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Effect of Protein Additives on Gelling Properties of Pacific White Shrimp (*Litopenaeus vannamei*) Meat

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Abstract: In this study, the effects of egg white (EW), whey protein concentrate (WPC) and bovine plasma protein (BPP) at various concentrations (0 - 3%) on properties of Pacific white shrimp gels were elucidated. For one-step heating (90°C/20 min) and two-step heating (40°C/30 min followed by 90°C/20 min), all protein additives showed inhibitory activity toward autolysis of Pacific white shrimp gels. The inhibition was more pronounced in one-step heated gel when the level of protein additive increased which was reflected in the greater extent of myosin heavy chain (MHC) retained. The addition of 0.5% BPP exhibited the most gel enhancing effect as indicated by the highest increase in breaking force of 211.9% and 283.3% for one-step and two-step heated gels, respectively. The addition of BPP resulted in a decrease in *L**-value of two-step heated gel and an increase in *b**-value. Conversely, EW increased the *L**-value, particularly at higher levels of addition.

Keywords: Pacific white shrimp, gelation, bovine plasma protein, egg white, whey protein concentrate

INTRODUCTION

Gelation of surimi proteins is a process involving unfolding and aggregation of proteins. During heating, the proteins unfold, exposing reactive surfaces and the neighboring protein molecules, which then interact to form intermolecular bonds. When sufficient bonding occurs, a three-dimensional network is formed, resulting in a gel (Lanier, 1991). During heating, surimi gel from some fish species undergo degradation associated with the weakening of the resulting gel. Tissue proteinases are involved in the breakdown of tissue proteins (Goll *et al.*, 1983). Activity of an alkaline proteinase and exopeptidase has been detected in the shrimp muscle (Doke and Ninjoor, 1987). Additionally, the presence of endogenous proteolytic enzymes in fish mince or surimi results in a decrease in gel strength with a brittle and non elastic gel (Alvarez *et al.*, 1995). To alleviate the problems associated with protein degradation caused by the endogenous proteinases, inhibitors and other additives have been used in surimi to improve the properties of surimi gels. Bovine plasma protein (BPP), porcine plasma protein (PPP), egg white and potato powders can be used as food grade inhibitors in surimi (Benjakul and Visessanguan, 2000; Lee *et al.*, 2000; Benjakul *et al.*, 2001). Nevertheless, information on the effect of protein additives on the gelling property of Pacific white shrimp is scanty and

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hence this study was conducted to investigate the preventive effects of EW, WPC and BPP on the hydrolysis of myofibrillar protein by endogenous proteinases in Pacific white shrimp meat to establish their effects on gelling properties of the resulting gels.

MATERIALS AND METHODS

Raw Material

Fresh Pacific white shrimps (*Litopenaeus vannamei*) with an average size of 55 - 60 shrimps kg^{-1} were purchased from a farm in Songkla Province. Samples were kept in ice using the shrimp/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 hour. Upon arrival, the shrimps were washed with clean water, immediately deheaded, peeled and deveined. The flesh was minced using a mincer with a hole diameter of 5 mm. The mince was placed in polyethylene bag and kept on ice during preparation (Thammatinna *et al.*, 2007). Shrimp mince was determined for moisture content according to AOAC method (AOAC, 1999).

Pacific White Shrimp Gel Preparation

To the Pacific white shrimp meat was added 2.5% NaCl, 150 mM $CaCl_2$ kg^{-1}, 5 mM PP kg^{-1} and 5 mM $MgCl_2$ kg^{-1}. The moisture content was then adjusted to 80% with iced water. Then protein additives (BPP EW and WPC) were added at levels of 0, 0.5, 1.0, 2.0 and 3.0% (w/w). The mixture was chopped for 4 min to obtain a homogenous sol. The sol was then stuffed into polyvinylidine casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. One-step heated gels were prepared by heating the sol at 90°C for 20 min. Two-step heated gels were made by incubating the sol at 40°C for 30 min, followed by heating at 90°C for 20 min. The gels were cooled in iced water and stored for 12 h at 4°C prior to analyses. Pacific white shrimp gels were then subjected to various analyses and measurements as follows.

Analyses

i. Breaking Force and Deformation

Texture analysis of surimi gels was carried out using a Model TA-XT2i texture analyzer (Stable Micro System, UK). Gels were equilibrated at room temperature (28 - 30°C) before analysis. Five cylindrical samples (2.5 cm in diameter) were cut into 2.5 cm lengths. Breaking force (strength) and deformation (cohesiveness/elasticity) were measured with the texture analyzer according to the method of Benjakul *et al.* (2003). A spherical probe with a diameter of 5 mm was pressed into the cut surface of a gel specimen perpendicularly at a constant depression speed (60 mm min^{-1}) until puncture occurred. The force to puncture into the gel (breaking force) and the distance at which the ball probe punctured into the gel (breaking distance or deformation) were both recorded.

ii. Color

The color of samples was measured in the *L* a* b** mode of CIE using a colorimeter (ColorFlex, Hunter Associates Laboratory, Reston, VA, USA). *L*, a*,* and *b** indicate lightness, redness/greenness, and yellowness/blueness, respectively.

iii. Determination of TCA-Soluble Peptides

TCA-soluble peptide content was determined according to the method of Morrissey *et al.* (1993). The sample (3 g) was homogenized with 27 ml of 5% TCA for 1 min at a speed of 11,000 rpm using a homogenizer (Model T25 basic, IKA, LABORTECHNIK, Selangor, Malaysia). The homogenate was kept in ice for 1 h and centrifuged at 5,000 x g for 5 min using a microcentrifuge (Model MIKRO20, Hettich ZENTRIFUGEN, Germany). The soluble peptide content in the supernatant was measured by the method of Lowry *et al.* (1951) and expressed as mmole tyrosine g^{-1} sample.

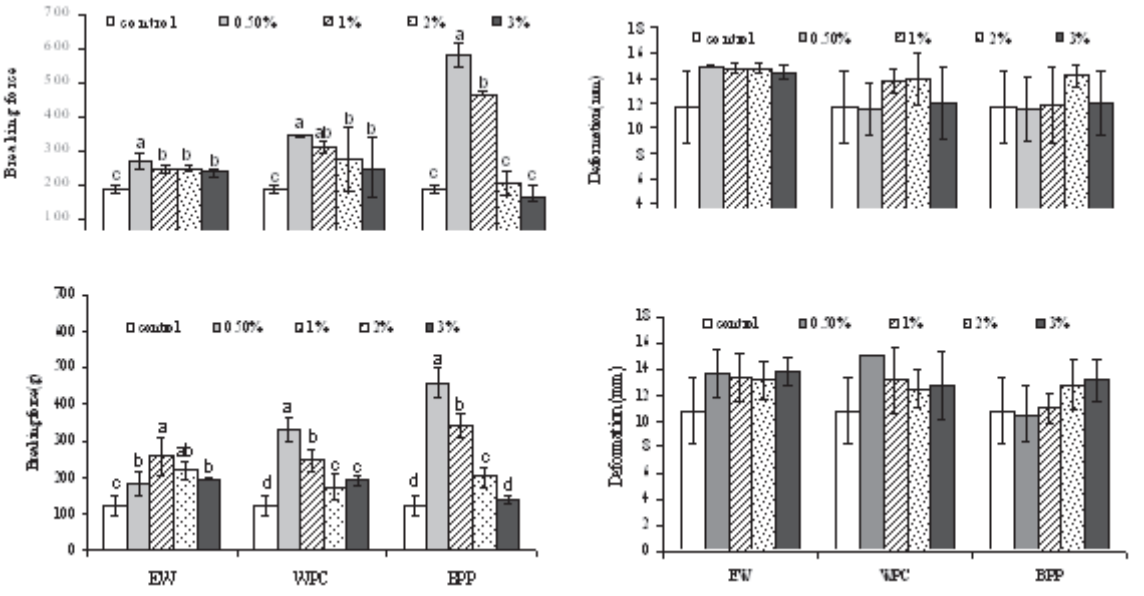

Figure 1: Breaking force and deformation of one-step (a) and two-step (b) heated gel from Pacific white shrimp meat treated with different types and concentrations of protein additives. Error bars indicate the standard deviation from five determinations. Different letters on the bars indicate significant differences (P<0.05). Percent values represent protein additive concentrations. Control: without protein additives

iv. Protein Patterns

Protein patterns of gels were determined using SDS-PAGE according to the method of Laemmli (1970) with 10% running gel and 4% stacking gel. To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85°C was added to the sample (3 g). The mixture was then homogenized for 2 min at a speed of 11,000 rpm using a homogenizer (Model T25 basic, IKA, LABORTECHNIK, Selangor, Malaysia). The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The samples were centrifuged at 8500 x g for 20 min to remove undissolved debris. Protein concentration was determined by the Biuret method (Robinson and Hodgen, 1940), using bovine serum albumin as a standard. Samples with the protein content of 20 μg were loaded onto the gel. Electrophoresis was conducted at 15 mA per plate. After separation, proteins were fixed and stained for 5 h in 0.125% Coomassie Brilliant Blue R-250 in 40% methanol and 10% glacial acetic acid. Gels were destained for 15 min with destaining solution I (50% methanol and 7.5% glacial acetic acid) and with destaining solution II (5% methanol and 7.5% glacial acetic acid) for 3 h.

v. Statistical Analysis

All experiments were run in triplicate and CRD (Completely Randomized Design) was used. Analysis of variance (ANOVA) was performed and means comparisons were carried out by Duncan's multiple range tests (Steel and Torrie, 1980). Analyses were conducted using

the SPSS software package (SPSS 11.0 for windows, SPSS Inc, Chicago, IL).

RESULTS AND DISCUSSION

Effects of Protein Additives on Textural Properties of Pacific White Shrimp Gels

Effects of EW, WPC and BPP at various concentrations (0, 0.5, 1, 2 and 3%) on properties of Pacific white shrimp gels prepared by one-step and two-step heating are depicted in Figures 1 a and b, respectively. Generally, two-step heated gel showed a lower breaking force than did the one-step heated gels. For one-step heated gel, breaking force increased as all protein additives at 0.5% were added. The marked decreases in breaking force were noticeable with increasing amounts added (P<0.05). Two-step heated gel showed similar results, except for gel added with EW, in which the highest breaking force was found when EW at 1% was added. The addition of 0.5% BPP increased breaking force by 211.9% and 283.3% for one-step and two-step heated gels, respectively. The addition of EW, WPC and BPP had no impact on deformation of gels prepared under both heating conditions (P>0.05). From the results, BPP was more effective in increasing gel strength of Pacific white shrimp gel than WPC and EW in all gels, regardless of heating conditions. Benjakul *et al.* (2004) reported that the addition of BPP and EW up to 3% increased gelling properties of lizard surimi regardless of heating condition. The effectiveness of BPP may be attributed to the proteinase inhibitory activity due to the presence of α_2M and kininogen (Hamann *et al.,* 1990; Morrisey *et al.,* 1993); plasma has protein cross-linking activity from both pig plasma transglutaminase (PTGase) and α_2-macroglobulin (α_2M) (Seymour *et al.*, 1997). This could contribute to strengthening of the gel by myosin and/or fibrinogen cross-linking and could also reduce the availability of myosin as a substrate for proteinase action (Lorand, 1983; Seki *et al.*, 1990; Kimura *et al.*, 1991; Sakamoto *et al.*, 1995). Fibrinogen and serum albumin also underwent aggregation synergistically with myosin during heating (Foegeding *et al.*, 1986). EW contains several proteinase inhibitors, namely ovomucoid, ovoinhibitor, ovomacroglobulin, which exhibit inhibitory activity against serine proteinase (Nakamura and Doi, 2000). A cysteine proteinase inhibitor, cystatin, was also found in EW. WPC was more effective in inhibiting cysteine proteinase rather than serine proteinase (Weerasinghe *et al.*, 1996).

Effects of Protein Additives on Color of Pacific White Shrimp Gel

L^*, a^* and b^* values of Pacific white shrimp gels to which EW, WPC and BPP were added at different heating conditions are shown in Table 1. The addition of all additives resulted in different changes in color values, depending on types of protein additives and heating conditions. For one-step heated gels, all protein additives resulted in the increase in b^*-value, indicating the increases in yellowness. Pigments in protein additives might contribute to such a change. Benjakul *et al.* (2001) found that the lower whiteness of gel was observed with plasma protein addition, because some hemoglobin as well as other pigments with a pale straw color were retained in the plasma. Reppon and Babbitt (1993) also found that L^* - value of arrowtooth flounder surimi decreased with addition of BPP.

Effects of Protein Additives on Pattern of Protein Degradation of Pacific White Shrimp Gels

TCA soluble peptide content in Pacific white shrimp gels can be used as an indicator of autolytic degradation of muscle protein. The autolysis was inhibited to a greater extent in one-step heated gel as protein additives levels increased (P<0.05) (Figure 2). Nevertheless, no differences in autolysis inhibition were observed in two -step heated gel when protein additives were used at levels ranging from 0.5 to 3%. However, the lower TCA-soluble peptide content was observed with the addition of protein additives.

Table 1
L a** and *b**- values of one-step and two-step heated gels from Pacific white shrimp meat treated with different types and levels of protein additives

Treatment	Concentration %	Color					
		*L**		*a**		*b**	
		one-step heated	one-step heated	one-step heated	one-step heated	one-step heated	one-step heated
EW	-	74.83±0.23c	80.06±0.51c	18.34±0.58b	19.91±0.46a	18.11±0.68b	21.20±0.17a
	0.5	80.31±0.33ab	80.12±0.45c	19.65±0.09a	19.40±0.46b	21.75±0.14a	20.66±0.20b
	1	80.17±0.08b	80.78±0.44b	19.57±0.17a	18.73±0.34c	21.74±0.41a	20.46±0.19bc
	2	80.61±0.31a	81.00±0.25b	19.45±0.33a	18.79±0.15c	21.53±0.45a	20.24±0.17c
	3	80.47±0.24ab	82.23±0.21a	19.23±0.09a	18.01±0.19d	21.45±0.20a	19.51±0.13d
WPC	-	74.83±0.23c	80.06±0.51a	18.34±0.58c	19.94±0.46b	18.11±0.68b	21.20±0.17c
	0.5	78.90±0.73b	79.03±0.25b	19.44±0.56b	20.66±0.23a	18.86±0.86b	21.93±0.19a
	1	79.21±0.66b	79.83±0.12a	20.18±0.58a	19.69±0.16b	22.06±1.01a	20.79±0.22d
	2	79.98±0.53a	79.74±0.15a	19.30±0.36b	19.08±0.35c	22.15±0.26a	21.52±0.31b
	3	80.10±0.29a	79.93±0.17a	19.11±0.20b	18.78±0.27d	21.49±0.15a	20.73±0.17d
BPP	-	74.83±0.23c	80.06±0.52a	18.34±0.58a	19.94±0.46a	18.11±0.68c	21.20±0.17d
	0.5	77.79±0.83a	79.59±0.15a	18.24±0.84a	18.16±0.06b	20.32±1.93b	21.91±0.08c
	1	78.21±0.30a	78.40±0.21b	16.42±0.59c	17.76±0.16c	17.98±1.14c	21.37±0.14d
	2	78.19±0.93a	77.08±0.37c	17.25±0.38b	17.15±0.20d	23.96±0.18a	22.36±0.33b
	3	76.67±0.60b	76.60±0.10d	16.12±0.39c	15.55±0.06e	24.63±0.40a	23.53±0.16a

Different letters in the same column within the same protein additive indicate significant differences (P<0.05)
Mean ± SD from triplicate determinations.

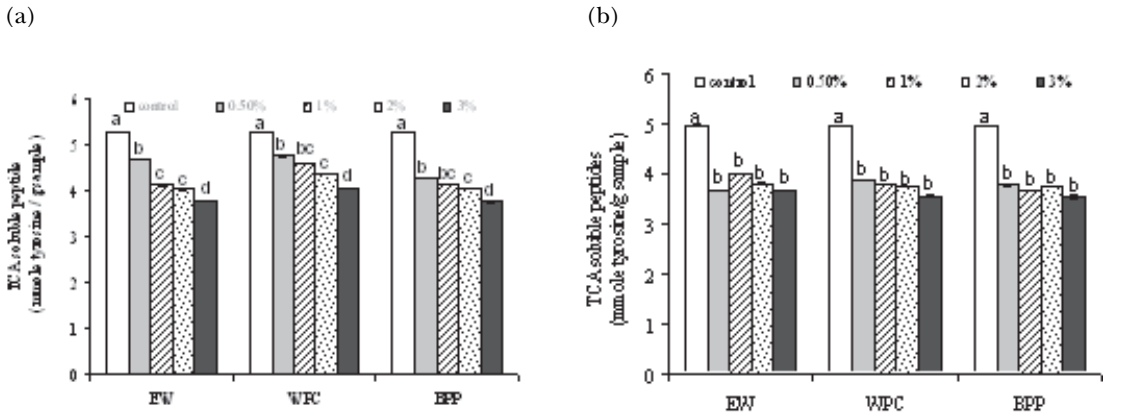

Figure 2: TCA soluble peptide content of one-step (a) and two-step (b) heated gels from Pacific white shrimp meat treated with different types and concentrations of protein additives. Error bars indicate the standard deviation from three determinations. Different letters on the bars indicate significant differences (P<0.05)
Control: without protein additives

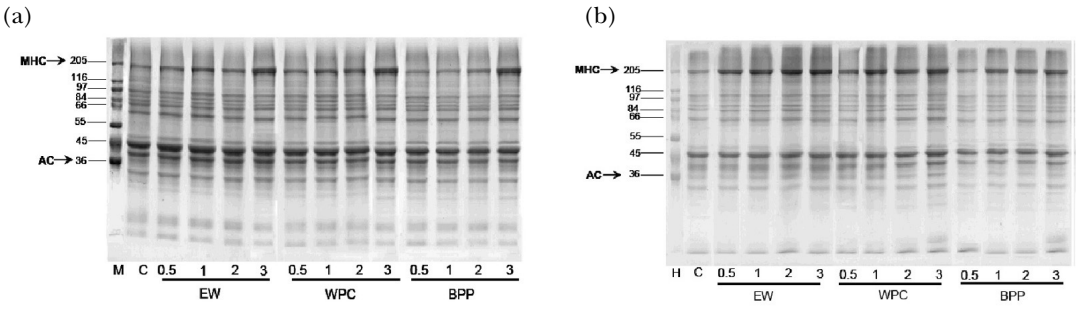

Figure 3: SDS-PAGE pattern of one-step (a) and two-step (b) heated gels from Pacific white shrimp meat treated with different types and concentrations of protein additives. M: high molecular weight marker; C: control (without protein additive); EW: egg white; WPC: whey protein concentrate; BPP: bovine plasma protein. Values on the X-axis represent protein additive concentrations (%).

Protein Pattern of Pacific White Shrimp Gels

Protein patterns of Pacific white shrimp gel added with different types and levels of protein additives are shown in Figure 3. The lowest intensity of MHC band in SDS-PAGE was observed in Pacific white shrimp gels without protein additives. For both heating conditions, MHC was retained more as the concentration of protein additives increased. MHC band intensity increased with increasing amount of protein additives. This might be caused by the inhibitory effect of protein additive on muscle protein degradation. However no changes in actin were observed for all treatments. Rawdkuen *et al.* (2007) also reported that chicken plasma protein (CPP) can prevent the degradation of MHC of modori gel (incubated at 70°C for 30 min followed by heating at 90°C for 20 min) from sardine (*Sardinella gibbosa*) surimi. Thus, protein additives could prevent proteolysis in the gel to some extent, leading to improved gel properties.

CONCLUSION

EW, WPC and BPP at appropriate levels effectively enhanced Pacific white shrimp gel strength. Generally an excessive amount of protein additives caused a decrease in gel strength. Although, BPP at a level of 0.5% (w/w) showed the highest inhibitory activity toward autolysis of Pacific white shrimp gel, it showed adverse effect on flavor and color of gel.

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