Effect of Whey Protein Concentrate on Gel-Forming Ability of Rohu (*Labeo rohita*)

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***Abstract*—The gel-forming ability of rohu (*Labeo rohita*) was investigated. Unwashed and washed gel was prepared under different setting conditions including kamaboko gel (40°C, 30 min) and modori gel (60 and 65°C for washed and unwashed gel, respectively, 30 min), then both gels were heated at 90°C for 20 min. The gel-forming ability was investigated using the folding test, gel strength, and water-holding capacity expressed by the expressible water content. The addition of whey protein concentrate (WPC) at 3% improved the folding test, gel strength, and water-holding capacity of unwashed modori gel. The addition of (WPC) at 2% increased the gel strength of unwashed kamaboko gel and improved the water-holding capacity. The increases in gel strength of both washed gels were found with WPC at 1%. The water-holding capacity of washed modori gel was improved at this concentration, while the addition of WPC did not affect the water-holding capacity of the washed kamaboko gel. Although WPC improved the modori gel, the increases were not as high as those for the kamaboko gel either unwashed or washed. Determinations of whiteness showed that the addition of WPC affected the whiteness of unwashed gel, while not affecting the washed gel.**

***Index Terms*—rohu (*Labeo rohita*), gel-forming ability, modori gel, kamaboko gel, whey protein concentrate**

I. Introduction

Rohu (*Labeo rohita*) is a freshwater fish species that is widely-used in aquaculture in Thailand. Based on the data for the year 2010, the amount of rohu produced in Thailand was 1167 tons, with a market value of 42 million Baht [1]. Nevertheless, the market value of rohu is considered low. Furthermore, rohu have small pin bones in their flesh, which hinders their utilization for cooking [2]. This might be the reason that rohu is less utilized than other fish. To expand utilization and the market value of rohu, the flesh could be processed by mincing or for use as surimi.

The degradation of myofibrillar protein by endogenous protease has been widely studied. The presence of this enzyme has an adverse effect on the gel-forming, producing too soft and mushy a gel. Ref. [3], [4] reported that the endogenous protease is activated at 50 - 70°C. This phenomenon is known as "modori", and causes destruction of the three-dimensional structure of the gel network [5], [6].

To overcome the softening of modori gel, a variety of food grade inhibitors have been used to inhibit the degradation of the myofibrillar protein, including beef plasma protein (BPP), egg protein, and whey protein concentrate (WPC) [7], [8], [5], [9]. From research, BPP contains α_2 – macroglobulin (α_2M) and kininogens that inhibit the endogenous protease activity [10]. Egg protein contains ovomucoid, which inhibits trypsin, and ovoinhibitors, which inhibit trypsin and chymotrypsin. However, BPP was prohibited because of the occurrence of mad cow disease and egg white has an undesirable egg-like odour and is expensive [7]. However, the addition of WPC can inhibit endogenous protease resulting from the protein that has a molecular weight of 100,000 Da. It can inhibit papain (cysteine protease) and trypsin (serine protease) [10]. In addition, WPC is "generally recognized as safe (GRAS)" by the U.S Food and Drug Administration [11] and had no adverse effect on the attributes of the gel [12], [5].

Whey protein concentrate (WPC) is obtained from cheese manufacturing and is commonly used as a thickener, whipping agent, emulsifier, whitener, or gelling agent [13]. Moreover, WPC has been used in various foods such as sausage, meat balls and low-salt fish products [14], [12], [5]. Furthermore, research has reported that the addition of WPC improved the gel strength in Pacific whiting, Alaska pollock [15], [12], bigeye snapper (*Priacanthus tayenus*), goatfish (*Mulloidichthys vanicolensis*), threadfin bream (*Nemipterus bleekeri*), and lizardfish (*Saurida tumbil*) [5]. Nevertheless, no study has reported on the addition of WPC to the gel from rohu. Consequently, the objective of this study was to investigate the effect of whey protein concentrate on the gel-forming ability of rohu (*Labeo rohita*).

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II. Materials and Methods

A. Materials

Rohu (*Labeo rohita*) was purchased from Klong Si Fish Market (Pathum Thani, Thailand). Fish were packed in an ice box and immediately transported to the laboratory.

Whey protein concentrate (WPC) was obtained from Open Country Dairy Ltd. (Wahora, New Zealand)

B. Preparation of Washed and Unwashed Minces

The fish were gutted and washed with tap water. The fillet was minced using a meat grinder (2 mm hole diameter; AT950A, Kenwood, England). The minced fish was divided into two groups, unwashed and washed. For preparation of the washed mince, iced water was added at a ratio of 4:1 (w/w), then the mixture was stirred for 5 min and left to stand without stirring for 5 min. After that, the mixture was filtered through cheesecloth. The washing process was repeated three times, twice with iced water and the third time with cold 0.3% (w/v) sodium chloride solution.

C. Preparation of Washed and Unwashed Gels

To prepare the gels, 2.5% salt was added to all the samples. WPC was added at concentrations of 1%, 2% and 3% with an untreated sample kept as a control. The moisture content was adjusted to 85% by the addition of iced water during the grinding of the mixture. Then, the batter was stuffed into cellulose casing (2.5 cm diameter) and tightly sealed at both ends. The temperature of the batter was maintained below 12^{o}C throughout the process. The batter was divided into two groups according to the type of gel: kamaboko (K) and modori (M) gel. The kamaboko gel was heated at 40^{o}C for 30 min followed by heating at 90^{o}C for 20 min. The modori gel was heated at 60^{o}C (washed gel) and 65^{o}C (unwashed gel) for 30 min and then heated at 90^{o}C for 20 min. After heating, all gels were immediately cooled in iced water until the core temperature of the samples fell below 10^{o}C. The samples were stored overnight at 4±2^{o}C prior to further analysis.

D. Determination of Folding Test

The gel samples (5 mm thickness, 2.5 cm diameter) were folded into halves and quarters. The procedure for the folding test followed [16].

E. Determination of Textural Properties

The textural properties of the gel samples (breaking force (g) and deformation (cm)) were determined using a texture analyzer (TA -XTPlus, Stable Micro Systems, Surrey, UK) with a spherical probe (5 mm diameter; 60 mm min^{-1} test speed). Gel samples were allowed to reach room temperature (≈30°C). Five cylinder-shaped samples 2.5 cm in length were prepared from each gel. The gel strength (g.cm) was expressed as the breaking force multiplied by breaking distance.

F. Determination of Expressible Water

The expressible water content was measured according to the method of [17] with slightly modification. Gel samples were cut into pieces of 0.5 x 1 x 0.5 cm^3, weighed (X) and placed between Whatman paper (No.1), with three pieces of paper at the bottom and two pieces on the top. A standard weight (5 kg) was placed on the gel samples for 2 min and then removed. The samples were weighed again (Y). Expressible water was calculated using the following equation and expressed as percentage of sample weight:

$$Expressible\ \ water\ \ (\%) = [(X - Y)/(X) \times 100 \qquad (1)$$

G. Determination of Whiteness

The whiteness of the gel samples (2.5 cm thickness, 2.5 cm diameter) were determined using a colorimeter (HunterLab, ColorFlex CX2687, USA). D65 illuminant was used as the light source. CIE L*, a*, and b* values were measured. Whiteness was calculated using the following equation [18]:

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

H. Statistical Analysis

The experiment was designed using a completely randomized design. Data were subjected to analysis of variance. A Duncan's new multiple range test was used to determine the differences between sample means at $P \leq 0.05$. All experiments were done in triplicate.

III. Results and Discussion

A. Effect of Whey Protein Concentrate on Textural Properties of Unwashed Rohu Gel

Folding test and gel strength of kamaboko (K) and modori (M) gel of unwashed rohu gel containing WPC at different levels (0-3%) are shown in Fig. 1. The concentration significantly affected the folding test and gel strength of modori and kamaboko gels ($P<0.05$). For modori gel, the folding test increased as the concentration increased. The increase was significantly greater with the addition of WPC at 2-3%, compared to that of the control ($P<0.05$). The gel strength of the modori gel increased as the concentration increased, but not significantly ($P>0.05$). No significant difference in the folding test of kamaboko gel was observed ($P>0.05$). The strength of the kamaboko gel increased as the concentration increased. The gel strength of gels with WPC above 2% was higher than that of the control. These results agreed with the findings in [7] that the addition of WPC improved the textural attributes of Pacific whiting surimi heat set gels. This may be because the addition of 3% WPC to the unwashed Pacific whiting mince reduced the relative enzyme activity by about 80% [7].

The characteristics of kamaboko and modori gel were investigated. As shown in Fig. 1, the kamaboko gel had a higher folding test and gel strength than the modori gel. The increased textural properties of gel set at 40^{o}C (kamaboko gel) may be due to endogenous transglutaminase (TGase). TGase catalyses the acyl transfer reaction between glutamine and lysine residues of muscle proteins, resulting in the formation of ε - (γ - glutamyl) lysine cross-links [19], [20]. In addition, setting gel at 65°C (modori gel) could be affected by endogenous protease. Previous studies [3], [4] have reported that the weakening of gel is caused by endogenous protease under heating at 50 - 70°C. Moreover, these enzymes degrade the myofibrillar protein, especially myosin, and prevent the development of the three-dimensional gel network, reducing the quality of the gel [21], [22], [19]. The modori gel improved with the addition of 3% WPC, though the textural properties were not as high as those of kamaboko gel. This finding implied that the addition of WPC at 3% and 2% could improve the textural properties of modori gel and kamaboko gel, respectively.

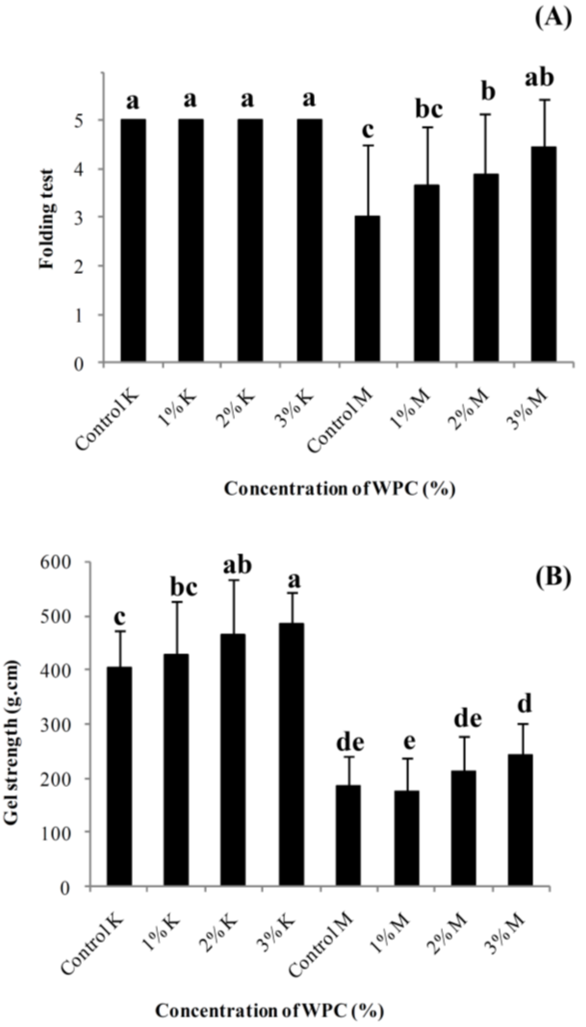

Figure 1. Folding test (A) and gel strength (B) of unwashed rohu gels containing whey protein concentrate at various concentrations.
K is kamaboko gel and M is modori gel. Bars represent the mean and standard deviation from five times determination. Different letters on each bar indicate significant differences (P≤0.05).

B. *Effect of Whey Protein Concentrate on Expressible Water of Unwashed Rohu Gel*

The expressible water content of unwashed kamaboko (K) and modori (M) rohu gel at different levels of WPC (0-3%) is shown in Fig. 2. For both of the gels, the expressible water content decreased as the concentration increased. For modori gel, the lowest expressible water content was obtained in gel with 3% WPC. Although the expressible water content of the kamaboko gel decreased as the concentration increased, no marked decrease was found with the addition of further WPC.

The results showed that the modori gel had a higher expressible water content than the kamaboko gel. This increase indicated the poor water-holding capacity of gel [5]. This result suggested that the modori gel network was poor in water-holding capacity compared with the kamaboko gel, which might be due to the role of endogenous protease that is activated under heating at 50 - 70°C [3], [4]. Nevertheless, the addition of 1% and 3% WPC improved the water-holding capacity of kamaboko and modori gels (P>0.05), respectively, compared with that of the control. This result might be due to the protein additives reducing the moisture content and increasing the density of the surrounding protein matrix, as its high water-holding capacity, causes swell and increases elasticity. [23]. This result coincided with those from the textural properties. WPC improved the modori gel containing at 3%, but the water-holding capacity was not as high as that of kamaboko gel. This finding suggested that the addition of WPC at 3% and 1% could improve the water holding capacity of modori and kamaboko gel, respectively.

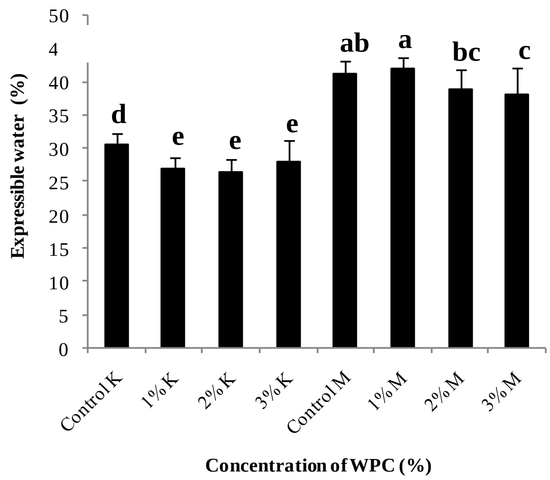

Figure 2. Expressible water of unwashed rohu gels containing whey protein concentrate at various concentrations.
K is kamaboko gel and M is modori gel. Bars represent the mean and standard deviation from three times determination. Different letters on each bar indicate significant differences (P≤0.05).

C. *Effect of Whey Protein Concentrate on Whiteness of Unwashed Rohu Gel*

The whiteness of unwashed kamaboko (K) and modori (M) gel of rohu at different levels of WPC (0-3%) are shown in Fig. 3. For both gels, the whiteness increased as the concentration increased. For modori gel, no marked increase was found in gels with the addition of further WPC. The highest whiteness of kamaboko gel was found with 3%WPC. From the results, the addition of 3% and 1% WPC increased the whiteness of kamaboko and modori gel (P>0.05), respectively, compared to that of control gel. These results revealed that gel samples might be affected by the light cream color of WPC. However, the result was not in agreement with [5], which reported that the whiteness of kamaboko gel of bigeye snapper and goatfish decreased with the addition of 3% WPC. The color of the WPC might affect the color of gels in different ways, depending on the native color of the mince.

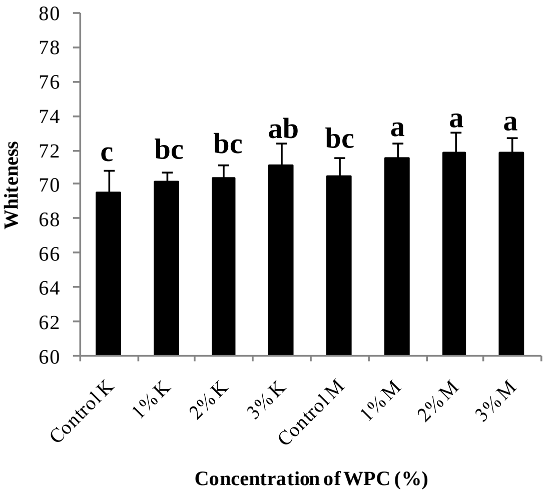

Figure 3. Whiteness of unwashed rohu gels containing whey protein concentrate at various concentrations.
K is kamaboko gel and M is modori gel. Bars represent the mean and standard deviation from three times determination. Different letters on each bar indicate significant differences (P≤0.05).

D. *Effect of Whey Protein Concentrate on Textural Properties of Washed Rohu Gel*

Fig. 4 shows the folding test and gel strength of washed kamaboko (K) and modori (M) gel of rohu containing WPC at different levels (0-3%). The concentration significantly affected the gel strength in both of the modori and kamaboko gel ($P<0.05$). For modori gel, the folding test slightly increased when compared with the control ($P>0.05$). No difference in folding test was found with further addition of WPC ($P>0.05$). The gel strength coincided with the folding test. The gel strength increased with the addition of WPC ($P<0.05$). However, no significantly difference in gel strength was observed with the increase in WPC above 1% ($P>0.05$). For kamaboko gel, no significant difference in folding test was observed ($P>0.05$). The gel strength increased with the addition of 1% WPC ($P<0.05$), compared with that of the control. However, the addition of further WPC did not increase the gel strength ($P>0.05$). This improvement of gel strength agreed with the findings of [5]. The addition of WPC improved the grade of bigeye snapper surimi from B to AA and enhanced the grade of threadfin bream surimi from B to A [5]. This may be due to the WPC having an inhibitory effect on the myofibrillar proteins of surimi or acting as an alternative substrate, which decreases the proteolytic activity [15].

The kamaboko gel had a higher folding test and the gel strength than the modori gel. This may be due to molecules of proteins unfolding slowly at 40°C, and then interacting with other molecules to form the three-dimensional network. As the gel is heated at 90°C, unfolded protein molecules become cross-linked and a more ordered structure is formed, strengthening the gel [24]. In addition, gel set at 65°C could be affected by the endogenous protease that remains within the myofibrillar protein after washing. Our results showed that washing appeared ineffective in removing the endogenous protease. This agreed with the findings of [25] that the protease level of lizardfish (*Saurida undosquamis*) muscle remained at 70% after washing. Ref. [26] suggested that the strength of maeso lizardfish (*Saurida undosquamis*) gel heated at 60 - 65°C was not improved by washing. Ref. [12] reported that Pacific whiting surimi gel set without inhibitors at 60°C for 30 min followed by 90°C, was too soft for textural measurement owing to the role of the proteinase. In addition, [6] reported that lizardfish surimi gel set at 65°C without inhibitors could not form a strong gel due to myosin heavy chain (MHC) loss. Although the modori gel was improved by WPC at 3%, the gel strength was not as high as that of kamaboko gel. This suggested that the addition of WPC at 1% could improve the textural properties of modori and kamaboko gel.

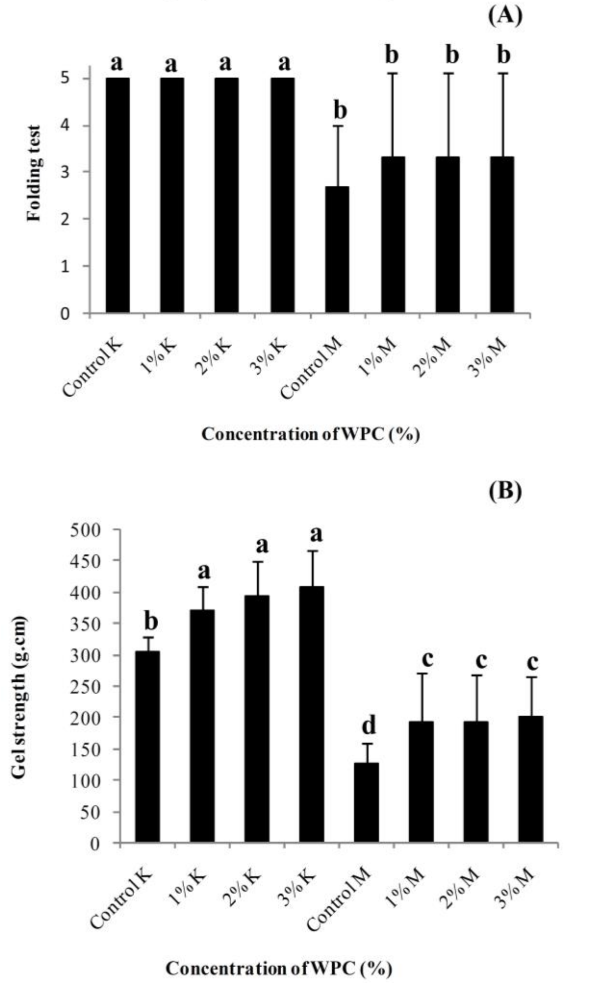

Figure 4. Folding test (A) and gel strength (B) of washed rohu gels containing whey protein concentrate at various concentrations.
K is kamaboko gel and M is modori gel. Bars represent the mean and standard deviation from five times determination. Different letters on each bar indicate significant differences (P≤0.05).

E. *Effect of Whey Protein Concentrate on Expressible Water Content of Washed Rohu Gel*

Fig. 5 shows the expressible water content of washed kamaboko (K) and modori (M) gel of rohu at different levels of WPC (0-3%). For modori gel, no difference in expressible water content was observed with addition of further WPC (P>0.05). However, the expressible water content decreased with the addition of 1%WPC (P<0.05), compared with that of the control gel. The results agreed with those for gel strength. Moreover, these findings support the finding of [5], that the addition of WPC improved the water-holding capacity of lizardfish surimi. For kamaboko gel, the concentration of WPC did not significantly affect the expressible water content of the gel (P>0.05). This might be due to setting at 40^{o}C followed by 90^{o}C strengthening the gel network, whereas the addition of WPC had no significant effect.

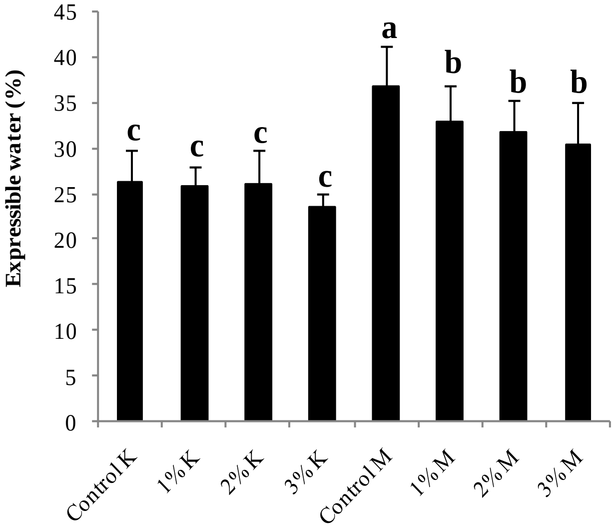

Figure 5. Expressible water of washed rohu gels containing whey protein concentrate at various concentrations.
K is kamaboko gel and M is modori gel. Bars represent the mean and standard deviation from three times determination. Different letters on each bar indicate significant differences (P≤0.05).

The results showed that the modori gel had a higher expressible water content than the kamaboko gel. These results coincided with those for the unwashed gel. A higher water-holding capacity was observed for the kamaboko gel network, which may be due to the greater strength of the gel network set at 40^{o}C. The unfolded protein molecules interacted with each molecule at 40^{o}C, and then cross-linked to form an ordered structure at 90^{o}C. These interactions strengthened the gel network, which retained more water [24], [27]. Moreover, the endogenous protease that remained within the myofibrillar protein might be activated at 60^{o}C, degrading the three-dimensional network, and preventing an ordered structure forming at 90^{o}C. Although WPC improved the modori gel at 1%, the water-holding capacity was not as high as that of kamaboko gel. This finding suggested that the addition of WPC at 1% could improve the textural properties of modori gel.

F. Effect of Whey Protein Concentrate on Whiteness of Washed Rohu Gel

Fig. 6 shows the whiteness of washed kamaboko (K) and modori (M) gel of rohu at different levels of WPC (0-3%). For both of gels, the addition of WPC did not significantly affect the whiteness. This may be because the washed mince already had a white color, therefore the addition of WPC did not affect the color. However, the result did not agree with those for the unwashed gel. Since the lightness (L*) of the unwashed gel was less than that of the washed gel (Data not shown), the addition of the light cream color WPC had a stronger effect on the color of unwashed gels.

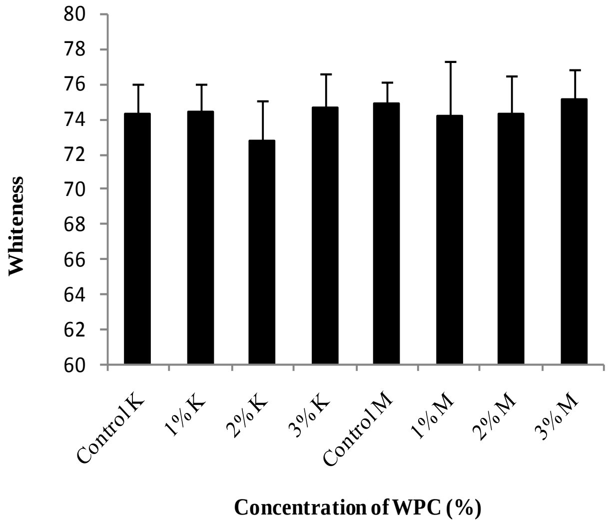

Figure 6. Whiteness of washed rohu gels containing whey protein concentrate at various concentrations.
K is kamaboko gel and M is modori gel.

IV. CONCLUSIONS

Our results conclude that the addition of WPC improves the textural properties and water-holding capacity of unwashed and washed rohu gel. The result for unwashed gel showed that the addition of WPC at 3% improved the modori gel, while the addition with 2%WPC improved the kamaboko gel, compared with the control. The result for the washed gel showed that the addition of WPC at 1% improved both gels. However, the increase for either unwashed or washed modori gel was not as high as kamaboko gel. The addition of WPC affected the whiteness of unwashed gel, but not affected washed gel.

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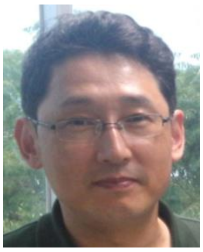

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