Could diet link to changes in gut microbiota? By E. Özcan[1,2], Ö. Güçlü-Üstündağ[1] and F.Y. Ekinci[1], *[1]Yeditepe University, Faculty of Architecture and Engineering, Food Engineering Department, Istanbul, Turkey, [2]University of Massachusetts Amherst, Department of Food Science, Amherst, MA.*

INTRODUCTION

The human gut is a highly colonized part of the human digestive system with diverse bacterial metabolic activity linked to regional and diet differences, which might originate from dietary compounds, including phenolic compounds[1,2]. Phenolic compounds are known for their abundance in many fruits, vegetables and beverages, and their bioactivity in human body[3]. Gut microbiota may cause changes in the chemical structure of phenolic compounds and produce microbial metabolites, which might have an influence on the composition of gut microbiota. The aim of this study was to investigate whether there are any changes in the phenolic composition of black tea extract induced by gut microbiota in *in vitro* gut fermentations.

The results of this study showed that human gut microflora have a potent capacity to metabolize black tea phenolics and increase their digestion by breaking them into more easily absorbed phenolic compounds such as pyrogallol, pyrocatechol, 4-hydroxyphenylacetic acid and 3-(3-hydroxyphenyl)propionic acid. At the end of the fermentation, there was a slight increase in the growth of *Lactobacillus* spp., and *Clostridium* spp. and decrease in the *Enterobacteria* and coliform organisms, but these changes were not significant ($p>0.05$) compared to control. Although this study provided valuable information about the interaction of black tea phenolics and human colonic microflora *in vitro*, culture independent techniques such as FISH and qPCR are needed to confirm the microflora profile at certain time points.

METHODS

In vitro fermentor cultures

Fecal fermentation was performed with fecal samples freshly collected from one healthy male volunteer in 135 mL of sterile basal medium[4] in 300 mL glass vessels at controlled pH conditions (pH 6.8) with slow agitation and N_2 sparging for 48 h at 37 °C. To observe the interaction of black tea and microbiota, freeze-dried black tea extract was inoculated into the fermentor vessels at a concentration of 3125 mg/L, yielding a final phenolic concentration of 1000 mg/L (R1) and in basal medium in without addition of black tea extract (R2) as control. The fermentations were performed in triplicate. Samples were taken at 7 time points (0, 4, 8, 10, 24, 30, 48 h) for analysis of microbial populations and phenolic compounds.

Plate Counting

To observe the changes of gut microbiota during 48 h under anaerobic conditions and the diet effect on bacterial community, the population of bacterial groups in fermentor cultures, collected from at time 0 and 48 h, was determined by plating on selective culture media as described previously[5].

GC-MS

The phenolic metabolite profiles of fermentor cultures were determined using GC-MS as described previously[6,7]. Briefly, 0.5 mL sample was centrifuged, mixed with the internal standard, acidified, and then extracted three times with ethyl acetate. The dried extracts were

derivatized with BSTFA and 10% TMCS and mixed with n-hexane prior to GC-MS analysis. Derivatized samples were analyzed using VF-5MS column (30 m x 0.25 mm i.d, coated with 5% Phenyl Methylpolysiloxane (film thickness: 0.1 µm) (Varian, Agilent Technologies, Netherlands)) on a Thermo ISQ mass spectrometer (Thermo Scientific, UK) equipped with a Thermo Model Trace GC Ultra gas chromatograph. Identification of the phenolic metabolites was carried out by comparing their ion spectrum with the NIST library and with that of targeted standards. Quantification was done using calibration standards.

Statistical Analysis

Data were subjected to a paired Student's t test for bacterial enumeration and repeated measure analysis of variance to observe the metabolite changes with time using Minitab 16.

RESULTS AND DISCUSSION

Plate Counting

The changes in bacterial community by black tea addition (R1) did not reveal conclusive effects (Figure 1). When compared to control (R2), after 48 h fermentation, the growth of total aerobes, *Lactobacillus* spp. and *Clostridia* group showed a slight increase upon black tea extract addition. On the other hand, a slight suppression was observed on the growth of total anaerobes, *Enterobacteria* and coliform. However, *Staphylococcus aureus* was not detected in either culture (R1 and R2). The changes in the bacterial groups were not significant when compared to control group ($p>0.05$). Contradictory results on the community changes in the presence of either individual phenolics or black tea extracts were reported in literature[8,9]. Black tea phenolics (catechin and epicatechin) and their metabolites (4-hydroxyphenylacetic acid) positively affected the growth of commensal *Clostridium* spp., *Bifidobacterium* spp. and *Lactobacillus* spp. but not pathogenic *Clostridium* spp.[8]. However, on the other hand, plate counting did not show conclusive evidence about the effects of phenolics on bacterial numbers in the intestine model whereas clear trends could be observed using culture independent methods[9].

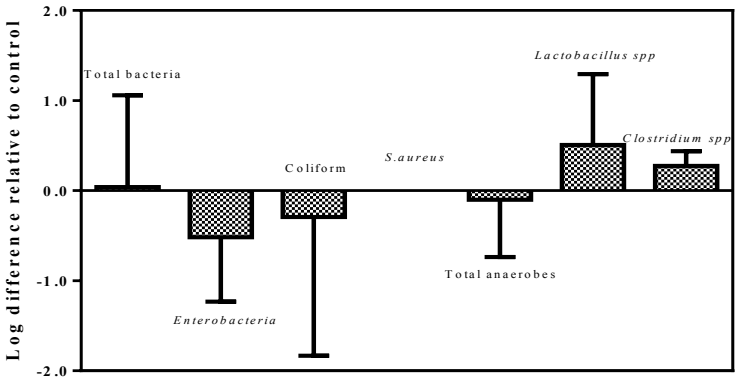

Figure 1. Selective enumeration of microorganism by plate counting. Changes in bacterial growth were calculated by comparing the number of a specific bacterial group in the fecal fermented sample with the number found in a control group, at 48th h.

GC-MS

GC-MS analysis of the fermentation samples of black tea extracts with fecal slurries (R1) showed mainly the appearance of pyrocatechol, pyrogallol, 4-hydroxyphenylacetic acid, and 3-(3-hydroxyphenyl) propionic acid. As stated in previous studies[10,11], pyrogallol and pyrocatechol are the main fermentation products of gallic acid and gallated catechins (EGCG, ECG) and theaflavins. Pyrocatechol and pyrogallol were initially absent at fermentor cultures, but they appeared at the 4th h and reached their highest concentrations (3.02 µg/mL and 95.45 µg/mL, respectively) after 24 h of incubation. While pyrocatechol concentration did not change after 24th h (from 3.02 to 2.77 µg/mL) ($p>0.05$) (Figure 2.a), pyrogallol decreased consistently until the end of incubation (17.09 µg/mL) (Figure 2.b), suggesting that pyrogallol might have also been utilized later in fermentation by gut microflora. 4-hydroxyphenylacetic acid and 3-(3-hydroxyphenyl)propionic acid, previously reported as the main metabolites of epicatechins, were initially present in all fecal slurries[11] and their concentration increased gradually during 48 h of fermentation (14.84 and 22.90 µg/mL, respectively) (Figure 2.c and Figure 2.d). In R2 samples, 4-hydroxyphenylacetic acid and 3-(3-hydroxyphenyl)propionic acid were also observed at 0th h, however, their concentration did not change over incubation period.

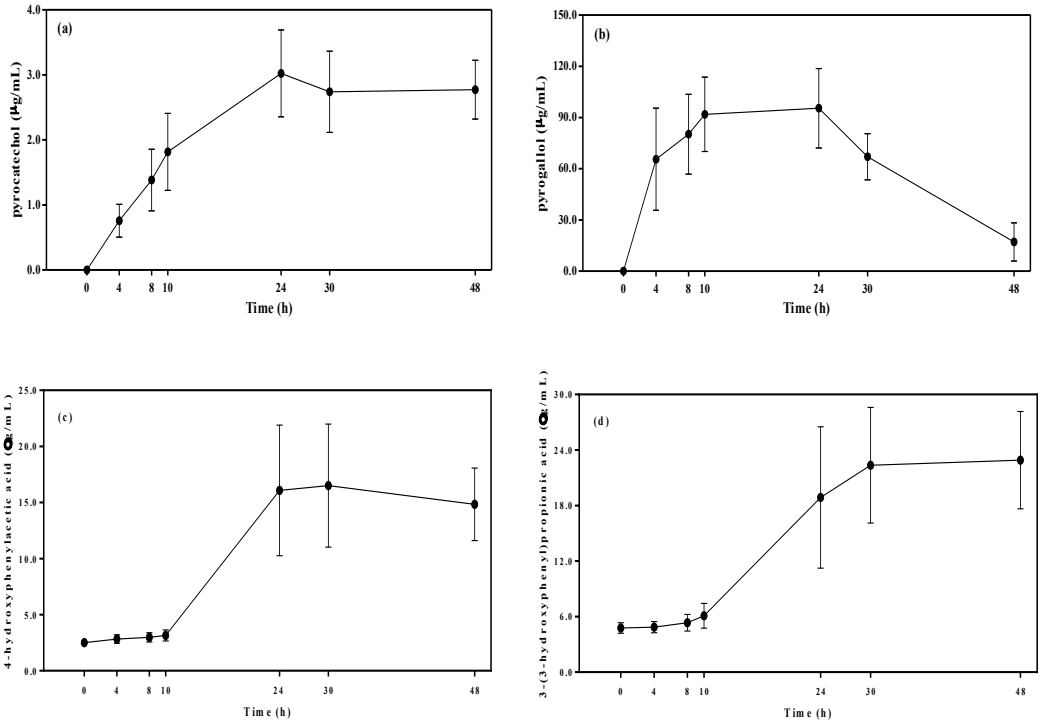

Figure 2: The composition of metabolites, pyrocatechol (a), pyrogallol (b), 4-hydroxyphenylacetetic acid (c), 3-(3-hydroxyphenylpropionic acid (d), of fecal fermentation of black tea during 48 hour period determined using GC-MS. Data are expressed as mean±SD of three individual fermentations (n=3).

In the study of Gao et al[12], the fate of phenolic precursors, especially catechins in black tea resulted in the production of 3-methoxy-4-hydroxyphenylacetic acid and smaller amounts of 4-hydroxyphenyl acetic acid, 3-3-hydroxyphenyl propionic acid, 3,4-dihydroxyphenyl acetic acid and 2,4,6-trihydroxybenzoic acid. Moreover, these results are in agreement with the findings in the gut fermentation studies performed with green tea and theaflavins, however the variance in the concentration and the appearance rate of each metabolite can be attributed to difference in the microbial communities of fecal for each donor[7, 11].

CONCLUSION

Although plate counting did not reveal conclusive effects on the changes and maintenance of microbiota by diet, GC-MS data showed that intestinal bacteria can metabolize black tea phenolics and break them into more easily absorbed phenolic compounds. Since bacterial enumeration was performed at the beginning and end of fermentations and applied only for the culturable cells, the changes in the bacterial communities during incubation period at specific time points could not be observed. Therefore, to observe the changes of the communities during fermentation, bacterial enumeration using culture independent methods such as FISH and qPCR need to be performed.

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