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Research Article

Effects of Diet and Flavor on the Gut Microbiota Diverge Between Family Members

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Abstract

In general, it is accepted that diet has one of the greatest impacts on the Gut Microbiota (GM). Diets cooked with various flavor might cause GM shifts. In addition, the effect of diet and flavor might diverge depending on the genotype, sex and age of the host. Clarifying these interactions can reveal the potential health implications associated with diet changes. We explored the effects of diet and flavor on the GM. A family of three members (father, mother, and daughter) was recruited and was reared on a designed experimental diet for 8 weeks. The diet was cooked with salty, sour, sweet and hot flavor ingredients in weeks 2, 4, 6 and 8, respectively, and each flavor ingredient treatment was separated by 1-week washout. Fecal samples were collected before and during experimental-diet and flavor ingredient treatments.

Health indicators (body weight, blood pressure, heart rate) were monitored daily. Blood levels of glucose, lipids and an inflammatory factor (C-reaction protein) were measured at the end of each treatment. Analyses of *16S rRNA* gene sequencing revealed that the experimental diet caused a significant change, salty and hot flavor ingredients caused obvious shifts, while sour and sweet flavor caused minor alterations in the GM. Importantly, the diet and flavor caused GM changes that were highly divergent among the family members. Flavor ingredient treatments also caused fluctuations in most health indicators but the fluctuations were only weakly correlated with GM changes. We concluded that dietary factors interacted with individual factors in driving GM variability.

Keywords: Gut microbiota; Diet; Flavor; Salty; Sour; Sweet; Hot

Introduction

There are thousands of different microbes inhabiting the gut of humans [1]. An individual may have up to several hundred species of microbes, and there is high degree of variation in the composition of the Gut Microbiota (GM) between individuals [2,3]. Gut microbes ferment undigested food components and produce many bioactive compounds that can improve or damage health [4]. There is growing evidence that the GM is associated with diseases not confined to the Gastrointestinal Tract (GIT) but the whole body and even psychiatric problems [5]. Nevertheless, there are many gaps in our understanding of the interactions between environmental factors, host genetics, gut microbes, and health [6]. The origins of individual variations, and the extent to which environmental/dietary factors or host genetics contribute to such variations, are crucial factors [7,8]. Understanding these questions can enable microbial profiles to be modulated. The human diet is often supplemented with specific taste-promoting ingredients, among which salt, vinegar, sugar/sweeteners, and chili peppers are used to generate salty, sour, sweet and hot tastes, respectively. These food seasonings with specific properties or phytochemicals also confer beneficial or harmful effects to health [9-12]. Some people may have one or a combination of two or more flavors supplemented in their daily diet. Other people accustomed to a bland diet may occasionally indulge themselves meals with specific tastes and the long-term and short-term taste preference may result in different health outcomes. However, there are gaps in our understanding of the interactions between taste preference/flavor ingredients, the GM, and health.

There are a few reports on GM changes in rats following a high-sodium diet except for one mini-review [13]. Animal tests and clinical trials on changes in the GM by sweeteners have been reviewed [12]. However, very few common (or even controversial) alterations to GM composition have been noted for rodents reared on a specific diet compared with those given to control-group rodents. Coconut water vinegar has been shown to alter the GM in mice due to an increase in abundance of Bacteroides and Akkermansia genera, which helped to overcome the obesity and inflammation caused by a high-fat diet [14,15]. A pepper-containing diet was reported to increase Lactobacillaceae and Acetobacteraceae abundance in *Drosophila melanogaster* of different genetic backgrounds [16]. Overall, more (especially human) data on GM changes due to taste ingredients is needed for full understanding of the interactions between taste preference/flavor ingredients, the GM, and health.

We explored changes in the GM in humans by diets cooked with salty, sour, sweet and hot flavor ingredients. The GM has been reported to be affected by host genetics, dietary factors, environmental factors, and even minor changes in lifestyle (e.g, sleeping, and exercise) [17-20]. A family of three members was recruited and an experimental

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diet was designed to reduce the variation caused by factors other than host genetics. We also explored diet-genetics interactions in the GM, as well as the correlation between GM profiles and health indicators.

Materials and Methods

Participants

A family of three members was recruited. All family members had a body mass index of 22 kg/m^2. The father and mother were aged 58 years, and the daughter was aged 35 years. The father and daughter were healthy. The mother had hypertension but her blood pressure was controlled to a normal range (systolic blood pressure <90 mmHg, diastolic blood pressure <120 mmHg) by oral administration of nifedipine. All participants were free of metabolic disease or gastrointestinal disease, and not taking antibiotics for ≥ 6 months before or during the study. The characteristics of participants at baseline were shown in (Table S1).

Study design

Participants were asked to complete a questionnaire on diet and living habits. This questionnaire included detailed descriptions of the types and amounts of foods and beverages consumed, smoking, sleeping and exercising. At baseline, fecal samples of all participants were collected three times within 1 week. Then, an experimental diet was created according to the following principles: (i) energy and nutrients were sufficient according to dietary guidelines for Chinese residents; (ii) food diversity was sufficiently rich for all participants to maintain rigorous dietary control for 8 weeks; (iii) the recipe was partially different from previous choices of food but acceptable to each participant (Table S2).

The study had a longitudinal design and lasted 8 weeks. During the study period, all participants were strictly consistent with living habits: they consumed the same experimental diet, beverages, as well as sleeping and exercising habits. The study started with the experimental diet for 1 week. Treatment with salty, sour, sweet and hot flavor ingredients started from weeks 2, 4, 6 and 8, respectively. Each treatment lasted for 1 week and was separated by a 1-week washout period. Fecal samples were collected at days 5, 6 and 7 of each treatment. Blood samples were collected the next morning at the end of each treatment. Body weight, blood pressure, and heart rate were recorded every morning throughout the study (Figure 1A).

Blood analyses

Whole blood (5 mL) was withdrawn from the peripheral circulation. It was centrifuged at 500 × g for 5 min at room temperature, and the supernatant (serum) was collected. The following serum indicators were determined by enzyme-linked immunosorbent assay kits according to manufacturer (Roche Diagnostics, Basel, Switzerland) instructions: glucose (catalog number: 04404483190), total cholesterol (05168538190), triglycerides (05171407190), high-density lipoprotein-cholesterol (05168805190), low-density lipoprotein-cholesterol (07005768190), alanine aminotransferase (05850797190), aspartate aminotransferase (05850819190), urea (05171873190), uric acid (05171857190), creatinine (06407137190) and C-reactive protein (04628918190).

Isolation of fecal microbiota DNA and *16S rRNA* sequencing

Fecal samples were snap-frozen in liquid nitrogen before storage at -80°C. The DNA of fecal microbiota was extracted using a Stool DNA Kit (Guangzhou IGE Biotechnology, Guangzhou, China) and the quality was assessed via gel electrophoresis. For each fecal sample, the V4 region of the *16S rRNA* gene was amplified using a pair of composite primers: forward primer (5′-GTGYCAGCMGCCGCGGTAA XXXXXXgTg(C/T)CAgC(A/C)gCCgCggTAA-3′) and reverse primer (5′-GACTGCAGGACTACNVGGGTWTCTAAT XXXXXXggACTAC(A/g/C/T)(A/C/g)gggT(A/T)TCTAAT-3′). This was a composite of an Illumina linker sequence (underlined), a unique six-base barcode (XXXXXX) and a universal bacterial primer V4F, V4R (italics). For each sample, a Polymerase Chain Reaction (PCR) mix (30 μL) was prepared (with all ingredients from Toyobo, Tokyo, Japan) containing 100 ng of a DNA template, 15 μL of KOD Neo Buffer (2x), 6.4 μL of dNTPs (2 mM), 0.6 μL of KOD Neo DNA Polymerase and 1 μL each of the composite primer pairs (10 μM). PCR conditions were 98°C for 3 min, followed by 20 cycles of 98°C for 45 s, 52°C for 30 s and 68°C for 30 s, and a final extension of 68°C for 5 min. PCR products were subjected to agarose-gel electrophoresis, and amplicons were purified using the Omega Gel Extraction Kit (Omega, Biel, Switzerland). Then, the purified amplicons were quantified and an equal amount DNA was sequenced on an Illumina Miseq platform to generate paired-end reads.

Statistical analyses

The raw pair-end reads were overlapped and merged to get raw tags by FLASH software (v1.2.7). Then, raw tags were filtered to get high-quality clean tags by Trimmomatic software (v0.33). Then,

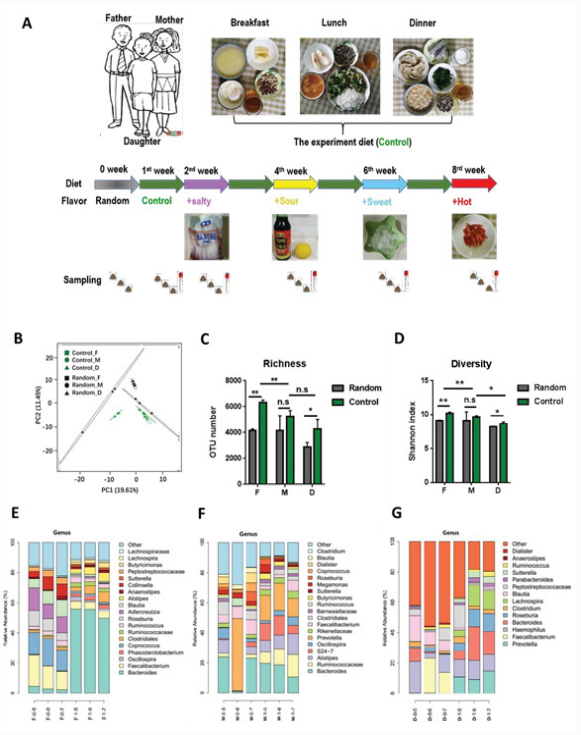

Figure 1: Study design and alterations in the GM profile after consumption of an experimental diet. (A) The study cohort comprised three members of a single family (father, mother, and daughter) conditioned with a new experimental diet for 8 weeks and four flavors with a 1-week interval. Fecal samples were collected at days 5, 6 and 7 of each treatment. Blood samples were collected at the end of each treatment. The Principal Co-ordinates Analysis (PCoA) score was based on Weighted UniFrac distance (B), the total abundance (C), α-diversity/Shannon Index (D), and genus composition (E-G) of all samples. Data were the mean ± SEM. Statistical analyses were according to two-way ANOVA and Bonferroni post hoc tests. n.s. not significant; *P<0.05; **P<0.01.

clean tags were imported into the software package Quantitative Insights into Microbial Ecology 2. Within QIIME, sequences were quality-filtered and denoised using the Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline. Taxonomy was assigned using a classifier (Greengenes) based on 99% sequence similarity to the V4 region of the *16S rRNA* gene. The composition and abundance of each sample at different taxa levels were summarized statistically. Metadata from QIIME was then exported for further analysis in R (V3.3.1). Alpha and beta diversity were computed using a rarefaction depth of sequences. Alpha diversity (Shannon's index and observed features) was evaluated based on the rarefied data and tested by the Wilcox rank-sum method. Beta diversity was calculated using weighted UniFrac distance by the R package VEGAN (V2.5-3), which were visualized further by principal co-ordinates analysis.

Statistical analyses were undertaken using Prism v7.0 (GraphPad, San Diego, CA, USA). Unless indicated otherwise, data were analyzed by two-way repeated-measures ANOVA, followed by Bonferroni post hoc tests. Data were the mean ± SD. $P<0.05$ were considered significant. GM similarity between samples and correlation between GM and health indicators were assessed using spearman correlation analysis.

Results

The GM profile was changed significantly by an experimental diet and the effect diverged among family members

In the week before and every week during the experiment, stool samples were taken from each family member three times. Then, *16S rRNA* gene sequencing was undertaken to analyze the abundance, diversity, and composition of the GM. The experimental diet was partially different and the food richness was increased compared with the diet consumed previously by all family members. The body weight and blood pressure remained stable (Table S1), suggesting that the experimental diet provided balanced nutrition and sufficient energy. The GM profile of the three participants was significantly different before the beginning of the experiment and was changed significantly by consumption of the experimental diet (Figure 1A). The richness and diversity of the GM were decreased by the experimental diet (Figure 1B and C). Reared on the experimental diet, 6 phyla, 11 classes, 13 orders, 24 families, 37 genera, and 34 species were detected by *16S rRNA* gene sequencing from the fecal microbiota of all participants (Figure S1). The intestinal type of the father and mother belonged to Bacteroides and the daughter to Prevotella [21]. When looking at GM composition at the genus level (the level to which can be annotated accurately by *16S rRNA* gene-sequencing data), the experimental diet caused changes that were obviously dependent upon the individual. For the GM of the father, the relative abundance of Bacteroides, Oscillospira, Alistipes, Phascolarctobacterium and Parabacteroides was increased significantly.

However, the relative abundance of Butyricimonas, *Faecalibacterium*, *Roseburia*, *Coprococcus*, *Blautia*, *Adlercreutzia*, *Ruminococcus*, *Collinsella*, *Streptococcus*, *Clostridium* and *Butyricicoccus* was decreased significantly (Figure 1E). For the GM of the mother, the relative abundance of *Alistipes*, *Sutterella*, *Dialister*, *Butyricimonas*, and *Sphingomonas* was increased significantly. However, the relative abundance of *Coprococcus*, *Blautia*, *Adlercreutzia*, *Ruminococcus*, *Collinsella*, and *Gemmiger* was decreased significantly (Figure 1F). For the GM of the daughter, the relative abundance of *Bacteroides*, *Prevotella*, *Roseburia*, *Sutterella*, *Lachnospira*, *Parabacteroides*, and *Megasphaera* was increased significantly. However, the relative abundance of *Faecalibacterium*, *Coprococcus*, *Blautia*, *Clostridium*, *Bifidobacterium*, *Streptococcus*, *Enterobacter*, *Shigella*, and *Escherichia* was decreased significantly (Figure 1G).

Overall, these results suggested that the GM profile changed with consumption of a new diet but that the individual variation was not narrowed down by the same diet. However, if reared on the same diet every day, the daily variation within an individual family member was reduced (Figure S2). This phenomenon reduced the number of interfering factors for subsequent tests on the effect of taste treatments.

The GM profile was altered by a salty flavor ingredient and the effect diverged among family members

To detect the effect of flavor on the GM, the three study participants continued to consume the experimental diet to make flavor ingredient the single dietary factor to be tested. For salty-flavor ingredient treatment, the daily intake of sodium in salt was increased from 2.3 g per person to 5.5 g per person from amount of internationally accepted as being beneficial the highest regional daily intake according to a global survey [22,23]. Blood pressure was increased: it was increased most in the mother, followed by the father (Table S1). These data are consistent with reports stating that older people and people suffering from hypertension are more sensitive to a high-salt diet [24]. These findings demonstrated that our salty-flavor ingredient treatment was effective.

The GM profile of the three study participants was changed by a salty flavor ingredient (Figure 2A). The GM richness of the three family members was changed divergently, among which that of the father was increased significantly (Figure 2B). The GM diversity of the three family members was changed divergently and non-significantly (Figure 2C). At the genus level, for the GM of the father, the relative abundance of *Butyricicoccus*, *Roseburia*, *Anaerostipes*, *Coprococcus*, *Clostridium* and SMB53 was increased significantly, whereas the relative abundance of *Alistipes* and *Butyricimonas* was decreased significantly. For the GM of the mother, the relative abundance of *Oscillospira*, *Clostridium* and SMB53 increased significantly, whereas the relative abundance of *Prevotella*, *Coprococcus*, *Phascolarctobacterium* and *Dialister* decreased significantly. For the GM of the daughter, the relative abundance of *Faecalibacterium*, *Coprococcus*, *Dialister* and *Megasphaera* increased significantly (Figure 2D). These results suggested that the GM profile was changed by a salty flavor ingredient but that the effect diverged among individuals.

The GM profile was altered by sour flavor ingredients and the effect diverged among family members

One week of consumption of an experimental diet was conducted to wash out the effects of a salty flavor ingredient before sour-flavor ingredient treatment. The blood pressure of all study participants returned to initial levels, suggesting that the washout was effective. The GM profile of the three family members was changed slightly by a sour flavor (Figure 3A). The richness and diversity of the GM of the study participants were changed divergently and non-significantly (Figure 3B and C).

At the genus level, for the GM of the father, the relative abundance of Oscillospira, SMB53 and *Akkermansia* was increased significantly, whereas the relative abundance of *Alistipes*, *Butyricimonas* and *Faecalibacterium* was decreased significantly. For the GM of the mother, the relative abundance of *Oscillospira* and *Sutterella* was

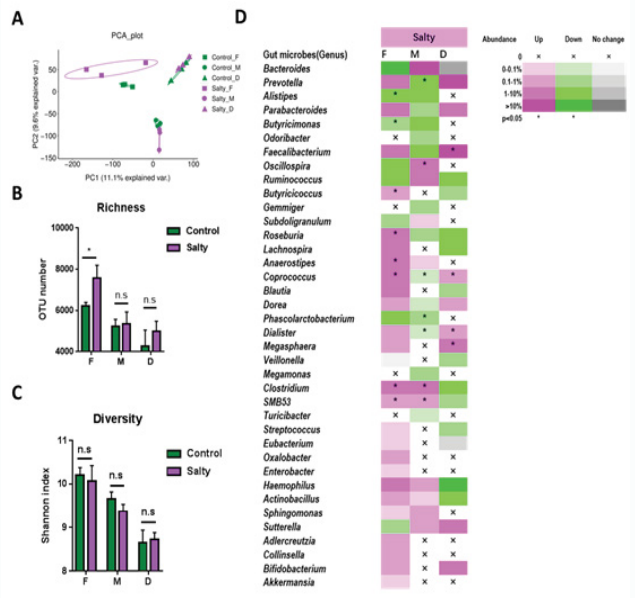

Figure 2: The GM profile was altered by a salty flavor ingredient. The Principal Co-ordinates Analysis (PCoA) score was based on Weighted UniFrac distance (A), the total abundance (B) and microbial α-diversity/ Shannon Index (C) of a control diet versus salty-flavor diet. (D) The GM was altered by a salty flavor ingredient at the genus level. Data were the mean ± SEM. Statistical analyses were according to two-way ANOVAs and Bonferroni post hoc tests. n.s. not significant; *P<0.05.

increased significantly, whereas the relative abundance of *Prevotella* and *Phascolarctobacterium* was decreased significantly. For the GM of the daughter, the relative abundance of *Faecalibacterium*, *Anaerostipes*, *Coprococcus* and *Dialister* increased significantly, whereas the relative abundance of *Megasphaera* and *Bifidobacterium* decreased significantly (Figure 3D). These results suggested that the GM profile was changed by sour flavor ingredients but that the effect diverged among individuals.

The GM profile was altered by a sweet flavor ingredient and the effect diverged among family members

One week of consumption of the experimental diet was conducted to wash out the effects of sour flavor ingredients before sweet-flavor ingredient treatment. The GM profile of the father was changed obviously by the sweet flavor whereas that of the mother and daughter was changed slightly (Figure 4A). The richness and diversity of the GM of the father were decreased significantly, whereas those of the mother and daughter were changed non-significantly (Figure 4B and C). At the genus level, for the GM of the father, the relative abundance of *Oscillospira*, *Akkermansia*, *Phascolarctobacterium* and SMB53 was increased significantly, whereas the relative abundance of *Alistipes*, *Butyricimonas*, *Parabacteroides* and *Blautia* was decreased significantly. For the GM of the mother, the relative abundance of *Oscillospira* and *Sutterella* was increased significantly, whereas the relative abundance of *Prevotella* was decreased significantly. For the GM of the daughter, the relative abundance of *Prevotella*, *Faecalibacterium*, *Anaerostipes*, *Coprococcus* and *Dialister* was increased significantly, whereas the relative abundance of *Lachnospira* and *Megasphaera* was decreased significantly (Figure 4D). These results suggested that the GM profile was changed by a sweet flavor ingredient but the effect diverged among individuals.

The GM profile was altered by hot flavor ingredients and

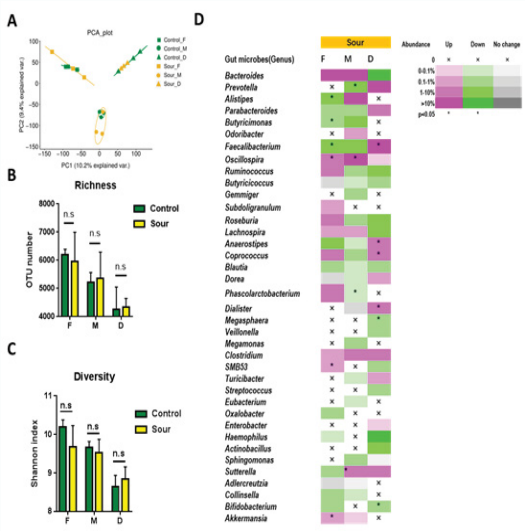

Figure 3: The GM profile was altered by sour flavor ingredients. The Principal Co-ordinates Analysis (PCoA) score was based on Weighted UniFrac distance (A), the total abundance (B) and microbial α-diversity/Shannon Index (C) of the control diet versus a sour-flavor diet. (D) The GM was altered by sour flavor ingredients at the genus level. Data were the mean ± SEM. Statistical analyses were according to two-way ANOVAs and Bonferroni post hoc tests. n.s. not significant; *P<0.05.

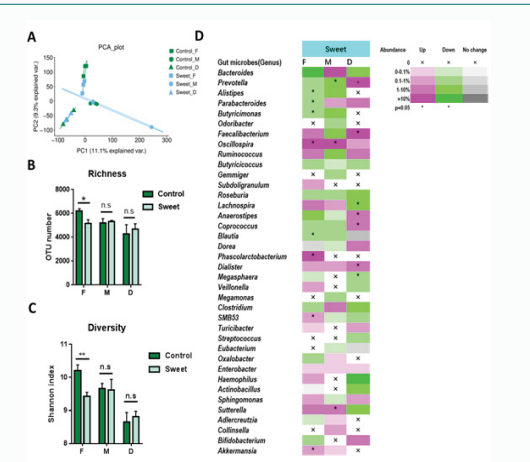

Figure 4: The GM profile was altered by a sweet flavor ingredient. The Principal Co-ordinates Analysis (PCoA) score was based on Weighted UniFrac distance (A), the total abundance (B) and microbial α-diversity/ Shannon Index (C) of a control diet versus a sweet-flavor diet. (D) The GM was altered by a sweet flavor ingredient at the genus level. Data were the mean ± SEM. Statistical analyses were according to two-way ANOVAs and Bonferroni post hoc tests. n.s. not significant; *P<0.05; **P<0.01.

the effect diverged among family members

One week of consumption of the experimental diet was conducted to wash out the effects of a sweet flavor ingredient before hot-flavor treatment. The GM profile of the family members was changed by a hot flavor ingredient (Figure 5A). The GM richness of all study participants was increased (Figure 5B). The GM diversity of the father and mother was decreased, among which that of the father was decreased significantly, but the GM diversity of the daughter was changed non-significantly (Figure 5C).

At the genus level, for the GM of the father, the relative abundance of Subdoligranulum, SMB53, and *Akkermansia* was increased significantly, whereas the relative abundance of *Alistipes*,

Butyricimonas and *Parabacteroides* was decreased significantly. For the GM of the mother, the relative abundance of *Bacteroides*, *Oscillospira* and *Sutterella* increased significantly, whereas the relative abundance of *Prevotella*, *Butyricimonas*, *Parabacteroides* and *Odoribacter* decreased significantly. For the GM of the daughter, the relative abundance of *Faecalibacterium*, *Anaerostipes*, *Coprococcus*, *Dorea*, *Dialister* and *Clostridium* increased significantly, whereas the relative abundance of *Lachnospira*, *Megasphaera* and *Bifidobacterium* decreased significantly (Figure 5D). These results suggested that the GM profile was changed by hot flavor ingredients but the effect diverged among individuals.

Flavor-induced GM alterations had no significant correlation with common health indicators

Weight, blood pressure, and heart rate were monitored daily during consumption of the experimental diet and flavor treatments. Blood samples were taken once at the end of each treatment. Then, levels of glucose, lipids (total cholesterol, triglycerides, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol), an inflammatory factor (C-reactive protein), liver-function indicators (alanine aminotransferase, alanine aminotransferase) and renal-function indicators (serum creatinine, uric acid, urea) were measured. Different taste ingredients had an impact on these health indicators. Salty taste ingredient, in addition to increasing blood pressure significantly, increased blood levels of glucose and low-density lipoprotein-cholesterol, decreased triglyceride levels, and caused divergent changes in other health indicators (Table S1). Sour, sweet, and hot taste ingredients had no significant effect on blood pressure or heart rate, but caused divergent fluctuations in blood levels of glucose, lipids, liver-function indicators, and kidney-function indicators (Table S1). Spearman correlation analyses revealed a very weak correlation between how taste affected the GM and health indicators (Figure 6). However, that the abundance of Oscillospira had a significant positive correlation with expression of C-reactive protein (Figure S3). These results suggested individual variations in the changes of health indicators caused by short-term taste ingredient

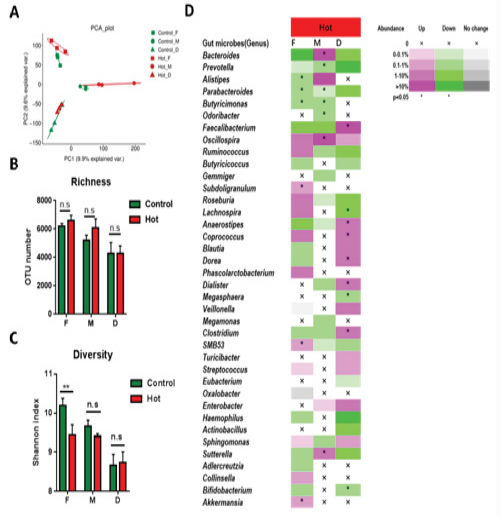

Figure 5: The GM profile was altered by hot flavor ingredients. The Principal Co-ordinates Analysis (PCoA) score was based on Weighted UniFrac distance (A), the total abundance (B) and microbial α-diversity/Shannon Index (C) of a control diet versus a hot-flavor diet. (D) The GM was altered by a hot flavor at the genus level. Data were the mean ± SEM. Statistical analyses were according to two-way ANOVAs and Bonferroni post hoc tests. n.s. not significant; *P<0.05; **P<0.01.

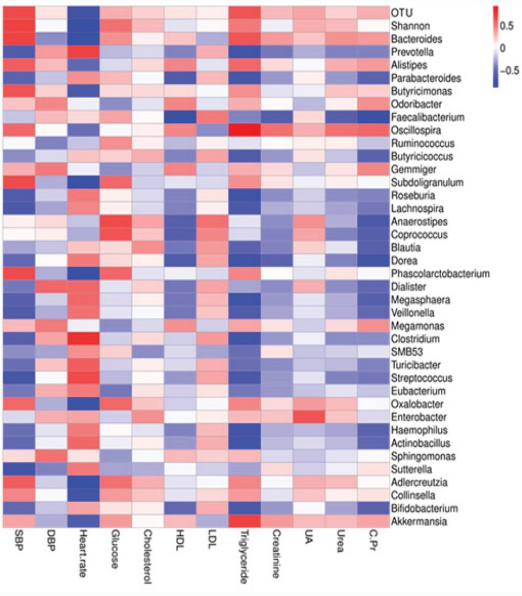

Figure 6: Spearman correlation between flavor ingredients-induced alteration in the GM and common health indicators. Red indicates a positive correlation and blue indicates a negative correlation. OTU, Optical Transform Units.

treatment. However, there was no significant causal relationship between the changes in health indicators and changes in GM caused by taste ingredient testing.

Discussion

Whether host genetics or environmental factors are more important in shaping of the GM, and the stability of the latter, are controversial concepts [25]; on the GM. (COVID-19) outbreak in China (when home isolation was required) the GM [26]. We showed that salty taste and hot taste ingredients had a greater impact on the GM than sour taste or sweet taste ingredients. This observation was probably because the on the GM regard to the GM [27]. Ingredients No common specific microbial taxon was enriched or depleted by the new diet or any of the flavor ingredients. In addition to biological factors, technology modulates the GM of individuals [28-32].

Parkinson's disease is not decreased compared with that of healthy controls (Dan et al. [31] common. These observations challenge the efforts to link the GM profile and health indicators. In the present study [33-38], the richness and diversity of the GM of the daughter were lower than those of the father and mother, but no indicators suggested that the daughter was less healthy [39-43]. We did not find significant correlations between the abundance, diversity or taxa of 37 genera of GM and general health indicators (blood pressure, blood glucose, blood lipids) except that the relative abundance of Oscillospira and expression of C-reactive protein were significantly positively correlated [44-46]. We need more data from more stringently controlled experiments to define a healthy GM profile because this research field is new [47-49].

Conclusion

The experimental diet caused a significant change, salty and hot flavor ingredients caused obvious alterations, while sour and sweet flavor ingredients caused minor shifts in the GM of the three family members. We speculate that dietary factors which have food ingredients with greater access to the colorectum, such as salt and chili peppers versus sucrose and vinegar, may have a greater impact

on the GM. Flavor treatments also caused fluctuations in most of the health indicators we tested. However, the diet and flavor caused changes in the GM and health indicators were highly divergent among the family members, and the fluctuations in health indicators were only weakly correlated with GM changes. We concluded that dietary factors interact with individual factors in driving GM variability.

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References

1. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207-14.
2. Human Microbiome Project Consortium. A framework for human microbiome research. Nature. 2012;486(7402):215-21.
3. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. Nature Med. 2018;24(4):392-400.
4. Donia MS, Fischbach MA. HUMAN MICROBIOTA. Small molecules from the human microbiota. Science. 2015;349(6246):1254766.
5. Thomas S, Izard J, Walsh E, Batich K, Chongsathidkiet P, Clarke G, et al. The host microbiome regulates and maintains human health: a primer and perspective for non-microbiologists. Cancer Res. 2017;77(8):1783-812.
6. Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ. Role of the microbiome in human development. Gut. 2019;68:1108-14.
7. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, et al. Human genetics shape the gut microbiome. Cell. 2014;159(4):789-99.
8. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, et al. Environment dominates over host genetics in shaping human gut microbiota. Nature. 2018;555(7695):210-15.
9. Farquhar WB, Edwards DG, Jurkovitz CT, Weintraub WS. Dietary sodium and health: more than just blood pressure. J Am Coll Cardiol. 2015;65(10):1042-50.
10. Ho CW, Lazim AM, Fazry S, Zaki U, Lim SJ. Varieties, production, composition and health benefits of vinegars: A review. Food Chem. 2017;221:1621-30.
11. Archer E. In defense of sugar: a critique of diet-centrism. Prog Cardiovasc Dis. 2018;61(1):10-9.
12. Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil A. Effects of sweeteners on the gut microbiota: a review of experimental studies and clinical trials. Adv Nutr. 2019;10(suppl_1):S31-S48.
13. Smiljanec K, Lennon SL. Sodium, hypertension, and the gut: does the gut microbiota go salty? Am J Physiol Heart Circ Physiol. 2019;317(6):H1173-82.
14. Beh BK, Mohamad NE, Yeap SK, Ky H, Boo SY, Chua JYH, et al. Anti-obesity and anti-inflammatory effects of synthetic acetic acid vinegar and Nipa vinegar on high-fat-diet-induced obese mice. Sci Rep. 2017;7(1):6664.
15. Mohamad NE, Yeap SK, Ky H, Ho WY, Boo SY, Chua J, et al. Dietary coconut water vinegar for improvement of obesity-associated inflammation in high-fat-diet-treated mice. Food Nutr Res. 2017;61(1):1368322.
16. Garcia-Lozano M, Haynes J, Lopez-Ortiz C, Natarajan P, Peña-Garcia Y, Nimmakayala P, et al. Effect of pepper-containing diets on the diversity and composition of gut microbiome of drosophila melanogaster. Int J Mol Sci. 2020;21(3):945.
17. Benedict C, Vogel H, Jonas W, Woting A, Blaut M, Schürmann A, et al. Gut microbiota and glucometabolic alterations in response to recurrent partial sleep deprivation in normal-weight young individuals. Mol Metab. 2016;5:1175-86.
18. Poroyko VA, Carreras A, Khalyfa A, Khalyfa AA, Leone V, Peris E, et al. Chronic sleep disruption alters gut microbiota, induces systemic and adipose tissue inflammation and insulin resistance in mice. Sci Rep. 2016;6:35405.
19. Mach N, Fuster-Botella D. Endurance exercise and gut microbiota: A review. J Sport Health Sci. 2017;6(2):179-97.
20. Monda V, Villano I, Messina A, Valenzano A, Esposito T, Moscatelli F, et al. Exercise modifies the gut microbiota with positive health effects. Oxid Med Cell Longev. 2017;2017:3831972.
21. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174-80.
22. Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibzadeh S, Engell RE, et al. Global sodium consumption and death from cardiovascular causes. N Engl J Med. 2014;371(7):624-34.
23. Van Horn L. Dietary sodium and blood pressure: how low should we go? Prog Cardiovasc Dis. 2015;58(1):61-8.
24. Graudal NA, Hubeck-Graudal T, Jurgens G. Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride. Cochrane Database Sys Rev. 2011;11:CD004022.
25. Carmody RN, Bisanz JE, Bowen BP, Maurice CF, Lyalina S, Louie KB, et al. Cooking shapes the structure and function of the gut microbiome. Nat microbiol. 2019;4(12):2052-63.
26. Carmody RN, Gerber GK, Luevano JM, Gatti DM, Somes L, Svenson KL, et al. Diet dominates host genotype in shaping the murine gut microbiota. Cell Host Microbe. 2015;17(1):72-84.
27. Hugenholtz F, de Vos WM. Mouse models for human intestinal microbiota research: a critical evaluation. Cell Mol Life Sci. 2018;75(1):149-60.
28. Brussow H. How stable is the human gut microbiota? And why this question matters. Environ Microbiol. 2016;18(9):2779-83.
29. Cani PD, de Vos WM. Next-generation beneficial microbes: the case of akkermansia muciniphila. Front Microbiol. 2017;8:1765.
30. Conlon MA, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. Nutrients. 2014;7(1):17-44.
31. Dan X, Mushi Z, Baili W, Han L, Enqi W, Huanhu Z, Shuchun L. Differential analysis of hypertension-associated intestinal microbiota. Int J Med Sci. 2019;16(6):872-81.
32. Debelius J, Song SJ, Vazquez-Baeza Y, Xu ZZ, Gonzalez A, Knight R. Tiny microbes, enormous impacts: what matters in gut microbiome studies? Genome Biol. 2016;17(1):217.
33. Derrien M, Belzer C, de Vos WM. Akkermansia muciniphila and its role in regulating host functions. Microb Pathog. 2017;106:171-81.
34. Haddad EN, Sugino KY, Tucker RM, Comstock SS. Gut enterotypes are stable during Bifidobacterium and Lactobacillus probiotic supplementation. J Food Sci. 2020;85:1596-604.
35. Heintz-Buschart A, Pandey U, Wicke T, Sixel-Döring F, Janzen A, Sittig-Wiegand E, et al. The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. Mov Disord. 2018;33(1):88-98.
36. Hughes DA, Bacigalupe R, Wang J, Rühlemann MC, Tito RY, Falony G, et al. Genome-wide associations of human gut microbiome variation and implications for causal inference analyses. Nat Microbiol. 2020;5(9):1079-87.
37. Kriss M, Hazleton KZ, Nusbacher NM, Martin CG, Lozupone CA. Low 520 diversity gut microbiota dysbiosis: drivers, functional implications and recovery. Curr Opin Microbiol. 2018;44:34-40.
38. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500(7464):541-6.
39. Li Y, Ning L, Yin Y, Wang R, Zhang Z, Hao L, et al. Age-related shifts in gut microbiota contribute to cognitive decline in aged rats. Aging (Albany NY). 2020;12(9):7801-17.
40. Ma ZS, Li W. How and Why Men and Women Differ in Their Microbiomes:

Medical Ecology and Network Analyses of the Microgenderome. Adv Sci (Weinh). 2019;6(23):1970140.

41. Martinez-Guryn K, Leone V, Chang EB. Regional diversity of the gastrointestinal microbiome. Cell Host Microbe. 2019;26(3):314-24.

42. Matenchuk BA, Mandhane PJ, Kozyrskyj AL. Sleep, circadian rhythm, and gut microbiota. Sleep Med Rev. 2020;53:101340.

43. Nuzum ND, Loughman A, Szymlek-Gay EA, Hendy A, Teo WP, Macpherson H. Gut microbiota differences between healthy older adults and individuals with Parkinson's disease: A systematic review. Neurosci Biobehav Rev. 2020;112:227-41.

44. Olatunji TL, Afolayan AJ. The suitability of chili pepper (*Capsicum annuum L.*) for alleviating human micronutrient dietary deficiencies: A review. Food Sci Nutr. 2018;6(8):2239-51.

45. Santos-Marcos JA, Haro C, Vega-Rojas A, Alcala-Diaz JF, Molina-Abril H, Leon-Acuña A, et al. Sex differences in the gut microbiota as potential determinants of gender predisposition to disease. Mol Nutr Food Res. 2019;63(7):e1800870.

46. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. Microbiome. 2016;4(1):33.

47. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. Front Microbiol. 2017;5:494.

48. Wang C, Huang Z, Yu K, Ding R, Ye K, Dai C, et al. High-salt diet has a certain impact on protein digestion and gut microbiota: a sequencing and proteome combined study. Front Microbiol. 2017;8:1838.

49. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, et al. Salt-responsive gut commensal modulates T_H17 axis and disease. Nature. 2017;551(7682):585-9.

Supplymentary Files

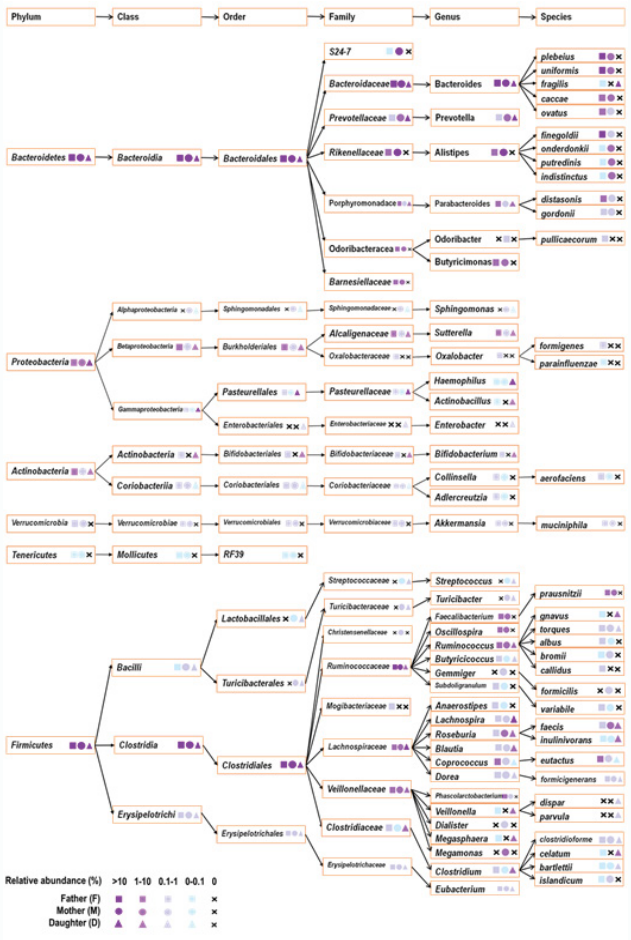

Figure S1: GM profile of family members shaped by the experimental diet at all taxa levels.

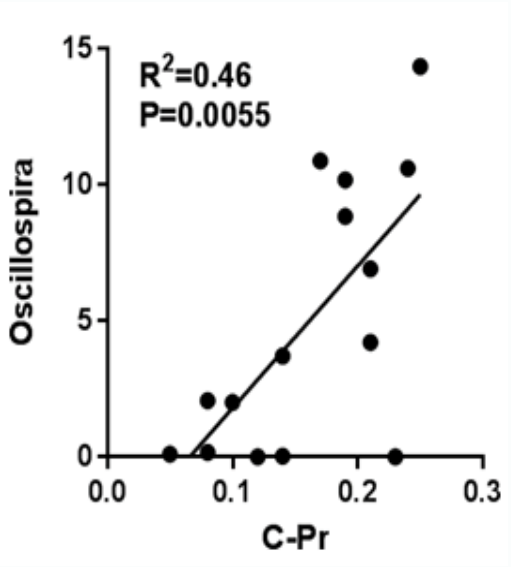

Figure S3: Relative abundance of *Oscillospira* and blood content of C-reactive protein were significantly positive correlated.

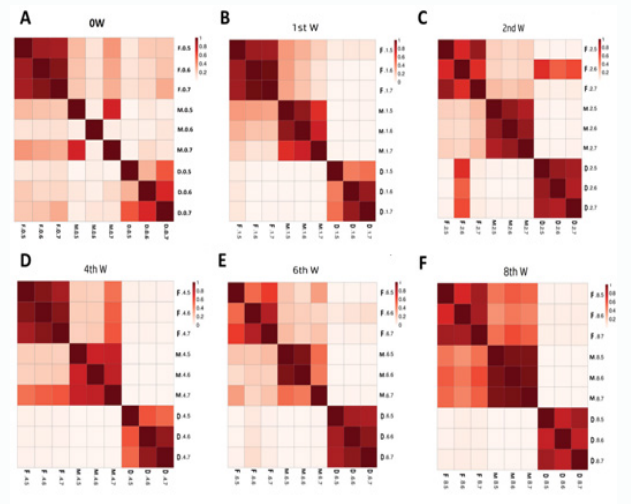

Figure S2: GM similarity affected by the same diet. (A-F) GM similarity of all samples along with the experimental diet by spearman correlation analysis. Shading indicates similarity.

Supplementary Table 1: Subject characteristics.

	1st week on the experiment diet			2nd week after salty flavor			4th week after sour flavor			6th week after sweet flavor			8th week after hot flavor		
	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D
Age, years	58	58	35	58	58	35	58	58	35	58	58	35	58	58	35
Sex	Male	Female	Female	Male	Female	Female	Male	Female	Female	Male	Female	Female	Male	Female	Female
BMI, kg/M^2	21.2	21.9	22.1	21.4	22.1	22.2	21.5	22	22.1	21.3	22.1	22.2	21.3	22.1	22.3
Body weight, kg, mean ± SE	61.2 ± 0.1	52.6 ± 0.2	58.0 ± 0.2	61.9 ± 0.4	53.1 ± 0.3	58.3 ± 0.2	62.0 ± 0.2	52.8 ± 0.2	58.1 ± 0.3	61.7 ± 0.1	53.0 ± 0.2	58.3 ± 0.3	61.7 ± 0.2	53.1 ± 0.1	58.4 ± 0.2
SBP, mmHg, mean ± SE	104.0 ± 2.9	103.4 ± 4.5	97.3 ± 8.0	110.3 ± 1.7	116.4 ± 5.5	97.6 ± 5.0	105.9 ± 3.0	99.4 ± 4.6	93.4 ± 3.4	103.6 ± 3.2	101.7 ± 2.2	98.1 ± 2.7	101.0 ± 4.3	103.4 ± 2.9	99.4 ± 1.9
DBP, mmHg, mean ± SE	68.4 ± 2.6	72.3 ± 1.7	67.9 ± 3.8	72.4 ± 1.3	74.9 ± 3.8	69.7 ± 3.5	69.1 ± 2.4	69.7 ± 3.4	69.6 ± 2.4	68.4 ± 3.3	71.1 ± 1.1	72.1 ± 2.2	69.1 ± 2.8	69.7 ± 3.1	73.3 ± 3.1
Heart rate, BPM, mean ± SE	57.1 ± 5.2	68.1 ± 1.5	83.5 ± 8.6	57.4 ± 4.1	71.7 ± 4.0	82.6 ± 6.1	63.6 ± 3.4	69.4 ± 2.3	80.4 ± 3.6	61.4 ± 3.3	72.4 ± 3.5	82.4 ± 5.2	60.6 ± 2.1	72.0 ± 4.0	86.4 ± 5.8
Glucose, mmol/L	5.3	5	4.7	5.3	5.1	4.9	4.4	4.9	5.3	5.1	4.9	5.3	5.1	4.8	4.9
Cholesterol, mmol/L	4.2	3.3	3.7	4.7	3.4	3.1	3.7	4	3.3	3.5	3.8	4.5	3.3	3.7	4.4
Triglyceride, mmol/L	1.9	1.4	1	1.3	0.9	1	1.3	1.2	1.1	1.3	1.6	1.3	1	0.9	1.1
HDL, mmol/L	1.2	1.3	1.5	1.3	1.4	1.2	1.4	1.1	1.2	1.4	1.4	1.2	1.3	1.5	1.3
LDL, mmol/L	2.5	1.7	1.2	3	1.8	1.7	1.1	2.5	1.7	1	1.2	2.9	1.8	1.2	2.7
ALT, U/L	13.8	26	13.6	12.1	67.8	20.2	12.3	9	18.5	12.9	15.7	12.7	24.7	12.4	12.9
AST, U/L	15.6	22.8	19.5	13	43.5	16.1	17.7	11.8	16.8	18.3	19.2	15.5	24.7	19.6	13.9
Creatinine, umol/L	46	46	71	50	55	47	69	47	50	75	77	49	54	77	41
UA, umol/L	273	196	304	285	204	175	293	265	199	273	296	268	208	277	242
Urea, mmol/L	3.7	3.1	3.9	3.5	3.2	2.6	3.8	3.5	2.8	4	4.3	3.6	2.9	3.8	3
C-reactive protein, mg/L	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.3	0.1	0.2	0.2	0.1

Supplementary Table 2: Food list of the experiment diet.

Daily intake (/people)									
		Recipe	Amount (Fresh /dry* weight)	Energy (KJ)	Protein (g)	Fat (g)	CHO (g)	Fiber (g)	Vitamin (mg)
Breakfast		Millet congee	40 g*	604.4	3.6	1.24	30.04	0.64	2.22
		Boiled egg	60 g	348.6	7.86	5.16	1.44	0	1.26
		Pistachios	25 g*	636	6	12.73	5.1	2.6	2.11
		cashew	25 g*	652.5	5.15	13.25	5.48	2.05	5.25
		Apple	100 g	227	0.4	0.2	13.7	1.7	3.66
		Black tea	500 ml	–	–	–	–	–	–
Food		Rice	50 g*	726.5	3.95	0.45	38.6	0.3	1.46
		Tomato	120 g	74.4	1.08	0.24	3.96	0	18.42
		/egg soup	/40 g	232.4	5.24	3.44	0.96	0	0.84
		Fried Broccoli	150 g	166.5	5.25	0.9	5.55	0	86.69
Lunch		Fried cucumber	50 g	32.5	0.4	0.1	1.45	0.25	4.87
		/mushroom	/30 g	32.1	0.66	0.09	1.56	0.99	0.92
		/pork	/50 g	300	10.15	3.1	0.75	0	3.16
Dinner		Sunflower seeds	25 g*	611.25	7.13	12.25	3.78	2.05	3.73
		Pear	100 g	211	0.3	0.1	13.1	2.6	6.4
		Black tea	500 ml	–	–	–	–	–	–
		Noodles	100 g*	1483	11.4	0.9	75.1	0.9	3.41
		Dumplings (wheat	70 g	317.1	2.73	0.28	15.96	0	0.41
		/carrot	20 g	26.6	0.2	0.04	1.62	0	2.76
		/celery	15 g	13.95	0.18	0.03	0.68	0.18	1.53
		/pork)	35 g	277.2	6.27	4.48	0.28	0	2.08
		Boiled Spinach	100 g	116	2.6	0.3	4.5	1.7	37.65
		Sunflower seeds	25 g*	611.25	7.13	12.25	3.78	2.05	3.73
		Milk	4 g*	80.8	0.8	0.89	2.02	0	1.09
		Pitaya	150 g	351	1.65	0.3	19.95	2.4	5.12
		Black tea	500 ml	-	-	-	-	-	-
		oil	32 g	1203.52	0.00	31.97	0.00	0.00	29.79
		salt	2.3 g Na	0.00	0.00	0.00	0.00	0.00	0.00
Sum of food nutrients				9335.57	90.11	104.69	249.34	20.41	228.54
	salty	+salt	3.2 g Na	0.00	0.00	0.00	0.00	0.00	0.00
Flavors	sour	+ lemon,	200 g	312	2.2	2.4	12.4	2.6	47.62
		vinegar	40 ml	264	0	0	15.28	0	–
	sweet	+Crystal sugar	60 g	1003.8	0.00	0.00	60	0.00	0.00
	hot	+ Chili pepper	6 g*	73.62	0.92	0.72	3.44	0.00	2.5