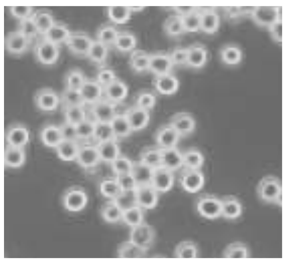




Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V.

Flavour Stability

Flavour Stability

- The retaining of the original character of the beer, from filling until consumption
- Beer is often stored for several weeks (even month) before it is sold
- Important ➔ flavour and taste of the beer constant over long period
- Changes ➔ divided in two groups:
  - Change of palatefullnes and bitternes, loss of the original harmony
  - Change of the aroma ➔ lightstruck and stale flavour
- Oxidation ➔ high oxygen content in wort and beer
- Thermal influences ➔ long boiling times, pasteurisation, high storage temperatures
- Influence of light (wave length of 350-500nm) ➔ lightstruck flavour

Journal-No. / Date of Arrival: # Sample:

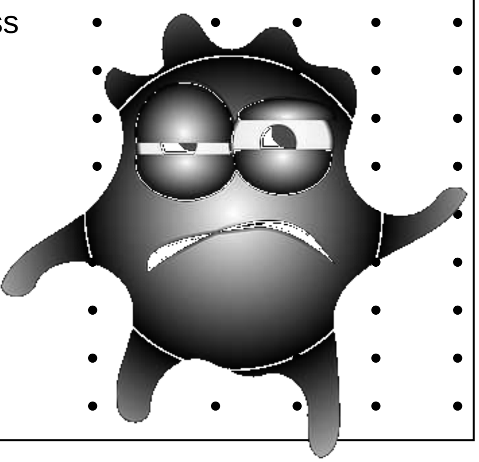
Storage time and storage temperature:	weeks			°C	
	0	4	8	0	28
	•	•	•	•	•

GENERAL IMPRESSION

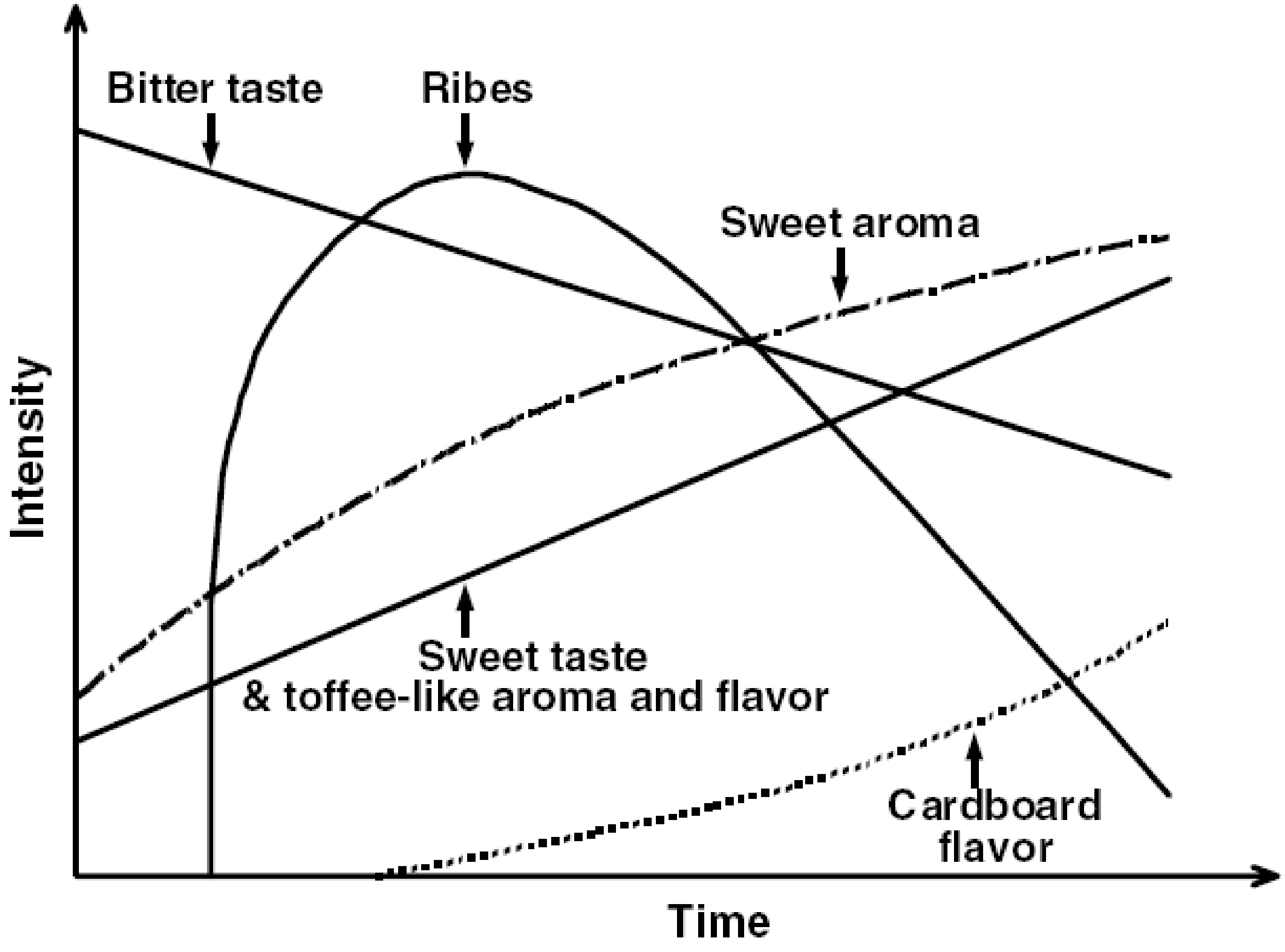
1	2	3	4	5	6	7	8	9
•	•	•	•	•	•	•	•	•

ODOUR		**1**	**2**	**3**	**4**	**5**
Quality		•	•	•	•	•
FLAVOUR						
Intensity		•	•	•	•	•
Quality		•	•	•	•	•
Palate fullness		•	•	•	•	•
Bitterness	Intensity	•	•	•	•	•
	Quality	•	•	•	•	•
Fizziness	Intensity	•	•	•	•	•
Fruitiness	Intensity	•	•	•	•	•
Harmony of the beer (palate fullness/bitterness)		•	•	•	•	•

OFF-FLAVOURS	not present	**1**	**2**	**3**	**4**
Oxidized, paper, cardboard	•	•	•	•	•
Oxidized, rancid	•	•	•	•	•
Oxidized, madeira/sherry	•	•	•	•	•
Scratchy, harsh, clinging bitterness	•	•	•	•	•
Adstringent	•			•	•
DMS, cabbage, celery	•			•	•
Diacetyl	•			•	•
Sulfury (-SH)					•
Sulfitic (SO_2)				•	•
Others.................	•			•	•
Others.................	•			•	•
Others.................	•	•	•	•	•



Flavour Changes during Storage



Dalgliesh1977

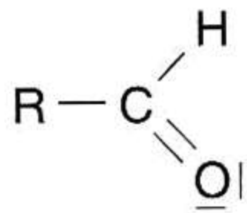
Possible Pathways on the Formation of Stale Flavour Aldehydes

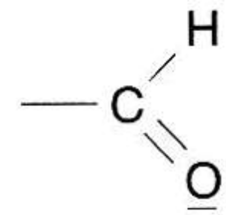
- Oxidation of higher alcohols
- Oxidative decomposition of bitter substances
- Maillard reaction and Strecker degradation
- Aldol condensation
- Secondary autoxidation of aldehydes
- Oxidation of unsaturated fatty acids
    - Photooxidation
    - Radical induced autoxidation
    - Enzymatic oxidation (lipoxygenase)

Carbonyl Compounds

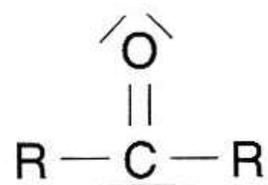
- Aldehydes and Ketones
- Characteristic → complete change of flavour, due to minor changes
- Very low flavour threshhold in ppb-range

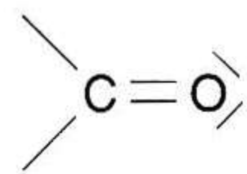
Aldehyde
Alkanale





Ketone
Alkanone





<u>Example:</u>
Moving a double bound one step along the carbon chain changes the flavour from cardboard-like to cucumber-like

Tech. Quart, **No. 3,** 135 – 141, 1974

Flavour Thresholds of chosen Aldehydes

Compound	Threshold [ppm]	Description
Acetaldehyde	25	Green leaves, fruity
Furfural	150	Paper, husk
n-Hexanal	0.35	Bitter, vinous
2*E*-Hexenal	0.6	Bitter, astringent, green leaves
3*Z*-Hexenal	0.02	Green leaves, freshly cut grass
5-Methylfurfural	20	Almonds, burnt/phenolic
n-Heptanal	0.075	Vinous, bitter, very unpleasant
n-Octanal	0.04	Orange peel, bitter, vinous
2*E*-Octenal	0.0002	Bitter, stale
2*E*-Nonenal	0.00011	Papery (cardboard), oxidized, stale
2*E*,4*E*-Nonadienal	0.0005	Oily, rancid
T-2-c-6-Nonadienal	0,00005	cucumber
2*E*-Decenal	0.001	Bitter, rancid, stale
2*E*,4*E*-Decadienal	0.0003	Oily, deep-fried

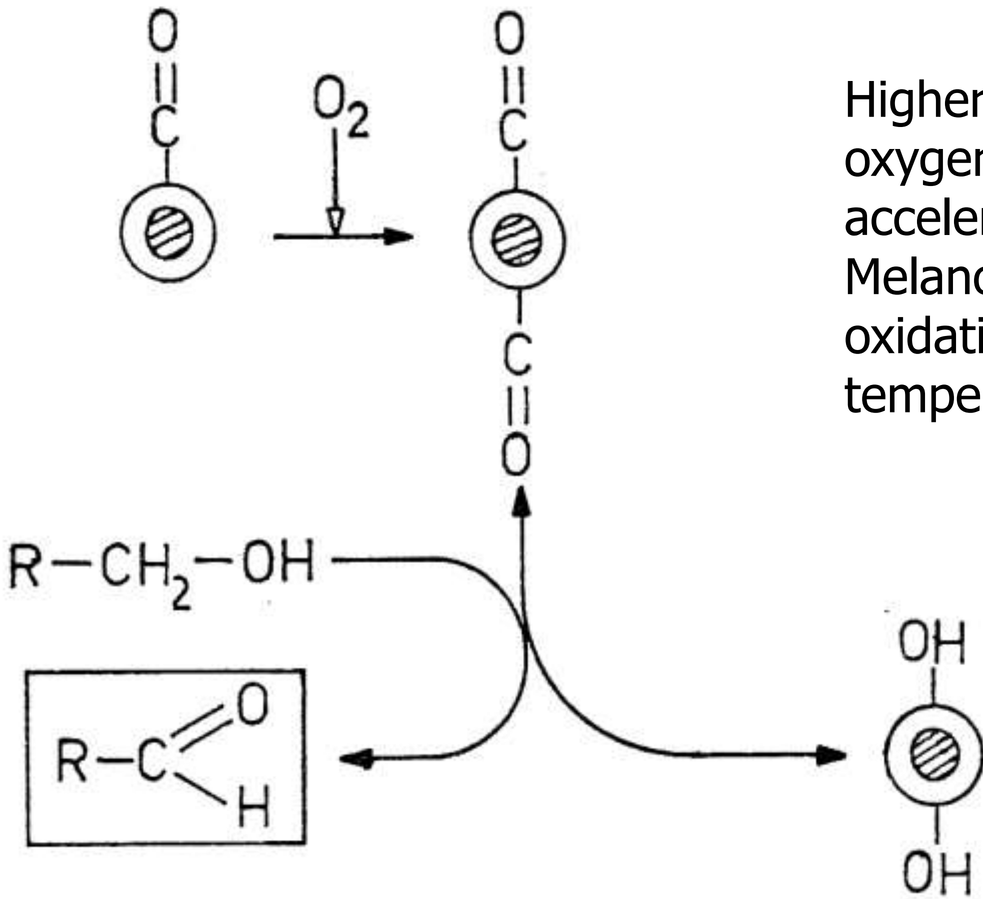
Monatsschr. F. Brauwiss., **No. 10,** 396 – 401, 1985

Changes in Aldehyde Values during Storage [ppb]

Compound	0 °C	30 °C			Threshold
	12 Weeks	4 Weeks	8 Weeks	12 Weeks	
2-Methylpropanal	6.1	20.0	30.6	42.4	1 000
3-Methylbutanal	1.8	3.1	4.2	5.2	1 250
3-Methylbutanal	12.2	17.2	20.7	24.4	600
Pentanal	0.3	0.6	0.7	0.8	500
Hexanal	1.0	1.8	2.1	2.5	350
Furfural	28.8	202.8	362.0	458.3	150 000
Methional	2.8	3.6	4.1	4.6	250
Phenylacetaldehyde	6.6	9.9	10.1	12.7	1 600
2*E*-Nonenal	0.01	0.02	0.02	0.03	0.11

Vesely *et al.* (2003)

Oxidation of Higher Alcohols by Melanoidins



Higher alcohols can be oxidized by oxygen. This reaction can be accelerated by the presence of Melanoidins. In addition to that this oxidation can be accelerated by higher temperatures and a low pH value.

Brew. Digest., **No. 4**, 48 – 57, 1972

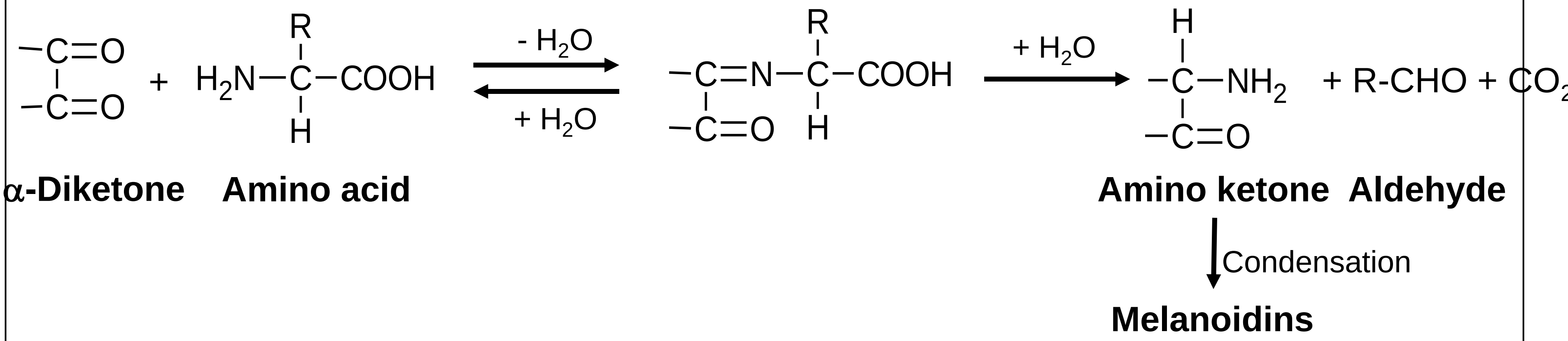
Oxidation of Higher Alcohols

Higher Alcohols	Aldehydes
Propyl	Propylaldehyde
Iso-butyl	Iso-butylaldehyde
n-butyl	n-butylaldehyde
Amyl	Amylaldehyde
Iso-amyl	Iso-amylaldehyde

Brauwelt **114, Nr. 10**, 159-161, 1974

FORMATION OF ALDEHYDES

Strecker Degradation

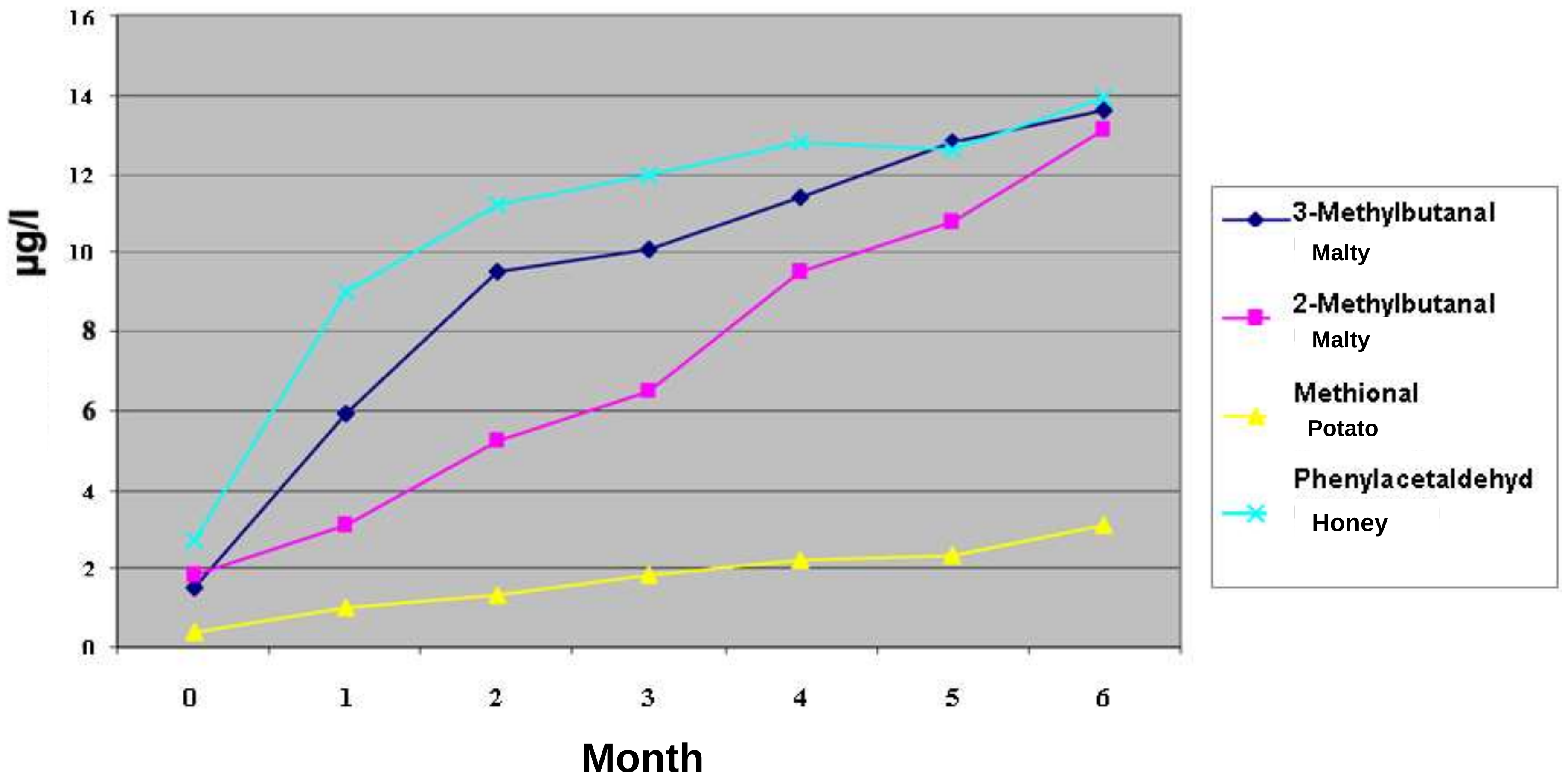


Amino acid	Corresponding aldehyde
Alanine	Acetaldehyde
Leucine	3-Methylbutanal
Isoleucine	2-Methylbutanal
Phenylalanine	2-Phenylethanal
Valine	2-Methylpropanal
Methionine	Methional
Glycine	Formaldehyde

Non-oxidative Aging of Beer (Strecker Aldehydes)

+ TBI as indicator for heat stress is giving a measure for amount of maillard products
  - Directly proportional to amount of HMF (Hydroxy-Methyl-Furfural)
  - Sugar and amino acids reacting
+ Amount of FAN has been found to have an impact on intensity of this „aging pathway“. Excessive amounts of FAN are as well not beneficial to taste, foam, bitterness.
+ Just consider the right amount of FAN to make the yeast happy. Todays understanding of yeast metabolism: about 100 – 140 ppm FAN is taken up. This can be easily calculated when knowing the wort and final beer FAN.
+ Raw material mix, mashing, yeast food (as additive)…. Has an influence on the FAN amounts.

Strecker Aldehydes during Storage



Important Strecker Aldehydes

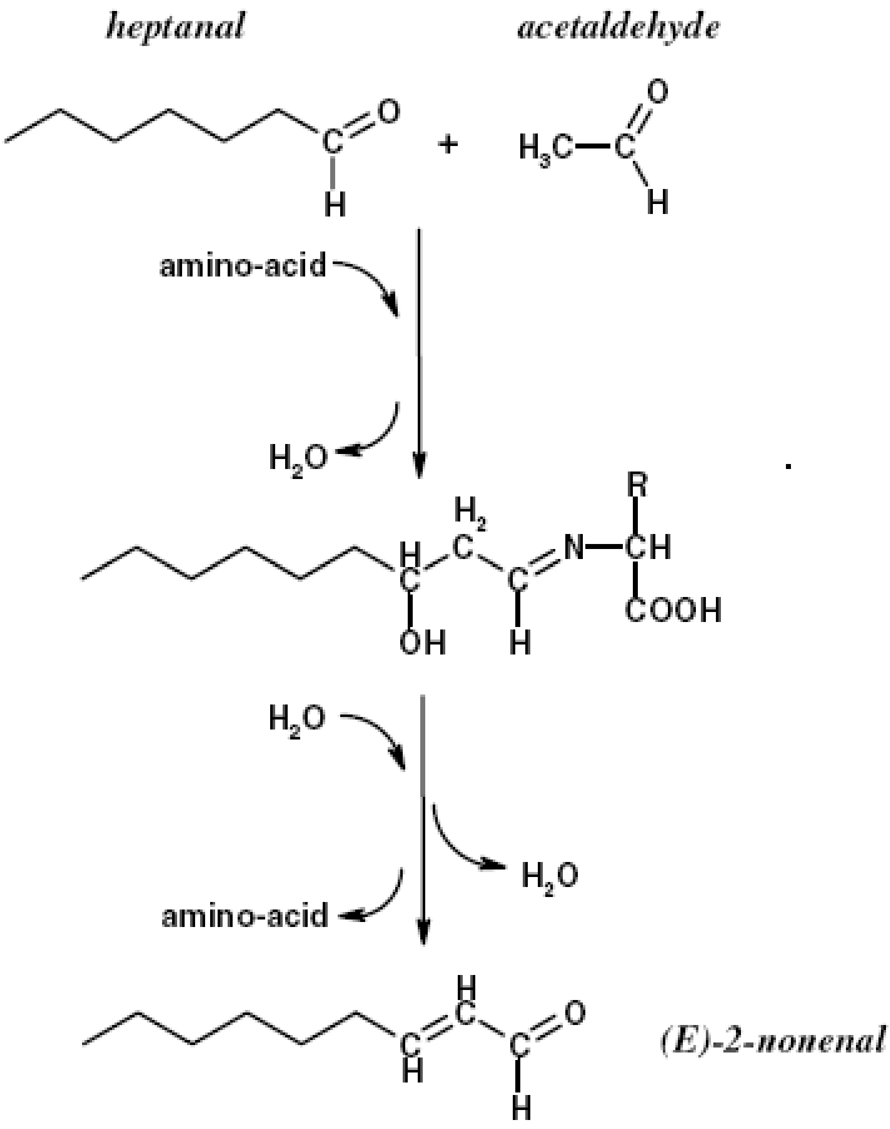
Precursor Aminoacid	Strecker-Aldehyd	Aroma
Leucin	3-Methylbutanal	malty
Isoleucin	2-Methylbutanal	malty
Methionin	Methional	potato, cooked
Phenylalanin	Phenylacetaldehyd	Honey-like, floral

EBC Proc. 29, 732-739,2003

Important Carbonyles

Amino acid	Carbonyl	Flavor
Glycin	Formaldehyde	Caramel
Alanin	Acetaldehyde	Caramel
Valin	Isobutanal	Breadcrum
Leucin	Isovaleral	Chocolate
Phenylalanin	Phenylacetaldehyd	Viola

Aldol Condensation of Acetaldehyde and Heptanal

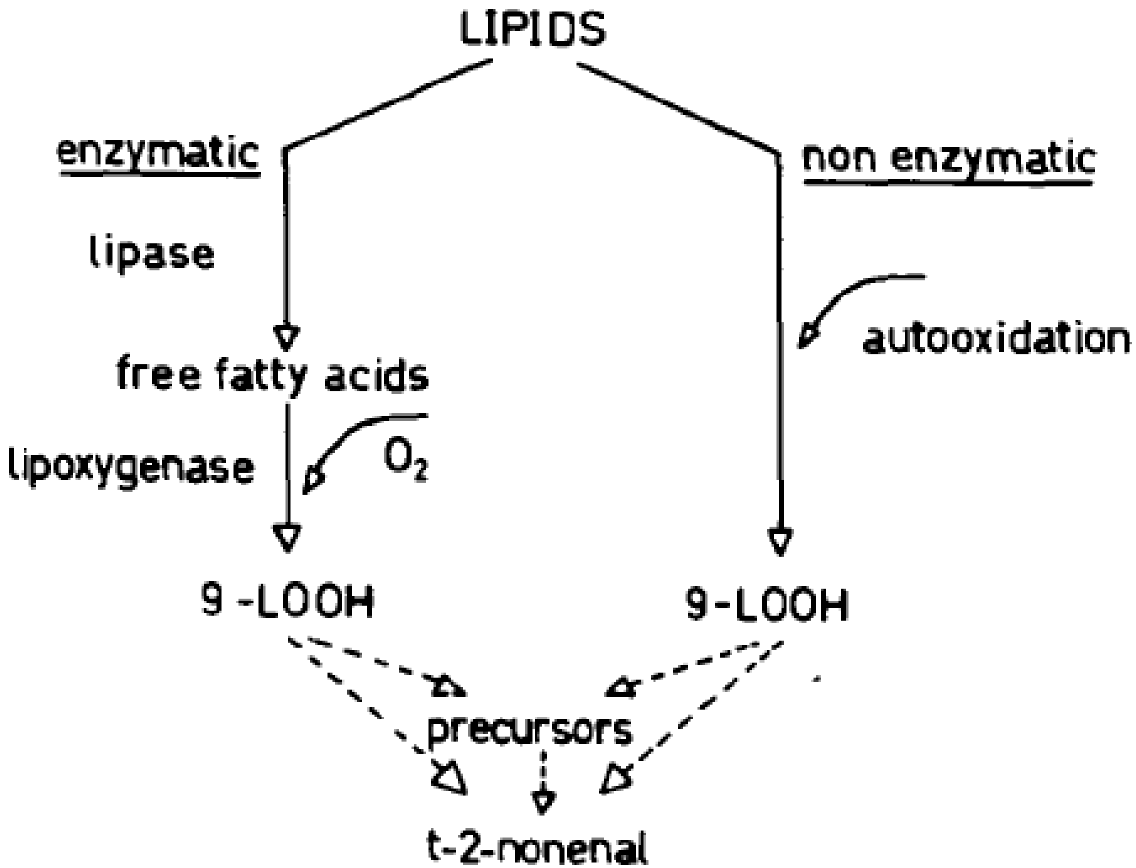


Hashimoto, Kuroiwa (1975)

Degradation of Lipids

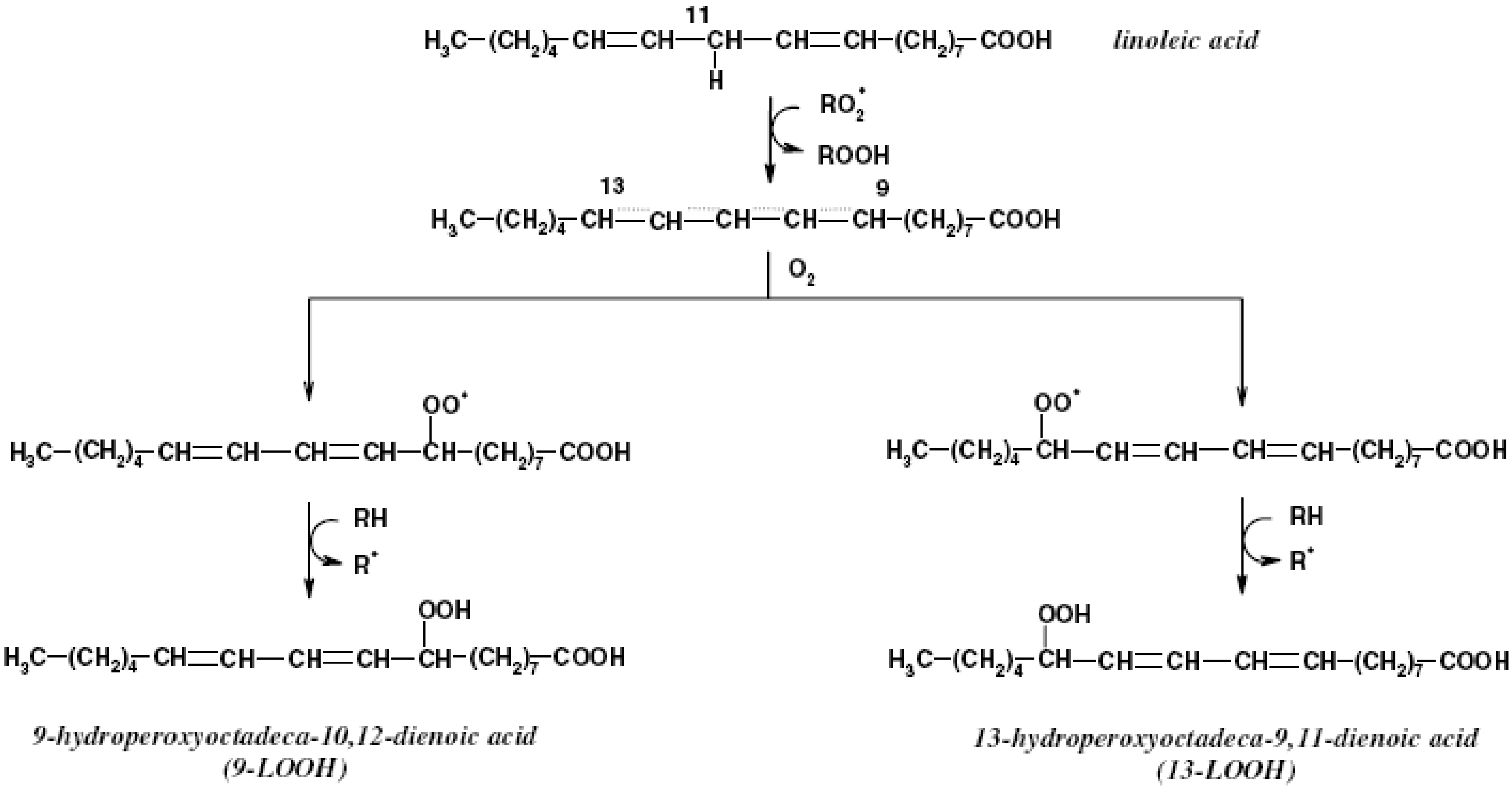
- Degradation of fatty acids ➔ aroma active components responsible for stale flavour
- High-molecular unsaturated fatty acids (linoleicacid and linolenicacid)
  ➔ Precusours for stale flavour
- Degradation autooxidative (chemical oxidation) or enzymatic oxidation
- Enzymatic degradation ➔ during mashing (lipoxidase activity
  ➔ 50-65°C) ➔degradation to hydroxyacids
  ➔ long chained unsaturated aldehydes
- Aldehydes ➔ cardboard flavour
- Aldehydes:
  – T-2-nonal, decanal, 2-octenal, 2,4-nonadienal

Possible Routes for the Formation of Trans-2-nonenal



ASBC-Journal, 1990

Formation of the Hydroperoxy Fatty Acids 9-LOOH and 13-LOOH by Autoxidation of Linoleic Acid



Belitz and Grosch, 1999

Photo Oxidation of Fatty Acids

$$Sen \rightarrow Sen^*$$

$$Sen^* + {}^3O_2 \rightarrow Sen + {}^1O_2$$

(singulet)

$$ {}^1O_2 + CH_3\text{-}(CH_2)_7\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$$

↓

$$CH_3\text{-}(CH_2)_6\text{-}CH{=}CH\text{-}CH\text{-}(CH_2)_7\text{-}COOH$$

(OOH on the marked CH)

OOH

↓

$$CH_3\text{-}(CH_2)_6\text{-}CH{=}CH\text{-}CH\text{-}(CH_2)_7\text{-}COOH$$

OH

$+{}^1O_2$ ↓

OOH

$$CH_3\text{-}(CH2)_5\text{-}CH{=}CH\text{-}CH\text{-}CH\text{-}(CH_2)_7\text{-}COOH$$

OH

↙ ↘

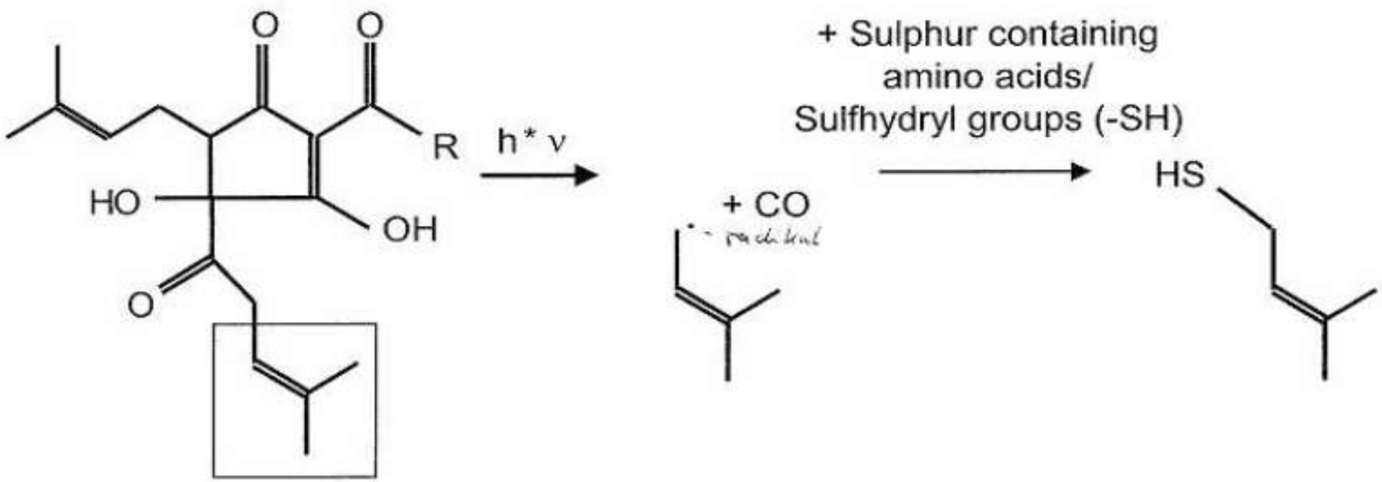
$$CH_3\text{-}(CH_2)_5\text{-}CH{=}CH\text{-}CHO$$

trans-2-nonenal

$$OHC\text{-}(CH_2)_7\text{-}COOH$$

9-oxonanoicacid

Formation of Lightstruck Flavour

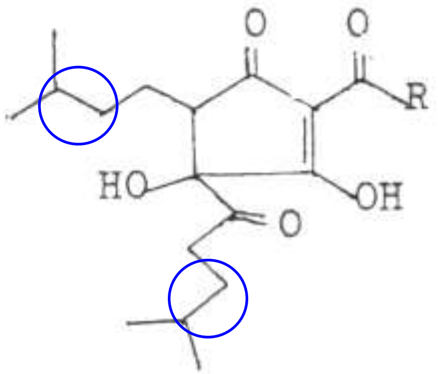


Isohumulone

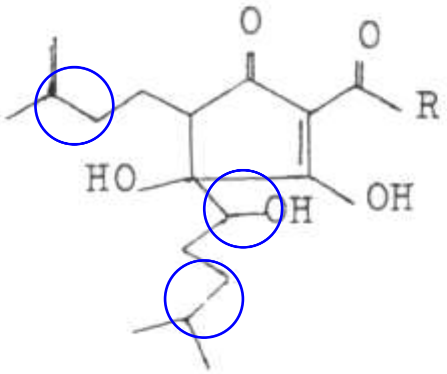
3-Methyl-2-butenyl-radical
+ Carbon monoxide

3-Methyl-2-butenyl-mercaptan

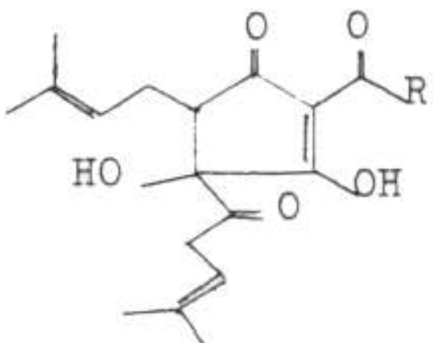
Structures of Iso- Alpha Acid and Reduced Iso- Alpha Acids



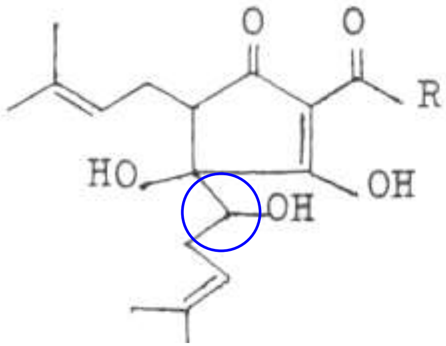
Tetra- Iso - alpha



Hexa-iso-alpha

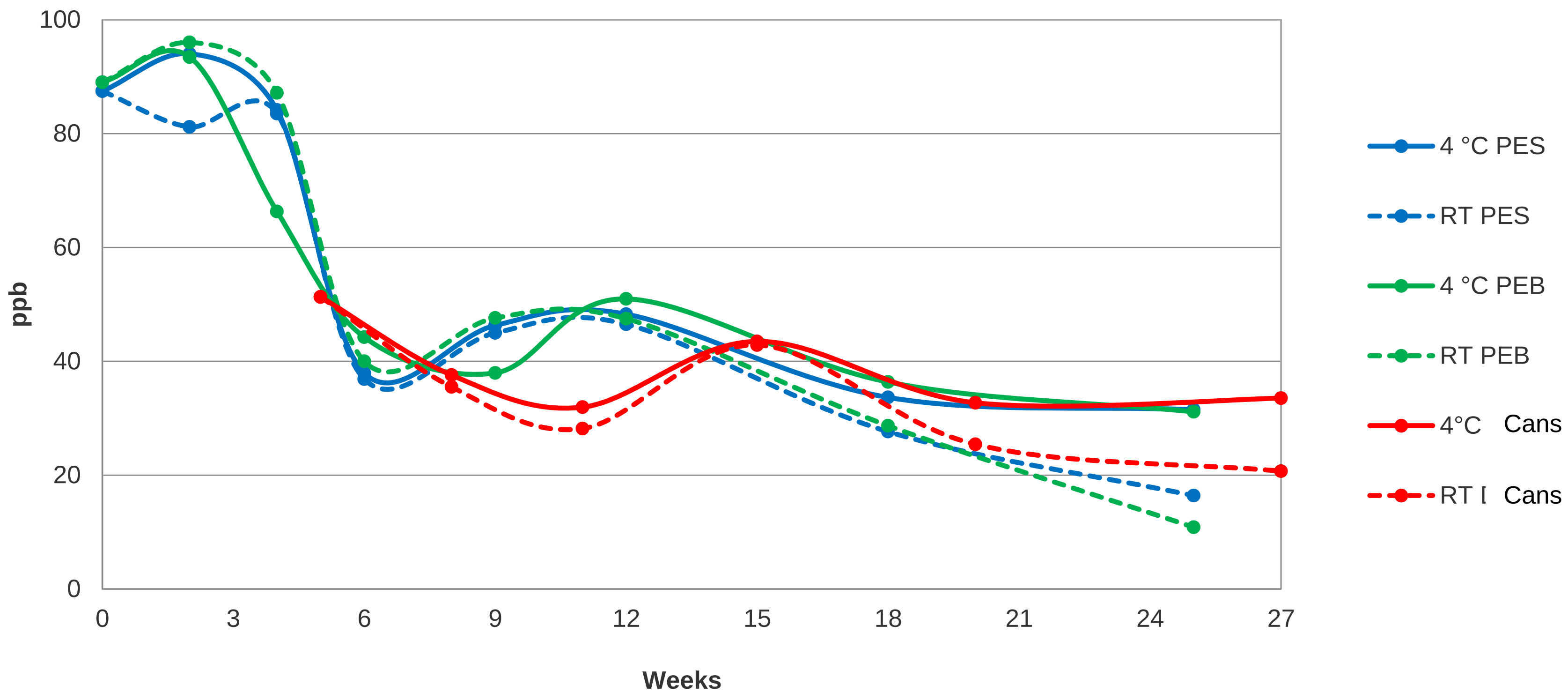


Iso alpha



Rho-iso-alpha

Geraniol (ppb)



Oxygen in the Brewing Process

- Brewhouse: oxygen uptake during mashing, pumping, lautering
- Boiling: kettles should only be filled from the bottom
- Closed wort boiling systems: ➔ lower yield in bitter substances but better flavour stability
- Open boiling systems: ➔ darker beers, harsher taste, less favourable polyphenolic composition
- Minimize oxygen uptake ➔ keep the oxygen limit low during fermentation, filtration and filling

  ➔ Pressurisation of the tanks, pipes and vessles with CO_2

  ➔ Evacuation and CO_2 pressurisation during filling

Wort Clarification

- High correlation between the clarification of wort and flavour stability
- The brighter the wort, the less fatty acids are in the wort
- Reasons: poorer precipitation during boiling, lower trub separation in the whirlpool
- Modern lauter tuns: reduction of the solid content in wort to 20-50 ppm
- Mash filters: reduction of the solid content in wort to 50-80 ppm
- Aging of beers, made of turbid worts ➔ much stronger
- Increase of acetone, t-2-butenal, iso-butanal,2-phenylacetaldehyd, iso-valeral, hexanal, t-2-nonenal, nonanal, t-2-t4-decadienal and t-2-t-4-undecadienal

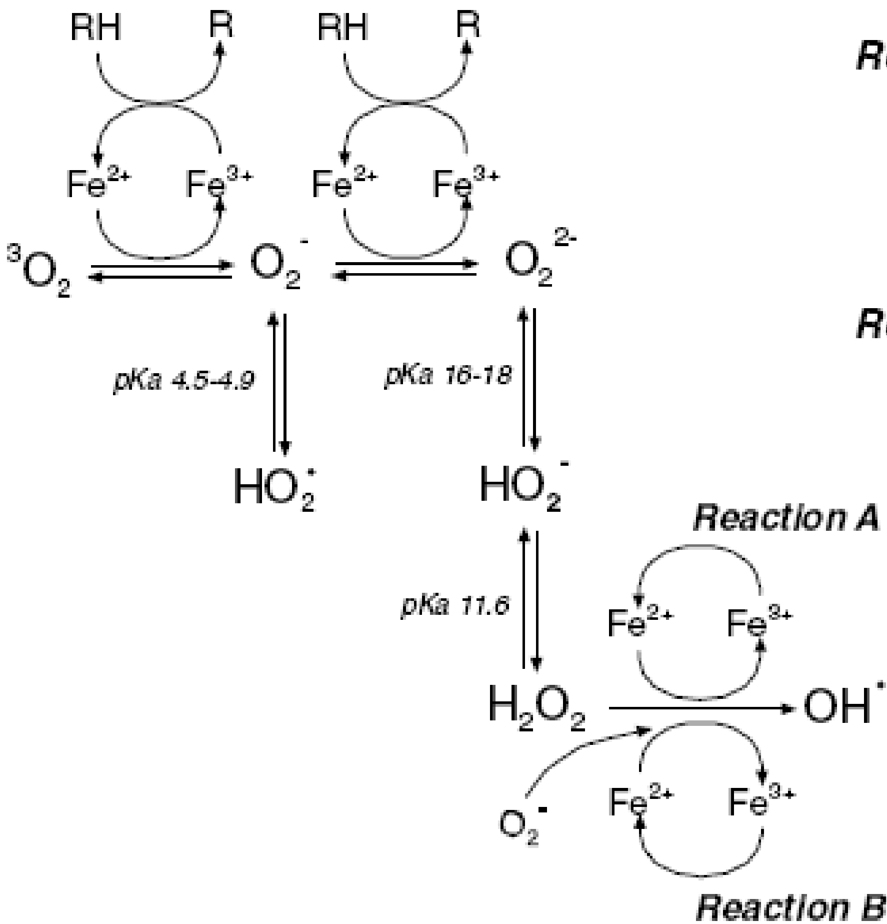
Thermal Influence

- Wort boiling, pasteurisation (flash pasteurizer, tunnel pasteur)
- Boiling: Melanoidines are formed
  - – amount Melanoidines depends on the time at high temperatures

Oxidation Reactions are intensified by:

- Higher temperatures
- Low pH-values
- But also co-factors like iron (Fe) and copper (Cu) from water, DE and malt accelerate oxidation reactions decisively (Fenton and Haber-Weiss-reactions)

Reactions Producing Reactive Oxygen Species (ROS) in Beer



Reaction A: Fenton reaction

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + O_2^- + 2H^+$$

$$\text{Net: } 2H_2O_2 \quad OH + OH^- + O_2^- + 2H^+$$

Reaction B: Haber-Weiss reaction

$$Fe^{3+} + O^{2-} \rightarrow Fe^{2+} + O_2$$

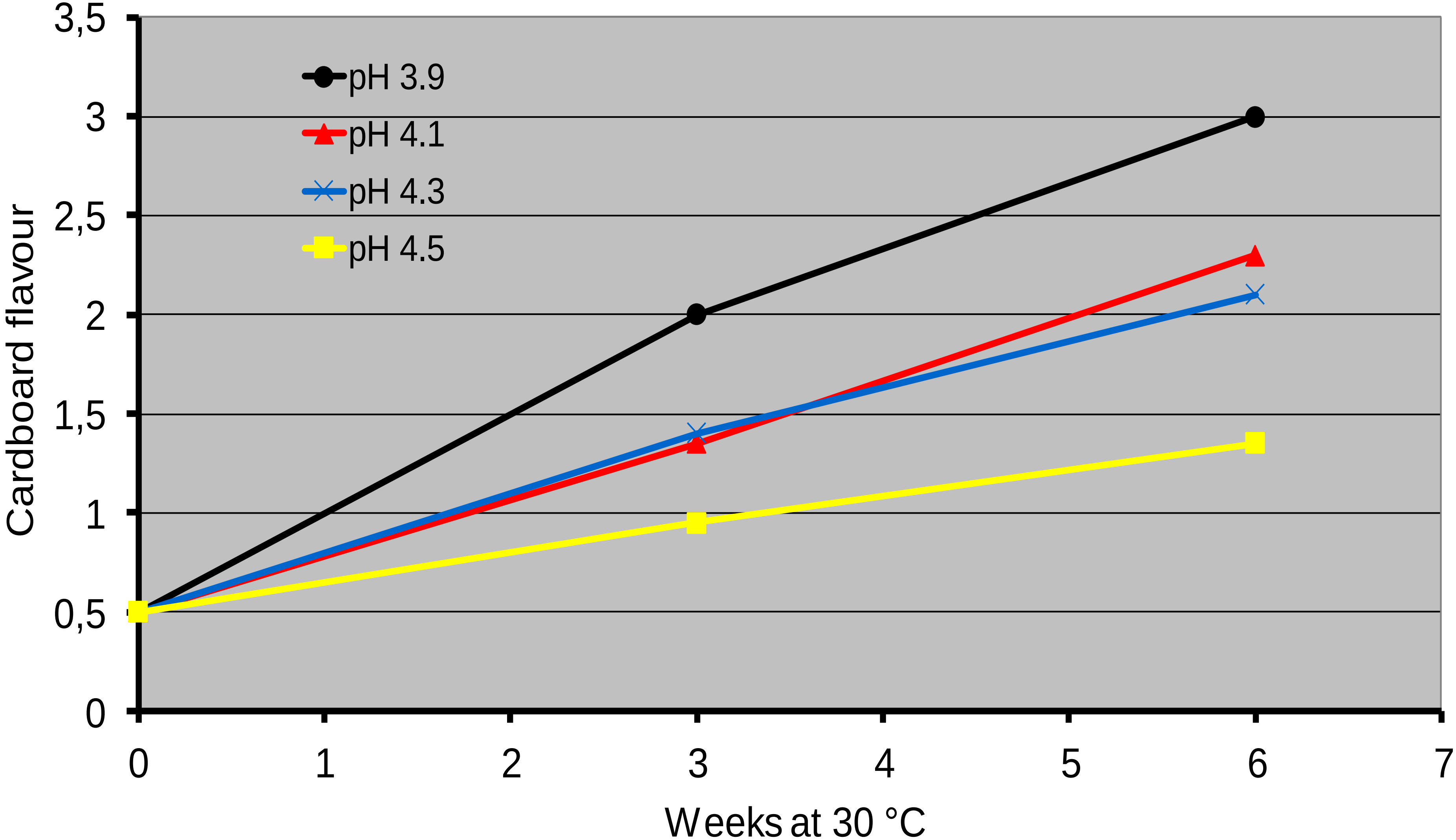
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$$

$$\text{Net: } O^{2-} + H_2O_2 \rightarrow O_2 + OH + OH^-$$

Kaneda et al., 1999

FLAVOUR STABILITY

Influence of pH on Beer Flavour Stability



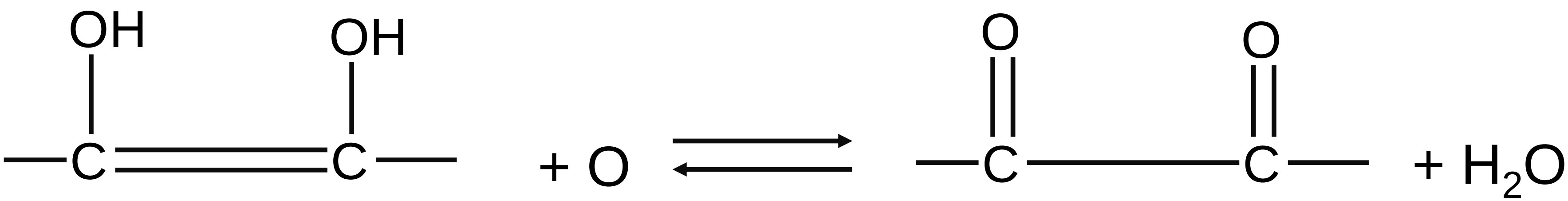
Anti-Oxidants –

They help, but do I want or need them?

+ Ascorbic acid
+ Sulfite products
+ Often in combination

ASCORBIC ACID (AH_2)

Dienol groups help to bind approx. 50 % of the oxygen

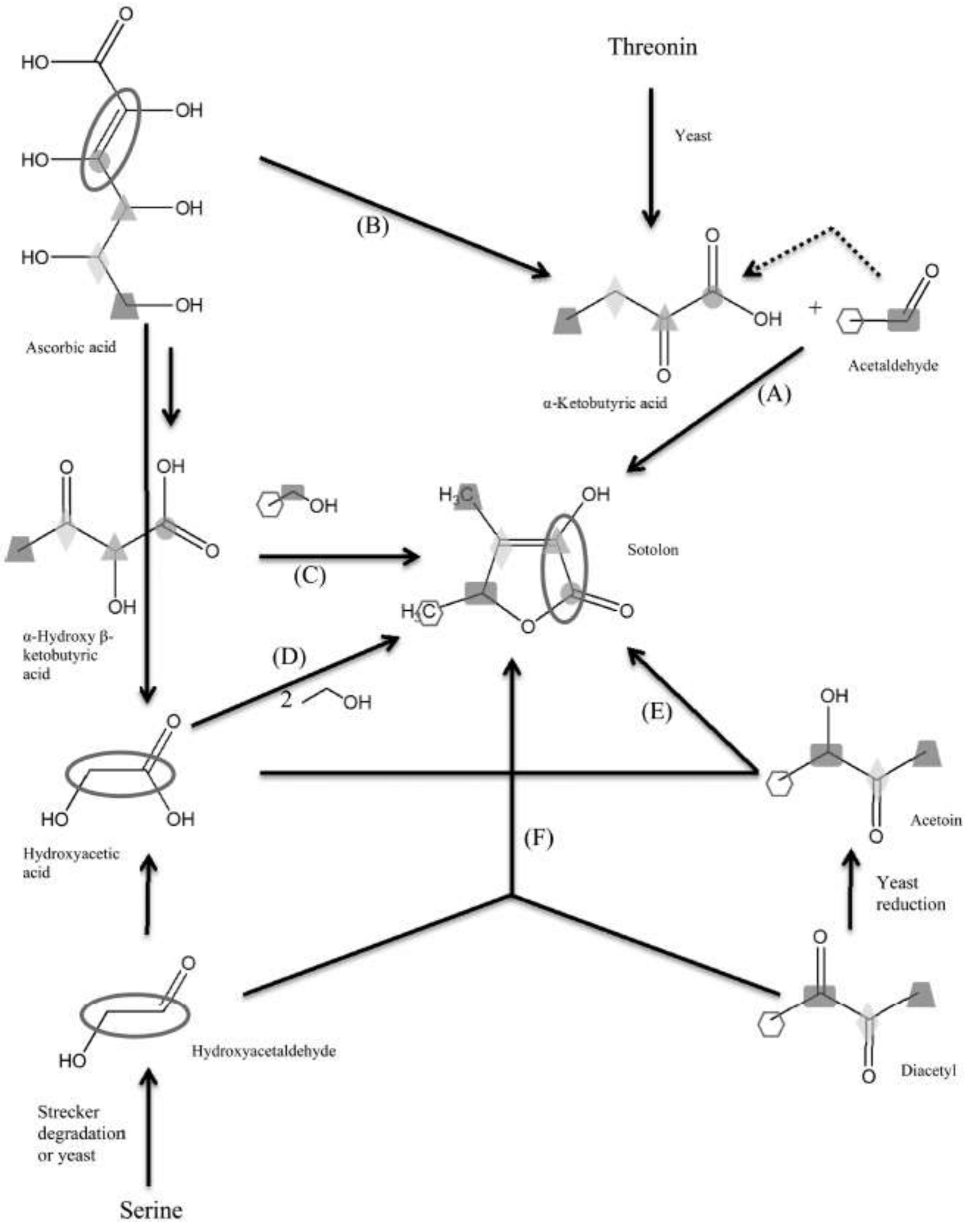


Ascorbic acid (AH_2)

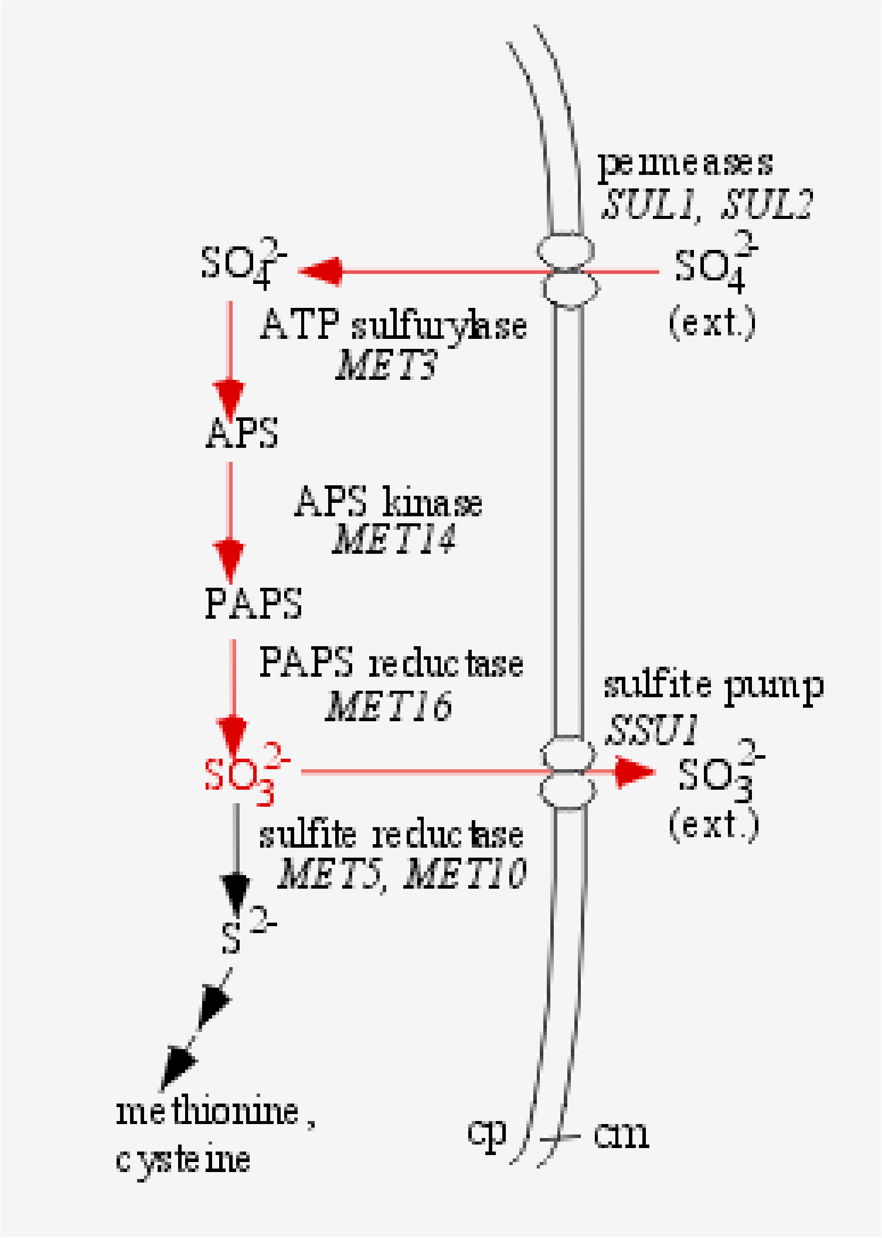
Dehydroascorbic acid (A)

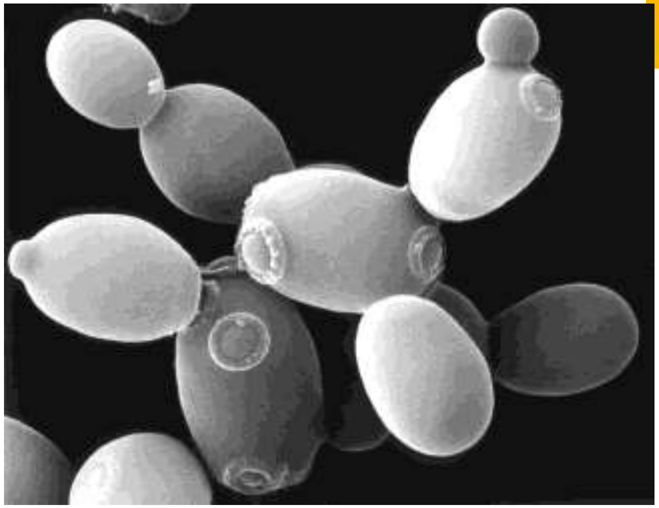
$$AH_2 + RH_2 + O_2 \rightleftharpoons A + R + 2\,H_2O$$

“Linked” reactions mean other reducing compounds (RH_2) are simultaneously oxidized. (R = oxidized organic compounds)



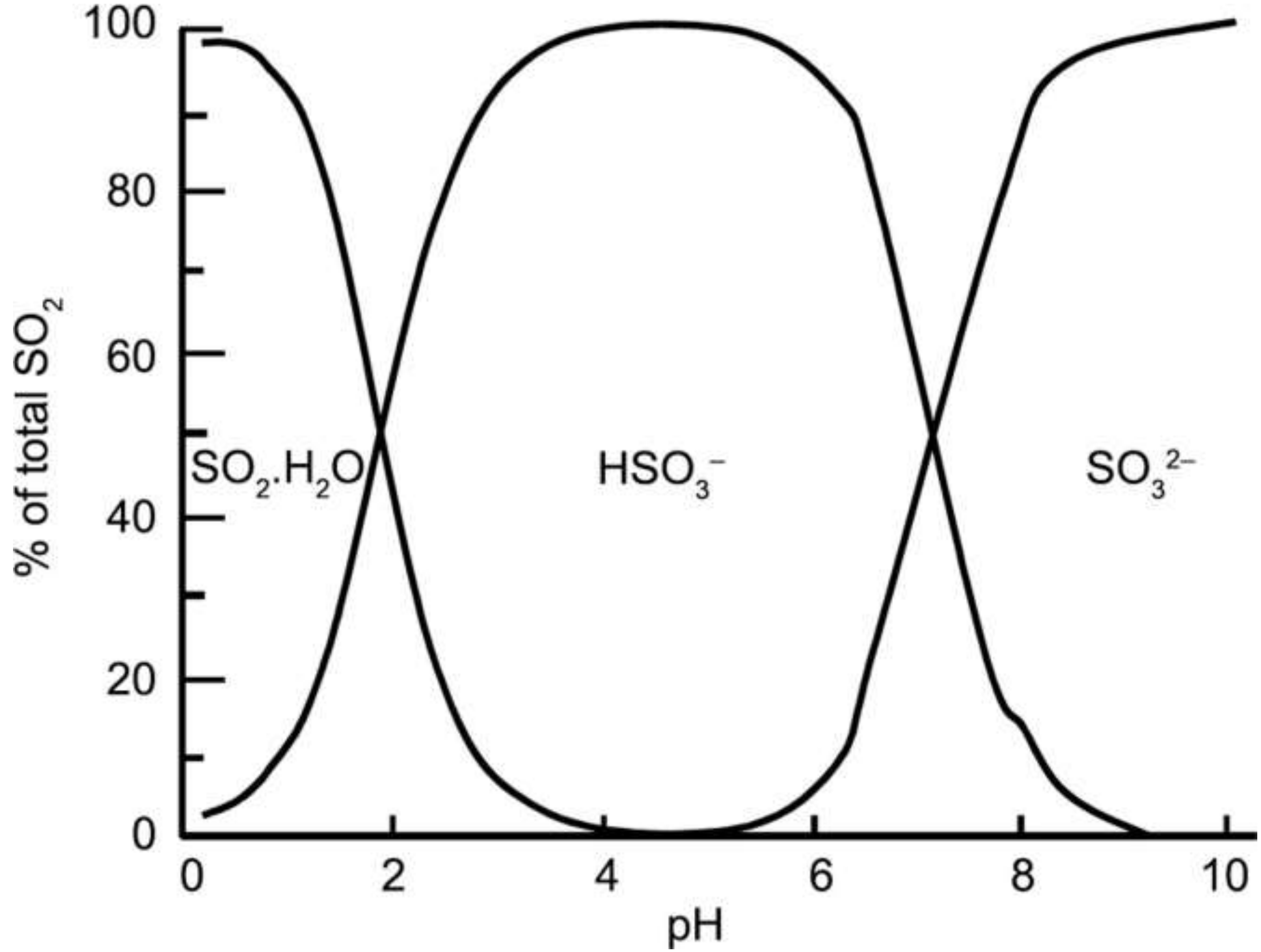
J. Agric. Food Chem. 2015, 63, 2886−2892

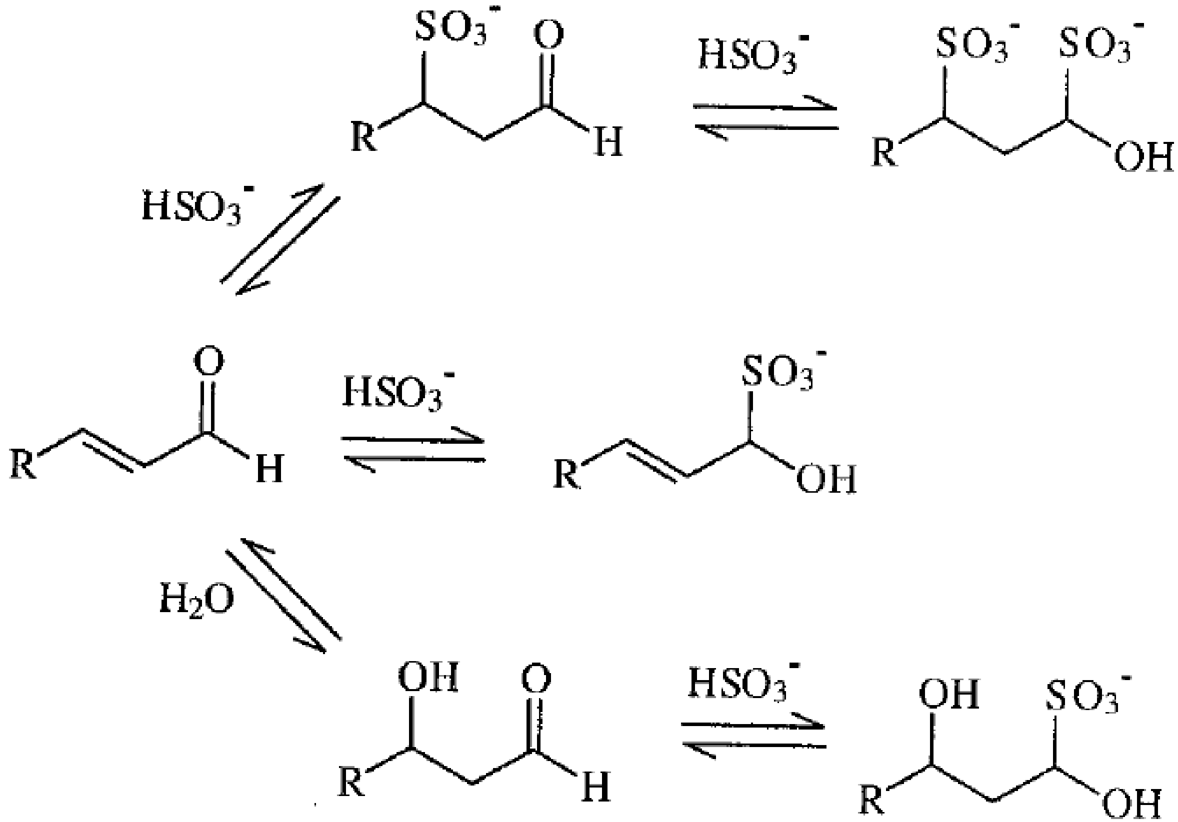




Sulfite Formation by Yeast

- SO_2 formation of yeast is genetically determined and amounts up to 10 ppm
- Can be increased e.g. by reduced aeration at pitching
- In general every measure that reduces growth increases SO_2 formation


100
80
60
40
20
0
% of total SO_2
$SO_2.H_2O$
HSO_3^-
SO_3^{2-}
0
2
4
6
8
10
pH



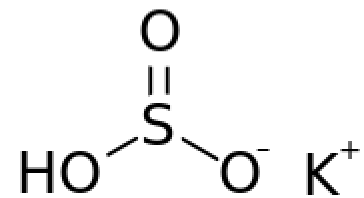
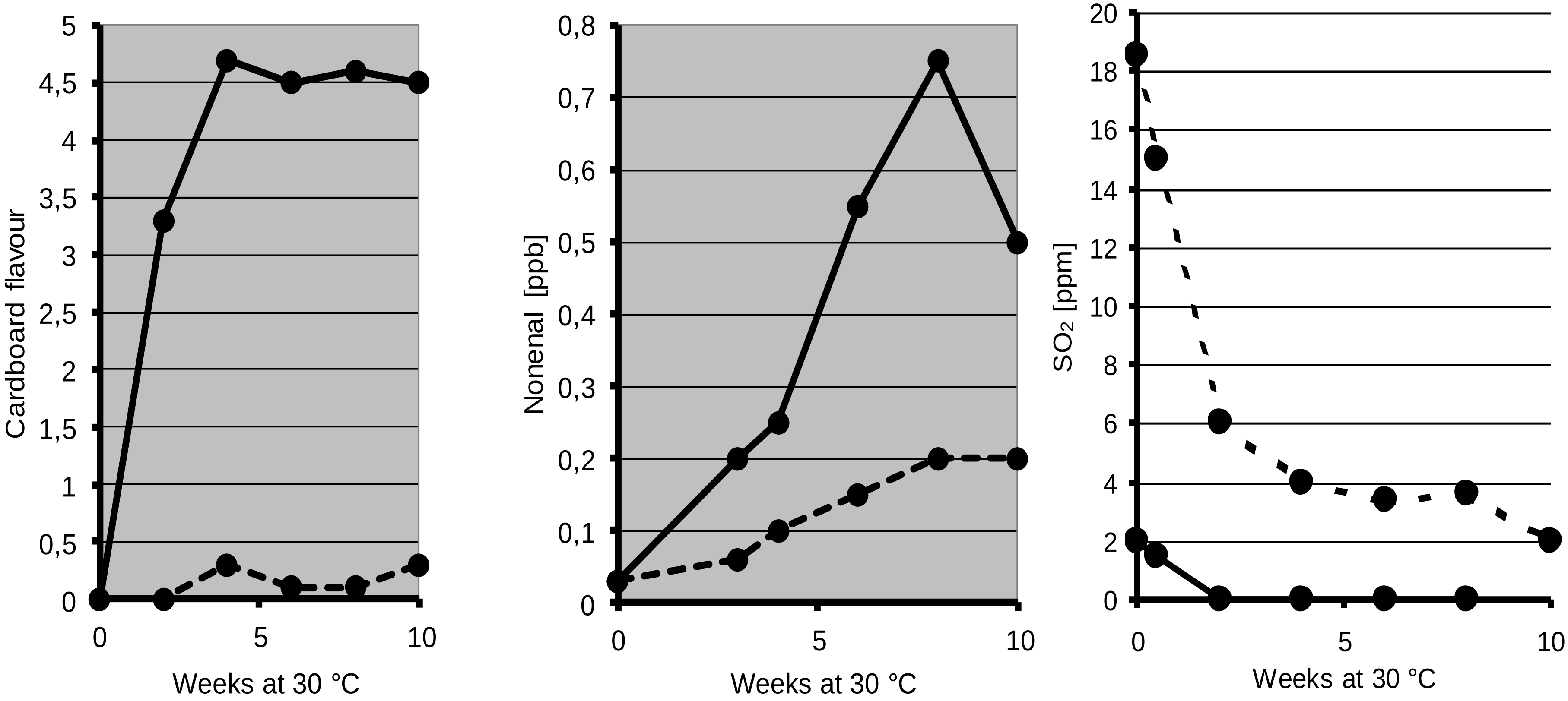


Fig. 2. *trans*-2-Nonenal-bisulfite equilibria (R = CH_3-$(CH_2)_5$-) as proposed by Barker et al (1).

Dufour, J.-P., Leus, M., Baxter, A. J., and Hayman, A. R.

FLAVOUR STABILITY

Flavour Stabilization of Bisulphite Addition to Beer



A) Effect on appearance of cardboard flavour
B) Effect on 2E-nonenal formation
C) Disappearance of sulphur dioxide during ageing

SO_2:
2 ppm ———
18 ppm – – –

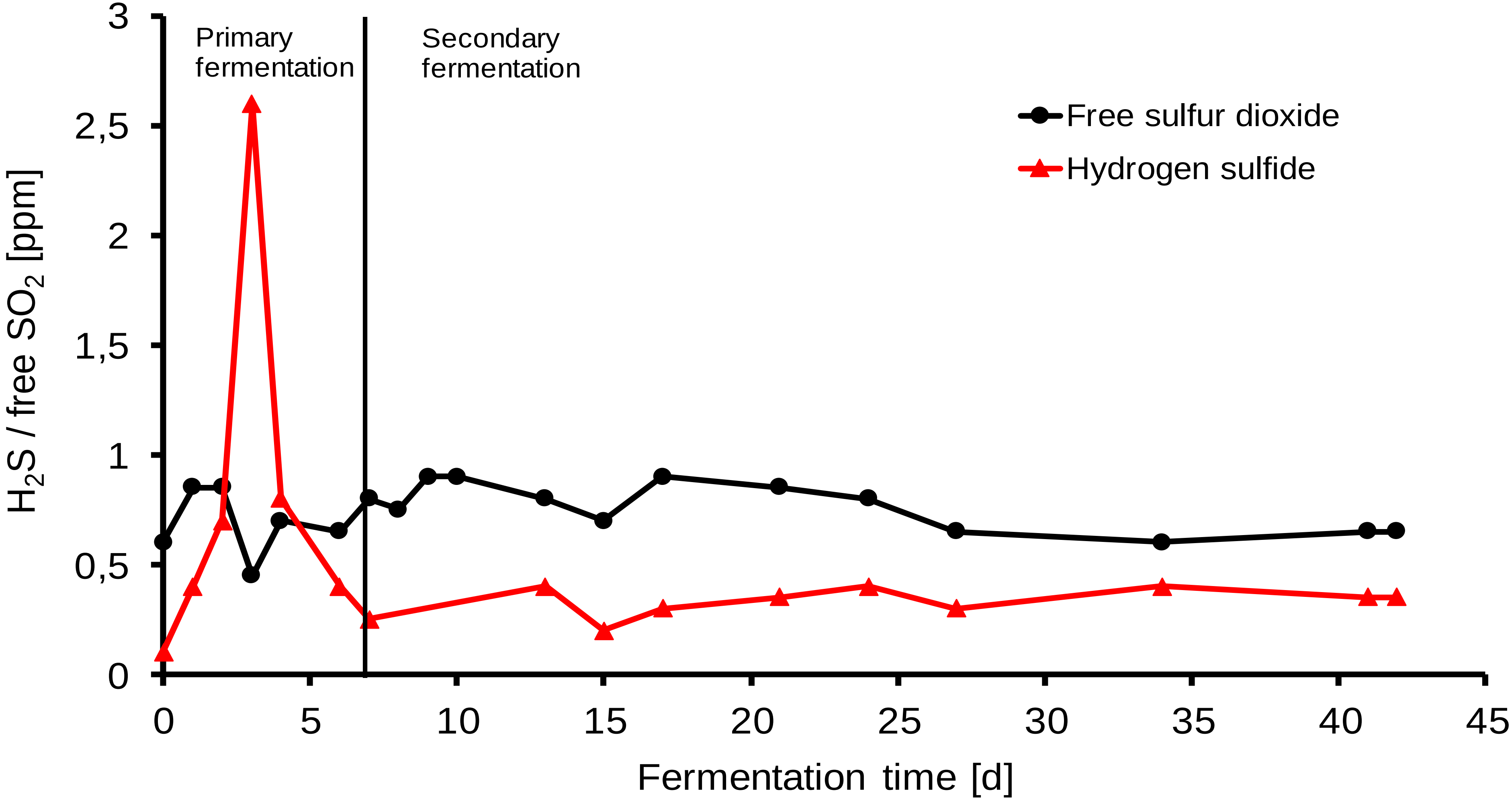
Important Influences on SO_2- Formation

- Wort composition (especially gravity)
- Oxygen content of pitching wort
- Yeast strain
- Physiological state of yeast

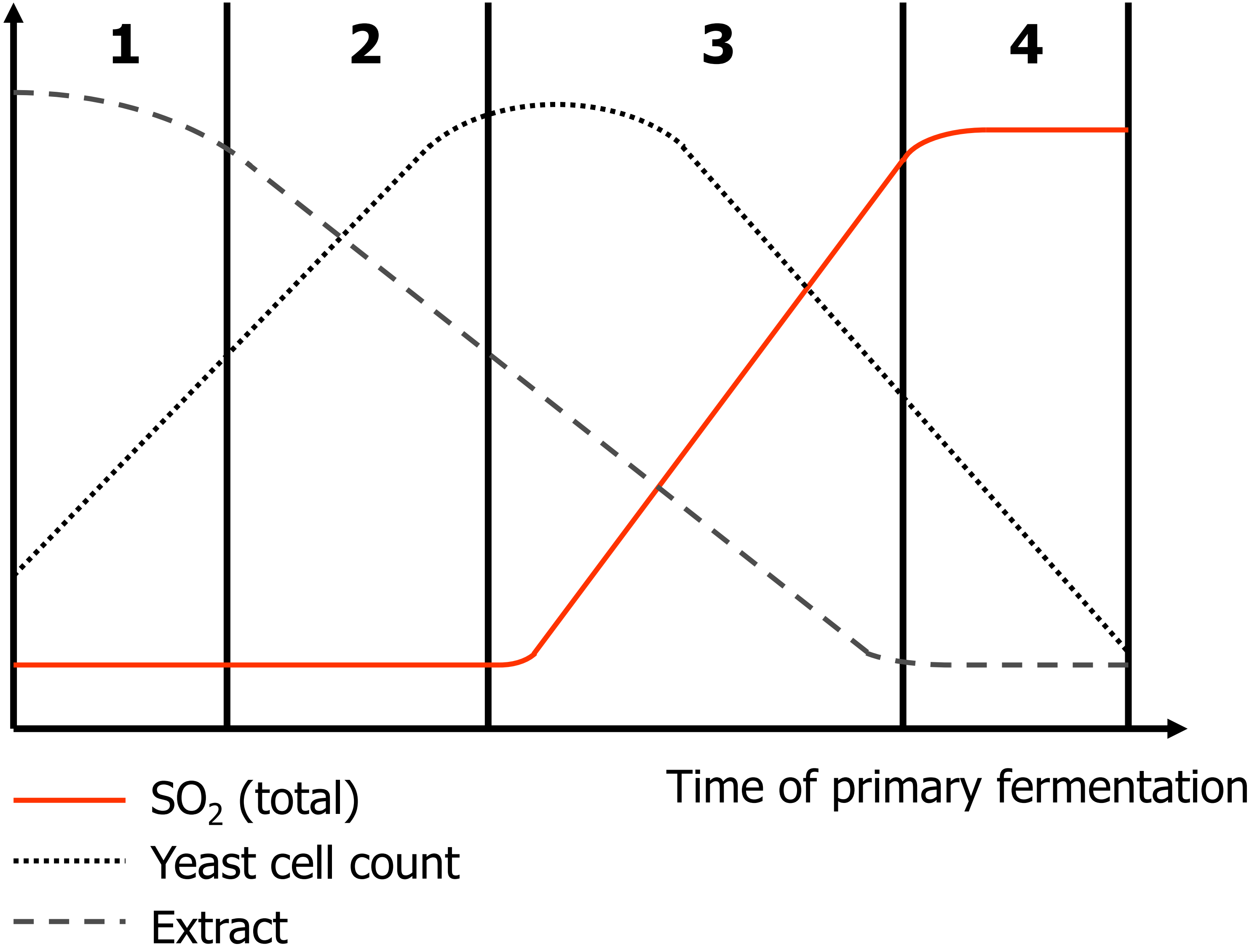
→ Influencing factors are similar to those of ester formation

FORMATION OF H_2S AND FREE SO_2 DURING FERMENTATION AND LAGERING

Pilsen-type beer

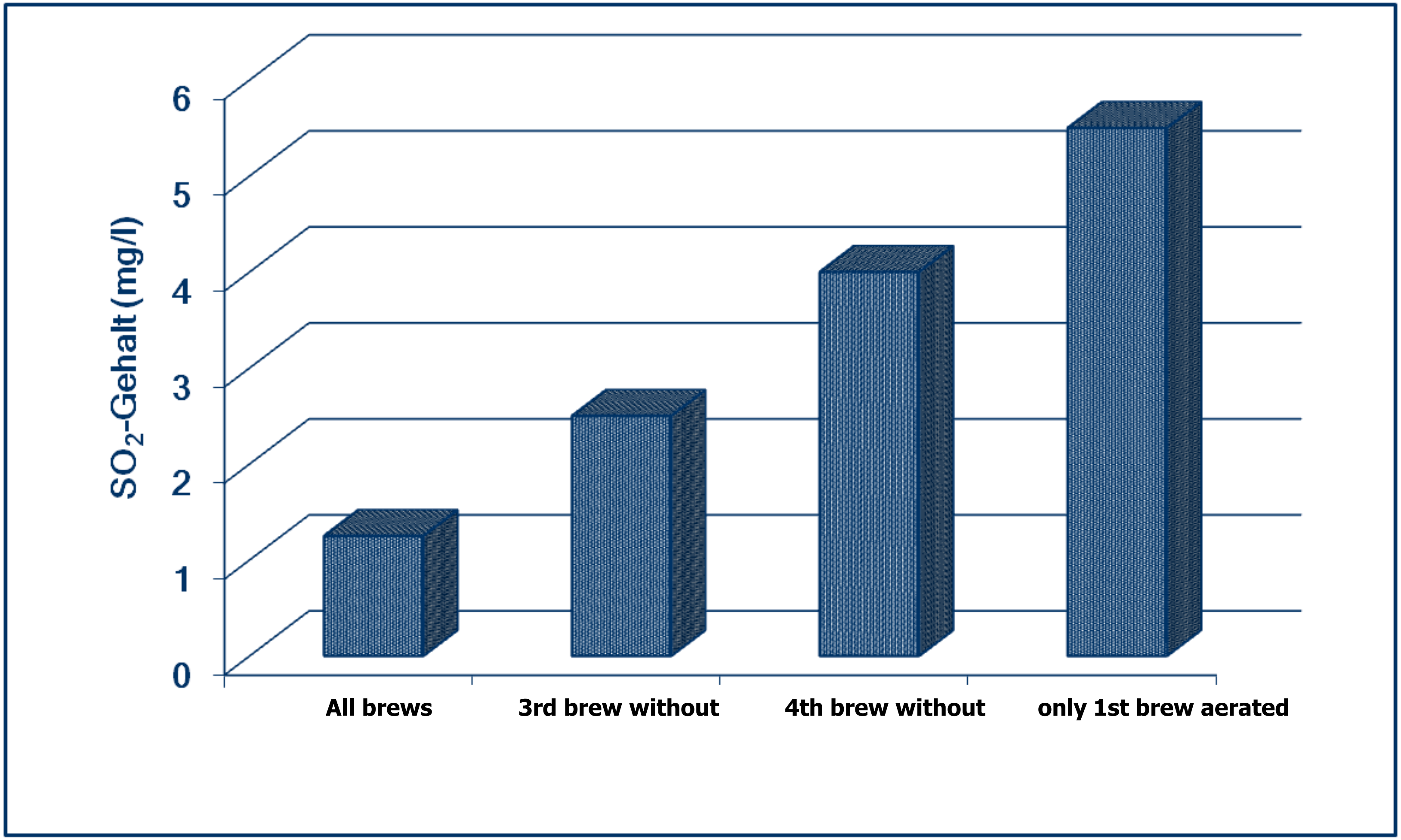


Phases of SO_2- Formation



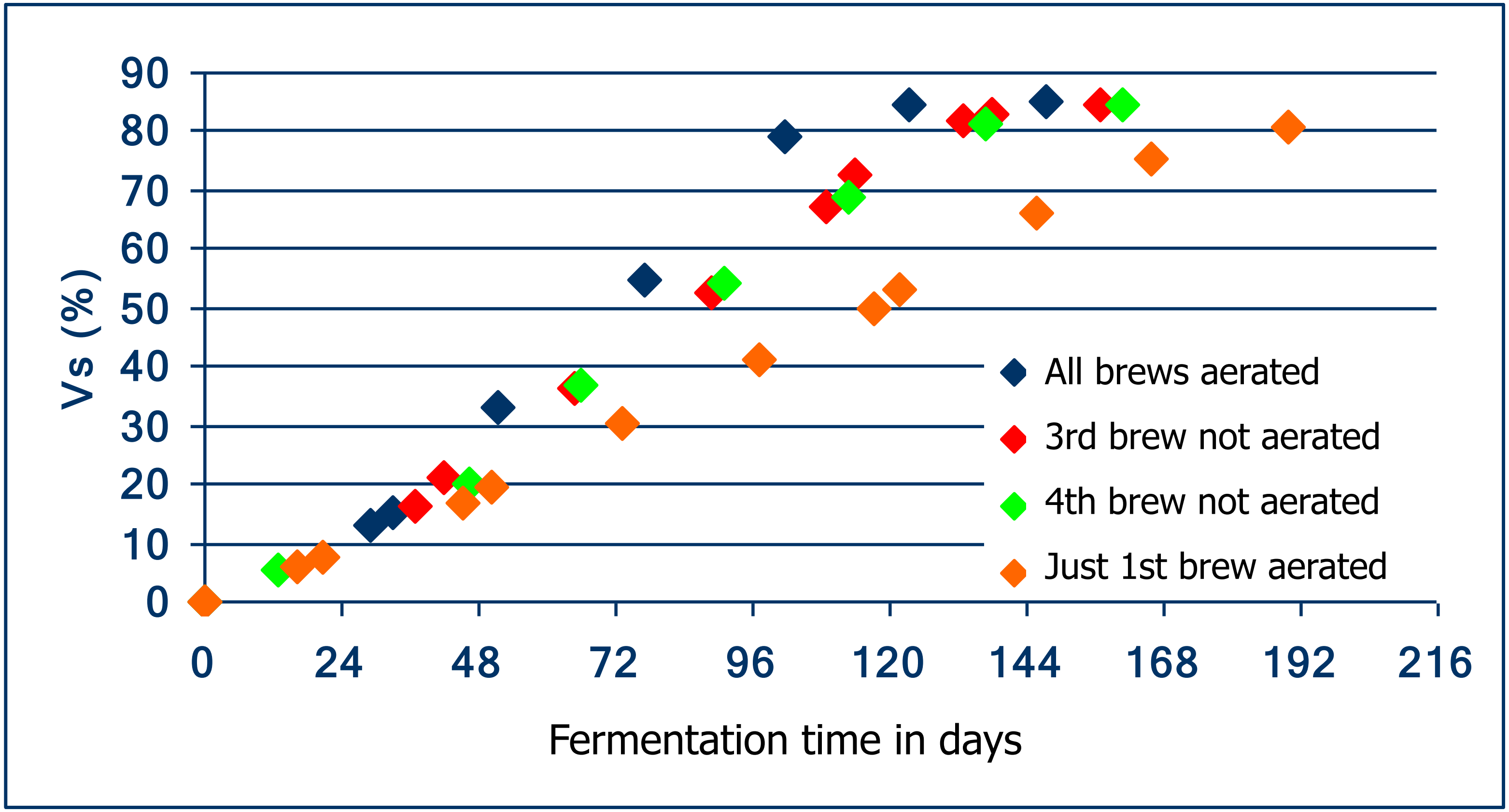
EBC Proc. 23, 1991

SO_2 -Content depending on Aeration Strategy



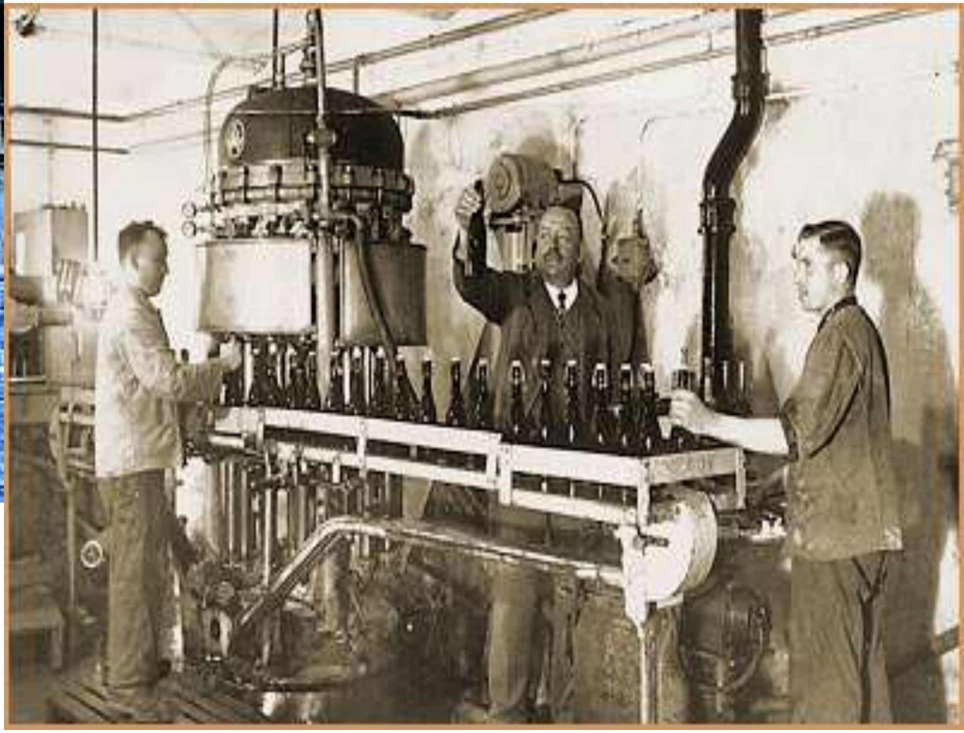
Source: C.Tenge

Aeration Strategy



Source: C. Tenge

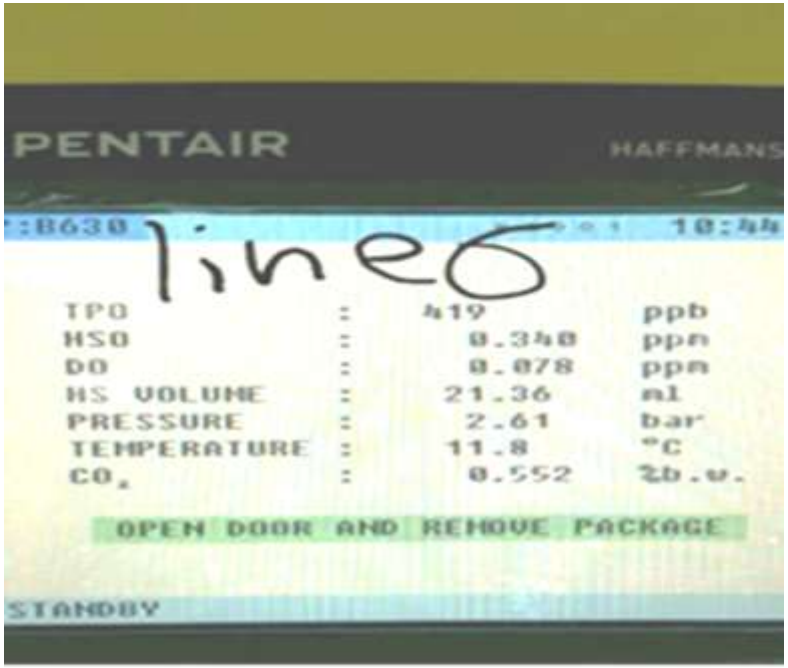
Influence of Packaging on Flavor Stability



Case Study from Brewery Consulting

- **Pick-up during filling**
- Random spot checks: 0.15 mg/L to > 0.4 mg/L (TPO)
- Target should be: < 0.1 mg/L

- TPO: Dissolved + headspace oxygen



Influences on Oxygen Pick-Up during Filling

- Filling pressure (high → high O_2)
- Filling tube
- Pre-evacuation (1x, 2x, 3x)
- CO_2 rinsing
- CO_2 in ring vessel
- Air in bottle neck
- **Jetter (!)**
- Time while not closed

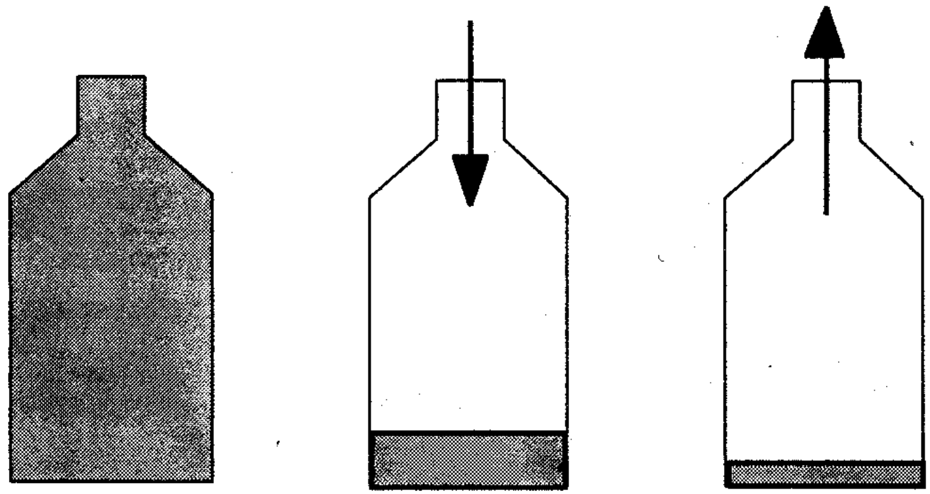
O_2 uptake through filler: 0.01…0.1 mg/l (goal: 0.05 mg/l)

Oxygen Reduction during Filling

e.g. 0.5 l bottle

Application of vacuum 90 % removal of air

Pre-pressurising using CO_2



Contents:	520 ml air at 1 bars	52 ml air at 1 bars	17 ml air at 3 bars

Jetter

Air in bottle neck: (500 ml bottle → 20 ml →4.2 ml O_2 → 5.6 mg O_2)

What can I do to improve my Stability?????

Simple Approaches with little Investment Costs…..

Raw Materials

- Barley
  - Low LOX, No LOX varieties (?!)
- Malt
  - Protein and FAN content
- Yeast
  - SO_2 – production
- Water
  - De-aeration (< 5 ppb possible)

Check List - Brewhouse

- Reducing heat stress (Strecker aldehydes; TBI)
  – Malt
  – Wort boiling
  – Whirlpool
  – Wort cooler performance

- Reduction of reaction partners
  – Lower FAN contents of malt and less FAN release during mashing

- Oxygen uptake (?!)
  – Milling
  – Mashing-in
  – Mashing (turbulent surface)
  – Lautering

Oxygen Check List - Cold block

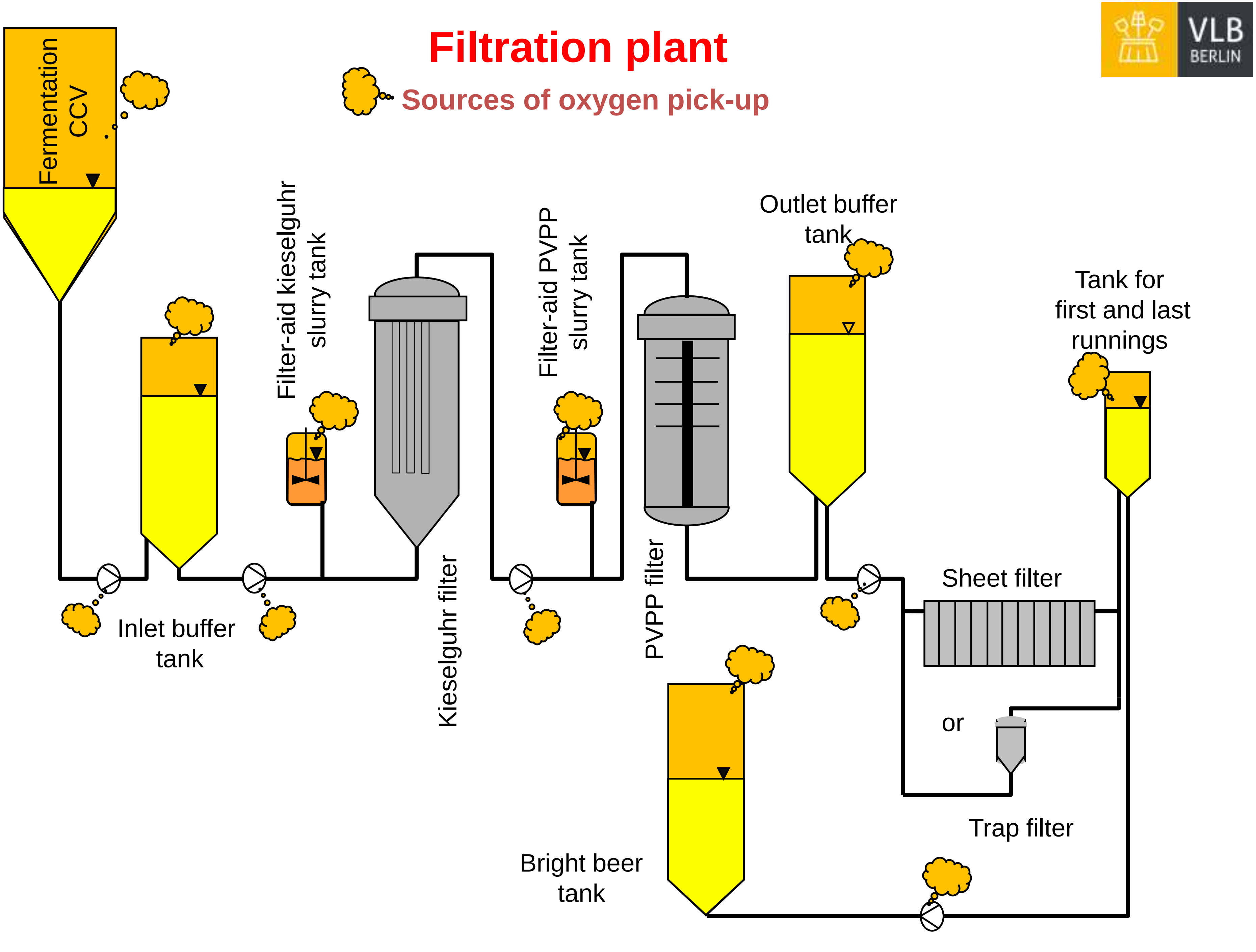
- Tank farm
  - Pre-pressurising and emptying with inert gas (N_2, CO_2)
  - Immediate pitching (after aeration)
  - Transfer into maturation tank without oxygen uptake
  - Check if connections to pump are tight (snorkeling of air)
  - Function of transfer pump´s mechanical shaft seal
  - Flow rate into tank too high → fountain formation

- Pipelines
  - „Snorkeling“ of air by untight connections
  - Avoidance of air cushions by filling hoses and pipes with (de-aerated) water backwards
  - Centrifuges

Oxygen Check List - Cold block

- Filter
  - De-aerated water, line de-aeration
  - Proper bleeding when sterilizing and before pre-coating
  - Inert gas and slow running stirrers in DE-dosing-vessel
  - Iron and cupper contents in DE
  - Pre- / post-runs to maturation, scrubbing with CO_2 or blending

- BBTs
  - Low oxygen in BBTs: CO_2-pressurizing from below
  - No oxygen approach: BBT filled with water and emptied with CO_2
  - Cleaning with acid under CO_2-athmosphere keeps tank oxygen-free



Filtration plant
Sources of oxygen pick-up
VLB BERLIN
Fermentation CCV
Inlet buffer tank
Filter-aid kieselguhr slurry tank
Kieselguhr filter
Filter-aid PVPP slurry tank
PVPP filter
Outlet buffer tank
Tank for first and last runnings
Sheet filter
or
Trap filter
Bright beer tank

Oxygen Check List - Packaging

Packaging

- Correct operation of jetter (check air in head space)
- Efficient pre-evacuation
- Machine construction
  - single chamber filler, multi chamber
  - Filling tube
  - Filling pressure
- Good removal of oxygen from filler ring channel
- Removal of coarse bubbles with CO_2 flow (bubble breaker) before can seamer
- Reduce stops of filler (open bottles)

The Bottom Line

- Avoid oxygen uptake as good as possible!
  - At any stage except for wort aeration
  - Maybe precursors for oxidation can be minimized
  - Optimize yeast and its production of natural antioxidants (SO_2)
  - The investment into a DO-meter is expensive but inevitable when optimizing the process
- Avoid heat stress and minimize the precursors for non-oxidative staling (FAN)
- Keep beer cold and try to minimize agitation of the product during transport

BUT: Beer is no beef jerky or dry pretzel! Its flavour will always change over time! You can only slow the process down by the above measures!

Thank you for your Attention!

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