Combined effect of low acid paste and modified atmospheric condition on quality changes of white shrimp, *Litopenaeus vannamei* during chilled storage

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**Abstract**

Antibacterial activity, total phenolic compound content, beta-carotene content and antioxidant activity of low acid paste, Tom-Yum paste was evaluated before subjected to apply to white shrimp as marinade agent. It was found that Tom-Yum paste could inhibit *S. aureus*, *E. coli* 0157:H7, *L. monocytogenes* and *P. fluorescens* with clear zone’s sizes of 21.2-24.8, 14.2-18.3, 11.2-12.6 and 7.0-9.0 mm, respectively. The beta-carotene, total phenolic compound content and IC$_{50}$ value of Tom-Yum paste were 676.46±52.41 µg/g dried sample, 1.00±0.07 g catechin/100 g dried sample and 2.17±0.16 mg dried sample/ml extract solution, respectively. The shelf-life of marinated shrimp kept in normal air and modified atmospheric packagings were 15-18 and 18-21 days, respectively, while shelf-life of raw white shrimp was only 9-12 days. Using marinating and modified atmosphere condition could provide more antibacterial and antioxidant properties resulted in increased white shrimp shelf-life.

**Introduction**

In the present time, most important commercial species shrimp cultivated in Thailand is Pacific white shrimp (*Litopenaeus vannamei*) as nearly 400,000 tons in 2006 (Wyban, 2007). However, shrimp is a very perishable product, and postmortem changes occur rapidly. A serious problem in shrimp processing and storage is rapid microbial spoilage due to digestible nutrients such as free amino acids and other soluble non-protein nitrogenous. Psychrophilic bacteria count, H$_2$S-producing bacteria, and enterobacteriaceae count of Pacific white shrimp stored in ice for 10 days were 5-6, 4-5 and 4-5 log CFU/g, respectively (Nirmal and Benjakul, 2009). Therefore loss of marketable value is more concerned and interested to find out the proper way to manage.

There is growing evidence that Thai cuisines promote antioxidant activity and have health benefits because of several herbs and spices. Diallyl disulfide and diallyl trisulfide in garlic were identified as primary active antioxidant compounds (Dwivedi _et al._, 1996; O’Gara _et al._, 2000; Tsao and Yin, 2001) and antibacterial activity (Siripongvutikorn _et al._, 2005). Some active compounds derived from galangal rhizome, lemongrass and kaffir lime leaves were reported as antitumors in the digestive tract (Division of Health Statistics, 1989; Murakami _et al._, 1995). Several researchers have reported that galangal extract showed antioxidant activity in a model system (Barik _et al._, 1987; Cheah and Abu-Hasim, 2000) and in pork meat (Juntachote _et al._, 2005). Using of natural product as preservative agent is more interesting to substitute synthetic one due to health concern.

Several spices, for example galangal rhizome, lemongrass, kaffir lime leaves and chili are used in Thai soup particularly Tom-Yum soup. The soup will end up with acid fruits such as lime juice, tamarind pulp or dried garcinia slice to make a sour taste. With a busy lifestyle therefore, convenience food as marinated product, a ready to cook goods, is more increasing. As marketing survey, shrimp marinated with low acid paste, Tom-Yum mixes are not now commercially available particularly packed in modified atmosphere condition. This present research are, therefore, aimed to evaluate the antibacterial and antioxidant properties of low acid paste, Tom-Yum
paste and its application on white shrimp and to monitor the shelf life of the marinated shrimp packed under modified atmospheric condition during chilled storage.

Materials and Methods

Materials

Fresh spices for making low acid paste, Tom-Yum mixed, consisted of chili (*Capsicum frutescens*), lemongrass (*Cymbopogon citralus* DC Stapt), kaffir lime leaves (*Citrus hystrix* DC), galangal rhizome (*Alpinia galanga*), garlic (*Allium sativum* L) and shallot (*Allium ascalonicum*, L). These were bought from a fresh market in Hat-Yai city, Songkhla Province, Thailand. Fresh Pacific white shrimp (*Litopenaeus vannamei*), size 60-70 shrimp/kg was purchased from shrimp farm and kept in ice during transportation.

Preparation of low acid paste, Tom-Yum mixed

All fresh spices were sorted, trimmed, washed, drained for 2 min, sliced, weighed according to the recipe and blended until it became a fine paste. The paste was aseptically sampled, sealed in sterilized polyethylene bag and kept at 4±2°C. The individual spice listed above and the paste were evaluated for moisture content, pH, total viable count, total phenolic content, beta-carotene, antioxidant and antibacterial activities.

Preparation of marinated shrimp with low acid paste, Tom-Yum mixed

The fresh shrimp was removed from ice, washed with chilled tap water, deheaded and peeled. Peeled shrimp was analyzed for total viable count, coliforms, *Staphylococcus aureus* (BAM, 2001), total volatile base nitrogen (TVB-N) and thiobarbituric acid reactive substances (TBARS).

The paste was mixed with the shrimp in the ratio of 1:3 (paste: shrimp). Marinated shrimp samples of about 80 g were packaged in Nylon/LDPE bags before subjected to pack in 6 conditions. A1; control, B1; normal package, C1; vacuum package, D1; *O*₂: *CO*₂: *N*₂ as 5:50:45, E1; *O*₂: *CO*₂: *N*₂ as 5:70:25 and F1; *O*₂: *CO*₂: *N*₂ as 5:90:5 were set and stored at 4±2°C.

All samples were taken every three days for physical, chemical and microbiological analyses and in the case of sensorial evaluation; both cooked shrimp and soup were separately tested by 30 panelists.

Physical, chemical, microbiological and sensorial analyses

Beta-carotene content

Beta-carotene content of the spices and the paste was extracted and analyzed using HPLC technique as modified by Siripongvutikorn *et al.* (2005).

Total phenolic content

Total phenolic content of spices and the paste was determined by the Folin-Ciocalteau method (Singleton and Rossi, 1965) using catechin (Sigma Chemical Co., St. Louis, USA) as the standard. Results were expressed as catechin equivalents.

Determination of free radical scavenging ability

The DPPH (1,1-diphenyl-2-picrilhydrazyl) radical scavenging activity of the methanolic extracts was determined using the method proposed by Von Gadow, Joubert, and Hansmann (1997). Results were expressed as half inhibitory concentration (IC₅₀) at which DPPH radicals were scavenged by 50%.

**pH**

Sample was blended with sterilized distilled water at a ratio of 1:10 (sample: water) and allowed to stand for 2 min before measuring the pH with a pH meter (Mettler 350, Singapore).

Moisture content

The moisture content determination method of all samples was followed by Gravimetric method (A.O.A.C., 2000).

Total volatile base nitrogen (TVB-N)

Two-gram samples of shrimp, and marinated shrimp were blended with 4% trichloroacetic acid, filtered with Whatman paper No.1 before being subjected to TVB-N analysis using the Conway method (Hasagawa, 1987).

Thiobarbituric acid reactive substances (TBARS)

Two-gram of chopped shrimp or marinated shrimp was homogenized with 10 ml of mixture of 0.375% TBA, 15% trichloroacetic acid and 0.25 N HCl (termed TBA reagent) for 2 min, then heated in boiling water for 10 min. The sample was cooled down, centrifuged at 8,000 rpm for 20 min before brought to measure for absorbance at 532 nm. Results were calculated and expressed as mg malondialdehyde/kg sample (Modified method of Buege and Aust, 1978).

Drip loss (%)

The marinated shrimp was washed with the tap water to get rid of the paste before subjected to absorb the water by filter paper (Whatman paper No.1). Then the shrimp was weighed and calculated the drip loss (%).
Cooking loss (%)

The marinated shrimp packed in Nylon/LLDPE was heated in boiling water until shrimp’s temperature was 75 °C for 20 seconds. Then the cooked shrimp packed in bag was cooled in ice water. The cooled shrimp was taken from the bag and rinsed with the tap water to get rid of the paste before subjected to remove the excess water by filter paper (Whatman paper No.1). The cooked shrimp was weighed and calculated the cooking loss (%).

The bacteriological analysis

The psychrophilic, mesophilic bacteria, yeast and mold, coliforms, lactic acid bacteria, Staphylococcus aureus, Clostridium perfrigens and Salmonella spp. were determined as procedure of BAM (BAM, 2001).

Antibacterial activity determination of spices and the paste

The blended sample was placed in a sterile cheese cloth and squeezed to get the crude juice before subject to filter with 0.45 micron acetate filter paper with aseptic technique. The filtrated juice was subjected to evaluate for antibacterial activity determination.

Two Gram-positive bacteria: Staphylococcus aureus and Listeria monocytogenes and two Gram-negative bacteria: Escherichia coli 0157:H7 and Pseudomonas fluorescens obtaining from Department of Medical Science, were used as test organisms. Two-loopful of each test organism was aseptically transferred into 5-ml BHI (Merck) and incubated at 35°C for 18 h. These overnight cultures were adjusted to final concentrations of 10<sup>6</sup> and 10<sup>7</sup> CFU/ml in fresh 9 ml BHI broth. One milliliter of this final concentration was taken into 7 ml BHI semi soft agar and then poured on 15 ml already gel Mueller Hinton agar (Merck) and allowed to solidify. Wells were made using 6-mm cork borer and 100 µl of each aqueous extract were filled into the wells. The plates were then incubated at 35°C for 24 h, while the plates inoculated with P. fluorescens were incubated in at room temperature (30°C). The plates were observed after 24 h for zones (mm) of inhibitions and these were measured using vernier caliper (Parente and Hill, 1992). The minimum inhibition concentration (MIC) was determined by well diffusion (Parent and Hill, 1992). The minimum bactericidal concentration (MBC) was determined by broth diffusion (Bakri and Douglas, 2005). The minimum bactericidal concentration was recorded in the plate with no growth or not over 10 colony forming unit.

Sensorial evaluation

Marinated samples were boiled in water at a ratio of 1:2 (sample: water). The Tom-Yum soup and shrimp were separately served to taste at 45-50°C to 30 experienced consumers who regularly eat Tom-Yum soup. The sensorial attributes of soup as color, flavor, taste, appearance and overall liking while attribute of cooked shrimp as texture, flavor and overall liking were evaluated using the 9-point hedonic scale. The meaning of 9-point hedonic scale was judged from “1 = extremely dislike to 9= extremely like”. Before the samples were brought to cooked and served to panelists, total viable count of all samples were evaluate and not exceed 10<sup>6</sup> CFU/g for microbiological safety.

Statistical analyses

Data were subjected to Analysis of Variance (ANOVA) and mean comparison was performed using the Duncan’s New Multiple Range Test. Statistical analyses were carried out using the SPSS statistical software version 11.

Results and Discussion

The initial quality of low acid paste, Tom-Yum mixed

Moisture content (%), pH and total viable count (reported as CFU/g) of spices used in low acid paste, Tom-Yum mixed and the paste were exhibited in Table 1. It pointed out that some spices used in paste were perishable sample based on low acid food (pH > 4.6), high moisture content and high TVC particularly galangal rhizome and lemon grass.

Beta-carotene content was highest in red chili followed by Tom-Yum paste and kaffir lime leaves (Table 2) similar to the result of Siripongvutikorn et al. (2005). Furthermore, galangal rhizome was highest in total phenolic content and IC<sub>50</sub> compared other samples. Similar to finding of Siripongvutikorn et al. (2008) who reported that galangal rhizome was highest in total phenolic content and DPPH activity, IC<sub>50</sub> in garcinia Tom-Yum paste. Interesting, total phenolic content may or may not correlate to antioxidant activity in each sample differently. Moreover, it pointed out that beta-carotene content did not responsible for antioxidant activity determined by DPPH assay.

Among the spices used in Tom-Yum mixed, only garlic bulb provided inhibition zone as shown in Table 3. While, Siripongvutikorn et al. (2005) reported that kaffir lime leaves possessed some antibacterial property by using disk diffusion method. It implied that organism variety, spices location, and method used may cause different results. After spices
were made to be the paste, however, antibacterial activity of Tom-Yum paste occurred. It confirmed that allicin, active compound derived from garlic bulb had enough antibacterial activity against all test organisms. In addition, MIC and MBC values of garlic were $15.15^{+0.52}$ to $24.87^{+1.40}$ and $70.32^{+0.52}$ to $140.65^{+0.82}$ µg/ml, respectively (Table 4). Based on antibacterial activity, \textit{S. aureus} was most sensitive organism to garlic juice as shown in Table 3 and 4 which similar to previous result (Siripongvutikorn et al., 2005).

Marinated shrimp with low acid paste, Tom-Yum mixed

Shelf-life of shrimp without marinated Tom-Yum paste, control was 12 days based on psychrophilic and mesophilic bacteria as standard limit ($10^{6-7}$ CFU/g (ICMSF, 1986) while shrimp marinated the paste and packed in normal air was 18 days (Table 5). Vacuum condition, mixed gas of $O_2$: $CO_2$: $N_2$ as 5:50:45 (D1), 5:70:25 (E1) could not retard microbial count when compared with normal package condition. However, increasing $CO_2$ to 90 (%) could reduce bacterial count then its shelf-life extended to 21 days. It could be pointed out that low acid paste, Tom-Yum mixed, played more antibacterial function compared with packing techniques when sample stored in chilled condition.

The pH value of shrimp with and without marinated Tom-Yum paste and packed in any conditions was in the range 6.4-6.7 throughout the storage (data did not show). This may due to buffering capacity from high free amino acid such as histidine and phosphate compounds (Suyama et al., 1986; Abe, 2000).

Drip loss increased as storage time increased as shown in Figure 1. Additionally, it was found that 90$\%$ $CO_2$ (F1) and vacuum (C1) samples caused more drip loss. This may be due to protein deterioration from dissolving of $CO_2$ to carbonic acid and expressible force from vacuum technique, respectively.
Table 4. Antibacterial activity measured as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of garlic juice

<table>
<thead>
<tr>
<th>Test organism</th>
<th>MIC (μg/ml)</th>
<th>MBC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>15.35±0.52</td>
<td>&gt;32.50±0.52</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>0.04±0.91</td>
<td>93.76±0.91</td>
</tr>
<tr>
<td>E. coli 0157:H7</td>
<td>0.50±0.30</td>
<td>140.02±0.32</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>0.50±1.40</td>
<td>93.76±0.61</td>
</tr>
</tbody>
</table>

Means ± SD from triplicate data of 2 different lots. **Means within a column with different letters are significantly different (p<0.05)

Table 5. Microbiological quality changes of marinated shrimp packed in various modified atmospheric conditions during chilled storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage (day)</th>
<th>Microbiological index (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>21</td>
<td>Yeast, Mold</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Psychrophile</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Mesophile</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>LAB</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td>B1</td>
<td>21</td>
<td>Yeast, Mold</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Psychrophile</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Mesophile</td>
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<td>12</td>
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<tr>
<td></td>
<td>9</td>
<td>S. aureus</td>
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<tr>
<td></td>
<td>6</td>
<td>90%</td>
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<tr>
<td></td>
<td>3</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>20%</td>
</tr>
</tbody>
</table>


Northcutt et al. (1994) addressed that changing of drip loss and cooking loss values of pork meat caused by pH value related to water holding capacity. However, Allen et al. (1998) reported that drip loss and cooking loss of chicken meat did not have any correlation with pH. Cooking loss in this present work tended to increase as increased storage time (Figure 2). Though, shrimp without marinating (A1) seemed to have lower cooking loss in the first day, there was no significantly difference in day after. It pointed out that marinating and packing condition may help to protect protein degradation caused by endogenous enzyme (autolysis) and exogenous enzyme (microbial enzyme).

As well know that TVB-N value is used for fish freshness index, however, many studies (Civera et al., 1999; Paarup et al., 2002) also reported that acceptable limits were species and storage conditions dependent. A chemical reference criterion in term of TVB-N for fishery products export of Thailand is not higher than 30 mg/100g sample (Department of Fisheries, 2004). From Figure 3, there was no significant difference in TVB-N values in all samples in first 9 days, however, A1 reached standard limit (<30 mg/100g sample) at 12 days of storage accordant with microbiological quality (Table 5). While, TVB-N of marinated shrimp packed in any modified atmospheric condition was under standard throughout storage. It confirmed that Tom-Yum pastes played an important role to control shrimp quality as antibacterial and antioxidant functions.

Juntachote et al. (2007) addressed that addition of dried galangal powder into cooked ground pork could inhibit the formation of TBARS value, peroxide value (POV), conjugated diene and hexanal content during storage at 5°C for 14 days. Surprising, marinated shrimp with Tom-Yum mixed pronounced more TBARS (Figure 4) when compared with raw shrimp (A1). Kilinc and Cakli (2004) reported that an increase of TBARS value in sardine marinated with citric solution was observed during storage. While, Cadun et al., (2005) reported that an increase of TBARS in marinated pink shrimp probably due to prooxidant, such as heme iron and non-heme iron from membrane leakage (Cheng et al., 2004). Standard limit of rancidity in oily food is 5-8 mg/kg malonaldehyde (Shamberger et al., 1977; Bergamo et al., 1998). Therefore, if based on this value it point out that marinated shrimp product could not fit for consumer consumption even first day. However, some researchers mentioned that chlorophyll in plant sample would be prooxidant in food system (Juntachote et al., 2005; Michalak, 2006). While, Odour-Odote and Obiero (2009) addressed that TBARS value is not good indicator for determination of smoked fish quality.

Based on consumer acceptability, it pointed out that shelf-life of marinated shrimp packed in any packaging conditions was longer when compared with microbiological aspect (Figure 5).
sensorial score did not change as storage time increased probably due to masking effect of the paste, Tom-Yum mixed, and antimicrobial activity particularly in marinated shrimp and packed under modified atmospheric conditions as D1, E1 and F1.

Conclusion

Garlic bulb and galangal rhizome played a major role for antibacterial and antioxidant activities, respectively in low acid paste, Tom-Yum mixed. Most sensitive organism to antibacterial agent was \textit{S. aureus}. Application of the paste to shrimp could retard growth of TVC and control TVB-N value. However, TBARS value of marinated shrimp was higher than that value of control. Shelf-life of shrimp with marinated the paste, Tom-Yum mixed, was extended from about 12 days to 18-21 days based on microbiological quality and TVB-N value. To sum up, shrimp with marinated the paste; Tom-Yum mixed, did not only possess more shelf-life but also could be used as convenience food that fit for new life style.

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