Malt Enzymes

The Key to Successful Brewing

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MBAC Ontario Technical Conference

January 31, 2014

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CANADIAN MALTING BARLEY TECHNICAL CENTRE

Introduction

- Malt → Barley Malt

- Why Barley
  - Availability? (not used for food)
  - Adaptability
  - Husk
  - Enzyme potential
  - Tradition
  - Breeding

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Introduction

- What does the brewer want that barley has to offer?
  - Enzymes
  - Proteins
  - Carbohydrates
  - Filter aid
  - ……

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Introduction

- Beer production (process of converting barley into beer) is probably the oldest example of applied biotechnology
- It involves endogenous enzymology in three main stages:
  - Malting (barley kernel)
  - Mashing (mash tun – bioreactor)
  - Fermentation (yeast cell)

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Introduction

- Biochemical changes during the entire brewing process mostly involve the action of different enzymes which are essential in catalyzing these changes
- Enzymes are present in mature barley (β-amylase and carboxypetidases), finished malt (α-amylase, limit dexrinase, proteases, glucanases, pentosanases) and yeast
- Malt derived enzymes will be the focus

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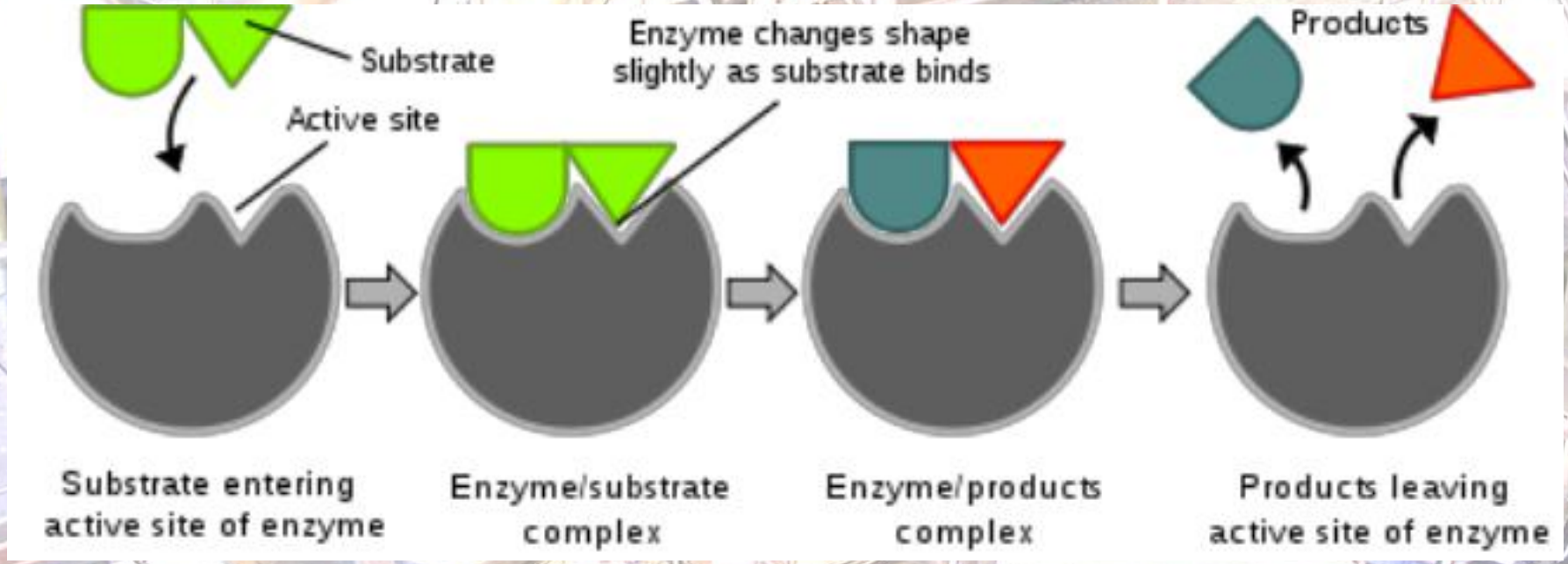
Introduction

- **Enzymes (which are proteins) cause reactions to happen without being destroyed in the process**



Example of "lock and key" description of enzyme action

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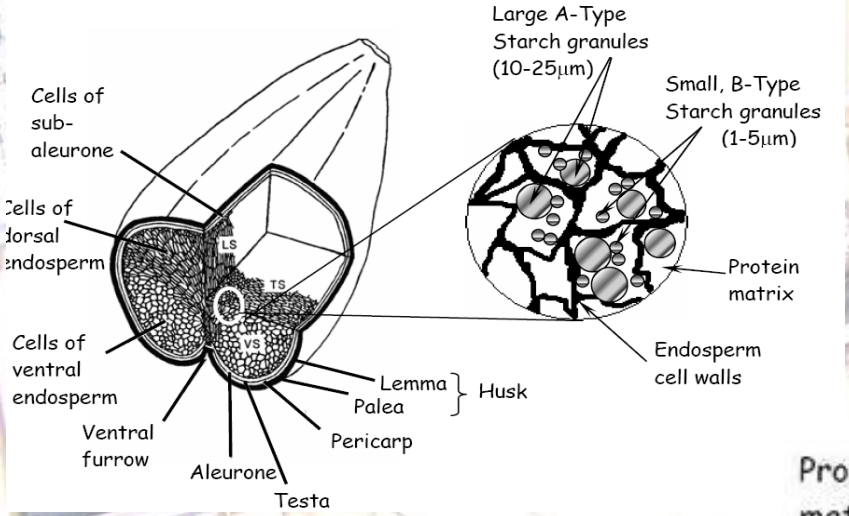
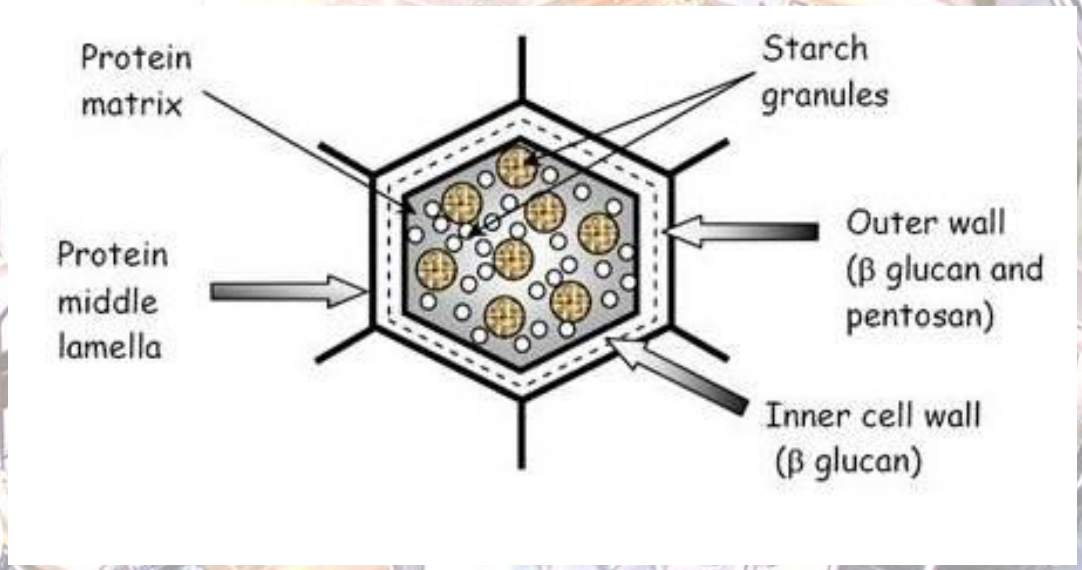
Substrates





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Enzymes

Protein

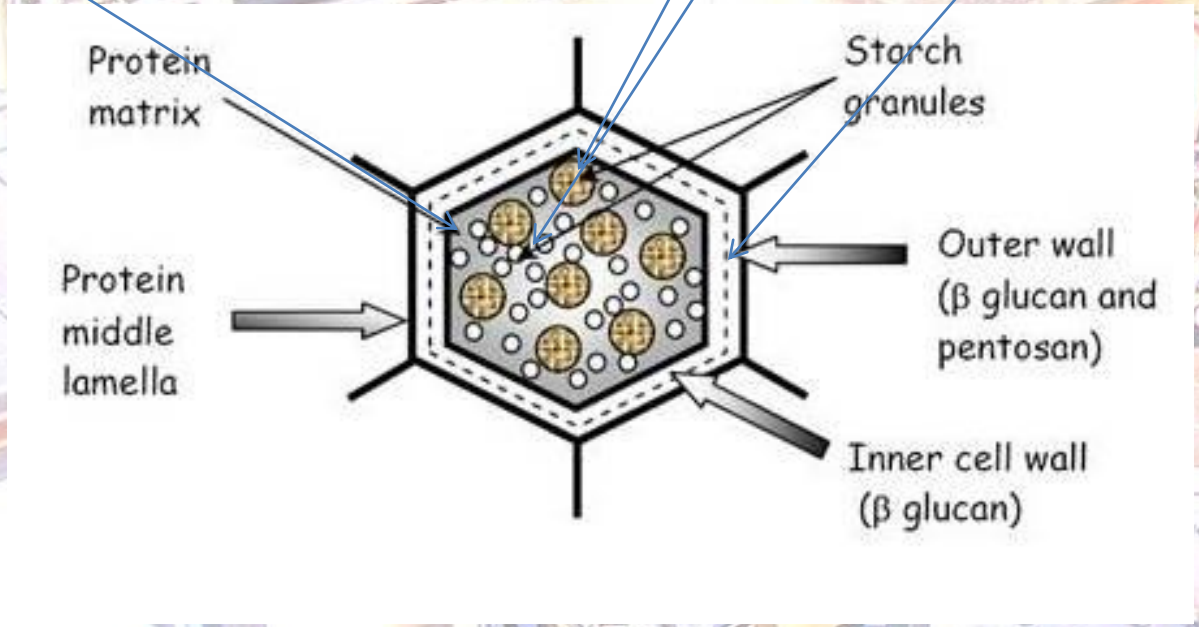
- Endo-peptidases
- Exo-petidases

Starch

- α-Amylase
- β-Amylase
- Limit dextrinase
- α-Glucosidase

Cell Wall

- Xylanase
- Arabinofuranosidase
- Feruloyl esterase
- Acetoxylan esterase
- Carboxypeptidases
- Endo-glucanases
- Exo-glucanases
- Glucosidases



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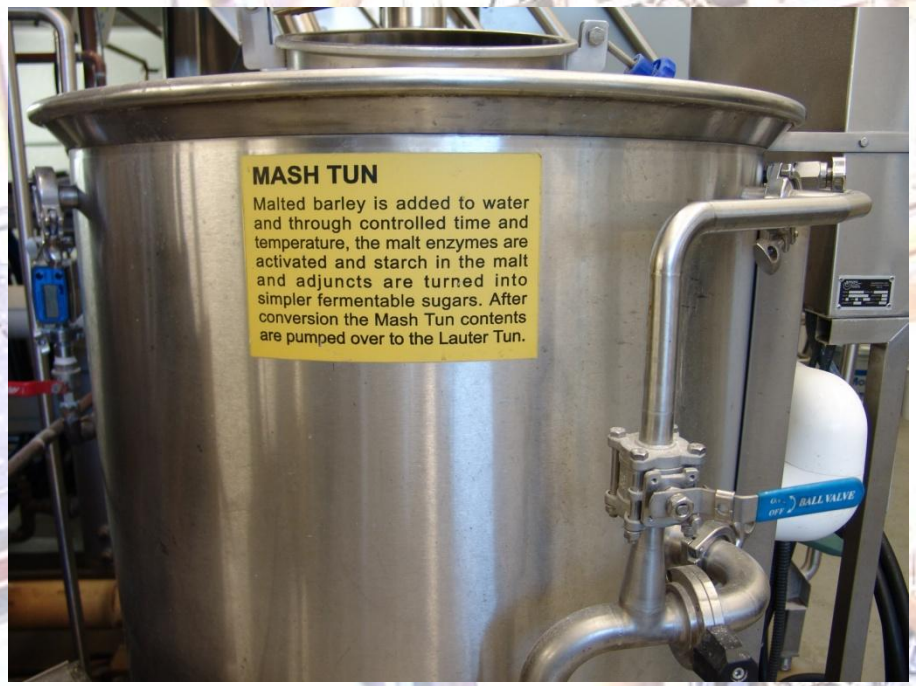
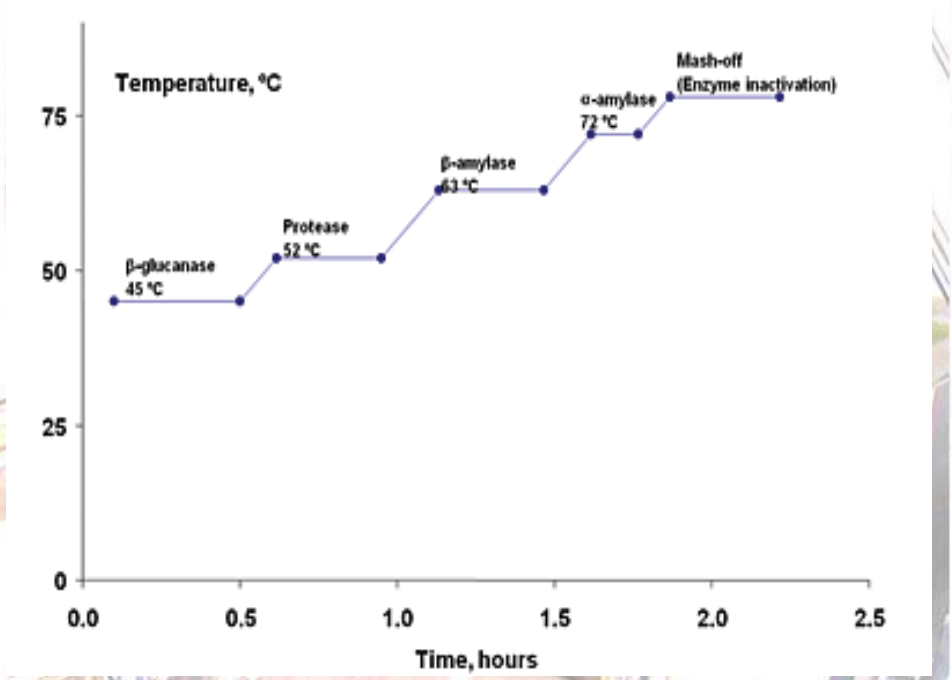
Mash Tun – Bioreactor (Enzyme Reactor)

Enzymes	Optimum (°C)	Deactivation (°C)
Glucanases	40-50	60
Proteinases	45-55	63
β-Amylase	60-65	70
α-Amylase	72-75	80





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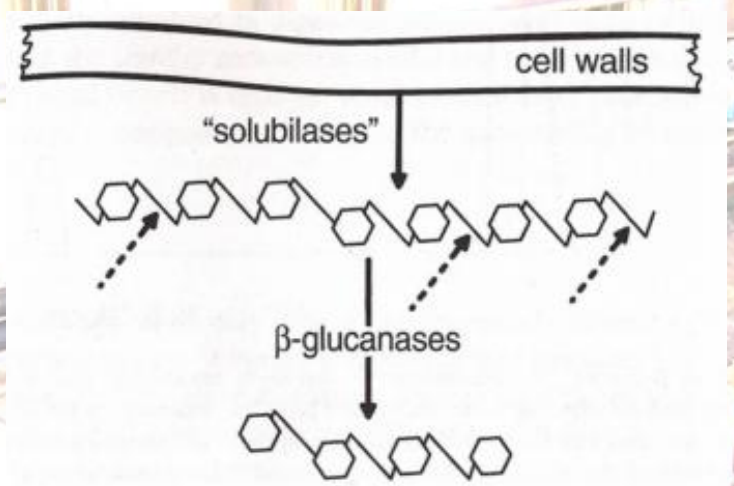
Enzymes

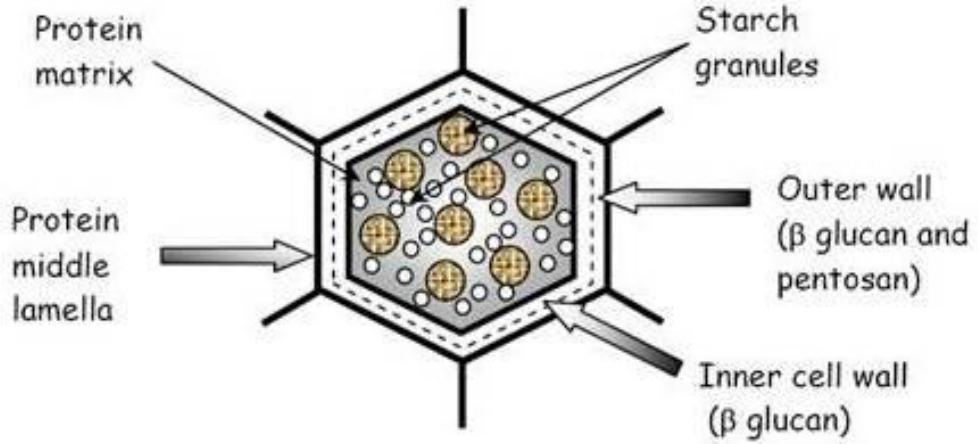
Solubilisation

Hydrolysis



Cell Wall

- Xylanase
- Arabinofuranosidase
- Feruloyl esterase
- Acetoxylan esterase
- Carboxypeptidases



- Endo-glucanases
- Exo-glucanases
- Glucosidases
- Xylosidase

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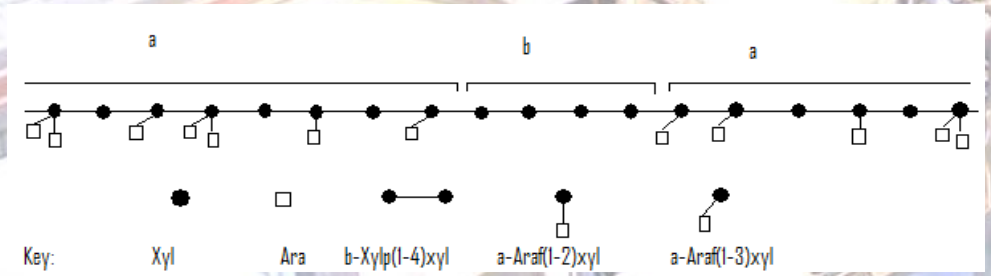
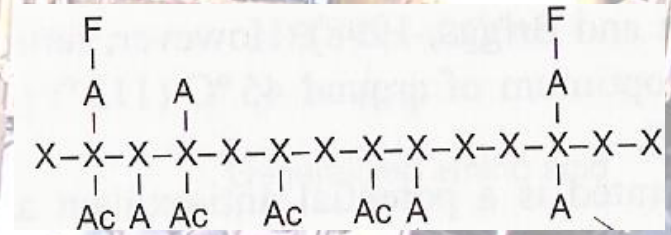
Hemicelluloses and gums

- They make up about 10% of barley.
- Gums are water soluble.
- Hemicelluloses are soluble in hot alkali.
- These fractions are a series of material with different solubility (distinction between hemicelluloses and gums is rather vague).
- If the extraction of gums is done with water of increasing temperature the quantity of gum increases at the expense of the residual hemicelluloses.
- Chemically there are two groups: β-glucans and pentosans (arabinoxylans).

Arabinoxylans (pentosans)

- They have chains of β-D-xylopyranose joined trough (1,4) links
- A xylose residue may be unsubstituted, or be substituted on C-2 , C-3 or in both positions with α-L-arbinofuranose units
- The substitution occur irregularly along the xylan backbone
- Some arabinose units are substituted with acetic and ferulic acid





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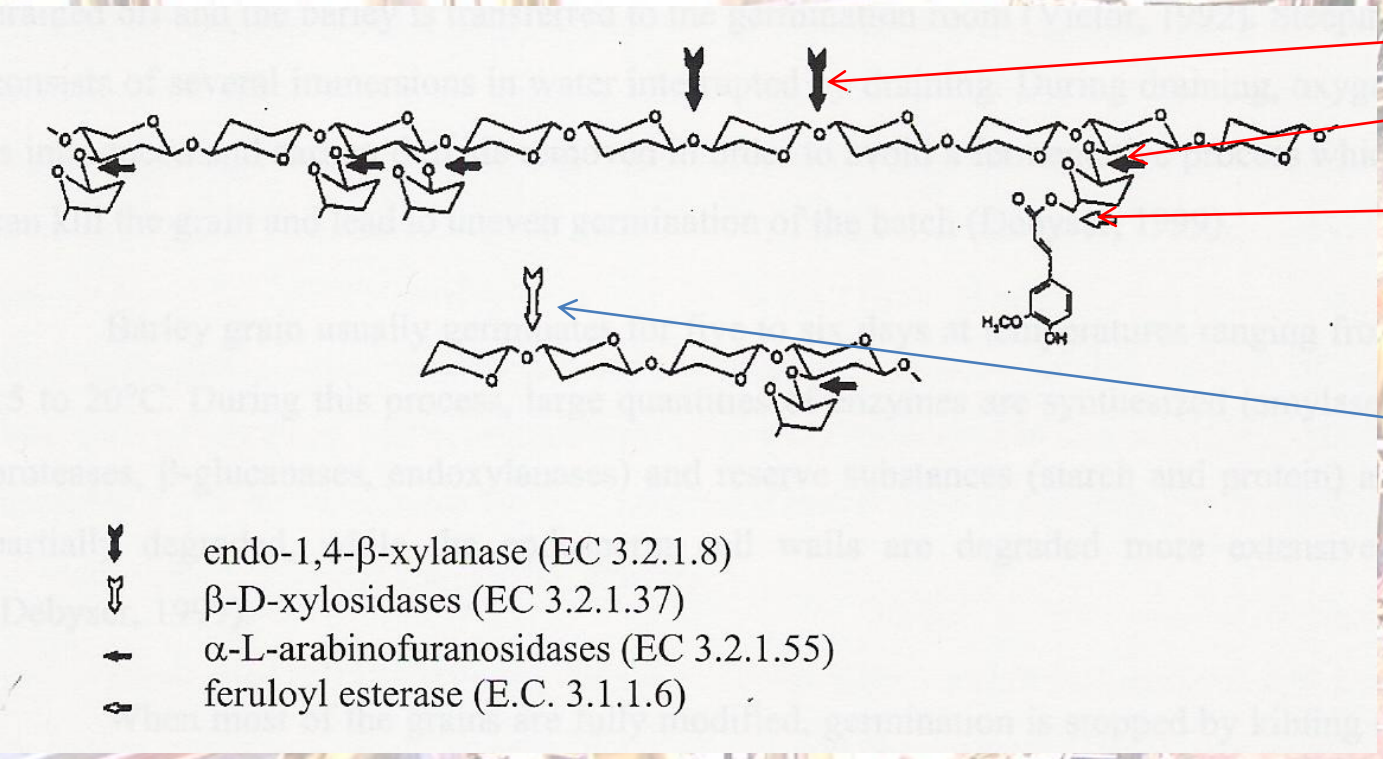
Arabinoxylan hydrolysis



Cell Wall

- Xylanase
- Arabinofuran-osidase
- Feruloyl esterase
- Acetoxylan esterase
- Xylosidase

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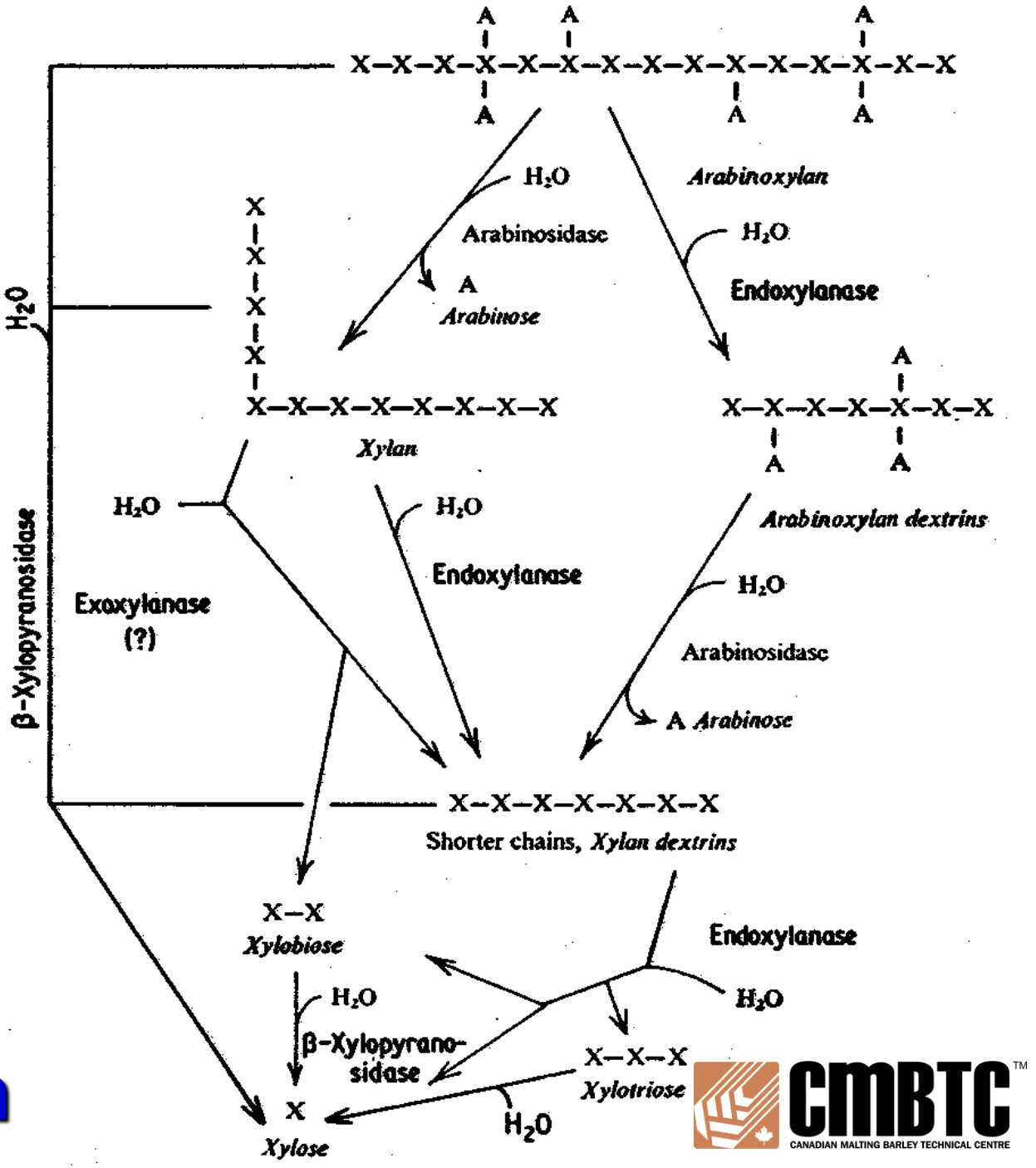
Enzymatic Degradation of Arabinoxylan



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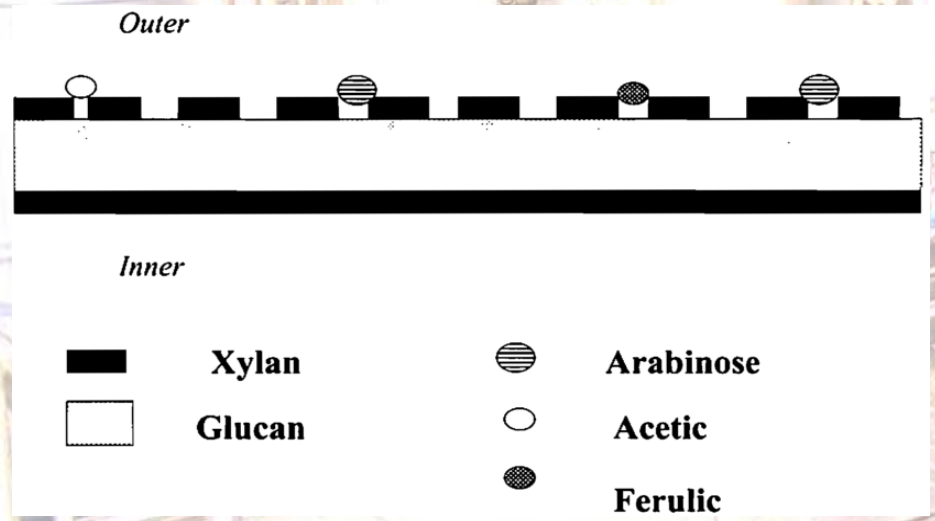
Endosperm cell wall



Bamforth, C.W., Kanauchi, M., 2001. JIB 107, 235-240

Cell Wall

- Xylanase
- Arabinofuranosidase
- Feruloyl esterase
- Acetoxylan esterase

- AX layer in the outer cell wall covers β-Glucans
- Outer AX layer is not complete (glucanases have access their substrate)
- Xylanolytic plus the enzymes that remove the arabynosyl-, feruloyl- and acetoyl- plugs all increase β-Glucan accessibility
- Another more substantial AX layer is buried deeper within the cell wall

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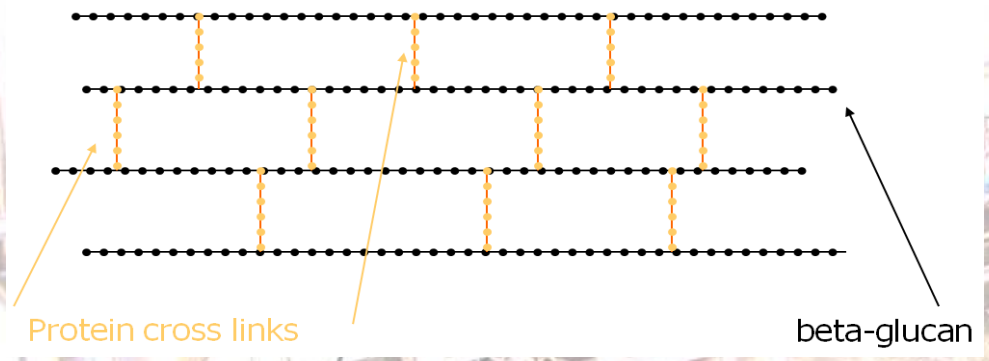
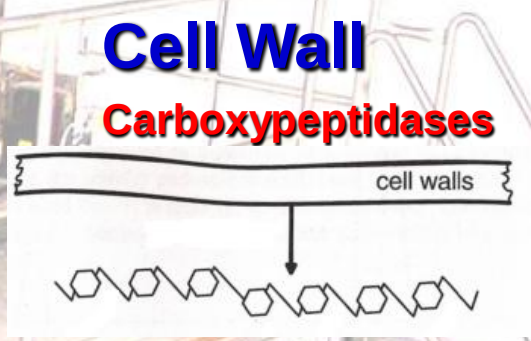
β-Glucan solubilase





- β-Glucan can be released from the cell wall trough the breakage of ester bond between glucan and protein
- β-Glucan solubilase (Carboxypeptidase) is more heat stable than Endo- β-glucanases
- 85% of the enzyme survives kilning
- Able to withstand 65°C for 1 hour
- May cause difficulties – release of HMW β-Glucan

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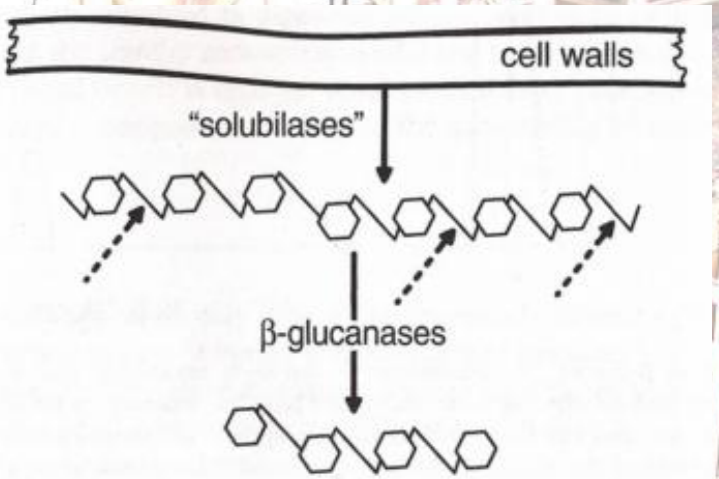
Hydrolysis



Cell Wall

- Xylanase
- Arabinofuranosidase
- Feruloyl esterase
- Acetoxylan esterase
- Carboxypeptidases
- **Endo-glucanases**
- **Exo-glucanases**
- **Glucosidases**

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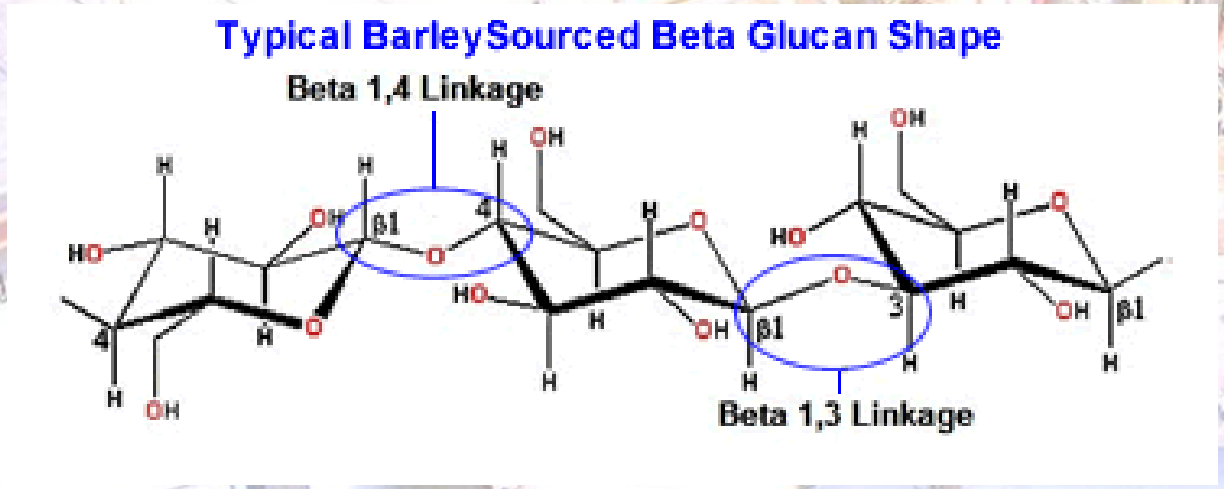
β-Glucans

- β-glucans consist of linear chains of β-D-glucopyranose units of various lenghts
- The chains are unsubtituted and consist of a mixture of β-(1,4) bonds (3 to 4 glucose units) separated by a single β-(1,3) bond
- Frequency of (1,3) to (1,4) links is about 3 to 7



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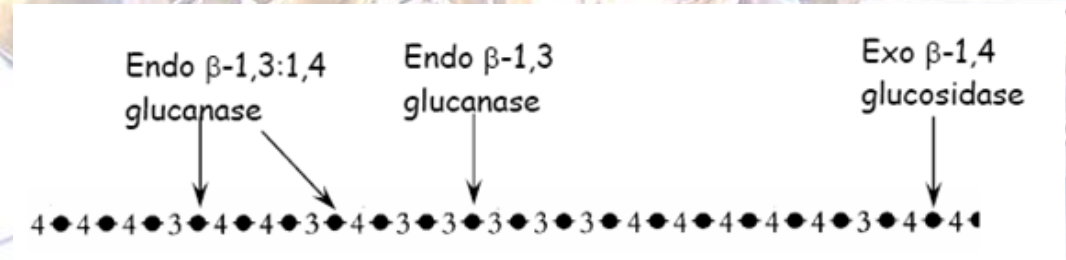
Enzymatic Degradation of β-glucan

- Endo β-1,3:1,4 glucanase – hydrolyses β-1,4 links adjacent to β-1,3 links
- Endo β-1,3 glucanase - hydrolyses β-1,3 links adjacent to β-1,3 links
- Endo β-1,4 glucanase - hydrolyses β-1,4 links adjacent to β-1,4 links
- Exo-glucanase – broad specificity
- β–glucosidase – hydrolyzing β–oligosaccharides



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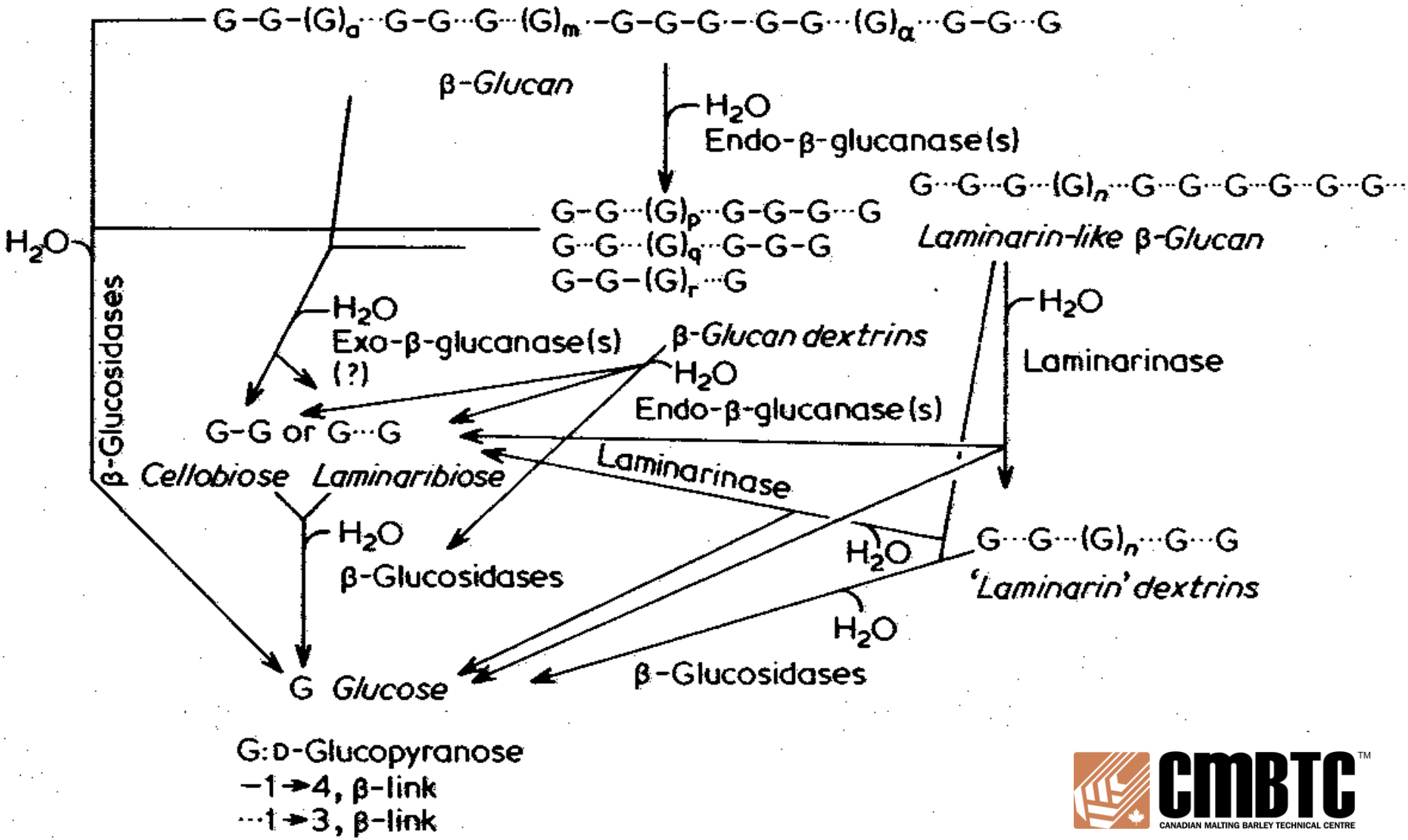
Enzymatic Degradation of Beta-glucan



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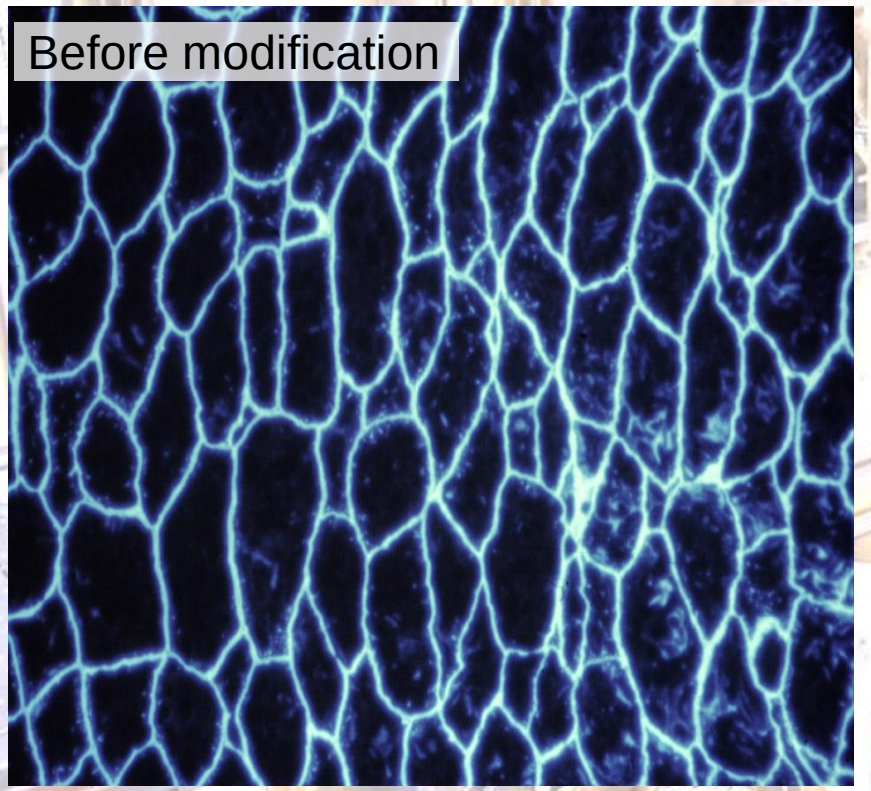
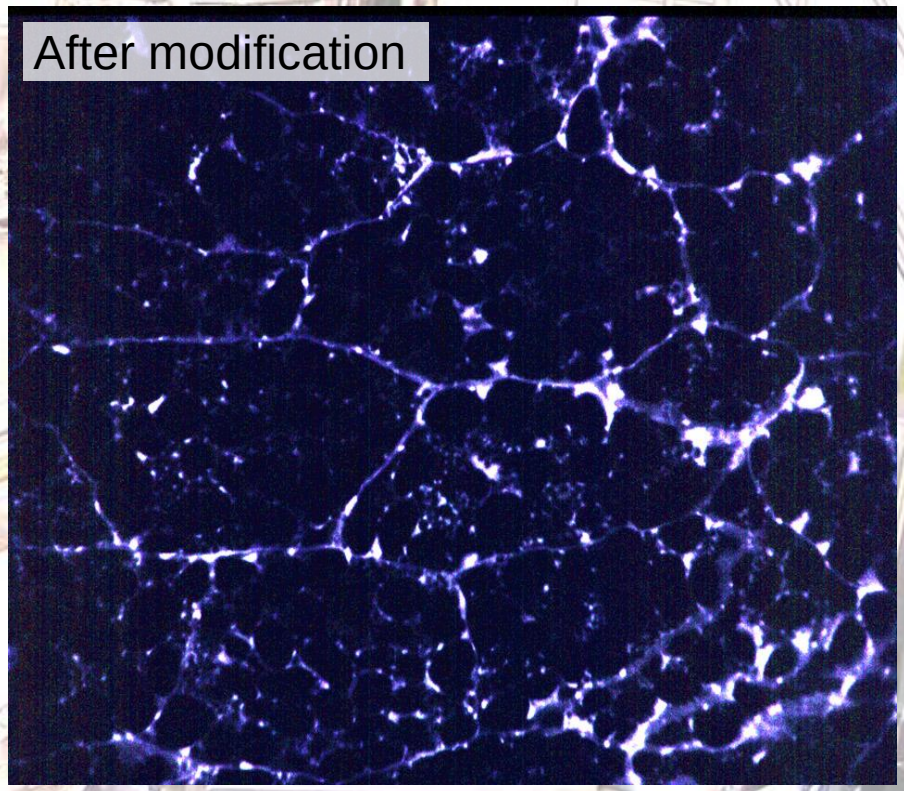
Breakdown of Barley β-glucan





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Impact of b-glucans and arabinoxylans

- NSP have been implicated in numerous processing problems (reduced lautering rates, low recovery of malt extracts, poor filterability of wort and beer, formation of gels, hazes and sediments in beer)
- AX received little attention in brewing literature
- AX contribute to beer foam stability and produce viscous aqueous solutions causing production problems
- NSP impact directly correlated to concentration, molecular weight and structure (all affected by enzyme activity)
- Synergetic role of b-glucans and arabinoxylans should be studied more closely

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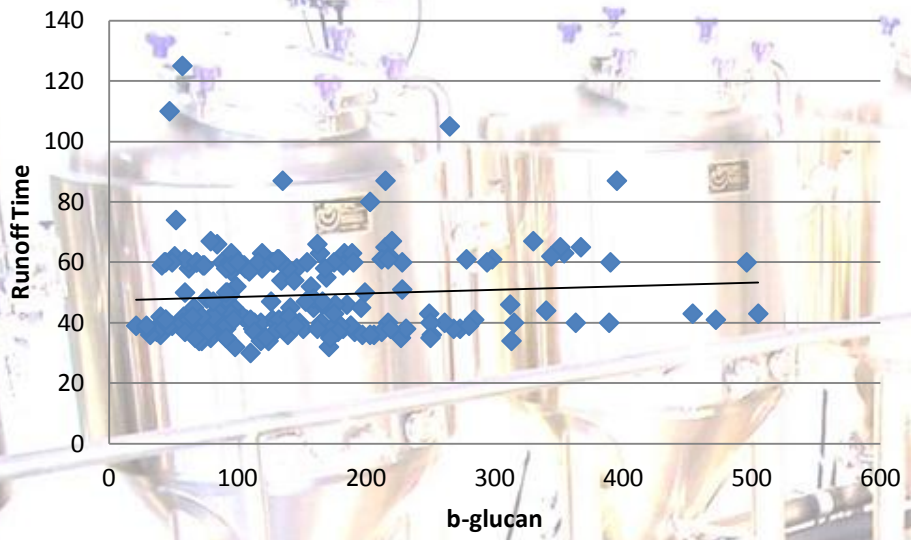
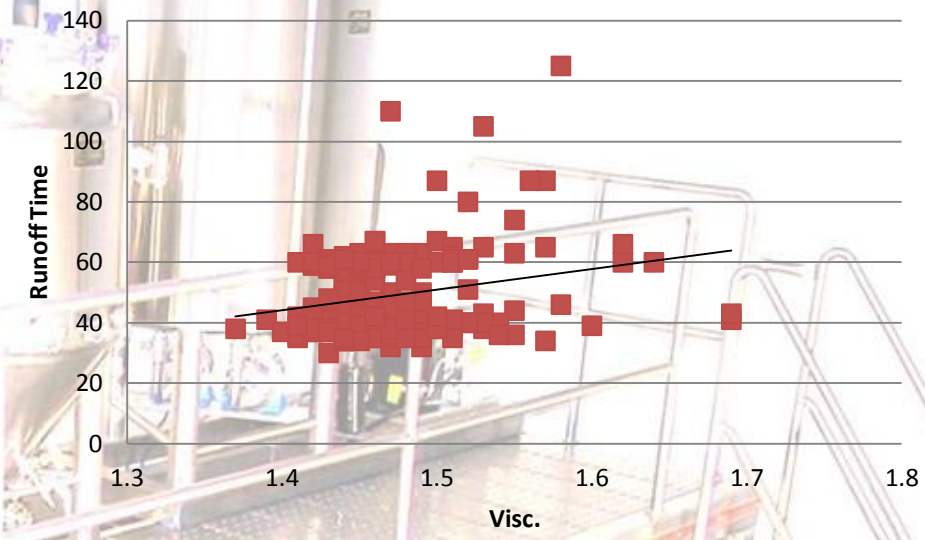
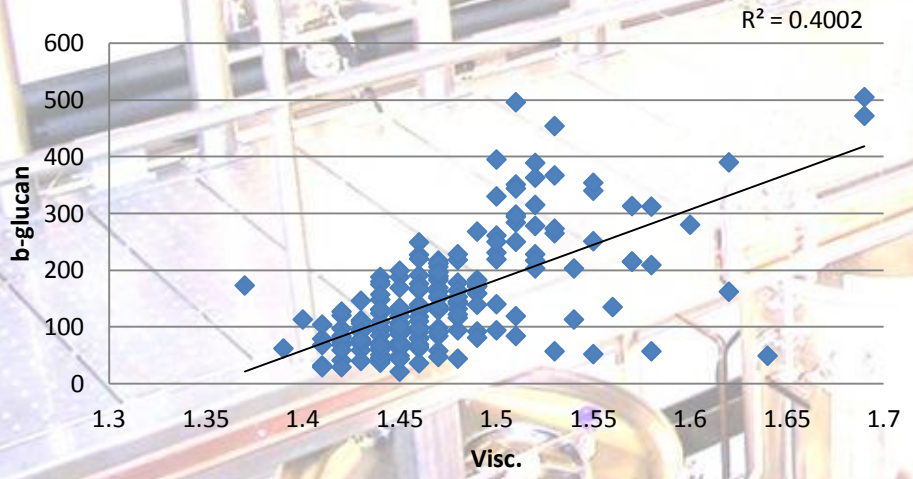




30 Varieties



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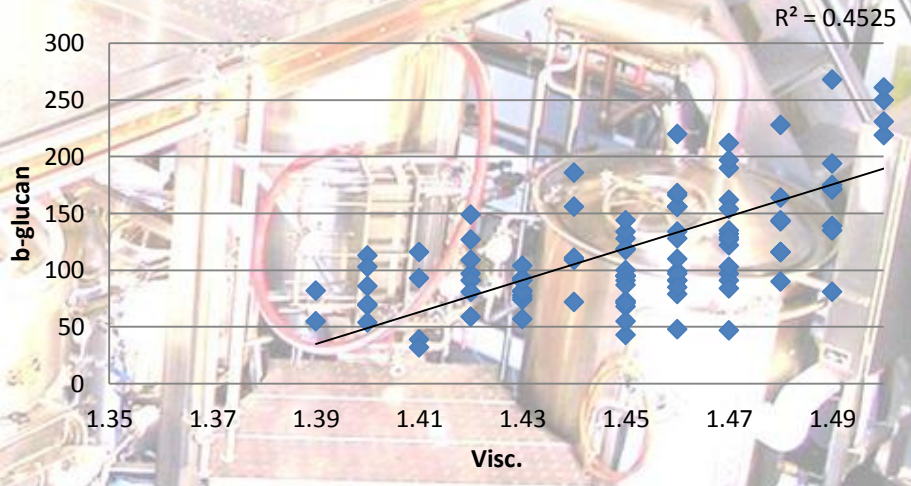

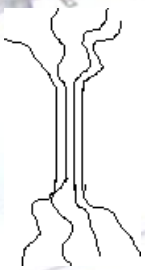

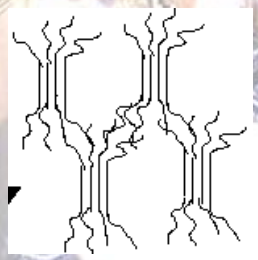

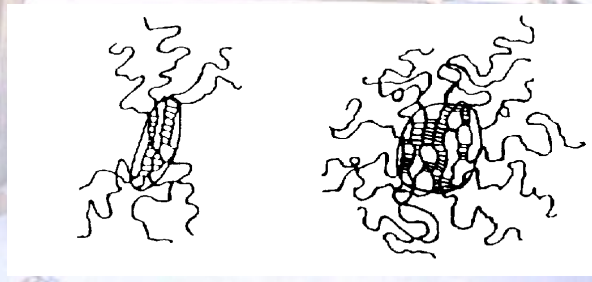

Protein Biochemistry

- Proteins represent 10-14% of the barley or malt kernel
- They can be:
  - Storage (hordeins and globulins)
  - Structural
  - Enzymes
- Only about 35 – 45% of the malt protein is solubilised

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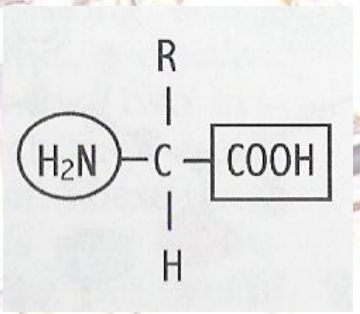
Protein Structure



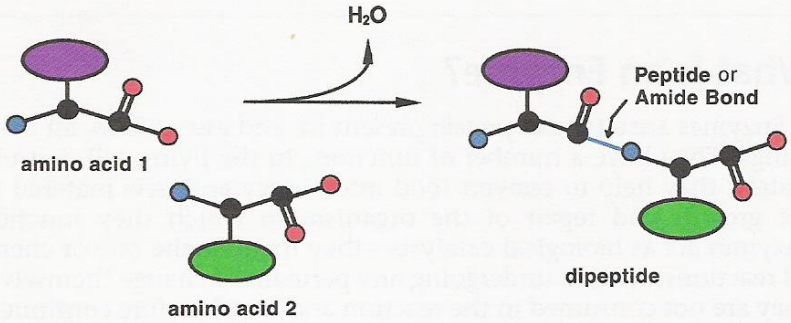


Fig. 1-1. Formation of a dipeptide from two amino acids joined with a peptide bond. Blue = nitrogen atom, black = carbon atom, red = oxygen atom.

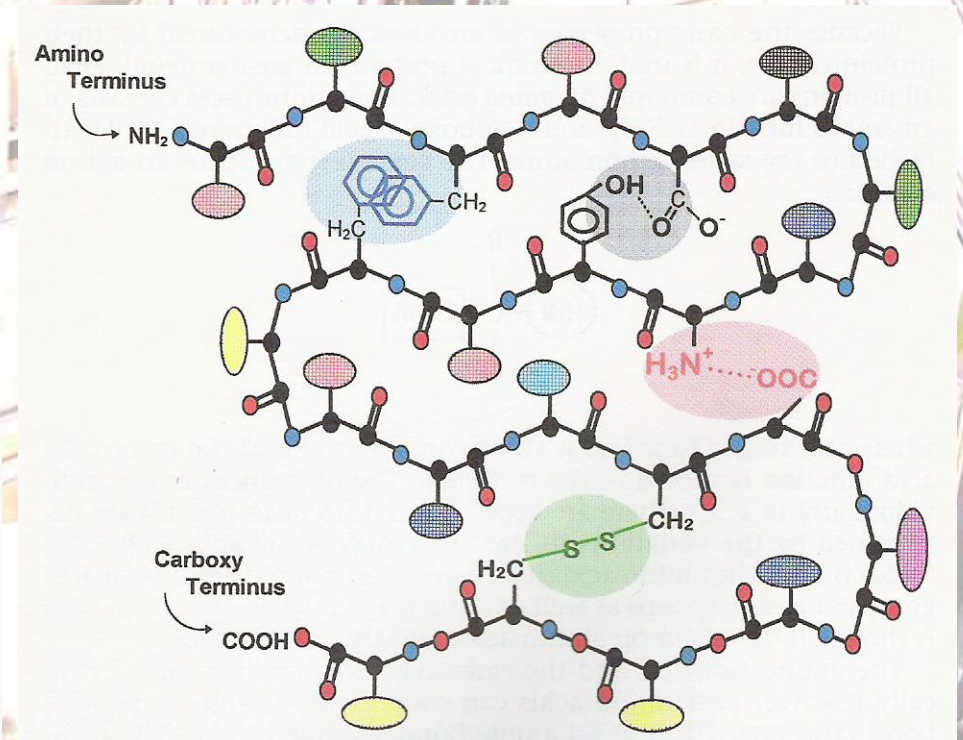


Fig. 1-2. A protein polymer composed of a linear combination of amino acids. Forces holding the protein in proper conformation include hydrophobic interaction (blue), disulfide bonds (green), hydrogen bonds (gray), and electrostatic interaction (red).

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Protein Structure

- Primary: refers to amino acid sequence
- Secondary: refers to local arrangement of polypeptides in 3D space (α-helix)
- Tertiary: refers to arrangement of entire polypeptide chain in space
- Quaternary: non-covalent association of subunits of protein

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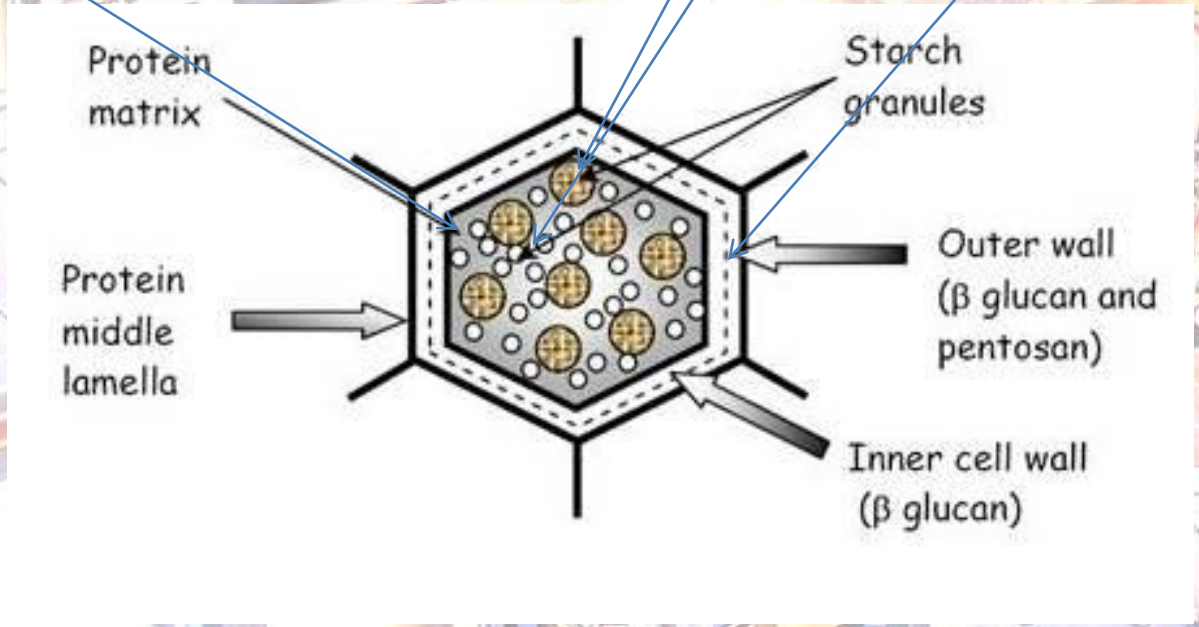

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Peptidases

- Barley proteins are initially solubilized by endopeptidases and further degraded by exopeptidases
- Exo-peptidases catalyze protein hydrolysis from the terminal amino acid
  - Aminopetidases not relevant in mashing (activities in basic pH range)
  - Carboxypeptidases are termotolerant (more than endo-petidases) and present in abundance

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Peptidases

- More than 40 endo-petidases have been identified in malt (classified as: cysteine-, metallo-, aspartic- and serine petidases)
- The efficacies of protein solubilisation

  cysteine ≈ metallo > aspartic > serine ≈ 0
- Cysteine and metallo-petidases predominant role in releasing SP
- Aspartic- petidases significant part in protein solubilisation
- Serine- petidases play no role in increasing SP levels
- All enzyme classes affect FAN formation

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Peptidases

- At the commercial mashing pH about 1/3 (32%) of SP in wort comes from ungerminated barley, about 1/2 (46%) is solubilised during malting and the rest is released during mashing
- Protein hydrolysis is mostly a function of proteolytic activity during malting
- Endogenous inhibitors are the main reason for limited proteolytic activity during mashing

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Types of Protein Material in Wort

- **PROTEINS**
  - Large molecules with unique identity
  - Lots of surplus protein is left behind in spent grain
  - Some soluble proteins play essential role as enzymes
- **POLYPEPTIDES**
  - Long chain sequences of relatively high molecular weight
  - Hydrophobic – make up beer foam
  - Acidic – can form hot and cold break with polyphenols, and if not removed can cause colloidal instability in beer
  - Contribute to texture and mouthfeel in beer

Types of Protein Material in Wort

- **PEPTIDES**
  - Short chain sequences of amino acids (2 – 10 units long)
  - Minor effect on body and mouthfeel of beer
- **AMINO ACIDS**
  - Make up 10 – 15% of TSN
  - Essential source of nutrients for yeast growth
  - Role in producing flavour active compounds in beer

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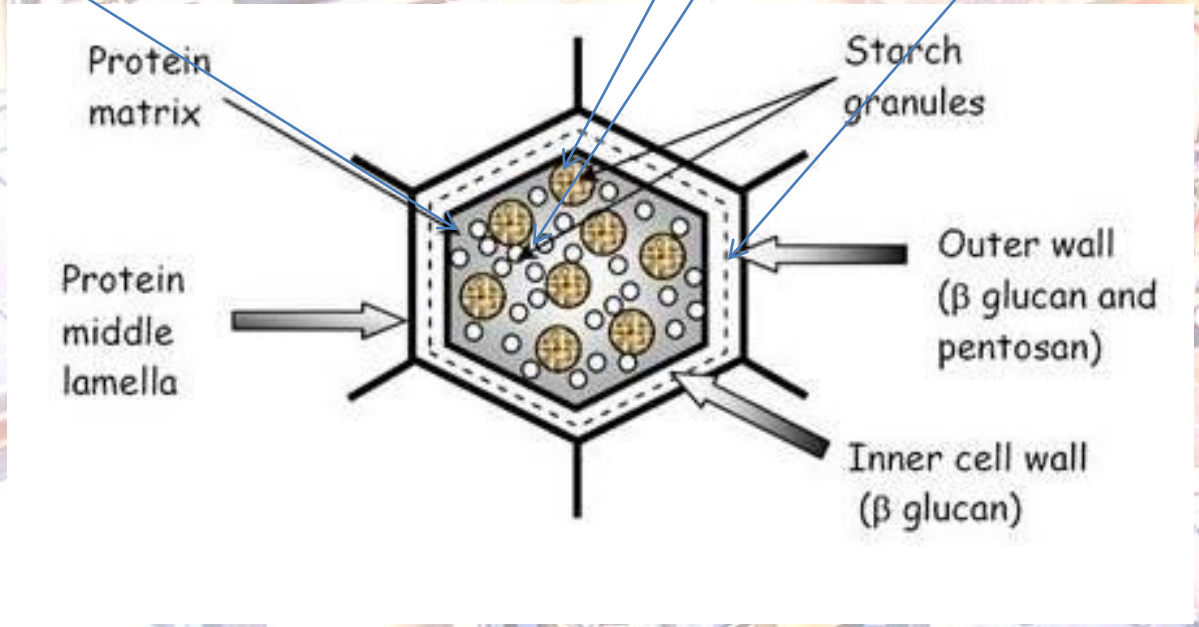

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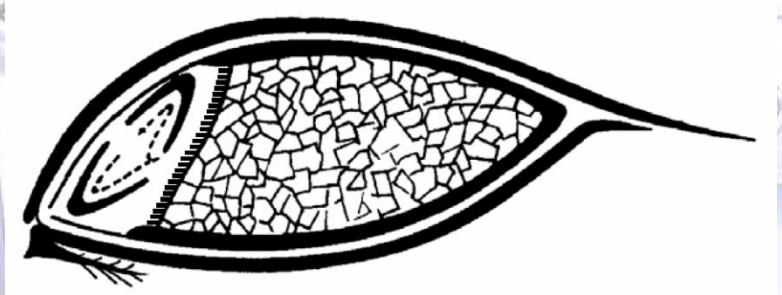
Starches

- Starch is produced by all green plants as an energy store
- It is the major component of barley grain (up to 65%)
- It provides the major part of the “brewers” extract:
  - Soluble sugars formed during malting,
  - Sugars and dextrins arising from hydrolysis during mashing
- Starch molecules are found in starch granules within the endosperm cells



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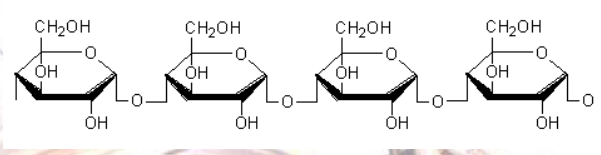
Starch Structure

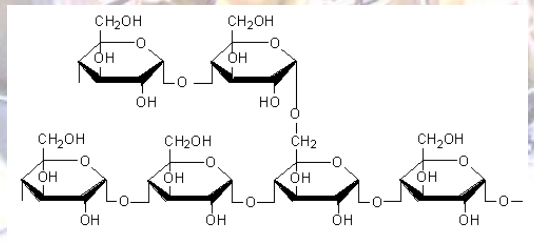
- Starch is a polysaccharide carbohydrate consisting of large number of glucose (monosaccharide) units
- The cereal starch is in granular form with two types of polysaccharide molecules: amylose and amylopectin
- Amylose makes up 22 – 26% of the polysaccharide
- Amylopectin the balance (74 – 78%)

non-reducing end



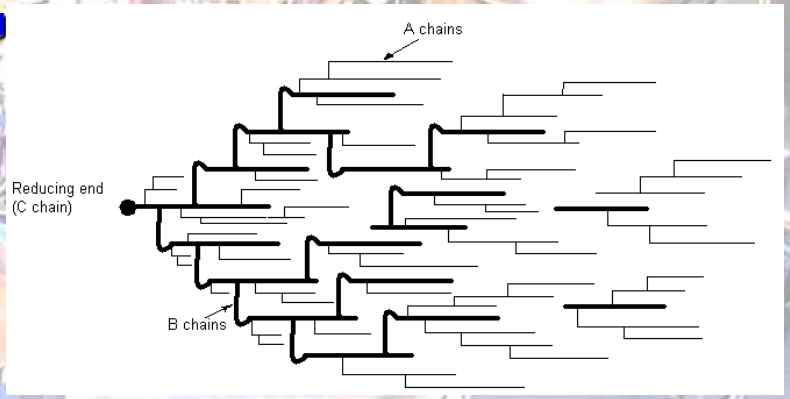
reducing end

non-reducing end



non-reducing end

reducing end



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Crystalline and Amorphous regions

- Both amylose and some A and B exterior amylopectin chains can form double helices (entwine)
- This promotes the formation of crystalline structures
- The degree of crystallinity influences the packing of helices within the molecule, which also influences the starch granule structure
- Barley is associated with 20-24% crystallinity
- Branching points constitute amorphous regions within the granules

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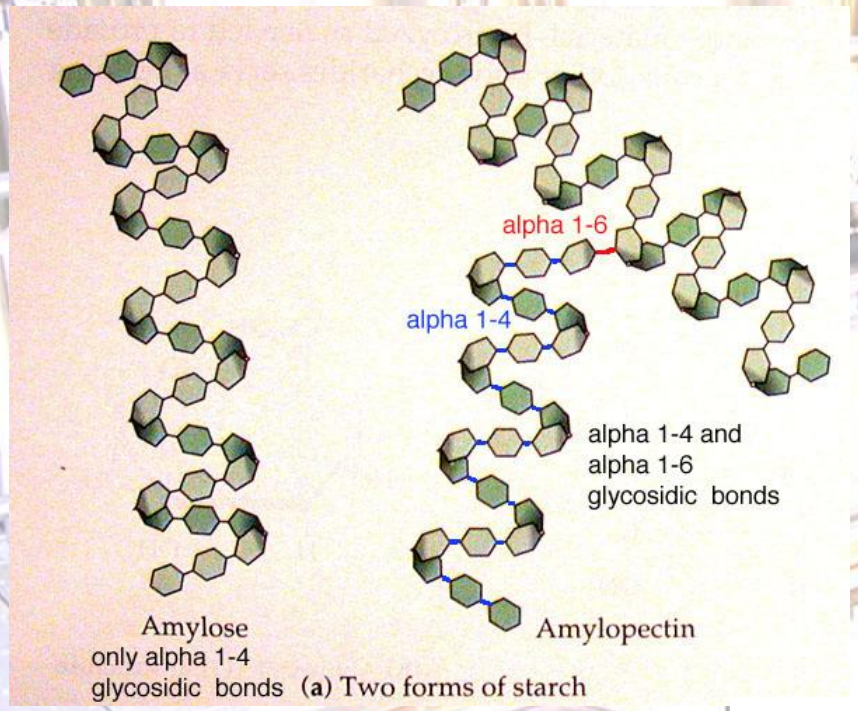
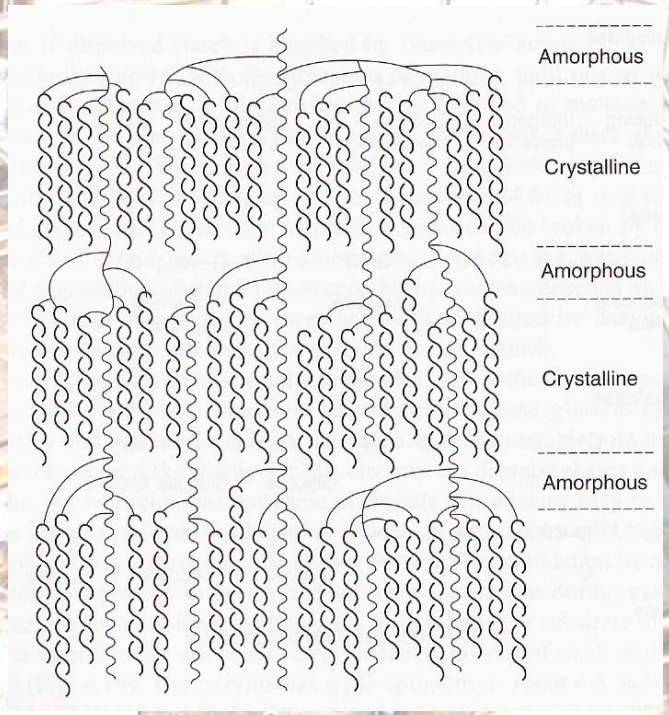
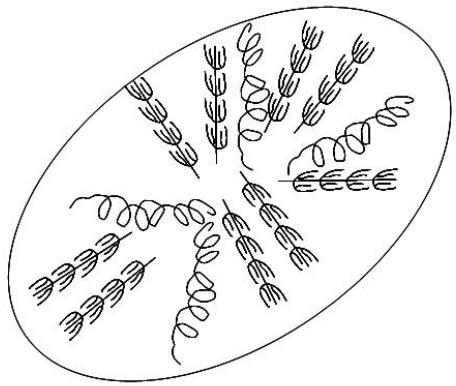
Visual Representation







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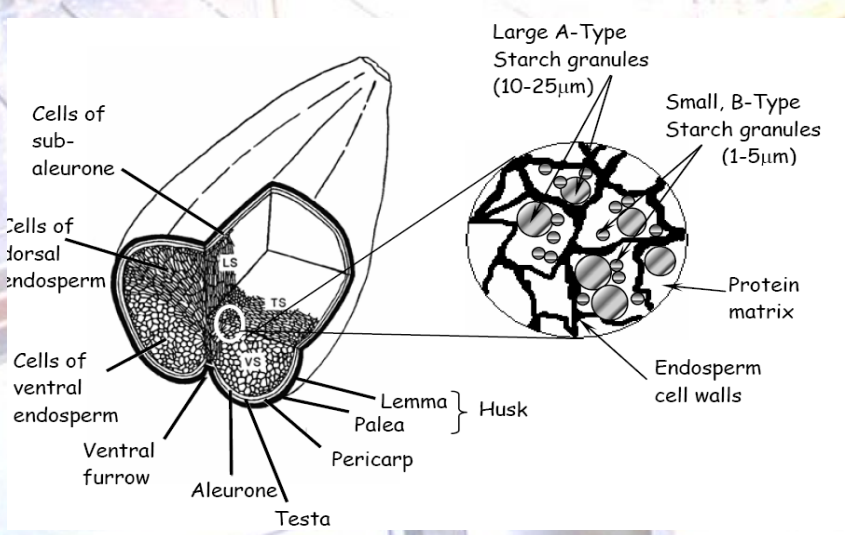
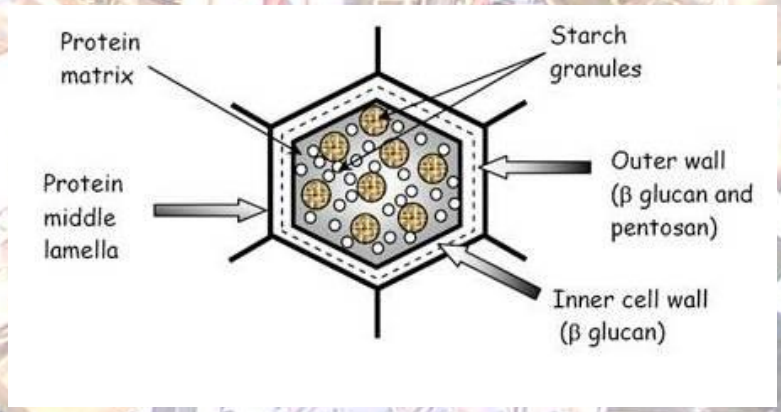
Barley type A and B starch granules

- Larger Type A granules (10-25µm) make up 10-20% of the granules by number but 85-90% by weight
- Smaller Type B granules (1-5µm) have higher gelatinization temperature
- During malting only 10% of starch is hydrolyzed (mostly Type B – greater surface to volume ratio)





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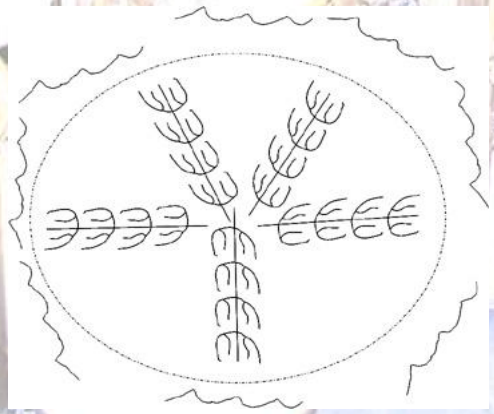
Starch gelatinization temperature

Source	Gelatinisation Temperature (°C)	Shape	Granule Size (µm)	
			B Type	A Type
Barley	61-62	Round/ Lenticular	1-5*	10-25
Wheat	52-54	Round/ Lenticular	1-5	15-25
Maize	70-80	Round/ Polygonal	10-15	
Rice	70-80	Polygonal/ Compound	2-8	9-30
Sorghum	70-80	Round/ Polygonal	10-12	
Oats	55-60	Polygonal/ Compound	2-10	
Rye	60-65	Spherical/ Lenticular	1-8	10-30

* Small starch granules of barley gelatinise at 75-80°C



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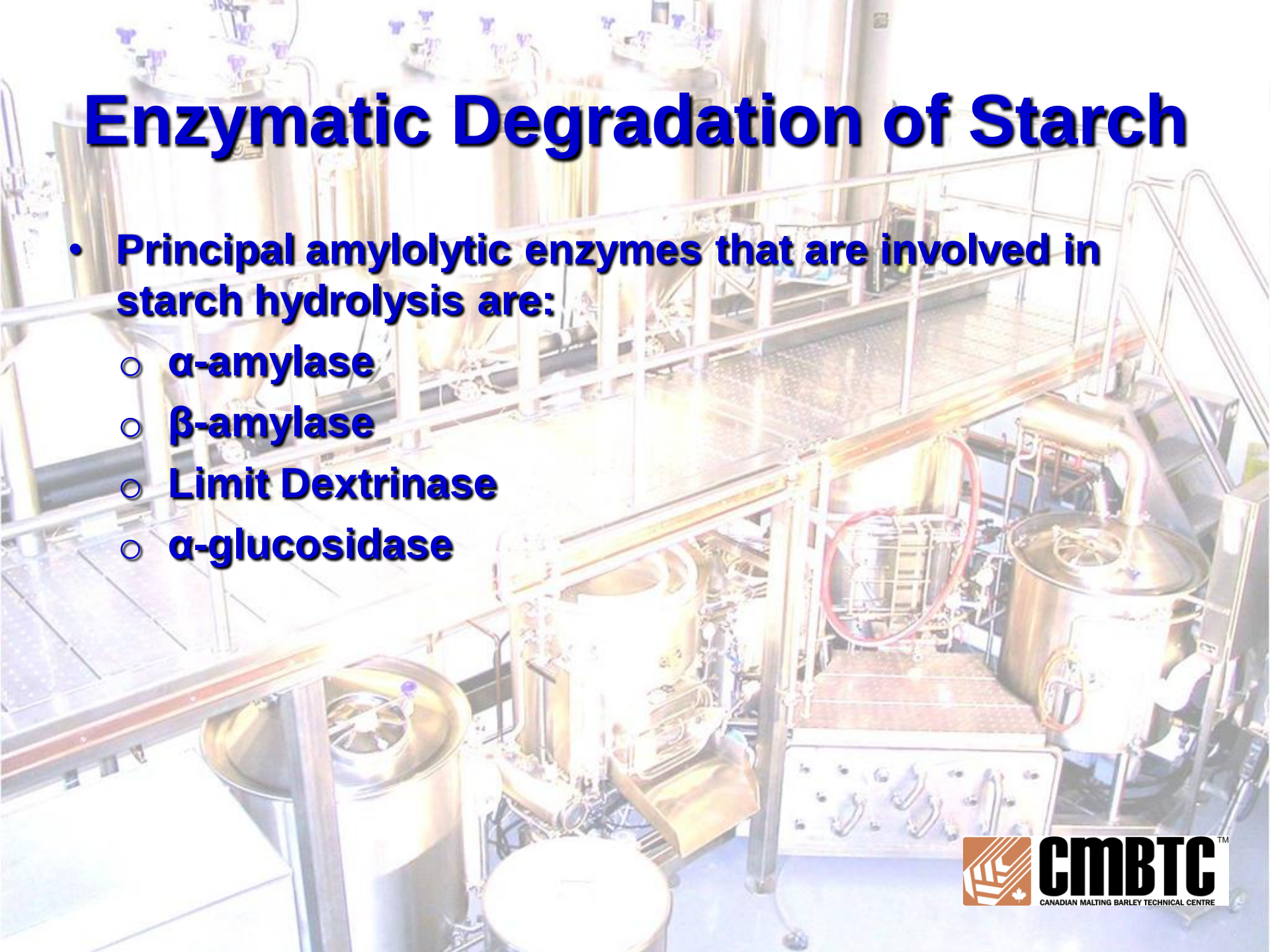
Enzymatic Degradation of Starch

- Principal amylolytic enzymes that are involved in starch hydrolysis are:
  - α-amylase
  - β-amylase
  - Limit Dextrinase
  - α-glucosidase



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α-amylase

- Malt α-amylase is an endoenzyme (working within the α-(1,6) bonds) attacking the α-(1,4) links within the starch chain producing: glucose, maltose and primarily a complex mixture of branched and unbranched oligosaccharides and dextrins
- When α-amylase is working within the α-(1,6) bonds it frees substrate (β-limit dextrin) that is then available for β-amylase to continue conversion to maltose
- Attack is slower at the chain ends and stops near α-(1,6) branch points
- Optimum pH (5.3-5.8), optimum T (70-75ºC), Inactivation T (75-80ºC) – Thermo-tolerant

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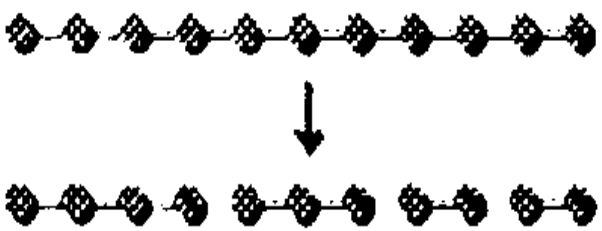
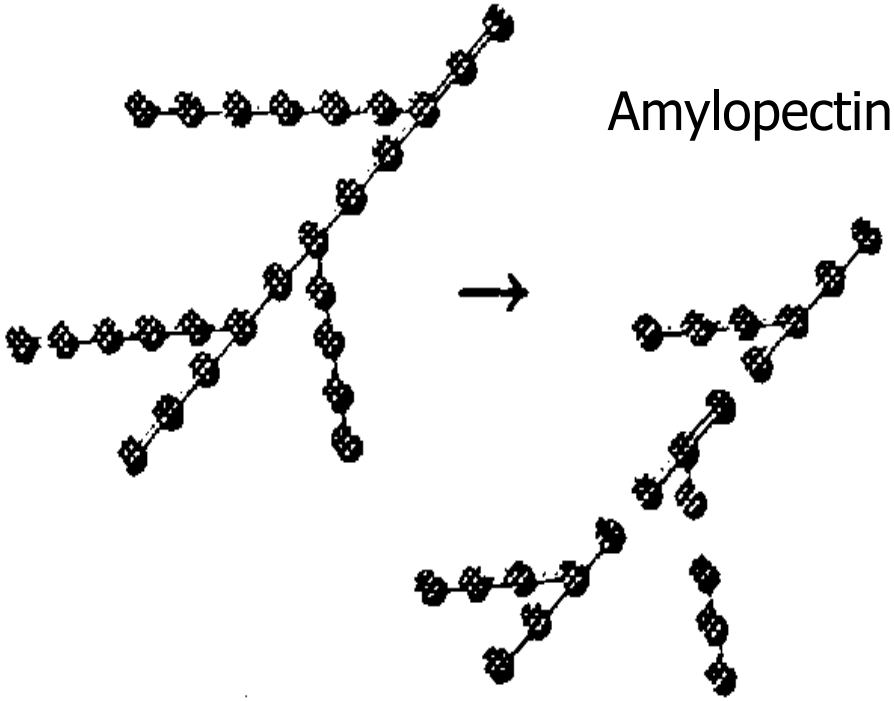
α-amylase



Amylose



Amylopectin

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α-amylase

- Extremely abundant so there is seldom a shortage
- Only in the highest adjunct grists
- Becomes limited for water only during very high gravities mashing (very low water/grist ratios)
- Inhibited by the high levels of sugars produced in such circumstances

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β-amylase

- β-amylase (present in barley) is an exoenzyme (works outside of the α-(1,6)links) attacking every second α-(1,4) links starting from non-reducing chain ends of amylose and amylopectin, producing maltose
- Enzyme does not hydrolyze α-(1,4) bonds near α-(1,6) branching points in amylopectin
- Amylopectin is degraded to maltose and β-limit dextrin where non reducing chain ends are 2 to 3 glucose residues away from branches
- Optimum pH (5.4-5.6), optimum T (63-65ºC), Inactivation T (68-70ºC)

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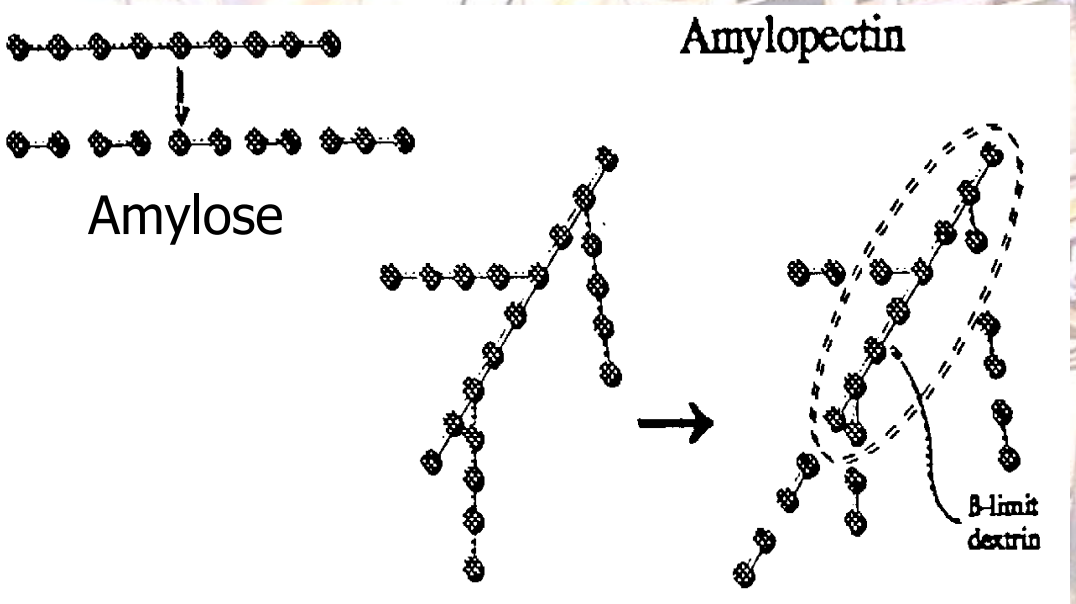
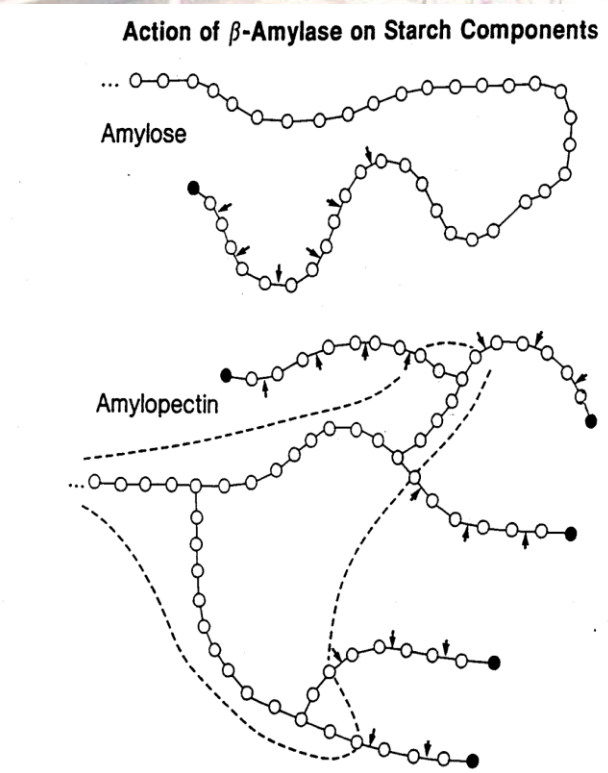
β-amylase





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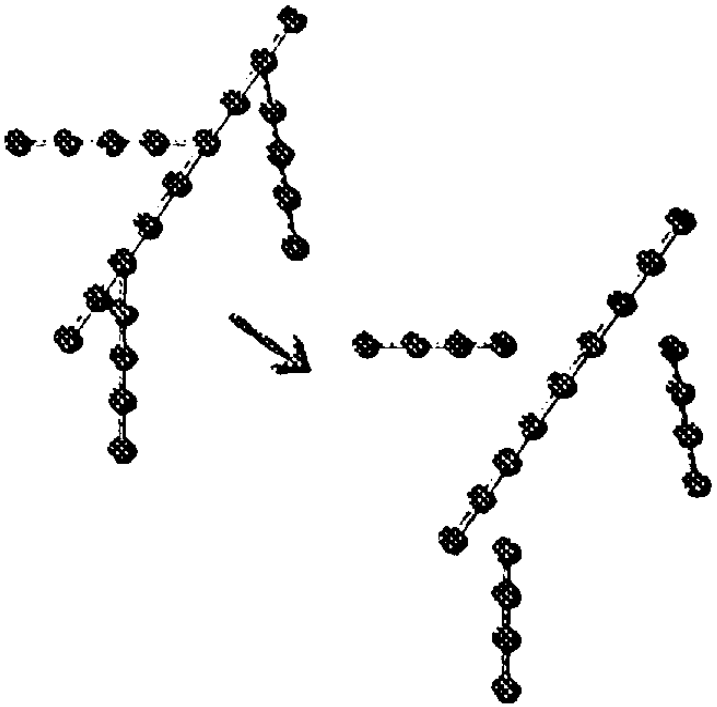
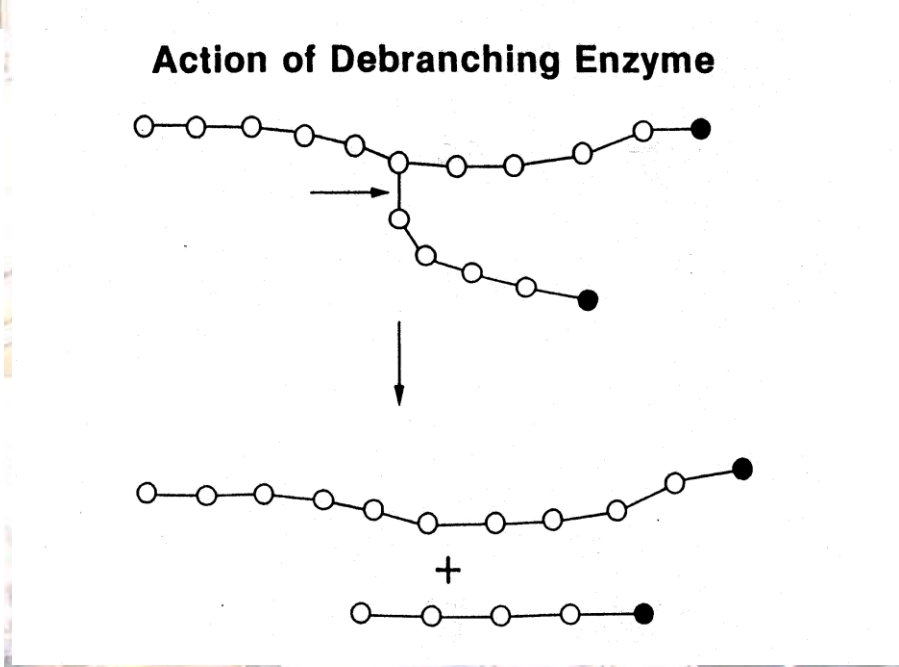
β-amylase

- β-amylase has higher affinity with higher molecular weight starch molecules
- It also has higher catalytic activity with bigger substrate molecules (converts them faster)
- Rapid action of α-amylase during mashing produces progressively more dextrins
- HMW amylose and amylopectin become limited

Limit Dextrinase

- **Limit Dextrinase is a debranching enzyme catalyzing the hydrolysis of α-1,6 –glucosidic linkages in starch**





Hydrolyzes alpha(1-6) links

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Limit Dextrinase

- Limit Dextrinase transcription (from a single gene in barley) reaches max after 5 days of germination
- It was suggested that prolonged germination would result in malt with more fermentable worts
- With lager malts with max kilning temp of 75°C enzyme survival was 75%
- With ale malts with max kilning temp of 95°C enzyme survival was 13%
- Limit Dextrinase thermostability is comparable to β-amylase

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Limit Dextrinase

- Reason for limited action during mashing is that it is in inactive bound form (79-92% latent state complexed with LMW inhibitor)
- Latent form is relatively heat stable - binding protects the enzyme which is steadily released during mashing
- Lowering the mash pH (from 5.5 to 5) significantly enhances fermentability of resulting wort
- By reducing the levels of dextrins in beer Limit Dextrinase also helps to prevent dextrin gel formation and reduces difficulties with filtration

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Limit Dextrinase

- Fermentability (%) *Full Malt* = 69.9 + 0.017*a + 9.602*b + 0.195*c + 0.007*d - 0.5375e - 0.0008d*e
  (R^2 = 0.91)
- Fermentability (%) *30% Rice adjunct* = 68.1 + 0.0188*a + 6.898*b + 0.1945*c + 0.0066*d + 0.393e - 0.0006d*e
  (R^2 = 0.82)

  where a= α-amylase,

  b=Total Limit Dextrinase,

  c= KI (%),

  d=Total β-amylase,

  e=β-amylase thermostability

Evans,D.E. et al. 2005 JASBC 63, 185-198

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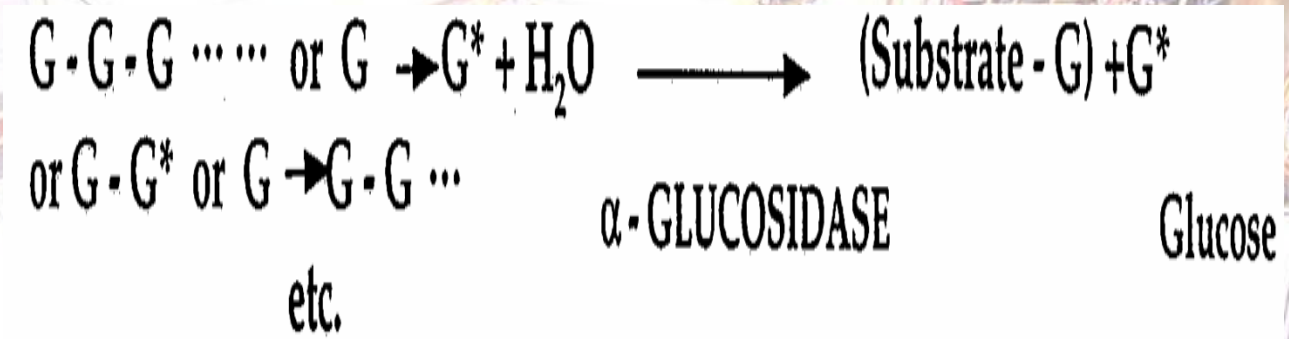
α-Glucosidase

- Hydrolyses the last α-1,4–glucosidic linkages at the nonreducing end of amylose and amylopectin, yielding glucose
- Termolabile and of limited significance during mashing
- May improve the efficiency of β-amylase by removing maltose (product inhibition)



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Other factors

Copeland	DP	a-Amyl	Con. Time
2004	119	45	11
2005	118	42	10
2006	128	42	13
2007	125	57	**23**
2008	137	52	12
2009	136	57	9
2010	112	43	16
2011	123	49	15
2012	131	61	16
2013	126	50	13.8

Barley properties

- Variety
- Environment
- Endosperm texture
- Starch granule size and gelatinisation
- Protein matrix
- Enzyme availability

Metcalfe	DP	a-Amyl	Con. Time
2004	139	58	9
2005	138	49	9
2006	142	55	12
2007	138	66	**21**
2008	147	63	10
2009	158	76	7
2010	152	64	10
2011	151	66	13
2012	158	69	17
2013	147	63	12

	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Average Protein	11.8	11.7	11.9	**12.3**	11.7	11.1	11.6	11.4	11.9	11.1
Average TKW	43.2	43.5	42.7	**39.4**	45.2	44.4	41.8	44.4	40.4	48.2

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In Haven
there is no Beer
therefore we drink it here!

Thank You!