

## Introduction

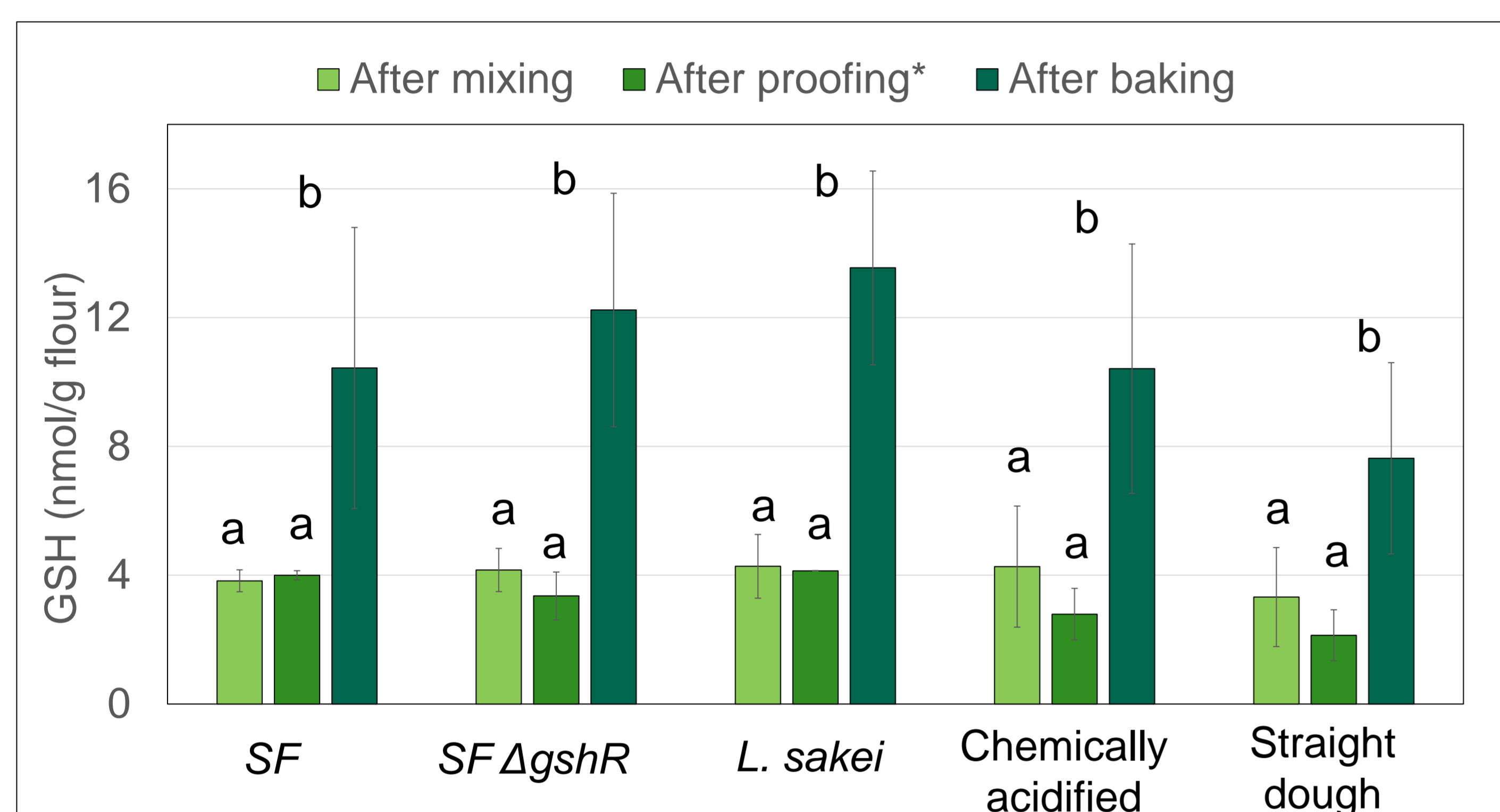
- Sourdoughs exert pH-dependent proteolytic effects that modify the structure of wheat proteins [1]
- Accumulation of thiols such as glutathione (GSH) also play a role by reducing the intermolecular disulfide bonds critical for tertiary structure [2]
- Modification of proteins by sourdough lactic acid bacteria (LAB) may reduce the bioactivity of wheat allergens, such as alpha-amylase/trypsin inhibitors (ATI) [3,4]

**Purpose:** to develop LC-MS/MS-based methods for the quantification of ATI CM3 and GSH in wheat sourdough to elucidate how their abundances might change over the course of the sourdough baking process for applications in food processing

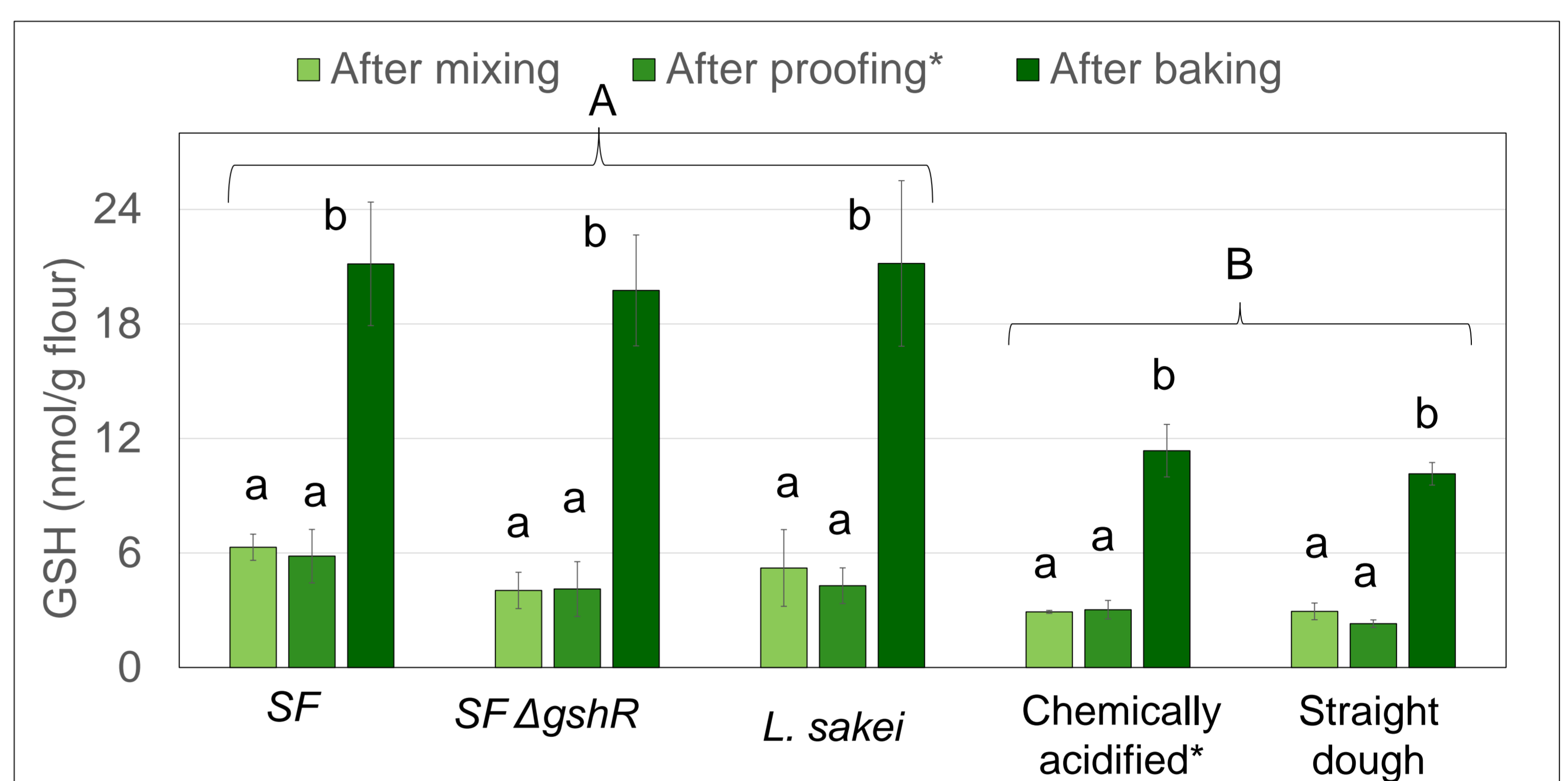
### Experimental Design

- Samples were fermented with: *Fructilactobacillus sanfranciscensis* (SF), *F. sanfranciscensis*  $\Delta$ gshR (SF  $\Delta$ gshR, lacks glutathione reductase and accumulates less thiols in sourdough), and *Lactilactobacillus sakei*; chemically acidified dough and straight dough served as controls
- Breads were baked using two wheat cultivars: Red Fife and Brennan; samples were analyzed from wheat flour, after mixing, after proofing, and after baking
- Method development and analysis were performed using LC-QTOF and LC-QTRAP mass spectrometers

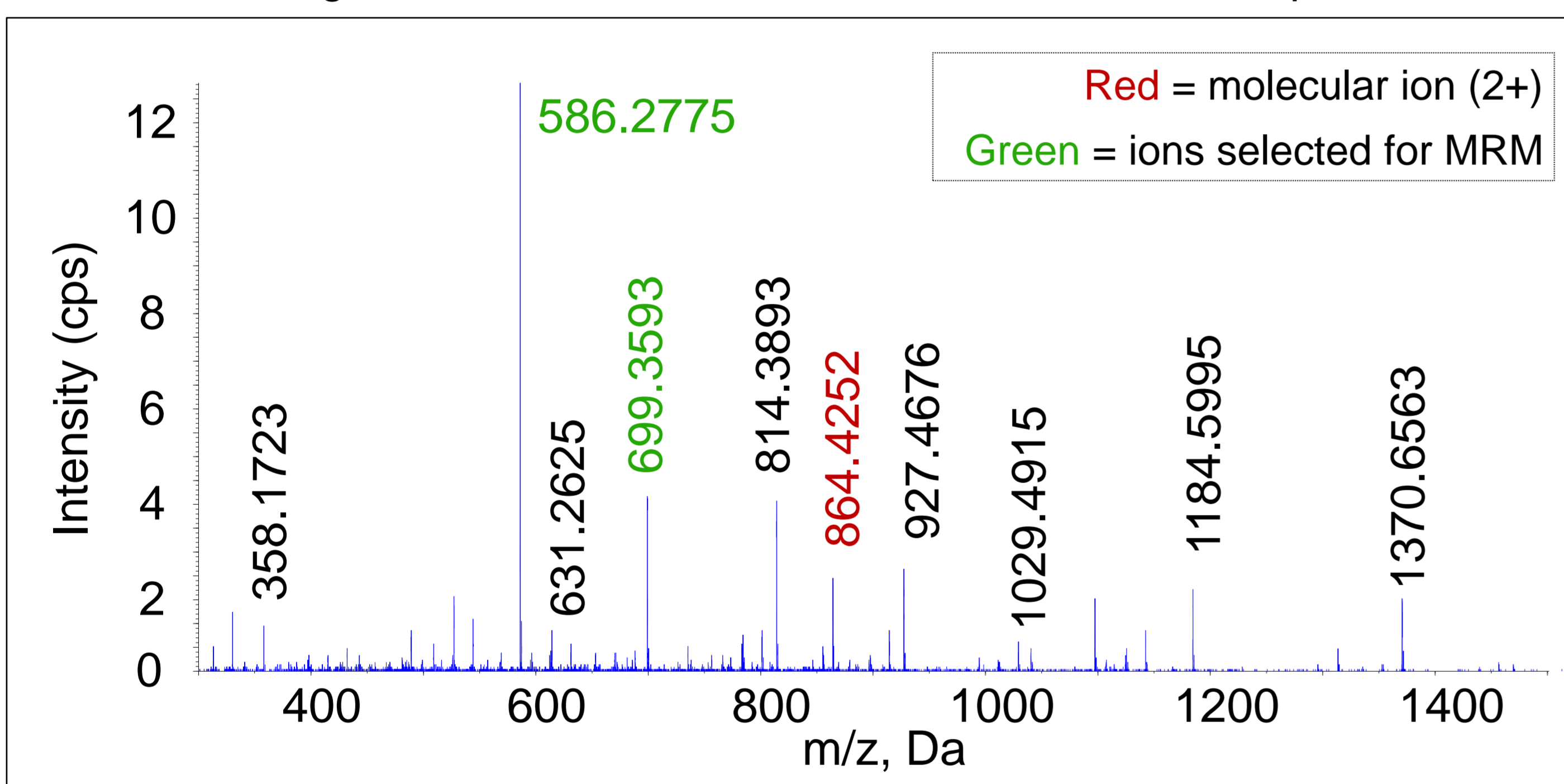
## Results



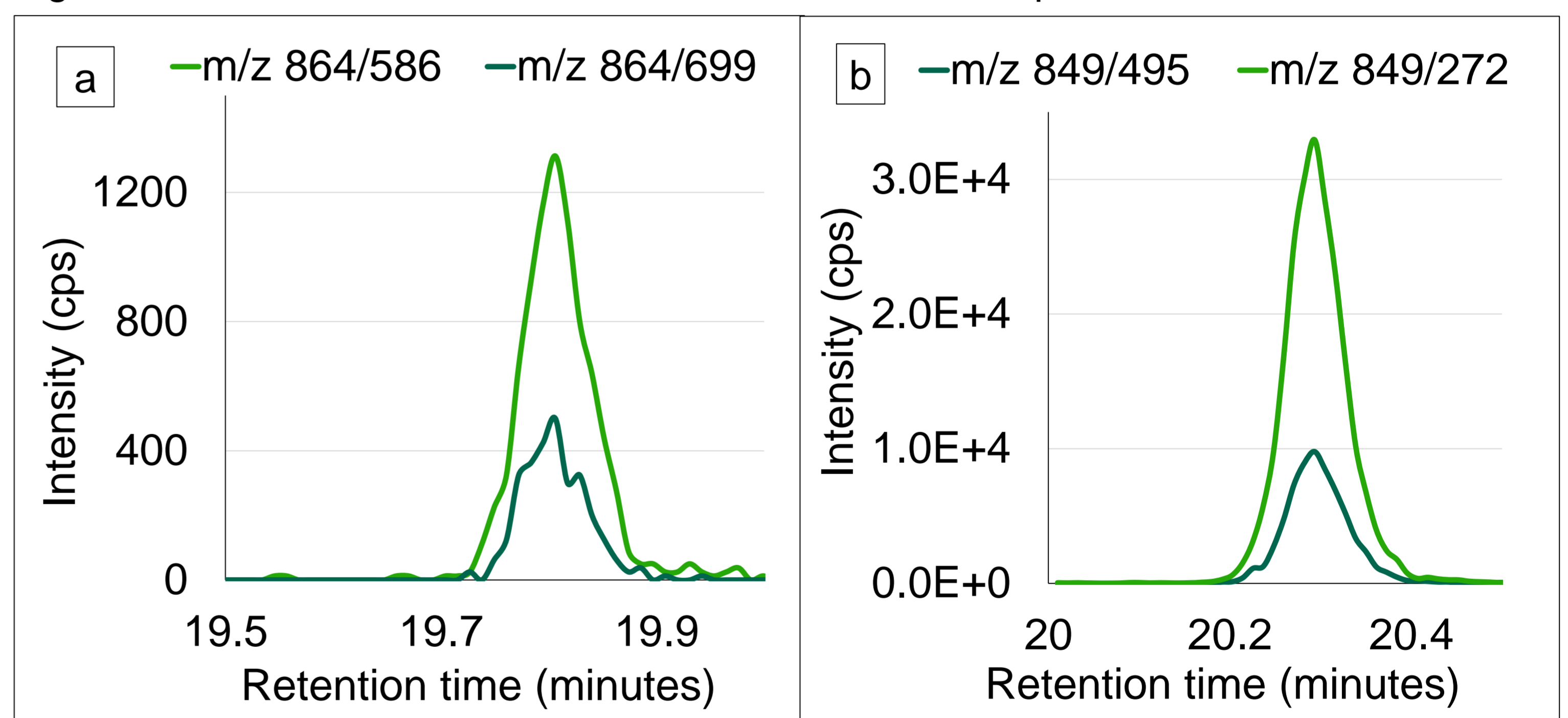
**Figure 1.** Concentration of glutathione during breadmaking in sourdough made with cv Red Fife. GSH was alkylated, extracted with 80% EtOH from sourdough and quantified by LC-MS/MS. Data was normalized to the internal standard and significant differences found at  $P < 0.05$ .  $n = 3$ , except for  $*n = 2$ .



**Figure 2.** Concentration of glutathione during breadmaking in sourdough made with cv Brennan. GSH was alkylated, extracted with 80% EtOH from sourdough and quantified by LC-MS/MS. Results were normalized to the internal standard and significant differences were found at  $P < 0.05$ .  $n = 3$ , except for  $*n = 2$ .



**Figure 3.** MS/MS spectrum of CM3 peptide SGNVGESGLIDLPG CPR. Wheat ATI was digested with trypsin and LC-QTOF was used to identify and select 2 CM3 peptides and their characteristic fragment ions for MRM quantification.



**Figure 4.** MRM chromatograms of CM3 peptides (a) SGNVGESGLIDLPG CPR and (b) YFIALPVPSQPVDPR. CM3 was extracted with 50 mM  $\text{NH}_4\text{HCO}_3$  from sourdough made with cv. Red Fife; the tryptic digest was analyzed by LC-MS/MS.

## Conclusions

- LC-MS/MS-based methods were developed and applied to quantify GSH and ATI CM3 in wheat sourdough
- GSH abundance in cv. Red Fife and cv. Brennan was approximately 35 nmol/g and 55 nmol/g, respectively
- GSH significantly increased after baking and, in the cv. Brennan samples, it was higher in breads fermented with LAB than in the controls

### References:

- [1] Gänzle, Michael G., et al. "Proteolysis in Sourdough Fermentations: Mechanisms and Potential for Improved Bread Quality." *Trends in Food Science & Technology*, vol. 19, no. 10, Oct. 2008, pp. 513–21. doi:10.1016/j.tifs.2008.04.002.
- [2] Joye, Iris J., et al. "Endogenous Redox Agents and Enzymes That Affect Protein Network Formation during Breadmaking – A Review." *Journal of Cereal Science*, vol. 50, no. 1, July 2009, pp. 1–10. doi:10.1016/j.jcs.2009.04.002.
- [3] Caminero, Alberto, et al. "Lactobacilli Degrade Wheat Amylase Trypsin Inhibitors to Reduce Intestinal Dysfunction Induced by Immunogenic Wheat Proteins." *Gastroenterology*, vol. 156, no. 8, June 2019, pp. 2266–80. doi:10.1053/j.gastro.2019.02.028.
- [4] Di Cagno, Raffaella, et al. "Gluten-Free Sourdough Wheat Baked Goods Appear Safe for Young Celiac Patients: A Pilot Study." *Journal of Pediatric Gastroenterology and Nutrition*, vol. 51, no. 6, Dec. 2010, pp. 777–83. doi:10.1097/MPG.0b013e3181f22ba4.