

Effect of Washing and Additives on Gel Formation of Squid Surimi

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Effects of washing and additives on the texture of squid surimi gel which has been known to hard to gelation due to high protease activities and many water solubles were studied by SDS-PAGE, compression test, jelly strength and transmission electron microscopy analysis (TEM).

Myosin (205 kDa) heavy chain was the major protein in water soluble fractions. It was impossible to make a gel after washing of the minced squid meat. These results suggested that squid (*Todarodes pacificus*) minced meat does not need a washing for good jelly products. 3.0% of bovine plasma protein (BPP) produced the hardest gel (16% harder than the control) among the additives including egg white (EW), potato extracts (PE) and transglutaminase-K (TG-K) by compression test ($P > 0.05$). Microstructure of control, 2% EW and 4% TG-K treated gels showed a sponge-like structure with more vacant space. Gels containing 3% BPP formed the most rigid and arranged networks. Those results indicates that poor gel-network formation was due to the degradation of myofibrillar proteins by proteases contained in the minced meat, which result in non-interlinkage.

Key words : squid, texture of gel, washing and additive effects, water soluble protein

Introduction

Squid (*Todarodes pacificus*) has not been used for gel products because of its low gel forming ability. There are many reports on the proteases in squid mantle which may cause myosin degradation and influence textural quality of cooked squid meat. They may be responsible for rapid autolysis and different muscle type of squid which are differ from those of other fin fishes (Konno and Fukazawa, 1991; Sugiyama et al., 1989). Rodger et al. (1984) demonstrated that some squids had so high protease activities in their mantle muscles and these protease activities could not maintain the high textural quality. Nagashima et al. (1992) suggested that metallo-protease and serine-protease existed in the squid mantle resulted in the changes of textural quality of cooked squid meat by myosin degradation.

Another reason the poor gel formation is thought to be the high level of water soluble components in

the squid mantle muscle. Kahn et al. (1974) reported that although squid has low fat and high protein content about 77~85% of the total protein is water solubles.

The objectives of this study were to characterize the effects of washing conditions on the gelation-behavior of minced squid mantle meat, to verify the inhibition of proteolysis by additives, and to enhance the gel formation of minced squid mantle meat by using food grade protease inhibitor.

Materials and Methods

Materials

Squid (*Todarodes pacificus*) samples, captured and kept in ice less than 8hrs, were purchased at a local seafood market. High grade (FA) Alaska pollock (*The- ragra chalcogramma*) frozen surimi containing 4% sucrose, 5% sorbitol, and 0.3% mixture (1:1) of

sodium tripolyphosphate and tetrasodium pyrophosphate, was obtained by courtesy of Daelim Seafood Company (Pusan, Korea) and used as control sample. 800 g of pollock surimi blocks were vacuum-packed, and kept at -30°C until used.

During gel preparation, different levels of additives were treated with 2.5% salt. All additive treatments were carried on a meat basis of 80% moisture. The food grade additives used in this study were potato extracts (PE), egg white (EW, Henningsen, Henning sen foods Inc.), bovine plasma protein (BPP, AMPC, Inc., Ames, IA) and microbial transglutaminase (TG-K, Ajinomoto, Inc, Tokyo, Japan).

The moisture and total nitrogen contents of minced but not washed squid and pollock surimi were determined following AOAC standard procedures (1984).

Preparation of heat-induced gels

In order to investigate the effects of washing on the protein loss from squid minced meat, skinned mantle was minced and blended four times at $9,000\times\text{g}$ for 30 sec with 3 times volume of icy deionized water. The homogenate was filtered through two layers of cheese cloth to remove connective tissues and then centrifuged at $3,000\times\text{g}$ for 10 min. The supernatant and meat residue from first washing were designated as first washing solubles and meat residue, respectively. The residue was resuspended with the 10 times volume of chilled (4°C) deionized water and centrifuged again at same condition mentioned above. These washing procedures were repeated three times to obtain each supernatant and meat residue. All of those supernatant and meat residue were used to study SDS-PAGE pattern and gel properties. In order to know the properties of non-washed squid minced meat, skinned mantle was minced with extruder type mincer and ground in stone mortar with various amounts of additives (PE, EW, BPP, TG-K). The ground meat was stuffed into a polyvinylidene casing (30 mm in diameter and 30 mm in height). Temperature of the surimi paste was maintained below 5°C .

The samples were heated at 90°C for 30 min. Heated gels were immediately chilled to 5°C using icy tap water and then stored at 5°C .

Texture profile analysis

After standing at $18 \pm 2^{\circ}\text{C}$ for 3 hours, each sample gel was cut into cylindrical type of blocks (30 mm in diameter and 30 mm in height). Three blocks from each sample were subjected to compression analysis (double bite) using a Model TM-M Instron Universal Testing Machine (Instron corp., Canton, MA). The data analysis was carried on using Bourne (1968) methods; hardness was defined as the force required for a specified degree of compression of its original length. Elasticity was calculated as the ratio of the base length of the second curve to the base length of the first curve and expressed as a percentage.

The jelly strength was measured using rheometer (Sun Science Co.) with a spherical plunger (5 mm in diameter). The breaking force, W (g), and depth of the indentation, L (cm), were determined and their product was defined as the jelly strength ($\text{g} \cdot \text{cm}$).

SDS-PAGE

Protein patterns of all samples were studied using SDS-PAGE described by Laemmli (1970). Sample solution with 5% sodium dodesyl sulfate solution was made according to Morrissey et al. (1993). Stacking and separating gels were 4% (w/v) and 7.5% (w/v) acrylamide, respectively. The amount of protein applied on the polyacrylamide gel was $60 \mu\text{g}$. Protein standards used to estimate the molecular weight were rabbit myosin (205 kDa), galactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine albumin (66 kDa), egg albumin (45 kDa), and carbonic anhydrase (29 kDa). Gels were fixed and stained with 0.125% Coomassie brilliant blue R-250 (Bio-Rad, Richmond, CA), and destained in a solution containing 25% ethanol and 10% acetic acid.

Transmission electron microscopy analysis

Small pieces of sample gels were cut with a razor blade (1 mm×1 mm×1 mm) and fixed for 2 hr in 1% osmium tetroxide solution buffered with 0.125 cacodylate buffer pH 7.2.

In order to remove unreacted osmium tetroxide and water, samples were immersed for 20 min in polyethylene oxide, and then soaked in polyethylene oxide and epon resin mixture (10 min each). After toluidine blue staining of sliced samples, the samples were cut into 60~90 nm thin sections using LKB Ultramicrotome (NOVA, Sweden). Electron microscopy was performed with final double stained sample (JEM 1200 EX-II, JEOL, Japan).

Results and Discussion

Changes of water soluble proteins during washing

In order to investigate the effect of washing on water soluble protein pattern of squid minced meat (80.85 ± 0.04% of moisture and 15.16 ± 0.12% of protein content), SDS-PAGE patterns of water solubles and meat residue at each washing were compared (Fig. 1).

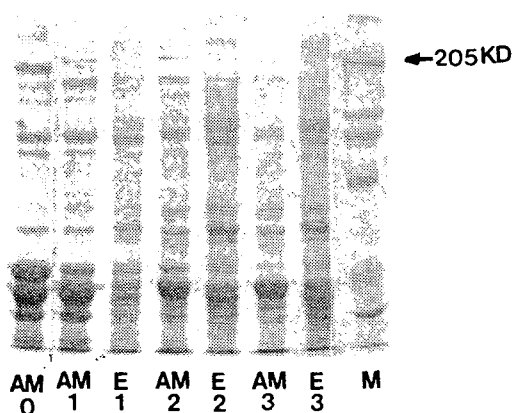


Fig. 1. 7.5% SDS-PAGE pattern of water solubles and meat extracts from minced squid. AM; Minced meat (pellet after centrifuge), E; wash extract (supernatant after centrifuge), 1, 2, 3; washing cycle, M; molecular marker protein

As shown on Figure 1, myosin heavy chain band (205 kDa) disappeared from minced meat, whereas many myosin bands showed up in water solubles comparing with control (AM0).

Washing procedure is very important for increasing myofibrillar protein content. (Toyoda et al., 1992; Ohshima et al., 1993).

Toyoda et al. (1992) reported that the number of required washing cycles depends on the type, composition, and freshness of the fish to be processed. Normally, after repeated washing, the gel strength increased because the soluble materials (blood, sarcoplasmic protein, odor, etc) were removed.

Our results indicated that the more washing treatment made the poorer gel strength and squid minced meat does not need washing procedure for good gel property. We could not present those gel strength data because of no gel formation.

Effect of additives on rheological properties of squid surimi

Various additives (PE, EW, BPP and TG-K) were added to prepared squid surimi pastes (without washing) and commercial Alaska pollock paste (74.85 ± 0.04% of moisture and 17.16 ± 0.12% of protein content) were used as control. PE, EW and BPP have been used as endogenous proteinase inhibitor during heating (Hamann et al., 1990; Morrissey et al., 1993) and TG-K has been used as a gel enhancer (Tsukamasa and Shimizu, 1991; Sakamoto, et al., 1994; Kurth and Rogers, 1984). Results in Figure 2 shows that 3% of BPP and PE were most effective in hardening than other levels. On the other hand, EW and TG-K were most effective at 5%, but a strong odor of boiled egg was detected from gels containing more than 3.0% of EW. PE could make harder gels than EW but these two did not result in the gel strength with 3% BPP. There were no relationship between each additive concentration and gel hardness except BPP ($P > 0.05$).

The elasticity of heat induced gels with various levels of additives were similar (Fig. 3). Between the

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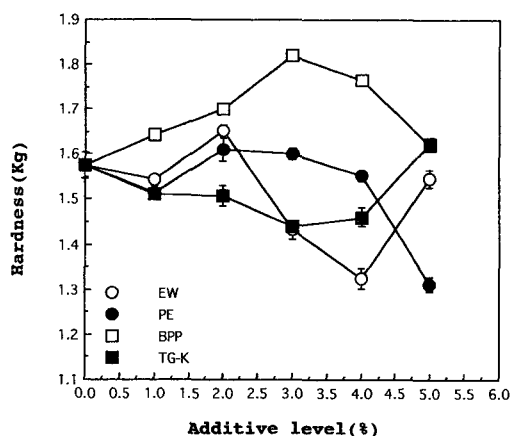


Fig. 2. Hardness of heat induced gels with concentrations of egg white(EW), potato extract(PE), beef plasma protein(BPP), microbial TGase(TG-K).

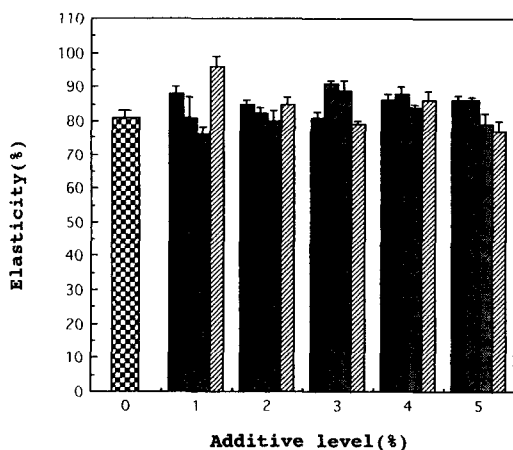


Fig. 3. Elasticity of heat induced gels with levels of egg white(EW), potato extract(PE), beef plasma protein(BPP) and microbial TGase(TG-K).

range from 1 to 3%, additives gradually increased the elasticity ($P < 0.05$, 0.936), but gels containing BPP were less ($p < 0.05$) elastic than those containing PE even at 4%.

1% level TG-K gave better gel elasticity than the control but addition of 1% to 5% TG-K did not increase the elasticity significantly ($p > 0.05$).

The jelly strength of heat induced gels were di-

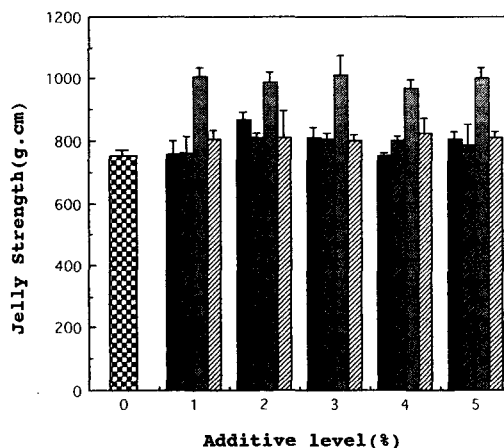


Fig. 4. Jelly strength of heat induced gels with concentrations of egg white(EW), potato extract(PE), beef plasma protein(BPP), microbial TGase(TGK).

ffer with the levels of additives (Fig. 4). BPP gave the highest jelly strength among the all additives at every concentration.

Many researchers have been reported the role of protease inhibitors (Morrissey et al., 1993; An et al., 1994). Beef plasma protein has been shown to inhibit active endogenous enzymes in Pacific whiting (*Merluccius productus*) for desirable gel properties (Park et al., 1994). Peng and Nielsen (1986) also reported that protein-protein interaction could occur between beta-conglycinin (a protein from soy protein isolates) and chicken myosin and could enhance gel strength. Those researchers demonstrated that the presence of conglycinin resulted in diminished aggregations of myosin heavy chain at the temperature ranges from 50 to 100°C.

Nagahisa et al. (1983) have reported that egg white improved the strength of Pacific whiting surimi based gels and those effects were presumably attributed by thiol ovinhibitors through the S-S crosslinking.

In our squid surimi study, however, the application of BPP resulted in the superior gel strength than the other additives, EW, PE or TG-K, at any levels.

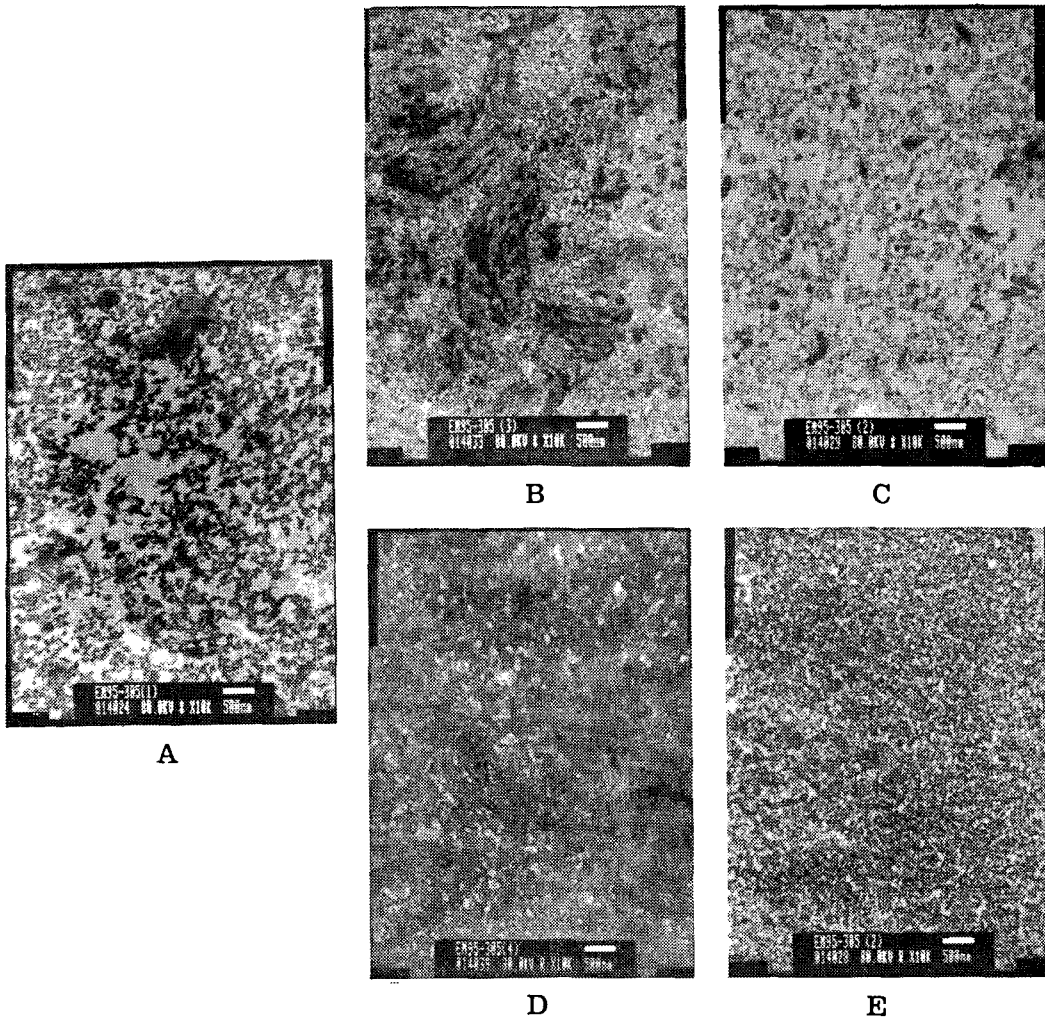


Fig. 5. TEM micrography of squid gel products with each inhibitor optimum formulation.
 A: add 2.5% NaCl, B: add 2% Egg white(include 2.5% NaCl), C: add 4% Tgase(include 2.5% NaCl), D: add 2% potato extract(include 2.5% NaCl), E: add 3% Bpp(include 2.5% NaCl).

Gel microstructure of squid surimi

Transmission electron micrographs of squid surimi gels prepared by conventional heat processing (90°C/30 min) with various additives were compared (Fig. 5). Gels containing no additives (2.5% NaCl), 2% egg white and 4% TGase showed a sponge-like microstructure with more vacant space. The surimi gel proteins appeared to be randomly aggregated with disordered networks. On the other hand, 3% of BPP produced more rigid and arranged networks.

The structure of gel containing 2% PE was more compact than gels containing other additives (control, 2% EW and 4% TGase), but jelly strength was similar in gels containing those additives.

Burgarella et al. (1985a, b) suggested that the function of starchy additives in gel formation was filling of the interstitial spaces of the myofibrillar protein network without myosin starch interaction which led gel weakening.

From the above results, potato extracts presuma-

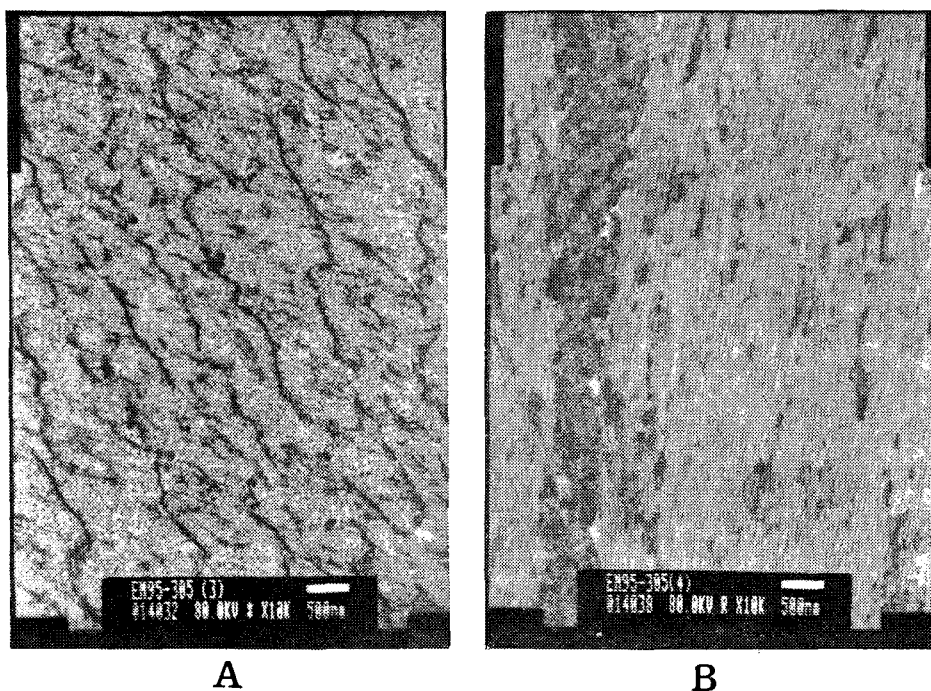


Fig. 6. TEM micrography of squid and Alaska pollack gel products with optimum formulation.

A: Squid kamaboko (add 2.5% NaCl, 2% Egg white, 2% potato extract, 3% Bpp, 5% Corn starch).

B: Alaska pollack kamaboko (add 2.5% NaCl, 2% Egg white, 2% potato extract, 3% Bpp, 5% Corn starch).

bly acted as a filler in the interstitial space of squid gel. Our results also indicated EW produced no rigid and compact structure compared to control and BPP gel.

Similar gel structure was noted in squid surimi gel compared with Alaska pollack gel under the optimal formulation and at processing condition (Fig. 6).

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