Pre-treatment of Pitching Yeast With Zinc

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ABSTRACT

Preconditioning of yeast with zinc during acid washing was a way of overcoming zinc deficiency in wort provided that the barm beer was not separated prior to pitch. This is because uptake of zinc by the yeast was not efficient during the acid washing process. The optimum point of zinc supplementation was directly to wort at the exact time of pitching. Zinc addition to hot wort can result in a decrease in the bioavailable zinc concentration in wort and poor fermentation.

Keywords: yeast washing, zinc acid washing

INTRODUCTION

Zinc is an essential micronutrient for yeast. Zinc is accumulated by yeast and concentrations of zinc below toxic levels, stimulate fermentation rate. The exact requirement of yeast for zinc as a macro trace element is strain dependent. Zinc is essential for the action of alcohol dehydrogenase and stimulates ethanol production. Zinc increases the rate of carbohydrate utilization by stimulating the uptake mechanism. This ion can accumulate in yeast and the concentration of the zinc in the medium from which yeast is obtained affects the response of that yeast to zinc levels in medium used for the subsequent fermentation.

Zinc is linked with several secondary phenomena. Higher concentrations of zinc stimulate ester production and zinc can induce flocculation in some ale strains. The presence of zinc at levels of 0.15–0.20 ppm has been associated with an increase in the concentration of beer octanoic, decanoic and dodecanolic acids. Zinc is reported to stabilize protein-membrane systems, enhance riboflavin synthesis, activate acid and alkaline phosphatases and stimulate uptake of maltose and maltotriose. Zinc does not increase yeast proliferation.

In high concentrations zinc exerts a toxic effect on yeast through activation of degradative enzymes, suppression of some secondary metabolism, disruption of membrane structures, induction of yeast autolysis, inhibition of transport systems for essential ions and nutrients and denaturation of enzymes.

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Although higher concentrations of zinc can be toxic to yeast, the presence of other ions in wort can alleviate the toxic effect of zinc. For instance, a concentration of zinc over 0.6 ppm can be toxic to yeast although if accompanied by a similar concentration of manganese the toxic effect of zinc is overcome.[41]

Zinc is the only cation that is commonly deficient in wort[4] and is always completely removed from wort by yeast[15]. The available concentration of zinc in wort can be less than its measured concentration due to the presence of natural chelating agents in wort[18] such as amino acids and polyphenols[16] although there has been evidence that yeast is capable of utilizing trub-bound zinc[17].

To avoid adsorption of zinc by naturally occurring chelating agents in wort, yeast could be potentially fortified with an appropriate concentration of zinc during storage or acid washing. This would enable uptake of zinc by yeast prior to fermentation. The barriers to such a process are the low storage temperature of yeast slurries, which decreases the yeast metabolic and transport activities, together with the relatively low concentrations of external sources of energy for the yeast to fuel active uptake of zinc ions. Yeast storage, although controlled, does vary in its duration. The duration of acid washing[17] on the other hand is very well defined and controlled. Therefore, the acid washing procedure, where applied, offers a suitable opportunity for the preconditioning of yeast with zinc ions. Preconditioning of yeast with magnesium ions during propagation has been successful[18].

The aim of this work was to test the possibility of yeast pre-treatment with zinc ions during acid washing and to determine the optimum time for the addition of zinc to fermentations pitched with yeast which has not been pre-treated with this ion.

**EXPERIMENTAL**

Lager yeast collected from full scale fermentations was acid washed and used to pitch (5g wet spun solids/L) lager wort (OG of 1060°). Fermentations were performed in EBC tall tubes (2L working capacity) at a constant 15°C. Fermentations were monitored for changes in present gravity (PG), pH and suspended cell count. When target PG (10115) was achieved, the cultures were chilled to 5°C. Once the yeast had visibly settled, the yeast crop size and viability were determined.

Acid washing of yeast slurries was performed at 0-4°C for 2-4 h within an initial pH range of 2.2-2.4. The slurries were continuously stirred during acid washing. Zinc was always added from stocks of sterile zinc sulphate heptahydrate solutions. When zinc was added to the slurry during acid washing, the concentration of zinc was always calculated to give a wort initial zinc concentration of 0.2 ppm assuming the yeast absorbed no zinc.

**RESULTS AND DISCUSSION**

A) Yeast preconditioning with zinc

The influence of acid washing in the presence of zinc on fermentation performance was tested. The control fermentation consisted of wort supplemented with zinc to 0.2 ppm and immediately pitched with acid washed yeast. The conditions of all fermentations are shown in Table 1.

### TABLE 1

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>Acid Washing</th>
<th>Wort supplementation</th>
<th>Pitching conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>No zinc during acid washing</td>
<td>Zinc not added to wort immediately prior to pitching</td>
<td>Slurry added directly</td>
</tr>
<tr>
<td>2</td>
<td>Zinc added to slurry immediately prior to pitching</td>
<td>Zinc not added to wort</td>
<td>Slurry added directly</td>
</tr>
<tr>
<td>3</td>
<td>Zinc present throughout acid washing</td>
<td>Zinc not added to wort</td>
<td>Slurry added directly</td>
</tr>
<tr>
<td>4</td>
<td>Zinc present throughout acid washing</td>
<td>Zinc not added to wort</td>
<td>Cells separated by centrifugation, pellet resuspended in wort and pitched</td>
</tr>
</tbody>
</table>

### FIGURE 1

**Preconditioning of yeast with zinc**

**Figure 1 Legend:** Fermentation 1 represents the control where zinc is added directly to wort just prior to pitching. In fermentation 2, zinc was added to the acid washed yeast slurry immediately before pitching. In fermentation 3, zinc was added to the yeast slurry at the start of acid washing. Fermentation 4 was similar to fermentation 3 but differed in that the barb beer was separated from the yeast prior to pitching. Fermentation 1-3 performed similarly but fermentation 4 was slower. This suggests that zinc uptake by yeast is poor during acid washing and that zinc carried over to the wort in the barb beer can overcome the zinc deficiency of wort.

Apart from “fermentation 4” the final zinc concentration was always calculated to be 0.2 ppm in wort. All fermentations performed similarly apart for the fermentation that involved separation of barb beer prior to pitching (Fig. 1). In fermentations 1-3, the final concentration of zinc in wort at pitching would have been 0.2 ppm, however, in fermentation 4, any unabsorbed zinc in the acid washing barb beer would not have been carried over into the fermentation but discarded with the supernatant.
Comparing fermentations 1, 2 and 3, no improvement in the fermentation performance was observed through preconditioning of yeast with zinc. In fermentation 4, carry over of zinc into the fermentation through the barm beer was avoided due to the separation of the yeast from the barm beer. The slower fermentation rate of fermentation 4 indicated that yeast did not accumulate zinc effectively during acid washing.

B) Optimizing zinc addition to wort

The aim of this experiment was to determine the optimal time for zinc supplementation of wort. The pitching yeast was acid washed in the absence of zinc. The fermentation conditions are shown in Table 2. For one fermentation, wort was heated to 60°C, supplemented with zinc and cooled to 15°C prior to pitching. All final wort concentration of zinc were calculated to be 0.2 ppm.

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>Time of zinc supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 h prior to pitching</td>
</tr>
<tr>
<td>2</td>
<td>2 h prior to pitching</td>
</tr>
<tr>
<td>3</td>
<td>1 h prior to pitching</td>
</tr>
<tr>
<td>4</td>
<td>Immediately prior to pitching (control)</td>
</tr>
<tr>
<td>5</td>
<td>1 h post pitching</td>
</tr>
<tr>
<td>6</td>
<td>Zinc addition to hot wort</td>
</tr>
</tbody>
</table>

All final wort concentration of zinc were calculated to be 0.2 ppm.

![Zinc Addition Time (h)](image)

**FIGURE 2**

Optimum time and temperature for the addition of zinc to wort.

**FIGURE 3**

Optimizing zinc addition to wort

**Legend:** The optimum regime for the addition of zinc to wort is to add the ion at the time of pitching. This provides the maximum amount of zinc for uptake by yeast.

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**CONCLUSION**

- Zinc is not effectively absorbed by yeast during acid washing.
- Supplementation of wort with zinc is best achieved by the addition of a source of zinc ions to wort immediately prior to, or at the exact time of, pitching.
- Addition of zinc ions to hot wort is not a suitable method for overcoming the zinc shortage in wort.
- Addition of zinc to yeast prior to pitch may be carried out provided that the barm beer is not separated.
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REFERENCES