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, 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.
A statistical model describing the random distribution of substituted xylopyranosyl residues in 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. 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
Volume: 88
Issue number: 4
ISSN (Print): 0021-9584
Ratings:
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
Når fisken dør på en smagfuld måde

General information
State: Published
Organisations: National Institute of Aquatic Resources
Authors: Nielsen, M. K. (Intern)
Pages: 60-70
Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: Fisk og Hav
Issue number: 59
ISSN (Print): 0105-9211
Ratings:
ISI indexed (2013): ISI indexed no
ISI indexed (2012): ISI indexed no
ISI indexed (2011): ISI indexed no
Original language: Danish
Links:
http://www.aqua.dtu.dk/Publikationer/Fisk-og-hav.aspx
Source: orbit
Source-ID: 226354
Publication: Research › Journal article – Annual report year: 2005

Seafood Enzymes

General information
State: Published
Organisations: Department of Systems Biology, Section for Aquatic Protein Biochemistry, National Institute of Aquatic Resources
Authors: Nielsen, M. K. (Intern), Nielsen, H. H. (Intern)
Pages: 379-400
Publication date: 2005

Host publication information
Title of host publication: Food Biochemistry and Food Processing
Volume: Chapter 17
Place of publication: Oxford
Publisher: Blackwell Publishing UK/USA
Editors: Hui, Y., Nip, W., Paliyath, G., Simpson, B.
ISBN (Print): 08-13-80378-0
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 184226
Publication: Education - peer-review › Book chapter – Annual report year: 2005

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
Issue number: 12
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.
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 68
Issue number: 5
ISSN (Print): 0099-2240
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 4.08
  Web of Science (2016): Indexed yes
  BFI (2015): BFI-level 2
  Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
  Web of Science (2015): Indexed yes
  BFI (2014): BFI-level 2
  Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
  Web of Science (2014): Indexed yes
  BFI (2013): BFI-level 2
  Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
  ISI indexed (2013): ISI indexed yes
  Web of Science (2013): Indexed yes
  BFI (2012): BFI-level 2
  Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
  ISI indexed (2012): ISI indexed yes
  Web of Science (2012): Indexed yes
  BFI (2011): BFI-level 2
  Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
  ISI indexed (2011): ISI indexed yes
  Web of Science (2011): Indexed yes
  BFI (2010): BFI-level 2
  Scopus rating (2010): SJR 1.887 SNIP 1.436
  Web of Science (2010): Indexed yes
  BFI (2009): BFI-level 2
  Scopus rating (2009): SJR 1.972 SNIP 1.528
  Web of Science (2009): Indexed yes
  BFI (2008): BFI-level 2
  Scopus rating (2008): SJR 2.156 SNIP 1.572
  Web of Science (2008): Indexed yes
  Scopus rating (2007): SJR 2.043 SNIP 1.647
  Web of Science (2007): Indexed yes
  Scopus rating (2006): SJR 2.054 SNIP 1.602
  Web of Science (2006): Indexed yes
  Scopus rating (2005): SJR 2.074 SNIP 1.653
  Web of Science (2005): Indexed yes
  Scopus rating (2004): SJR 2.108 SNIP 1.648
  Web of Science (2004): Indexed yes
  Scopus rating (2003): SJR 2.097 SNIP 1.821
  Web of Science (2003): Indexed yes
  Scopus rating (2002): SJR 2.046 SNIP 1.754
  Web of Science (2002): Indexed yes
  Scopus rating (2001): SJR 1.989 SNIP 1.736
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

Publication information
Journal: European Food Research and Technology
Volume: 213
Issue number: 1
ISSN (Print): 1438-2377
Ratings:
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 0.742 SNIP 0.882 CiteScore 1.81
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.732 SNIP 0.822 CiteScore 1.55
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.828 SNIP 0.908 CiteScore 1.71
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.791 SNIP 0.901 CiteScore 1.71
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.872 SNIP 1.038 CiteScore 1.68
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
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
ISSN (Print): 0022-5142
Ratings:
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
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)
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.
**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 gadoid 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**

**Period:** 01/11/1997 → 31/10/1998

**Number of participants:** 6

**Project participant:**
- Nielsen, Michael Krogsgaard (Intern)
- Berner, Lis (Intern)
- Espe, Marit (Ekstern)
- Poulsen, Marita (Ekstern)
- Einarsson, Sigurdur (Ekstern)

**Financing sources**

**Source:** Unknown

**Name of research programme:** U podem

**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