A Practical Approach to Controlling the Formation of *trans*-2 Nonenal & Sensory Perception of Papery Off-Notes in Packaged Beer, a Six Sigma Approach

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Abstract

The formation of *trans*-2 nonenal, (E)-2-nonenal, in packaged beer during shelf life is one parameter that brewers seek to control as this aldehyde imparts undesirable papery off-notes. One mechanism generally accepted by brewers is that flavor inactive bound forms of *trans*-2 nonenal produced in brewhouse operations survive to packaged beer and free *trans*-2 nonenal is liberated during its shelf life, where it is eventually perceived organoleptically. In utilizing a structured problem solving approach to assist in the control of both free and bound *trans*-2 nonenal, a cross functional team followed the Define-Measure-Analyze-Improve-Control (DMAIC) process to uncover practical brewing factors that influence the formation of *trans*-2 nonenal. Both wort and beer samples were analyzed using a head space solid-phase micro extraction, on fiber derivitization in combination with GCMS, for both free and bound forms of *trans*-2 nonenal. Through a series of experiments it was determined that some practical factors can influence (p-val < 0.05) the formation of *trans*-2 nonenal in wort such as: mash-in pH, mash-in temperatures, whirlpool stand times, wort boiling times, and timing when hops are added whereas other factors tested (p-val > 0.05) were deemed not significant (sparge water temperature, number of lauter tun deep bed rakes, and sweet wort clarity). In a series of confirmation experiments with the significant factors optimized, we were successful in reducing the wort *trans*-2 nonenal levels by 75% with concomitant improvements (p-val < 0.01) in sensory papery scores at 8 weeks of ambient (24 °C) storage. However, the sensory results in the confirmation trial were still deemed too high leading us to also question the masking effects of other compounds in beer. Using a full factorial design, dimethyl sulfide and iso-amyl acetate was added to stale-papery beer and the test runs were evaluated on our sensory panels. Based on the results, it was found that both compounds exhibited a masking effect (p-val < 0.05) and we suspect the interaction between these two compounds may also be important. Thus, controlling the formation of papery off-notes in beer can be viewed as having multiple avenues to drive improvements, all of which must be considered when addressing papery-off notes.

Outline

- What is 6 Sigma (**DMAIC**)
- **D**efine Phase
  - Y-vars
- **M**easure Phase
  - Papery Gage R&R
  - SPME Fiber Absorption
  - Flow Chart: X-vars
  - C&E Matrix – Prioritization
- **A**nalyze Phase
  - Brewing Trials
- **I**mprove Phase
  - 4 RED X's OPTIMIZED SIMULTANEOUSLY
  - DMS & IAA Masking Effects
  - Additional Investigations
- **C**ontrol Phase

What is 6 Sigma

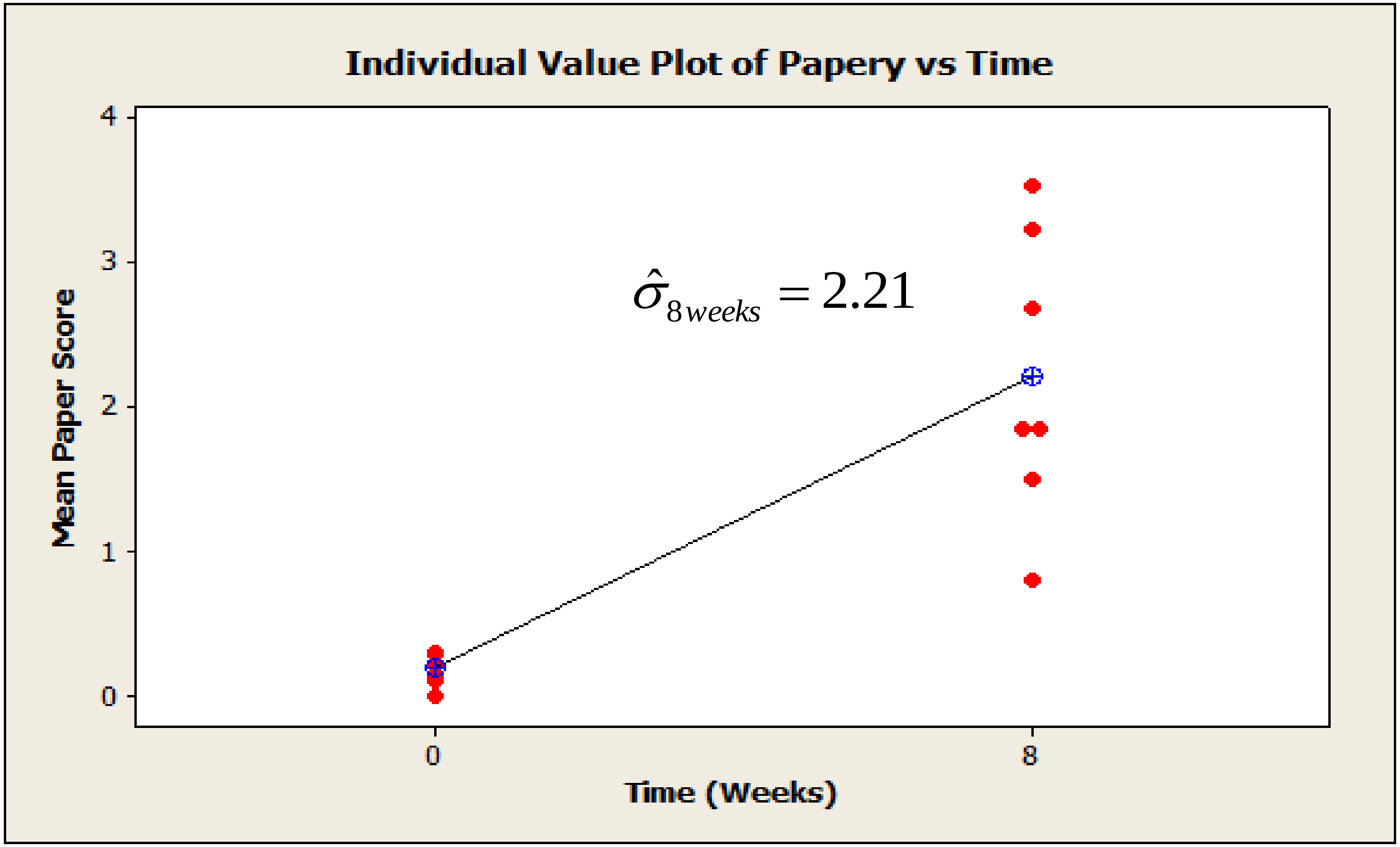
- Methodology for improving key processes
- Pioneered by Motorola in 1980s
- Team Based structured problem solving process
- Deploys "black belts" trained in statistical and quality management tools to facilitate
- Based on

$$Y - \text{vars} = f\left\{X - \text{vars}\right\}$$

Define Phase

- Papery Off-Notes formed after 8 weeks at 75 °F

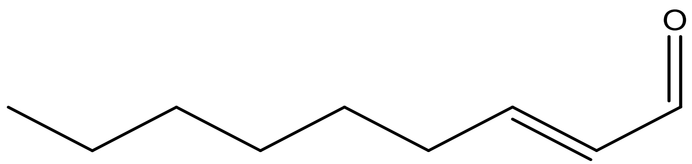
  Y_1 = Mean Papery Score Panels at 8 weeks



Define Phase

- Investigation into compounds associated with papery

  Y_2 = free *trans*-2 nonenal at 8 weeks



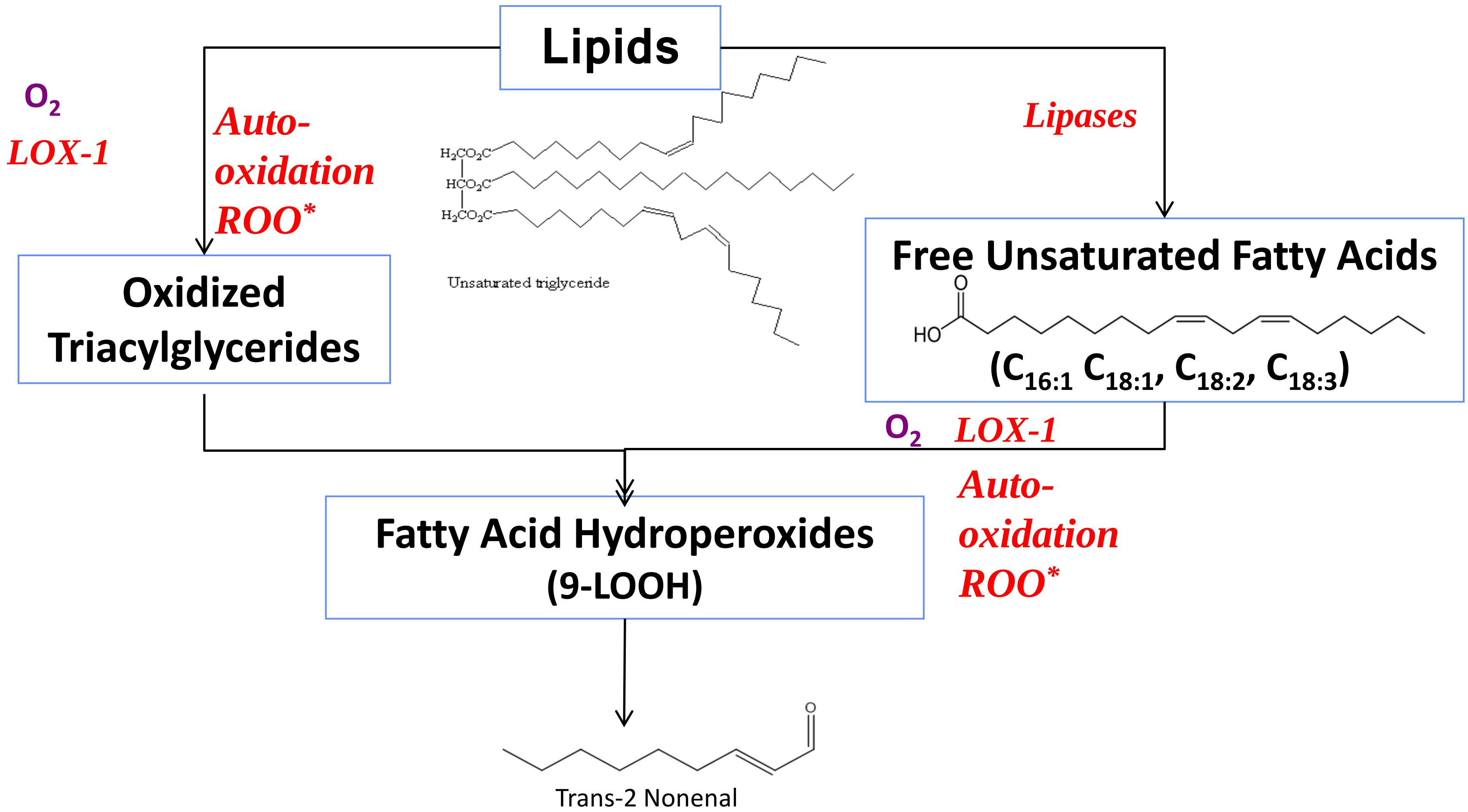
- Reported flavor thresholds (μg/l)

  0.030 - Saison *et al.* (2009)

  0.050 - Van Eerde & Strating (1981)

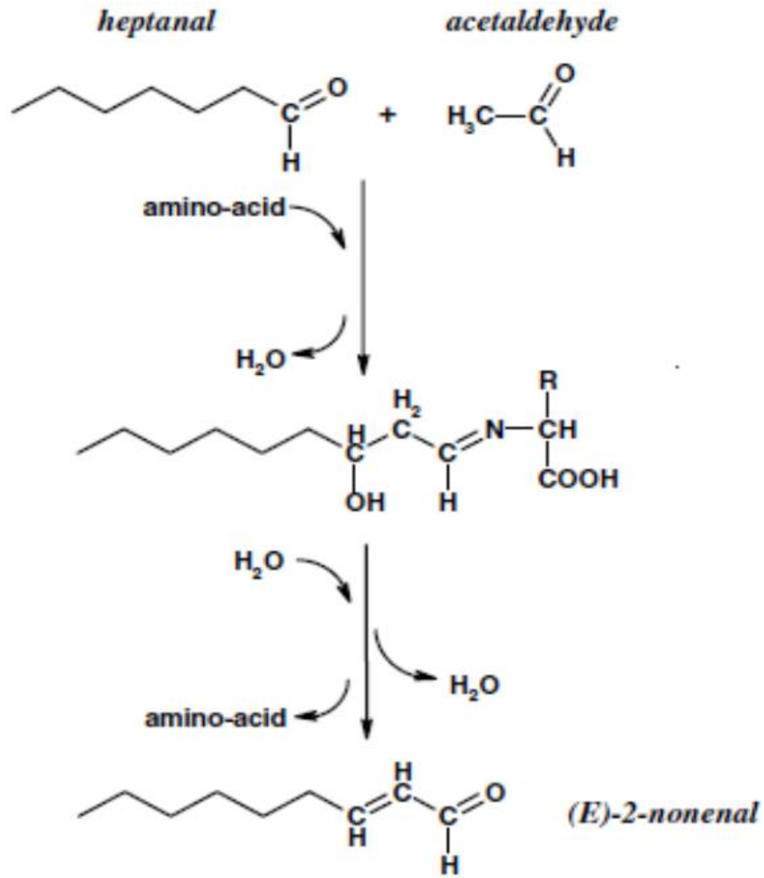
  0.110 - Meilgaard (1975)

Mechanisms of *trans*-2 Nonenal Formation



Mechanisms of *trans*-2 Nonenal Formation

- Aldol condensation (Hashimoto & Kuroiwa, 1975)



Fate of *trans*-2 Nonenal

- Yeast can only reduce "free" *trans*-2 nonenal to flavor neutral nonenol through reductase enzymes

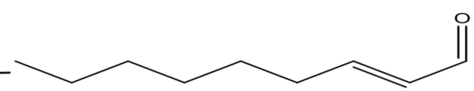
  (Eg. NADPH-dependent aldo-ketoreductases).

- Bound Forms:

  Imine Complexes – Lysine & Proteins- Brewhouse

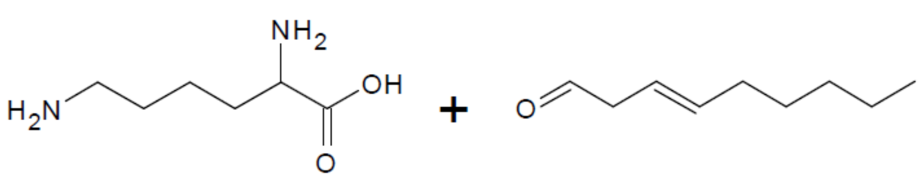
  Sulphite Adducts – Formed during fermentation

*t*2N can form an imine complex (Schiff Base) and be protected during Fermentation



*t*2N can form reversible & irreversible bisulfite adducts

*t*2N can be reduced by yeast to nonenol

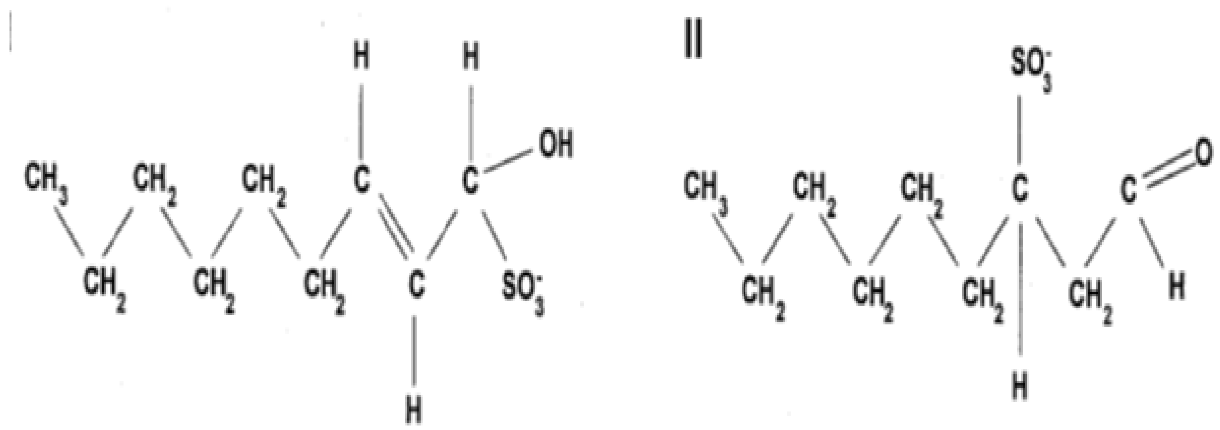


Lysine

trans-2-Nonenal

Reapparance of free *trans*-2 Nonenal

- Acid hydrolysis of Schiff base products
- Dissassociation of bisulphite complexes.



- Acetaldehyde ← SO_2-t2N Adducts (Burger *et al*. 1954; Barker *et al*. 1983)

$$CH_3CH_2OH + {}^{\bullet}OH \rightarrow CH_3C^{\bullet}HOH + H_2O$$
$$CH_3C^{\bullet}HOH + O_2 \rightarrow CH_3C(OO^{\bullet})HOH$$
$$CH_3C(OO^{\bullet})HOH \rightarrow CH_3CHO + O_2^{\bullet -} + H^+$$

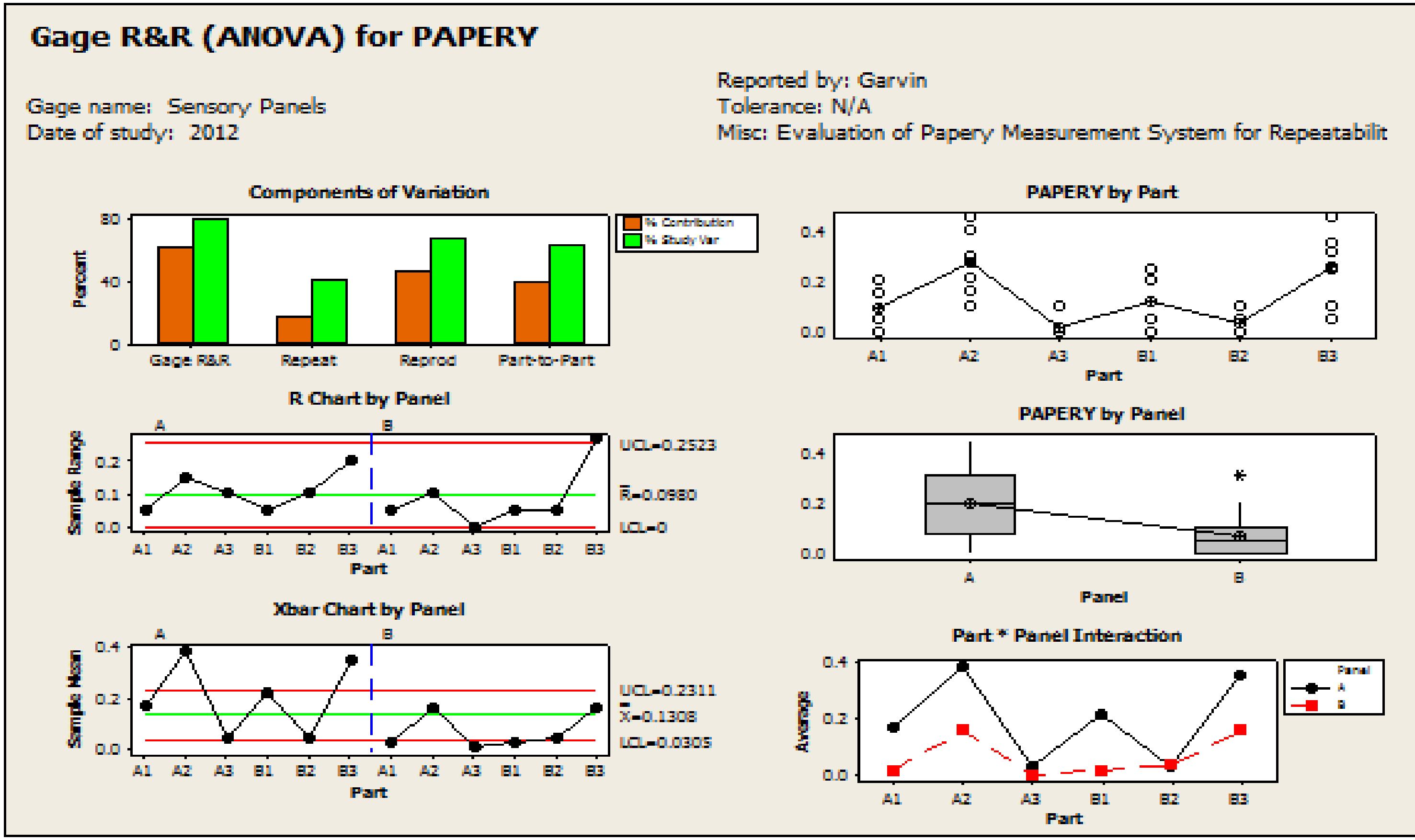
Hydroxyl radical mediated oxidation of ethanol results in acetaldehyde formation

Measure Phase

- Descriptive Sensory Analysis for Papery
  Trained Panelists, 0-10 Scale, n ≥10
- Gage R&R
  - 6 Samples
  - Two panels (trained/calibrated panelist)
  - n=3 replicates
  - ANOVA Method for repeatability/reproducibility
- Discrimination ratio criteria

$$D_R = \sqrt{\frac{2\hat{\sigma}^2_{8week}}{\hat{\sigma}^2_{\text{repeatability}}} - 1} > \quad 4$$

Papery Gage R&R Results



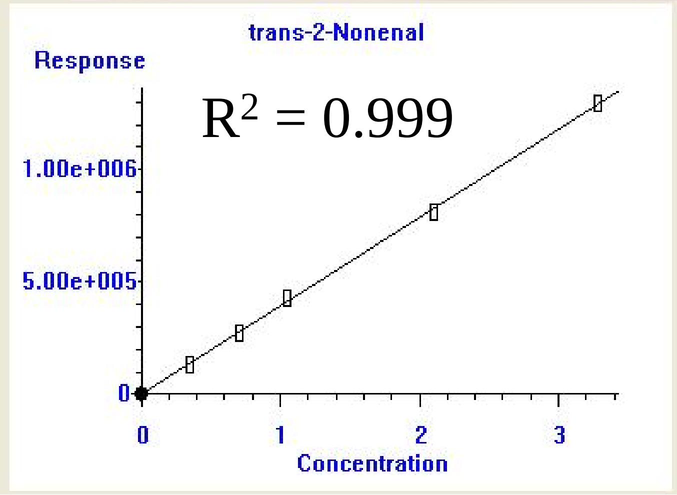
$$\hat{\sigma}_{repeatabilty} = 0.064$$

$$\hat{\sigma}_{reproducibility} = 0.106$$

$$D_R = \sqrt{\frac{2\hat{\sigma}^2_{8week}}{\hat{\sigma}^2_{\text{repeatability}}} - 1} = \sqrt{2\frac{(2.21)^2}{(0.064)^2} - 1} = 48.8$$

Measurement of *trans*-2 Nonenal

- Method based on Vesley *et al*. (2003) in conjunction with in-house modification and optimization
- Adsorption - SPME fiber coated with PFBHA as the derivitisation agent for selective on-fiber reaction (Poster R. Ortiz: A-91)
- Separated by GC (Agilent 7890A) and detected by electron ionization mass spectrometry (Agilent 5975C)
- Internal standard 3-Fluorobenzaldehyde

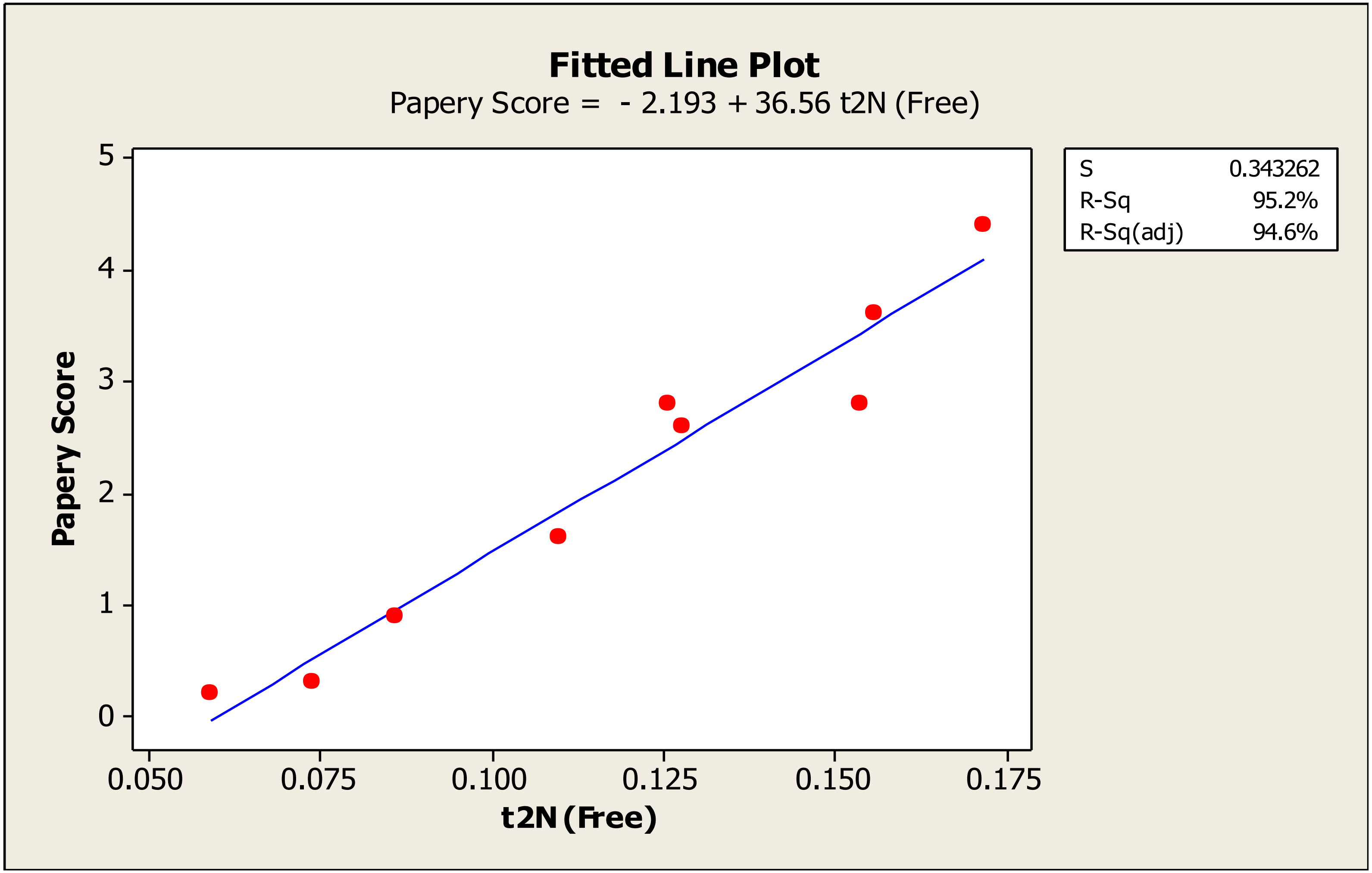


Measurement of *trans*-2 Nonenal

- 6 Sigma Notation:
  Y-vars = consumer perceives
  y-vars = variables with correlation to Y-vars

- Bound form *trans*-2 Nonenal (beer and wort) determined using method of Drost *et al*. (1990)
  - pH adjust to 4.0 (wort only)
  - purged with N_2
  - boiled 120 minutes
- Wort Nonenal Potential y_{wort}
- Beer Total *trans*-2 Nonenal y_{beer}

Preliminary Correlation



Measure Phase – List Potential X-vars

Process Inputs	Class	Proces Step/Purpose	Process Outputs
Foundation Water (40 bbls)	C	Lautering (Separate out extract from solids and sparge out sugar from grains	Kettle Wort Clarity (Turbidity - Lipids)
Foundation Water Temperature	M		Wort Volume (1040 bbls)
Rake Speed During Lauter Tun Filling	C		Wort Gravity
Start of Volrlauf (Time)	C		Wort pH
Vorlauf Time (min)	C		
Vorlauf Speed (gals /min)	C		
Set Point (Turbidity - EBC 40) to start first runnings	C		
Initial Delta P across the bed (Good filter bed)	M		
Turbidity Meter Calibration	SOP		
Bed Exposure to air	SOP		
Sparge (2nd Runnings) Water Volume ()	C		
Sparge (2nd Runnings) Water pH	M		
Sparge (2nd Runnings) Water Temperature (78C)	C		
Sparge (2nd Runnings) water Flow Rate (gals/min)	C		
Sparge (3nd Runnings) Water Volume ()	C		
Sparge (3nd Runnings) Water pH	M		
Sparge (3nd Runnings) Water Temperature (78C)	C		
Sparge (3nd Runnings) water Flow Rate (500 gals/min)	C		
Bed Raking (Rake Hgt is controlled by Delta P level)	C		
Deep Bed Rakes (If DP > 30")	C		

Measurement Phase – Prioritization

- Over 200 Process Inputs were documented (malt storage → packaging)
- Brainstormed 5 potential mechanisms

  - inputs could be involved in multiple mechanisms
  - weighted for importance ω_i

Mechanism Involved	Importance
t2N (hydroperoxides) formation/reduction in Process	9
Reversible Sulphite Adducts	6
Schiff Base Products	7
(Acetaldehyde + SO2-t2N adducts)	5
Aldol Condensation (acetaldehyde + Heptanal)	3

- Technique: Cause & Effect Matrix Analysis

Measurement Phase – Prioritization

- Input X-Variable Correlation, ρ_{ij}

Correlation Scale Matrix	
0	No possible C&E relationship
1	Perhaps Indirectly Related
3	Some known Association
6	Documented - Suggested
9	Well known documentation & Tested

- Variable Importance Rating

$$VIR_j = \sum_i \varpi_i \rho_{ij}$$

- SIMAC Classification

Symbol	Description
C	Controllable (PID, Recipe Parameter)
A	Has variability but can be ADJUSTED Through Recipe Changes
M	Only MEASUREABLE
I	IMPOSSIBLE to measure
S	SOPs

Cause & Effect Matrix Analysis

Project Name Papery Off Note Improvements

Cause and Effect Matrix - Prioritization of the X-vars

X-variables	Process Step	Process Inputs	SIMAC Class	Y1	Y2	Y3	Y4	Y5	Variable Importance Rating	Rank Rating
Rating - Importance to Papery Notes in Pkg Beer				9	6	7	5	3		
				t2N (hydroperoxides) formation/reduction in Process	Reversible Sulphite Adducts	Schiff Base Products	(Acetaldehyde + SO2-t2N adducts)	Aldol Condensation (acetaldehyde + Heptanal)		
120	Wort Boiling	Whirlpool Stand Time (10min)	C	6	9	9	0	0	171	1
96	Lautering	Sparge (3nd Runnings) Water Temperature (78C)	C	9	6	6	1	1	167	2
113	Wort Boiling	Total Wort Boil Time (75min)	C	9	6	6	0	0	159	3
105	Wort Boiling	Wort pH	A	6	3	9	3	0	150	4
86	Lautering	Set Point (Turbidity - EBC 40) to start first runnings	C	9	6	3	1	1	146	5
114	Wort Boiling	Wort Boiling Temperature	M	9	0	9	0	0	144	6
92	Lautering	Sparge (2nd Runnings) Water Temperature (78C)	C	6	6	6	1	1	140	7
99	Lautering	# Deep Bed Rakes (Deep Bed Rakes when DP > 30")	M	9	6	3	0	0	138	8
102	Wort Boiling	Hops Addition Time Point	C	9	6	3	0	0	138	8
154	Fermentation	Early Onset of SO2 production	M	6	9	0	6	0	138	8
137	Fermentation	Wort Lipids (UFAs - C18:2)	M	9	6	0	3	0	132	11
45	Mashing	Mash pH - At Mash Tun Full	M	6	6	6	0	0	132	11
91	Lautering	Sparge (2nd Runnings) Water pH	A	6	1	9	1	1	131	13
95	Lautering	Sparge (3nd Runnings) Water pH	A	6	1	9	1	1	131	13
131	Wort Cooling	Wort Solids (% Trub)	M	6	9	3	0	0	129	15
48	Mashing	Speed mash agitator (during grain in)	C	9	0	6	0	0	123	16
49	Mashing	Speed mash agitator (during Proteolytic stand)	C	9	0	6	0	0	123	16
139	Fermentation	Yeast Storage Time (before Pitching)	SOP	6	6	0	6	1	123	16
160	Fermentation	Yeast that is pitched on the 2nd FV (longer storage) (Viability or Temperature induced issues)	M	6	6	0	6	1	123	16
84	Lautering	Vorlauf Time (min)	C	6	3	6	1	1	122	20
47	Mashing	Proteolytic rest time (min)	C	9	1	3	1	1	116	21
94	Lautering	Sparge (3nd Runnings) Water Volume ()	C	6	6	3	0	0	111	22
98	Lautering	Bed Raking (Rake Hgt is controlled by Delta P level)	C	6	6	3	0	0	111	22
38	Mashing	Mash in Water Temperature	?	9	1	3	0	0	108	24
44	Mashing	Mash-in Temperature (Set Pt 50C vs Actual)	C	9	1	3	0	0	108	24

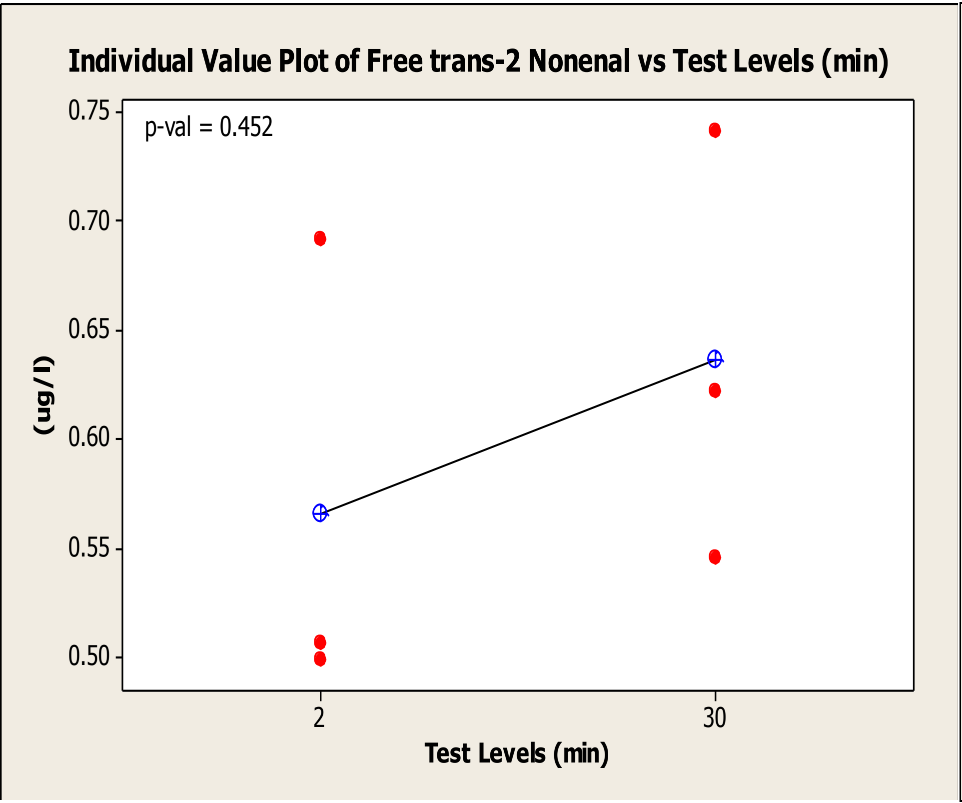
Analyze Phase -

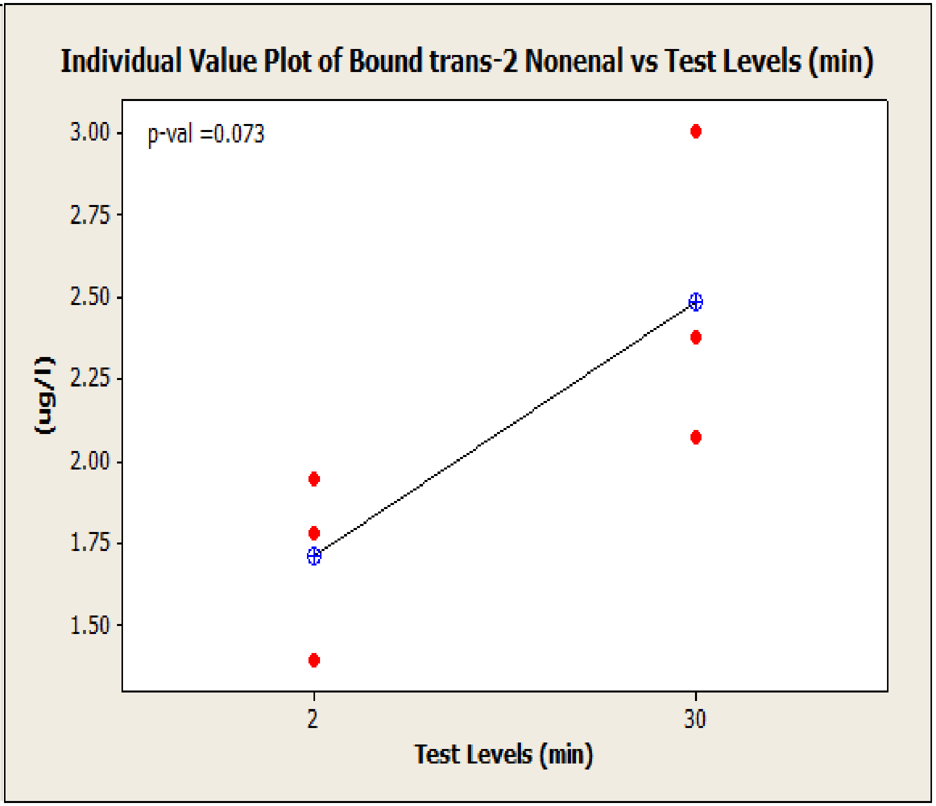
- Narrowed Down List of X-variables

X-Variable	SIMAC Classification	Low (-) Level	High (+) Level	Current Practice
Whirlpool Rest Time	C	2 min	30 min	10 min
Sparge Water Temperature	C	75C	81C	80C
Wort Boiling Time	C	75 min	95 min	75 min
EBC Turbidity (Collection of First Worts)	C	20	100	40
Deep Bed Rakes (#)	M	0	2	Range 0 - 2
Mash in Temperature	C	50 C	62 C	50 C
Mash pH at Mash Tun Full (Acidification)	A	Acidified	No Adjustments	No Adjustments

- One-Way ANOVA Designs
  - y-var = Wort *trans*-2 nonenal (free & bound)

Analyze Phase – WP Rest Time

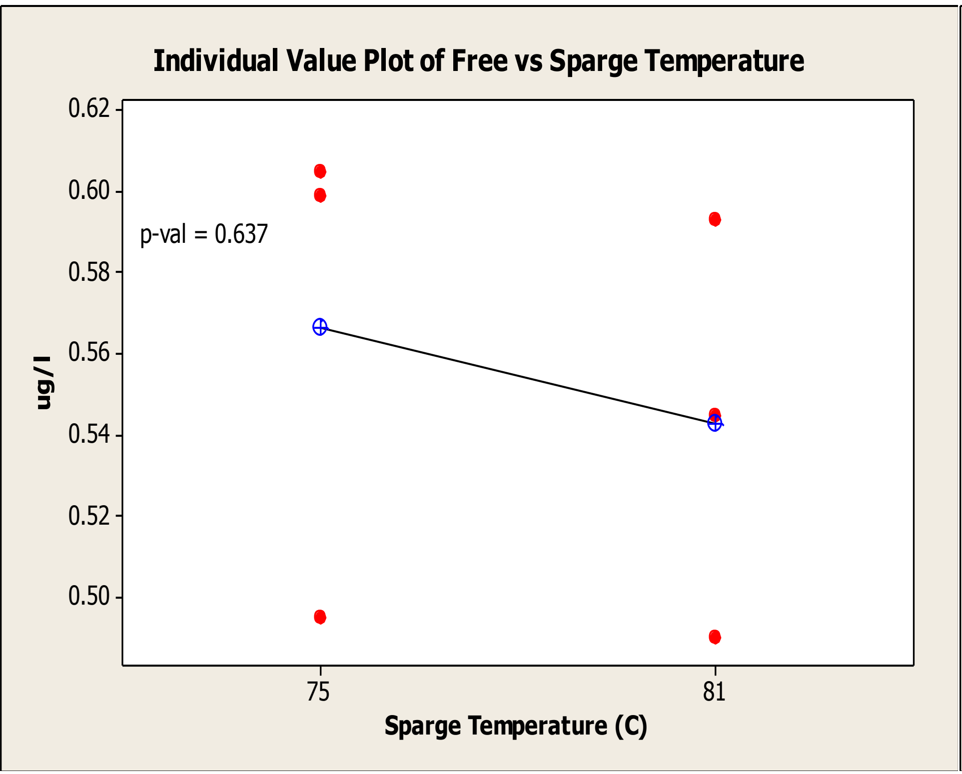


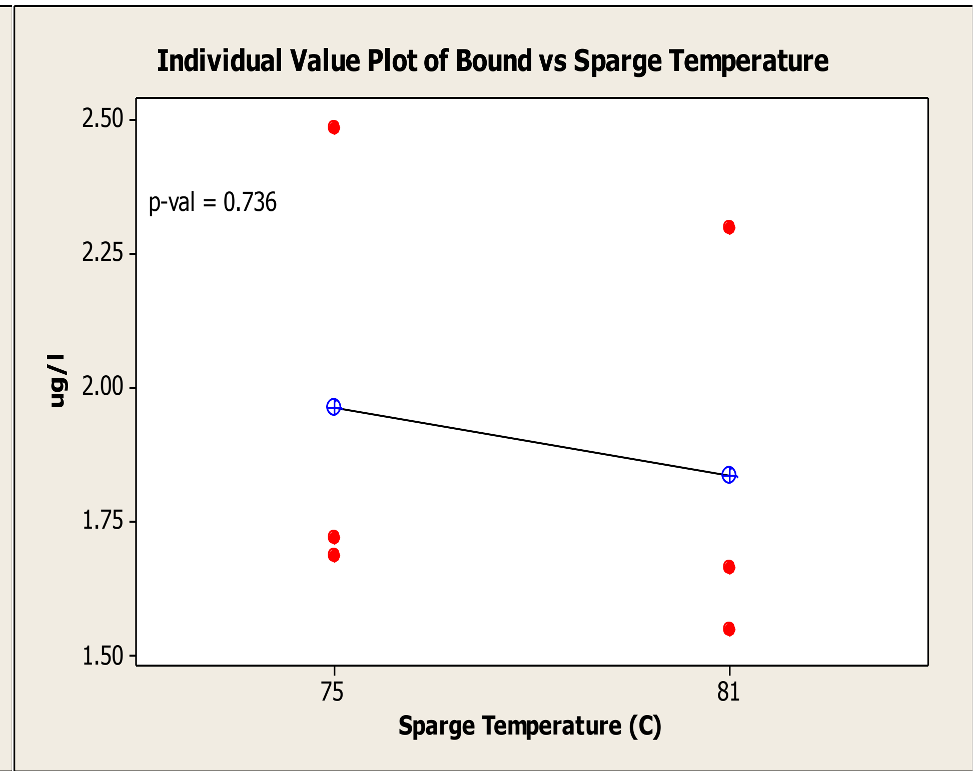


Conclusions:

Evidence exists that a shorter WP Rest will result in lower bound trans-2 nonenal

Analyze Phase – Sparge Water Temperature

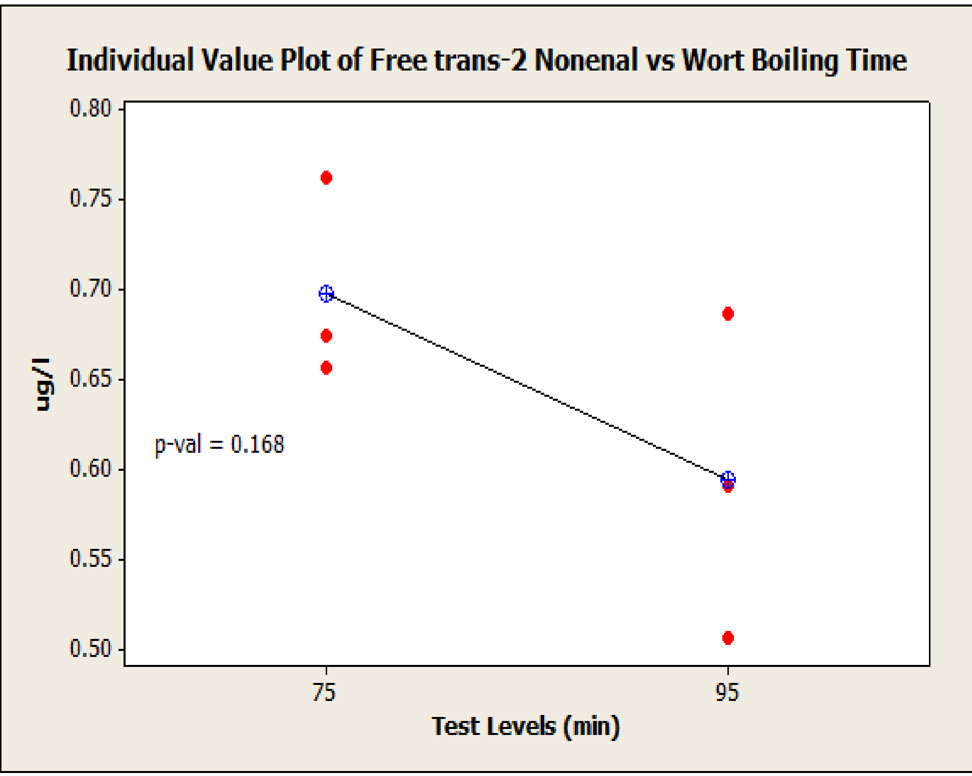


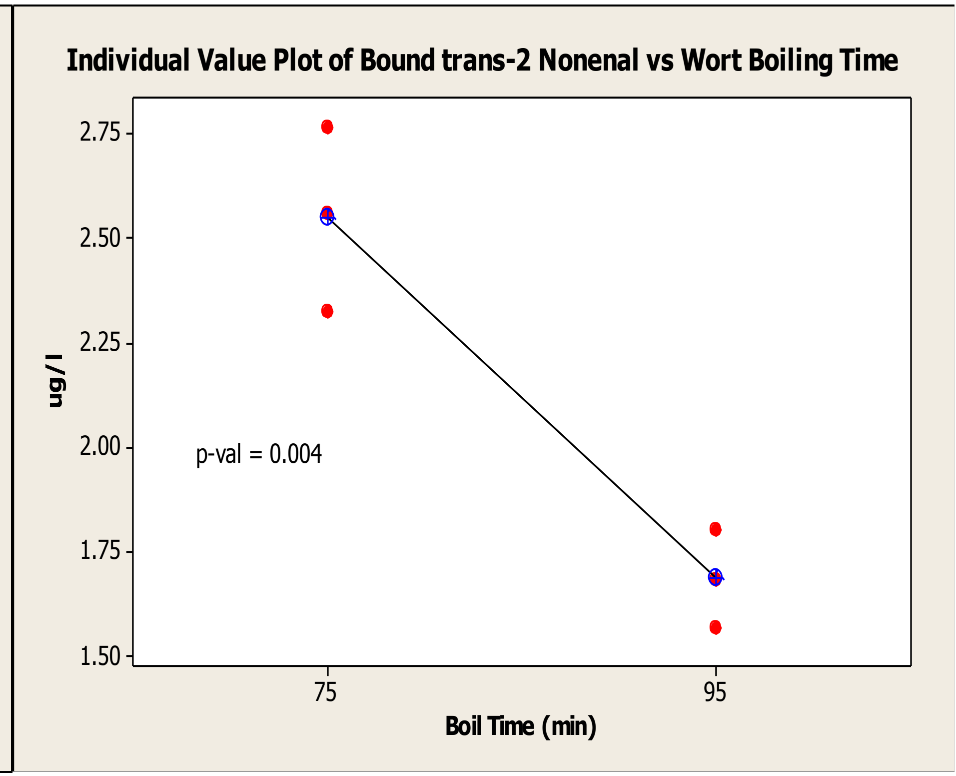


Conclusions:

Lack of evidence that sparge water temperature affects either free or bound trans-2 nonenal

Analyze Phase – Wort Boiling Time

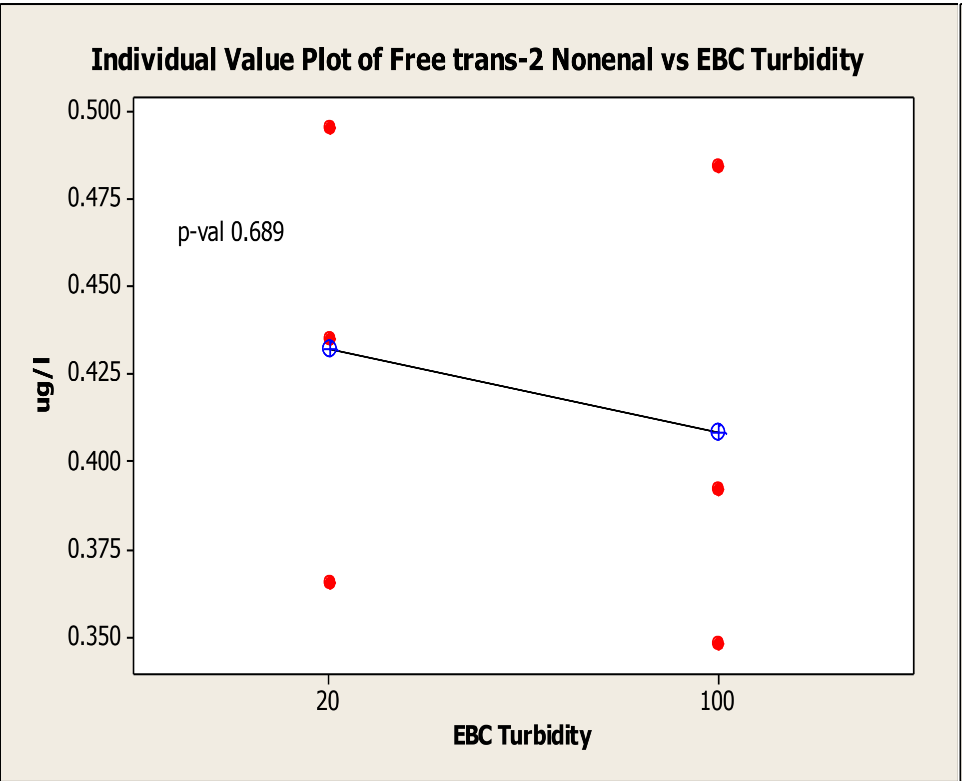


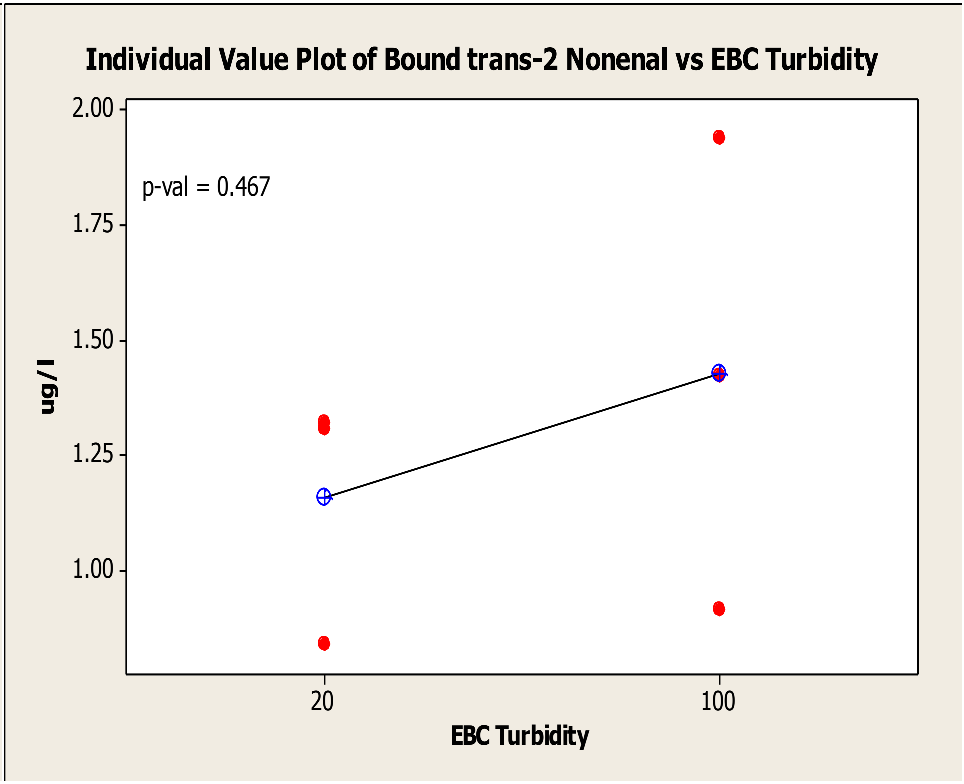


Conclusions:

Evidence exists that longer wort boiling time lowers bound trans-2 nonenal

Analyze Phase – Turbidity Set Point for First Wort Collection

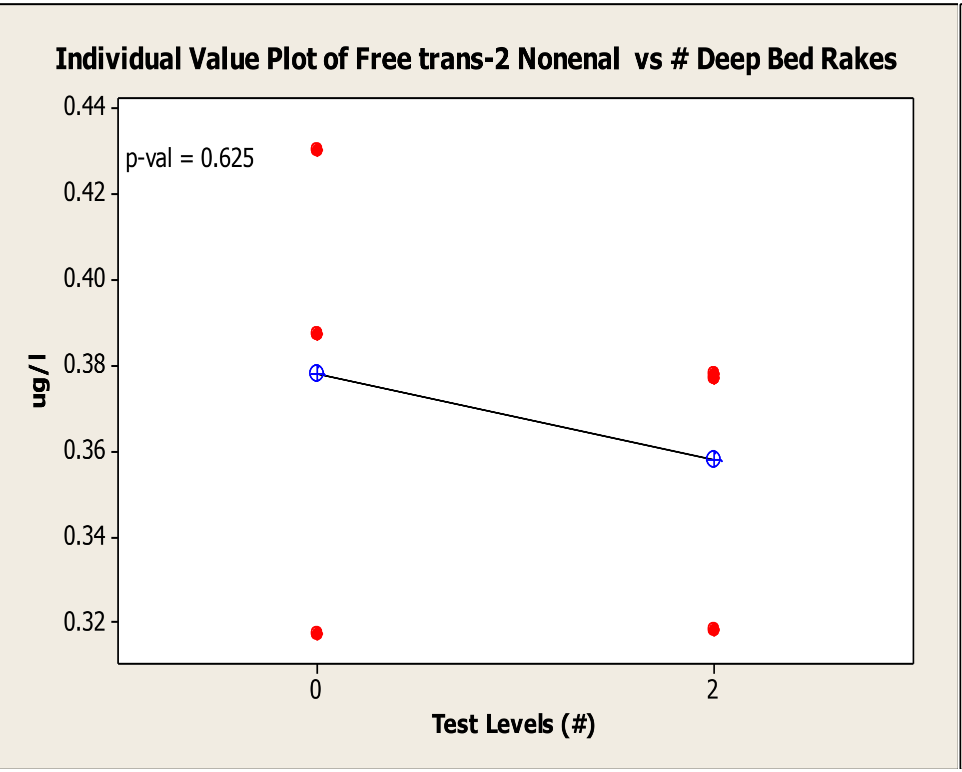


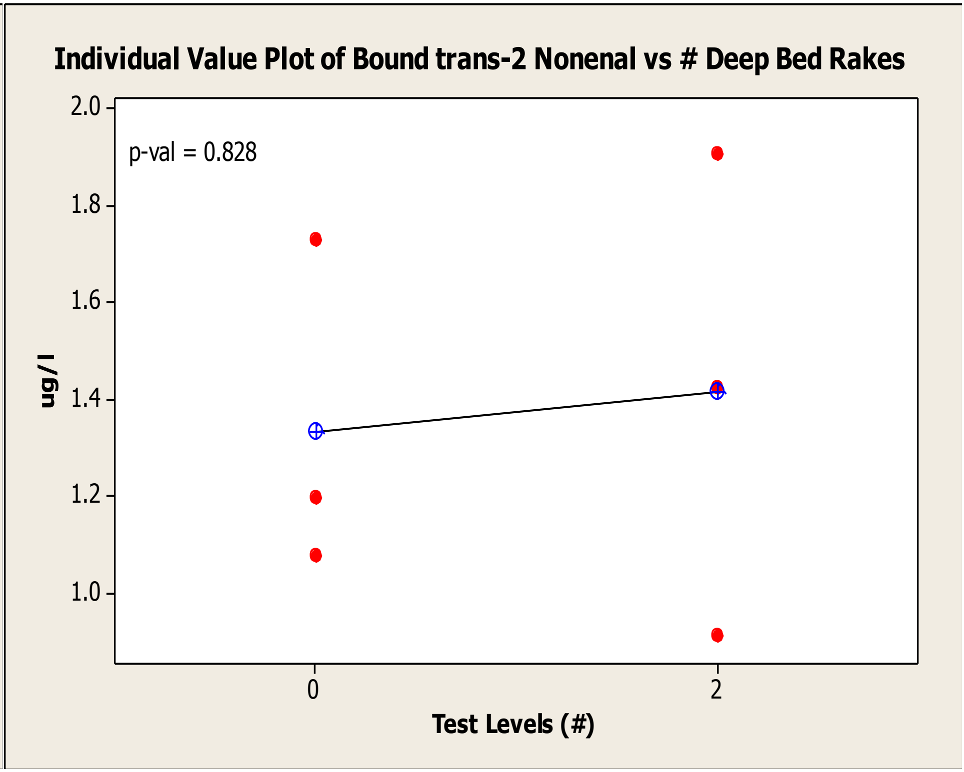


Conclusions:

Lack of evidence that first wort clarity affects either free or bound trans-2 nonenal

Analyze Phase – Deep Bed Rakes

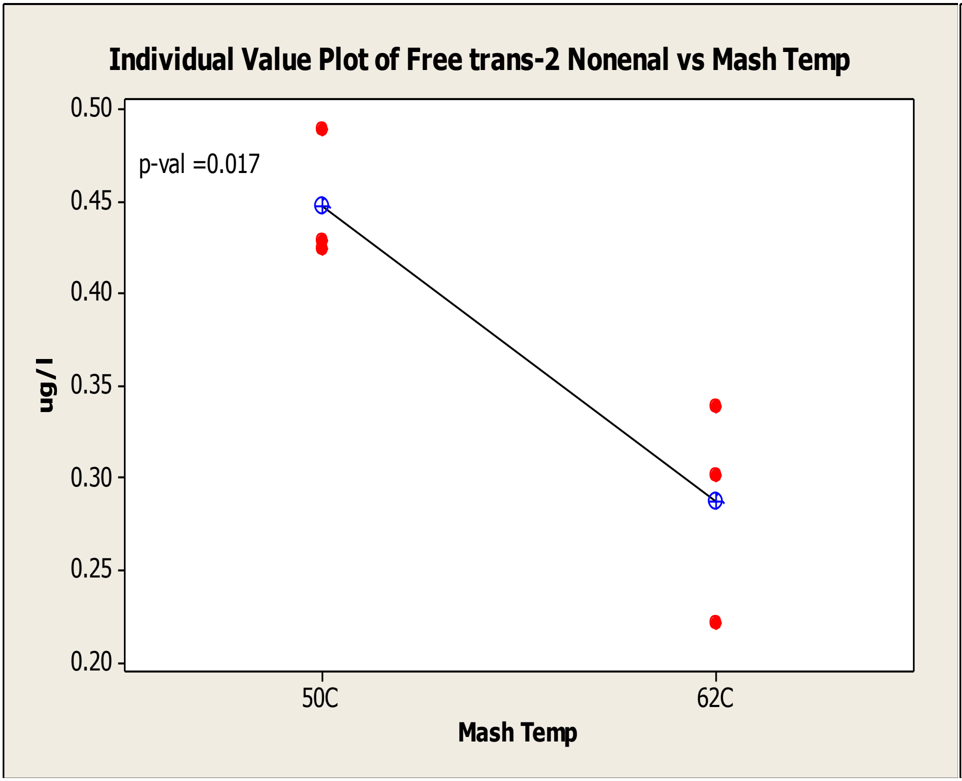


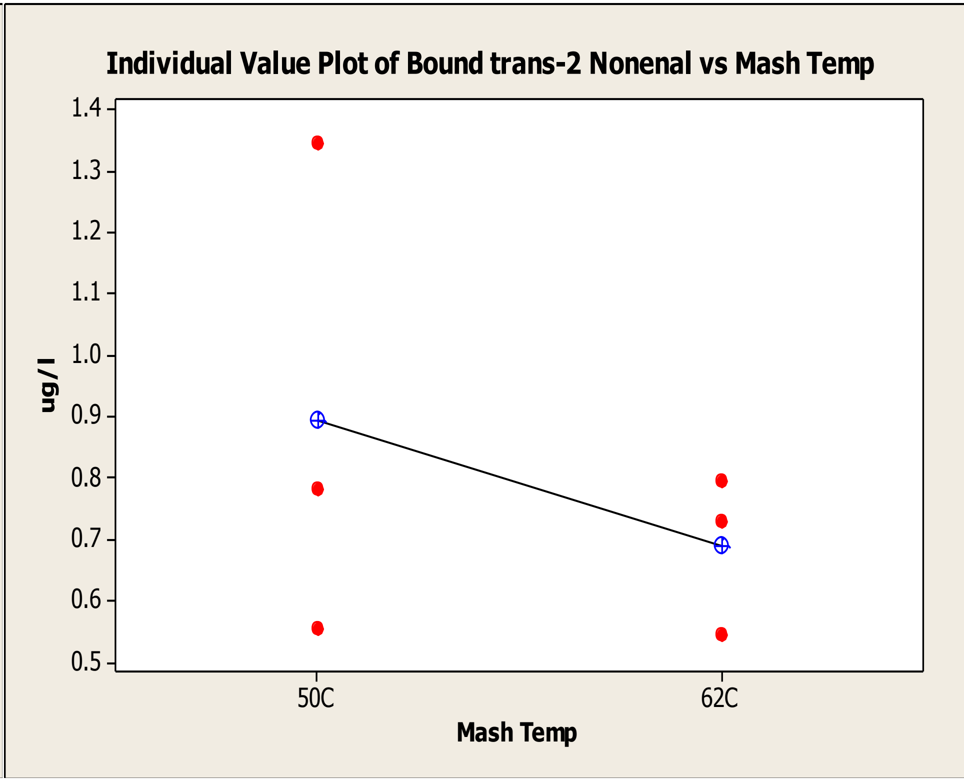


Conclusions:

Lack of evidence that the number of deep bed rakes affects either free or bound trans-2 nonenal

Analyze Phase – Mash-In Temperature



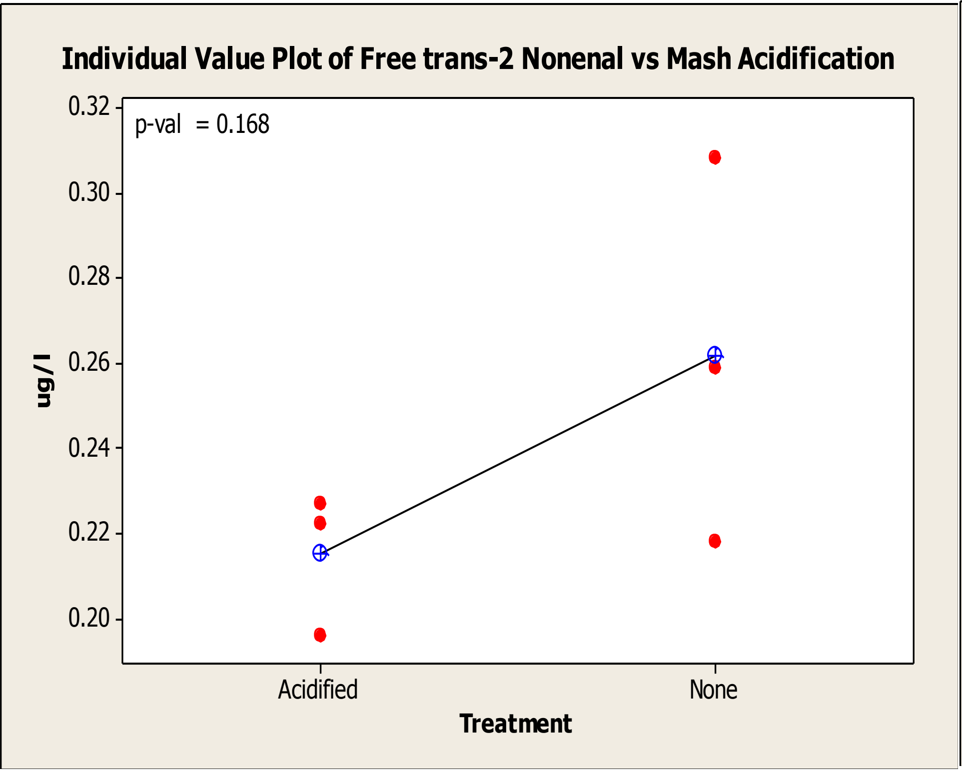


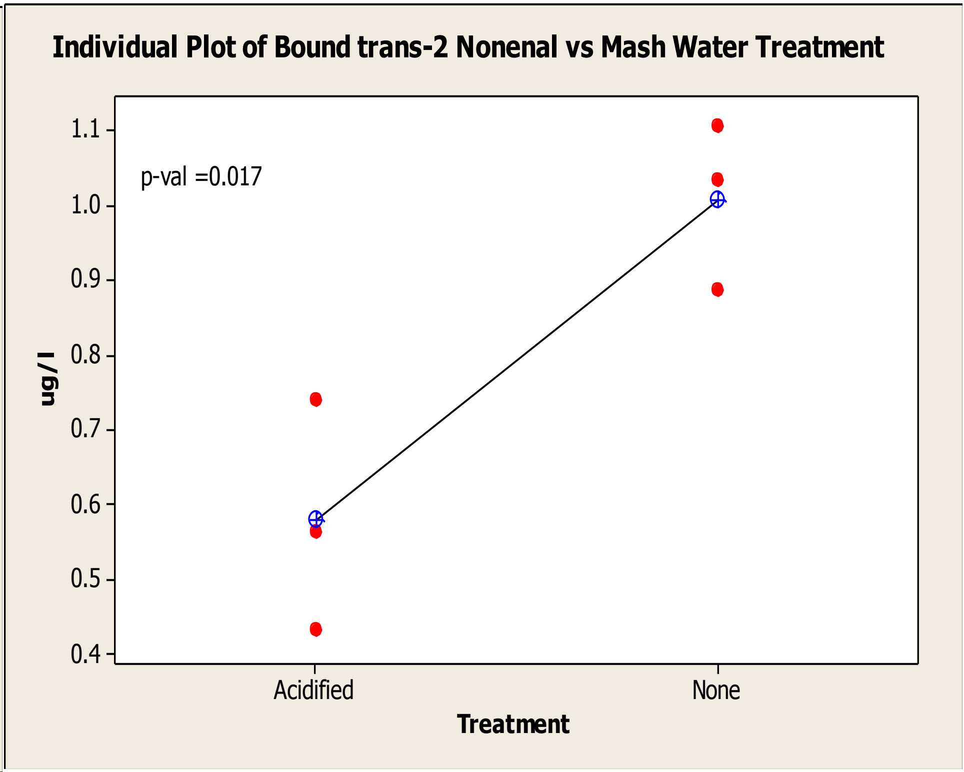
Conclusions:

There is evidence to support that a warmer mash-in temperature lowers free trans-2 nonenal

Analyze Phase – Mash-In pH

Mean pH levels were reduced by 0.27 units with H_3PO_4





Conclusions:

There is evidence to support that a lower mash-in pH lowers bound trans-2 nonenal

Analyze Phase – Trial Recap

	Effect	
X-Variable	**Free *trans*- 2 Nonenal**	**Bound *trans* -2 Nonenal**
Whirlpool Rest Time	-	SHORTER REST LOWERS
Sparge Water Temperature	-	-
Wort Boiling Time	-	LONGER BOIL LOWERS
EBC Turbidity (Collection of First Worts)	-	-
Deep Bed Rakes (#)	-	-
Mash in Temperature	WARMER MASH-IN LOWERS	-
Mash pH at Mash Tun Full (Acidification)	-	LOWER pH @ MASH-IN LOWERS

RED X's Statistically Significant @ $\alpha = 0.10$ Level

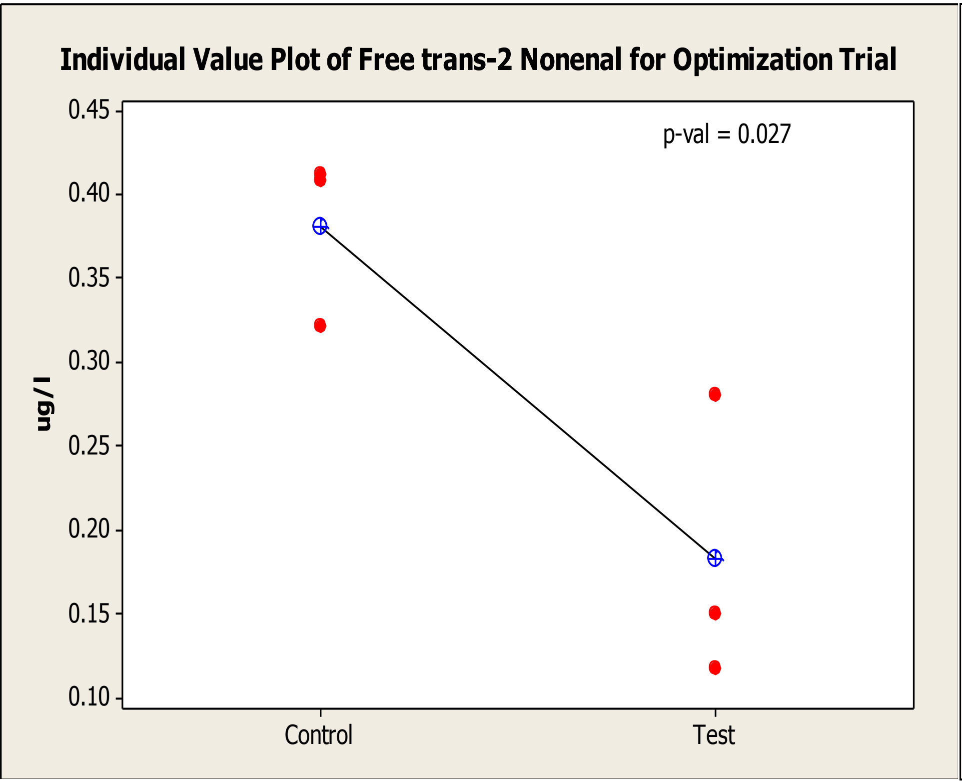
Improve Phase – Optimization Trial

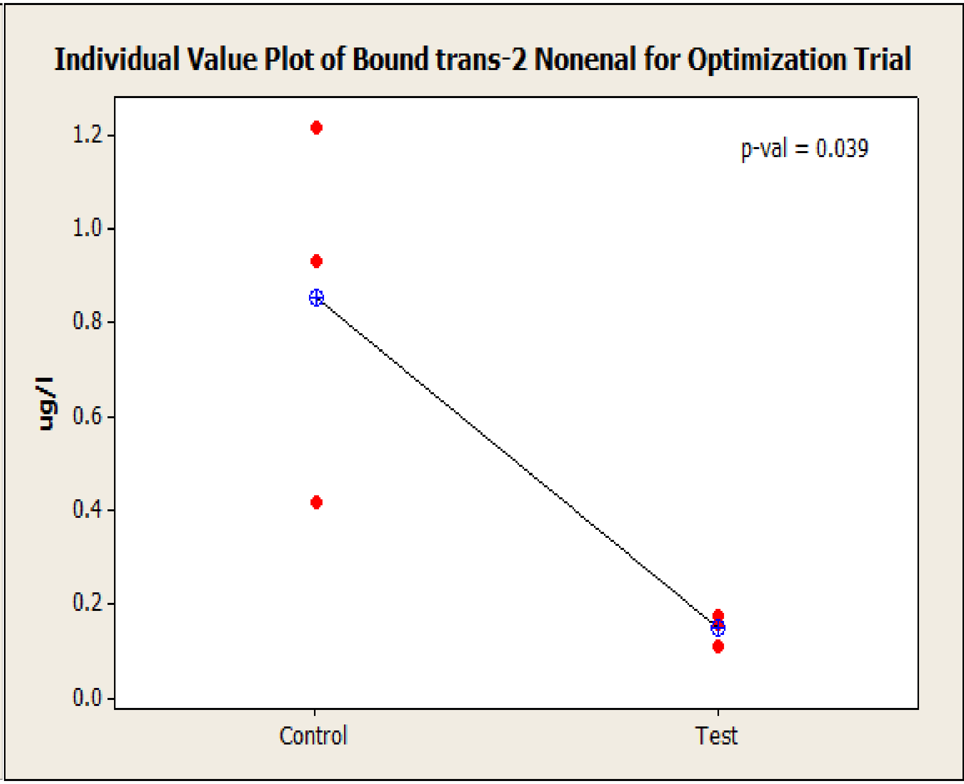
- During the Improve Phase of DMAIC, solutions are implemented, results are evaluated
- Trials executed by setting the Red X-vars simultaneously to their optimal levels and batches were isolated to packaging

X-Variable	Optimal Setting
Whirlpool Rest Time	2 Minutes
Wort Boiling Time	90 Minutes
Mash in Temperature	62 C
Mash pH at Mash Tun Full (Acidification)	Acidified

- Sensory & *trans*-2 Nonenal evaluation (Y-vars) during storage (ambient 75 °F)

Improve Phase – Wort Profile

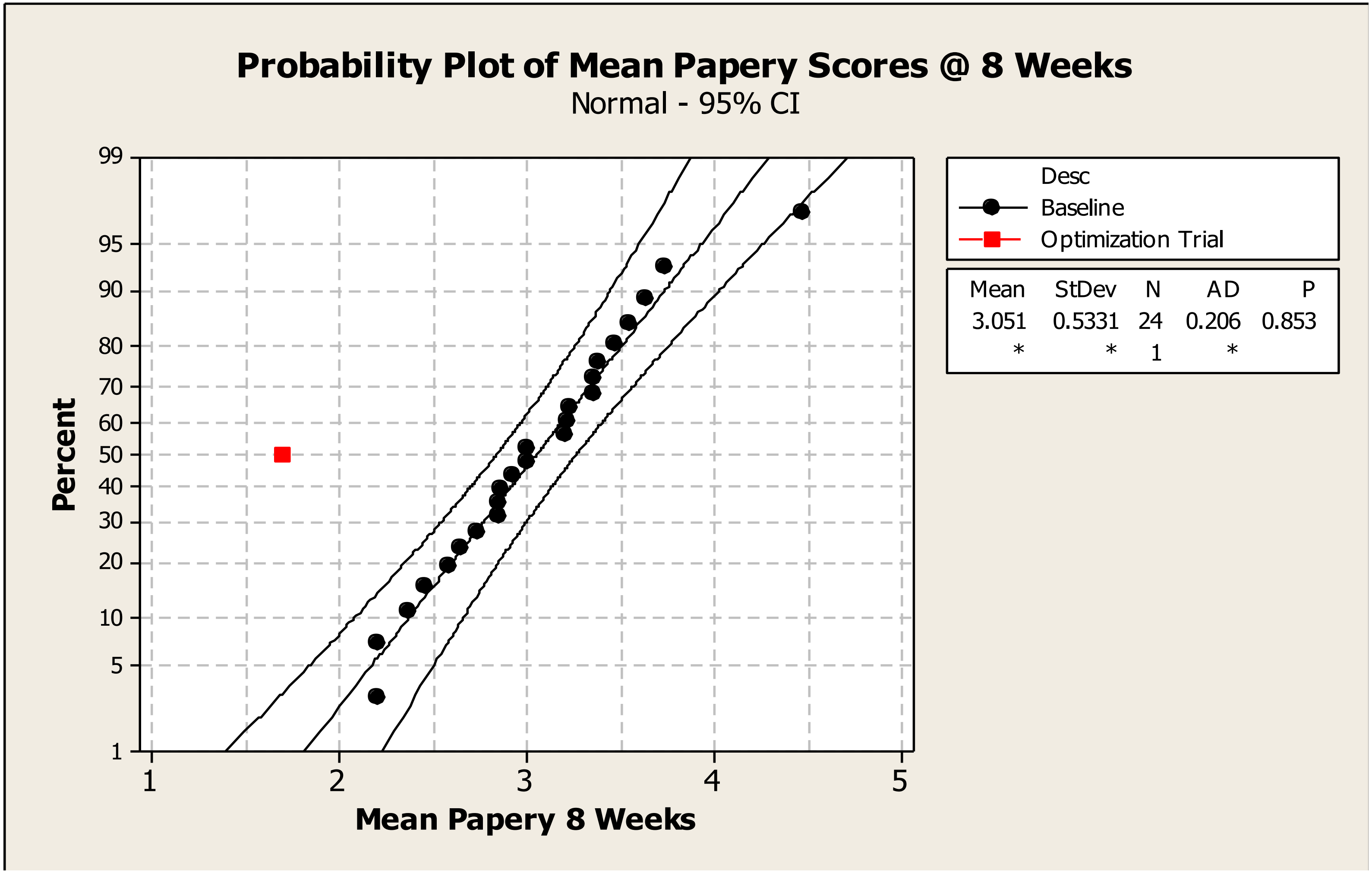




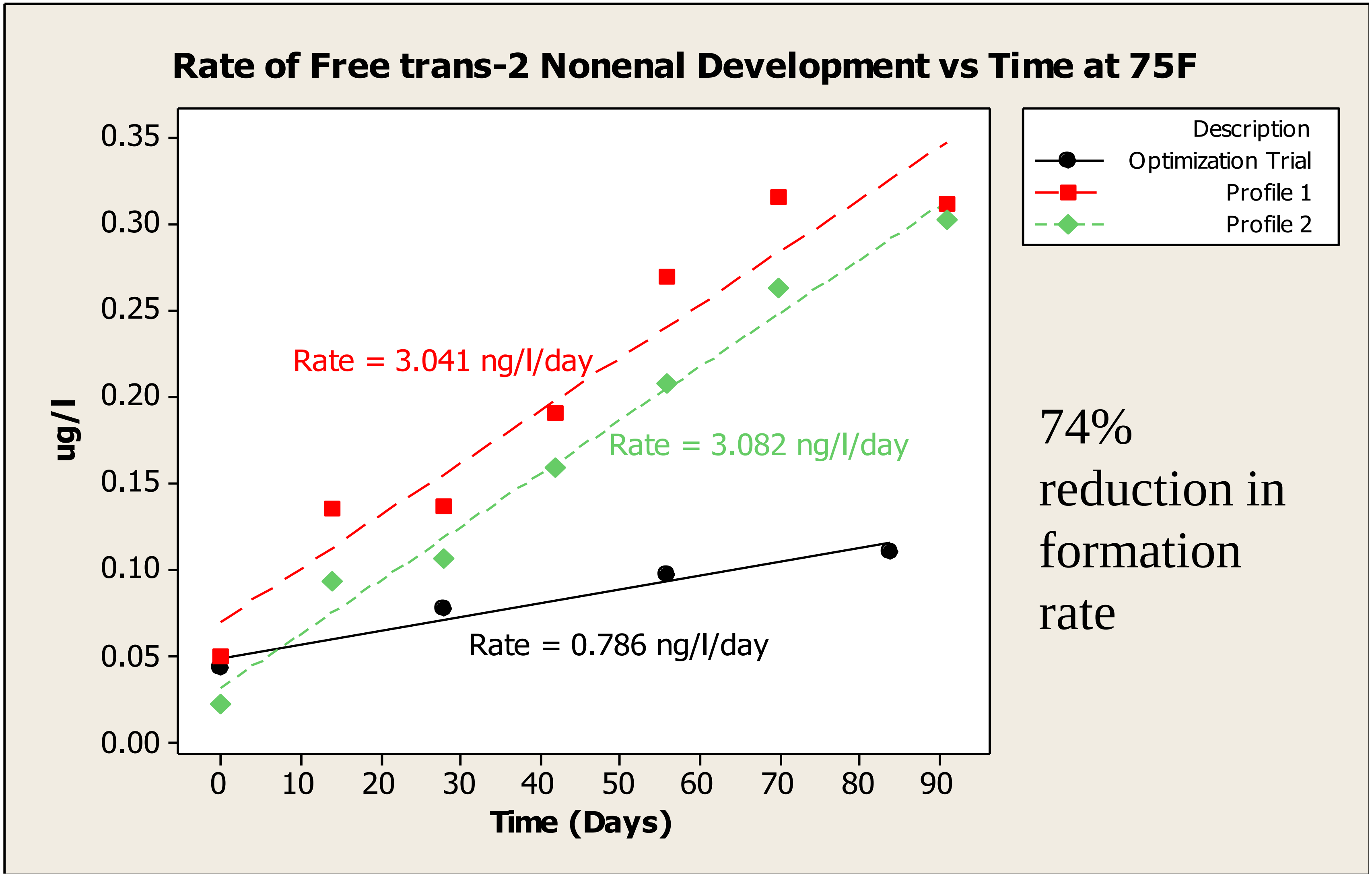
Conclusions:

The results of the Optimization Trial are significantly lower in both free and bound trans-2 Nonenal

Improve Phase – Comparison to Baseline

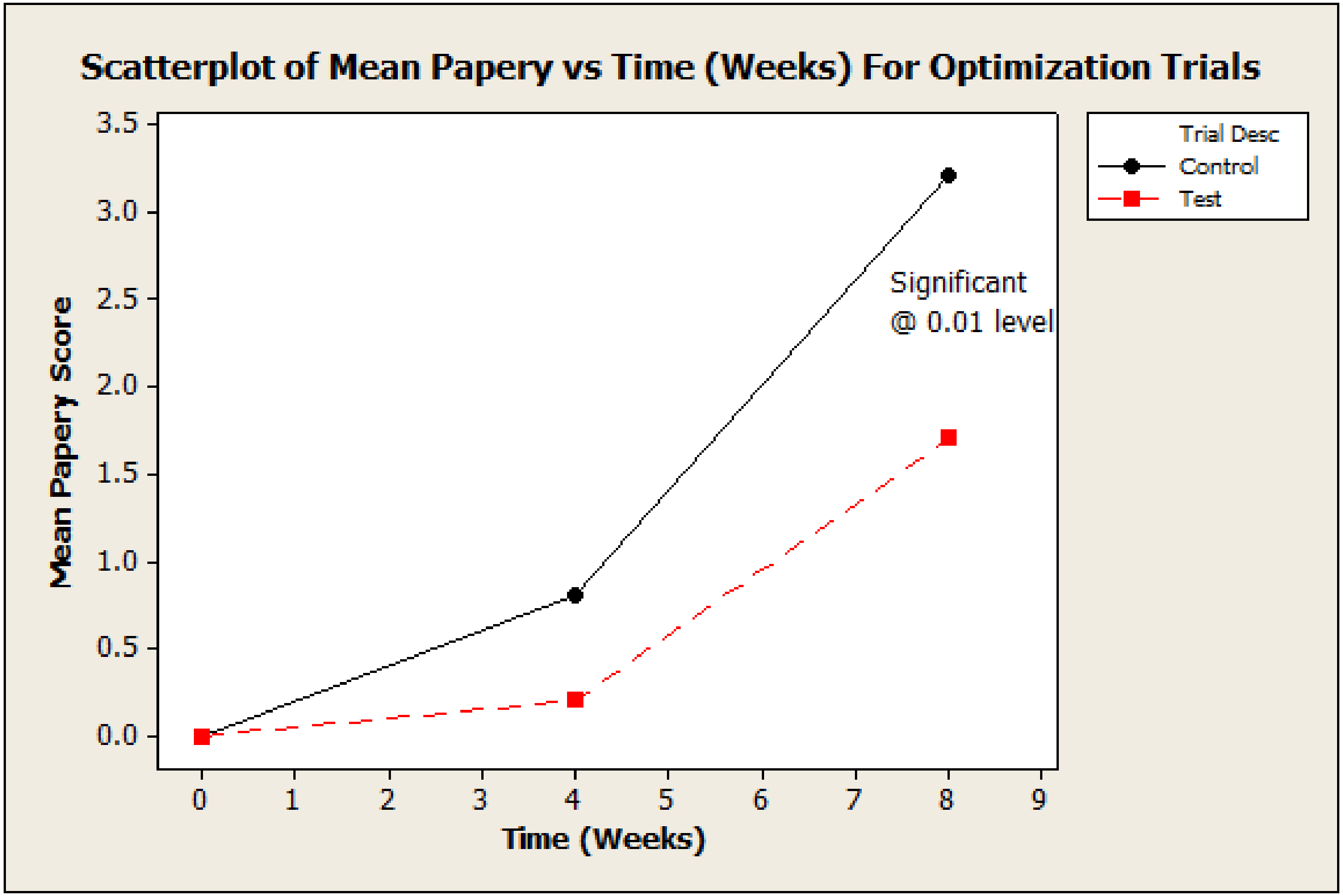


Comparison of Kinetics



74% reduction in formation rate

Improve Phase – Packaging Profiles



Improve Phase – Masking Compounds

- Research Question: What effect does IAA and DMS and their interaction have on the sensory perception of Papery
- Test Design: Base Design 2^2 factorial with replication (eight samples total)

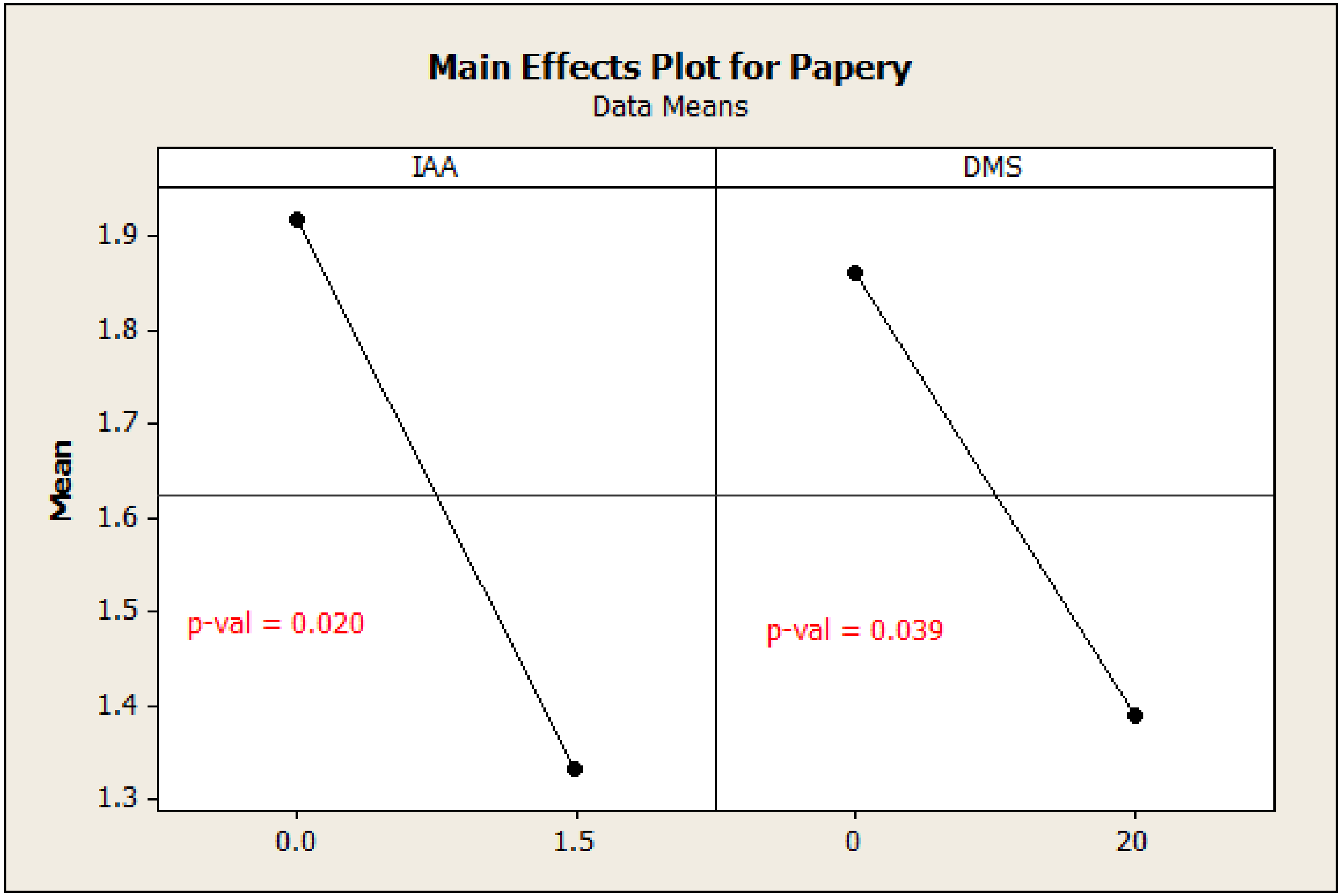
		Low (-)	High (+)
Factor 1	IAA	0 added	+1.5 ppm
Factor 2	DMS	0 added	+20 ppb

Test Design

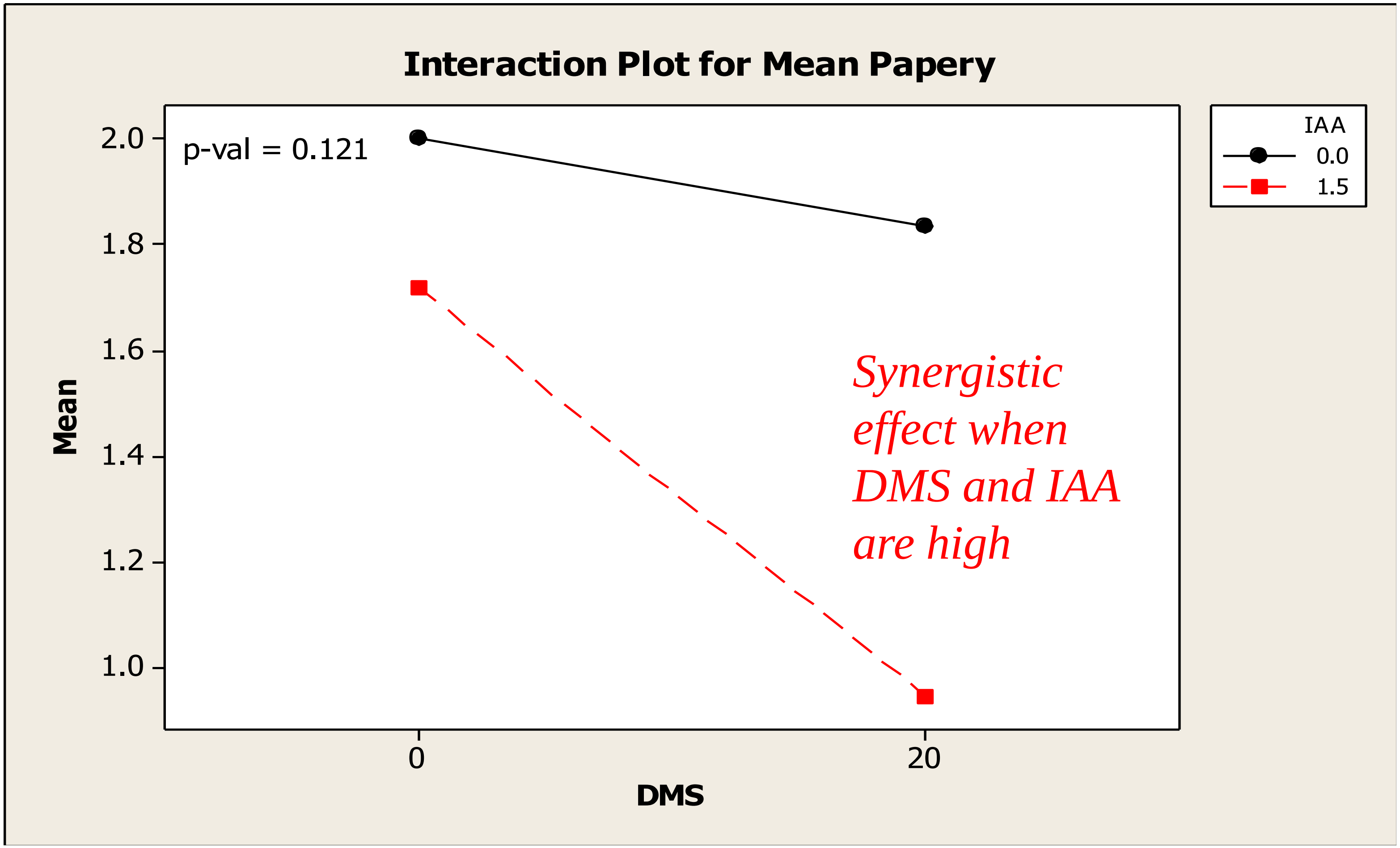
Test Number	IAA	DMS	Papery
1	-	-	2.11
2	+	-	2
3	-	+	1.78
4	+	+	1
5	-	-	1.89
6	+	-	1.44
7	-	+	1.89
8	+	+	0.89

- Panelists:
  - 9 Advanced tasters - blind scaling validation on Papery/t2N with an $R^2 \geq 0.8$.
- Beer stored 6 weeks ambient with significant papery notes were presented blind in a randomized complete block design, with each panelist assessing each of the eight samples. Response variable was the panel mean papery.

Improve Phase – Masking Compounds

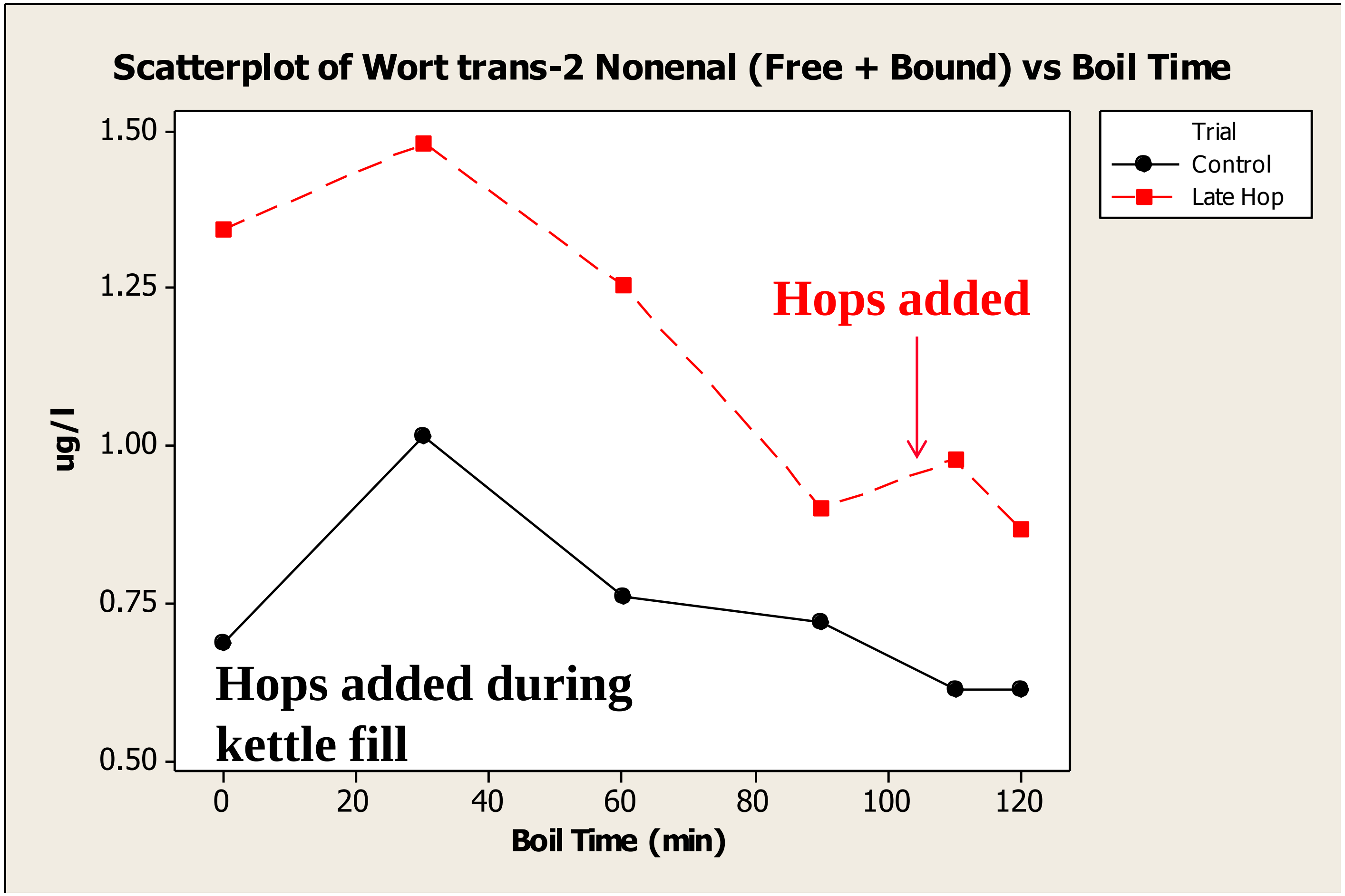


Improve Phase – Masking Compounds



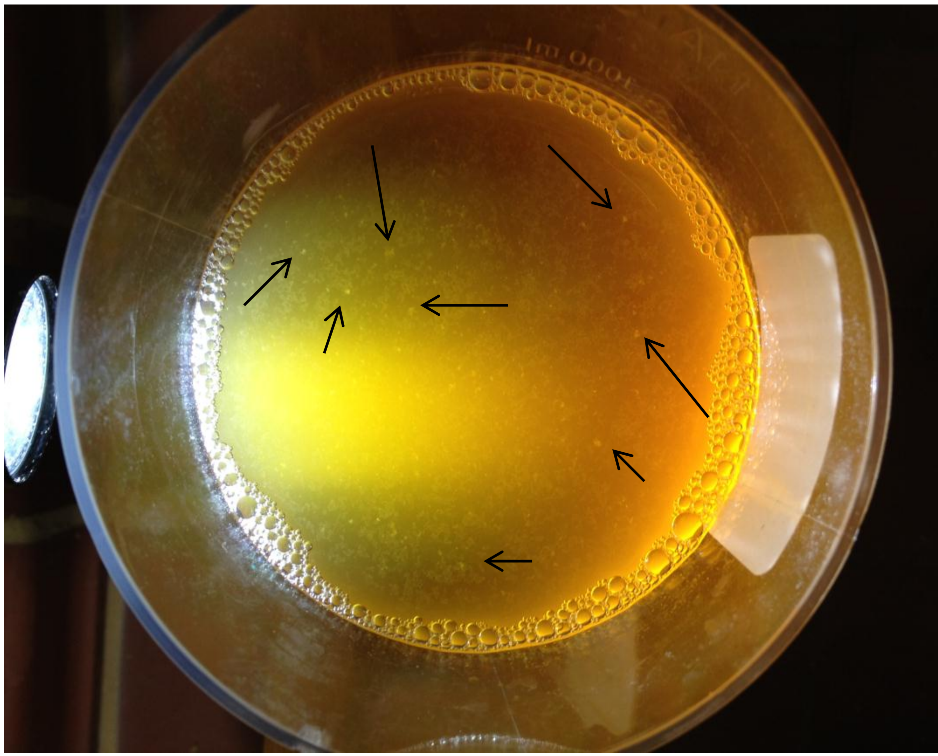
Additional Investigations – Hop Timing

- Lemursieau *et al.* (2001) - Hop Products
- Hop Addition Timing

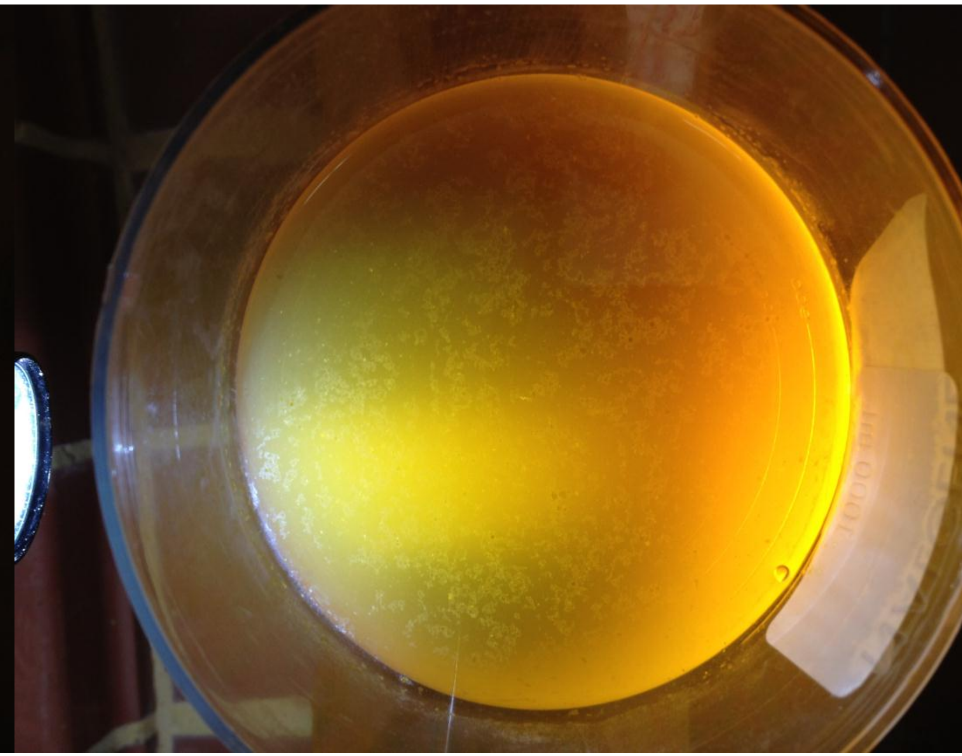


Additional Investigations – Hop Timing

Late Hop - EOB

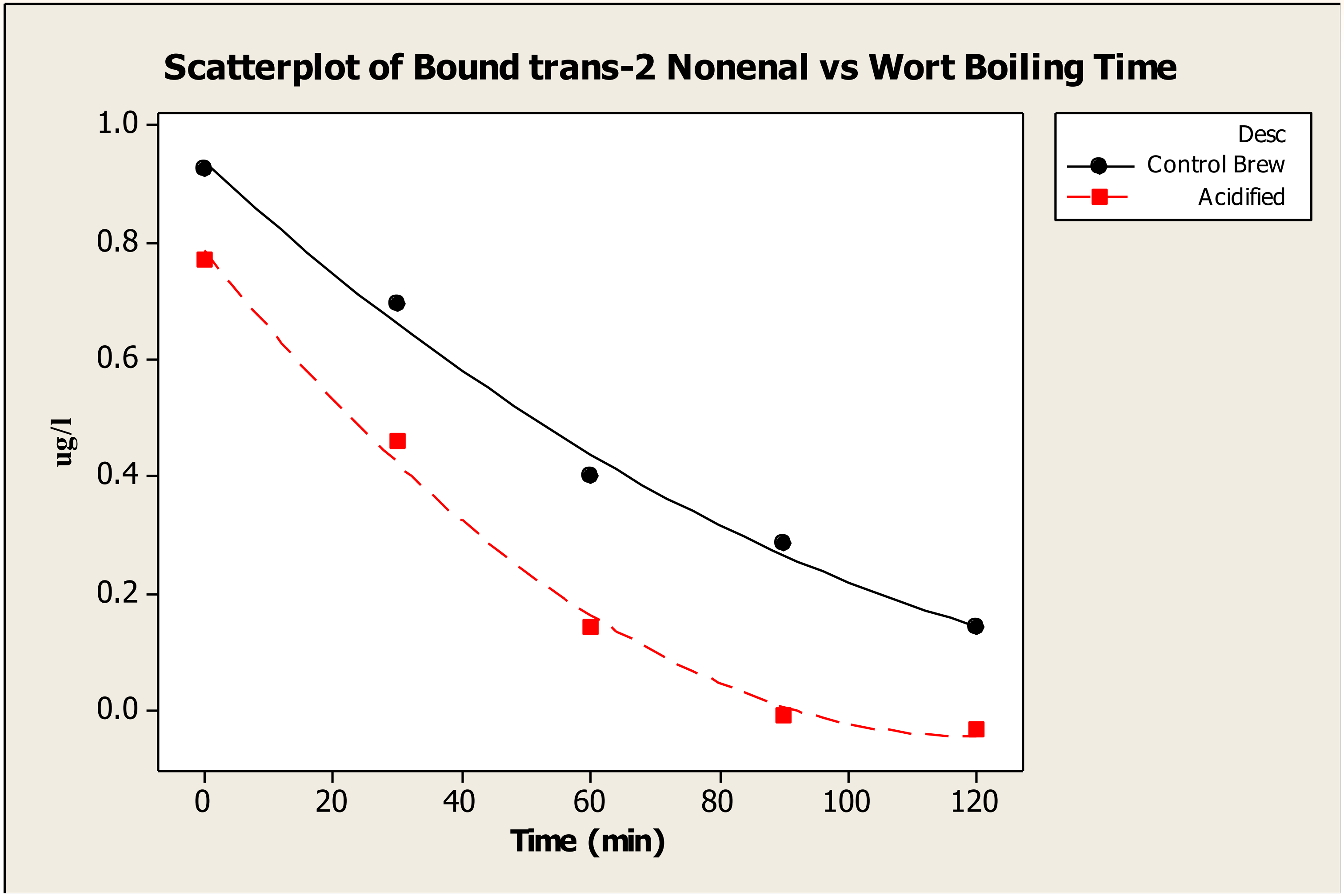


Early Hop - EOB



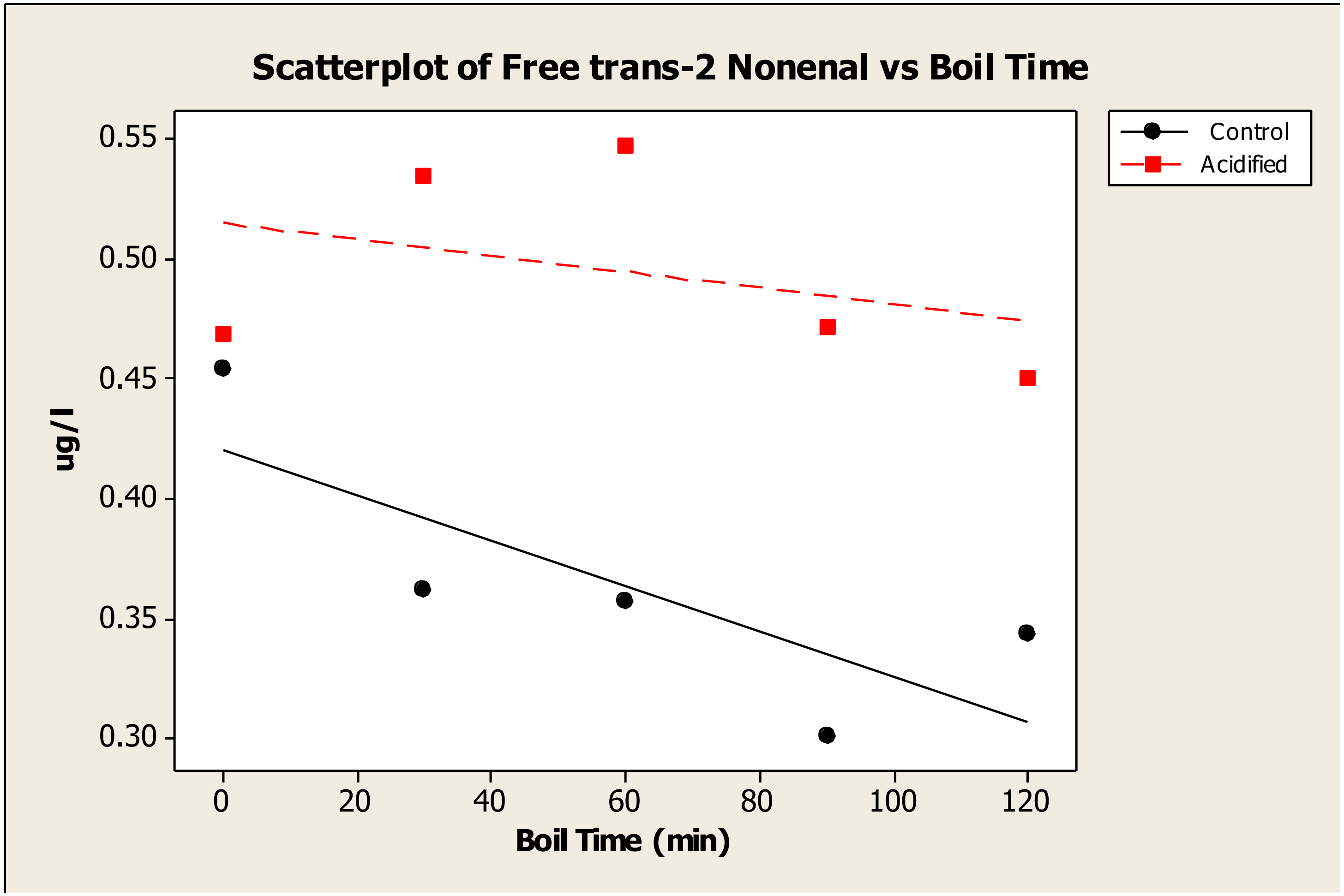
Late-hop supernatant had visually higher particulate (protein flocks) in suspension compared to the early hop brew

Additional Investigations – Boil Time



Bound *trans*-2 Nonenal continues to precipitate out during the boil – Schiff Base Products complexing with Trub

Additional Investigations – Boil Time



Free *trans*-2 Nonenal appears to not change much

Control – Update Your Recipes

Y-Vars (What we are trying to Control That is Important to the Customer)

		Specifications							
Critcal To Quality Y-Variables	Objective Y-var (min. max, target)	LSL	Target	USL	Sample Size	Frequency	By Who	Where Recorded	Reaction Plan
1 Mean Papery Score @ 8 Weeks	Minimize		0	1.2	1 6 pk	1/month	Sensory	Compusens	N/A
2 Freshly Pkg Total t2N (ppb) @ 8 weeks	Minimize		0	0.4	1 can	1/month	Labs	LIMS	N/A
3									

INPUTS KPIVs X-Variables (How are we going to control the outputs)

						Specifications						
Verified X-Factor	Process Step	Effect on Y-vars	How is it to be controlled (SPC, SOP, Audit, PID, Parameter)	Measurement Method	LSL	Target	USL	Sample Size	Frequency	By Who	Where Recorded	Reaction Plan (QFR)
Mash in pH	Mash Tun	As mash in pH increases t2N increases	SPC	Lab Probe	5.3	5.4	5.5	500 mls	1 per day	QC lab	LIMS	Adjust H3PO4
Mash in Temperature	Mash Tun	Increase Temp reduces t2N	PID	In line Temp Proble	59 C	60 C	61 C	N/A	Every Brew	Brewing	MES	
Boil Time (6.25% total Evap)	Wort Boiling	Longer boil reduces bound t2N	Parameter	Control System	89	90	91	N/A	Every Brew	Brewing	MES	
WP Rest Time	Wort Clarification	If WP rest increases, t2N increases	Parameter	Control System	1	2	3	N/A	Every Brew	Brewing	MES	
Hop Addition Timing	Wort Boiling	If HOPS are added later into the boil wort t2N levels tend to increase	SOP	(bbls hops are added)	15% KFV	25% KFV	50% KFV	N/A	Every Brew	Brewing	MES	

Summary

- DMAIC Application was effective
- Controlling the formation of *trans*-2 Nonenal and Papery off-notes can be achieved in the brewhouse

$$Y_{t2N} = F(Mash\, pH, Mash\, in\, temp, Hops\, add, Boil\, Time, WP\, \mathrm{Stand})$$

- We have evidence that other compounds (ie. yeast derived) can mask *trans*-2 Nonenal

- ***BE CAREFUL CUTTING DOWN BOIL TIME***

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