced inflammation in rats and also in previous published studies [9-12]. Reductions of intestinal inflammation and irritation contribute to the overall well-being and reduce abdominal pain.

In general, nutritional treatment of diarrhea would be preferable to conventional drug treatments that include steroid treatment that have severe side-effects, pain medication.

In contrast to chemical laxatives (e.g. polyethylenglycol) and fiber treatments that are available, mango combines the benefits of fiber, polyphenolics (with multiple benefits), sorbitol and a wonderful taste. Overall, the high incidence of constipation in the US, specifically in an elderly population and the composition of mango strongly suggest that the consumption of mango would be highly beneficial in individuals with constipation.

Overall Study Objective: While mango shares many of its health benefits with other fruits and vegetables, mangoes contain several unique polyphenolics and combinations of these. Mangos are high fiber, have considerable amounts of polyphenolics and also contain sorbitol that may contribute to increased bowel movements in constipated individuals.

Significance: Overall, the proposed objective would generate new, highly potent data for the mango board which would promote the consumption of fresh mango, specifically considering the potential for increasing marked saturation. As for previous studies, the preparation of press releases into different media outlets is planned as well as scientific publications.

Hypotheses:

We hypothesize that the consumption of fresh mango decreases the frequency and severity of constipation in affected elderly individuals over a period of 2-4 weeks. Based on the content of polyphenolics and sorbitol in addition to the fiber, we further hypothesize that the consumption of mango have superior effects compared to a solution of fiber that is comparable to the composition of fiber in mango.

Study Approach:

Based on the presented literature, we performed a short-term study investigating the efficacy of the consumption of fresh mango on intestinal regularity and gastro-intestinal well-being. The study was carried out after approval by the Institutional Review Boards (IRB) at Texas A&M University and was registered at www.Clinicaltrials.gov upon initiation.

Study Treatments

Mangos: Commercially available mangos of the variety Keitt was obtained from a fruit-wholesaler. These mangos have been imported from Mexico as commercial produce and gone through USDA inspection. Upon arrival, mangos were stored in a fruit-storage at the Horticulture Department, Texas A&M University until ripening. Upon ripening, mangos were processed according to GMP guidelines by the Food Science and Nutrition Department, Texas A&M University. In brief, intact mangos underwent a wash in bleach-solution, were deseeded, peeled, cut and frozen under vacuum in food storage bags (250-400g) within 6h of deseeding. Bags are stored at -30ºC. Temperature is monitored daily.

Control Fiber: Control subjects receive a control fiber that has a comparable fiber composition to mango that they dissolved in a drink.

*Subject Population and Recruitment***:** Subjects with frequent constipation were recruited through advertisements and clinical contacts through the study physician. Informed written

consent was obtained by the research personnel before the study begins. Approximately 36 individuals per group are expected to undergo the safety screening in order to obtain 24 subjects per group completing this study (considering screening failure and potential drop-outs). We expect a maximum of 50 subjects for the initial screening.

Inclusion criteria: Male or female subjects, age 18-79 years (see Protection of Human Subjects) with frequent constipation

Exclusion criteria: history of acute cardiac event, stroke, or cancer, within the last 6 months, recurrent hospitalizations, drug treatment of any of the listed conditions within the last 6 months, abuse of alcohol or substance within the last 6 months, currently smoking more than 1 pack/week, seizures, liver or renal dysfunction, pregnancy or lactation, allergy against mangos hepatitis B, C, or HIV, regular exercise (>60 minutes, ≥ 5 times/wk), due to association of antioxidant and anti-inflammatory effects and moderate exercise.

Moreover, subjects that are receiving drug treatment against constipation, including steroids were excluded from this study.

Study Schedule:

Subjects attended a familiarization session where their eligibility for this study was assessed. Subjects were randomized into the mango or control fiber group.

Subjects consumed 300g of mango daily for four weeks or the equivalent fiber amount.

Subjects filled in a Bowel specific Index Questionnaire and an overall digestive wellness questionnaire at study begin and after that weekly. A blood sample was collected at study begin and every week for four weeks.

Research Team: The proposed studies was performed by Dr. Susanne Talcott, molecular nutrition and pharmacometrics, Dr. Mick Deutz, MD, metabolic clinician, Dr. Steve Talcott, phytochemist, Dr. Hongwei Zhao, bio-statistician, TAMU. Dr. Andrew Dupont, MD, gastroenterologist, Consultant.

Assessed Biomarkers:

Primary outcome:

Frequency/Severity of intestinal irritation/constipation before and after the study (Bowel Index)

Secondary outcome:

Inflammation markers, hematocrit, Vit-D status, erythrocyte sedimentation, blood cell counts, hs-CRP in plasma, 72h dietary questionnaire, wellness questionnaire, cholesterol panel (the more feces is excreted the less cholesterol the body can recycle, therefore cholesterol is investigated as well).

Analysis of intestinal microbiota: Fecal samples were obtained from subjects and stored at -80ºC until analysis. Total DNA was extracted and purified using a bead-beating phenol-chloroform method as previously described (Suchodolski et al., 2010). Quantitative real-time PCR (qPCR) was performed to initially investigate changes in specific bacterial groups. (Garcia-Mazcorro et al., 2012b).

Evaluation of constipation symptoms. Constipation symptoms were evaluated weekly throughout the study duration. The evacuation categorization was based on stool consistency and shape according to the scale of Bristol [13]. 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 [14]. The AGACHAN score was performed 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 [14, 15].

Plasma preparation and analysis. For each sample collection session (before and after the 4-week treatment period), a 10-mL blood sample was collected using Vacutainer® system and K_2EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA). For the plasma preparation, tubes were centrifuged at 1,500 $\times$ g for 10 minutes at 4 ºC. Plasma samples were then stored at -80 ºC until analysis. Inflammatory biomarkers, hormones and adipokines were assessed in plasma samples from the treated subjects according to the methodologies described below.

Inflammatory biomarkers The concentration of the inflammatory biomarkers interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10) and tumor necrosis factor alpha (TNF-α) were assessed 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. All determinations were performed in duplicate and the results were expressed as pg/mL.

Gastrin, adipokines and metabolic hormones. Gastrin concentration was performed by ELISA kit acquired from Abcam (Cambridge, UK). All samples were analyzed in duplicate and the results were expressed as pg/mL. A adipokine panel (Adiponectin, resistin and plasminogen activator inhibitor-1) and a metabolic hormone panel consisting of 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). were assessed 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. All determinations were performed in duplicate.

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). Grinded 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 were transferred to vials for the GC/MS injection. Dry matter weights of fecal samples were used to normalize the concentration of SCFA. SCFA results are expressed as μmol/mL [16].

Endotoxins - EndoLISA test. Endotoxin levels in the stool samples were measured by EndoLISA (Hyglos, Germany), according to the manufacturer's instructions. Grinded feces (0.2g) were 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 fluorescence reader using excitation/emission wavelengths of 380/440 nm (BMG Labtech, Durhan, NC).

Statistical analyses. All data were analyzed by using SAS versing 9.3 (SAS Institute Inc., Cary, NC). Baseline demographics from the two treatment groups and nutritional intake were compared with Student's t-test. The main variables of interest, biochemical and pro-inflammatory markers were compared between the two treatment groups and the two different times using a mixed effects model. In order to investigate the influence of baseline characteristics on biochemical and pro-inflammatory markers, a type 3 tests of fixed effects model was also fit on the changes of these markers with group, time, time*group, gender, and one of the six food record measurements (calories, fat, carbohydrates, cholesterol, dietary fiber and protein). All response variables were log-transformed. A 5% significance level was used for all interpretations.

RESULTS

A total of 36 subjects have entered and successfully completed the entire study. 12 subjects withdrew for different reasons, e.g. scheduling, before beginning the study after signing the informed consent form. Subjects were randomly assigned to the fiber or mango group. Overall, the compliance in the mango group was higher compared to the control group. **Table 1** shows the distribution of subjects in control and mango groups regarding gender, age, height, weight and body mass index (BMI). No differences were found between control and mango groups.

Table 1 – Distribution of subjects: gender, age, height, weight and body mass index.

Group	Control	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

Table 2 shows the constipation parameters analyzed throughout the four weeks of nutritional intervention. Both mango and fiber improved the constipation markers analyzed. However, mango treatment exhibited better improvement (by increasing evacuation categorization and decreasing AGACHAN score. Please see also **Figure 1**.

Table 2 – Evacuation categorization and AGACHAN score of subjects from control and mango groups.

Variable	Group	Control			Mango			P value
		Baseline	4 week	Δ	Baseline	4 week	Δ	
Evacuation categorization	Mean	-1.8	-1.5	0.3	-1.5	-0.2	1.3	
	SD	1.3	1.4		1.3	1.4		
AGACHAN score	Mean	11.0	6.4	-4.6	12.1	4.9	-7.2	
	SD	3.2	5.2		3.6	4.6		

The food intake (calories, fat, cholesterol, carbohydrates, dietary fiber and protein) data is shown in **Table 3.** There were no differences on the food intake profile of subjects in the control or in the mango group.

Table 3 – Daily intake obtained from 72-hour food questionnaires

Variable	Group	Control			Mango			P value
		Baseline	4 week	Δ	Baseline	4 week	Δ	
Calories (kcal)	Mean	2566	2273	-292	2319	1939	-379	0.8094
	SD	1028	761		640	345		
Fat (g)	Mean	106	96	-10	97	86	-11	0.8094
	SD	51	37		47	18		
Cholesterol (mg)	Mean	297	312	15	371	316	-55	0.4262
	SD	169	154		204	196		
Carbohydrates (g)	Mean	319	279	-40	266	222	-44	0.9177
	SD	134	109		94	55		
Dietary fiber (g)	Mean	29	28	-1	23	18	-4	0.3190
	SD	13	10		10	8		
Protein (g)	Mean	93	81	-12	106	81	-25	0.8506
	SD	36	34		43	23		

Table 4 shows the inflammatory biomarkers, gastrin, adipokines and metabolic hormones levels at baseline and after 4 weeks of nutritional intervention. Statistical analysis found that subjects who received mango for 4 weeks exhibited higher change in gastrin levels than the subjects in the control group (after adjustment for gender and the food record variables). Changes in PYY levels were also higher in mango group subjects when compared to the control group (after adjustments for gender, fat, carbohydrates, fiber and protein).

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 control and mango groups.

Variable	Group	Control			Mango			P value
		Baseline	4 week	Δ	Baseline	4 week	Δ	
Inflammatory biomarkers								
IL-1β (pg/mL)	Mean	10.14	9.51	-0.63	9.23	8.70	-0.53	0.6294
	SD	5.66	4.00		4.86	2.53		
IL-6 (pg/mL)	Mean	14.24	15.12	0.88	15.17	11.67	-3.50	0.0120
	SD	8.76	6.60		7.25	4.64		
IL-10 (pg/mL)	Mean	48.69	47.25	-1.44	46.06	38.95	-7.11	0.0307
	SD	46.55	32.28		23.76	16.08		
TNF-α (pg/mL)	Mean	5.13	4.71	-0.42	6.38	4.81	-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	0.11	1.70	1.92	0.22	0.0288
	SD	0.16	0.18		0.24	0.24		
Adiponectin (μg/mL)	Mean	22.15	25.54	3.39	22.03	20.78	-1.25	0.2432
	SD	13.21	13.72		12.63	12.05		
Resistin (ng/mL)	Mean	52.34	51.67	-0.67	54.97	45.33	-9.64	0.2569
	SD	34.87	23.57		28.46	21.28		
PAI-1 (ng/mL)	Mean	45.35	50.14	4.79	56.97	48.93	-8.03	0.1247
	SD	21.55	27.03		34.06	28.34		
C-Peptide (pg/mL)	Mean	1461.36	1513.35	52.05	1317.74	1384.25	66.51	0.5669
	SD	586.38	539.58		908.34	878.02		
GIP (pg/mL)	Mean	95.31	102.29	6.98	72.21	86.17	13.96	0.7314
	SD	66.44	56.13		51.43	49.24		
GLP-1 (pg/mL)	Mean	211.51	240.31	28.81	214.54	243.70	29.16	0.9025
	SD	108.37	137.78		91.72	135.87		
Glucagon (pg/mL)	Mean	17.16	17.51	0.35	17.71	18.29	0.58	0.7561
	SD	3.72	3.40		3.98	4.24		
Insulin (pg/mL)	Mean	217.32	236.54	19.22	195.52	241.80	46.28	0.5448
	SD	130.15	130.02		112.10	163.39		
Leptin (ng/mL)	Mean	3.84	4.42	0.58	5.30	5.05	-0.25	0.3498
	SD	2.21	2.61		3.64	2.43		
MCP-1 (pg/mL)	Mean	60.07	64.54	4.48	60.56	59.91	-0.25	0.4814
	SD	16.21	18.42		19.97	28.59		
PYY (pg/mL)	Mean	60.51	58.79	-1.72	67.57	73.99	6.42	0.2432
	SD	19.83	16.06		33.95	21.04		

Table 5 shows the levels of SCFA (acetic acid, propionic acid, butyric acid, isovaleric acid and valeric acid) in stool samples from this 4-week nutritional intervention, as well as endotoxin concentrations. No differences were found on these parameters thoughout the time or treatment

Variable	Group	Control			Mango			P value
		Baseline	4 week	Δ	Baseline	4 week	Δ	
Short chain fatty acids								
Acetic acid (μmol/mL)	Mean	50.78	24.81	-25.97	46.03	52.08	6.05	0.1453
	SD	62.61	14.19		24.38	30.49		
Propionic acid (μmol/mL)	Mean	25.65	22.15	-3.50	16.07	20.31	4.24	0.1350
	SD	17.95	11.25		6.42	12.52		
Butyric acid (μmol/mL)	Mean	31.25	23.45	-7.80	24.77	26.40	1.64	0.2204
	SD	21.65	13.33		31.01	20.79		
Isobutyric acid (μmol/mL)	Mean	3.79	2.72	-1.06	3.07	3.23	0.16	0.2063
	SD	3.53	1.72		1.97	1.52		
Isovaleric acid (μmol/mL)	Mean	2.77	2.67	-0.11	2.61	2.93	0.32	0.1929
	SD	1.97	1.84		2.02	1.53		
Valeric acid (μmol/mL)	Mean	6.41	4.47	-1.94	3.19	4.61	1.42	0.0336
	SD	4.51	3.28		2.16	4.24		
Endotoxins								
EndoLISA (log EU/mg)	Mean	1.10	1.29	0.18	0.90	0.47	-0.43	0.0247
	SD	0.97	0.87		1.18	1.26		

Table 5 – Levels of short chain fatty acids and endotoxins at baseline and 4 weeks of the treatment for the control and mango groups.

After controlling for gender, time, group, time*group, and food record measurements (calories, fat, carbohydrates, cholesterol, dietary fiber and protein), statistically significant effect was found for carbohydrates consumption on IL-10 (-0.1981, p=0.045). In addition, there were statistically significant of calories, fat, and carbohydrate effects on the reduction in IL-6 with parameters values of -0.2959, -0.3105, -0.3080 respectively and p-values respective of 0.0416, 0.0424, and 0.0352. Calories, fat, cholesterol, carbohydrate, fiber, and protein intake were significant on Gastrin (0.08129 (p=0.0208), 0.07886 (p=0.0270), 0.07919 (p=0.0287), 0.08030 (p=0.0155), 0.08711 (p=0.0142), 0.08112 (p=0.0262) respectively) (**Table 6 and Figure 2**)

Table 6. The fixed effects modeling relating the baseline covariates to the before and after differences of biochemical and pro-inflammatory markers.

	Parameter	Changes (log)	P-value	Group x Time interaction	
				Changes (log)	P-value
IL-10	Carbohydrate	0.001050 ± 0.000518	0.0578	-0.1981 ± 0.09198	0.0450*
IL-6	Calories	0.000202 ± 0.000090	0.0383*	-0.2959 ± 0.1349	0.0416*
	Fat	0.002765 ± 0.001422	0.0677	-0.3105 ± 0.1422	0.0424*
	Carbohydrate	0.001298 ± 0.000718	0.0873	-0.3080 ± 0.1352	0.0352*
Gastrin	Calories	0.000029 ± 0.000022	0.2001	0.08129 ± 0.03210	0.0208*
	Fat	0.000108 ± 0.000338	0.7537	0.07886 ± 0.03275	0.0270*
	Cholesterol	6.331E-6 ± 0.000080	0.9379	0.07919 ± 0.03331	0.0287*
	Carbohydrate	0.000365 ± 0.000164	0.0387*	0.08030 ± 0.03005	0.0155*
	Fiber	0.002335 ± 0.001523	0.1427	0.08711 ± 0.03210	0.0142*
	Protein	0.000187 ± 0.000442	0.6779	0.08112 ± 0.03348	0.0262*
Acetic acid	Calories	-0.00032 ± 0.000182	0.1034	0.7134 ± 0.3322	0.0485*
	Fat	-0.00349± 0.002935	0.2528	0.7429 ± 0.3472	0.0492*
	Carbohydrate	-0.00217± 0.001534	0.1773	0.7178 ± 0.3324	0.0474*
Valeric acid	Fat	-0.00503± 0.003551	0.1767	1.0084 ±0.3864	0.0197*
	Cholesterol	-0.00065± 0.000884	0.4729	0.9410 ±0.3971	0.0316*
	Carbohydrate	-0.00192± 0.001977	0.3460	0.9741 ±0.4174	0.0340*
	Fiber	-0.00383± 0.01722	0.8269	0.9583 ±0.4191	0.0372*
	Protein	-0.00357± 0.004848	0.4724	0.9191 ±0.4102	0.0406*
Endolisa	Calories	0.000415 ± 0.000377	0.2884	-1.4109 ± 0.5654	0.0247*
	Fat	0.003964 ± 0.005852	0.5085	-1.4458 ± 0.5934	0.0278*
	Cholesterol	-0.00095± 0.001450	0.5245	-1.4734 ± 0.6163	0.0304*
	Carbohydrate	0.005875 ± 0.002823	0.0550	-1.4120 ± 0.5111	0.0145*
	Protein	-0.00726± 0.007964	0.3766	-1.5389 ± 0.6239	0.0262*

Mean ± Standard error. *p<0.05.

Overall Results:

1. The consumption of fresh-frozen Mango was more efficacious in the treatment of chronic constipation in young adults (where chronic constipation most commonly occurs) compared to a commercially available fiber treatment. Both treatments were equal in fiber content and the additional benefits are likely to be attributed to the content of tannins and other polyphenols in mango.

2. In addition to improving symptoms and severity of constipation, the mango treatment also improved biomarkers for inflammation and the production of short chain fatty acids by the intestinal microflora.
3. The production of endotoxins is not only correlated with intestinal inflammation but also with aging, diabetes, chronic inflammation associated with obesity and degenerative diseases, including neuro-degenerative diseases. Mango significantly decreased the production of entotoxins in healthy individuals which is of major significance and should be followed up in a population with increased endotoxin production due to the potential of improving systemic inflammation and symptoms associated with aging.
4. Study participants consumed the mango treatment more regularly than the fiber treatment that was skipped more frequently.

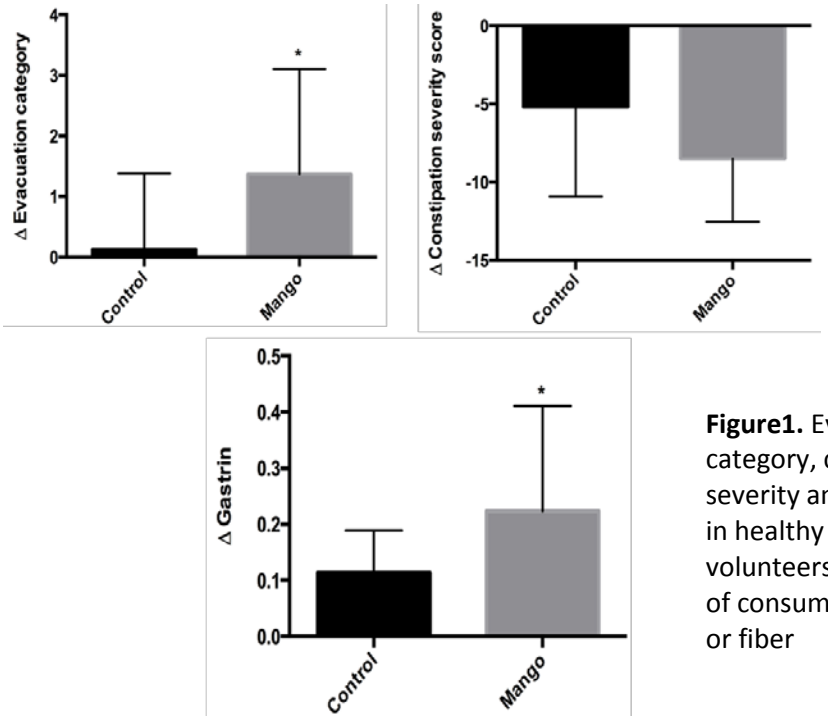

Figure1. Evacuation category, constipation severity and gastrin levels in healthy human volunteers after 4 weeks of consumption of mango or fiber

Benefits to the Mango Industry

Based on these data, mango is more effective in the treatment of chronic constipation compared to a fiber treatment and also had a better treatment adherence.

It is of great significance that even in healthy individuals the production of endotoxins and chronic inflammation was reduced and this has great relevance in the treatment of aging-related symptoms in future research studies.

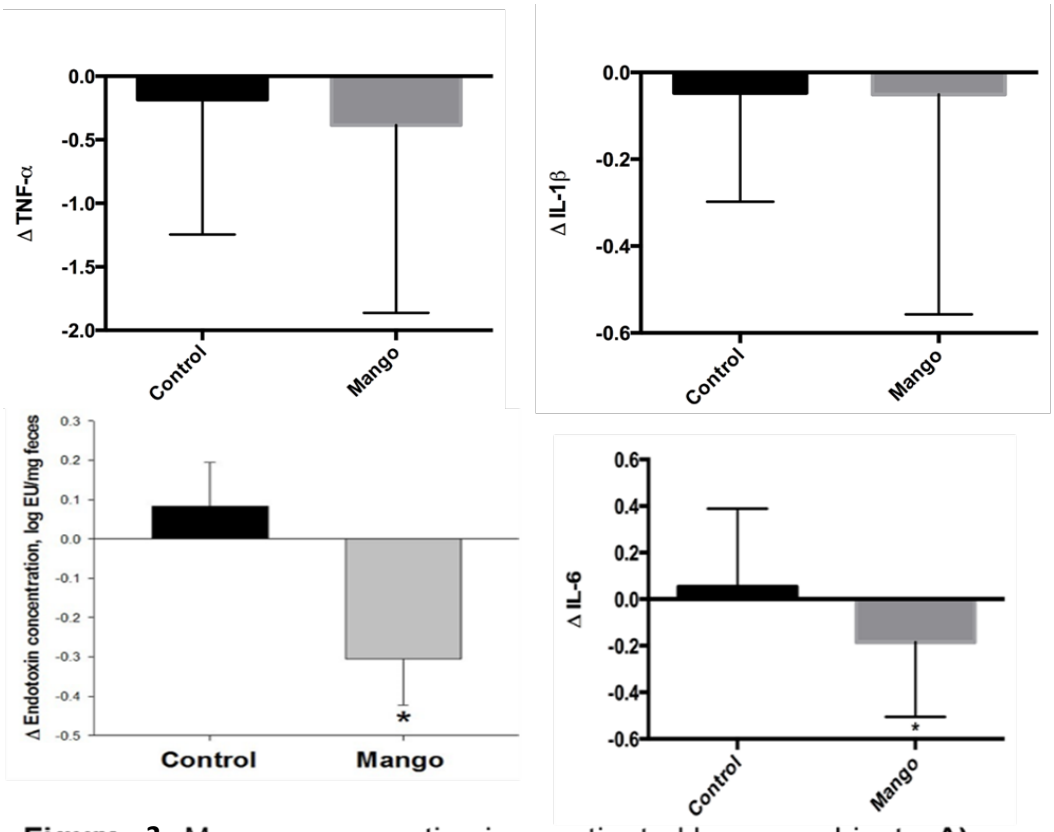

Figure 2 Mango consumption in constipated human subjects**. A)** Endotoxin production was determined in fecal samples after 4 weeks of consuming mango or a fiber-based control. B) Corresponding plasma concentrations of IL-6. Values are mean ± SEM. Different letters = significant differences between groups (n = 10; Kruskal-Wallis test; $p<0.05$).

References

1. Johanson JF, Sonnenberg A, Koch TR: **Clinical epidemiology of chronic constipation**. *Journal of clinical gastroenterology* 1989, **11**(5):525-536.
2. Sonnenberg A, Koch TR: **Epidemiology of constipation in the United States**. *Diseases of the colon and rectum* 1989, **32**(1):1-8.
3. Higgins PD, Johanson JF: **Epidemiology of constipation in North America: a systematic review**. *The American journal of gastroenterology* 2004, **99**(4):750-759.
4. Krammer H, Schlieger F, Singer MV: **[Therapeutic options of chronic constipation]**. *Der Internist* 2005, **46**(12):1331-1338.
5. McCrea GL, Miaskowski C, Stotts NA, Macera L, Varma MG: **A review of the literature on gender and age differences in the prevalence and characteristics of constipation in North America**. *Journal of pain and symptom management* 2009, **37**(4):737-745.
6. Stewart WF, Liberman JN, Sandler RS, Woods MS, Stemhagen A, Chee E, Lipton RB, Farup CE: **Epidemiology of constipation (EPOC) study in the United States: relation of clinical subtypes to sociodemographic features**. *The American journal of gastroenterology* 1999, **94**(12):3530-3540.

7. Islam MS, Sakaguchi E: **Sorbitol-based osmotic diarrhea: possible causes and mechanism of prevention investigated in rats**. *World J Gastroenterol* 2006, **12**(47):7635-7641.
8. Knapp BK, Parsons CM, Swanson KS, Fahey GC, Jr.: **Physiological responses to novel carbohydrates as assessed using canine and avian models**. *Journal of agricultural and food chemistry* 2008, **56**(17):7999-8006.
9. Biasi F, Astegiano M, Maina M, Leonarduzzi G, Poli G: **Polyphenol supplementation as a complementary medicinal approach to treating inflammatory bowel disease**. *Curr Med Chem* 2011, **18**(31):4851-4865.
10. Ferretti G, Bacchetti T, Masciangelo S, Saturni L: **Celiac disease, inflammation and oxidative damage: a nutrigenetic approach**. *Nutrients* 2012, **4**(4):243-257.
11. Qin B, Dawson HD, Schoene NW, Polansky MM, Anderson RA: **Cinnamon polyphenols regulate multiple metabolic pathways involved in insulin signaling and intestinal lipoprotein metabolism of small intestinal enterocytes**. *Nutrition (Burbank, Los Angeles County, Calif* 2012, **28**(11-12):1172-1179.
12. Younes-Sakr L, Senesse P, Laurent C, Rouanet JM, Rugani N, Cristol JP, Gaillet S: **Validation of a surgical technique for rat intestinal irradiation: potential side effects prevention by dietary grape phenolics**. *Dig Dis Sci* 2012, **57**(10):2562-2570.
13. Lewis SJ, Heaton KW: **Stool form scale as a useful guide to intestinal transit time**. *Scand J Gastroenterol* 1997, **32**(9):920-924.
14. Agachan F, Chen T, Pfeifer J, Reissman P, Wexner SD: **A constipation scoring system to simplify evaluation and management of constipated patients**. *Dis Colon Rectum* 1996, **39**(6):681-685.
15. Waitzberg DL, Logullo LC, Bittencourt AF, Torrinhas RS, Shiroma GM, Paulino NP, Teixeira-da-Silva ML: **Effect of synbiotic in constipated adult women - a randomized, double-blind, placebo-controlled study of clinical response**. *Clin Nutr* 2013, **32**(1):27-33.
16. Noratto GD, Garcia-Mazcorro JF, Markel M, Martino HS, Minamoto Y, Steiner JM, Byrne D, Suchodolski JS, Mertens-Talcott SU: **Carbohydrate-free peach (Prunus persica) and plum (Prunus domestica) juice affects fecal microbial ecology in an obese animal model**. 2014.