ผลการยับยั้งเชื้อจุลินทรีย์บนผิวหน้าไส้กรอกเปรี้ยวหมู ของสารสกัดจากขิงและกระเทียม

Antimicrobial Effects of Ginger and Garlic Extracts on Microbial Growth on Surfaces of Thai Fermented Pork Sausages

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการยับยั้งเชื้อจุลินทรีย์บนผิวหน้าไส้กรอกเปรี้ยวหมูของสารสกัดจากขิงและกระเทียม โดยทำการศึกษาวิธีการใช้สกัด 2 วิธี (วิธีการจุ่ม และการฉีดพ่น) และศึกษาผลของระดับความเข้มข้นของสารสกัดทั้งสองชนิด (ร้อยละ 0 50 และ 100) ผลการวิจัย พบว่า วิธีการใช้สารสกัดไม่มีผลต่อปริมาณจุลินทรีย์ทั้งหมดและยีสต์บนผิวหน้าไส้กรอกเปรี้ยวหมู และเชื้อราบนผิวหน้าไส้กรอกเปรี้ยวหมูมีความไวต่อฤทธิ์การยับยั้งจุลินทรีย์จากสารสกัดทั้ง 2 ชนิดมากกว่าเชื้อจุลินทรีย์ทั้งหมดและยีสต์ ทั้งนี้เมื่อใช้สารสกัดทั้ง 2 ชนิด ที่ระดับความเข้มข้นเดียวกันพบว่าสารสกัดกระเทียมมีผลในการยับยั้งเชื้อจุลินทรีย์มากกว่าสารสกัดจากขิง

คำสำคัญ: สารสกัดจากขิง, สารสกัดจากกระเทียม, การยับยั้งการเจริญเชื้อจุลินทรีย์, ไส้กรอกเปรี้ยว

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ABSTRACT

This study aims to investigate the antimicrobial effects of ginger and garlic extracts on microbial growth on surfaces of Thai fermented pork sausages. Two application methods (dipping and spraying) and concentrations (0% 50% and 100%) of crude extracts were evaluated. The results show that the application method did not affect the number of total bacteria and the yeast on the surfaces of the sausages. Moulds were more susceptible to the antimicrobial effects of both the ginger and garlic crude extracts. When applied at the same concentration, garlic extract was more effective than ginger extract.

Key words: ginger extract, garlic extract, antimicrobial effect, Thai fermented sausage

INTRODUCTION

Thai fermented sausage is a very popular dish, particularly in the North and Northeast region of Thailand. The production process is relatively simple and can be consumed as a snack. The main ingredients include minced pork / beef, lard, cooked rice, garlic, salt, pepper, sugar and food additives (mono-sodium glutamate and nitrite).

The sausage mix is stuffed in pork intestine or other edible casings, and tied into 1-3 inches in length (Jatupornpipat and Keatikumjorn, 2007). Traditionally, after stuffing, the sausages are placed on hanging rails and left to ferment in an open-air area for good ventilation at room temperature for a few day. The fermentation is stopped when the pH of the sausages reaches 4.6, which is one important control point. The formation of lactic acid during the fermentation stage is critical as it contributes to pathogen control and the sourness characteristic of the product. The sausages are cooked (by grilling, frying, or roasting) before consumption (Thai Industrial Standards Institute, 1994).

One challenge often encountered during fermentation stage by small and medium-sized entrepreneurs (SMEs) is the growth of microorganisms on the surfaces of the sausages as they do not have a well-controlled fermentation environment. High relative humidity, warm temperature, and high contents of moisture and nutrients in the sausages are optimal conditions for the growth of aerobic mesophiles on the surfaces (casing) of the product. Such contamination is highly undesirable as it creates extremely undesirable visual effects and could affect food safety. The manufacturers generally brush or rinse the sausages to get rid of those unwanted microorganism colonies before packing.

Various herbal extracts have been reported for their antimicrobial effects. Ethanolic extracts of ginger extract is found to be effective in inhibiting the growth of *Escherichia coli* and *Salmonella typhi*. Garlic has long been recognised for its antimicrobial

activity as it shows a strong inhibitory effect on the growth of *Candida* sp. (Cavallito and Bailey, 1944) and antifungal activity (Ankri and Mirelman, 1999). Allicin in garlic could inhibit *Staphylococcus* sp. (Gonzalez-Fondoz *et.al*., 1994; Uzodike and Igwe, 2005) Garlic extract is also reported to be highly effective in inhibiting the growth of *A. niger*, *Cryptococcus* sp., *Aspergillus flavus*, and *Caldosporium herbarum* (Gupta and Ravishankar, 2005; Tagoe *et al*., 2010). Applications of ginger, garlic, and onion extracts have been reported to help prolonging the shelf-life of meat products (Tagoe *et al*., 2010).

The present study aims to investigate the use of garlic and ginger crude extracts as a means to control the growth of microorganisms on the surface of fermented sausages in an attempt to find a solution to the aforementioned problem of Thai SMEs.

MATERIALS AND METHODS

1. Materials

1.1 Sausage production

Ingredients for sausage production were purchased from local markets in Muang District, Loei Province, Thailand. All chemicals were obtained from Merck (Bangkok, Thailand) unless otherwise stated. Culture media were purchased from HiMedia (Mumbai, India).

1.2 Preparation of ginger and garlic crude extracts

1.2.1 Ginger crude extract was prepared according to the method described by Pattanawadee *et al*. (2006) with modifications. Fully mature ginger was cleaned and rinsed with hypochlorite solution before cutting into thin slices. Sliced ginger was dried in an hot-air oven at 50 ^{o}C for 24 h. Dried ginger (10 g) was ground into powder before addition of 100 ml of 50% (v/v) ethanol. The mixture was agitated by a shaker at 250 rpm for 24 h at ambient temperature. Ginger residues were separated by filtration and the filtrate was concentrated (to 2-3 ml) under vacuum. The extract residue was diluted with water to get a final volume of 25 ml before centrifugation at 3000 x *g* for 15 min, followed by filtration through 0.2 µm membrane filter. The extract was kept in a sterilised container at refrigeration temperature (5$\pm$2 ^{o}C) until use.

1.2.2 Garlic crude extract was prepared by the methods described by Sood *et al*. (2003); Tangnoo and Tuntakom (2006); Pongmanee and Sanampol (2007) with modifications. Garlic (100 g) was cleaned, soaked in 70% (v/v) alcohol for 3 min and rinsed with distilled water. The cleaned garlic was finely ground in a food processor before addition of 50 ml distilled water. The mixture was mixed and left at ambient temperature for 24 h. Garlic residue was separated from the mixture by filtration. The filtrate was passed through a 0.2 µm membrane filter and kept in a sterilised container at refrigeration temperature (5$\pm$2 ^{0}C) until use.

2. Methods

2.1 Application methods of ginger and garlic crude extracts on the surfaces of fermented sausages

Sausages were prepared according to the method described by Siripahanakul *et al*. (2007). Ginger and garlic crude extracts were diluted with water (50% v/v) before application onto the surfaces of the sausages. Two application methods - dipping the sausages into extract solution, and evenly spraying the crude extracts onto the sausages were investigated to compare with control. The extract treated sausages were left to ferment at ambient temperature. Monitoring of colour (Hunter Lab spectrophotometer) and microbial counts (total bacteria, yeasts and moulds) on the surfaces of the sausages by swab test method (FSAI, 2006), as well as pH and titratable acidity of the sausages were performed every 12 h until the pH reached 4.6 or titratable acidity reached $1.0 \pm 0.1\%$ (w/w as lactic acid). Sausages without the treatment of the crude extracts were used as a control. The experiment was performed in triplicate. Data analysis was performed according to the method described in Statistical Analysis Section below. The best extract application method was selected for the subsequent experiment.

2.2 Effect of crude extract concentration on microbial growth on the surfaces of fermented sausages

Sausages were newly prepared and treated with ginger or garlic crude extracts at two different concentrations (50% v/v dilution with water and undiluted). Monitoring of quality parameters (pH, titratable acidity, colour and microbial counts) during the fermentation time course was performed in the same fashion as described in the previous experiment. Sausages without the treatment of the crude extracts were used as a control. The experiment was performed in triplicate. Data analysis was performed according to the method described in Statistical Analysis Section.

2.3 Statistical Analysis

Analysis of variance with completely randomised design was used in statistical analysis. Differences between mean values of the treatments were subsequently analysed by Fisher's least significant difference. SPSS software was used for the analyses.

RESULTS AND DISCUSSION

1. Application methods of ginger and garlic crude extracts on the surfaces of fermented sausages

Applications methods of ginger and garlic crude extracts did not affect the fermentation pattern of the sausages. The initial pH of all samples (at 0 h) was between 5.5 and 6.0 and gradually decreased to 4.6 within 48 h of fermentation. Initial titratable acidity (expressed as lactic acid, w/w) were approximately 0.7% for garlic-treated samples and 0.8% for ginger-treated sample. Within 48 h of fermentation, the value of titratable acidity for all samples was between 1.2-1.4%. The changes of pH and titratable acidity over the time course of the fermentation stage was similar to previously reported experiment and was expected (Siripahanakul *et al*., 2007). Comparison of colour measurement (L, a, b scales) revealed that the treatments of ginger and garlic crude extracts did not affect the colour of the sausages. Throughout the course of fermentation courses, colour measurement showed that the

L value of the sausages decreased from 57 (t = 0 h) to 49 (t = 48 h); as values increased from 4 (t = 0 h) to 14 (t = 48 h); b values increased from 14 (t = 0 h) to 20 (t = 48 h).

Total numbers of viable cells, yeasts, and moulds (cfu/cm^2) detected on the surfaces of the sausages treated with ginger and garlic crude extracts (50% v/v dilution in water) by dipping and spraying method are shown in Tables 1 and 2. It was found that total bacterial and yeast numbers on the surfaces of the sausages treated by both extracts were less than those of the control throughout the course of fermentation.

In all treatments, the numbers of mould counts were less than 30 cfu / cm^2 throughout the fermentation course. This could be because of high moisture content on the sausages' surfaces especially at the beginning of the fermentation, which was not optimal for the growth of moulds (Mizakova *et al*., 2002). The majority of microorganisms found on the sausages' surfaces were bacteria, followed by yeasts (Andersen, 1995).

As can be seen in Table 1, the number of detected microorganisms on the surfaces of sausages treated with ginger crude extract by dipping and spraying method did not differ. The same pattern was seen in sausages treated with garlic crude extract as well (Table 2). Yeasts were susceptible to the treatment of both crude extracts, especially the garlic extract. Garlic crude extract was found to be highly effective in inhibiting the growth of yeasts. Compare to the yeast count number detected on the surfaces of control sausages at 48 h of fermentation time, reduction of 1-log and 4-log cycles in yeast cells were observed on the surfaces of ginger-treated and garlic-treated sausages, respectively (Tables 1 and 2). With regards to the crude extract application method, dipping and spraying did not differ in terms of the numbers of viable cells and yeasts detected on the sausages' surfaces ($p > 0.05$). Since both methods were found to be equally effective, spraying is a more preferred method as it requires less amount of extract than dipping per application.

Table 1 Total numbers of viable cells, yeasts, and moulds (cfu/cm^2) detected on the surfaces of sausages treated with ginger crude extract (50% v/v dilution in water) by dipping and spraying method and control

Time (h)	Control			Dipping method			Spraying method		
	Total bacteria	Yeasts	Moulds	Total bacteria	Yeasts	Moulds	Total bacteria	Yeasts	Moulds
0	5.50×10^4	< 30	< 30	1.62×10^4	< 30	< 30	5.90×10^4	< 30	< 30
12	6.30×10^6	< 30	< 30	6.10×10^5	< 30	< 30	6.25×10^5	< 30	< 30
24	5.45×10^7	2.95×10^3	< 30	1.98×10^7	1.55×10^3	< 30	1.00×10^7	1.90×10^3	< 30
36	7.20×10^8	7.70×10^3	< 30	8.50×10^7	1.08×10^3	< 30	8.25×10^7	1.70×10^3	< 30
48	2.05×10^9	4.96×10^4	< 30	5.80×10^8	4.80×10^4	< 30	5.89×10^8	5.00×10^3	< 30

Table 2 Total numbers of viable cells, yeasts, and moulds (cfu/cm^2) detected on the surfaces of sausages treated with garlic crude extract (50% v/v dilution in water) by dipping and spraying method and control

Time (h)	Control			Dipping method			Spraying method		
	Total bacteria	Yeasts	Moulds	Total bacteria	Yeasts	Moulds	Total bacteria	Yeasts	Moulds
0	7.10×10^4	< 30	< 30	3.20×10^4	< 30	< 30	2.70×10^4	< 30	< 30
12	5.50×10^6	2.25×10^3	< 30	3.10×10^5	< 30	< 30	3.00×10^5	< 30	< 30
24	2.04×10^6	4.86×10^4	< 30	4.20×10^6	< 30	< 30	3.95 x010^6	< 30	< 30
36	3.03×10^8	5.11×10^4	< 30	5.70×10^6	< 30	< 30	5.90×10^6	< 30	< 30
48	2.95×10^8	2.72×10^5	< 30	2.10×10^7	< 30	< 30	2.40×10^7	< 30	< 30

2. Effect of crude extract concentration on microbial growth on the surfaces of fermented sausages

Investigation into the effects of two different concentrations of ginger and garlic extract (50% v/v dilution in water, and undiluted) on the growth of microorganisms on sausages' surface by spraying method was performed. Fermentation was stopped at 48 h for a ginger-treated set and at 36 h for a garlic-treated set as the pH and titratable acidity of the samples reached the end point (pH ≤ 4.6 and titratable acidity ≥ 1.0% w/w, as lactic acid). Total numbers of viable cells, yeasts, and moulds (cfu/cm^2) detected on the surfaces of sausages sprayed with ginger and garlic crude extracts are shown in Tables 3 and 4.

Table 3 Total numbers of viable cells, yeasts, and moulds (cfu/cm^2) detected on the surfaces of sausages sprayed with ginger crude extract (50% v/v dilution in water, and undiluted) and control

Time (h)	Control (0% extract)			diluted extract (50% v/v)			Undiluted extract (100% v/v)		
	Total bacteria	Yeasts	Moulds	Total bacteria	Yeasts	Moulds	Total bacteria	Yeasts	Moulds
0	2.79×10^4	< 30	< 30	1.69×10^4	< 30	< 30	1.55×10^4	< 30	< 30
12	1.43×10^6	8.60×10^2	< 30	4.70×10^5	< 30	< 30	4.71×10^5	< 30	< 30
24	1.46×10^7	6.75×10^3	< 30	6.36×10^6	2.72×10^3	< 30	6.56×10^6	2.82×10^2	< 30
36	2.62×10^8	8.06×10^3	< 30	2.02×10^7	4.36×10^3	< 30	2.10×10^7	4.30×10^3	< 30
48	7.93×10^8	1.92×10^5	< 30	6.49×10^7	3.15×10^4	< 30	6.42×10^7	3.12×10^4	< 30

Table 4 Total numbers of viable cells, yeasts, and moulds (cfu/cm^2) detected on the surfaces of sausages sprayed with garlic crude extract (50% v/v dilution and undiluted), and control

Time (h)	Control (0% extract)			diluted extract (50% v/v)			Undiluted extract (100% v/v)		
	Total bacteria	Yeasts	Moulds	Total bacteria	Yeasts	Moulds	Total bacteria	Yeasts	Moulds
0	3.00×10^4	< 30	< 30	6.10×10^3	< 30	< 30	2.52×10^3	< 30	< 30
12	8.90×10^5	2.30×10^2	< 30	3.15×10^5	< 30	< 30	1.34×10^5	< 30	< 30
24	4.65×10^7	3.40×10^3	< 30	1.47×10^6	< 30	< 30	1.51×10^5	< 30	< 30
36	7.46×10^7	2.49×10^4	< 30	5.70×10^6	< 30	< 30	1.60×10^5	< 30	< 30

The microbiological results showed that viable cell counts on the surfaces of ginger-treated sausages at both -concentrations were approximately 1-log cycle less than those of the control (Table 3). The microbial numbers resulting from the application of undiluted ginger crude extract, however, did not differ from those from the application of the diluted extract.

It is clearly shown in Table 4 that the antimicrobial activity of garlic crude extract was more potent at higher concentration. Applications of 50% diluted and undiluted garlic extract decreased the viable cell numbers of microorganisms grown on the surfaces of sausages by 1-log and 3-log cycles, respectively, after 36 h of fermentation. The garlic extracts at both concentrations were highly effective in inhibiting the growth of yeasts, as was seen earlier in the previous section. The growths of yeasts were completely suppressed throughout the fermentation course.

Garlic aqueous extract has been reported to possess high antimicrobial activity against certain pathogens (El-Astal, 2004). The results obtained from this study were comparable to the studies previously reported. Indu *et al*. (2006) reported that antimicrobial activity of garlic extract against a wide range of pathogens (*Escherichia coli*, *Salmonella paratyphi*, *S. mgulani*, *S. bareily*, *S. enteritidis*, *S. senftenberg*, *S. typhimurium*, *S. weltevreden*, *S. Worthington*, and *Aeromonas hydrophila*) was dependent on the concentration of the extract used. In another study by Shobana *et al*. (2009), observation of higher inhibitory effects at higher concentrations of water-extracted garlic solutions against *E. coli*, *Proteus mirabilis*, *S. typhi*, and *Shigella flexineri* was also reported.

Although the results (Tables 1 to 4) showed both ginger and garlic crude extracts exhibited antimicrobial activities, the antimicrobial potency of both extracts differed considerably. The differences in antimicrobial potency of ginger and garlic extracts could be due to the difference in extract preparation process, as well as the difference in the profile of active compound presented in each extract (Figure 1). Ginger has

been reported to have relatively low potency in inhibiting the growth of microorganisms (Snyder, 1997). Nevertheless, Yushau *et al*. (2008) reported an improvement in inhibitory effects of ginger extracts again microbial growth at higher extract concentrations. In this study, such observation was not the case. This may be because the preparation method used to obtained ginger crude extract was not optimal to obtain a higher yield of active components.

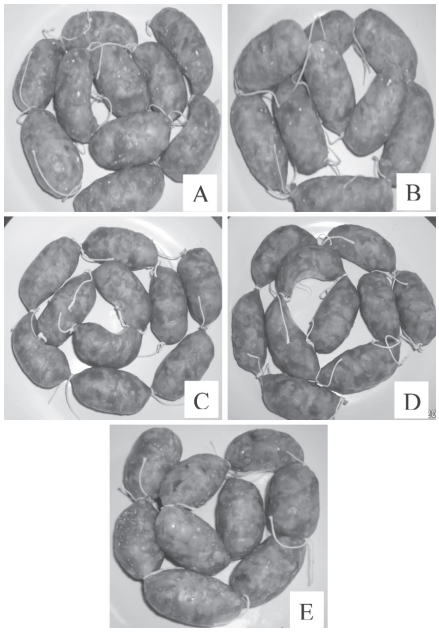

Figure 1 Microorganism growth (white spot) on the surfaces of sausages sprayed with crude extract of;

A : ginger 50% v/v diluted extract

B : ginger Undiluted extract

C : garlic 50% v/v diluted extract

D : garlic Undiluted extract

E : Control (0% extract)

CONCLUSION

Ginger and garlic crude extracts were able to reduce the number of viable cells and yeasts grown on the surfaces of Thai fermented pork sausages. Garlic crude extract was more effective in inhibiting the growth of microorganisms, especially on yeasts, than ginger extract. Reduction of microbial and yeast numbers on the surfaces of sausages was observed by application of 50% diluted extracts. Spraying undiluted garlic extract into the surface of sausages before fermentation stage could significantly reduce the numbers of unwanted aerobic mesophiles on the surface of the product. The findings from this study could be applied to resolve the problem Thai fermented sausage produced by SMEs.

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