# Fate of Deoxynivalenol and Deoxynivalenol-3-β-D-glucopyranoside during wheat processing

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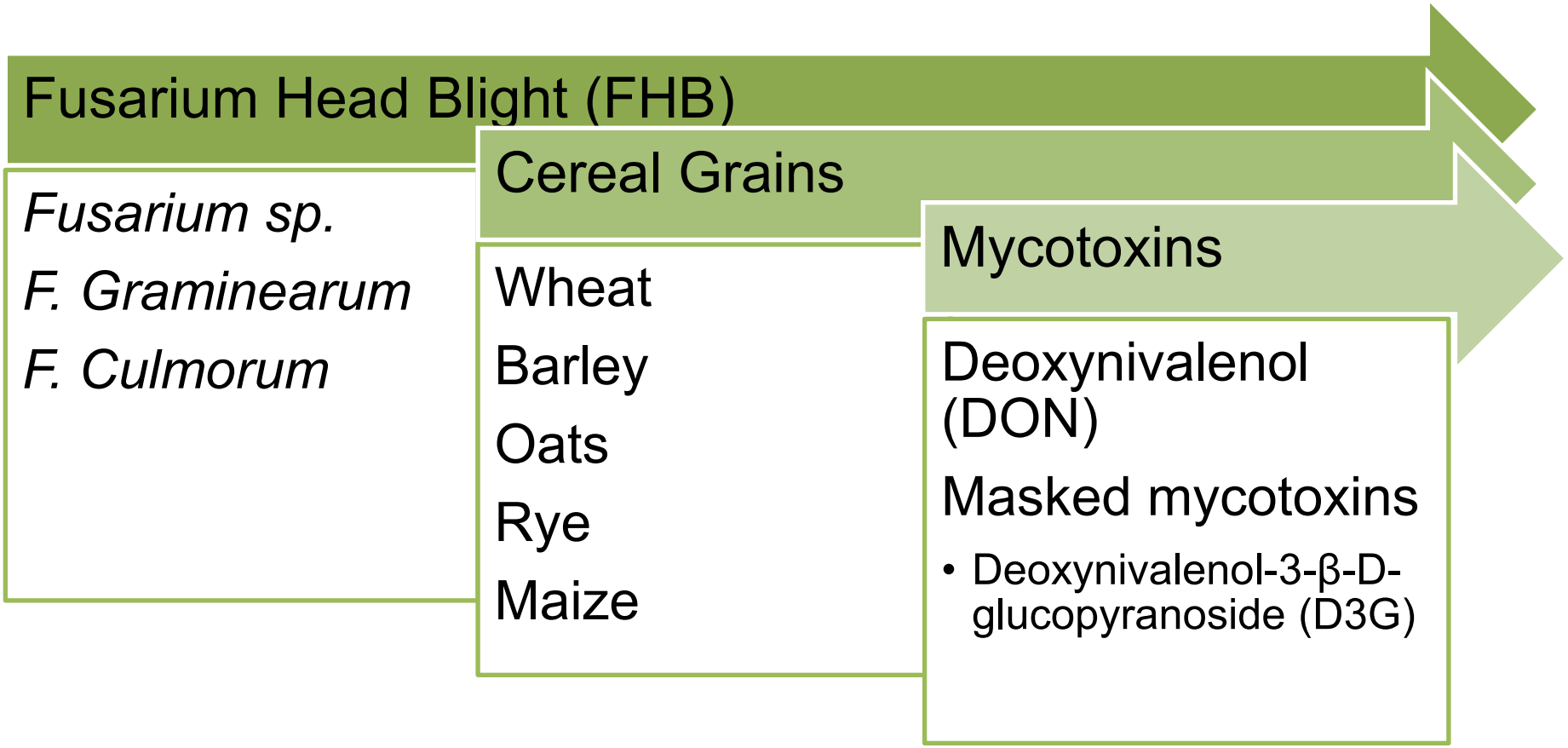
# Introduction



# Mycotoxins

| DON | D3G |
| --- | --- |
| Secondary metabolite produced by fungi | Formed by Fusarium-infected plants as a detoxification process |
| Toxic effects in animals, livestock and humans | Less toxic than DON |
| Very stable | May be hydrolyzed to release DON |

# Mycotoxins and Processing

- Sorting and Cleaning
- Milling
- Brewing
- Enzymes
- Thermal Processing
  - Baking
  - Extrusion

# Mycotoxins and Processing

- Biscuits (Generotti et al., 2017)
  - DON and D3G reduction
    - pH,
    - Baking time and
    - Formulation ingredients (Milk)

# Mycotoxins and Processing

- Baking Additives (Vidal et al., 2018)
- DON and D3G in cakes
  - DON decreased by 40% after baking
  - D3G increased by >100% after baking
  - DON and D3G not affected by presence of 14 common bakery additives

# Mycotoxins and Processing

## Bread-making Enzymes (Vidal et al., 2017)

- DON reduced during baking without enzymes
- D3G reduced during fermentation but increased after baking
- α-Amylase and xylanase prevented decrease in DON
- Increase of deepoxy-deoxynivalenol (DOM-1) during baking

# Mycotoxins in Bread Baking

- Studies show high variation in results
  - Does bread baking result in an increase or decrease in mycotoxins?
- Review of 14 bread baking studies (Wu et al., 2017)

| Relationship | DON | D3G |
|---|---|---|
| Increase | 4 | 1 |
| Decrease | 9 | 2 |
| No Change | 1 | -- |

# Methods

- Hard red spring wheat with moderate susceptibility to FHB
- The wheat had been inoculated with fusarium inoculum
- Amylase activity: Megazyme tablet test kit, AACC approved method 22-05 (AACC-I, 2009)
- Endo-protease activity: Protazyme tablets from megazyme (Ichinose et al., 2001).
- Xylanase activity: Megazyme tablet test kit (Courtin et al., 2005)

# Methods

## Enzyme Treatments

| Enzyme | Buffer | Concentration | pH | Temperature |
|---|---|---|---|---|
| α-Amylase | MOPS/$CaCl_2$ | 50mM/5mM | 6.5 | 60ºC |
| Cellulase | Sodium acetate | 50mM | 4.7 | 45ºC |
| Protease | Sodium phosphate | 50mM | 7.0 | 50ºC |
| Xylanase | Sodium acetate | 50mM | 4.7 | 40ºC |

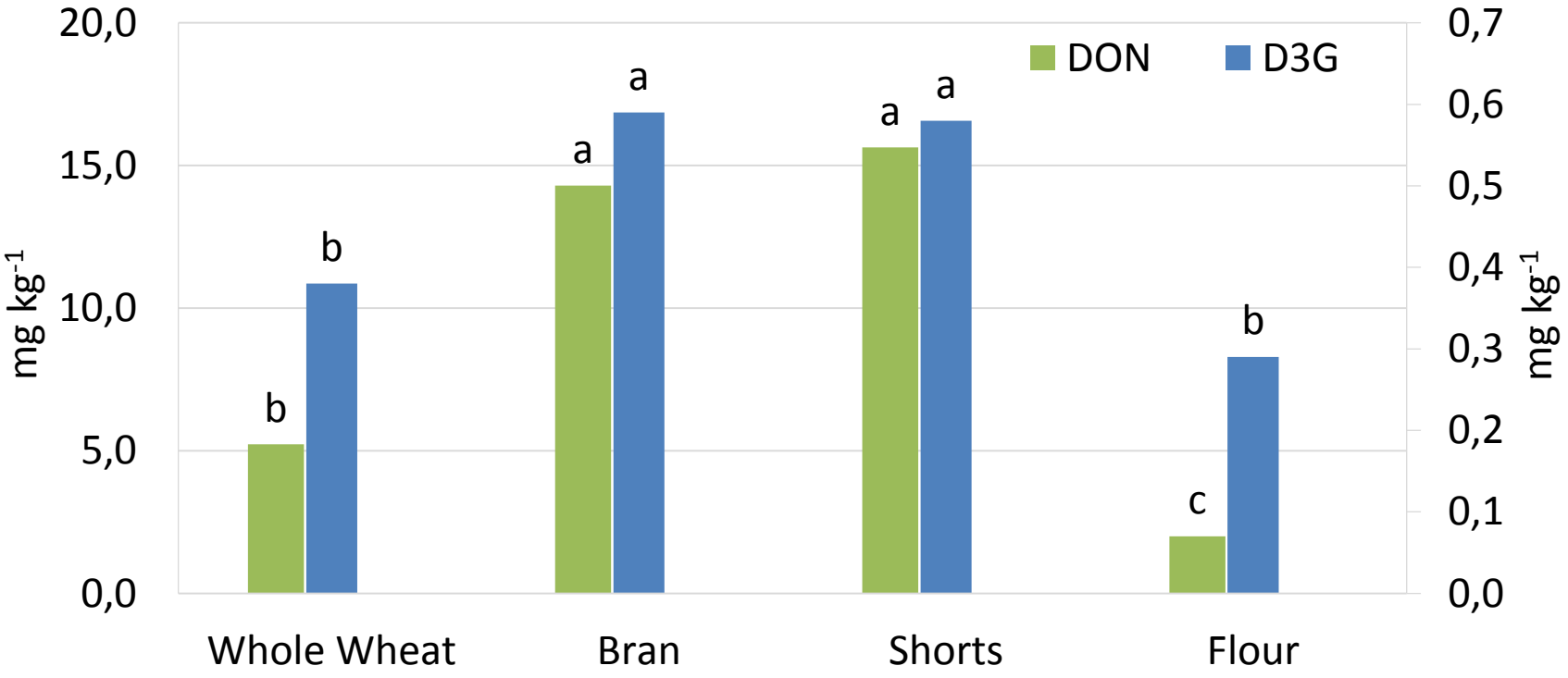
- Wheat composite with ≈5 μg $kg^{-1}$ DON was treated with enzymes,
- Wheat was also incubated with buffer only for a control to each enzyme treatment

# Methods

- Milling: Tempered to 16.5% moisture, Buhler MLU – 202 laboratory mill
- Baking: AACC approved method 10-09.01 (AACC-I, 2009)
- Deoxynivalenol: Extraction with acetonitrile:water (84:16 v/v), Derivatization and analysis with GC-ECD (Tacke and Casper 1996)
- Deoxynivalenol-3-glucoside: Extraction with acetonitrile:water:acetic acid (79:20:1 v/v), HPLC-MS (Vendl et al., 2009 and Simsek et al., 2012)
- ANOVA was conducted with SAS v9.1 and LSD ($P<0.05$) was used for mean separation.

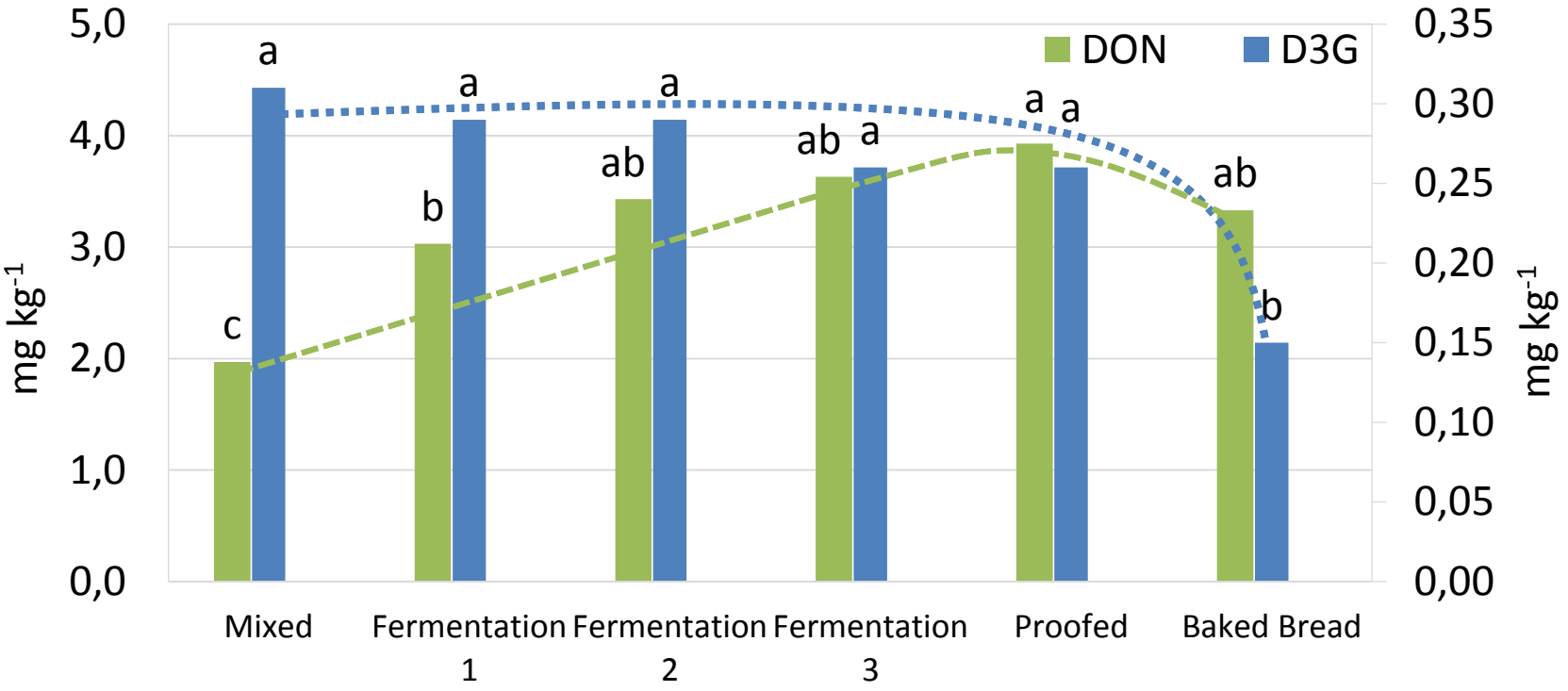
# RESULTS

# Milling: Mycotoxins




DON: Deoxynivalenol; D3G: Deoxynivalenol-3-Glycoside; Columns with the same color and letter are not significantly different P<0.05, mean separation was done using least significant difference (LSD)

# Baking: Mycotoxins



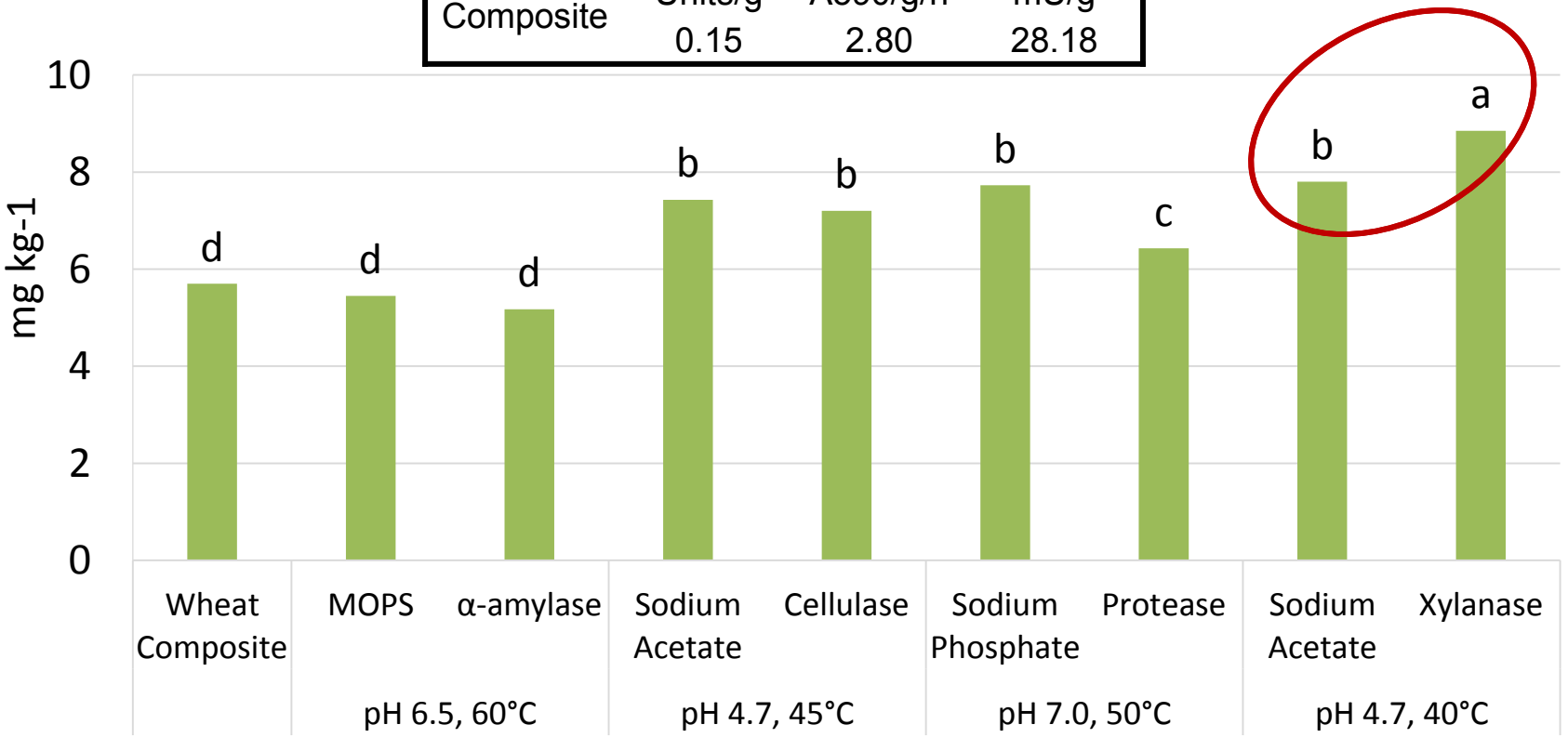

*D3G: Deoxynivalenol-3-Glycoside; DON: Deoxynivalenol; Columns with the same color and letter are not significantly different P<0.05, mean separation was done using least significant difference (LSD); Fermentation 1: 105 minutes, Fermentation 2: 165 minutes and Fermentation 3: 190 minutes

# Baking: Mycotoxins

- Other mechanism besides conversion of D3G to DON?
- Wheat contains embedded DON (Berthiller et al., 2009)
- Increased enzyme activity during fermentation may release DON from these biopolymers

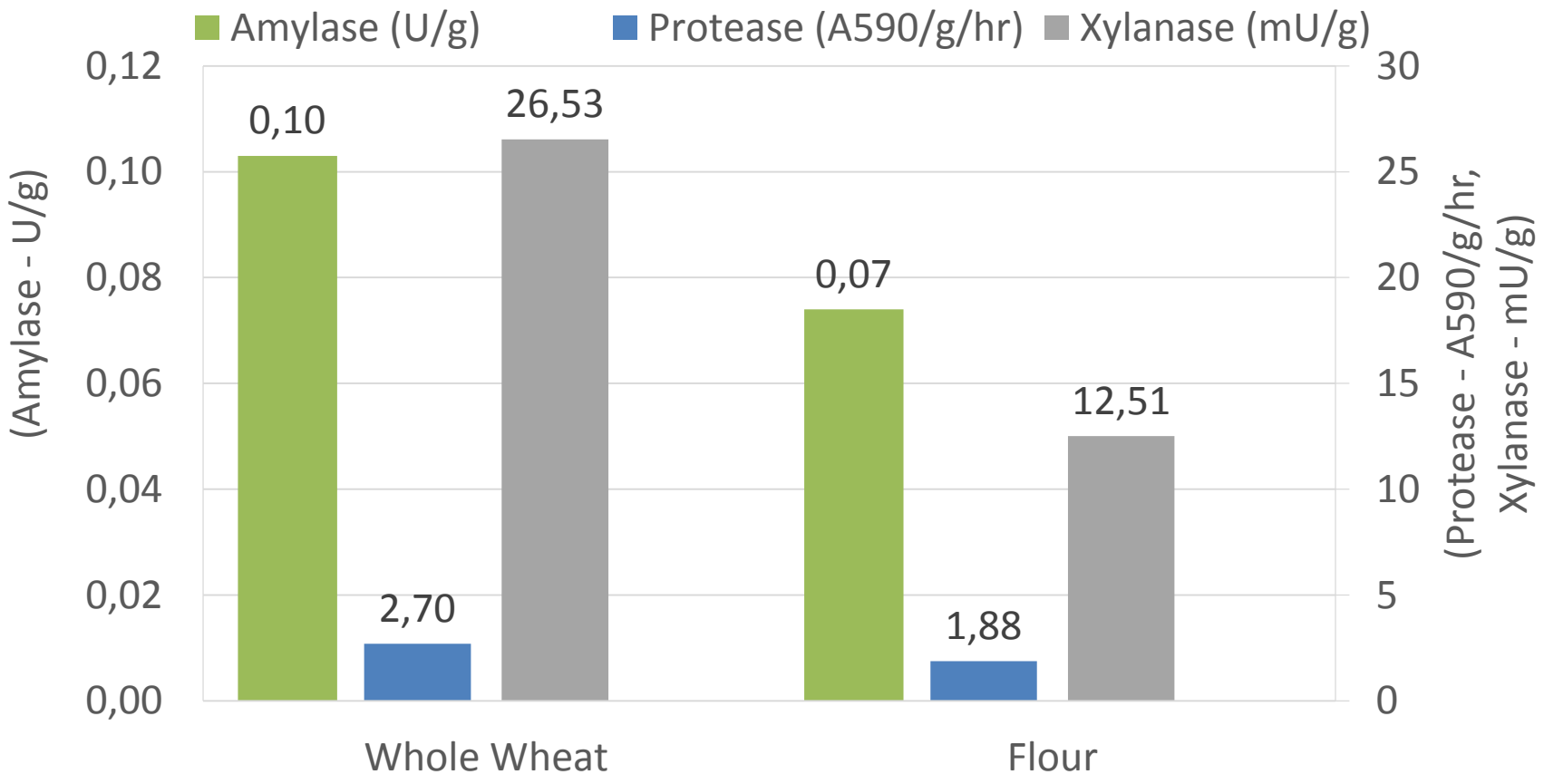
# Enzyme Treatment: DON

| Wheat Composite | Amylase Units/g | Protease A590/g/h | Xylanase mU/g |
|---|---|---|---|
| | 0.15 | 2.80 | 28.18 |

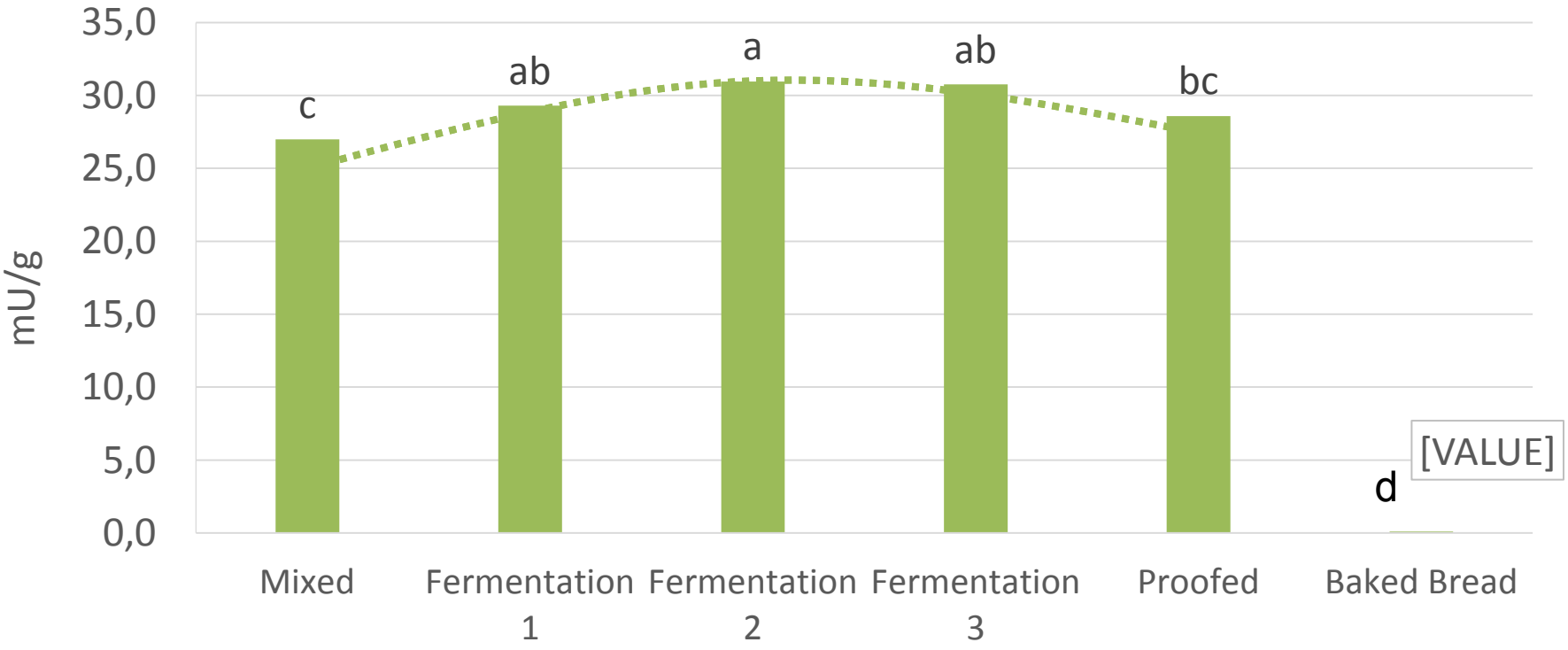



*Columns with the same letter are not significantly different P<0.05, mean separation = least significant difference (LSD)

# Milling: Enzyme Activity



# Baking: Xylanase Activity




*Columns with the same letter are not significantly different P<0.05, mean separation was done using least significant difference (LSD); Fermentation 1: 105 minutes, Fermentation 2: 165 minutes and Fermentation 3: 190 minutes

# Conclusions

## Enzyme Hydrolysis: DON Content

| | |
|---|---|
| Amylase | • No significant (P<0.05) change |
| Cellulase | • Significantly (P<0.05) higher but not different from buffer |
| Protease | • Significantly (P<0.05) higher but lower than buffer |
| **XYLANASE** | • Significantly (P<0.05)higher than control and buffer |

# Conclusions

Standard lean formula

bread baking method

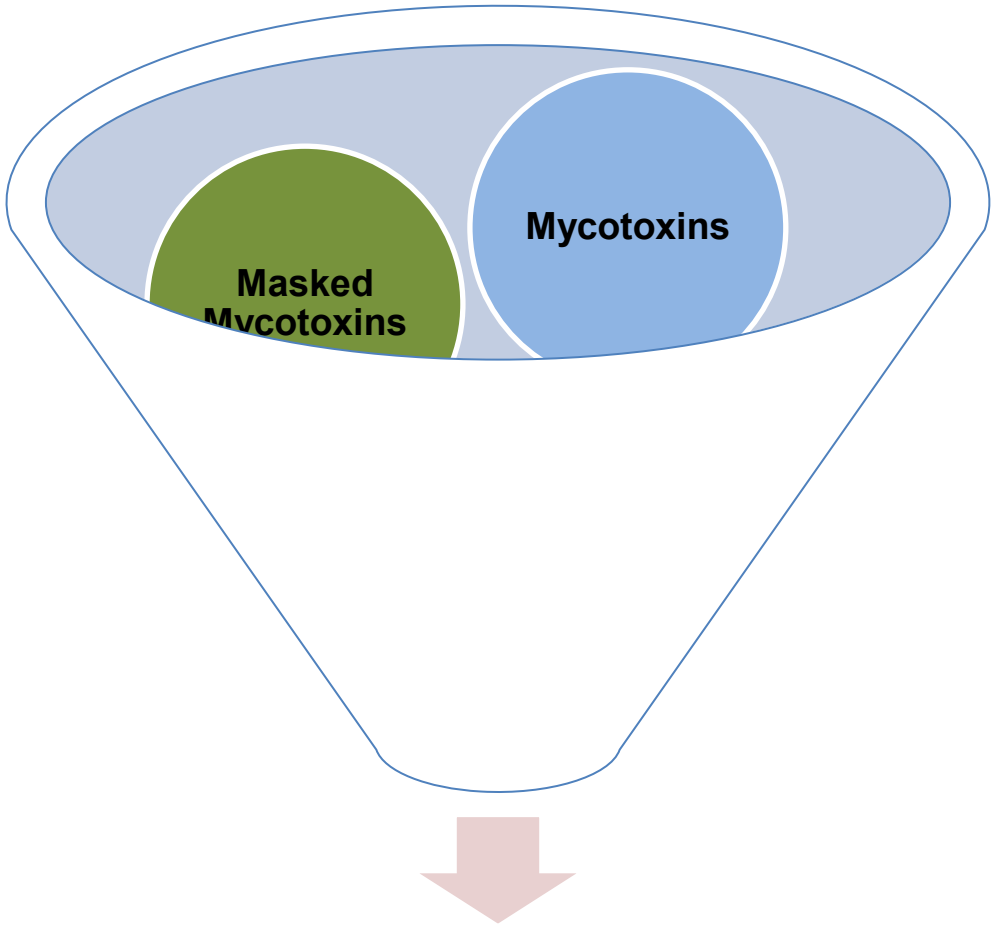
## Fermentation

- Increase in DON
- Decrease in D3G

## Baked bread

- Significant ($P<0.05$) decrease in D3G
- Slight but insignificant ($P<0.05$) decrease in DON

# Take Home Message




**Final Product?**

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# Thank You

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