Enzymatic production of wheat and ryegrass derived xylooligosaccharides and evaluation of their in vitro effect on pig gut microbiota

This study examines enzymatic production of linear xylooligosaccharides (XOS) and branched arabinoxylooligosaccharides (AXOS) from monocotyledonous biomass, wheat straw and ryegrass, and compares the in vitro effects of these XOS and AXOS on pig gut microbiota. XOS and AXOS were obtained from the biomass by treatment with different endo-1,4-β-xylanases. XOS of DP2-6 from wheat straw, obtained after treatment with Aspergillus niger endo GH11, suppressed growth of Clostridium perfringens and resulted in a high level of lactic acid production when fermented in vitro by pig fecal microbiota. Analogously, XOS ryegrass produced in the same way also suppressed Cl. perfringens growth, and more so than the corresponding ryegrass AXOS, but AXOS exhibited a more pronounced stimulation of lactic acid bacteria growth than XOS. The prebiotic potential, i.e., suppression of Cl. perfringens and stimulation of lactic acid bacteria growth, for the ryegrass oligosaccharides was as follows: XOS, produced by A. niger endo-1,4-β-xylanase (GH 11) ≥ AXOS, produced by Thermotoga maritima and Cellvibrio mixtus endo-1,4-β-xylanase s (GH10) > AXOS, produced by Trichoderma viride and Aspergillus aculeatus endo-1,4-β-xylanase s (GH11). These results indicate that wheat straw as well as green grass biomass such as ryegrass have potential as new sources of putative prebiotics for pig feed.

General information
State: Accepted/In press
Organisations: Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, Aarhus University
Authors: Dotsenko, G. (Intern), Meyer, A. S. (Intern), Canibe, N. (Ekstern), Thygesen, A. (Intern), Nielsen, M. K. (Intern), Lange, L. (Intern)
Pages: 11
Publication date: 2017
Main Research Area: Technical/natural sciences

Publication information
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Scopus rating (2016): CiteScore 0.8 SJR 0.324 SNIP 0.334
Scopus rating (2015): SJR 0.172 SNIP 0.109
Scopus rating (2014): SJR 0.192 SNIP 0.121
Scopus rating (2013): SJR 0.321 SNIP 0.275
ISI indexed (2013): ISI indexed no
Scopus rating (2012): SJR 0.27 SNIP 0.234
ISI indexed (2012): ISI indexed no
A statistical model describing the random distribution of substituted xylopyranosyl residues in arabinoxylooligosaccharides is suggested and compared with existing experimental data. Structural diversity of arabinoxylooligosaccharides of various length, originating from different arabinoxylans (wheat flour arabinoxylan (arabinose/xylose, A/X = 0.47); grass arabinoxylan (A/X = 0.24); wheat straw arabinoxylan (A/X = 0.15); and hydrothermally pretreated wheat straw arabinoxylan (A/X = 0.05)), is semiquantitatively approximated using the proposed model. The suggested approach can be applied not only for prediction and quantification of arabinoxylooligosaccharides' structural diversity, but also for estimate of yield and selection of the optimal source of arabinoxylan for production of arabinoxylooligosaccharides with desired structural features.
Enzyme kinetics and identification of the rate-limiting step of enzymatic arabinoxylan degradation

This study investigated the kinetics of multi-enzymatic degradation of soluble wheat arabinoxylan by monitoring the release of xylose and arabinose during designed treatments with mono-component enzymes at different substrate concentrations. The results of different combinations of α-l-arabinofuranosidases (EC 3.2.1.55), one derived from Aspergillus niger (AFAn) and one from Bifidobacterium adolescentis (AFBa), respectively, a β-xylosidase (EC 3.2.1.37) from Trichoderma reesei, and an engineered D11F/R122D variant of Bacillus subtilis XynA endo-1,4-β-xylanase (EC 3.2.1.8) were examined. The two selected α-l-arabinofuranosidases catalyze liberation of arabinose residues linked 1→3 to singly (AFAn) or doubly (AFBa) substituted xyloses in arabinoxylan, respectively. When added to arabinoxylan at equimolar levels, the AFBa enzyme catalyzed the release of more arabinose, i.e. had a higher rate constant than AFAn, but with respect to the xylose release, AFAn – as expected – exhibited a better synergistic effect than AFBa with β-xylosidase. This synergistic effect with AFAn was estimated to increase the number of β-xylosidase catalyzed cuts from ∼3 (with β-xylosidase alone) to ∼7 in each arabinoxylan substrate molecule. However, the synergistic effects between β-xylosidase and the α-l-arabinofuranosidases on the xylose release were low as compared to the effect of xylanase addition with β-xylosidase alone, which increased the xylose release by ∼25 times in 30min, to a yield equivalent to ∼104 β-xylosidase catalyzed cuts in each arabinoxylan substrate molecule. At equimolar addition levels of the four enzymes, the xylanase activity was thus rate-limiting for the β-xylosidase catalyzed depolymerization to release xylose from arabinoxylan. The work provides clues to design efficient enzymatic degradation of arabinoxylan into fermentable monosaccharides.

General information
State: Published
Organisations: Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, Danisco AS
Authors: Rasmussen, L. E. (Intern), Xu, C. (Intern), Sørensen, J. (Forskerdatabase), Nielsen, M. K. (Intern), Meyer, A. S. (Intern)
A Laboratory Exercise To Understand the Importance of Enzyme Technology in the Fruit-Processing Industry: Viscosity Decrease and Phenols Release from Apple Mash

In a 4-h laboratory exercise, students accomplish a series of enzymatic macerations of apple mash, assess the viscosity of the mash during the maceration, extract the juice by centrifugation, and measure the levels of antioxidant phenols extracted into the juice after different enzyme treatments. The exercise shows the impact of enzyme-catalyzed plant cell-wall degradation on the viscosity of apple fruit mash and on the extraction of antioxidant phenols into experimentally
prepared apple juice. The exercise also demonstrates that pectinolytic and cellulolytic enzymes have different effects on the viscosity of apple mash. Depending on the academic skills and background of the students, various aspects of quantitative enzyme activity assessment and advanced data analysis of decay curves can be included in the postexercise discussions and reporting of the data.

General information
State: Published
Organisations: Center for BioProcess Engineering, Department of Chemical and Biochemical Engineering, BioChemical Engineering
Authors: Pinelo, M. (Intern), Nielsen, M. K. (Intern), Meyer, A. S. (Intern)
Pages: 499-502
Publication date: 2011
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Chemical Education
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.405 SNIP 0.892 CiteScore 1.39
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.388 SNIP 0.978 CiteScore 1.24
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.38 SNIP 1.013 CiteScore 1.13
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.349 SNIP 1.017 CiteScore 0.83
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.315 SNIP 0.896 CiteScore 0.56
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.337 SNIP 0.867 CiteScore 0.52
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.303 SNIP 0.803
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 0.291 SNIP 0.584
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.285 SNIP 0.787
Scopus rating (2007): SJR 0.272 SNIP 0.91
Scopus rating (2006): SJR 0.272 SNIP 0.758
Scopus rating (2005): SJR 0.217 SNIP 0.973
Scopus rating (2004): SJR 0.269 SNIP 0.951
Scopus rating (2003): SJR 0.281 SNIP 1.025
Scopus rating (2002): SJR 0.274 SNIP 0.332
Scopus rating (2001): SJR 0.335 SNIP 0.772
Scopus rating (2000): SJR 0.415 SNIP 0.883
Scopus rating (1999): SJR 0.427 SNIP 1
Quantitative relationship between trimethylamine-oxide aldolase activity and formaldehyde accumulation in white muscle from gadiform fish during frozen storage

The accumulation of formaldehyde and the resulting deterioration of seafood products during frozen storage are primarily caused by the enzymatic activity of trimethylamine oxide aldolase (TMAOase). A screening of muscle samples from 24 species showed TMAOase activity in only the nine gadiform species that were analyzed. Enzyme activities in the major white muscle of gadiform fish showed large variations between species as well as between individuals. A frozen storage experiment showed a similarly large variation in the rate of formaldehyde accumulation, which could be accounted for by the endogenous white muscle in situ TMAOase activity. This TMAOase activity also correlated with the rate of
insolubilization of otherwise high ionic strength soluble protein. A simple model describing the accumulation of free formaldehyde during frozen storage of gadiform fish is proposed. The model is based on a storage time-dependent decay of substrate-saturated white muscle TMAOase activity.

**General information**

State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Process and Product Technology
Authors: Nielsen, M. K. (Intern), Jørgensen, B. (Intern)
Pages: 3814-3822
Publication date: 2004
Main Research Area: Technical/natural sciences

**Publication information**

Journal: Journal of Agricultural and Food Chemistry
Volume: 52
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ISSN (Print): 0021-8561
Ratings:
- BFI (2018): BFI-level 2
- Web of Science (2018): Indexed yes
- BFI (2017): BFI-level 2
- Web of Science (2017): Indexed yes
- BFI (2016): BFI-level 2
- Scopus rating (2016): CiteScore 3.45 SJR 1.291 SNIP 1.344
- Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 2
- Scopus rating (2015): SJR 1.236 SNIP 1.253 CiteScore 3.23
- Web of Science (2015): Indexed yes
- BFI (2014): BFI-level 2
- Scopus rating (2014): SJR 1.278 SNIP 1.421 CiteScore 3.25
- Web of Science (2014): Indexed yes
- BFI (2013): BFI-level 2
- Scopus rating (2013): SJR 1.423 SNIP 1.479 CiteScore 3.44
- ISI indexed (2013): ISI indexed yes
- Web of Science (2013): Indexed yes
- BFI (2012): BFI-level 2
- Scopus rating (2012): SJR 1.43 SNIP 1.471 CiteScore 3.2
- ISI indexed (2012): ISI indexed yes
- Web of Science (2012): Indexed yes
- BFI (2011): BFI-level 2
- Scopus rating (2011): SJR 1.384 SNIP 1.446 CiteScore 3.1
- ISI indexed (2011): ISI indexed yes
- Web of Science (2011): Indexed yes
- BFI (2010): BFI-level 2
- Scopus rating (2010): SJR 1.408 SNIP 1.392
- Web of Science (2010): Indexed yes
- BFI (2009): BFI-level 2
- Scopus rating (2009): SJR 1.317 SNIP 1.303
- Web of Science (2009): Indexed yes
- BFI (2008): BFI-level 2
- Scopus rating (2008): SJR 1.361 SNIP 1.324
- Web of Science (2008): Indexed yes
- Scopus rating (2007): SJR 1.249 SNIP 1.439
- Web of Science (2007): Indexed yes
- Scopus rating (2006): SJR 1.358 SNIP 1.418
- Web of Science (2006): Indexed yes
- Scopus rating (2005): SJR 1.286 SNIP 1.521
Role of acetate in production of an autoinducible Class IIa Bacteriocin in Carnobacterium piscicola A9b

Carnobacterium piscicola strain A9b isolated from cold smoked salmon inhibits growth of the food-borne pathogen Listeria monocytogenes partly due to the production of a proteinaceous compound (L. Nilsson, L. Gram, and H. H. Huss. J. Food Prot. 62:336-342, 1999). The purpose of the present study was to purify the compound and describe factors affecting its production, with particular emphasis on food-relevant factors. Amino acid sequencing showed that the compound is a class IIa bacteriocin with an N-terminal amino acid sequence identical to that of carnobacteriocin B2. The production of the bacteriocin was autoinducible, and the threshold level for induction was 9.6 x 10(-10) M. We also report, for the first time, that acetate acts as an induction factor, with a threshold concentration of 0.3 to 12 mM. Acetate could not act as an inducer during the late exponential phase of C. piscicola A9b. The induction of bacteriocin production showed a dose-dependent relationship at acetate concentrations of up to 10 to 20 mM (depending on the growth medium) and at a concentration of 1.9 x 10(-8) M for the bacteriocin itself; a saturation level of bacteriocin specific activity was reached at these concentrations of induction factors. The combined use of both inducers did not enhance the saturation level of bacteriocin production compared to that seen with the use of each inducer alone. Increasing NaCl and glucose concentrations negatively influenced the efficiency of acetate as an induction factor. Based on the results, carnobacteriocin B2 was used as an induction factor to manipulate the production of bacteriocin in cold smoked salmon juice and thus improve the ability to inhibit L. monocytogenes.
Localization of formaldehyde production during frozen storage of European hake (Merluccius merluccius)

The formation of dimethylamine and formaldehyde from trimethylamine N-oxide by the enzyme trimethylamine N-oxide demethylase in whole hake during frozen storage was studied. The objective was to check if there were parts of the muscle with a higher production of dimethylamine and formaldehyde, and if the presence of kidney during frozen storage had any significant influence on the production. Three variables were examined through one year storage. The first was anatomical location, considering the red muscle and three zones of white muscle, one located right over the kidneys, the dorsal part over the viscera, and the tail. The second variable was the temperature of storage, -11 degreesC or -18 degreesC. Finally, the influence of kidneys during storage, comparing fish with and without kidneys, was also evaluated.

No differences were found in dimethylamine and formaldehyde production between fish with and without kidneys stored at -18 degreesC. However at -11 degreesC the amounts of dimethylamine and formaldehyde detected in fish without kidneys were, in some cases, higher than in those with kidneys. Kidney removal does not have a statistically significant influence on DMA and FA production in frozen storage hake. Differences in dimethylamine and formaldehyde values among different anatomical locations were found, especially in those stored over one year. It was found that, in general, the white muscle located right over the kidneys produced more dimethylamine than other parts of the fish.

General information

State: Published
Organisations: Section for Aquatic Process and Product Technology, National Institute of Aquatic Resources
Pages: 43-47
Publication date: 2001
Main Research Area: Technical/natural sciences
A sensitive trimethylamine-N-oxide aldolase assay in two steps without deproteinisation

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Process and Product Technology
Authors: Nielsen, M. K. (Intern), Havemeister, W. (Ekstern), Rehbein, H. (Ekstern), Sotelo, C. (Ekstern), Jørgensen, B. (Intern)
Pages: 197-200
Publication date: 2000
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of the Science of Food and Agriculture
Volume: 80
Issue number: 2
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BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
HNMR analysis of water in muscle

General information
State: Published
Organisations: National Institute of Aquatic Resources
Authors: Nielsen, M. K. (Intern), Scherfig, U. (Ekstern), Pedersen, H. (Ekstern)
Quantitative relation between in vitro TMAOase activity and in situ formaldehyde formation

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Process and Product Technology
Authors: Nielsen, M. K. (Intern), Jørgensen, B. (Intern)
Number of pages: 379
Publication date: 2000

Host publication information
Title of host publication: Proceedings of 29th WEFTA Meeting, 10 - 14 October, 1999 - Leptocarya - Pieria, Greece
Place of publication: Thessaloniki
Publisher: Greek Society of Food Hygienists and Technologists
Main Research Area: Technical/natural sciences
Conference: 29th WEFTA Meeting, Thessaloniki, Greece, 10/10/1999 - 10/10/1999
Source: orbit
Source-ID: 229398
Publication: Research › Conference abstract in proceedings – Annual report year: 2000

TMAO-aldolase i fiskeprodukter: En nøgle til reduktion af kvalitetsproblemerne knyttet til formaldehyd og dimethylamin

General information
State: Published
Organisations: National Institute of Aquatic Resources
Authors: Nielsen, M. K. (Intern)
Number of pages: 25
Publication date: 1998

Publication information
Publisher: Nordisk Ministerråd, København
Original language: Danish
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 226926
Publication: Research › Report – Annual report year: 1998

Relation between TMAOase activity and content of formaldehyde in fillet minces and bellyflap mince from gadoid fishes

General information
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Organisations: National Institute of Aquatic Resources, Section for Aquatic Process and Product Technology, Section for Aquatic Protein Biochemistry
Number of pages: 114-118
Publication date: 1997
Main Research Area: Technical/natural sciences

Publication information
Journal: Informationen für die Fischwirtschaft aus der Fischereiforschung
Ionic liquids: a green solution to enzymatic lipophilisation of bioactive compounds

Flavonoids are found in a variety of foods and beverages. They are commonly regarded as antioxidants or bio-
antioxidants for lipids in food or biological systems. Flavonoids are water-soluble compounds and limited in antioxidative
functions due to limited mass transfer and/or insufficient partitioning in the real heterophasic food or biological systems.
Therefore, lipophilisation of the compounds is one of the best solutions to improve their functions. Enzymatic approach
offers the best possibility for the lipophilisation of flavonoids due to milder conditions and specificity. However no traditional
organic solvents are suitable to provide an efficient reaction system due to lower solubility and other aspects. Ionic liquids
have been emerged in sight recently as green solvent due to negligible vapour pressure. Thus ionic liquids provide a
potential solution for the system development in enzymatic flavonoids lipophilisation. In this project, the major objectives
are to develop an efficient system for the acylation of flavonoids to obtain flavonoids esters as well as process optimisation
for product separation and purification. A few interesting flavonoids will be selected for the process development. In order
to reduce the experimental workload for the screening of numberless ionic liquids, activity coefficient model will be
validated to predict the related properties, acting as pre-screening. To better understand the impact of ionic liquids on the
enzyme activity, water distribution in the reaction system will be evaluated through NIR and 1H-NMR. Eventually a
preliminary product evaluation will be conducted also concerning antioxidation performance of the products in LDL and
liposome model systems.

Food Biotechnology and Engineering Group

Department of Systems Biology
Period: 01/09/2005 → 28/02/2009
Number of participants: 3
Project ID: 45789
Project participant:
Guo, Zheng (Intern)
Thomsen, Kaj (Intern)
Nielsen, Michael Krogsgaard (Intern)

Financing sources
Source: Forskningsrådene - STVF
Name of research programme: Forskningsrådene - STVF
Amount: 3,573,724.00 Danish Kroner

Project
TMAO aldolase in fish products. A key to reduction of the quality problems connected with formaldehyde and dimethylamine.
The formation of formaldehyde and dimethylamine are main factors in the reduction in quality of lean fish like cod during frozen storage. They are formed from trimethylamine-oxide, catalysed by the enzyme trimethylamine-oxide aldolase (TMAOase; EC 4.1.2.32) which is situated mainly in the inner organs like gall bladder, spleen and kidney. The presence of the enzyme in other marine species is not thoroughly described, and it is to be expected that TMAOase activity may be the cause of formaldehyde formation and quality deterioration in other products than those formed from lean fish. Products of commercial importance to the Nordic fish industry were screened for TMAOase activity. TMAOase was almost only found in gandoide fishes. The TMAOase activity concentrations varied much between individuals. Results from the frozen storage experiment showed that the formation of formaldehyde at -10°C was both proportional to the TMAOase activity and the storage time. Therefore TMAOase activity concentration can be used as a selection criteria to sort out individuals less suitable to frozen storage.

National Institute of Aquatic Resources
Number of participants: 6
Project participant:
Nielsen, Michael Krogsgaard (Intern)
Berner, Lis (Intern)
Espe, Marit (Ekstern)
Poulsen, Marita (Ekstern)
Einarsson, Sigurdur (Ekstern)

Project Manager, organisational:
Jørgensen, Bo Munk (Intern)

Financing sources
Source: Unknown
Name of research programme: Ukendt
Amount: 624,000.00 Danish Kroner

Project
Purification and characterization of TMAOase of saithe and hake.
The intracellular distribution of the enzyme TMAO aldolase (EC 4.1.2.32) is determined from detergent-treated tissue extracts. The enzyme is isolated and purified by chromatography and its properties are studied. Thereby, greater knowledge is gained of the factor that determines the formation of dimethylamine and formaldehyde in frozen fish. This knowledge forms a basis for the possibility of influencing the process that is considered important for quality deterioration during frozen storage.

National Institute of Aquatic Resources
Bundesforschungsanstalt für Fischerei
Universidad de Vigo
Period: 01/04/1995 → 31/03/1998
Number of participants: 6
Project participant:
Nielsen, Michael Krogsgaard (Intern)
Jessen, Flemming (Intern)
Berner, Lis (Intern)
Rehbein, Hartmut (Ekstern)
Gonzalez-Sotelo, Carmen (Ekstern)
Project Manager, organisational:
Jørgensen, Bo Munk (Intern)

Financing sources
Source: Unknown
Name of research programme: Ukendt
Amount: 1,300,000.00 Danish Kroner
Project