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Human gut microbiota fermentation of cooked eggplant, garlic, and onion supports distinct microbial communities

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Abstract

Heating and cooking vegetables not only enhances their palatability but also modifies their chemical structure, which in turn might affect their fermentation by resident gut microbes. Three commonly consumed vegetables that are known to undergo chemical browning, also known as Maillard reaction, during cooking – eggplant, garlic, and onion – were each fried, grilled, or roasted. The cooked vegetables were then subjected to an *in vitro* digestion-fermentation process aimed to simulate the passage of food through the human oro-gastro-intestinal tract. In the last step, the undigested fractions of these foods were anaerobically fermented by the complex human gut microbiota. We assessed the structure of microbial communities maintained on each cooked vegetable by high-throughput 16S rRNA gene amplicon sequencing, measured the levels of furosine, a chemical marker of the Maillard browning reaction, by HPLC, and determined the antioxidant capacities in all samples with ABTS and FRAP methods. Overall, vegetable type had the largest, statistically significant, effect on the microbiota structure followed by the cooking method. Onion fermentation supported a more beneficial community including an expansion of *Bifidobacterium* members and inhibition of Enterobacteriaceae. Fermentation of cooked garlic promoted *Faecalibacterium* growth. Among cooking methods, roasting led to a much higher ratio of beneficial-to-detrimental microbes in comparison with grilling and frying, possibly due to the exclusion of any cooking oil in the cooking process.

Keywords: vegetables, cooking, gut microbiota, antioxidants, human microbiome, furosine

Introduction

Our diet shapes the structure and function of the gut microbiome [1] with contributions from the eaten dietary compounds [2], food additives [3], food preparation methods, and various processing techniques [4]. Long-term dietary patterns as well as sudden shifts in ingested foods were shown to determine the community structure and the functionality of gut microbiota [5, 6]. Recent findings also point towards the role of food preparation methods in affecting the accessibility of dietary compounds to intestinal microbes [4].

Cooking enhances palatability, microbial safety, and flavor profile of the foods. Mechanistically, cooking modifies the physicochemical structure of the foods, which in turn

affects the nutritional properties of the diet [7]. Because cooking has extensive effects on the chemical composition of the foods, it also determines the bio-accessibility of macronutrients and other molecules [8], which in turn affect the gut microbial ecology. For example, cooking can remove harmful-to-microbes molecules introduced by agriculture [9] or naturally occurring phytochemicals such as phenolic acids, capsaicin, and allicin [10]. Cooking can especially affect the phenolic compounds, which are the phytochemicals known to possess antioxidant properties, an important trait of vegetables and related to their effectiveness against oxidative stress [11]. On the one hand, cooking has been shown to increase the bioavailability of phenolic compounds due to cell rupture and leakage of molecules [8]. On the other hand, some of these compounds are degraded during heat treatment [12].

In addition, cooking can chemically generate novel molecules that have either an inhibitory effect on some microbial species such as furanic compounds [13], or a beneficial prebiotic-like effect recently attributed to melanoidins [14, 15]. Both products are generated during the course of the Maillard reaction or chemical browning [13]. Higher cooking temperature and longer time favors the development of Maillard reaction [16], going through different stages that can be monitored by the determination of specific molecules such as furosine, an early stage Maillard reaction indicator [10].

Accordingly, different cooking methods as well as different cooking parameters (time, temperature) are bound to have an effect on gut microbial ecology [9]. For example, subjecting foods to the intense cooking techniques such as grilling and roasting increased the abundances of beneficial gut bacteria such as *Ruminococcus spp.* and *Bifidobacterium spp.* compared to the foods that had been boiled [4, 17]. In fried meats, *Clostridium* spp. showed higher abundance than in their boiled counterpart [18]. Toasting bread impaired the growth of several *Clostridium* species, with the magnitude of inhibition associated with toasting time [4]. Melanoidins were also recently shown to influence gut microbiota structure and function [14, 15]. Such effect was specific to the food from which the melanoidins were extracted, linking the biochemical variability of melanoidins with unique microbial responses in the gut.

The standalone studies that explore the impact of different cooking methods of different foods on human gut microbiota have therefore become a vital and relatively unexplored [9] area. Hence, with this research we aimed to answer the following two questions: (1) do different cooking methods applied to the same microbiota-friendly foods (vegetables) result in a different

microbial community, and (2) does longer cooking time (traditionally known as "well done" technique) have any effect? The objective of this study was to infer how different cooking techniques and intensities applied to onion, garlic, and eggplant, three commonly consumed vegetables that differ in their macronutrient contents (Table 1), could affect their availability and fermentation by the human gut microbiota. These three vegetables are usually cooked similarly, contain fair amount of free sugars amenable to Maillard reaction transformation, and contain compounds that have been shown previously to affect gut microbes (phenolic acids in eggplant [12] and allicins in garlic and onion [10]). Finally, to the best of our knowledge, these chosen vegetables have not been studied in this context.

Materials and methods

Vegetables and cooking conditions.

Garlic *(Allium sativum)*, eggplant (*Solanum melongena*), and onion (*Allium cepa* L.) were purchased at local markets in Granada, Spain. Vegetables were washed and peeled. Vegetables were cut in different sizes to achieve comparable texture during cooking. Onions were finely diced (brunoise cutting), eggplants were cut into sticks (julienne cutting) and garlic cloves were crushed. The culinary treatments chosen were grilling (G), roasting (R), and frying (F) (see Table 2 for detailed information about all treatments). Additionally, for each culinary process, two processing times were applied; the usual (normal; No) and the prolonged processing time consisting of a 50% longer cooking exposure (well-done; WD). Extra virgin olive oil (EVOO) was used as a medium for grilling and frying. The utensils used for food preparation were the following: stainless steel spoons, forks, knives, a frying pan, and a portable oven (1500W). All these utensils were purchased from Centro Hogar Sanchez (Granada, Spain). Cooking times and proportions were based on the work of Ramírez-Anaya and colleagues [19] and adapted to our equipment and laboratory conditions. Cooked samples were homogenized and stored under nitrogen atmosphere at -80^{o}C in order to avoid oxidations. All cooking procedures were carried out in duplicate. Note that due to an accident, the collected grilled eggplant (normal cooking time) and grilled garlic (well done) samples were lost, and the described below analyses were not carried out on those samples.

In vitro digestion and fermentation.

All cooked vegetables were subjected to an *in vitro* digestion process followed by an *in vitro* fermentation to mimic physiological processes in the human gut. The *in vitro* digestion method was carried out according to the previously described protocol [20]. The oro-gastro-intestinal (OGI) digestion of each 5g sample was composed of an oral phase (5 minutes at 37^{o}C with alpha-amylase 75 U mL^{-1} and salts mimicking human saliva, pH 7.0, total mixture volume: 10 mL), a gastric phase (2 hours at 37^{o}C with pepsin 2000U mL^{-1} and salts mimicking gastric fluid composition, pH 3.0, total volume 20 mL) and an intestinal phase (2 hours at 37^{o}C with pancreatin 13.4 mg mL^{-1} and 10mM bile salts, pH 7.0, total volume 40 mL). Salt composition of each fluid is provided in Supplementary Table 1. Activities of all enzymes were confirmed prior to the digestion experiments by following the procedure described by Brodkorb et al [12]. Enzyme activity was stopped by immersing OGI digested samples in ice for 15 minutes. Digested samples were centrifuged, after which the 90% of the supernatant (fraction approximated to be absorbed in the small intestine) was removed, and the remaining 10% of the soluble fraction together with the entire pellet (undigested fraction that would reach the colon) was used for an *in vitro* fermentation.

Fecal samples from four healthy adult donors were collected and mixed together to account for the inter-individual variation in fecal microbiota composition. The collection of fecal material has been approved by Wright State University Institutional Review Board (protocol 06360), and all donors consented to the stool collection. The exclusion criteria were (1) taking antibiotics or probiotics within the three months before the start of the study, (2) a diagnosis of chronic gastrointestinal disorders or any other chronic disease, and (3) not following a standard omnivorous diet. The body mass index of the participants was within the range of 21.3-23.1. The *in vitro* fermentation was performed in oscillating, sealed 15-mL tubes at 37ºC for 20 hours. For this procedure, we used 0.5 grams of the pellet obtained after the OGI digestion, and 10% of the OGI supernatant as we did previously [21]. A total of 7.5 mL of fermentation medium consisting of peptone (14 g l^{-1}), cysteine (312 mg l^{-1}), hydrogen sulfide (312 mg l^{-1}), and resazurin (0.1% v/v) was added to each fermentation tube. Fecal material was mixed with sterile phosphate buffered saline at a 1:2 ratio. Two mL of this inoculum were added to the fermentation tube. Nitrogen gas was then bubbled into the tubes for at least 2 minutes to create an anaerobic environment. Anaerobicity of the tubes was assured by the color change of the resazurin indicator [22]. After 20 hours of incubation at 37ºC, microbial activity was slowed by immersing the tubes in ice for 15

minutes, followed by the centrifugation of the tubes to collect the pellets. The latter were stored at -80ºC until further analysis.

Microbiota profiling.

Prokaryotic genomic DNA (gDNA) was isolated from fermented samples using ZR bacterial/fungal DNA kit (Zymo Research, USA) following manufacturer's instructions. The V1V2 hypervariable region of the prokaryotic 16S rRNA gene was amplified using complementary 16S gene sequence AGRGTTYGATYMTGGCTCAG as the forward primer and 16S gene complementary sequence GCWGCCWCCCGTAGGWGT as the reverse primer. Forward primer incorporated a 6-7 nucleotide barcode for sample multiplexing on the sequencer. In PCR amplifications, 25 ng of the starting gDNA material was first subjected to 4 cycles of linear elongation with the forward primer only in order to reduce sample-to-sample PCR bias [23], followed by 25 cycles of traditional exponential PCR. Amplicon sequencing was carried out on the Ion Torrent Personal Genome Machine (Thermo Fisher) using a 318 Chip v2. After quality filtering, we obtained 12,106 reads per sample on average (minimum 8,637, maximum 14,687 reads). All sequence reads were processed in QIIME [24] following our standard pipeline [25] to obtain the 16S rRNA gene copy number adjusted, rarefied taxon counts. The dataset of cumulative genus counts among all samples is provided in Supplementary Table S2. This final dataset was used for all downstream analyses using R, MATLAB, and PYTHON scripts that are described elsewhere [26]. Statistical tests (one-way analysis of variance (ANOVA) unless otherwise stated) were carried out in SPSS v19.

Furosine assay.

Furosine is a chemical that is formed in the beginning of the multi-step Maillard reaction, and furosine levels correlate with the thermal damage of foods during cooking [4]. Furosine determination was performed following the previously described method [27]. Fermented samples (0.125 g) were added to 4 mL of 7.95M HCl, high-purity N_2 gas was bubbled through the solution for 2 mins, and the samples were hydrolyzed at 120°C for 23 hours in a Pyrex screw-cap vial with PTFE-faced septum. The hydrolysate was filtered with a medium-grade paper filter. A 0.5 mL portion of the filtrate was applied to a Sep-Pak C_{18} cartridge (Waters, Milford, USA) pre-wetted with 5 mL of methanol and 10 mL of deionized water, and was then eluted with 3 mL of

3M HCl. A 50 µl portion of the solution was analyzed using ion-pair RP-HPLC, which consisted of a Waters model 600 quaternary gradient bomb and a UV/VIS detector model 200 (Konik, Barcelona, Spain) set to 280 nm. The analytical column was a C_{18} column (250 mm × 4.6 mm I.D., Altech) maintained at 32°C. The mobile phase was 80:20 ratio of water : acetonitrile containing 5 mM sodium heptanosulphonate, and 0.1% of formic acid. Running time was 15 min. Quantification was performed by the external standard method, using a commercial standard of pure furosine, within the range 0.01−1000 mg L^{-1}. The analysis was performed in duplicate and the results were expressed as µg of furosine per gram of sample.

Antioxidant measurements.

Radical scavenging capacity and Fe (III) reducing capacity of samples were measured using ABTS (2,29-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) and FRAP (ferric reducing ability of plasma) methods, respectively, as described below.

ABTS. The ABTS radical scavenging capacity was determined following the described procedure [28]. Briefly, ABTS radicals were produced by reacting an ABTS stock solution (7 mM) with potassium persulphate (2.45 mM) and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. This ABTS:persulfate solution was diluted with ethanol and water mixture (50:50 v/v) to an absorbance of 0.70 ± 0.02 measured at 730 nm wavelength. After mixing 20 µL of sample or Trolox standard and 280 µL of diluted ABTS:persulfate solution in the wells of a transparent 96-well polystyrene microplate (Biogen Científica, Spain), the antioxidant reaction was monitored on a FLUOStar Omega microplate reader equilibrated to 37ºC. Calibration was performed with a Trolox stock solution ranging from 0.01 to 1.0 mg mL^{-1}. The results were expressed as mmol Trolox equivalents per kg of sample.

FRAP. The Fe (III) reducing capacity was measured as originally described [29]. The procedure was adapted to a microplate reader (FLUOStar Omega, BMG Labtech). Briefly, 20 µl of each sample were placed in duplicate in the 96-well microplate and mixed with 280 µl of freshly prepared FRAP reagent (25 mL of 0.3 mM sodium acetate pH 3.6, 2.5 mL of 20 mM ferric chloride and 2.5 mL of 40 mM TPTZ). The antioxidant reaction was monitored at 37ºC. Trolox was used to construct a calibration curve ranging from 0.01 to 4.0 mg of Trolox mL^{-1}. Results were expressed as mmol Trolox equivalents per kg of sample.

Prebiotic Index calculation.

We calculated an expanded version of microbiota Prebiotic Index (PI, [30]) for all samples. Total beneficial microbes consisted of combined abundances of *Akkermansia*, *Bifidobacterium*, *Eubacterium*, *Faecalibacterium*, *Lactobacillus*, *Roseburia*, and probiotic *Streptococcus*. Total detrimental microbes combined the abundances of *Clostridioles difficile*, Desulfovibrionaceae, Enterobacteriaceae, *Fusobacterium*, and *Helicobacter*.

Results

Fermentation of cooked eggplant, garlic, and onion supports different communities of human gut microbiota.

Using a combination of *in vitro* oro-gastro-intestinal enzymatic digestion followed by batch culture anaerobic microbial fermentation, we assessed the structure of human gut-derived microbiota communities grown on three cooked vegetables – eggplant, garlic, and onion. These vegetables are commonly consumed, are usually cooked with the same culinary techniques, and undergo Maillard reaction during cooking [31]. At the same time, the chosen vegetables have a range of macronutrient distributions and contents as shown in Table 1.

Canonical correspondence ordination analysis (CCA) was employed to compare the relative contributions of vegetable type, cooking method, and cooking duration to the overall variance of the microbiota community structure (Figure 1A). Vegetable type had the highest impact on microbiota structure ($p<0.001$), followed by cooking method ($p<0.001$) and cooking duration ($p<0.05$). A number of different microbial genera were associated with each vegetable as shown in Figure 1A, though most of these were numerically minor members of the microbial communities.

Furosine and antioxidants are weakly associated with microbiota structure.

We have measured the levels of furosine (an indicator of the early steps of the Maillard reaction) and total antioxidant capacity in the same set of samples (Table 3). As hypothesized, the levels of furosine were consistently higher in the well-done samples compared to their normally cooked counterparts because prolonged exposure of foods to high temperatures should lead to an increased formation of Maillard reaction products. Though the variability of furosine amounts

among different samples was high (coefficient of variation CV=117.7%), fried samples on average contained higher furosine levels than roasted and grilled samples, possibly because the carbonyl group of fatty acids in the extra virgin olive oil reacted with an amino group of amino acids to form Amadori products [32]. Antioxidant (AOX) levels showed much lower variation among different samples (CV 21.2% for AOX_{ABTS} and 31.4% for AOX_{FRAP}; see Table 3). In comparison to the CCA analysis described above, UniFrac phylogenetic distance-based redundancy ordination analysis (dbRDA) utilizing furosine and AOX values as continuous explanatory variables indicated that furosine and antioxidant amounts were only weakly associated with the microbiota structure (Figure 1B). Samples were largely distributed in the RDA space according to the cooked vegetable type, even though it was not used as one of the explanatory variables in this analysis.

Differences in the human gut microbiota structure among samples.

Overall, there were no statistically significant differences among sample groups in microbial community richness (based on the calculation of the Shannon's H' index) and evenness (Simpson's E index) established by the one-way ANOVA evaluation of the genus abundance dataset (data not shown). However, specific taxa displayed a differential abundance among the subsets of samples as shown in Figures 2A and 2B. Based on ANOVA tests, classes Actinobacteria and Gammaproteobacteria differed in their abundance among different vegetable types, with Actinobacteria being more prevalent in fermented onion samples, and Gammaproteobacteria prevailing in eggplant samples. Erysipelotrichia were statistically more abundant in roasted samples, and Verrucomicrobia increased with longer cooking duration (Figure 2A).

These findings at the microbial class level were also reflected in the genus abundance dataset. The levels of *Bifidobacterium* (class Actinobacteria), *Escherichia*/*Shigella* (class Gammaproteobacteria), *Faecalibacterium*, *Ruminococcus* (both class Clostridia), *Parabacteroides*, and *Phocaeicola* (both class Bacteroidia) were statistically significantly different among the fermented vegetables ($p_{ANOVA} < 0.01$, see Figure 2B). Additionally, *Coprococcus* (class Clostridia) abundance depended on the cooking method (3.8%, 4.0% and 2.0% relative abundance among F, G, and R samples, respectively; $p_{ANOVA} < 0.001$), whereas the

level of *Akkermansia* (class Verrucomicrobia) was higher in the well-done samples compared to the normally cooked ones (9.1% vs 5.0%, $p_{ANOVA} = 0.005$).

The abundances of the above-mentioned genera among different sets of samples are displayed in more detail on the violin plots shown in Figure 3A. Fermentation of the cooked onion supported the expansion of *Bifidobacterium*; eggplant fermentation favored *Escherichia*, *Phocaeicola*, and *Ruminococcus*; and garlic promoted the growth of *Faecalibacterium* and *Enterobacter* (class Gammaproteobacteria). Fermentation of the roasted samples benefitted *Fusicatenibacter* (class Clostridia) but inhibited *Coprococcus* (Figure 3A).

Onion fermentation and roasting promote highly beneficial microbiota structure.

We calculated an expanded version of the gut microbiota Prebiotic Index (PI, [30]) for all samples, which is visualized in Figure 3B. The fermentation of cooked onion promoted a strongly beneficial community (beneficial-to-detrimental PI ratio of 4.0, $p < 0.001$), primarily due to its support of *Akkermansia* and *Bifidobacterium* members and its inhibition of Enterobacteriaceae numbers (see Figure 3A). Garlic samples also sustained a beneficial community structure (2.1 PI ratio, $p < 0.01$) via the expansion of *Faecalibacterium*. Eggplant fermentation was much less beneficial (PI ratio of 1.1) due to its maintenance of higher Enterobacteriaceae.

Among the cooking methods, fermenting roasted vegetables promoted by far the most beneficial community (4.0 PI ratio, $p < 0.001$) due to its support of *Akkermansia*, *Bifidobacterium*, and *Faecalibacterium* members (Figure 3B). Because fermentation of well-done samples maintained higher *Akkermansia* and *Bifidobacterium*, this led to a higher Prebiotic index value (2.8 PI ratio vs 1.5 for normally cooked samples).

Discussion and conclusions

Most consumed vegetables are only partially digested in the upper gastrointestinal tract and become available to the colonic microbiota. By simulating the enzymatic oro-gastro-intestinal digestion process *in vitro* in combination with the digest fermentation by the human distal gut microbiota, we assessed the impact of each cooked vegetable on the microbiota community structure. Overall, each vegetable promoted a unique microbial community (see Figure 1A). The type of vegetable affected microbiota more than the cooking method or cooking duration, likely

because each vegetable provided a unique combination of nutrients to microbiota, thus promoting different community members. The amount of dietary fiber, a food component uniquely available for fermentation by gut microbes, is higher in onion compared to the other two vegetables (see Table 1). It is therefore not surprising that among the three cooked vegetables onion appears to stimulate the most beneficial community structure (see Figure 3B) by promoting the expansion of *Bifidobacterium* and *Akkermansia*, gut microbial taxa with known beneficial properties [33, 34], while limiting the growth of Enterobacteriaceae that comprise many pathogenic members [35]. In contrast, the raw eggplant contains the highest macronutrient fraction of proteins and fats based on the available nutritional information (30% in eggplant compared to 18% in garlic and 15% in onion, see Table 1), and this was associated with less beneficial microbial community structure due to the growth of Enterobacteriaceae on the cooked eggplant samples.

Microbiota structure also depended on the cooking method applied to the tested vegetables (Figures 1A and 3A). Roasting, which did not use any oils for cooking, promoted a highly beneficial community, whereas the ratio of "good" to "bad" microbes was much lower for grilled and especially for fried samples (Figure 3B). We have shown previously that fermentation of fatty acids including unsaturated oils present in the olive oil promoted the expansion of *Bilophila* and enteric bacteria, considered detrimental to human health [36]. At the same time, oleic and linoleic acids which constitute approximately 70% and 10% of EVOO contents, respectively, inhibit the growth of probiotic *Bifidobacterium* [22]. Our present data are consistent with those findings.

Increasing the cooking time by 50% generally had the lowest effect on the overall community structure (Figure 1A). Nevertheless, the extended cooking promoted higher *Akkermansia* counts and as a result showed a better ratio of beneficial to detrimental microbes in comparison with normally cooked samples (Figure 3). As could be expected, longer cooking time increased the levels of furosine, an indicator of the early steps of the Maillard reaction, in most samples (Table 3). Intermediates formed in the early Maillard reaction steps such as fructosyl-lysine, glucosyl-lysine, hydroxymethylfurfural, and furfural can be metabolized by certain bacteria and can thus provide additional fuel to these organisms [37, 38]. However, we did not find evidence for the encoding of the corresponding enzymatic reactions in the reference genomes of *Akkermansia muciniphila* and *Akkermansia glycaniphila* available in the METACYC

and KEGG genome databases. Thus, it is not clear whether *Akkermansia* is capable of utilizing these Maillard compounds directly.

A potential limitation of this study is that, due to an accident, two samples (grilled eggplant - normal cooking time and grilled garlic - well done) were lost before they could be analyzed. Because we could not generate new samples without potentially introducing a confounding factor (e.g., due to the use of different batch of olive oil, vegetable variety, etc), a decision was made not to remake these samples at a later time. Thus, we could not obtain microbial and metabolite data for these two combinations of the vegetable, cooking type, and cooking length out of the total of 18 combinations. All other combinations were analyzed in replicates, and the measured values had low spread between duplicates (see Table 3 for examples). Robust statistical approaches have been utilized throughout our analyses to test the significance of any differences as shown, and the slightly decreased N value did not have a profound effect on our ability to confirm our findings statistically.

In conclusion, we provide evidence that not only the type of consumed vegetables but also how they are cooked can have a substantial impact on the gut microbial community. Due to the close relationship between diet and gut microbiota, these results open a door for future personalized nutrition strategies, where not only specific foods are recommended but also how they should be prepared. However, this study also comes with its limitations mainly related to the limited number of vegetables investigated, and that it was carried out under *in vitro* conditions. Therefore, further studies translating our findings into clinical and *in vivo* settings are needed.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Sumudu Rajakaruna: Investigation, Methodology, Formal analysis, Writing – original draft. Sergio Pérez-Burillo: Investigation, Methodology, Formal analysis, Writing – original draft. José Ángel Rufián-Henares: Conceptualization, Funding acquisition, Writing - review & editing. Oleg Paliy: Formal analysis, Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

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Tables

Table 1. Macronutrient content of studied vegetables [*], per 100 g

Vegetable	Eggplant	Garlic	Onion
Energy, kcal	15	138	44
Carbohydrates, g	3.4	28.4	8.6
Sugars	2.0	8.5	5.1
Dietary fiber	1.4	1.0	3.1
Protein, g	1.2	6.1	1.4
Fat, g	0.2	0.1	0.1

[*] Source: Stance4Health food database [39].

Table 2. Cooking conditions

Cooking method	Grilling	Frying	Roasting
Temperature	220-250^{o}C	180^{o}C	180^{o}C
Duration (normal) [*]	3+3 mins	8 mins	10 mins
EVOO[†]:vegetables	0.5:1	5:1	-

[*] Cooking times for well-done aliquots were increased by 50%

[†] EVOO = extra virgin olive oil

Table 3. Measurements of furosine and anti-oxidant capacities (AOX) in all samples [*].

Vegetable	Cooking method	Cooking time	Furosine	AOX_{ABTS}	AOX_{FRAP}
Eggplant	Roasted	Normal	7.06±0.27	8.41±0.56	3.14±0.18
Eggplant	Roasted	Well-done	6.07±0.06	12.03±0.54	1.85±0.11
Eggplant	Fried	Normal	113.99±2.40	6.80±1.08	3.72±0.10
Eggplant	Fried	Well-done	123.17±3.38	13.47±0.67	3.57±0.17
Eggplant	Grilled	Well-done	29.38±0.98	7.42±0.20	1.56±0.15
Garlic	Roasted	Normal	9.93±0.12	12.79±1.09	1.67±0.15
Garlic	Roasted	Well-done	11.70±0.69	13.00±0.02	2.44±0.17
Garlic	Fried	Normal	6.32±0.47	13.37±0.48	1.45±0.31
Garlic	Fried	Well-done	22.03±0.95	11.04±0.08	3.10±0.03
Garlic	Grilled	Normal	10.23±0.51	11.90±0.21	2.64±0.20
Onion	Roasted	Normal	0.95±0.03	11.08±0.19	2.12±0.13
Onion	Roasted	Well-done	50.08±1.33	9.79±0.36	1.66±0.18
Onion	Fried	Normal	15.23±0.19	7.95±0.55	1.75±0.27
Onion	Fried	Well-done	38.34±0.69	8.21±0.34	2.34±0.01
Onion	Grilled	Normal	9.95±0.45	11.11±0.17	1.78±0.06
Onion	Grilled	Well-done	42.09±0.26	9.59±0.27	2.28±0.08

* Data are shown as arithmetic mean ± standard deviation.

Figure legends

Figure 1. Constrained ordination analyses of the genus abundance dataset. Panels A and B display the output of the canonical correspondence analysis (CCA, panel A) and UniFrac phylogenetic distance-based redundancy analysis (dbRDA, panel B). For CCA, vegetable type, cooking method, and cooking time were used as categorical explanatory variables that constrained the variability of each dataset. For dbRDA, measurements of furosine and antioxidant capacity served as continuous explanatory variables. The percent of dataset variability explained by each axis is shown in parentheses in axis titles. Samples are colored according to the vegetable type. For constraining categorical variables, the position of each class centroid is indicated with a diamond. For constraining continuous variables, the arrows indicate the vector of that variable gradient in the reduced space, and the arrow length is proportionate to the amount of the variance explained. CCA triplot laos visualizes the position of genera (shown as small green cicrles) in relation to the explanatory variable centroids and samples. The analysis of variance of the CCA and dbRDA outputs provided the relative contribution of explanatory variables to the overall variance of the genus abundance dataset; ***: $p < 0.001$, *: $p < 0.05$.

Figure 2. Comparisons of microbiota community composition among samples. Panel A displays the microbial community structure at the class level. Each stacked column represents the average values among all samples within the corresponding group. Classes with statistically significant difference ($p \leq 0.01$) in their abundance are highlighted with an asterisk. Panel B displays the microbial community structure at the genus level for each sample type for the most abundant genera. Each column represents an average between two independent samples. Classes and genera are ordered according to the phylum (see legends). Genera with statistically significant differences ($p \leq 0.01$) in their abundance among vegetable types are shown with an asterisk in the legend.

Figure 3. Differences in microbiota members among samples. The relative abundances of the statistically differentially abundant genera are illustrated on violin plots shown in panel A. Genera are ordered based on the comparison (first vegetable type, then cooking method, then cooking time) and their phylum, and are abbreviated as follows: Bif - *Bifidobacterium*, Par – *Parabacteroides*, Pho - *Phocaeicola*, Fae – *Faecalibacterium*, Rum – *Ruminococcus*, Ent – *Enterobacter*, Esh – *Escherichia/Shigella*, Cop – *Coprococcus*, Fus – *Fusicatenibacter*, Akk –

Akkermansia. Class coloring follows the color scheme shown in Figure 2. Violins are colored as shown in the legend. Each violin depicts the density distribution of the genus abundances among all samples of that group; vertical bar and white circle represent 25%, 75%, and 50% percentile of values, respectively. Individual abundances are shown as colored circles. Panel B shows the cumulative relative abundances of beneficial and detrimental members of each community as defined by the Prebiotic Index calculation. Error bars display the standard error of the mean. Stars above the comparisons denote the statistical significance of the beneficial-to detrimental ratio: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$.

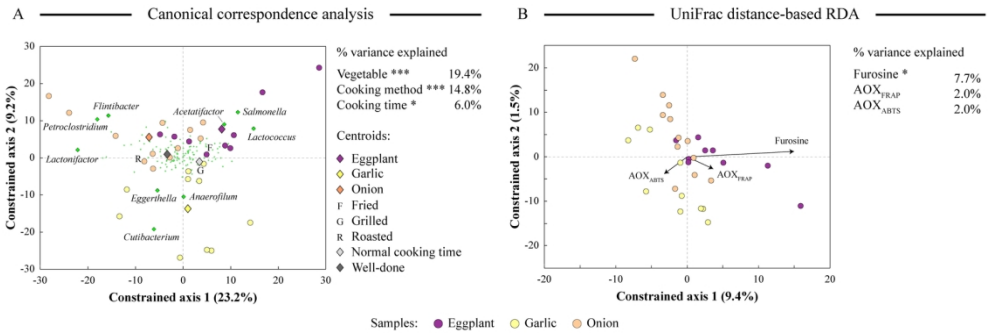

Figure 1

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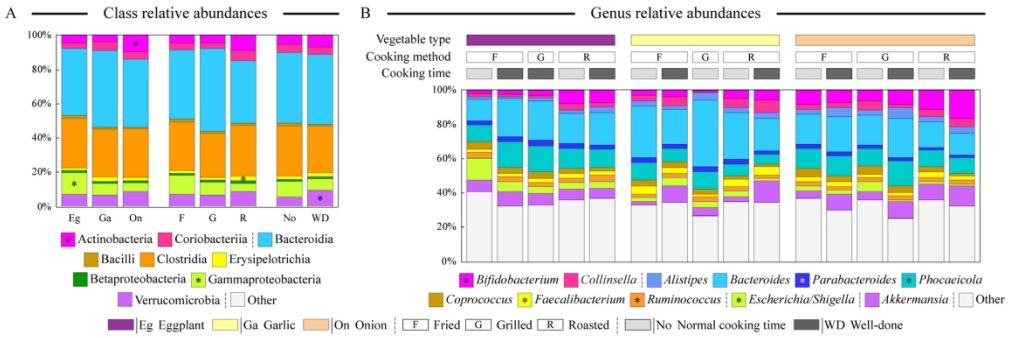

Figure 2

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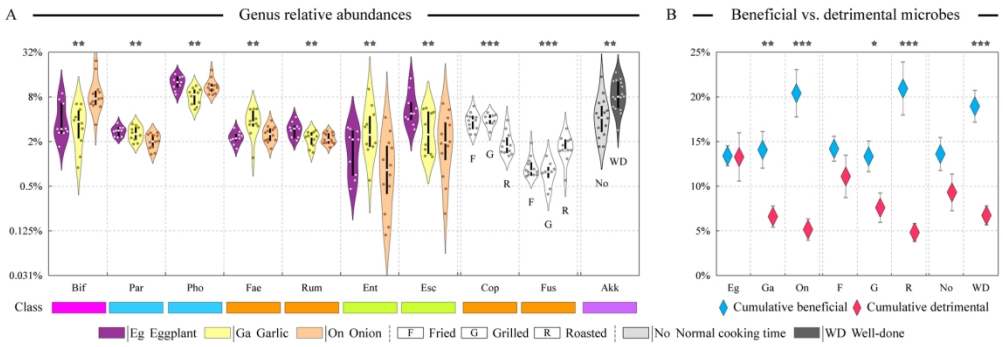

Figure 3

160x54mm (300 x 300 DPI)