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Impact of food additives on human gut microbiota and intestinal inflammation

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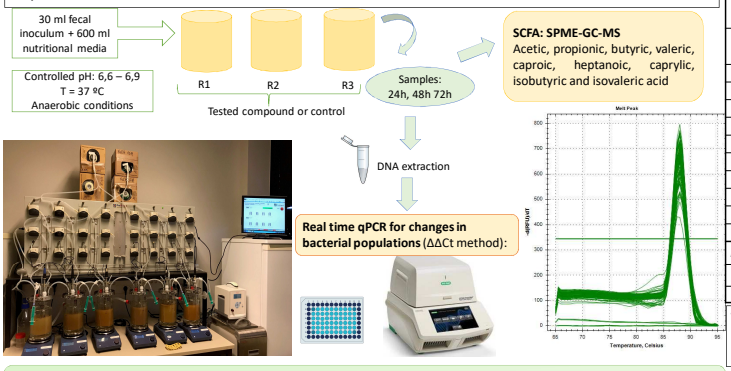
Introduction

Some food additives have been related with adverse effects on health by the onset of microbial dysbiosis in animal models. The effects of food additives on human intestinal microbiota composition and function are less known. Accumulating evidence demonstrates a contribution of dietary emulsifiers in the increase of prevalence of diseases associated with intestinal inflammation, such as inflammatory bowel disease (IBD; Naimi et al., 2021). Two emulsifiers, polysorbate 80 and carboxymethylcellulose, profoundly impact intestinal microbiota leading to gut inflammation (Frolkis et al., 2013; Maaser et al., 2017; Chassaing et al., 2017; Naimi et al., 2021). A study carried out in mice demonstrated that some mucolytic bacteria, such as *Mucispirillum schaedleri, Ruminococcus* and *Anaeroplasma* have been directly correlated with fibrosis induction while other bacteria such as *Oscillospira* and *Coprococcus* were negatively correlated with it (Jacob *et al.,* 2018).

The aim of this work is to determine how food additives influence intestinal microbiota toward a dysbiosis, inflammation, and finally the subsequent formation of fibrosis in the context of IBD.

Materials and Methods

Six common food additives (polysorbate 80, titanium dioxide, sucralose sodium nitrite, maltodextrin and carrageenan) were tested *in vitro* using batch culture models of intestinal microbiota, using the SHIME ® system to simulate the intestinal microbiota inhabiting the human colon for 72 hours. The tested concentrations for the additives, were based on the acceptable daily intake (ADI) or on the estimated daily exposure, assuming in both cases an average body weight of 70 kg. A pool of fecal samples from five healthy donors was used to inoculate the system with the microbiota. Changes in microbiota were assessed every 24h using qPCR methods targeting bacterial groups involved in short chain fatty acid (SCFA) production or in inflammation. In addition, the SCFA production was assessed using SPME-GC/MS .

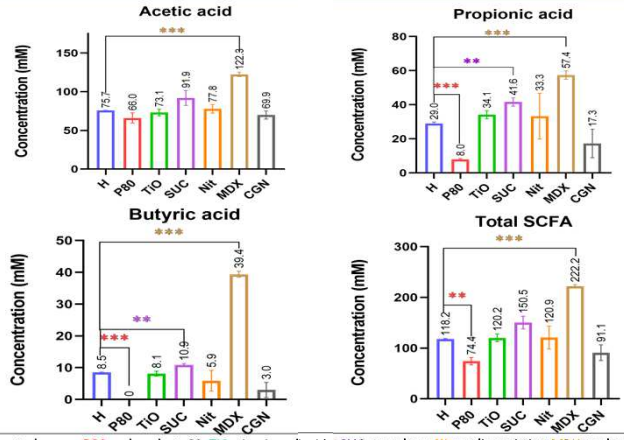


Results – Microbial changes

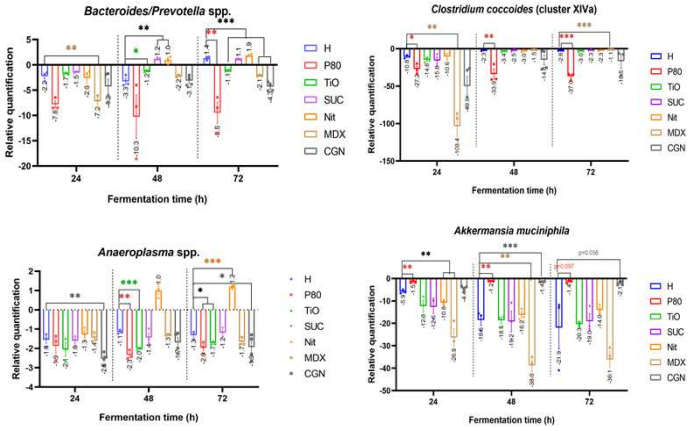
Treatment	Polysorbate 80			Titanium dioxide			Sucralose			Sodium nitrite			Maltodextrin			Carrageenan		
Fermentation time (h)	**24h**	**48h**	**72h**	**24h**	**48h**	**72h**	**24h**	**48h**	**72h**	**24h**	**48h**	**72h**	**24h**	**48h**	**72h**	**24h**	**48h**	**72h**
Firmicutes																		
Bacteroidetes																		
Actinobacteria																		
γ - Proteobacteria																		
Bifidobacterium spp.																		
Bacteroides/Prevotella spp.																		
Streptococcus spp.																		
Enterococcus spp.																		
Clostridium coccoides (cluster XIVa)																		
Clostridium leptum (cluster IV)																		
Oscillospira spp.																		
Anaeroplasma spp.																		
Coprococcus spp.																		
Ruminococcus spp.																		
Fusobacterium spp.																		
Lactobacillus spp.																		
Bilophila spp.																		
Escherichia/Shigella spp.																		
Butyryl Coenzime A																		
Butyrate kinase																		
Faecalibacterium prausnitzii																		
Akkermansia muciniphila																		
Mucispirillum schaedleri																		

Table 1: Relative changes observed using qPCR (ΔΔ method) regarding specific bacterial groups. The effect of food additives was compared with control group (healthy donors – no additives added). refers to a decreasing or increasing trend respectively (0,05 < p < 0,1). means significative decrease or increase (p < 0,05).

Results – SCFA production after 72h of fermentation



H: control group; P80: polysorbate 80; TiO: titanium dioxide; SUC: sucralose; Nit: sodium nitrite; MDX: maltodextrin; CGN: carrageenan (*) $p < 0,05$; (**) $p < 0,01$ and (***) $p < 0,001$. No significant differences were observed regarding the production of branched – chain fatty acids (BCFA)



Impact of food additives on the relative quantity of *Bacteroides/Prevotella* spp., *Clostridium coccoides, Anaeroplasma* spp. and *Akkermansia muciniphila* after 24, 48 and 72h of anaerobic fermentation. **Legend**: H: control group; P80: polysorbate 80; TiO: titanium dioxide; SUC: sucralose; Nit: sodium nitrite; MDX: maltodextrin; CGN: carrageenan. (*) $p < 0,05$; (**) $p < 0,01$ and (***) $p < 0,001$.

Discussion and Conclusion

At the end of the fermentation period (72h), the most relevant changes were for polysorbate 80, who dramatically decreased butyrate and propionate production. This was consistent with qPCR results, where significant decreases of *Clostridium cluster XIVa* (butyrate producing bacteria) and *Bacteroides/Prevotella* spp. (propionate producing bacteria) were observed. Maltodextrin increased acetic, propionic and butyric acids production as well as the total SCFA production, this could be explained by the increase of several bacterial groups such as *Bifidobacterium, Streptococcus* and *Enterococcus* and the preservation of *Clostridium cluster XIVa* species. Sucralose significantly increased the production of propionic and butyric acid. The addition of sodium nitrite induced an increase in the quantity of *Anaeroplasma* genus, which have been linked with increased intestinal fibrosis. However, this compound promoted the growth of *Coprococcus* spp. that have been associated with reduced fibrosis. The growth of *Enterococcus* genus was higher than the control group with all additives tested. It is interesting to note that this genus has been associated with intestinal inflammation. The genus *Escherichia / Shigella* has been detected in higher proportions in patients with ulcerative colitis. In this study, the addition of titanium dioxide, sucralose, sodium nitrite and carrageenan promoted its growth. Some members of *Bacteroides* genus have been negatively associated with fibrosis. The use of polysorbate 80, titanium dioxide, maltodextrin and carrageenan decreased the relative quantity of *Bacteroides / Prevotella* members showing a negative impact of these compounds.

In conclusion, the use of some food additives could enhance the growth of bacterial groups considered deleterious for human health or impact negatively some bacterial species known as health promotors and increase the risk of inflammatory bowel diseases or intestinal fibrosis.

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