IMPROVEMENT OF THE OXIDATIVE STABILITY OF BEER

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A new research project at Department of Food Science, University of Copenhagen (former KVL), has recently been launched to improve the oxidative stability of beer in collaboration with Danish microbreweries and DHI Group. The overall goal is to increase knowledge on how interactions between thiol-containing proteins and yeast in beer affect the stability of beer.

INTRODUCTION

The overall purpose of the research project is to make it possible for breweries not using 'active yeast' in the bottles to produce beer that is oxidative stable for up to one year without the addition of sulfite or ascorbic acid. This is to be achieved by increasing our knowledge about how especially thiol-containing proteins affect the oxidative stability of beer and how the yeast during fermentation affect the content of reducing (antioxidative) components in the final beer. This knowledge will be communicated to Danish microbreweries through the Society for Danish Breweries (Danske Bryghuse) and DHI and will make it possible for the microbreweries by suitable selection of composition of malt, yeast strain, and fermentation conditions to predict and improve the stability of the final beer.

The stability of bottled beer is very often limited by oxidative processes. Heat, light, and oxygen are all factors that promote oxidation and which hereby limits the stability of beer[1]. Sulfite, which is produced by the yeast during fermentation, has decisive influence on the stability of pilsner[2]. Sulfite works as an antioxidant, and it has been shown that when sulfite in beer is depleted, the resistance towards oxidative reactions disappear. In Denmark, it is permitted to improve the stability of beer by adding sulfite in concentrations up to 50 mg/l (incl. sulfur dioxide)[3]. However, sulfite has been associated with allergy[4]. Another strategy to improve the stability of beer is to add oxygen-consuming compounds.

Oxygen is either introduced into the bottle during bottling or penetrates the bottle slowly between the bottle and the crown. Some breweries add ascorbic acid for removal of oxygen in the bottles, while 'active yeast' is often used for bottled beer among microbreweries.

PROTEIN THIOLS: ANTIOXIDANTS IN BEER?

It has recently been shown that thiol-containing proteins work as important mediators in relation to the antioxidative role of sulfite in beer[5]. Hydrogen peroxide, H_2O_2, can react with thiol-containing proteins, Pr-SH, to produce protein sulfenic acids, Pr-SOH (reaction 1), and in doing so proteins buffer against hydrogen peroxide formation in beer. In the presence of sulfite, SO_3^{2-}, or other small thiol-containing compounds, the protein sulfenic acids can be reduced (via disulfides, Pr-SS-R (reaction 2)) to give the original Pr-SH (reaction 3), which will commit to more cycles with hydrogen peroxide, or possibly give protein

thiosulfate (also called Bunte salt) (reaction 4). This mechanistic model of the antioxidative defence in beer requires, apart from sulfite, the presence of thiol-containing proteins as e.g. glutathione and thioredoxin.

$Pr\text{-}SH + H_2O_2$	→	$Pr\text{-}SOH + H_2O$	(1)
$Pr\text{-}SH + H_2O_2$	→	$Pr\text{-}SS\text{-}R + H_2O$	(2)
$Pr\text{-}SH + H_2O_2$	→	$Pr\text{-}S^- + RSSO_3^-$	(3)
$Pr\text{-}SS\text{-}R + SO_3^{2-}$	→	$Pr\text{-}SSO_3^- + RS^-$	(4)

The reaction between thiol-containing proteins from beer and hydrogen peroxide is very sparsely described e.g. in relation to effects of pH, temperature, reactivity between the different reactants in the above equations (what is the rate of reaction between H_2O_2 and Pr-SH and between H_2O_2 and SO_3^{2-}?), and stoichiometry (to what extent are Pr-SOH and Pr-SSO_3^- formed?). It is also unknown how different malt types affect the content of thiol-containing proteins and the reducing capacity of the beer. Characterisation of the mechanistic model described above will help us to understand if sulfite or thiol-containing proteins act as the primary antioxidant in beer, and if it is possible to avoid addition of sulfite to beer and still obtain oxidative stable beer.

DOES YEAST PRODUCE ANTIOXIDANTS?

So far, it has been assumed that yeast only produces the reducing compounds for intracellular use, but recently it has been shown that yeast used for production of rice wine is capable of excreting thioredoxin[6]. However, production of thioredoxin and other reducing compounds in brewers' yeast are extremely sparsely described, and it is unknown how growing conditions during beer fermentation (wort composition, work strength, dissolved oxygen, temperature, and pH) affect the production and secretion of the reducing compounds in different brewers' yeast strains. Finally, it is unknown if a larger secretion of reducing compounds from brewers' yeast gives an improved oxidative stability of beer proteins.

It is well-known that yeast produce sulfite during fermentation. However, during beer fermentation the yeast cells are exposed to a long range of stress conditions, e.g. oxidative stress as a result of wort aeration. The presence of oxygen →

results in an increased content of free oxygen radicals in the yeast cells with oxidative damage as a consequence. As response to this stress, the yeast synthesizes different reducing (antioxidative) compounds as for example the compounds in the glutaredoxin and thioredoxin systems: glutathione, glutaredoxin and thioredoxin[7]. Many physiological functions have been suggested for these compounds, among others repair of oxidative damaged proteins. The synthesis pathways of these compounds have been established and the genes which code for the enzymes that are involved in the synthesis of these compounds are known in laboratory yeast[8]. In brewers' yeast the presence and expression of these genes are basically unknown. However, a new investigation shows that the expression of the most important of these genes are being upregulated in a S. cerevisiae brewers' yeast strain during the first hour of industrial beer fermentation.

RESULTS AND IMPLEMENTATION

This project will characterise how the oxidative stability of beer is affected by thiol-containing proteins and yeast strains including fermentation conditions. Interactions between all three factors will be investigated by controlled laboratory brews, and the most important conclusions from the laboratory experiments will be tested in full-scale brews.

In the recent years, DHI and the Society for Danish Breweries have run ERFA groups and work shops for the Danish Microbreweries concerning technical topics of general interest. Remembering that the overall goal for this project is to prolong the oxidative stability of beer with no active yeast up to one year, the results will be highly interesting and the results from the project will be communicated to the Danish brewery industry in this regime.

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