From vegetable refuse to health promoting food

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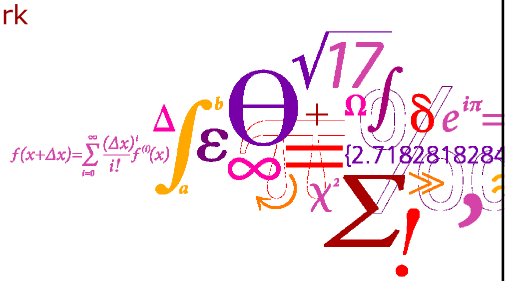

Enzymatic Production of Dietary Fibers and Prebiotics

WHY ? WHAT ? HOW ? → SCIENTIFIC PROGRESS

- The new problems and the new advanced tech opportunities
- Enzymatic biorefining of byproduct materials
- Dietary fibres and prebiotics
- Conversion of byproduct streams to fibres and prebiotics
  - Example: Potato pulp from starch processing
- Technology driven provision of new basic understanding

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The new problems

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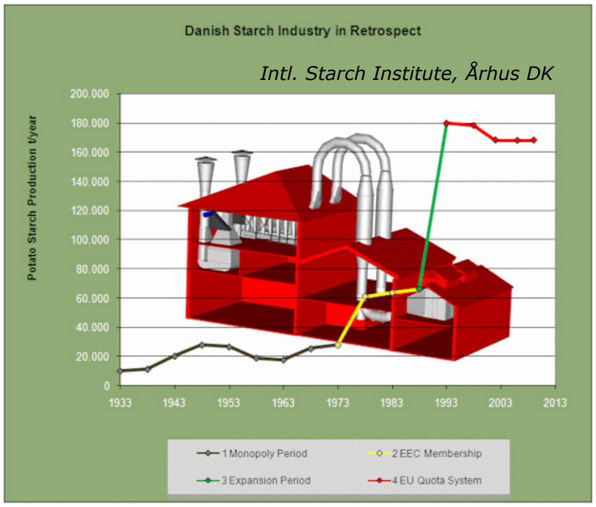

STIMULUS: BYPRODUCT UPGRADING TO HEALTH PRODUCTS

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New technological tools and opportunities

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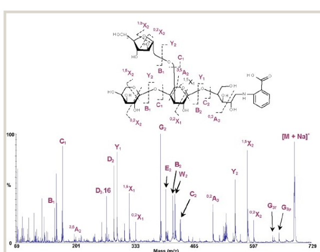
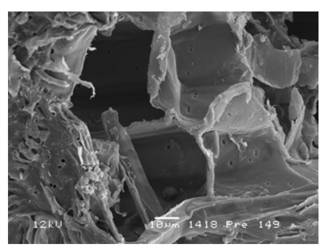
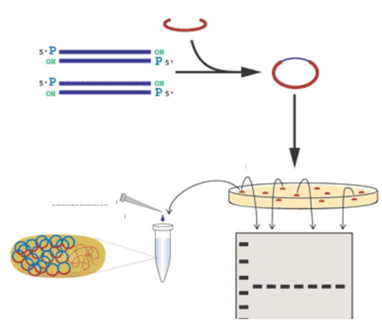




Enzyme	CAZy family	*A. niger*	*A. nidulans*	*A. fumigatus*
Glycosyl hydrolases				
β-glucosidase	GH1	3	3	5
β-galactosidase	GH2	0	3	2
β-mannosidase	GH2	3	3	2
β-glucuronidase	GH2	3	3	1
Exo-β-d-glucosaminidase	GH2	0	0	2
β-glucosidase	GH3	12	15	12
β-xylosidase	GH3	2	3	2
β-*N*-acetylhexosaminidase	GH3	3	2	3
β-1,4-endoglucanase	GH5	4	4	6
β-1,4-endomannanase	GH5	1	6	2
Exo-1,3-β-glucanase	GH5	4	3	2
Endo-1,6-β-glucosidase	GH5	0	1	1
Endoglycoceramidase	GH5	1	1	2
Cellobiohydrolase (non-reducing end)	GH6	2	2	1
Cellobiohydrolase (reducing end)	GH7	2	2	2
β-1,4-endoglucanase	GH7	0	1	2
β-1,4-endoxylanase	GH10	1	3	4
β-1,4-endoxylanase	GH11	4	2	3
Xyloglucan-active β-1,4-endoglucanase	GH12	3	1	4
α-amylase	GH13	4	3	2
α-1,6-glucosidase	GH13	2	2	5
α-1,3-glucan synthase	GH13	5	2	3
GPI-anchored α-amylase-like	GH13	3	3	3
Intracellular α-amylase-like	GH13	2	1	1
Glycogen debranching enzyme	GH13	2	2	1
Glucoamylase	GH15	2	2	5
Endomannanase	GH26	1	3	0
α-galactosidase	GH27	5	3	5
Endopolygalacturonase	GH28	7	3	4
Endorhamnogalacturonase	GH28	6	1	2
Exopolygalacturonase	GH28	4	3	3
Exorhamnogalacturonase	GH28	3	1	2
Xylogalacturonan hydrolase	GH28	1	1	1
α-l-fucosidase	GH29	1	0	0



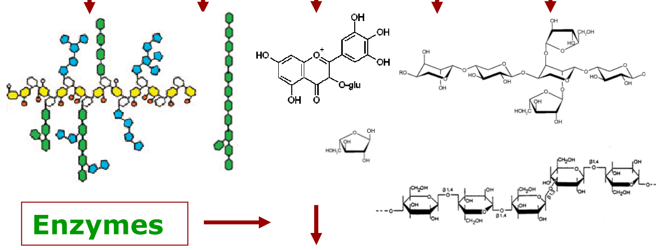
Bio-upgrading of byproduct plant materials

"Biorefining"

Enzymatic catalysis → New transformations, molecular level tailoring

Precise, rational value addition

↓ Energy use
↓ Water use



Enzymes →

New functional products for health

New understanding of mech. & effects

Dietary Fibers and Prebiotics: Gut health

Dietary fibers
Plant material, polysaccharides and lignin, that are resistant to human digestive enzymes

Prebiotics
Indigestible carbohydrates that selectively stimulate the growth and/or activity of beneficial bacteria of the intestinal flora

Probiotics
Beneficial bacteria in the gut, mainly *Bifidobacteria* and *Lactobacilli*

Bioactivity beyond the gut microbiota
Stand-alone effects of prebiotics and dietary fibers
Dendritic cell responses, lipid regulation, sugar response, mineral absorption, weight loss….

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Multiple classical actions of dietary fibers

The benefits of a high fiber diet in relation to chronic disease widely recognized by epidemiological evidence

Component	Action
”Cellulose”	Holds water, ↓ colonic pressure, ↓ transit time, slows postprandial absorption
”Hemicellulose”	Holds water, ↑ stool bulk, may bind bile acids, ↓ colonic pressure, ↓ transit time, slows postprandial absorption
”Pectins, gums”	slow gastric emptying, may bind bile acids, ↑ colonic fermentation
”Lignin”	May bind trace minerals and ↑ their excretion May increase fecal steroid levels

Dietary fibers and prebiotics

Issues

- Multiple actions, complex mechanisms
- Complex relations between actions and health/disease
- Very diverse chemical structures have been assessed
- Many inconsistent data
- Exact role of specific carbohydrate structures in prevention of specific diseases is still scarcely understood !
- Novel effects of isolated fibers and prebiotics, e.g. weight control

Hypotheses

- Inconsistent data partly due to varying chemistry of tested products
- New clues via molecular design of fibers from plant materials

Biological Prodn of Dietary Fibers and Prebiotics

To produce designed nutrifunctional fibers and prebiotics by enzyme technology from plant byproduct streams

To develop the required technologies:

Enzyme technology, selective hydrolysis and synthesis technologies

Enzyme development (cloned mono-enzymes), reaction optimization

Substrate and product separation and purification technologies

Scale-up and process design, pilot plant production process

To establish a platform for assessing the prebiotic and other gastrointestinal effects of novel oligomers and polymers

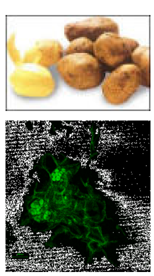
To evaluate any immunological effects of the developed fibers/ prebiotics – from *in vitro* to human studies

Collaboration: Danisco A/S, Lyckeby AmBa, Herlev Hospital, DTU

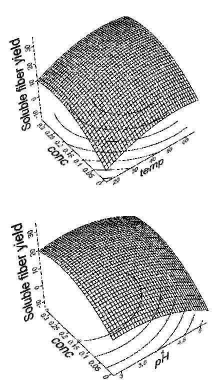
Example

Enzymatic production of potato fibers

Potato pulp	Starch removal	Fiber residue	Enzymatic catalysis	Separation	Bioactive component



Fungal pectinase preparation →



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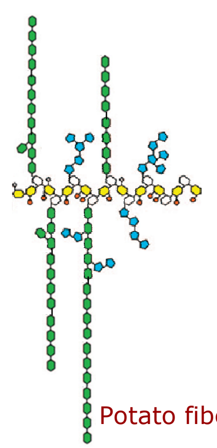

Potato pulp | Reaction optimization | Potato fibers

Nutr Res 27: 152-160 (2007)
Biochem Eng J 43: 106-112 (2009)
Enz Microb Tech 46: 297-303 (2010)

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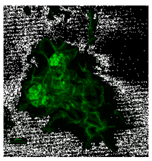

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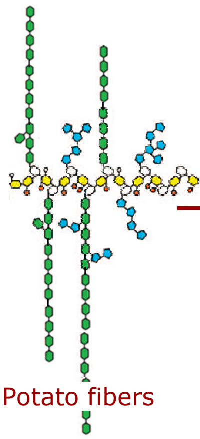

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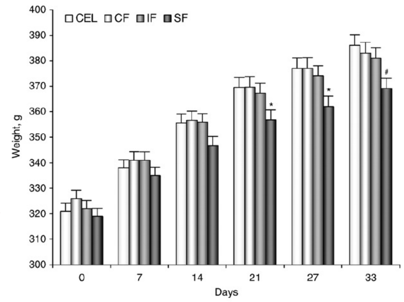

Fig. 1. Weight (g) development during ad libitum feeding of the experimental diets. Values are expressed as least square means with their SEM. *Significantly different from CEL ($P < .05$); #significantly different from all other diets ($P < .05$) (n = 10).

Potato pulp

Potato fibers

↓ weight gain in rat model

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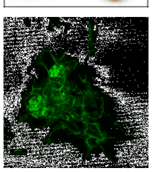
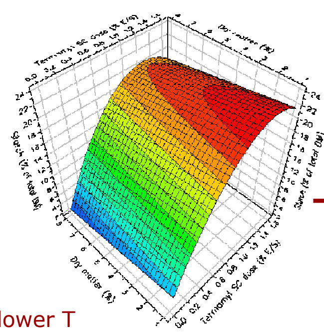


Minimal starch removal treatment →

Higher DM

Less enzyme

Shorter time, lower T



→ Fiber residue

Further upgrading and production scale-up

Potato pulp

Reaction optimization

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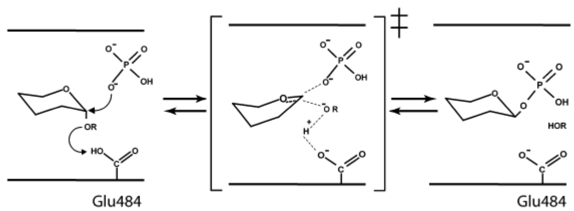
Enzymatic production of new oligos

Byproduct stream	Enzymatic hydrolysis	Separation	Enzymatic catalysis	Separation	New oligos

New enzymes
GH65 maltose
phosphorylase

Hydrolysate →

New reactions
New products



Acceptor	Product	Optimum time (h)	Yield (%)
Glucose	α-Glc*p*-(1,4)-Glc*p*	5	61
Glucosamine	α-Glc*p*-(1,4)-GlcN*p*	2	69
N-acetyl glucosamine	α-Glc*p*-(1,4)-GlcNAc*p*	2	38
Mannose	α-Glc*p*-(1,4)-Man*p*	5	61
Xylose	α-Glc*p*-(1,4)-Xyl*p*	2	40
L-Fucose	α-Glc*p*-(1,4)-L-Fuc*p*	5	39

FEBS J 276: 7353-7365 (2009)

Biological Prodn of Dietary Fibers and Prebiotics

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