D-32: Effect of higher dissolved carbon dioxide on acetate ester production by *Saccharomyces cerevisiae* and on alcohol acetyltransferase activity during beer fermentation

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Elevated dissolved carbon dioxide (DCO_2) in fermenting liquid, suppressed the production of acetate ester, which often occurred in the industrial scale high capacity beer fermenting vessels, such as in cylinder conical tanks and affected the flavour stability. DCO_2 had significant influence on the suppression of ester production. Fermentation was carried out in 100 L pilot scale tanks with 80 L wort. All malt wort having 15 w/w% was used as fermentation medium. Wort dissolved oxygen was adjusted to 10 ppm before pitching and temperature was maintained at 12°C throughout the experiment. Wort was pitched at the rate of 15 x 10^6 cells/ml using *Saccharomyces cerevisiae*, brewing yeast.

The concentration of DCO_2 of fermenting liquid was changed by the external supply of CO_2 pressure at the level of 0.75 atm and 1.5 atm. except in the control tank. Pressure adjustment was done stepwise within 2 h by external CO_2 supply when the cell growth transmitted from exponential phase to the stationary phase, in order to avoid any influence on yeast growth by increased DCO_2. 2.0 g of yeast cells pressed by filter paper was collected periodically from each fermentation tank and analysed for cell membrane bound alcohol acetyl transferase activity after disruption by a Freeze-Blast method at _140°C. Cell membrane bound enzyme was harvested by triton X-100 treatment 0-2°C.

Crude alcohol acetyltransferase (AAT) was further purified by membrane filtration and gel filtration chromatography and biochemical characters of the enzyme were studied. The activity of AAT was not affected by the increased DCO_2 at the maximum growth stage, although the production of acetate esters were significantly suppressed at a rate of 12.8% and 28% respectively in fermenting liquid having 0.45 and 0.58% w/w DCO_2. Most fractions of the enzyme were found as membrane bound and biochemical characterization

studies showed that molecular mass of the enzyme was 312,000, optimally active at pH 7.0 and 25°C. Michaelis constant measured using acetyl CoA as substrate was 2.12×10^{-5} M and the inhibition constant for oleic acid and linoleic acid were respectively 4.2×10^{-6} M and 2.56×10^{-7} M. Enzyme had a narrow pH stability range (7.0 - 7.5). Results suggest that the suppression mechanism of acetate ester production by DCO_2 was different from that of unsaturated fatty acids or sterols.