ผลของน้ำมันหอมระเหยในกำรยืดอำยุกำรเก็บรักษำกุ้งแช่เย็นเก็บรักษำภำยใต้สภำวะ

Effects of Holy Basil Oil on Shelf Life of Chilled Shrimp under Modified Atmosphere Packaging

น้ำมันหอมระเหยในกำรยืดอำยุกำรเก็บรักษำกุ้งแช่เย็นเก็บรushmanภำยใต้สภำวะ

ABSTRACT

This study aimed to investigate the inhibitory effect of essential oils extracted from holy basil (Ocimum sanctum, L.) on the growth of bacteria (Escherichia coli, Staphylococcus aureus, Salmonella Typhimurium, Vibrio parahaemolyticus and Shewanella putrefaciens) and to establish the possibility of its application with modified atmosphere during shrimp refrigerated storage (8 ± 2°C). Firstly, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using broth dilution technique. The results showed that MIC and MBC ranging from 5 to 10 mg/mL and 20 to 25 mg/mL, respectively. In the second part, two portions of shrimps: untreated samples and treated samples (shrimps soaked in holy basil oil) were packaged under normal (air) and modified atmospheric (60%CO₂ + 40%N₂) conditions. The results showed that essential oil in cooperation with modified atmospheric technique was the most effective to maintain shrimp quality during chill storage.

Key words: holy basil oil, modified atmosphere, shrimp
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INTRODUCTION

The increased demand for shrimp in the world market has encouraged many researchers to enter into the practice of shrimp preservation. In Thailand, White shrimps (*Penaeus vannamei*) are exceptionally popular because of its fast growth and high production yield. Since consumers expect that the foods they purchase and consume will be safe and of high quality, microbial contamination plays a major role as critical quality index. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Typhimurium*, and *Vibrio parahaemolyticus* have been reported as common spoilage and pathogenic bacteria found in shrimp products. Different preservation and storage methods are applied to maintain the shrimp quality (Mielink et al., 1999; Chaijan, 2004; Wan Norhana et al., 2010). Among such techniques, essential oil which can be safely used to inhibit the growth of bacteria (Burt, 2004). Holy basil (*Ocimum sanctum*) has been known to have antibacterial property. Singh et al. (2005) found that its essential oil showed good antibacterial activity against *S. aureus*, *Bacillus pumilus* and *Pseudomonas aeruginosa*. According to Joshi et al. (2009), it could inhibit the growth of *S. Typhi*. However, the practical application of essential oils from holy basil in foods is limited due to the strong flavor which imparts to foods and also to its interaction with some food ingredients (Burt, 2004). For these reasons the preservative effect of essential oils may be achieved by using at low concentration in combination with other preservation technologies such as low temperature (Skandamis and Nychas, 2001). Modified atmospheric packaging is considered as an effective method. It is well known as a method for extending the shelf-life of a variety of foods, including seafood (Soccol and Oetterer, 2003). Therefore, the objectives of the present study were to investigate the antimicrobial activity of holy basil oil on some pathogenic and spoilage microorganisms and the effect of the essential oil added individually or in combination with MAP on shrimp qualities and shelf life.

MATERIALS AND METHODS

1 Material

1.1 Essential oil

Holy basil oil (*Ocimum sanctum, Linn.*) (extracted by steam distillation) was purchased from Thai-China Flavours and Fragrances Industry Co., Ltd., Thailand.

1.2 Microorganisms

The tested organisms used in this study were as follows: *E. coli*, *S. aureus*, *S. Typhimurium* (Department of Microbiology, Faculty of Science, KMUTT, Thailand), *V. parahaemolyticus* TISTR 1596 (Thailand Institute of Scientific and Technological Research, Thailand) and *S. putrefaciens* (Department of Medicine Science, Thailand).
1.3 Shrimps

Fresh white shrimps (*Penaeus vannamei*) were purchased from a local fish market in Samutsakorn province. Raw shrimps (100 - 110 shrimps/kg) were packed on ice and transported to the laboratory within 30 minutes. After deheading, peeling, deveining and washing with tap water, shrimps were ready for an experiment.

2 Determinations of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum inhibitory concentration (MIC) of holy basil oil was tested by broth dilution method (Hussain *et al.*, 2008). Holy basil oil was firstly dissolved in nutrient broth (containing 1% of Tween 80) and serially diluted. A suspension of each tested microorganism ($10^6$ CFU/mL approximately) was transferred to each tube to achieve the final essential oil concentrations of 30, 25, 20, 15, 10, 5, 2.5, 1.25 or 0.62 mg/mL. Tubes containing only bacterial suspension were used as a control. All samples were incubated at $35 \pm 2^\circ C$ for 24 h. Inhibition of bacterial growth in the tubes containing tested oil was determined by comparison with growth in the blank control tubes. MIC values were determined as the lowest concentration of oil inhibiting visible growth of each microorganism. The assay to assess minimum bactericidal concentration (MBC) was performed by subculture of the tubes showing no apparent of growth in a sterile nutrient agar. The lowest concentration of the essential oil at which inoculated microorganisms were 99.9% killed (no visible growth on agar) was taken as MBC values.

3 Effect of holy basil oil in combination with modified atmosphere packaging on qualities of chilled shrimp

Raw peeled shrimps were divided into 2 portions and treated differently: untreated samples and samples soaked in holy basil oil in the ratio of 1:2 (shrimp:essential oil (w/v)) for 15 min (treated samples). Each portion was separated into 2 batches and packaged differently: under atmospheric condition in polyethylene (PE) pouch (54 µm in thickness; oxygen and carbon dioxide permeability of 3804.95 and 8310 cm$^3$/m$^2$.24hr at 0% relative humidity (RH), 23°C, respectively; water vapor permeability of 6.38 g/m2.24hr at 90% RH, 38°C) and under modified atmospheric condition of 60%CO$_2$ + 40%N$_2$ in nylon/ polyethylene (nylon/PE) pouch, (106 µm in thickness; oxygen and carbon dioxide permeability of 46.20 and 140 cm$^3$/m$^2$.24hr at 0% relative humidity (RH), 23°C, respectively; water vapor permeability of 3.89 g/m2.24hr at 90% RH, 38°C). All samples were kept at $8 \pm 2^\circ C$ and randomly taken on day 0, 4, 7, 11, 14, 18 and 21 for analysis of quality and storage life.

4 Analytical methods

4.1 Microbiological analysis

Shrimp sample (25 g) was removed aseptically and transferred to 225 mL of sterile 0.1% peptone water solution for total viable count (TVC). The sample was homogenized for 60 seconds. A 10-fold dilution was prepared using sterile peptone water. For microbial enumeration, 0.1 mL of serial dilution of
shrimp meat homogenates were spreaded on the surface of plate count agar (Oxoid, England). The samples were incubated at 35 ± 2 °C for 24 h.

4.2 Chemical analysis

Determination of trimethylamine (TMA) and total volatile base nitrogen (TVB-N) content were determined using Conway micro-diffusion method (Hasegawa, 1987).

pH value was recorded using pH meter (Metrohm, Switzerland). Samples (10 g) were thoroughly homogenized with 100 mL of distilled water and the homogenate was used for pH determination.

5 Statistical evaluations

Analysis of variance of the data was performed using the ANOVA procedure by Duncan's multiple range test. Significant differences ($P<0.05$) between mean values of triplicate samples were determined.

RESULTS AND DISCUSSION

1 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Experimental results from Table 1 indicated that each of the strains tested was effectively sensitive in low concentration of holy basil oil. MIC and MBC values were different for each strain. S. Typhimurium, S. aureus and V. parahaemolyticus showed the lowest MIC values (5 mg/mL) while it was 10 mg/mL against S. putrefaciens and E. coli. MBC values of holy basil oil were the highest against S. Typhimurium, S. putrefaciens and E. coli (25 mg/mL) while the lower MBC values were obtained against V. parahaemolyticus and S. aureus (20 mg/mL of MBC).

Table 1. Minimum inhibitory concentrations (MIC) and Minimum bactericidal concentrations (MBC) of the holy basil oil against some bacteria

<table>
<thead>
<tr>
<th>Tested bacteria</th>
<th>Holy basil oil (mg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>MIC*</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>10</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5</td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td>5</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>5</td>
</tr>
<tr>
<td>Shewanella putrefaciens</td>
<td>10</td>
</tr>
</tbody>
</table>

The antimicrobial activity of holy basil oil is related to eugenol, which is the main biologically active component in holy basil oil (Kumar et al., 2010). According to Burt (2004), the oil binds to the cell surface and then penetrate to phospholipid bilayer of the cytoplasmic membrane and membrane bound enzymes thus inhibiting metabolism system. Simultaneously, damages as pores or deformity might occur in cell wall due to the loss of structural integrity and the ability of the membrane to act as a permeability
barrier. The results also demonstrated that gram-negative bacteria were somewhat less inhibited than gram-positive bacteria. This is related to the outer membrane of gram-negative bacteria which constitutes with lipopolysaccharide. This constituent impedes the penetration of essential oil thus preventing bacterial cell wall and cytoplasmic membrane from damage (Bezić et al., 2003).

2 Effect of holy basil oil in combination with modified atmosphere packaging on qualities of chilled shrimp

2.1 Microbiological analysis

Figure 1 illustrated the growth curve of total viable counts (TVC) in all samples. The initial count was $3.44 \pm 0.64$ log CFU/g. According to the results, the number of microorganisms of untreated shrimps packaged under atmospheric condition continuously increased as storage time increased. Extension of the lag phase and decrease in growth rate during the logarithmic phase were observed for shrimps treated with holy basil oil packaged under both atmospheric and MAP conditions. Total counts of treated shrimps packaged under atmospheric condition significantly increased their growth rate while untreated and treated shrimps packaged under MAP condition showed gradual growth after day 4. In agreement of its slightly shorter lag and log phase, the extent of increase in microbial growth rate of treated shrimps packaged under atmospheric condition was considerably higher than that of shrimps packaged under MAP condition, both untreated and treated with essential oil. This suggested that MAP by bacteriostatic action of $\text{CO}_2$ was more effective than antibacterial activity of holy basil oil. As described by Smith et al. (1990), 20-60% $\text{CO}_2$ was required for effectiveness against microorganisms. The $\text{CO}_2$ penetrated microbial membrane thus lowering intracellular pH and resulting in metabolism failure (Venugopal, 2006).

![Figure 1](image)

**Figure 1.** Changes in total viable counts of shrimps stored at $8 \pm 2^\circ\text{C}$; •: untreated shrimps (control), X: shrimps treated with holy basil oil, ▲: untreated shrimps packaged under MAP ($60%\text{CO}_2 + 40%\text{N}_2$) and ●: shrimps treated with holy basil oil and packaged under MAP ($60%\text{CO}_2 + 40%\text{N}_2$)

For holy basil oil, its antibacterial activity was supported by the results obtained by MIC and MBC analysis. However, the oil concentration used in food is limited by its strong flavor. In this study, shrimps were treated with oil only at MBC concentration. This was a reason why microbial counts of treated
shrimps were higher than those of untreated shrimps packed under MAP condition. The result in this study also supported that the association of essential oil and MAP condition slightly improve inhibitory action in chilled shrimp. As defined by the ICMSF (1986), fishery product is considered good quality until the TVC exceeds a value of 7 log CFU/g. TVC of untreated and treated shrimps packaged under atmospheric condition remained below the limit and reached 5.26 ± 1.15 log CFU/g on day 4 and 6.29 ± 0.52 log CFU/g on day 7, respectively, whereas untreated and treated shrimps packaged under MAP reached 6.28 ± 0.23 log CFU/g on day 14 and 6.86 ± 0.12 log CFU/g on day 18, respectively.

2.2 Chemical analysis

Determination of TVB-N and TMA provided some valuable information about their quality changes. TVB-N and TMA values of 5.61 ± 1.21 mg/100 g and 2.28 ± 0.06 mg TMA-N/100g, respectively were found at the beginning of storage (Figure 2a-2b). It was observed that the amount of TVB-N was higher than TMA throughout storage period. This is because TVB-N represents the sum of basic nitrogenous compounds under the analysis conditions including TMA, dimethylamine (DMA) and ammonia (Huss, 1995). TVB-N and TMA of untreated and treated shrimps packed under atmospheric condition significantly increased throughout storage period (P<0.05). However the increase of both parameters in treated shrimps was slightly slower than those of the untreated. In Fishery products, TVB-N and TMA contents are indicators which reflect bacterial activity and spoilage. According to Thailand Industrial Standards (TIS) (1986), the TVB-N and TMA values of fishery product should be less than 30 mg N/100 g and 10 - 15 mg TMA-N/100 g, respectively. On the basis of TVB-N limit, the untreated and treated shrimps were rejected on the days 14 and 18 whereas the other spoilage indicator, TMA, exceeded the upper acceptability limit on days 11 and 14, respectively. MAP delayed the onset of both TVB-N and TMA in shrimps. The slightly lower TVB-N and TMA values were observed after 4 day storage before gradually increase afterwards. This was due to bacteriostatic effect of CO₂ as previously described. The essential oil in combination with MAP provided the superior results since TVB-N and TMA levels of shrimps remained below 30 mg/100 g and 15 mg TMA-N/100g until day 18 of storage. This suggests that essential oil and MAP provide synergistic, inhibitory effects to each other.

The pH changes in shrimps were parallel to the development of TVB-N and TMA (Figure 2c). The initial pH of shrimps was 6.67 ± 0.16 for all samples. The increases of pH values were rapid in untreated shrimps packaged under atmospheric condition. This was resulted from deamination of nitrogenous compounds by microbial activity. Slightly lower rate of pH increment was observed for shrimps treated with holy basil oil and packaged under atmospheric condition, untreated and treated shrimps packaged under MAP condition, respectively. On day 4, the untreated and treated shrimps packaged under MAP condition had lower pH values from day 0. This was partly due to dissolution of CO₂ in the aqueous phase of the muscle tissue, resulting in the formation of carbonic acid (Masniyom, 2011).
CONCLUSIONS

Holy basil oil provided the antimicrobial activity against all tested microorganisms with MIC and MBC ranging from 5 to 10 mg/mL and 20 to 25 mg/mL, respectively. Experimental results indicated that MAP provided higher efficiency for antimicrobial activity as compared with essential oil. Based on microbiological standards, shrimps packaged under MAP condition had shelf life of 14 days which was longer than that of shrimps treated with holy basil oil packaged under atmospheric condition (7 days). A combination of those two approaches was successful in quality preservation by extending shrimp shelf life by 18 days as compared to the untreated samples packaged under atmospheric condition (4 days).

REFERENCES


