How do green and black coffee brews and bioactive interaction with gut microbiome affect its health outcomes? Mining evidence from mechanistic studies, metagenomics and clinical trials

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Abstract

Background:

The gut microbiome has become a hot topic in recent years with increasing reports on the positive role of a well-balanced gut microbiota composition for one's health and well-being. A number of dietary factors can modulate gut composition, although few publications have focused on common daily beverages impact on the gut microbiome. Coffee is a worldwide beverage consumed mostly as black coffee that is originally derived from green coffee beans post roasting. To enhance the taste and aroma, green coffee is typically roasted and to further affect its chemical composition and rationalize for the different health outcomes. Roasted seeds contain a high caffeine levels versus phenolic acids *i.e.*, chlorogenic acid enrichment in green coffee suggestive that they interact differently with gut microbiota and to affect its metabolism.

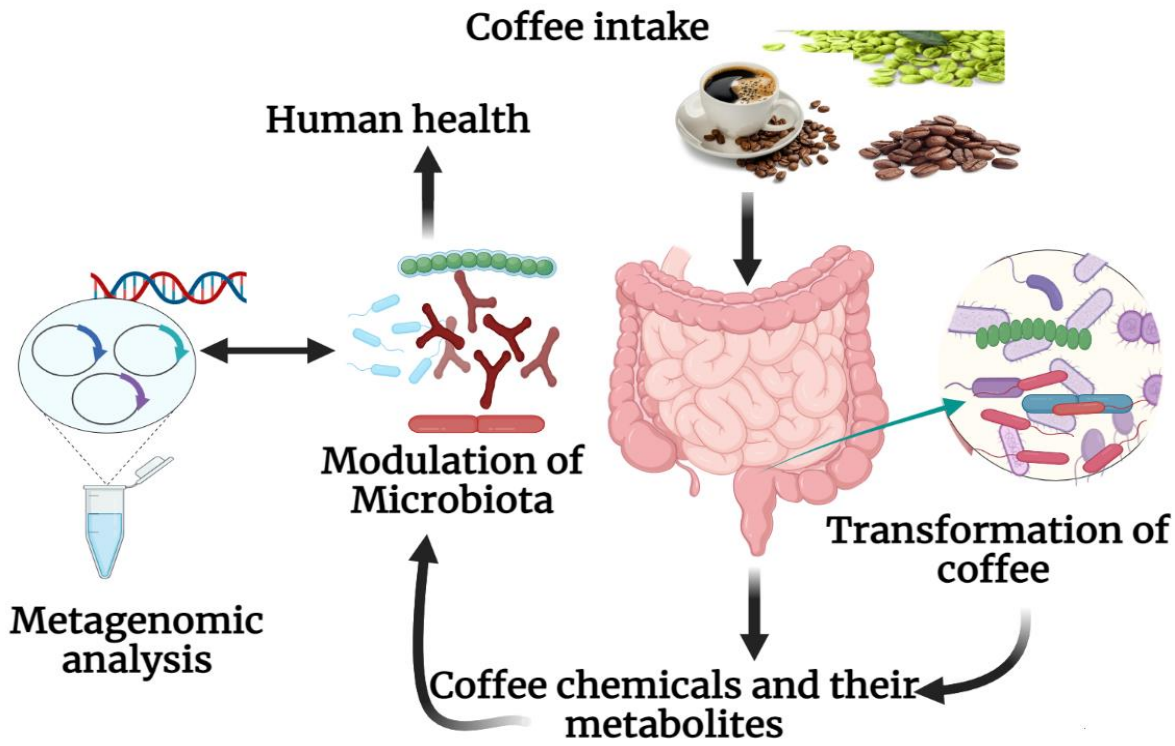
Scope and approach:

The present review provides a mechanistic insight on the effects of black and green coffee chemicals on the gut microbiome. We present herein the first comprehensive review of how coffee natural bioactive such as caffeine and chlorogenic acid and its process derived chemicals *i.e.*, melanoidins can specifically influence gut homeostasis, and likewise *via* gut microbiota-mediated coffee chemicals metabolism.

Key findings and conclusions:

The role of gut microbiota in affecting coffee chemicals and the potential of mining metagenomics data to uncover gut microbiome community and carbohydrate active enzyme (CAZyme) profile associated with coffee consumption are presented for the first time. Moreover, our metagenomics analysis *in silico* showed a decrease in abundance in either *Desulfofarcimen* or *Mycoplasma* genera, confirmed the basic coffee-gut microbial enzymes repertoire found in the literature and highlights for the first time the coffee CAZyme biomarkers encoded by the human gut microbiome.

Keywords: gut microbiota; coffee; probiotic bacteria; biotransformation; metagenomics analysis; microbial CAZyme



1. Introduction

Coffee is one of the most frequently consumed beverages worldwide with a myriad of health benefits. Globally, almost 7 million tons of green coffee beans were produced in 2010, with 160 million packs produced in 2018 (Angeloni et al., 2020). *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee) are the two main types of coffee, Arabica coffee is a high-altitude species native to Ethiopia, Sudan, and northern Kenya, whereas Robusta coffee is a lowland plant native to tropical Africa west of the Rift Valley (Davis et al., 2011).

Green coffee beans *i.e.*, the raw material used to make roasted coffee and coffee drinks are obtained from the seeds of coffee cherries following a series of operations to remove the outer layers (the skin, pulp, mucilage, and parchment), followed by drying to a final water content of 10.0-12.0 %. (de Melo Pereira et al., 2019). Roasted coffee accounts for approximately 80% of global coffee consumption, while the remaining 20% is unclear. For example, roasted Robusta is widely used for instant coffee production due to its high extractability of soluble solids, such as carbohydrates, followed by soluble proteins, melanoidins, caffeine, and chlorogenic acids. (Moeenfard & Alves, 2020), as evidenced by **(Fig. 1).**

Levels of nutrients and bioactive chemicals in green coffee range from (55.0-65.5%) in carbohydrates, lipids (10.0-18.0%), nitrogen-containing compounds (11.0-15.0%), purine alkaloids (0.8-4.0%), chlorogenic acids (6.7- 9.2%) and minerals (3.0-5.4%). Other compounds are found at low level include non-volatile aliphatic acids (citric, malic and quinic acids) and phenols (Mussatto, Machado, et al., 2011). The principle reason associated with the high coffee consumption rate lies in the different types of coffee preparation that satisfy the various customers need, with regards to its sensorial properties and moreover physiological effects (Higdon & Frei, 2006). Physiological effects in black and green coffee is mostly ascribed to its antioxidants and bioactive compounds such as caffeine, chlorogenic acids, nicotinic acid, tannic acid, trigonelline, and pyrogallic acid (Xu et al., 2019). Compared to black coffee, green coffee represents a rich source of chlorogenic acids (CGAs), with much higher CGAs levels than other resources as potatoes, apples, and chines parsley. The major antioxidants of CGAs in coffee beans may be classified into three categories based on their phytochemical properties: caffeoylquinic acids (CQA), feruloylquinic acids (FQA), and dicaffeoylquinic acids (diCQA) (Xu et al., 2019).

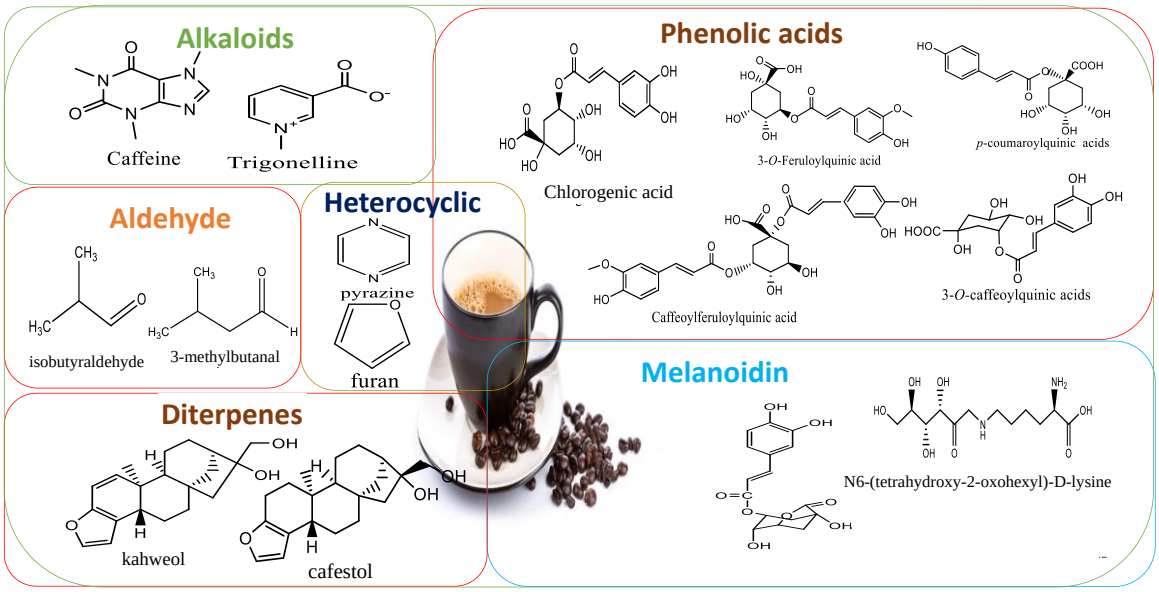


Fig. 1 Chemical structures of the major bioactive classes detected in green and black coffee.

Roasting is one of the most complex and significant phases of the coffee production chain and to affect green coffee composition dramatically. During the roasting process, many

chemical reactions, such as hydrolysis, polymerization and pyrolysis contribute to changes in sensorial and biological effects (Leme et al., 2019). Colorful compounds, such as melanoidins, which are dark and have a high molecular weight, are produced during the roasting process as a result of the complex chemical transformations of the Maillard and the caramelization reactions. Further, during roasting process chlolrogenic acids are partially hydrolyzed and then incorporated into coffee melanoidins through non-covalent or covalent bounds, and leading to reduced levels compared to green beans (Ludwig et al., 2012).

Based on the level of coffee roasting and the extraction process, these antioxidants are known to be degraded and engaged in the chemical transformations to produce diverse CGAs derivatives to impart a distinct flavor, quality, and bioactivities of black coffee (Xu et al., 2019). Numerous investigations have demonstrated asides from coffee central nervous system (CNS) stimulant action, that increased coffee consumption has various favorable impacts on liver disorders, clinical type 2 diabetes, and Parkinson's disease. Nevertheless, the impact of coffee chemicals on the gut microbiota has not been well investigated, and warrants for more future work to identify the true repertoire of coffee consumption gut microbiome interaction and how it mediates further for coffee systematic health effects.

Metagenomics studies on the gut metagenome have provided key insights of commensal microbial communities and their functional catalogue that allow us knowing how to manipulate the structure and functions of our microbiota, how they affect the health and function of their hosts and how we could improve human health through prevention and treatment of diseases. High-throughput functional metagenomics screening is used to encode carbo-hydrate-active enzymes (CAZymes) through identifying genes in the human gut microbiome (Prakash & Taylor, 2012; Ufarté et al., 2016). Enzymes mining *in silico* from microbiome gut metagenomes has been employed widely to highlight the abundance and variety of microbial CAZymes associated to different diet consumptions such as yoghurt (Roy et al., 2020), milk and solid food (Ye et al., 2019). However, the relationship between coffee intake and the composition of the gut microbiota has yet to be studied in *silico*. This study presents a pipeline of four steps to gain more detailed information about gut microbial functional compositional changes and their associated impact related to coffee consumption.

2. Microbiota and gut homeostasis

The gut microbiota of higher animals show large variation and exceedingly active, with approximately 10 trillion microbial cells and 1000 microbial strains (Sommer & Bäckhed, 2013). Metagenomics studies have revealed that the human body encompasses genetic material is 90% of microbial origin and 10% human. The human gastrointestinal tract contains at least 10^{12} microorganisms/ml of luminal content and *ca*. 15,000 bacterial species. This microorganisms are called gut microbiota, established during the first year of life and is strongly influenced by external factors, including the mode of birth (natural or caesarean), early postnatal nutrition (breastfeeding or nutritional formulas), GI infections (bacteria and parasites), the use of antibiotics, and diet (Pimentel et al., 2013).

Bacteroidetes, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* are the four primary phyla of microorganisms that make up the human microbiota. *Firmicutes* and *Bacteroidetes* account for more than 90% of the relative abundance of the gut microbiome, and their connection is critical for gut homeostasis, whereas *Actinobacteria* and *Proteobacteria* account for the remaining 10%. (Binda et al., 2018). Inside the human body, gut microbiota plays a pivotal role by competitive inhibition of pathogens, as it sustains good intestinal health, energy production from nutrient biotransformation (Bäckhed & Crawford, 2010), regulation of lipid metabolism, metabolism of vitamins and absorption (Younes et al., 2001). Asides gut microbiota improve the intestinal immune system from childbirths, and regulate the growth of the intestinal mucosa (Lee et al., 2015).

Gut microbiota affects the physiology of the hosts ranging from energy metabolism to immunological responses. Growing evidence suggests that changes in gut microbiota composition, often known as gut dysbiosis, have a role in the development of metabolic syndrome, including non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and diabetes mellitus, particularly in terms of inflammation linked with obesity. (Nishitsuji et al., 2018). Considering that, gut microbiota is very much influenced by dietary habits and environmental factors, alteration of the gut microbial community composition among humans occur leading to damage of the intestinal epithelial integrity and concurrent with many gastrointestinal diseases upon disruption. Recent research has linked coffee consumption to changes in gut microbiota composition. Additionally, an *in vitro* study found that CGAs significantly increased the number of beneficial bacteria such as *Bifidobacterium*

spp. and the *Clostridium coccoides-Eubacterium rectale* group (Younes et al., 2001; Bäckhed & Crawford, 2010).

Most studies have focused on the effect of CGAs on gut microbial composition, while less is known regarding the synergistic response of intestinal epithelial integrity and microbiota to CGAs. Moreover, studies analyzed fecal samples, which might not reflect the full intestinal or cecal microbiome scenario (Younes et al., 2001; Bäckhed & Crawford, 2010). Microbiota dysbiosis has been linked to metabolic dysregulation (e.g., obesity, inflammatory bowel disease (IBD)), disease risk factors (e.g., coronary heart disease), and even the etiology of various diseases (e.g., autism, cancer) (Moco et al., 2012). It is still unclear whether these etiologies cause differences in coffee gut microbiota interaction compared to normal individuals.

3. Coffee brews and bioactive impact on gut microbiota composition through *in vitro* and *in vivo* studies

The influence of coffee compounds on gut microbiota has been studied using a variety of techniques, including quantitative PCR with particular gene primers (qPCR), 16S rRNA gene sequencing with a universal 16S rRNA bacterial primer, and genome sequencing. Various experimental approaches have been used, including *in vitro* fecal fermentation, mice, mice inoculated with human microbiota, and humans. (Mansour et al., 2020).

Different studies have examined changes in the makeup of gut bacteria following coffee ingestion. These changes can occur by several mechanisms to include: the direct effect of caffeine that promotes gastroesophageal reflux, stimulation of the gall bladder contraction and colonic motor activity (Preda et al., 2019), or through an indirect action of various coffee metabolites on the intestinal habitat such as CGAs as well as other metabolites (Umemura et al., 2004; Wei et al., 2021). The influence of coffee chemicals on gut microbiota composition was revealed mostly based on *in vitro* studies or mechanistic studies using static batch fermentations with fecal slurries or less from clinical trials as illustrated in the next subsections.

3.1. Evidence from mechanistic studies

Due to the presence of oral bacteria within gut microbiota, changes in the oral microbiota may lead to a shift in the gut microbiota. (Pérez-Burillo, Mehta, et al., 2019). Based on the

similarities between tea and coffee, as both are caffeinated beverages, studies have shown that the intake of tea polyphenol compounds leads to gut dysbiosis. Inside the colon, *Clostridium perfringens* were found to be inhibited, while *Bifidobacterium spp.* showed increase based on counting the bacterial colonies from the fecal sample after tea polyphenol treatment, and further translated by in the production of SCFAs i.e., acetic and propionic acids (Delgado-Andrade et al., 2017). In contrast, no significant effect on the overall microbiota was observed with roasted or green coffee consumption (Pérez-Burillo, Mehta, et al., 2019) suggestive that phenolics account for such differential response as caffeine is abundant in both plants..

More recently, green coffee consumption was reported to lead to an increase in *Firmicutes* and *Actinobacteria* relative abundance, while a decrease in *Bacteroidetes* was detected at the phylum level **(Table 1)**. In addition, SCFAs producing bacteria, i.e., *Roseburia*, *Faecalibacterium*, *Eubacterium rectale* group, *Blautia*, *Coprococcus*, and *Bifidobacterium longum* showed an increase concurrent with a decrease in *Prevotella* that overall accounted for the increase in SCFA levels (Preda et al., 2019) Delgado-Andrade et al., 2017). Likewise, moderate consumption of coffee for 3 weeks in a healthy population was reported to increase *Bifidobacterium* (Sales et al., 2020), occasionally likewise linked to a decrease of pathogenic *Clostridium* and *Escherichia coli* (Vollmer et al., 2017; Benitez et al., 2019).

Consumption of coffee was associated with a decrease of *Clostridium* Cluster XI and *Bacteroides*/*Prevotella*, whereas other studies revealed increase of *Enterobacteriaceae.* For instance, coffee and galacto-oligosaccharide (GOS) consumption effect on human gut microbiota suggest for an antibiotic effect, with GOS content to significantly decrease *E. coli* and *Clostridium spp*. population, whereas increase in *Bifidobacterium spp*., was evident (Nakayama & Oishi, 2013) and in accordance with results of (Sales et al., 2020). Coffee may have a more significant role in human health through influencing the growth of some colon bacteria types. For instance, the increase of *Bifidobacterium spp.* growth may be involved in preventing colon cancer *via* inhibiting the growth of some colon cancer cells (Mills et al., 2015), as illustrated in **(Table 1)**.

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Table 1. The effects of coffee or coffee chemicals on microbiota in human studies

No.	Study design, treatment	Phylum	Genus	Detection methods	References
1	Human fecal samples, Nescafe ´Green Blend (80·8 mg CGAs) Nescafe´ Gold Blend (33·9 mg CGAs) Nescafe´ Original (33·8 mg CGAs)	↑ Actinobacteria ↑ Firmicutes	↑ *Bifidobacterium spp.* ↑ *Clostridium coccoides–Eubacterium rectale*	LC–MS, HPLC	(Mills et al., 2015)
2	Human fecal samples, chlorogenic acid, caffeic acid, rutin and quercetin	↑ Actinobacteria ↓ Bacteroides ↑ Firmicutes	↑ *Bifidobacterium longum* ↓ *Bacteroides thetaiotamicron* ↑ *Lactobacillus rhamnosus*	LC-MS, RT-PCR	(Stalmach et al., 2010)
3	Human fecal samples, green coffee		Non identified bacteria	HPLC- HR-MS/MS	(Farag, Hegazi, et al., 2020)
4	Human, coffee and CGAs	↑ Actinobacteria ↑ Firmicutes	↑ *Bifidobacterium sp.* ↑ *Clostridium coccoides* *Eubacterium rectale*	LC-MS, HPLC	(Tomas-Barberan et al., 2014)
5	Human fecal samples, coffee fibre (0.5 g), and inulin	↑ Bacteroides	**Coffee fibre & inulin** ↑ *Prevotella group,*	FISH-FC	(Gniechwitz et al., 2007)

			↑ *B. Fragilis* **Coffee fibre** ↑ *B. Vulgatus*		
6	Human fecal samples, MOS (0.5 g/ cup)	↑ Actinobacteria ↓ Firmicutes	↑ *Bifidobacterium sp.* ↓ *Lactobacillus sp.*	Anaerobic culture	(Umemura et al., 2004)
7	Human fecal samples, coffee, 3 cup/day for 3 weeks	↔ Bacteroidetes ↔ Firmicutes ↑ Actinobacteria	↑ *Bifidobacterium sp.,* ↑ *Lachnospira* ↑ *Roseburia* ↔ *Prevotella*	RT-PCR DGGE, FISH	(Jaquet et al., 2009)
8	Human fecal samples, 200 μM of C-QA	↓ Bacteroidetes ↑ Firmicutes ↑ Actinobacteria	↑ *Bifidobacterium spp.* ↓ *Bacteroides-Prevotella* ↑ *Lactobacillus spp*	HS-SPME, (GC–MS	(de Cosío-Barrón et al., 2020)
9	Human fecal samples, *C. arabica* aqueous extracts and *C. canephora*, probiotic bacteria,	↑ Firmicutes ↑ Actinobacteria ↓ Proteobacteria	↑ *Lactobacillus rhamnosus* ↑ *Lactobacillus acidophilus* ↑ *Bifidobacterium animalis subsp. lactis* ↑ *Bifidobacterium animalis* ↓ *Escherichia coli*	HPLC-DAD	(Benitez et al., 2019)

10	Human fecal samples, spent coffee	↑ Bacteroidetes ↑ Firmicutes ↓ Actinobacteria ↓ F/B	↑ *Barnesiella* ↑ *Odoribacter* ↑ *Coprococcus* ↑ *Butyricicoccus* ↑ *Intestinimonas* ↑ *Pseudoflavonifractor* ↑ *Veillonella* ↓ *Faecalibacterium* ↓ *Ruminococcus* ↓ *Blautia* ↓ *Butyricimonas* ↓ *Dialister* ↓ *Collinsella* ↓ *Anaerostipes*	HPLC	(Pérez-Burillo, Pastoriza, et al., 2019)

LC–MS : (Liquid chromatography–mass spectrometry, HPLC: (High performance liquid chromatography), RT-PCR: (Reverse Transcription Polymerase Chain Reaction), HR-MS/MS: (High Resolution Mass Spectrometry), FISH-FC: (fluorescence in situ hybridization combined with flow cytometry), DGGE: Denaturing Gradient Gel Electrophoresis), HS-SPME: (Headspace-solid phase microextraction), GC–MS: (Gas chromatography–mass spectrometry) and HPLC-DAD: (High-performance liquid chromatography (HPLC) with a diode-array detector). F/B: the ratio of Firmicutes over Bacteroidete

The arrows represent the change in the microbial population: ↑ (increase in the microbial population) ↓ (decrease in the microbial population)

↔ (no change)

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3.2. Evidence from animal trials

Studies have typically used a high-fat diet (HFD) to induce obesity/diabetes to determine the effect of coffee chemicals on gut microbiota especially considering green coffee known slimming effect, with different types of constituent's preparations, dosages, and durations treatment (**Table 2**). Coffee compounds have been found to have an effect on the gut flora without changing its overall count (Cowan et al., 2014). Compared to the group of mice to have consumed water instead of coffee, the number of bacteria was equal although the composition was entirely different (Nakayama & Oishi, 2013).

Chronic coffee consumption has been shown to modify gut microbiota in high fat diet-fed rats, as well as to reduce the growth in the ratio of *Firmicutes* to *Bacteroidetes* (Cowan et al., 2014). In mice, the effects of coffee and coffee galacto-oligosaccharide (GOS) on gut microbiota and host responses were explored, and it was shown that after drinking coffee and GOS, there was a considerable increase in total bacteria counts in the proximal colon. Although *E. coli* and *Clostridium spp*. decreased in the proximal colon, *Bifidobacterium spp*. Showed an increased (Nakayama & Oishi, 2013).

In a recent study, daily coffee consumption for 16 weeks protected non-alcoholic steatohepatitis (NASH) without altering obesity in Tsumura Suzuki obese diabetic (TSOD) mice, a model of metabolic syndrome with evident gut dysbiosis and consequent modification of the type and amount of SCFAs. The impact of coffee was not able though to restore the gut microbial equilibrium, but it induced shift in other bacterial genera. Caffeine and chlorogenic acid, on the other hand, enhanced the profile of SCFAs in inactivated plasma in TSOD mice, whereas coffee itself had no impact (Nishitsuji et al., 2018).

231 **Table 2. The effects of coffee brews and chemicals on microbiota in mice.**

No.	Study design, treatment	Phylum	Genus	Detection methods	References
1	7 Pathogen-free A/J mice, 8 weeks of age, one week rodent diet CE-2, 23 °C, coffee (500 µL day-1)+GOS (2000 mg kg-1 day-1)	↓ *Proteobacteria* ↓ Firmicutes ↑ Actinobacteria	↓ *Escherichia coli* ↓ *Clostridium spp.* ↓ *Enterococcus faecalis* ↑ *Bifidobacterium spp.*	RT-PCR	(Nakayama & Oishi, 2013)
2	Mice, HFD, 12 weeks, 50 mg/kg of caffeic acid	↑ Actinobacteria ↑ Bacteroidetes	↑ *Muribaculaceae* ↓ *Lachnospiraceae*	16S rRNA-PCR	(J. Xu et al., 2020)
3	Rat, HFD, 10 weeks, caffeinated coffee at 20g/L	↑ Firmicutes ↓ Bacteroidetes ↑ F/B	↑ *Enterobacteriaceae* ↑ *Clostridium leptum* ↑ *Bifidobacterium spp.* ↓ *Bacteroides/Prevotella*	qPCR	(Cowan et al., 2014)

4	TSOD mice, MF, 16 weeks, coffee 0.5%, chlorogenic acid 0.5, caffeine 0.5%	↑ Firmicutes ↓ Bacteroidetes ↑ F/B	↑ *Blautia* ↑ *Coprococcus*	16S rRNA-PCR	(Nishitsuji et al., 2018)
5	Rat, HFD, 8 weeks, 5% spent coffee ground	↑ Firmicutes ↓ Bacteroidetes ↓ F/B	↑ *Lachnospiraceae* ↓ *Clostridium*	16S rRNA- qPCR	(Bhandarkar et al., 2020)
6	Mice, HFD, 12 weeks, 4-hydroxy-3-methoxycinnamic acid (HMCA)	↑ Bacteroidetes ↓ Firmicutes	↑ *Coriobacteriaceae* ↓ *Lactobacillaceae* ↓ *Lachnospiraceae*	16S rRNA- RT-PCR	(Ohue-Kitano et al., 2019)

GOS: (galacto-oligosaccharide), HFD: (high-fat diet), qPCR: (quantitative real-time polymerase chain reaction), TSOD: (Tsumura Suzuki obese diabetes) and MF: (moderate or basal diet).

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4. Human gut microbiota-mediated coffee components biotransformation

A wide range of biological processes have been demonstrated to be influenced by the gut microbial population, including gut maturation and angiogenesis, (Stappenbeck et al., 2002), innate immunity development (Singh et al., 2019; Moco et al., 2012), production of vitamins i.e. vitamin K and B (LeBlanc et al., 2013), biotransformation of endogenous and exogenous chemicals xenobiotic (Blaut & Clavel, 2007), dietary energy harvest, and recently, regulation of the host fat storage (Mokkala et al., 2020). Interaction of gut microbiota with food metabolism is well-documented (Farag, et al., 2020). The gut microbiota complements the function of the liver and gut mucosal enzymes participating in nutrients digestion and metabolism (LeBlanc et al., 2013; Huang et al., 2020).

Increasing attention has been directed towards determining how a diet can influence both the composition and metabolism of the gut microbiota though scarce data are available concerning the mechanisms involved in coffee chemicals metabolism by gut microbiota. Carbohydrate-rich diets have a significant effect on the numbers of viable butyrate-producing bacteria in the gut, *i.e.,* clostridia clusters IV and Xi'an (*Ruminococcus*/*Faecalibacterium* and *Roseburia*/*Eubacterium* respectively, which comprise over 50% of the bacteria in the human colon). Butyrate is the preferred energy source for colonic epithelial cells and is thought to play an important role in maintaining colon health in humans by activating apoptosis and cell cycle arrest as well as inhibits aberrant colonic epithelial cell proliferation in diabetics (Blaut & Clavel, 2007; Mokkala et al., 2020).

Coffee chemicals biotransformation is achieved *via* the diverse microbial community residing in the human colon *i.e.,* formation of theophylline which further metabolized into xanthine from transformation of caffeine by *Pseudomonas putida* affecting the abundance of *Firmicutes, Cyanobacteria, Bacteroides and Lactobacillus.* Likewise, modulating the growth of *Bifidobacterium spp, Actinobacteria* as results of biotransformation of caffeic acid to dihydrocaffeic acid by *Peptostreptococciis sp., and Clostridium perfringens*. Similarly, changing the F/B ratio and increasing the frequency of *Bifidobacterium spp., Lactobacillus,* and *Enterococcus* following *Lactobacillus* transformation of monooligosaccharides (MOS) into short-chain fatty acids (SCFAs). Furthermore, *Bifidobacterium spp*. fermented melanoidins into pyrogallol, 2-(3,4-dihydroxyphenyl) acetic acid, and 3-(3,4-

263 dihydroxyphenyl) propionic acid, increasing the proliferation of *Bifidobacterium* and
264 *Faecalibacterium* as demonstrated in **(Fig. 2)**.

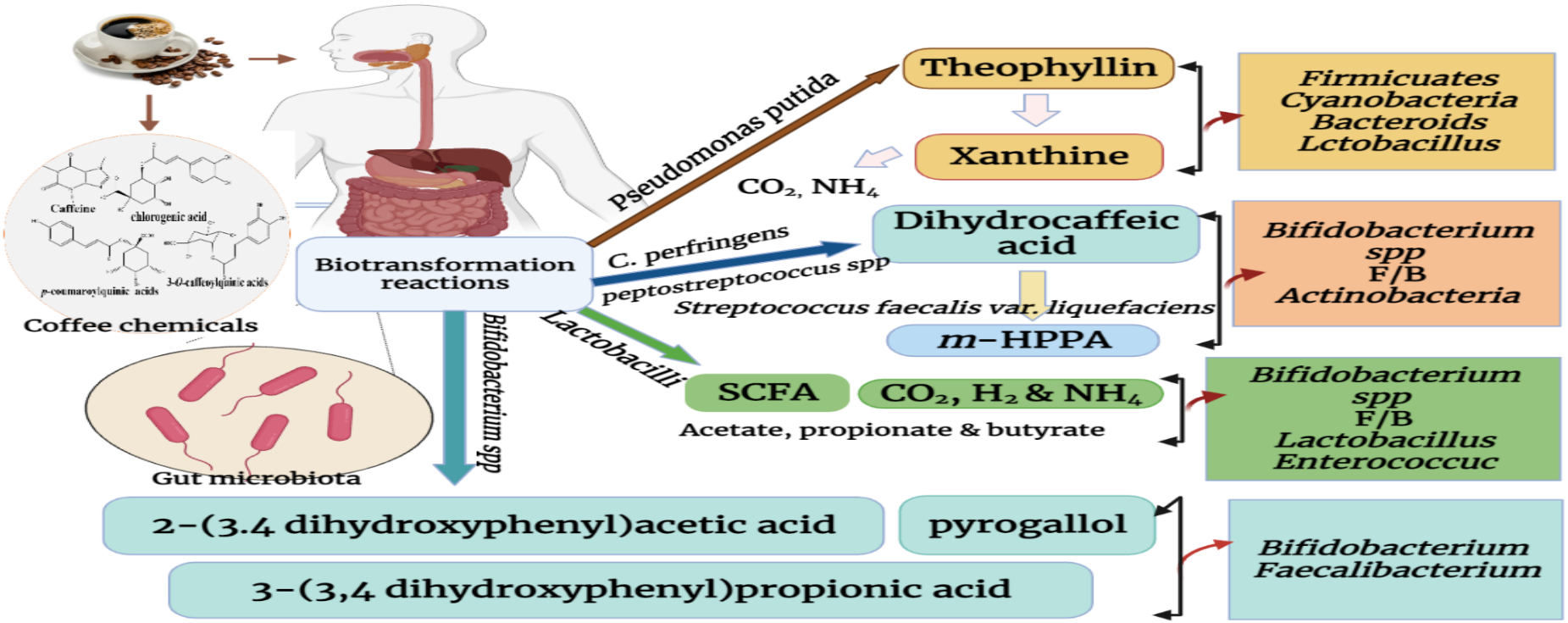


Fig. 2. An overview of the different coffee chemicals biotransformation with gut microbiota. Caffeine is converted by *Pseudomonas putida* into theophylline, which is then converted into xanthine, affecting *Firmicutes*, *Cyanobacteria*, *Bacteroides*, and *Lactobacillus*. *Peptostreptococciis sp*. *Clostridium perfringens* transform caffeic acid to dihydrocaffeic acid, which is further dehydroxylated to *m*-HPPA *via* a mixed culture of *Escherichia coli* and *Streptococcus fecalis* var. liqiiifaciens, modulating the growth of *Bifidobacterium spp*, *Actinobacteria* and altering the F/B ratio. MOS are catabolized by *Lactobacilli* into SCFA, CO_2, H_2 and NH_4, which increase abundance of *Bifidobacterium spp.*, *Lactobacillus*, *Enterococcus* and altering the F/B *ratio*. *Bifidobacterium spp* also ferment melanoidins into pyrogallol, 2-(3, 4-dihydroxyphenyl) acetic acid and 3-(3, 4-dihydroxyphenyl) propionic acid), resulting in promoting the growth of *Bifidobacterium* and *Faecalibacterium.*

Due to the complexity of coffee molecules, minor absorption only occurs in the small intestine, and it reaches the large intestine to be digested by gut microbiota. For instance, chlorogenic acids isomers that have been inadequately absorbed in the upper gastrointestinal tract *i.e.*, around 1/3 absorbed in the small intestine, while the rest entering the large bowel un metabolized (Stalmach et al., 2010). Indeed, gut microbiota encompass a complex machinery of chemical reactions to include; demethylation, dehydroxylation, ester cleavage, reduction, isomerization, ring fission, decarboxylation and other reactions (Cuervo et al., 2016). Secondary by-products that are generated from these biotransformation reactions may further alter the composition of gut microbiota. It has been reported that significant increase in the growth of *Bifidobacterium spp.*, (*Actinobacteria* phylum) occurred after ingestion of 80 mg CGAs enriched in green coffee. Such increase has long been linked to improved gut health *via* an increase in saccharolytic metabolism as well as the generation of short chain fatty acids (SCFAs) i.e., acetate and lactate, which have anti-pathogenic properties (Parkar et al., 2013).

Roasted coffee, on the other hand, has the ability to affect gut microbiota differently than green coffee due to the high presence of melanoidins, which can act as fiber-like molecules in the gut, *i.e.*, prebiotics. (Jiménez-Zamora et al., 2015). Given that, chemical reactions that occurs by gut microbiota may vary in context to the chemical structure of coffee constitutions. In the next subsections, we will address the reaction of each coffee bioactive class inside the gut and its ultimate effect on gut homeostasis and further systemic health outcomes.

4.1. Alkaloids

Coffee contains two types of alkaloids: purine alkaloids such as caffeine (1, 3, 7-*N*-trimethylxanthine) and theobromine (3, 7-*N*-dimethylxanthine); and pyridine alkaloids such as trigonelline (1-*N*-methylnicotinic acid) (Eckman et al., 2010). Purine alkaloid caffeine occurs naturally in coffee seed and is documented at significant levels in more than 60 plants *i.e.*, kola nut (*Cola acuminate*), cacao bean (*Theobroma cacao*), yerba mate (*Ilex paraguariensis*), and guarana berries (*Paullinia cupana*). However, roasted coffee seeds (*Coffea arabica* and *Coffea robusta*), and tea leaves (*Camelia siniensis*) are considered the world's primary sources of dietary caffeine (Eckman et al., 2010).

Caffeine is a water-soluble alkaloid that belongs to the xanthine family and has a variety of biological functions, including a psychoactive stimulant action of the central nervous system

(de Melo Pereira et al., 2020). Caffeine as a major alkaloid in green coffee appeared to undergo mostly demethylation type reactions in response to the incubation with *ex vivo* gut microbiome culture. Nevertheless, it degrades slowly due to the removal of the three methyl groups, which leads ultimately to the formation of xanthine (Jasiewicz & Sierakowska, 2020).

Caffeine catabolism normally starts with its conversion to theophylline, which is catalysed by N7-demethylase through demethylation reaction *via Pseudomonas putida*, by breaking down caffeine into carbon dioxide and ammonia to harvest energy and cellular building blocks (Kim et al., 2019). Theophylline, a caffeine analogue found at lower levels it's also further catalysed into different steps ending with formation of xanthine, which is converted to CO_2 and NH_3 *via* uric acid, allantoin and allantoate through the traditional purine catabolism route. *Pseudomonas*, *Serratia*, *Rhodococcus,* and *Klebsiella spp., a*re the most common bacterial genera involved in caffeine degradation (Jasiewicz & Sierakowska, 2020; Kim et al., 2019), as illustrated in **(Fig. 3)**.

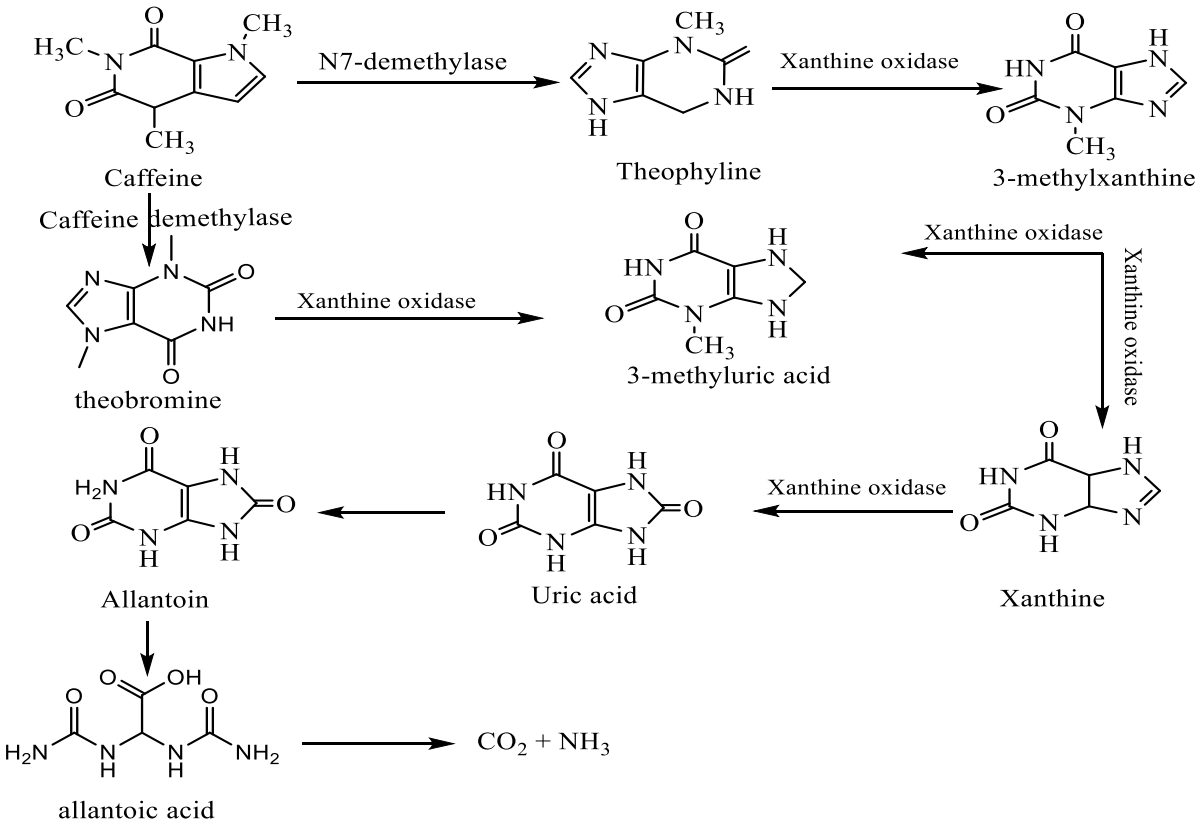


Fig. 3. Caffeine biotransformation by *Pseudomonas putida* and its underlying enzyme mechanisms

Caffeine can likewise modify the microbiome composition, it was reported that caffeine intake decreases the abundance of *Lactobacillus* in rats as well as the growth of *Bacteroidetes* versus enhancement of *Cyanobacteria* multiplication (Kleber Silveira et al., 2018). Also, xanthine metabolism was found to affect gut microbiota in resistance to high-fat diet induced obesity rat with decrease in *Bacteroida* versus an increase in *Clostridia*, *Oscillospira* and *Ruminococcus* phyla (Wei et al., 2021).

4.2. Phenolic acids

Phenolics are the main determinant of antioxidant potential found at high levels in plant-derived foods. The recovery of phenolic compounds from coffee industry by-products and their antioxidant activity has been investigated recently (Campos-Vega et al., 2015). With regards to coffee metabolism inside the human body, phenolic acids that are not completely digested in the small intestine are subjected to the action of human gut microbiota in the colon to afford catabolic by-products that are absorbed systemically (Vollmer et al., 2017). Such bio-transformed metabolites play a role in dietary phenolics biological effects and/or fate (Marín et al., 2015). The major phenolic acids present in coffee are chlorogenic acids (CGAs) which are a family of non-flavonoid molecules composed of quinic acid esterified with cinnamates.

The key classes of CGAs in coffee are caffeoylquinic acids (CQAs), feruloylquinic acids (FQA), *p*-coumaroylquinic acids (pCoQA), dicaffeoylquinic acids (diCQA), and caffeoylferuloylquinic acids (CFQA) (González et al., 2020), illustrated in (**Fig. 1**). The gut microbiota acts as a powerful bioreactor able to break the complex structures of polyphenols into different low-molecular-weight molecules, which are then readily absorbed and to exert diverse biological functions. Catabolism typically start with a hydrolysis step into aglycones and extensively metabolizes the aglycones into various aromatic acids that are well absorbed through the colon wall barrier (Santhakumar et al., 2018).

The fraction of CGAs that reach the small bowel and the colon is subjected to hydrolysis and extensive metabolism by gut microbiota. Based on its vast gene pool within gut bacteria, the intestinal microbiota has a large metabolic potential and to catalyze many reactions in the course of chlorogenic acid conversion such as; demethylation, dehydroxylation, ester cleavage, reduction, isomerization, ring fission, decarboxylation, etc… (Cuervo et al., 2016). For example, cleavage of the ester linkage between quinic and caffeic acid occurs, with released

caffeic acid may be absorbed intact or, more probably further metabolized to *O*-methylated, sulphated and glucuronidated derivatives (Stalmach et al., 2010; Neielsn et al., 2018). Gut bacteria, that mediates for the C-ring fission in chlorogenic acid include *Eubacterium oxidoreducens*, *E. ramulus*, *E. casseli avus*, *Clostridium orbiscidens*, and others belonging to the *Butyrivibrio* genus (Marín et al., 2015).

Eubacterium, *Enterococcus*, *Micrococcus*, *Fusobacterium*, *Streptococcus*, *Peptostreptococcus*, and *Chrostridium* are the most well-known bacterial genera involved in the metabolism of CGAs and other polyphenols (Farah & Duarte, 2015). Chlorogenic acid and other phenolic compounds entering the colon can be used as additional growth substrates by obligate or facultative anaerobic bacteria, and aaccording to the type of bacterial genus involved in the biotransformation processes, different by-products are formed. For example, bacterial esterases from *Escherichia coli*, *Bifidobacterium lactis*, and *Lactobacillus gasseri*, may liberate cinnamic acid moiety from chlorogenic acid molecules, resulting in caffeic and ferulic acids that can be absorbed or subsequently converted to other metabolites (Parkar et al., 2013).

Gut microbiota has been found to transform caffeic acid *via* a number of different bacteria to other phenolic derivatives. *Peptostreptococciis sp*., and *Clostridium perfringens* isolated from human feces were found capable of reducing caffeic acid to dihydrocaffeic acid, which is further dehydroxylated into *m*-HPPA *via* a mixed culture of *Escherichia coli* and *Streptococcus fecalis*. In contrast, *Streptococcus fecitim* decarboxylates caffeic acid to 4-vinylcatechol (Vollmer et al., 2017), as illustrated in **(Fig.4)**.

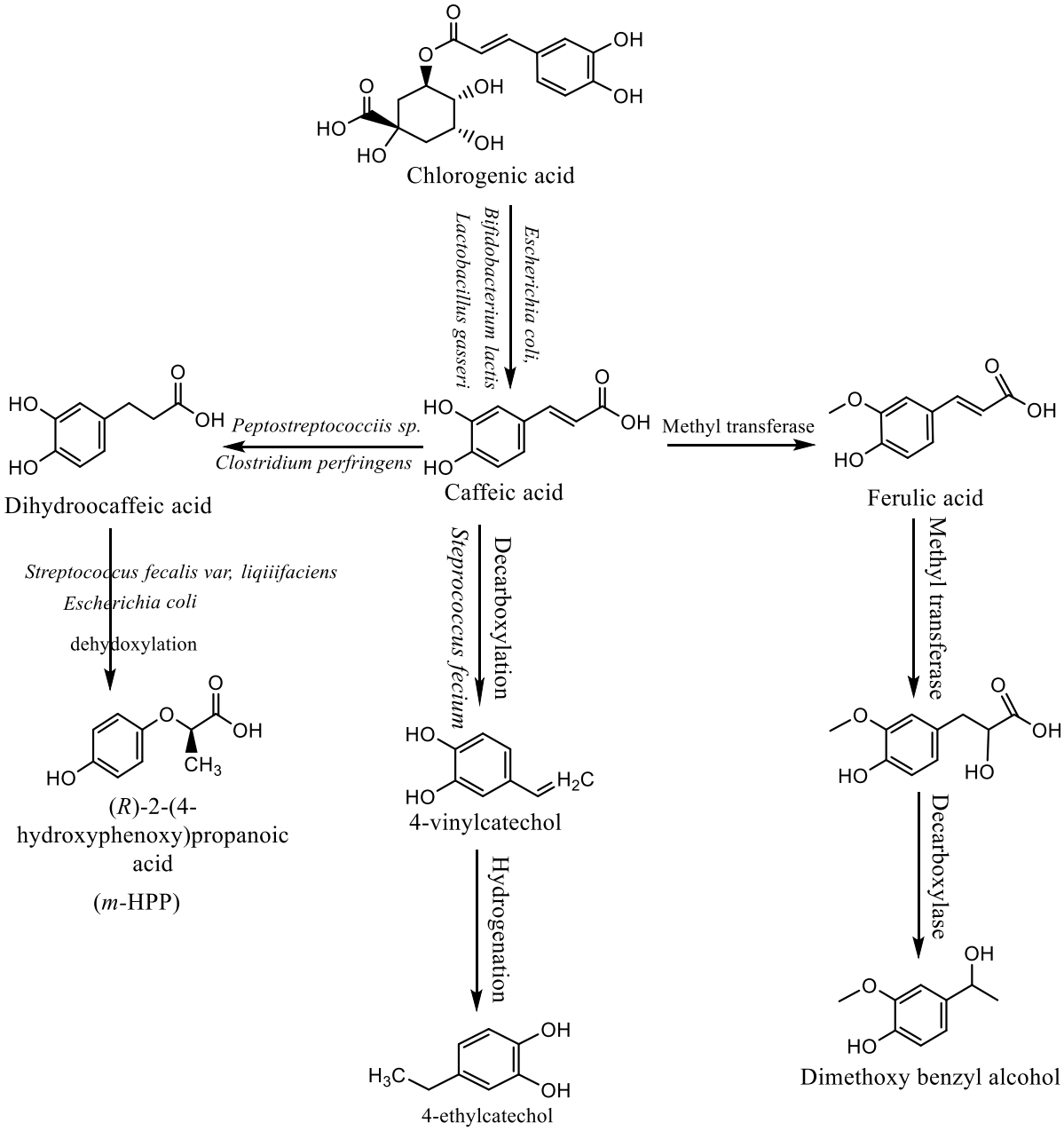


Fig. 4. Biotransformation reaction products of chlorogenic acid *via* various gut microbiota and underlying reaction mechanisms

The hydroxycinnamtes (caffeic and ferulic acids) released *via* the deesterification of CGAs or present freely in coffee brews can be further catabolized *via* gut microbiota into dihdroxyphenyl-ethanol methyl ether and methylenedioxy cinnamic acid methyl esters (Farag, Hegazi, et al., 2020). Additionally, hydroxybenzoic acid was identified as the main biotransformed product of hydroxycinnamtes in espresso and green coffee post incubation with human gut microbiome (Ludwig et al., 2013). Studies have also revealed that *Escherichia coli*,

Bifdobacterium lactis, *Lactobacillus gasser* have the ability to yield vanillin from ferulic acid moiety in cinnamates (Marín et al., 2015).

An *in vitro* study in which espresso coffee was incubated with human fecal and CGAs breakdown products were monitored using high-performance liquid chromatography-mass spectrometry (HPLC-MS) and gas chromatography-mass spectrometry (GC-MS) revealed that CGAs were rapidly degraded by the colonic microflora over the 6-h incubation period. 11 Catabolites were identified including caffeic and ferulic acids, with a transient maximal response at 1 h. In contrast, dihydrocaffeic acid, dihydroferulic acid, and 3-(3-hydroxyphenyl) propionic acid were the major end products, comprising 75–83% of the total catabolites, whereas the remaining 17–25% consisted of 6 minor catabolites. The biotransformation of coffee cinnamates *i.e.*, CGAs is typically catabolized by the action of bacterial esterase's such as *Escherichia coli*, *Bifidobacterium lactis*, and *Lactobacillus gasser* (Ludwig et al., 2013). The bacteria metabolize caffeic acid to yield 3-hydroxyphenylpropionic acid through a series of reactions starting from de-esterification, double bond reduction, and dihydroxylation following that, β-oxidation shortens the side-chain, resulting in the production of benzoic acid.

Coffee bio-transformed products showed a substantial influence on enhancing the proliferation of *Bifidobacterium spp*. and modifying or decreasing the *Firmicutes* to *Bacteroidetes* ratio in the gut microbiota. *Bifidobacteria* defend the gut mucosa against bacterial invasion by inhibiting pathogens such as *Salmonella* through lumen acidification and competitive exclusion by preventing pathogenic occupancy of epithelial colonization sites through nutritional competition which present an added value for coffee phenolics (Stalmach et al., 2010; de Melo Pereira et al., 2020).

4.3. Spent coffee *i.e.*, mannooligosaccharides

Spent coffee ground (SCG) is the waste that accumulates after coffee consumption, or it can be defined as the residue obtained during the brewing process (Campos-Vega et al., 2015). The accumulation of SCG as a result of increasing coffee consumption across the world is of increasing attention, with *ca*. 6 million tons of SCG produced yearly (Vítězová et al., 2019). Given the massive amounts of SCG, there is debate about whether it exert nutritional value and can be utilized or to be exploited for industrial uses. SCG contains high levels of organic components (fatty acids, cellulose, hemicellulose, lignin, and other polysaccharides) that may

be used as a source of value-added products. SCG has been used for various application including waste water treatment (Vítězová et al., 2019), biodiesel production (Caetano et al., 2012), sorbent for removal of metal ions (Fiol et al., 2008), renewable energy source (Tun et al., 2020), and as a reducing agent (Han et al., 2021).

In contrast, the discharge of wasted coffee grounds into the environment as a result of rising coffee consumption pollutes the ecosystem since its breakdown requires a huge amount of oxygen (Hardgrove & Livesley, 2016). Because of the inclusion of phenols, caffeine, and tannins, which are very hazardous to numerous biological processes, SCG use and management presents a major issue. Warm treatment, microbial biodegradation, and aerobic fermentation have all been used to decrease the toxicity of SCG (Hao et al., 2018; Brachi et al., 2021). Spent coffee grounds are particularly rich in polysaccharides, with galactomannans amounting for ca. half of their overall composition, while arabinogalactans and cellulose account for the other half (Mussatto, Carneiro, et al., 2011). Mannans are the main polysaccharide component of SCG, and to account for its high viscosity, which has a detrimental impact on the technical processes involved in instant coffee production (Campos-Vega et al., 2015).

Galactomannans from roasted coffee infusions are composed of a backbone of β- (1→4)-linked mannopyranosyl units, which are partially substituted with single galactopyranosyl residues at the O-6-position. While arabinogalactans are composed of a β- (1→3)-linked galactose backbone substituted at the O-6 position with arabinose and/or galactose residues and have a 0.4/1 arabinose/ galactose ratio (Campos-Vega et al., 2015). Galactomannans and arabinogalactans ingested with coffee beverages include polysaccharides, mannooligosaccharides, oligosaccharides, and associated dietary substances that are not degraded by human digestive enzymes. Consequently, they reach the colon and potentially serve as substrates for the colonic microbiota to function as prebiotic (Pérez-Burillo, Mehta, et al., 2019).

Colonic microbiota performs a key function in the degradation of polysaccharides *via* its fermentable activity into short-chain fatty acids (SCFAs) i.e., acetate, propionate, and butyrate, as well as gases such as H_2, CH_4, and CO_2 (Benitez et al., 2019). Production of SCFAs lowers the colonic pH, impeding the growth of certain pathogenic species and supporting the growth

of *Bifidobacteria* and other lactic acid bacteria that are considered to be beneficial for human health (Gniechwitz et al., 2007).

Short-chain carbohydrates called mannooligosaccharides (MOS) are formed from coffee galactomannans by acid, alkaline, or enzyme hydrolysis. (Ludwig et al., 2013; Pérez-Burillo, Mehta, et al., 2019). MOS are categorized as prebiotic non-digestible short chain oligosaccharides because of their selective fermentation by gut microbes, especially *Lactobacilli, Bifidobacteria* etc. and beneficial short chain fatty acid (SCFAa) production. Prebiotic effects of MOS detected for a subset of bacterial phyla of the human gut microbiota may also be explained by the utilization of the attached sugar moieties. The growth enhancement of *Bifidobacterium, Lactobacillus* and *Enterococcus* species by MOS may serve as such example (gen Suryawanshi & Kango, 2021).

MOS obtained from spent coffee grounds was incubated with human faecal samples in an *in vitro* study, which proved its prebiotic action by promoting the proliferation of beneficial genera such *Barnesiella, Odoribacter, Coprococcus, Butyricoccus, Intestinimonas, Pseudoflavonifractor,* and *Veillonella*. Furthermore, SCFAs has shown a rise in 5-(hydroxymethyl) and polyphenols in a dose-dependent manner (which are either produced or released from the spent coffee grounds matrix during hydrolysis). In contrast, the quantity of other beneficial genera, such as *Faecalibacterium*, *Ruminococcus*, *Blautia, Butyricimonas, Dialister*, *Collinsella*, and *Anaerostipe*s was reduced, which might adversely influence MOS prebiotic activity (Pérez-Burillo, Pastoriza, et al., 2019).

In a similar research, the effect of a coffee mix drink containing MOS on defecation circumstances and fecal microbiota composition in healthy human volunteers was investigated. Results proved that the ingestion of two cups of coffee mix drink containing MOS substantially enhanced the number of days of defecation and frequency of defecation per week compared to the placebo drink, suggesting that coffee mix containing MOS might be useful for improving defecation conditions and or bowel functions (Santhakumar et al., 2018; Pérez-Burillo, Pastoriza, et al., 2019).

4.4. Maillard products i.e., melanoidins

Although coffee brews include significant amounts of structurally complicated compounds resulting from Maillard process (melanoidins), it is impossible to distinguish between melanoidins and poly- or oligosaccharides since carbohydrates are assumed to be important components of coffee melanoidins. Melanoidins and dietary fibers have comparable physiological features in that they are both indigestible, have high water holding capacities, and may adsorb organic compounds such as bile acids (Pérez-Burillo et al., 2020). Melanoidins are colorful polymers that occur during the later stages of the Maillard reaction (MR) and are found in a variety of thermally processed foods, including black coffee. Basically, MR occurs in heat-treated foodstuffs by an irreversible combination of sugar and amino acids or proteins (Goya et al., 2015), as typical to occur during coffee roasting.

Formation of melanoidins occurs from the covalent linkage between galactomannans and arabinogalactans, polyphenols, proteins and free amino acids during roasting of coffee green seeds (Vitaglione et al., 2012). Inside the GIT, dietary melanoidins escape gastrointestinal digestion (similar to fiber) and to reach the colon where they become substrates for the gut microbiota (Gniechwitz et al., 2007; Farah & Duarte, 2015). High level of melanoidins were found in roasted coffee, and fermented by gut microbiota, possibly to likewise affect the gut microbial consortium composition. SCFAs are produced as a result of melanoidins fermentation by gut bacteria (mainly as acetate and lactate) which can inhibit harmful bacteria development. (Kumar & Chandra, 2006).

The impact of coffee species, roasting degree and decaffeination on the *in vitro* probiotic bacterial growth was studied with aqueous extracts of both *C. arabica* and *C. canephora.* Results revealed enhanced growth of all tested probiotic bacteria (*Lactobacillus rhamnosus* GG ATCC 53103, *Lactobacillus acidophilus* LA-5 DSM 13241, *Bifidobacterium animalis subsp. lactis* BB12 DSM 15954 and *Bifidobacterium animalis* CNCM-I 2494), whereas growth of *E. coli* ATCC 25922 showed inhibition (Sales et al., 2020). CGAs alongside with polysaccharides appeared as the major components responsible for the coffee prebiotic action for *Lactobacillus rhamnosus* GG ATCC 53103, *Lactobacillus acidophilus* LA-5 DSM 13241, and *Bifidobacterium animalis* CNCM-I 2494. In contrast, *Bifidobacterium animalis subsp. lactis* BB12 DSM 15954, preferred melanoidins as their primary substrate (Sales et al., 2020).

Melanoidins have been reported to enhance prebiotic activities to encourage the formation of beneficial genera such as *Bifidobacterium* and *Faecalibacterium* concurrent with the production of SCFAs (Pérez-Burillo et al., 2020). Additionally, melanoidins encompass different phenolic compounds depending on the food source, consequently identification of phenolic compound from melanoidin biotransformation could aid in the identification of potential antioxidant and prebiotic activity (Pérez-Burillo et al., 2020). Coffee melanoidins reduce *Streptococcus mutans'* adherence to the tooth surface, which leads to reduced biofilm production and prevents dental plaque growth. The antibacterial action of coffee melanoidins was shown to be stronger against Gram-positive bacteria, such as *Staphylococcus aureus*, than Gram-negative bacteria, such *as Escherichia coli*, which is likely related to the fragility of the Gram-positive bacterial cell wall (Rufián-Henares & Pastoriza, 2015). Whether incorporation of coffee melanoidins in oral care products could present potential health benefits has yet to be determined.

The specific mechanism underlying melanoidins' antimicrobial activity is unknown, however other theories have been proposed, including a reduction in glucose and oxygen absorption or inhibition of microbe carbohydrates catabolizing enzymes. The major metabolites resulting from the fermentation of coffee melanoidins by gut microbiota are acetate and propionate, likely derived from their polysaccharides backbone (Reichardt et al., 2009).

An *in vitro* study investigated melanoidins bioavailability from different dietary sources including coffee post *in vitro* fermentation led to the detection of pyrogallol, 2-(3,4-dihydroxyphenyl) acetic and 3-(3,4-dihydroxyphenyl) propionic acids as biotransformed metabolites of melanoidins entrapped phenolics. Results further showed that melanoidin's antioxidant activity was similarly impacted favorably by gut microbiota fermentation (Pérez-Burillo et al., 2020).

5. Coffee consumption and diseases prevention mediated *via* gut microbiota interaction

The effect of diet and dietary habits on the host gut microbiota is increasingly recognized, and with such interaction found to affect both coffee chemicals and gut homeostasis (Harakeh et al., 2020). Several studies have reported that both black and green coffee extracts can affect gut microbiota activities *via* the modulation of different metabolic pathways (Farag, et al., 2020). Coffee metabolites alteration by gut microbiota can in turn affect the host metabolism.

Changes in gut microbiota composition, for example, might have an impact on the gut microbiome-brain axis and the host's inflammatory responses (Farag, et al., 2020).

While coffee extract inhibited some beneficial microbiome, it stimulated other genera appearing to exert a somewhat prebiotic effect. For example, it was found to increase the levels of butyrate producer bacterium i.e., *Anaerostipes*, *Butyricimonas*, and *Faecalibacterium.* The produced butyrates in turn exhibit a protective role against inflammatory diseases like ulcerative colitis. It is also known that coffee demonstrates protective effect against many certain diseases *i.e.*, obesity, immunity disorders, inflammatory bowel syndrome (Zafar & Yaddanapudi, 2020). In the next subsections, **(Fig. 5)**, illustrations on how coffee consumption could affect the gut microbiota composition to mediate for coffee health effects in different diseases will be presented with focus on gut-liver axis and gut-brain axis.

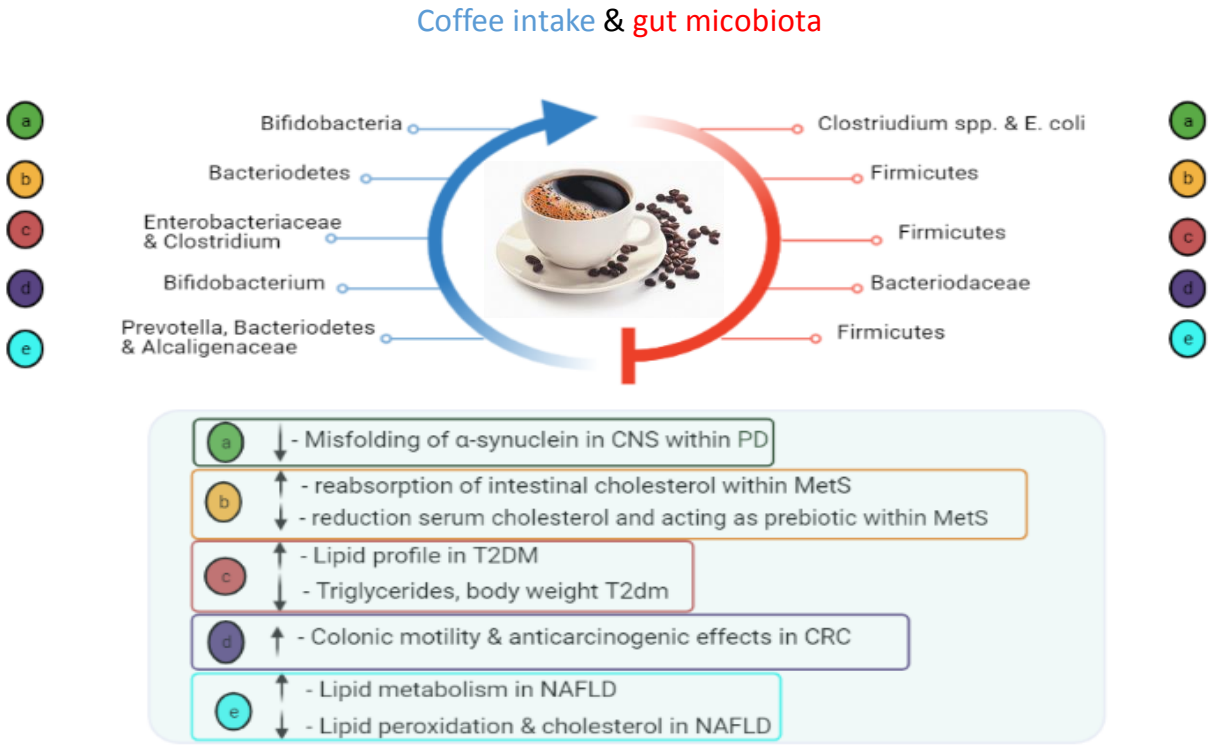


Fig. 5. The influence of coffee consumption and its implications in multiple diseases, where letters resemble the effect of coffee chemical composition in each disease. Where **a** denotes for Parkinson disease (PD); **b** for metabolic syndrome (MetS); **c** for diabetes mellitus type 2 (T2DM); **d** for colon rectal cancer (CRC) and **e** for non-alcoholic fatty liver disease (NAFLD).

5.1. Parkinson disease

Parkinson's disease (PD) is a persistent disease condition in which neurodegeneration occur resulting in movement disorders, mainly slowness of movement and other non-motor symptoms (Zafar & Yaddanapudi, 2020). PD affects 0.1% of the people worldwide at any time including 1% of people above sixty years old (Zafar & Yaddanapudi, 2020). Several studies have shown that PD is linked to intestinal microbiome changes, and that coffee drinking reduces PD risk (Delgado-Andrade et al., 2017; Pérez-Burillo, Mehta, et al., 2019a).

Coffee intake can change the composition of the intestinal microbiota resulting in gut dysbiosis associated with decrease in intestinal inflammation, which may contribute to less misfolding of α-synuclein in the enteric nervous system, decreasing the risk of PD by limiting the distribution of protein to the central nervous system (CNS). Coffee consumption in both mice and humans has also been associated with substantial increase in *Bifidobacteria*, to exert anti-inflammatory properties. For instance, daily consumption of 3 cups of coffee for 3 weeks by 16 healthy adult volunteers showed increase in the metabolic activity of *Bifidobacteria* spp., (Jaquet et al., 2009) as illustrated in (Table 1). Similarly, increase in the abundance of *Bifidobacteria* spp., in mice post consumption of 500 µL of coffee and 2000 mg kg GOS per day has also been reported (Nakayama & Oishi, 2013) as illustrated in (Table 2). This is attributed to the GOS content in coffee found to stimulate *Bifidobacteria* that would convert this oligosaccharide to lactic acid and form ATP. Coffee can also promote bacteria connected to an increased risk for PD in some forms of chronic GI illnesses such as those caused by *Helicobacter pylori* (Mulak & Bonaz, 2015).

Reduced abundance of *Prevotellaceae* family bacteria concurrent with an increased abundance of *Enterobacteriaceae* bacteria was observed in Parkinson's patients. Coffee-enriched dietary fiber was found to exhibit significant effect on intestinal microbiota mostly *via* its metabolized SCFAs that alter *Bacteroides* and *Prevotella* species abundance. In conjunction with and reduction in *Clostridium* and *Escherichia coli* in the intestinal mucosa of the PD, the use of coffee led to an increase in anti-inflammatory *Bifidobacteria*. Such gut microbiota dynamic changes suggest for several action mechanisms for the protective role of coffee against PD (Scheperjans et al., 2015).

5.2. Metabolic syndrome

Metabolic syndrome is an accumulation of many conditions that altogether increase the risk of a person experiencing insulin resistance and diabetes mellitus, atherosclerotic cardiovascular disease, neurological and vascular complications such as a stroke. Coffee was reported in several meta-analysis studies to exert beneficial effects on both visceral fats and diabetes mellitus. It was also reported to enhance the overall metabolic status by improving glucose level, liver triglycerides, insulin resistance thus reducing weight gain (Caro-Gómez et al., 2019), and to support that daily coffee consumption is linked with a lower risk of metabolic syndrome (Xu et al., 2020; Bhandarkar et al., 2020).

One of the possible mechanisms of coffee protective effects against metabolic syndrome is mediated *via* affecting gut microbiota composition *i.e*., 6 genus level that are protective against metabolic syndrome. Human gut microbiota composition is much altered in metabolic syndrome patients as exemplified by a reduction of *Bacteroidetes* population concurrent with the abundance of *Firmicutes* (Binda et al., 2018; Ohue-Kitano et al., 2019). It is stated that ingestion of coffee grounds reverses these alterations in intestinal microbiosis by reducing the fraction of *Firmicutes* to *Bacteroidetes* (Bhandarkar et al., 2020).

Coffee melanoidins and polyphenols readily reach the colon acting as prebiotic and to further interact with gut microbiota increasing the abundance of *Alcaligenaceae* which suggest for higher reabsorption of intestinal cholesterol and a reduction in serum cholesterol levels (Vitaglione et al., 2019). Another prebiotic function in coffee seeds includes mannan sugars modulating the microbiota resulting in enhanced immunity and better health effects. Degrading enzymes in mannans contribute to intestinal microbiota metabolism through the generation of simple monosaccharides/oligosaccharides fractions, which are the oxidation products of mannans. Their significance may be contributed to their effects on regulating the body weight and lowering blood pressure, glucose and cholesterol levels (Singh et al., 2018).

Chlorogenic acid, which is another major coffee component, improved the diversity of intestinal microbiota that ultimately boost total body metabolism. Chronic chlorogenic acid consumption in dietary foods therefore may have beneficial effects in case of inflammation and metabolic changes. About a third of the chlorogenic acid in the coffee is transmitted through the intestine and metabolized through microbiota (as mentioned in section 4.2. and **(Fig.4**),

which has an effect on the *in vivo* and *in vitro* composition of microbiota, and which reverse the changes in obesity and metabolic syndrome that may occur (Cowan et al., 2014; Yang et al., 2020).

5.3. Type II diabetes

Diabetes mellitus (DM) is a metabolic disease in which the patient suffers from polydipsia, polyuria, and weight loss as a result of elevated blood glucose level above normal ranges; with the main two subtypes are type 1 and type 2. Type 1 is caused by defective insulin secretion and is more common in elderly, while type 2 is caused by defective insulin action and is believed to be more abundant at younger ages (Sapra & Bhandari, 2020). Due to the significant morbidity and mortality related with type 2 diabetes, numerous measures are being made to reduce the risk of acquiring the disease, one of which is nutrition-based therapies. The consumption of coffee has been shown to lower the risk of type 2 diabetes. A dose response meta-analysis of 30 prospective trials with a total of 53018 individuals found that increasing daily coffee consumption by one cup reduced the incidence of type 2 diabetes by 6% (Carlström & Larsson, 2018). One of the proposed explanations for coffee's health benefits on type 2 diabetes is the influence of coffee polyphenols, such as chlorogenic acid and flavanols, as well as their metabolites, on the gut microbiota, which affects the glycemic response and exerts anti-diabetic activity (Williamson, 2020; Márquez Campos et al., 2020; Walker et al., 2020).

Intestinal microbial communities can influence the rate of fat deposition and utilization, insulin resistance and diabetes. It is widely perceived that gut microbiota contributes to the overall body metabolism through energy balance, carbohydrate consumption, and low-grade inflammation in obesity and associated metabolic disorders, such as type 2 DM. Consequently, any change in intestinal microbiota has been shown to modify insulin resistance for patients with metabolic syndrome (Fagherazzi et al., 2016; Kerimi et al., 2020).

Coffee polyphenols are reported to stimulate the growth of certain phyla *i.e*., *Akkermansia muciniphila* and *Bifidobacterium spp*, which in turn alter endogenous and exogenous substances metabolism and to exert a protective role against DM (Cornelis et al., 2018; Xu et al., 2020; Le et al., 2015). Chlorogenic acid, on the other hand, has been shown to promote the growth of *Bifidobacterium spp*., *Clostridium coccoides*, and *Eubacterium rectale*. Both bacterial species are well known to negatively correlate with DM (Singh et al., 2018; Vitaglione et al., 2019).

A pilot randomized study determining the short-term effect of regular coffee consumption for 12 weeks showed noticeable direct effect on metabolic state and diabetes mellitus. It was reported that the daily consumption of 200 mg caffeine and chlorogenic acid resulted in 3.6 kg weight reduction in diabetic patients partially mediated *via* increasing the gut *Bifidobacteria* (Mansour et al., 2020). Also, daily coffee consumption for 10 weeks decreased *Firmicutes/Bacteroidetes* ratio and to increase the levels of *Enterobacteriaceae* and *Clostridium* phylla, concurrent with 50% lower triglycerides level, lower body weight and an improved lipid profile (Cowan et al., 2014).

5.4. Cancer and Inflammation

Inflammation is a type of immunological response to a certain toxic compound or a pathogen that leads to the response of inflammation that may finally lead to cancer if untreated (Carlström & Larsson, 2018; Sapra & Bhandari, 2020). Coffee drinking was reported to decrease inflammatory markers and consequently associated with lower cancer risk (Loftfield et al., 2015). One of the possible mechanisms for this relationship is the well-known effect of coffee on gut microbiome. For example, in case of colorectal cancer there is a strong evidence that gut microbiota has a strong role in shaping the inflammatory response and controlling cancer occurrence and further metastasis (Brennan & Garrett, 2016).

Colon microbiota ferment the dietary fibers in the coffee producing SCFAs to modulate cytokines production and to further exert a protective role against inflammation, with such action found more evident in case of spent coffee grounds more enriched in dietary fibers than instant coffee (López-Barrera et al., 2016). Diet is closely linked to colon cancer risks through several pathways, including dietary effects on gut microbiome. Coffee or/and its components are linked to changes in intestinal microbiota *i.e.*, increasing *Bifidobacterium*, reducing *Bacteroidaceae* and other effects which results in better colonic motility and further anti-carcinogenic effects *via* apoptosis induction of HT-29 colon cancer cells (Le et al., 2015; Cornelis et al., 2018).

5.5. Fatty liver disease

Fatty liver disease is defined by the presence of more than 5% hepatic steatosis either without secondary cause such as non-alcoholic fatty liver disease or with the presence of other

cause like chronic use of medication or heavy alcohol consumption (Budryn et al., 2017; Adeshirlarijaney & Gewirtz, 2020).

Caffeine is reported in many meta-analyses to lessen the risk of liver fibrosis, cirrhosis and fatty liver disease (Chen et al., 2017; Singh et al., 2019). One of the possible mechanisms is through modulation of gut-liver access (Feng et al., 2019). For example, coffee intake increases *Alcaligenaceae* which in turn have a role in lipids metabolism and is negatively associated with lower cholesterol levels (Vitaglione et al., 2019). Chlorogenic acid improves gut microbiota diversity and subsequently improve the overall body metabolism which in turn is reflected on lowering fat accumulation and improving the liver health (Bhandarkar et al., 2019).

High coffee intake of around 45–500 ml daily is found to increase levels of *Prevotella, Porphyromonas* and *Bacteroides* reflected with less lipoperoxidation and incidence of fatty liver in these heavy coffee consumers. It was found that the modulation of gut microbiota accompanied with coffee intake is strongly associated with an antioxidant and less lipogenesis effect (Binda et al., 2018; Kim et al., 2019).

Hydroxy methoxycinnamic acid that is present in coffee was found to be metabolized by gut microbiome to hydroxy methoxyphenyl propionic acid as illustrated in **(Fig.4),** which modulates gut microbes responsible for metabolic status in the host and to ultimately regulate lipid metabolism in the liver as manifested by increase in *Bacteroidetes* versus a decrease in *Firmicutes* (Ohue-Kitano et al., 2019). In addition, coffee in general and certain processed coffee products such as coffee silverskin extract enriched in dietary fibers demonstrated beneficial effects on lipid metabolism likely due to the generated SCFAs (Iriondo-Dehond et al., 2019).

6. Mining the alteration in Gut microbiome and CAZymes profile with coffee through comparative metagenomics analysis

The CAZymes' repertoire represents the key protagonist in defining the nutritional status of the individuals. Malnutrition and improper nutrition are associated to gut microbiota dysbiosis, which might contribute to the development of many food-diseases. Little is known about which microbial communities and CAZymes related to coffee consumption are present in the human gut, therefore we follow a methodology of four steps to identify the microbial

CAZYme's signature that helps to discriminate between coffee consumer and non-consumer (control) metagenome samples.

6.1. Human gut metagenomics data selection

Publicly available reference metagenomics data on stool samples were downloaded from NCBI BioProject PRJNA289586 (Heintz-Buschart et al., 2016), as .fastq files using the 'fasterqdump' command in the NCBI SRA-Toolkit v2.10.8 software. The samples are SRS1369966, SRS1369963, SRS1369964 and SRS1369954 with accession numbers SRR3313057, SRR3313090, SRR3313079 and SRR3313102, respectively. Our samples belonged to four adults whose ages ranged from 57 to 60, where the first two samples concern two people whose last meal consisted of coffee, while the remaining two samples were considered as controls for our comparative analysis.

6.2. Raw data quality assessment and trimming

The raw sequence data was first quality checked with the objective to have an idea whether it has any problems of which we should be aware before doing any further analysis. The raw reads were then trimmed to exclude host sequences and all those sequences that could exist in bad orientation and do not meet the standard quality scores. Quality of reads was checked by FastQC, version 0.11.9, while paired-end reads were trimmed using Trimmomatic, version 0.40 (Bolger et al., 2014).

6.3. Metagenome Assembly

Metagenome assembly is the process of constructing microbiomes' genomes by transforming noisy DNA segments found in sequence data into accurate, longer, contiguous sequence fragments. Our samples were assembled using MetaSPAdes, version 3.13.0, with default parameters (Nurk et al., 2017).

6.4. Gene prediction and CAZymes annotation

The four assembled sequences were first submitted to Prodigal (PROkaryotic DYnamic Programming Genefinding ALgorithm), version 2.6.3, to anticipate protein-coding genes associated with bacterial and archaeal genomes in GFF3 (General Feature Format) file format (Hyatt et al., 2010). CAZymes annotation was then done using dbCAN2 tool, which integrates three state-of-the-art tools; (i) HMMER searches against the dbCAN HMM (hidden Markov model) database; (ii) DIAMOND searches against the CAZy pre-annotated CAZyme sequence

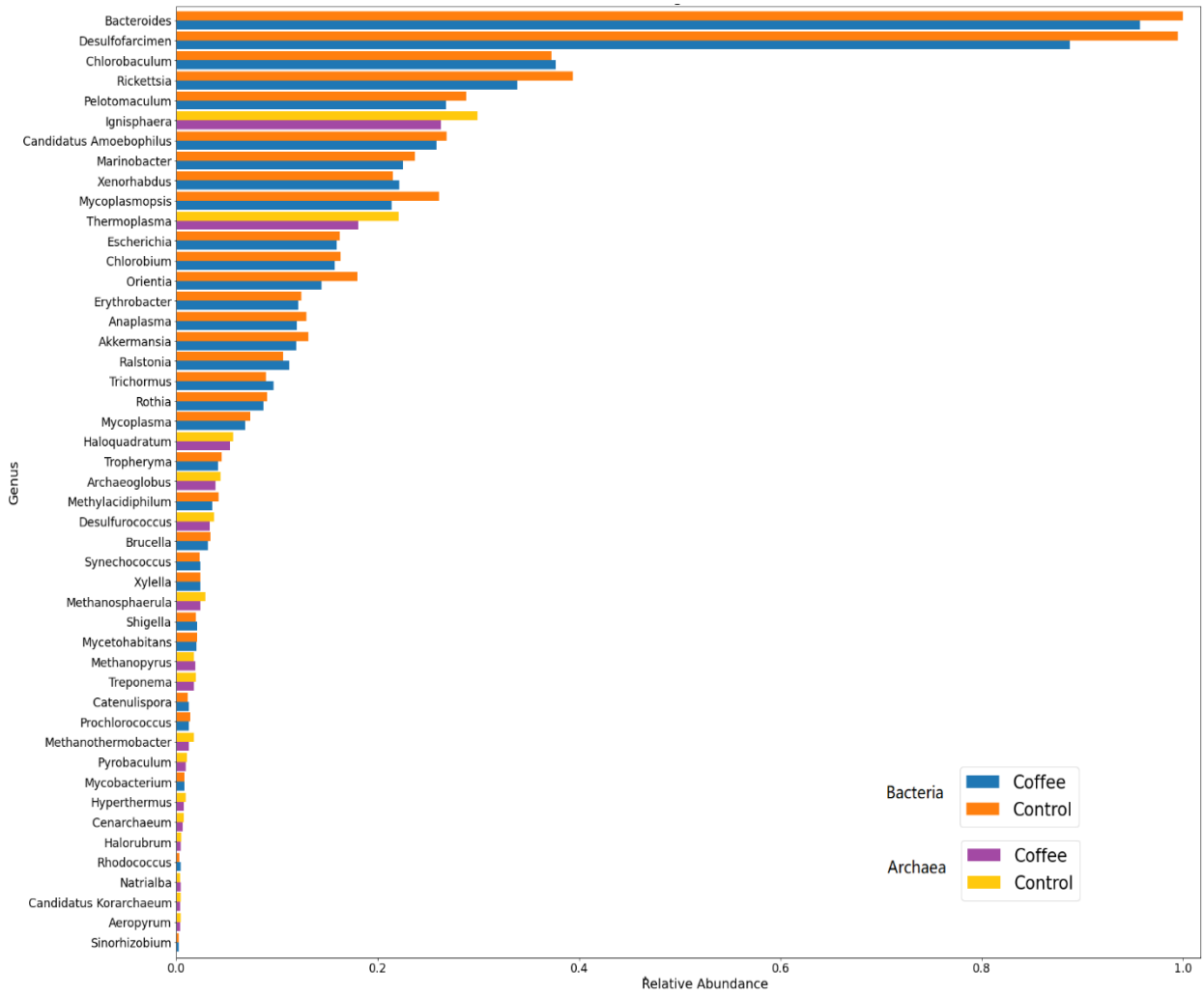
454 database and (iii) Hotpep searches against the conserved CAZyme short peptide database
455 (Zhang et al., 2018). The predicted CAZymes are divided into five classes: carbohydrate-
456 binding modules (CBMs), carbohydrate esterases (CEs), glycoside hydrolases (GHs),
457 glycosyltransferases (GTs) and polysaccharide lyases (PLs). In our study, we only considered
458 those CAZymes that were anticipated by the three tools for the same gene. The results of this
459 step are threefold:

460 **6.4.1. Microbial community abundance**

461 **(Fig. 6)** reveals the microbiome relative abundance profile per genus in coffee
462 consumer's vs controls. We can note that *Bacteroides* and *Ignisphaera* represent the most
463 abundant genus for Bacteria and Archaea, respectively. No clear association between archaea
464 and human disease has been described to date (Eckburg et al., 2003), therefore and from here,
465 we only discuss results related to Bacteria.

466 Compared to the group of non- coffee consumers, there are remarkable decrease in
467 abundance of many genera such as; *Bacteroides, Desulfofarcimen* and *Mycoplasma*.
468 Decreasing in the relative abundance of *Bacteroides* was indeed reported in literature
469 (Stalmach et al., 2010) and in (**Table 3**). The fact that coffee causes a decrease in abundance
470 in either *Desulfofarcimen* or *Mycoplasma* is our new finding, which is not reported before in
471 the literature and suggestive that our metagenomics in identifying novel hits. Sulfate-reducing
472 bacteria (SRB) plays an important role in intestinal hydrogen and sulfur metabolism. IBD is
473 linked to the increase of intestinal H_2S. *Desulfofarcimen* is a SRB and decrease in its abundance
474 led to an obvious reduction of sulfate (Watanabe et al., 2018). *Mycoplasma* species were found
475 in patients with neurodegenerative diseases and behavioral disorders (Garth, 2007) and they
476 also cause inflammatory diseases, including IBD (Chen et al., 2001). As a result, these findings
477 confirm once more that coffee consumption helps in decreasing the risk of PD (Moco et al.,
478 2012) and IBS (Singh et al., 2019), as previously discussed in sections 5.1 and 5.4.

479 On the other hand, there is a slight increase in the abundance of *Ralstonia* and
480 *Trichormus*. The *Ralstonia solanacearum* produces extracellular polysaccharide (EPS)
481 (Milling et al., 2011) that can increase certain host immune responses in mammalian gut
482 (Makino et al., 2006), while the *Anabaena azollae* bacteria (*Trichormus*) was used as a
483 biofertilizer in coffee plantation (Anand, et al 2006). Further studies should be conducted to
484 determine the relationship between *Ralstonia* and *Trichormus* with the human gut.



485

486 **Fig. 6**. Microbial relative abundance per kingdom, genus and class (coffee vs control)

487 **6.4.2. Microbial CAZymes abundance**

488 **Fig.7** depicts the presence and absence of CAZymes' composition per class group. As
489 can be noted that GH (71.05%) and GT (17.15%) are the most abundant CAZyme families
490 overall. The relative abundance of the CBM, CE, GH, GT and PL families differed significantly
491 between the coffee and control samples. The 6 most abundant CAZyme subfamilies (GH2,
492 GT2, GH3, GH13, GH43 and GH92) are present in both groups. However, coffee presents only
493 enrichment of the GH92 subfamily and reduction of the remaining subfamilies in comparison
494 to the control group. It was also clear that coffee samples lack of CBM3, CBM50, CBM61,
495 CBM78, GH11, GH44, GH87 and GH128 subfamilies (green boxes), and the CBM76, GT1,
496 GT10, GT25, GT38, GT107, GH121 and PL6 subfamilies are absent33 in control samples (red

boxes) and only present in coffee samples. Theses CAZyome profiles represent the gut microbiome signatures that help to discriminate between coffee and control samples.

6.4.3. Prediction of enzymatic functions related to coffee

Our experiments reported that there were 11 CAZyme subfamily-groups encountered only in coffee samples to include: GH5_17, CBM76_1, GH13_3, GH13_17, GH3_32, GH13_74, GH121_1, GT25_8, GT38_1, GT107_1 and PL6_1. These functionally relevant groups of proteins and their corresponding enzymatic functions (EC numbers) are detailed in (**Table 3**). The colon in the "EC Number" column refers to the sum of the number of conserved peptides in each characterized protein in the group. The higher the value, the more proteins in the group have the enzymatic activity represented by the EC number (Busk et al., 2017). In family GH5 group 17 there are 191 conserved peptide matches to enzymes characterized as mannan endo-β-1, 4-mannosidase (EC 3.2.1.78). GH5_17 were found in the two samples SRR3313057 (57) and SRR3313090 (90). Mannan benefits to the human health were discussed in section 5.2.

Cellulase (3.2.1.4) performs hydrolysis of cellulose during drying of coffee beans and it is used as a treatment for phytobezoars (Kramer & Pochapin, 2012). A xyloglucan-specific endo-β-1, 4-glucanase (EC 3.2.1.151) is an enzyme that catalyses the chemical reaction and it is used in feed applications with the objective to digest substrates that cannot be hydrolysed by endogenous enzymes. α-1,4-glucan: phosphate α-maltosyltransferase (EC 2.4.99.16) is the defining enzyme of a bacterial α-glucan biosynthetic pathway and is a genetically validated anti-tuberculosis drug target (Syson et al., 2011).

Pullulanase (EC 3.2.1.41) belongs to α-amylase class of enzymes and it is used in the starch processing industries and the production of ethanol and sweeteners (Print et al., 2015). Starches contribute, in the upper human gut, in the transport of probiotic organisms thus encouraging the immune response and suppressing potential pathogens (Murillo et al., 2015). α-Amylase (EC 3.2.1.1) is a digestive and anti-diabetes (Proença et al., 2019) enzyme that has the responsibility of helping human body process carbohydrates into simple sugars, providing it with more energy.

The GH13_18 matches sucrose phosphorylase enzyme (EC 2.4.1.7). The majority of GH13 18 is found in the lactic acid bacteria group, which is well-known for its numerous health

benefits (e.g. probiotic, oral health, etc.) (Tauzin et al., 2019). Coffee produces glycosidases (EC 3.2.1.-) enzymes, which contribute to solve many health problems. Deficiency of glycosidases can result in lactose intolerance or lysosomal storage diseases. The most common lysosomal storage disease is called Gaucher's disease. This disorder is characterized by anaemia, liver/spleen enlargements, progressive brain damage, and seizures (Ngo, 2012). Uridine diphosphate galactose (UDP-galactose) (EC 2.4.1) is an intermediate in the production of polysaccharides, which is the main compound responsible for coffees' prebiotic effect as detailed in section 4.3.

Alpha3-sialyltransferase 3 (EC 2.4.99.-) is a group of enzymes that degrade sialic acids, which are involved in variety of human diseases, including atherosclerosis (Varki, 2008). A poly (α-L-guluronate) lyase (EC 4.2.2.11) is an enzyme that catalyses the chemical reaction and it is produced by several organisms, including bacteria, fungi, viruses, and algae. Some bacteria are industrially essential enzymes used in food, biofuel, and biomedical industries. Dietary alginate has many beneficial health effects as its inclusion in food reduces the rate of nutrient absorption, and potentially lowers risks associated with the glycemic response and/or cardiovascular disease. In general, alginates have shown immunomodulatory, antimicrobial, antioxidant, prebiotic, antihypertensive, antidiabetic, antitumor, anticoagulant, and other activities (Ngo, 2012; Tauzin et al., 2019).

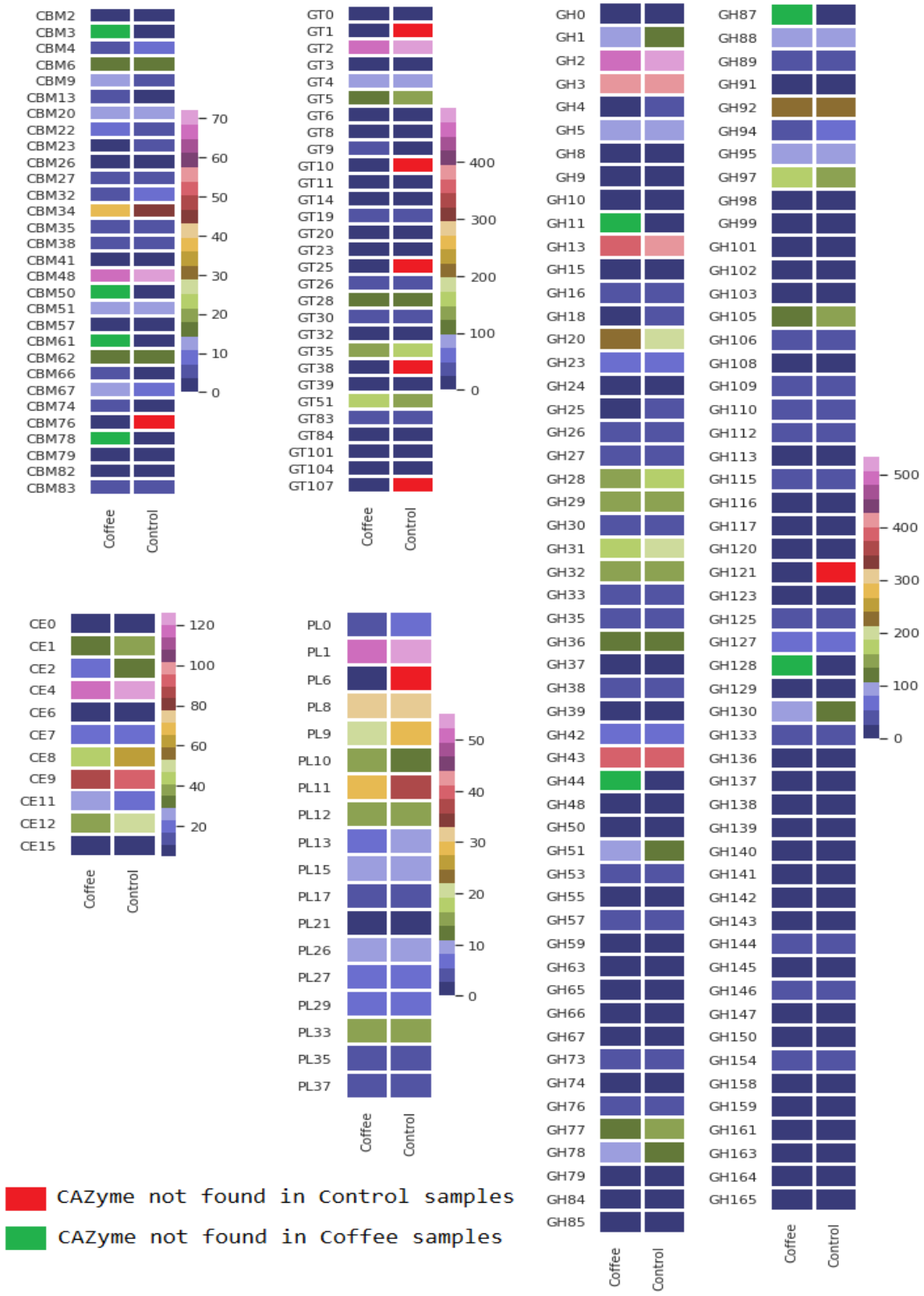
545

Table 3. Predicted enzymes detected as markers in coffee samples

CAZyme Subfamily	Group	File	Species	Genus	Kingdom	EC Number	Enzyme Name
GH5	17	57	*Thermoplasma volcanium* GSS1	*Thermoplasma*	Archaea	3.2.1.78:191	mannan endo-β-1,4-mannosidase
GH5	17	90	*Thermoplasma volcanium* GSS1	*Thermoplasma*	Archaea	3.2.1.78:191	mannan endo-β-1,4-mannosidase
CBM76	1	90	*Escherichia coli* UMN026	*Escherichia*	Bacteria	3.2.1.151:133; 3.2.1.4:128	Xyloglucan-specific endo-beta-1,4-glucanase; Cellulase
GH13	3	57	*Erythrobacter litoralis* HTCC2594	*Erythrobacter*	Bacteria	2.4.99.16:130	α-1,4-glucan: phosphate α-maltosyltransferase
GH13	17	57	*Anaplasma phagocytophilum* HZ	*Anaplasma*	Bacteria	3.2.1.41:150; 3.2.1.-:70	Pullulanase
GH13	32	57	*Chlorobium tepidum* TLS	*Chlorobaculum*	Bacteria	3.2.1.1	α-Amylase
GH13	18	57	*Escherichia coli* UMN026	*Escherichia*	Bacteria	2.4.1.7:70	Sucrose phosphorylase
GH121	1	57	*Chlorobium tepidum* TLS	*Chlorobaculum*	Bacteria	3.2.1.-:24	Glycosidases
GT25	8	57	*Nostoc azollae* 0708	*Trichormus*	Bacteria	2.4.1.-:12	glucosylceramide β-1,4-galactosyltransferase (UDP-galactose)
GT38	1	57	*Orientia tsutsugamushi Boryong*	*Orientia*	Bacteria	2.4.99.-:280	α-3-sialyltransferase 3
GT107	1	57	*Escherichia coli* UMN026	*Escherichia*	Bacteria	2.4.99.-2:39; 2.4.99.-:25	α-3-sialyltransferase 3

PL6	1	57	*Bacteroides fragilis* NCTC 9343	*Bacteroides*	Bacteria	4.2.2.11	poly(α-L-guluronate) lyase / G-specific alginate lyase

546



547

548 **Fig. 7**. Microbial CAZymes abundance per class (Coffee vs Control).

6. Conclusion & future directions

Coffee is consumed as a universal beverage due to its nutritional value and positive physiological effects. Coffee is an important source of many nutritive chemicals including fats, carbohydrates, minerals, vitamins, along with phytonutrients such as caffeine and chlorogenic acid (CGAs). Processing of coffee further extend its chemical composition by providing other new chemicals such as melanoidins of potential health benefit *i.e.*, antioxidant and prebiotic actions though concurrent by a reduction in coffee native CGAs levels. Coffee consumption has also been linked to beneficial effects on the gastrointestinal system and gut microbiota through increasing beneficial bacteria population such as *Bifidobacterium* as mediated *via* its bioactive constituents. Further studies need to be performed to clarify this results being limited by small donor number and to be confirmed for which exact coffee type and or brewing methods. Large-scale comparison regarding coffee different sources and brewing methods on gut homeostasis is still lacking in literature. A complete colonic model, containing pre-digestion of the coffee, would ensure the validity of the results and provide a rapid screening tool for identifying best preparations. Both black and green coffee extracts affect gut microbiota activities *via* the gut microbiome brain axis which in turn affect the host metabolism and to exert protective role against Parkinson's disease, metabolic syndrome, diabetes mellitus Type II, fatty liver, colon cancer and inflammation. Further studies need to be done to explore how to achieve the maximum benefits of these coffee protective actions and what is the recommended daily consumption to achieve such level.

Metagenomics analysis is an important tool for the investigation of the complex microbial communities associated with the human gut. In this study, we present a better understanding of the abundance and diversity of microbial genus in samples of coffee consumers. Our *in silico* results showed that coffee led to a decrease in the abundance of *Desulfofarcimen* or *Mycoplasma genus*, which helped to sustain a healthier human gut. In addition, CAZyme's biomarkers were further annotated to distinguish between coffee and controls samples. For example, a mannan-metabolising enzyme that helps regulating the body weight and lowering blood pressure, glucose levels and cholesterol levels appeared as marker for coffee group. Another avenue for future work is to focus on adaptive diet based on coffee consumption that takes in consideration, genes, sex, age, ethnic, geographical origin and

lifestyle factors with the objective to develop effective and safe nutrition plans that could be tailored to individual variations.

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