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ScienceDirect
Food Chemistry 106 (2008) 1077–1084
Food Chemistry
www.elsevier.com/locate/foodchem

Whey protein concentrate: Autolysis inhibition and effects on the gel properties of surimi prepared from tropical fish

Saroat Rawdkuen [a,*], Soottawat Benjakul [b]

[a] Food Technology Program, School of Agro-Industry, Mae Fah Luang University, Muang, Chiang Rai 57100, Thailand
[b] Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

Received 22 April 2007; accepted 12 July 2007

Abstract

Effects of whey protein concentrate (WPC) on autolysis inhibition and gel properties of surimi produced from bigeye snapper (*Priacanthus tayenus*), goatfish (*Mulloidichthys vanicolensis*), threadfin bream (*Nemipterus bleekeri*) and lizardfish (*Saurida tumbil*) were investigated. WPC (0–3%) showed inhibitory activity against autolysis in all surimi at both 60 and 65 °C in a concentration-dependent manner. Myosin heavy chain (MHC) of surimi was more retained in the presence of WPC. Breaking force and deformation of kamaboko gels of all surimi increased as added levels of WPC increased ($P < 0.05$). This was associated with lower levels of protein degradation, as evidenced by the decrease in trichloroacetic acid-soluble peptide content ($P < 0.05$). WPC at 3% (w/w) significantly decreased the whiteness of gels. However, water-holding capacity of kamaboko gels was improved with increasing concentration of WPC. The microstructure of surimi gels generally became denser with the addition of WPC.
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Keywords: Surimi gel; Gel weakening; Proteinase; Whey; Gel strength

1. Introduction

Whey protein concentrate (WPC) has commonly been used as a protein supplement, foam stabiliser, filler/water binder, thickening, emulsifying and gelling agent (Morr & Foegeding, 1990). It can be used to improve texture and nutritional value of a variety of foods, such as sausages, meat balls and low-salt fish products (Giese, 1994; Ulu, 2004; Uresti, Tellez-Luis, Ramirez, & Vazquez, 2004).

Proteolytic degradation of myofibrillar proteins has an adverse effect on gel-forming properties of surimi. The presence of indigenous proteinases brings about the gel softening of surimi from some fish species, e.g. threadfin bream (Kinoshita, Toyohara, & Shimizu, 1990), arrowtooth flounder (Wasson, Babbit, & French, 1992), Pacific whiting (Seymour, Morrissey, Peter, & An, 1994), lizardfish and bigeye snapper (Benjakul, Visessanguan, & Tueksuban, 2003; Benjakul, Visessanguan, & Leelapongwattana, 2003). The active proteinases in fish muscle, which soften the surimi gel, vary with fish species. An, Weerasinghe, Seymour, and Morrissey (1994) reported that cathepsins B and L were the most active cysteine proteinases in Pacific whiting fish fillets and surimi, respectively. Myofibril-associated proteinases in lizardfish were cysteine and serine proteinases, while a serine proteinase was found in muscle of bigeye snapper (Benjakul, Visessanguan, & Tueksuban, 2003; Benjakul, Visessanguan, & Leelapongwattana, 2003). To improve the physical properties and prevent the textural degradation of surimi gels, various food-grade inhibitors, such as egg white, beef plasma protein (BPP) and potato powder have been used (Akazawa, Miyauchi, Sakurada, Wasson, & Reppond, 1993; Benjakul, Visessanguan, Tueksuban, & Tanaka, 2004). However, the use of BPP has been prohibited, due to the occurrence of mad cow disease. In addition, some BPP preparations result in off-flavours at concentrations greater than 1% (w/w). Egg white is expensive and has an undesirable egg-like odour, while off-colour

* Corresponding author. Tel.: +66 5391 6752; fax: +66 5391 6739.
E-mail address: saroat@mfu.ac.th (S. Rawdkuen).

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doi:10.1016/j.foodchem.2007.07.028

problems may be encountered when potato powder is used (Akazawa et al., 1993). Therefore, alternative food-grade proteinase inhibitors for surimi production are still needed. Previous studies have shown that WPC addition increases the shear strain of surimi gels prepared from Pacific whiting and Alaska pollock (Chang-Lee, Lampila, & Crawford, 1990; Park, 1994; Piyachomkwan & Penner, 1995; Weerasinghe, Morrissey, Chung, & An, 1996). Furthermore, some researchers have reported that WPC may inhibit protein degradation in Pacific whiting surimi (Akazawa et al., 1993; Piyachomkwan & Penner, 1995). Addition of WPC had no adverse effect on sensory attributes of gels formed from Pacific whiting surimi (Weerasinghe et al., 1996). Nevertheless, no information regarding the use of WPC has been reported in surimi from tropical fish. Therefore, the objective was to investigate the effects of WPC on autolysis and gel properties of surimi produced from tropical fish.

2. Materials and methods

2.1. Chemicals and surimi

Whey protein concentrate (WPC: Proliant 8600) was obtained from I.P.S. International Co., Ltd. (Bangkok, Thailand). Bovine serum albumin (BSA) was obtained from Fluka Chemika–BioChemika (Buchs, Switzerland). L-Tyrosine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium dodecyl sulphate (SDS), *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) and Coomassie Blue R-250 were procured from Bio-Rad Laboratories (Hercules, CA, USA).

Frozen surimi grade B (breaking force of 100–400 g; deformation of <8 mm), freshly produced from bigeye snapper (*Priacanthus tayenus*), goatfish (*Mulloidichthys vanicolensis*), threadfin bream (*Nemipterus bleekeri*) and lizardfish (*Saurida tumbil*), were purchased from Man A Frozen Foods Co., Ltd. (Songkhla, Thailand) and kept at −20 °C not more than two months before use.

2.2. Effect of whey protein concentrate on autolytic activity of surimi

2.2.1. Autolytic activity assay

Autolytic activity assay was performed according to the method of Morrissey, Wu, Lin, and An (1993). WPC at concentrations of 0%, 0.5%, 1%, 2% and 3% (w/w) was added to surimi (3 g) and mixed thoroughly. Samples with and without WPC were incubated at 60 and 65 °C for 60 min, since 60–65 °C was the temperature range giving the maximal autolysis of tropical fish (Benjakul, Visessanguan, & Tueksuban, 2003; Benjakul, Leelapongwattana, & Visessanguan, 2003). Then, 27 ml of cold 5% (w/v) trichloroacetic acid (TCA) were added to terminate the autolysis reaction. The mixture was centrifuged at 8000*g* for 5 min using a Mikro 20 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). The concentration of the soluble peptides released in the supernatant was measured by using the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). %Inhibition was calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{tyrosine released (without WPC)} - \text{tyrosine released (with WPC)}}{\text{tyrosine released (without WPC)}} \times 100$$

2.2.2. SDS–polyacrylamide gel electrophoresis

To determine the electrophoretic profile of the incubated surimi systems, 27 ml of 5% SDS (85 °C) were added instead of 5% TCA to the samples subjected to autolysis in the absence and in the presence of WPC at 60 and 65 °C, as mentioned previously. The mixtures were homogenised at a speed of 11,000 rpm for 1 min using an IKA Labortechnik homogeniser (Selangor, Malaysia). The homogenate was incubated at 85 °C for 60 min, followed by centrifugation at 10,000*g* for 5 min, using a Mikro 20 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). The supernatant was then subjected to SDS-PAGE according to the method of Laemmli (1970). Supernatants were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4% SDS, 20% glycerol and 10% βME) and boiled for 3 min. The samples (20 μg protein) were loaded into the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protean II unit (Bio-Rad Laboratories Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% (v/v) methanol and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.3. Effect of whey protein concentrate on gel properties of surimi

2.3.1. Surimi gel preparation

Frozen surimi was partially thawed at 4 °C for 4–5 h, cut into small pieces with an approximate thickness of 1 cm and then placed in the mixer (Moulinex Masterchef 350, Paris, France). The moisture content of samples was then adjusted to 80% (w/w) and salt (2.5%, w/w) was added. WPC, at different concentrations (0%, 0.5%, 1%, 2% and 3% w/w), was added. The mixture was chopped for 4 min at 4 °C. The paste was stuffed into a polyvinylidine chloride casing with a diameter of 2.5 cm and both ends were sealed tightly. The paste was incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min in a water bath (Memmert, Schwabach, Germany). After heating, all gels were immediately cooled in iced water for 30 min and stored at 4 °C overnight prior to analysis. The gel was referred to as "kamaboko gel". Protein patterns of surimi gel samples, after solubilization with 5% SDS, were determined by SDS-PAGE, as described above.

2.3.2. Texture analysis

Texture analysis of kamaboko gels was carried out using a Model TA-XT2 texture analyser (Stable Micro System, Surrey, UK). Gels were equilibrated at room temperature (25–30 °C) before analysis. Five cylindrical samples (2.5 cm in length) were prepared and tested. Breaking force (strength) and deformation (cohesiveness/elasticity) were measured by the texture analyser equipped with a spherical plunger (5 mm diameter), with a depression speed of 60 mm/min and 60% compression.

2.3.3. Determination of whiteness

Three gel samples from each treatment were subjected to whiteness measurement using a HunterLab (ColorFlex, Hunter Associates Laboratory, VA, USA). Illuminant C was used as the light source of measurement. CIE L^*, a^* and b^* values were measured. Whiteness was calculated using the following equation (Park, 1994):

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

2.3.4. Determination of expressible moisture

Expressible moisture was measured according to the method of Ng (1987). Cylindrical gel samples were cut to a thickness of 5 mm, weighed (X) and placed between two pieces of Whatman paper (No. 1) at the bottom and one piece of paper on the top. A standard weight (5 kg) was placed on the top of the sample for 2 min, and then the sample was removed from the papers and weighed again (Y). Expressible drip was calculated and expressed as percentage of sample weight as follows:

$$\text{Expressible drip } (\%) = [(X - Y)/X] \times 100$$

2.3.5. Determination of autolysis in surimi gel

To 2 g of finely chopped gel samples, 18 ml of 5% TCA were added and homogenised for 2 min using an IKA Labortechnik homogeniser (Selangor, Malaysia) at a speed of 11,000 rpm for 2 min. The homogenate was incubated at 4 °C for 1 h and centrifuged at 8000g for 5 min, using a Mikro 20 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). TCA-soluble peptides in the supernatant were measured according to the Lowry method (Lowry et al., 1951) and expressed as micromole tyrosine/g sample.

2.3.6. Scanning electron microscopy (SEM)

Microstructure of kamaboko gels was determined using a scanning electron microscope (SEM) (JEOL JSM-5800 LV, Tokyo, Japan). Kamaboko gels from bigeye snapper, goatfish, threadfin bream and lizardfish surimi, without and with 3% WPC, with a thickness of 2–3 mm, were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h. The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with a serial concentration of 50%, 70%, 80%, 90% and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, PA, USA). The specimens were observed with a SEM at an acceleration voltage of 10 kV.

2.4. Statistical analysis

Completely randomized design was used throughout the study. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel & Torrie, 1980). Analysis was performed using a SPSS package (SPSS 10.0 for windows, SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Effect of whey protein concentrate on autolytic activity of surimi

Inhibition of surimi autolysis at 60 and 65 °C, in the absence and the presence of WPC at levels of 0.5%, 1%, 2% and 3%, is shown in Fig. 1A and B, respectively. In general, a greater degree of inhibition was observed when the levels of WPC added increased at both 60 and 65 °C ($P < 0.05$). At the same WPC concentration, different percentages of inhibition were observed among different species. This suggested that proteinases in surimi might be

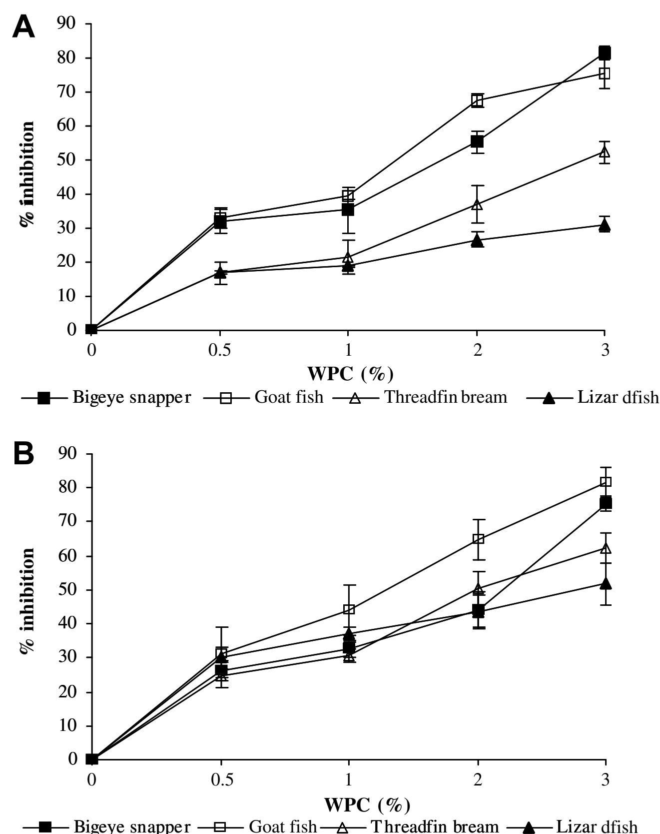

Fig. 1. Effect of whey protein concentrate at different concentrations on autolysis inhibition of surimi from some tropical fish. Samples were incubated for 60 min at 60 °C (A) and 65 °C (B). Bars represent the standard deviations from triplicate determinations.

different in terms of types and amount. Generally, WPC showed the highest level of inhibition for surimi goatfish compared to other species ($P < 0.05$). WPC exhibited the lowest level of inhibition against autolysis in lizardfish surimi at both incubation temperatures, compared to other species ($P < 0.05$). Regardless of added WPC levels, a higher proteolytic activity remaining in the lizardfish surimi was observed, compared with surimi from other species. Thus, it was postulated that lizardfish surimi contained a higher amount of indigenous proteinases than did other surimi. Suwansakornkul, Itoh, Hara, and Obatake (1993) reported that both serine and cysteine proteinases were responsible for MHC degradation of washed lizardfish mince. Benjakul, Visessanguan, and Tueksuban (2003) also reported that a cysteine proteinase was found to be a major sarcoplasmic proteinase in lizardfish muscle. With the addition of 3% WPC, 81.4%, 75.6%, 52.3%, 31.2% and 75.3%, 81.5%, 62.4%, 51.9% inhibition against autolysis were observed in bigeye snapper, goatfish, threadfin bream and lizardfish surimi incubated at 60 and 65 °C, respectively. From the result, WPC showed no marked differences in autolysis inhibition pattern when surimi was incubated at 60 °C (Fig. 1A) and 65 °C (Fig. 1B). It was found that WPC showed the greatest inhibitory effect on autolysis of surimi from goatfish, as indicated by the highest % inhibition. However, at some levels of WPC added, slight differences in the efficacy of autolysis inhibition between the two temperatures were noticeable for the same surimi tested. Piyachomkwan and Penner (1995) reported that WPC may protect myofibrillar proteins of surimi by acting as a true inhibitor or by serving as an alternative substrate, which effectively decreased the proteolytic activity on myosin *per se*. Akazawa et al. (1993) also found that increasing WPC concentration to 3% reduced the enzyme activity of Pacific whiting surimi by approximately 80% compared with the control (without WPC). WPC was reported to contain both serine and cysteine proteinase inhibitors (Weerasinghe et al., 1996). Due to the differences in proteolytic activity among surimi from different fish species, different levels of WPC are needed to reduce proteolysis.

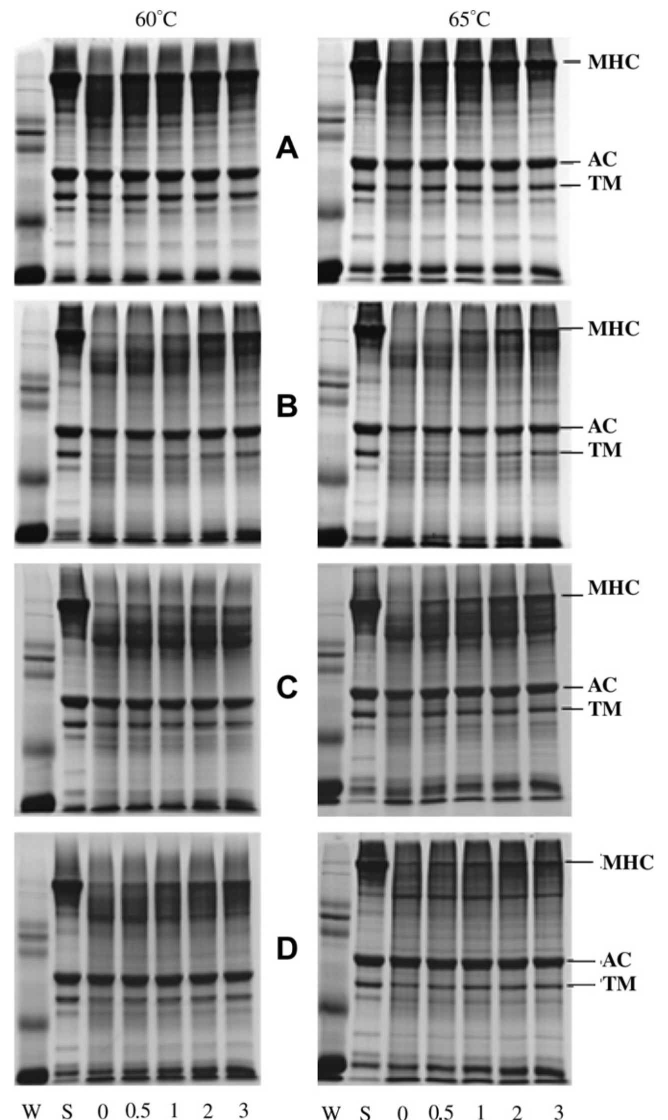

Fig. 2. Autolysis pattern of surimi containing added whey protein concentrate at different levels. Samples were incubated for 60 min at 60 and 65 °C. W: whey protein concentrate; S: surimi; MHC: myosin heavy chain; AC: actin; TM: tropomyosin. Numbers denote the amounts of WPC added (%). (A) bigeye snapper, (B) goatfish, (C) threadfin bream, (D) lizardfish.

3.2. Effect of whey protein concentrate on autolysis pattern of surimi

Autolysis patterns of surimi from different fish species incubated at 60 and 65 °C, in the absence and presence of WPC at different levels, are depicted in Fig. 2. MHC was found as the major protein band, followed by actin and tropomyosin in all surimi (lane 2). When the surimi was incubated at 60 or 65 °C for 60 min, the MHC band almost disappeared, except in surimi from bigeye snapper, which still showed high density of the MHC band. However, actin in all species was found to be resistant to proteolysis. The susceptibility to autolysis of tropomyosin varied with species. Tropomyosin of surimi from bigeye snapper was more resistant to degradation, compared to other species. An et al. (1994) reported that MHC, β-tropomyosin and troponin-T were more susceptible to degradation than actin when Pacific whiting surimi was incubated at 55 °C up to 1 h. Benjakul, Visessanguan, and Tueksuban (2003) reported that degradation of muscle proteins, especially MHC, in both washed and unwashed lizardfish mince, occurred at temperatures ranging from 60 to 65 °C. MHC band intensity of all samples generally increased as the concentration of WPC increased. The result indicted that WPC could inhibit the degradation of MHC to some extent, as evidenced by the more retained MHC. However, the efficacy in preventing the degradation of MHC varied with surimi. The result was in agreement with the varying inhibitory activity of WPC against autolysis of different surimi (Fig. 1). However, bigeye snapper surimi showed

the highest level of retention of MHC at all levels of WPC added, compared with others. When WPC at 2% was added, the MHC band was more retained in both bigeye snapper and goatfish surimi, than that found in the control surimi (without WPC addition). It was noted that a very small MHC band was retained in the control goatfish surimi, while a larger MHC band was found in the control bigeye snapper surimi. This suggested a greater autolytic activity in goatfish surimi. The more retained MHC in goatfish surimi treated with 2% WPC confirmed the high efficiency of autolysis inhibition of WPC. From the result, the degradation caused by indigenous heat-activated proteinases could be impeded to some extent by the addition of WPC. Piyachomkwan and Penner (1995) reported that autolysis was undetectable in Pacific whiting surimi containing 2% added WPC.

3.3. Effect of whey protein concentrate on textural properties of surimi gels

Breaking force and deformation of kamaboko gel of surimi from four fish species containing added WPC at the levels of 0–3% are shown in Fig. 3. The lowest breaking force and deformation were observed in gels containing no WPC ($P < 0.05$). For surimi from bigeye snapper, breaking force of kamaboko gel increased with increasing WPC concentrations ($P < 0.05$). However, no marked increases in breaking force were found in other surimi gels with the addition of WPC above 2% ($P > 0.05$). Breaking force of kamaboko gels, produced from bigeye snapper, goatfish, threadfin bream and lizardfish surimi containing 3% added WPC, increased by 61.22%, 55.69%, 81.84% and 80.89%, respectively, and deformation increased by 18.37%, 12.24%, 27.68% and 31.42%, respectively, compared to those of the control gel (without WPC). For the deformation, the increases were noticeable with the addition of WPC up to 1%. However, no difference in deformation was observed ($P > 0.05$) between the gels of lizardfish surimi without and with 0.5% WPC. No changes in deformation were found with further increasing amount of WPC. From this result, WPC at 3% could improve the grade of bigeye snapper surimi from B (breaking force 332 g) to AA (breaking force 535 g) and increased the grade of threadfin bream surimi from B (breaking force 227 g) to A (breaking force 413 g). The result revealed that WPC was effective in increasing gel strength of all surimi. This might be associated with the inhibitory effect of WPC on autolysis (Figs. 1 and 2). Bigeye snapper and threadfin bream contain serine proteinase as a major indigenous enzyme associated with myofibrillar proteins, while lizardfish muscle contains both serine and cysteine proteinases (Benjakul, Leelapongwattana, & Visessanguan, 2003; Benjakul, Visessanguan, Tueksuban, & Tanaka, 2004; Kinoshita et al., 1990). For surimi produced from goatfish and lizardfish, though WPC did not improve the gel strength to the higher grade, breaking force was increased by more than 50%, compared with that of gels without WPC. Weerasinghe et al. (1996) reported that WPC inhibited both serine and cysteine proteinases by using papain and trypsin inhibitory activity assay. Akazawa et al. (1993) found that the addition of WPC resulted in an improvement in heat-set gel texture and an apparent inhibition of autolysis of Pacific whiting surimi. Apart from the inhibition of proteolysis during gel formation caused by indigenous proteinases, WPC might function as the filler in the gel matrix. This gel strengthening could take place in concert with the retardation of protein degradation.

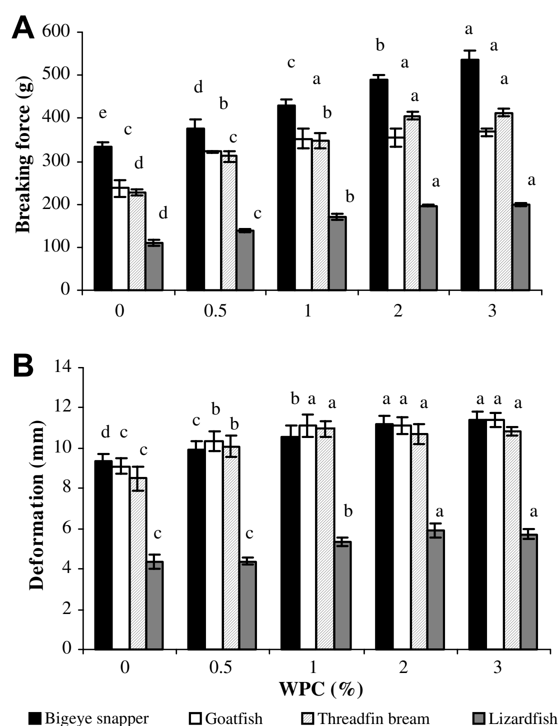

Fig. 3. Breaking force (A) and deformation (B) of kamaboko gels containing added whey protein concentrate at different levels. Bars represent the standard deviations from five determinations. Different letters within the same fish species indicate significant differences ($P < 0.05$).

3.4. Effect of whey protein concentrate on whiteness of surimi gel

The whiteness of kamaboko gels containing added WPC at different levels and control (without WPC) is shown in Table 1. The whiteness of kamaboko gels containing added WPC decreased to the some extent, compared with the control. However, no changes in whiteness were noticeable in kamaboko gel from lizardfish ($P > 0.05$). From this result, the highest whiteness was obtained in kamaboko gels from threadfin bream ($P < 0.05$), followed by the gels from bigeye snapper, lizardfish and goatfish surimi, respectively. For the gels of surimi from bigeye snapper and goatfish, the addition of WPC up to 1% and 2% showed no effect on whiteness ($P > 0.05$). On the other hand, addition of 3% WPC resulted in a decrease in whiteness of all kamaboko gels ($P < 0.05$), except the gels from lizardfish

Table 1
Whiteness[A] of kamaboko gels containing added whey protein concentrate at different concentrations

Samples	Bigeye snapper	Goatfish	Threadfin bream	Lizardfish
Kamaboko gel (K)	$79.62 \pm 0.50^{a,*}$	73.14 ± 0.30^{a}	82.92 ± 0.36^{a}	77.34 ± 0.10^{a}
K + 0.5% WPC	79.20 ± 0.28^{a}	72.72 ± 0.72^{a}	82.30 ± 0.24^{b}	77.45 ± 0.36^{a}
K + 1% WPC	79.15 ± 0.22^{a}	72.40 ± 0.31^{a}	82.31 ± 0.04^{b}	77.18 ± 0.18^{a}
K + 2% WPC	78.40 ± 0.49^{b}	72.49 ± 0.15^{a}	81.62 ± 0.25^{c}	77.24 ± 0.10^{a}
K + 3% WPC	78.26 ± 0.03^{b}	71.48 ± 0.16^{b}	80.86 ± 0.45^{d}	77.24 ± 0.26^{a}

[A] Values are given as means ± SD from triplicate determinations.
[*] Different superscripts in the same column indicate significant differences ($P < 0.05$).

surimi, which showed no changes in whiteness ($P > 0.05$). Since WPC is predominantly light cream-coloured in nature, it might reduce the whiteness of surimi gel slightly, especially when a higher amount is used. Thus, WPC at a low level can be used as a protein additive in surimi to improve the gel strength without causing marked changes in whiteness.

3.5. Effect of whey protein concentrate on expressible moisture of surimi gel

The expressible moisture content of kamaboko gels containing added WPC at different levels is shown in Table 2. The expressible moisture content significantly decreased as the level of WPC added increased ($P < 0.05$). The lowest expressible moisture content was found in the gel sample containing 3% added WPC. This indicated that water-holding capacity of kamaboko gel was improved with the addition of WPC. Among surimi gels produced from the four fish species, lizardfish surimi showed the highest expressible moisture at all levels of WPC used. The result showed that the gel of lizardfish surimi was poor in water holding, which might be associated with the inferior gel network. However, the expressible moisture content was decreased with WPC addition. Decrease in expressible moisture content indicated an increase in water-holding capacity of the gel. The high water-holding capacity of protein additives causes them to swell and augment elasticity by reducing the moisture content of the mixtures and increasing the density of surrounding protein matrix (Niwa, Wang, Kanoh, & Nakayama, 1988). Gomez-Guillen and Montero (1996) also concluded that adding hydrocolloids (2% iota-carageenan, egg white, soy protein) and then incubating the mixture at 37 °C for 30 min and 90 °C for 50 min, considerably increased water-holding capacity of sardine mince gel.

3.6. Effect of whey protein concentrate on the degradation of surimi gel

For all surimi, no difference in TCA-soluble peptides was noticeable between gels treated with 0.5% and 1% WPC ($P > 0.05$) (Fig. 4). Also, no marked differences in TCA-soluble peptides were found in gels from goatfish and threadfin bream surimi treated with 2% and 3% WPC ($P > 0.05$). TCA-soluble peptide contents of kamaboko gels produced from bigeye snapper, goatfish, threadfin bream and lizardfish surimi containing 3% added WPC decreased by 90.91%, 93.33%, 91.30% and 66.67%, respectively, compared with that of the control kamaboko gel.

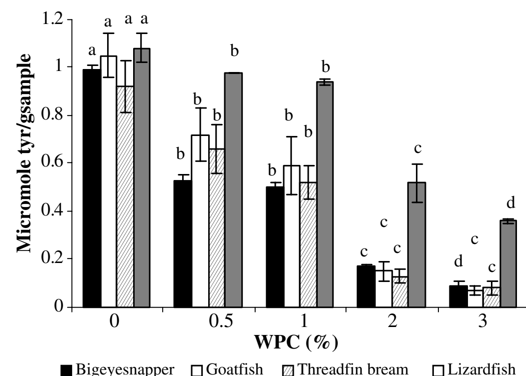

Fig. 4. TCA-soluble peptide content in kamaboko gels containing added whey protein concentrate at different levels. Bars represent the standard deviations from triplicate determinations. Different letters within the same fish species indicate significant differences ($P < 0.05$).

Table 2
Expressible moisture[A] of kamaboko gels containing added whey protein concentrate at different concentrations

Samples	Bigeye snapper	Goatfish	Threadfin bream	Lizardfish
Kamaboko gel (K)	$3.68 \pm 0.04^{a,*}$	3.97 ± 0.09^{a}	3.68 ± 0.12^{a}	11.61 ± 1.06^{a}
K + 0.5% WPC	3.56 ± 0.03^{b}	3.73 ± 0.16^{ab}	3.49 ± 0.09^{b}	8.64 ± 0.56^{b}
K + 1% WPC	3.49 ± 0.03^{c}	3.71 ± 0.21^{ab}	3.30 ± 0.07^{c}	5.67 ± 0.42^{c}
K + 2% WPC	3.31 ± 0.04^{d}	3.59 ± 0.17^{b}	3.20 ± 0.08^{cd}	5.28 ± 0.33^{cd}
K + 3% WPC	3.07 ± 0.03^{e}	3.24 ± 0.07^{c}	3.12 ± 0.06^{d}	4.53 ± 0.17^{d}

[A] Values are given as means ± SD from triplicate determinations.
[*] Different superscripts in the same column indicate significant differences ($P < 0.05$).

This result indicated that the addition of WPC at a level of 3% might be effective enough to prevent proteolysis in surimi gel of most fish species tested, except for the gel from lizardfish surimi. Setting at 40 °C accelerates the proteolysis in surimi from some tropical fish (Benjakul, Chantarasuwan, & Visessanguan, 2003; Benjakul, Visessanguan, & Chantarasuwan, 2004). From these results, the highest TCA-soluble peptide content was observed in the sample without WPC addition. This result was in agreement with the lowest breaking force and deformation of the gels without WPC addition (Fig. 3). The results reconfirmed that the improved gel strength of surimi from the addition of WPC was associated with a reduction in the extent of proteolysis that occurred.

3.7. Effect of WPC on microstructure of surimi gel

The microstructures of kamaboko gels from bigeye snapper, goatfish, threadfin bream and lizardfish surimi, without and with 3% WPC, were visualised by SEM, as shown in Fig. 5. The microstructure of kamaboko gel without WPC showed a well-structured matrix with a highly interconnected network of strands and fine three-dimensional protein network. However, the protein network of kamaboko gel containing 3% added WPC seemed to be more compact with smaller clusters of aggregated protein, than that of the control gel. These observations suggested that WPC might distribute uniformly as the filler in the ordered network. From the micrograph, the microstructure of kamaboko gel from bigeye snapper was finer and denser than others, while the gel structure of lizardfish consisted of a large number of pores and loose structure. Barbut and Foegeding (1993) indicated that fine-stranded gels are usually formed by an ordered association of protein molecules.

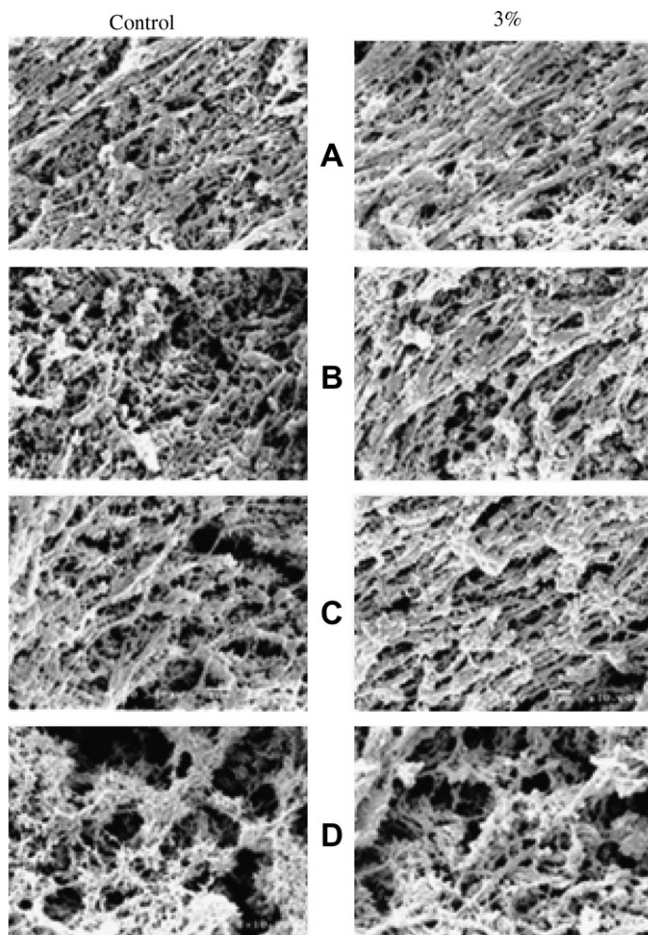

Fig. 5. Electron microscopic image of kamaboko gels containing 3% added whey protein concentrate and control (without whey protein concentrate) (Magnification: 10,000×). (A) bigeye snapper, (B) goatfish, (C) threadfin bream, (D) lizardfish.

4. Conclusion

Autolysis of surimi from some tropical fish caused by heat-activated proteinase was partially inhibited by the addition of WPC. The addition of WPC at concentrations up to 3% (w/w) increased the breaking force and deformation of kamaboko gels and improved the grade of surimi from some fish species.

Acknowledgement

The authors thank I.P.S. International Co., Ltd. (Bangkok, Thailand) for providing the whey protein concentrate.

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