Evaluation of chemical treatments and ozone on the viability of *Cryptosporidium parvum* oocysts in fruit juices

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(ABSTRACT)

Cryptosporidium parvum is a protozoan parasite historically associated with waterborne and more recently foodborne outbreaks of diarrheal illness. Contamination of certain foods, such as unpasteurized apple cider, with infective oocysts may occur as oocysts are shed in the feces of common ruminants like cattle and deer that graze in and around orchards. Cryptosporidiosis can result in a severe illness for previously healthy individuals and a life-threatening illness in immunocompromised individuals. Disease occurs after the ingestion of small infective oocysts (4 to 5 ?m in size). The relatively thick membrane of the oocysts allows them to be resistant to chlorine and many other environmental pressures, making oocysts difficult to inactivate.

In this study, alternative treatments to pasteurization were evaluated for their ability to inhibit *C. parvum* oocyst viability in fruit juices. Oocyst viability was analyzed with a cell culture infectivity assay, using a human illeocecal cell line (HCT-8) that is most similar to human infection. The percent inhibition of infection by each treatment was determined along with the corresponding log reduction for the treatments found to be most effective. Infection by treated oocysts was compared to that of control untreated oocysts. Cell monolayers were infected with 10^6 treated oocysts or a series of 10-fold dilutions. Parasitic life stages were visualized using an immunohistochemistry system and 100 microscope fields counted per monolayer. Organic acids and H_2O_2 were added on a wt/vol basis to apple cider, orange juice, and grape juices. Malic, citric, and tartaric

acids at concentrations from 1%-5% inhibited *C. parvum* infectivity of HCT-8 cells by up to 88%. Concentrations ranging from 0.025%-3% H_2O_2 were evaluated where addition of 0.025% H_2O_2 to each juice resulted in a >5 log reduction of *C. parvum* infectivity as determined with an MPN-based cell culture infectivity assay. Treating apple cider, orange juice, and grape juice with ozone for a time period of 30 seconds up to 15 minutes at 6? and 22?C (0.9 g/L flow rate) inhibited *C. parvum* viability to > 90% as monitored in the cell culture assay. It is hypothesized that oocyst wall proteins that are necessary for infection are oxidized by the reactive oxygen species generated from the decomposition of the ozone and hydrogen peroxide treatments. These treatments or combinations thereof may offer potential alternatives to traditional pasteurization for fruit juices to successfully inhibit *C. parvum* viability.

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DEDICATION

I would like to dedicate this dissertation to my beloved and missed father, Karl Kniel, who passed away on March 3, 1993. A special thanks to my Dad who instilled in me a love for science along with the ability to question science and myself. I only wish that he could be here to read this and to continue to question science himself.

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