

High Temperature Tolerant Fish Protein Gel Using Transglutaminase and Sodium Ascorbate

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ABSTRACT

To improve the textural properties of canned fish balls, we studied the influences of commercial transglutaminase (TGase) and sodium ascorbate on gelling property of threadfin bream surimi retorted at 116°C for 30 min (F_0 7.8 min). The treatment conditions were 0.2 % TG-B, 0.2 % TG-AK, 0.1 % TG-K, 0.2 and 0.3 % sodium ascorbate and 0.1 % TG-K mixed with 0.2 % sodium ascorbate. The test samples were measured for gel strength, whiteness, water holding capacity, SDS-PAGE and sensory preference. It was found that thermal processing decreased gel strength, whiteness and water holding capacity. However, TGase significantly improved the gel strength of heated gel whereas sodium ascorbate showed less effect on gel strength and lowered whiteness. Addition of 0.2 % TG-B to fish balls improved gel strength and water holding capacity; and resulted in the high preference scores for appearance, color, flavor, texture and overall liking at the level of moderate liking after heat treatment.

Key words: transglutaminase, sodium ascorbate, gelling property, fish ball, canned product

INTRODUCTION

Texture is an important property of food products made from fish meat pastes such as kamaboko and fish ball. One of the methods to improve textural properties of food is by addition of TGase. TGase enhances the formation of ϵ -(γ -glutamyl) lysine crosslinking reaction of the myosin heavy chain during setting of salted fish paste thereby contributing to firmer texture (Matsumoto and Noguchi, 1992; Motoki and Seguro, 1998). This crosslinking also occurs during high temperature treatment. Sakamoto *et al.* (1995) detected ϵ -(γ -glutamyl)lysine crosslinking in canned sardine and canned tuna. They suggested that this crosslinking could also

be generated during thermal treatment of protein without TGase activity.

Ascorbate is known as a cross-linking agent. The gel-strengthening properties of ascorbate have been reported to increase cross-linkages through oxidation of sulfhydryl (-SH) groups in proteins (Lee *et al.*, 1992a). Sodium ascorbate significantly improved gel cohesiveness and sensory firmness of fiberized products with a maximum strengthening effect at 0.2 % level (Lee *et al.*, 1992b).

According to the commercial sterility of low acid canned food (LACF), it is mandatory to process all LACF to an F_0 value equal to or greater than 3 min. In practice, the process is usually higher than this minimum $F_0 = 3$ min owing to some probability of underprocessing and spoilage

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caused by heat resistance organisms. Depending on the product and the climatic conditions of storage, a typical F_0 value used operationally for fish and meat products generally varies in the range of 5-20 min (Bratt, 1995; Pflug and Christensen, 1980). However, during longer heating at high temperature, the gel strength of protein gel decreases due to a loss of total SH plus SS groups that occur by oxidation to cysteic acid or splitting to hydrogen sulfide (Nakai and Li-Chan, 1988; Yamazawa *et al.*, 1979). H_2S formation originated from the free reacting SH groups of actomyosin starts at about 80°C and increases exponentially with rising temperature (Hamm and Hofmann, 1965). Shie and Park (1999) reported that the higher the heating temperature (at the study range of 75° to 93°C) and longer time, the lower was the shear strain and whiteness.

The objective of this experiment is to investigate the influence of both commercial TGase and sodium ascorbate on fish jelly textural properties, whiteness, water holding capacity, SDS-PAGE and sensory quality before and after canning with the aim of improving the textural properties of canned fish jelly products.

MATERIALS AND METHODS

Raw materials

Frozen threadfin bream SA grade surimi obtained from Apitoon Enterprise Industry Co., Ltd. was cut into 1 kg each, packed in polyethylene bags and kept at -18°C during experiment for 4 mo. Three kinds of commercial TGase (Ajinomoto Co., Ltd) were used namely: TG-B comprised 0.5% TGase, 2.5% sodium polyphosphate, 2.5% sodium pyrophosphate, 2.0% silicon dioxide and 92.5% milk protein and other; TG-AK comprised 0.6 % TGase, 60.0% trisodium phosphate, 39.4% soybean protein and other; TG-K comprised 1.0% TGase, 75.0% calcium lactate and 24.0% dextrin and other. These TGase were produced by microorganisms and stable up to

40°C (Ajinomoto, n.d.)

Gel preparation

Frozen surimi was thawed at 4°C overnight. The formula of salted fish paste was 100 g surimi, 2.5 g sodium chloride, 0.2 g garlic powder, 0.2 g pepper and ice for adjusting moisture content to 78 %. Surimi was chopped with the above ingredients and TGase or sodium ascorbate for 10 min in a food processor (MARA KF 1692). The concentrations of TGase and ascorbate used were 0.2 % TG-B, 0.2 % TG-AK, 0.1 % TG-K, 0.2 and 0.3 % sodium ascorbate and 0.1 % TG-K mixed with 0.2 % sodium ascorbate. The salted fish paste was then stuffed into a cylindrical case (2.5 cm × 2.5 cm) and was formed into 2.5 cm diameter fish balls. They were incubated at the setting temperature of 40°C for 20 min and subsequently heated at 90°C for 20 min. After cooling the gels in ice-water bath for 5 min, they were kept at 4°C overnight prior to canning.

Canning treatment

75 g of fish ball were packed in 307 × 113 can and filled with 115 ml of 2 % NaCl solution. After seaming, the cans were heated in an overpressure retort FMC 091-3 (USA) at 116°C for 30 min. F_0 was calculated from heating data collected by data logger (Pressica 2001E) using the general method (Bigelow *et al.*, 1920).

Texture analysis

The penetration test was determined as described in MFRD (1987) with a minor modification. A 5 mm diameter spherical probe was allowed to penetrate at the speed of 1.1 mm/sec using a texture analyzer TA-HD (Stable Micro System, Surrey, UK). The peak force point was called a breaking strength (g) and the distance from the starting point to the peak force point was called a deformation (cm). Gel strength (g. cm) was the multiplication of breaking strength by deformation.

Whiteness measurement

Six samples from each treatments were measured for the degree of lightness L^* , redness a^* and yellowness b^* using a Chroma meter CM-3500d (Minolta, Japan). Whiteness was calculated as $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$ (Lanier, 1992).

Water holding capacity

The water holding capacity was determined as described by Motohiro (1981) with minor modification. Fish ball (cut into cubes of $1.5 \times 1.5 \times 0.5 \text{ cm}^3$) was pressed between filter papers at 10 kg/cm^2 for 2 min. The moisture remaining in the sample was expressed as the percentage of water holding capacity.

SDS-PAGE

0.25 g gel was solubilized in 10 ml of 8 M urea-2 % SDS-0.05M sodium phosphate buffer (pH 6.8) containing 10 % 2-mercaptoethanol (Merck, AR grade). SDS-PAGE was carried out on the 5% acrylamide continuous gel according to Weber and Osborn (1969). The pattern of protein as polymer, myosin heavy chain (MHC) and actin (AC) was evaluated.

Sensory evaluation

Fish ball was evaluated for appearance, color, odor, flavor, texture and overall liking by 20 untrained panels using a 9-point hedonic scale.

Statistical analysis

Treatments were conducted in duplicate. Gel strength, whiteness and water holding capacity data were analysed by complete block design. Sensory data was analysed by randomized complete block design. The differences of means were resolved by Duncan's new multiple range test (DMRT).

RESULT AND DISCUSSION

Commercial TGase and sodium

ascorbate were subjected to fish ball prior to heat treatment and the results of gel strength, whiteness and water holding capacity are shown in Figure 1.

Gel property

Before canning, all TGase fish balls had the higher gel strength than control (Figure 1a) owing to the formation of $\epsilon-(\gamma\text{-glutamyl})\text{lysine}$ crosslink enhancing by TGase (Matsumoto and Noguchi, 1992; Motoki and Seguro, 1998). 0.3 % sodium ascorbate sample showed no difference in gel strength from control whereas that of 0.2 % sodium ascorbate was slightly lower than control.

After canning, the gel strength of all samples decreased. The decrease in gel strength of canned fish balls might be owing to a loss of total SH plus SS groups occurs by oxidation to cysteic acid or splitting to hydrogen sulfide (Nakai and Li-Chan, 1988; Yamazawa *et al.*, 1979). Hydrogen sulfide was found to be the most prominent off-flavor component of retorted Kamaboko, by means of sensory investigation and chemical analysis (Yamazawa *et al.*, 1979).

However, the gel strength of fish balls mixed with 0.2 % TG-B and mixture of 0.1 % TG-K and 0.2 % sodium ascorbate decreased to 75.8 % and 83.87 % of the original gel strength, respectively and was higher than those of control and the other samples ($p < 0.05$) which decreased to the range of 52.6 % to 57.7 %. As for the lower effects of TG-AK and TG-K alone on the gel strength of canned fish ball, this might suggest that some bonds between some ingredients such as milk protein in TG-B and fish protein had greater thermal tolerance than those bonds in TG-AK and TG-K. Tsai *et al.* (1996) reported that TGase can enhance the crosslinking between various proteins such as casein and soybean, casein and myosin, soybean and myosin, whey protein and protein. This action was useful in improving the texture of surimi products. Mixture of 0.1 % TG-K and 0.2 % sodium ascorbate also affected the gel strength. It might be a combination effect between sodium

ascorbate and a major component (calcium lactate) in TG-K. Gel strength of canned ascorbate samples decreased due to the S-S bond enhanced from

sodium ascorbate addition that split to hydrogen sulfide at high temperature (Nakai and Li-Chan, 1988; Yamazawa *et al.*, 1979).

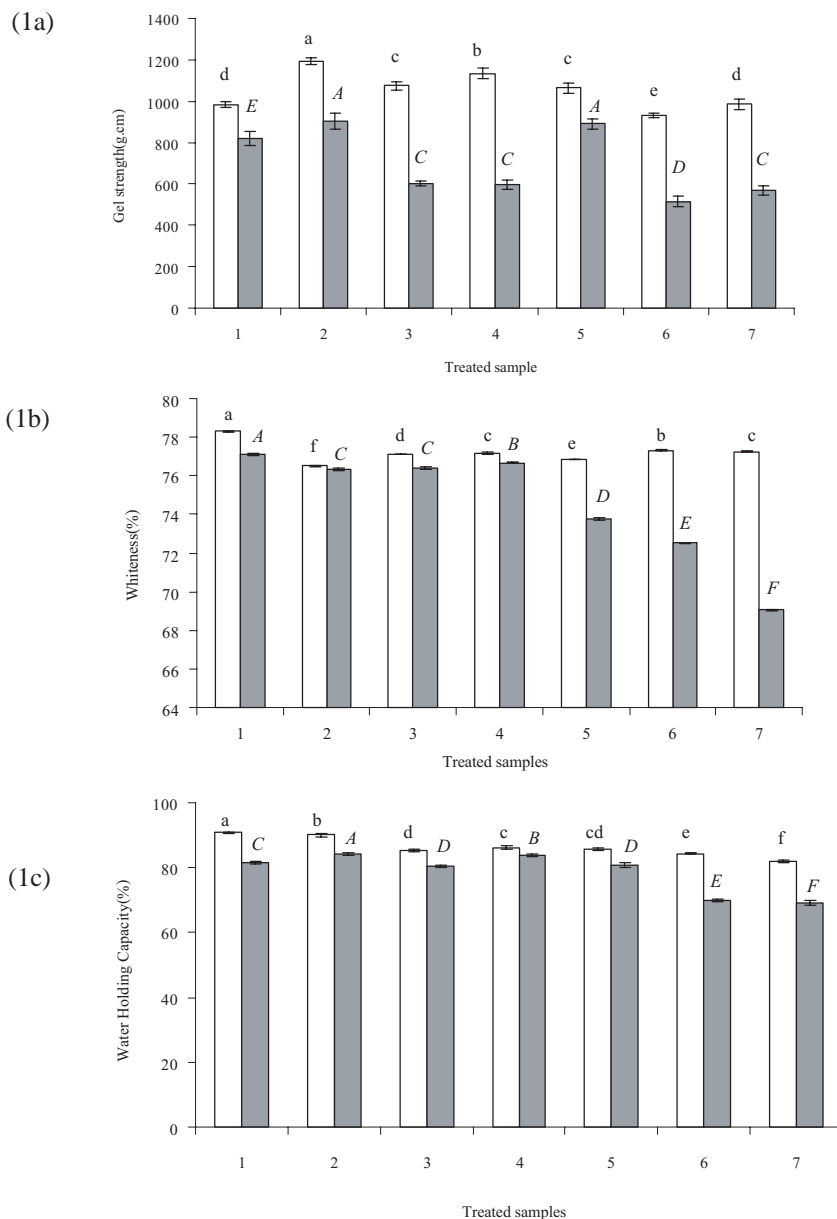


Figure 1 Gel strength (1a), whiteness (1b) and water holding capacity (1c) of canned fish ball sterilized at 116°C for 30 min. Treated samples: (1) control, (2) 0.2 % TG-B, (3) 0.2 % TG-AK, (4) 0.1 % TG-K, (5) 0.1 % TG-K with 0.2 % sodium ascorbate, (6) 0.2 % sodium ascorbate, (7) 0.3 % ascorbate. a-e or A-F means (n = 6) with different letters are significantly different (p < 0.05) □ before canning ■ after canning

Whiteness

Before canning, all treated samples showed slightly lower whiteness than the control, but after canning, the decrease of whiteness was clearly visible in the ascorbate added sample, because of its yellowness (Figure 1b). Nishimura *et al.* (1994) also reported that heated-induced gel with 0.2 % ascorbic acid heated at 90°C for 10 min gave darker color after incubation at 40°C for 10 min due to the influence of ascorbic acid oxidation.

Water holding capacity

Water holding capacity of control sample was the highest before canning but only slightly higher than TG-B sample (Figure 1c). After canning, TG-B fish ball presented the highest in water holding capacity. It was shown that after canning, the water holding capacity of TGase fish ball decreased less than that of ascorbate fish ball. Lee *et al.* (1992b) also reported that fish gel mixed with 0.2% sodium ascorbate steamed at 90°C more than 20 min was denatured and showed the changes in protein network structure resulting in a loss of water holding capacity.

SDS-PAGE

Figure 2 shows SDS-PAGE patterns of proteins extracted from fish ball samples before and after canning. All solubilized samples were

reduced by 2-mercaptoethanol in order to reduce disulfide bonds to sulfhydryl groups. Prior to canning, it was shown that the MHC intensities were higher in the control and ascorbate samples showing disulfide bond occurring. For TGase samples, TGase enhances myosin polymerization through isopeptide of (γ -glutamyl)lysine linkage and urea and 2-mercaptoethanol added in SDS buffer cannot reduce this linkage, therefore, MHC polymers were located tightly on the top gel resulting in the thinner MHC remaining. After canning, MHC and actin bands almost disappeared in all samples that agreed with a decrement in gel strength. It seems that disulfide bond is sensitive to severe heat as shown in ascorbate sample. High temperature caused damage to protein in which the formation of 'isopeptides' by reaction of the α -amino group of lysine is probably included (Hofmann, 1977) and formed hydrogen sulfide (Nakai and Li-Chan, 1988; Yamazawa *et al.*, 1979). The decrement of actin band was lower than MHC as same as the results of Hofmann (1977) and Runglerdkriangkrai (1999).

Sensory evaluation

Hedonic scores of fish ball before and after canning are shown in Table 1 and 2 respectively. Before canning, preference scores of all samples were not significantly different

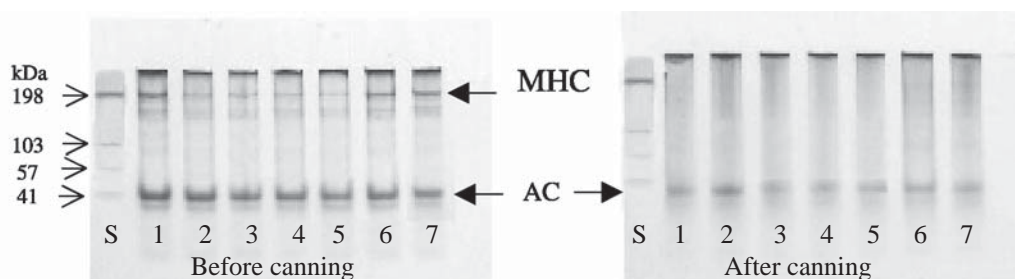


Figure 2 SDS-PAGE with β -mercaptoethanol of canned fish ball mixed with TGase and sodium ascorbate sterilized at 116°C for 30 min.; (S) Standard protein (1) control, (2) 0.2 % TG-B, (3) 0.2 % TG-AK, (4) 0.1 % TG-K, (5) 0.1 % TG-K with 0.2 % sodium ascorbate, (6) 0.2 % sodium ascorbate, (7) 0.3 % ascorbate; MHC (Myosin heavy chain), AC (Actin)

($p \leq 0.05$) in odor, flavor, texture and overall liking but the appearance and color scores of ascorbate samples both before and after canning were lower since their colors were darker and yellower. All canned samples showed no differences for odor scores. While TGase samples showed higher scores in flavor and texture than the control and ascorbate samples due to the gel strength and water holding capacity decrement. Overall liking scores of TGase samples alone were higher than the others at the level of moderate liking.

CONCLUSIONS

TGase significantly improved of the texture of canned fish jelly products whereas sodium ascorbate showed less effect on texture and lowered whiteness. Addition of 0.2 % TG-B was suitable for improving the gel strength and water holding capacity and gave the good whiteness of canned fish ball. Moreover, the overall liking score was still rated in moderate liking.

Table 1 Hedonic scores of fish ball mixed with TGase and sodium ascorbate before canning.

Characters	Means \pm Standard deviation					
	Appearance	Color	Odor ^{ns}	Flavor ^{ns}	Texture ^{ns}	Overall liking ^{ns}
Control	7.7 ^a \pm 0.6	8.0 ^a \pm 0.5	7.0 \pm 1.3	7.1 \pm 0.8	7.6 \pm 0.8	7.5 \pm 0.8
0.2% TG-B	7.9 ^a \pm 0.6	8.1 ^a \pm 0.5	7.1 \pm 0.8	7.1 \pm 0.7	7.3 \pm 1.2	7.3 \pm 0.9
0.2% TG-AK	7.7 ^a \pm 0.5	7.9 ^a \pm 0.5	6.7 \pm 0.9	6.9 \pm 0.8	7.4 \pm 1.0	7.3 \pm 0.8
0.1% TG-K	7.9 ^a \pm 0.8	7.9 ^a \pm 0.8	6.9 \pm 1.1	6.7 \pm 0.7	7.4 \pm 1.1	7.3 \pm 0.8
0.1% TG-K + 0.2%	6.9 ^b \pm 0.9	6.9 ^b \pm 0.5	7.1 \pm 0.9	6.6 \pm 0.5	7.4 \pm 0.9	7.1 \pm 0.8
Sodium ascorbate						
0.2% Sodium ascorbate	7.0 ^b \pm 0.8	7.1 ^b \pm 0.5	7.3 \pm 0.9	6.9 \pm 0.7	7.7 \pm 0.8	7.3 \pm 0.8
0.3% Sodium ascorbate	7.2 ^b \pm 0.9	7.1 ^b \pm 0.7	6.8 \pm 0.9	7.0 \pm 0.7	7.4 \pm 0.7	7.3 \pm 0.6

^{ab} Means in the same column with different superscripts are significantly different ($p \leq 0.05$)

^{ns} Means of all treatments are not significantly different ($p > 0.05$) by DMRT.

Table 2 Hedonic scores of fish ball mixed with TGase and sodium ascorbate after canning at F_0 value 7.8 min.

Characters	Means \pm Standard deviation					
	Appearance	Color	Odor ^{ns}	Flavor ^{ns}	Texture ^{ns}	Overall liking ^{ns}
Control	7.6 ^a \pm 0.5	7.8 ^{ab} \pm 0.5	6.7 \pm 0.8	6.9 ^b \pm 0.7	7.2 ^b \pm 0.6	6.9 ^b \pm 0.6
0.2% TG-B	7.8 ^a \pm 0.6	7.9 ^a \pm 0.5	6.9 \pm 0.7	7.4 ^a \pm 0.5	7.8 ^a \pm 0.4	7.3 ^a \pm 0.4
0.2% TG-AK	7.7 ^a \pm 0.6	7.7 ^{ab} \pm 0.7	7.1 \pm 0.7	7.3 ^a \pm 0.5	7.8 ^a \pm 0.7	7.6 ^a \pm 0.6
0.1% TG-K	7.6 ^a \pm 0.7	7.6 ^{ab} \pm 0.6	7.0 \pm 0.7	7.4 ^a \pm 0.5	7.6 ^a \pm 0.5	7.3 ^a \pm 0.5
0.1% TG-K + 0.2%	6.7 ^b \pm 0.8	7.5 ^b \pm 0.6	6.7 \pm 1.0	7.4 ^a \pm 0.5	7.7 ^a \pm 0.5	6.9 ^b \pm 0.5
Sodium ascorbate						
0.2% Sodium ascorbate	6.8 ^b \pm 0.8	6.1 ^c \pm 0.5	6.8 \pm 1.0	7.2 ^a \pm 0.5	6.6 ^c \pm 0.8	6.5 ^c \pm 0.6
0.3% Sodium ascorbate	7.0 ^b \pm 0.9	6.3 ^c \pm 0.4	6.7 \pm 1.0	6.8 ^b \pm 0.7	6.3 ^c \pm 0.6	6.2 ^c \pm 0.5

^{ab} Means in the same column with different superscripts are significantly different ($p \leq 0.05$)

^{ns} Means of all treatments are not significantly different ($p > 0.05$) by DMRT.

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