EVALUATION OF INHIBITORY EFFICIENCY FOR *LISTERIA* AND *STAPHYLOCOCCUS* USING CHINESE MAHOGANY EXTRACT SPRAY COATING ON THE SURFACE OF SLICED PORK HAM

H-C Wei[1], Rommanee Thammasena[1], C-W Fu[1], P-Y Li[1], Y-H Tang [1] Y-C Chang[1], H-Y Chiu[1], Ch-F Hsiao[1] and D-C Liu [1,*]

[1] Department of Animal Science, National ChungHsing University, Taichung, 40227, Taiwan ROC

[*]Corresponding author email: dcliu@ dragon.nchu.edu.tw

Abstract –The results showed that the extract of Chinese mahogany hold higher total polyphenol and total flavonoids contents. The minimum inhibitory concentration (MIC) of extract for two target bacteria (*Listeria monocytogenes* BCRC 14845 and *Staphylococcus aureus* BCRC 11863) was 1.95 μg/mL and 7.81μg/mL individually. In this study *L. monocytogenes* BCRC 14845 and *Staphylococcus aureus* BCRC 11863 were inoculated separately on sliced hams then spray coated with 0, 125, 250 and 500 μg/mL of extracts. During 5 day storage, the count of *L. monocytogenes* of all lots were increased with storage time. A significant difference was found between treatments and control at day 1. Moreover, all sliced ham treated with extracts were significantly lower count than control at all time points during storage (5 days). The similar results also found in *Staphylococcus aureus*. Although the growth of *L. monocytogenes* was better than *S. aureus* during storage at 15℃ but use of Chinese mahogany extract exhibited the same inhibitory ability to this two target bacteria in this study.

Key words- total phenol, flavonid, minimum inhibitory concentration.

I. INTRODUCTION

Consumption of ready-to-eat (RTE) foods has considerably increased due to their convenience. But RTE meat products were easily polluted with microorganism during preparation and resulted in outbreak of foodborne illness. Synthetic preservatives play an important role in RTE meat products, but they always know as negative response for human health. At present, a lot of scientific studies are looking for nature antimicrobial compounds from plants to replace synthetic preservatives in food system (Cabeza *et al*., 2010; Zhang *et al*., 2009; Ha and Kang, 2015). Chinese mahogany *(Toona sinesis)* was rich in polyphenolic compounds that has excellent antimicrobial and antioxidant activities (Chang *et al*., 2006；Wang *et al*., 2007；Liu *et al*., 2009).

Therefore, the aims of this study were 1) to determine the total phenol and flavonoids concentrations in Chinese mahogany extract and the minimum inhibitory concentration of Chinese mahogany extract for *Listeria monocytogenes* and *S. aureus* . 2) to screen the different levels of Chinese mahogany extracts (0, 125, 250 and 500 μg/mL) spray coating on sliced ham to control the growth of *L .monocytogenes* and *S. aureus* stored at 15℃ for 5 days.

II. MATERIALS AND METHODS

Preparation of Chinese mahogany extracts

Fresh Chinese mahogany was purchased from local herb farm and was dried by 55℃ for 16hr. 5% dry sample was extracted with 50% ethanol for 80min by ultrasonication. and the extract will to determine total phenol content and flavonoids.

Determination of MIC

The minimum inhibitory concentration (MIC) of extract was detected by broth microdilution method of Murray *et al*.(2007).

Preparation of spraying solution

Spraying solutions were prepared as 0, 125, 250 and 500 μg phenol/mL of Chinese mahogany extract, individually.

Treatments

Sliced pork hams were manufactured as the procedures of Liu (2002). *L. monocytogenes* BCRC 14845 and *Staphylococcus aureus* BCRC 11863 were target bacteria in this study. 1 mL of target bacteria (10^3 CFU/mL) was inoculated on the surface of slice ham and covered on the surface by swab method then hold 10 min. 5 mL different levels of spraying solution of Chinese mahogany extract, individually, were spray-coated on the surface of hams. Finally, samples were vacuum package and stored at 15℃ for 5 days.

Determination of *L. monocytogenes*

The count of *L. monocytogenes* was determined by modified method of Geornaras *et al*. (2004), 10 cm^2 of slice ham was sampled and soaked in sterilized peptone solution then shook in stomacher for 30sec. PALAMC agar was used for *L. monocytogenes* and incubated in anaerobic condition for 48-72hr. at 35℃.

Determination of *Staphylococcus aureus*

The count of *Staphylococcus aureus* was determined by modified method of Islam *et al*. (2001), 10 cm^2 of slice ham was sampled and soaked in sterilized peptone solution then shook in stomacher for 30sec. Baird-Parker agar (Merck, Germany) was used for *Staphylococcus aureus* and incubated in aerobic condition for 48-72hr. at 35℃.

III. RESULTS AND DISCUSSION

Phenol and flavonids content

Total phenol and flavonoids content of extract from Chinese mahogany treated by ultrasonication at 20℃ for 80 min was 17831.45 μg/mL and 1952.93 μg/mL, individually. This result was slightly lower as Lin (2011) reported that the extract of Chinese mahogany can be reached the maximum of 18538.18 μg/mL in phenol content and 2168.75 μg/mL in flavonoids when extracted by shaking method at 20-25℃ for 24hr.

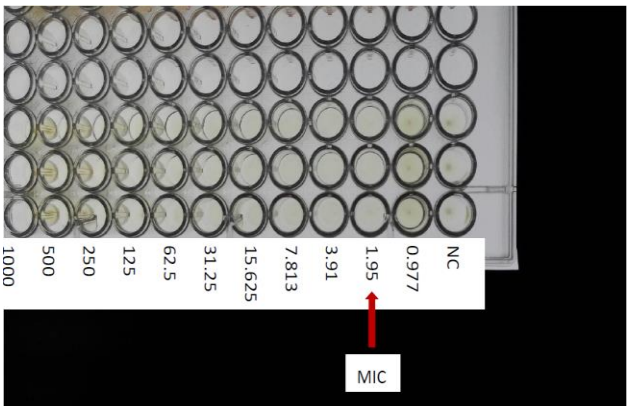
The minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of Chinese mahogany (*T. sinesis*) extract for *L. monocytogenes* was showed as Figure 1. The diluted concentration (phenol) of Chinese mahogany extract from 0.977 to 1000μg/mL was loaded into 96 cell incubation plate in order, . The non-inhibitory can be detected by clear solution and no precipitate on the button of cell. According to the two index in figure1, 1.95 μg/mL was the minimum inhibitory concentration for *L. monocytogenes* in this study .

Based on the index, 7.81257 μg/mL was the minimum inhibitory concentration for *S. aureus.*

This result also indicated that inhibitory efficiency for *L. monocytogenes* was better than *S. aureus* when Chinese mahogany (*T. sinesis*) extract was used as spray coating solution in this study.

Figure 1. The minimum inhibitory concentration of Chinese mahogany for *L. monocytogenes*.



The inhibitory efficiency for pathogen bacteria in slice pork hams

In this research, slice pork hams were used as samples and the initial loading count of *L. monocytogenes* and *S. aureus* was 2.31-2.55log CFU/10cm^2 and 2.19-2.57 log CFU/10cm^2 , individually.

The data of Table 1 showed that the count of *L. monocytogenes* in all sliced pork hams increased with time. The count of control increased significantly from day 0 (2.55 log CFU/10cm^2)to day5 (6.17 log CFU/10cm^2). In general, the count of treatments also increased with time but slower than the control. Before day3 the count of control and treatments was from 3.74 to 4.61 log CFU/10cm^2 . However, at the end of storage (day 5), the count of control showed signal increase to 6.17(log CFU/ 10cm^2) but all treatments were 4.45-4.98(log CFU/ 10cm^2). On the whole this result indicated that a good inhibitory efficiency for *L. monocytogenes* while slice ham were sprayed with 125μg/mL or more than 125μg/mL of Chinese mahogany extract .

Table 1 Change of *L. monocytogenes* count(log CFU/ $10cm^2$)of slice pork hams sprayed coating with different levels of Chinese mahogany extract at 15℃for 5 days

	ppm			
(days)	Control	125	250	500
0	2.55 ^{a,D}	2.41 ^{a,C}	2.31 ^{a,D}	2.43 ^{a,C}
1	3.18 ^{a,C}	2.71 ^{bc,C}	2.90 ^{ab,C}	2.53 ^{c,C}
3	4.61 ^{a,B}	3.92 ^{bc,B}	4.06 ^{b,B}	3.74 ^{c,B}
5	6.17 ^{a,A}	4.98 ^{b,A}	4.45 ^{c,A}	4.97 ^{b,A}

mean±S.D., n=9
a-d：Means within the same row without the same superscript are significantly different ($p<0.05$).
A-D：Means within the same column without the same superscript are significantly different ($p<0.05$).

The data of Table 2 was showed the growth of *S. aureus* on the surface of slice hams during 5 days at 15℃. At the initial (0day), the count of control was significantly higher than all treatments. The count of *S. aureus* in all lots increased with time during storage, however all treatments had a slower growth rate than control. A significantly different condition was found between control and treatments at the third day and the end of storage. In general, Chinese mahogany extract showed a good inhibitory efficiency for *S. aureus* when used as spraying solution in slice hams.
Analysis of the data in Table 1 and 2, although the growth of *L. monocytogenes* was better than *S. aureus* during storage at 15℃ but the inhibitory efficiency of Chinese mahogany extract exhibited the same ability to this two target bacteria in this study.

Table 2. The changes in *Staph. aureus* count(log CFU/ $10cm^2$) of sliced pork hams spray coating by different levels of Chinese mahogany extract during storage at 15±2℃

Time	ppm			
(days)	Control	125	250	500
0	2.57 ^{a,C}	2.49 ^{ab,B}	2.42 ^{b,B}	2.19 ^{c,C}
1	2.61 ^{a,C}	2.50 ^{ab,B}	2.50 ^{ab,B}	2.33 ^{c,C}
3	3.13 ^{a,B}	2.54 ^{c,B}	2.75 ^{b,A}	2.76 ^{bc,B}
5	4.11 ^{a,A}	3.10 ^{b,A}	2.72 ^{b,A}	3.12 ^{b,A}

mean±S.D., n=9
a-c：Means within the same row without the same superscript are significantly different ($p<0.05$).
A-D：Means within the same column without the same superscript are significantly different ($p<0.05$).

IV. CONCLUSION

In MIC Chinese mahogany (*T. sinesis*) extract showed excellent inhibition for *L. monocytogenes* and *S. aureus*. In application case of spraying solution for sliced pork ham, also demonstrated better retarded the growth of the target bacteria.

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