

ASSESSMENT OF THE ANTI- AND PRO-OXIDANT ACTIVITY OF SPECIALITY MALTS

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Delaying flavour staling, which is the greatest challenge for brewers, can be achieved by choosing suitable raw materials, particularly malt. Prooxidant malt compounds are mainly the enzymes involved in the degradation of lipids. The antioxidant activity of malt results from polyphenols and Maillard reaction products. The choice of malting barley and malting process can lead to malt with low 'oxidant' and high 'antioxidant potentials', thereby delaying the risk of beer staling, and avoiding the use of chemical exogenous antioxidant compounds. Malt antioxidants originate either from the barley or from the malting process. In the malting process, Maillard reaction products occur during the kilning step. The concentration of Maillard reaction products in malt will mainly depend on the temperature and time of kilning. Intensively kilned malts are rich in melanoidins, which also give beer its specific colour. It has been shown that intermediate reductone compounds, as well as high molecular weight melanoidins, possess antioxidant properties (Boivin *et al.*, 1996; Takashio and Shinotsuka, 1998).

We have developed two complementary methods to assess the anti- and pro-oxidant activity of malts. The first one is based on AAPH-induced lipid oxidation (Liégeois *et al.*, 2000). The oxidation of lipids initiated with the water-soluble azo compound, AAPH [2,2'-azobis(2-amidinopropane)dihydrochloride], proceeds as follows: azo radical initiators generate free radicals by their spontaneous thermal decomposition and are able to initiate lipid peroxidation, even at 37°C. As the lipid chosen is linoleic acid, products resulting from peroxidation are the conjugated diene hydroperoxides which absorb at 234 nm. In the absence of radical initiator, the rate of spontaneous oxidation at 37°C can be considered as negligible. The addition of AAPH induces an oxidation, which starts at a constant rate of conjugated dienes formation, so that the absorbance increases at a constant rate to a maximum.

When an antioxidant is added, oxidation is delayed and an inhibition time can be determined. When the inhibition time is over, oxidation proceeds at the same rate as in the absence of inhibition. This assay measures the antioxidants as free radical scavengers.

When applied to several antioxidant compounds, the measured inhibition time was proportional to the concentration of the additive, except for sulphites, which did not inhibit AAPH-induced oxidation of linoleic acid. Among the antioxidants tested, phenolic compounds proved to be the most efficient.

When applied to malts, the method was very rapid and reproducible and the malt concentration showed a linear relationship with antioxidant activity. A comparison of inhibition times obtained for pale malt and speciality malt, which contain similar levels of polyphenols, showed that the malt kilned at higher temperature gave a longer inhibition time (16.3 min against 24.8 min, table 1). Therefore, measuring the antioxidant activity of malt against AAPH-induced oxidation seems to take account of the presence of Maillard reaction products. Similar results were obtained all along the brewing process (figure 1).

The second method used to assess the antioxidant and prooxidant activity is based on electron spin resonance spectroscopy (Uchida and Ono, 1996). Free radicals in beer and wort can be detected during an oxidative forcing test using a spin trap, *N*-tert-butyl- α -phenylnitron (PBN). The detected free radicals were identified as hydroxyl radicals. By monitoring in detail the progress of hydroxyl radical generation, hydroxyl radicals revealed to be generated just after starting the forcing test and reached a maximum after around 2 hours.

However, in the presence of sulphites, hydroxyl radicals were generated after a definite period of time, called 'lag time of hydroxyl radical generation'. Ascorbic acid appears to be prooxidant as it regenerates iron and copper ions for the Fenton reaction. This method measures antioxidants as 'hydroxyl radical scavengers' as well as prooxidants as 'hydroxyl radical generators'.

Table 1. Comparison of antioxidant activity, measured as inhibition time (T_{inh}) of the AAPH-induced linoleic acid oxidation, obtained for pale malt and speciality malt. The final malt concentration in the assay is 133.3 mg/L. * Assay duplicates. ** Extraction duplicates.

	T_{inh} (min)		Average (min)
	*	**	
Pale malt 2.8°EBC	15.8	16.2	16.3
	16.6		
	16.4	16.4	
Speciality malt 152°EBC	24.1	24.2	24.8
	24.4		
	25.9	25.4	
	24.9		

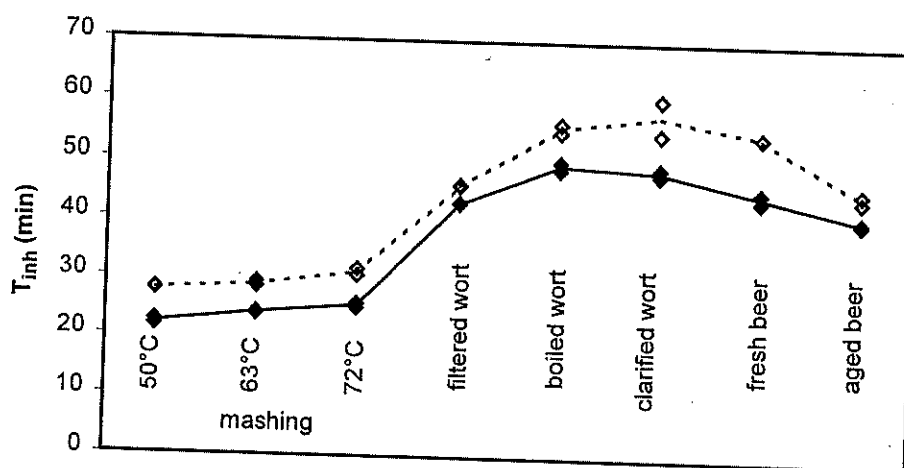


Figure 1. Antioxidant activity profile during the brewing process, measured as inhibition time (T_{inh}) of the AAPH-induced linoleic acid oxidation. (—) mashing of 100% pale malt (2.8°EBC); (---) mashing of 90% pale malt (2.8°EBC) and 10% speciality malt (152°EBC).

References

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WHEY-PROTEINS AND MINERAL BINDING A COMPARATIVE STUDY WITH REGARD TO NORWEGIAN BROWN CHEESE

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Norwegian Brown Cheese

Traditional Brown Whey Cheese (BWC) is a heat treated whey protein concentrate product. BWC fortified with iron has been reported in a Norwegian study (Borch-Iohnsen *et al.*, 1994). The results from this study showed high bioavailability of iron in humans compared with traditional iron supplement.

BWC is a typical Maillard reaction product - high heat treated, whey concentrate product containing whey proteins (β -lactoglobulin, α -lactalbumin, bovine serum albumin, lactoferrin), lactose, Fe^{2+} ($FeSO_4$), fat, small amount of casein and milk salts.

Whey proteins form melanoidins by high heat treatment with a reducing sugar like lactose. Melanoidins are produced through Maillard reactions and may act as amphoteric chelators (Homma *et al.*, 1997). In the present work we have studied binding and release of the Fe-complex under different condition (pH, temperature, pepsin hydrolysis).

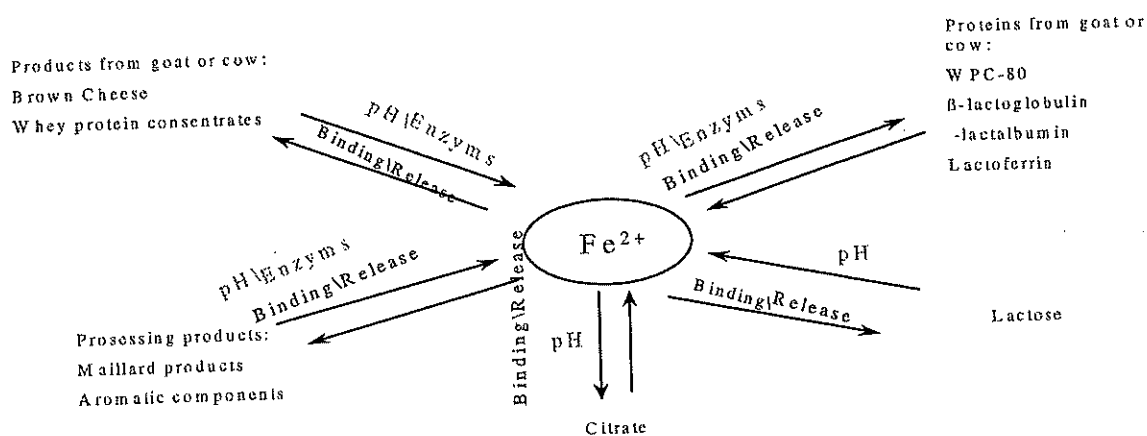


Figure 1. Model Experiments Performed

Methods

Different methods were used to measure the iron content in the cheese or in different fractions:

- water soluble protein fraction,
- water insoluble protein fraction (the brown nitrogen containing precipitate).

The methods used to study the binding/release of soluble Fe were:

- | | |
|---------------------------|-------------------------------------|
| Estimation of total iron: | Atomic absorption spectrophotometry |
| Estimation of ionic iron: | Bathophenanthroline method |

Results

- 1) Iron is associated to the water-insoluble-brown product.
- 2) Release of ionic iron seemed to be pH- dependent, in the water soluble fraction.
- 3) Degradation by gastric enzymes *in vitro* showed release of ionic iron to the water soluble phase. Increased pH from 2 to 5.8 seemed to decrease the amount of ionic iron.

Further experiments

- Degradation by gastric enzymes by *in vitro* and *in vivo* experiments.
- Effect of different conditions simulated as in the gut (pH, other components/acids, lactic acid bacteria).
- Identification of the water-insoluble-brown-Fe complex.
- Binding / release of iron.
- Antioxidant status of the products.

References

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