The effect of amylolytic activity and substrate availability on sugar release in non-yeasted dough

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Introduction

Bread ingredients

The four main ingredients of bread dough are:

Wheat flour

Salt

Water

Yeast

Fermentable sugars in dough

Different sources of fermentable sugars in dough:

❖ Damaged starch: 5-8%

❖ Fructan: 1-3%

❖ Free sugars: glucose
fructose
sucrose
maltose } < 1%

α -Amylase

β -Amylase

Literature:

- ❖ α -Amylase the **limiting factor** for sugar release, β -amylase is present in adequate levels (*Drapron and Godon, 1987; Martínez-Anaya et al., 1996; Cauvain and Young, 2007*)
- ❖ α -Amylase is added to wheat flour at the miller to increase the level of fermentable sugars till a Hagberg falling number of ± 300 seconds is reached (*Hebeda and Zobel 1996; Goesaert et al., 2005; Cauvain and Young, 2007*)

What are the limiting factors for sugar release in dough?

α -Amylase

β -Amylase

Substrate availability

Approach

Two different flour types with different amylolytic background

Approach

Step 1: Comparison of sugar release in dough samples prepared of Bilux and Bison flour

Step 2: Analysis of sugar release in dough samples prepared from Bilux flour (lowest α -amylase activity) with additional enzymes/substrate

Bison and Bilux (14% moisture content)
Addition of enzymes (α/β amylase)
Addition of substrate (damaged starch)

Dough (1.5 % salt)

Incubation (30°C)
0 – 60 – 120 – 180 min

Sugar analysis

High Performance Anion Exchange chromatography (HPAEC)

Results and conclusions

2 different flour types with different amylolytic background

	Bilux	Bison
HFN (s)	477 ± 18	332 ± 8
α -Amylase activity (Amylzyme Units ($\Delta A_{590}/g$ dm.h))	1.97 ± 0.1	6.67 ± 0.4
Damaged starch content (% dm)	6.78 ± 0.1	5.22 ± 0.1
Active β -amylase fraction (Units/g dm*) Non-bound	518 ± 13	499 ± 35

α -amylase addition at the miller necessary

α -amylase addition at the miller **not** necessary

* 1 unit = amount of enzyme needed to release one micromole of maltose from soluble starch in one minute at pH 6 and 40°C

Comparison of sugar release

Bilux **Bison**

❖ *Release of maltose is not higher in dough prepared from flour with higher α -amylase activity*

Inhibition and addition of α -amylase

Results of comparison of sugar release in dough samples prepared from Bilux and Bison flour:

α -amylase is not the only limiting factor for sugar release in dough

❖ Inhibition of α -amylase in Bilux flour: 100 μ mol EDTA/g dm

❖ Addition of α -amylase (*Aspergillus oryzae*; Fungamyl) to Bilux flour

μ L α -amylase/g dm	0	0.02	0.2	2
α -Amylase activity (AU)	2	39	235	3075

Amylzyme Units (AU) = $\Delta E_{590}/g \text{ dm.h}$

Results

Sugar release with inhibition of α -amylase

- ❖ Addition of 100 μ mol EDTA/g dm almost completely inactivates α -amylase (as indicated by Amylazyme test)

- ❖ Release of maltose with inhibition of α -amylase activity after 180 min is **1.7 % (Bilux)** and **1.3% (Bison)**

Sugar release with addition of α -amylase

- ❖ Addition of varying amounts of α -amylase (*Aspergillus oryzae*) to Bilux flour to increase the α -amylase activity

$\mu\text{L } \alpha\text{-amylase/g dm}$	0			
$\alpha\text{-Amylase activity (AU)}$	2			

Results

Sugar release with addition of α -amylase

- ❖ Addition of varying amounts of α -amylase (*Aspergillus oryzae*) to **Bilux flour** to increase the α -amylase activity

$\mu\text{L } \alpha\text{-amylase/g dm}$	0	0.02		
$\alpha\text{-Amylase activity (AU)}$	2	39		

Sugar release with addition of α -amylase

- ❖ Addition of varying amounts of α -amylase (*Aspergillus oryzae*) to **Bilux flour** to increase the α -amylase activity

μ L α -amylase/g dm	0	0.02	0.2	
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Results

Sugar release with addition of α -amylase

- ❖ Addition of varying amounts of α -amylase (*Aspergillus oryzae*) to **Bilux flour** to increase the α -amylase activity

μ L α -amylase/g dm	0	0.02	0.2	2
α -Amylase activity (AU)	2	39	235	3075

Results

Sugar release with addition of β -amylase

Results of comparison of sugar release in dough samples prepared from Bilux and Bison flour:

α -amylase is not the only limiting factor for sugar release in dough

❖ Addition of β -amylase (Barley) to Bilux flour

$\mu\text{L } \beta\text{-amylase/g dm}$	0	2	20
$\beta\text{-amylase activity (Units/g dm)}$	518	538	716

1 Unit = amount of enzyme needed to release one micromole of maltose from soluble starch in one minute at pH 6 and 40°C

Addition of damaged starch

Results of comparison of sugar release in dough samples prepared from Bilux and Bison flour:

α -amylase is not the only limiting factor for sugar release in dough

❖ Substitution of Bilux flour with different amounts of damaged wheat starch

NWS = native wheat starch

BMWS = ball milled wheat starch (damaged for $\pm$ 80%)

Conclusion

2 different flour types with different amylolytic background

Bilux

- ❖ Low α -amylase activity
- ❖ High HFN
- ❖ High amount of damaged starch

Bison

- ❖ Higher α -amylase activity
- ❖ Higher HFN
- ❖ Lower amount of damaged starch

→ *Substrate availability is the limiting factor in Bison dough samples, although the damaged starch is not totally degraded (5.2% starch vs. 1.9% of maltose released)*

→ *α -Amylase activity is the limiting factor in Bilux dough samples*

What are the limiting factors for sugar release in dough?

α -Amylase

β -Amylase

Substrate availability

*Increasing α -amylase activity in dough samples with limited α -amylase activity **strongly increases** the release of maltose*

What are the limiting factors for sugar release in dough?

- ❖ *β-Amylase activity in wheat flour is not a limiting factor*
- ❖ *β-Amylase is very important for the release of sugars in dough, since **1.7% of maltose** is released by β-amylase alone in Bilux dough when α-amylase is inhibited*

What are the limiting factors for sugar release in dough?

α -Amylase

β -Amylase

Substrate availability

*Even when α -amylase activity is high, sugar release can be **limited** by the availability of damaged starch*

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