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Original Article

Development of green curry paste marinade for white shrimp (*Litopenaeus vannamei*)

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Abstract

Green-curry paste with additional amount of garlic was developed and its consumer acceptability was investigated. Quality changes in the marinated white shrimp stored at 4±2°C for 15 d was monitored. Total viable count in the marinated shrimp was 10^6 cfu/g while that of un-marinated shrimp (control) was 10^7 cfu/g at the end of the storage. Total volatile basic nitrogen (TVB-N) of the marinated shrimp and the control increased from 7.39 and 7.69 mgN/100 g sample to 48.95 and 125.15 mg/100 g sample, respectively, at the end of the storage. Trimethylamine nitrogen (TMA-N) of the marinated shrimp increased from 1.01 mgN/100 g sample to 12.88 mgN/100 g sample, while that of the control increased from 3.08 mgN/100 g sample to 35.76 mgN/100 g sample on day 15. Consumer acceptability of the marinated shrimp decreased as storage time increased but the score was still higher than borderline (>5) at the end of the storage.

Keywords: green curry paste, white shrimp, marinating, shelf life

1. Introduction

Green curry or sweet green curry made from fresh spices and flavored with cumin, is the Thai second most-ordered dish among consumers, both in Thailand and worldwide, because of its taste, flavor and appearance (Office of the National Culture Commission, 1999; http://www.thaiwaysmagazine.com/thailand/thailand_dining.html). The curry paste usually consists of green chili, galangal rhizome, shallot, cumin, lemon grass, kaffir lime, garlic and black pepper. The spices/ingredients used in the curry paste may differ from home to home or region to region. Many ingredients used in the curry paste have been found to contain antimicrobial, antioxidant, and have medicinal value. Garlic, one of the ingredients, is reported to possess allicin, a highly reactive volatile compound, which is unstable in the presence of heat (Ankri and Mirelman, 1999). It has antimicrobial and antioxidant compounds with health benefits (Nishimura *et al*., 2000). Galangal rhizome, lemon grass, and kaffir fruit peels have been reported to be effective in inhibiting tumors in the digestive tracts (Division of Health Statistics, 1989; Murakami *et al*., 1994; Murakami *et al*., 1995). Therefore, green curry might be considered a functional food.

In modern lifestyle, there are smaller families with less in-home cooking activity. Ready-to-cook convenient foods such as marinated products are becoming quite popular among these groups of consumers. Marinades may help prolong shelf life and maintain the texture of some fresh meat such as pork or chicken. Marinating shrimp with green-curry paste may serve the same purpose, particularly when the curry paste has higher garlic content. However, higher garlic in the recipe may alter the product acceptability. The objectives of this research, therefore, were to develop a green curry paste having maximum acceptable garlic content as a marinade for fresh white shrimp, and to monitor the shelf life of the marinated shrimp during chilled storage.

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2. Materials and Methods

2.1 Materials and reagents

Fresh spices for green curry paste consisted of big finger chili (*Capsicum annuum),* green stage, lemon grass (*Cymbopogon citratus*), galangal (*Alpinia galangal* L.), phak wan ban (*Sauropus androgyus* L.), shallot (*Allium ascalonicum* L.), garlic (*Allium sativum*), root of coriander *(Corriandrum sativum)*, kaffir fruit peel (*Citrus hystrix)*, and hot chili (*Capsicum frutescens*), green stage. These were bought from a fresh market in Hat Yai city, Songkhla Province, Thailand. Black pepper, coriander seeds, and cumin powder were bought as prepared spices already packaged from a grocery store. Fresh white shrimp (*Litopenaeus vannamei)*, size 80-90 shrimp/kg was purchased from fresh market and kept in ice during transportation.

All chemicals, reagents, and media were of analytical grade from Sigma Chemical Co. (St Louis, MQ, USA) and Merck (Darmstadt, Germany).

2.2 Methods

1) Green curry paste preparation

All fresh spices were sorted, trimmed, washed, drained for 2 min, sliced, weighed according to the recipe and blended with black pepper, coriander seeds and cumin powder until it became a fine paste. The paste was aseptically sampled, sealed in sterilized polyethylene bag and kept at 4±2°C. The green curry paste formula has been applied for patent, therefore, it cannot be revealed here. The paste was subjected to the analysis for pH, a_w, total viable count and coliform counts. Initial sensory acceptability of the paste was evaluated by five experienced panelists.

2) Green curry paste with additional amounts of garlic

The curry paste from section 2.2.1 was divided into five groups and fresh garlic was added at 0 (F1), 10 (F2), 15 (F3), 20 (F4), and 25% (F5), w/w, before being subjected to evaluation of the intensity of garlic and spice flavor and paste color by 12 panelists who had been previously briefed on the attributes to be evaluated. Descriptive Method was used for the evaluation with the scale from 0 to 10. The sensory acceptability of the curry was then evaluated by 30 experienced panelists, using 9-point hedonic scale. The curry paste containing the highest garlic content with no negative effect on consumer preference was selected for subsequent marinating experiments.

3) Shrimp preparation

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) as described in section 2.2.5, and total volatile basic nitrogen (TVB-N) and trimethylamine (TMA-N) by the Conway method (Speck, 1976) as described in section 2.2.7.

4) Marinating the shrimp with the curry paste

The selected curry paste described in section 2.2.2 was mixed with the shrimp in the ratio of 1:2 (curry paste: shrimp). Marinated shrimp samples of about 50 g each were packaged in polyethylene bags sealed and stored at 4±2°C for 15 d. Non-marinated shrimp was used as control. Samples were taken every three day for analysis as described in section 2.2.3, and in the case of marinated shrimp the samples were also subjected to sensory evaluation as described in section 2.2.8.

5) Bacteriological analysis

5.1) Total viable count

Shrimp sample of 25 g was added to 225 ml of sterilized 0.1% peptone water and macerated in a stomacher. Serial dilutions were made and 1 ml of each dilution were pour-plated using Plate Count Agar (PCA), allowed to be solid and incubated at 35°C for 24 h. Total viable count was expressed in cfu/g sample.

5.2) Coliform counts

Lauryl Tryptose Sulphate broth (LTS) was used to determine coliforms counts at the dilutions of 10^1 to 10^3 with Durham tubes to detect gas production. Subsequently, the contents of the positive tubes were inoculated into Brilliant Green Lactose Bile broth (BGLBB) and incubated at 35°C for 24 to 48 h to confirm coliforms presence. Contents of the positive tubes from BGLBB were then inoculated into EC broth (EC) and incubated at 44.5°C for 24 to 48 h to detect fecal coliforms. Contents of the positive tubes from EC broth were streaked on EMB agar (EMB) and an IMViC test was used to confirm *E. coli.*

6) Physical analysis

6.1) Color determination

Color of the curry paste was measured using a color meter (Hunter lab Universal Software). The color was expressed in L*, a*, and b* values, where L means degree of lightness of the samples and ranges from 0-100, a means redness to greenness (+ is red, - is green) and b means yellowness to blueness (+ is yellow, - is blue).

6.2) Water activity

Water activity of the curry paste was determined using the Novasina water activity meter (Thermoconstanter Novasina TH200, Switzerland).

6.3) pH

Samples was blended with sterilized distilled water at a ratio of 1:5 (sample: water) and allowed to dissolve for 2 min before measuring their pH measured with a pH meter (Mettler 350, Singapore).

7) Chemical analysis

Two-gram samples of shrimp were blended with 4% Trichloro acetic acid, filtered with Whatman paper No 1 before being subjected to TVB and TMA analyses using the Conway method (Hasegawa, 1987).

8) Sensory evaluation

Samples (curry paste and marinated shrimp with curry paste) were cooked in coconut milk at a ratio of 1:2 (sample: coconut milk). The curry soup made from the marinated shrimp was served at 45-50°C to 12 well-briefed panelists to evaluate the intensity of garlic flavor, spice flavor and color on the scale of 10, 10 being the most intense. For the soup from marinated shrimp, its acceptability was evaluated by 30 experienced consumers using the 9-point hedonic scale.

9) 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.

3. Results and Discussion

The basic green-curry paste formulated in the lab was given acceptability score of >6 by five experienced panelists. Some panelists indicated that they would prefer the soup to be hotter. The paste color was light green-yellow with L*, a* and b* values of 28±0.2, -7.9±0.1 and 32.4±0.3, respectively. The curry paste may be classified as low acid food (pH 5.4-5.6), having high water activity (a_w 0.99) and high bacterial load (10^6 cfu/g), making it a highly perishable product. Siripongvutikorn *et al.* (2005) reported that galangal, chili and lemon grass used as ingredients in Thai curries were heavily contaminated with bacteria. Therefore, these ingredients may be the major sources of microorganisms in the curry paste. However, no coliforms or *S. aureus* were found in the paste samples.

Increasing the garlic content in the curry paste to 20% (F4) did not affect the sensory acceptability of the product (Tables 1 and 2). In fact, F4 had the highest sensory score among all the samples, including the control (Table 2). High garlic content had another benefit that it reduced bacterial count in the curry paste from $10^{5\text{-}6}$ cfu/g to $10^{4\text{-}5}$ cfu/g.

The pH, TVB, TMA, and TVC of fresh white shrimp were 6.4-6.6, 5.4-5.6 mgN/ 100g sample, 1.1-1.3 mgN/100g sample and 3.6-4.2x10^6 cfu/g, respectively. Shrimp marinated with the selected curry paste (F4) had lower pH, TVB, TMA, and TVC than the un-marinated shrimp (control) when stored at 4±2°C (Figures 1, 2, 3 and Table 3). The decrease in pH of marinated shrimp during the first 6 d may be due to the pH of the paste, which was about 5.4-5.6.

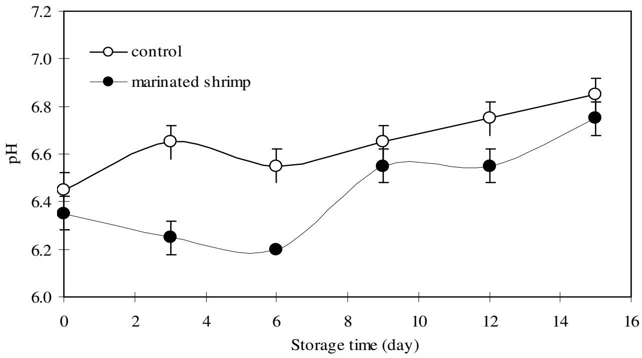

Figure 1. pH of un-marinated and marinated shrimp stored at 4±2°C

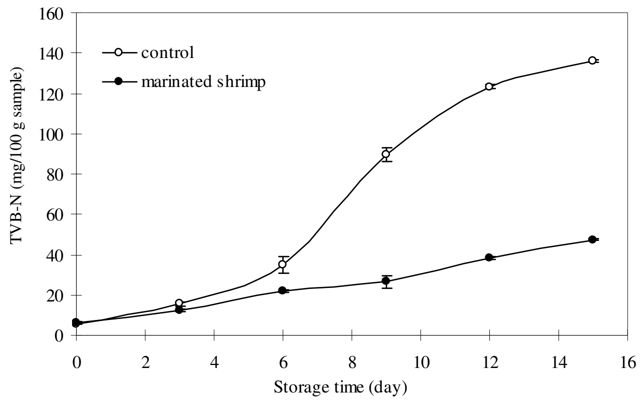

Figure 2. Changes in TVB-N (mgN/100g sample) of unmarinated and marinated shrimp stored at 4±2°C

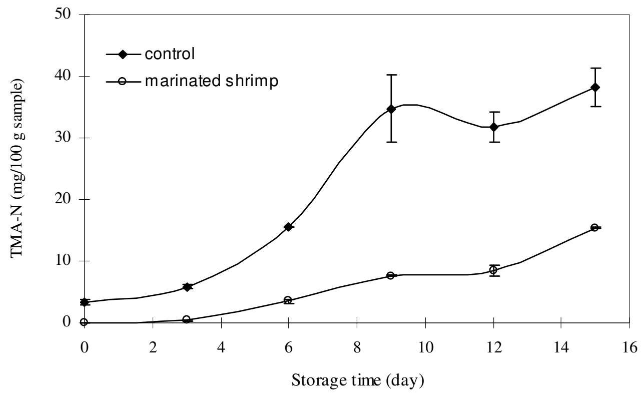

Figure 3. Changes in TMA-N (mgN/ 100g sample) of unmarinated and marinated shrimp stored at 4±2°C

Table 1. Color and flavor intensity of curry paste containing varying amounts of garlic made into soup and evaluated by 12 panelists

Attribute	Intensity[1]				
	F1	F2	F3	F4	F5
Color	4.5±1.9[a]	4.8±2.1 [a]	4.6±1.5 [a]	5.5±1.3 [a]	3.6±2.1 [a]
Spice flavor	6.1±1.3 [a]	5.6±1.7 [a]	5.1±1.9 [a]	5.2±0.8 [a]	4.8±1.2 [a]
Garlic flavor	4.1±2.1 [a]	3.8±2.1 [a]	4.9±1.8 [a]	4.8±1.3	4.2±1.6 [a]

[1] means ($\bar{x}$) ± SD were evaluated by 9-point hedonic scale
[a] means within a row with the same letter is not significantly different (p>0.5)
F1 = basic formula
F2 = basic formula plus 10% garlic
F3 = basic formula plus 15% garlic
F4 = basic formula plus 20% garlic
F5 = basic formula plus 25% garlic

Table 2. Sensory scores of the soup made with curry paste from marinated shrimp samples, containing varying amounts of garlic, as evaluated by 30 experienced panelists

Attribute	Sensory Score[1]				
	F1	F2	F3	F4	F5
Color	6.9±1.7 [a]	6.5±1.8 [ab]	6.6±1.1[ab]	7.3±1.2[a]	6.4±2.1 [ab]
Flavor	6.7±1.1 [a]	6.5±1.7 [a]	6.7±1.9 [a]	7.1±0.8 [a]	6.3±1.2 [ab]
Taste	6.3±1.9[a]	6.2±1.5[a]	6.5±1.9[a]	6.6±1.5[a]	5.9±2.1[a]
Appearance	7.2±1.2 [a]	6.5±1.31 [a]	6.4±1.8 [a]	6.8±1.1 [a]	6.2±1.6 [a]

[1] means ($\bar{x}$) ± SD were evaluated by 9-point hedonic scale
[a-b] means within a row with the different letters are significantly different (p<0.5)
F1 = basic formula
F2 = basic formula plus 10% garlic
F3 = basic formula plus 15% garlic
F4 = basic formula plus 20% garlic
F5 = basic formula plus 25% garlic

Table 3. Total viable count in marinated shrimp and un-marinated shrimp kept at 4±2°C for 15 days

Storage time (day)	un-marinated shrimp	marinated shrimp
0	3.85×10^6	5.23×10^5
3	7.14×10^6	6.21×10^5
6	3.78×10^7	3.14×10^6
9	6.47×10^7	7.14×10^6
12	$>10^8$	5.28×10^7
15	nd	9.28×10^7

nd : not determined

However, as the storage proceeded, pH of both samples slightly increased but did not reach 7 after 15 d. This might be caused by the buffering capacity of some compounds produced in the shrimp, e.g. histidine, amine, phosphate and nucleotide (Okuma and Abe, 1992)

In fish TVB-N lower than 25-30 mgN/100g sample is

Table 4. Sensory scores of the soup made from marinated shrimp kept at 4±2°C for 15 days

Attribute	Sensory Score[1]					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Color	7.74±0.75[a]	6.92±1.23[b]	7.36±0.99[ab]	7.16±1.00[b]	6.88±1.51[b]	6.68±0.96[b]
Flavor	7.58±1.13[a]	7.20±1.09[ab]	7.18±1.00[ab]	7.28±1.05[a]	6.74± 1.19[b]	6.96± 1.12[b]
Taste	7.08±1.19[a]	7.16±1.00[a]	6.98±1.19[a]	6.76±1.25[ab]	5.93±1.68[b]	5.96±1.47[b]
Appearance	7.28±1.09[a]	6.98±0.98[ab]	7.08±1.10[ab]	6.66±1.48[b]	6.58±1.68[b]	6.74± 1.10[b]

[1] means ($\bar{x}$) ± SD were evaluated by 9-point hedonic scale
[a-b] means within a row with the different letters are significantly different (p<0.5)

considered acceptable (Cadun *et al.*, 2005). If the same rule applied to shrimp it could be seen that shelf life of un-marinated and marinated shrimp was 6 and 12 days, respectively (Figure 2). However, it has been reported that some shrimp species were very high in proteinase enzyme, free amino acid and TVB-N (Cadun *et al.*, 2005), leading to rapid spoilage. Similarly Ruiz-Capillas *et al*. (2003) reported that initial TVB in Norway lobster was high, 27.73 mg/100 g and passed the established threshold of unfitness for human consumption (40 mg/100 g) at 14 days. Since TVB-N is derived from protein and non-protein nitrogen by the function of indigenous and exogenous enzymes, the more hydrolysis takes place the higher the TVB-N produced and the shorter the shelf life. It is possible that curry paste suppressed the hydrolysis of the shrimp, thus increased the shelf life of the marinated shrimp. Ozogul and Ozogul (2000) reported that fish kept in aerobic condition spoiled faster than those kept under anaerobic condition. Aerobic *Pseudomonas sp* and *Shewanella putrefaciens* are considered spoilage bacteria in chilled fish (Gram and Huss, 1996). Marinating the shrimp with curry paste might reduce the aerobic condition surrounding the shrimp by coating its surface. Moreover, allicin from garlic in the curry paste might also help inhibit both spoilage and pathogenic bacteria (Siripongvutikorn *et al*., 2005; Arora and Kaur, 1999).

TMA-N, derived from TMAO, is considered a spoilage indicator since this compound is usually detected after fish has been kept for a number of days. However, this indicator is not useful for freshwater fish since there is no or very little TMAO in freshwater fish as compared to seawater fish, which use TMAO as an osmoregulator. Hebard *et al.* (1982) reported that if TMA-N was higher than 5 mgN/100 g sample the fish would not be safe for consumption. Similar to TVB-N, TMA-N in this experiment was very high in both control and marinated samples, reaching the above limit by day 3 and day 6, respectively (Figure 3). Considering the sensory results, which indicated that the shrimp samples were still acceptable by day 15 at 4±2°C (Table 3), it could be argued that TVB-N and TMA-N may not be suitable spoilage indicators or these threshold values may be shifted up for white shrimp.

Sensory scores slightly decreased as storage time increased (Table 4). Regardless of the chemical indices such as TVB-N and TMA-N, shrimp marinated with the curry paste was still acceptable after 15 d by slightly like to moderate like for color, flavor, taste and appearance; score 5.96-6.96 by 9-point hedonic scale. The TVC value of 10^6 cfu/g appears to support this result. The fact that the initial bacterial load in the curry paste itself was very high could explain why the paste was not as effective in suppressing bacterial growth as it should be. Thorough washing and some pretreatments such as blanching and pasteurizing or hurdle technique of the curry paste ingredients could improve its anti-microbial property. These steps will be introduced in the next experiment.

4. Conclusion

The green curry paste with the addition of 20% garlic (w/w) maintained its sensory acceptability. The paste showed antimicrobial property, leading to an extended shelf life of white shrimp when marinated with the curry paste. Applying GMP or hurdle technique to the preparations of the ingredients and the paste to reduce their microbial load may improve the antimicrobial activity of curry paste further.

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