EVALUATION OF ANTIBACTERIAL ACTIVITY OF *TOONA SINENSIS* EXTRACTS USING IN TAIWANESE STYLE SAUSAGE

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Abstract –The utilization of extracts of *Toona sinensis* in Taiwanese style sausage to inhibit bacteria and prolong the shelf life during storage at high refrigerated temperature (15°C) was the purpose of this research. The results showed that the total plate count, anaerobic plate count, lactic acid bacteria count, mold and yeast count and VBN value of all treatments increased ($p<0.05$) with storage time, the sausage treated with 250 ppm *T. sinensis* (Te_{250}) and 125 ppm *T. sinensis* (Te_{125}) showed the lower bacteria count and VBN value during storage and the results were similar to the results of NaL. Sausages treated with NaL, Te_{125} and Te_{250} had higher pH values than that of KS during storage at 15°C. In conclusion, extracts of *Toona sinensis* possessed high total flavonoids and total phenolics contents and also showed high antibacterial ability, especially extract of *T. sinensis* with ethanol.

Key Words –Total plate count, Anaerobic plate count, Lactic acid bacteria count, Mold and yeast count and VBN value

I. INTRODUCTION

Taiwanese style sausage is a semi-dry sausage made with the larger particle size of ground pork curing with backfat, high sugar content, rice wine and some spices, which produces a unique aroma after curing. However, the shelf life of this product is a short time due to high sugar content, low temperature and short time for drying and makes it susceptible to bacterial growth. *Toona sinensis*, is also known as Chinese toon or Chinese Mahogany in class Magnoliopsida, Meliaceae family; it widely in Asia. Several phytochemical compounds have been isolated from *T. sinensis* including vitamin B and C, phenolic compounds, flavonoids and limonoids. *T. sinensis* leaves have been reported to possess antioxidative activity including high DPPH radical scavenging activities, superoxide anion radicals, reducing power and metal chelation (Hseu *et al.,* 2008; Cheng *et al.,* 2009). *T. sinensis* leave increased the immune response in tilapia (*Oreochromis mossanbicus*) against *Aeromonas hydrophila* pathogen (Wu *et al.,* 2010). In addition, rutin from *T. sinensis* can increase resistance against *Vibrio alginolyticus* pathogen on white shrimp (Hsieh *et al.,* 2008). *Houttuynia cordata*, is also known as the fishy smell herb. It belongs to the Sauraceae family, and has been widely cultivated in Southeast Asia and in East Asia (Toda, 2005). The water extract of *H. cordata* possesses strong antioxidative activities in the reducing power and scavenging activities against DPPH radical, superoxide radical and hydroxyl radical, as well as strong herbicide power (Tian *et al.,* 2011). It can inhibit the growth of *Salmonella typhimurium* (Kim *et al.,* 2008) and mycelium and spore germination of *Fusarium oxysporum* and *Colletotrichum capsici* (Puttawong and Wongroung, 2009).

II. MATERIALS AND METHODS

Herb extracts and antibacterial agent: herb preparation- 7 g of freeze dry powder of *T. sinensis* extract with ethanol was dissolved with 100 mL sterilized water and added into the sausage formula based on meat and fat weight. Sodium lactate (NaL)- 2.5% NaL (Sodium-L-lactate; 60% (w/w) solid contain, pH 5.3) was added into the sausage formula based on meat and fat weight, which represented as NaL treatment. Potassium sorbate (KS)- 0.1% KS was added into the sausage formula based on meat and fat weight, which represented as KS treatment. The Taiwanese style sausage was prepared by mixing of 75% ground pork ham and 25% diced backfat with non-meat ingredients based on formula in table 1. The groups were divided into 7 treatments including control group (Con), 0.1% potassium sorbate

(KS), 2.5% sodium lactate (NaL), 125 ppm of 7% of *T. sinensis* (Te_{125}) and 250 ppm of of 7% of *T. sinensis* (Te_{250}). A total of 30 packs which was 180 g of sample with vacuum package was prepared for each treatment and stored at 15 ºC for 8 weeks. At week 0, 1, 2, 3, 4 and 8, three bags of each treatment were taken for analyses including microbiological quality, total volatile basic nitrogen (VBN), pH value and one bag for sensory evaluation. Data were analyzed using the ANOVA system of Statistical Analysis System's Procedures (SAS , 2002) with a 5% level of significant. Means were separated using the Duncan's new multiple range test.

Table 1 The formula of Taiwanese style sausage

	Con	NaL	KS	Te_{125}	Te_{250}
Pork ham	75	75	75	75	75
Pork backfat	25	25	25	25	25
Salt	1.6	1.6	1.6	1.6	1.6
Sugar	8	8	8	8	8
Polyphosphate	0.15	0.15	0.15	0.15	0.15
Sodium nitrite	0.015	0.015	0.015	0.015	0.015
MSG	0.8	0.8	0.8	0.8	0.8
Spices	0.35	0.35	0.35	0.35	0.35
Wine	2	2	2	2	2
NaL	-	2.5	-	-	-
KS	-	-	0.1	-	-
T. sinensis	-	-	-	0.0125	0.025

III. RESULTS AND DISCUSSION

1 Total plate count

The number of total plate count of Taiwanese style sausage treated with *Toona sinensis* extracts stored at 15°C was showed in figure 1. The total plate count (TPC) of all treatments significantly increased ($p<0.05$) with storage time. During storage at 15°C, TPC value of each treatment was significantly different ($p<0.05$), except of at week 0 the count of all treatment were around 3.51-3.87 log CFU/g. Noticeably, sausage treated with sodium lactate (NaL) had lower TPC at any storage periods than other treatments, the count of this treatment around 3.51-7.92 log CFU/g during storage at 0 weeks to 8 weeks. Sausage containing *T. sinensis* (125 and 250 ppm) also showed similar results to NaL and TPC value was 3.60-8.14 log CFU/g in Te_{125} and 3.40-8.13 log CFU/g in Te_{250} during storage. However, KS had higher TPC value at 3.79-7.96 log CFU/g,but lower than the control group (3.59-8.14 log CFU/g).

In general, sausages treated with *T. sinensis* had lower microbial counts than sausage treated with *H. cordata* in this study, was probably due to *T. sinensis* had higher contents of flavonoids and phenolics than *H. cordata*. This result was similar to the result of Liu *et al.* (2009) found *Toona sinensis* can reduce the value of the total plate count in fresh chicken sausages when treated with the dose of 500, 1000 and 1500 ppm than treating with rosemary at the same concentrations due to *T. sinensis* had higher total phenolic contents than rosemary. In addition, sausages treated with NaL in this study showed the lowest total plate count value during storage and this result also was similar to the result of Lamkey *et al*. (1991) reported that 3% of sodium lactate can extend the lag phase in fresh pork sausage for 10 days. Lin and Lin (2002) found 3% of sodium lactate had the lowest total plate count value in low-fat Chinese-style sausage stored under refrigerator (4°C). In this study we also found that addition of potassium sorbate seemed to possess low antibacterial activity in sausages during storage and this result agreed with the result of Hsu and Sun (2006) who added 0-0.2% of potassium sorbate in Chinese style emulsified meatball after stored at 4-7°C for 7 days, potassium sorbate did not have an antimicrobial effect on total plate count and mold and yeast count.

2 Anaerobic plate count (ANP)

The analysis of anaerobic plate count of Taiwanese style sausages stored at 15°C were shown in figure 2. Anaerobic plate count of all treatments increased with increasing storage time ($p<0.05$), ANP at weeks 1, 2, 3, 4 and 8 were significantly different ($p<0.05$) between treatment. Sausages treated with NaL demonstrated effective inhibition on ANP during storage at 15°C and ANP was 3.21-6.42 log CFU/g. During storage for 8weeks, ANP of sausages treated with Te_{250} had the similar changes with NaL and ANP was 3.36-7.69 log CFU/g. During storage at weeks 3, KS also was found to have the highest ANP count (8.20 log CFU/g) and indicated low antibacterial activity on Taiwanese style sausage. On the other hand,

NaL showed the best activity on against the growth of anaerobic bacteria and the results was similar to the result of Lin and Lin (2002) found that sodium lactate had the lower anaerobic count than potassium sorbate during storage for 9 weeks. Herbs extract from *T. sinensis* showed moderated on against the growth of anaerobic bacteria during storage for 3 week and the cause was to use low concentration (125-250ppm) in this study.

3 Lactic acid bacteria count

The analysis of lactic acid bacteria count of Taiwanese style sausage with herbs extracts and vacuum package were showed in figure 3. lactic acid bacteria count (LAB) for all treatments were significantly increased ($p<0.05$) with increasing storage time and significantly different ($p<0.05$) between treatment at weeks 2 during storage . At weeks 2, all treatments had LAB value lower than the control group and increased to nearly control group at after weeks 3. However, the sausage treated with NaL and Te_{250} had lower LAB than other groups. Noticeably, LAB of NaL was higher than Te_{250} at weeks 4 and weeks 8 and the number was 7.50 and 7.52 log CFU/g respectively. Analysis of Lactic acid bacteria count of all treatments , *T. sinensis* showed excellent activity to inhibit the growth of lactic acid bacteria, especially at 250 ppm had bacteria count lower than other groups.

4. pH values

The change of pH of sausages treated with herb extracts were showed in figure 4. pH value of all treatment were significantly decreased ($p<0.05$) with increase of storage time, the pH was reduced due to the growth of lactic acid bacteria in sausages. Sausage with NaL trended to keep stable pH until week 2 and then decreased, and the pH value was higher than other groups. Sausages with *T. sinensis* had higher pH values than KS. However, the sausage with all treatments had pH values higher than control group during the storage period. Moreover, the pH value of each treatment at the beginning and the end of storage were shown as control 6.44-4.42, NaL 6.49-5.25, KS 6.45-4.74, Te_{125} 6.46-4.72. Te_{250} 6.48-4.77, respectively.

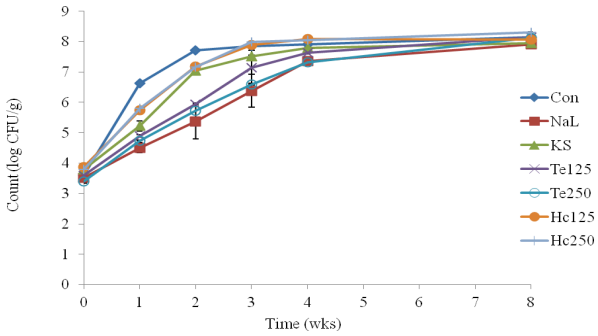

Figure 1. The changes in total plate count of Taiwanese style sausage treated with *T. sinensis* during storage at 15°C.

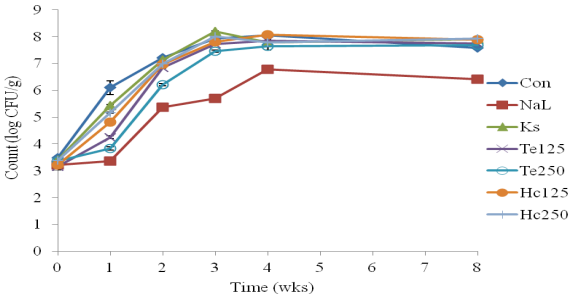

Figure 2. The changes in anaerobic plate count of Taiwanese style sausage treated with*T. sinensis* during storage at 15°C.

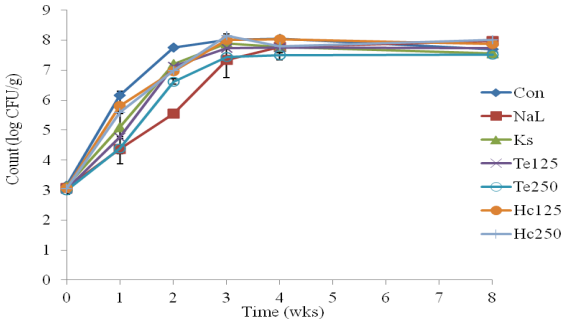

Figure3. The changes in lactic acid bacteria count of Taiwanese style sausage treated with *T. sinensis* during storage at 15°C.

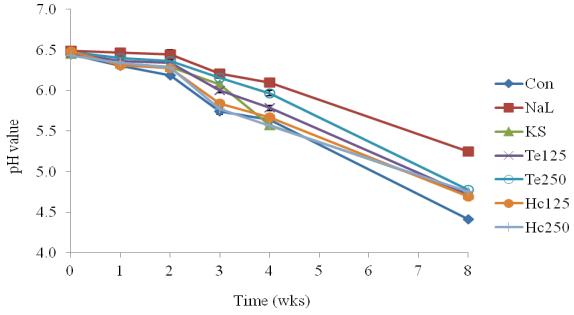

Figure4. The changes in pH value of Taiwanese style sausage treated with *T. sinensis* during storage at 15°C.

5. volatile basic nitrogen (VBN)

The increasing of volatile basic nitrogen (VBN), which is the result from decomposition of protein during storage with microorganisms, can use an index of loss of meat product freshness (Liu *et al.,* 2009). In this study, VBN values, as well as the bacterial counts, increased significantly during storage period (figure 5). In general, all treatments had lower VBN values than control group (37.11 mg%) during storage period but the values of sausage with KS had higher values than control at the ending of storage and the value was 37.75 mg%. The VBN of sausages with NaL and *T. sinensis* $_{250}$ and *T. sinensis* $_{125}$were significantly lower than the control and KS at the ending of storage and value was 30.09 mg%, 32.12 and 31.34 mg%, respectively.

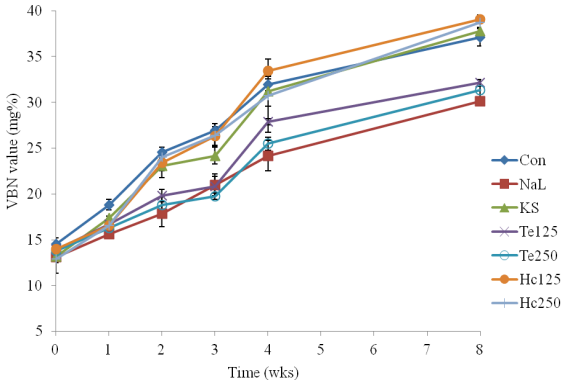

Figure5. The changes in VBN value of Taiwanese style sausage treated with *T. sinensis* during storage at 15°C.

IV. CONCLUSION

In conclusion, sausages treated with *T. sinensis* extracts showed better antibacterial activity than that of potassium sorbate and the same as sodium lactate in the study. Therefore, extracts of *T. sinensis* will be suitable to replace sodium lactate or potassium sorbate in sausage for inhibiting the growth of bacteria in the future.

REFERENCES

1. Cheng, K. W., R. Y. Yang, S. C. S. Tsou, C. S. C. Lo, C. T. Ho, T. C. Lee & M. F.Wang. (2009). Analysis of antioxidant activity and antioxidant constituents of *Chinese toon*. Journal of Functional Foods I: 253-259.
2. Hseu, Y. C., W. H. Chang, C. S. Chen, J. W. Liao, C. J. Huang, F. J. Lu, Y. C. Chia, H.K. Hsu, J. J. Wu & H. L. Yang. (2008). Antioxidant activities of *Toona sinensis* leaves extracts using different antioxidant model. Food and Chemical Toxicology 46: 105-114.
3. Hsieh, T. J., J. C. Wang, C. Y. Hu, C. T. Li, C. M. Kuo & S. L. Hsieh. (2008). Effects of rutin from *Toona sisnensis* on the immune and physiological responses of white shrimp (*Litopenaeus vannamei*) under *Vibrio alginolyticus* challenge. Fish & Shellfish Immunology 25: 581-588.
4. Hsu, S. Y. & L. Y. Sun. (2006). Effects of salt, phosphate, potassium sorbate and sodium erythorbate on qualities of emulsified meatball. Journal of Food Engineering 73: 246-252.
5. Lamkey, J. W., W. F. Leak, W. B. Tuley, D. D. Johnson & R. L. West. (1991). Assessment of sodium lactate addition to fresh pork sausage. Journal of Food Science 56: 220-223.
6. Lin, K. W. & S. N. Lin. (2002). Effects of sodium lactate and trisodium phosphate on the physicochemical properties and shelf life of low-fat Chinese-style sausage. Meat Science 60: 147-154.
7. Liu, D. C., R. T. Tsau. Y. C. Lin, S. S. Jan & F. J. Tan. (2009). Effect of various levels of rosemary or Chinese mahogany on the quality of fresh chicken sausages during refrigerated storage. Food Chemistry 117: 106-113.
8. Puttawong, S. & S. Wongroung. (2009). Plucao (*Houttuynia cordata*) Thunb. and sabsua (*Eupatorium odoratum* L.) extracts suppress *Colletotrichum capsici* and *Fusarium oxysporum*. Asian Journal of Food Agro-Industry. Special issue: S381-S386.
9. Tian, L. M., Y. Zhao, C. Guo & X. B. Yang. (2011). A comparative study on theantioxidant activities of an acidic polysaccharide and various solvent extracts derived from herbal *Houttuynia cordata*. Carbohydrate Polymers 83: 537-544.
10. Toda, S. (2005). Antioxidative effects of polyphenols in leaves of *Houttuynia cordata* onprotein fragmentation by copper-hydrogen peroxide in vitro. Journal of Medicinal Food 8: 266-268.
11. Wu, C. C., C. H. Liu, Y. P. Chang & S. L. Hsieh. (2010). Effects of hot-water extract of *Toona sinensis* on immune response and resistance of *Aeromonas hydrophila* in *Oreochromis mossambicus*. Fish & Shellfish Immunology 29: 258-263.