THE POTENTIAL USE OF ORGANIC ACIDS IN BEEF PRESERVATION INDUSTRY

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All praise to ALLAH the ALMIGHTY for His bless and guidance that had helped me in completing this project…

And also to my beloved family especially my father and my mother,

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ABSTRACT

Acetic acid and lactic acid can inhibit growth of Gram negative bacteria particularly a pathogenic species. In food industries acetic acid and lactic acid were used as preservative. Current study is to immerse beef carcasses in acetic acid and lactic acid with *E. coli* strains BL21 (DE3), DH5α and an unknown strain. Beef carcass pieces were immersed in the *E. coli* suspension which contained (10^7 CFU/ml) for 30 seconds and left for 2 hours to allow *E. coli* to settle down on the surface of beef carcass. The beef carcass pieces were thereafter immersed in 1.0, 2.0 and 3.0% solutions of acetic acid and 1.5, 2.5 and 3.5% solutions of lactic acid for 30 seconds, then tap water was used to wash the samples. A clean specimen 5 x 5 cm was put on the surface of each sample. The count of *E. coli* on beef surface immersed in 1.0, 2.0 and 3.0% of acetic acid reduced by 0.87, 1.33 and 1.73 log CFU/ cm^2, respectively also reduced by 1.1, 1.9 and 2.47 log CFU/cm^2 for 1.5, 2.5 and 3.5% of lactic acid, respectively. The results showed that concentration of acetic acid and lactic acid had a significant effect to reduce *E. coli* count. The results also proved that lactic acid had greater effect on non-pathogenic *E. coli* rather than acetic acid.

Key words: Acetic acid, Lactic acid, Acid resistance bacteria, *E. coli*.

ABSTRAK

Asid asetik dan asid laktik boleh menghalang pertumbuhan bakteria Gram negatif terutamanya spesies patogenik. Dalam industri makanan asid asetik dan asid laktik telah digunakan sebagai pengawet. Kajian semasa adalah untuk merendam kepingan daging asid asetik dan asid laktik dengan *E. coli* strain BL21 (DE3), DH5α dan stain yang tidak diketahui. Keping an daging lembu telah direndam dalam penggantungan *E. coli* yang mengandungi (107 CFU / ml) selama 30 saat dan dibiarkan selama 2 jam untuk membolehkan *E. coli* menetap di permukaan daging. Kepingan daging lembu itu seterusnya direndam dalam 1.0, 2.0 dan 3.0% larutan asid asetik dan 1.5, 2.5 dan 3.5% larutan asid laktik selama 30 saat, kemudian air bersih digunakam untuk mencuci sampel. Spesimen bersih berukuran 5 x 5 cm diletakkan di permukaan setiap sampel. Kiraan *E. coli* pada permukaan daging lembu yang direndam dalam 1.0, 2.0 dan 3.0% asid asetik masing-masing dikurangkan sebanyak 0.87, 1.33 and 1.73 log CFU/ cm^2, begitu jugalah dengan daging yang direndam dalam asid laktik masing-masing dikurangkan sebanyak 1.1, 1.9 dan 2.47 log CFU/cm2 bagi 1.5, 2.5 dan 3.5% asid laktik. Hasil kajian menunjukkan bahawa kepekatan asid asetik dan asid laktik mempunyai kesan yang signifikan untuk mengurangkan kiraan *E. coli*. Keputusan juga membuktikan asid laktik mempunyai kesan yang lebih besar terhadap bukan patogen *E. coli* dan bukan asid asetik.

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LIST OF ABBREVIATION

SYMBOLS	DESCRIPTION
AA	Acetic Acid
AR	Acid Response
ATR	Acid Tolerance Response
CFU	Colony Forming Unit
cm2	Centimeter Square
g	Gram
GFP	Green florescent Protein
GFPUV	Green florescent Protein Ultra Violet
GRAS	Generally Recognized as Safe
h	Hour
IUPAC	International Union of Pure and Applied Chemistry
L	Liter
LA	Lactic Acid
L-Lactic acid	Levorotatory Form Obtained by Biological Fermentation of Source
Log	Logarithm
LSD	Least Significance of Difference
M	Molar
mg	Milligram

MIC	Minimum Inhibiter Concentration
ml	Milliliter
mM	Millimole
MRD	Maximum Recovery Diluent
^{o}C	Degree Centrigrade
OH	Hydroxyl
PBS	Phosphate Buffer Saline
pH	Power of Hydrogen
pHi	Interior Cell Power Hydrogen
pKa	Acid Dissociation Constan
PMF	Proton Motive Force
rpm	Round Per Minute
S.E	Standard Error
spp	Species
SS	Sigma Factors
TSB	Tryptic Soy Broth
TSI	Triple Sugar Iron Agar
VFA	Volatile Fatty Acid
ΔpH	Difference Between Two pH Value

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CHAPTER 1

INTRODUCTION

1.1 Overview

Nowadays food safety is a serious issue must be considered because of the wide diversity of food types. The most common ways to preserve food is the use of safe acid percentage to inhibit and kill microbes. The food fermentation is the oldest method which has been used to preserve the food since the beginning appearance of civilization. The numerous of Gram negative bacteria have presented the progression for acid resistance, as well when the acid stress was used alone the specific proteins were synthesized that keep the safety of the *E. coli* cells from much load of acidity, moreover the acid stress alone is not enough to protect and provide food safety, because the other factors such as acid type, ionic strength and pH also have significant roles to protect the food from contamination by *E. coli*. (Lu, Breidt and Perez-Diaz 2011).

Treating food with organic acids such as acetic acid and lactic acid does not decrease the pH and damage the environmental of the organism only, but in addition to this, the organic acids use the poisonous components as an extra pressure to inhibit even to kill the organisms. Low pH takes part the greatest effect to confuse the environment of microbes and with the other factors such as temperature and osmosis make the multiple obstacles to disrupt and inhibit the microbial survival (Perpetuo, Souza and Nascimento, 2011). There are different types of acidulant which must be considered during their using in food acidification due to their toxicity effects to the

food (Presser, Ratkowsky and Ross, 1997). On the other hand the effect of low pH of acids leads to decrease the stability of microbes and adds the further stress with other environmental stresses.

In this study three non-pathogenic *E. coli* strains BL21 (DE3), DH5α and an unknown strain were used to investigate their acid tolerance to acidified beef carcass which can be used as a model to explain the acid resistance in non-pathogenic strains of *E. coli.* The acidity condition to preserve the food against microbes must be in the specific range without the toxicity for human. In the United States and Europe the large concentration of pickles approximately 3.6% wt/vol were investigated to preserve the food but the taste of the food was changed to acid taste (Ozeki, Kurazono and Saito, 2003). For this reason acidified food must be arranged according to the taste, smell and even the color of the food, while in the recent years flavor direction has gradually shift to moderate acidulent (Lu *et al.*, 2011).

The effect of organic acids as the antimicrobial agents comes from the low pH and specificity interaction of the organic acids . Low pH acidic condition of organic acids are able to change the composition of the necessary macromolecules such as phospholipid and protein on the cell surface and consequently these macromolecules lose their ability to bind the required molecules that the cell needs (Beales, 2004).

One of the most important step must be performed for food safety against Gram negative bacteria is inactivation the acid tolerance response system in these organisms. Enterohemorrhagic *E. coli* are one of the Gram negative bacteria that have effective acid tolerance response against acidic condition; this ability in *E. coli* is required to be inactivated (Elder et al., 2000). Currently, scientists have been identified the acid tolerance response in *E. coli* while they tested the medium of *E. coli* with mildly acidic condition (R. Buchanan, Edelson and Sapers, 1999).

1.2 Problem Statement

E. coli is one of the microbes which easily grow on the food. Preservation food against contamination with this organism requires acidification of the food with organic acid. However the organic acids, containing acetic, citric, and lactic acids, in a broth medium can encourage the survival of *E. coli* O157:H7, compared to its survival in non-acidified control medium held at the same temperature (Conner, Kotrola and Mikel, 1997). Thus, the study of using organic acid in a medium containing *E. coli* may become a helpful step to further explain the response of *E. coli* to organic acids containing acetic acid and lactic acid.

1.3 Research Objectives

1. To validate antimicrobial activity of acetic acid and lactic acid against *E.coli*.

2. To determine the minimum inhibitory concentration (MIC) of acetic acid and lactic acid against *E.coli*.

3. To compare the effect of different concentration of acetic acid and lactic acid on *E. coli* in beef.

1.4 Research Significance

The significant from this research where the effect of different concentrations of acetic acid and lactic acid as a control means of *E. coli* survival can be established. In addition it is possible to predict and discover the novel effective concentration of acetic acid and lactic acid to kill non-pathogenic strains of *E. coli*.

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