ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

DAGRL
Department of Agricultural and Food Sciences

Optimization of grain fibres and phytochemicals for health benefits

Prof. Laura Nyström

Institute of Food, Nutrition and Health

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World Food System

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- **Food Process Engineering**
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Apr 2010

Food Biochemistry Group

Group Members:

- Prof. Dr. Laura Nyström
- Dr. Giuseppe Manzardo
- M.Sc. Monika Risel-Seyda

Post doctoral scientist:

- Dr. Joanna Brys

PhD Students:

- M.Sc. Audrey Faure
- M.Sc. Eszter Mandak

Administrative, Technical and IT Support:

- Teresa Probanowski-Angst
- Aida Huber-da Costa
- David On

- 1-2 MSc students, 3-5 BSc students

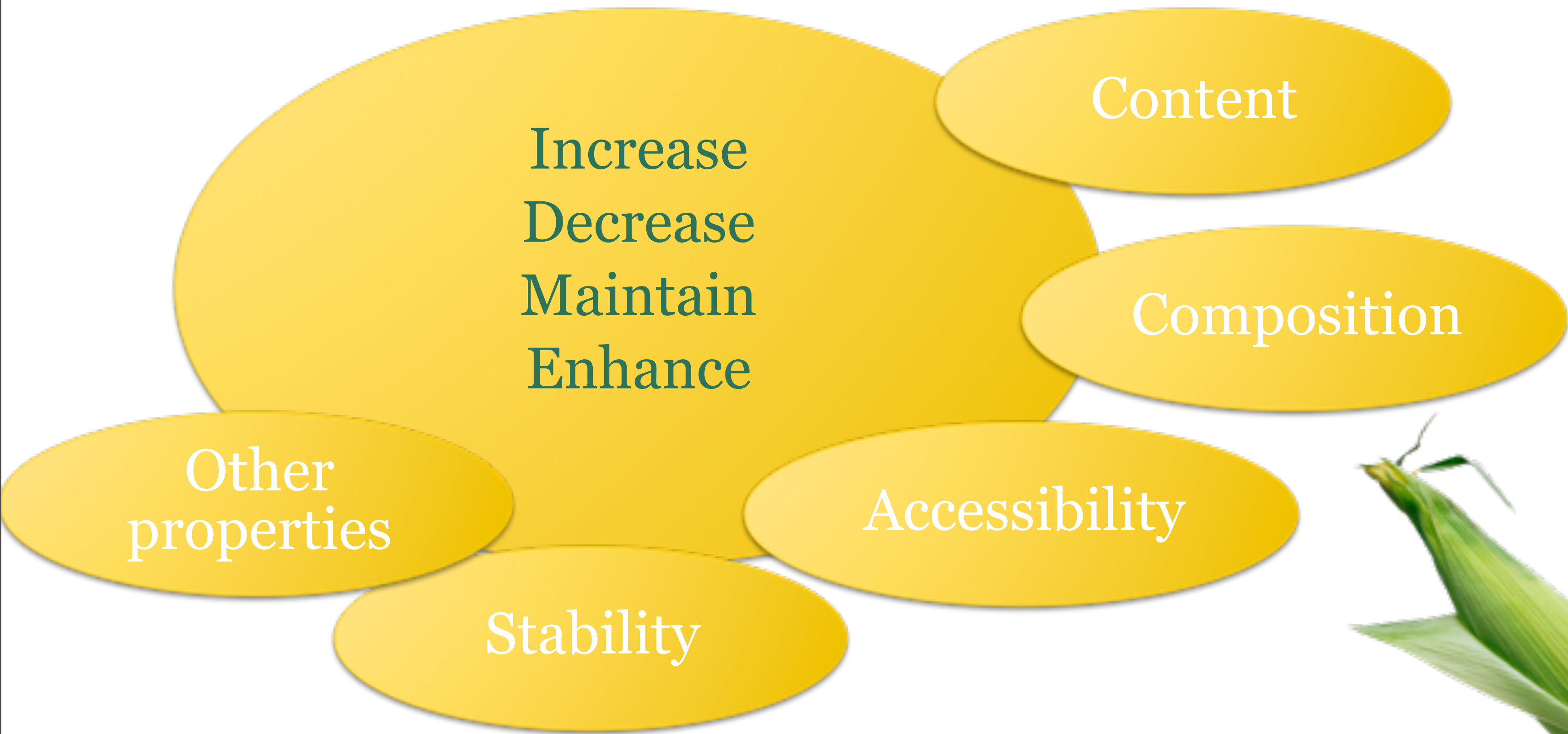
Themes of interest

- Reactions that occur in food processing, preparation and storage
- Way to control and utilize the reactions for better and safer foods
- In-depth knowledge on the composition of foods, chemistry and other properties of food constituents
- Further focus on the analytical methods to study the foods (composition, reactions etc.)

Key areas of competence

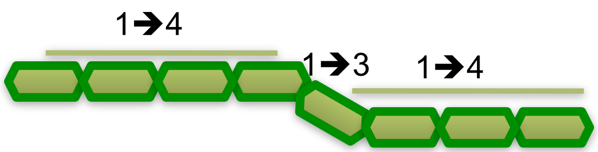
- Analytical methods for the analysis of food constituents
  - Content
  - Composition
- Enzymatic and chemical reactions in food processing and storage and analytical methods to monitor the resulting changes
- Cereal chemistry
  - Dietary fibre components and their properties
    - Beta-glucan
  - Co-passengers of dietary fibre
    - Plant sterols
    - Phytates

Optimization of nutritional quality



Stability of beta-glucan in aqueous systems

- Beta-glucan as the only dietary fibre is allowed a health claim for maintenance of healthy cholesterol levels
  - Decreasing blood pressure, attenuation of glucose and insulin responses, immunomodulatory effects, satiety control also studied
- Health benefits considered (mainly) to be dependent on viscous properties of BG
  - Molecular weight, concentration and beta-glucan source affect viscosity
  - Role of low molecular weight BG unclear
  - Sensory properties altered
  - Possibilities to use BG as a hydrocolloid in foods?
- Instabililty in aqueous systems (beverages, semisolid foods) due to radical mediated degradation
  - Spontaneous reaction caused by redox chain reaction



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β-Glucan – Radical Mediated Degradation

- Scission of polysaccharides can be initiated by reactive oxygen species (ROS) such as hydroxyl radicals (·OH)
- Shown that β-glucan is degraded in presence of ascorbic acid and $Fe(II)SO_4$
- Viscosity loss is closely related to degradation of β-glucan

Fenton Reaction

(1) $Fe^{2+} + H_2O_2 \rightarrow \cdot OH + OH^- + Fe^{3+}$

Role of Ascorbic Acid

(2) $AH_2 + Fe^{3+} \rightarrow A + 2H^+ + Fe^{2+}$

(3) $AH_2 + O_2 \rightarrow A + H_2O_2$

AH_2 = Ascorbic acid
A = Dehydroascorbic acid

Effect on Polysaccharides

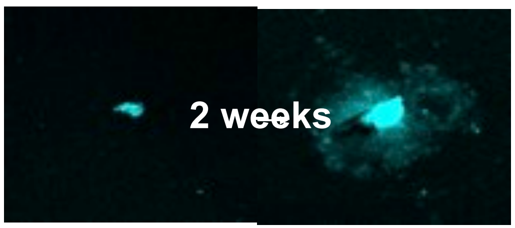
(4) $RH + \cdot OH \rightarrow R\cdot + H_2O \rightarrow$ degradation

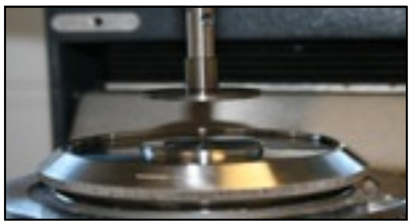
RH = Polysaccharide
R· = Polysaccharide radical

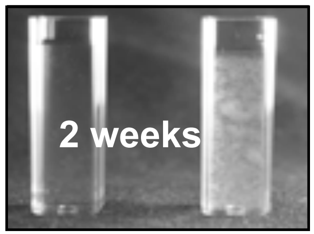
Fry SC, 1998; Kivelä R et al., 2009

Radical mediated degradation of beta-glucan

- Development of analytical methods to study the reaction in complex food systems
  - Radical formation: amount and type of radicals formed (EPR spectroscopy)
  - Changes in MW
  - Effects of various raw materials, and other matrix effects
- Find ways to inhibit or control the reaction in foods
  - Antioxidants and oxidation inhibitors
  - Control by structural and compositional features
  - Different processes – baking, freezing, thawing etc.
  - Interactions with other polymers (proteins, resistant starch etc.)
- Ways to utilize the reaction technologically or chemically?







Inhibition of Radical-Mediated Degradation

MSc Thesis Linda Münger

(1) Addition of Antioxidants

- Free radical scavengers → formation of antioxidant radical with low reactivity

$$R\bullet + AH \rightarrow RH + A\bullet$$

R• = Radical
AH = Antioxidant

- Phenolic acids, polyphenols, sugars, sugar alcohols, some amino acids

(2) Addition of Preventive Inhibitors

- Substances that deactivate one of the substrate of the Fenton reaction
- **Iron chelators**
  - Organic acids (phytic, citric, malic, tartaric and oxalic acid)
- **H_2O_2 decomposer**
  - Catalase ($2H_2O_2 \rightarrow O_2 + 2H_2O$)

Pokorny J et al., Antioxidants in food, 2001

Method

β-glucan (0.6% in H_2O)

⇩

pH-adjustment: 4.5 ± 0.05

⇩

Sample preparation for EPR

0.5 ml β-glucan solution containing 2 % ethanol

40 mM POBN
50 µM $FeSO_4$
250 µM ascorbic acid

+ potential inhibitor

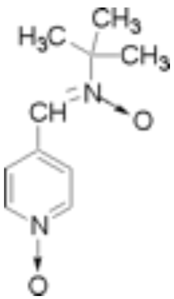
⇩

Samples are measured every 20 minutes starting at 1 min until 100 min

⇩

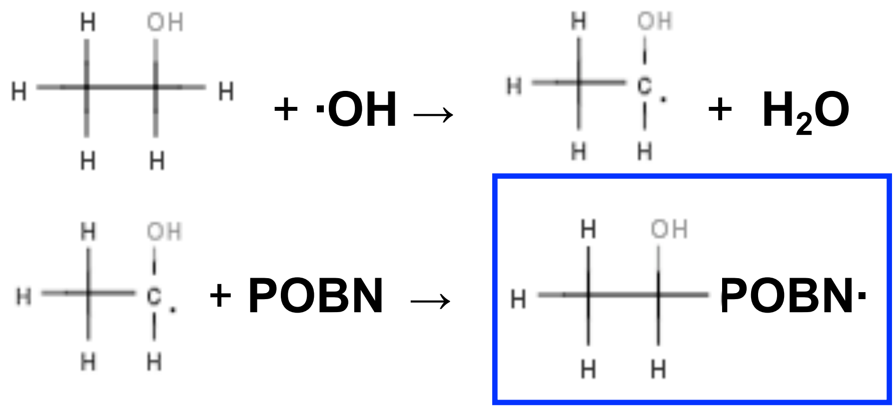
Evaluation of spectra → determination of relative amplitude

Spin trapping with POBN



α-(4-Pyridyl *N*-oxide)-*N*-*tert*-butylnitrone

spin trap → preferably forms adducts with carbon-centred radicals



β-glucan radical might not trapped by POBN due to steric reasons

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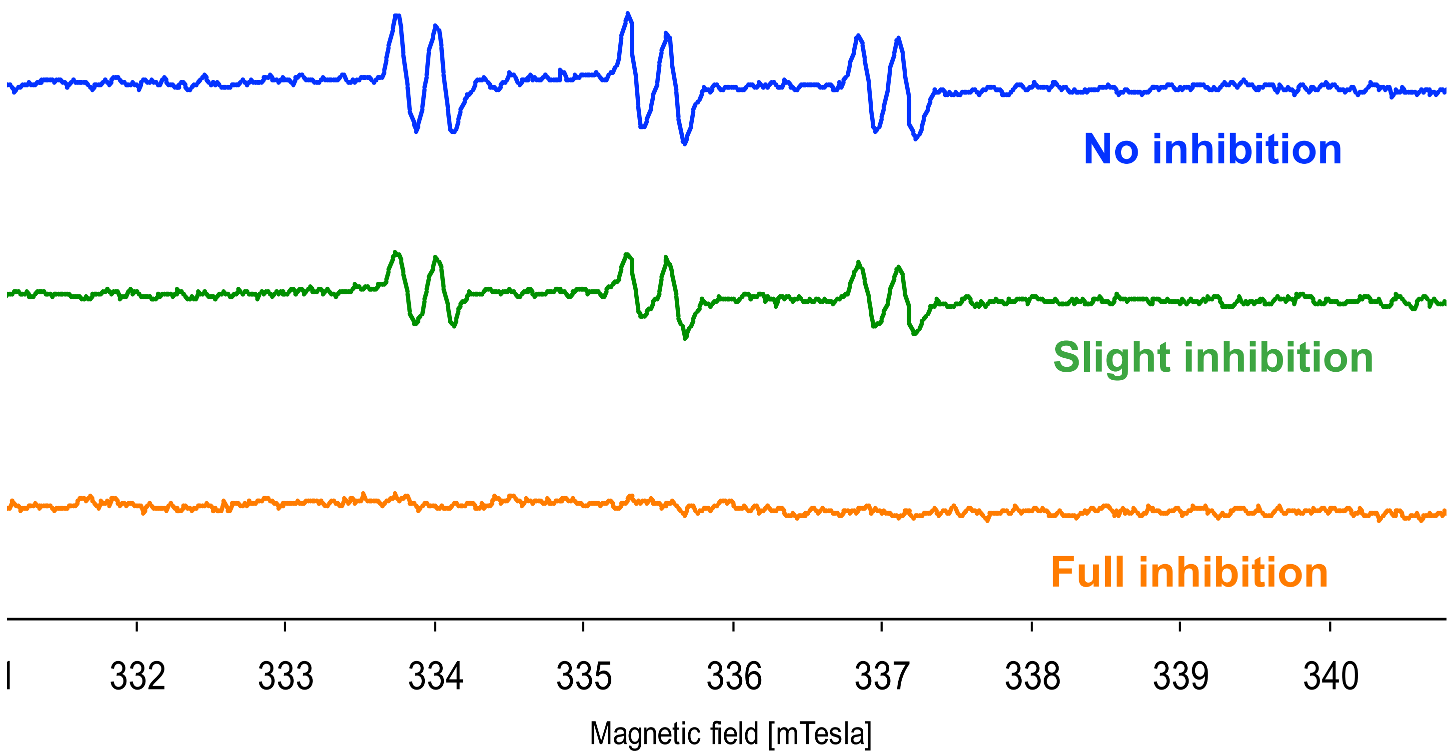
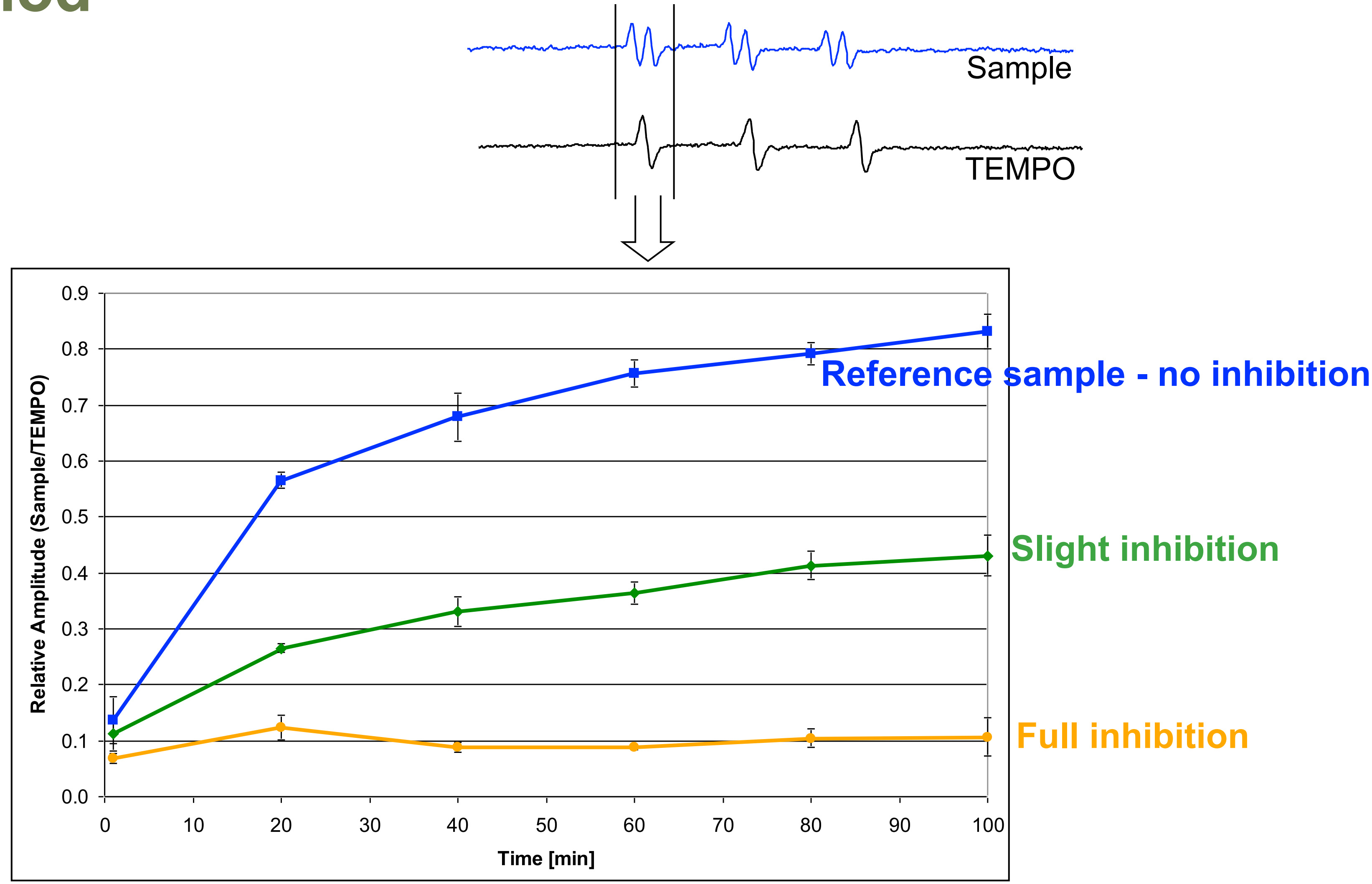
EPR Spectra


No inhibition
Slight inhibition
Full inhibition
332
333
334
335
336
337
338
339
340
Magnetic field [mTesla]

Method


Sample
TEMPO
Relative Amplitude (Sample/TEMPO)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
10
20
30
40
50
60
70
80
90
100
Time [min]
Reference sample - no inhibition
Slight inhibition
Full inhibition

Inhibition of Radical Formation (100 min)

	Potential Inhibitor	Inhibition [%]		Potential Inhibitor	Inhibition [%]
Monosaccharides	Glucose (1M)	86	Organic acids + phytic acid	Citric acid (10 mM)	95
	Fructose (1M)	69		Malic acid (10 mM)	93
	Ribose (1M)	88		Tartaric acid (10 mM)	97
	Deoxyribose (1M)	87		Oxalic acid (10 mM)	99
	Arabinose (1M)	67		Phytic acid (2.2 mM)	96
	Rhamnose (1M)	64			
Disaccharides and sugar alcohols	Sucrose (1M)	96	Phenolic acids	Ferulic acid (200 mM)	51
	Maltose (1M)	93		P-coumaric acid (200 mM)	71
	Lactose (1M)	37		Caffeic acid (200 mM)	75
	Mannitol (1M)	76		4-Hydroxybenzoic acid (200 mM)	97
Amino acids	Methionine (100 mM)	89	Polyphenols	Epicatechin (10 mM)	10
	Cysteine (100 mM)	93		Catechin (10 mM)	-2
	Cystine (100 mM)	-91		Rutin (10 mM)	42
	Histidine (100 mM)	-55		Quercetin (10 mM)	92
	Tyrosine (100 mM)	0.2	Others	Catalase (3000 U/ml)	100
	Tryptophan (100 mM)	97		Superoxide dismutase (580 U/ml)	94
	Phenylalanine (100 mM)	93		Potassium disulfite (100 μM)	95

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Conclusions

- Different oxidation inhibitors vary significantly in their effectiveness to inhibit radical formation in β-glucan solutions.
- Even relatively low concentrations inhibit radical formation significantly
- Most effective and long lasting inhibition obtained with enzymes (catalase and superoxide dismutase)

➔ Antioxidant mixtures and synergism

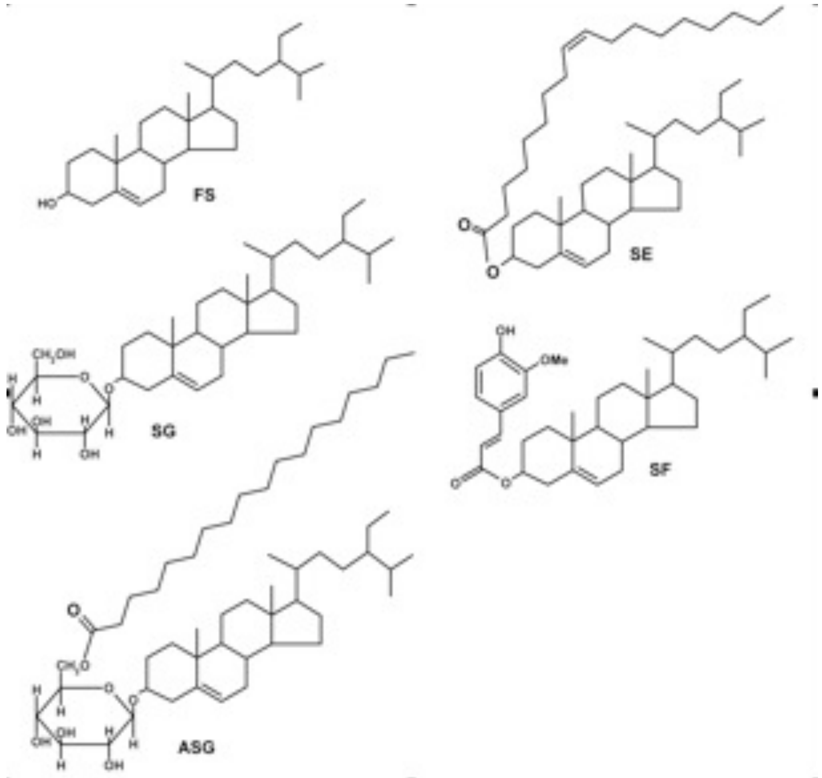
➔ Links to changes in MW

Plant sterols and their conjugates

- Cereals, beans, pulses, seeds and some vegetables contain plant sterol conjugates with potential health benefits in addition to the well-established cholesterol lowering effect.

- Chemical properties of these sterol conjugates are different and hence they could be introduced to foods with lower fat content

- Importance of natural intake levels, additional health benefits of conjugates



Themes in plant sterol research

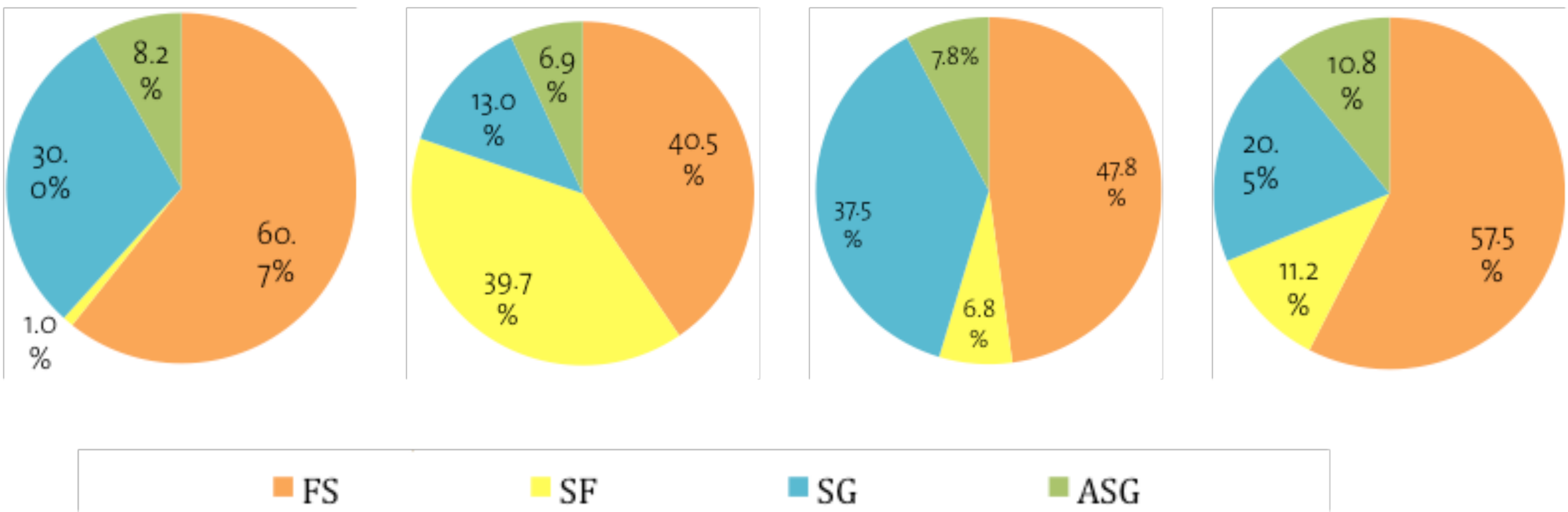
- Contents of SF and SG in foods and the effects of processing on the content
- Bioaccessibility of sterols conjugates from complex cereal foods
- Enzymatic and chemical reactions of SF involved in food processing and consumption
- Antioxidant activity of SF
- Other possible biological activities of SF&SG

Changes in steryl conjugates during cereal processing

MSc Thesis Mirjam Grüter

Conjugate composition in non-processed grains*

Barley | Rice | Rye | Wheat

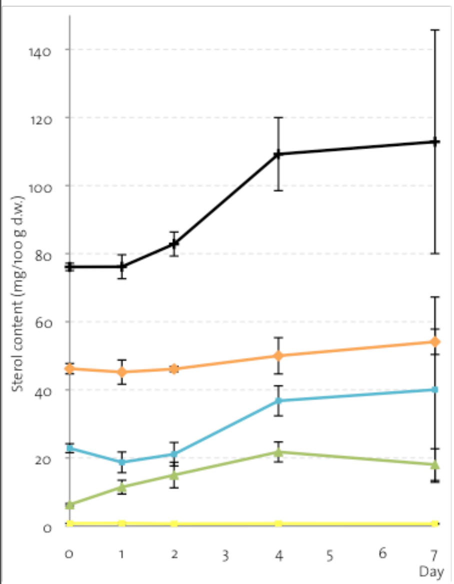


* SE (Steryl fatty acid esters) not included

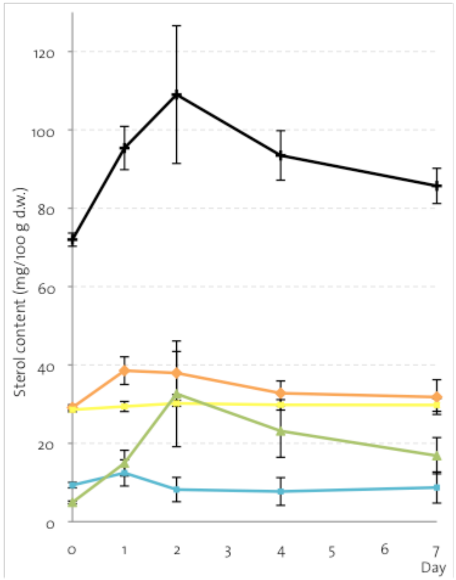
Germination

- Germination of grains at two temperatures 15°C and 25°C
- Sampling at 0, 1, 2, 4 and 7 days
- Extraction of lipids with accelerated solvent extraction (ASE)
- Analysis of steryl conjugates using Diol-HPLC with RI-detection (FS, SG, ASG) and UV-detection (SF)

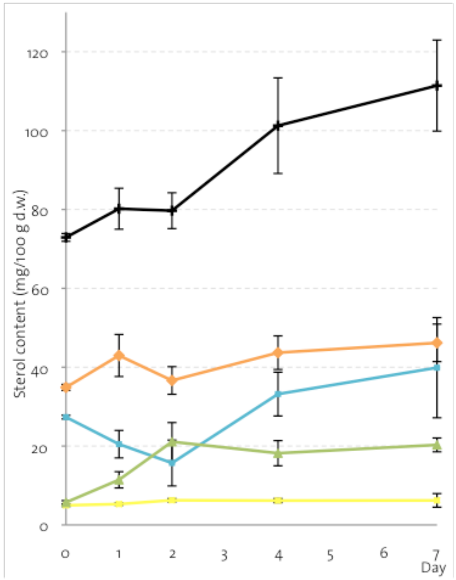
Changes in processing: Germination



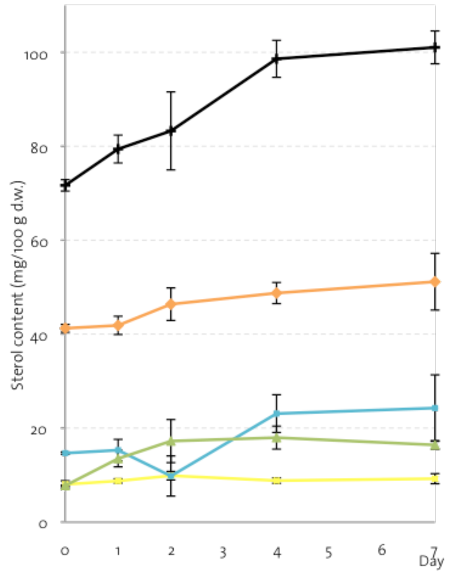
Barley



Rice



Rye



Wheat

Free sterols Steryl ferulates Steryl glycosides Acylated steryl glycosides

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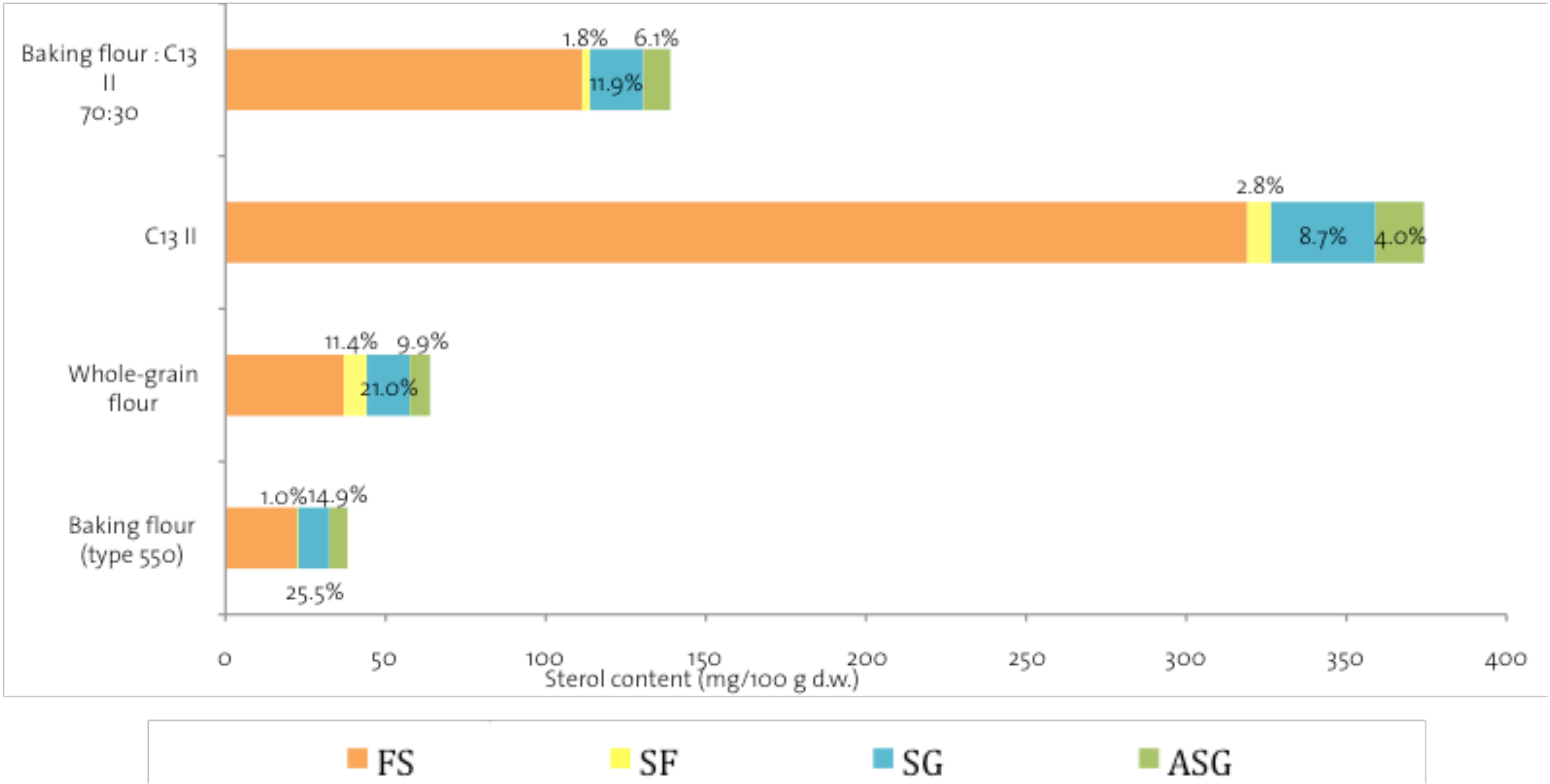
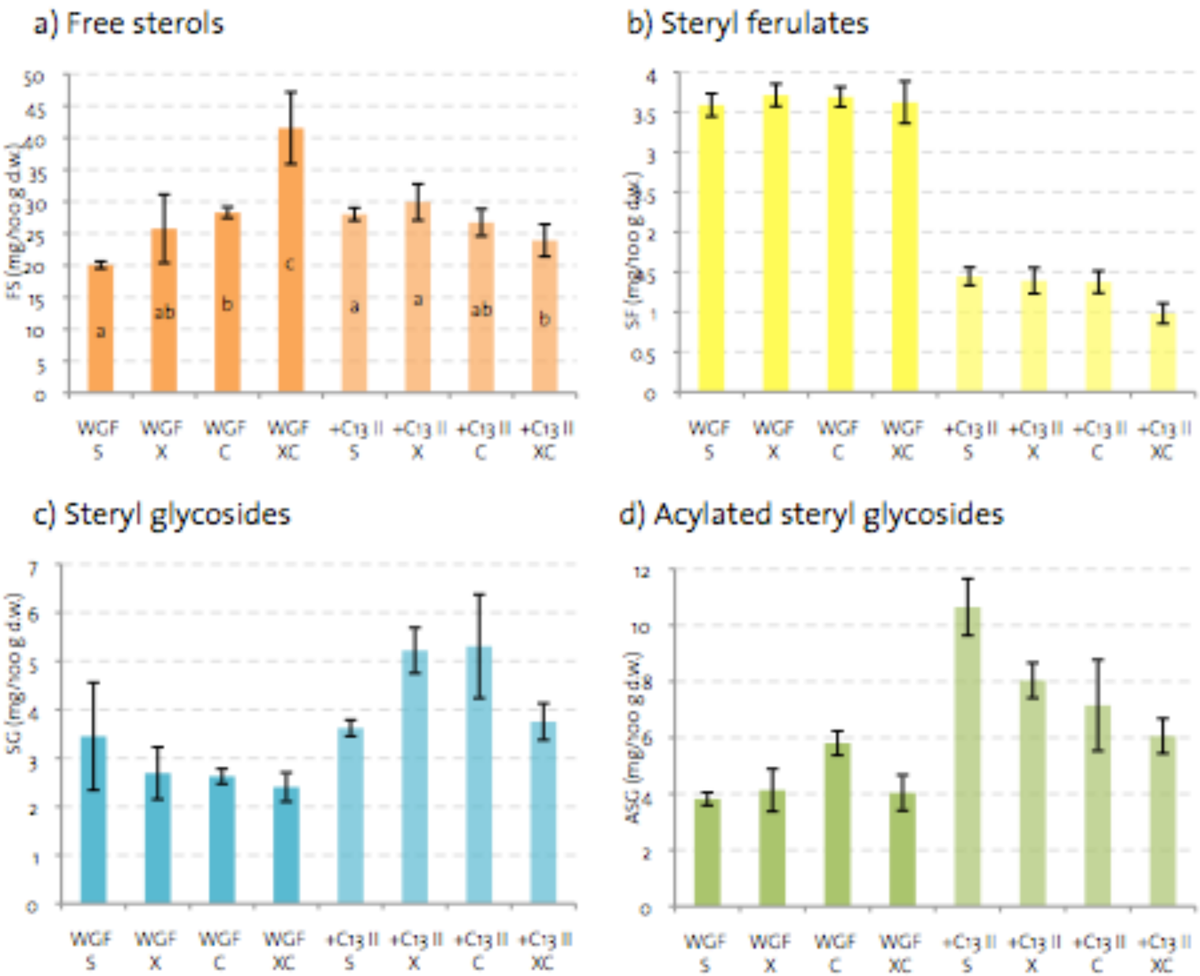
Baking: Raw material composition



Changes in sterol conjugates in enzyme aided baking



WGF= whole grain flour bread

+C13 II = bread with baking flour+ fraction C13 (70:30)

S= standard

X= xylanase

C= cellulase

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Changes in steryl conjugates in processing

- Overall changes in the content of steryl conjugates during processing (germination, enzyme-aided baking) are small
- Changes in bioaccessibility?
- Raw material selection has a larger impact on the content of steryl conjugates
  - Selection of grain and variety
  - Utilization of milling techniques and milling fractions

Phytates

- Phytic acid (IP6, inositol hexaphosphate) is the main antinutritional component in pulses and (wholegrain) cereal products responsible for decreasing mineral availability, especially Fe and Zn
- Metal-binding effect can, on the other hand, protect the foods from oxidative damage
- Lower phytates (IP3-IP4) may have some anti-cancer effects for which the importance of food sources is increasingly studied
- Improved analytical methods to analyse various inositol phosphate forms
- Establishing phytate database for contents in relevant foods