Gluten Quantitation in Fermented Sourdough Using a Multiplex-Competitive ELISA

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Abstract

Background:

Gluten from wheat, barley, and rye can cause adverse reactions in individuals with celiac disease, who need to follow a strict gluten-free diet. Here we report a multiplex-competitive ELISA for the quantification of gluten in fermented sourdough.

Methods: Gluten-incurred sourdough were prepared using four types of sourdough starters. The starter cultures were combined with rice flour, water, incurred with 8, 20, and 100 ppm wheat gluten, and fermentation was carried out for 72 hrs. Samples collected every 24 hours were analyzed by a multiplex-competitive ELISA.

Results:

The average gluten recovery was between 55-195% for all samples. The coefficient of variation (%CV) ranged between 2-31%.

Implications:

The average % recovery for most sourdough samples were within acceptable range of 50-150%. Few samples showed recovery up to 200%. The %CV for most samples were ≤ 20%. These results indicate that the multiplex-competitive ELISA can provide accurate and precise quantitation of wheat gluten in fermented sourdough.

Introduction

- Gluten is a protein found in cereals such as wheat, barley, and rye. It is responsible for causing adverse reactions in individuals with Celiac disease (CD).
- There is no cure for CD. Currently, the only effective treatment for CD is a strict, lifelong gluten-free diet.
- To be considered a gluten-free food, there are regulations established by the FDA.
- The FDA limits the amount of gluten in foods to less than 20 ppm (mg/kg) in order to be considered gluten-free.
- Accurate and precise testing is needed to comply with gluten-free regulations and to guarantee that foods labeled “gluten-free” meet the requirements to be gluten-free.
- Currently, there are accurate and reliable methods to quantify intact gluten, however, quantifying gluten in fermented or hydrolyzed products is not possible with these methods.
  - The main reason is lack of suitable calibrant(s) and variable proteolysis depending on the type of fermentation/hydrolysis.
  - Quantifying the level of remaining gluten protein/peptides in fermented or hydrolyzed foods, in terms of equivalent amounts of intact gluten proteins, is challenging.
- As a step forward in this direction, a multiplex-competitive ELISA has been developed and successfully applied to the quantitation of gluten in fermented dairy products.
- The multiplex-competitive ELISA uses a gluten-incurred yogurt calibrant.
- In this study, gluten quantitation in fermented sourdough was investigated using the multiplex-competitive ELISA.

Materials and Methods

Wheat gluten-incurred sourdough preparation:

Sourdough samples containing 8, 20, and 100 ppm gluten were prepared by incurring wheat gluten into a mixture of rice flour, starter, and water, and fermenting the sourdough for up to 72 hours. Four different starter cultures were used to prepare sourdough; Cultures for Health (CH), Positively Probiotic (PP), NW Ferments (NW), and Suzie-Uie (Suzie). Samples were taken every 24 hours during fermentation for analysis. S2, S3 and S4 samples in Figures 2 and 3 represent sourdough samples collated after 24, 48, and 72 hours of fermentation.

Multiplex-competitive ELISA:

Gluten-incurred sourdough samples were analyzed by using a previously developed multiplex-competitive ELISA (Panda et al., 2022) using a gluten-incurred yogurt calibrant, and 6 gluten specific antibodies (Table 1). Adjustments were made to the calibration curves: standards ranged from 0, 0.24, 0.74, 2.2, 6.6, and 20 ppm. Total Gluten was an antibody used that differs from the original protocol. Samples were extracted in a 1:10 dilution using UD buffer (105 mM sodium phosphate, 75 mM NaCl, 2.5% Difco skim milk powder, 0.05% Tween 20, pH 7.4) and mixed on a shaker for an hour at room temperature. Samples were centrifuged at 3,000 g for 10 minutes. Two samples taken from sourdough with the same fermentation time, gluten concentration, and starter cultures were analyzed per ELISA plate, with two replicates per sample, for a total of 4 replicates per antibody.

Statistical Analysis:

Results were analyzed using Prism GraphPad, and standard curves were established using a four parameter logistic (4PL) regression (Figure 1). The estimated concentrations of gluten in the sourdough samples were interpolated from the standard curve. The estimated concentrations were adjusted for ten-fold dilution, then % recovery (calculated by dividing the concentration of gluten obtained from analysis by the amount of gluten we expected), and the coefficient of variation (%CV) were determined.

Table 1. Conjugated antibodies used in multiplex-competitive ELISA and dilutions.

Antibody (name used)	Type [a]	Target	ELISA kits [d]	Manufacturer	Dilution [e]
G12	MAb	QPQLPY	AgraQuant Gluten G12	Romer Labs	1 to 3
R5	MAb	QQPFP, QQQFP, LQPFP, QLPFP	RIDASCREEN Gliadin Competitive	R-BioPharm, AG	1 to 50
2D4	MAb	Deamidated gliadin	Microbiologique Gluten Sandwich	Pi Bioscientific Inc.	1 to 20
MIoBS	PAb	Gliadin	Wheat/Gluten (Gliadin) MIoBS	Morinaga Institute of Biological Sciences, Inc.	1 to 2
Skerritt	MAb	HMW [b] glutenin	AllerTek Gluten	ELISA Technologies Inc.	1 to 5
Total Gluten	MAb/ PAb	R5, HMW gluten-in, HMW secalin, LMW[c] glutenin	RIDASCREEN Total Gluten	R-BioPharm, AG	1 to 10

[a] MAb- Monoclonal antibody, PAb- Polyclonal antibody; [b] HMW-high molecular weight; [c]LMW- Low molecular weight; [d] Name of the ELISA test kits with the conjugate antibodies used in the multiplex-competitive ELISA; [e] Dilution done on a volume/volume basis using PBS

Results and Discussion

Figure 1. Calibration curves for the six gluten specific antibodies used in the multiplex-competitive ELISA. Absorbances measured at a wavelength of 450 nm. The average antibody responses and estimated gluten concentration values from the Total Gluten and MIoBS antibodies were used to obtain the final gluten concentrations of the sourdough samples.

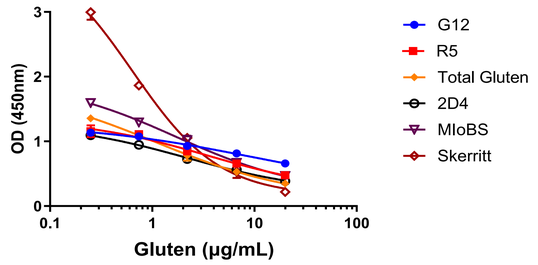


Figure 2. Percent recovery of wheat-incurred sourdough prepared with Culture for Health starter.

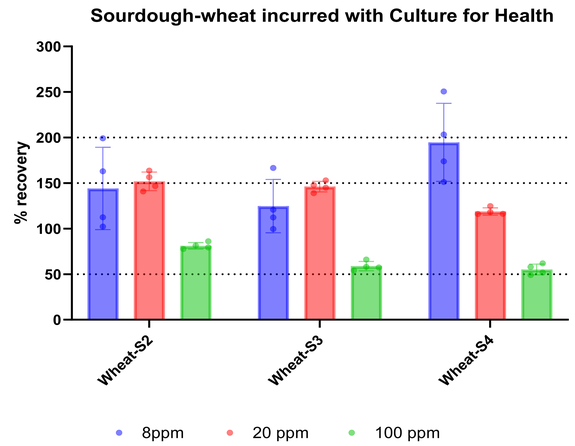
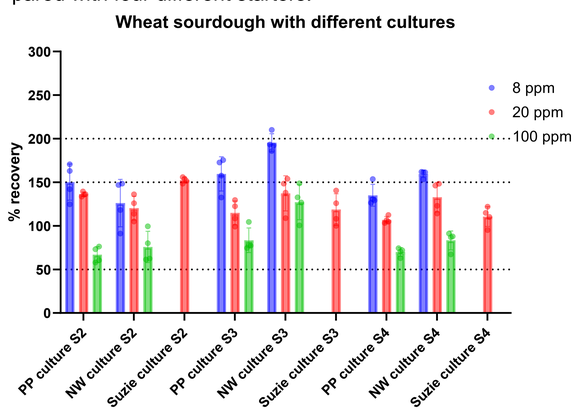


Figure 3. Percent recovery of wheat-incurred sourdough prepared with four different starters.



Conclusion

- For ELISA-based allergen detection methods, the acceptable range of % recovery is from 50-150%. For most of the fermented samples in this study, the average % recovery was within that range with very few samples showing recovery around 200%. According to the AOAC SMPR for gluten in oats, a range of 50-200% recovery is acceptable, and the average % recoveries for all samples in this study were within this range (Boison et al., 2018).
- For the coefficient of variation, the acceptable value of %CV is less than 20% as per FDA Foods Program Chemical Methods Validation Guidelines and many allergen specific AOAC SMPRs. For most samples in this study, the %CV was less than or around 20%. A few samples have %CV above 20% but the maximum %CV observed was around 30%.
- Samples with 8 ppm gluten tended to have higher recovery of gluten compared to 20 ppm and 100 ppm samples.
- Looking at the 20 ppm samples, which is the maximum amount of gluten allowed in foods to be considered gluten-free by the FDA, almost all of the samples resulted in average % recovery between 100-150%, which fits in the acceptable 50-150% range for % recovery.
- These results indicate that the multiplex-competitive ELISA can provide accurate and precise gluten quantitation from fermented sourdough prepared using four different starter cultures.
- Currently, the effects of heat treatment on gluten quantitation in sourdough is being investigated Other variations in sourdough preparation (e.g., fermentation temperature, starter to flour ratio, effects of enzymes) are also being investigated.

References

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