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Research Findings On Haze Formation From Transition Metals And Some Tips For Addressing This Problem

Frank-Jürgen Methner | MBAA Brewing Summit 2014 | Chicago

Flavor and Haze Stability

Haze Stability is important with respect to consumers‘ expectations and beer quality

Origin of Haze in Beer

- Biological haze
  - Yeasts
  - Beer spoilage bacteria, e.g. Lactobacilli, Pediococcus, Megasphaera, Pectinatus
- Organic or inorganic haze
  - Retrograded starch (α-glucans)
  - Pentosans / β-glucans (barley or spoilage organisms)
  - Calcium oxalate
  - Filter aids
- Colloidal haze formation
  - Interaction of haze active proteins and polyphenols have been identified as one main reaction for haze formation during storage
  - PVPP and silica gel currently stabilize beer
  - Nevertheless haze still increases during aging.

Influencing Factors on Haze Stability

oxygen (air)
(formation of ROS)

temperature
(acceleration of chemical reactions)

metal ions
(catalytic factor, complex formation)

Reaction Products of Fenton- / Haber-Weiss-Reaction and Complex formation by Metallic Ions

light
(formation of ROS)

alcohol
(polarity - bonding strength of metallic-ion-polyphenol-protein complex)

pH-value
(metallic ion bonding sites and speed of the Fenton-Reaction System)

Fenton- and Haber-Weiss Reaction System

Fenton-Reaction:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{-} \quad (k_1 = 63\ M^{-1}sek^{-1})$$

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

Net: $$2\ H_2O_2 \xrightarrow{Fe} OH^{\bullet} + OH^{-} + O_2^{-\bullet} + 2\ H^{+}$$

Haber-Weiss-Reaction:

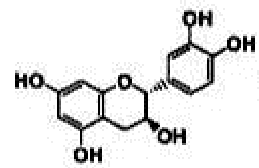
$$Cu^{2+} + O_2^{-\bullet} \longrightarrow Cu^{+} + O_2$$

$$Cu^{+} + H_2O_2 \longrightarrow Cu^{2+} + HO^{\bullet} + OH^{-}$$

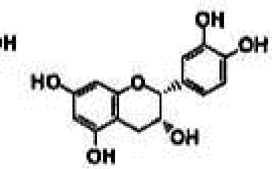
Net: $$O_2^{-\bullet} + H_2O_2 \xrightarrow{Cu} O_2 + OH^{\bullet} + OH^{-}$$

Beer Haze Compounds

- High molecular degradation products of proteins
  - 10 - 60 kDa
  - Prolin rich fraction

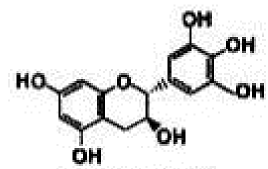


(+)-Catechin



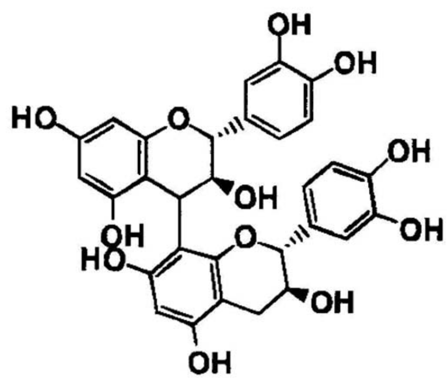
(-)-Epicatechin

- Phenolic compounds
  - Flavan-3-ole
    (Catechin, Epicatechin, Gallocatechine,...)
  - Polymers (Dimeres, Trimeres);
    => Proanthocyanidines

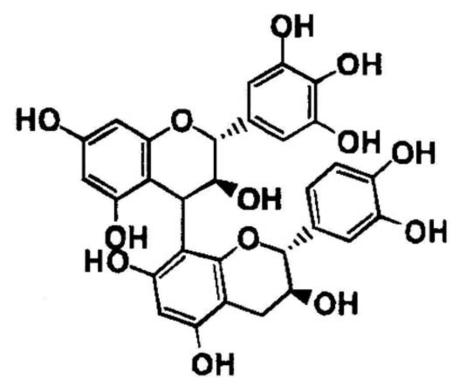


Gallocatechin

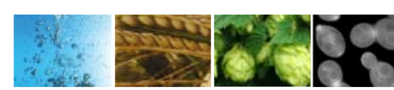
- Carbohydrate (α- / β-Glucan)
- Mineral substances
- Metal ions



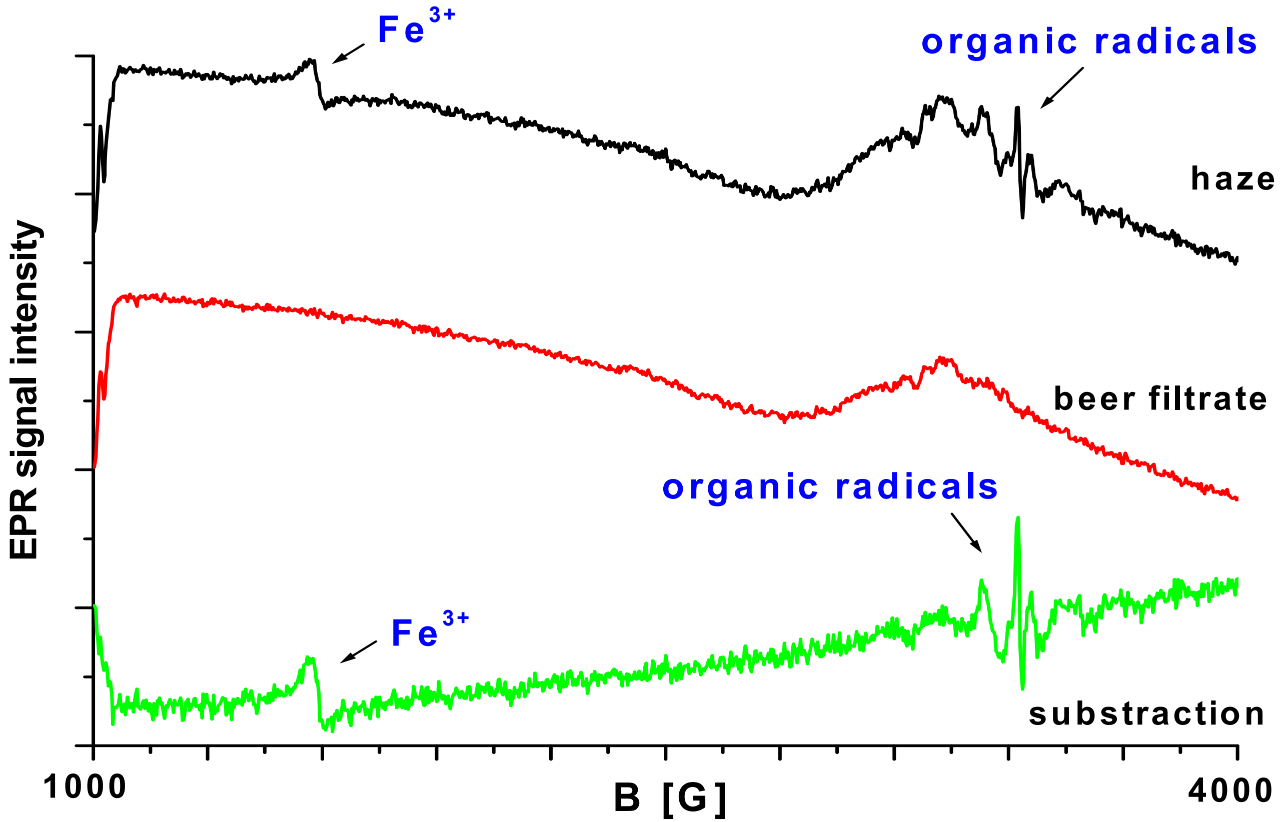
Procyanidin B3



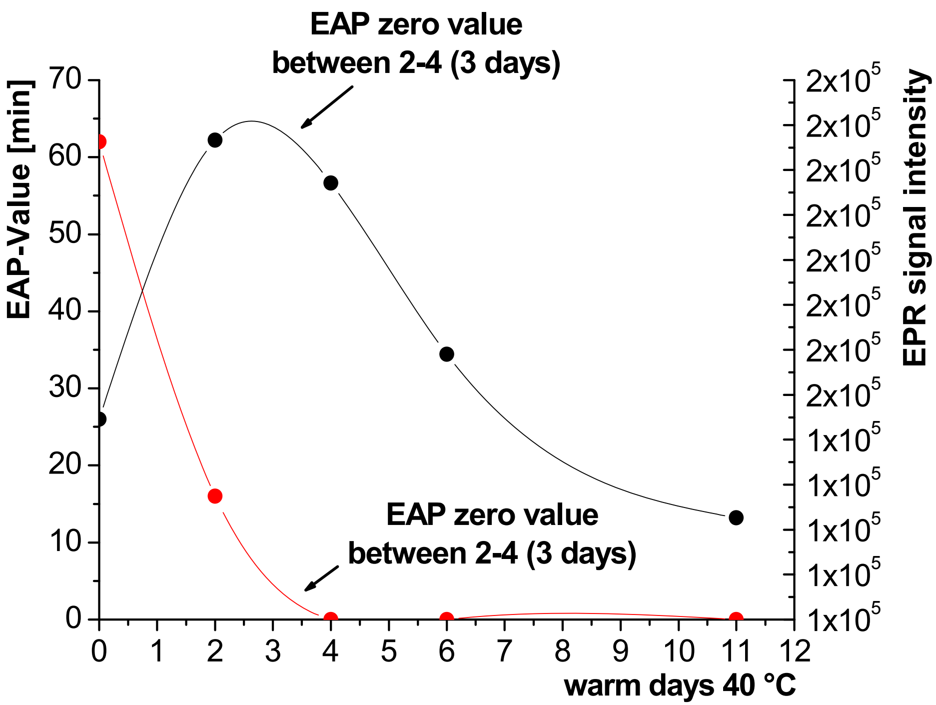
Prodelphinidin B3

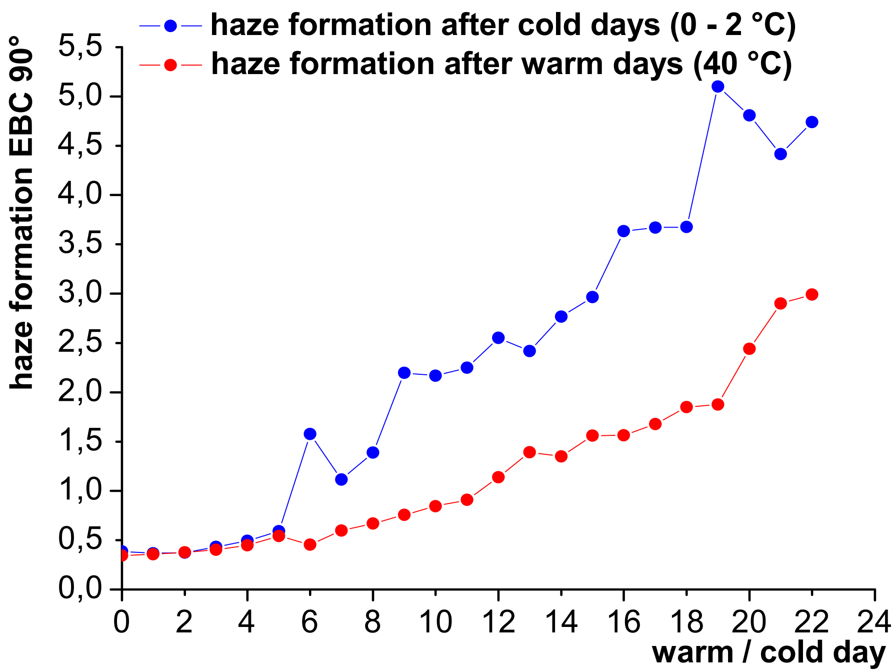


EPR-Spectra – Sweep Wide 3000 G



Influence of Air in Bottle Headspace





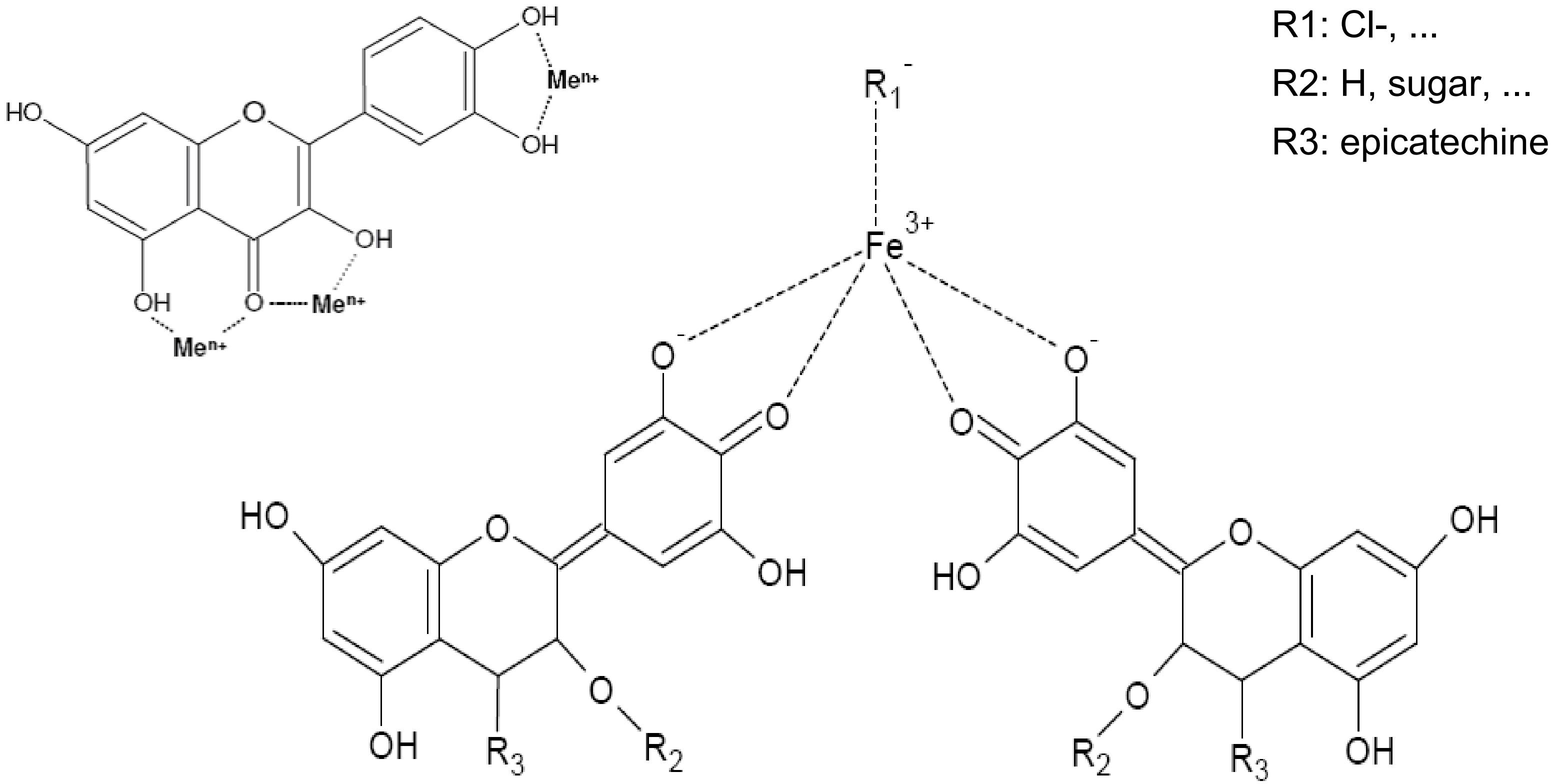
Chill Haze Formation

Beer with a low EAP-value (2 ºC)

Addition of $Iron^{3+}$ ($FeCl_3$)

Addition of $Iron^{2+}$ ($FeSO_4$)

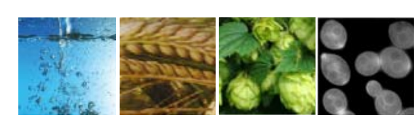
Possible Complex Formation Between Oxidized Polyphenol-Protein-Complexes and Iron^{3+}



R1: Cl-, ...

R2: H, sugar, ...

R3: epicatechine, ...



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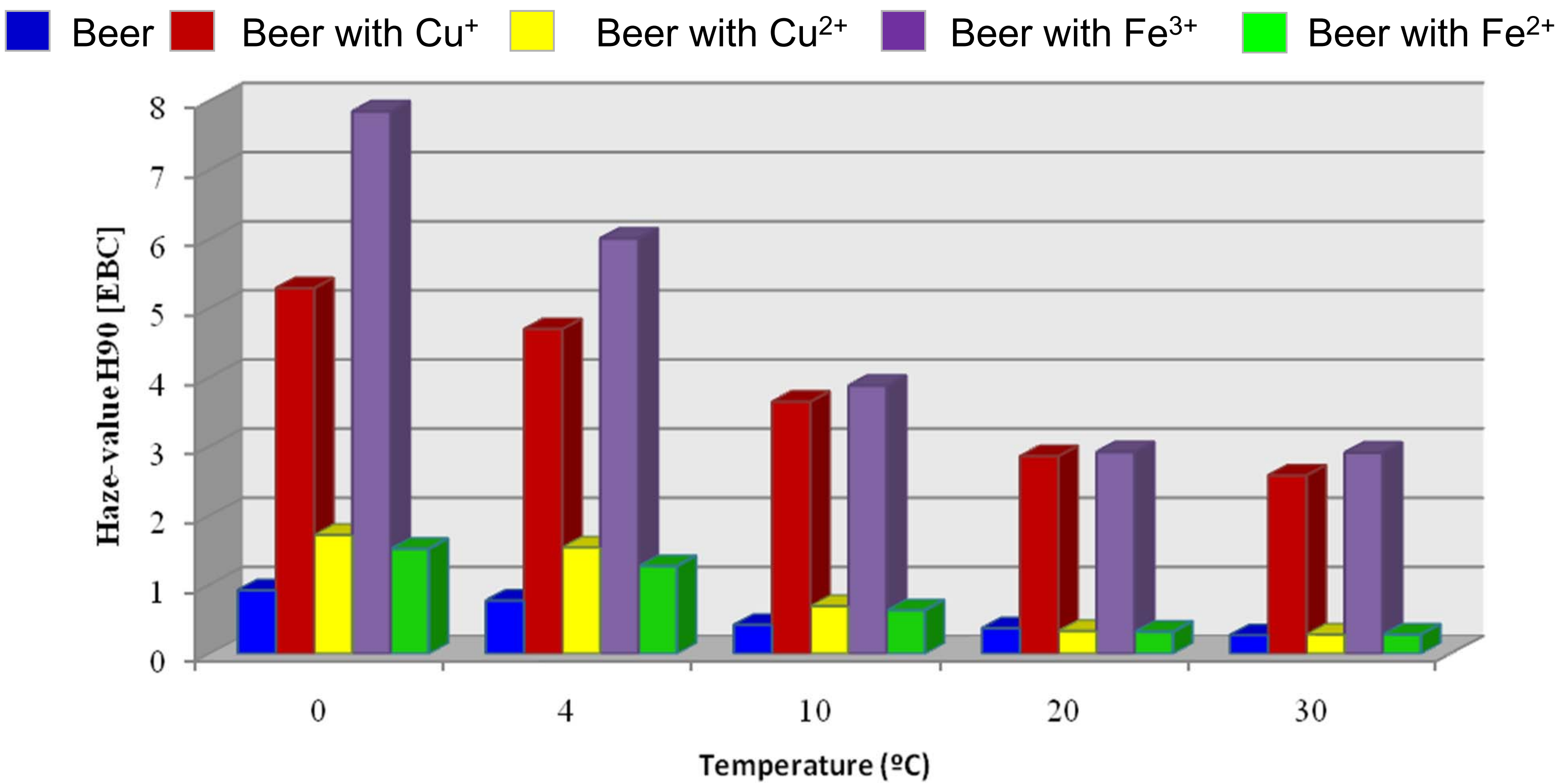
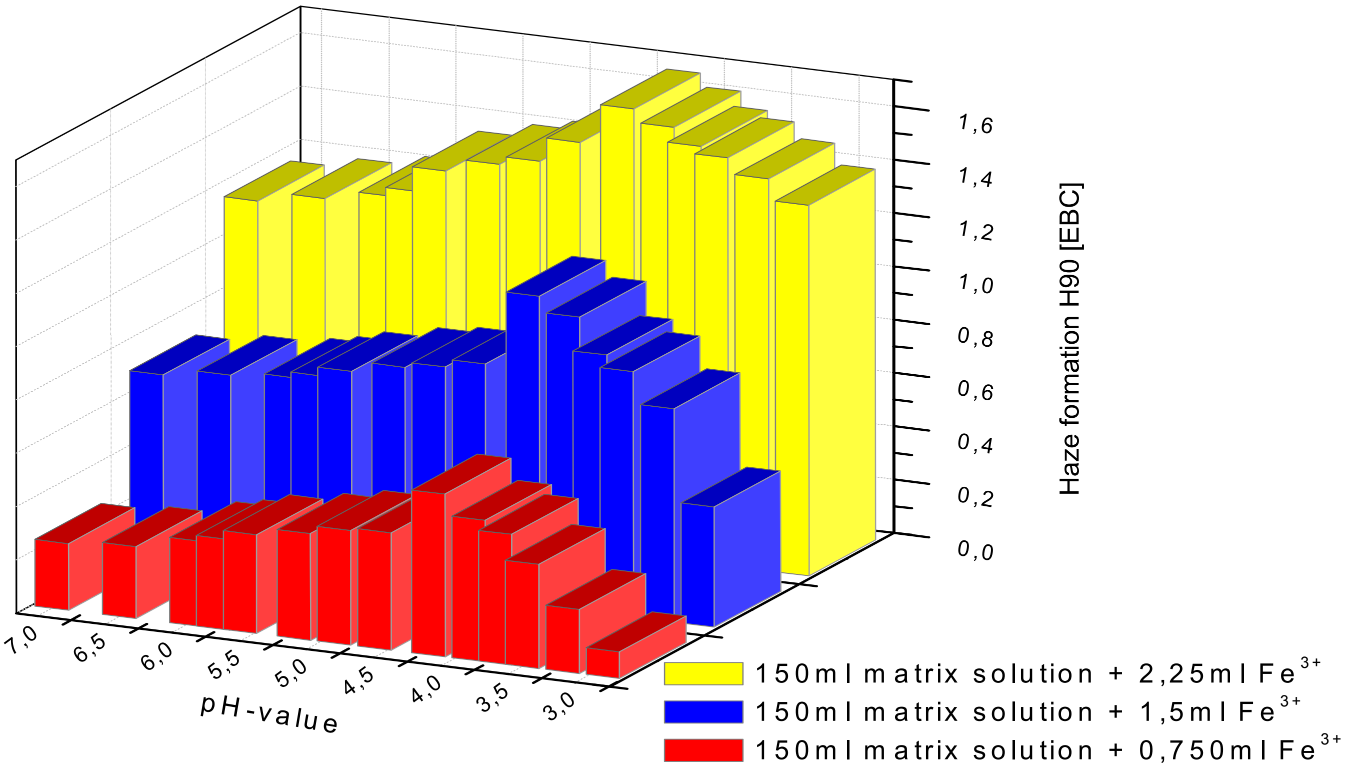
Haze Formation After Addition of Metal Ions



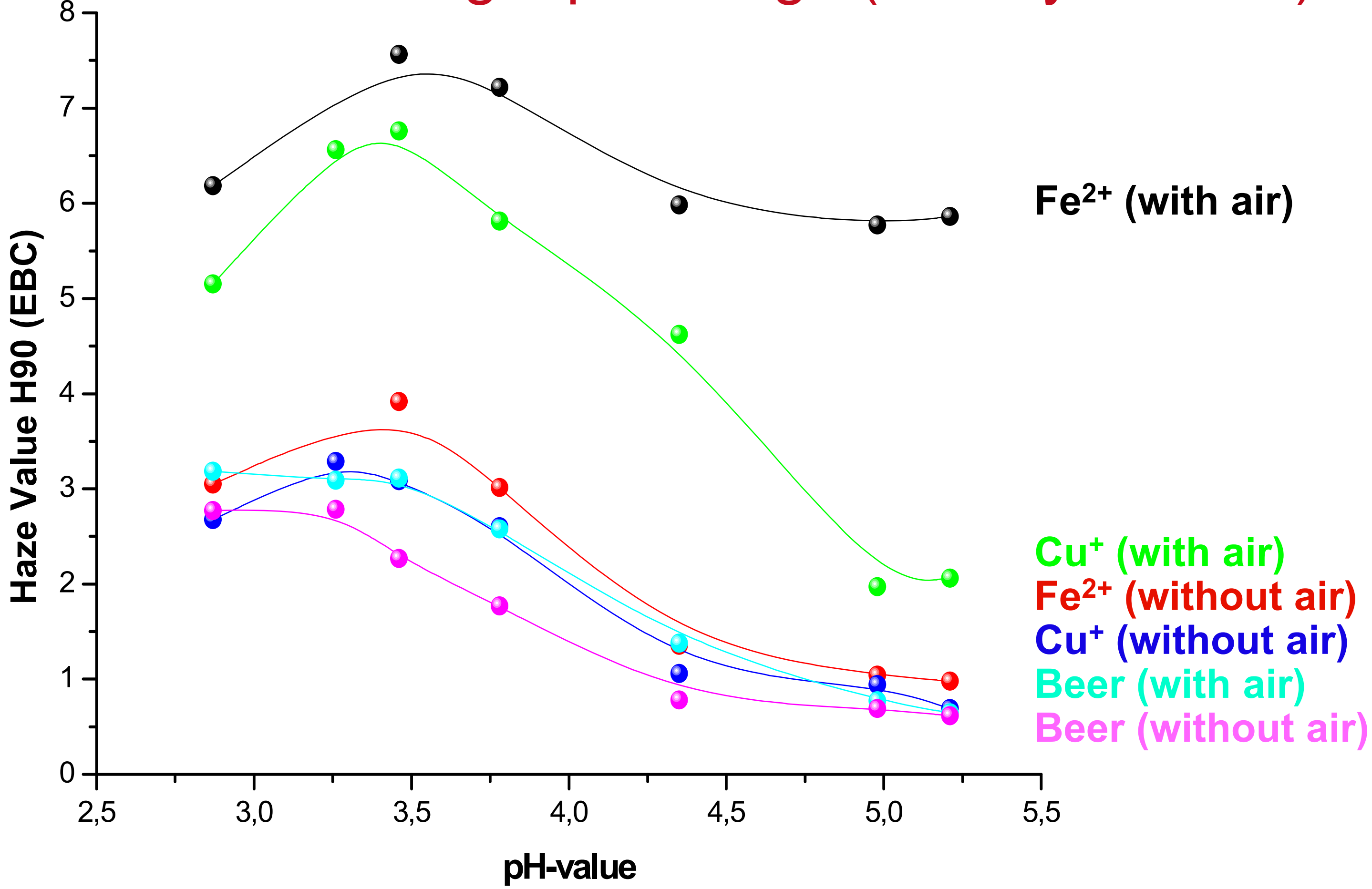
1 ml 0,01 M metal ion solution / 150 ml beer

Haze Model Solution

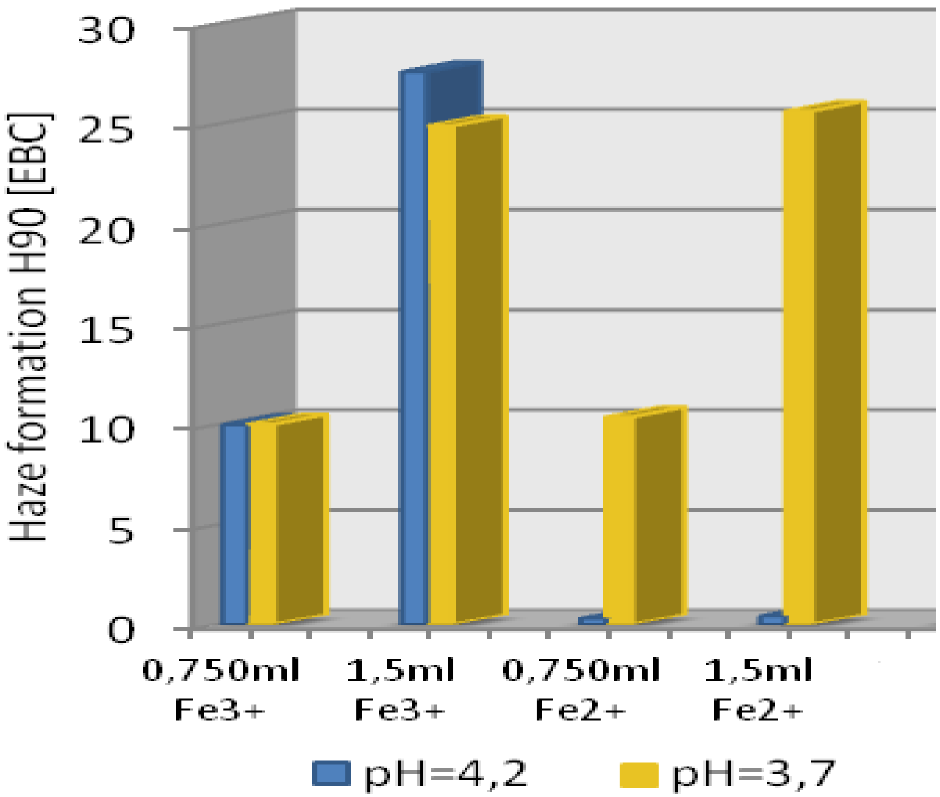


Effect of Fe^{3+} (0,01 M) and pH-value on the haze formation in a gliadin (25 mg/L) / hop tannin extract (5 mg/L) - solution (0.2 M phosphate buffer) – according to Siebert et al.

Haze Formation Along a pH Range (12 Days - 40°C)



pH Dependent Haze Formation With Iron Ions in a Gliadin / Hop Extract Model Solution



pH dependent bonding sites in iron complexes

Fe[3+], FeOH[2+], Fe(OH)2[+]

$[Fe(H_2O)_6]^{3+}$, $[Fe(H_2O)_5OH]^{2+}$,$[Fe(H_2O)_4(OH)_2]^{+}$

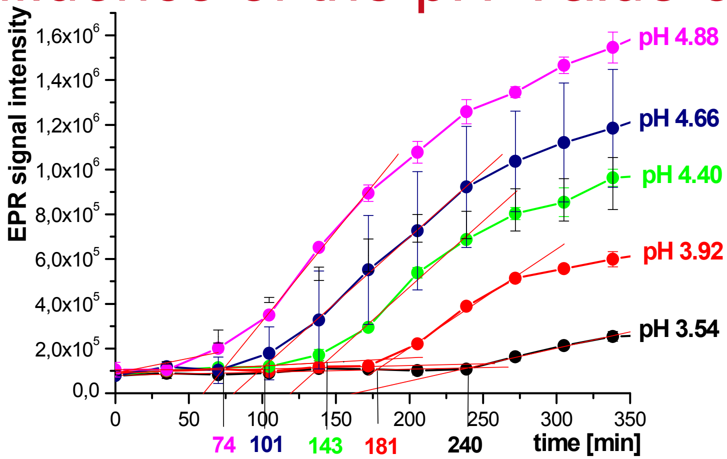
——— increase pH-value ———>

Fe[2+], FeOH[+]

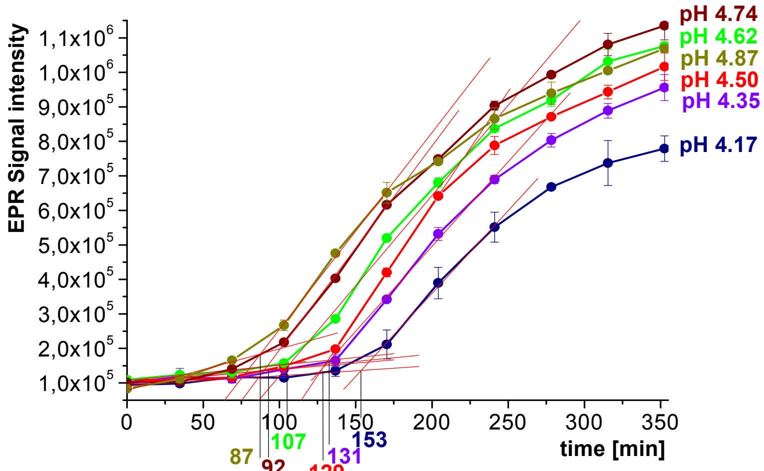
$[Fe(H_2O)_6]^{2+}$, $[Fe(H_2O)_5OH]^{+}$

0,75 - 1,5 ml Fe^{2+} / Fe^{3+} 0,1 M => 150 ml gliadin / tannic acid Solution (100 / 20 mg /l)
phosphate buffer **pH 4.2 & 3.7**; 0.2 M

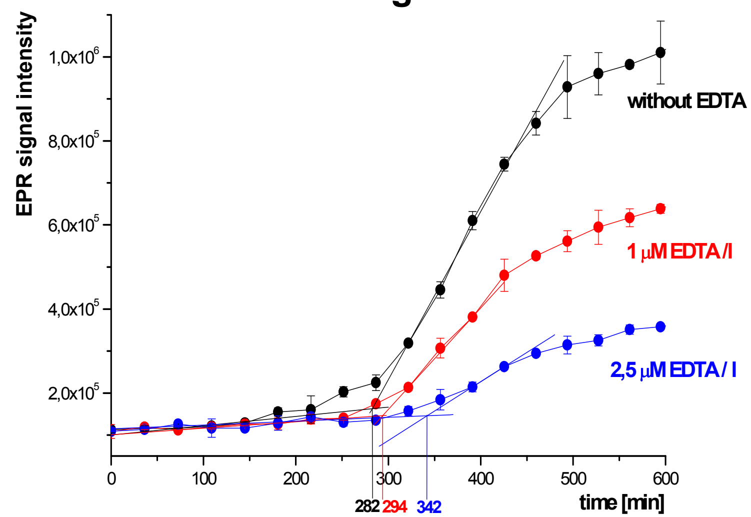
Influence of the pH-Value on the Oxidative Stability

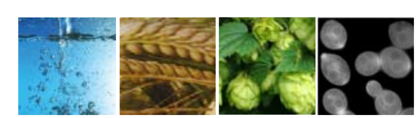


Influence of pH on the EAP-value and radical generation

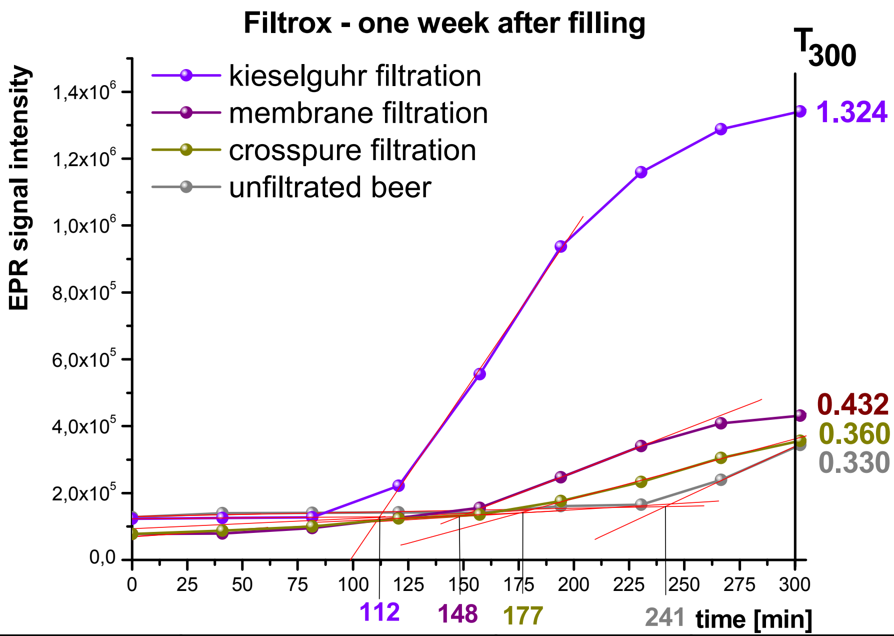


Influence of EDTA on the EAP-value and radical generation



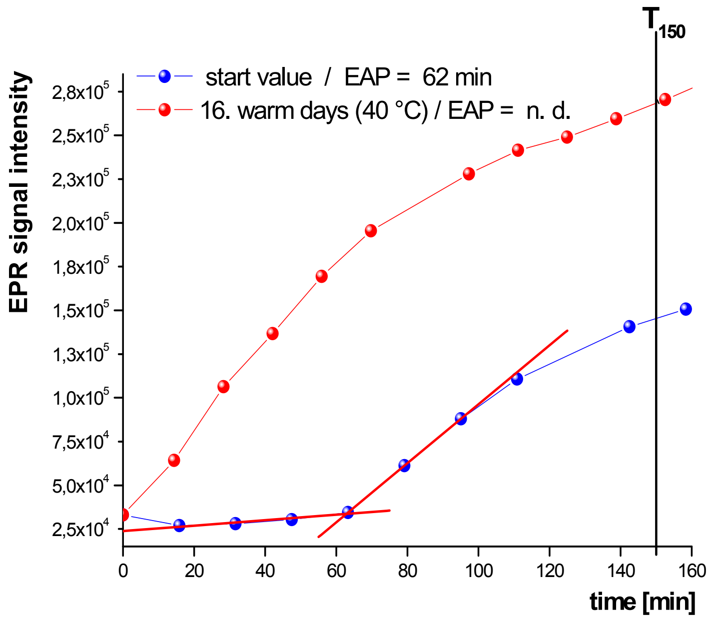


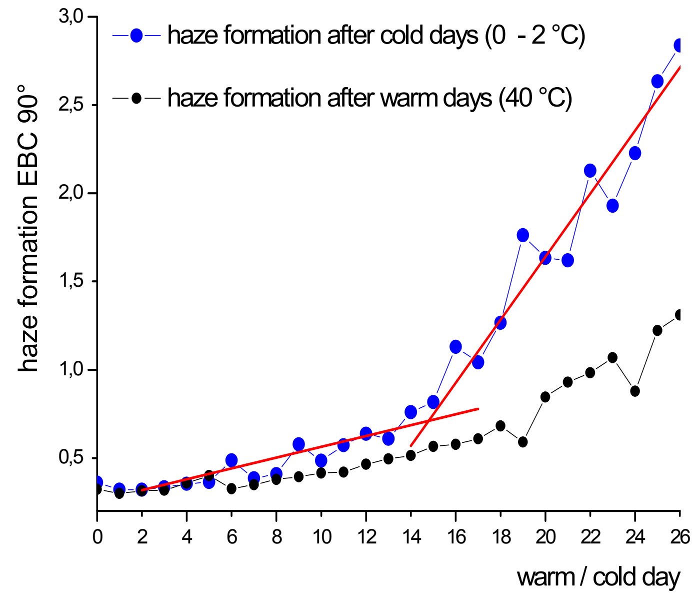
Influence of Filtration on Radical Generation and Iron Content



	unfiltered beer	Kieselguhr	Crosspure	Membrane
Fe [µg/L]	--	63	14	17
pH-Value	*4.29*	*4.35*	*4.33*	*4.34*
EAP-value [min]	241	112	177	148
T_{300}	1.32	0.33	0.36	0.43

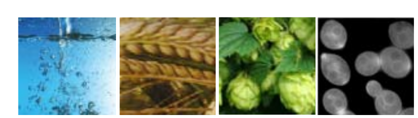
EAP-Value and Haze Formation



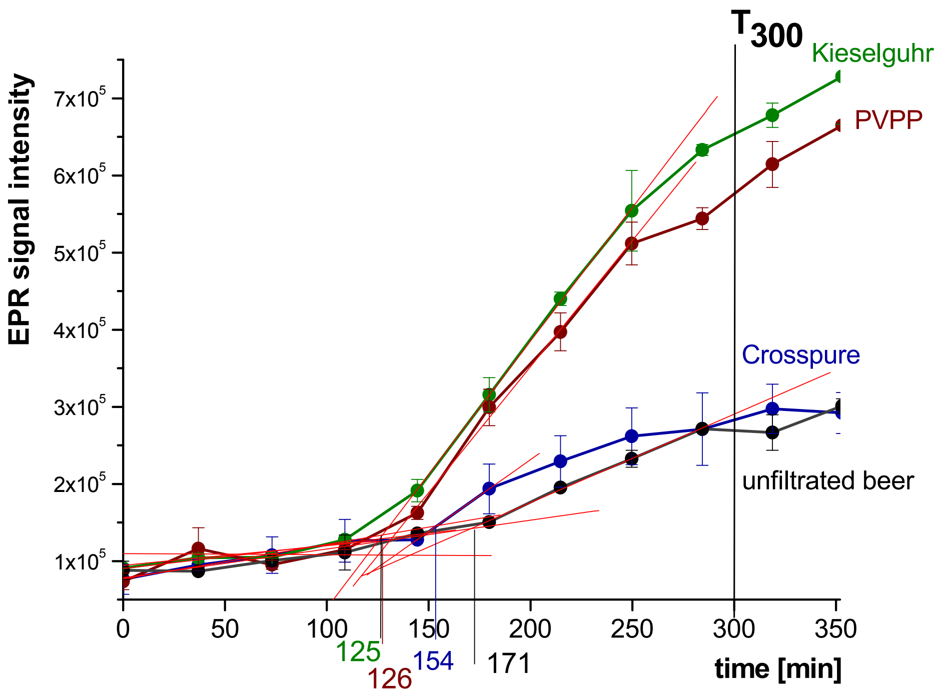


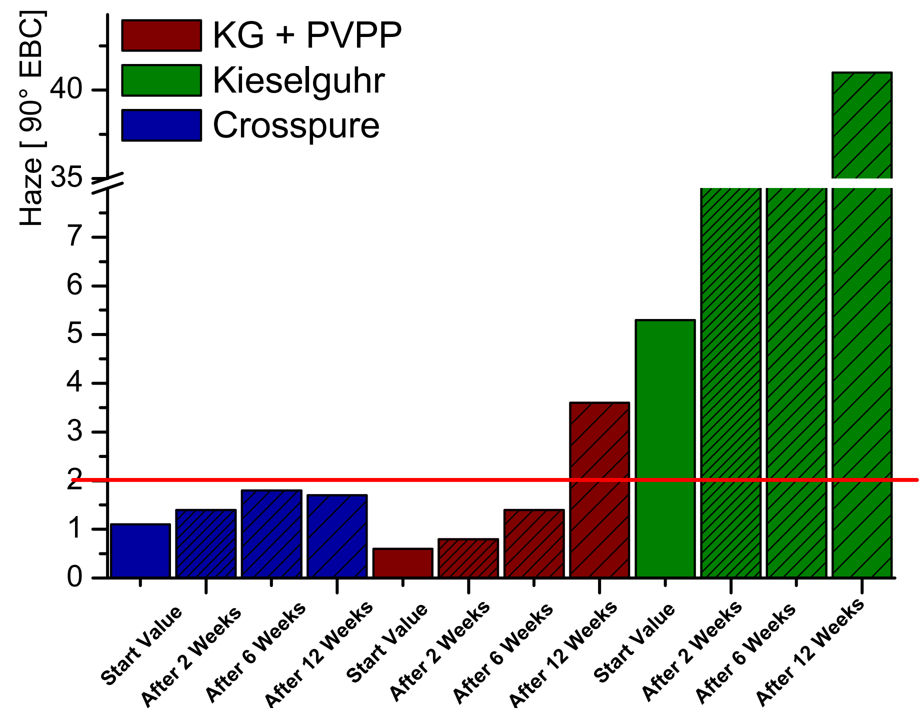
- <u>Haze measurements:</u>
- chill haze at 0 ° C
- permanent haze at 20 ° C

- <u>Storage conditions:</u>
- - warm storage 24 h at 40 ° C
- - cold storage 24 h at 0 – 2 ° C



Influence of Kieselguhr and Crosspure Filtration on Haze Formation and Oxidative Stability





Conclusion

- After the consumption of the EAP, the reactive hydroxyl-radicals and metal ions like iron-(III) are generated by the Fenton- / Haber Weiss reaction system in beer
- Due to the complex formation among oxidized polyphenol-protein-complexes and iron-(III)/copper-(I)-ions the development of chill haze can be observed
- Chill haze is dependent on the temperature and pH-value
  - Lower temperatures (< 5° C) and pH values result in an increase of chill haze formation, based on the stronger bonding power and pH-dependent availability of bonding sites in the metallic complexes.
  - At higher pH ranges, the lower participation of metal ions in complexes results in a higher availability for oxidative processes leading to an earlier but lower haze formation in beer.
- Stabilized organic radicals in chill haze can react with each other resulting in the formation of covalent bonds which describes one probable way of the conversion from chill haze to permanent haze