INVESTIGATING CONSUMER PERCEPTIONS OF HEALTHFULNESS IN SOURDOUGH BREADS AND THE EFFECT OF SOURDOUGH FERMENTATION ON GLUTEN PROTEIN

COLORADO STATE UNIVERSITY EXTENSION

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Food Structure & Function Lab

Introduction

Sourdough is a bread product fermented by communities of wild bacteria and fungi known as a starter culture[1]. Sourdoughs have been shown to have certain favorable properties compared to straight doughs, such as improved shelf life[2], flavor[3], and texture[4]. Previous work has examined the effects of specific starter organisms on bread quality, but the relationships between whole microbiomes and dough/bread physicochemical properties are currently unknown. The objective of this study was to investigate the relationship between physicochemical properties of sourdough breads and the microbiomes of their starter cultures. Sourdough starters representing 20 distinct microbiomes were selected to represent a diverse subset of relative abundance and diversity of bacteria and fungi. These starters were used to prepare fermented wheat doughs and breads which were analyzed for their physicochemical properties. These properties were then compared to the microbiomes of the starters.

Objectives

- Survey gluten-sensitive consumers regarding their sourdough consumption habits and sourdough's effect on the symptoms of their gluten-mediated pathology
- Develop a survey for Colorado bakeries probing sourdough knowledge/practices
- Create outreach materials for CSU Extension summarizing findings from consumer survey above connecting them to ongoing work in the Food Structure and Function Lab
- Analyze the bacterial and fungal microbiomes present in 20 core sourdough starters[5]
- Measure the physical, chemical, and molecular properties of dough and bread made from the starters
- Investigate correlations between sourdough starter culture microbiomes and the physicochemical properties of the dough/bread

Materials & Methods

Consumer Survey A survey was developed for a target population of gluten-sensitive individuals above 18 years of age. It received IRB approval under Protocol #3606. The survey used the Qualtrics platform and was disseminated via social media. Analysis was carried out using Prism 9 software and one-way ANOVA where responses could be coded on a Likert scale and Chi-squared Fisher's test where they could not.

Bioinformatics Pipeline Raw sequence reads were obtained from NCBI BioProject Accession No. PRJNA589612 and analyzed with the R package DADA2 v3.14 pipeline[6]. Amplicon sequence variants (ASVs) were assigned taxonomy using the Silva database v132[7]. Taxonomic composition for mapped reads above 1% were plotted using the R package ggplot2 v3.3.5[8]. Principal component analysis was performed on center-log transformed data to visualize microbiota composition by samples. Beta diversity was estimated using Aitchison distances from center log ratio transformed data. All analyses were conducted in R v4.1.0. Chimeras were detected and removed, and remaining ASVs were assigned taxonomy using the Silva database v132 for bacteria and the UNITE database for fungi[9].

Sample Preparation Sourdough starter cultures provided by Ben Wolfe (Tufts University) were thawed and propagated to 225 g using flour and sterilized water. Bread doughs were made by mixing 400 g of flour with 200 g of starter and 225 g of sterile water, then proofed at 37°C (98.6°F) for 24h. Bread loaves were baked at 126°C (350°F) for 40 min.

Gluten Extraction Gluten was extracted in a solution of NaCl and 70% ethanol via centrifugation and lyophilized, then stored at -80°C until later use.

SDS-PAGE Proteins were separated by molecular weight via electrophoresis on a polyacrylamide gel according to the Laemmli procedure[10], then imaged to compare relative intensity of bands.

Ninhydrin Assay Free amino acids in dough samples at 1 mg/ml were interpolated from a standard curve of leucine from absorbance measured at 570nm[11]. A solvent of 10% ethanol at pH 1.5 was used for both samples and standards.

Results

Preliminary survey results indicate similar levels of gluten knowledge among diagnosis groups, but different sourdough consumption habits. Categories include respondents with celiac disease (CD), non-celiac gluten sensitivity or similar (NCGS), and those who have not been diagnosed (Undiagnosed).

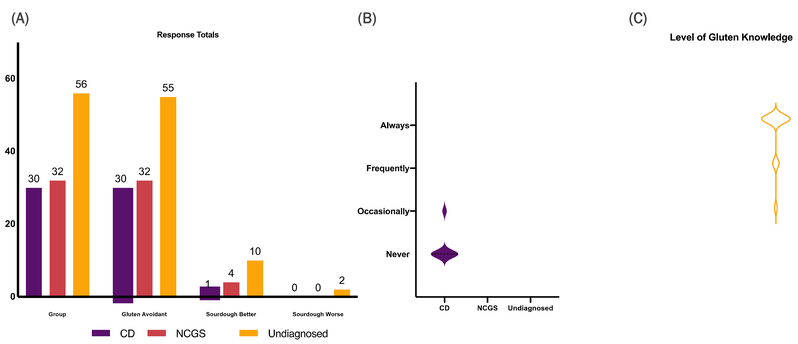

Figure 1: Visualization of survey responses per diagnosis category. (A) Total responses per diagnosis category, (B) frequency of sourdough consumption per diagnosis category, and (C) level of gluten knowledge per diagnosis category.

Results

Long-form answers reveal that among gluten-sensitive individuals who eat sourdough, reduced abdominal pain is the principal response, taste dominates respondents' motivations for choosing sourdough, a change in eating habits is the most significant effect of a gluten sensitivity, and that most respondents feel supported in their management of symptoms and get information from labels and ingredient lists. Each answer is counted as a separate response even in the cases where more than one answer was listed by the same respondent.

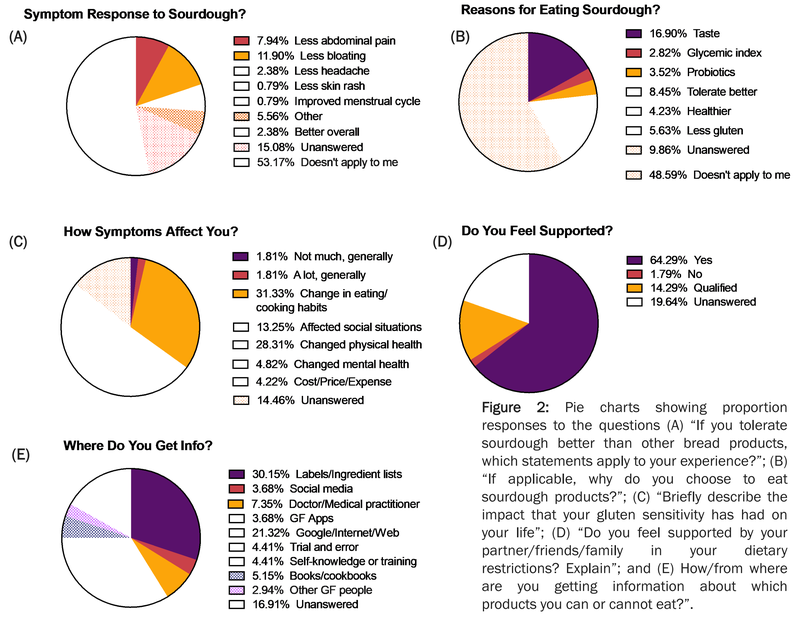

Figure 2: Pie charts showing proportion responses to the questions (A) "If you tolerate sourdough better than other bread products, which statements apply to your experience?"; (B) "If applicable, why do you choose to eat sourdough products?"; (C) "Briefly describe the impact that your gluten sensitivity has had on your life"; (D) "Do you feel supported by your partner/friends/family in your dietary restrictions? Explain"; and (E) How/from where are you getting information about which products you can or cannot eat?".

Word clouds reveal how each diagnosis category describes their experience in each diagnosis group: celiac disease (CD), non-celiac gluten sensitivity (NCGS), or undiagnosed.

Figure 3: The top 20 words in responses to the question "Briefly describe the impact that your gluten sensitivity has had on your life" in diagnosis category, (A) celiac disease, (B) non-celiac gluten sensitivity, and (C) undiagnosed respondents.

A PCA plot of variables (A) demonstrates strong positive correlations between titratable acidity and water activity, and it shows strong negative correlations between compression and loaf volume as well as between pH and both water activity and titratable acidity. An Individual Factor Map (B) shows that while most samples cluster together, showing similarities, sample 24 falls outside the cluster and has less relationship to the other samples.

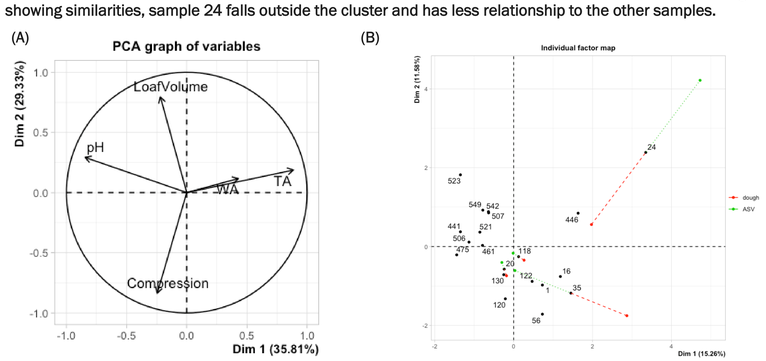

Figure 4: (A) Principal Component Analysis of dough and bread physical and chemical properties in which the angle between vectors indicates how closely properties are correlated, and the length of the vector indicates the strength of the influence that loading value or characteristic has on the plotted dimension. (B) Individual Factor Map of all samples in which the closer samples appear on the plot indicates greater similarity between those samples; this plot takes into account both observed physicochemical and molecular properties of the samples as well as the samples' microbiomes.

Results

Sourdough samples show differences in degree of gliadin breakdown compared to one another and to yeast control, but no differences in free amino acid content

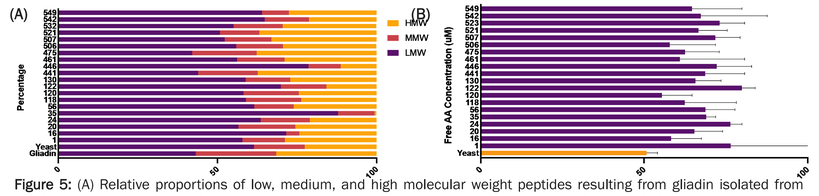

Figure 5: (A) Relative proportions of low, medium, and high molecular weight peptides resulting from gliadin isolated from yeast- and sourdough-fermented dough, compared against a pure gliadin standard, and (B) concentration of free amino acid in yeast- and sourdough-fermented dough as measured by ninhydrin assay.

Conclusions

Preliminary survey results (n=118) indicate a significant difference in the frequency of sourdough consumption between diagnosis group (p=0.0011 between undiagnosed consumers and those with CD p=0.0067 between undiagnosed consumers and those with NCGS).

- No significant difference in the diagnosis groups' change in symptoms with sourdough consumptions (p=0.896)
- No significant difference in the level of gluten knowledge between diagnosis groups—overall high knowledge (p=492)

Survey results indicate respondents with celiac disease do not consume sourdough, but some gluten-sensitive individuals who have not been diagnosed with celiac disease choose to consume sourdough.

- Taste is the main reason for eating sourdough, but some respondents believe it has health-promoting traits

Respondents largely feel supported in their restrictions and symptom management.

- Most responses were yes (64.29%) or a qualified yes (14.29%) to this question

Fungal populations appear to be driving biochemical outcomes (data not shown).

- PCA of fungal ASVs against physicochemical properties show greater clustering than when plotting bacterial ASVs against physicochemical properties, which show only loose associations

Relative ratio of low, medium, and high molecular weight peptides from gluten extracted differs among fermented dough samples, as assessed by SDS-PAGE and densitometry.

- This is indicative of differences in enzymatic potential and supports the hypothesis of breakdown of immunogenic gluten peptides
- No statistically significant difference in free amino acid concentration between any samples

Physical and chemical differences among sourdoughs suggest potential for functional differences in gluten proteins.

- Physicochemical differences exist between all sourdough samples and between sourdough and yeast control

Project outputs included Extension outreach materials (newsletter, blog posts) and a training seminar for Extension agents into which these results have been incorporated.

Future & Ongoing Work

- Survey will continue until n=1000 responses
- Microbes present in the samples will be investigated for the presence of hydrolytic enzymes
- The presence/absence of celiac-specific immunostimulatory peptides will be measured in sourdough-fermented dough based on known peptide standards using LC-MS
- Immunostimulatory effects of sourdough fermented gluten will be measured *in vitro*
- The strength of the associations between bacterial and fungal microbiota and physicochemical properties that appear in PCA plots will be assessed with Redundancy Analysis

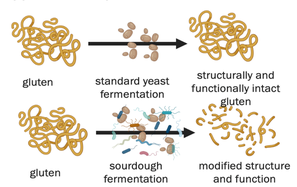

Figure 6. Hypothesized influence of sourdough fermentation on gluten structure/function. Organisms present in sourdough microbiomes generate enzymes capable of breaking down the gluten protein. Unique microbiomes are thought to differ in degree of gluten proteolysis. Gluten proteolysis is hypothesized to affect the functional properties of gluten on dough and bread as well as its immunostimulatory effect on celiac disease pathology.

References

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Acknowledgements

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