Starch Isolation from Selected Wheat Varieties as Affected by Nitrogen Fertilization Intensity and the Relation to Protein Content and Gluten Composition

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Contents

• Description of wheat field trials on nitrogen fertilisation – induction of different protein levels in harvested grain:
  3 vegetation periods (2003/2004/2005) - 6 German wheat varieties of 3 quality levels – 3 fertilisation intensities (high, adapted, low)

• Requirements of modern wheat starch isolation utilizing flour/water systems

• Centrifugal separation of flour components as main separation principle

• Results on gluten and starch recovery on laboratory and small scale technical level

• Results in gluten composition (by Osborne fractionation and RP-HPLC) and relation to gluten recovery

• Conclusions
SEM of Wheat Flour Particle
Experimental Design of Field Trials

3 Harvests:
- 2003
- 2004
- 2005

3 Baking classes:
- A
- B
- C

6 Cultivars:
- Batis Applaus
- Flair Maltop
- Hybnos Contra

3 N-Fertilization levels [kg N/ha]:
- high:
  - 2003: 210
  - 2004: 180
  - 2005: 150
- adapted:
  - 2003: 147
  - 2004: 126
  - 2005: 105
- low:
  - 2003: 105
  - 2004: 90
  - 2005: 75
Climatic Situation During the Studied Vegetation Periods (2003 – 2005)
Effect of Fertilizing on Grain Yield and Protein Content

<table>
<thead>
<tr>
<th>Harvest</th>
<th>Yield [dt/ha]</th>
<th>Protein [% d.m.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>37,2</td>
<td>15,3</td>
</tr>
<tr>
<td>2004</td>
<td>63,9</td>
<td>9,6</td>
</tr>
<tr>
<td>2005</td>
<td>46,6</td>
<td>12,2</td>
</tr>
</tbody>
</table>
Wheat Milling Diagram - Miag "Multomat" Used in Flour Production

Flour type 550 → Ash content 0.53 – 0.61 [% d.m.]

B = breaking
C = milling
f = flour
Sieves:
10 x 200 number of sieves x aperture size (μm)

BfEL
Bundesforschungszentrum für Ernährung und Lebensmittel
Relationship Between Grain and Flour (T 550) Protein Content

Grain Protein Content [% d.m.]

Flour Protein Content [% d.m.]

\[ y = -1.13 + x \]

\[ r^2 = 0.994 \quad *** \quad (n=54) \]

cultivars:

A: Applaus
B: Batis
C: Flair
D: Maltop
E: Contra
F: Hybnos
### Extended Catalogue of Characteristics Describing Suitability of Wheat Flour for Industrial Starch Extraction

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>~ 15.0</td>
</tr>
<tr>
<td>Protein (N conversion factor: 5.7; %d.b.)</td>
<td>~ 12.0</td>
</tr>
<tr>
<td>Minerals (% d.b.)</td>
<td>~ 0.63</td>
</tr>
<tr>
<td>Lipids (% d.b.)</td>
<td>~ 1.5</td>
</tr>
<tr>
<td>Fibers (% d.b.)</td>
<td>~ 1.5</td>
</tr>
<tr>
<td>Starch (% d.b.)</td>
<td>~ 80.0</td>
</tr>
<tr>
<td>Moist gluten (g)</td>
<td>~ 28.0</td>
</tr>
<tr>
<td>Amylogram peak viscosity (BU)</td>
<td>~ 500</td>
</tr>
<tr>
<td>Falling number (s)</td>
<td>~ 250</td>
</tr>
<tr>
<td>Starch potential* (%)</td>
<td>min. 70</td>
</tr>
<tr>
<td>Starch granules &lt;10 µm (%)</td>
<td>max. 30</td>
</tr>
</tbody>
</table>

*to be determined by the “Mixer-test”
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet gluten (g)</td>
<td>32.0 ± 6.33</td>
<td>18.35 – 46.4</td>
</tr>
<tr>
<td>Dry gluten *</td>
<td>12.0 ± 2.11</td>
<td>7.65 – 16.3</td>
</tr>
<tr>
<td>(Protein content**)</td>
<td>(90.3 ± 2.9)</td>
<td>85.3 – 96.3</td>
</tr>
<tr>
<td>A Starch *</td>
<td>65.4 ± 4.5</td>
<td>51.1 – 73.65</td>
</tr>
<tr>
<td>(Protein content**)</td>
<td>(0.29 ± 0.04)</td>
<td>0.195 – 0.395</td>
</tr>
<tr>
<td>B Starch *</td>
<td>11.7 ± 2.5</td>
<td>8.0 – 15.3</td>
</tr>
<tr>
<td>(Protein content**)</td>
<td>(3.69 ± 1.12)</td>
<td>1.96 – 6.85</td>
</tr>
<tr>
<td>Fibers *</td>
<td>1.94 ± 0.89</td>
<td>0.91 – 4.69</td>
</tr>
<tr>
<td>Process water (L)</td>
<td>9.2 ± 1.9</td>
<td>5.8 – 10.9</td>
</tr>
<tr>
<td>Dry substance *</td>
<td>0.15 ± 0.06</td>
<td>0.07 – 0.32</td>
</tr>
<tr>
<td>Soluble protein ***</td>
<td>20.3 ± 3.7</td>
<td>13.4 – 31.4</td>
</tr>
</tbody>
</table>

* All figures % dry weight basis
** N conversion factor 6.25
Principles of Wheat Starch Separation

**Substrate**
- Dough

**Separation principle**
- Extraction by washing
  - Dough extractor
- Centrifugal separation
  - Hydrocyclone or Decanter centrifuge

**Separation technique**
- Traditional
- Modern

**Products of separation**
- Traditional:
  - Gluten
  - A Starch + B Starch (incl. Pentosans)
  - Liquid phase

- Modern:
  - Gluten
  - A Starch
  - B Starch
  - Liquid phase (incl. Pentosans)
Three Phase Decanter Separation - Idealized Results of a Spin Test
Phase Formation in a „Centrifugation Test“ with Flour/Water Mixtures of Inferior and Acceptable Separation Capability
## Products of Industrial Wheat Wet Milling

(Composition following German „Guidelines for Starch and Particular Starch Products“, 1976)

<table>
<thead>
<tr>
<th>Product</th>
<th>Moisture</th>
<th>Proteins (f=6.25)</th>
<th>Minerals</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>„A starch“</td>
<td>max. 14%</td>
<td>max. 0.5% s.b.</td>
<td>max. 0.3% s.b.</td>
<td>max. 0.1% s.b.</td>
</tr>
<tr>
<td>„B starch“</td>
<td>max. 14%</td>
<td>max. 5.0% s.b.</td>
<td>max. 1.0% s.b.</td>
<td>max. 0.5% s.b.</td>
</tr>
<tr>
<td>“Gluten/„vital gluten“</td>
<td>max. 5%</td>
<td>min. 80% s.b.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Fibres**: no standards („feed component“)
- **“Pentosans”**: no standards („feed component“)
- **Process liquid**: waste water containing soluble carbohydrates, minerals, and proteins, in general
Laboratory Gluten Recovery as Affected by Flour Nitrogen Concentration

\[ y = 9.46 \cdot x - 8.43 \]
\[ r^2 = 0.91 \]
Laboratory A Starch Recovery as Affected by Flour Nitrogen Concentration
Technical Wheat Flour Separation into its Components
A Starch, B Starch, Fibres, and Gluten

Wheat flour

Continuous dosage

Shear mixer

Mohno pump

Mass flow measurement

Decanter

Gluten washing

1 Gluten washing

2 Gluten washing

Gluten

Starch fraction

A Starch B Starch

Gluten fraction

Vibration sieving (75 µm)

Tap water 35°C

Vibration sieving (75 µm)

Centrifuge

Tap water

Shear mixer

Mohno pump

Mass flow measurement

Decanter

Gluten fraction

Gluten washing

Gluten
Selected Steps of Small Scale Wheat Wet Milling

1. Decanter overflow – Gluten/Liquid phase
2. Decanter concentrate – Starch
3. Flour water mixing
4. Starch slurry sieving
5. Gluten washing
Survey of Wheat Flour Samples Separated into Fractions of Wheat Wet Milling on Small Scale (15 kg)

<table>
<thead>
<tr>
<th>Fertilization intensity</th>
<th>High</th>
<th>Adapted</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batis</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Applaus</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Flair</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Maltop</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Hybnos</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Contra</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Relation Between Small Scale Dry Gluten Recovery and Flour Protein Content

Flour Protein Content [% d.m.] (N x 5.7) vs. Dry Gluten Recovery [% d.m.]

- 0  5  10  15  20
- 0  5  10  15  20
Exemplary Weighted Statistics for Multiple Isolation of Flour Components in Small Scale

<table>
<thead>
<tr>
<th>Starch fraction</th>
<th>Mean [%]</th>
<th>Standard deviation</th>
<th>Coefficient of variation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluten</td>
<td>15.33</td>
<td>0.437</td>
<td>2.85</td>
</tr>
<tr>
<td>A Starch</td>
<td>58.03</td>
<td>3.27</td>
<td>5.64</td>
</tr>
<tr>
<td>B Starch</td>
<td>20.30</td>
<td>3.61</td>
<td>17.77</td>
</tr>
</tbody>
</table>
Composition and classification of wheat gluten proteins

Gluten
- approx. 31% HMW Subunits
- approx. 13% LMW Subunits
- approx. 56% Gliadins
  - α/β-type Gliadins
  - γ-type Gliadins
  - ω-type Gliadins

HMW Prolamins
- 80-120 kDa

S-rich Prolamins
- 35-60 kDa

S-poor Prolamins
- 30-80 kDa

Shewry & Miflin:
v. Dijk & Bekkers
Modified Osborne-Fractionation* of Gluten Proteins

Extraction and Removal of Albumins & Globulins
Soerensen Puffer pH 7.6; RT

Extraction of Gliadins
60% Ethanol; RT

Extraction of Glutenins
Solution for Reduction (50% 1-PrOH/2 m Urea/0.05 m Tris/HCl pH 7.5/1% DTE); 60°C

Relation Between Gluten HMW:LMW Ratio and Flour Nitrogen/Protein Content

\[
y = 0.023 + 0.147 \times \quad r^2 = 0.720^{***} (n=36)
\]
Relation Between Technical Scale Dry Gluten Recovery and Flour HMW: LMW Ratio
Summary and Conclusions

• In the fertilization trial wheat samples of high variability in protein quantity and quality were produced due to the climatic conditions in the production periods. Grain/flour protein followed well fertilization intensity.

• On laboratory scale gluten and A starch as well as all other technologically interesting fractions could be isolated. Nevertheless, plant development as affected by fertilization played an important role.

• In the applied small scale technical procedure, only a limited number of samples could be wet milled because of ceased gluten development or poor gluten aggregation.

• On the basis of grain material with high and low fertilization, Osborne fractionation and investigation of respective gluten fractions by RP-HPCL revealed so far that processing ability seems to depend on the relation of high (HMW) and low molecular (LMW) glutenins. All samples having a ratio below approximately 0.3 could hardly be processed.
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Laboratory A Starch Recovery as Affected by Flour Nitrogen Concentration

![Graph showing the relationship between Flour Nitrogen Concentration and A Starch Recovery]

- **Regression Equation:**
  - $r^2 = 0.51$

- **Data Points:**
  - 2003
  - 2004
  - 2005

- **Species:**
  - Batis
  - Applaus
  - Flair
  - Maltop
  - Hybnos
  - Contra

- **Legend:**
  - Orange line: Regr. N conc. vs A Starch recovery
  - Dashed line: Prediction limits (95%)