WORLD BREWING CONGRESS
August 13–17, 2016 • Denver, Colorado, U.S.A.
#ElevateBeer

The comparison of DP enzyme release and persistence with the production of yeast fermentable sugars during a modified IoB 65^{o}C & the Congress mashes

Dr Evan Evans
The Tassie Beer Dr
Tasmania, Australia

Dr Glen Fox
University of Queensland
Queensland, Australia

The key enzymes hydrolysing starch into fermentable sugars during mashing

1. α-amylase – relatively high mash thermostability?
2. β-amylase – relatively low mash thermostability?
3. limit dextrinase – relatively low mash thermostability?

While α-amylase is fully soluble (free),

β-amylase & limit dextrinase have:

- Free, bound and latent forms

Mashing protocol comparison summary

Mashing characteristic	"Commercial"	"Congress"	"Modified IoB"
Malt milling	"6 roller mill"	0.2mm disc	0.7mm disc
Mash temperature	65°C	45-70°C	65°C
Grist:Water ratio	1:3	1:4 then 1:6	1:3 then 1:6
Calcium addition	Yes ~2.0mM	No	Yes, 3.0mM
Saccharification time	variable	50-60min	60min
Final mash temperature	78-80°C	70°C	78°C
Lautering Temperature	Hot lauter	Cool "lauter"	Cool "lauter"
Wort boiling	Trub removed	Trub remains	Trub remains
Wort gravity	>10°P	~8°P	~8°P
Residual gravity after fermentation	Av 2.11°P Range 1.19-4.22°P	0.7-1.3°P	0.5-1.2°P

The mechanics of starch hydrolysis during mashing?

1. Know your substrate

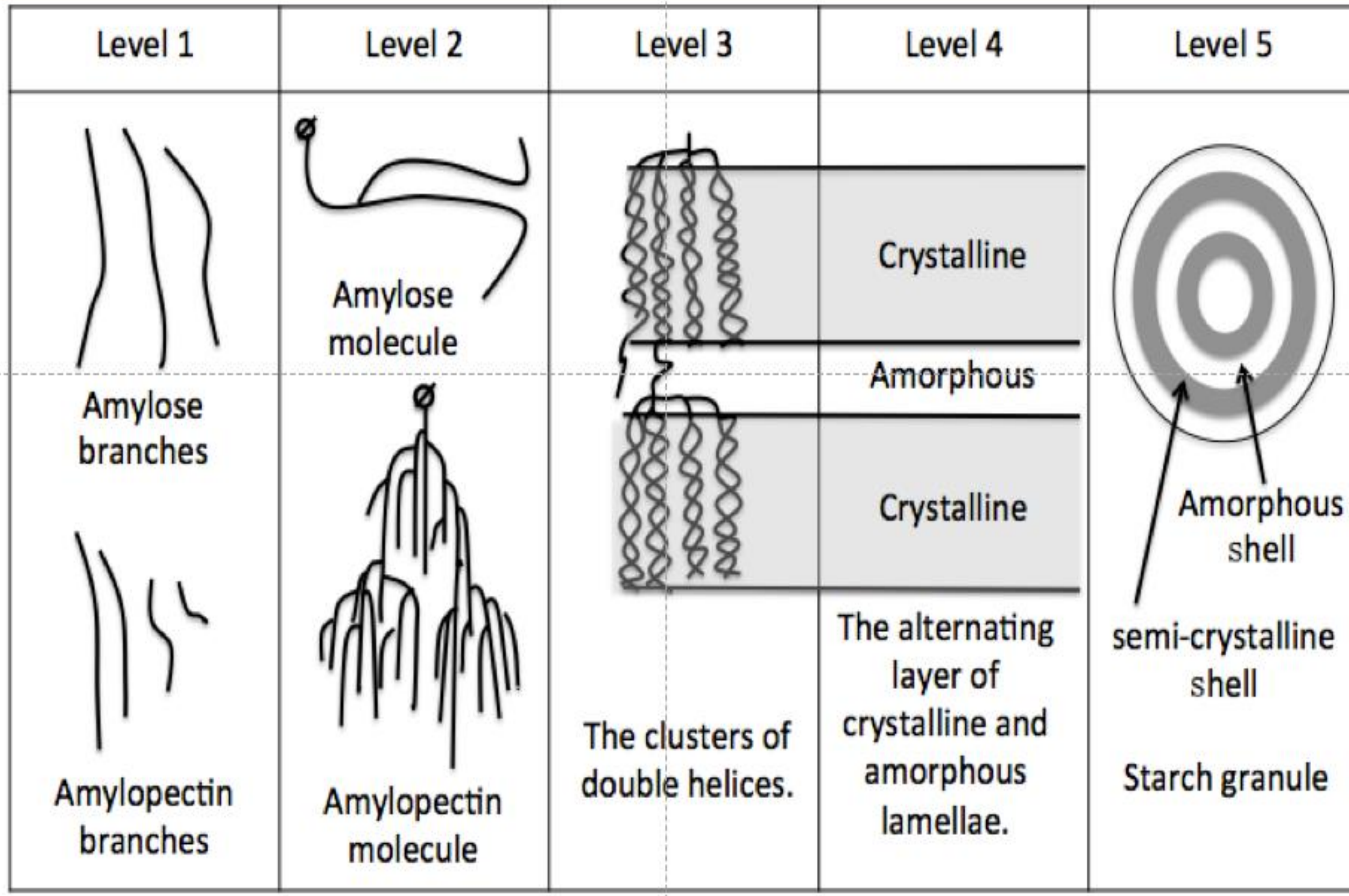
and

2. Know your enzymes

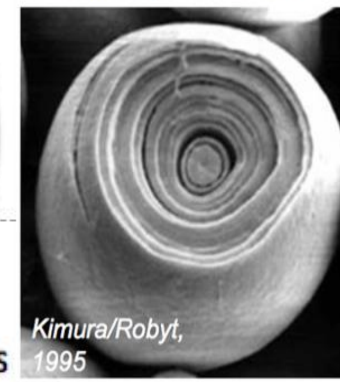
Hierarchical levels of starch structure in cereal grain

THE UNIVERSITY OF QUEENSLAND AUSTRALIA | QAAFI Queensland Alliance for Agriculture and Food Innovation

Centre for Nutrition & Food Sciences
Provided by Dr Glen Fox



Kimura & Robyt 1995, Carb Poly 277: 87-107



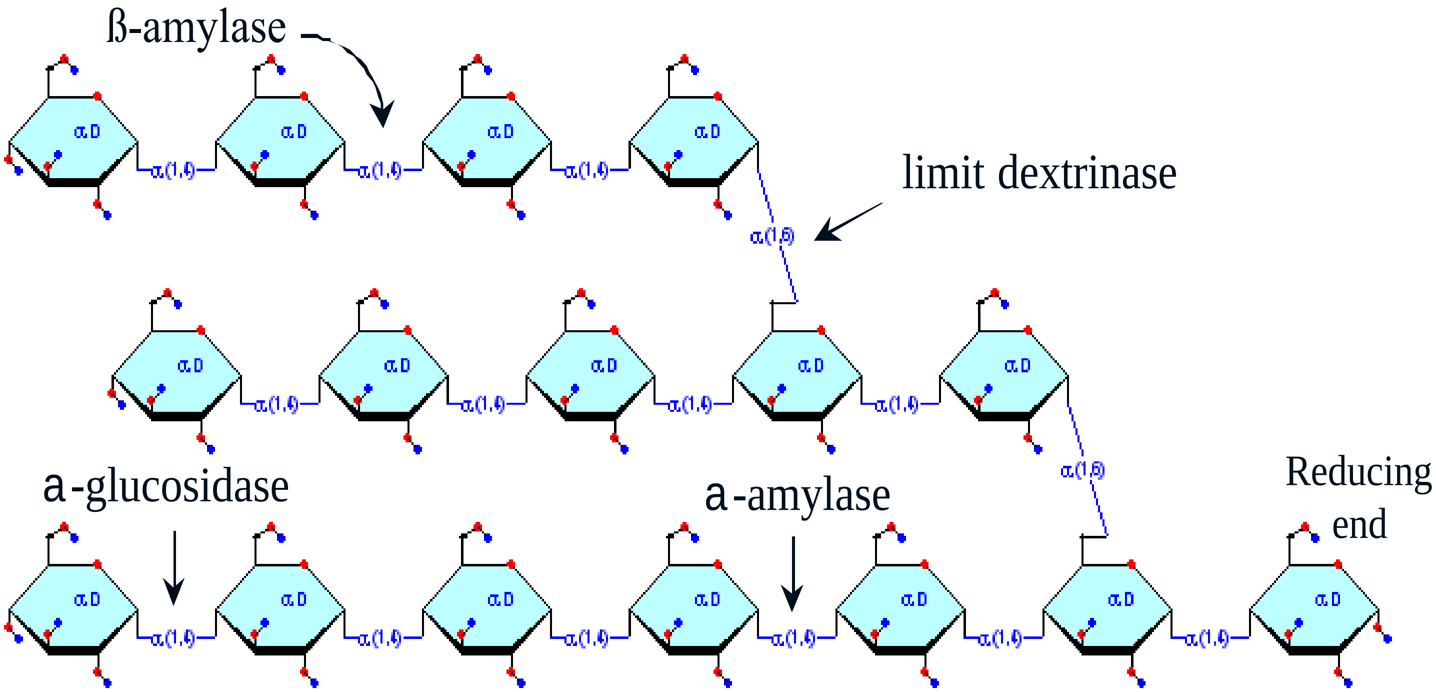
Typical gelatinization temperatures (Tp) of cereal starches

Origin of Starch	Gelatinization Temp range (°C)
Barley malt	**62-67**
Barley	60-62
Barley - small granules	51-92
large granules	60-65
Wheat	52-66
Rice	**61-82**
Rice: short grain	**65-68**
Rice: long grain	**71-74**
Maize	62-77
Sorghum	69-75

(Briggs et al., Brewing: science and practice, 2004).

Gelatinized starch degraded most efficiently by the DP enzymes

Starch is degraded by the four diastase enzymes

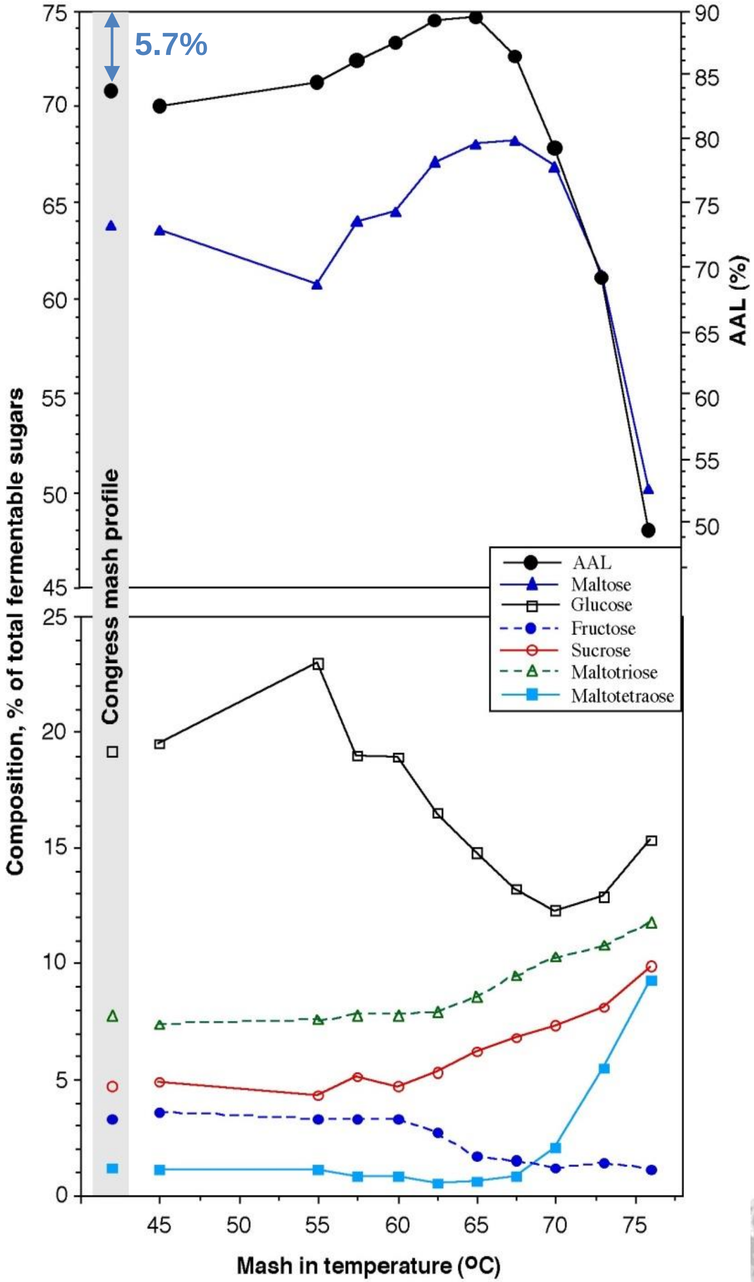


Composition of fermentable extract produced by a 65^{o}C, modified Congress mash protocol, (wort gravity ~8-9 ^{o}P)

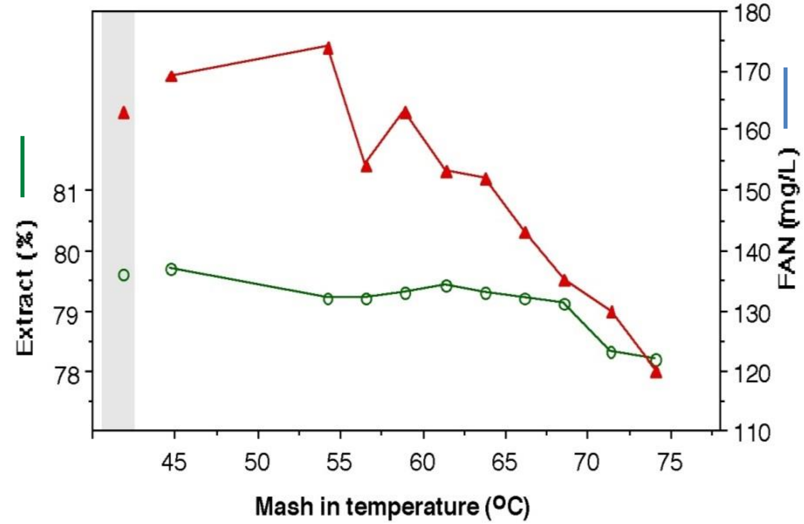
(Evans et al., 2005, J Am Soc Brew Chem 63: 185-198).

Sugars	Mean level (mmole/L)	Fermentable extract (%) mean	Fermentable extract (%) range
Glucose	**34**	**16.9**	**12.5-22.9**
Fructose	4	1.9	1.4-3.1
Sucrose	11	5.3	1.5-6.6
Maltose	**138**	**67.6**	**61.0-70.5**
Maltotriose*	16	7.9	5.1-9.5
Maltotetrose**	1	0.5	0.0-2.0
Total	204	100.1	

*** Partially fermentable depending on yeast strain, ** poorly fermentable sugar**



The influence of mash in temperature on AAL, FAN and sugar composition



<u>Flavor implications</u>

- Acetate esters increase as wort maltose level decreases

See: Evans, E, Collins, H, Eglinton, J, & Wilhelmson A (2005). *J. Am. Soc. Brew. Chem., 63: 185-198.*

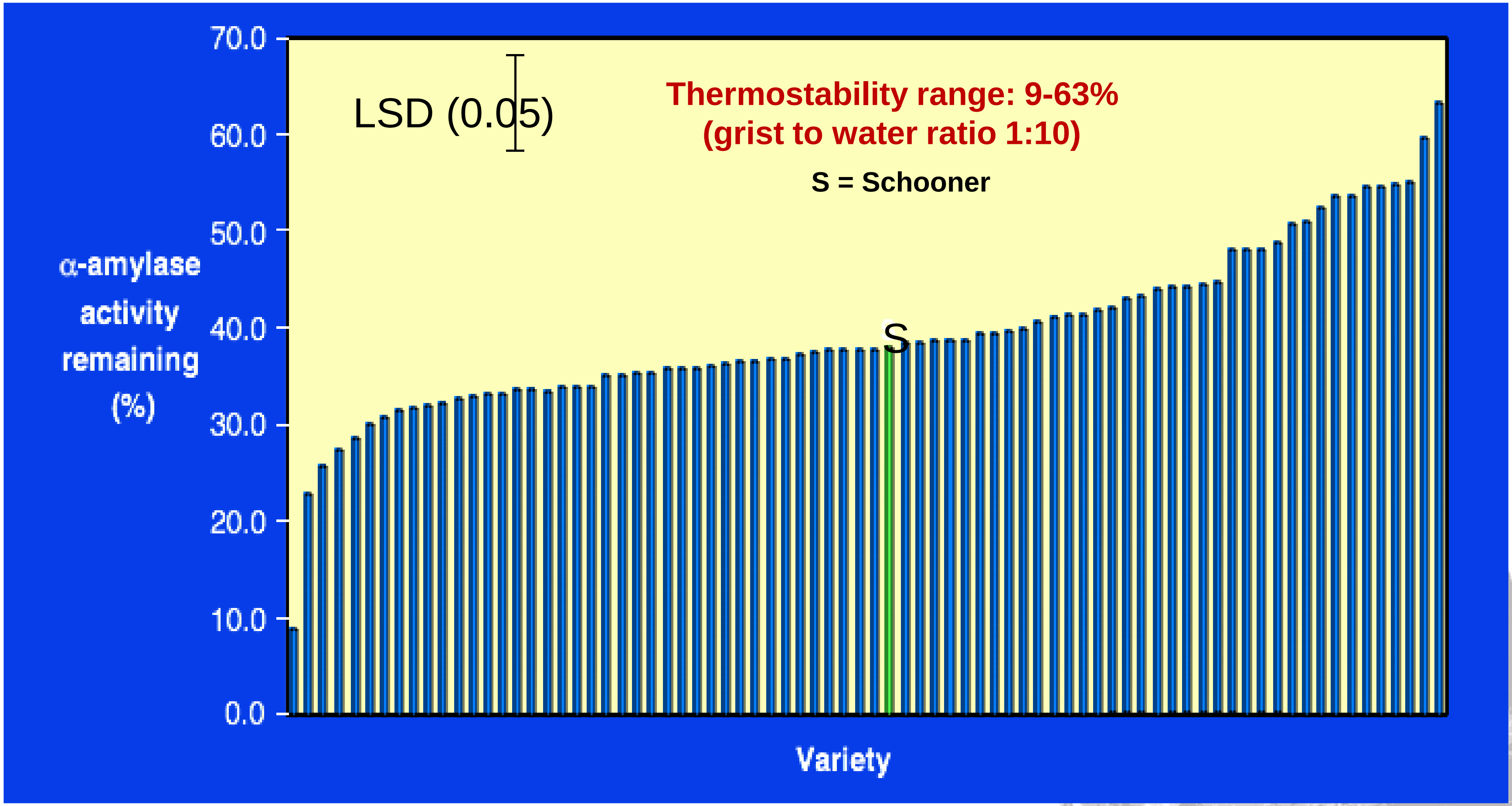
Prediction of Fermentability & Biological Meaning

Useful parameters for 80-90% prediction :

- α-Amylase: Primary starch attack to produce substrates for β-amylase and limit dextrinase & fermentable sugars.
- Limit dextrinase: α-1,6-glycosidic bond cleavage to produce substrates for β-amylase and fermentable sugars.
- β-Amylase: determinant of maltose production.
- β-A thermostability: maintain β-amylase activity.
- KI: enzyme protection & access of enzymes to starch.
- Starch (RVA): gelatinization temp, enzyme access to starch substrate.

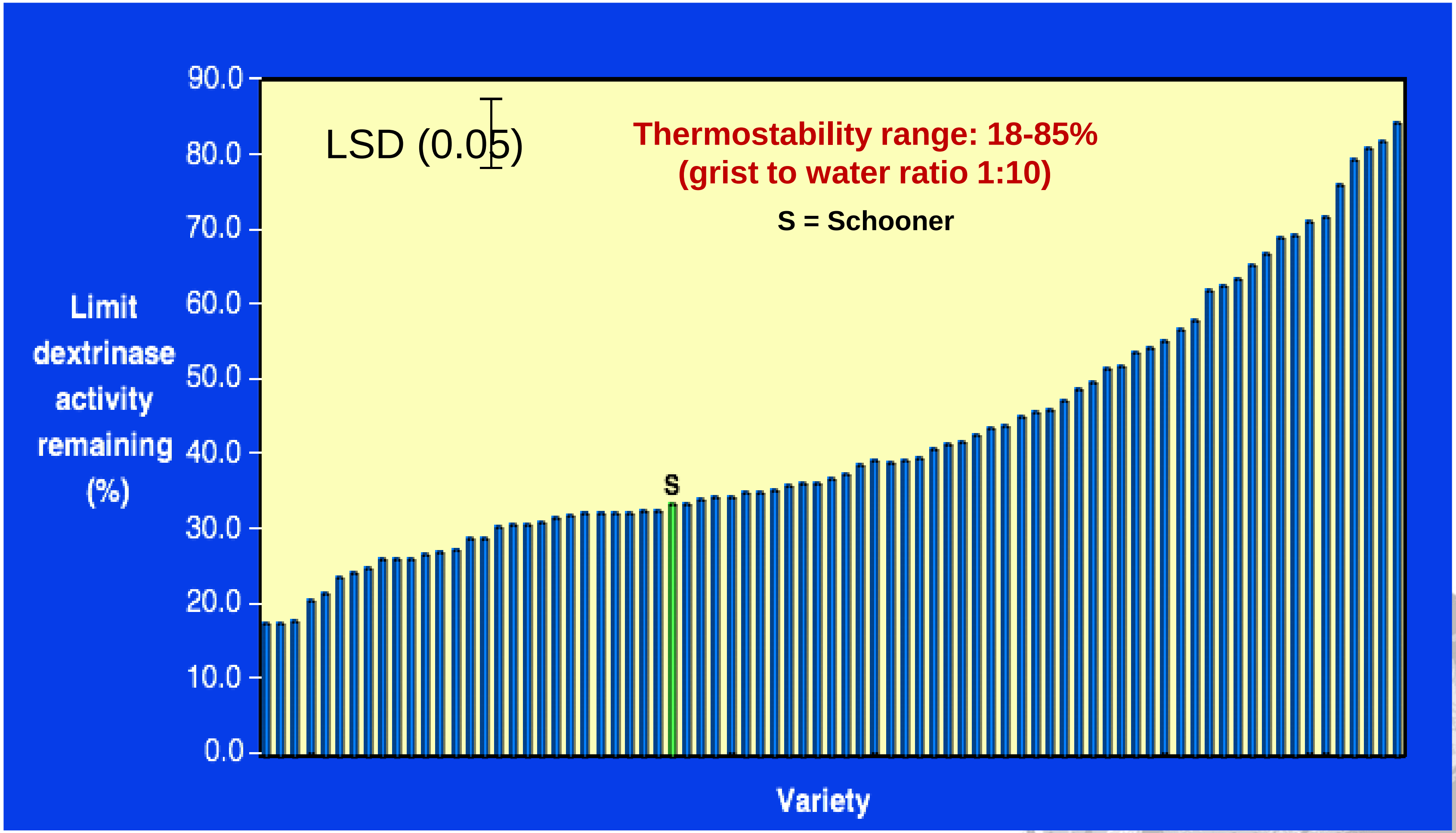
The relative thermostability of the DP enzymes

Remaining α-amylase activity from malt extracts from 78 varieties incubated at 72.5^{o}C for 10min



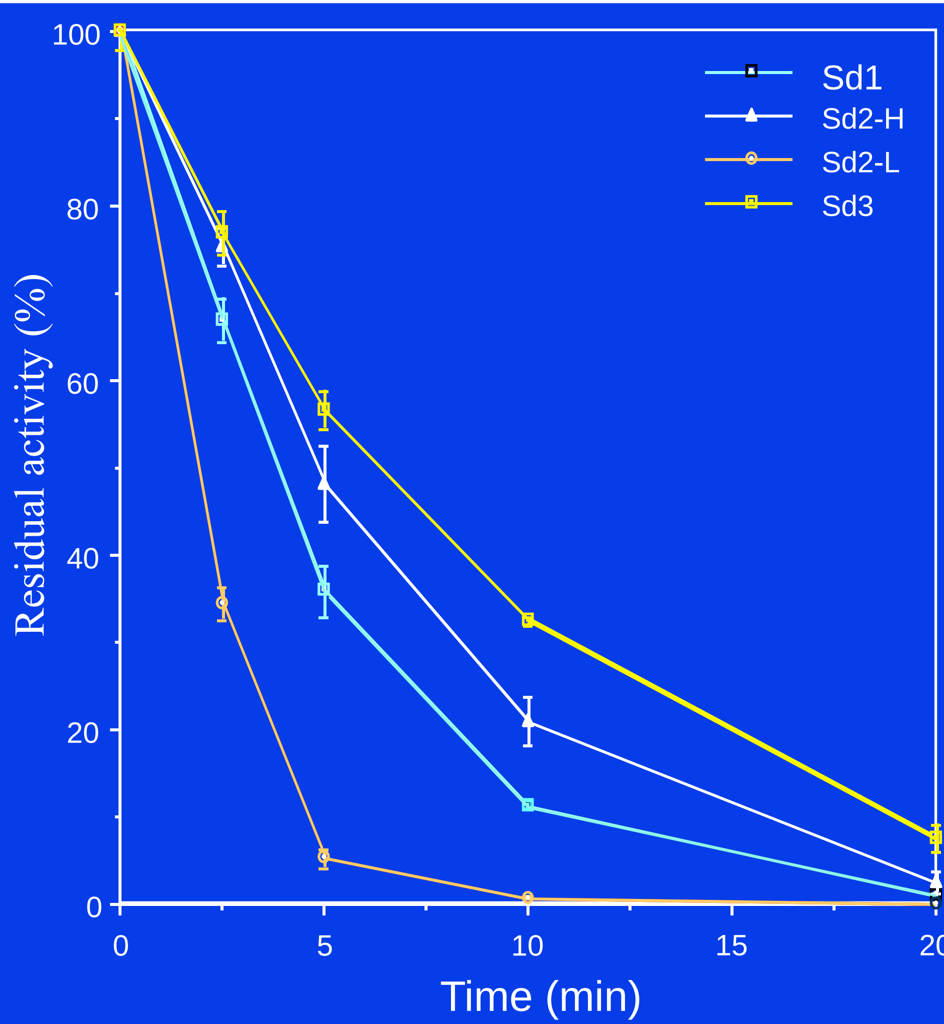
See Evans, van Wegen, Ma, Eglinton (2003) J. Am. Soc. Brew. Chem, 61: 210-218

Remaining limit dextrinase activity from malt extracts from 78 varieties incubated at 57.5°C for 15min



See Evans, van Wegen, Ma, Eglinton (2003) J. Am. Soc. Brew. Chem, 61: 210-218

Relative Rates of Irreversible Thermal Inactivation of β-amylase in Barley Extracts at 60°C (grist :water 1:10)



Sd2L: low β-amylase thermostability ie Schooner, Stirling, Clipper

Sd1: intermediate β-amylase thermostability, ie Baudin, Gairdner AC Metcalfe, Harrington, Morex

Sd2H: high β-amylase thermostability, ie Buloke, Flagship, Haruna nijo, Latrobe Hindmarsh.

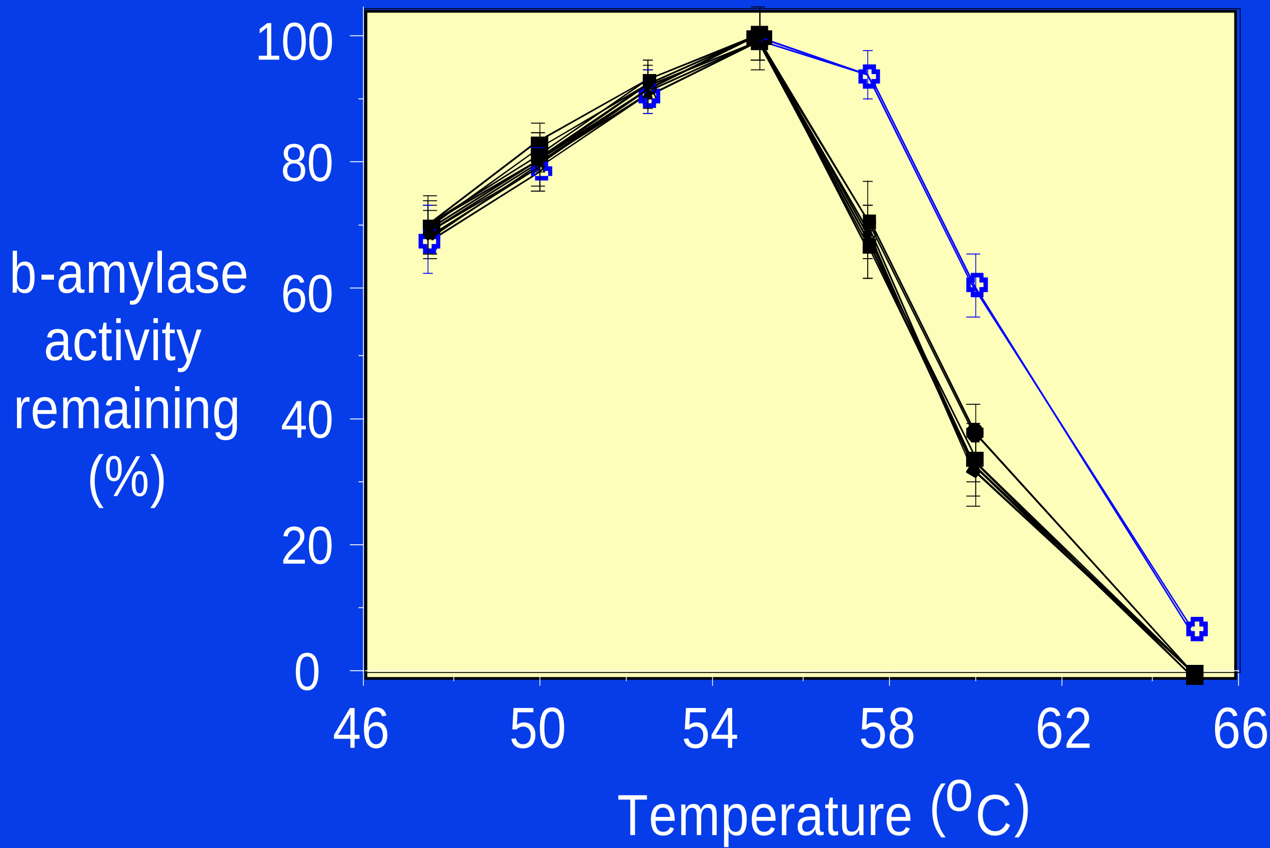
See Eglinton, Langridge & Evans (1998), J. Cereal Sci. 28: 301-309.

Comparison of the amino acid substitutions between the four barley β-amylase enzyme types

Impact	Sd2L[1]	Sd1[2]	Sd2H[3]	Sd3[4]
IEF, bound/free, K_m	Arg115	Cys115	Arg115	Arg115
	Asp165	Glu165	Asp165	Glu165
Thermostability	Val233	Val233	Ala233	Ala233
	Ser254	Ser254	Ser254	Thr254
Thermostability	Leu347	Ser347	Ser347	Ser347
	Val430	Ala430	Ala430	Ala430
IEF, Thermostability	Gln472	Gln472	Gln472	Lys472
	Ile527	Ile527	Met527	Ile527
C-terminal G489		Thermostability, K_m		

See Ma, Langridge, Logue, & Evans, (2001) Mol Gen. Genet. 266: 345-352.

Effect of assay temperature on the activities of mutant and wild type barley β-amylases (Extracted from Ma *et al*., 2001)



Sd2H only

V-A233 = true thermostability

Sd2H and Sd1

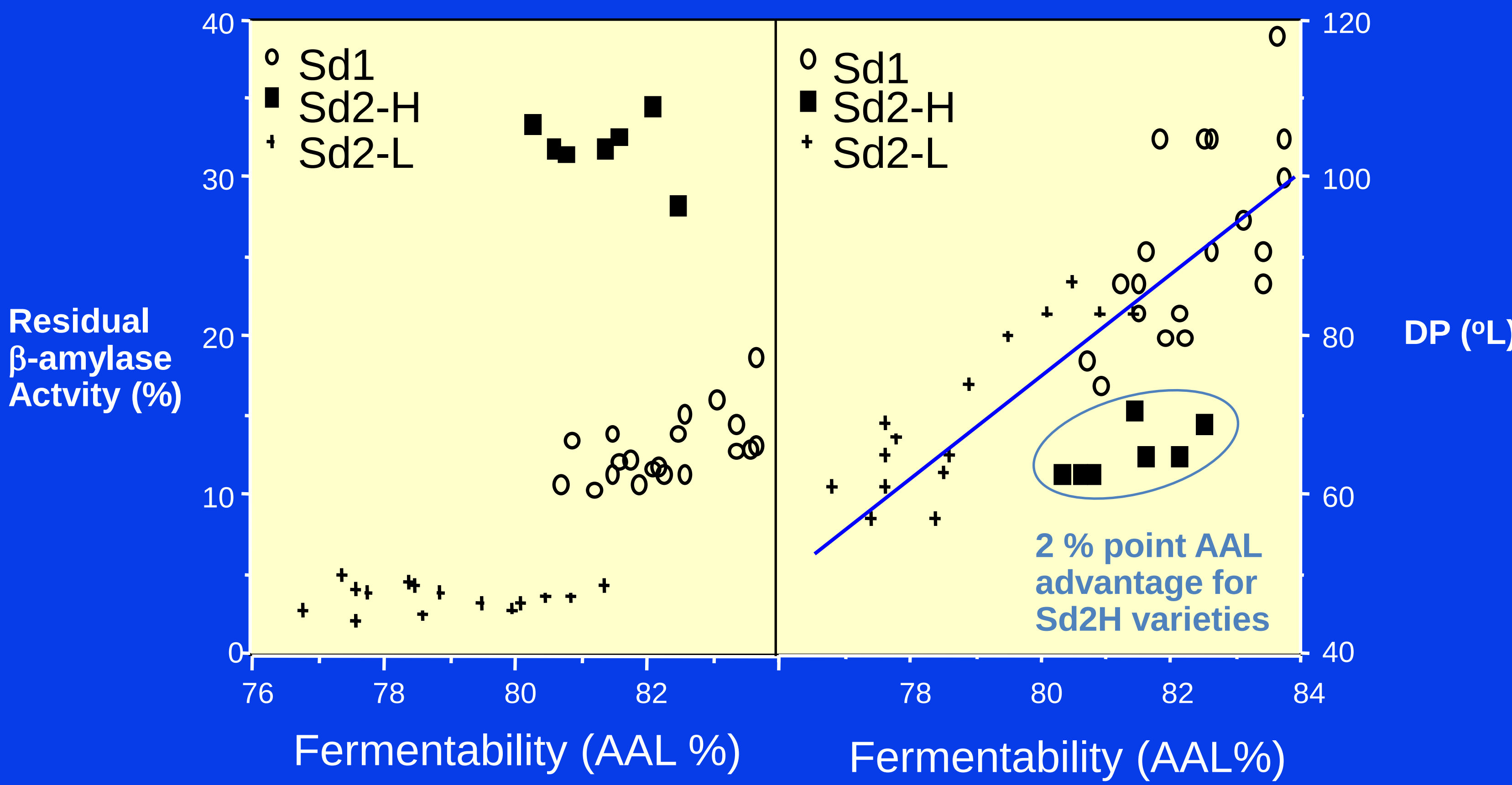
L-S347 = enzyme activity recovery on cooling

Sd2L-V233A, Sd2L-R115C, Sd2L-D165E, Sd2L-L347S, Sd2L-V430A

See Ma, Langridge, Logue, & Evans, (2001) Mol Gen. Genet. 266: 345-352.

Relationship between β-amylase thermostability and wort fermentability in 42 commercial samples

(Extracted from Eglinton et al., 1998)



What is the actual persistence of DP enzymes in the mash?

Mash/extract thickness:

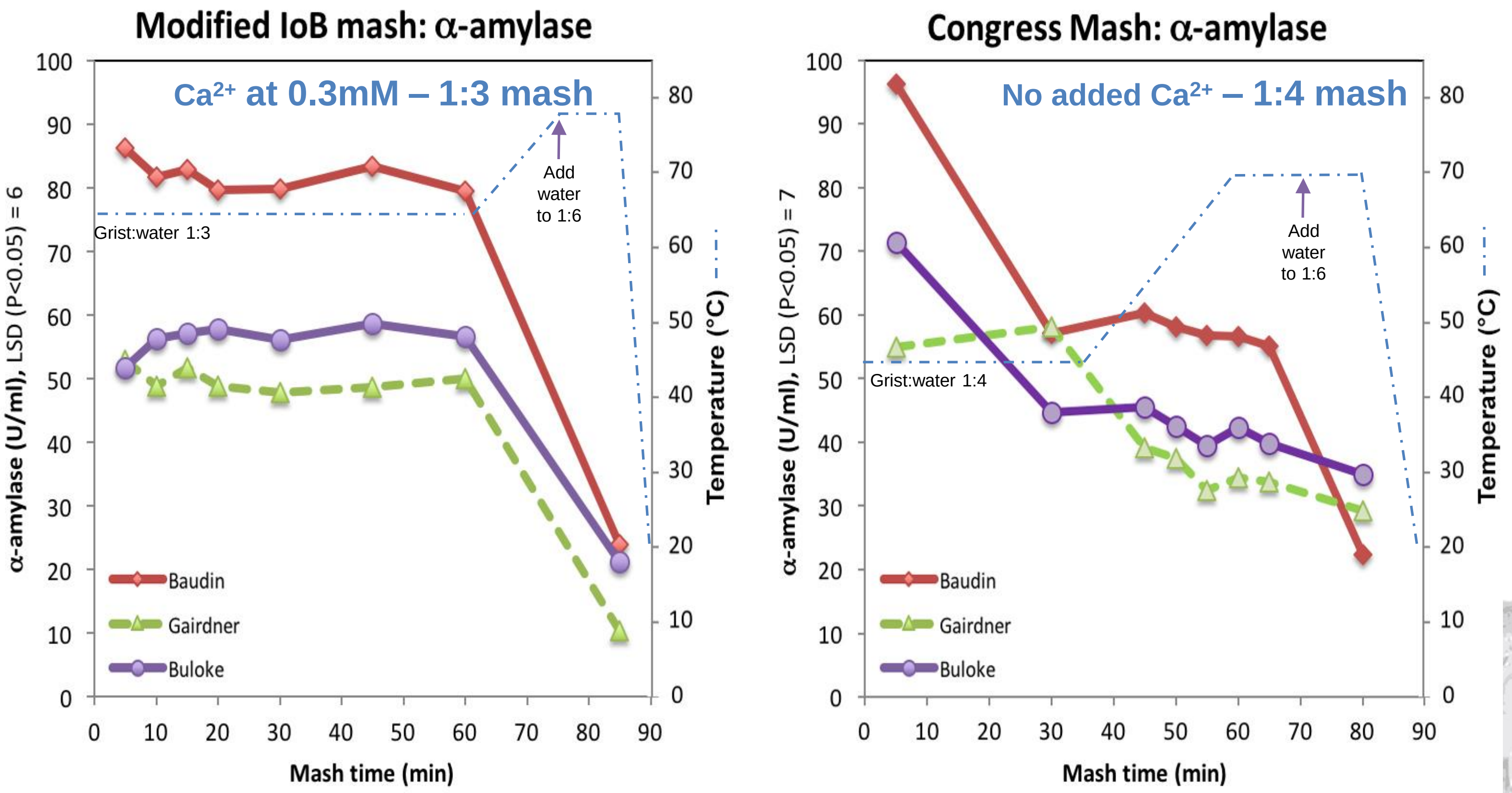
- **Enzyme extracts: 1:10**
- **Congress: 1:4**
- **Modified IoB: 1:3**

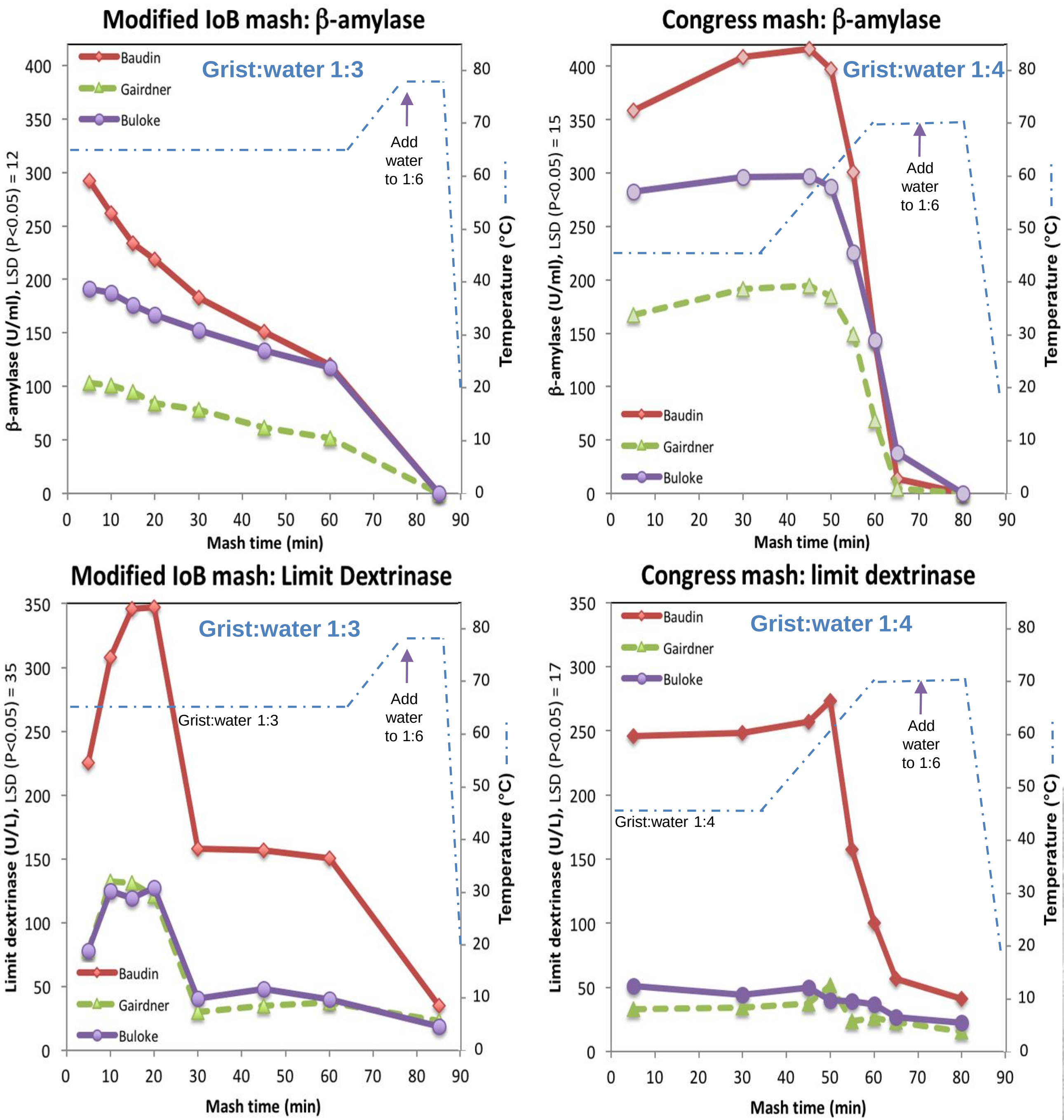
Quality characteristics of malts used

Variety	Moist (%)	Total protein (%)	Total β-amy (U/g dw)	β-amy activity Rem (%)	β-amy Free Act (%)	α-amy (U/g dw)	α-amy activity Rem (%)	Total LD (U/Kg dw)	LD activity Rem (%)	LD Free Act (%)
Baudin	4.7	11.0	1048	33 Sd1	87	395	53.4	815	61.8	20.4
Buloke	4.7	11.7	745	48 Sd2H	90	292	53.3	655	53.9	8.5
Gairdner	3.7	11.7	604	35 Sd1	83	255	50.3	410	48.9	12.2

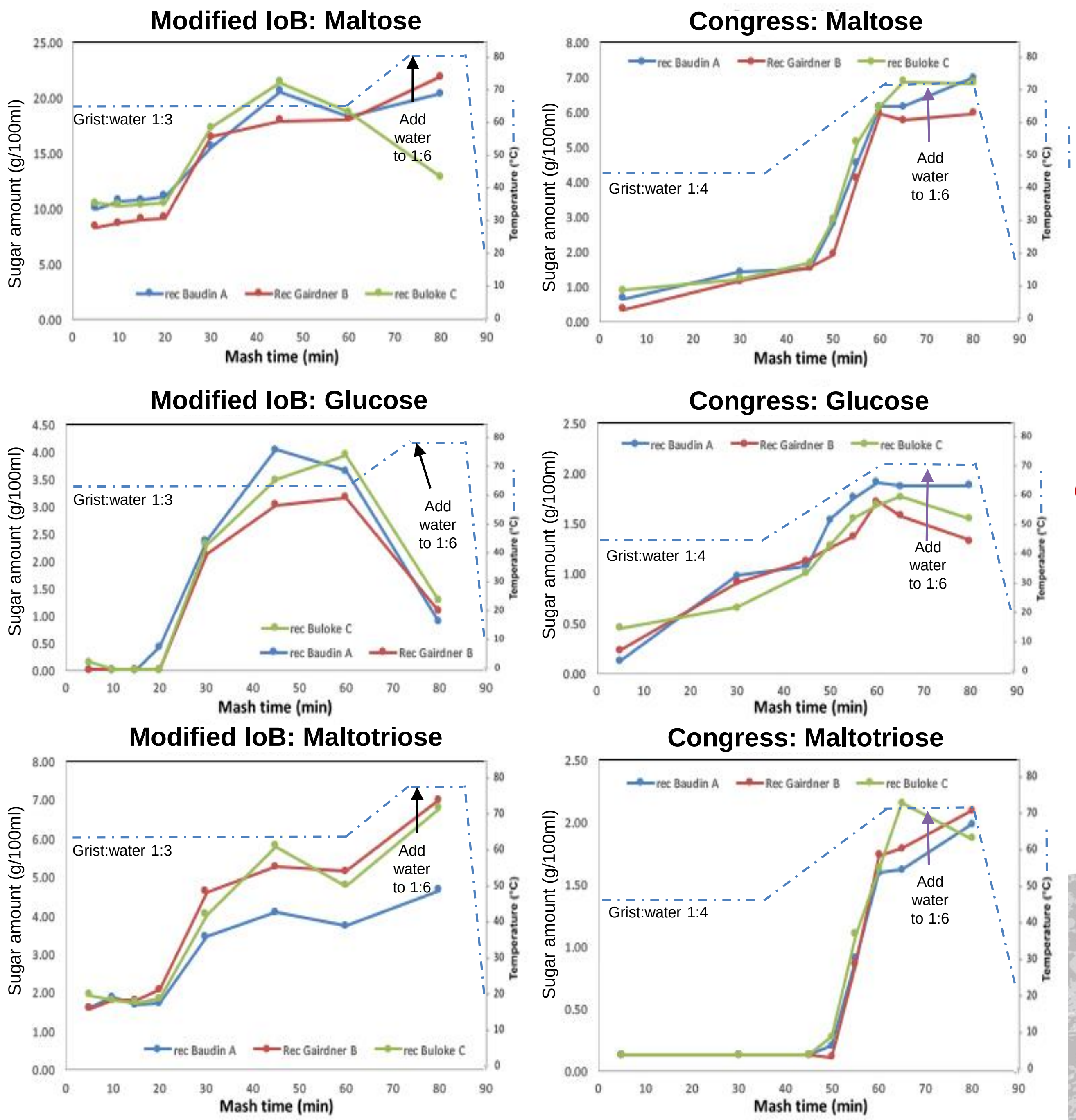
Variety	Congress KI (%)	Mod IoB* Extract (% Dwt)	Congress AAL (%)	Mod IoB* AAL (%)
Baudin	52.8	79.4	92.4	95.2
Buloke	38.0	78.9	91.1	92.2
Gairdner	37.3	78.4	86.7	87.1

What happens to DP enzymes during mashing?





Modified IoB mash: β-amylase
Grist:water 1:3
Baudin
Gairdner
Buloke
Add water to 1:6
β-amylase (U/ml), LSD (P<0.05) = 12
Temperature (°C)
Mash time (min)
Congress mash: β-amylase
Grist:water 1:4
Baudin
Gairdner
Buloke
Add water to 1:6
β-amylase (U/ml), LSD (P<0.05) = 15
Temperature (°C)
Mash time (min)
Modified IoB mash: Limit Dextrinase
Grist:water 1:3
Grist:water 1:3
Add water to 1:6
Baudin
Gairdner
Buloke
Limit dextrinase (U/L), LSD (P<0.05) = 35
Temperature (°C)
Mash time (min)
Congress mash: limit dextrinase
Grist:water 1:4
Grist:water 1:4
Add water to 1:6
Baudin
Gairdner
Buloke
Limit dextrinase (U/L), LSD (P<0.05) = 17
Temperature (°C)
Mash time (min)



Congress sugar levels a work in progress

Conclusions

- Heat ~55-60^{o}C releases bound and latent β-amylase & limit dextrinase.
- β-amylase and limit dextrinase persist longer in mash than expected.
- Fermentable sugar generation lags DP enzyme activity persistence.
- Modified IoB & Congress mash programs produce different spectrums of DP enzyme activity (& fermentable sugar generation?).
- Thicker mashes assist preservation of thermolabile enzymes in mash.
- What about other malt enzymes (i.e., lipoxygenase, proteases etc)?
- Practical implications for modification of temperatures during mashing with respect to concentration of sugars, lipids and other components.

Acknowledgments

Assistance:

Australian Maltsters,
Loraine Watson (QAAFI), Jenifer Wanders (UQ),
Hugh Dunn, Jon Luff (Pilot Malting Australia)

Funding:
Grains Research and Development Corporation