



Discolouring of grape juice concentrate: Causes and possible ways of inhibition

By: M.J. Loedolff

Promoter: Prof. L. Lorenzen

Sponsors: Winetech, NRF

Introduction

- ✦ Applications of grape juice concentrate
- ✦ Manufacturing process
- ✦ Problems experienced
- ✦ Scope of work
- ✦ Objectives

Applications of grape juice concentrate

- ◆ Sweetening of table wines
- ◆ Wine production in countries not suitable for vineyard cultivation
- ◆ Base of juices and cooled drinks
- ◆ Food sweeteners
- ◆ Baked goods
- ◆ Baby foods, yoghurts and ice creams
- ◆ And more

Background: Manufacturing process

✦ Boiling in open pots: “Moskonfyt”

✦ Historically/Currently

- ◆ Distillation processes

- Multiple effect evaporation

- ◆ Freeze concentration

✦ New developments

- ◆ Centrifugal evaporation processes

- ◆ Reverse osmosis

Problems experienced

- ✦ Tartrate instability
- ✦ Sugar crystallisation
- ✦ Fermentation
- ✦ Foul tastes and offensive odours
- ✦ Discolouring or Browning of juice

Scope of work

✦ KVV plant at Robertson

- ✦ Experience browning problems during storage of juice concentrate
- ✦ Increased operating cost
- ✦ Increased solid and liquid waste production

✦ Plant has since stopped production

- ✦ Marginal profits

Objectives

- ✦ Background and Literature study
- ✦ Development of method of analysis
- ✦ Investigate effect of conventional process on juice
- ✦ Compare effects of three adsorption products on juice
- ✦ Suggest a possible change in process to:
 - ◆ Minimise juice treatment
 - ◆ Minimise waste production
 - ◆ Ensure longer storage life of product
- ✦ Comparison of operating cost of conventional vs suggested process

Background and literature study

- ✦ Background

 - ✦ Conventional process

- ✦ Literature study

 - ✦ Browning and methods of dealing with it

 - ✦ The chemistry of browning reactions

 - ✦ Favourable conditions

- ✦ Adsorption products chosen

Conventional process

Cellar:

Stage 1 Harvesting/Crushing and SO₂-addition (3 levels)
[Transportation or Storage]



GJC Plant:

Stage 2 Direct concentrate, storage AND/OR desulphurisation

Stage 3 1st Concentration

Stage 4 Protein stabilization and decolourisation

Stage 5 Filtration **Stage 6** Cooling

Stage 7 Tartrate Stabilisation **Stage 8** Filtration

Stage 9 2nd Concentration and storage

Additional Steps:

Stage 10 Blending

Stage 11 Pasteurisation

Stage 12 Drum Filling



Browning and methods of dealing with it

✧ Prevention

- ◆ Formaldehyde (Canterelli, et al. (1971))
- ◆ Enzymes (Kelly and Finkle (1969))
- ◆ Ion exchange (Peterson and Caputi (1967))
- ◆ Anti-oxidant type preservatives (Panagiotakopoulou and Morris (1991))
- ◆ Honey (Lee and other researchers (1987 onwards))

✧ Cure

- ◆ Adsorption products (Bru et al. (1995), Escolar et al.(1995), Mennet and Nakayama (1969))

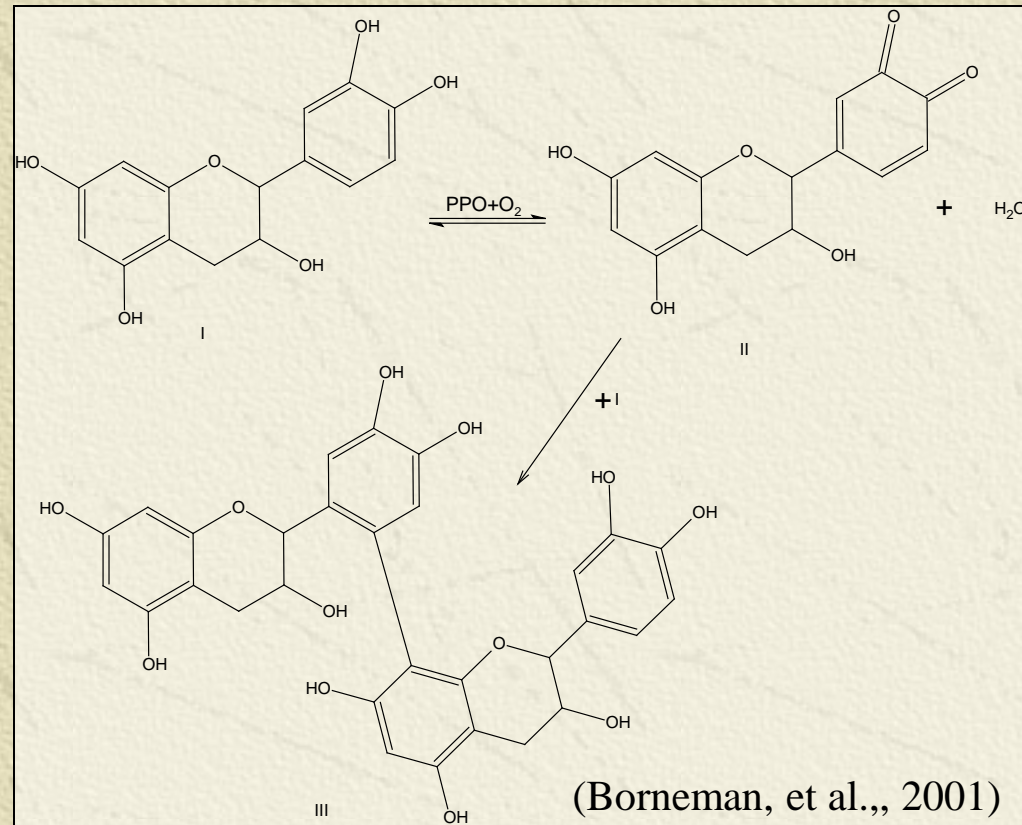
The chemistry of browning reactions

✦ Four pathways to browning*

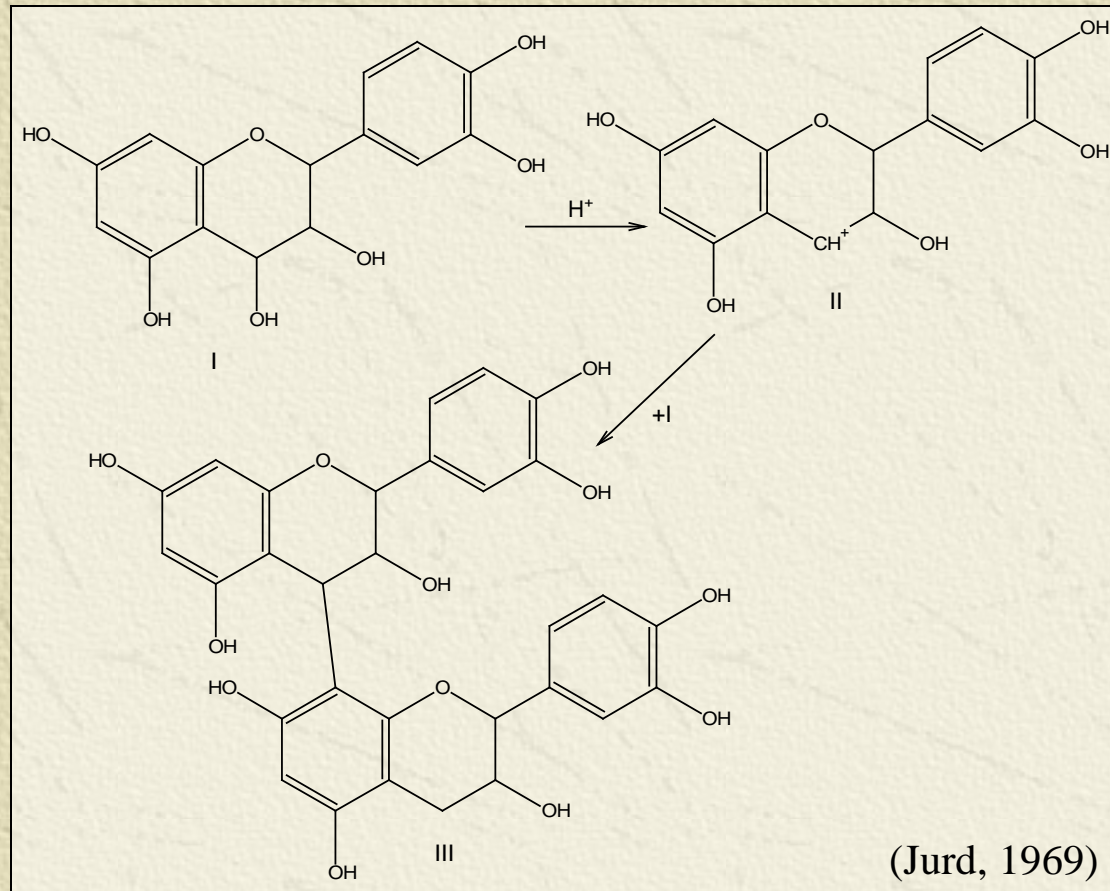
- ✦ Enzymatic Oxidative Browning
- ✦ Non-Enzymatic Oxidative Browning
- ✦ The Maillard Reaction
 - 5-Hydroxymethylfurfural
- ✦ *Caramelisation*

(*Collectively mentioned by researchers Kramling & Singleton (1965), Dutson & Orcutt (1984), Mayen et al. (1997) and Garza et al. (1999))

Enzymatic Oxidative Browning



Non-Enzymatic Oxidative Browning



The Maillard reaction

✦ Early stage

- ✦ Condensation of reducing sugar with amino acid to form Amadori or Heyns rearrangement products

✦ Advanced stage

- ✦ Degradation of Amadori or Heyns rearrangement products *via* four to five pathways

✦ Final stage

- ✦ Formation of brown nitrogenous polymers and co-polymers

5-Hydroxymethylfurfural

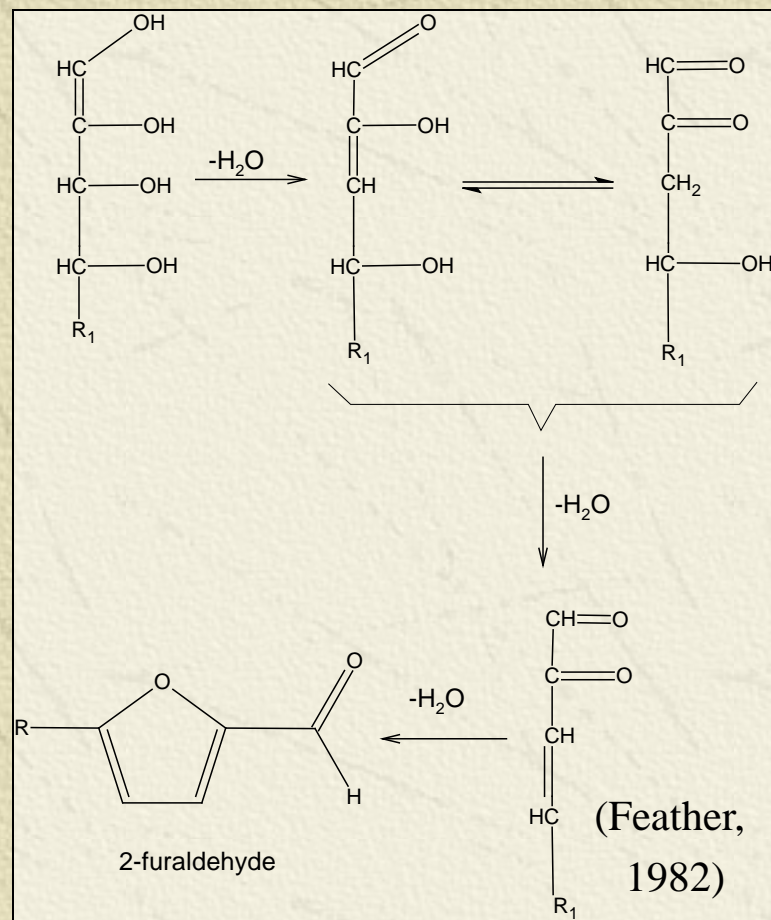
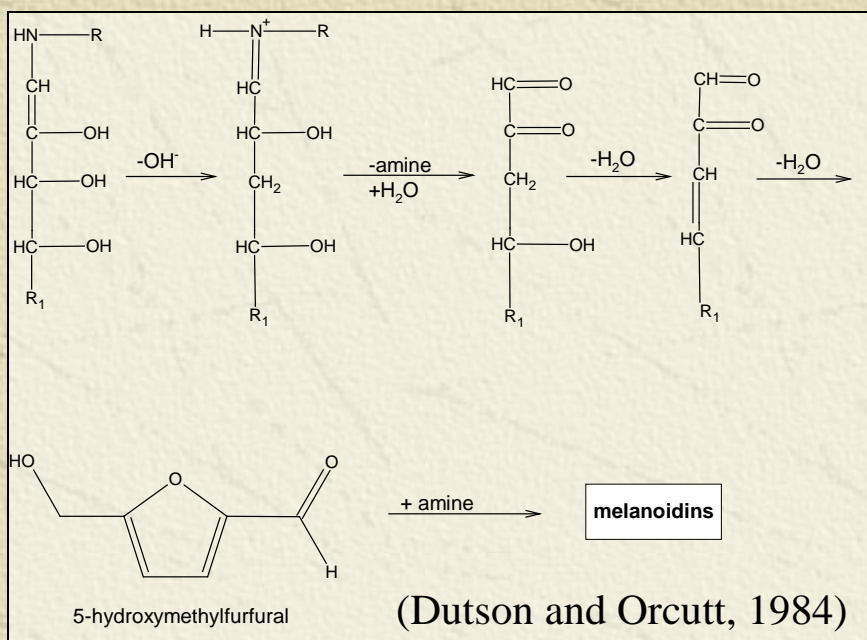
- ✦ 5-HMF – Indicator of browning potential (Gomis et al. (1991))
- ✦ Two pathways to 5-HMF formation

Formation of 5-HMF

Two Pathways:

✦ Amine Assisted

✦ Acid Catalysed



Favourable conditions

Browning Reaction	Preferred Environment
Enzymatic Oxidative browning	Mild temperatures, mild acidic environment
Non-Enzymatic Oxidative Browning	Acidic environment, high temperature
The Maillard Reaction	Acidic environment, high temperature
Caramelisation	Acidic environment, very high temperature, low water content

Adsorption products chosen

- ✦ CA1 – Chemically activated carbon powder
- ✦ SA4 – Steam activated carbon powder
- ✦ PVPP – Polymeric adsorbant (Polyclar V)

Analysis: Method development

✦ Method development

✦ Trials and results

✦ Selected method of analysis

Method development

Motivation

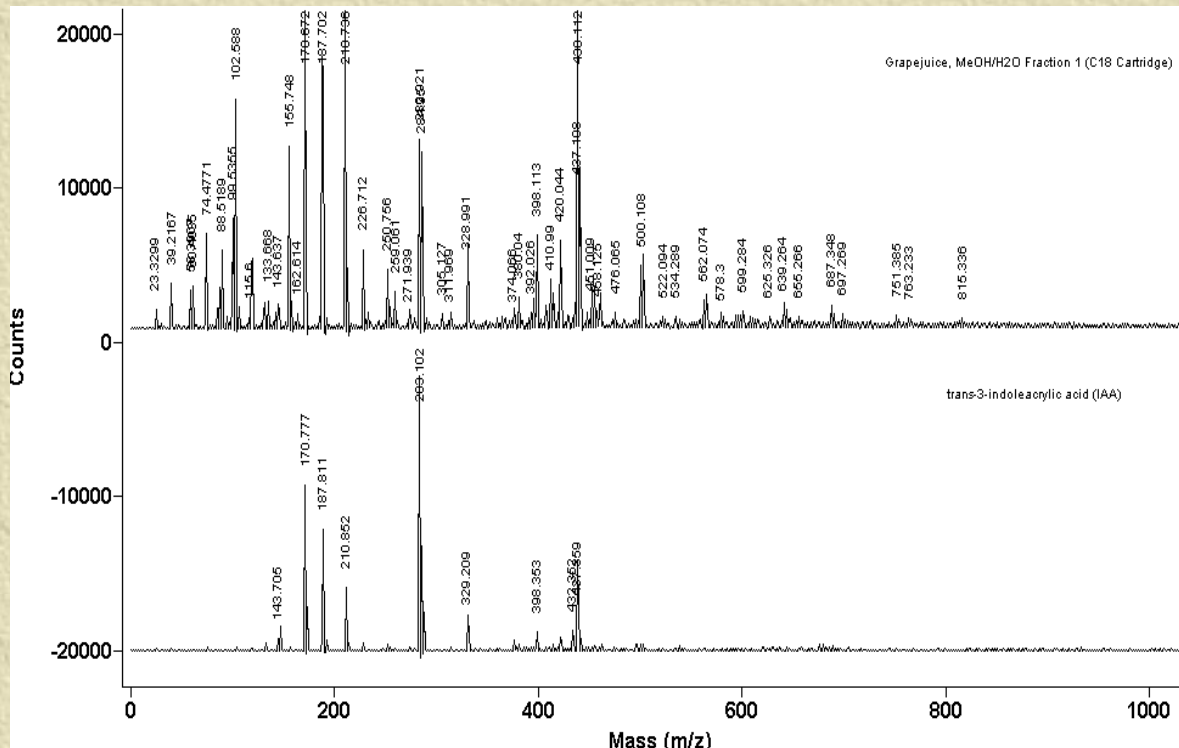
- ✦ Simultaneous qualification and quantification of several grape juice phenolics and 5-Hydroxymethylfurfural (5-HMF)
- ✦ Cost of existing 5-HMF quantification analysis

Trials and results

- ✦ Matrix assisted laser de-ionisation time-of-flight (MALDI-TOF)
- ✦ HPLC for sake of interest (UCT)
- ✦ HPLC followed by -ESI-MS-MS
- ✦ HPLC followed by APcI-MS-MS
- ✦ Selected method of analysis

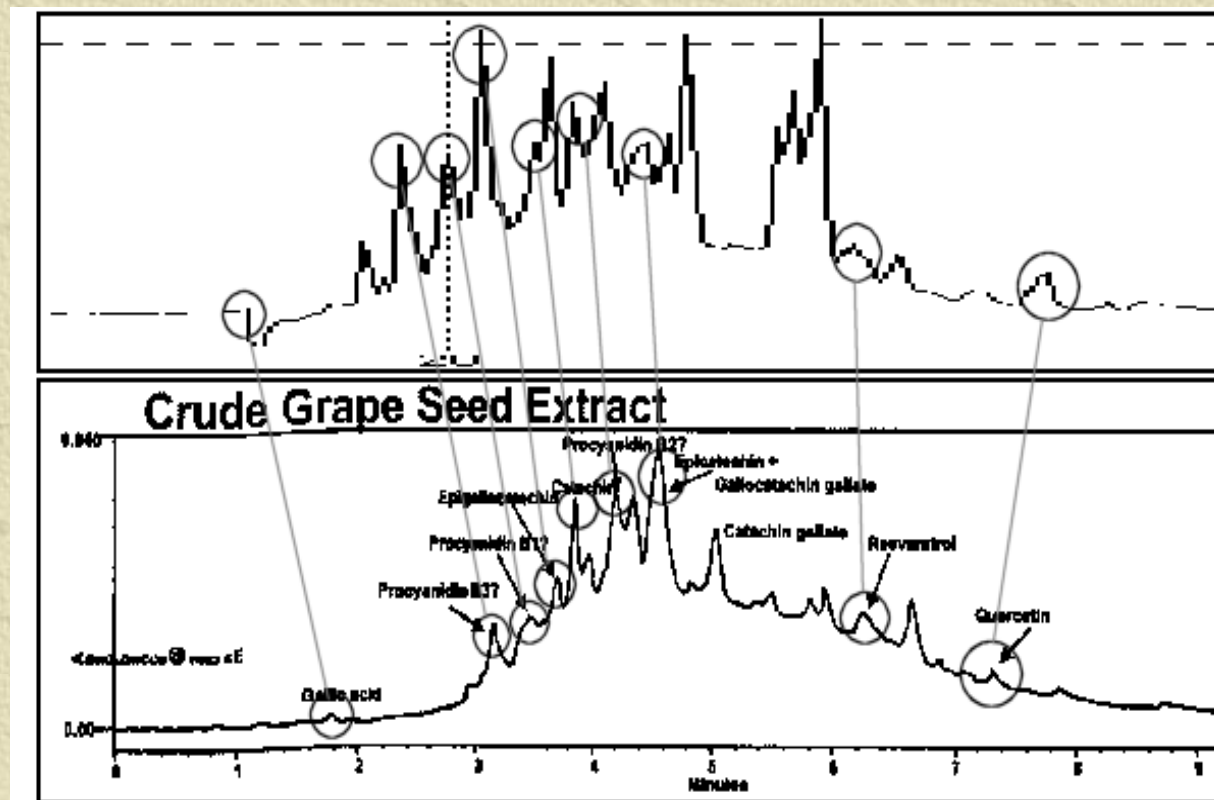
MALDI-TOF

- ✦ To determine size and mobility
- ✦ Difficult to distinguish
- ✦ Confirmed size



HPLC at UCT

- ✦ To determine change in phenolic content during storage
- ✦ No significant difference detected, however:



-ESI-MS-MS

- ✦ Good fragmentation of phenolics
- ✦ Poor fragmentation of 5-HMF

Substance	Mr g/mol	m/z of molecular- ion	m/z of fragment- ion
Quercetin	302	301	151
Catechin	290	289	109
Epicatechin	290	289	109
Caffeic Acid	180	179	135
Vanillic Acid	168	167	91
Gallic Acid	170	169	125
Resveratrol	228	227	143

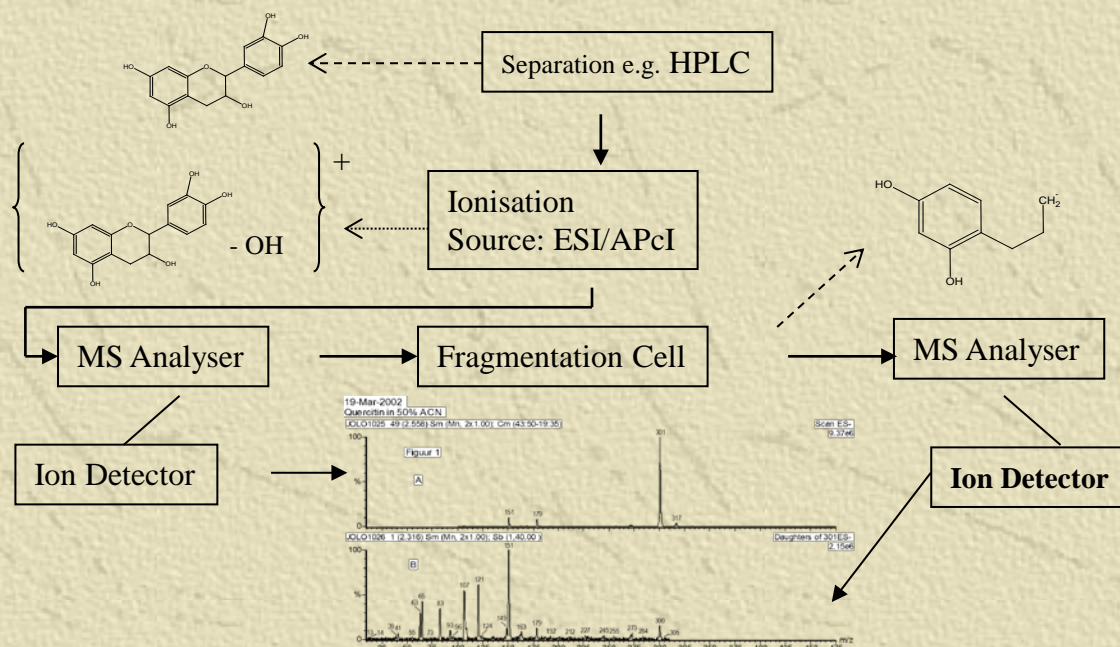
APcI-MS-MS

- ✦ Good fragmentation of phenolics and 5-HMF
- ✦ Technical problems – long storage periods – poor repeatability

Substance	Mr g/mol	m/z of molecular-ion	m/z of fragment ion
Quercetin	302	303	69
Catechins	290	291	139
Caffeic Acid	180	181	89
Vanillic Acid	168	169	65
Gallic Acid	170	171	81
Reveratrol	228	229	107
HMF	126	127	53

Selected method of analysis

- ✦ HPLC followed by Positive electron spray ionisation (+ESI) followed by dual mass spectrometry (MS-MS)
- ✦ 5-HMF only



Experimental

- ✦ Effect of conventional process on 5-HMF
 - ◆ Samples taken after all production stages
- ✦ Effect of heat treatment on protein stable and protein unstable juices
- ✦ Comparison of adsorption products
 - ◆ Optimum conditions for adsorption products
 - ◆ Product profiles
- ✦ General observations during experimental work

Effect of conventional process on 5-HMF

-
- ✦ Sampling after all stages of production
 - ✦ Three different batches:
 - ◆ RA - Direct concentrate, reconstituted to 20°Balling, 400mg/L SO₂ (Base juice)
 - ◆ RB - Direct concentrate, reconstituted to 35°Balling, skipping desulfurization (120mg/L SO₂)
 - ◆ RC - SO₂-juice, 1200mg/L SO₂
 - ✦ Ethyl acetate extraction, drying, storage and analysis

Effect of heat treatment

- ✦ 500ml samples of RA (stable and unstable)
- ✦ Boiled at 100°C under total reflux
- ✦ Sampled every 20 minutes for 3 hours
- ✦ Ethyl acetate extraction, drying, storage and analysis
- ✦ Performed twice for both stable and unstable juice - repeatability

Comparison of adsorption products

✦ To determine:

- ✦ Effect of protein stability under various conditions
- ✦ Efficiency of HMF adsorption
- ✦ Most cost effective and environmentally friendly product

✦ Subdivisions

- ✦ Product profiles
- ✦ Optimum conditions for products

Product profiles

✦ 6 Hours at 55°C

Sample/Tech.	CA1	SA4	PVPP
1	0.5g	0.5g	0.05g
2	1.0g	1.0g	0.35g
3	2.5g	2.5g	0.6g

Optimum conditions for products

✦ Product dosages

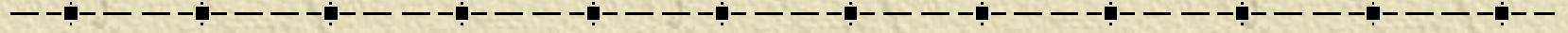
Product	Dosage (g/Litre)
CA1	4
SA4	4
PVPP	0.5

✦ Experimental conditions

Time/ Temp	Room(20°C)	40°C	60°C	80°C
½ Hour	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP
1 Hour	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP
3 Hour	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP
6 Hour	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP

General observations during experimental work

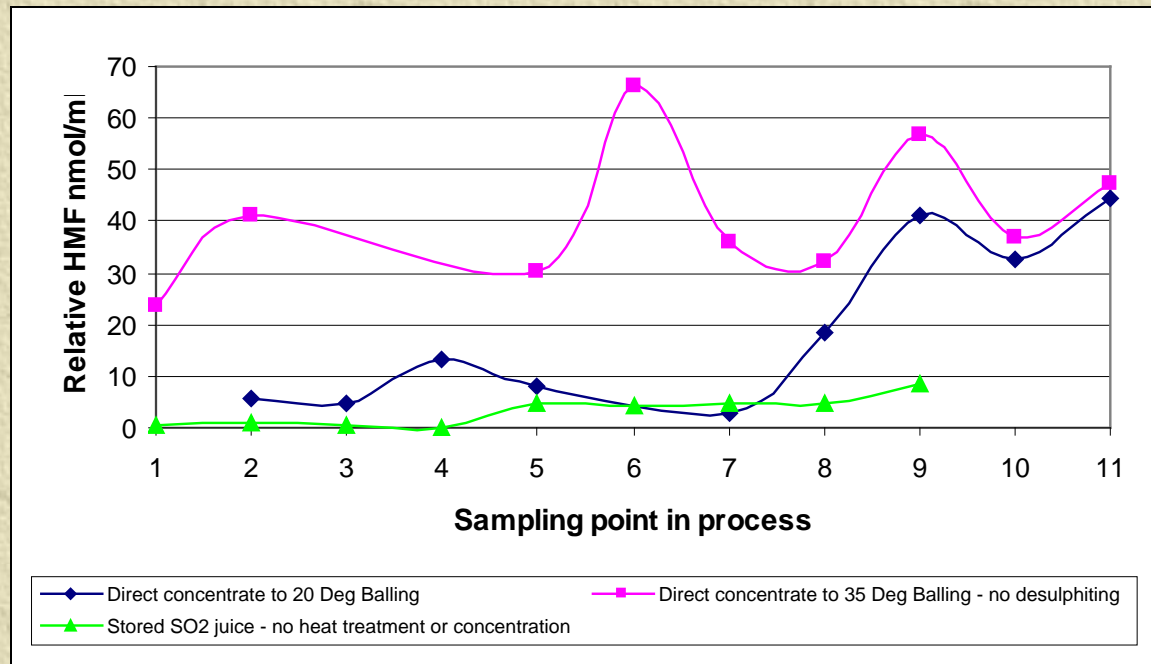
- ✦ Heated juice samples + SA4 = hydrogen sulphide-like smell and foaming
- ✦ Heated juice samples + CA1 = foaming only
- ✦ Colour:
 - ◆ SA4 – yellow to greenish
 - ◆ CA1 – yellowish to colourless
 - ◆ PVPP – bright yellow
- ✦ PVPP settled better on unstable juice



Results and discussions

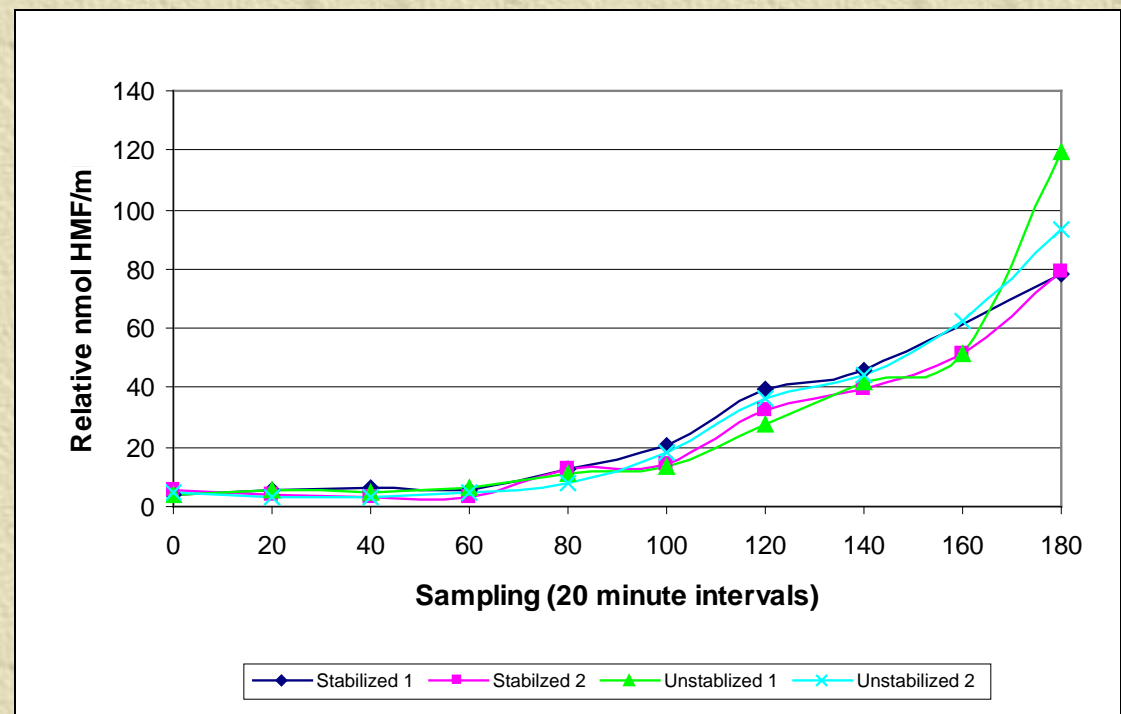
Results: Effect of conventional process on 5-HMF

- ✦ Less SO_2 = more 5-HMF
- ✦ Storing juice on SO_2 instead of concentrating it



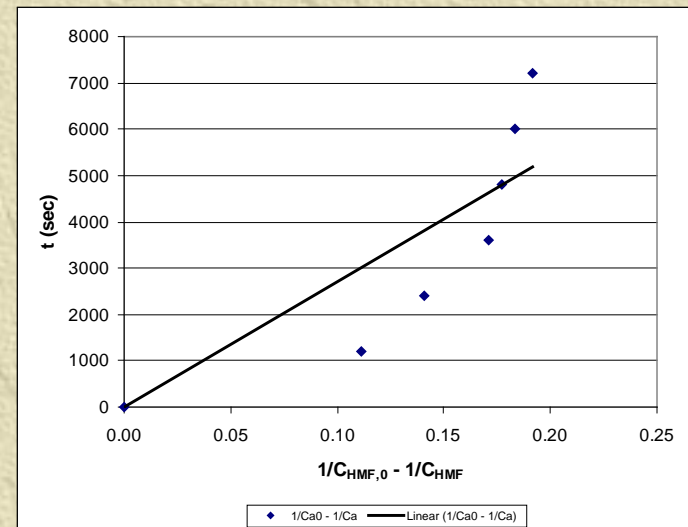
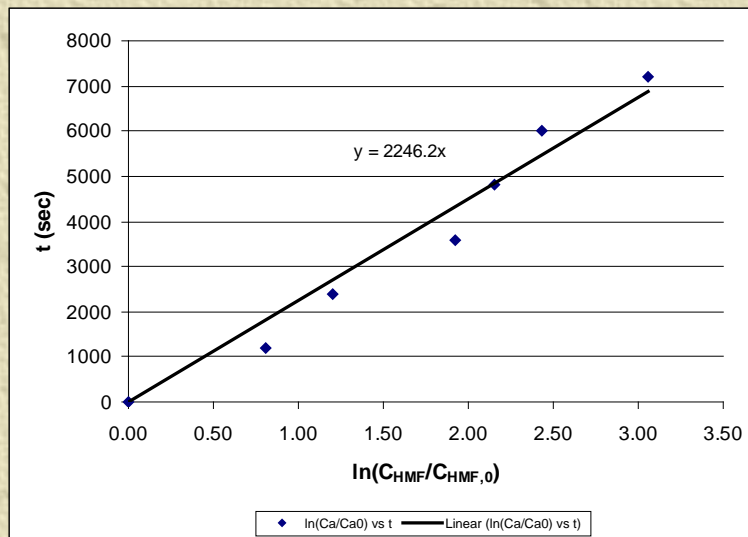
Results: Effect of heat treatment

- ✦ Good repeatability, good linearity (R^2 for stable and unstable juices 0.984 and 0.931, respectively)
- ✦ Lag phase
- ✦ 5-HMF formation = time dependant
- ✦ No conclusions regarding stability



Results: Effect of heat treatment (Continued...)

- ✦ Time > 1 hour – 1st order reaction rate
- ✦ Time < 1 hour – 5-HMF remains constant



Results: Comparison of adsorption products

✦ 6 hours at 55°C

✦ 5-HMF removal efficiency

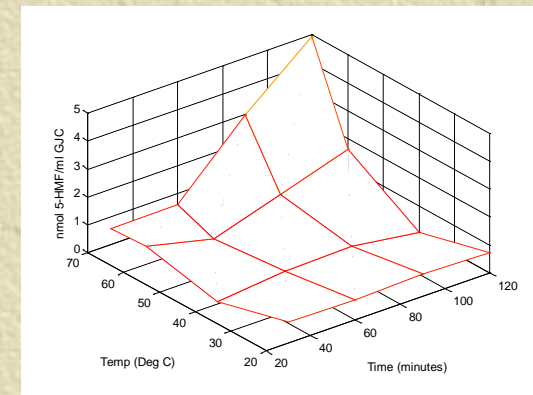
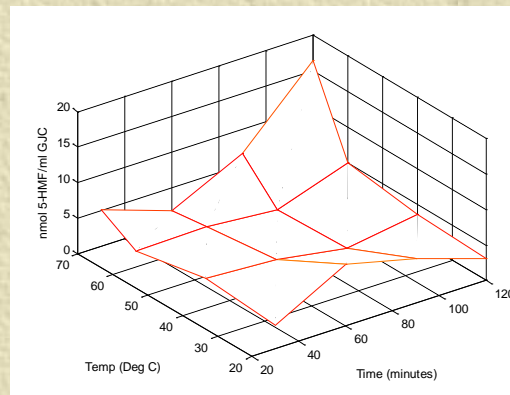
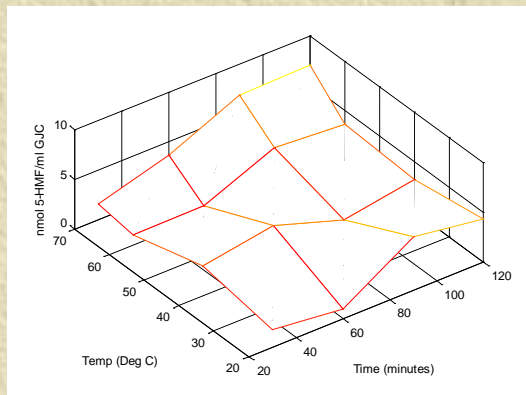
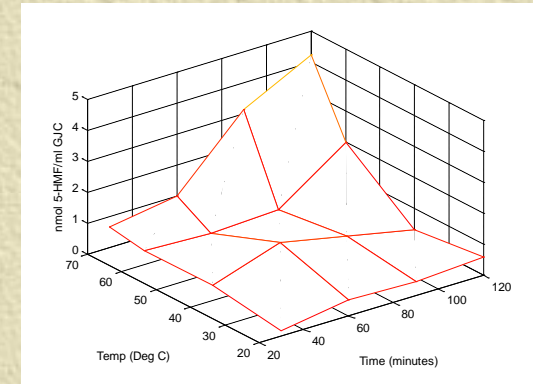
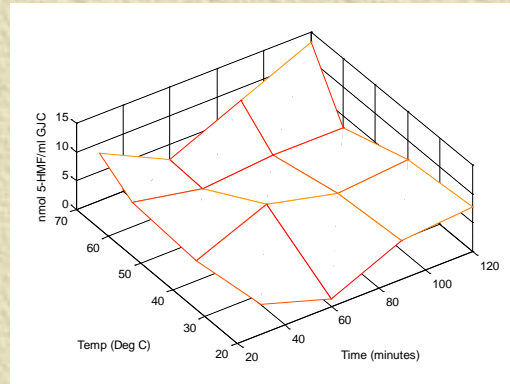
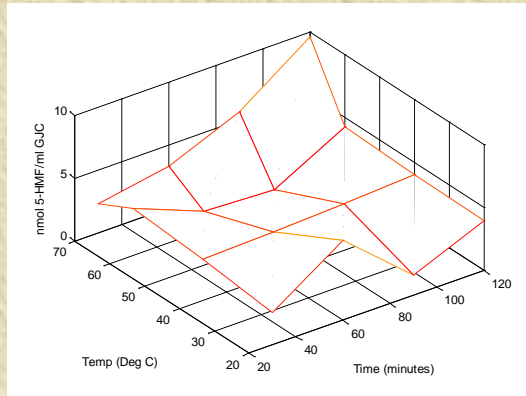
Dosage	CA1		SA4		Dosage	PVPP	
g/L	Stable (%)	Unstable (%)	Stable (%)	Unstable (%)	g/L	Stable (%)	Unstable (%)
0	NA	NA	NA	NA	0	NA	NA
1	44%	24%	83%	75%	0.1	56%	64%
2	42%	20%	89%	88%	0.7	53%	72%
5	46%	46%	87%	85%	1.2	43%	71%

Results: Optimum conditions for products

CA1

PVPP

SA4



Results: Summary

- ✦ Direct concentrate vs SO₂-juice (Boston and Boyacioglu, 1997)
- ✦ No concrete conclusions regarding protein stability, however no significant difference
- ✦ Heat treatment and heat exposure
 - ◆ Non-enzymatic oxidative browning (Bozkurt et al., 1999)
 - ◆ One hour lag phase (Quintas et al., 2003)
 - ◆ First order kinetics after one hour

Evaluation of conventional process – possible improvements

✦ To recap:

- ✦ Minimise juice treatment
- ✦ Minimise waste production
- ✦ Ensure longer storage life

✦ Main objective – reduce heat treatment

✦ Possible ways:

- ✦ Alterations to conventional process
- ✦ Alternative adsorption products
- ✦ Alternative concentration technologies

Alterations to conventional process

- ✦ Increase storage capacity + SO₂ addition
 - ◆ Once-off concentration – less heat exposure
- ✦ Protein stabilization before concentration
 - ◆ Possibly less contact time/heat exposure
 - ◆ Less solid waste
 - Less powdered activated carbon (PAC)
 - Less filter media
 - Less PAC contaminated bentonite

Alternative adsorption products

✦ SA4 instead of CA1

- ✦ Reduction in solid waste
- ✦ SA4 more easily reactivated

Alternative concentration technologies

-
- ✦ Reverse osmosis
 - ✦ Centrifugal evaporation
 - ✦ Combination of the two

Disadvantage:

- ✦ High capital cost

Advantages:

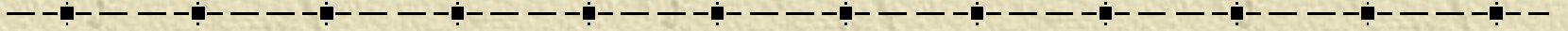
- ✦ Heat treatment reduced by 90%
- ✦ Superior product
- ✦ Possible reduction in solid waste

Cost comparison

✦ RO followed by CE = R12,000,000

✦ Alternative adsorption product

	CA1	SA4	PVPP
Cost/kg	R13.60	R22.50	R260.00
Dosage/L	4g	2g	0.5g
Annual dosage	160,000kg	80,000kg	20,000kg
Annual cost	R2,160,000	R1,800,000	R5,200,000



Conclusions

Conclusions: In general

✦ Significant amount of research

- ◆ Four browning pathways
- ◆ Causes of browning, reaction kinetics, etc.
- ◆ Ways of prevention/cure

✦ +ESI-MS-MS has potential

✦ From experimental:

- ◆ Heat and exposure time
- ◆ Protein stability

Conclusions: Most likely browning reaction

- ✦ Caramelisation
- ✦ Enzymatic oxidative browning – enzyme-catalysed oxidation
- ✦ The Maillard reaction – amine assisted degradation of sugars
- ✦ Non-enzymatic oxidative browning – most likely

Conclusions: Changes to conventional process

Minimise juice treatment:

- ✦ SO₂ addition and storage
- ✦ Alternative concentration technology

Minimise waste production:

- ✦ Protein stabilisation
- ✦ Other adsorption product (e.g. SA4)

Ensure longer storage life:

- ✦ Less heat treatment – Alternative concentration technology

(Possible) Future Work

- ✦ Improvement of method of analysis
- ✦ Continue laboratory and pilot scale

Thanks

-
- ✦ Prof. Leon Lorenzen
 - ✦ Dr. Thinus van der Merwe
 - ✦ Technical personnel
 - ✦ Sponsors Winetech and NRF
 - ✦ You

Questions??

?

Now boys...don't do anything to enhance the browning reactions

