Effects of Transglutaminase and Kappa - carrageenan on the Physical and Sensory Qualities of Fish (*Pangasius hypophthalmus*) Patties

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Abstract

The objective of this research was to study the effect of transglutaminase (TGase) and κ-carrageenan (CG) on the physical and sensorial qualities of a fish patty compared with control (with 0.2 % phosphate). A 3 × 3 full factorial in Complete-Randomized-Design was performed using TGase at 0, 0.3 and 0.6 % with CG at 0, 0.5 and 1 % (w/w), respectively. The results indicated that cooking loss of fish patties containing TGase alone at 0.3 % and 0.6 % was not different from the control. On the contrary, the cooking loss was likely to decrease when 0.3 % TGase was applied with CG at high level compared to other formulations. Regarding the percentage of expressible moisture content (% EMC), the results showed that increasing the level of CG alone has no significant effect on the % EMC ($P > 0.05$), whereas using TGase with CG yielded fish patty with lower % EMC ($P > 0.05$). The lowest % EMC value was found in sample containing TGase/CG at 0.3/1.0 %, (w/w). It had lower Δ cooking loss and higher hardness (4.37 N) than the control (3.14 N) with the overall like score of 6.5 (slightly to moderately like). Providing the health benefit of developed fish patty to consumers, the consumers' purchase intention increased from 60 % to 79 %. This study indicated that TGase and CG could be applied together at the appropriate amount for producing fish patty with selected comparable quality with the control.

Keywords: fish patty, transglutaminase, κ-carrageenan, physical quality, consumer acceptability

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1. Introduction

Increasing on demand for minimally processed, safe and stable products has stimulated the interest in research on alternative technological approaches [1]. Phosphate is a food additive as GRAS (Generally Recognized as Safe) [1]. It enhances the effect of salt properties by improving the water binding capacity, cohesion, and yield of meat products [2].

However, the demand for meat products made without added phosphate is increasing [3]. The presence of excessive amounts of phosphates in the diet may affect the balance of some mineral contents such as calcium, iron and magnesium in the human body as well as can cause the risk of bone diseases [2]. Moreover, higher concentrations of phosphate affect the flavour of the finish product. Transglutaminase (TGase) initiates the formation of covalent bonds between glutamine and lysine residues in proteins [1, 3, 4]. The addition of TGase can improve the thermal stability of meat and fish proteins, imparting desirable properties to reconstructed products during heating [1].

Several researchers reported the positive effect of TGase on the texture of various products such as restructuring pork shoulder, chicken meat, beef and fish [4-6]. In addition, dietary fiber, for instance, carrageenans, pectins and konjac have been used by the meat industry for their gelling properties. The capacity to form a gel and its characteristics depend on various factors such as concentration, temperature, presence of certain ions and pH. Thermo-irreversible gels are used to bind diced meat pieces as cold-set binders and produce restructured meat products [7]. The addition of dietary fibre could improves the nutritional value, textural quality and cooking yield of meat product by increasing water and fat binding capacities [8, 9]. There have been various studies regarding the application of either TGase or dietary fiber in meat products. The TGase was reported to enhance gel strength of meat product after cooking at low-salt levels, while some dietary fiber contributed to water-binding ability improvement and cold-set gel formation. Therefore, combining of TGase with dietary fiber would be the potential advantage of improving the quality of patty product at lower salt level more than using TGase alone [10].

Pangasius hypophthalmus is one of the major fish species in the Mekong River fishery, one of the largest and most important fisheries in the world [11]. However, according to its soft texture, it was still underutilised to produce processed food. The study on the quality of underutilised fish fillet due to its texture by using both enzyme and dietary fiber without phosphate added was limitedly reported. Therefore, objectives of this study were 1) to study the effects of transglutaminase and carrageenan on the quality of underutilised fish fillet and 2) to evaluate the consumer acceptability on the developed fish patty product.

2. Materials and Methods

2.1 Materials

Fish fillet from *Pangasianodon hypophthalmus* was provided by Charoen Pokphand Foods Public Company Limited, Thailand. All ingredients used in the formulation were food grade. Transglutaminase (TGase) with actively of 100 unit/g dry matter was supplied by Ajinomoto Co., (Thailand) Ltd., Bangkok, Thailand and consisted of 99 % maltodextrin and 1 % enzyme on a mass basis.

2.2 Methods

2.2.1 Amino acid profiles of fish (*Pangasianodon hypophthalmus*) fillet

Amino acid profiles of fish fillet were analysed according to AOAC [12].

2.2.2 Determination of pH and TVB-N value of raw material

The pH of fish fillet as raw material was determined according to Choi *et al.* [13] using a pH meter (CyberScan 2500, EUTECH instrument, Singapore). The TVB-N value was measured in duplicate following the method of Hong and Chin [10].

2.2.3 Preparation of Fish patties

To prepare the fish patties, fish fillet was thawed at 4ºC for 16 h prior to processing. It was then minced using a grinder. Two hundred grams of each treatment was prepared and 0.25 % of salt was added to the batters. The levels of TGase (0, 0.3, and 0.6 %, w/w) and carrageenan (CG at 0, 0.5, and 1.0 %, w/w) added to the formulations were determined using factorial 3 × 3 in CRD design. The batter was mixed using a hand-blender at low speed (approximately 12,000 rpm) for 2 min. After that, the batters were stuffed into cylindrical aluminium moulds with a diameter of 65 mm and height of 12 mm. Each sample was then heated on the pan to a final internal temperature of 72ºC, and thereafter cooled down until reached a core temperature of 25ºC. Then patties were subjected to the selected measurements. The fish patty containing 0.2 % sodium tri-polyphospate without TGase and CG was used as the control.

2.2.4 Measurement of fish patty physical property

Cooking loss of fish patties was determined with six measurements for each treatment/batch and calculated by the weight differences between of each patty before and after cooking as the following equations:

$$\text{Cooking loss (\%)} = \frac{[(\text{weight of raw sample (g)} - \text{weight of cooked sample (g)})] \times 100}{[\text{weight of raw sample (g)}]} \quad (1)$$

$$\Delta\,\text{Cooking loss (\%)} = \frac{[(\text{cooking loss of sample (\%)} - \text{cooking of the control (\%)})] \times 100}{[\text{cooking of the control (\%)}]} \quad (2)$$

Colour profiles of fish patties were measured in quadruplicates using a Hunter Lab colour meter (ColorFlex E2, Hunter Associates Laboratory, Reston, VA, USA) and reported as the L* value (metric lightness), a* (redness/greenness), and b* (yellowness/blueness).

Textural profile analysis (TPA) of fish patties (core temperature of 72ºC) was evaluated in quadruplicates using a texture analyser (LF500, Lloyd instruments Ltd., Fareham, UK). The fish patty slice (30 × 30 × 10 mm^3) was compressed to 30 % deformation using a compression probe (diameter of 48 mm) at a cross-head speed of 50 mm/min. The value of hardness, springiness, cohesiveness and gumminess were determined.

2.2.5 Measurement of expressible moisture content

Fish patty samples were determined for expressible moisture content with triplicate measurements following the method of Kaewudom *et al.* [4]. The 5 mm thickness of cylindrical gel samples, weighted accurately (X), were placed between three pieces of Whatman paper No.4 at the bottom and two pieces on the top of the sample. The standard weight (5kg) was placed on the top and held for 2 min. Subsequently, the samples were removed from the papers and weighed (Y). Expressible moisture content was calculated by using the following equation:

$$\text{Expressible moisture content (\%)} = [(X - Y)/X] \times 100 \qquad (3)$$

2.2.6 Evaluation of sensory property

The only selected fish patties were used for sensory evaluation with the criteria of low cooking loss (positive value) as well as hardness values comparable with the control. The respondents were regular consumers who consume fish with no allergic to seafood products. They were served with 3-digit code of samples as counter-balance followed the randomized block design. The sample for each session was not over 4 samples. Respondents (n = 30) provided a liking score for appearance, overall flavour, softness, and overall like using the 9-point hedonic scale. They were provided with white bread and water to rinse their mouths before testing and between samples.

2.2.7 Evaluation of consumer acceptability

Consumer acceptability was conducted in partitioned booths illuminated with cool, fluorescent lights. Consumers (n = 60) who were not allergic to fish or sea food product were recruited. Each consumer was served with the selected fish patty formulation coded with a 3-digit number comparing with the control. They were provided with white bread and water to cleanse their palate before testing and between samples. They were asked to rate acceptability of appearance, texture softness, flavour and overall like using the 9-point hedonic scale on a paper ballot. Consumers were asked to provide the purchase intention before and after providing the benefit of product about using natural food additives. The 5-point Likert scale was used (1= definitely not purchase, 3 = neither purchase nor not purchase and 5 = definitely purchase).

2.2.8 Statistical analysis

All results were subjected to analysis of variance (ANOVA). The Duncan's Multiple Range Test (DMRT) was performed for post-hoc multiple comparison. Statistically significant difference was established at alpha=0.05.

3. Results and Discussion

3.1 Amino acid profile of fish fillet (*Pangasius hypophthalmus*)

Fish fillet from *Pangasius hypophthalmus* was too soft to make processed fish product such as fish ball or fish patty according to its weak protein structure. The fillets could be transformed into high-value products by the restructuring technology [13]. These technologies are used to obtain novel products using additives to improve the mechanical and functional properties.

However, several biochemical and physicochemical considerations regarding muscle proteins must be taken into account to obtain high-quality products. The amino acid profile was conducted to measure focusing on lysine and glutamine amino acid which are the main substrate of transglutaminase (TGase).

The results indicated that *Pangasius hypophthalmus* contained amino acid as shown in Table 1. The highest amount of amino acid found in the fish fillet was glutamic acid and lysine (1369.08 and 903.47 mg/100g), respectively. Moreover, the methionine was found to be at least

175.34 mg/100g. Therefore, TGase could be used to improve the texture of the finish product made from *Pangasius hypophthalmus* fillet, which contains substrates (glutamic acid and lysine) of the enzyme. The TGase catalyses an acyl transfer reaction between protein-bound glutaminyl residues and primary amine [1, 3] and can improve the thermal stability of meat and fish proteins, imparting desirable properties to reconstructed products during heating [1].

Table 1. Amino acid profiles (mg/100g) of fish fillet (*Pangasius hypophthalmus*)

Amino acid	Amount (mg/100g)
Alanine	685.46
Arginine	651.25
Aspartic acid	998.65
Cystine	138.57
Glutamic acid	1,369.08
Glycine	412.06
Histidine	449.22
Hydroxylysine	ND
Hydroxyproline	ND
Isoleucine	407.73
Leucine	791.89
Lysine	903.47
Methionine	175.34
Phenylalanine	380.61
Proline	387.32
Serine	443.16
Threonine	501.73
Tryptophan	203.93
Tyrosine	456.21
Valine	412.39

*ND = Not detected

3.2 pH and total volatile basic nitrogen (TVB-N) of fish fillet

The results showed that the fillet of *Pangasius hypophthalmus* was in the basic condition (pH 8.37) which still meet the quality standard of white fish meat (pH should be above 7.0). Total Volatile Bases Nitrogen (TVB-N) is one of the most widely used methods to estimate the degree of decomposition of fish. The level of TVB-N for white fish is generally considered to be fresh if the TVB-N is less than 20 mg/100 g sample [15]. The results showed that TVB-N of the raw material was of 8.34 mg/100 g which was in good quality and conformed to the standard [15].

3.3 Physicochemical property of the fish patties

The result indicated that pH of the fish patty increased when TGase and CG was added in the different levels as shown in Table 2. As the level of TGase and CG increased, the pH of the fish patties increased. This may be attributed to both enzyme and additive themselves having pH for TGase and CG normally ranged from 7.0 - 10.0 and 6.0 - 8.0, respectively [16].

In respected to the cooking loss, the tendency of decrease in a discrepancy of cooking loss (Δ cooking loss) was observed. Increasing the level of TGase decreased in the Δ cooking loss among all samples comparing with the control. The TGase was reported to induce the acyl transfer between acyl donors to acyl acceptor, in which ε-(γ-glutamyl) lysine linkage could be formed which contributed to the increase in gel strength [4]. Similar results were observed in the study of Moreno *et al.* [17], who reported that the water holding capacity of restructured fish gel from hake increased when 1 % MTGase was added. Trespalacios and Pla [6] also reported that the addition of 0.5 % MTGase, to beef homogenates significantly decreased expressible moisture content as well as the cooking loss. The results indicated that 0.3 % TGase was sufficient to reduce the cooking loss of the samples.

Table 2. The effect of transglutaminase (TGase) and carrageenan (CG) on the selected quality of fish patties

Sample[A] TGase (%)	CG (%)	pH	Δ Cooking loss (%)[B]	L*	a*	b*
Control		8.60±0.02c	0.0	69.51±1.18a	-2.82±0.09c	7.90±0.17ab
0.0	0.0	8.27±0.02e	5.9	68.28±0.52a	-2.84±0.14c	6.25±0.35b
0.3	0.0	8.55±0.02d	11.4	69.08±1.77a	-2.72±0.19bc	7.14±0.65ab
0.6	0.0	8.50±0.02d	2.8	69.08±1.54a	-2.81±0.26c	6.77±0.49b
0.0	0.5	8.58±0.02c	22.4	69.67±1.13a	-2.70±0.09abc	7.73±0.49ab
0.3	0.5	8.52±0.01d	17.7	69.05±0.18a	-2.58±0.09abc	6.52±0.52b
0.6	0.5	8.79±0.03b	-7.9	69.79±0.25a	-2.32±0.03a	6.44±0.25b
0.0	1.0	8.50±0.02d	-21.6	68.24±0.70a	-2.45±0.09abc	8.02±0.21ab
0.3	1.0	8.57±0.05c	-5.1	69.06±1.44a	-2.38±0.16ab	8.38±1.54ab
0.6	1.0	9.08±0.04a	5.6	67.25±1.36a	-2.46±0.06abc	9.23±1.50a

Mean ± standard deviation from six measurements for pH and cooking loss and triplicate measurement for colour profiles. Means with the different letters in a column were significant different ($P < 0.05$).

[A]The sample with phosphate served as the control. Sample with 0 % TGase and 0 % CG was the sample without phosphate.

[B](+) Δ cooking loss showed higher cooking loss but (-) Δ cooking loss showed lower cooking loss than the control.

Without TGase, the sample containing only CG at the highest level (1 %) had the lowest Δ cooking loss (-21.6 %). However, when only 0.5 % CG was applied, it had no effect on the reduction of Δ cooking loss of the samples. When the highest level of TGase (0.6 %) and CG (1 %) was incorporated in the formulation, the higher Δ cooking loss was noticeable. This may be attributed to the greater interaction between protein molecules with the denser network in the patty matrix. This led to more free water released from network [18] resulting in higher cooking loss of the sample.

The determination of colour profiles showed that there were no significant differences in lightness of the sample obtained with and without TGase and CG. It was probably due to the colour of CG that was white powder (L^* = 90.28), and the inactivation step of enzyme during cooking. The result was similar to the study of Kaewudom *et al.* [4] who reported that there was no difference in whiteness of surimi gel from threadfin bream (*Nemipterus bleekeri*) with increasing levels of MTGase.

In addition, the sample containing TGase and CG at the appropriate levels obtained texture profiles comparable to the control. In the presence of only TGase, the hardness value of sample containing 0.3 % TGase was lower than other concentrations (Table 3).

Table 3. The effect of transglutaminase (TGase) and carrageenan (CG) on the textural quality of fish patties

Sample		Hardness (N)	Cohesiveness index	Springiness	Gumminess
TGase (%)	CG (%)				
Control		3.14±0.30abc	0.51±0.14ab	0.82±0.02a	1.58±0.39ab
0.0	0.0	3.75±1.25ab	0.46±0.13ab	0.83±0.08a	1.66±0.73ab
0.3	0.0	1.94±0.67c	0.43±0.10ab	0.79±0.03a	0.88±0.49b
0.6	0.0	3.23±0.64abc	0.28±0.09b	0.84±0.03a	0.92±0.36b
0.0	0.5	4.04±0.69ab	0.54±0.11a	0.85±0.06a	2.16±0.55ab
0.3	0.5	3.28±1.39abc	0.42±0.13ab	0.83±0.06a	1.41±0.89ab
0.6	0.5	2.46±0.68bc	0.47±0.15ab	0.83±0.07a	1.17±0.55ab
0.0	1.0	4.56±1.17a	0.48±0.16ab	0.84±0.05a	2.25±1.04ab
0.3	1.0	4.37±1.63a	0.57±0.21a	0.85±0.07a	2.57±1.54a
0.6	1.0	3.11±0.60abc	0.59±0.14a	0.84±0.10a	1.90±0.79ab

Mean ± standard deviation from six measurements for pH and cooking loss and triplicate measurements for color profiles. Means with the different letters in a column were significant different ($P < 0.05$).

Comparing among all samples containing only CG, the level of CG had significant effect on hardness of fish patties. As the level of CG increase, hardness and gumminess value of fish patty significantly increased ($P < 0.05$). This result was in agreement with the study of Cardoso *et al.* [19], who stated that the CG acted as a simple filler of the myofibrillar protein gel, when increasing CG the hardening effect on restructured fish with dietary fibre was considerably observed. Cierach *et al.* [20] also found that CG caused a reduction in cooking loss and increased hardness and gumminess of the low-fat frankfurter. Moreover, Brewer [21] revealed that incorporated into low-fat meat formulations, CG improved the textural characteristics of the product by decreasing toughness and increasing juiciness.

When TGase mixed with CG, the result showed that as the levels of TGase and CG increased, the hardness of fish patties was significantly increased. However, increasing TGase up to 0.6 % did not cause further hardening effect on fish patties more than at 0.3 %. In the presence of 0.6 % TGase and 1.0 % CG, the fish patty had higher Δ cooking loss than the control. This result is consistent with Min and Green [9] who reported that the addition of MTGase increased textural properties such as binding strength, hardness, cohesiveness, chewiness, and springiness, but decreased cooking yield of the patties made from channel catfish (*Ictalurus punctatus*).

3.4 Expressible moisture content

The loss of water may result in shrinking of the gels, changing texture and reducing quality of food. Therefore, water holding capacity is an important criterion in evaluation of the acceptability of food gels [22]. Expressible water content of samples added with CG and TGase at different levels was significantly different as summarized in Figure 1. The expressible moisture content of fish patty decreased as the level of TGase increased. The result showed that the addition of TGase was able to hold water in the patty as observed by the decrease in expressible moisture content.

Additionally, when TGase and CG was incorporated together, it could induce protein cross linking at the appropriate level. The results showed that 0.3 % TGase and 1.0 % CG was sufficient to decrease the expressible moisture content. However, TGase at 0.6% with CG at 1.0 % had no effect on the reduction of expressible moisture content MTGase at the level above 0.4 unit/g did not cause the reduction in expressible moisture content in gel from unwashed mince

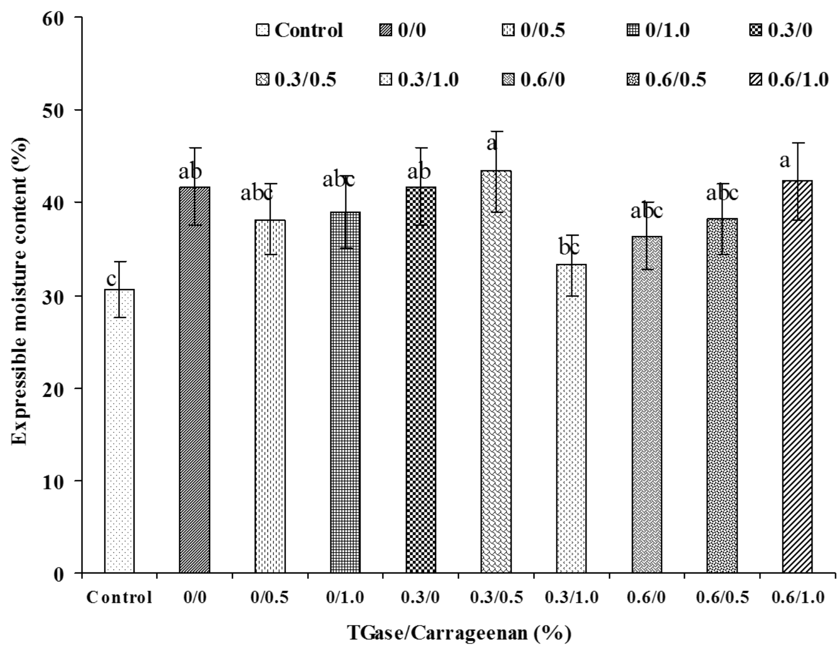

Figure 1. Effect of TGase and CG on the expressible moisture content (EMC) among fish patty formulations compared with the control (sample with1% phosphate)
X/Y = the level of TGase (0, 0.3, and 0.6 %)/CG (0, 0.5 and 1.0 %), for example, 0.3/0.5 = 0.3 % TGase with 0.5 % CG

Indian mackerel fish [15]. However, Zhu *et al.* [23] indicated that the formation of cross-linking was not enhanced significantly in the treatments, and non-covalent interactions could be important roles in producing the microstructure of the gel.

3.5 Sensory quality

The selected fish patty formulations were screened and selected with the criteria of low cooking loss and expressible water comparing with the control. The result showed that TGase and CG significantly affected sensorial quality of fish patty products ($P < 0.05$). The sample without either

TGase or CG had lower score of appearance acceptance (5.9 vs 5.6) (Table 4). However, there were no significant effect on the texture and overall like of all formulations compared to the control.

However, the addition of TGase and CG had no effect on the appearance acceptance and a reduced effect on flavour acceptance among all samples. The similar effect was found as the previous study of Dimitrakopoulou *et al.* [2], who reported that transglutaminase significantly affected ($P<0.05$) the overall acceptability of the restructured cooked pork shoulder while it had no effect on the colour, odour and taste of the product.

Additionally, comparing between TGase 0.3 % and 0.6 % at all levels of CG, the result showed that there was no significant difference of all sensory attributes. Thus, the addition of TGase could be incorporated with CG at 0.3 % and 1.0 %, respectively to produce fish patties from *Pangasius hypophthalmus* with desirable texture, cooking loss and acceptability compared with the control. These levels of TGase and CG would be used to further evaluate consumers' acceptability of the developed fish patty.

Table 4. Preliminary sensory evaluation screening fish patty with different levels of TGase and carrageenan (CG) (n = 30)

Sample		Appearance	Texture	Flavour	Overall like
TGase (%)	CG (%)				
Control		6.4 ±1.0ab	6.3±1.1a	6.2±1.4a	6.5±1.1a
0.0	0.5	5.9±1.5ab	6.1±1.3a	5.4±1.8b	6.4±1.2a
0.3	0.0	6.2±1.3ab	6.3±1.2a	5.7±1.6ab	6.8±1.1a
0.3	1.0	6.5±0.9a	6.4±1.1a	6.4±1.2a	6.6±1.1a
0.6	0.0	5.6±1.3b	6.3±1.1a	5.2±1.4b	6.2±1.2a
0.6	1.0	6.4±1.2ab	6.7±1.0a	6.3±1.3a	6.6±1.2a

Mean ± standard deviation with the different letters in the same column was significant different ($P < 0.05$) based on 9-point hedonic scale.

3.6 Consumer acceptability of the developed fish patty

The addition of TGase and CG could yield the product without negative effect on consumers' acceptability compared with the control (with phosphate) as shown in Table 5. There was no significant difference for all sensory attributes between fish patty containing TGase mixed with CG and the control sample. The acceptance score of all attributes ranged from 6.5 - 6.8 (slightly to moderately like) (Table 5).

Table 5 Consumers' acceptability of the final developed fish patty compared with the control (n = 60)

Sample	Appearance	Texture	Flavour	Overall like
Control	6.7±1.2a	6.8±1.5a	6.5±1.3a	6.8±1.2a
Developed product	6.7±1.4a	6.6±1.6a	6.4±1.2a	6.5±1.4a

*Mean ± standard deviation with the different letters in a row were significant different ($P < 0.05$) based on 9-point hedonic scale.

**The developed product contained TGase and CG 0.3 % and 1.0 %, respectively without added phosphate.

The purchase intention of consumers was evaluated in this study. The result indicated that before getting information of health benefit of the developed product, consumers' intend for purchasing this product was only 60 %. On the contrary, when consumers are provided with information about health benefit of the product as containing no chemical additives, it would increase willingness for purchasing the developed fish patty up to 79 % which is 19 % over the test before the information was provided (Figure 2). Therefore, TGase and CG would be potential alternative food additives to produce the fish patty with comparable textural quality, acceptability as well as the opportunity to attract consumers' attention with the control.

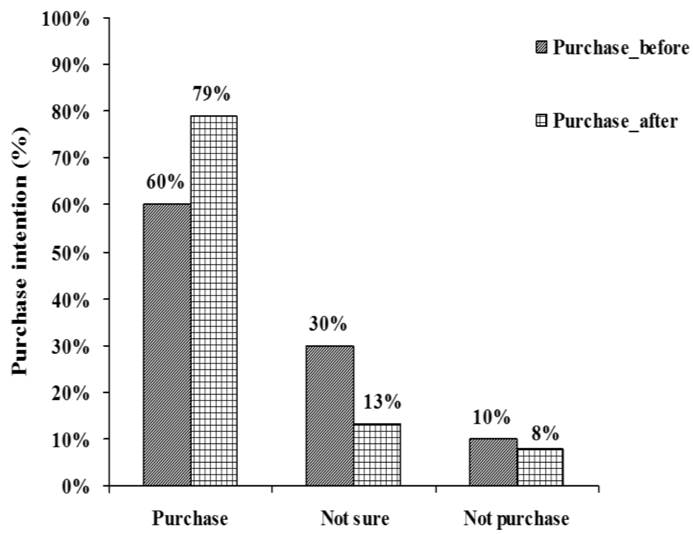

Figure 2. The purchase intention of consumers on fish patties with TGase and CG before (▨) and after (⊞) receiving the information on health benefit (n = 60)

4. Conclusions

Based on the results, it could be concluded that TGase and CG affected the physical and sensory qualities of patty from underutilized fish fillet. The results indicated that TGase was able to enhance hardness of the samples at the highest level, while increasing CG contributed to water-binding ability of the samples by lowering cooking loss and expressible moisture content compared with the control. Therefore, combining of TGase and CG would be the potential additive to improve the quality of fish patty without the addition of phosphate. TGase (0.3 %) and CG (1.0 %) was appropriate to produce fish patty with acceptable sensory quality and comparable texture to the control. Consumers' purchase intention was higher when they were provided with the health benefit of additives used in the formulation. This product could be used as the raw material for making alternative healthy product. However, the quality of the product during the storage condition needs further investigation.

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