Effect of Alcohol and Frozen Foods Consumption on the Gut Microbiome

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Abstract

Human gut microbiomes can be heavily influenced by their diet and consuming certain types of foods which creates a predictable shift in the existing microbiome landscape of the host[1]. Alcohol consumption– both moderate and severe– has been shown to change the microbiome composition and abundance of bacteria such as proteobacteria in the GI tract, which could potentially raise levels of endotoxins in the bloodstream[2]. There is limited research on the effect of frozen foods on the microbiome, but studies show that the process of freezing may negatively affect the nutritional quality of the food[3]. However, the effects of the consumption of alcohol and frozen foods on college students' microbiomes is not well studied. Here we show the amount of frozen foods and alcohol a person consumes per week and its impact on the gut microbiome. The consumption of both frozen foods and alcohol does not impact their microbiome but it depends on the servings too. The ANCOM results showed no association between variables and microbiome. This analysis serves to be an initial foray into further understanding the impacts alcohol and frozen food consumption has on the gut microbiome of college students and possible correlation between the food and disease.

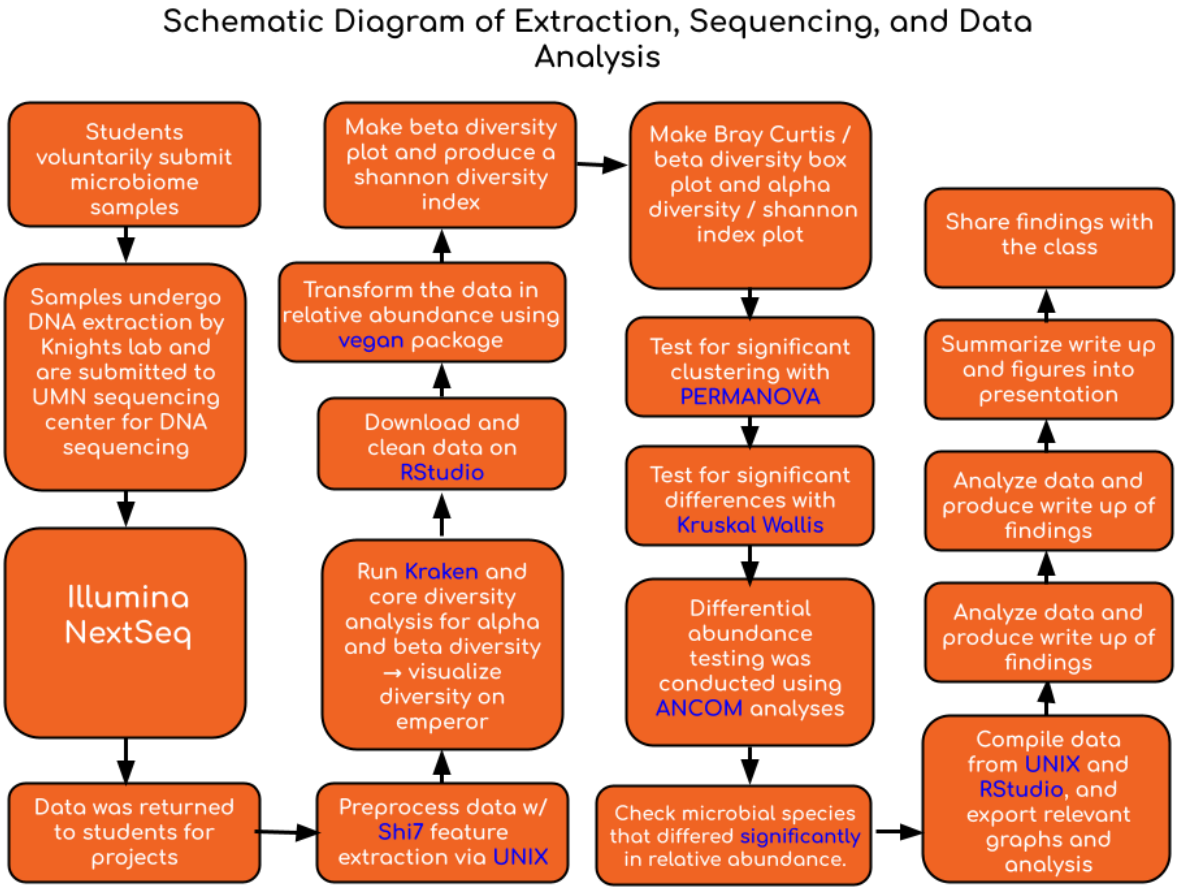
Summary of previous research on your topic

Chronic alcohol consumption can negatively alter a microbiome composition by causing bacterial dysbiosis in the GI tract. Samples from the GI tract of alcoholics show that alcohol use can lower the levels of Bacteroidetes and raise the level of Proteobacteria. Some studies have found that dysbiotic microbiome may correlate with higher levels of endotoxin in the blood, intestinal hyper-permeability and increased circulation of bacterial byproducts into the bloodstream. Alcohol abuse can result in higher levels of *proteobacteria* and lower levels of *Clostridia*[2]. The alcohol type can also impact the microbiome. Gin can increase *Bacteroides* and *Clostridium* and decrease *Prevotellaceae*. Red wine was found to increase diversity and *Firmicutes* and *Bacteroidetes*[4].

Freezing fruits can disrupt enzyme and Vitamin C[4] stability. Studies have found that Vitamin C supplementation increases *Bifidobacterium* bacteria in the GI tract, which can increase immune response during infections[5]. It is hypothesized that a diet heavily reliant on frozen fruit as its

main source of Vitamin C, would produce a microbiome with minimal *bifidobacterium* and therefore weaken a person's ability to fight possible infections.

Description of Approach



Findings and Discussion

The gut microbiome composition and diversity did not appear to be significantly impacted by alcohol consumption or frozen food consumption in this study. Alpha diversity analysis using the Shannon's diversity index revealed that alcohol intake had no significant influence on gut microbiome diversity (Figure 1a; $p = 0.271$). This aligns with prior research that did not find a clear effect of moderate alcohol intake on alpha diversity[6]. Frozen food consumption also did not

significantly alter alpha diversity (Figure 1b; $p = 0.299$), consistent with a past study that found no change after increased intake of commercially processed foods[7].

Analysis of microbiome clustering patterns using weighted Bray-Curtis PCoA also showed no significant clustering associated with alcohol as indicated by PERMANOVA test (Figure 2a; $p = 0.471$) or frozen food consumption (Figure 2b; $p = 0.486$). This indicates that overall microbiome structure was largely conserved regardless of these dietary factors.

At the species level, 215 microbial taxa were initially identified with significantly differing abundances between frozen food consumption groups ($p < 0.05$) and 260 microbial taxa between alcohol consumption groups ($p < 0.05$) based on ANCOM-BC analysis. However, after FDR correction, none of these associations remained significant (Figure 3; $q > 0.05$). The lack of significance after adjusting for multiple comparisons suggests these initial apparent associations may reflect false discoveries rather than robust diet-related taxonomic differences.

While no significant effects were found here, some contrary studies have reported alterations to the gut microbiome related to alcohol intake[8] or frozen food consumption[9]. Further research in larger cohorts is warranted to clarify any potential subtle impacts of these dietary factors. But overall, the current analysis does not show strong evidence for associations between frozen food intake, alcohol consumption, and characteristics of the gut microbiome.

Conclusion

The analysis revealed no significant difference in alcohol and frozen food consumption depending on the servings and their impact on the gut microbiome. A factor that could have affected the data is the interpretation of frozen food. In literature, frozen food is often defined as ultra-processed foods. This does not cover foods like frozen produce or homemade meals frozen for long-term storage. Therefore, the definition needed to be differentiated in the survey. Additionally, how participants viewed their alcohol consumption at the time of the survey could have affected the data results. For example, participants might have only been thinking about their consumption during the week the survey was sent out, not in the long term and this could have affected the data results.

Methods

Raw sequence reads were inspected for quality using FastQC. Primer sequences were trimmed and low quality reads were filtered using SHI7 with a phred score of 30. Taxonomic classification was conducted by aligning reads with Kraken2[10] database using Bowtie2. The Kraken2 classifier was utilized to assign taxonomic labels based on the alignment results.

Alpha and beta diversity metrics were calculated in QIIME2[11] to examine community richness, evenness, and dissimilarity across groups. Species-level taxonomic tables were rarefied for equal

subsampling depth prior to analysis. Alpha diversity was visualized using Shannon index boxplots. Beta diversity principal coordinate analysis (PCoA) Bray-Curtis plots were generated to examine clustering by categories. Permutational multivariate analysis of variance (PERMANOVA) was conducted using the vegan adonis2 function to test if categories displayed significantly different centroid positions.

To investigate differences in taxon abundance between groups, ANCOM-BC[12] for Analysis of Compositions with Bias Correction was utilized. Necessary packages were loaded in RStudio including ape, dplyr, haggis, ggplot2, ggpubr, multcompView, labdsv, and ANCOM. A TreeSummarizedExperiment object joining metadata and abundances was constructed with tse. ANCOM-BC was then run and repeated for alcohol and frozen foods variables.

Figures

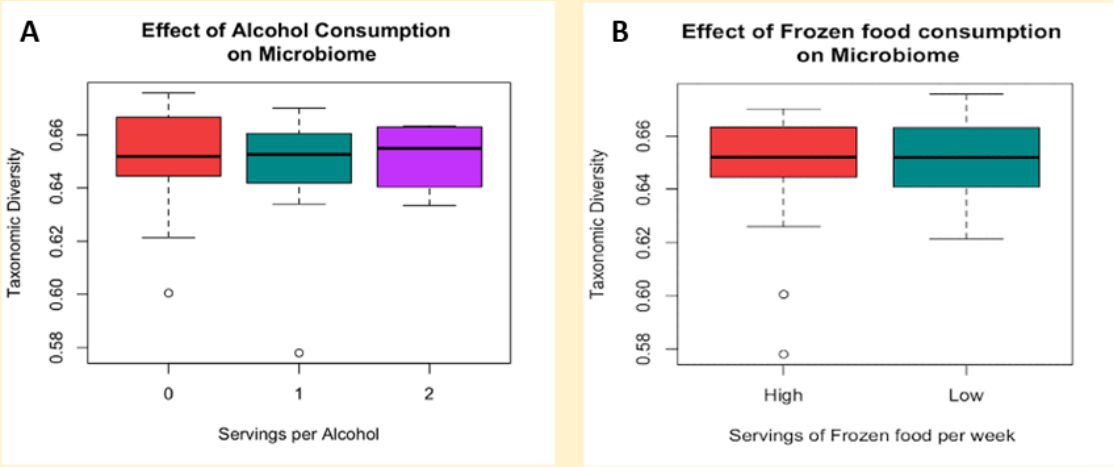
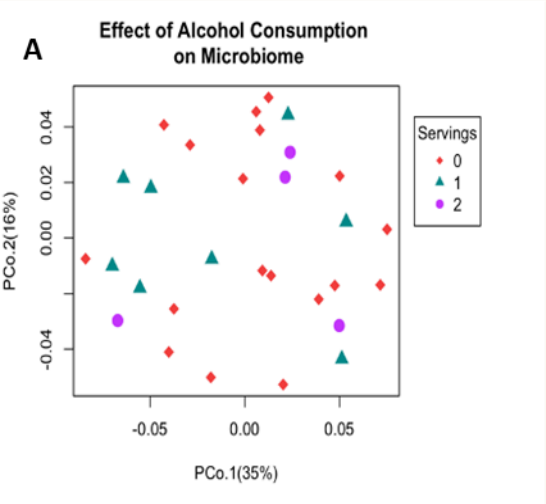


Figure 1a & 1b: **Alpha Diversity of Alcohol and Frozen Foods Consumption and its Effect on Gut Microbiome -** Alpha diversity revealed no difference in the alcohol consumption and gut microbiome with Kruskal Wallis p value 0.26 and in figure 1b frozen foods consumption had no effect of gut microbiome with Wilcoxon test p-value 0.299.



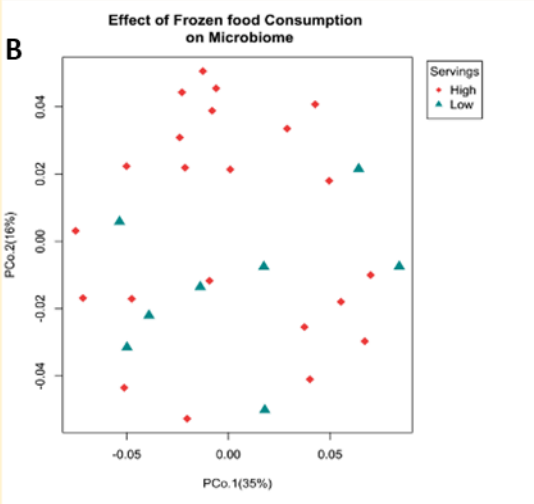


Figure 2a & 2b: Bray Curtis Beta Diversity of Alcohol and Frozen Foods Consumption and its Effect on Gut Microbiome - Beta diversity reveled no significant clustering in alcohol consumption and permonova value was 0.471 and figure 2b frozen foods consumption showed no significant clustering with permonova value of 0.486.

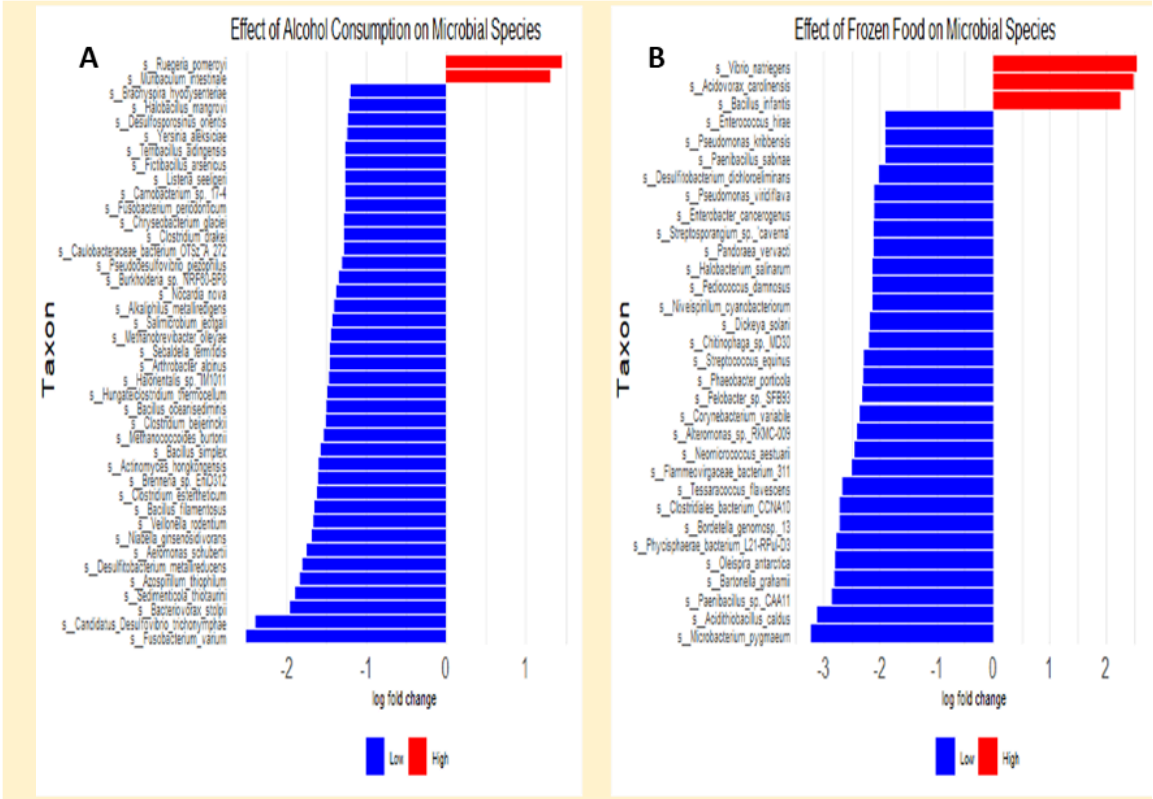


Figure 3a: ANCOM Analysis of Alcohol and Frozen Foods Consumption and its Effect on Gut Microbiome - ANCOM analysis revealed several microbial species that differed significantly ($p < 0.05$) in relative abundance between consumption of alcohol. These discriminating taxa were then plotted in a bar plot to illustrate these differences in log fold change. All taxa have q-values above 0.05, indicating no significant differences with controlled false discovery rates.

Figure 3b: ANCOM analysis revealed several microbial species that differed significantly ($p < 0.05$) in relative abundance between consumption of frozen foods. These discriminating taxa were then plotted in a bar plot to illustrate these differences in log fold change. All taxa have q-values above 0.05, indicating no significant differences with controlled false discovery rates.

Contributions

Everybody contributed equally to the project, data processing, data analysis, figure creation and helped each other wherever required.

Acknowledgements

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