Catalase inhibition by metal ions for stabilizing hydrogen peroxide to improve disinfection efficiency in fresh-cut lettuce wash water

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Introduction
The presence of catalase in (waste)water of biological origin can decrease the performance of disinfection or oxidation processes with H2O2. Studies show that certain metals (including Cu, Zn, Ag) can inhibit catalase in plants and animals, a process which depends on the metal, the metal concentration, the tissue and species [1]. Furthermore, Ag, Cu, and to lesser extent Zn have antimicrobial properties [2-4]. In this study, the use of Bacsan® (containing a.o. Cu, Ag, and Zn ions) to inhibit catalase was assessed in order to improve the stability of H2O2, and as such improve the disinfection efficiency.

Materials & methods
The content of the Bacsan® solution (Labola, Norway) was analyzed with inductive coupled plasma emission spectrometry to determine the actual metal ion concentrations. H2O2 was measured with a test kit. Bacsan®, at concentrations 0, 2 and 10 mg/L Bacsan-Cu (10 mg/L Cu, 0.9 µg/L Ag, 2.8 mg/L Zn), and H2O2 at 500 mg/L were used. Artificial lettuce wash water was made by homogenizing iceberg lettuce in tap water to obtain a desired level of chemical oxygen demand (COD). All experiments were executed at 4°C. Total psychrotrophic plate count and inoculated Escherichia coli were measured in the experiments. Tests with catalase were executed in 0.05 M phosphate buffer with catalase from bovine liver.

Results
The Bacsan® significantly reduced the catalase activity, and lowering the pH enhanced the inhibition of catalase. Bacsan® improved the stability of H2O2 in artificial lettuce wash water and increased the disinfection efficiency of total psychrotrophic plate count and Escherichia coli. COD concentration had detrimental effect on the H2O2 stability and disinfection efficiency. The Cu alone does not explain the greater stability of H2O2 as the combination of 10 mg/L Cu2+ (from CuSO4) with H2O2 gave a lesser inhibition of catalase and a more rapid decomposition of H2O2 in artificial lettuce wash water than Bacsan® (10 mg/L Cu ions) with H2O2, yet significant inhibition of catalase and increased H2O2 stability compared to solely adding H2O2. As such, the inhibition was most likely due to the combination of metal ions present in the Bacsan®.

Discussion
The results in this study showed i) catalase can be inhibited by a metal ion mixture (10 mg/L Cu, 0.9 µg/L Ag, 2.8 mg/L Zn), ii) lowering the pH reduces the catalase activity, iii) the use of Bacsan® improves the stability of H2O2 and the bacterial disinfection efficiency of H2O2, iv) H2O2 (combined or not with Bacsan®) is a slow acting water disinfectant and as such not suited for use in a vegetable washing process to avoid cross-contamination of bacteria. However, it has potential for offline disinfection (disinfection before reuse) of fresh-cut lettuce wash water if it can be shown that Cu is not transferred to the lettuce in quantities that hold a risk towards the consumer.

References