

# *Feruloyl Esterases: Potential in Grain Processing*

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Fifth Grain Conference Norwich 2008

Stuart West

 BIOCATALYSTS

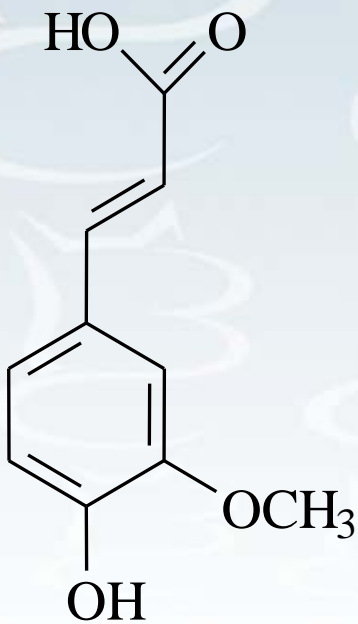


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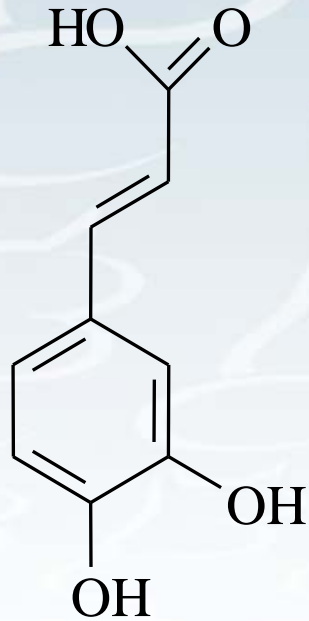
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- Different types of Ferulic acid esterases
- Potential use in baking
- Use in brewing
- Use in wheat & barley waste processing
- Potential in bioethanol production

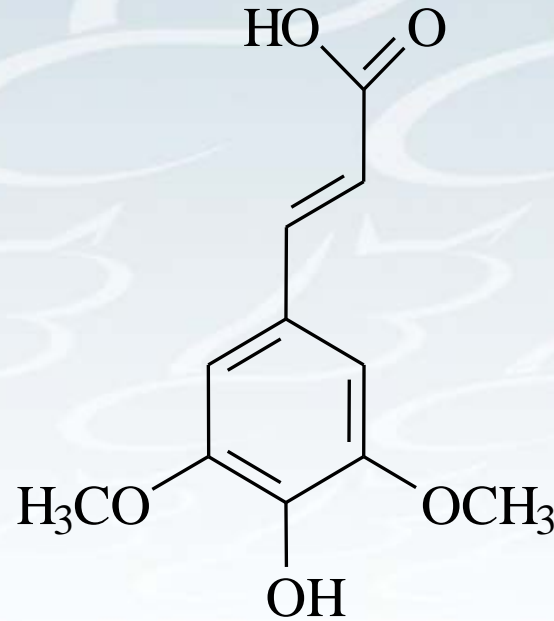
# Hydroxycinnamic acids



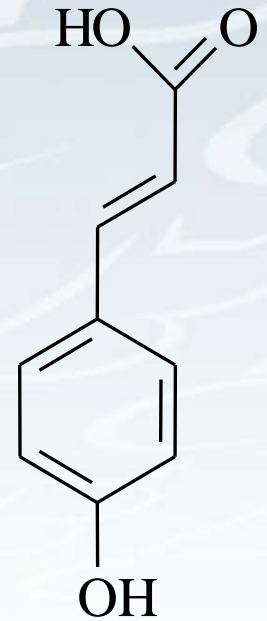
Ferulic Acid



Caffeic Acid

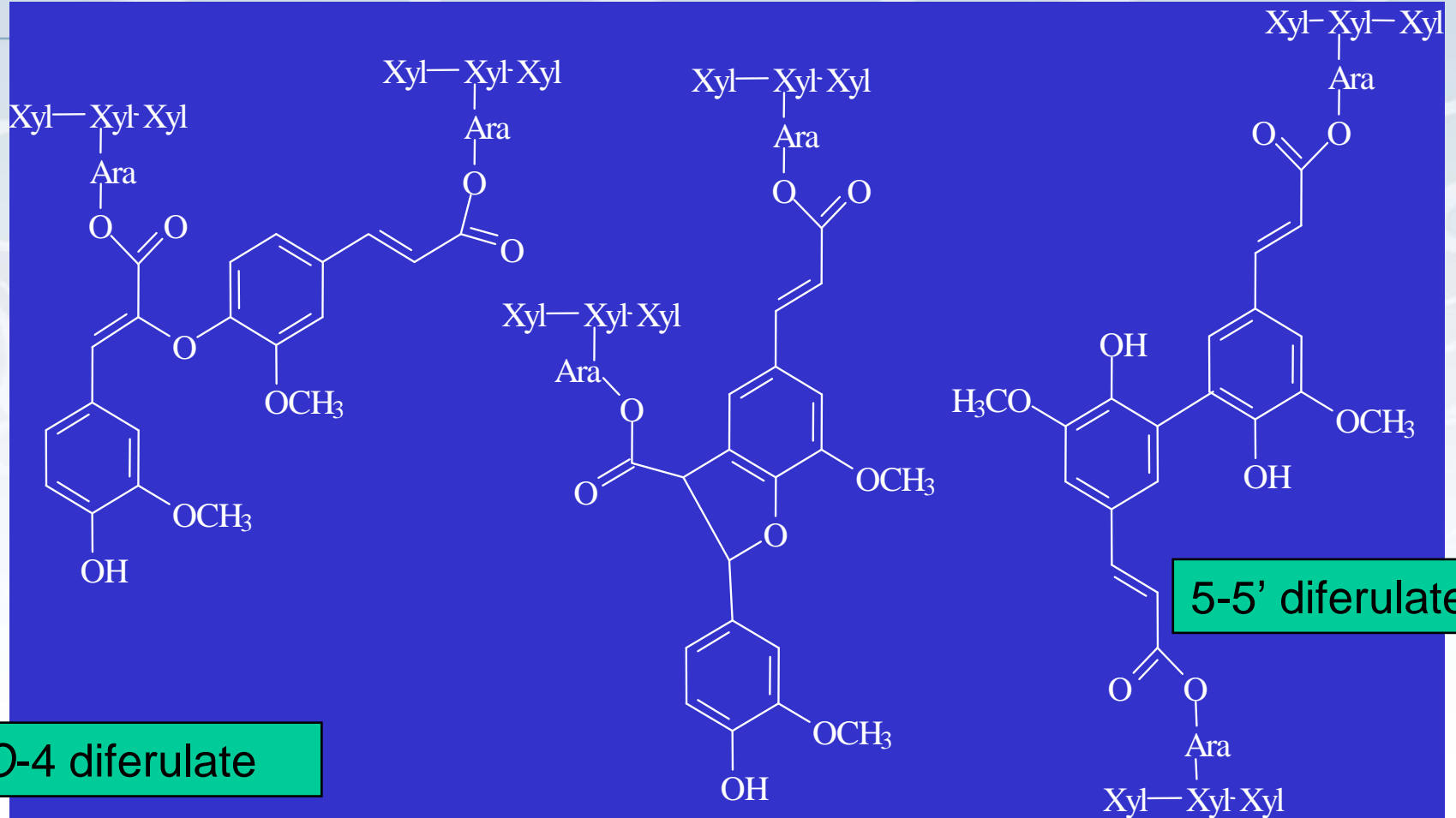


Sinapic Acid



*p*-Coumaric Acid

# Dimeric ferulates



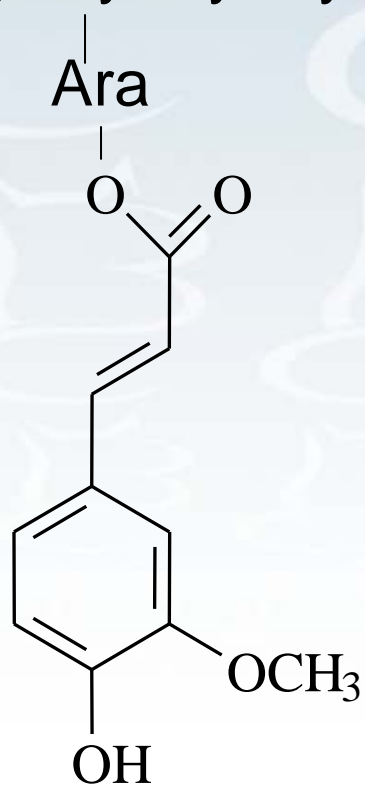
8-O-4 diferulate

5-5' diferulate

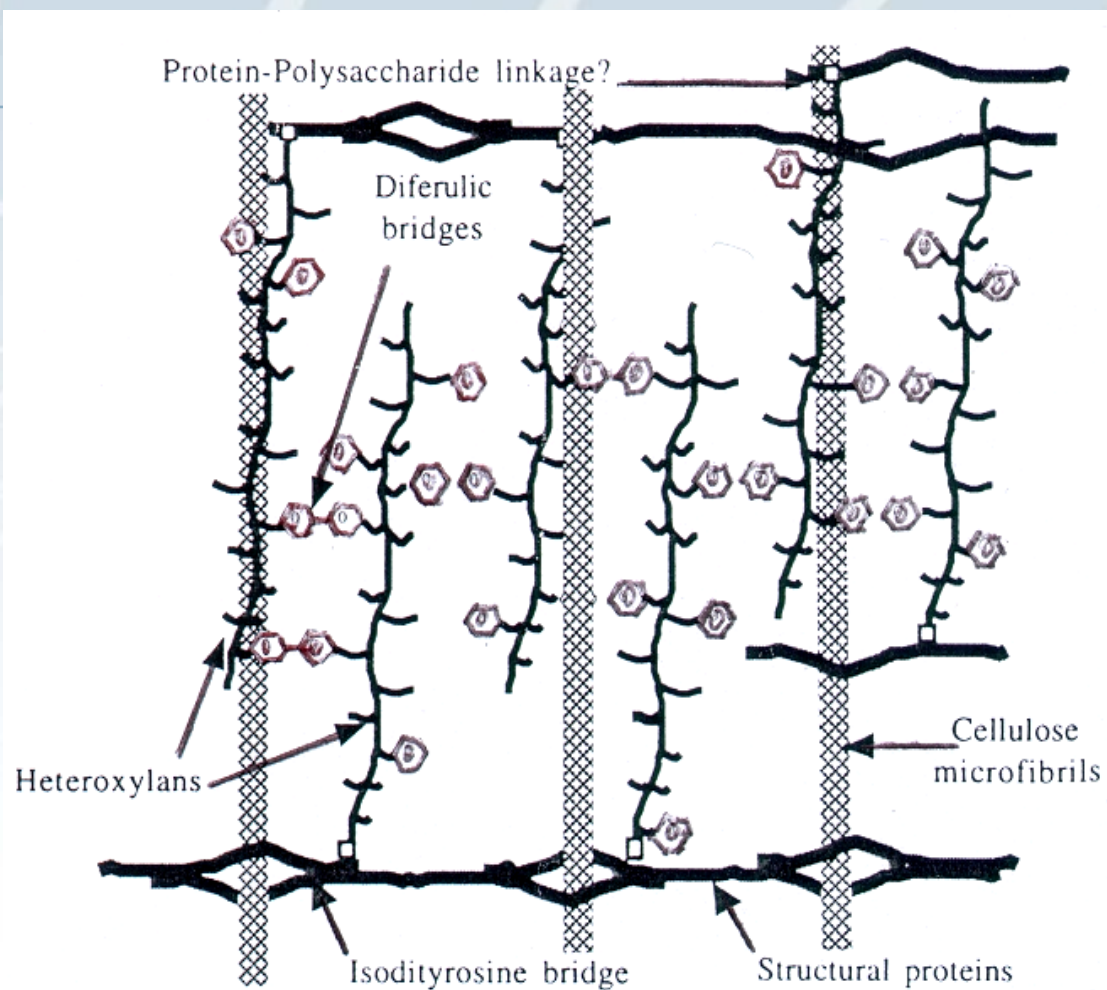
8-5' Benzofuran diferulate

# Ferulic Acid Esterase (EC 3.1.1.73)

Xyl-Xyl-Xyl-Xyl



Ferulic Acid



# Current & potential uses/benefits of ferulic acid

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- 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

# A devoted conference

## Ferulate'08

**An International Conference on Hydroxycinnamates and Related Plant Phenolics**  
**August 25-27, 2008 - Minneapolis/St. Paul, USA**

**The meeting**  
Hydroxycinnamates such as ferulic acid, p-coumaric acid, sinapic acid, cinnamic acid and their methyl derivatives such as methylferulate were already known in 1830 when the first Ferulate conference was held in Ferula'06. Hydroxycinnamates and related plant phenolics exhibit a broad range of physiological roles in the plant and they are also of huge interest as potential bioactive compounds in our diets, as factors influencing food processing, as flavor precursors, as factors for the fungal degradation of food and for food preservation.

Ferulate'08 aims to provide a 10-day update and also aims to broaden the focus on related compounds that are related to hydroxycinnamates. In eight different sessions we will highlight:

- general aspects of hydroxycinnamates
- hydroxycinnamates in plants
- hydroxycinnamates in food
- hydroxycinnamates in food processing
- hydroxycinnamates in food preservation
- hydroxycinnamates in food safety
- hydroxycinnamates in food quality
- hydroxycinnamates in food nutrition
- hydroxycinnamates in food bioactivity

Our sessions are planned to give insights into the role of hydroxycinnamates and related plant phenolics in the following fields:

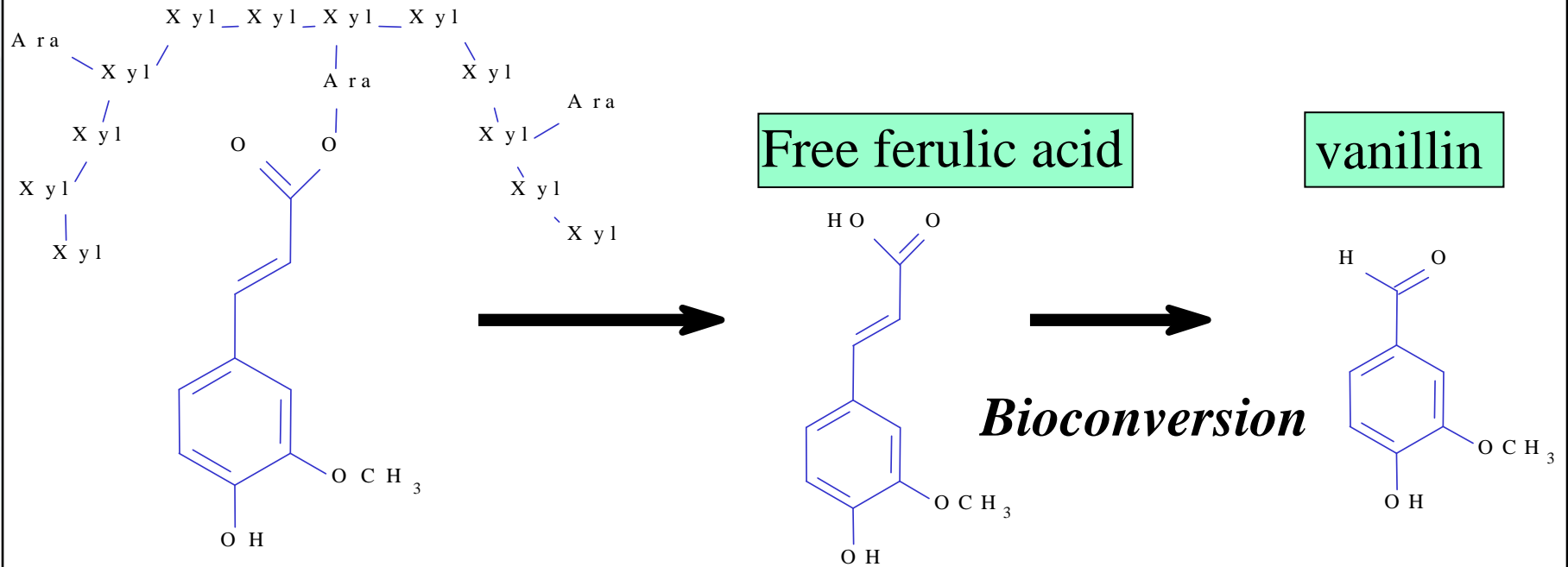
- general and analytical chemistry
- synthesis and role in plant structure
- plant nutrition
- biosynthesis of phenolics
- health and food processing
- food preservation
- food safety and quality
- bioactivity
- general aspects

**Scientific Committee**  
Wolfgang Brandl, University of Minnesota, St. Paul, USA (chair) • John Eklund, University of Wisconsin, Madison, USA • Greg Lurie, Institute of Food Research, Norwich, United Kingdom • Philip Harris, University of Adelaide, Australia • Max Ziegler, University of Minnesota, St. Paul, USA • Peter Klason, Biotech Center of Food Chemistry, Germany, Germany • Gary Birkbeck, University of Minnesota, St. Paul, USA

UNIVERSITY OF MINNESOTA

# Enzyme systems for Artificial Vanilla production

Arabinoxylan  
Containing ester-linked  
ferulic acid



# Ferulic acid Esterase Specificities

## Type A

Active on

MFA

MSA

5,5' diFA

## Type B

Active on

MFA

MCA

MpCA

## Type C

Active on

MFA

MCA

MpCA

MSA

## Type D

Active on

MFA

MCA

MpCA

MSA

5,5' diFA

*A. niger* FAE A

*Tal. stipitatus*

FAE A

*P. funiculosum*

FAE B

*Tal. stipitatus*

FAE B

*A. niger* FAE B

*Tal. stipitatus*

FAE C

*P. funiculosum* FAE A

*P. fluorescens* XYL D

FAE D

# Typical properties of FAEs discovered to date

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- Enzyme type
- pH optima
- Temperature optimum
- MW
- Carboxylic esterases
- 4-7
- 45-60
- 25 -40 kDa

# Production routes to ferulic acid

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

# F AE types and synergy with xylanases

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- 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

# FAE synergy for oat hulls

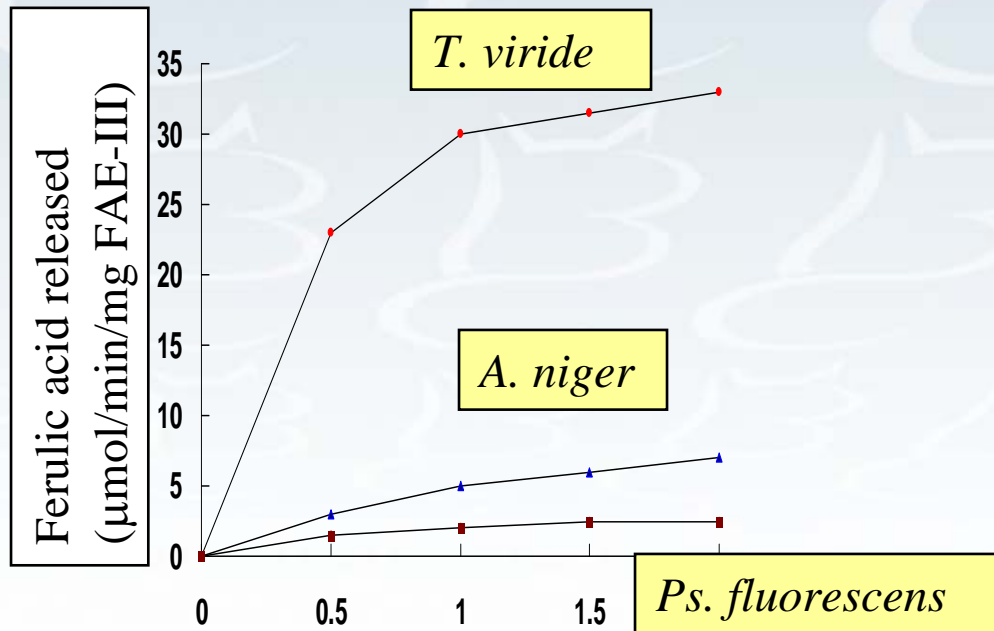
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- Release of reducing sugars
  - Cellulase alone: 39%
  - Xylanase alone: 33%
- Combination of cellulase, xylanase & FAE  
69% of reducing sugars released

(From Yu et al, 2003)

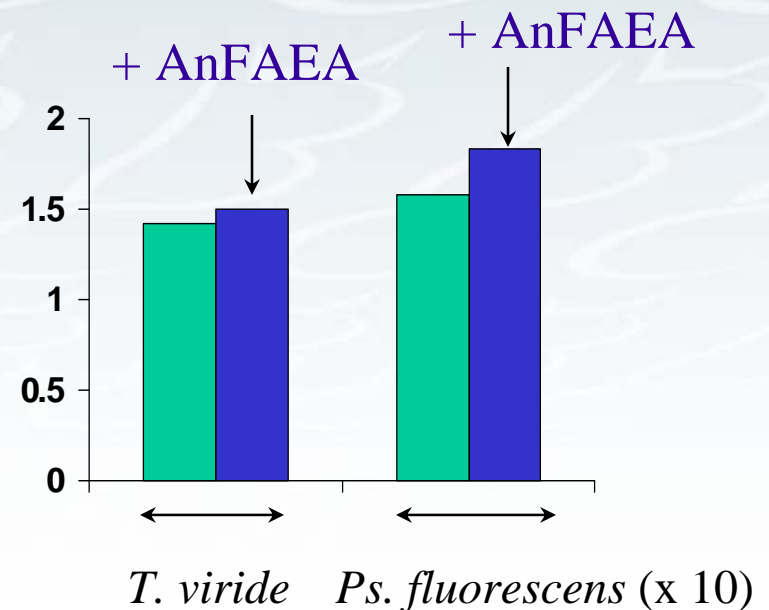
# Biproduct heterosynergy

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 ( $\mu\text{mol}/\text{assay}$ )

**FAEC (mU)**

**6.5**

**65**

**650**

**6500**

**65000**

**Xylanase  
(D222P)**

**0.1g**

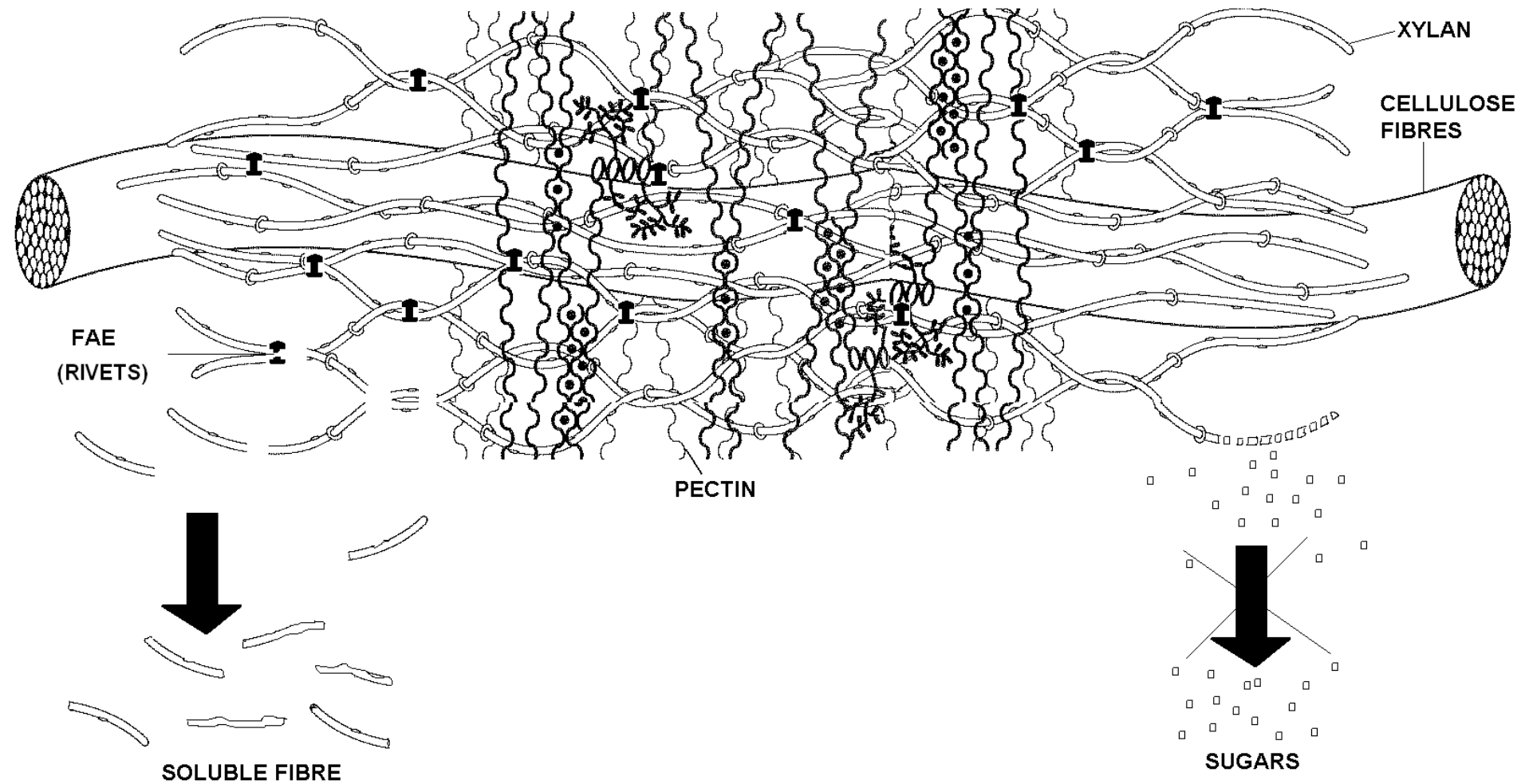
**Control  
(no  
enzyme)**



# Upgrading of wheat bran

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- 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



# FAE in brewing

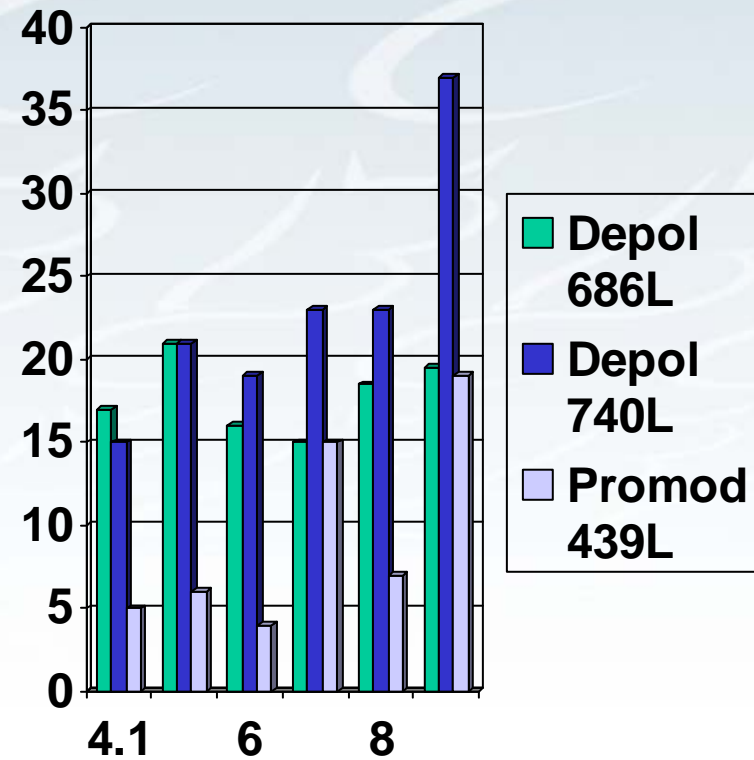
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- 'Glucan gums/particles' do not contain only glucans
- Removal of xylan micro-particles are crucial for cold-filtered beer



# Solubilisation of brewers' spent grain

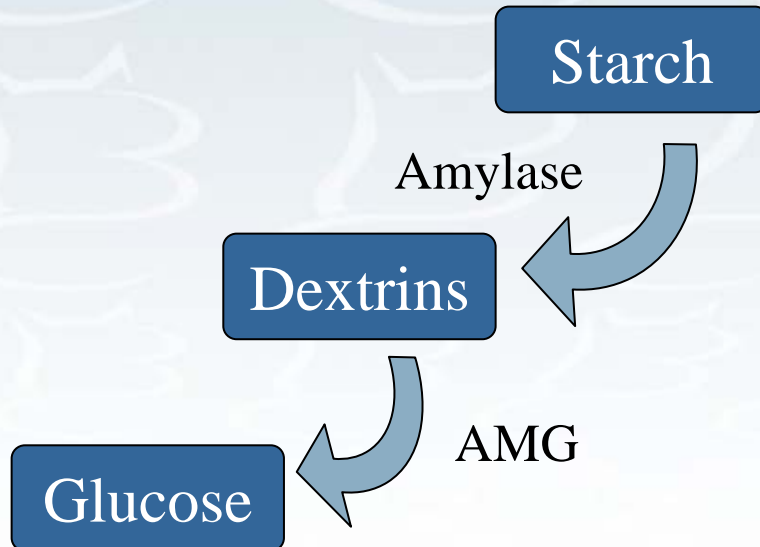
- Graph of % solids release against pH
- All protein (~18% released by P439L)
- Both D740L & D686L had 2 pH optima
- (From Faulds, Robertson & Waldron)



# Two step production of Bio-ethanol

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- Ferment glucose to ethanol

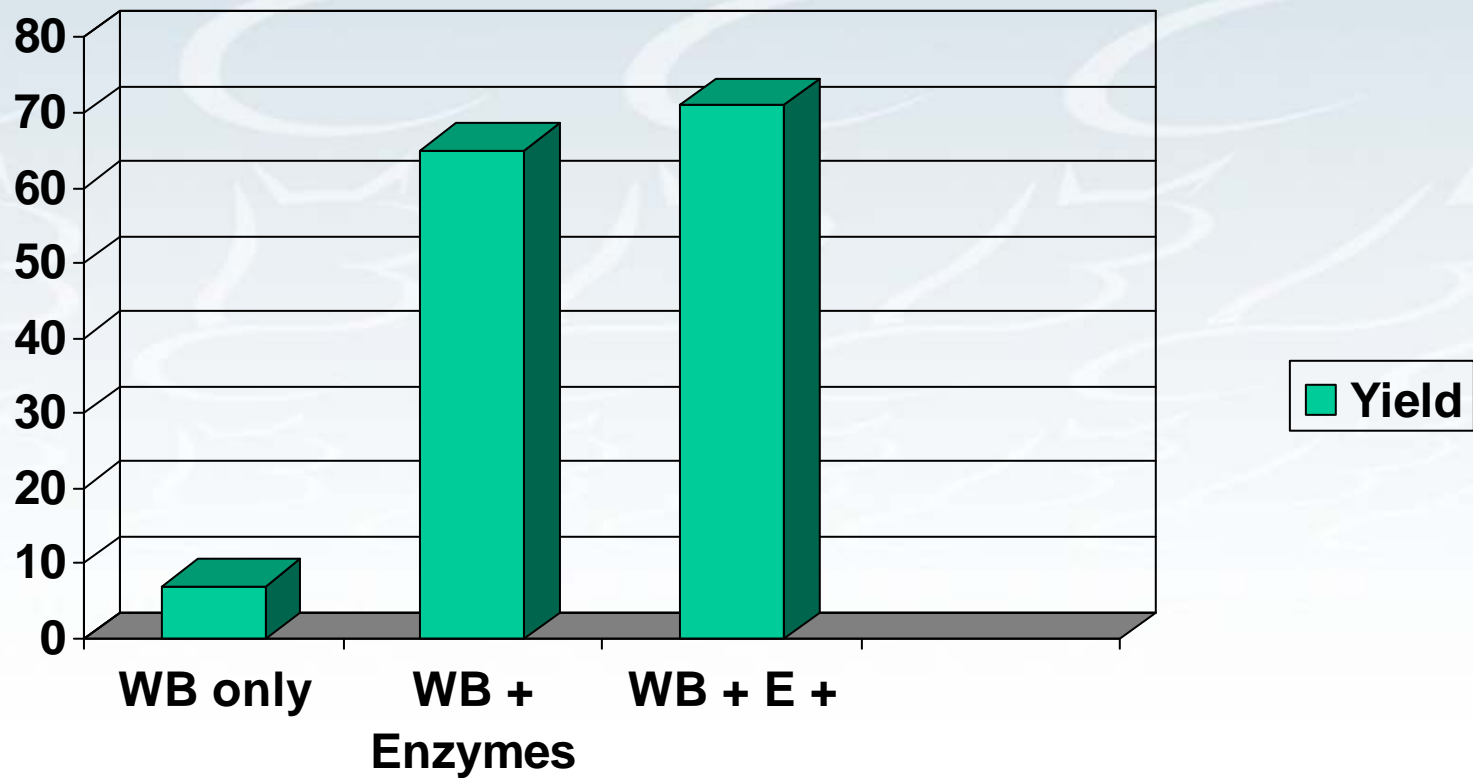


# Simultaneous saccharification & fermentation

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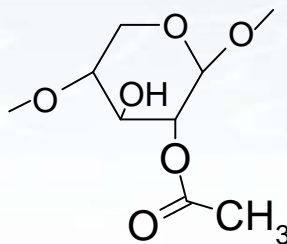
- Production of lactic acid from wheat bran

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

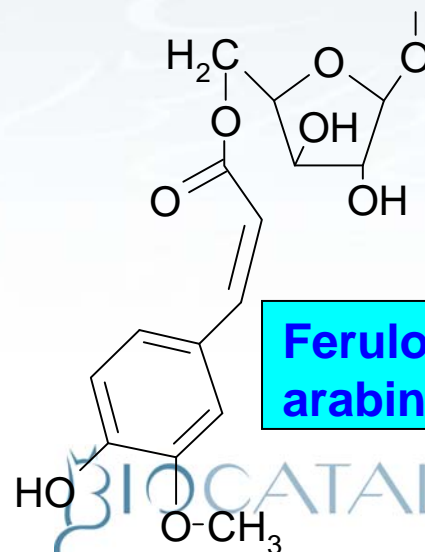


# Deacetylation reactions

- 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

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- 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

# Conclusions

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- 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