Antimicrobial Activity of Green Tea Extract (*Camellia Sinessis*) on Refrigerated Ground Pork

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Abstract—**Antimicrobial activity of green tea extract at 0, 3, 6, 9, 12, 15 and 20 mg/ml against 17 bacteria strains was studied *in vitro* by spot-on-lawn method. Only 4 strains, *Listeria innocua* ATCC 33090^T, *Brochothrix campestris* NBRC 11547^T, *staphylococcus aureus* TISTR 118 and *Aeromonas hydrophila* TISTR 1321 were inhibited. Moreover, examination on the effect of green tea extract on microbial in refrigerate ground pork was done by mixing ground pork with different concentration of green tea extract at 0, 50, 100, 200, 300 and 400 mg/kg (extract/ ground pork) before keeping at 4 ºC in oxygen-permeable cling film. Total microbial count, psychotropic bacteria, Total coliform and *Escherichia coli* were detected by sampling after 10 min., 1, 3 and 5 days during storage. There were no significant different of total bacterial count, psychotropic bacteria, Total coliform and *E. coli* found among green tea extract concentration. However, their number of total microbial count and psychotropic bacteria increased with long-storage. On the contrary, the rate of total coliform growth was lower and *E. coli* was suppressed after 1, 3 and 5 days storage.**

Index Terms— **Antimicrobial, green tea extract, ground pork**

I. INTRODUCTION

Consumption of contaminated food cause millions of people worldwide suffer from food born diseases, and illness each year (Karabudak, Bas & Kiziltan, 2008). Recently, consumers demand quality food products with safety and long shelf-life. These demands lead the public concern about natural antibacterial used to preserve foods. Although, good management, sanitation and hazard analysis critical control point (HACCP) program can be applied as a basis tools for contaminated reduction along production chain (McMullen, 2000) but the numbers of food-borne illnesses and intoxications are still on the increase (Soomro, Masud & Anwaar, 2002).Tea from *Camellia Sinessis* leaves are the most widely beverage consumed. Moreover, green tea components show antimicrobial activities against fungi, virus, *Salmonella, Staphylococcus*, *Campyrobacter* and *Pseudomonas aeroginosa* (Jazani, Shahabi, Ali & Zartoshti, 2007). Therefore, the objectives of this study were to evaluate the antibacterial activity of green tea extract and compare the shelf-life extension of ground meat with addition of green tea extract.

II. MATERIALS AND METHODS

A. Preparation of green tea extract

Green tea extract in powder form was obtained from Mahidol University, Thailand. Each 100 mg of green tea powder contained cathechins 12.94 mg.

B. Detection of antibacterial activity

The antibacterial activity was carried out by spot-on-lawn method (Ennahar, Sonomoto & Ishizaki, 1999). The different amount of extracted powder at 0, 1, 3, 6, 9, 12, 15 and 20 mg/ml were put in heated water and shook at 70 °C for 20 min., before applied to microbial plates in solution form. The two layers of agar plate were prepared and 5 ml of soft agar (0.8-1% agar) was added to make top layer which seeded with 10 µl of freshly grown bacterial strain (about 10^7 CFU/ml). Antibacterial activity was tested by spotting 10 µl of solution onto the top surface of agar plate. Inhibition zone was observed, after overnight incubation at proper temperature. List of bacterial strains, media and their growth condition was shown in Table 1.

C. Microbiological analysis in ground pork

The effect of green tea extract on microbial in refrigerate ground pork was investigated. Ground pork was prepared by mixing lean pork ham (70%) and back fat pork (30%), mincing twice using meat grinder before external fat and connective tissue were removed. Then, mixed ground pork was randomly sampled for initial microbes. Six different concentrations of green tea extracted powder at 0, 50, 100, 200, 300 and 400 mg/kg (powder/meat) were added into fresh ground pork and wrapped in oxygen-permeable cling film. All samples were then stored in chill room with the light on at 4 °C. Total microbial count, psychotropic bacteria, total coliform and *E. coli* were detected by sampling mixed ground pork after 10 min., 1, 3 and 5 days during storage. All microbial determination was carried out according

to AOAC (2006) and expressed as log $_{10}$ colony forming unit (cfu/g of meat). Total coliform and *E. coli* were evaluated using Chromocult (Merck, Germany).

III. RESULTS AND DISCUSSION

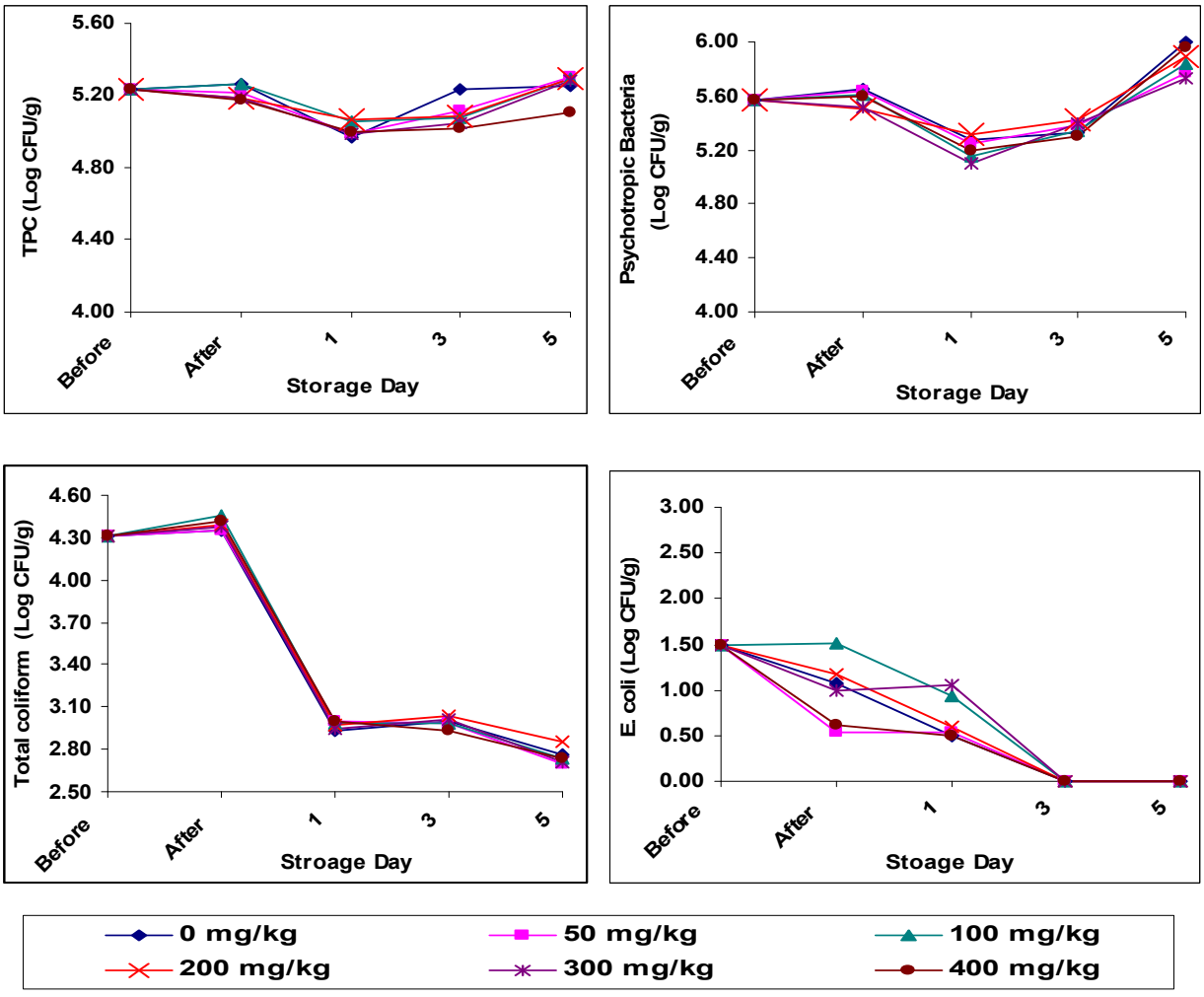
Antimicrobial activity of green tea extract at 0, 1, 3, 6, 9, 12, 15 and 20 mg/ml against 17 bacteria strains was studied *in vitro* by spot-on-lawn method has shown in Table 1. The minimum concentration of green tea extract in solution form could inhibit *L. innocua* ATCC 33090^T, *Br. campestris* NBRC 11547^T, *S. aureus* TISTR 118 and *A. hydrophila* TISTR 1321 at concentration of 3, 12, 20 and 15 mg/ml, respectively. Interestingly, it was noticed that green tea extract had no effect on lactic acid bacteria which reported to be food spoilage bacteria as *L. innocua* and *Br. campestris*. However, some useful species of lactic acid bacteria account for an important part of the intestinal microflora of animal and human. It also found as microflora in many fermented food (Zacconi, Scolari, Fraioli & Sarra, 1999); Stile & Holzapfel, 1997). While, *S. aureus* and *A. hydrophila* are pathogenic bacteria often found in food including fresh meat. Ibrahim & Mac Rae (1991) reported that *A. hydrophila* and *L. innocua* were prevalence in fresh meat (fish, shellfish, poultry, and raw meat). The potential role of *A. hydrophila* is human gastrointestinal infections (Daskalov, 2006). The fatal case of *L. innocua* was first report by Perrin, Bemer & Delamare (2003). A 62-year-old woman was admitted to the hospital with a 3-day history of right-upper-quadrant abdominal pain. Later, it was found that *L. innocua* could lead to a fatal outcome. However, Jazani et al. (2007) reported that various studies have shown significant suppressive effects of green tea against many microorganisms, for example *Salmonella* Typhimurium, *Salmonella* Typhi, *Shigella dysenteriae, Yersinia enterocolitica, E. coli, S. aureus, campyrobacter jejini* and *P. aeroginosa.*

Table 1 Antibacterial activity of green tea extract against bacteria

Bacteria strains	Media	Temp. (°C)	Antibacterial activity Concentration (Mg/ml)							
			0	1	3	6	9	12	15	20
Lactic acid bacteria										
Lactobacillus plantarum ATCC 14917^T	MRS	30	-	-	-	-	-	-	-	-
Lactobacillus sakei subsp. *sakei* JCM 1157^T	MRS	30	-	-	-	-	-	-	-	-
Lactobacillus sakei TISTR 890	MRS	37	-	-	-	-	-	-	-	-
Leuconostoc mesenteroides subsp.*mesenteroides* JCM 6124 ^{T}	MRS	30	-	-	-	-	-	-	-	-
Leuconostoc mesenteroides subsp.*mesenteroides* TISTR 942	MRS	30	-	-	-	-	-	-	-	-
Enterococcus faecalis JCM 5803 ^{T}	MRS	37	-	-	-	-	-	-	-	-
Enterococcus faecalis TISTR 888	MRS	37	-	-	-	-	-	-	-	-
Lactobacillus salivarius K4	MRS	37	-	-	-	-	-	-	-	-
Lactobacillus salivarius K7	MRS	37	-	-	-	-	-	-	-	-
Other gram positive bacteria										
Listeria innocua ATCC 33090 ^{T}	TSB-YE	37	-	-	+	-	-	-	-	-
Brochothrix campestris NBRC 11547 ^{T}	TSB-YE	26	-	-	-	-	-	+	-	-
Staphylococcus aureus TISTR 118	TSB-YE	37	-	-	-	-	-	-	-	+
Other gram negative bacteria										
Salmonella Typhimurium TISTR 292	TSB-YE	37	-	-	-	-	-	-	-	-
Escherichia coli TISTR 890	TSB-YE	37	-	-	-	-	-	-	-	-
Pseudomonas fluorescens JCM 5963 ^{T}	TSB-YE	26	-	-	-	-	-	-	-	-
Pseudomonas fluorescens TISTR 358	TSB-YE	26	-	-	-	-	-	-	-	-
Aeromonas hydrophila TISTR 1321	NB	30	-	-	-	-	-	-	+	-

ATCC = American Type Culture Collection, Rockville, Md
JCM = Japanese Culture of Microorganism, Wako, Japan
NBRC = National Institute of Technology and Evaluation (NITE) Biological Resource Center
TISTR = Thailand Institute of Scientific and Technological Research
MRS = de Man, Rogosa and Sharpe (Merck, Germany)
TSB-YE = Tryptic soy broth + 0.6%Yeast extract (Merck, Germany)
NB = Nutrient broth (Merck, Germany)

The result of green tea extracted powder effect on microbial in ground pork showed that there were no significant different of total microbial count, psychotropic bacteria, total coliform and *E. coli* found in the ground pork among green tea extract concentrations. However, their number of total mirobial count and psychotropic bacteria increased with long-storage, especially in day 5 (Fig.1(a) and (b)). On the other hand, the rate of total coliform growth was lower and *E. coli* was not exhibiting (below the limit of detection) after 1, 3 and 5 day's storage ((Fig.1(c) and (d)). Although, green tea extract could inhibit bacterial as shown Table 1, this study showed that the extract had no potential to decreased microbial numbers on ground pork throughout storage. Since antimicrobial property of this extract was specific to few strains and concentrations of the extract tested could be too low to complete the microbial number. Bañón, Díaz, Rodríguez, Garrido & Price, (2007) reported that after applied green tea to beef patties, total viable count and total coliform increased throughout storage. Whereas, Adam & Moss (1995) reported that coliform and *E. coli* were mesophile typical growing 20-40 °C. As a result of this, it was observed that low temperature had lethal effect on coliform and *E. coli*.



Before = Before added green tea extract ; After = After added green tea extract

Figure 1 Effect of green tea extract on microbial in ground pork

IV. CONCLUSION

Only *L. innocua*, *Br. campestris*, *S. aureus* and *A. hydrophila* could be inhibited by green tea extract at 3-20 mg/ml for antimicrobial activity test. However, there were no significant different of total bacterial count, psychotropic bacteria, total coliform and *E. coli* found in therefrigerated ground pork.

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