Evaluation of Short Chain Fatty Acids (SCFAs) intestinal absorption, following digestion and fermentation of a novel medical device containing partially-hydrolyzed Guar gum plus simethicone

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Abstract

Irritable Bowel Syndrome (IBS) is a common disease characterized by alternate symptoms (diarrhea and constipation) and intestinal gas overproduction. A new medical device (Fibergone®), containing Partially Hydrolyzed Guar Gum (PHGG) and Simethicone (SM) has been proposed for managing patients with bowel disorders. PHGG acts also as a prebiotic so increasing the Short-Chain Fatty Acid (SCFA) production, useful for intestinal physiology. This *in vitro* study investigated the effects exerted by PHGG+SM on SCFA production and their intestinal absorption following *in vitro* digestive process and fermentation model. An *in vitro* model evaluated the digestive process and fermentation using simulated digestive fluids and a human intestinal epithelium *in vitro* model derived from based on intestinal adenocarcinoma Caco-2 cells (ATCC, HTB-37TM) and organized as a functional monolayer on Transwell® inserts. PHGG+SM was added in experiments and compared with a control (non-treated). SCFA production and absorption were assessed. Viability and barrier integrity of the intestinal epithelium model were also evaluated. PHGG+SM significantly ($p<0.05$) increased SCFAs content after fermentation, indicating that this medical device is effectively fermented at the large intestine level. However, in relation to SCFAs bioavailability, their absorption did not increase compared to the non-treated condition in the light of the physiological contribution of SCFAs resulting from the microflora. PHGG+SM did not affect intestinal epithelium apparent permeability (Papp) and viability. This *in vitro* study documented that partially hydrolyzed guar gum combined with simethicone significantly affects short-chain fatty acids production and consequently could be fruitfully employed in managing patients with intestinal disorders.

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Introduction

Chronic bowel diseases are widespread in the general population, significantly affect quality of life and have a relevant socio-economic burden. With a prevalence of over 20% in the western world, Irritable Bowel Syndrome (IBS) represents the disease most frequently managed by doctors.[1] IBS is more common in females and young adults,[2] and it is based on functional symptoms, including pain, discomfort, and impaired bowel movement pattern (*i.e.*, alternating diarrhea and constipation). Several comorbidities combine with IBS, such as inflammatory and functional gastrointestinal disorders, mental illnesses, and somatic syndromes.[3]

The natural history of patients with IBS usually presents recurrent episodes of intestinal symptoms alternating with phases of inactivity; equally, the intensity of symptoms varies considerably over time. The leading phenotypes are patients with prevalent constipation and/or diarrhea.[4,5] In addition, bloating, colic, and flatulence, caused by dysbiosis, are bothersome symptoms for IBS patients.

From a pathogenic point of view, IBS depends on different factors, including stress, infections, antibiotic overuse, dysbiosis, malfunctioning brain-gut axis, immune dysfunction, and disturbing somatic perception.[6-8] Nevertheless, the diagnosis is mainly based on recognizing typical symptoms, a thorough history and physical examination and excluding primary diseases.[4,8] IBS management is a compelling task that requires time and commitment.[7,8]Although no treatment can modify the natural history of IBS patients, appropriate diet, emotion control, and medications are medical therapy's mainstay.

As a consequence, it is preferable to use alternative strategies in the long run. This approach includes prescribing natural products, mainly fiber-rich food, as safe and effective. Within this context of natural products, Guar gum represents an attractive option. Guar gum derives from the seeds of *Cyamopsis tetragonoloba*, a typical legume from the Indian area. The Partially Hydrolyzed Guar Gum (PHGG) is widely used as it has high water-solubility and low viscosity.[9]

PHGG exerts a dual mechanism of action, such as blocking diarrhea and laxative.[10] This double activity modulates bowel movement patterns and makes PHGG particularly useful in IBS patients, regardless of the leading symptom. In particular, PHGG regularizes bowel function, hydrates and lubricates stools, and increases stool volume.

PHGG is also a prebiotic as it promotes the growth of Lactobacilliand Bifidobacteria, and, consequently, stimulates the production of Short-Chain Fatty Acids (SCFA). SCFA have relevant anti-inflammatory and immunomodulant activity and are widely used in IBS.[11]

For this reason, a new medical device (Fibergone®) has been proposed as an add-on option in IBS management. This medical device combines PHGG with Simethicone (SM), an antifoam agent.[12] Therefore, this multicomponent product could be helpful in treating both altered bowel movements and intestinal gas overproduction.

To date, there is no evidence about the efficacy of these two combined substances on SCFA metabolism. The present study is aimed to evaluate the production and intestinal absorption of three SCFA (*i.e.*, acetic, propionic and butyric acid) following digestion and fermentation of this medical device using an *in vitro* integrated approach.

Materials and Methods

The evaluation of SCFAs production and intestinal absorption was performed by exposing the medical device containing PHGG and SM to an integrated approached based on *in vitro* digestion with fermentation and a cell-based intestinal *in vitro* model.

Digestive process and fermentation

Digestive process

The medical device (PHGG+SM) was exposed to *in vitro* digestion procedure designed to simulate the physiological process in human oral, gastric, and intestinal compartments.[13] Briefly, 5 mL of either PHGG+SM containing about 670 mg of partially hydrolyzed Guar gum and 16 mg of SM or distilled water (ddH_2O) used as negative control were added to 5 mL of simulated saliva (pH 7) and kept under constant agitation at 37°C for 2 min. Following incubation, the resulting digests were mixed with 10 mL of simulated gastric juice, and the pH was adjusted to 3. The resulting mix was maintained at 37°C for 120 min under constant agitation. Subsequently, 20 mL of simulated intestinal juices were added, and the pH was corrected to 7. As for the previous step, the resulting digests were incubated for 120 min at 37°C under constant agitation. At the end of the digestive process, the resulting digests were vacuum-dried overnight at room temperature and prepared for the fermentation step. The composition of the digestive fluids is reported in Table 1.

Fermentation

The fermentation step was performed according to the method described by Carlson *et al.*[14] Briefly, 0.5 g of dried samples of digestion with or without PHGG+SM were rehydrated in sterile trypticase peptone fermentation media in a 100 mL serum bottle, capped, and incubated for 12 h at 4°C to limit microbial growth. The PHGG content was about 280 mg. After incubation, rehydrated digests were warmed to 37°C for 2 h. Meanwhile, freshly collected fecal samples from a healthy donor who has not taken antibiotics for at least 6 months and assumes a regular Western-type diet were mixed with phosphate buffer solution with a 6:1 ratio of phosphate buffer to fecal sample. After mixing, the fecal slurry was combined with a prepared reducing solution (2.52 g cysteine hydrochloride,16 mL 1 N NaOH, 2.56 g sodium sulfide non-anhydride, 380 mL dd H_2O). Following fecal inoculum addition, 0.8 mL Oxyrase® was added to each rehydrate digest to ensure anaerobic conditions, flushed with CO_2, sealed, and then immediately placed at 37°C. Fecal inoculum without digests was prepared as a blank fermentation (Blank) for SCFA comparison. The baseline pH of the fermentation media was measured and, if necessary, corrected to mimic the environment of

Table 1. Composition of the digestive simulated fluids.

Constituent	Stock conc. g L^{-1}	Stock conc. mol L^{-1}	SSF pH 7 Vol. of stock mL	SSF pH 7 Conc. in SSF mmol L^{-1}	SGF pH 2.5* or 5** Vol. of stock mL	SGF pH 2.5* or 5** Conc. in SGF mmol L^{-1}	SIF pH 7 Vol. of stock mL	SIF pH 7 Conc. in SIF mmol L^{-1}
KCl	37.3	0.5	15.1	15.1	6.9	6.9	6.8	6.8
KH_2PO_4	68	0.5	3.7	3.7	0.9	0.9	0.8	0.8
$NaHCO_3$	84	1	6.8	13.6	12.5	25	42.5	85
NaCl	117	2	-	-	11.8	47.2	9.6	38.4
$MgCl_2(H_2O)_6$	30.5	0.15	0.5	0.15	0.4	0.1	1.1	0.33
$(NH_4)_2CO_3$	48	0.5	0.06	0.06	0.5	0.5	-	-
$CaCl_2(H_2O)_2$	44.1	0.3	-	1.5 (0.75^)	-	0.15 (0.075^)	0.7	0.6 (0.3^)
Other constituents (enzyme and bile salts)			-		Pepsin		Trypsin in pancreatin	Bile salts
			-		2000 U/mL		100 U/mL	10 mM

*pH in fasted condition; **pH in fed condition; ^corresponding Ca^{2+} concentration in the final digestion mixture; SSF, simulated salivary fluid; SGF, simulated gastric fluid; SIF, simulated intestinal fluids applied during the digestive *in vitro* process proposed by Minekus *et al.*[13]. The volumes are calculated for a final volume of 500 mL of each simulated fluid.

the distal colon (about pH 6.83). A 1 M solution of NaOH and HCl were used for pH correction. Following 24 h incubation, the fermentations were centrifuged at 3200g for 15 min at 4 °C to remove the bacteria, and the resulting supernatants (*i.e.*, bio-accessible fraction) were collected and stored at -80°C.

Quantification of SCFAs

The quantification of SCFAs (*i.e.*, acetic, propionic, and butyric acid) produced by fermentation was performed by Flame Ionization Detector – Gas Chromatography (FID-GS). The Limit of Detection (LOD) and Limit of Quantification (LOQ) for the analyzed short-chain fatty acids are reported in Table 2. The analysis was accomplished using a gas chromatograph (Agilent Technologies 6850 GC, Santa Clara, CA, USA) equipped with a split/splitless injector and FID. A GC 6890N equipped with a mass spectrometer detector 5973 (both from Agilent Technologies, SantaClara, CA, USA) was also used to confirm the identity of the analytes in the real samples analyzed. The capillary chromatographic column used was a Nukol column (30 m, 0.25 mm i.d., 0.25 µm film thickness). The GC injector was maintained at 280°C. The injection was performed in splitless mode (splitless time 3 min). The oven temperature was initially set at 80°C for 2 min, programmed at a rate of 5°C/min to 190°C that was held for 6 min, resulting in a total run time of 30 min. The carrier gas was hydrogen at a flow rate of 3.70 mL/min. The FID temperature was maintained at 220°C. MS operational conditions were: electron ionization (EI) at 70 eV; transfer line and ion source temperature: 230°C; quadrupole temperature: 150°C; and mass range: m/z 25–300. The identity of SCFAs detected in the real samples was confirmed by comparing their retention times and mass spectra with those of authentic standards and with reference spectra from the US National Institute of Standards and Technology.[15]

Intestinal epithelium *in vitro* model

The intestinal absorption of produced SCFAs was determined using an *in vitro* human intestinal model based on adenocarcinoma Caco-2 cells (ATCC, HTB-37TM) organized as a functional monolayer on Transwell® inserts. Briefly, Transwell® inserts are characterized by two compartments, apical (or lumen) and basolateral (or serosal), separated by a microporous membrane (Figure 1). The monolayers of Caco-2 grown on the microporous membranes consist of polarized cells with morpho-functional characteristics typical of enterocytes, such as the presence of microvillus, occluding junctions (tight junctions) and the glycoprotein-P (P-gp) (Figure 2).

Evaluation of the impact of digested formulations on the intestinal epithelium viability

The impact of digested and fermented formulation on the viability of the intestinal epithelium was evaluated through a dose-response curve. The fermented formulation was serially diluted in the fermentation supernatant without digests (Blank), containing the amount of SCFAs physiologically present during the digestive process. The resulting fermentation concentrations (from 0 to 100 %) were added to the apical compartment of the intestinal epithelium *in vitro*. At the same time, HBSS (Hanks' Balanced Salt Solution) buffer was placed in the basolateral compartment. The supernatant of Blank fermentation was used as a negative control. After 3 h incubation, intestinal epithelia viability was evaluated using the Methylthiazol Tetrazolium Salts (MTS) assay, which is based on the reduction of MTS tetrazolium compound by viable cells to generate a colored formazan product that can be quantified by measuring the absorbance at 490 nm.[16] Therefore, cell viability is directly proportional to absorbance. In parallel, the digested formulations impact on the intestinal epithelium barrier integrity was evaluated by measuring the Trans-Epithelial Electrical Resistance (TEER).[17]

Evaluation of SCFAs intestinal absorption

Based on dose-response curve, the highest non-toxic bio-accessible fraction of fermented PHGG+SM obtained after dilution with the supernatant of Blank fermentation that contains physiological SCFAs from microflora, were added to the apical side of the *in vitro* intestinal epithelium. At the same time, HBSS was placed in the basolateral compartment. Following 3 h exposure, apical and basolateral fractions were collected, and their acetic, propionic, and butyric acid content was determined by Gas Chromatography with Flame Ionization Detector (GC-FID), according to the previously described method. The amount of SCFAs loaded in the apical com-

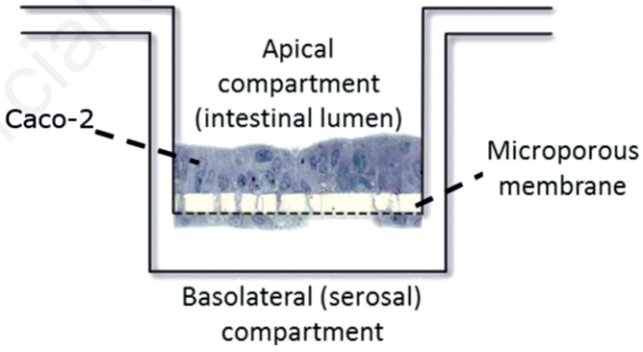

Figure 1. Representative image of *in vitro* intestinal epithelium model, with Caco-2 monolayer cultured in Transwell® inserts.

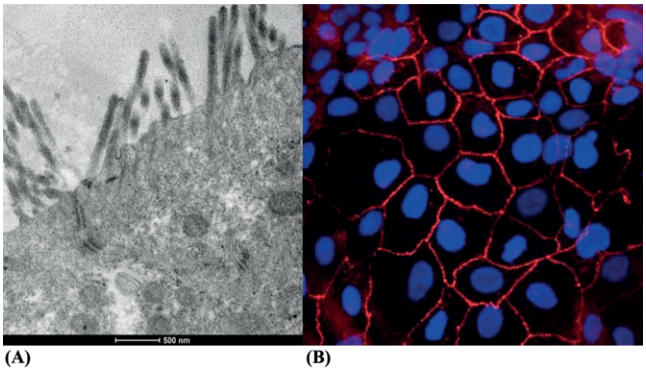

Figure 2. (A) Electron micrograph of enterocytes forming the Caco-2 monolayer and showing microvilli and tight junctions; (B) Confocal microscope image showing tight junctions (red) and nuclei (blue).

Table 2. Limit of detection (LOD) and limit of quantification (LOQ) of analysed short chain fatty acids (SCFAs) according to the applied quantification method such as by gas chromatography with flame ionization detector (GC-FID).

SCFA	LOD (µg/mL)	LOQ (µg/mL)
Acetic acid	2.9	8.9
Propionic acid	1.9	5.7
Butyric acid	5.2	15.7

partment at the beginning of the intestinal absorption experiment was determined for both blank and PHGG+SM fermentations. SCFAs bioavailability was expressed as amount (µg) and percentage of absorption (%) compared to the SCFAs amount added in the apical compartment at the beginning of the absorption experiment.

To evaluate intestinal bioavailability of SCFAs released from PHGG+SM following fermentation, intestinal epithelia were exposed for 3 h to its diluted bio-accessible fraction (17%), and SCFAs content was measured in both apical (lumen) and basolateral (serosal) compartments. Bioavailability – the amount of acetic, propionic, and butyric acids in the basolateral/serosal compartment – was then calculated and expressed as the amount (µg) and absorption percentage (%) for the amount of the analyzed SCFAs initially added in the apical compartment.

Viability and barrier integrity of the intestinal epithelium model

After exposure to the digested and fermented formulation, the intestinal epithelium cell viability and barrier integrity were evaluated. Cell viability was assessed using the MTS assay, which is based on the reduction of MTS tetrazolium compound by viable cells to generate a colored formazan product that can be quantified by measuring the absorbance at 490 nm. Therefore, cell viability is directly proportional to absorbance. Barrier integrity was evaluated by measuring the cell monolayer's TEER and assessing its permeability to Lucifer Yellow, a polar tracer unable to pass through intact tight junctions.

The apparent permeability coefficient (P_{app}, cm/min) was calculated with the following formula: $P_{app} = (\Delta CV)/(\Delta tAC0)$, where $\Delta C/\Delta t$ is the flow of the molecule being transported across the monolayer during the incubation time (mM/min), V is the volume of the basolateral compartment (cm^3), A is the area of the membrane (cm^2), C0 is the initial concentration of the molecule in the apical compartment.

Before measuring SCFAs bioavailability, the impact of digested and fermented formulation on intestinal epithelium viability was assessed. To this aim, Caco-2 monolayers were exposed to increasing concentrations of the bio-accessible fraction of PHGG+SM fermentation, diluted with the Blank supernatant containing the amount of SCFAs physiologically present during the digestive process, and dose-response curves were obtained.

Statistical analysis

All data are presented as mean±Standard Deviation (SD) of three independent experiments. In addition, a T-test analysis was performed to determine if statistically significant differences between treatments were present. The T-test is a statistical method used to test differences between two means. The differences between groups are considered significant at $p<0.05$. All statistical analyses were performed with OriginLab software.

Results

SCFAs release following PHGG+SM digestion and fermentation – "bio-accessible fractions"

Bio-accessibility refers to the amount of active principle released or produced from its matrix in the gastrointestinal tract and available for absorption. In this case, acetic, propionic and butyric acids are produced during dietary fiber bacterial fermentation at the large intestinal level. For this reason, the SCFAs bio-accessibility was measured at the end of the fermentation phase. As indicated in Figure 3, a significant increase in SCFAs content is observed following PHGG+SM fermentation compared to the non-treated condition, indicating that this medical device is effectively fermented at the large intestine level. Following PHGG+SM fermentation, acetic and propionic acids are the more abundant SFCAs (about 56 % and 12 %) (Figure 3A and 3B) with a larger increase compared to digestive fluids (1.6 and 1.9 PHGG+SM /DF ratio). The butyric acid fold-change is 1.1 (about 6 and 7 mg in DF and PHGG+SM, respectively) (Figure 3A and 3B), though the increase is still significant when compared to non-treated condition.

Impact of digested formulations on intestinal epithelium viability

As shown in Figure 4 the bio-accessible fraction of PHGG+SM fermentation significantly decreases the viability of Caco-2 monolayers starting from a 50 % bio-accessible fraction concentration (Caco-2 monolayer viability<70 %) (Figure 4).

The PHGG+SM bio-accessible fraction concentration significantly alters the intestinal epithelium permeability from 25%, as highlighted in Figure 5. The first concentration that allows for a significant recovery of the intestinal epithelium barrier integrity is 17% (about 120%). Taking into consideration that SCFAs absorption could be due to active and/or passive mechanisms, intestinal mucosa integrity is highly recommended. Although no impact on intestinal epithelium viability was observed at 25%, intestinal epithelia were exposed to a PHGG+SM fermentation bio-accessible fraction concentration of 17% to guarantee intestinal mucosa integrity.

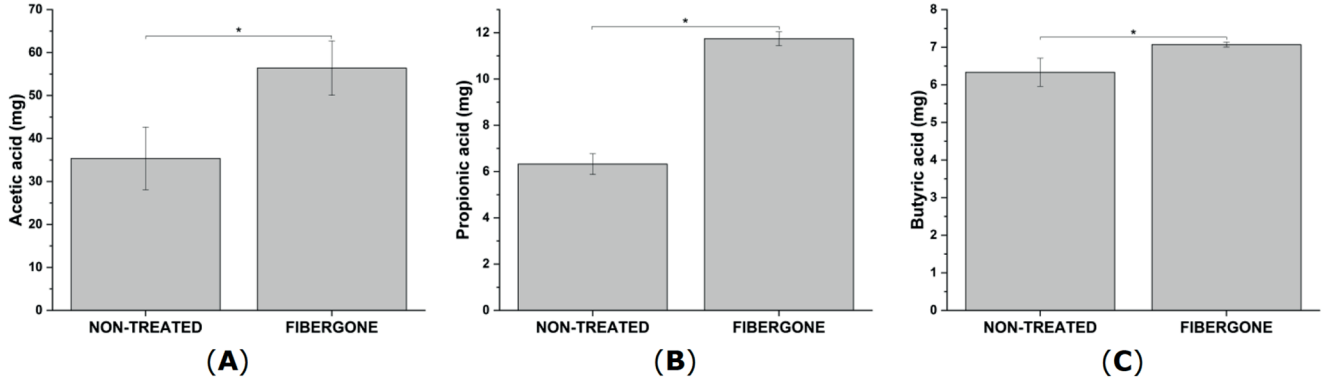

Figure 3. Bio-accessibility of acetic acid (A), propionic acid (B) and butyric acid (C), expressed as amount (mg), following fermentation of non-treated and Partially-Hydrolyzed Guar Gum plus Simethicone (PHGG/SM) digestions. *p<0.05.

Intestinal absorption of SCFAs following PHGG+SM fermentation

Table 3 reports the amount (µg) of each SCFAs contained in the diluted bio-accessible fraction of the fermented digest in the presence and absence of PHGG+SM in the apical compartment at the beginning of the absorption experiment. The presence of PHGG+SM did increase the absorption of acetic acid, propionic acid, and butyric acid; the mean increase rate was about 10%.

As shown in Figure 6A, a significantly higher amount of acetic acid is absorbed at the intestinal level than the other considered SCFAs (*i.e.*, propionic and butyric acids). The absorption trend is reversed when the percentage absorption is considered, since the percentage of propionic and butyric acids absorption is significantly

Table 3. Amount of short chain fatty acids (SCFAs), including acetic, propionic and butyric acids) added in the apical compartment at the beginning of the intestinal absorption experiment. The results are expressed as mean±standard deviation.

SCFAs	Non-treated µg	PHGG+SM µg
Acetic acid	486.9±11.0	548.3±59.8
Propionic acid	110.5±1.8	127.1±3.1
Butyric acid	112.8±1.3	132.0±0.8

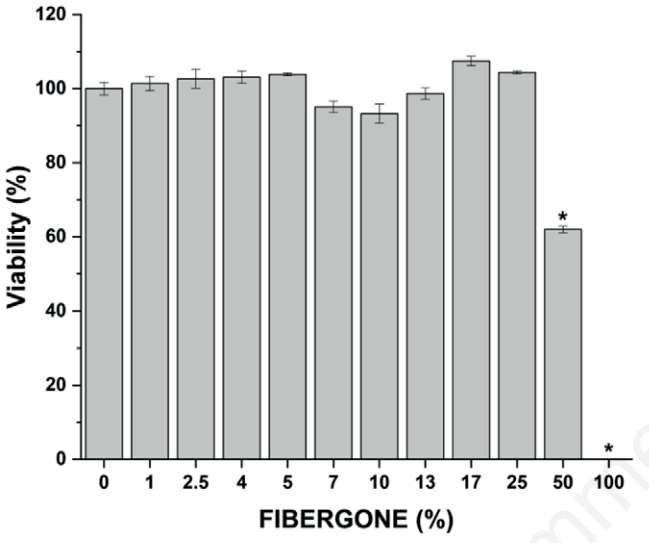

Figure 4. Impact of Partially-Hydrolyzed Guar Gum plus Simethicone (PHGG/SM)increasing bio-accessible fraction concentrations on intestinal mucosa viability, evaluated by Methylthiazol Tetrazolium Salts (MTS)assay. *<70% viability.

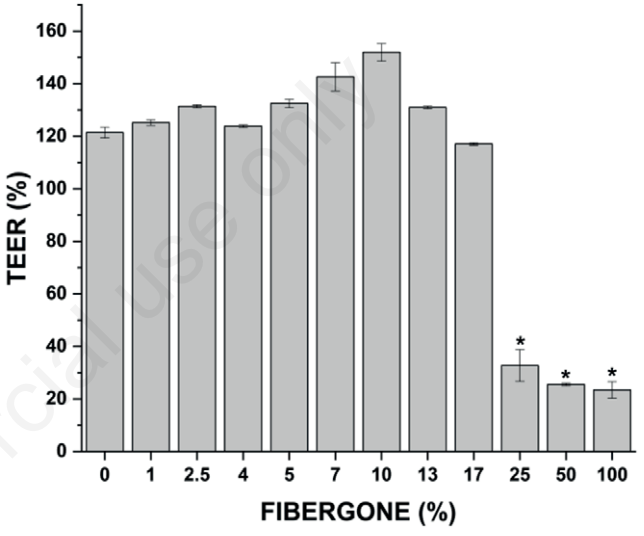

Figure 5. Intestinal epithelium model barrier integrity recovery, following 3 h exposure to increasing concentration of Partially-Hydrolyzed Guar Gum plus Simethicone (PHGG/SM) fermentation bio-accessible fraction and 24 h recovery. Data are expressed as % of Trans-Epithelial Electrical Resistance (TEER). *p<0.05.

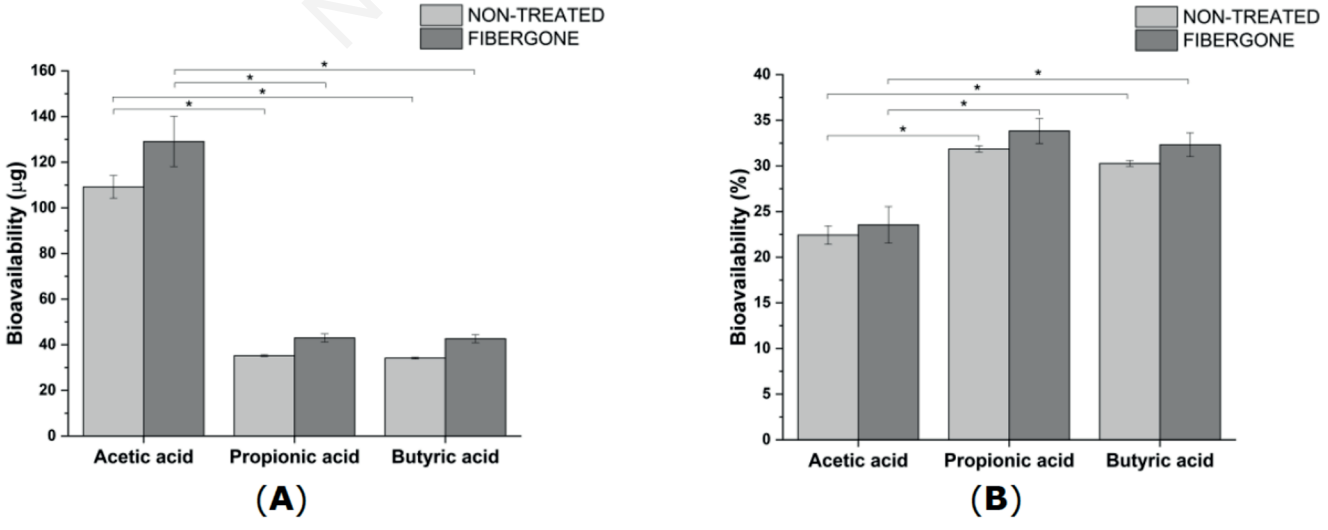

Figure 6. Short Chain Fatty Acids (SCFAs) bioavailability, expressed as amount (µg) (A) and percentage (%) (B).

higher than that of acetic acid (Figure 6B and Table 6). However, no statistically significant differences in acetic, propionic, and butyric acid percentage intestinal absorption were observed between non-treated, and PHGG+SM exposed intestinal epithelia.

Impact of digested formulations on intestinal epithelium viability and integrity

Besides absorption evaluation, the impact of digested and fermented PHGG+SM on the intestinal epithelium model viability was monitored. As expected, no significant alterations in intestinal epithelium viability are observed (Figure 7A). As for the viability, no significant alteration of the intestinal epithelium apparent permeability (P_{app}) was highlighted following treatment with the digested formulation (Figure 7B). However, even if the result was not significant, the difference suggests a valuable effect.

As expected from its limited effect on the intestinal epithelium's apparent permeability, digested and fermented PHGG+SM reduced TEER only temporarily, and its values fully recovered within 24 h, following the same trend observed for the non-treated condition (Figure 8).

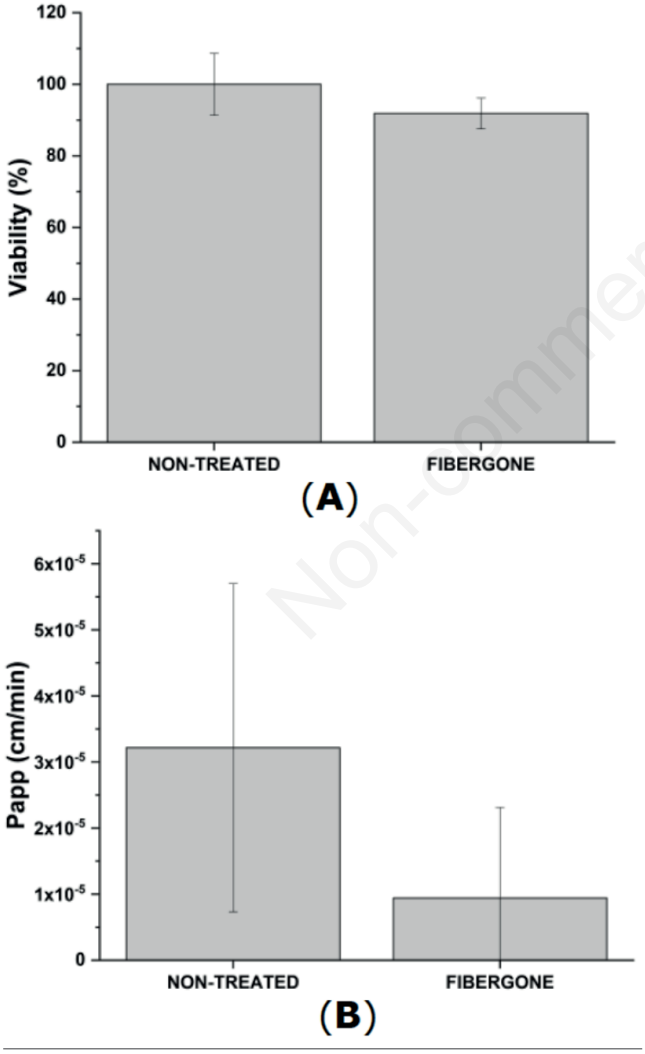

Figure 7. Intestinal epithelium viability (A) and apparent permeability (Papp) (B) following exposure to digestive fluids (DF; control) and diluted digested and fermented Partially-Hydrolyzed Guar Gum plus Simethicone (PHGG/SM) for 3 h.

Discussion

IBS is a medical condition characterized by symptom recurrence, such as symptomless periods alternated to symptom relapse. Several therapeutic options are available for patients with IBS, including psychotropic agents. However, IBS is not yet curable, as any treatment can change its natural history. Therefore, the duration of medical therapy is unpredictable, though it may be long-lasting in most patients. As a result, also important adverse reactions may emerge over time.

For this reason, alternative and complementary treatments are welcome in IBS management. In this regard, the new medical device containing PHGG and SM could represent an attractive option due to its mechanisms of action. SM is an anti-foaming agent that successfully reduces intestinal gas overproduction so, relieving pain, bloating, and flatulence.[18] Simethicone is a silicone compound that acts as a non-systemic surfactant, decreasing the surface tension of gas bubbles present in the gastrointestinal tract. Simethicone induces coalescence and dispersion of the gas bubbles allowing their removal from the digestive tract as flatulence or belching. Simethicone causes the gas bubbles to accumulate and therefore pass more easily either through the upper digestive or lower digestive opening. Simethicone does not appear to reduce the actual production of intestinal gas.

PHGG exerts multifaced effects in IBS such as regulation of bowel movements, role as prebiotic, improvement of stool characteristics, and increase of SCFA production.[19-22] In particular, SCFAs, mostly butyrate, enhance the growth of Lactobacilli and Bifidobacteria and play a central role in the physiology and metabolism of the colon. SCFAs effect on cell proliferation, differentiation, apoptosis, mucin production, immune function, mineral absorption, lipid metabolism, and gastrointestinal peptides has been well documented.[23] Therefore, adequate SCFAs quantity is also necessary for IBS patients.

The present study served as proof of the concept regarding the possible impact of a new medical device containing PHGG and SM on SCFAs metabolism. Moreover, this study was the first and, presently, the only research concerning the *in vitro* effects

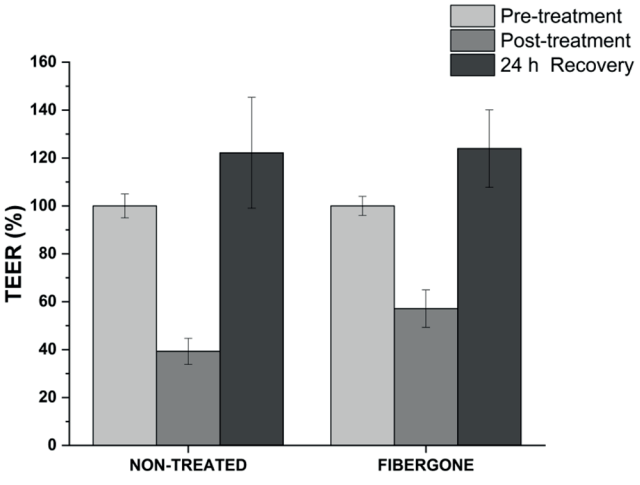

Figure 8. Trans-Epithelial Electrical Resistance (TEER) trend following 3 h exposure to digested and fermented Partially-Hydrolyzed Guar Gum plus Simethicone (PHGG/SM).

provided by this compound. As a result, there is the need that other studies should be conducted to confirm and expand the obtained outcomes.

The results from this *in vitro* study demonstrated that the two components, PHGG and SM in a well-defined ratio, lead to interesting outcomes. The findings suggest that this combination may be effectively fermented at the large intestine level, inducing a significant increase in the SCFAs. Among them, acetic acid was the most produced, though its relative absorption is lesser than propionic and butyric acids. Following *in vitro* digestion and fermentation, no significant differences in the intestinal absorption of acetic, propionic, and butyric acids are observed between blank- and PHGG+SM-treated epithelia.

Although the use of an *in vitro* model could be limited by the absence of other physiological mediators involved in SCFAs formation and absorption, it provides a good model to predict what occurs *in vivo*. Therefore, further studies by using clinical settings should be conducted to confirm these findings. In this regard, two very recent studies explored the effectiveness of this compound in patients with IBS. The first study included a large group of Greek gastroenterologists.[24] The study recruited 1190 patients with IBS who took the compound (PHGG 5 g and simethicone 100 mg) one sachet daily for three months). A questionary evaluated IBS symptoms (abdominal pain, distention, flatulence, constipation, diarrhea, urgent bowel movement, and incomplete bowel movement) scored on a seven-level scale. Patients were evaluated at baseline and after 4, 8, and 12 weeks.

The medical device significantly reduced all symptoms ($p<0.001$). Constipation and gas-related symptoms (flatulence, distention, and abdominal pain) were the IBS complaints that obtained the highest reduction. The treatment was well tolerated and received high compliance (more than three-quarters took the product for more than 90 days as satisfied).

The second clinical study was conducted in Italy.[25] Fifty patients with IBS randomly took this medical device as add-on therapy (in addition to standard treatment for IBS: diet and appropriate medications) or only the same standard IBS treatment. The add-on therapy lasted 8 weeks. The Visual Analogue Scale (VAS) measured the perception of symptom severity (abdominal pain, distention, flatulence, constipation, diarrhea, and incomplete bowel movement). The medical device significantly reduced abdominal pain, distension, and flatulence in comparison with standard therapy alone ($p<0.001$, 0.042, and <0.01, respectively) after 8 weeks. Interestingly, abdominal distension was significantly improved just after 4 weeks ($p=0.031$). The treatment was also well tolerated.

Prospectively, well-designed studies, such as randomized and controlled, should be performed to confirm these preliminary experiences and to better define the mechanism of action *in vivo*. In particular, appropriate studies should investigate the SCFA metabolism change after ingestion of PHGG-SM and effects on intestinal microbiota. Namely, the produced SCFAs could exert these benefits in managing patients with intestinal disorders.

Conclusions

This *in vitro* study documented the partial hydrolysis of Guar gum combined with simethicone by intestinal microbiota, with the subsequent formation of SCFAs, such as acetic, propionic and butyric acids.

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