

Listening in: yeast trafficking in reductants - implications for beer stability

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1.0 Listening to What?

Some day-traders explain that playing the stock market is a little like surfing. It's not necessary for surfers to understand the physics of simple harmonic motion to pick a good wave, a.k.a. a good buy. Just recognise the shape, the frequency, the likely trends and balance that against experience.

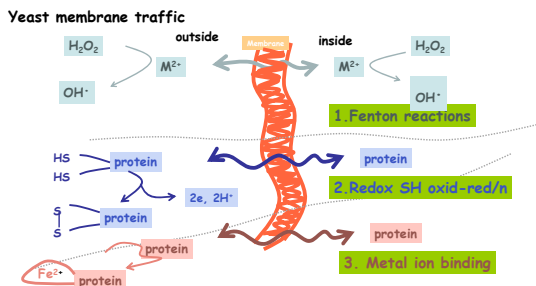
Applied science is a bit like that. Businesses don't have too much time for the fundamentals. But we often have experience on our side, and we can listen and we can learn. We can imagine ourselves looking out to sea, trying to recognise patterns or frequencies, just to stretch the metaphor, and isn't that just a little bit like listening in?

We would like to convince you that by 'listening in' to macromolecular frequencies, we can gain insights that point to new ways of stabilizing beer, ways of breaking away from the old shibboleths. Akin to what the Japanese brewers have done with Happoshu and the 3rd-beers? Shown how to make a quality product without malt?

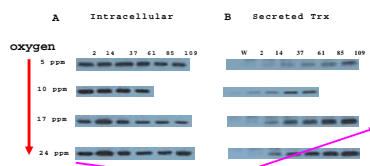
Listening-in to membrane traffic has led us to develop a novel finings agent for stabilising beer. So novel, so good, so effective that it can do the things that isinglass and collagen can do. And not only that - stabilise beer at the same time.

2.0 Listening Devices

We can for starters listen to the membrane traffic. We can track the traffic. Walk the talk. It's quite loud. There's lots going on, in both directions, both in and out. Just look below at some proteins and metal ions, and that is not the half of it. Not to mention all those small redox active molecules like SO₂ and glutathione.



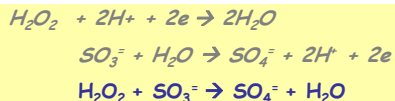
Look at how thioredoxin a small protein that has very reactive thiol groups, is secreted from brewers' yeast. The more oxygen added to wort at pitching the more goes out over time (x-axis) and the less remains in the cytoplasm.



The stain = thioredoxin, the more stain the more thioredoxin is present. Yeast were suspended in wort with 5-24ppm O₂; the yeast were sampled for 109h; thioredoxin was detected using 'Westerns'.

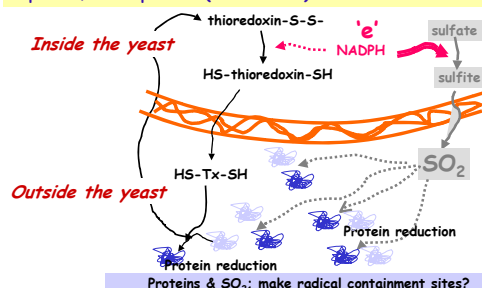
3.0 Short-Wave

Reactive O₂ leads to H₂O₂. (Remember the ROS sequence O₂-> H₂O₂-> OH[·]). Yeasts secrete SO₂ and the two react, so that the peroxide is destroyed. That we can all accept happens in beer. Choppy waves, short term effects, till the SO₂ runs out - but for the long term? NOoo..

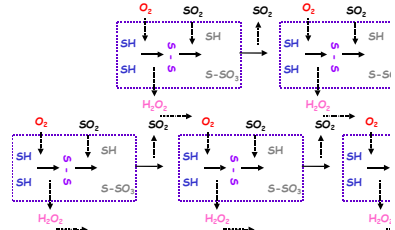


4.0 Permanent-Wave (La Cage aux Folles)

H₂O₂ is, surprisingly, not terribly reactive. It's more like ascorbate rather than a super reactive moiety like hydroxyl radical. It wanders around and takes it's time to damage and cause staling. H₂O₂ is likely produced by oxidation of protein thiol groups. Peroxide can be destroyed at reactive protein sites as shown below. SO₂ reactivity can be affected by binding to protein; redox proteins (rich in thiols) are best.



Protein disulfides (-S-S-) react with SO₂ and generate reactive thiols. A cyclical series of reactions involving oxidation of vicinal thiols, destruction of H₂O₂ with SO₂, sulfiteolysis of the disulfide bridge back to the starting thiols and so on can be imagined. Once the SO₂ is depleted the thiol groups become irreversibly oxidised, the protein is denatured, and hydrogen peroxide will dissipate and damage other compounds in the beer. The message? It pays to keep beer proteins healthy and functional.



'La Cage' in a 2D matrix

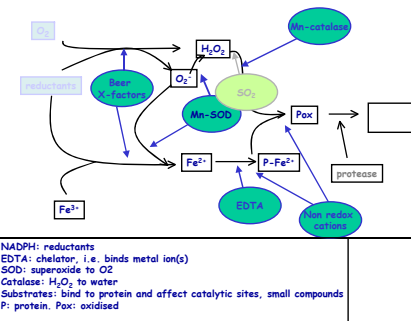
5.0 The Sounds of Silence

But what's missing? There is, of course something missing in the scheme above. Did you guess? (Answer: transition metal cations!)

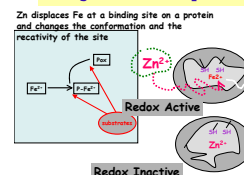
(It is beginning to look as though protein events have an important role to play in the formation of free radicals such as the putative hydroxyl radical. It is also known that protein oxidation results in the formation of carbonyls and free radicals. It is also clear that bound metal ions can play a significant role in enhancing or inhibiting the rates of these protein-centric reactions. Redox cations like Cu and Fe and Mn certainly. But non redox cations like Zn can also have an effect if they are able to bind and even displace Fe or some of these others. They may cause conformational changes which in turn render previously catalytic sites inactive - and perhaps with better outcomes as far as staling goes.)

6.0 NEW-Wave

NEW-wave / long term solutions? Any chance? Yes. Consider our own mortality. As we age proteins oxidise and accumulate carbonyls. This can be accelerated, by smoking. And it can be slowed down fortunately, by drinking red wine and beer as well. Fe²⁺ is particularly effective in catalysing protein oxidation when it is bound.



Dislodge the Fe²⁺ (with a chelator) or distort the protein by binding another cation, like Zn and it is possible to slow down the oxidation. This can be shown by many measurements including free radical trapping using ESR techniques.



There are many ways of reducing cations in beer - kettle finings such as carrageens have an effect, so too do tannins, tannic acids, kettle hopping, using yeast that have very active uptake systems for Fe and Cu, using lagering more effectively, and avoiding DE.

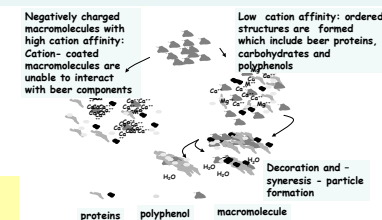
Another way is using a new storage finings agent we have developed - **BrewFINE**. This plant-based agent was developed as a replacement for isinglass and collagen.

7.0 Fining the Blues Away

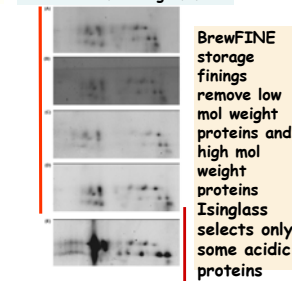


Isinglass replacements - treated H₆ beer

BrewFINE finings agents consistently increase the Ea values (time to detect hydroxyl free radicals) in fined beer by (>)70%. The only comparable effect is obtained with extra strong, non-approved chelating agents.



Isoelectric focusing of flocs



8.0 BrewFINE targets thiol-rich proteins taking advantage of their REDOX activity, and CATION-BINDING functionality to reduce BOTH in finished product => MORE STABLE BEER. It was developed from an understanding of REDUCTANT TRAFFICKING.