# Antibacterial Activity of Protein Hydrolysates from Processed Mangoes against Human Pathogens

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***Abstract*—This research aimed to investigate the antibacterial activity of peptides from 4 types of processed mangoes including pickled mango, pickled mango with chilli, mango sheets and mango sheets with chilli by broth dilution method. Antibacterial activity was assayed against human pathogens such as *E. coli, S. aureus, P. aeruginosa, B. subtilis* and *K. aerogenes*. The results showed that mango sheets with chilli showed high activity against all pathogens except *E. coli* 8739. While all processed mangoes can inhibit the growth of *S. aureus* 6538 and *S. aureus* 6538P. Then, a proper amount of consumption will protect consumers from pathogens.**

***Index Terms*—processed mangoes, protein hydrolysates, antibacterial activity**

## I. Introduction

Microbes are mainly common behind infectious diseases which involve in about half of the deaths in human worldwide. In many developing countries, significant morbidity and mortality due to diarrhea act as a major problem. The gastrointestinal infections caused by wide range of bacterial pathogenic such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella aerogenes* are most common [1]. Antibiotic and synthetic drugs have been mainly selected for cure. After repeatedly used for long time, it will help the development of antibiotics resistance and side effects may be occurred. Therefore, effective medicine from natural product was interested approach for these days.

Antimicrobial Peptides (AMPs) from edible plants are one of interesting choices. AMPs are oligopeptides that interact with microbes by penetration of microbial lipid membranes due to their amphiphilic characteristic and high density of positive charged, causing pore formation and cell lysis at the end [2]. Usually AMPs show broad classes of targets including viruses, bacteria, fungi, and parasites. They are commonly found in living organisms both prokaryote and eukaryote [3].

Medicinal plants with their biologically compounds hold an important status of being the main sources of pharmacology in lately years [4]. Around 80% of individuals from developed countries use traditional herb medicine, containing compounds isolated from parts of plants like leaves, seeds and fruits. Many plants have been utilized by reason of their antimicrobial qualities, which are because of their compounds released in the secondary metabolism. According to the WHO, medicinal plants would be the best source to replace synthetic drugs with antimicrobial properties [5]. Ethnobotanical studies conducted in Nigeria and other parts in the world have reported variety of plants that are used in the therapy of infectious diseases, including *Mangifera indica* [6].

Mango (*Mangifera indica* L.) is one of the most significant tropical fruits marketed and consumed all over the world with its delightful taste and extraordinary source of nutrients and bioactive compounds. They are highly perishable, consequently, large quantities of mango are lost annually in many parts of the world including Thailand. To lower postharvest losses of mango fruits, processing is recommended. Moreover, previous work reported that vitamin C and β-carotene still remained in minimally processed or dried products [7]. Then processed mango is an interesting source of edible AMPs.

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For this reason, the goal of this research is to study the antimicrobial activity of processed mango after hydrolyzed with pepsin, an endopeptidase in human stomach that shorten proteins into smaller peptides, against gastrointestinal pathogens. The bioactive peptide will be identified and processed mango might be used as a potential medicinal food in the future.

## II. Methodology

### A. Plant Material

Four samples of processed mangoes were collected from representative of processed mango community in Chachoengsao, Thailand. These were Pickled mango, Pickled mango with chilli, Mango sheets and Mango sheets with chilli as shown in Fig. 1.

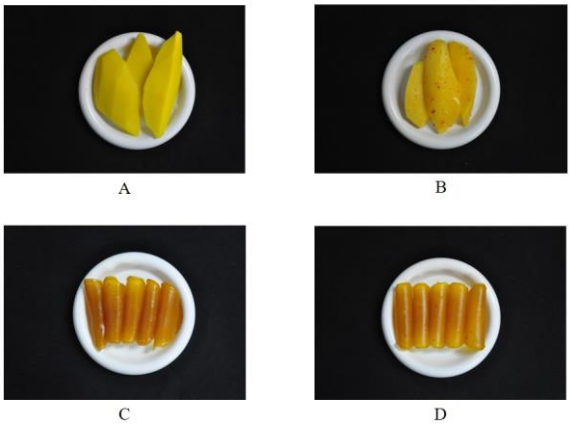


Figure 1. Processed mango samples; a) Pickled mango, b) Pickled mango with chilli, c) Mango sheets and d) Mango sheets with chilli.

### B. Preparation of Protein Hydrolysate

Total protein from 50g of samples were extracted using 200mM sodium acetate, pH 3.7, heat at 121°C followed by hydrolysis with pepsin at 37ºC for 16 h. The crude hydrolysate was filtrated with cheesecloth. The total protein concentration of the supernatant was measured by the method of Lowry using bovine serum albumin as standard.

### C. Antibacterial Activity Determination

The antibacterial activity was determined against *Escherichia coli* DH5a, *Escherichia coli* 8739, *Staphylococcus aureus* 6538, *Staphylococcus aureus* 6538P, *Pseudomonas aeruginosa* 9027, *Bacillus subtilis* 6633 and *Klebsiella aerogenes* 13048 using broth dilution method in triplicate. While the starting OD at 600nm of all bacterial was 0.05, protein hydrolysates were added and mixed well. The sterile water and tetracycline were used as negative control and positive control, respectively. The final concentration of protein hydrolysate and tetracycline was 100µg/ml.

## III. Results and Discussion

The total protein concentration of all samples was measured by Lowry assay before hydrolysis with pepsin and the result showed that percentage of protein by weight of all samples were 0.77-0.90 % as presented in Table I.

TABLE I. The Total Protein of All Samples

| sample | % protein by weight |
|---|---|
| Pickled mango | 0.80 |
| Pickled mango with chilli | 0.77 |
| Mango sheets | 0.90 |
| Mango sheets with chilli | 0.80 |

Antibacterial activity of protein hydrolysates from processed mango samples were tested by broth dilution method, while sterile water was used as positive control and tetracycline was negative control. Protein hydrolysates from all samples were able to inhibit the growth of *S. aureus* 6538, *S. aureus* 6538P and *B. subtilis* 6633 within 7h after exposure. Both mango sheets and mango sheets with chilli were able to inhibit the growth of *E. coli* DH5α while only mango sheets with chilli showed antibacterial activity against *P. aeruginosa* 9027 and *K. aerogenes* 13048 as shown in Table II and Fig. 2.

TABLE II. Average of Optical Density at 600nm of Pathogens at 6h

| Human pathogen | Optical density at 600nm at 6h | | | | | |
|---|---|---|---|---|---|---|
| | Negative | Positive | Pickled mango | Pickled mango with chilli | Mango sheets | Mango sheets with chilli |
| *E. coli* DH5α | $0.53^{d}$ | $0.20^{a,b}$ | $0.33^{c}$ | $0.35^{c}$ | $0.22^{b}$ | $0.19^{a}$ |
| *E. coli* ATCC 8739 | $0.92^{e}$ | $0.29^{a}$ | $0.63^{c}$ | $0.67^{d}$ | $0.62^{c}$ | $0.51^{b}$ |
| *S. aureus* ATCC 6538 | $0.68^{c}$ | $0.21^{a}$ | $0.29^{c}$ | $0.29^{c}$ | $0.27^{b,c}$ | $0.26^{b}$ |
| *S. aureus* ATCC 6538P | $0.68^{c}$ | $0.24^{a}$ | $0.25^{a}$ | $0.23^{a}$ | $0.34^{b}$ | $0.28^{a,b}$ |
| *P. aeruginosa* ATCC 9027 | $0.50^{e}$ | $0.25^{b}$ | $0.40^{d}$ | $0.41^{d}$ | $0.31^{c}$ | $0.21^{a}$ |
| *B. subtilis* ATCC 6633 | $1.04^{e}$ | $0.21^{a}$ | $0.51^{d}$ | $0.50^{c,d}$ | $0.49^{c}$ | $0.41^{b}$ |
| *K.aerogenes* ATCC 13048 | $0.61^{c}$ | $0.17^{a}$ | $0.59^{b,c}$ | $0.67^{d}$ | $0.56^{b}$ | $0.21^{a}$ |

Note: Means followed by the same letter in the column are not statistically different by the test of Duncan at 5% of probability

TABLE III. Inhibition Percentage at 6h

| Human pathogen | Inhibition percentage at 6h | | | |
|---|---|---|---|---|
| | Pickled mango | Pickled mango with chilli | Mango sheets | Mango sheets with chilli |
| *E. coli* DH5α | 36.93 | 34.22 | 58.92 | 64.78 |
| *E. coli* ATCC 8739 | 31.49 | 27.66 | 33.01 | 45 |
| *S. aureus* ATCC 6538 | 56.65 | 57.54 | 59.79 | 61.76 |
| *S. aureus* ATCC 6538P | 62.94 | 66.86 | 49.66 | 58.87 |
| *P. aeruginosa* ATCC 9027 | 19.91 | 18.84 | 38.21 | 57.25 |
| *B. subtilis* ATCC 6633 | 51.15 | 52.01 | 53.48 | 60.41 |
| *K. aerogenes* ATCC 13048 | 3.28 | -9.07 | 8.14 | 65.79 |

The inhibitory percentage of 50% or higher on at least one targeted microbe was shown in Table III. Protein hydrolysates from mango sheets and mango sheets with chilli showed satisfying result of *E. coli* DH5α growth inhibition, but no sample could inhibit *E. coli* ATTC8739. Mango sheet with chilli is the only one hydrolysate sample that suppress growth of *P. aeruginosa* and *K. aerogenes*, while hydrolysates from all processed mango sample could suppress the growth of *S. aureus* and *B. subtilis.*

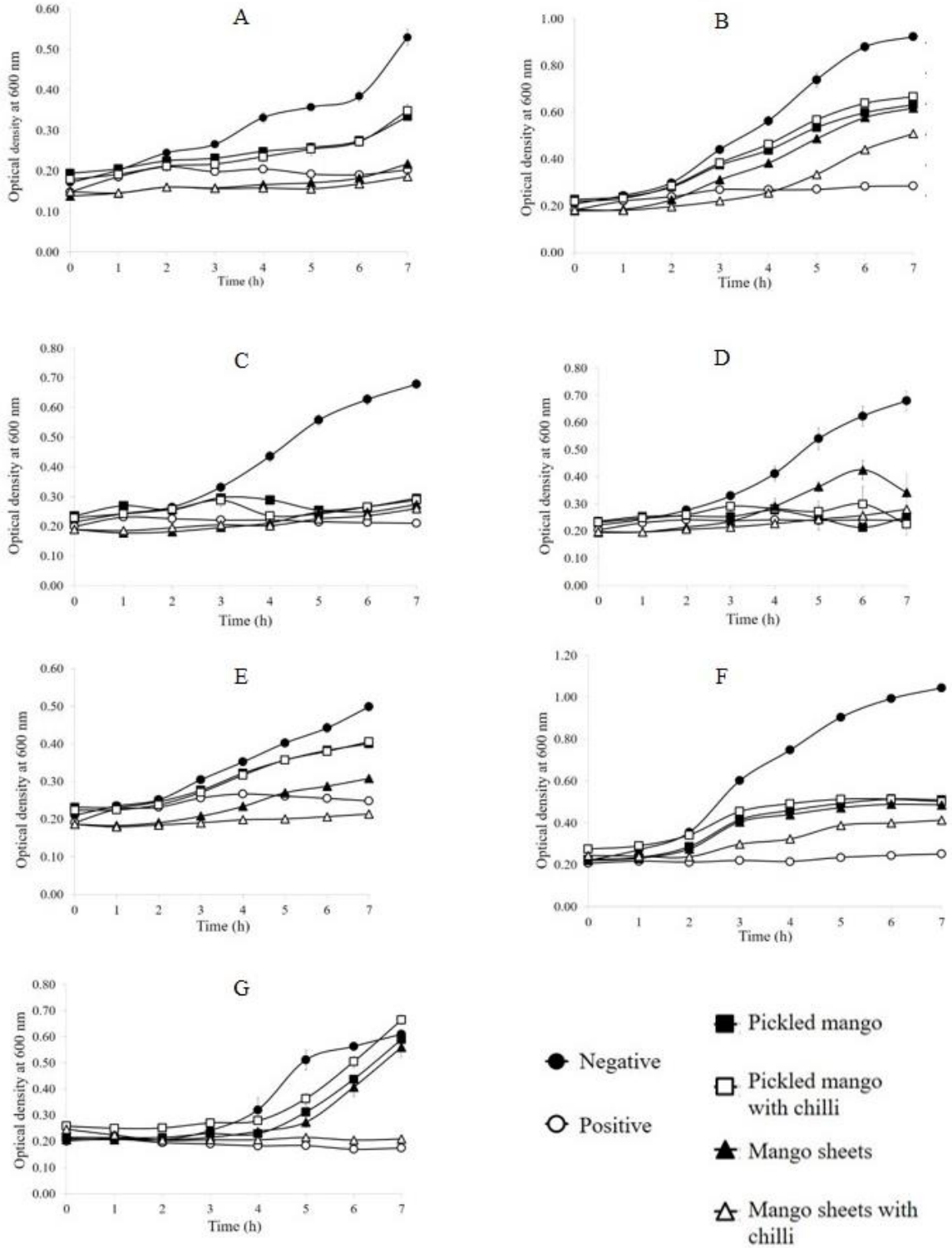


Figure 2. Antibacterial activity of protein hydrolysates from 4 processed mango samples against bacteria. *a) Escherichia coli* DH5a, b) *Escherichia coli* 8739, c) *Staphylococcus aureus* 6538, d) *Staphylococcus aureus* 6538P, e) *Pseudomonas aeruginosa* 9027, f) *Bacillus subtilis* 6633, and g) *Klebsiella aerogenes* 13048.

In human venter, high concentration of hydrochloric acid and proteolytic enzyme called pepsin implement protein digestion. Pepsin works best in a strong acidic condition (pH in stomach is about 2 and pepsin working

pH range is 1-4). Additional digestion to particular amino acids occurs in small intestine and the duration of the stomach usually pass into the small intestine is within 2-6h after meal. After digestion with pepsin in the experiment, the protein hydrolysates from all processed mangoe samples were able to inhibit growth of *S. aureus* in 6 h. The toxin from this bacterium places in human small intestine and causes irritation and swelling. This causes the symptoms of abdomain pain, dehydration, diarrheal and fever. Pickled mango and Pickled mango with chilli inhibit growth of *S. aureus* and *B. subtilis*, while Mango sheets can inhibit *E. coli* DH5α. The mango sheets with chilli showed the highest antimicrobial activity, it inhibited all human pathogenic bacteria used (*E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis* and *K. aerogenes*) except *E. coli* 8739. Diarrheal illness, Urinary Tract Infection (UTI) and food poisoning, that usually occur in Thailand and south east Asia countries for long time, were caused by infection of these pathogens. Thus, the risk from infection of these pathogen will be decreased after eating these processed mango products.

Similar results of the report from Nigeria, mango leaf extract expressed the highest antibacterial activity on all the five test bacterial samples (*Shigella flexneri*, *Pseudomonas fluorescens*, *E. coli*, *S. aureus* and *B. spp*.) with inhibition zones spanning from 18 to 24 mm at a concentration of 25 and 12.5 mg/ml and showed no sensitivity at concentration 6.25, 3.125 and 1.5625 mg/ml. *P. aeruginosa* was the least susceptible pathogen. *E. coli* was the least susceptible organism with the exclusion of *S. aureus* with no susceptibility but resistance [4]. Years after that, agar disc diffusion and broth dilution assay of Thai mango (Keaw Morakot, Nam Doc Mai and Mahajanaka) against human pathogens was reported [8]. Leaf extracts from all tested *M. indica* varieties could suppress the growth of *S. epidermidis*, *S. aureus*, Methicillin-Resistant *S. aureus* (MRSA), *Propionibacterium acnes* and *P. aeruginosa*. Beside mango leaves, peel aqueous extracts showed positive results against microorganisms. Both hot and cold aqueous phase of mango showed high antimicrobial effect against *S. aureus*, *E. coli*, *P. aerogenes*, *B. cereus* and *C. albicans* evaluated by agar well diffusion method [9].

## IV. Conclusion

Processed mango especially mango sheets were able to inhibit growth of *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis* and *K. aerogenes* the gastrointestinal infected pathogens. While pickled mango showed positive results only with *S. aureus* and *B. subtilis*. As a result, it could be concluded that the protein hydrolysates from processed mango possess remarkable antibacterial activity against some human pathogens, then these products might be used as effective medicinal food.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Thitiporn and Sittiruk conducted the research; Yodying analyzed the data; Thitiporn wrote the paper; all authors had approved the final version.

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