

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: The inhibitory effects of essential oil against germination and outgrowth of *Clostridium perfringens* spores in meat products.

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COOPERATORS: (if any)

SUMMARY: The food industry is interested in developing bacterial spore-inactivation strategies alternative to conventional heat processing technologies, that meet consumer expectations for increased food safety, extended shelf life and improved food quality. Alternative technologies consider the use of natural antimicrobial compounds in foods. One of the attractive antimicrobial compounds that have been used as flavoring agent is plant Essential Oil (EO). It is aromatic and volatile liquid extracted from plant material as secondary metabolites. It possesses antimicrobial properties against broad range of microorganisms such as bacteria, fungi, parasites, and viruses. The crude EOs are classified as 'Generally Recognized as Safe (GRAS)' for consumption. These include, clove, oregano, thyme, nutmeg, basil, mustard, and cinnamon. Intensive research has been focused on the antimicrobial activity of EOs and their constituents against various food-borne pathogens. However, information on the inhibitory effect of EOs against *Clostridium perfringens* cells and spores is limited. Thus, extensive studies are needed to optimize the use of EOs in meat industry to control *C. perfringens* contamination. Moreover, there are gaps on the fundamental knowledge of whether EOs inhibit the initiation of spore germination or prevent the outgrowth of germinated spores, which are essential basis in developing strategy of using EO as an antimicrobial agent in food products.

OBJECTIVES: The objectives of this proposal are: i) to determine the potential antimicrobial activities of different EO compounds on spore germination, outgrowth, and subsequent vegetative growth of *C. perfringens* clinical isolates and ii) to validate the effectiveness of EO in controlling the germination and outgrowth of *C. perfringens* spores in meat products under abusive storage temperature.

PROCEDURES: We evaluated the effect of different EOs on the germination rate of *C. perfringens* spores from 6 different human clinical isolates. The spores of an initial OD₆₀₀ of ~ 1.0 will be heat-activated (at 75-80°C for 15 min), and then inoculated into a 96-well microtiter plate containing broth medium supplemented with various concentrations of the tested compounds, and incubated at 37 °C. Spore germination was recorded by monitoring the decrease in OD₆₀₀ at 10 min intervals for up to 120 min using the plate reader.

SIGNIFICANT ACCOMPLISHMENTS: Our result showed that the incorporation of EOs including cinnamaldehyde, eugenol, or allyl isothiocyanate at various concentrations into growth medium inhibited the germination and outgrowth of spores of *C. perfringens* 3 food poisoning strains (SM101, NCTC8239 and E13) and 3 non-food-borne strains (F4969, NB16 and B40) during 60 min of incubation at 37 °C. This inhibition effect is dependent on the concentrations of EOs and the significant effect was obtained with concentrations higher than 0.1%. However, spore germination of NFB isolates was more susceptible to eugenol, as germination of NFB spores was

inhibited significantly with lower concentrations of eugenol (0.05%) as compared to germination of FP spores at the same concentrations. Collectively, our results indicate that the inhibition of *C. perfringens* spore germination and outgrowth is dependent on the concentration of EOs as well as the isolation source. Further research on validating the effectiveness of EOs in controlling the germination and outgrowth of *C. perfringens* spores in meat products are currently undergoing.

BENEFITS & IMPACT: The result of this proposal will: i) determine the impact of EO compounds on inhibiting germination and outgrowth of spores of various *C. perfringens* isolates; ii) define the optimal conditions for inhibitions of *C. perfringens* growth with EOs in meat products,; and iii) establish the essential basis for further development of strategies to produce high-quality and safe meat products.

ADDITIONAL FUNDING RECEIVED: none

FUTURE FUNDING: Will explore funding from USDA.