学 位 論 文 の 要 旨

論文題目　Plant extracts, antimicrobials and ultraviolet light as hurdle technology in controlling *Alicyclobacillus acidoterrestris*
（*Alicyclobacillus acidoterrestris* の制御における植物エキス、抗菌剤、紫外線の複合的効果）

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DISSERTATION SUMMARY

The fruit juice industry has been driven by the consistent demands of consumers for products that cater to their health and well-being. Nonetheless, a persistent spoilage microorganism *Alicyclobacillus acidoterrestris* has plagued the industry in recent years and has caused considerable losses for some. This spore-forming bacterium is considered as the most important spoiler for fruit juices and acidic beverages due to its thermophilic and acidophilic nature, which means that it can resist and survive typical thermal pasteurization regimes that are applied in fruit juice production. Its survival and contamination ultimately result in products that have unacceptable quality due to the off-flavors and off-odors that are produced, resulting in substantial economic losses due to food wastage. Hurdle technology is a novel concept that involves use of multiple, mild physical or preservation methods that can result in process schedules with optimal efficacies. In this work, a hurdle technology combining the use of UV-C processing with traditional and natural antimicrobials in order to control *A. acidoterrestris*. was investigated and proposed.

Study 1: Effects of diluents, temperature and pH on the enumeration and growth kinetics of *Alicyclobacillus acidoterrestris* in standard growth media

The efficacies of distilled water, saline solution, peptone water and phosphate buffered saline as diluents on the enumeration and viability of *A. acidoterrestris* were investigated. In typical microbiological experiments, diluted samples may be kept for a short period of time prior to plating during intensive experimental runs, which could possibly affect the outcome of enumerated counts. Distilled water proved to be the most ideal diluent as it was economic yet effective enough to maintain organism viability. Interestingly, it was observed that the common peptone water is actually unfavorable as a diluent for *A. acidoterrestris*. It must be noted that there have been very little studies that have reported the same observation. In the succeeding phase, the influences of temperature and pH on the growth behavior of *A. acidoterrestris* vegetative cells in yeast starch glucose broth (YSGB) were also determined and quantified. Variations in growth behavior included the presence or absence of growth lag as well as differing growth rates and population changes. It is important to note that some temperature-pH combinations of the experimental design even resulted in the inactivation of vegetative cells, even though the operable region of the experimental design was based on optimum ranges in published literature. Out of the three growth kinetic response parameters, the model for lag time was not significant. Model fitting and analysis showed temperature and pH were factors that directly influence the other growth kinetic parameters. Model validation ultimately revealed acceptable performance for the population change model and limited success for the growth rate model. The results established in the first study may be used in the preparation of specific YSG growth media with the desired temperature and pH conditions that will allow for the control of the growth kinetic parameters of *A. acidoterrestris* vegetative cells. Also, the results obtained in this study inadvertently provided additional information as to how *A. acidoterrestris* vegetative cells might be inhibited by environmental control and product formulations. Based on the results of this study, it is believed that different strains of *A.*

acidoterrestris ought to have different optimum temperature and pH growth parameters.

Study 2: Antimicrobial efficacies of plant extracts and antimicrobials against *Alicyclobacillus acidoterrestris*

In this study, 27 ethanolic plant extracts were screened for potential antimicrobial activity against *Alicyclobacillus* spp. Licorice and sage extracts were found to be the two most effective ones. The combined efficacies of the two chosen plant extracts with glycine and sodium acetate were also investigated. Positive additive effects were observed for licorice extracts with glycine or sodium acetate and sage extract with glycine. However, sage extract with sodium acetate exhibited adverse effects, even up to antagonism. Prior to survival studies in apple juice, germination studies of *A. acidoterrestris* spores were conducted, and the results showed that *A. acidoterrestris* spores could germinate and grow in diluted apple juice even up to concentrations of 90%, but not at the undiluted level. 50% apple juice was chosen as it was without unwanted inhibitory effects or stresses at the onset of incubation. Based on calculated FIC indices, the tested ideal combination of licorice extract with glycine or sodium acetate resulted in sporicidal activity against *A. acidoterrestris* in apple juice. For sage extract with glycine, only a temporary sporostatic effect was observed prior to eventual germination, growth and proliferation of *A. acidoterrestris* spores. The results presented in this study may be used in further research dealing with the shelf-life extension of fruit juices through the application of similar hurdle technologies with natural antimicrobials.

Study 3: Influence of plant extracts and antimicrobials on the subsequent UV resistance of *Alicyclobacillus acidoterrestris*

The effects of physicochemical stress exposures and their resulting physiologies on the UV-C resistance of *A. acidoterrestris* were investigated. Microorganisms are exposed to different environments during food production, and these unwanted exposures to different physicochemical stresses can induce adaptive mechanisms that could enhance their survival and further affect their resistance to the kill step during processing. In this study, inactivation

behaviors of *A. acidoterrestris* mainly followed first-order kinetics or a log-linear trend for both vegetative cells and spores. It was also observed that the UV-C resistance of cells treated in YSGB was higher than those treated in apple juice. Heterologous adaptation was observed for heat-stressed cells but was limited to those treated in the YSGB suspending medium. More importantly, spore cells were deemed to be the most resistant physiology as it was the least susceptible to UV-C radiation. This study also investigated the possible hurdle technology between UV-C processing and the application of antimicrobials such as plant extracts, glycine and sodium acetate. It was observed that spore cells were rendered to be more susceptible to UV-C in all treatment combinations of these antimicrobials. While it is generally believed that using plant extracts may impart adverse effects on the sensory qualities of a food product, colorimetry findings revealed that the treatment of plant extracts and antimicrobials have indeed significantly changed the color profile of apple juice, but the color changes brought about by the treatments were still within acceptable limits. Sensory analysis further showed that the developed hurdle technology resulted in apple juice with acceptability ratings that were generally a bit lower but did not significantly differ from the control. The results obtained from this work may be used in the establishment of UV-C process schedules for better shelf-life extension of apple juice.

Considering the economic importance of fruit juices as well as the challenges posed by *A. acidoterrestris* to the fruit and beverage industry, along with the implications of various food-, process-, and microorganism-related factors pertinent to food processing, food quality and food safety, the present work has proposed a hurdle technology that makes use of natural and traditional antimicrobials in line with a novel food processing technique in controlling the spoilage microorganism and shows promise as a possible alternative to currently employed thermal processing methods.