

Effect of food additives on the quality of white shrimp (*Litopenaeus vannamei*)¹Nor Salasiah, M. and ²Jirarat, T.¹Food Technology Research Centre, Malaysia Agricultural Research Development Institute, 43400

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DOI:[https://doi.org/10.26656/fr.2017.2\(6\).114](https://doi.org/10.26656/fr.2017.2(6).114)**Abstract**

The effects of three additives: sodium tripolyphosphate (STPP), sodium bicarbonate (NaHCO₃) and microbial transglutaminase (MTGase) with the addition of 2% sodium chloride (NaCl) at different concentrations (2, 5 and 8%) and times (10, 30 and 60 minutes) at 4±2°C on the quality of frozen-thawed white shrimp (*Litopenaeus vannamei*) were studied. In average, compared to control, different concentrations and immersion times of three additives with 2% NaCl significantly (p<0.05) affected the water holding capacity (WHC), cooking weight loss (CL), weight gain (WG), moisture content (MC) and microbial load (TVC) of white shrimps. The combined effect of 2% NaCl with all additives showed synergistic effect on reducing CL, increased WHC and yielded a good quality of frozen-thawed white shrimps. Treatment by immersions of shrimp in 5% MTGase with 2% NaCl for 30 minutes improved the quality as compared to control. Furthermore, these treatments increased weight gain (16%) and WHC up to 94%, lowered CL to 10% and TVC 1.78 log cycle.

1. Introduction

The white shrimps *Litopenaeus vannamei* is one of the dominant economically-important products of Thailand. Frozen shrimp export generating export revenue for Thailand economy. Changes in texture of thawed muscle are a negative economic factor. Processing of frozen shrimp products encompasses a wide array of pre and post-treatments to create higher value addition. Shrimp muscle increases in firmness by heat processing and becomes too firm, lack of juiciness and unpalatable when heating temperature exceeds 70°C (Mizuta *et al.*, 1999). There is a possibility of increasing the quality of frozen-thawed shrimp by using food additives.

Sodium tripolyphosphate (STPP) has been widely accepted as an additive in seafood and the most popular choice to improve water holding capacity, texture, stabilise colour and reduce cooking loss. STPP is limited to a maximum 0.5 g/100 g sample in the final product according to Europe, Canadian and Brazilian regulations for seafood products (Gonçalves and Ribeiro, 2008). The use of non-phosphate ingredients such as sodium bicarbonate (NaHCO₃) and microbial transglutaminase (MTGase) have an advantage over using phosphate. MTGase is an enzyme with the ability

to cross-link proteins through covalent bonds. It improves solubility, water holding capacity and texture quality of fish products (Motoki and Seguro, 1998) MTGase increase breaking force of white shrimp gel and lowered the expressible moisture content with increasing MTGase amount (Tammattinna *et al.*, 2007). Sodium bicarbonate is a chemical compound that able to increase the weight gain of fresh shrimp up to 10% and lowered CL to 20% by dipping at 8% concentration (Henderson *et al.*, 1990).

This study analyzed the effects of food additive treatments with different treatment conditions (food additive concentration and immersion time) on the quality of frozen-thawed white shrimp to maximize the water holding capacity (WHC), increase weight gain (WG) and moisture content (MC), improve the texture, reduce cooking loss (CL) and total viable count (TVC) of the shrimp. The treatment with non-phosphate additive could be an alternative way to improve the quality of frozen shrimp.

2. Materials and methods**2.1 Materials**

White shrimps (90-100 count/kg) were procured

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from Charoan Pokphand Food Public Company, Thailand. The untreated white shrimps were dressed in headless, peeled and deveined style and frozen prior to transportation and treatments. The untreated frozen white shrimp were thawed in chill storage (0-4°C) for overnight (12 hrs) before the treatments.

2.2 Food additives treatment

The treatments were done using three additives: Sodium tripolyphosphate (STPP) with 2% sodium chloride (NaCl), Sodium bicarbonate (NaHCO₃) with 2% NaCl and Microbial transglutaminase (MTGase; Activa TG-HP®) with 2% NaCl at different concentrations (2, 5 and 8% w/w) and different immersion times (10, 30 and 60 mins) at 4±2°C with 1:2 shrimp: brine solution ratio. The experiment was designed using 3³ factorial in Completely Randomized Design (CRD) with two replicates to analyze the effects of food additives immersion on the quality of white shrimp (Table 1). Food grade NaCl, NaHCO₃ and STPP was procured from CT Chemical Co., Ltd and MTGase (Activa® TG-HP) was supplied by Ajinomoto Co. (Thailand) Ltd.

2.3 Texture measurement

Shear force determination was carried out at room temperature (28±2°C). The shear force of samples was measured using texture analyser (Stable Micro System; TA-XT2i, England) with a Warner-Bratzler blade. The operating parameter used was the cross-head speed of 10 mm/s. The maximum force to cut at the centre of the second abdominal segment about 1 mm at 45° angle of the shrimp was recorded as the shear force (Mallick *et al.*, 2010). The measurement was replicated ten times for each sample.

2.4 Water holding capacity (WHC)

WHC was characterized by measuring the expressible moisture (EM). EM was determined using a modification of the filter paper press method (Schubring *et al.*, 2003). The sliced samples were pressed between paired filter sheets (Schleicher and Schuell, 7x7 cm) and parallel plates using a texture analyser. A 25 kg load cell and a crosshead speed of 1.70 mm/s were used. Samples were pressed to 75% deformation and held at that point for 15s. WHC was calculated as %WHC = {1-[initial weight-final weight]/initial weight} x 100 (Díaz-Tenorio *et al.*, 2007).

2.5 Cooking weight loss (CL)

The treated shrimp was cooked with hot water at 100±2°C for 5 mins with 1:5 shrimp: water ratio. CL was calculated based on the weight of the sample before and after cooking (Erdogdu *et al.*, 2007). Equation used is

shown as %CL= (A-B)/A x 100. Where A and B is the weight before and after cooking, respectively.

2.6 Weight gain (WG)

WG was calculated based on the weight of the sample before and after pre-treatment. %WG equals (B-A)/Ax100. Where A and B is the weight before and after pre-treatment, respectively (AOAC, 2005).

2.7 Moisture content (MC)

Moisture content was determined by drying 10 g of the sample in air oven at 105°C for 5-12 hrs to constant weight (AOAC, 2005).

2.8 Microbiological analysis - total viable count (TVC)

A total of twenty-five grams of food sample were diluted to 225 mL of sterile 0.1% peptone water and masticated using laboratory paddle blender (Stomacher®). Serial dilutions were made and one mL of each appropriate dilution was pour plated using plate count agar (Merck), allowed to gel to cool and incubated at 37°C for 24-48 hrs (McLandsborough, 2005).

2.9 pH analysis

The pH value was recorded using a pH meter Consort C860 (Consort, Turnhout, Belgium). Each measurement was replicated three times with two replicates. Thirty grams of shrimp was homogenized with 150 mL distilled water at sample to water ratio 1:5 (w/v) and allowed to dissolve for 2 mins before the analysis. The pH meter was calibrated with standard buffers of pH 4.0 and 7.0 before the measurement (Siripongvutikorn *et al.*, 2008).

2.10 Colour analysis

Colour of the raw and treated shrimps, the gravy (green curry) and the homogenized sample (marinated shrimp in green curry) were measured using a Chromameter CR-400 (Minolta, Osaka, Japan). The color was expressed in CIE Lab system L*, a*, and b* values, where L* denotes lightness on a 0-100 scale from black to white, a* (+) red or (-) green and b* (+) yellow or (-) blue (Schubring *et al.*, 2003). This instrument was calibrated with white reference tiles (Y=93.5; x = 0.3132; y = 0.3198) before the analysis. The shrimp was placed on the white tray above the light sources and will be measured directly. Glass cell containing the green curry and homogenized sample of shrimp with green curry was placed above the light sources and L*, a*, b* values were then recorded. Five readings were done for each sample with two replicates (Mallick *et al.*, 2010).

Table 1. Pre-treatment using 3³ Factorial in Completely Randomized Design (CRD) of twenty-seven treatments.

Std	Run	Block	Factor 1 % Percentage	Factor 2 Time (mins)	Factor 3 Preservatives
41	1	Block 1	8	10	MTGASE
13	2	Block 1	2	30	STTP
7	3	Block 1	2	20	STTP
29	4	Block 1	8	20	sodium bicarbonate
35	5	Block 1	8	30	sodium bicarbonate
21	6	Block 1	5	10	sodium bicarbonate
1	7	Block 1	2	10	STTP
27	8	Block 1	5	20	sodium bicarbonate
33	9	Block 1	5	30	sodium bicarbonate
53	10	Block 1	8	30	MTGASE
45	11	Block 1	5	20	MTGASE
9	12	Block 1	5	20	STTP
49	13	Block 1	2	30	MTGASE
19	14	Block 1	2	10	sodium bicarbonate
23	15	Block 1	8	10	sodium bicarbonate
5	16	Block 1	8	10	STTP
43	17	Block 1	2	20	MTGASE
51	18	Block 1	5	30	MTGASE
17	19	Block 1	8	30	STTP
3	20	Block 1	5	10	STTP
15	21	Block 1	5	30	STTP
47	22	Block 1	8	20	MTGASE
31	23	Block 1	2	30	sodium bicarbonate
11	24	Block 1	8	20	STTP
25	25	Block 1	2	20	sodium bicarbonate
37	26	Block 1	2	10	MTGASE
39	27	Block 1	5	10	MTGASE
50	28	Block 2	2	30	MTGASE
28	29	Block 2	5	20	sodium bicarbonate
10	30	Block 2	5	20	STTP
8	31	Block 2	2	20	STTP
4	32	Block 2	5	10	STTP
54	33	Block 2	8	30	MTGASE
20	34	Block 2	2	10	sodium bicarbonate
12	35	Block 2	8	20	STTP
32	36	Block 2	2	30	sodium bicarbonate
14	37	Block 2	2	30	STTP
46	38	Block 2	5	20	MTGASE
34	39	Block 2	5	30	sodium bicarbonate
44	40	Block 2	2	20	MTGASE
22	41	Block 2	5	10	sodium bicarbonate
36	42	Block 2	8	30	sodium bicarbonate
52	43	Block 2	5	30	MTGASE
26	44	Block 2	2	20	sodium bicarbonate
16	45	Block 2	5	30	STTP
48	46	Block 2	8	20	MTGASE
38	47	Block 2	2	10	MTGASE
40	48	Block 2	5	10	MTGASE
6	49	Block 2	8	10	STTP
42	50	Block 2	8	10	MTGASE
18	51	Block 2	8	30	STTP
24	52	Block 2	8	10	sodium bicarbonate
30	53	Block 2	8	20	sodium bicarbonate
2	54	Block 2	2	10	STTP

min: minutes. The pre-treatment was done using the same raw material for every batch in the same replicates, the pre-treatment was replicated two times.

Factor A : Percentage of preservative : a1= 2% , a2 = 5% and a3 = 8%

Factor B : Time immersion: b1 = 10 mins, b2 = 30 mins and b3 = 60 mins

Factor C : Additives: C1= STPP and 2%NaCl, C2 = NaHCO₃ (sodium bicarbonate) and 2% NaCl and C3= MTGase (Activa® TG-HP) and 2% NaCl.

Control: Un-treated shrimps.

3. Results

Effects of concentration and immersion time of each type of additives on physical properties and microbiology analysis are shown in Tables 2 and 3. On average, the results showed that both concentration and immersion time had a significant effect ($p < 0.05$) for all attributes of the treated shrimp when compared to control. Among the three additives with 2% NaCl, MTGase gave better results for all studied parameters. Immersion white shrimp in 5% MTGase for 30 mins increased the WG (15.65%), MC (84.00%) and WHC (94.50%), lowered CL to 10.15% and reduced TVC for about 1.78 log cycle. The shear force value for this sample was not significantly different with control ($p > 0.05$). For NaHCO₃ and STPP, when compared within their own group, 5% of concentration and 60 mins immersion reduced TVC and CL, increased WG and MC.

3.1 Weight gain (WG)

Overall, WG was increased with increasing concentration of the additives with 2% NaCl and immersion time ($p < 0.05$) up to 15.74% compared with control. In contrast, some treated shrimps (treatments no. 7-8, 12, 15-18, 22-23 and 25-27) did not show an increase in WG as the concentration and immersion time increased (Table 2). Higher concentration of the additives could lead to yield loss due to excessive solubilisation or disruption of protein filaments that increases expressible moisture and reduces water holding capacity.

3.2 Texture

The shear force value for treated shrimp was not significantly different from control (Table 2). When toughening of the treated shrimp was reduced, firmness and juiciness increased, and the shrimp texture becomes more elastic (chewy). The shear force value was decreased when the moisture content was increased. The elastic texture was dominant and provided fibrous characteristic on the sample treated with MTGase+2% NaCl. Faithong *et al.* (2006) reported that the shrimp immersed in a higher concentration of phosphate (2-3%) and salt (2-3%) for 8 and 10 hrs at 5°C resulted in higher shear force.

According to physical observation, the texture of the raw shrimp muscle (control) was softer than the treated shrimp, but the shear force value for control was higher because of the ventral nerve code at below abdominal segment inside the shrimp meat. The ventral nerve code was toughed and stronger than the ventral nerve code in treated shrimp. This ventral nerve code usually was not removed by the manufacturer. The graph of the shear

force was increased when the Warner-Bratzler blade cut at the ventral nerve code inside the shrimp meat and gave a higher value of the shear force.

Improvement of water holding capacity in treated shrimp with MTGase at 4°C resulted in a good texture of shrimp which reduced toughness (shear force range 15.35-18.09 N) and increased firmness, juiciness and gel-forming ability (elastic texture) that provided fibrous characteristic. Motoki and Seguro (1998) reported that the activity of MTGase was inactivated after heating to above 70°C. This is due to the optimum temperature for enzymatic activity was 50°C, and MTGase fully sustained its activity even at 50°C for 10 mins. On the other hand, it lost activity within a few minutes on heating to 70°C. MTGase still expressed activity at 10°C and still retained some activity at temperatures just above the freezing point.

Electrophoretic pattern SDS-PAGE of white shrimp reported by Sriket *et al.* (2007) showed that myosin heavy chain (MHC) was the dominant protein component (57-64%), actin was found as a second dominant protein. The actin and myosin (actomyosin) react as a substrate, gelled by an enzyme (MTGase) through the cross-linked protein and result in thermal stability of protein (Motoki and Seguro, 1998).

3.3 Total viable count (TVC)

The treatment with 5% MTGase + 2%NaCl for 30 mins results in the lowest microbial load for only 2.92 ± 0.01 log CFU/g compared with the other treatments. This treatment lowers the microbial load for about 1.78 log cycle compared with the control sample (4.70 log CFU/g) (Table 2). The result showed that only the samples treated with MTGase had a significant lower TVC value compared with control samples. This might be due to the pH value range from 8.17 to 11.15 (Table 2), which was not suitable for microbial growth. Carballo *et al.* (2006) reported that MTGase/caseinate (1.5 g/100 g) using cold binding systems for restructured meat inhibited the rapid growth of bacteria.

3.4 Cooking weight loss (CL)

The lowest CL value was found in sample treated with 5% MTGase + 2%NaCl for 30 minutes (10.15%) that was much lower than the control sample which had 51.13% CL (Table 2). It was also found that treated shrimp had a lower CL when the concentration of the additives and immersion time was increased ($p \leq 0.05$). Only the sample treated with 8% MTGase + 2% NaCl for 60 mins and 2%STPP + 2%NaCl for 30 and 60 mins had a higher CL value as the immersion time increased. Treatments with longer immersion time reduced the water retention and increased water loss during cooking.

Table 2. Effects of three additives with 2% NaCl on the qualities of white shrimps.

Treatment	Level (%)	Time (mins)	Additives +2% NaCl	Weight gain (%)	Shear force (N)	TVC (log cfu/g)	Cooking loss (%)	WHC (%)	Moisture content (%)	pH
Control	0	0	Untreated	0.00 ⁱ	18.81 ± 2.01 ^{ab}	4.36 ± 0.28 ^{ab}	51.13 ± 1.27 ^a	87.50 ± 1.21 ^{kl}	81.41 ± 0.79 ⁱ	7.16 ± 0.33 ⁱ
1	2	10	MTGase	11.26 ± 0.51 ^{efg}	16.76 ± 3.23 ^{ab}	3.30 ± 0.03 ^{efghi}	17.30 ± 0.13 ^d	89.93 ± 0.65 ^{hi}	83.06 ± 1.06 ^{defgh}	8.17 ± 0.24 ^{efghi}
2	2	30	MTGase	11.22 ± 0.35 ^{efgh}	16.73 ± 1.18 ^{ab}	3.46 ± 0.40 ^{defghi}	15.74 ± 0.68 ^{def}	91.09 ± 0.59 ^{gh}	84.52 ± 1.37 ^a	8.80 ± 0.05 ^{cdefg}
3	2	60	MTGase	14.45 ± 0.10 ^b	17.75 ± 1.84 ^{ab}	3.26 ± 0.20 ^{fghi}	16.43 ± 0.08 ^{de}	92.68 ± 1.71 ^{ef}	84.27 ± 1.48 ^{abc}	9.24 ± 0.49 ^{cdef}
4	5	10	MTGase	15.65 ± 0.13 ^a	15.88 ± 4.71 ^b	3.24 ± 0.07 ^{fghi}	14.93 ± 1.17 ^{efg}	92.91 ± 0.88 ^{def}	82.72 ± 0.70 ^{fghij}	9.30 ± 0.12 ^{cde}
5	5	30	MTGase	15.68 ± 0.02 ^a	17.14 ± 2.88 ^{ab}	2.92 ± 0.01 ⁱ	10.15 ± 0.11 ^k	94.50 ± 1.42 ^{ab}	84.00 ± 0.97 ^{abcd}	9.29 ± 1.36 ^{cde}
6	5	60	MTGase	15.77 ± 0.09 ^a	15.35 ± 2.17 ^b	3.05 ± 0.01 ^{ghi}	10.59 ± 0.27 ^{jk}	94.04 ± 1.32 ^{abcd}	83.50 ± 1.28 ^{abcdefg}	9.54 ± 1.05 ^{bcd}
7	8	10	MTGase	11.28 ± 0.42 ^{ef}	18.09 ± 3.21 ^{ab}	3.22 ± 0.08 ^{fghi}	11.69 ± 1.33 ^{ijk}	93.49 ± 1.90 ^{abcde}	82.96 ± 1.80 ^{defghi}	9.90 ± 0.74 ^{bc}
8	8	30	MTGase	11.62 ± 0.26 ^{de}	16.19 ± 0.37 ^b	2.97 ± 0.12 ^{hi}	10.58 ± 0.06 ^{jk}	94.40 ± 1.39 ^{abc}	81.17 ± 0.90 ⁱ	10.55 ± 0.81 ^{ab}
9	8	60	MTGase	13.10 ± 0.82 ^c	15.35 ± 0.81 ^b	3.07 ± 0.60 ^{ghi}	15.18 ± 0.60 ^{ef}	94.68 ± 0.21 ^a	82.12 ± 1.91 ^{hijkl}	11.15 ± 0.12 ^a
10	2	10	NaHCO ₃	8.00 ± 0.78 ^{ij}	17.75 ± 1.14 ^{ab}	3.60 ± 0.58 ^{bcdefghi}	19.03 ± 1.55 ^c	89.31 ± 1.39 ^{ij}	83.99 ± 1.19 ^{abcd}	8.02 ± 0.02 ^{ghi}
11	2	30	NaHCO ₃	13.28 ± 0.28 ^c	17.43 ± 0.49 ^{ab}	4.22 ± 0.08 ^{abcd}	14.00 ± 0.28 ^{fgh}	87.21 ± 1.99 ^l	83.11 ± 0.67 ^{defgh}	8.06 ± 0.37 ^{fghi}
12	2	60	NaHCO ₃	11.07 ± 0.60 ^{efgh}	17.27 ± 0.12 ^{ab}	3.68 ± 0.34 ^{bcdefghi}	15.63 ± 0.52 ^{def}	85.96 ± 1.65 ^m	83.80 ± 0.08 ^{abcde}	8.09 ± 0.40 ^{efghi}
13	5	10	NaHCO ₃	11.11 ± 0.56 ^{efgh}	19.87 ± 1.01 ^a	4.21 ± 0.24 ^{abcd}	12.49 ± 0.19 ^{hi}	93.26 ± 1.33 ^{bcde}	83.64 ± 0.79 ^{abcdef}	8.42 ± 0.01 ^{defgh}
14	5	30	NaHCO ₃	13.25 ± 0.25 ^c	16.77 ± 1.50 ^{ab}	4.31 ± 0.10 ^{abc}	12.59 ± 0.28 ^{hi}	93.69 ± 1.24 ^{abcde}	84.33 ± 0.94 ^{ab}	8.68 ± 0.11 ^{defg}
15	5	60	NaHCO ₃	12.60 ± 0.99 ^{cd}	17.92 ± 4.43 ^{ab}	3.49 ± 0.39 ^{cdefghi}	12.57 ± 0.28 ^{hi}	93.17 ± 1.37 ^{cde}	83.73 ± 0.74 ^{abcdef}	8.71 ± 0.10 ^{defg}
16	8	10	NaHCO ₃	8.55 ± 0.21 ⁱ	16.89 ± 1.54 ^{ab}	3.41 ± 0.42 ^{defghi}	14.39 ± 0.44 ^{fg}	92.91 ± 1.42 ^{def}	82.87 ± 1.09 ^{efghi}	8.64 ± 0.04 ^{defg}
17	8	30	NaHCO ₃	10.35 ± 0.09 ^{fgh}	17.93 ± 3.16 ^{ab}	4.03 ± 0.15 ^{abcdef}	12.22 ± 0.09 ^{hij}	93.45 ± 1.73 ^{abcde}	83.46 ± 1.29 ^{bcdefg}	8.71 ± 0.08 ^{defg}
18	8	60	NaHCO ₃	13.62 ± 0.73 ^{bc}	17.70 ± 3.22 ^{ab}	3.68 ± 0.46 ^{bcdefghi}	12.32 ± 0.07 ^{hij}	93.50 ± 1.28 ^{abcde}	83.36 ± 1.26 ^{bcdefg}	8.72 ± 0.09 ^{defg}
19	2	10	STPP	10.90 ± 0.38 ^{efgh}	17.78 ± 3.59 ^{ab}	4.17 ± 0.24 ^{abcd}	13.24 ± 0.18 ^{ghi}	91.09 ± 0.32 ^{gh}	83.01 ± 1.31 ^{defghi}	7.20 ± 0.12 ⁱ
20	2	30	STPP	10.93 ± 0.48 ^{efgh}	18.20 ± 2.14 ^{ab}	4.56 ± 0.40 ^a	23.22 ± 0.22 ^b	91.00 ± 0.30 ^{gh}	83.30 ± 1.35 ^{cdefg}	7.33 ± 0.15 ^{hi}
21	2	60	STPP	11.64 ± 0.75 ^{de}	17.94 ± 5.13 ^{ab}	4.09 ± 0.08 ^{abcde}	23.47 ± 0.35 ^b	89.56 ± 0.56 ^{ij}	83.78 ± 1.27 ^{abcde}	7.38 ± 0.20 ^{hi}
22	5	10	STPP	6.74 ± 0.79 ^k	17.34 ± 0.72 ^{ab}	3.80 ± 0.45 ^{abcdefg}	17.37 ± 0.10 ^d	89.52 ± 0.56 ^{ij}	82.04 ± 0.83 ^{ijkl}	7.38 ± 0.53 ^{hi}
23	5	30	STPP	8.81 ± 0.80 ⁱ	18.24 ± 1.42 ^{ab}	3.79 ± 0.97 ^{abcdefgh}	14.93 ± 0.36 ^{efg}	91.71 ± 0.93 ^{fg}	83.26 ± 1.41 ^{cdefg}	7.69 ± 0.71 ^{fghi}
24	5	60	STPP	11.56 ± 0.92 ^{def}	18.07 ± 1.10 ^{ab}	3.44 ± 0.10 ^{defghi}	11.76 ± 0.83 ^{ijk}	88.61 ± 1.48 ^{jk}	82.57 ± 0.25 ^{ghijk}	7.69 ± 0.87 ^{fghi}
25	8	10	STPP	7.07 ± 0.39 ^{jk}	17.53 ± 2.02 ^{ab}	3.42 ± 0.44 ^{bcdefgh}	13.29 ± 0.14 ^{gh}	91.75 ± 0.88 ^{fg}	82.53 ± 1.29 ^{ghijk}	7.68 ± 0.61 ^{fghi}
26	8	30	STPP	9.98 ± 0.74 ^b	18.02 ± 1.10 ^{ab}	4.11 ± 0.23 ^{abcde}	12.24 ± 1.27 ^{hij}	88.82 ± 1.61 ^{ij}	81.75 ± 0.73 ^{ijkl}	7.79 ± 0.76 ^{fghi}
27	8	60	STPP	10.02 ± 1.05 ^{gh}	17.36 ± 0.64 ^{ab}	3.95 ± 0.13 ^{abcdef}	12.29 ± 1.20 ^{hij}	88.42 ± 1.55 ^{jk}	81.69 ± 0.36 ^{kl}	7.90 ± 0.76 ^{fghi}

Means in the same column followed by the same letter are not significantly different (p>0.05).

This may be due to an excessive solubilisation and disruption of protein filaments resulting an increase in CL value.

Benjakul *et al.* (2008) reported that the CL of black tiger shrimp and white shrimp increased sharply after being heated for a longer time for more than 0.5 to 3 mins ($p < 0.05$). Cooking loss of frozen fish products after thawing was reduced by a combination of MTGase in tumbling or injection of frozen fish products (Motoki and Seguro, 1998). Tammatinna *et al.* (2007) reported that more water retained in the gel network of white shrimp with the increasing MTGase (Activa® TG-K) amount added (0.2-0.8 unit/g sample).

Erdogdu *et al.* (2007) explained that cook losses were highly depending on the concentration and time of immersion in sodium tripolyphosphate (STPP) solution ($p < 0.05$). The diffused amount of STPP was around 0.25% in the meats dipped in the 2% STPP solution after 30 mins of dipping time which made 2% STPP not effective to reduce cook losses.

Faithong *et al.* (2006) reported that the combined effect of 2% STPP with 1-3% salt for 10 hours at $< 5^{\circ}\text{C}$ showed a synergistic effect on reducing cooking loss and increase raw weight gain of white shrimp ($p < 0.05$) when compared with sample treated with only phosphate. There was no significant effect with tetrasodium pyrophosphate (TSPP) and sodium hexametaphosphate (SHMP) in reducing cooking weight loss.

3.5 Water holding capacity (WHC)

Overall, the WHC of treated shrimp was increased ($p \leq 0.05$) from 87.50% for the control sample up to 94.68% (Table 2). The result showed that when pH increased the WHC value was also increased (Table 2). Only three samples (treatment number 11, 24 and 27) did not show an increase in WHC compared with control ($p > 0.05$). For treatments with MTGase, the WHC was increased when the concentration and immersion time increased. Too much absorption of water will increase the muscle protein solubility and lowered the WHC.

Tammatinna *et al.* (2007) reported that lowered expressible moisture was noticeable with increasing MTGase (Activa® TG-K) amount added from 0.2 to 0.8 unit/g sample ($p < 0.05$). For NaHCO_3 and STPP at higher concentration and longer immersion time, the WHC did not increase. This is due to the absorption of too much water that increased the muscle protein solubility and thus, lowered WHC.

Pietrasik and Li-Chan (2002) reported that in cooked restructured meat products, gel firmness and water-holding capacity (WHC) significantly ($p < 0.01$) increased

by the addition of 0.5% MTGase in high salt (2%) compare to the sample without MTGase.

3.6 Moisture content (MC)

Compared with control, MC of treated shrimp was increased when the concentration and immersion time increased ($p \leq 0.05$). The MC increased from 81.41% (control) to 84.52% (Table 2). Only five samples (treatments number 8, 9, 22, 26 and 27) were not significantly different from control. Higher concentration and longer immersion time might cause protein denaturation and lower the moisture content. Faithong *et al.* (2006) reported that moisture content of the sample treated with 1-3% salt and 2% STPP for 10 hrs at $< 5^{\circ}\text{C}$ was significantly lower than that of the control (without salt).

3.7 pH analysis

Treated shrimp with MTGase and NaHCO_3 significantly increased the pH value of white shrimp up to 11.15 and 8.72 respectively (Table 2). The pH value was increased when the concentration of the additives and immersion time increased. For shrimp treated with 2% MTGase + 2% NaCl for 10 mins, 2% NaHCO_3 for 10, 30 and 60 mins and all treated shrimp using STPP + 2% NaCl, the pH value was not significant different from control ($p > 0.05$).

Ünal *et al.* (2004) reported that there were two diffusion mechanisms taking place. Where the meat samples naturally had high amounts of orthophosphates the compound diffuses into the solutions, while STPP diffuses into the meat samples. The STPP diffusion into the meat samples was relatively slower compared with the orthophosphate diffusion out of the meat samples until the water-protein-STPP complex barrier formation on the surface of the meat samples was completed (Tenhet *et al.*, 1991). Moreno *et al.* (2010) reported that MTGase is active and stable at a wide pH range of 4-9 and the optimum pH was at 5-8.

3.8 Colour ($L^* a^* b^*$)

Table 3 shows that the colour ($L^* a^*$ and b^*) value for treated shrimp was significantly different from control ($p \leq 0.05$). Treatments with MTGase increased a^* value (+ = red) of treated shrimp, while treatments with STPP and NaHCO_3 increased b^* value (+ = yellow). The a^* and b^* values increased in treated shrimp due to the interaction of pigments with the food additives. Pietrasik and Li-Chan (2002) reported that MTGase addition had no significant influence on the b^* value of restructured meat, the addition of 0.5% MTG increased lightness and redness of gels containing 8% meat protein and 2% egg albumin with 0.5% K-carrageenan, respectively.

Table 3. Effects of three additives combination with 2% NaCl on colour L* a* b*

Treatment	Level (%)	Time (mins)	Additives +2% NaCl	L*	a*	b*
Control	0	0	Un-treated	51.67±2.01 ^a	-0.10±0.92 ^{de}	-3.55±1.42 ^{bcdef}
1	2	10	MTGase	46.98±1.24 ^{bcde}	3.76±0.22 ^{ab}	-2.46±4.11 ^{abc}
2	2	30	MTGase	47.42±1.09 ^{bcd}	4.35±2.06 ^a	-1.32±1.29 ^{ab}
3	2	60	MTGase	46.16±0.99 ^{bcdef}	3.95±1.32 ^{ab}	-1.35±2.12 ^{ab}
4	5	10	MTGase	45.88±1.48 ^{cdef}	4.04±1.28 ^{ab}	-2.97±2.03 ^{abcde}
5	5	30	MTGase	45.35±0.19 ^{cdef}	5.41±3.68 ^a	-1.86±2.10 ^{abc}
6	5	60	MTGase	46.65±1.46 ^{bcdef}	4.79±2.85 ^a	-1.36±2.40 ^{ab}
7	8	10	MTGase	46.25±1.40 ^{bcdef}	4.23±1.65 ^a	-0.89±2.61 ^a
8	8	30	MTGase	47.45±0.86 ^{bcd}	4.94±1.65 ^a	-2.77±1.36 ^{abcd}
9	8	60	MTGase	46.72±0.69 ^{bcdef}	4.08±2.71 ^{ab}	-2.48±0.93 ^{abc}
10	2	10	NaHCO ₃	46.60±4.33 ^{bcdef}	1.80±1.94 ^{cd}	-4.08±1.77 ^{cdefg}
11	2	30	NaHCO ₃	46.41±4.25 ^{bcdef}	0.49±1.17 ^{cde}	-5.60±3.20 ^{ghijkl}
12	2	60	NaHCO ₃	46.83±1.36 ^{bcdef}	0.33±0.55 ^{de}	-6.10±2.43 ^{ghijkl}
13	5	10	NaHCO ₃	45.98±1.54 ^{cdef}	0.83±1.00 ^{cde}	-5.36±1.48 ^{ghij}
14	5	30	NaHCO ₃	44.73±3.51 ^{def}	1.30±2.16 ^{cde}	-7.72±1.12 ^{jkl}
15	5	60	NaHCO ₃	45.48±3.10 ^{cdef}	0.73±1.76 ^{cde}	-7.59±1.66 ^{ijkl}
16	8	10	NaHCO ₃	43.59±1.96 ^{ef}	2.31±1.63 ^{bc}	-6.55±2.75 ^{ghijkl}
17	8	30	NaHCO ₃	44.87±2.83 ^{def}	0.92±1.29 ^{cde}	-7.77±1.07 ^{jkl}
18	8	60	NaHCO ₃	43.22±1.00 ^f	1.04±1.60 ^{cde}	-8.07±1.12 ^{kl}
19	2	10	STPP	47.88±1.21 ^{bcd}	-0.13±0.01 ^{de}	-5.61±0.81 ^{ghijk}
20	2	30	STPP	48.63±0.49 ^{abc}	-0.28±0.28 ^e	-5.17±0.13 ^{fghi}
21	2	60	STPP	49.81±2.68 ^{ab}	-0.47±0.52 ^e	-5.00±0.56 ^{defgh}
22	5	10	STPP	46.58±2.67 ^{bcdef}	0.05±0.72 ^{de}	-6.17±2.79 ^{ghijkl}
23	5	30	STPP	46.40±2.18 ^{bcdef}	0.96±1.22 ^{cde}	-6.06±2.96 ^{ghijkl}
24	5	60	STPP	46.00±0.13 ^{cdef}	0.11±0.71 ^{de}	-8.12±1.14 ^l
25	8	10	STPP	44.38±0.69 ^{def}	0.49±1.33 ^{cde}	-6.60±2.86 ^{hijkl}
26	8	30	STPP	45.19±1.43 ^{cdef}	0.23±0.95 ^{de}	-7.28±1.00 ^{hijkl}
27	8	60	STPP	44.76±3.70 ^{def}	0.23±1.11 ^{de}	-8.51±2.40 ^l

Means in the same column followed by the same letter are not significantly different ($p>0.05$).

Benjakul *et al.* (2008) reported that the L* a* b* value of white shrimp was increased when heating time was longer than 0.5 to 1 mins ($p>0.05$).

4. Discussion

4.1 Effect of 2% NaCl combination with MTGase, STPP and NaHCO₃

When the frozen-thawed shrimp were submerged in the additives solution with 2% NaCl, water molecules started diffusing into the shrimp meat. The role of the 2% NaCl was to enhance protein-water interaction. Part of the additives and 2% NaCl molecule anchored to positively charged groups of proteins, while the rest scavenged for free water molecule and presumably created a gradient concentration to allow more water to propagate into shrimp muscle. Protein composition and conformation have significant effects on water holding capacity (WHC). Combination of 2% NaCl with the three types of additives have a synergistic effect to enhance the WHC and significantly increased the weight gain, moisture content and reduced cooking loss ($p\leq 0.05$) (Tables 2 and 3).

Shults and Wierbicki (1973) reported that the combination of 1-5% salt to 0.5% phosphates namely; tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP) solution reduce the shrinkage of chicken breast after cooking and increased the water holding capacity compared to sample treated with phosphate without salt. Phosphate and salt were found to have a synergistic effect on reducing cooking loss. When WHC increased, the ionic strength was also increased which caused the swelling of muscle fibre and increased extractability and solubility of myofibrillar protein (Liu and Xiong, 1997).

Damodaran and Kinsella (1982) reported that salt increased the functional properties of protein due to hydrophobic and electrostatic interactions which resulted in thermal stability of proteins. Faithong *et al.* (2006) reported that the effect of 1-3% salt combination with 2% tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP) on shear force was found to depend on salt concentration; the higher shear force value was with the shrimp immersed in a higher salt concentration. In cooked restructured meat, the water-holding capacity

(WHC) was increased by the addition of 0.5% MTGase in high salt (2%), but not in low salt products (Pietrasik and Li-Chan, 2002).

4.2 Selection of suitable food additives for the pre-treatment

The most suitable food additive that had been selected as the treatment was the MTGase (Activa® TG-HP) at 5% concentration with 2% NaCl for 30 mins. Immersion white shrimp in 5% MTGase with 2% NaCl for 30 mins increased the WG (15.68%), MC (84.00%) and WHC (94.50%), lowered CL to 10.15% and reduced TVC for about 1.44 log CFU/g compared with control. The shear force value for this sample was not significantly different from control. Only the pH value of treated shrimp (5% MTGase with 2% NaCl for 30 mins) needed to be adjusted because the pH of treated shrimp was 9.30. If the beginning of pH value of marinated shrimp in green curry was higher the final pH of the product after storage will be too high. Dziezak (1990) reported that higher pH value will shorten the shelf-life of the product and failures as sliminess, translucency and fat decomposition will be observed.

The decision had been made to reduce the pH value of the treated shrimp before it was mixed with green curry. The raw shrimp was rinsed using running tap water before the pre-treatment with the 5% MTGase and 2% NaCl for 30 mins at 4±2°C. For the second step, after the pre-treated shrimp was drained, it was rinsed with water again for 30 s and drained before immersion in 0.5% citric acid for 5 mins at 4±2°C. After that, the treated shrimp was drained and mixed with green curry. The pH of the green curry was 5.10±0.03.

Finally, the average pH value of 120 g marinated shrimp with green curry was 7.68. This product was categorized as a low acid food, which is suitable to maintain a good quality of shrimp texture. Citric acid was certified as GRAS (Generally Recognized as Safe) status according to US FDA (Food and Drug Administration) and the maximum daily dosage limit is 8 grams (FDA, 2012).

Low acid food products are important for product like marinated shrimp in green curry to improve the quality of the shrimp by increasing the WHC, MC, WG and improve the texture. Xiong *et al.* (2002) reported that high acid food like marinated food in acidic solutions (citric acid, lemon juice) caused protein denaturation, resulting in decreased water binding ability of myosin, actin and other myofibrillar proteins. Prawns marinated in sodium tripolyphosphate solution (pH 7.0) did not show a significant change in muscle toughness. But immersion in calcium chloride (CaCl₂) at pH 7.0,

citric acid (pH 3.0) and lemon juice (pH 3.0) increased (p<0.05) the muscle toughness of marinated prawn (Xiong *et al.*, 2002).

5. Conclusion

Dipping frozen-thawed white shrimp in food additives was an important step to get a good quality of final process product. The treatments with non-phosphate additives can be an alternative way to improve the quality of frozen white shrimp. Overall, the treatments increased the WG (15.65%), MC (84.00%) and WHC (94.50%), lowered CL to 10.15% and reduced TVC for about 1.78 log cycle. Immersion white shrimp in 5% MTGase for 30 mins improved the quality when compared with control.

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