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Department of Nutrition and Food Science

# Mango in the Promotion of Intestinal Regularity in Subjects with Constipation

**FINAL Report**


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## 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 $K_2$EDTA 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-1$\beta$), interleukin 6 (IL-6), interleukin 10 (IL-10) and tumor necrosis factor alpha (TNF-$\alpha$) 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 $\mu$mol/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.

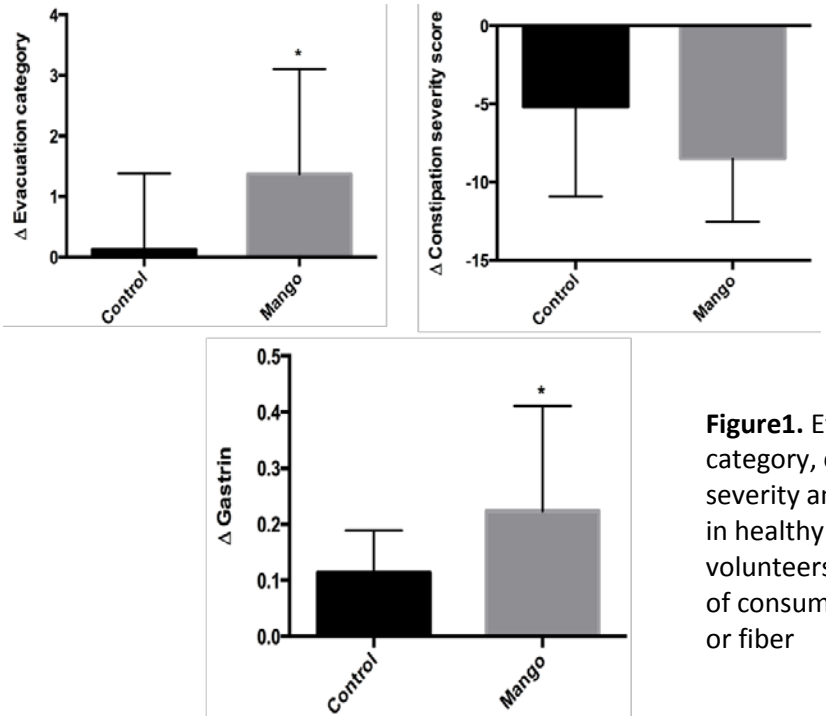


**Figure1.** Evacuation category, constipation severity and gastrin levels in healthy human volunteers after 4 weeks of consumption of mango or fiber

**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.

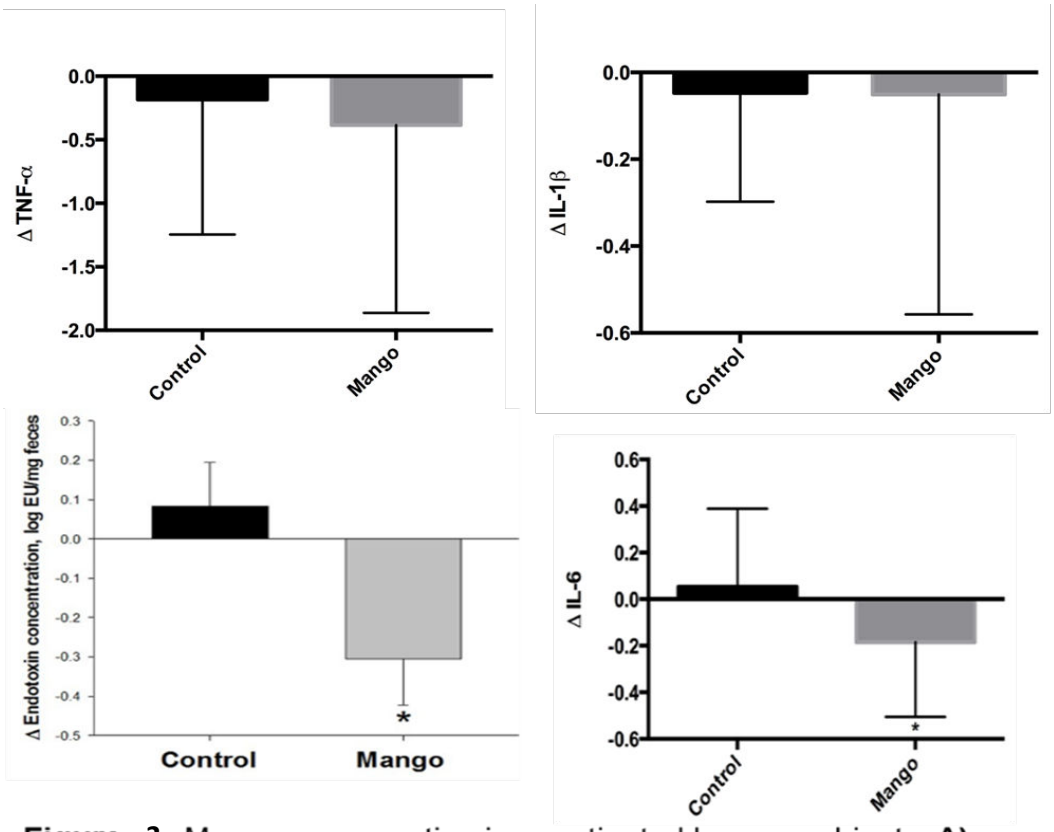


**Figure 2** Mango consumption in constipated human subjects**. A)** Endotoxin production was determined in fecal samples after 4 weeks of consuming mango or a fiber-based control. B) Corresponding plasma concentrations of IL-6. Values are mean ± SEM. Different letters = significant differences between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

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