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Elucidating Comminution Steps to Enhance the Value of Surimi from Tropical Fish

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2

3 Elucidating comminution steps to enhance the value of surimi from tropical fish

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Abstract

Biochemical and rheological properties of surimi were examined based on: 1) salting time (from 18 to 3 min) while maintaining 21 min for total chopping time; 2) total chopping time (from 6 to 21 min) while salting during the final 3 min. Extending salting time significantly increased breaking force and penetration distance while chopping time extension with fixed salting time did not. Salt soluble proteins decreased when salting time decreased; however, this trend performed contrarily against chopping time. A relationship between gel texture and salt soluble proteins was not found. Oxidation of sulfhydryl groups could occur during the chopping process when chopping without salt was extended. A degree of protein unfolding, as noted by surface hydrophobicity, behaved differently against chopping and salting time. Dynamic rheology demonstrated that total chopping time affected denaturation of the myosin tail region more than salting time.

Key words: Threadfin bream surimi, chopping, salting, texture, protein solubility

48 **Introduction**

49 Gelation is one of the most important functional properties of surimi and can be affected
50 by many factors such as fish species, comminution conditions (chopping time and temperature),
51 and the amount of salt. Mixing surimi with salt could obtain high gel strength, provide salty
52 taste, and prevent growth of microorganism. Salt could greatly magnify the gel texture when
53 surimi was set at optimum temperature for a specific period (Kim & Park, 2008; Niwa, Nowsad
54 & Kanoh, 1991). Fish protein isolate (FPI), on the other hands, demonstrated higher gel texture
55 when salt was not added during chopping (Kim & Park, 2008). FPI gel texture obtained from
56 some fish species is also superior to conventional surimi chopped with salt (Perez-Mateos,
57 Amato & Lanier, 2004; Yongsawatdigul & Park, 2004). The addition of salt in FPI, where
58 protein was chemically unfolded during the pH shift, was thought to be unnecessary and could
59 induce protein aggregation (salting-out) prior to gel network formation, which is undesirable
60 according to traditional surimi processing.

61 The effect of chopping conditions on gelation properties was extensively studied
62 (Douglas-Schwarz & Lee, 1988; Esturk, Park & Thawornchinsombut, 2004; Poowakanjana,
63 Mayer & Park, 2012a). These studies suggested the maximum gel strength could be obtained
64 when chopping conditions (temperature) correlate to the environmental habitat of each fish
65 species. Threadfin bream, which is warm water fish, exhibited superior gel texture when
66 finished chopping at temperatures around 25-30 °C. To obtain the specific final chopping
67 temperature, however, surimi needed to be chopped for longer time. Since salt is added at the
68 beginning of the chopping process using partially thawed surimi, any conformational change of
69 myofibrillar protein in the surimi paste is based on not only chopping temperature but also long
70 chopping time that allows proteins to be in contact with salt. As the chopping process is

71 extended, proteins can become more unfolded and subsequently aggregated due to the presence
72 of salt similar to FPI. In addition, physical unfolding (chopping) may not be able to denature
73 protein structure as much as chemical unfolding (pH-shift processing) resulting in improved gel
74 texture.

75 Since fish proteins have unique features due to their thermal stability, temperature factor
76 must be disregarded to be able to compare the effect of chopping time and salting time. The
77 temperature of surimi increases rapidly when salt is not added during chopping. Therefore,
78 cooling down using a circulating chiller is necessary to avoid negative effects of temperature.
79 Therefore, tropical surimi such as threadfin bream (TB) surimi is favored due to its high thermal
80 stability and no sign of setting if the temperature was controlled below 25 °C for up to 4 hrs.

81 As mentioned earlier, the effect of chopping temperature on the texture of gel made from
82 fish muscle is quite unique. However, for land animal muscle, gel texture is less dependent on
83 this temperature factor (Ugalde-Benitez, 2012). The textural properties of gels made from land
84 animal meat are highly correlated to its degree of salt extraction and chopping time. Long time
85 chopping resulted in higher salt soluble protein concentrations and better gel texture (Gillett,
86 Meiburg, Brown & Simon, 1977; Liu & Xiong, 1997). However, in the case of fish muscle, no
87 clear pattern has been demonstrated as affected by various processing or biological factors.
88 Therefore, our objective was to investigate the biochemical and rheological properties of surimi
89 proteins from tropical fish by determining the effect of chopping duration and with salting time.
90

91 **Materials and Methods**

92 **Surimi**

93 Threadfin bream (TB) ("SA" grade: approximately 2 frozen months old with 6.0%
94 sucrose and 0.2% sodium tripolyphosphate as cryoprotectants) surimi was obtained from Mana
95 Frozen Foods, Bangkok, Thailand.

96

97 **Chemicals**

98 All chemicals were purchased: Potassium chloride (KCl) from VWR International (West
99 Chester, PA, USA); Tris-HCl from J.T. Baker Chemical Company (Phillipsburg, NJ, USA);
100 Bradford reagent from Bio-Rad Laboratory (Hercules, CA, USA); Ethylenediaminetetraacetic
101 acid (EDTA), 5-5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and anilinonapthalene-8-sulfonic
102 acid (ANS) from Sigma Chemical Company (St. Louise, MO, USA).

103

104 **Surimi paste preparation**

105 Threadfin bream (TB) surimi was cut into small blocks (~ 1,000 g) and kept at -18 °C
106 until used. Surimi was partially thawed and cut into cubes (~ 2 cm). Chopping was done using a
107 vacuum silent cutter (UM 5 Universal, Stephan Machinery Corp, Columbus, OH, USA)
108 equipped with cooling jacket. Two sets of chopping process were developed and executed. For
109 the first set, surimi was chopped for 21 total minutes while 2% salt was added for the last 3, 6, 9,
110 12, 15, and 18 min. The abbreviation for this experiment set is [21/X_2] where X_2 refers to the
111 actual chopping time with salt. For the second set, surimi was chopped for 6, 9, 12, 15, 18, and
112 21 min while 2% salt was added during the final 3 min of chopping. The abbreviation for this set
113 is [X_1/3] where X_1 refers to the total chopping time. Low speed chopping (1800 rpm) was
114 applied during the first 3 min and surimi was adjusted to 78% moisture content by adding ice.
115 After that, high speed chopping (3,600 rpm) was applied until finished. At high speed, the silent

116 cutter was connected to a vacuum pump (40-60 kPa) to remove air pockets developed during
117 chopping and circulating coolant running at -5 °C. Chopping temperature was controlled at 15
118 °C or below to avoid the effect of temperature since gel texture of TB surimi could be improved
119 when chopped at high temperature (20-25 °C) (Esturk et al., 2004; Poowakanjana et al., 2012a).
120
121
122 **Salt soluble protein (SSP)**
123 SSP was measured as outlined by Thawornchinsombut and Park (2006) with a slight
124 modification. The paste sample was taken immediately from the silent cutter after chopping.
125 Three grams of paste were homogenized at speed 1 with 27 mL of 0.6 M KCl in 20mM Tris-HCl
126 buffer (pH 7) for 1 min using a homogenizer (model GLH-115, PG 700, Fisher Scientific,
127 Pittsburgh, PA, USA). The homogenized samples were then centrifuged at 10,000 × g (Sorvall
128 RC-5B, Newtown, CT, USA) at 4 °C for 30 min. After centrifugation, the supernatant was
129 diluted to approximately 1 mg protein/mL with 0.6 M KCl in 20mM Tris-HCl buffer (pH 7)
130 before measuring salt soluble protein. Bradford's dye reagent was diluted 5 times and then
131 diluted reagent (5 mL) was added to 100 μL of sample solution. Sample was allowed to stand at
132 room temperature for 20 min before measuring the absorbance at 595 nm (UV-VIS
133 Spectrophotometer; UV 2401PC, Shimadzu Co, Kyoto, Japan). The protein concentration of the
134 extracted SSP was then determined using bovine serum albumin as a standard. Three readings
135 per treatment were recorded to calculate the mean value of SSP concentration as mg of proteins
136 per mL of sample volume. Each treatment was measured at least in duplicate.
137
138 **Surface reactive sulfhydryl (SRSH) content**

139 SRSH content was determined using Ellman's reagent [5-5′-dithiobis-(2-nitrobenzoic
140 acid): DTNB] (Ellman, 1959). After determining the SSP, protein concentration of the
141 supernatant was adjusted to approximately 1 mg protein/mL with 0.6 M KCl in 20mM Tris-HCl
142 buffer. A sample (0.5 mL) was mixed with 2 mL 0.6 M KCl in 20mM Tris-HCl buffer, and 50
143 μL of 0.1 M sodium phosphate buffer (pH 7.2) containing 10 mM DTNB and 0.2 mM
144 ethylenediaminetetraacetic acid (EDTA). The resulting mixture was left at room temperature for
145 15 min before measuring absorbance at 412 nm (UV-VIS Spectrophotometer; UV 2401PC,
146 Shimadzu Co., Kyoto, Japan). Reactive SH groups were determined using a molar extinction
147 coefficient of 13,600 $M^{-1}cm^{-1}$. Three readings were recorded for calculating the mean value of
148 total sulfhydryl content as mol per 10^5g protein. Each treatment was measured at least in
149 duplicate.
150

151 **Surface hydrophobicity (*S_0*)**

152 Using surimi paste, protein S_0 of the supernatant was determined using an 1-
153 anilinonaphthalene-8-sulfonate (ANS) probe according to the method of Alizadeh-Pasdar and Li-
154 Chan (2000). The ANS stock solution contained 8 x 10^{-3} M ANS in 0.1 M phosphate buffer (pH
155 7.4). The protein concentration of supernatant was diluted to 0.05, 0.1, 0.2, and 0.4 mg/mL
156 using 0.6M KCL in 20mM Tris-HCl buffer (pH 7). Four milliliters of samples were mixed with
157 20 μL of ANS stock solution and left at room temperature for 10 min before reading on a
158 luminescence spectrophotometer (Perkin Elmer LS-50B, Norwalk, Conn., U.S.A.). The samples
159 were measured at wavelengths of 390 nm and 470 nm ($\lambda_{excitation}$, $\lambda_{emmision}$), respectively. The
160 protein S_0 was calculated from the initial slope of the net relative fluorescence intensity versus
161 protein concentration. Each treatment was measured at least in duplicate.

Oscillatory dynamic measurement

The rheological properties of surimi paste were measured through temperature sweep (20 to 90 °C at a heating rate of 1 °C/min) using a CVO-100 dynamic rheometer (Malvern Instruments Limited, Worcestershire, UK). The paste was placed between a cone (4°, 4 cm diameter) and plate leaving a gap of 150 µm. A plastic cover with moistened sponge (trapper) was used to prevent sample drying during heating. The oscillatory mode was applied with a fixed frequency at 0.1 Hz. Shear stress was set at 100 Pa which was determined based on the linear viscoelastic range of the samples. Samples were tested at least in duplicate.

Gel preparation and fracture gel analysis

The paste prepared above was packed into a polyethylene bag and subjected to a vacuum machine (Reiser VM-4142; Roescher Werke GMBH, Osnabrueck, Germany) to remove air that was introduced when the paste was put into the polyethylene bag. The paste was extruded, using a sausage stuffer (model 14208, The Sausage Maker, Buffalo, NY, USA), into a nylon tube (Nylatron MC 907; Quadrant Engineering Plastic Products, Reading, PA, USA) with a 3.0 cm inner diameter and ≈ 15.0 cm length. The paste was ohmically cooked at a voltage gradient of 12.62 V/cm with settings of 250 V and 10 kHz. The sample temperature reached 90 °C in ≈ 34-36 sec and the sample was held at 90 °C for 1 min. Gels, after putting in a plastic bag, were then immediately submerged in cold ice/water for 15 min and stored overnight in a refrigerator (4 °C). The next day gels were equilibrated to room temperature for an hour prior to gel testing.

Gel samples were cut into 30 mm long and subjected to the puncture test using a Texture Analyzer (TA-XT plus, Texture Technologies Corp, NY, USA). A spherical probe (5 mm

185 diameter) penetrated into the center of gels at a penetration speed of 1 mm/sec. Breaking force
186 (g) and penetration distance (mm) at gel fracture were recorded to determine fracture gel
187 properties (gel hardness and cohesiveness, respectively). At least ten specimens were tested per
188 treatment.

189

190 **Statistical analysis**

191 Experimental data were subjected to analysis for the average and standard deviation,
192 respectively. Statistical significance at a level of $p < 0.05$ of sample means for salt soluble
193 protein, reactive sulfhydryl content, and gel texture was determined using ANOVA and Tukey's
194 test in SPSS (version 13) software package (SPSS Inc., Chicago, IL, USA).

195

196 ## Results and Discussion

197 Tropical surimi TB was selected because it possesses higher thermal stability. Chopping
198 time and temperature rise are in a linear relationship (Poowakanjana & Park, 2012b). Due to
199 temperature rise during chopping, chopping was frequently halted to control the temperature at
200 15 °C or below. Any conformational changes of the protein might be dependent on not only
201 chopping or salting time but also the aggregation or dissociation of fish proteins through the
202 function of endogenous transglutaminase (TGase) or protease, respectively. However, the effect
203 of two enzymes, TGase and protease, during chopping was not considered in this study. The
204 optimum active conditions of TGase and protease for tropical fish threadfin bream was
205 reportedly 25 ºC for 4 hrs or 40 ºC for 2 hrs (Yongsawatdigul, Worratao & Park, 2002) and 55-
206 70ºC (Yongsawatdigul, 2011), respectively.

207 Throughout this study, the term "total preparation time" was the whole chopping process
208 including the actual chopping time and the waiting time when sample temperature needed to be
209 cooled down. The term "time of salt presenting" was the total time (chopping and waiting) after
210 salt addition. The term "chopping time" [X_1] refers to the actual duration that surimi was
211 mechanically chopped, and "salting time" [X_2] refers to the actual chopping time after salt
212 addition. Numbers shown in descending order on the bar graph indicate final chopping
213 temperature (°C), total preparation time (min), and time of salt presenting (min), respectively.
214

215 **Salt soluble protein (SSP)**

216 In the production of muscle food products, salt is added to extract salt soluble
217 myofibrillar proteins during the comminution process. The concentration of salt greatly affected
218 the gelling ability of the muscle protein. Many studies demonstrated the relationship between
219 salt concentration and gel strength. Kubota, Tamura, Matsui, Morioka, and Itoh (2006)
220 suggested 3% (NaCl) for the optimum gel strength of walleye pollock surimi. Okada (1999)
221 reported that optimum concentration of salt used in kamaboko gels was around 5 - 7.5%.
222 Increased salt concentration could result in better gel texture (salting in). However, gel texture
223 diminishes if salt addition continues beyond the optimum level (salting out). This was because
224 unfolded proteins became aggregated resulting in a cluster of proteins in the matrix and not
225 dissolved in high ionic strength solution (Stefansson & Hultin, 1994).
226 In the current study, SSP of [21/X_2] increased gradually, but significantly ($p < 0.05$) as
227 the X_2 (salting time) increased to 15 min (Fig. 1). It seemed that more protein was extracted and
228 solubilized in salt solution as salting time increased. However, this observation might not be true
229 based on the SSP of [6/3] sample (Fig. 2). Surimi with 6 min total chopping time and 3 min

230 salting time, which was supposed to unfold surimi proteins at the least degree, demonstrated the
231 highest SSP at 70.53 mg/mL. The concentration of SSP [21/X_2] indeed decreased when salting
232 time was reduced from 18 min [21/18] (58.14 mg/mL) to 3 min [21/3] (44.18 mg/mL) (Fig. 1).
233 Based on Fig. 1 and Fig.2, it is clear that longer chopping time and more salt soluble protein
234 extraction are not in agreement. SSP decreased significantly ($p < 0.05$) (Fig. 2) when chopping
235 was extended to 18 min, while salting time was fixed to 3 min at the final stage,. We may
236 assume SSP concentration indicates the degree of protein unfolding and/or its subsequent
237 association.

238 This assumption was explored further by measuring changes of SSP between the two
239 different chopping systems: [21/X_2] vs. [X_1/3]. The latter system demonstrated decreased SSP
240 concentrations as total chopping time increased from 6 min to 21 min (Fig. 2). But salting time
241 was 3 min in the final stage and the time of salt presenting due to the temperature control 15°C
242 was 12 min except [6/3]. This is probably due to aggregation of unfolded proteins as a result of
243 longer total preparation time (8 min to 218 min). Chopping and holding longer (due to
244 temperature control) without salt were rigorous enough to severely unfold proteins and induce
245 their subsequent association resulting in reduced SSP concentration. The former system;
246 [21/X_2], demonstrated that SSP significantly decreased ($p < 0.05$) when salting time was reduced
247 from 18 to 3 min while maintaining total chopping time at 21 min (Fig. 1). The decreased salting
248 time was basically substituted by chopping time without salt. The increased total preparation
249 time from 110 min to 218 min (due to temperature control) likely explains this reduction in SSP
250 (Fig. 1). In addition, longer chopping and holding time (218 min) with final 3 min salting [21/3]
251 demonstrated the lowest SSP (Fig. 1 and Fig. 2). Longer chopping time without salt was likely
252 to be a key factor affecting decreased SSP. Mechanical cutting (without salt) probably damages

253 proteins to a greater degree than chemical unfolding (with salt). This observation can be applied
254 to the [X_1/3] system (Fig. 2) in which chopping time without salt was extended from 3 to 18 min
255 resulting in significantly decreased SSP ($p < 0.05$).
256 In the industrial chopping method where salt was added at the beginning, it was assumed
257 that proteins were unfolded (denatured) and solubilized by salt during chopping. Fish proteins
258 chopped without salt are physically unfolded and can be lead to random aggregation. The degree
259 of unfolding/association as shown by decreased SSP is certainly related to longer total
260 preparation time (218 min for [21/3] compared to 110 min for [21/18]) (Fig. 1) and (218 min for
261 [21/3] compared to 8 min for [6/3]) (Fig. 2).
262 In our previous study with TB surimi (Poowakanjana et al., 2012b), salting was made at
263 the second minute of 6-21 min chopping treatments. The 21 min treatment demonstrated SSP
264 reduction by 18% while chopping temperature was maintained at 15°C or lower like in the
265 current study. As compared with the current study, SSP reduction by 24% from [21/18] to [21/3]
266 (Fig. 1) and by 37% from [6/3] to [21/3], it is shown that extended chopping could cause SSP
267 reduction for fish proteins. But chopping without salt would result in more SSP reduction than
268 when chopped with salt. A relationship between SSP and gel texture will be discussed in more
269 detail later.
270 Meat scientists suggested that gel strength is always related to salt soluble protein in
271 which higher SSP results in higher gel texture (Camou & Sebranek, 1991; Samejima,
272 Egelandsdal & Fretheim, 1985; Smith, 1988). However, this relationship could not be applied to
273 fish muscle. The explicit phenomena against the relationship between SSP and gel strength is
274 the solubility of pH-shifted fish protein, either prepared by acidic or alkaline extraction. Fish
275 protein isolate (FPI) always demonstrated lower SSP than that of surimi. However, many studies

276 reported the better gel qualities obtained from FPI (Kristinsson & Liang, 2006; Park, 2009;
277 Perez-Mateos et al., 2004; Yongsawatdigul et al., 2004). The effect of chopping time on SSP
278 (Poowakanjana et al., 2012b) and gel texture (Poowakanjana et al., 2012a) of surimi paste from
279 three fish species was studied. Chopping process was slightly different; that is, chopping was
280 done for 6, 9, 12, 15, 18, and 21 min in total while 2% salt was added at the second minute of
281 chopping. The chopping time with salt presenting was 5, 8, 11, 14, 17, and 20 min, respectively.
282 The result suggested that the longer the chopping time, the lower the SSP. However, gel strength
283 behaved differently depending on species; threadfin bream (warm water fish) showed improved
284 gel texture while Alaska pollock (cold water fish) showed poor gel texture when chopping time
285 was extended. Therefore, we think SSP can predict the degree of protein denaturation more
286 effectively than gel texture. Higher SSP might indicate the presence of protein in a more native
287 form. It also confirms that a significantly lower SSP from FPI is due to a greater chemical
288 denaturation by the use of NaOH and HCl. Although FPI protein refolds back by neutralizing
289 the pH to 7, the pH-treated protein does not refold back to the original native form
290 (Thawornchinsombut, Park, Meng & Li-Chan, 2006).

291

292 **Surface reactive sulfhydryl (SRSH) content**

293 The major purpose of chopping surimi prior to cooking is to denature (unfold) the
294 protein. Therefore, increased SH content due to the exposure of buried SH groups is expected.
295 However, SRSH group could be reduced if fish protein was comminuted at relatively high
296 temperature depending on the thermal stability of the fish species. Poowakanjana et al. (2012b)
297 observed decreased SRSH content of Alaska pollock and threadfin bream surimi when chopped
298 at higher than 10 and 20 °C, respectively. This was possibly due to disulfide formation that

299 could occur during paste preparation (chopping and holding). In the current study, a significant
300 decrease ($p < 0.05$) in SRSH content of [21/X_2] samples was obtained when salting time was
301 reduced (18 min to 3 min), but the total preparation time was extended from 110 min to 218 min
302 (Fig. 1). A similar trend was observed from [X_1/3] samples where the chopping time was
303 extended beyond 12 min while salting time was maintained for the final 3 min. This indicates
304 fish myofibrillar proteins were not solubilized when chopped without salt. Instead, they
305 aggregated resulting in buried SRSH groups inside the protein cluster. Moreover, elevated
306 temperature due to chopping without salt is likely to accelerate the formation of disulfide bonds.
307 Poowakanjana, et al. (2012a) used Raman spectroscopy to determine the structural
308 change in surimi paste as affected by various chopping conditions. They found that disulfide
309 formation could occur during long chopping time (when temperature was strictly controlled) and
310 the rate of oxidation significantly increased when chopping was done at higher temperature.
311 Chen, Hwang, and Jiang (1989) observed the rate of myosin oxidation increased at higher
312 storage temperature. This suggested that increased temperature and physical chopping with or
313 without salt are likely to accelerate disulfide formation in surimi paste. Higher SRSH content in
314 [21/18] compared to [21/3] (Fig. 1) demonstrated that the protein structure of TB surimi seemed
315 less susceptible to disulfide formation when chopping was done with salt. However, it should be
316 noted that SRSH content did not change when chopping time was extended from 6 min to 12 min
317 as shown in [X_1/3] samples (Fig. 2). Under this condition, salting was maintained equally for the
318 final 3 min while chopping time without salt increased from 3 to 9 min. In this case, decreased
319 SRSH content should have been obtained due to the fact that chopping without salt triggered the
320 formation of disulfide bonding in the surimi paste. In fact, SRSH content did not change because
321 the sample preparation was done in a relatively short time (73 min for [12/3]). As shown in Fig.

322 1 and 2, the thiol oxidation was highly noted when the sample preparation time extended longer
323 than 73 min (125 min for [21/15] and 122 min for [15/3]).
324
325 **Surface hydrophobicity (S_0)**
326 ANS probe is widely used to determine the surface hydrophobicity of extracted protein
327 from both land animal and fish muscle. Decreased S_o, indicating aggregation, was found from
328 extracted soluble beef (Farouk, Wieliczko & Merts, 2003) and pork protein (Lacroix,
329 Smoragiewicz, Jobin, Latreille & Krzystyniak, 2000) during storage time. Unfolded tertiary
330 structure of cod actomyosin subjected to either pH 2.5 or 11 followed by neutralization to pH 7.5
331 exhibit higher S_o than control treatment (pH 7.5) (Kristinsson & Hultin, 2003). As suggested by
332 Li-chan, Nakai, and Wood (1985), high S_o demonstrated protein with mild denaturation and was
333 not accompanied by aggregation.
334 The S_0 of [21/X_2] and [X_1/3] are shown in Fig. 3. Protein structure of surimi paste based
335 on its hydrophobic interaction behaved differently between the two chopping methods
336 (maintaining equal chopping time vs. maintaining equal salting time). The S_0 of [21/X_2]
337 increased significantly ($p < 0.05$) when salting time was extended from 3 to 9 min while the S_0 of
338 [X_1/3] did not change during chopping for 6 to 15 min with salting at the last 3 min. The trend
339 then leveled off as salting time was maximized to 18 min for [21/X_2]. When chopping without
340 salt was extended beyond 15 min [X_1/3], the reduction of S_0 was noted, indicating hydrophobic
341 domains were buried within protein clusters. This suggested that hydrophobic domains can be
342 exposed only when salt is added at the earlier stage of chopping.
343 It was interesting to observe the gradual reduction of S_0 as chopping time extended
344 beyond 9 min to 21 min while maintaining 3 min of equal salting time at the final stage [X_1/3].

345 This result was opposite compared to [21/X_2] samples and was also in disagreement with our
346 previous study (Poowakanjana et al., 2012b) in which surface hydrophobicity of Alaska pollock,
347 Pacific whiting, and threadfin bream surimi paste increased significantly while chopping time
348 was extended from 6 to 21 min. It should be noted, however, that chopping method was slightly
349 different. In the previous work salting was done at the second minute of chopping for all
350 samples and then chopping continued until total chopping time reached the target duration, while
351 in the current study, salt was added at the final 3 min of various chopping.
352

353 **Oscillatory dynamic measurement**

354 Oscillatory rheograms of [21/X_2] and [X_1/3] are shown in Fig. 4 and 5. Overall, G'
355 increased during heating from 10-90 °C. Extending chopping time without salt did not improve
356 the elastic modulus (G') (Fig.5). However, increased G' was obtained when salt was added at the
357 early stage of chopping from approximately 80 kPa [21/3] to 120 kPa [21/18] (Fig. 4). The
358 dynamic rheogram of [21/X_2] exhibited the maximum G' at around 70-75 °C indicating the
359 completion of gelation. This G' pattern then decreased as heating continued to 90 °C. It should
360 be noted that this rheological behavior does not signify the weakening of surimi gel at
361 temperature between 75-90 °C. Reed and Park (2011) suggested that this decreased G' at the end
362 point might be due to the slippage between cone/plate and the sample once fish proteins
363 completed gelation.

364 The significant difference of G' formation at temperatures between 30 and 40 °C was
365 demonstrated in [X_1/3] samples (Fig. 5). The increased G' in this region was reported to be due
366 to the role of light meromyosin (LMM) forming a semi gel (Egelandsdal, Fretheim & Samejima,
367 1986; Fukushima, Satoh, Yoon, Togashi, Nakaya & Watabe, 2005). LMM swelled and formed a

368 weak matrix which is dismantled when heated up beyond 40 °C. Disrupted semi gel caused G' to
369 decrease. This peak around 38 °C became smaller as chopping time without salt extended (Fig.
370 5). No peaks around 38 °C were shown when chopping time with salt was extended (Fig. 4).
371 This suggested that extended chopping could damage the myosin tail regions that swell out due
372 to the mechanical chopping. A similar trend was observed from Alaska pollock (AP), Pacific
373 whiting (PW), and threadfin bream (TB) surimi as the formation of G' concomitantly vanished at
374 temperatures between 30-45 °C during heat-induced gelation (Poowakanjana et al., 2012a).
375 The destabilization of LMM as affected by long chopping time could be supported by
376 Raman spectroscopy. Carew, Asher, and Stanley (1975) suggested that the peak at 1304 cm^{-1}
377 was assigned for fibrous alpha-helical structure of LMM. Poowakanjana et al. (2012a) observed
378 decreased intensity of this peak when AP, PW, and TB were subjected to long chopping time
379 regardless of chopping temperature.
380 The onset of G' rising was another tool to determine how much protein unfolded. The
381 onset of G' value indicated where surimi paste started to form a gel (Egelandsdal et al., 1986).
382 Low onset of G' suggested that protein needs less energy to unfold prior to gelation.
383 Tadpitchayangkoon, Park, Mayer, and Yongsawatdigul (2010) studied the structural change of
384 sarcoplasmic proteins subjected to various pH-shift methods. They reported the onset of G'
385 rising correlated well with the DSC thermogram in which the sarcoplasmic proteins with higher
386 onset of G' rising would thermally unfold at higher temperature. In the current study, the onset
387 of G' rising decreased from 46.70±0.28 to 45.30±0.00 °C when chopping time was extended
388 from 6 to 21 min (Fig. 5), and from 45.30±0.00 to 44.75±0.35 °C when salting time was
389 maximized to 18 min. This indicated that longer chopping and increased salting time could
390 unfold protein structure at lower temperatures. The onset of G' rising for AP, PW, and TB

391 surimi as affected by various comminution conditions was also revealed by Poowakanjana et al.
392 (2012a). They suggested that surimi subjected to longer chopping time would have onset of G'
393 rising at lower temperatures as well.
394

395 **Fracture gel analysis**

396 Gel texture was described as hardness by breaking force and cohesiveness by penetration
397 distance. A significant increase in gel texture ($p < 0.05$) was obtained only when salting time
398 was extended (Fig.6). In other words, adding salt at the early stage resulted in better gel texture.
399 Gel strength could additionally increase as long as chopping was controlled to not exceed 15 °C.
400 This was in agreement with the previous study (Poowakanjana et al., 2012a). They suggested
401 that as the final chopping temperature for TB surimi elevated from 5 to 15 °C, gel hardness
402 (breaking force) and gel cohesiveness (penetration distance) increased by 27% and 20%,
403 respectively. There was a correlation between SSP (Fig.1) and gel texture (Fig. 6) in this case.
404 However, decreased SSP in [X_1/3] samples (Fig. 2) was not correlated to the respective gel
405 texture (Fig. 7). Extended chopping time while salt was added for the final 3 min resulted in a
406 significant ($p < 0.05$) decrease in SSP (Fig. 2), but reduction in gel texture was not significant (p
407 > 0.05) (Fig. 7). This supported the finding in this study that no distinctive relationship between
408 SSP and gel texture exists for fish proteins. As a consequence, SSP concentration in fish
409 proteins may not a true indicator for gel texture.
410 The result of gel texture was not correlated well with surface hydrophobicity. It was
411 believed that comminution unfolds the protein structure mechanically with cutting and
412 chemically with salt. As a result, unfolded proteins would aggregate in a well-organized
413 structure leading to better gelation properties (Egelandsdal, Martinsen & Autio, 1995). However,

414 the current study suggested that there was no clear relationship between degree of protein
415 unfolding and gel texture. Gel hardness and cohesiveness increased significantly ($p < 0.05$)
416 when salting time increased from [21/9] to [21/18] (Fig.6). However, there was no significant
417 difference ($p > 0.05$) in their surface hydrophobicity. The S_0 as obtained from [X_1/3] samples
418 (Fig. 3) decreased significantly ($p < 0.05$) when total chopping time was longer than 9 min;
419 nonetheless, their gel cohesiveness remained stable (Fig. 7). This indicated that gelation
420 properties did not always depend on degree of protein unfolding.

421 It was worthwhile to deeply discuss degree of protein unfolding based on the nature of
422 samples used at measurement. According to the previous studies, degree of protein unfolding
423 measured from solid state (paste) using Raman spectroscopy (Poowakanjana et al., 2012a) and
424 liquid state (paste that was extracted in 0.6 M KCl with 10 times dilution) using ANS probe
425 (Poowakanjana et al., 2012b) exhibited different patterns. Raman spectra assigned for tyrosine
426 and tryptophan indicated that protein was not unfolded when surimi was chopped for longer
427 time. In contrast to surface hydrophobicity using an ANS probe, degree of protein unfolding
428 significantly increased as the chopping time extended. This was probably because proteins in
429 solid state (paste) had no space to expose their hydrophobic domain comparing to those in liquid
430 state with proper dilution.

431 The relationship between gel texture of [X_1/3] samples (Fig. 7) and their SRSH content
432 (Fig. 2) should be noted as well. Normally, decreased SRSH content gave rise to the formation
433 of disulfide bonds, which would strengthen the gel texture. Indeed, no significant change ($p <$
434 0.05) in gel texture was observed from [X_1/3] samples even though SRSH content declined. This
435 was because the SH groups were buried inside the protein cluster due to the aggregation of

436 protein upon chopping without salt as mentioned earlier. On the other hand, the stabilized gel
437 strength of [X_1/3] samples confirmed that SH groups were not oxidized to disulfide bonds.
438 Another factor behind similar gel qualities of TB surimi treatments was the chopping
439 temperature. Comminution condition at 15 °C was not too extreme for this tropical fish species
440 due to its high thermal stability. As suggested by Poowakanjana et al. (2012a), long chopping at
441 25 to 30 °C improved gel texture of TB surimi. However, it may not be conclusive that gel
442 strength of [X_1/3] could increase vigorously if chopping was done at higher temperatures (25-30
443 °C). It was because salting procedures were different. Chopping without salt at high
444 temperature is possibly not appropriate as the protein starts to aggregate at the beginning of the
445 chopping process. High chopping temperature without salt may be able to unfold proteins
446 rapidly for subsequent aggregation resulting in impaired gel texture.
447

448 ## Conclusion

449 Total chopping time increased beyond 12 min including salting for the final 3 min
450 resulted in significantly decreased SSP. Since SSP was not correlated well with gel texture
451 values, SSP is not a true indicator of gel texture for fish proteins. In other way, SSP values may
452 denote the degree of protein denaturation and its subsequent aggregation, not gelation properties.
453 In addition, gel texture did not correlate well with the surface reactive sulfhydryl content or
454 surface hydrophobicity. Applying salt at the beginning was able to unfold the protein structure
455 to a greater extent as the onset of G' rising occurred at lower temperature. Fish proteins, when
456 chopped without salt for a long time, would enter the aggregation process quickly rather than
457 staying solubilized. Aggregated protein clusters formed prior to cooking are likely to contribute
458 to random coagulation resulting in lower gel texture. The optimum chopping process to obtain

459 the highest gel qualities for threadfin bream surimi was to add salt at the early stage of chopping
460 and chop for long time (21 min).
461

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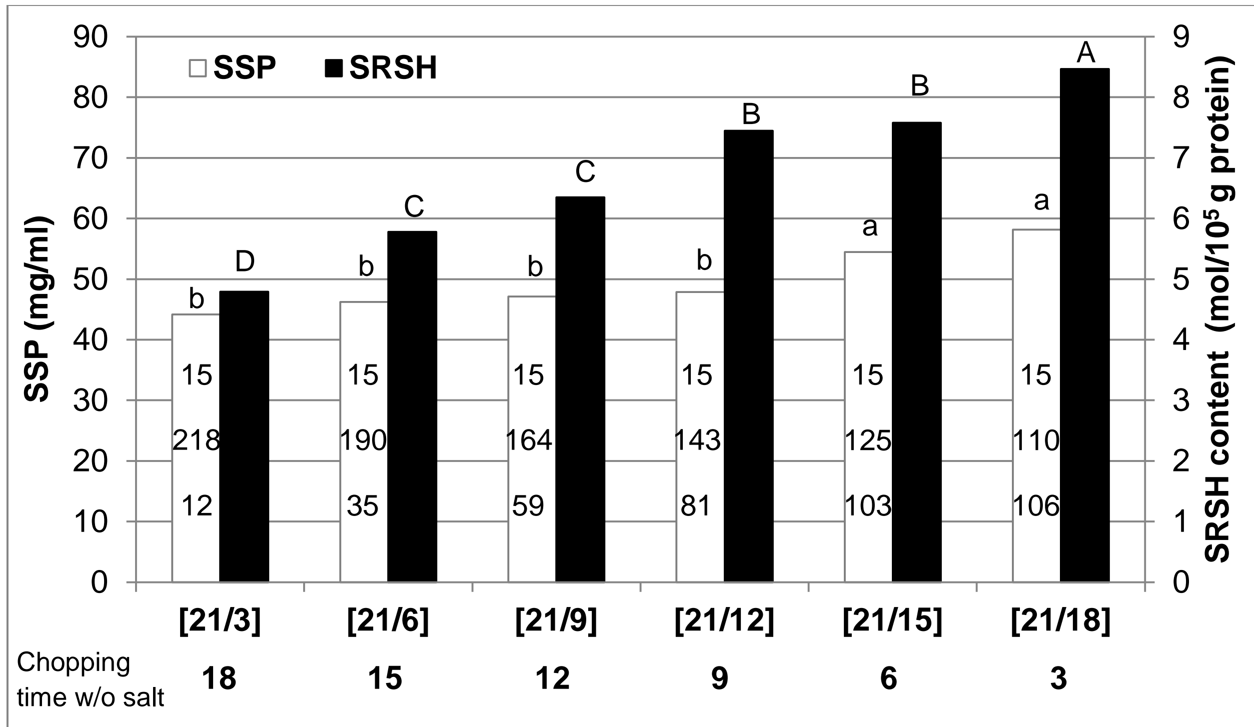

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563 Fig. 1: SSP and SRSH content of [21/X_2] TB paste as affected by various comminution
564 conditions. Different letters on each bar represents significant differences ($p < 0.0$) within the
565 same quality parameter. Numbers appeared vertically on the bar graph indicate the final
566 chopping temperature (°C), the total preparation time (min), the time of salt presenting (min),
567 respectively. [X_1/X_2] denotes the sample was chopped for X_1 min (total chopping) and salt was
568 added for the final X_2 min (salting time). [21/3] (Fig. 1) and [21/3] (Fig. 2) were the same sample
569 prepared with the same 21 min total chopping with 3 min salting at the final stage.

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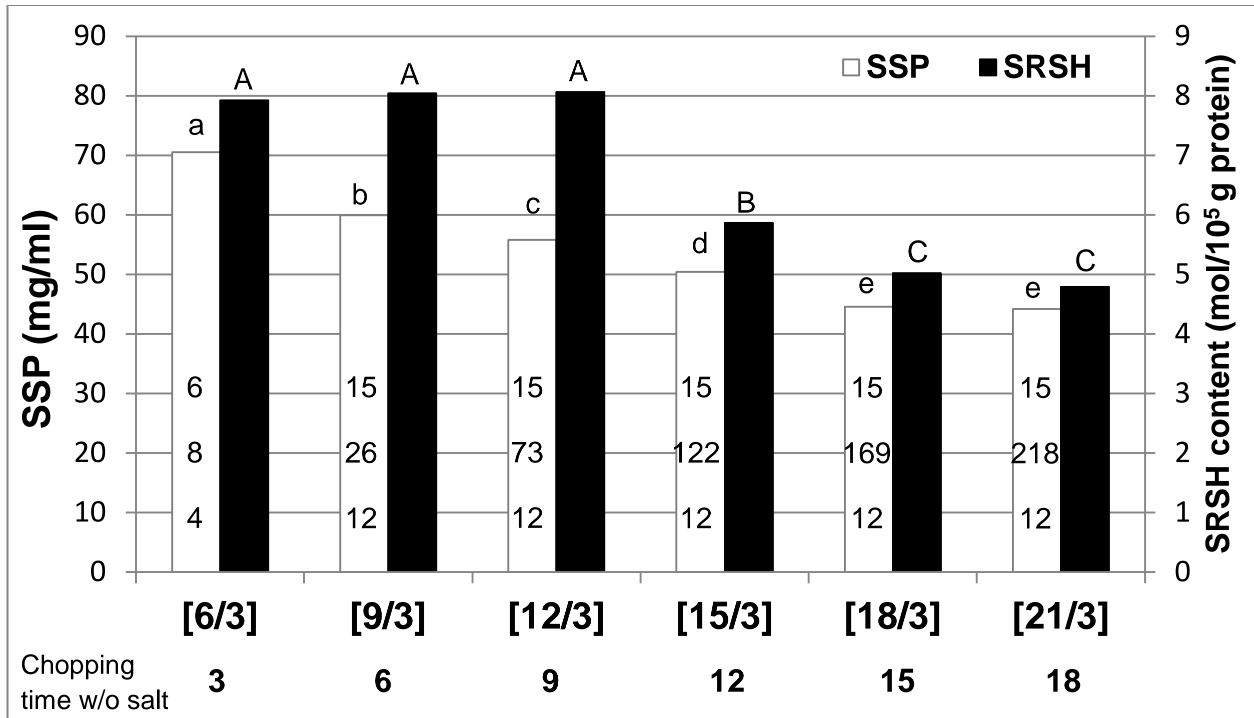

577 Fig. 2: SSP and SRSH content of [X_1/3] TB paste as affected by various comminution
578 conditions. Refer to Fig. 1 for codes.

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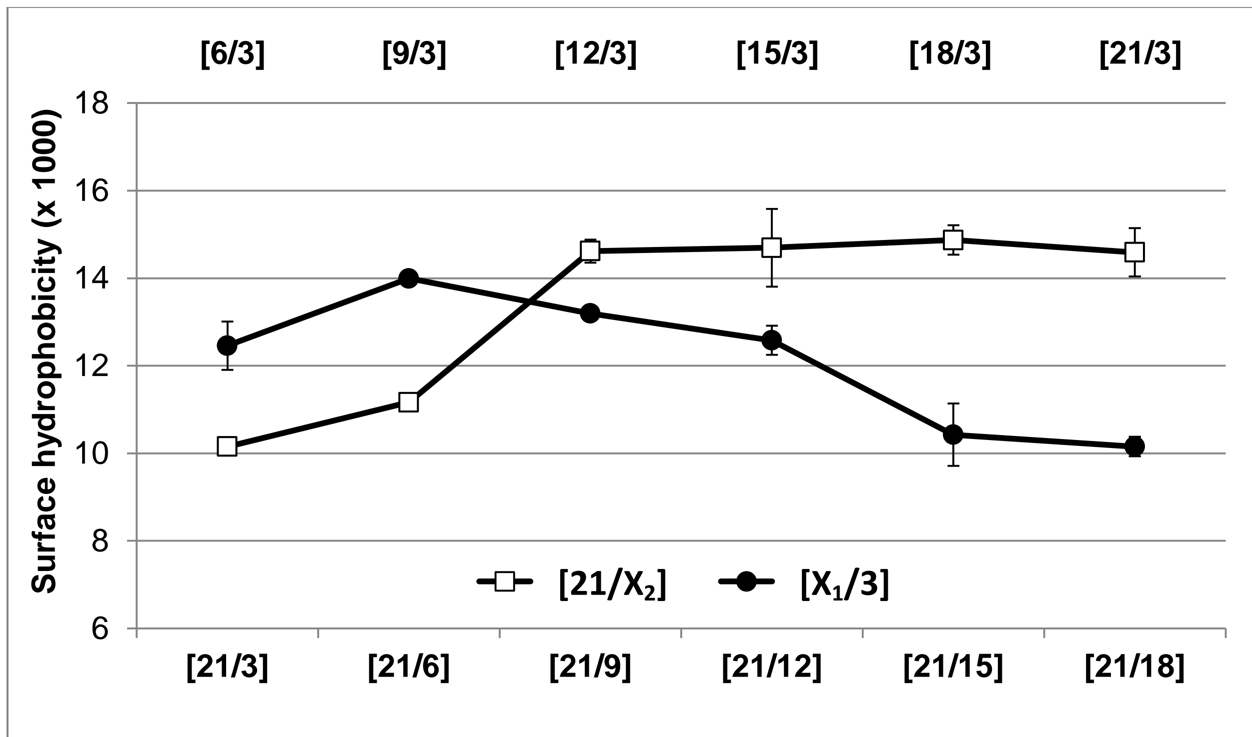

588 Fig. 3: Surface hydrophobicity of [21/X_2] and [X_1/3] TB paste as affected by various
589 comminution conditions.

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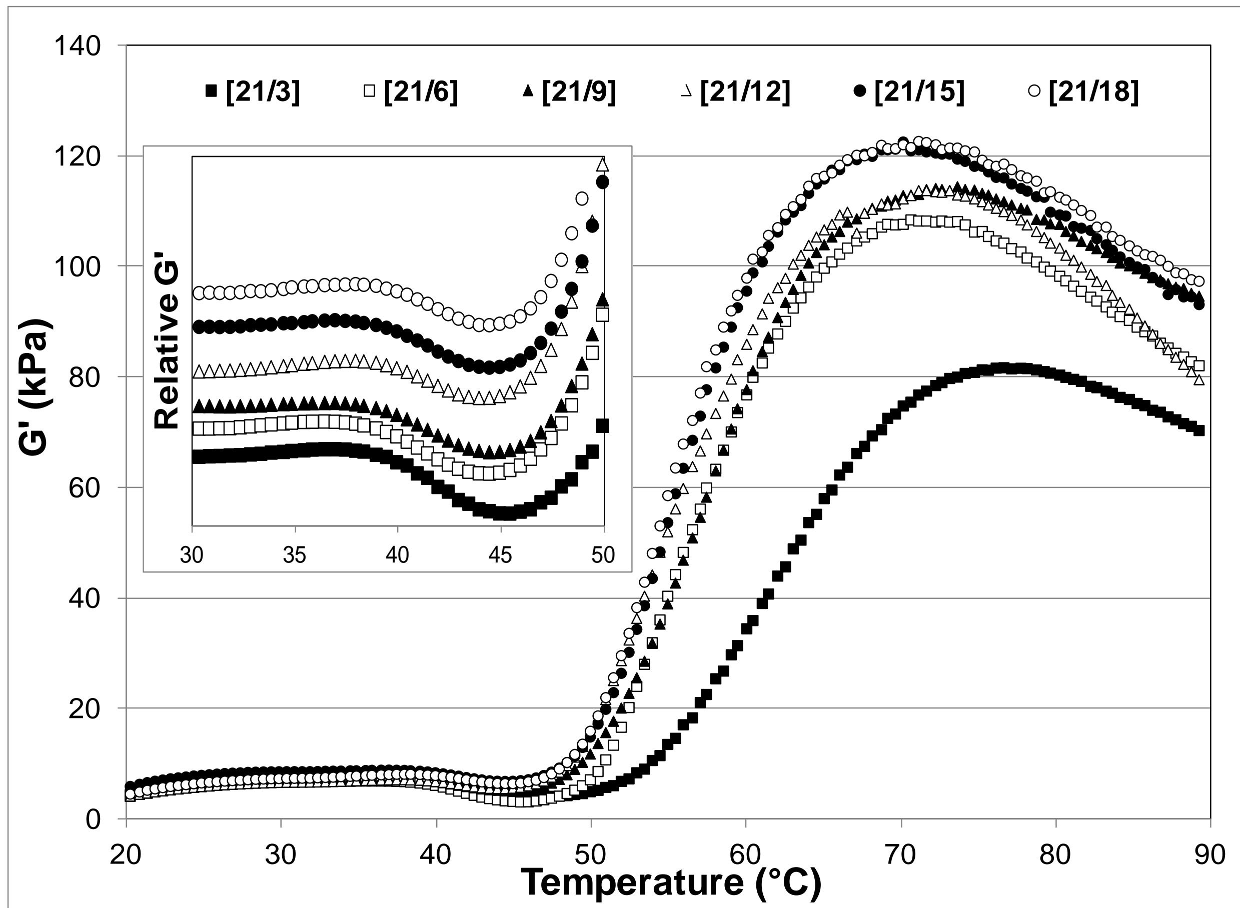

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Treatments	[21/3]	[21/6]	[21/9]	[21/12]	[21/15]	[21/18]
Onset of G' rising (°C)	45.30 ± 0.00	45.15 ± 0.35	44.1 ± 0.28	44.00 ± 0.00	44.40 ± 0.00	44.75 ± 0.35

599 Fig. 4: Dynamic rheology of [21/X_2] TB paste as affected by various comminution conditions.

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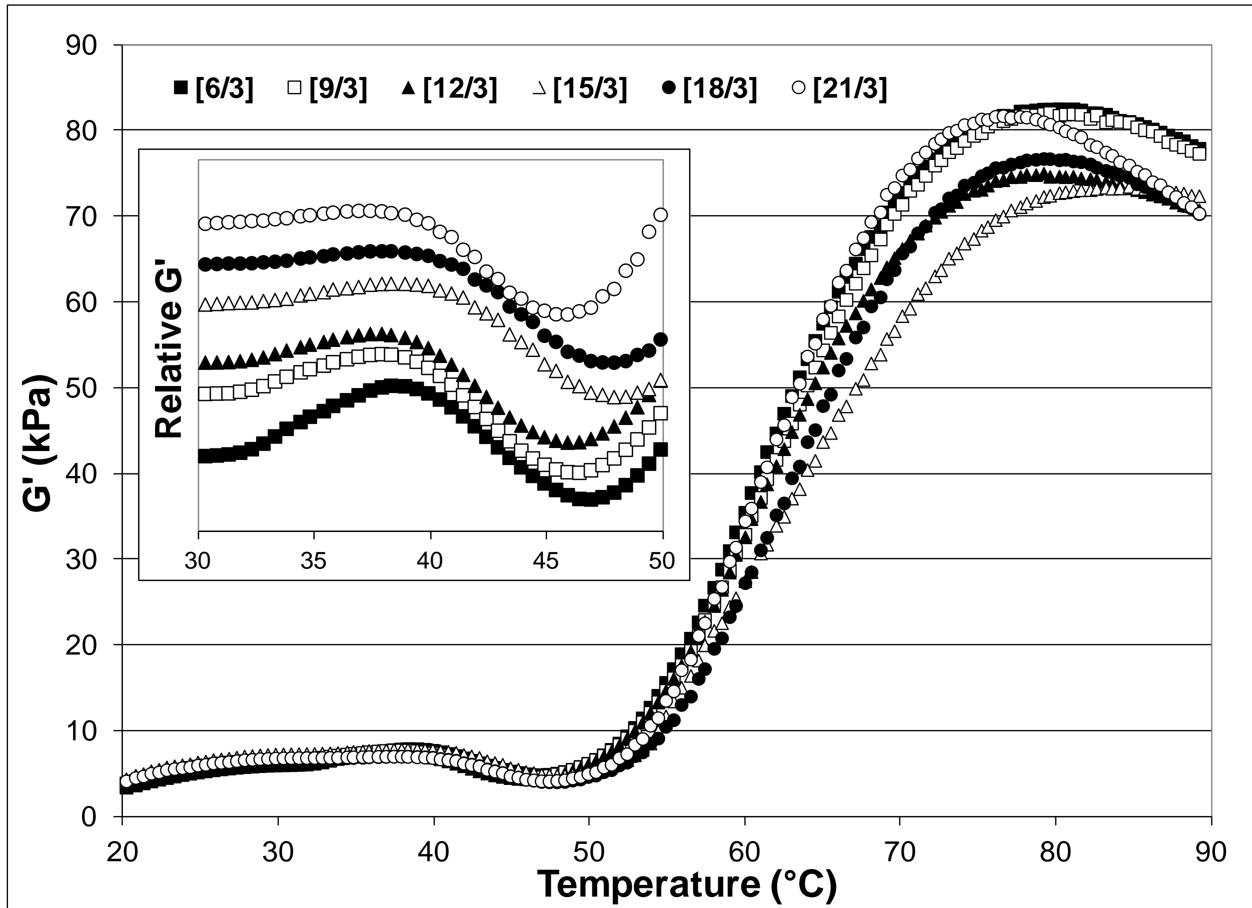

602

Treatments	[6/3]	[9/3]	[12/3]	[15/3]	[18/3]	[21/3]
Onset of G' rising (°C)	46.70 ± 0.28	45.95 ± 0.78	46.15 ± 0.35	45.50 ± 0.28	45.65 ± 0.07	45.30 ± 0.00

603 Fig. 5: Dynamic rheology of [X_1/3] TB paste as affected by various comminution conditions.

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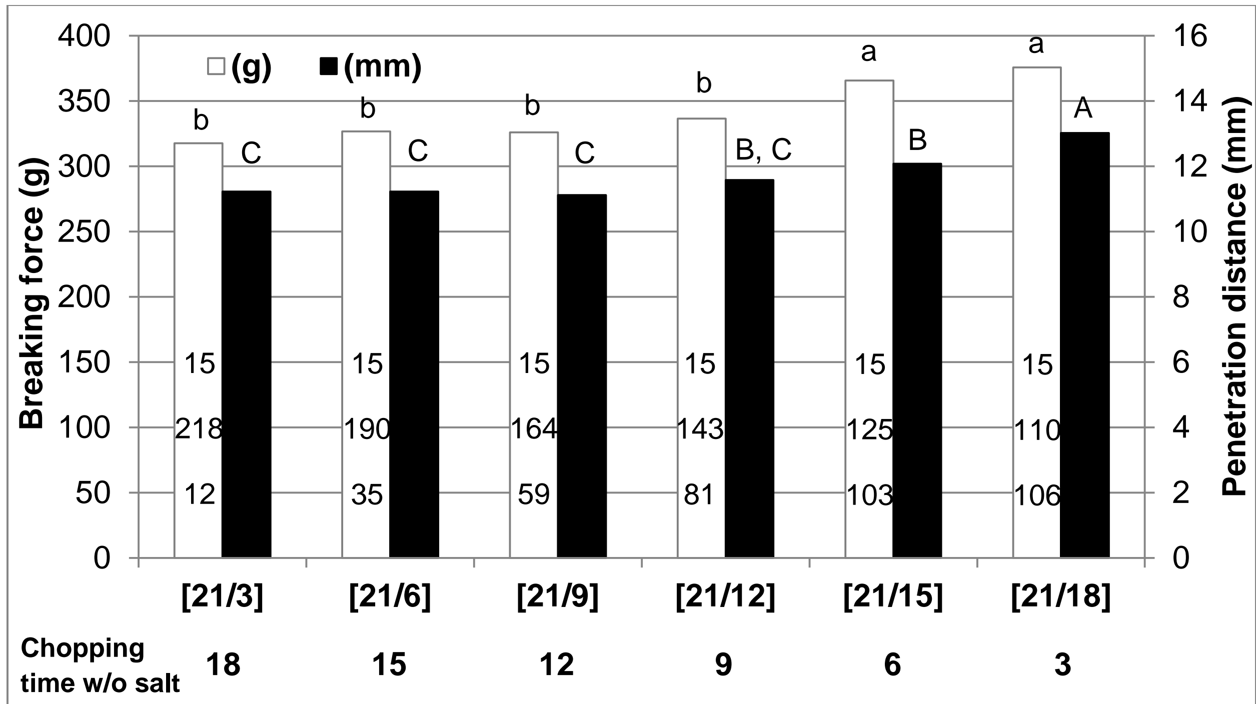

608 Fig. 6: Gel hardness and cohesiveness of [21/X_2] TB gels as affected by various comminution
609 conditions. Refer to Fig. 1 for codes.

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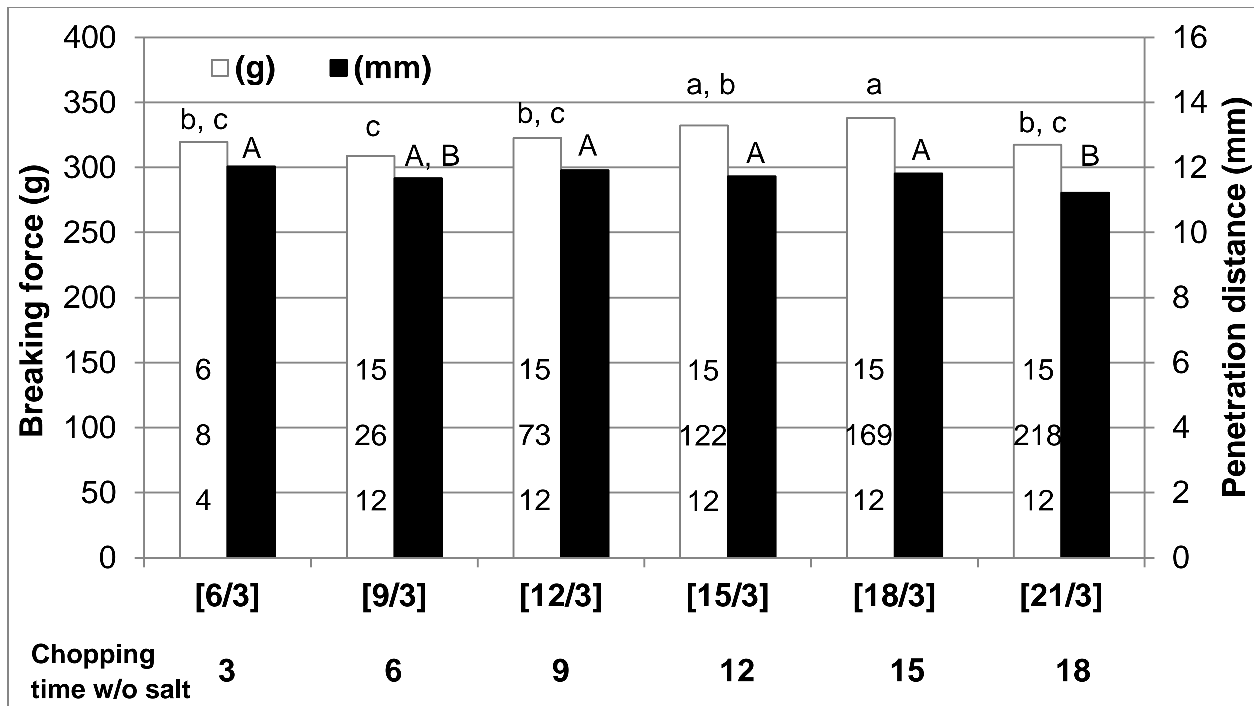

620 Fig. 7: Gel hardness and cohesiveness of [X_1/3] TB gels as affected by various comminution
621 conditions. Refer to Fig. 1 for codes.