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# Effect of different salts on dewatering and properties of yellowtail barracuda surimi

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## Abstract

Washing is an important process for surimi production, in which undesirable components in fish mince are removed, while myofibrillar proteins are concentrated. However, dewatering is less effective for some fish species. The use of appropriate salt can be a means to increase dewatering and simultaneously improve the gelling property of surimi. The impact of 0.45% NaCl containing CaCl<sub>2</sub> or MgCl<sub>2</sub> at various levels (0, 4, 8, 12, 16, and 20 mM) as the third washing media on dewatering of washed mince and gel-forming ability of surimi produced from yellowtail barracuda (*Sphyraena flavicauda*) was investigated. When CaCl<sub>2</sub> or MgCl<sub>2</sub> was incorporated into the washing media, the contents of Ca or Mg ions in washed mince increased ( $p < 0.05$ ), whereas the pH of washed mince slightly decreased ( $p < 0.05$ ). At the same concentration, a higher dewatering of mince was observed when CaCl<sub>2</sub> was used, compared with MgCl<sub>2</sub> ( $p < 0.05$ ). Differential scanning calorimetry indicated that the stability of myosin decreased when higher concentrations of both salts were used ( $p < 0.05$ ), while no difference in the stability of actin was obtained. Washing mince with 0.45% NaCl containing 20 mM MgCl<sub>2</sub> yielded increases in breaking force of the gel of resulting surimi for both one-step and two-step heating processes by 46% and 33%, respectively, compared with the control (without CaCl<sub>2</sub> or MgCl<sub>2</sub> incorporation in washing media). The whiteness of the gel slightly decreased when the mince was washed with MgCl<sub>2</sub> ( $p < 0.05$ ). Microstructure revealed that a gel possessing a fine network with improved water-holding capacity was formed when the third media containing 0.45% NaCl and 20 mM MgCl<sub>2</sub> was used. The use of 0.45% NaCl containing 20 mM MgCl<sub>2</sub> was recommended to increase dewatering efficacy and improve gel strength of surimi from yellowtail barracuda by rendering a fine and ordered gel network.

## Background

Fish has been used as the raw material for surimi production. In Thailand, common species, e.g., threadfin bream, bigeye snapper, have been continuously decreasing in quantity (Yanpakdee et al. 2009). Recently, yellowtail barracuda has gained interest as a new raw material for surimi production, due to its high yield and white color (Yasir and Benjakul 2012). However, during dewatering process by screw press, the moisture content of resulting washed mince remains at a level above the standard (77.0%). To enhance the dewatering efficacy, 0.03% to 0.6% NaCl was incorporated in wash water (Okada and Tomoto 1986). Na<sup>+</sup> and Cl<sup>-</sup> are able to bind with the opposite charge of amino acids in proteins, leading to the less repulsive force between adjacent protein molecules. As a consequence, protein molecules align more closely with the concomitant migration of water

from the space between protein molecules. Additionally, salt might compete with protein in binding with water, termed the 'salting out' effect. As a result, proteins could interact with each other at a higher extent, thereby repelling water from the mince. Nevertheless, excessive moisture content is still obtained in yellowtail barracuda surimi even though NaCl is incorporated. Therefore, the use of other salts in conjunction with NaCl could pave the way for effective dewatering of washed mince. Solberg et al. (1990) washed cod mince using water containing CaCl<sub>2</sub> and MgCl<sub>2</sub> in all three washing steps. In the third washing step, 0.15% NaCl was added. The dewatering was found to be much more efficient when CaCl<sub>2</sub> was used rather than MgCl<sub>2</sub>. The highest breaking force of the gel was found when washing with 5 mM CaCl<sub>2</sub> was implemented. The deformation of the gel decreased when the concentration of CaCl<sub>2</sub> increased. Dalgleish and Hunt (1995) reported that the binding of cations on protein molecules is based on the formation of a short-range hydration repulsion. When the charge of the protein molecule was neutralized, two protein molecules approached each other, thereby repelling the water from the protein molecules.

The presence of salts strongly affects the strength, deformability, and appearance of protein gels (Lupano et al. 1992; Kuhn and Foegeding 1991). Divalent cations, such as Ca<sup>2+</sup> and Mg<sup>2+</sup>, can be used to form protein gels. These ions form cross-links between negatively charged groups of protein molecules (Damodaran 1996). Additionally, calcium ion has been known as the activator of endogenous transglutaminase, which can induce the non-disulfide covalent bonds in the gel network, particularly during setting. This leads to the improved strength of surimi gel (Benjakul et al. 2003). The objective of this study was to investigate the effect of the third washing media (including 0.45% NaCl containing CaCl<sub>2</sub> or MgCl<sub>2</sub> at various levels) on dewatering and gel-forming ability of surimi produced from yellowtail barracuda (*Sphyræna flavicauda*).

## Methods

### Chemicals

Calcium chloride (CaCl<sub>2</sub>) and magnesium chloride (MgCl<sub>2</sub>) were obtained from Ajax Finechem Pty Ltd. (Taren Point, New South Wales, Australia).

### Fish sample

Yellowtail barracuda (*S. flavicauda*) with an average weight of 80 to 90 g were caught from Songkhla-Pattani Coast along the Gulf of Thailand. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (*w/w*) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, within 2 h. Upon arrival, the fish were washed with tap water, beheaded, eviscerated, and used for washed mince and surimi preparation.

### Preparation of surimi and surimi gel

Prepared fish were subjected to deboning using a deboner with a hole diameter of 3 to 4 mm. The mince obtained was then washed with cold distilled water (5°C to 8°C) for the first and second washing using a mince/water ratio of 1:3 (*w/v*). For the third washing, cold washing media, 0.45% NaCl containing CaCl<sub>2</sub> or MgCl<sub>2</sub> at different levels (0, 4, 8, 12, 16, and 20 mM), were used. The mixture was stirred gently for 3 min, and the washed mince was filtered through two layers of cheesecloth. After the third washing, the washed mince

was centrifuged at  $700 \times g$  for 5 min with a model CE 21 K basket centrifuge (Grandiumpiant, Belluno, Italy). The washed mince was mixed with 4% (*w/w*) sorbitol and 4% (*w/w*) sucrose and was referred to as 'surimi.' The surimi was kept at  $-18^{\circ}\text{C}$  until used.

To prepare the surimi gel, the frozen surimi was thawed using running tap water until the core temperature reached  $0^{\circ}\text{C}$ . To the surimi, 2.5% (*w/w*) NaCl was added, and the moisture was adjusted to 80%. The mixture was chopped for 3.5 min at  $4^{\circ}\text{C}$  to obtain a homogeneous sol. The sol was then stuffed into a polyvinylidene casing with a diameter of 3.6 cm, and both ends of the casing were sealed tightly. Surimi gels were prepared by two heating conditions: (1) one-step heating ( $90^{\circ}\text{C}$  for 20 min) and (2) two-step heating ( $40^{\circ}\text{C}$  for 30 min, followed by  $90^{\circ}\text{C}$  for 20 min). The gels were cooled in iced water and stored overnight at  $4^{\circ}\text{C}$  prior to analysis.

#### **Determination of calcium and magnesium contents**

Calcium and magnesium contents of washed mince were determined according to the method described by Mader and Thompson (1969). The samples were ashed, dissolved in 20% nitric acid, and boiled on a hot plate for 15 min before analysis by inductively coupled plasma optical emission spectrometry with a model Optima 4300 DV (PerkinElmer, Norwalk, CT, USA).

#### **Determination of pH**

The pH of washed mince was determined according to the method described by Benjakul et al. (2002). The samples were homogenized using an IKA Labortechnik homogenizer (Rawang, Selangor, Malaysia) with nine volumes of distilled water (*w/v*) at a speed of 1,300 rpm for 2 min. The pH of the homogenate was measured using a pH meter (Cyberscan500, Eutech Instruments, Singapore).

#### **Thermal properties of washed mince**

The thermal transition of yellowtail barracuda mince proteins was determined by differential scanning calorimetry (DSC; Model DSCM, PerkinElmer, Norwalk, CT, USA). The samples (15 to 20 mg wet weight) were placed in DSC hermetic pans, ensuring good contact between the sample and the pan bottom. An empty hermetic pan was used as a reference. The samples were scanned at  $10^{\circ}\text{C}/\text{min}$  over the range of  $20^{\circ}\text{C}$  to  $100^{\circ}\text{C}$ .  $T_{\text{max}}$  was measured and denaturation enthalpies ( $\Delta H$ ) were estimated by measuring the area under the DSC transition curve.

#### **Determination of moisture content**

The moisture content of washed mince was determined according to the method of AOAC (1999).

#### **Texture analysis**

The texture of surimi gels was determined according to the method of Benjakul et al. (2002). Gels were equilibrated and evaluated at room temperature ( $28^{\circ}\text{C}$  to  $30^{\circ}\text{C}$ ). Five cylinder-shaped samples with a length of 2.5 cm were prepared and subjected to determination. Breaking force and deformation were measured using a texture analyzer (TA-XT2,

Stable Micro Systems, Godalming, Surrey, UK) equipped with a spherical plunger (diameter 5 mm, depression speed 60 mm/min).

#### Determination of whiteness

The color of surimi gels was determined using a JP7100F colorimeter (Juki Corp., Tokyo, Japan).  $L^*$  (lightness),  $a^*$  (redness/greenness), and  $b^*$  (yellowness/blueness) were measured, and whiteness was calculated as described by Park (1994) as follows:

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

#### Expressible moisture content

The expressible moisture content of surimi gels was measured according to the method of Ng (1987). Cylindrical gel samples were cut into a thickness of 5 mm, weighed ( $X$ ), and placed between three pieces of Whatman paper (no. 1) at the bottom and two pieces 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 moisture content was calculated and expressed as the percentage of sample weight as follows:

$$\text{Expressible moisture content} = [(X - Y) / X] \times 100.$$

#### Microstructure

Surimi gel samples ( $0.25 \times 0.25 \times 0.25 \text{ cm}^3$ ) were prepared. The specimens were then fixed with 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.2, for 2 h at room temperature. The specimens were rinsed with distilled water and dehydrated in graded ethanol solutions with serial concentrations of 25%, 50%, 75%, 95%, and 100%. Dehydration was conducted for 1 h in each solution, except for 100% ethanol, in which the dehydration was performed twice. The specimens were dried using  $\text{CO}_2$  as transition fluid (Balzers model CPD 030, Balzers Process Systems, Balzers, Liechtenstein). The dried specimens were mounted on aluminum stubs and sputter-coated with gold. The prepared samples were examined on a JSM 5200 scanning electron microscope (JEOL, Ltd., Akishima, Japan) at a magnification of  $\times 10,000$  (15 kV).

#### Statistical analysis

A completely randomized design was used throughout the study. Data were subjected to analysis of variance. Comparison of means was carried out using Duncan's multiple-range test (Steel and Torrie 1980). Analysis was performed using SPSS package (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, USA).

## Results and discussion

### Chemical composition of washed mince

#### Calcium and magnesium contents

When  $\text{CaCl}_2$  or  $\text{MgCl}_2$  in the third washing media increased, calcium and magnesium contents in the resulting washed mince increased ( $p < 0.05$ ; Table 1). This result is in agreement with Solberg et al. (1990) who reported that higher contents of calcium and magnesium were obtained when the mince was washed with a higher concentration of both salts. At the same level of both salts used, a higher calcium content was found in

**Table 1 Effect of the different third washing media on calcium and magnesium contents of yellowtail barracuda washed mince**

Ions	Samples	Ion content (g/kg of sample, dry basis)
Ca <sup>2+</sup>	Unwashed mince	1.77 ± 0.02 b
	Mince washed with 0.45% NaCl	1.74 ± 0.03 a
	Mince washed with 0.45% NaCl + 4 mM CaCl <sub>2</sub>	2.97 ± 0.01 c
	Mince washed with 0.45% NaCl + 8 mM CaCl <sub>2</sub>	3.82 ± 0.02 d
	Mince washed with 0.45% NaCl + 12 mM CaCl <sub>2</sub>	4.43 ± 0.02 e
	Mince washed with 0.45% NaCl + 16 mM CaCl <sub>2</sub>	5.13 ± 0.01 f
	Mince washed with 0.45% NaCl + 20 mM CaCl <sub>2</sub>	5.84 ± 0.03 g
Mg <sup>2+</sup>	Unwashed mince	1.52 ± 0.01 c
	Mince washed with 0.45% NaCl	0.68 ± 0.02 a
	Mince washed with 0.45% NaCl + 4 mM MgCl <sub>2</sub>	1.29 ± 0.02 b
	Mince washed with 0.45% NaCl + 8 mM MgCl <sub>2</sub>	1.75 ± 0.02 d
	Mince washed with 0.45% NaCl + 12 mM MgCl <sub>2</sub>	2.11 ± 0.01 e
	Mince washed with 0.45% NaCl + 16 mM MgCl <sub>2</sub>	2.61 ± 0.02 f
	Mince washed with 0.45% NaCl + 20 mM MgCl <sub>2</sub>	3.03 ± 0.02 g

Values are means ± SD from triplicate determinations. Different lowercase letters within the same column under the same ion determined indicate significant differences ( $p < 0.05$ ).

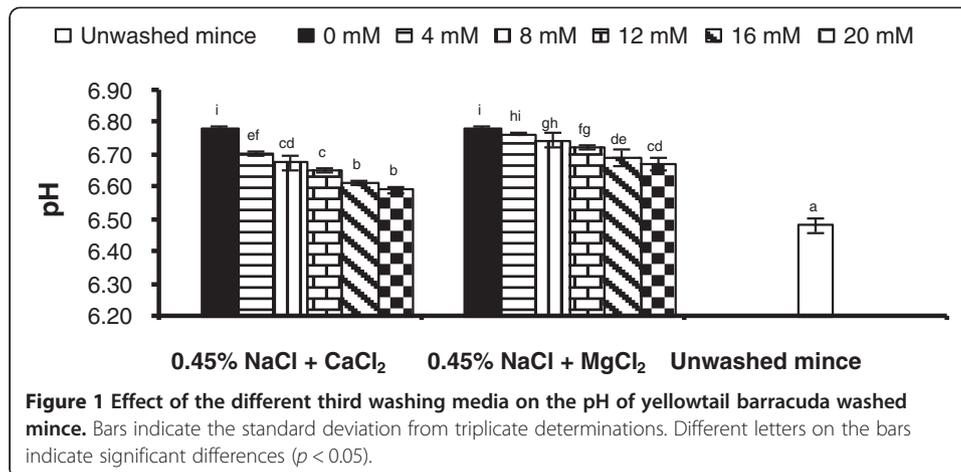
washed mince than magnesium content. This might be due to the different affinities for protein molecules between Ca<sup>2+</sup> and Mg<sup>2+</sup>, where Ca<sup>2+</sup> showed higher affinity toward muscle protein than Mg<sup>2+</sup>. The preferential interaction of the protein with various ions increased in the order of Ca<sup>2+</sup> > Mg<sup>2+</sup> > Na<sup>+</sup>, respectively (Arakawa and Timasheff 1984). Na<sup>+</sup> cation preferred to interact with water, leading to increased surface tension of washing water, whereas Ca<sup>2+</sup> and Mg<sup>2+</sup> (divalent cations) preferred to bind with protein via ionic interaction. When the preferential interactions between divalent cations and proteins overcame salt exclusion, those ions were retained in the washed mince. Thus, the concentration of both salts in washing media directly affected their residue in washed mince.

#### **pH**

The pH of washed mince varied, depending on the type and concentration of ions in the third washing media (Figure 1). Generally, the pH of washed mince slightly decreased when the mince was washed with 0.45% NaCl containing increasing concentrations of CaCl<sub>2</sub> or MgCl<sub>2</sub> (0 to 20 mM;  $p < 0.05$ ). Washing with 0.45% NaCl could increase the pH of mince from 6.48 to 6.78. Washing might remove acidic compounds, especially lactic acid, in the mince. The slight differences in the pH of mince washed with CaCl<sub>2</sub> or MgCl<sub>2</sub> at the same levels were observed. Mince washed with media containing MgCl<sub>2</sub> had a slightly higher pH than those washed with media comprising CaCl<sub>2</sub>. Therefore, both CaCl<sub>2</sub> or MgCl<sub>2</sub> affected the final pH of washed mince and might determine the physico-chemical properties of proteins in washed mince.

#### **Moisture content**

The dewatering of mince washed with 0.45% NaCl containing CaCl<sub>2</sub> or MgCl<sub>2</sub> at various levels was monitored by measuring the moisture content of washed mince (Table 2). Washing mince with 0.45% NaCl containing CaCl<sub>2</sub> or MgCl<sub>2</sub> at the third washing step



enhanced dewatering ability, compared with washing with only 0.45% NaCl. A higher decrease in moisture content was obtained in mince washed with the media containing CaCl<sub>2</sub>, compared with that using MgCl<sub>2</sub>. The result indicated that dewatering of mince washed with the media containing CaCl<sub>2</sub> was much more efficient than that using MgCl<sub>2</sub> ( $p < 0.05$ ). The differences in dewatering might be associated with the different abilities of ions in interacting with the charged groups of proteins. This is in agreement with Dalglish and Hunt (1995) who reported that the binding of cations on protein molecules was based on the formation of a short-range hydration repulsion. When the charge of the protein molecule was neutralized, two protein molecules approached each other, thereby repelling the water from the protein molecules. Ca<sup>2+</sup> might bind with protein via ionic interaction more potentially than Mg<sup>2+</sup>, leading to greater charge neutralization. This contributed to the lowering of the water-binding ability of protein when the washing media containing Ca<sup>2+</sup> were used. Based on the Hofmeister series, Mg<sup>2+</sup> has a higher ability to precipitate proteins than Ca<sup>2+</sup>. Nevertheless, Ca<sup>2+</sup> seemed to bind with water more effectively than Mg<sup>2+</sup> in the present study. As a result, water was more removed from proteins by the

**Table 2** Effect of the different third washing media on the thermal property of yellowtail barracuda washed mince

Samples	Moisture content (%)
Unwashed mince	80.57 ± 0.10 a
Mince washed with 0.45% NaCl (control)	84.80 ± 0.15 f
Mince washed with 0.45% NaCl + 4 mM CaCl <sub>2</sub>	84.15 ± 0.08 de
Mince washed with 0.45% NaCl + 8 mM CaCl <sub>2</sub>	83.68 ± 0.05 c
Mince washed with 0.45% NaCl + 12 mM CaCl <sub>2</sub>	83.12 ± 0.09 b
Mince washed with 0.45% NaCl + 16 mM CaCl <sub>2</sub>	83.04 ± 0.22 b
Mince washed with 0.45% NaCl + 20 mM CaCl <sub>2</sub>	82.78 ± 0.34 b
Mince washed with 0.45% NaCl + 4 mM MgCl <sub>2</sub>	84.18 ± 0.30 e
Mince washed with 0.45% NaCl + 8 mM MgCl <sub>2</sub>	83.94 ± 0.15 cde
Mince washed with 0.45% NaCl + 12 mM MgCl <sub>2</sub>	83.78 ± 0.02 cd
Mince washed with 0.45% NaCl + 16 mM MgCl <sub>2</sub>	83.68 ± 0.07 c
Mince washed with 0.45% NaCl + 20 mM MgCl <sub>2</sub>	83.57 ± 0.08 c

Values are means ± SD from triplicate determinations. Different lowercase letters within the same column indicate significant differences ( $p < 0.05$ ).

former. This was plausibly due to the differences in concentration and proteins presented in fish mince, in comparison with hen egg white proteins, originally used for this series.

### Thermal property

$T_{max}$  and  $\Delta H$  determined by DSC of mince washed with the third washing media containing 0.45% NaCl incorporated with  $CaCl_2$  or  $MgCl_2$  at various concentrations are shown in Table 3. A DSC thermogram was used to indicate the transition of myosin and actin in the muscle (Akahane et al. 1985). In the present study, the peaks of myosin and actin were found with  $T_{max}$  of 51.75°C and 70°C, respectively. However, when  $CaCl_2$  or  $MgCl_2$  was present in the washing media,  $T_{max}$  of myosin shifted to lower temperatures. Salt was found to decrease the stability of proteins in washed mince. Myosin in mince washed with 0.45% NaCl containing  $CaCl_2$  possessed less stability than that in mince washed with  $MgCl_2$  when the same level was used. Generally, when a higher concentration of both ions was used, the stability of myosin was lowered ( $p < 0.05$ ). No difference in  $T_{max}$  of actin was observed when  $CaCl_2$  or  $MgCl_2$  was used in the third washing media. Nevertheless,  $\Delta H$  of samples washed with media containing 12 and 16 mM  $CaCl_2$  slightly increased. Therefore, regardless of concentrations, both types and concentrations of ions affected the thermal stability of myosin at different degrees. Salts influence the electrostatic interaction by providing charged and polar groups and affect the hydrophobic interaction via the modification of the water structure (Von Hippel and Schleich 1969; Franks and England 1975). The degree to which the structure is affected depends on the nature of cations and anions and follows the Hofmeister series (Von Hippel and Schleich 1969). Cations and anions of higher order in the series (e.g.,  $Ca^{2+}$  and  $SCN^-$ ) could reduce the energy required to transfer the non-polar groups into water. This transfer would weaken the intramolecular hydrophobic interaction and enhance the unfolding tendency of proteins (Von Hippel and Schleich 1969). The Hofmeister series originated from the ranking of various ions toward their ability to precipitate the mixture of hen egg white proteins and are related with the ability to bind with water (salting out).

**Table 3 Effect of the different third washing media on the thermal property of yellowtail barracuda washed mince**

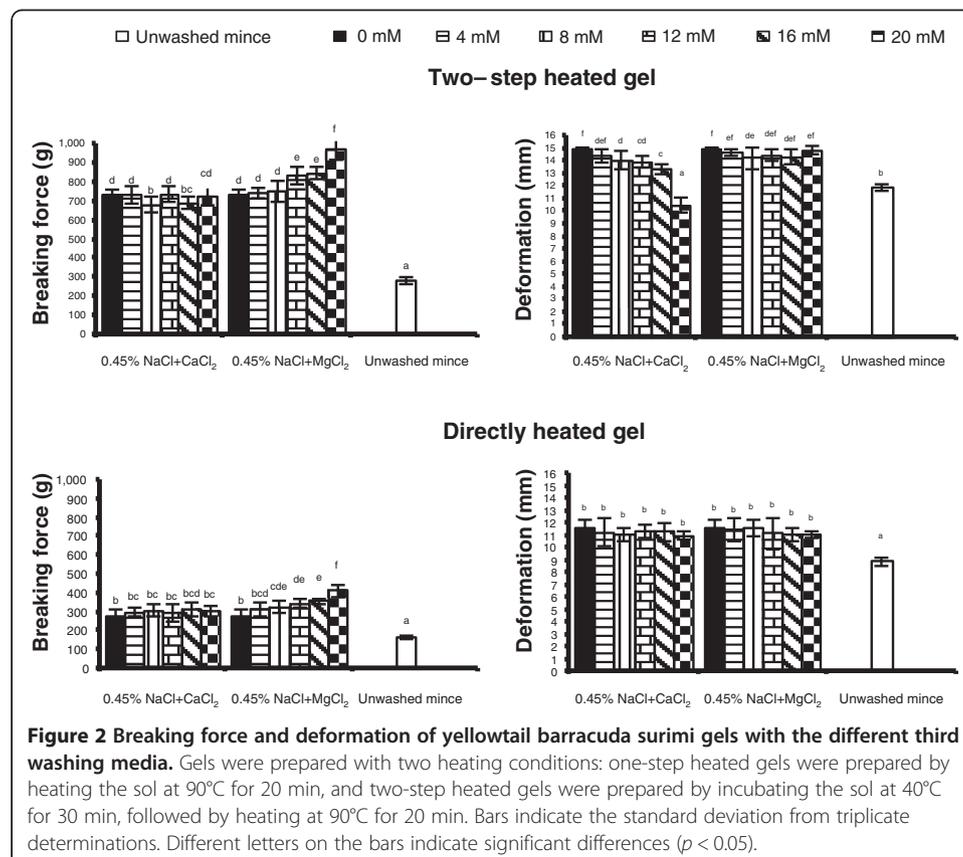
Samples	Peak I		Peak II	
	$T_{max}(^{\circ}C)$	$\Delta H$ (J/g)	$T_{max}(^{\circ}C)$	$\Delta H$ (J/g)
Mince washed with 0.45% NaCl (control)	51.75 ± 0.35 c	1.50 ± 0.07 bcd	70.00 ± 0.00 abc	0.33 ± 0.13 a
Mince washed with 0.45% NaCl + 4 mM $CaCl_2$	51.33 ± 0.24 bc	1.74 ± 0.07 def	69.67 ± 0.47 ab	0.44 ± 0.06 ab
Mince washed with 0.45% NaCl + 8 mM $CaCl_2$	51.00 ± 0.24 b	1.03 ± 0.05 a	70.08 ± 0.12 abc	0.33 ± 0.04 a
Mince washed with 0.45% NaCl + 12 mM $CaCl_2$	50.33 ± 0.24 a	1.90 ± 0.03 ef	70.00 ± 0.24 abc	0.50 ± 0.00 b
Mince washed with 0.45% NaCl + 16 mM $CaCl_2$	50.00 ± 0.47 a	1.94 ± 0.06 f	69.75 ± 0.35 abc	0.50 ± 0.03 b
Mince washed with 0.45% NaCl + 20 mM $CaCl_2$	49.75 ± 0.12 a	1.57 ± 0.18 bcd	69.50 ± 0.47 a	0.45 ± 0.04 ab
Mince washed with 0.45% NaCl + 4 mM $MgCl_2$	51.25 ± 0.12 bc	1.47 ± 0.06 bc	70.42 ± 0.59 bc	0.40 ± 0.00 ab
Mince washed with 0.45% NaCl + 8 mM $MgCl_2$	51.58 ± 0.12 bc	1.52 ± 0.21 bcd	70.42 ± 0.35 bc	0.40 ± 0.00 ab
Mince washed with 0.45% NaCl + 12 mM $MgCl_2$	51.00 ± 0.24 a	1.68 ± 0.10 bcde	70.50 ± 0.00 c	0.42 ± 0.09 ab
Mince washed with 0.45% NaCl + 16 mM $MgCl_2$	50.25 ± 0.35 a	1.44 ± 0.01 b	69.58 ± 0.35 a	0.42 ± 0.02 ab
Mince washed with 0.45% NaCl + 20 mM $MgCl_2$	50.33 ± 0.00 a	1.69 ± 0.05 cde	69.92 ± 0.12 abc	0.39 ± 0.03 ab

Values are means ± SD from triplicate determinations. Different lowercase letters within the same column indicate significant differences ( $p < 0.05$ ).

### Properties of surimi gel

#### Breaking force and deformation

Breaking force and deformation of the gel from yellowtail barracuda surimi prepared using different washing media (0.45% NaCl containing  $\text{CaCl}_2$  or  $\text{MgCl}_2$  at various levels) with two different heating conditions are depicted in Figure 2. In general, surimi gels prepared using the two-step heating process showed higher breaking force and deformation, compared with directly heated gels ( $p < 0.05$ ), regardless of salts added in the washing media. During setting, endogenous transglutaminase was able to induce cross-linking via non-disulfide covalent bonds, leading to gel strengthening (Benjakul et al. 2003; Kumazawa et al. 1995). Additionally, the alignment of proteins gradually took place, in which an ordered network could be formed during setting. Surimi prepared from mince washed with 0.45% NaCl containing 20 mM  $\text{MgCl}_2$  yielded the gel with the highest breaking force for both heating conditions ( $p < 0.05$ ), in which the breaking force increased by 46% and 33%, compared with that of the control (without  $\text{CaCl}_2$  or  $\text{MgCl}_2$  incorporated in washing media).  $\text{MgCl}_2$  has been reported to dissociate the actomyosin complex (Konno 1992). Free myosin undergo aggregation orderly, in which the strong network is developed. Generally, no differences in deformation of gels were observed ( $p > 0.05$ ), except for that obtained from the two-step heating, which decreased when the concentrations of  $\text{CaCl}_2$  in the washing media increased ( $p < 0.05$ ).  $\text{Ca}^{2+}$  is required for the activation of endogenous transglutaminase, which induces the formation of  $\epsilon$ - $\gamma$ -glutamyl-lysine linkage isopeptide associated with the stronger network (Kumazawa et al. 1995). This might contribute to the lowered elasticity of the gel as  $\text{CaCl}_2$  added in the washing media increased. Xiong



and Brekke (1991a) reported the increase in salt-soluble proteins and the improved gel strength for breast and leg chicken muscle when  $\text{CaCl}_2$  and  $\text{MgCl}_2$  were added at 5 mM. Furthermore, Solberg et al. (1990) reported that the gelling properties of cod surimi were strongly dependent on the type of ion present in the washing media. In the present study,  $\text{CaCl}_2$  in the washing medium had no impact on the breaking force of surimi gel, regardless of concentration in the washing media used. This might be due to the sufficient  $\text{Ca}^{2+}$  used for full activation of endogenous transglutaminase in the mince. As a result, further increase in  $\text{CaCl}_2$  had no effect on the breaking force of resulting surimi gels. It was suggested that both divalent cations,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , were able to form salt bridges with protein chains. Nevertheless,  $\text{Mg}^{2+}$  plausibly interacted with proteins in a fashion which favored the development of a fine and ordered network. As a result, gel with higher gel strength could be obtained.

#### Whiteness

Surimi gels prepared from mince washed with different third washing media using both heating conditions had higher whiteness, compared with the unwashed mince gel ( $p < 0.05$ ). During washing, several components, including blood, etc., were leached out, thereby improving the color of washed mince. It was noted that whiteness was slightly decreased when the mince was washed with 0.45% NaCl containing higher  $\text{MgCl}_2$  levels ( $p < 0.05$ ; Table 4), compared with that of the control (without  $\text{CaCl}_2$  or  $\text{MgCl}_2$ ). However, no marked difference in the whiteness of surimi gels was found when  $\text{CaCl}_2$  was added in washing media, irrespective of concentrations used. The effect of metal ions on browning was found to depend on the type of amino acid and heating time, as well as on the type of metal ion. It is known that a transition metal ion catalyzes the Maillard reaction by the oxidative pathway (Morita and Kashimura 1991).  $\text{Ca}^{2+}$  ion exhibited less effect on accelerating the Maillard reaction (Morita and Kashimura 1991). Thus,  $\text{Ca}^{2+}$  incorporation in washing media had a lower negative impact on the whiteness of surimi gel in comparison with  $\text{Mg}^{2+}$ .

**Table 4 Effect of the different third washing media on whiteness and expressible moisture content of yellowtail barracuda surimi gels**

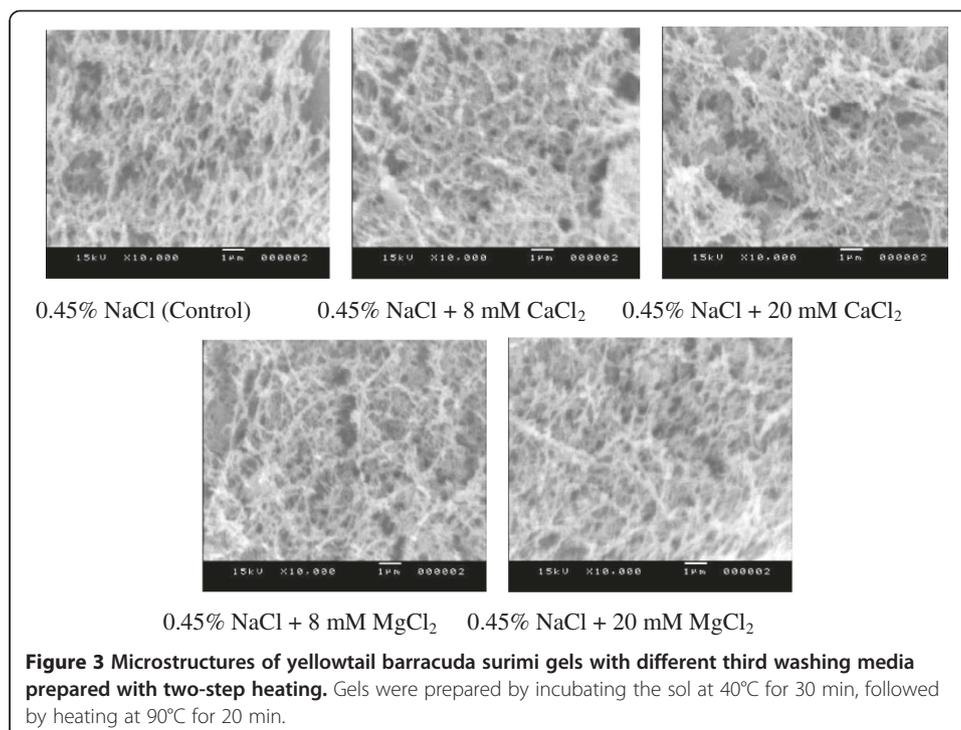
Samples	Whiteness		Expressible moisture content (%)	
	40°C/90°C	90°C	40°C/90°C	90°C
Unwashed mince	69.98 ± 0.94 a	70.04 ± 0.41 a	8.52 ± 1.32 d	9.56 ± 0.52 c
Mince washed with 0.45% NaCl (control)	78.24 ± 0.46 d	79.90 ± 0.77 ef	6.61 ± 1.27 abc	6.41 ± 0.72 ab
Mince washed with 0.45% NaCl + 4 mM $\text{CaCl}_2$	77.63 ± 0.56 c	79.54 ± 0.40 cd	7.56 ± 1.10 cd	7.03 ± 0.42 b
Mince washed with 0.45% NaCl + 8 mM $\text{CaCl}_2$	78.44 ± 0.40 de	78.70 ± 1.26 bc	6.70 ± 0.51 abc	6.19 ± 0.42 ab
Mince washed with 0.45% NaCl + 12 mM $\text{CaCl}_2$	78.32 ± 0.67 d	79.52 ± 0.86 cd	7.06 ± 0.39 bc	7.11 ± 0.68 b
Mince washed with 0.45% NaCl + 16 mM $\text{CaCl}_2$	79.02 ± 0.57 f	80.13 ± 0.55 ef	7.64 ± 0.96 cd	6.29 ± 0.84 ab
Mince washed with 0.45% NaCl + 20 mM $\text{CaCl}_2$	78.93 ± 0.50 ef	80.70 ± 0.25 f	7.78 ± 0.76 cd	6.24 ± 0.46 ab
Mince washed with 0.45% NaCl + 4 mM $\text{MgCl}_2$	77.66 ± 0.41 c	79.54 ± 0.35 cd	6.29 ± 0.39 ab	6.64 ± 0.40 ab
Mince washed with 0.45% NaCl + 8 mM $\text{MgCl}_2$	77.51 ± 0.53 c	79.50 ± 0.52 cd	6.55 ± 1.02 abc	6.91 ± 0.90 ab
Mince washed with 0.45% NaCl + 12 mM $\text{MgCl}_2$	76.47 ± 0.60 b	78.69 ± 0.42 bc	6.18 ± 0.25 ab	6.12 ± 0.92 ab
Mince washed with 0.45% NaCl + 16 mM $\text{MgCl}_2$	77.28 ± 0.63 c	78.50 ± 0.29 b	5.97 ± 1.08 ab	5.90 ± 1.09 a
Mince washed with 0.45% NaCl + 20 mM $\text{MgCl}_2$	76.58 ± 0.45 b	78.58 ± 0.47 b	5.87 ± 0.79 a	5.89 ± 0.78 a

Values are means ± SD from triplicate determinations. Different lowercase letters within the same column indicate significant differences ( $p < 0.05$ ).

Regardless of heating condition, higher whiteness was found in the gel prepared by one-step heating (direct heating), compared with those with two-step heating ( $p < 0.05$ ). Non-enzymatic browning might take place at a higher extent with longer exposure time used for two-step heating (Benjakul et al. 2003).

#### **Expressible moisture content**

Slight decreases in expressible moisture contents of gels with both heating conditions were found when the gels were produced from washed mince using higher  $MgCl_2$  levels in the third washing media, compared to the control gel ( $p < 0.05$ ; Table 4). Surimi gels prepared using media containing  $CaCl_2$  had similar expressible moisture contents when they were prepared by direct heating ( $p > 0.05$ ). However, slightly higher expressible moisture contents of gels were obtained when two-step heating was implemented ( $p < 0.05$ ). Regardless of heating condition, a higher expressible moisture content was found in gels from surimi prepared using media containing  $CaCl_2$  compared to those with  $MgCl_2$ . Therefore, not only the type and concentration of ions in washing media but also heating conditions affected the expressible moisture contents of gels. Terrell et al. (1981) indicated that magnesium was the best additive for decreasing moisture losses in raw and cooked beef. This response may be due to the effect of magnesium on increasing myosin extractability (Nayak et al. 1996). Xiong and Brekke (1991b) reported that moisture loss of chicken breast and leg gels was minimized when  $CaCl_2$  and  $MgCl_2$  at concentrations less than 5 mM were used but increased at greater concentrations of both salts. Both divalent cations affected gelation by changing the extraction and protein-protein interaction of salt-soluble proteins. Therefore, divalent ions in washing media showed an impact on the water-holding capacity of gels in conjunction with heating condition.



### Microstructure

Gel microstructures of surimi prepared from yellowtail barracuda mince washed with different third washing media, including 0.45% NaCl, 0.45% NaCl containing 8 and 20 mM CaCl<sub>2</sub>, or 8 and 20 mM MgCl<sub>2</sub>, are illustrated in Figure 3. There were no marked differences in the microstructure of gels produced from mince washed with 0.45% NaCl containing 8 mM CaCl<sub>2</sub> or MgCl<sub>2</sub>. However, it was noted that the gel from surimi prepared using 0.45% NaCl containing 20 mM MgCl<sub>2</sub> as washing medium had a finer gel network and less number of voids, as compared to the control gel. This ordered gel network correlated with the highest breaking force and deformation (Figure 2) as well as the lowest expressible moisture content (Table 4). On the other hand, surimi with 0.45% NaCl containing 20 mM CaCl<sub>2</sub> as washing medium had a fine network, but large voids were noticeable.

### Conclusion

The different types and concentrations of salts in the third washing medium affected the dewatering ability of washed mince and gel-forming ability of yellowtail barracuda surimi. Washing mince with the third medium, 0.45% NaCl containing 20 mM MgCl<sub>2</sub>, could improve the dewatering process and gel-forming ability of surimi from yellowtail barracuda, in which a fine and ordered network could be formed.

### Abbreviations

CaCl<sub>2</sub>: Calcium chloride; DSC: Differential scanning calorimetry; MgCl<sub>2</sub>: Magnesium chloride;  $\Delta H$ : Denaturation enthalpies.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

SB formulated the hypothesis and designed the studies. KL carried out the experimental and analyzed. SB and KL prepared the manuscript. SM and ABE made the comments and discussed on the results. All authors read and approved the final version of the manuscript.

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