IMAGING THE STRUCTURE OF BREAD AND DOUGH

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Research Institutes of Sweden
Bioscience and Materials
Agrifood and Bioscience

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Introduction to microstructure work at RISE Agrifood and Bioscience

Microstructure of bread and dough
- Different structural components
- Different techniques
- Different types of bread

How to use microstructure in product development
- Example from frozen stored bread and dough

AGENDA

A large variety of different preparation techniques of food and other “soft” material structures

Microscopy analysis at different length scales

Dynamic processes (e.g. visualising phenomena over time like foam formation, aggregation, phase separation, disintegration, migration, etc.)

Knowledge of materials and their properties
Instrumentation

<table>
<thead>
<tr>
<th>Instruments</th>
<th>Preparation equipment</th>
<th>Accessories</th>
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<tbody>
<tr>
<td>Light microscope (LM)</td>
<td>Balzer freeze-etching unit, BAF 400T</td>
<td>Specially designed microfluidic flow cells for microscopy observation</td>
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<tr>
<td>Nikon Microphot FXA, structural level: 2mm-0.5mm</td>
<td>Plunge freezing device</td>
<td>Micro injection tool</td>
</tr>
<tr>
<td>Confocal Laser Scanning Microscopes (CLSM) Leica SP5 and SP2, structural</td>
<td>Cryo-stage to the TEM</td>
<td>Heating/cooling cell for CLSM</td>
</tr>
<tr>
<td>level: 2mm-0.2mm</td>
<td>Leica Cryostate, CM 1900</td>
<td>Microtensile test for CLSM</td>
</tr>
<tr>
<td>Transmission electron microscope (TEM) LEO 906E 120 kV, structural level:</td>
<td>Ultramicrotome, RMC</td>
<td>Hybrid Detector at CLSM SP5</td>
</tr>
<tr>
<td>20nm-5nm</td>
<td>Tissue processor for plastic embedding, Leica EM TP</td>
<td>Mass transport, measuring of local diffusion (FRAP, RICS)</td>
</tr>
<tr>
<td>At RISE in Stockholm and Borås Confocal Raman, SEM, IR, AFM, ToF-SIMS, XPS</td>
<td></td>
<td>Image Analysis (Using both commercial and in-house developed software)</td>
</tr>
<tr>
<td>ESEM Quanta FEG ESEM 200 mod EDX-analys, 20mm-2nm</td>
<td></td>
<td></td>
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<tr>
<td>TEM Tecnai 200kV, structural level: 20nm-2nm</td>
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<tr>
<td>FIB-SEM</td>
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<td>RAMAN spectroscopy</td>
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<td>At Chanders:</td>
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<tr>
<td>X-ray Tomograph</td>
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RISE at MAX IV and ESS – Applied research using x-rays and neutrons

- RISE will enable utilization of MAX IV and ESS by industry as a tool in material and product development
- RISE “pilots” guide industry, from their material challenge, through measurements to final material solution
- RISE will integrate x-ray and neutron techniques in their toolbox and use them in combination with our exciting instruments and methods to solve industrial and societal challenges
- RISE is currently expanding it’s capabilities by employing three specialists and an area coordinator
- Project leaders: Niklas Lorén and Claes Holmqvist
Microstructure of a croissant
- Length scales in microscopy

X-ray tomography of croissantant

Length scales in microscopy

3D-visualisation using x-ray tomography

Gluten protein is red and starch granules are green in CLSM

In LM starch is shown in violet (amylose dark blue and amylopectin pink/brown, protein in light green and fat in brown

A granule surrounded by the gluten in TEM
Microstructure of bread using microscopy at different length scales (LM, TEM)

For brittle materials, e.g. bread, snacks, that often containing a lot of cereals, careful fixation followed by plastic embedding and analyse in LM is the solution.

LM and TEM preparation

1. **Fixation** - (days)
   Formaldeyde/glutaraldehyde

2. **Fixation 2** - (hours)
   Oso4

3. **Dehydration**
   Graded ethanol series

   ![Ethanol Series](https://via.placeholder.com/150)

4. **Embedding in plastic**

5. **Thin-sectioning**

6. **Staining**

For brittle materials, e.g. bread, snacks, that often containing a lot of cereals, careful fixation followed by plastic embedding and analyse in LM is the solution.

LM is the outstanding method for distinguish between amylose and amylopectin.
Microscopy on dough

CLSM

Yeast cells
starch granules

gluten

LM

Yeast cells
starch granules
gluten

Different types of bread

"normal" white wheat bread

glutenfree
Example of dynamic study - Dough - proofing

Developing of a bubble and deformation of material components during proofing

FROZEN BREAD AND DOUGH
April 2011-Mars 2014

Camilla Öhgren, RISE
Maud Langton, SLU

Research Institutes of Sweden
Bioscience and Materials
AGRIFOOD AND BIOSCIENCE
PARTICIPANTS:
Dafgårds (SIK) RISE Swedish Board of Agriculture
Fazer Chalmers
Lantmännen SLU
Ewalco
Norlander-Zeelandia
Jästbolaget
JBT
Sveba-Dahlen

Partly proofed dough – x-ray tomography at SLU, Uppsala
Partly proofed dough – x-ray tomography at SLU, Uppsala,

BACKGROUND

Today, more and more bread is frozen at the bakery...

- What are the quality differences between freshly baked bread and the frozen alternatives, and what causes such differences?
- Do the consumers notice the differences?
- How can quality loss be avoided or minimized by changing the baking methods (freezing rate, kneading time) and the recipe (fiber, emulsifiers, enzymes etc)?
Quality changes during storage - e.g. freshly baked aroma and texture

- Freshly baked aroma
- Stored as dough
- Stored as bread

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Aroma</th>
<th>Taste</th>
<th>Texture by finger</th>
<th>Texture in mouth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stored as dough</td>
<td>Stored as bread</td>
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What is the problem?....... volume decrease!!

Rye/wheat

Sweat wheat

Peaked buns!
Extra proofing…?

How are the yeast?

Wheat/rye dough
92% dead

Sweat dough
46% dead

Stored unproofed for 10 month in the freezer
More reasons?

Wheat/rye dough  Wheat/rye dough

Sweat dough  Sweat dough

Sugar content?

Effect of sugar content

Gluten
Starch granules

Sugar content
Effect of freezing

Fresh

Frozen stored dough

Why lower volume after frozen storage?

- Dead yeast
- Sugar,
- Ice crystals – deterioration of the gluten network
- Ice crystals – dead yeast
- Loss of water
- Or combinations
- ...

Solutions:
- Increase kneading time
- Avoid storage >-15°C
- Avoid too long freeze storage
- Yeast stays alive longer in freezer with small amounts of sugar available, 0.4%
