Feruloyl Esterases: Potential in Grain Processing

Fifth Grain Conference Norwich 2008

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WALES QUALITY CENTRE CANOLFAN AHSAWDD CYMRU
MANUFACTURING PRIZE WINNERS 2000

BSI REGISTERED FIRM

THE QUEEN'S AWARDS FOR ENTERPRISE: INNOVATION 2003

Contents

- Different types of Ferulic acid esterases
- Potential use in baking
- Use in brewing
- Use in wheat & barley waste processing
- Potential in bioethanol production

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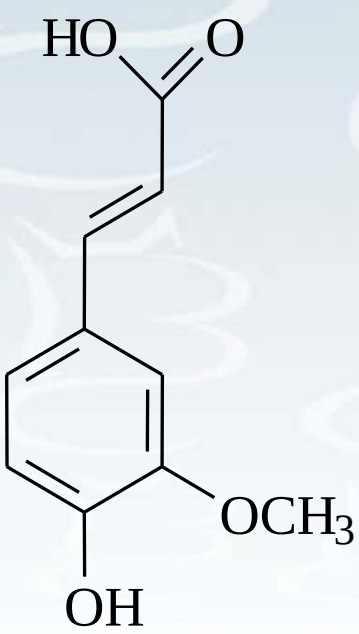
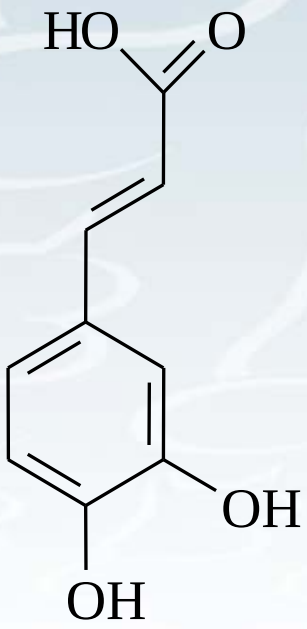
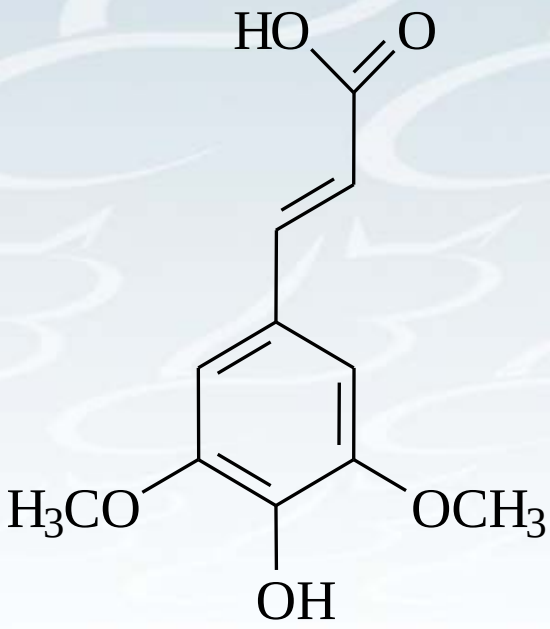
Hydroxycinnamic acids



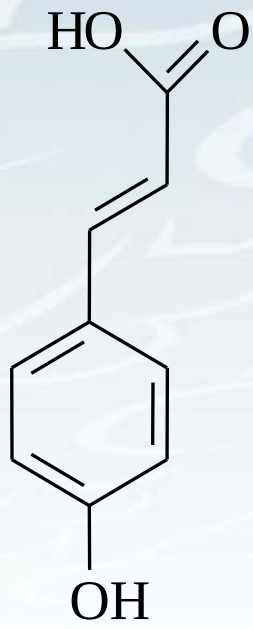
Ferulic Acid



Caffeic Acid



Sinapic Acid



p-Coumaric Acid

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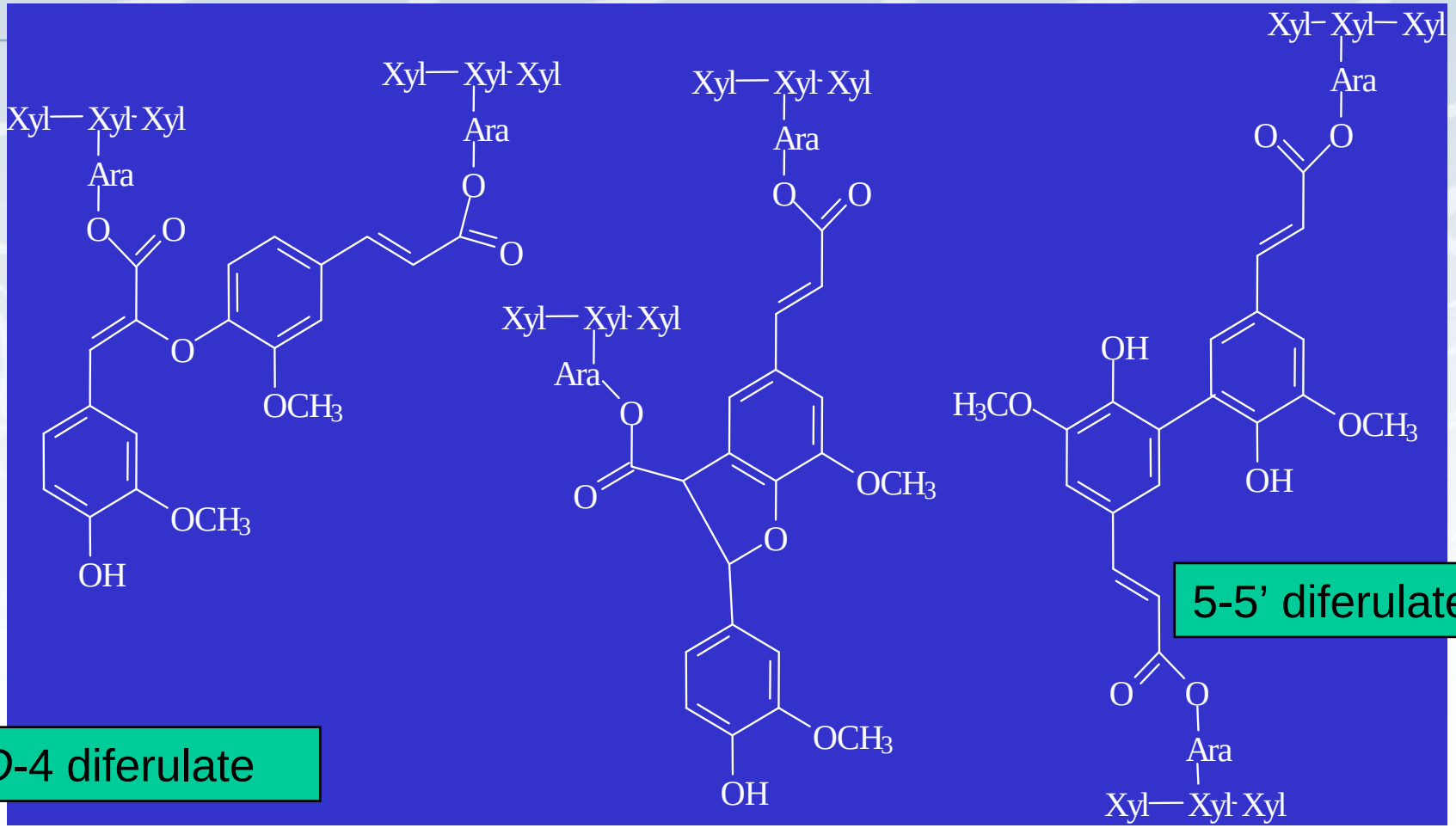
Dimeric ferulates



8-*O*-4 diferulate

5-5’ diferulate

8-5’ Benzofuran diferulate

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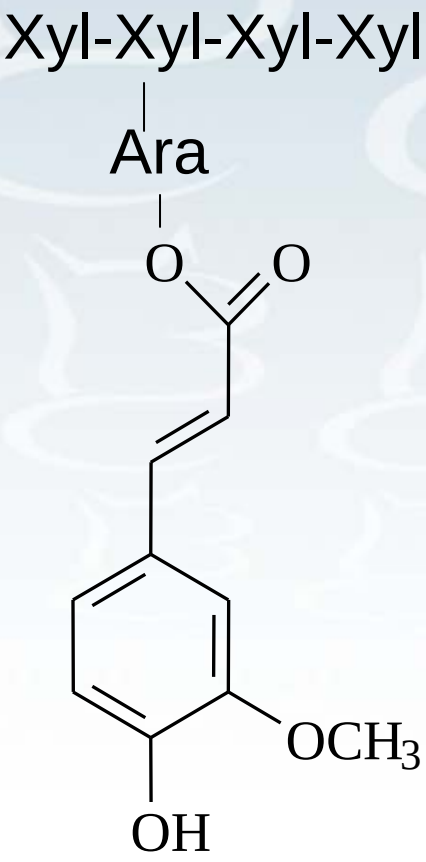
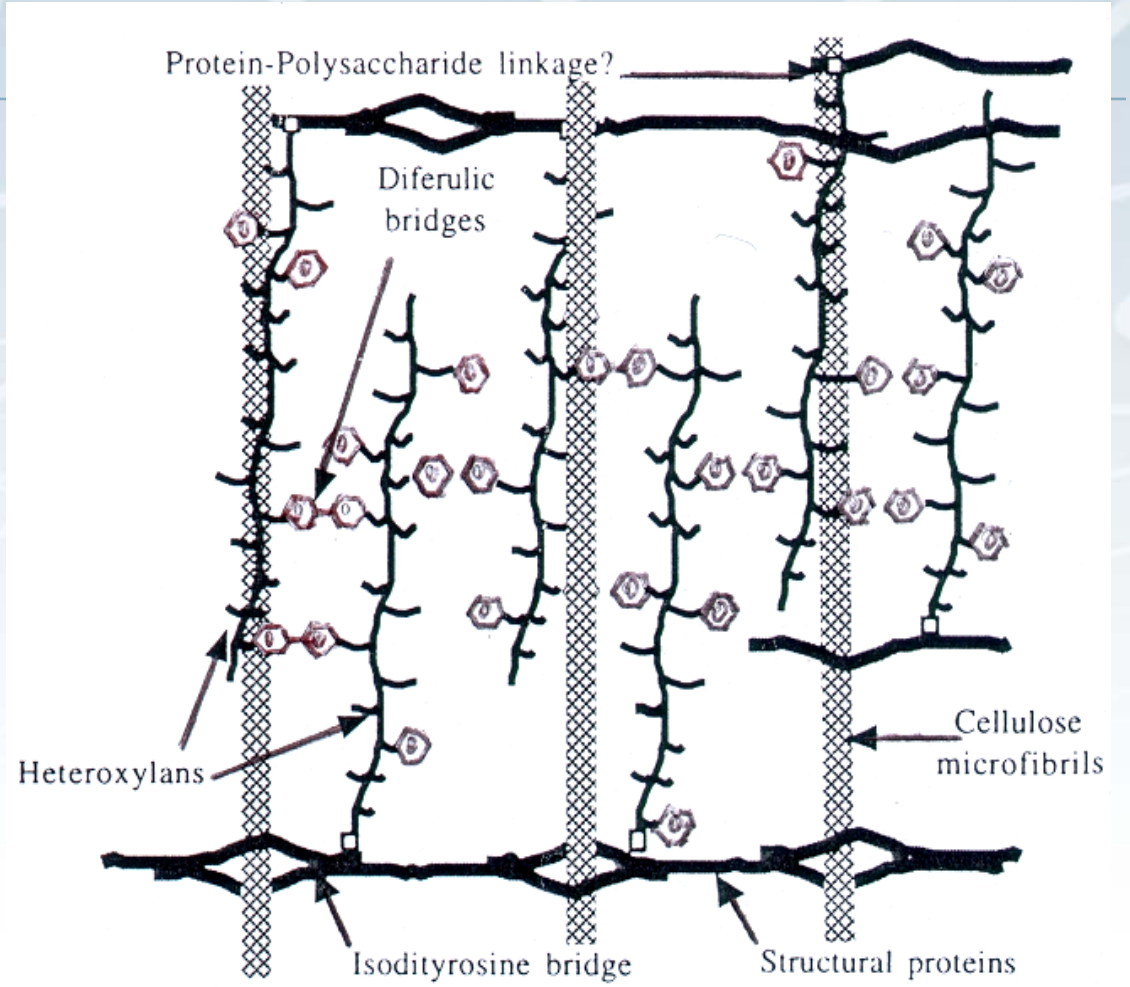
Ferulic Acid Esterase (EC 3.1.1.73)



Ferulic Acid



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Current & potential uses/benefits of ferulic acid

- Food anti-oxidant
- UV absorber in sun creams
- Production of vanilla
- Inhibition or prevention of cancers
  (breast, colon, lung, stomach & tongue)
- Stimulation of the immune system
- Prevention of brain damage by Alzheimer's proteins
- Lowering cholesterol production

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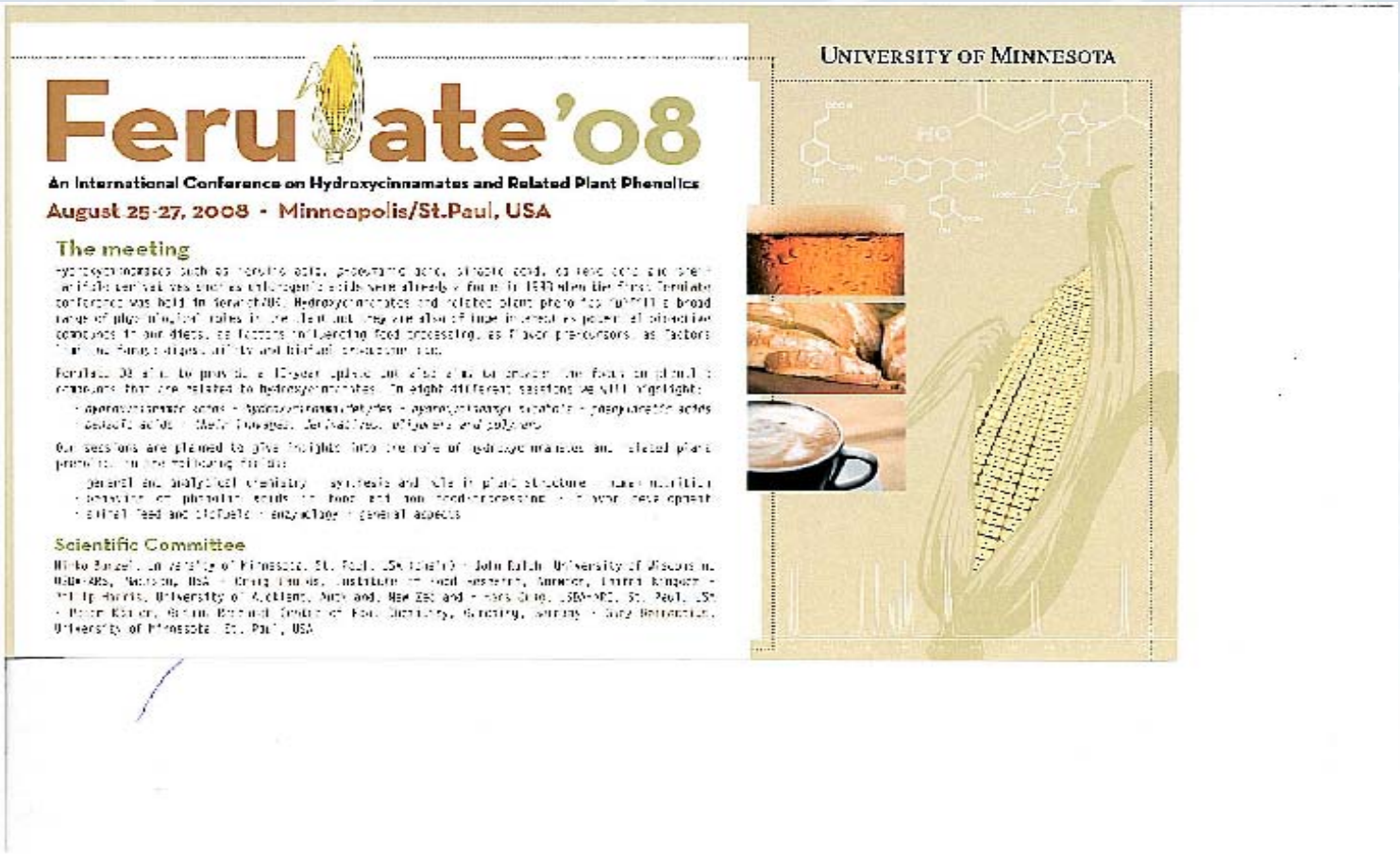
A devoted conference



UNIVERSITY OF MINNESOTA

Feru ate'08

An International Conference on Hydroxycinnamates and Related Plant Phenolics

August 25-27, 2008 · Minneapolis/St.Paul, USA

The meeting

Scientific Committee

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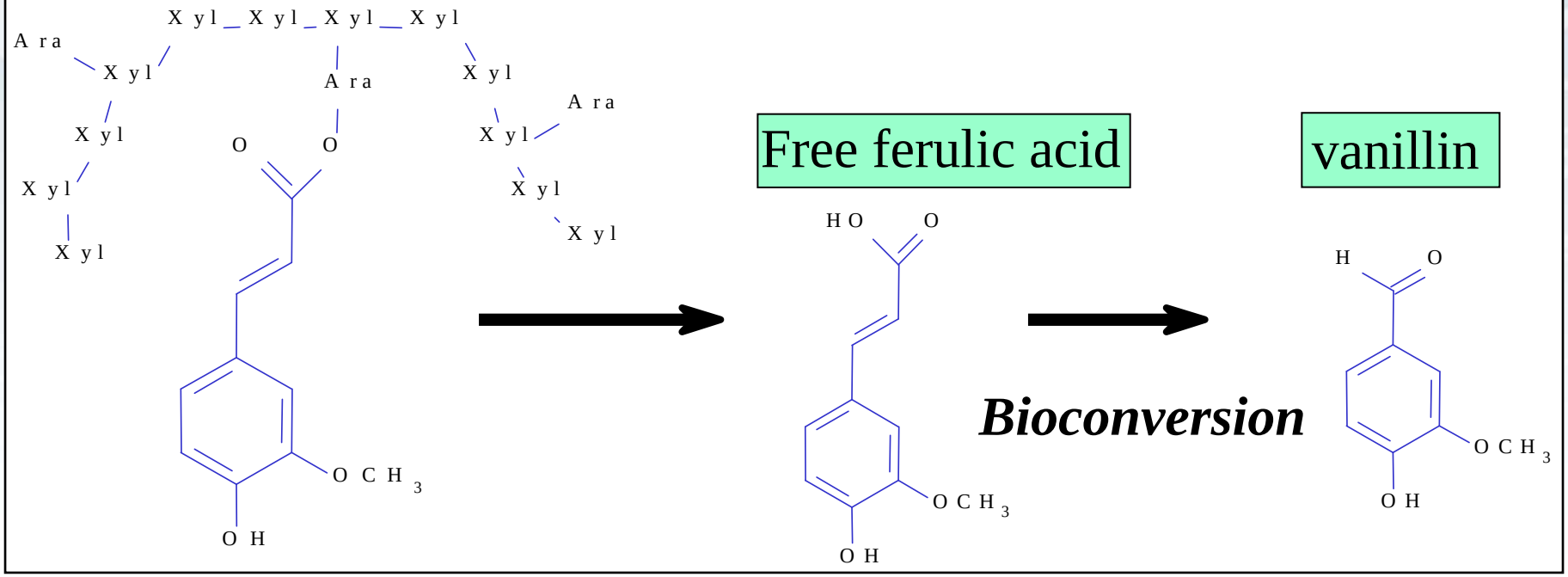
Enzyme systems for Artificial Vanilla production

Arabinoxylan
Containing ester-linked
ferulic acid



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Ferulic acid Esterase Specificities

Type A	Type B	Type C	Type D
Active on	Active on	Active on	Active on
MFA	MFA	MFA	MFA
MSA	MCA	MCA	MCA
5,5’ diFA	MpCA	MpCA	MpCA
		MSA	MSA
			5,5’ diFA
A. niger FAE A	*P.funiculosum* FAE B	*A. niger* FAE B	*P. funiculosum* FAE A
Tal. stipitatus FAE A	*Tal. stipitatus* FAE B	*Tal. stipitatus* FAE C	*P.fluorescens* XYL D FAE D

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Typical properties of FAEs discovered to date

- Enzyme type
- pH optima
- Temperature optimum
- MW

- Carboxylic esterases
- 4-7
- 45-60
- 25 -40 kDa

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Production routes to ferulic acid

- From γ-oryzanol (rice bran oil, ~2%)
- Alkaline hydrolysis eg wheat bran (0.5%)
- Enzyme release

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FAE types and synergy with xylanases

- Types B & C are similar, only release monomers, C broader specificity than B
- Types A & D are similar, release dimers, D broader specificity than A
- FAEs work synergistically with xylanases
- Synergistic level is different for different types of xylanase

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FAE synergy for oat hulls

- Release of reducing sugars
  - Cellulase alone: 39%
  - Xylanase alone: 33%
- Combination of cellulase, xylanase & FAE
  - 69% of reducing sugars released

(From Yu et al, 2003)

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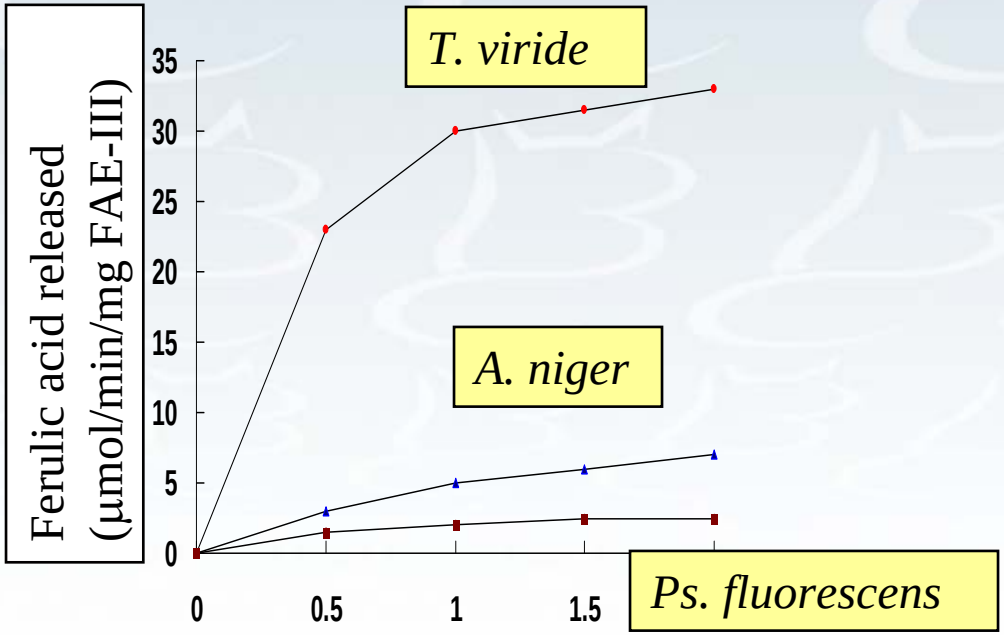
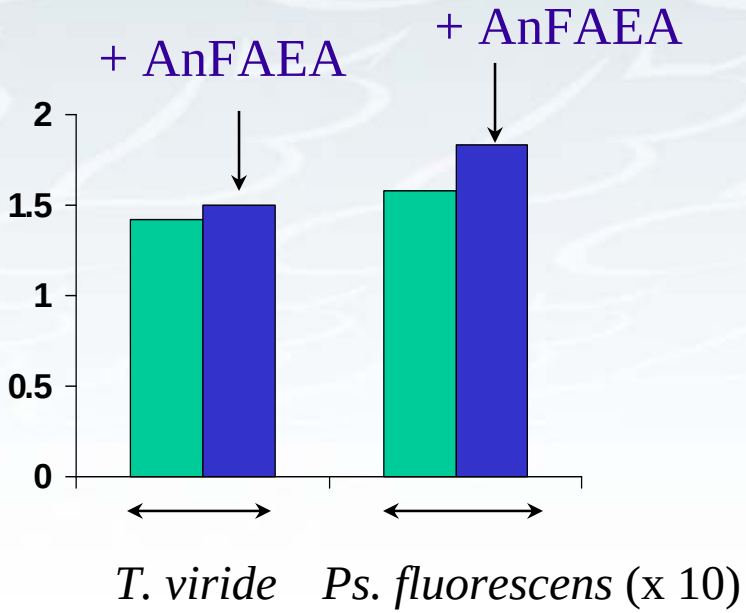
Biproduct heterosynergy

Effect of different xylanases on yield of ferulic acid from wheat bran (constant amount of FAEA from *A. niger*).



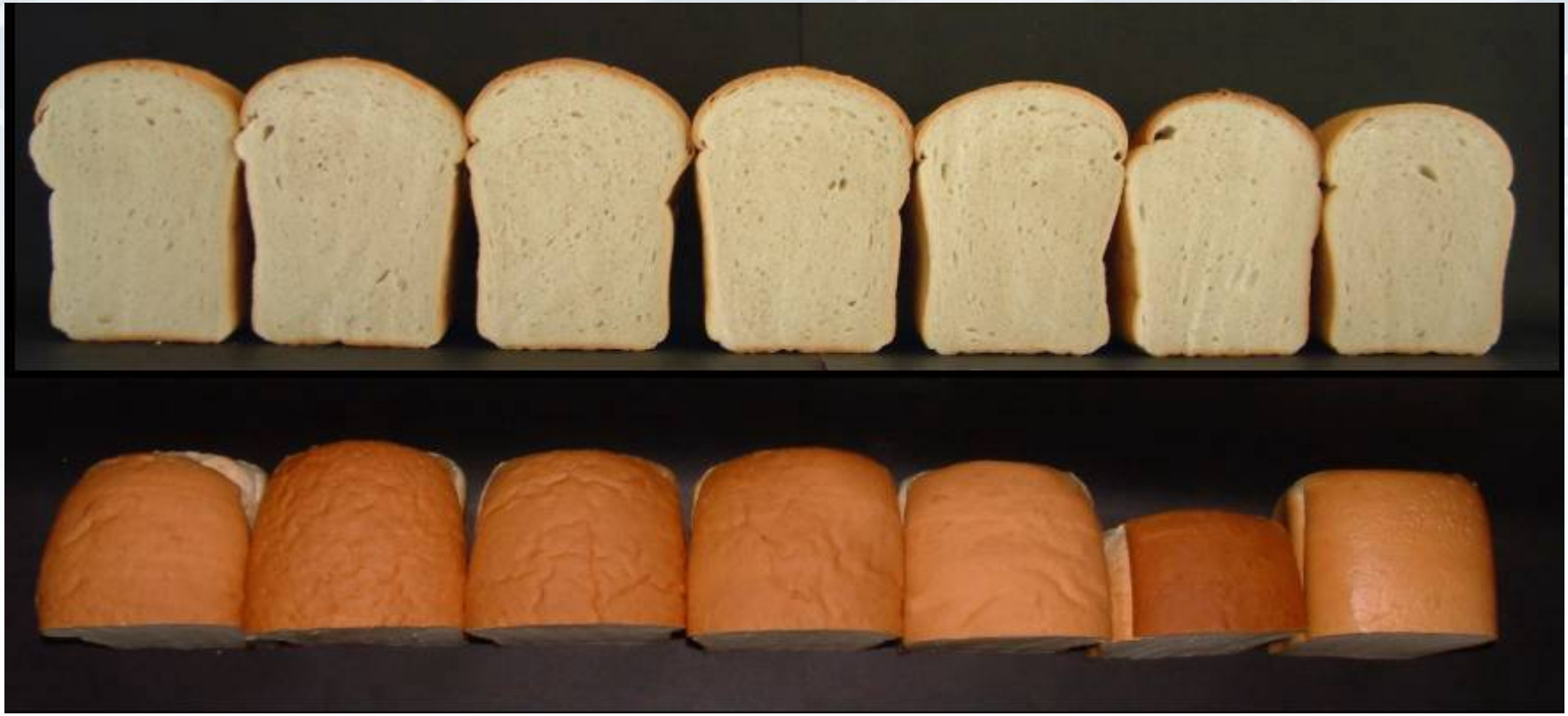
xylanase in assay (units of activity on oat spelt xylan)

AnFAEA slightly increases the amount of sugars ($X + X_2 + X_3 + X_4$) hydrolysed from wheat bran by xylanases.



Amount of ($X + X_2 + X_3 + X_4$) produced (µmol/assay)

FAEC (mU)					Xylanase (D222P) 0.1g	Control (no enzyme)
6.5	65	650	6500	65000		



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Upgrading of wheat bran

- Wheat bran is a good source of insoluble fibre
- Big current focus is on soluble fibre
- A hemicellulase/FAE mix can convert much of the insoluble fibre into soluble fibre without producing high levels of monomers

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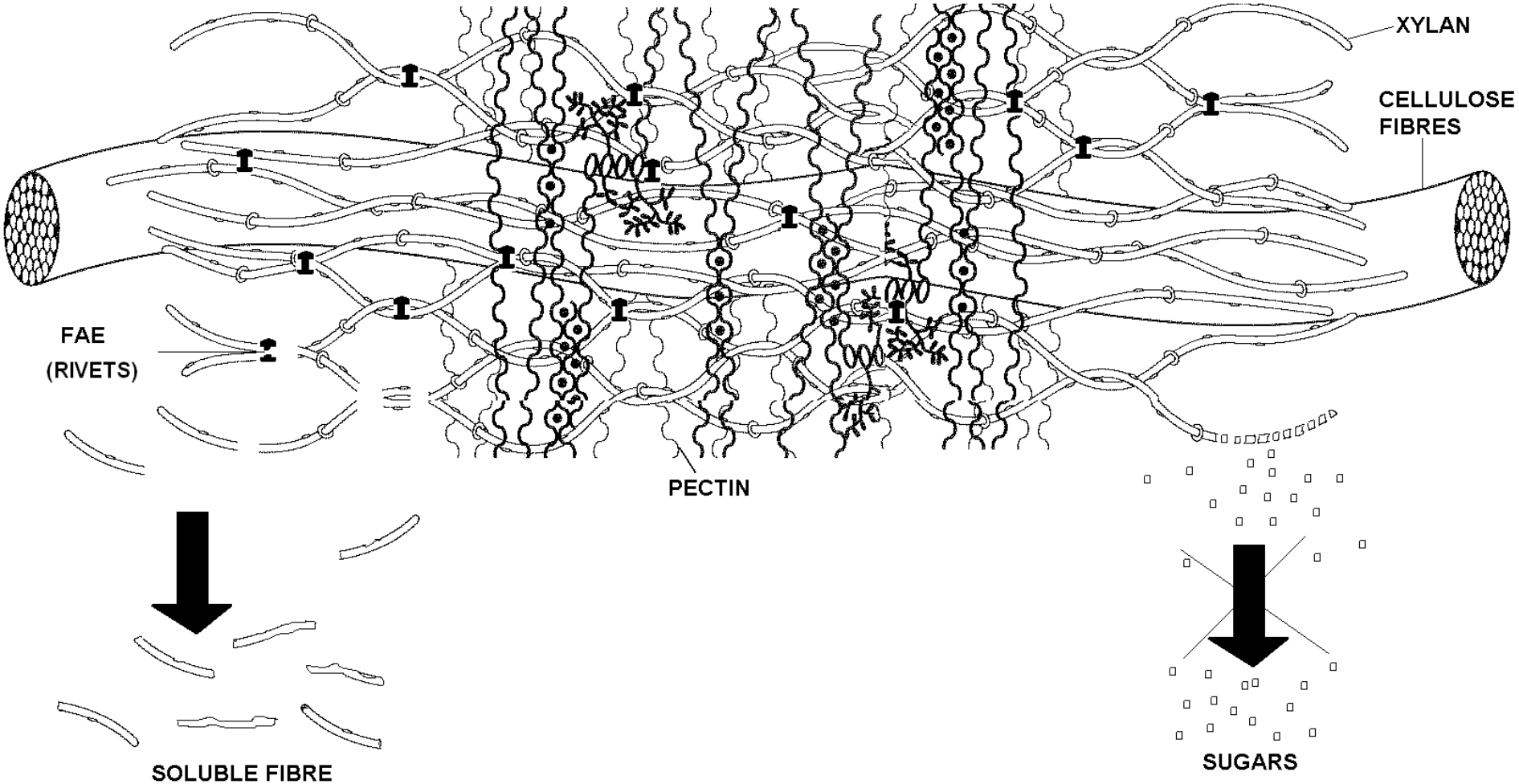

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FAE in brewing

- ‘Glucan gums/particles’ do not contain only glucans
- Removal of xylan micro-particles are crucial for cold-filtered beer

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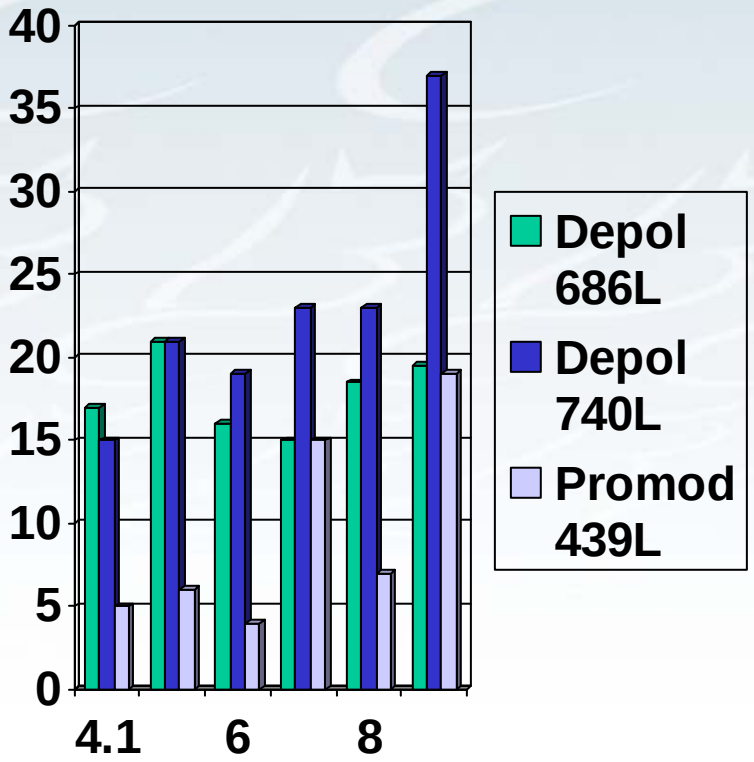
Solubilisation of brewers' spent grain

- Graph of % solids release against pH
- All protein (~18% released by P439L)
- Both D740L & D686L had 2 pH optima
- (From Faulds, Robertson & Waldron)



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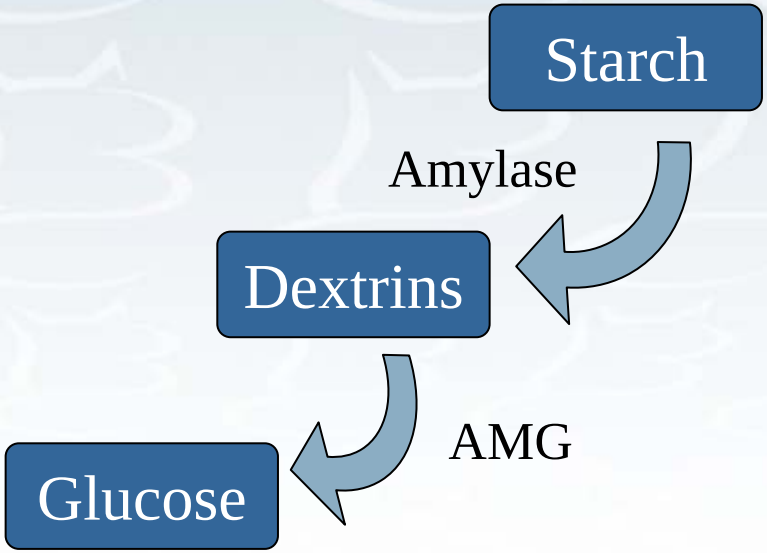
Two step production of Bio-ethanol

- Ferment glucose to ethanol



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Simultaneous saccharification & fermentation

- Production of lactic acid from wheat bran

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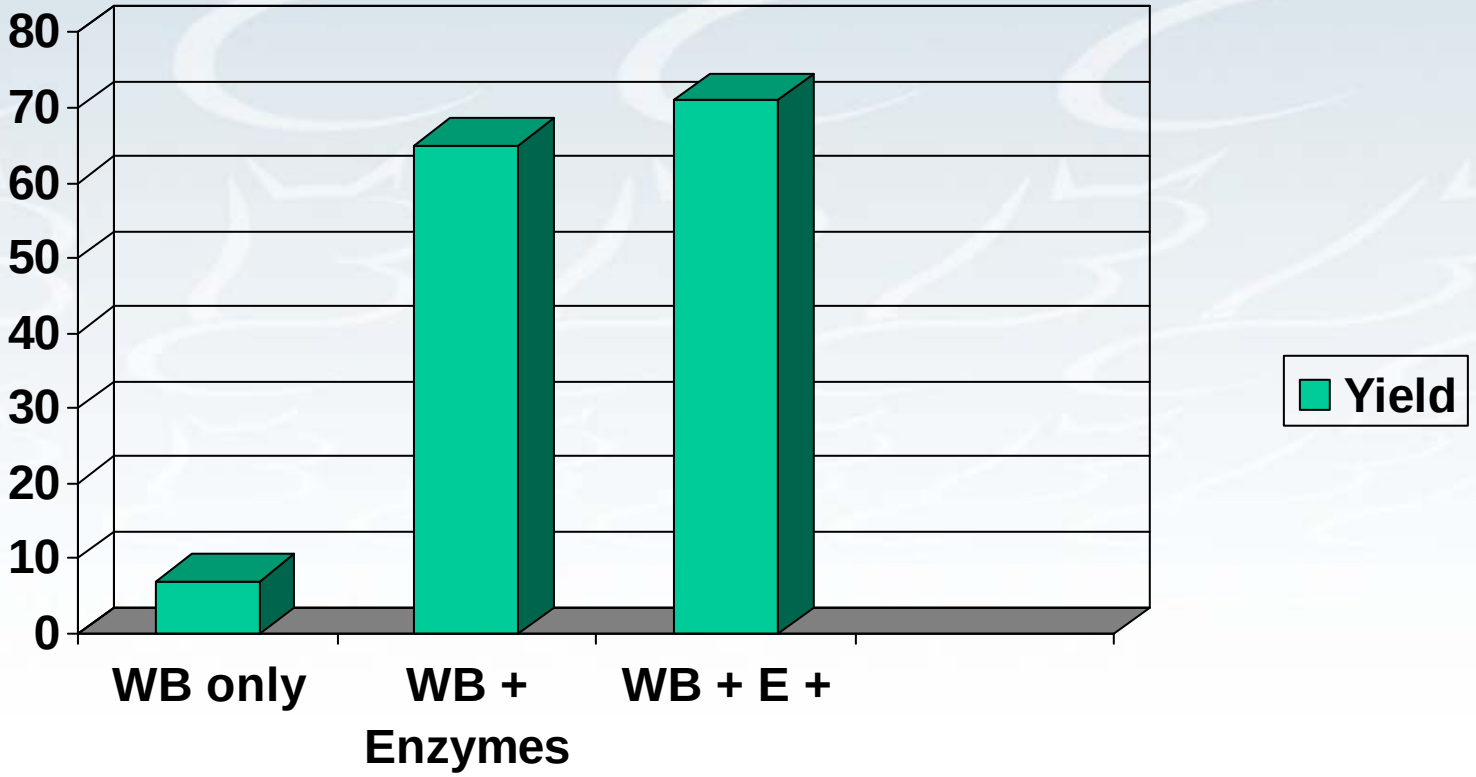
% lactic acid yield from wheat bran by SSF (calculated carbon)



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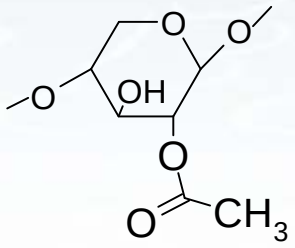
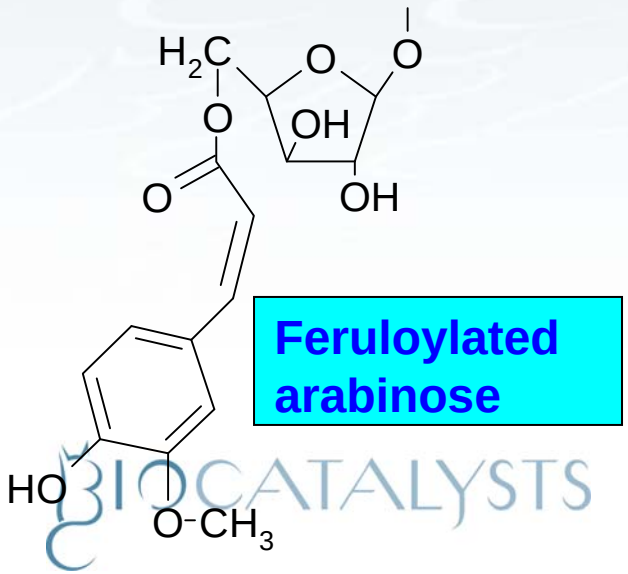
Deacetylation reactions

- Certain plant cell wall polysaccharides also contain acetyl esters, which again can hinder the cell wall decomposition.
  - wheat bran contains 0.77% (w/dry weight) acetate
  - brewer's spent grain is 0.2% acetylated.

- Almost all of the feruloyl esterases described in the literature can also remove these acetate esters from a chemically-acetylated xylan.



Acetylated xylose



Feruloylated arabinose

Commercial availability of Ferulic acid esterases

- Wild types (mixed source) available in some products as a side activity eg Depol 686L (Biocatalysts)
- Work on cloned enzymes by
  - Aspergillus type A by Danisco/Genencor
  - Talaromyces type C by Biocatalysts

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Conclusions

- Ferulic acid is a valuable chemical which can be made using ferulic acid esterase
- Ferulic acid esterases have many potential applications in food processing and also in bio-ethanol manufacture from cellulosic sources
- Cost effective sources are currently being developed by several companies

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