INHIBITION OF GEL WEAKENING OF THREADFIN BREAM SURIMI USING THAI LEGUME SEED PROTEINASE INHIBITORS

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Received for Publication November 5, 1999
Accepted for Publication May 10, 2000

ABSTRACT

Partially purified proteinase inhibitors from cowpea (Vigna unguiculata (L.) Wasp), pigeon pea (Cajanus cajan (L.) Millsp.) and bambara groundnuts (Voandzeia subterranea (L.) Thou) effectively inhibited sarcoplasmic modori-inducing proteinase extracted from threadfin bream muscle in a concentration dependent manner. Incorporation of these proteinase inhibitors into threadfin bream surimi partially inhibited autolytic degradation and increased the gel force and deformation. Combination of setting and incorporating proteinase inhibitors from cowpea and bambara groundnut var. HY at the level of 30 kunits/g resulted in an increase in gel force and deformation by 60% and 26%, respectively. However, the lightness and whiteness of surimi gels decreased slightly when the proteinase inhibitor was added at a level of 30 kunits/g.

INTRODUCTION

Gel-weakening phenomenon or “modori” observed at temperatures above 50°C
is a major concern in surimi gel manufacture. Much attention has been paid to surimi produced from muscle of the soft-textured fish species which contain high levels of endogenous proteinases or have poor gelling ability. Modori refers to severe degradation of myofibrillar proteins, especially that of myosin, by the action of endogenous heat-activated proteinases (An et al. 1996; Greene and Babbitt 1990; Chang-Lee et al. 1989; Boye and Lanier 1988) and/or the thermal behavior of myofibrillar proteins (Niwa 1992). This phenomenon generally results in soft texture and substantial decrease in gel strength, which is the most detrimental factor to market surimi for seafood analog products (Momssey et al. 1993).

Threadfin bream is widely used in Thailand and other countries in the Southeast Asia as an important raw material for surimi production. However, it is known as a typical modori-susceptible species. Threadfin bream contains at least two types of sarcoplasmic modori-inducing proteases, Sp-50-MIP and Sp-60-MIP, which are optimally active at 50°C and 60°C, respectively (Kinoshita et al. 1990a). The proteinases are responsible for gel weakening. Toyohara et al. (1990) reported that sarcoplasmic fluid contained gel degradation inducing factor (GIF) as well as heat stable alkaline proteinase (HAP). GIF showed a strong proteolytic activity against the myosin heavy chain only in the presence of 2.5% NaCl. However, HAP from threadfin bream showed negligible gel degradation activity in the presence or absence of NaCl (Toyohara et al. 1990; Kinoshita et al. 1990b). Toyohara et al. (1990) reported that the gel degradation-inducing factor (GIF), not heat stable alkaline proteinase (HAP), plays an important role in the breakdown of myosin heavy chain in threadfin bream muscle. Although the washing process can remove some of these proteinases, some may still remain in surimi and affect the overall gel quality.

To prevent the detrimental effect caused by the proteolytic activity, protease inhibitors have been widely used to maximize the gel strength of surimi. The most commonly used inhibitors are beef plasma protein (BPP), egg white, potato powder and whey protein concentrate (Chang-Lee et al. 1989; Hamann et al. 1990; Morrissey et al. 1993; Weerasinghe et al. 1996). Although plasma proteins effectively inhibited the proteolysis, the use of plasma in foods has not been accepted by several ethnic groups and has drawbacks associated with off-color, off-flavor, and high cost. Proteinase inhibitors from plant origin may be used as an alternative processing aid in surimi processing. Various proteinase inhibitors have been found in plant foodstuffs (Belitz and Weder 1990). Oryzacystatin from rice grain has inhibitory activity against heat-activated arrowtooth flounder protease (Izquierdo-Pulido et al. 1994). Spinach leaf extract showed inhibitory effect on modori-inducing proteinase in threadfin bream surimi (Toyohara et al. 1992). Some legume seed extracts were found to inhibit fish muscle proteinases (Garcia-Carreno et al. 1996). Recently, Benjakul et al. (1999) reported that the extract from some Thai legume seeds inhibited fish proteinase. Benjakul et al. (2000) partially purified and characterized trypsin inhibitors from cowpea, pigeon pea and bambara
groundnuts. However, the effect of proteinase inhibitor extract from legume seeds on the surimi gel has not been studied. The objective of this study was to evaluate the proteinase inhibitors from some Thai legume seeds as an additive to prevent modori phenomenon of surimi from threadfin bream.

**MATERIALS AND METHODS**

**Chemicals**

N-α-Benzoyl-DL-arginine-p-nitroanilide (BAPNA), trypsin from bovine pancreas, dimethylsulfoxide were purchased from Sigma Chemical Co. (St. Louis, MO). Ammonium sulfate was obtained from Merck (Darmstadt, Germany).

**Inhibitor Extract from Legume Seeds**

Cowpea (*Vigna unguiculata* (L.) Wasp.) and pigeon pea (*Cajanus cajan* (L.) Millsp.) were purchased from the market in Hat Yai. Two varieties of bambara groundnut (*Voandzeia subterranea* (L.) Thou), typical (T) and Hat Yai (HY), were obtained from the Seed Research Center, Hat Yai, Songkhla, Thailand.

The seed extracts were prepared according to the method of Benjakul *et al.* (2000). Dry seeds were ground using a coffee mill to a particle size of 20 mesh. The seed flour was defatted by mixing with 5 volumes of hexane (w/v) for 10 min and filtered through Whatman No.1 filter paper. The sediment was rinsed with hexane 3 times to remove the residual oil and the defatted seed flour was air-dried at ambient temperature (28-30C). The defatted seed flour was mixed with 0.15 M NaCl at a ratio of 1:10 (w/v) and shaken at 180 rpm at room temperature for 1 h for cowpea and 3 h for pigeon pea and bambara groundnuts. The extracts were recovered by centrifuging at 5,000xg for 30 min. To purify the proteinase inhibitors, the extracts were heated at 90C for 10 min and cooled down with iced water. The coagulated debris was removed by centrifuging at 8,000xg for 5 min. The supernatants were subjected to ammonium sulfate precipitation with 30-65% saturation. The precipitated proteins were dialyzed overnight in distilled water. The dialyzed fraction was freeze-dried and used for further study.

**Trypsin Inhibitory Activity Assay**

Trypsin inhibitory activity was determined using BAPNA as a substrate according to Benjakul *et al.* (2000). One unit of trypsin inhibitor activity was defined as the amount of inhibitor, which reduces the trypsin activity by one unit. One unit of trypsin activity was defined as an increase of 0.01 A₄₁₀ due to p-nitroaniline released.
Preparation of Modori-inducing Proteinases (MIP)

Sarcoplasmic modori-inducing proteinases (Sp-MIP) were prepared by centrifuging 50 g of finely chopped fillets of threadfin bream at 3,000xg for 30 min at 4°C. The supernatant was used as Sp-MIP.

Proteinase Inhibition Assay

The inhibition on Sp-MIP was run according to the method of Benjakul et al. (1999). Diluted Sp-MIP solution (100 μL) was incubated with 100 μL of partially purified inhibitors from various legume seeds at different final concentrations. The mixture was incubated at ambient temperature for 20 min and the residual activity was determined using threadfin bream actomyosin prepared according to the method of Benjakul et al. (1997). The assay was conducted in 50 mM Tris-HCl, pH 7.5 with 40 mM CaCl₂ at 37°C for 20 min. The inhibitory activity was reported as percent activity inhibited.

Autolytic Activity Assay

Surimi or surimi paste sol was incubated at 60°C for 2 h and the degradation products were measured according to method of Morrissey et al. (1993). Surimi gels were homogenized with 27 mL of 5% (w/v) trichloroacetic acid. The homogenate was placed on ice for 1 h and centrifuged at 5,000xg for 5 min. Tyrosine in the supernatant was determined and expressed as μmol tyrosine/g gel. The inhibition of autolysis was determined according to Benjakul et al. (1999).

Electrophoresis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was carried out by the method of Laemmli (1970) using 4% stacking gel and 10% separating gel. Sample (3 g) was homogenized with 5% (w/v) SDS in a final volume of 30 mL. The homogenate was incubated at 85°C for 1 h to dissolve the proteins. The debris was removed by centrifuging at 3,500xg for 5 min at room temperature. Protein (20 μg) was applied on the gel. The proteins were separated at the constant voltage of 100 V using Mini-Protean II system (Bio-Rad Laboratories, Hercules, CA). The proteins were stained with 0.125% Coomassie brilliant blue R-250 and destained in 25% ethanol and 10% acetic acid. High molecular weight standards (Sigma Chemical Co., St. Louis, Mo) included rabbit muscle myosin (205,000), E. coli β-galactosidase (116,000), rabbit muscle phosphorylase b (97,000), rabbit muscle fructose-6-phosphate kinase (84,000), bovine serum albumin (66,000), bovine liver glutamic dehydrogenase (55,000), chicken egg albumin (45,000) and rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36,000).
Protein Determination

Protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin was used as a standard.

Surimi Gel Preparation

Frozen high-grade threadfin bream surimi (Grade A) was obtained from Man-A Frozen Foods Co., Ltd, Songkhla, Thailand. Frozen surimi was tempered for 1 h at room temperature, followed by cutting into small pieces with the thickness of approximately 0.5-1 cm. The surimi was placed in a mixer (National Model MK-K77, Tokyo, Japan) and dry proteinase inhibitor was added at levels of 10 and 30 kunits. All treatments were adjusted to 80% moisture with a final concentration of 2.5% NaCl. The mixture was chopped for about 5 min to obtain homogeneous paste. The surimi paste was stuffed into a polyvinylidine casing with a diameter of 2.5 cm. Both ends of the casing were sealed. Modori gel was prepared by incubating surimi sol at 60°C for 30 min, followed by heating at 90°C for 20 min. The suwari gel was made by incubating the sol at 40°C for 30 min, followed by heating at 90°C for 20 min. The gels were then cooled down in iced water.

Texture Analysis

Texture analysis of surimi gel was performed using a texture analyzer Model TA-XT2 (Stable Micro Systems, Surrey, England). Gels were equilibrated and tested at room temperature. Six cylinder-shaped samples with 2.5 cm in length were prepared. The breaking force and deformation were measured using the texture analyzer equipped with a cylindrical plunger (diameter, 5 mm; depression speed, 60 mm/min).

Color Evaluation

Color of five surimi gel samples from each treatment was measured using a colorimeter JP7100F (Juki Corp., Tokyo, Japan). Results were expressed as L, a, and b. Whiteness was calculated according to the method of Park (1994) as follows:

\[
\text{Whiteness} = 100 - [(100-L)^2 + a^2 + b^2]^{1/2}
\]

Statistical Analysis

Analysis of variance was performed and mean comparisons were carried out using Duncan's multiple range test (Steel and Torrie 1980).
RESULTS AND DISCUSSION

Effect of Inhibitors on Modori-inducing Proteinases

Partially purified proteinase inhibitors from cowpea, pigeon pea, bambara groundnut var. T and HY had the specific activity of 13.48, 15.33, 6.77 and 7.70 kunits/mg protein, respectively. All inhibitors showed inhibitory activity against Sp-60-MIP and Sp-50-MIP from threadfin bream (Fig. 1 and 2). Inhibition of proteolytic activity was found to be dependent on the source and concentration of inhibitors used. When tested against Sp-60-MIP, the proteinase inhibitor from pigeon pea exhibited the highest inhibition (c.a. 20%) at the concentration of 5-10 units/ml. However, the proteinase inhibitor from cowpea exhibited the highest inhibition (>70%) at concentrations above 500 units/ml. The differences in inhibition efficiency of different legume seed proteinase inhibitors were presumed to be due to the differences in specificity toward proteinases tested. Generally, proteinase inhibitors from all legume seeds tested were found to be less effective towards Sp-50-MIP in which approximately 30-35% of proteolytic activity was inhibited at the highest concentration of inhibitor tested (Fig. 2). Sp-50-MIP shares common properties with Sp-60-MIP in respect to NaCl requirement, optimal pH, specificity to substrate and inhibitors (Kinoshita et al. 1992). However, discrepancies in their susceptibility to legume seed inhibitors are probably due to the differences in the molecular and catalytic properties among inhibitory components present in the partially purified fraction from various legume seeds (Benjakul et al. 1999).

Effect of Proteinase Inhibitors on Surimi Gel Degradation

Autolytic degradation of surimi gels indicated the presence of the endogenous proteinases in threadfin bream surimi (Benjakul et al. 1999). Since threadfin bream had no myofibril associated proteinases (Toyohara et al. 1990), it was thought that autolytic activity exerted in degradation of muscle proteins was possibly due to the remaining sarcoplasmic proteinases from washing process during surimi production. Incorporation of the partially purified proteinase inhibitors from legume seeds markedly decreased the extent of autolytic degradation in surimi gels prepared by incubating at 40C or 60C prior to heating at 90C (Fig. 3). The extent of inhibition was shown to be dependent on the source and concentration of inhibitors. At the same concentration used, proteinase inhibitor from pigeon pea showed the lowest inhibitory activity. Proteinase inhibitors from bambara groundnuts var. HY, var. T and cowpea showed the highest inhibitory activity at 10 kunits/g. Inhibition efficiency of the proteinase inhibitors generally increased with the amount used. Benjakul et al. (1999) reported that the crude extracts from cowpea and soybean partially inhibited the proteolytic activity of the washed threadfin bream mince incubated at 50C or 60C.
FIG. 1. EFFECT OF PARTIALLY PURIFIED PROTEINASE INHIBITORS FROM VARIOUS LEGUME SEEDS ON THE THREADFIN BREAM 60-MIP ACTIVITY

The partially purified proteinase inhibitors were added into Sp-MIP solution at different final concentrations. The residual activity was measured using actomyosin as a substrate. The assay was run at 60°C for 20 min and the soluble degradation products were determined.
FIG. 2. EFFECT OF PARTIALLY PURIFIED PROTEINASE INHIBITORS FROM VARIOUS LEGUME SEEDS ON THE THREADFIN BREAM Sp-50-MIP ACTIVITY

The partially purified proteinase inhibitors were added into Sp-MIP solution at different final concentrations. The residual activity was measured using actomyosin as a substrate. The assay was run at 50°C for 20 min and the soluble degradation products were determined.
FIG. 3. EFFECT OF PARTIALLY PURIFIED INHIBITORS FROM VARIOUS LEGUME SEEDS ON THREADFIN BREAM SURIMI DEGRADATION IN THE PRESENCE OF 2.5% NaCl
Partially purified proteinase inhibitors were added into surimi at levels of 10 and 30 kunits/g. Surimi samples were incubated at 60°C for 2 h, and the degradation products were determined.

Similar inhibition on autolytic degradation was observed between surimi gels preincubated at 40°C and 60°C prior to heating at 90°C. Preincubation of surimi at 35-40°C in the presence of salt is performed for the high temperature setting or “suwari”. The cross-linking of myosin heavy chain can be accelerated by endogenous transglutaminase, resulting in the enhanced gel properties (Seki et al. 1998). However, some degradation products have been found in ‘suwari’ gels. Our results are in agreement with Lee et al. (1990a, b), who reported that a considerable degree of proteolysis occurred at the optimum temperature in surimi pastes from threadfin bream and hoki. Formation of 150 kDa component, which might be a proteolytic fragment of myofibrillar proteins, occurred during the incubation at 25°C (Takeda and Seki 1996). Recently, Ando et al. (1998) found that the polymerization and degradation of MHC occurred simultaneously in the process of setting. Theoretically, a lesser extent of degradation was expected for surimi gels preincubated at 40°C in comparison to those preincubated at 60°C, an optimal temperature at which MIPS are highly active in hydrolyzing muscle proteins (An et al. 1996). To prevent gel softening, food grade inhibitors have been widely used in surimi (Wasson et al. 1992; Morrissey et al. 1993).
Electrophoretic Study of Surimi Gels

A slight decrease in intensity of myosin heavy chain (MHC) band in surimi gels without the added proteinase inhibitors (the control) indicated that myosin degradation was caused by endogenous proteinases. Conversely, no detectable changes in MHC band were observed in the surimi gels added with proteinase inhibitors (Fig. 4 and 5). Therefore, the results suggested that proteolysis in surimi samples was inhibited to some extent by the proteinase inhibitors from legume seeds. Myosin heavy chain bands were protected from degradation even at 60°C, at which the proteinase showed the highest activity. Myosin degradation observed in the control indicated the presence of some proteinases, particularly sarcoplasmic proteinases remained after washing process. Myosin is considered as the most important component contributing to formation of surimi gels. The presence and concentration of intact myosin are related to the gel strength of surimi (Niwa 1992; Morrissey et al. 1993). Degradation of myosin, a typical characteristic of modori phenomenon, mainly causes a loss of surimi gel strength.

For suwari gel (40/90°C), less proteolysis of myosin heavy chain was observed in the control (Fig. 5), compared to those found in modori gel (60/90°C) (Fig. 4). No detectable changes in MHC band were found in gel samples added with proteinase inhibitors.

Effect of Proteinase Inhibitor on Surimi Gel Strength

Corresponding to the extent of autolytic degradation, the lowest force and deformation were observed with the surimi gels prepared without proteinase inhibitors, suggesting the occurrence of gel weakening in both heating conditions (Fig. 6 and 7). However, force and deformation of surimi gels heated under both conditions increased substantially by adding partially purified proteinase inhibitors from all legume seeds. Under 60/90°C condition, the surimi gels added with proteinase inhibitors from cowpea and bambara groundnuts showed the higher breaking force compared to other samples (P<0.05) (Fig. 6). The increases in force by 76, 79 and 87% were observed with surimi gels added with proteinase inhibitors from cowpea, bambara groundnut var. T and HY at the level of 30 kunits/g, respectively. Compared to other samples with added proteinase inhibitors from other seeds, lower force and deformation were observed with surimi gels with added proteinase inhibitor from pigeon pea (P<0.05). The breakdown of myofibrillar proteins inhibits the development of a three-dimensional gel network of surimi (An et al. 1996). This result clearly indicates that a modori phenomenon occurred in the control, whereas proteinase inhibitors could suppress this phenomenon. Toyohara et al. (1992) reported that the extract from spinach (10%) effectively inhibited the modori phenomenon in threadfin bream mince and resulted in a considerable increase in gel strength.
FIG. 4. EFFECT OF PARTIALLY PURIFIED PROTEINASE INHIBITORS ON PROTEIN PATTERN OF THREADFIN BREAM SURIMI GEL PREPARED BY HEATING AT 60/90C

P, C, BT and BH represent pigeon pea, cowpea, bambara groundnut var. T and var. HY, respectively. Number 1 and 3 designate 10 and 30 kunits of trypsin inhibitors/g surimi, respectively.
FIG. 5. EFFECT OF THE PARTIALLY PURIFIED PROTEINASE INHIBITORS ON PROTEIN PATTERN OF THREADFIN BREAM SURIMI GELS PREPARED BY HEATING AT 40/90°C
P, C, BT and BH represent pigeon pea, cowpea, bambara groundnut var. T and var. HY, respectively. Number 1 and 3 designate 10 and 30 kunits of trypsin inhibitors/g surimi, respectively.
Higher force and deformation were observed with surimi gels prepared under 40/90C heat treatment (Fig. 7), than those prepared under 60/90C heat treatment (Fig. 6). Surimi gel with added proteinase inhibitors from cowpea and bambara groundnut var. HY at the concentration of 30 kunits/g had the highest force and deformation (P<0.05). The increases in force and deformation of gels, when both proteinase inhibitors were added at level of 30 units/g, were estimated to be 60% and 26%, respectively. The gels added with proteinase inhibitors from pigeon pea
FIG. 7. EFFECT OF THE PARTIALLY PURIFIED PROTEINASE INHIBITORS ON FORCE AND DEFORMATION OF THREADFIN BREAM SURIMI GELS PREPARED BY HEATING AT 40/90°C

At the concentration of 10 kunits/g showed no significant differences in force compared to the control (P>0.05) but had the higher force when 30 kunits/g was used (P<0.05). The result indicated that the proteinase inhibitors from pigeon pea had the lowest inhibition on autolysis under modori and suwari conditions. Ni et al. (1999) reported that actomyosin gelation was improved by the combined effects of transglutaminase and proteinase inhibitors. Therefore, it is concluded that proteinase inhibitors from the selected legume seeds effectively inhibited the
proteolytic degradation of surimi gels and greatly improved the textural properties of minced fish gel from threadfin bream, particularly for the gels prepared by setting at 40°C, followed by heating at 90°C.

**Color Evaluation of Surimi Gels**

Color characteristic of surimi gels were largely dependent on the types and amounts of the legume seed proteinase inhibitors added (Table 1 and 2). Lowest L-value and whiteness were observed in the samples added with partially purified proteinase inhibitor from cowpea (P<0.05). Overall, the decreases in lightness (L), greenness (-a), yellowness (b), and whiteness were observed when the higher concentration of proteinase inhibitors were added (P<0.05). With a difference in seed coat color among the legume seeds tested, undesirable components in the proteinase inhibitor extracts, e.g. pigments, can impart changes in color characteristics of surimi gels. However, no significant differences in color were observed between gels heated with the two different conditions (P>0.05). Park (1994) reported a decrease of L value with an increase in b value of Pacific whiting surimi gel added with 1% BPP.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>Whiteness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78.40±0.40 cd</td>
<td>-1.48±0.04 ab</td>
<td>9.74±0.08 de</td>
<td>76.26±0.36 cd</td>
</tr>
<tr>
<td>P-10K</td>
<td>78.40±0.10 cd</td>
<td>-1.43±0.00 b</td>
<td>9.49±0.15 cd</td>
<td>76.36±0.06 cd</td>
</tr>
<tr>
<td>P-30K</td>
<td>77.74±0.09 c</td>
<td>-1.54±0.05 a</td>
<td>9.72±0.23 de</td>
<td>75.66±0.06 c</td>
</tr>
<tr>
<td>C-10K</td>
<td>76.66±0.39 b</td>
<td>-1.04±0.03 d</td>
<td>9.12±0.04 b</td>
<td>74.92±0.35 b</td>
</tr>
<tr>
<td>C-30K</td>
<td>73.62±0.66 a</td>
<td>-0.63±0.02 f</td>
<td>7.98±0.08 a</td>
<td>72.43±0.63 a</td>
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<tr>
<td>BT-10K</td>
<td>78.79±0.14 d</td>
<td>-1.52±0.05 a</td>
<td>9.65±0.02 cd</td>
<td>76.60±0.05 d</td>
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<tr>
<td>BT-30K</td>
<td>76.91±0.12 b</td>
<td>-1.23±0.02 c</td>
<td>9.45±0.14 c</td>
<td>74.99±0.13 b</td>
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<tr>
<td>BHY-10K</td>
<td>78.25±0.70 cd</td>
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<td>9.92±0.20 e</td>
<td>76.05±0.63 cd</td>
</tr>
<tr>
<td>BHY-30K</td>
<td>75.90±0.55 b</td>
<td>-0.92±0.04 e</td>
<td>9.19±0.06 b</td>
<td>74.81±0.52 b</td>
</tr>
</tbody>
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*Mean±SD of triplicate determinations
Different letters in the same column indicate the significant differences (P<0.05).*
TABLE 2.
COLOR OF THREADFIN BREAM SUWARIS GELS CONTAINING PARTIALLY PURIFIED PROTEINASE INHIBITORS FROM THE THAI LEGUME SEEDS

<table>
<thead>
<tr>
<th>Treatments</th>
<th>L</th>
<th>A</th>
<th>b</th>
<th>Whiteness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.97±0.06 e</td>
<td>-1.58±0.05 a</td>
<td>9.70±0.16 e</td>
<td>75.63±0.12 c</td>
</tr>
<tr>
<td>P-10K</td>
<td>77.97±0.62 e</td>
<td>-1.53±0.04 a</td>
<td>9.48±0.20 de</td>
<td>75.96±0.63 c</td>
</tr>
<tr>
<td>P-30K</td>
<td>74.60±0.11 b</td>
<td>-1.41±0.05 b</td>
<td>9.51±0.11 de</td>
<td>72.84±0.07 b</td>
</tr>
<tr>
<td>C-10K</td>
<td>75.87±0.73 c</td>
<td>-1.15±0.04 cd</td>
<td>8.78±0.11 b</td>
<td>74.29±0.69 b</td>
</tr>
<tr>
<td>C-30K</td>
<td>73.34±0.26 a</td>
<td>-0.72±0.08 e</td>
<td>8.14±0.18 a</td>
<td>72.11±0.29 a</td>
</tr>
<tr>
<td>BT-10K</td>
<td>78.06±0.30 e</td>
<td>-1.53±0.02 a</td>
<td>9.18±0.11 c</td>
<td>76.16±0.32 c</td>
</tr>
<tr>
<td>BT-30K</td>
<td>76.76±0.29 d</td>
<td>-1.16±0.25 c</td>
<td>9.36±0.07 cd</td>
<td>74.91±0.28 bc</td>
</tr>
<tr>
<td>BHY-10K</td>
<td>76.02±0.32 c</td>
<td>-1.08±0.02 d</td>
<td>9.47±0.15 de</td>
<td>74.12±0.30 b</td>
</tr>
<tr>
<td>BHY-30K</td>
<td>74.14±0.26 b</td>
<td>-0.59±0.04 f</td>
<td>9.30±0.18 cd</td>
<td>72.51±0.21 a</td>
</tr>
</tbody>
</table>

*Mean±SD of triplicate determinations
Different letters in the same column indicate the significant differences (P<0.05).

CONCLUSION

Partially purified proteinase inhibitors from cowpea and bambara groundnut var. HY can be used effectively as a proteinase inhibitor in threadfin bream surimi. Gel strength of surimi containing proteinase inhibitors was improved by the combination of proteolysis inhibition and setting.

ACKNOWLEDGMENT

This work was supported by The Thailand Research Fund for the Project No. PDF/04/2541 to Dr. Sootawat Benjakul under postdoctoral program. The authors are indebted to Professor N.F. Haard, University of California, Davis for his invaluable guidance.

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