FRIEND OR FOE? A TALE OF MALTING BARLEY FREE AMINO NITROGEN CONTENT AND IMPACT ON BEER FLAVOR

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Minneapolis, MN

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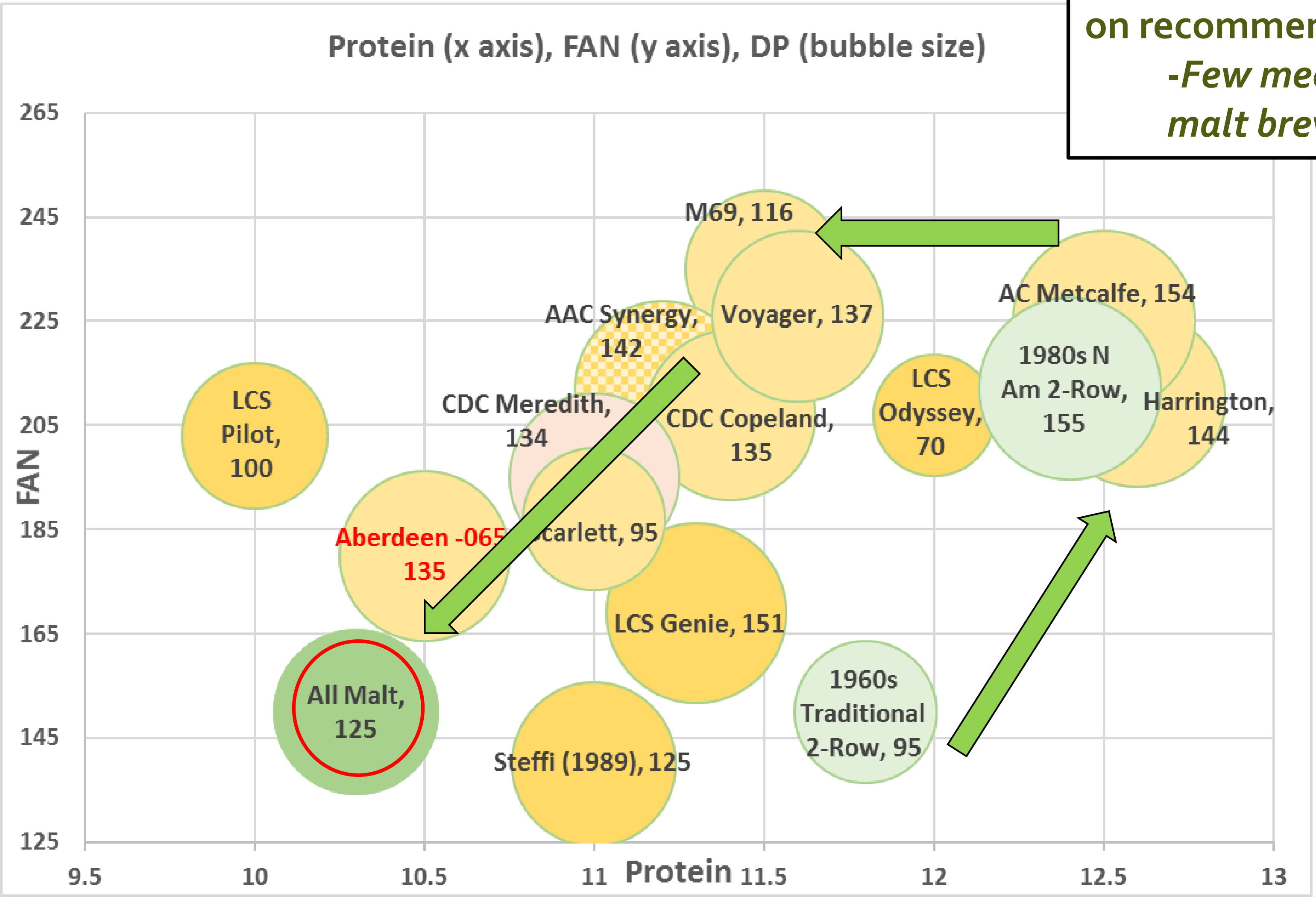
The Evolving North American Barley Variety Basket

AMBA: 20 malting barley varieties on recommended variety list

-Few meet criteria for all malt brewing…



2014 Brewer's Association White Paper:

BREWERS ASSOCIATION

MALTING BARLEY CHARACTERISTICS FOR CRAFT BREWERS

EXECUTIVE SUMMARY

The brewing industry is evolving rapidly, and the barley malt supply chain should likewise evolve rapidly to meet the very different needs of all-malt beer brewers. Brewers Association member craft brewers have identified malt supply mismatch as a potential impediment to growing their brands. To produce all-malt beer brands, craft brewers seek barley malts with

> distinctive flavors and aromas
> lower free amino nitrogen ("FAN")
> lower Total Protein
> lower Diastatic Power ("DP")
> lower Kolbach Index (ratio of Soluble Protein to Total Protein, or "S/T")

Such malts differ significantly from the current suite of available barley malts produced in North America. The demand for such malts will grow significantly as craft production increases.

INTRODUCTION

The U.S. brewing industry is currently undergoing profound and lasting structural change. Craft brewers are changing the face of the industry at all levels: retail, wholesale, production and supply. As of March 2014 there are over 2,800 breweries operating in the U.S., likely producing over 10,000 beer brands. This diversity presents challenges as well as opportunities for wholesalers and retailers, and unprecedented choice for beer consumers. The U.S. beer market is arguably the most diverse on Earth.

In July 2012, Brewers Association staff attended a North Dakota-based barley field course offered by the Institute of Barley and Malt Sciences (IBMS) and North Dakota State University (NDSU). Brewers Association staff learned at that U.S. craft brewers were already using over 20% of all malt consumed by U.S. brewers, belying their relatively small production share (at that time, around 6% of U.S. beer production). In response, barley grower and North Dakota Barley Council Chair Doyle Lentz replied "then craft brewers better figure out how to communicate with growers."

The Brewers Association couldn't agree more. This paper is offered in the spirit of communication with and by growers, academics, breeders, maltsters, dealers and brewers, and all other barley and malting industry stakeholders. These collected ideas and perspectives represent a single snapshot in time of an incredibly complex industry that continues to evolve rapidly.

RECENT HISTORY AND CURRENT STATUS OF BREWING AND MALTING INDUSTRIES

During the middle half of the 20th century (1930s to 1980s) the U.S. beer market was generally characterized by -

- Increased product homogeneity and dominance of adjunct lager styles
- Decreasing number of brewing companies via attrition and consolidation
- Increasing dominance of a relatively small number of brands of adjunct lagers
- Slowly decreasing original gravities
- Relatively homogenous barley malt needs

During this time breeders made important advances in yield, disease/stress tolerance, extract and digestibility – attributes that yielded high quality malt for a growing brewing Industry. Nonetheless, prior to the advent of craft brewery companies in the late 1970s and early 1980s, barley growers and the malting industry responded to relatively uniform brewer needs by developing a relatively small number of high diastatic power, high FAN malt varieties suitable for adjunct brewing.

By definition, the malt used to produce beer was nearly all consumed by large producers of adjunct lagers.

https://www.brewersassociation.org/attachments/0001/4752/Malting_Barley_Characteristics_For_Craft_Brewers.pdf

2014 BA White Paper

EXECUTIVE SUMMARY

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- **distinctive flavors and aromas**
- **lower free amino nitrogen ("FAN")** TARGET ~125 ish
- **lower Total Protein**
- **lower Diastatic Power ("DP")**
- **lower Kolbach Index (ratio of Soluble Protein to Total Protein, or "S/T")**

Such malts differ significantly from the current suite of available barley malts produced in North America. The demand for such malts will grow significantly as craft production increases.

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FAN – Free Amino Nitrogen

- FAN provides one of the main sources of nitrogen for yeast metabolism during wort fermentation.
- FAN comprises individual amino acids and small peptides (di and tri-peptides).

Impacts of FAN:

- Brewers look to malt FAN to determine yeast potential growth and fermentation efficiency: YEAST VIGOR and FERMENTATION RATE.

- Insufficient yeast nutrient leads to difficult fermentation and possible sulfur and other off flavor formation.

- Excessive FAN levels in beer contribute to negative flavor attribute formation, lowering overall beer quality and hence flavor stability.
  - Excess FAN serve as nutrients for microbial growth.

MALT FAN:

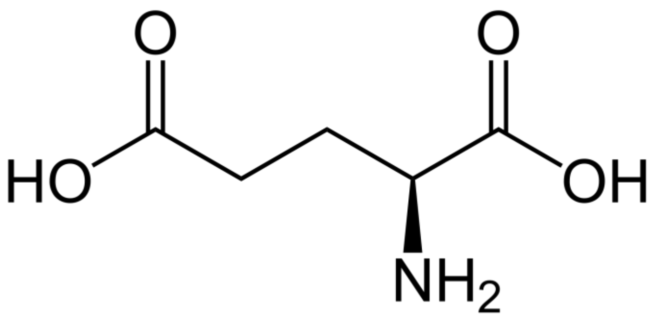
- Malt COA gives the total **FREE AMINO NITROGEN** content, but does not indicate the quality of ***assimilable*** amino acids.

- Malt amino acid profiles strongly influence beer flavor potential and flavor stability therefore:

Amino acids profiles assist to bridge the gap between malt/beer flavor characteristics and the barley genome……DARK MATTER (T. Nielsen, 2018)!

The Ordered Uptake Issue:

- Of the 20 essential amino acids wort contains 19 amino acids that yeast assimilate
- Uptake occurs in **preferential order** at **varying rates** depending on **yeast strain**



Glutamic Acid

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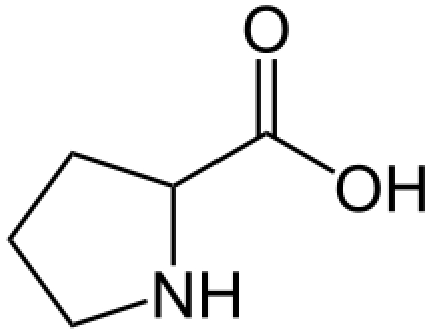
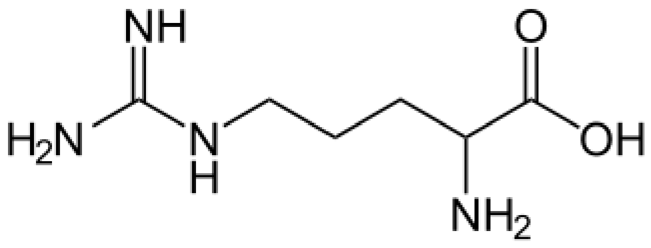


Proline

The Ordered Uptake Issue:

- Three Classes based on **Vitality of YEAST**:
  - Class 1 – Unimportant – PROLINE
  - Class 2 – VITAL
  - Class 3 – Crucial! – ARGININE
- Four Groups based on **ASSIMILATION RATE**:
  - Group A – Rapid - ARGININE, GLUTAMIC ACID
  - Group B- Moderate
  - Group C – Slow
  - Group D – UN-Assimilable – PROLINE
  - Pierce (1987)



Arginine

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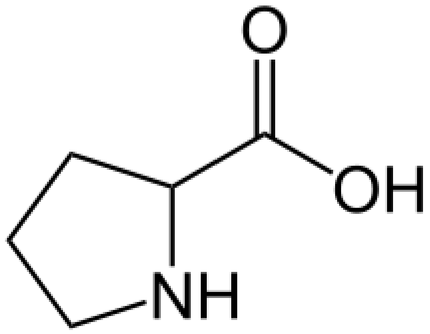


Proline

The Ordered Uptake Issue:

Crucial and Rapid
-Lysine
-Arginine

Vital and Moderate:
-Valine
-Isoleucine

Vital and Slow:
-Glycine
-Phenylalanine
-Tyrosine
-Alanine
-Tryptophan

FIX (1999)

Table 1.3 **Amino Acids in Brewing**

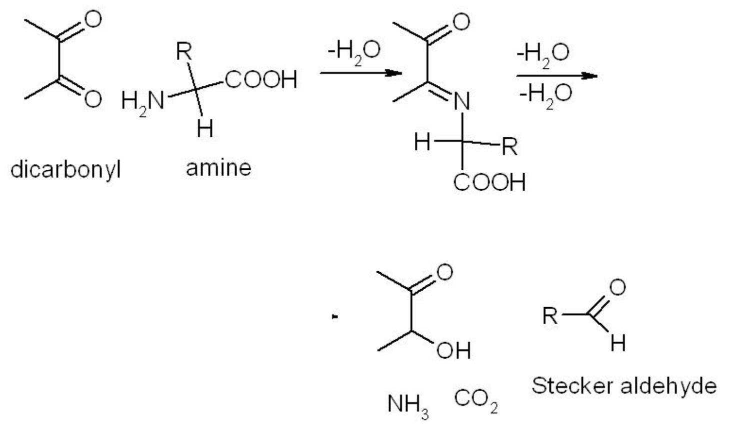
	Absorption rate			
	Group A: Rapid	**Group B: Moderate**	**Group C: Slow**	**Group D: Largely unabsorbed**
Class 1: Unimportant amino acids	Glutamic acid Glutamine Aspartic acid Asparagine Serine Threonine			Proline
Class 2: Vital amino acids		Valine Isoleucine	Glycine Phenylalanine Tyrosine Alanine	
Class 3: Crucial amino acids	Lysine Arginine	Leucine Histidine	Tryptophan	

FLAVOR IMPACT!!!

FLAVOR MATTERS! DAMN IT!

- Strecker Degradation of amino acids:
  - Breakdown of amino acids and dicarbonyl compounds
  - Amino acids are decarboxylated and deaminated to aldehydes
  - Dicarbonyls become alpha-aminoketones or amino-alcohols
  - Aldehydes condense to aldols and form furans, pyrazines, pyrroles, oxazoles, thiazoles as well as other heterocycle compounds == FLAVOR/AROMA = MEATY/BREADY

Strecker Degradation



Rich nutty, meaty flavors

Proline	**Pyrrolidine, 1-pyrroline NO STRECKER ALDEHYDE**	**Important intermediates for bread-like aromas**
Cysteine	**Mercaptoacetaldehyde, acetaldehyde, H_2S, ammonia**	**Important intermediates for meat-like aromas**

3. Sulphur containing amino acids such as Cysteine or cystine form **H_2S, NH_3 etc by Strecker Degradation**

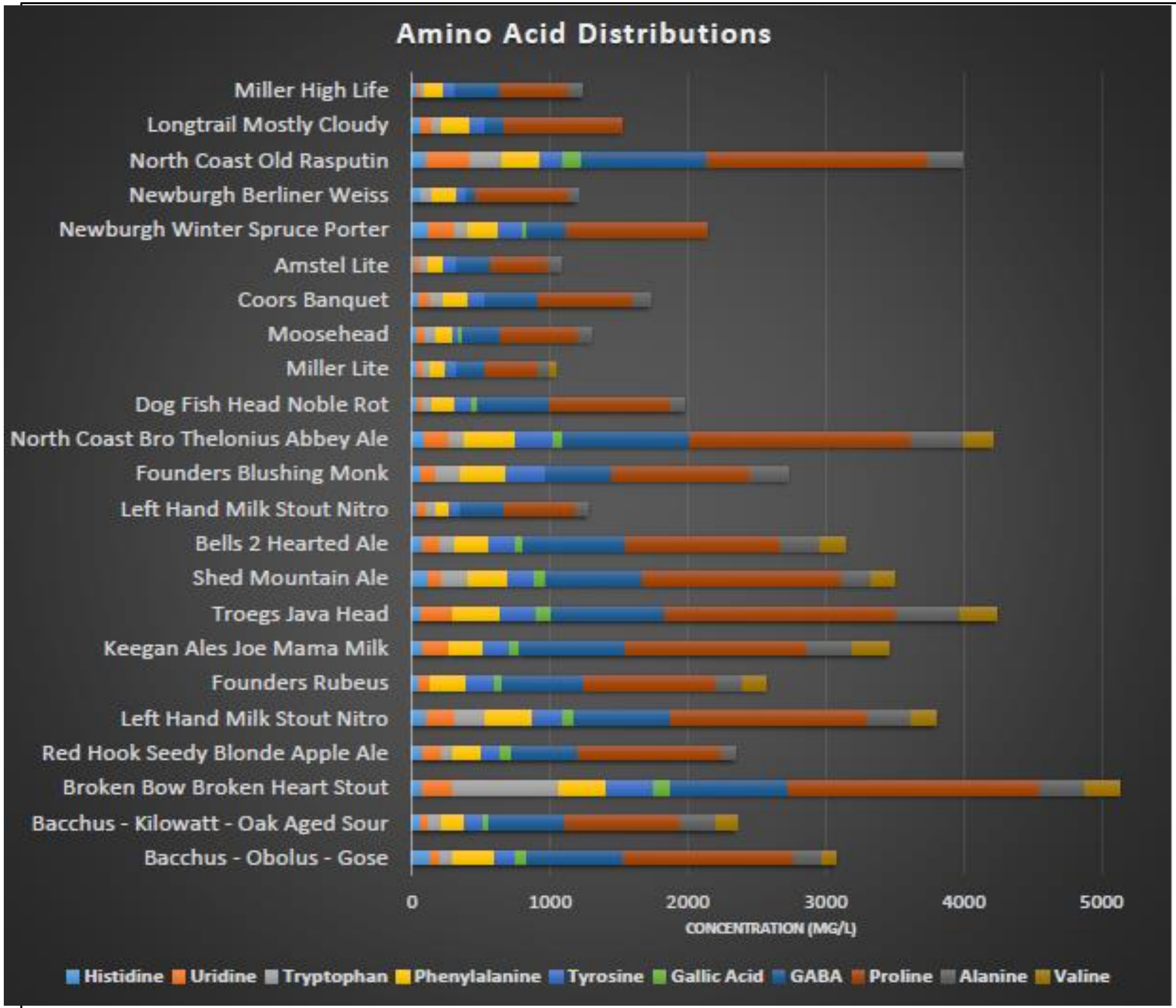
Shahidi et al (1998)

Strecker Degradation of Amino-Acids and Resultant Flavor Compounds

(Examples from literature - Meilgaard, 1975; Ho, 1996)

Amino Acid	Strecker Aldehyde	Threshold (PPM)	Odor Characteristics
Alanine	Acetaldehyde *CH3CHO*	10	Pungent, green, sweet
Valine	Isobutyraaldehyde *(CH3)2CHCHO*	(1.0)	Pungent, green, fruity/banana @ v. low conc. Extremely diffusive
Leucine	Isovaleraldehyde *(CH3)2CHCH2CHO*	(0.6)	Acrid-pungent, fruity/ pleasant @ v. low conc.
Isoleucine	2-Methylbutanal *CH3CH2CH(CH3)CHO*	1.25	Roasted cocoa or coffee like. Fruity@ v. low conc.
Phenylalanine	Phenylacetaldehyde *C6H5CH2CHO*	(1.6)	Powerful, pungent, floral, sweet
Methionine	Methional *CH3S.CH2CH2CHO*	(0.25)	Onion-meat-like, cooked potato

- Reaction catalysed by diketones / indirect need for O_2
- In beer, reaction favoured by high temperatures and high pH (4.3 - 4.6)
- May happen in the bottle even at room temperature



-Amino acid levels vary from **1000 mg/L** in commercial light beers to **5000 mg/L** in specialty/craft beers

-Strecker amino acids (**Alanine** and **phenylalanine** present in significant levels!)

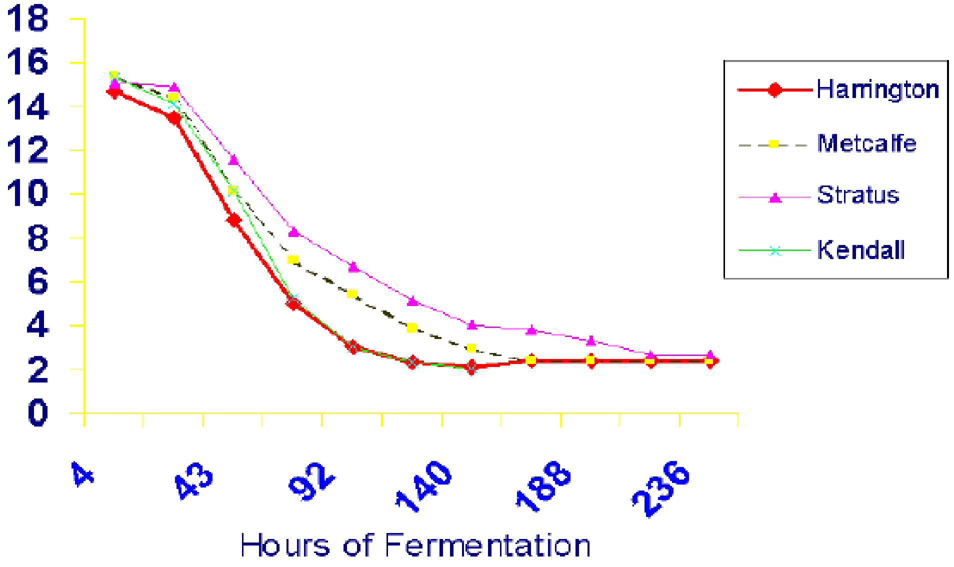
(Edwards, wBC 2016)

FAN PAST….
Amino Acid Uptake: A Commercial Study and Insights
(Yin et al. 2001)

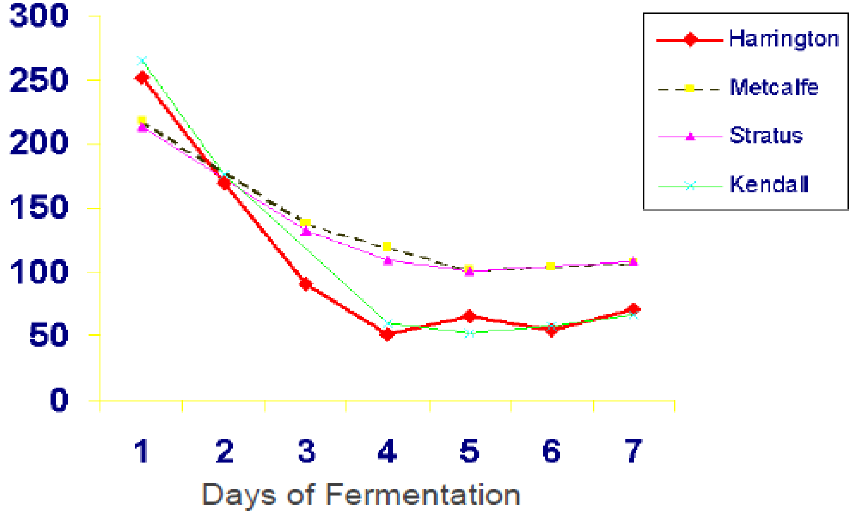
Patterns of Sugars and Free Amino Acid Uptake during Commercial Scale Fermentation

Case Study 1

Plato reduction



FAN reduction



- Some Varieties leaves high levels of FAN at end of fermentation, which could serve as precursor for other undesirable reactions

Source: Yin, IGB Africa, 2001

Case Study 1

Table V. Data of sugar spectrum analysis by HPLC of fermented wort samples at different stages of fermentation ("g per 100 ml of wort") for new variety trials

Brewer's wort samples		Harrington	Metcalfe	Kendall	Stratus
Glucose (DP1)	*Day 1 (0 hr)*	1.14	1.12	1.16	1.14
	Day 4 (70 hr)	0.02	0.31	0.03	0.02
	Day 7(142 hr)	0.01	0	0.01	0.34
Maltose (DP2)	*Day 1 (0 hr)*	*6.99*	*6.52*	*7.10*	*5.72*
	Day 4 (70 hr)	*1.98*	*2.74*	*2.18*	*3.16*
	Day 7(142 hr)	0.26	0.69	0.30	1.02
Maltotriose (DP3)	*Day 1 (0 hr)*	*2.02*	*1.82*	*1.98*	*1.67*
	Day 4 (70 hr)	*0.91*	*1.01*	*0.97*	*1.16*
	Day 7(142 hr)	0.40	0.94	0.63	0.62
Dextrins and oligo-saccharides (DP>3))	*Day 1 (0 hr)*	*2.80*	*2.51*	*2.53*	*2.57*
	Day 4 (70 hr)	*2.80*	*2.35*	*2.53*	*2.56*
	Day 7(142 hr)	2.80	2.35	2.53	2.46

Maltose utilization appears to be slower in some varieties even amino acids still available. Overall difference on 7th day within 1 g/100ml.

Source: Yin, IGB Africa, 2001

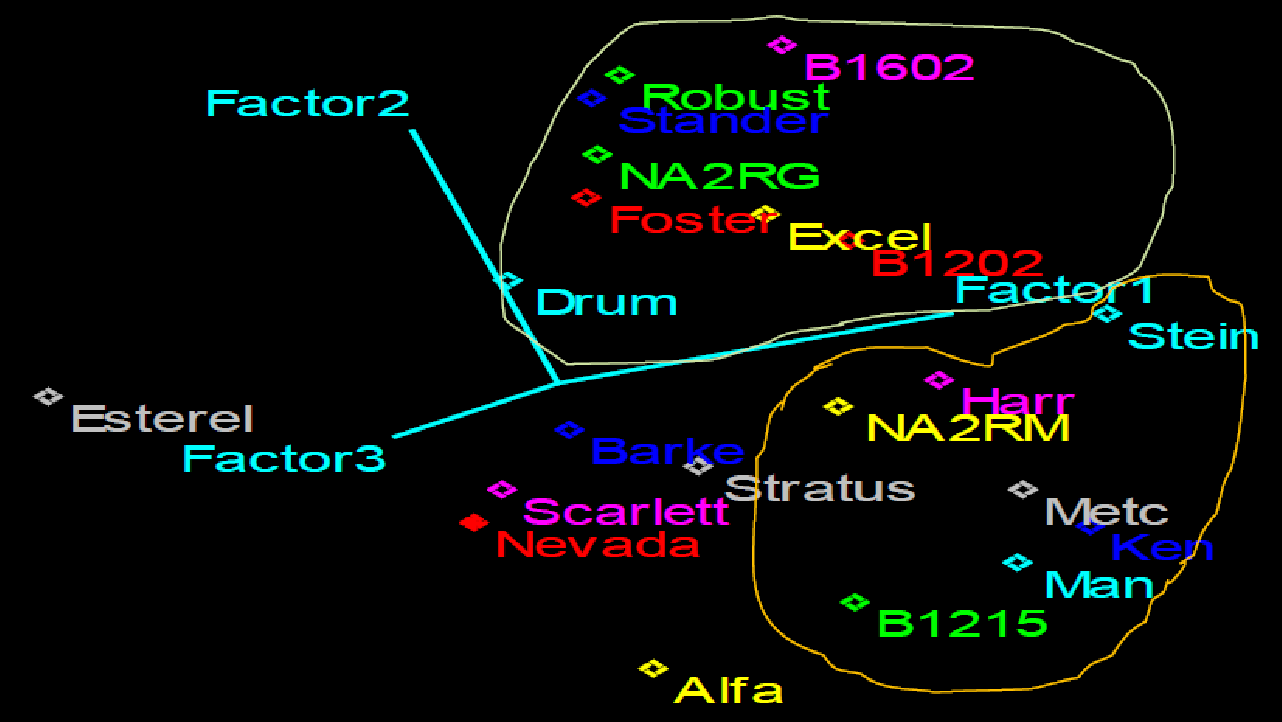
Amino Acid Metabolism during Fermentation

by Barley Variety (Case Study 1)

	Day 1 Fermentation				Day 4 Fermentation			
	Harrington	Kendall	Mecalfe	Stratus	Harrington	Kendall	Mecalfe	Stratus
Histidine	267	275	281	273	58	42	133	120
Arginine	1042	1103	795	721	41	47	156	147
Leucine	1044	1084	1074	949			236	188
Lysine	401	408	418	382		5	13	11
Subtotal-III crucial AA	2754	2870	2568	2325	99	94	538	466
Glycine	129	130	143	100		12	23	18
Alanine	1145	1111	1061	1131	151	42	913	828
Tyrosine	434	465	477	448	130	92	343	259
Valine	877	855	836	796	46		497	425
Isoleucine	492	485	494	447			150	131
Phenylalanine	681	701	688	609	52	36	370	294
Tryptophan	130	122	124	115	111	98	148	128
Subtotal-II important AA	3888	3869	3823	3646	490	280	2444	2083
Aspartic Acid	88	68	66	72	178	181	181	203
Glutamic Acid	192	181	166	198	17	22	24	33
glutamine	362	342	338	314	134	92	299	255
Serine	579	615	653	555		56	40	16
Threonine	448	455	465	536	14	14	14	
Methionine	189	203	194	188			25	23
Cysteine	60	70		36	13	16	15	14
Subtotal- others	1918	1934	1882	1899	356	381	598	544
Proline	3323	3223	3199	2501	2981	3294	3460	2455
Total AA, excl Pro	8560	8673	8273	7870	945	755	3580	3093

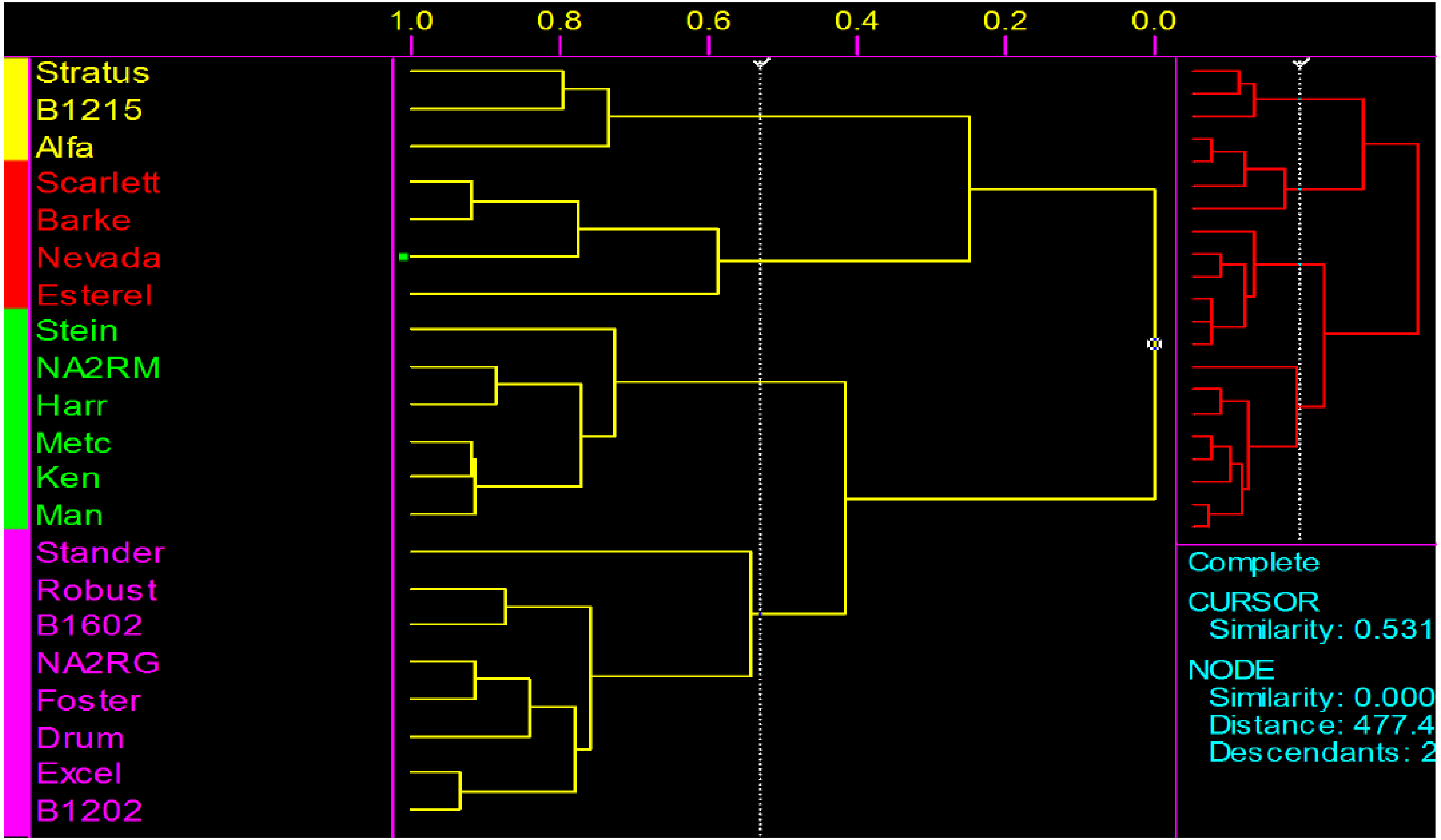
Significant difference in group III on Day 1. Total residual level of AA is variety dependent; some are substrates for Strecker reaction, as marked by "←"

PCA Distribution of Varieties by Wort Amino Acids



Barley type and growing geography affect the pattern of amino acid composition

Cluster Analysis of Varieties by Amino Acids



Geography and barley type (2R/6R) are dominant factors (with some exceptions)

FAN FUTURE….
Amino Acid Uptake: A Commercial Study and Insights
(The Force, 2018)

Rahr Technical Center

MALTING BARLEY EVALUATION REPORT

VARIETY: MAJOR NA TWO-ROWED
CROP YEAR: 2016

Date: Jan 18, 2018

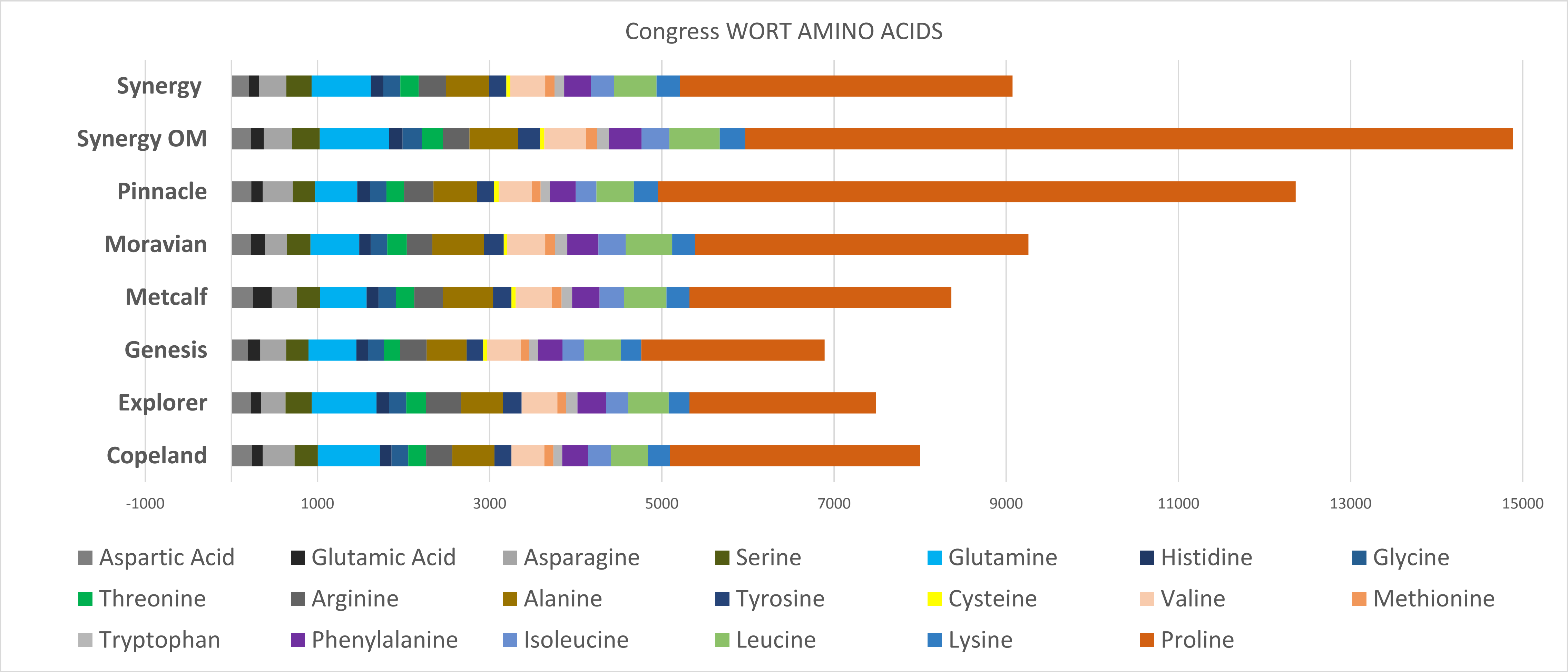
Barley Variety Evaluation

Malt Analysis Crop 16 – Shakopee, MN

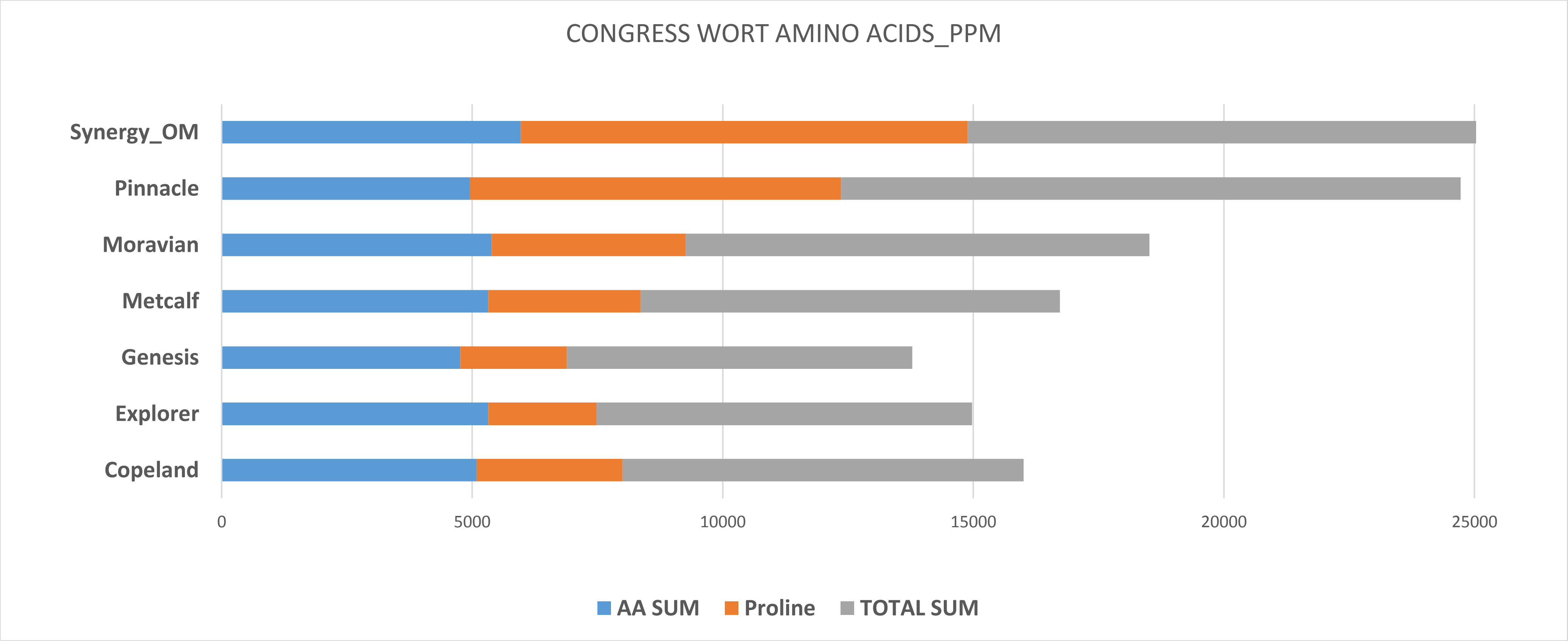
Variety 2016	Alpha Amylase	Beta Glucan	Color Skalar	NTU	Dias Power	Fine Grind	FAN	Moisture	pH	Sol Tot Pro Ratio	Soluble Protein	Total Protein (Leco)	Viscosity
Copeland	50.8	98	1.56	8.5	102	80.4	170	3.24	5.95	41.7	4.76	11.41	1.52
Genesis	58.7	124	2.13	19.0	103	83.7	162	3.88	5.96	44.5	4.65	10.45	1.52
Synergy	63.0	46	1.65	6.6	105	86.1	194	4.32	5.93	50.5	5.15	10.20	1.48
Explorer	66.7	57	1.91	14.7	101	85.1	181	4.10	5.89	43.9	4.85	11.06	1.52
Metcalfe	59.9	63	1.95	11.0	118	83.1	187	4.70	5.81	45.8	5.19	11.34	1.52
Pinnacle	54.2	105	2.20	27.1	106	83.7	161	3.68	5.84	46.2	4.67	10.10	1.52

Overmodified

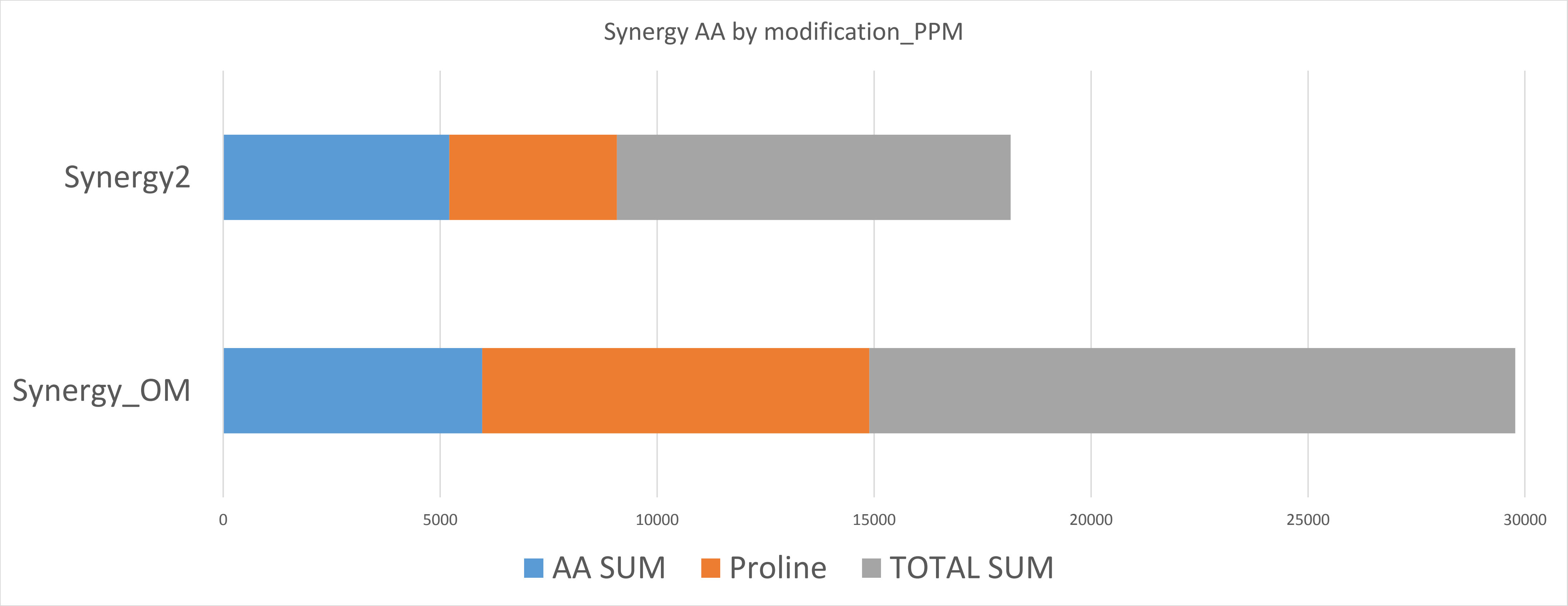
Congress Wort Amino Acid by Variety



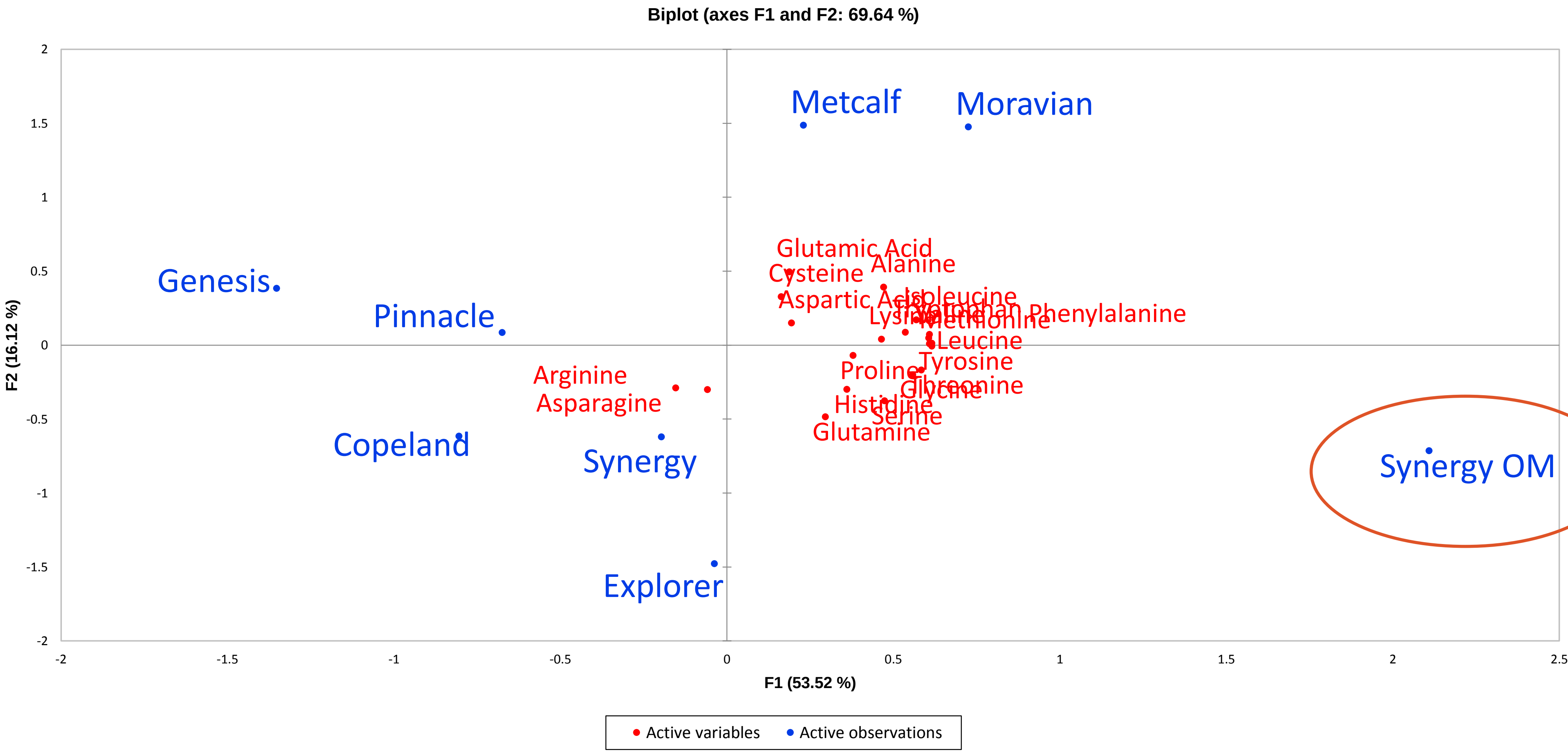
Congress Wort Amino Acids PPM



Synergy by Modification



AA in CONGRESS WORT BY VARIETY



Pilsener Pilot-brewing Trials

Malts produced from the malting trials were pilot brewed in RTC's 300L Pilot Brewery. Malt samples (5) were fermented and the final beer bottled.

Milling

- Mill setting: 1.3mm/0.85mm

Mash Tun

- 100% malt brew – 50 kg of malt and 150L of water added to mash tun
- Mash in at 50ºC, hold for 5 min
- Raise to 62ºC, hold for 20 min
- Raise to 72ºC, hold for 20 min
- Raise to 78ºC
- Pump over to Lauter Tun

Lauter Tun

- Vorlauf for 20 minutes
- Rakes not used for entire lautering
- 160L sparge water at 78ºC

Brew Kettle

- First hop (Kazbek) boiled for 50 min – 8oz
- Second hop (Kazbek) boiled for 30 min – 8 oz
- Third hop (Saaz) boiled for 10 min – 16 oz
- T90 pellets unless specified

Fermentation and Finishing

- Cooled to 10ºC, pitched with lager yeast at ~20 million cells per mL
- Fermented to attenuation (8-12 days) at 12ºC
- Cooled and stored at -0.5 ºC for 21 days
- Filtered through a 5 µm pad filter system, carbonated to 2.5 volumes CO_2 , and packaged

Main Brewhouse Observations

for pilot brewing trials (3 Hl)

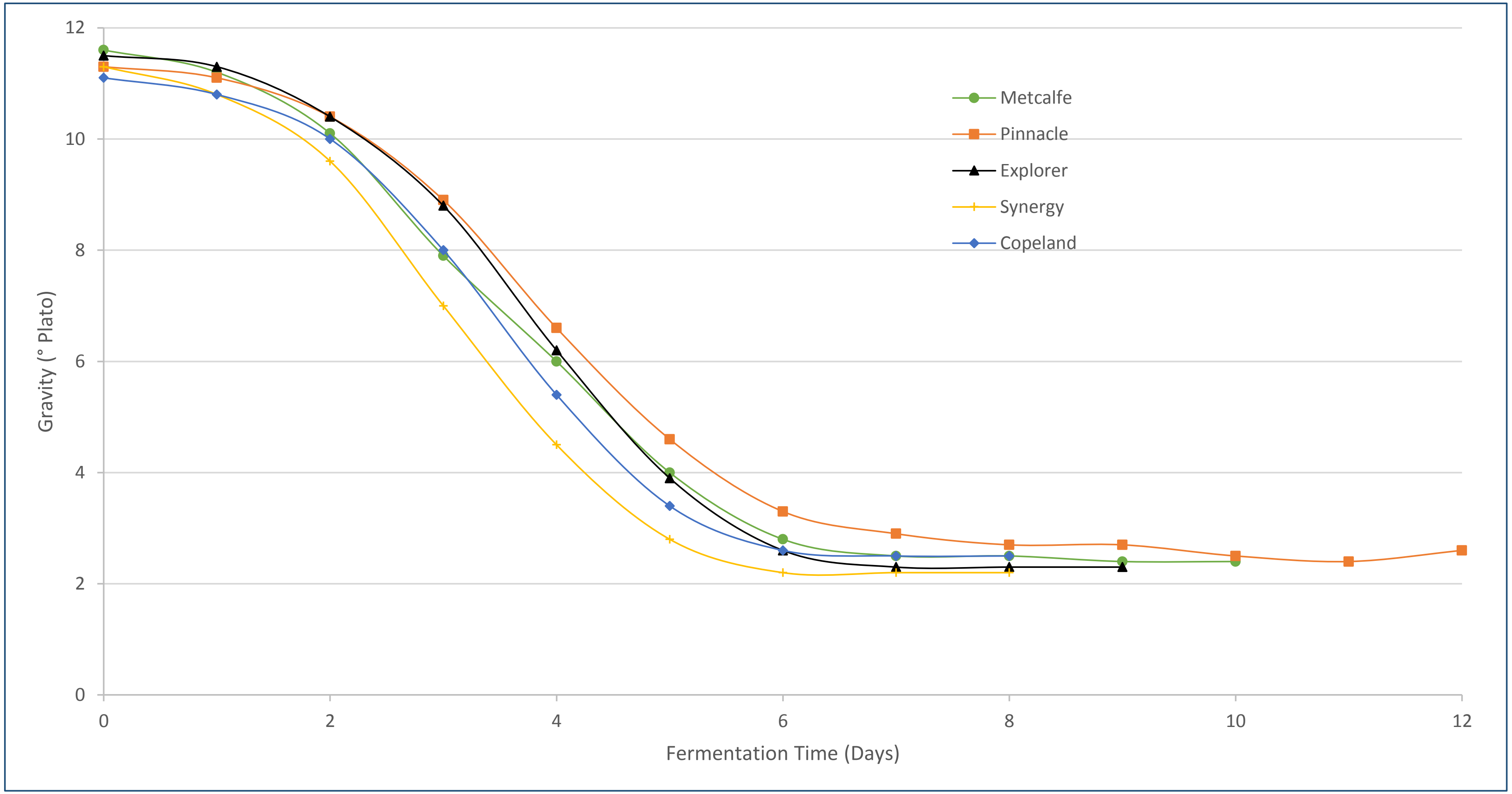
Parameter	Average	Metcalfe	Copeland	Explorer	Synergy	Pinnacle
Conversion time (min.)	**45**	45	45	45	45	45
Time to clear (min.)	**20**	20	20	20	20	20
Lautering time (min.)	**116**	114	116	114	**118**	114
Malt Material Yield (%)	**~72%**	70%	70%*	72%	73%*	73%
Wort pH	**5.5**	5.35	5.6	5.51	5.59	5.49
Wort Colour (SRM)	**4.42**	5.17	3.84	4.51	4.41	4.17
Days to attenuate	**9**	8	8	9	**8**	**12**

Fermentation Performance:

Day	Metcalfe				Pinnacle				Explorer				Synergy				Copeland			
	° Plato	% ABV	FAN (ppm)	pH	° Plato	% ABV	**FAN (ppm)**	pH	° Plato	% ABV	**FAN (ppm)**	pH	° Plato	% ABV	**FAN (ppm)**	pH	° Plato	% ABV	**FAN (ppm)**	pH
0	11.6	0	**241**	5.35	11.3	0	**210**	5.49	11.5	0	**246**	5.51	11.3	0	**228**	5.59	11.1	0	**203**	5.60
1	11.2	0.11	**239**	5.09	11.1	0.04	**212**	5.27	11.3	0.06	**246**	5.27	10.8	0.15		5.00	10.8	0.09		5.22
2	10.1	0.73	**227**	4.77	10.4	0.46	**187**	4.78	10.4	0.53	**214**	4.77	9.6				10.0			
3	7.9	1.93		4.53	8.9				8.8				7.0				8.0			
4	6.0				6.6				6.2				4.5	3.65		4.31	5.4	3.02		4.41
5	4.0				4.6	3.67		4.35	3.9	4.17		4.28	2.8	4.58	**137**	4.30	3.4	4.11	**122**	4.37
6	2.8	4.80		4.38	3.3	4.36	**130**	4.35	2.6	4.86	**151**	4.28	2.2	4.93		4.31	2.6	4.63		4.36
7	2.5	4.95	**162**	4.41	2.9	4.63		4.36	2.3	5.06		4.30	2.2	4.97		4.53	2.5	4.66		4.57
8	2.5	5.02		4.43	2.7	4.74		4.57	2.3	5.10		4.54	2.2	4.99		4.50	2.5	4.68		4.55
9	2.4	5.07		4.65	2.7	4.80		4.53	2.3	5.12		4.49								
10	2.4	5.08		4.59	2.5															
11					2.4															
12					2.6	4.86		4.56							**139**				**125**	
13							**135**				**154**									
14			**168**																	

MALT	FAN
Copeland	125
Explorer	154
Metcalf	168
Synergy OM	139
Pinnacle	135

Pilot Fermentation Charts - Plato



While Synergy shows rapid and high fermentability, Pinnacle less vigorous in plato reduction

Pilot Fermentation Charts - FAN

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Copeland	125
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Metcalf	168
Synergy OM	139
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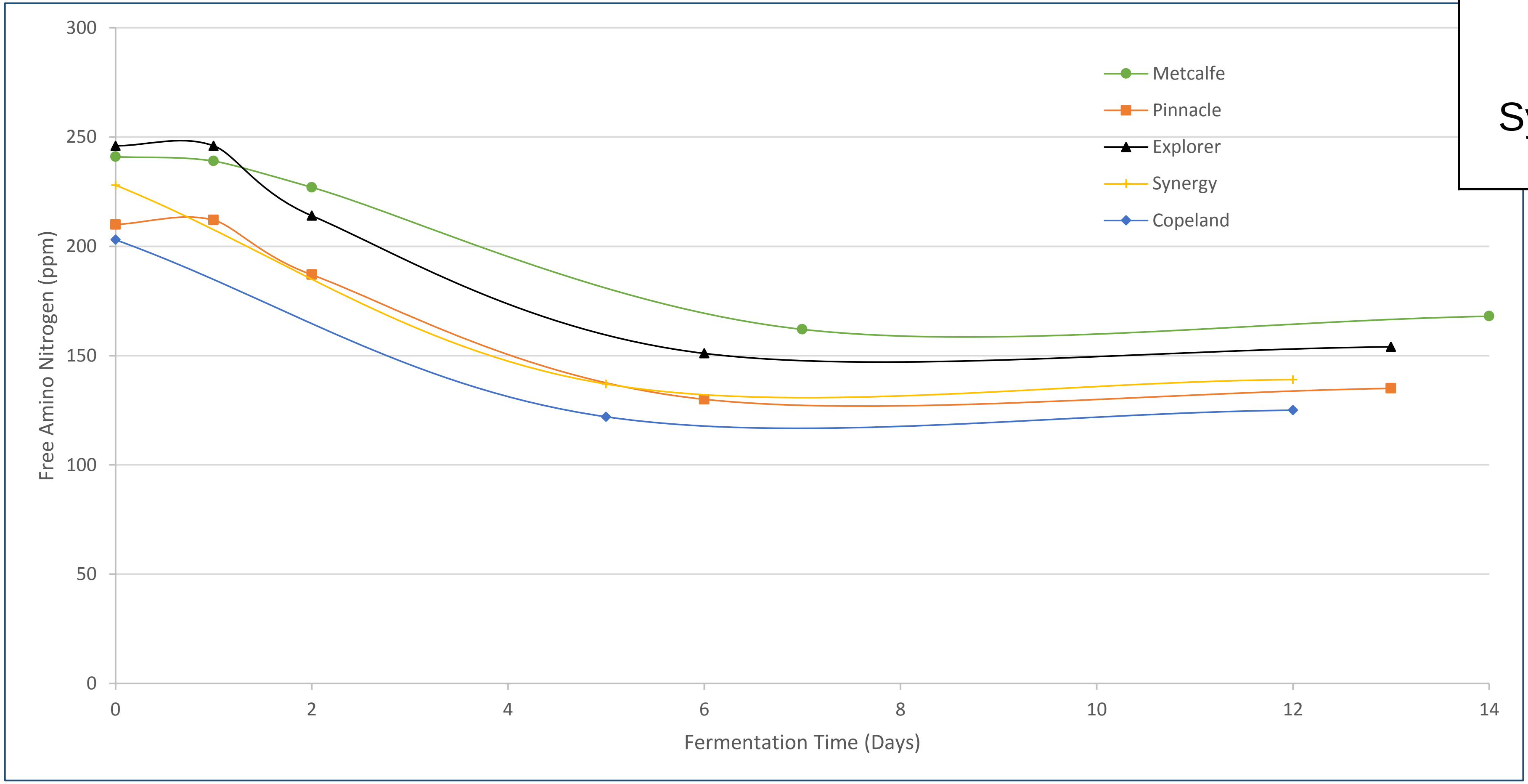
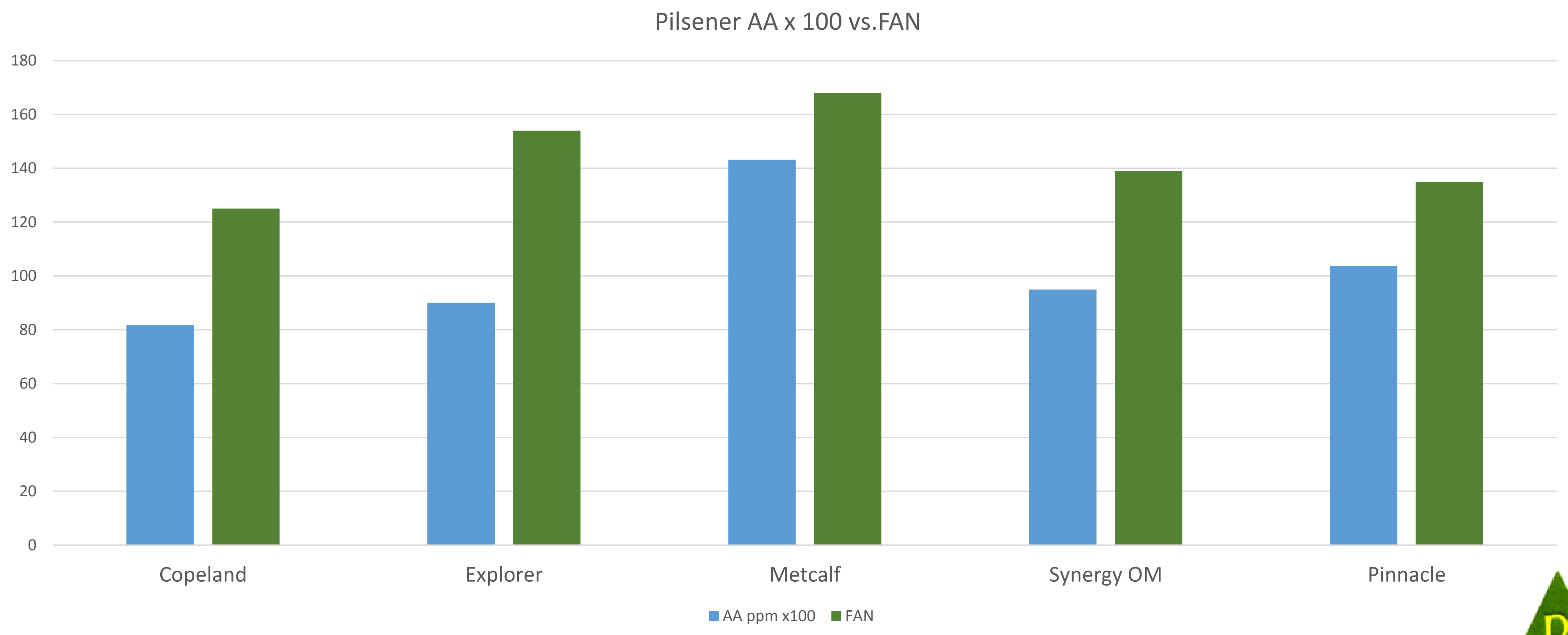
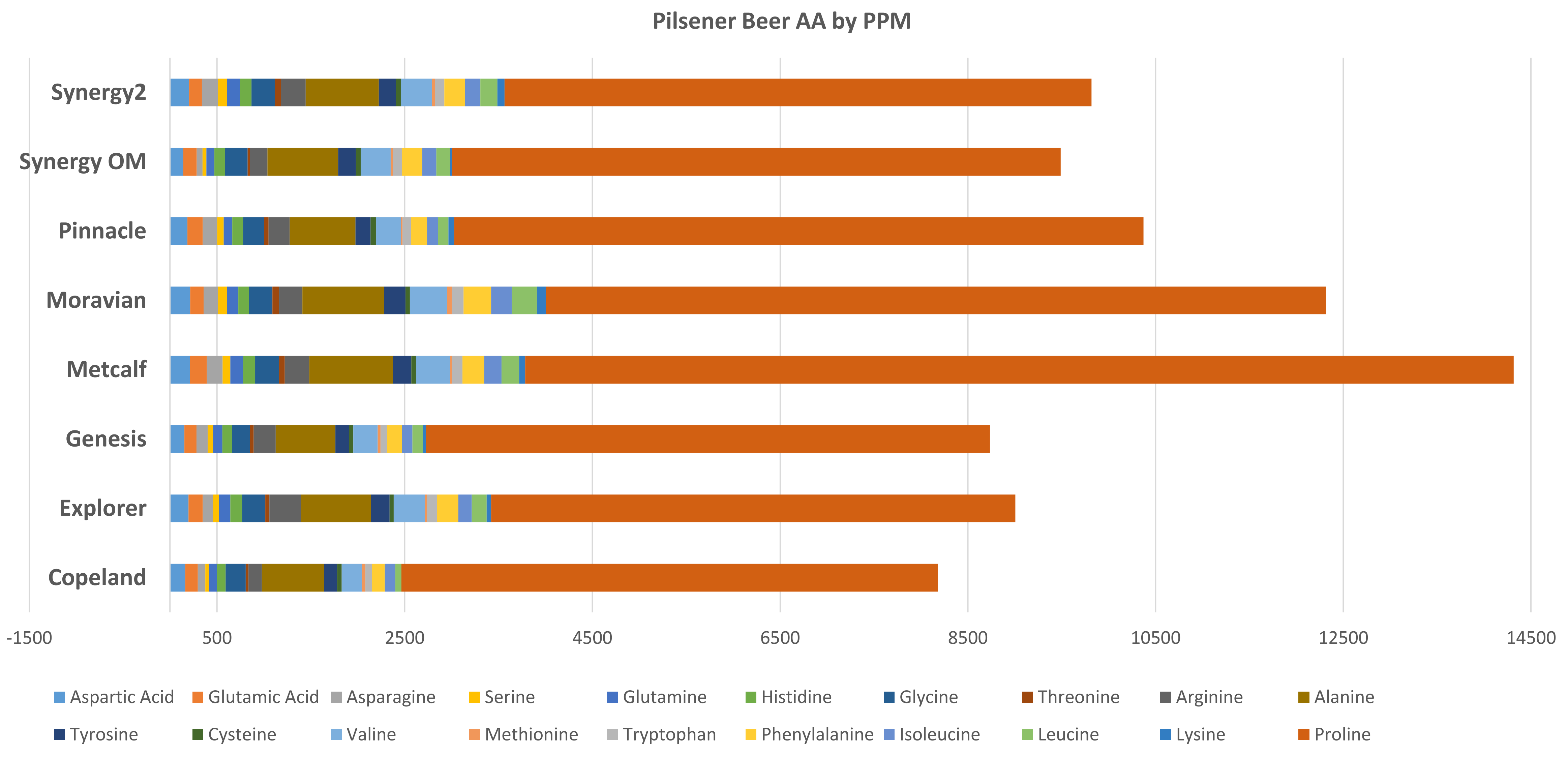


Copeland demonstrate lowest FAN, while Metcalfe and Explorer kept FAN high throughout the fermentation

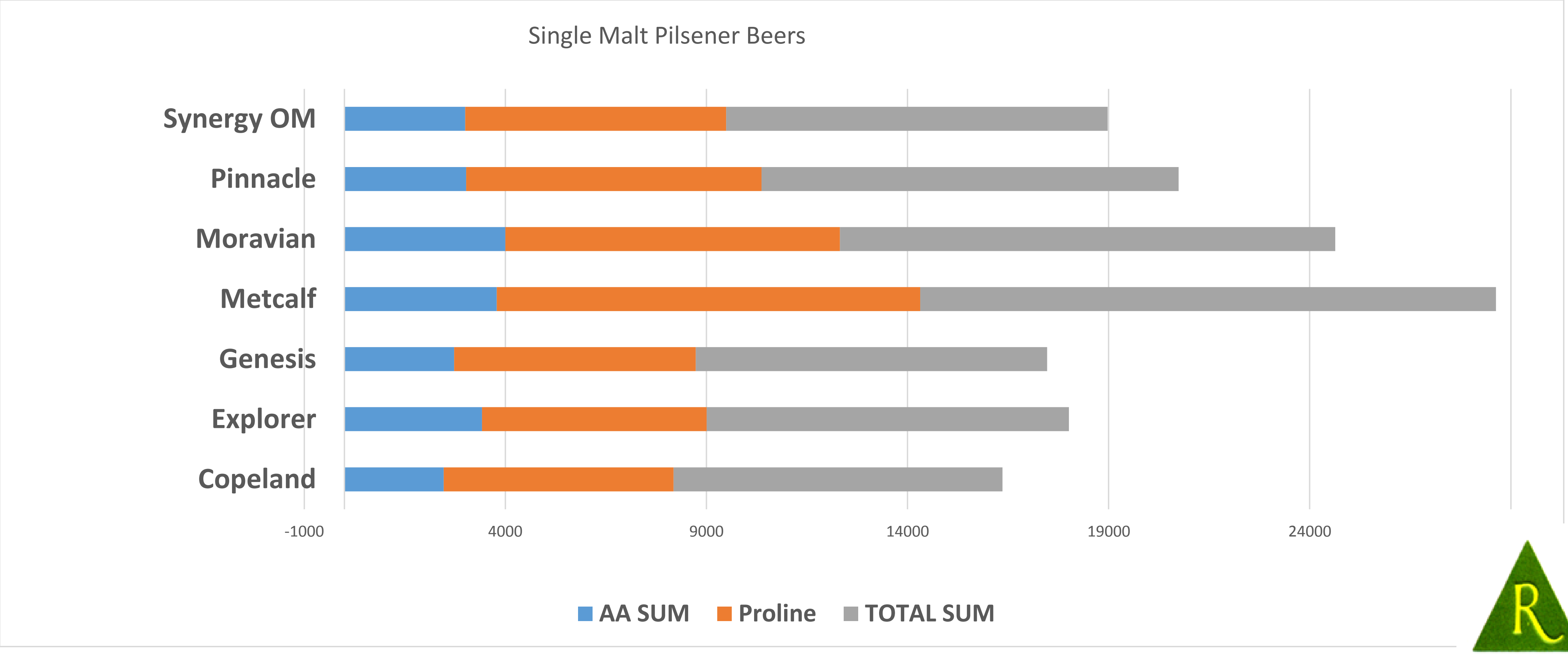
Total Amino Acid (ppm x 100) vs. FAN



Amino Acid Profile (PPM) - Pilsener



Amino Acid Breakdown



Pilsener Beer – Amino Acid PPM

CRUCIAL	Histidine	Arginine	Leucine	Lysine	SUM
Copeland	93.71	143.25	65.66	0.00	**302.62**
Explorer	127.37	341.34	158.46	47.86	**675.03**
Genesis	104.06	233.77	110.83	35.08	**483.74**
Metcalf	125.16	261.22	188.57	64.93	**639.88**
Moravian	113.81	248.96	**266.08**	94.92	**723.77**
Pinnacle	115.09	224.65	113.92	61.47	**515.13**
SynergyOM	111.70	184.60	146.45	21.81	**464.55**
Synergy	117.67	263.66	180.82	77.09	**639.24**

DARK MATTER	Methionine
Copeland	37.76
Explorer	22.44
Genesis	30.61
Metcalf	22.89
Moravian	**51.64**
Pinnacle	**17.23**
SynergyOM	24.77
Synergy2	31.94

Important	Glycine	Alanine	Tyrosine	Valine	Isoleucine	Phenylalanine	Tryptophan	SUM
Copeland	213.57	662.47	139.81	213.88	111.26	**137.53**	70.95	**1549.45**
Explorer	245.06	741.91	198.20	330.16	142.49	**226.90**	109.23	**1993.95**
Genesis	187.98	636.96	146.05	259.56	112.97	**158.86**	67.79	**1570.16**
Metcalf	255.17	889.56	197.46	362.06	182.91	**233.79**	108.26	**2229.21**
Moravian	249.76	869.93	227.81	397.04	221.22	**293.61**	122.59	**2381.96**
Pinnacle	223.92	699.98	162.38	264.29	114.55	**171.45**	89.70	**1726.28**
SynergyOM	241.29	753.75	189.08	318.03	148.99	**216.71**	96.86	**1964.72**
Synergy2	249.03	778.50	183.12	331.53	164.12	**221.07**	99.12	**2026.49**

SUMMARY

- Amino acid profile affects beer flavor and flavor stability…..
  - Barley Variety?
  - Malt Type?
  - Modification?
  - Beer?
  - Yeast?
- Just scratching the surface to this Dark Matter……..

……..to be continued.

THANK YOU for your KIND attention!