

Research Paper

Effects of NaCl Solution on the Quality Control of 'Surimi' From Anchovy (*Clupeonella spp.*) During the Frozen Storage

ABSTRACT

The effect of washing on shelf-life of surimi of *Clupeonella spp.* with cold-water, NaCl solution of 1.5 and 3 percent strength was investigated. The shelf-life of surimi was investigated by measuring total volatile base-nitrogen (TVB-N), peroxide value, total count of bacteria and sensory test for 80 days. The results showed the changes of TVB-N for control, 1.5% and 3% NaCl solution were 35, 20 and 19 mg N/100 g, respectively. Washing with NaCl solution 3 percent sample showed the high amount of extracted protein. The minced, which was washed by NaCl solution of 3 percent strength, revealed the sensory score was between 5-8 at all the time. However, on day 80, the total plate counts in control, samples washing with 1.5% and 3% NaCl solution were 9.4, 7.5 and 7.3 log CFU/g, respectively and the level was slightly higher than the permissible limit after 60 days.

Key words: *Clupeonella spp.*, Surimi, Minced meat, NaCl solution, Anchovy

1 INTRODUCTION

High demand for surimi for the production of a large variety of minced fish analogues and the recent decline in the Alaskan pollack fishery, substantial efforts are being made in many countries to study the suitability of other fish species for surimi production. [1] The measurement of proximal composition, total volatile base-nitrogen (TVB-N), peroxide value (PV), total count of bacteria and sensory test helps to identify the shelf life of surimi prepared using anchovy (*Clupeonella spp.*). Surimi is the Japanese word ("suru" bring the meaning of "to process or mash", and "mi" means "meat"), in short, surimi can be described as minced meat, processed and stabilized of myofibrillar protein from fish muscle that is used in the preparation of imitation seafood [2]. Once the protein is solubilised by salt and water and heated, these proteins cross-link to form the continuous matrix of a surimi hydrogel. Viscoelasticity is always an important quality indicator for surimi [3]. Initially, surimi was mostly made from white-fleshed fish like Alaska Pollock (*Theragra chalcogramma*) or Pacific Whiting (*Merluccius productus*) [1]. There are a number of species that are now utilized for commercial surimi processing, which functional and compositional properties vary depending on the species used [1,2,4]. Because of high demand for surimi for the production of a large variety of minced fish analogues and the recent decline in the Alaskan pollack fishery, substantial efforts are being made in many countries to study the suitability of other fish species for surimi production. Fish like cod, hake, whiting, Atlantic menhaden, croaker, Chilean mackerel and New Zealand hoki are found to be suitable [5].

Anchovy (*Clupeonella spp.*) is the most abundant species of fish in the Caspian Sea. Three species of anchovy are important commercially in the Caspian Sea, together accounting in the past decade for > 80 % of the total catch, as well as being a crucial part of the food chain [6]. Anchovy meat due to its special taste is not popular with all people. Other researchers have shown that the water-soluble protein and non-protein nitrogen (NPN) have a significant effect on the taste of this fish [7,8]. Therefore, the objective of this study was to investigate the effects of

35 washing (NaCl solution, 1.5 and 3%) and common cryoprotectants (comprising 4% sucrose and
36 4% sorbitol) on the functionality of anchovy surimi during long-term frozen storage at -20 °C.

37 **2 MATERIALS AND METHODS**

38 **2.1. Materials**

39 Magnesium oxide (MgO), methyl red, methylene blue, boric acid and hydrochloric acid (HCl)
40 were purchased from Sigma (St. Louis, MO, USA) and Sodium chloride (Merck, Germany). All
41 other chemicals used were of analytical grade.

42 **2.2. Methods**

43 **2.2.1. Sample preparation**

44 Fresh anchovy (*Clupeonella spp.*) fish (100 kg; weight range: 6-10 g) were captured in the
45 Caspian sea near the Bandar Anzali coast, transported to the central market of Bandar Anzali
46 (Iran) and finally carried out to National Fish Processing Research Center. Throughout this
47 process (10 h), the fish were maintained in ice flake. Upon arrival to National Fish Processing
48 Research Center, ten fishes were separated and analyzed as starting raw fish, while the
49 remaining fish were cleaned and minced with blander machine. The whole minced fish were
50 divided into three batches (3×25 kg). To prepare surimi by the conventional washing process,
51 fish mince was washed with cold (4 °C) water (control) and cold NaCl solution (1.5% NaCl (w/v)
52 and 3% NaCl (w/v)) using a washing media/mince ratio of 3:1 (v/w). The washed mince was
53 subjected to dewatering by covering with cheesecloth and squeezing in screw until the mince to
54 come out. A cryoprotectant consisting of 4% (w/w) sucrose (ADM Food Additives Division,
55 Decatur, Ill., U.S.A.) and 4% (w/w) Neosorb sorbitol (Roquette America, Keokuk, Iowa, U.S.A.)
56 was mixed with both 1.5% and 3% NaCl washed solution samples. Stabilized minces were then
57 packed (1 kg per bag) in 0.090-mm nylon, freezer bags (China) and frozen at -20 °C in a
58 double-contact plate freezer. All mince samples were transported for 4 h to the food processing
59 Pilot Plant, University of Tehran, Karaj, Iran. Upon arrival, sample bags were stored at -20°C in
60 chest freezer. Periodically, two bags of each type of mince were randomly evaluated for quality
61 every 15 days until 80 days of storage.

62 **2.2.2. Proximate composition**

63 Moisture, protein (N×6.25), fat, and ash contents of the minced fish were determined for each
64 group in duplicate following AOAC methods [9].

65 **2.2.3. Determination of Total volatile basic nitrogen (TVB-N)**

66 Total volatile basic nitrogen (TVB-N) value was estimated by the method Goulas and
67 Kontominas [10]. A 10 g sample of fish flesh was mixed with 50 ml of distilled water using a
68 Moulinex mixer. The mixture was quantitatively transferred with 200 ml of distilled water into a
69 500 ml round bottom flask and was distilled after the addition of 2 g of MgO and one drop of
70 silicone to prevent foaming. A 250 ml Erlenmeyer flask containing 25 ml of 3% aqueous solution
71 of boric acid, 0.04 ml of methyl red and methylene blue mixed indicators was used for the
72 titration of ammonia as the distillate receiver. Distillation was continued until a final volume of
73 125 ml of distillate was obtained. The boric acid solution turned green when made alkaline by
74 the distilled total volatile basic nitrogen (TVBN). This was titrated with an aqueous 0.1 N
75 hydrochloric acid solution. Complete neutralization was achieved when the color of the distillate
76 turned pink upon addition of a further drop of hydrochloric acid. The quantity of TVB-N in
77 mg/100 g of fish flesh was calculated:

$$78 \quad \text{TVB - N (mg N/100g)} = \frac{V \times C \times 14}{W} \times 100$$

79 Where (V) is the volume of hydrochloric acid added, (C) its concentration and (W) weight of
80 sample in gram.

81 **2.2.4. Measurement of expressible moisture**

82 Mince cylinders (1×1 cm) were weighed (A), placed between 2 pieces of weighed Whatman No.
83 42 filter paper, and put on a Pyrex watch glass. Initial load of 500 g was applied on the top of
84 the sample for 5 min, followed with another 500 g loading for an additional 20 min. After
85 pressing with the loads, samples were weighed (B). The drip under pressure was determined as
86 (A) - (B) and calculated against sample weight (A) as a percentage [11].

87 **2.2.5. Determination of pH**

88 The muscle was homogenised in distilled water in a ratio 1:10 (w/v) and the measurement was
89 made in a digital pH-meter model DELTA 320 at room temperature. All reading was performed
90 in triplicate.

91 **2.2.6. Peroxide value (PV)**

92 According to IUPAC [12], peroxide value is expressed in terms of milliequivalents (meq) of
93 active oxygen per kilogram of sample that oxidizes potassium iodide under condition of the test.
94 It is determined by titrating iodine liberated from potassium iodide with sodium thiosulphate
95 solution. About 0.3 g of fat was weighed into a 250 mL flask with stopper and dissolved in 10 ml
96 chloroform. Then 25 mL acetic acid added to the mixture. Then 1 mL of saturated KI solution
97 was added and immediately stopper was put and kept in dark place for 5 min. Finally, 75 mL
98 distilled water was added and then shaken. Sample was titrated with 0.01 N, Na₂S₂O₃ solution
99 until yellow color roughly disappeared. After that, 1mL of starch solution (1.0 %) was added and
100 titration continued until dark blue color was disappeared. A blank test was also done, without
101 lipid with the same condition.

$$102 \quad \text{Peroxide value (meq/1000g)} = \frac{1000 (V_1 - V_2) N}{W}$$

103 Where V₁ is the volume in milliliters of the sodium thiosulphate solution of normality N used for
104 the determination, V₂ is the volume of the sodium thiosulphate solution used for the blank test,
105 W is the weight (g) of the test sample, N is the exact normality of the sodium thiosulphate
106 solution.

107 **2.2.7. Total plate count (TPC)**

108 Twenty-five grams of fish slices was aseptically weighed and homogenized in the stomacher
109 bags (BAGMIXER® 400, Model P) with 225 ml sterile peptone water for 1 min. The
110 homogenized sample was serially diluted using 9 ml peptone water. Further serial dilutions were
111 made and 0.1 ml of each dilution was pipette onto the surface of the plate count agar (Merck), in
112 triplicates, after which they were incubated for 2 days at 30 °C [9].

113 **2.2.8. Sensory evaluation**

114 Minced meat was steam cooked without salt, cooled and assessed for sensory quality (overall
115 acceptability) by 10 trained panellists using 10-point Hedonic scale [13].

116 2.3. Statistical analysis

117 Data collected were analyzed by one-way analysis of variance (ANOVA). The one-way ANOVA
118 was used to analyse the effect of days by different washing method on the surimi. The Turkey's
119 test was used for mean comparison when a significant variation was found by the ANOVA test.
120 The significance of results was at $P < 0.05$. The software used was SPSS version16 for
121 windows (SPSS Inc. 2008).
122

123 3 RESULTS AND DISCUSSION

124 Proximate composition of the minced fish was calculated are given in table 1. The moisture
125 content was found to be 74.4 %. The fat content was estimated to 5.19% whereas the protein
126 content was 19.4 g/100 g minced fish. The total ash content was estimated and found to be
127 1.19 %.
128
129

Table 1. Proximate compositions of fish minced

Characteristics	Anchovy (<i>Clupeonella</i> <i>sp.</i>)
Protein (%)	19.40 ± 1.03
Fat (%)	5.19 ± 0.87
Moisture (%)	74.20 ± 2.52
Ash (%)	1.19 ± 0.08

131

132 Total volatile basic nitrogen (TVB-N) values for anchovy are presented in Fig. 1. The initial
133 TVBN value of anchovy samples on day 0 (8.8 mg N/100 g) is indicative of freshness of raw fish
134 material. This value is in good agreement with that of Fan et al. [14], who reported that the initial
135 TVB-N content in raw chub mackerel was 9.96 mg N/100 g. Similar TVB-N value was reported
136 for fresh hake: 10.44 mg N/100 g [15]. As results show, the TVB-N level increased gradually
137 with time of storage, for samples either with or without washing with NaCl solution 1.5 and 3 %.
138 TVB-N increase is expected because it is related to bacterial spoilage [16]. This increase was
139 significantly lower ($P < 0.05$) in the 1.5% NaCl solution samples than in the 3% samples and
140 can be attributed to the preservative effect of salt.

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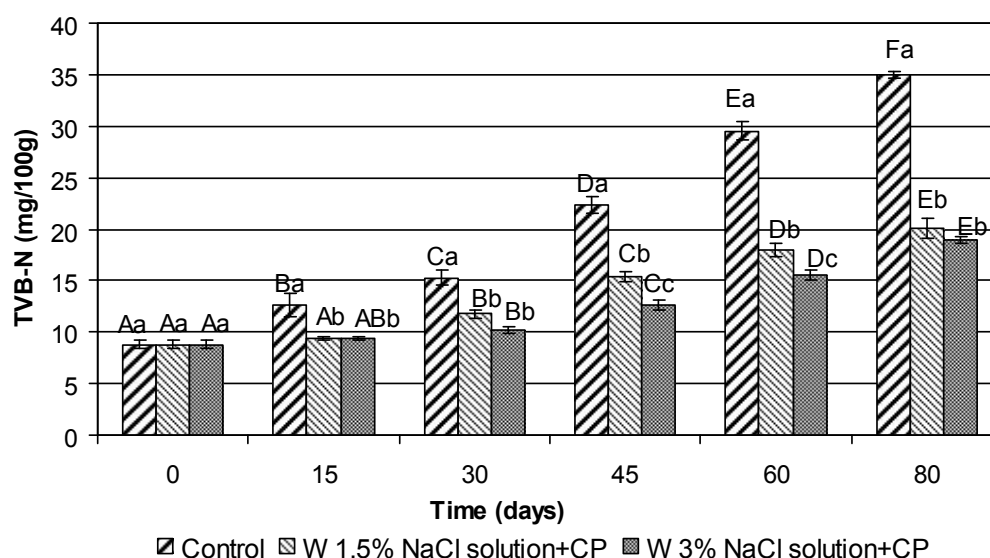


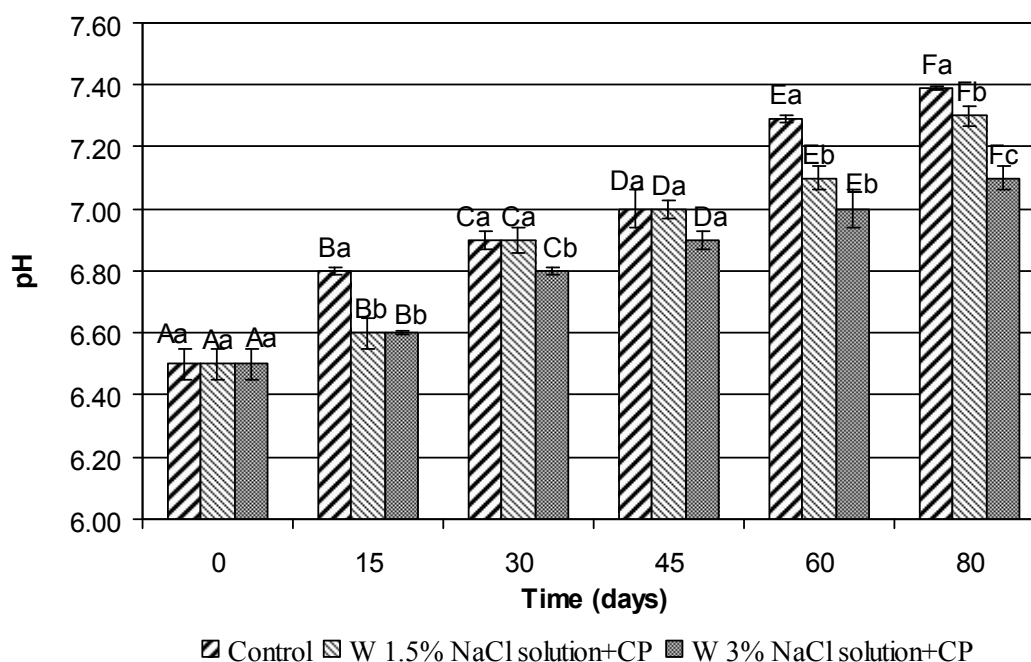
Fig. 1. Effects of washing and cryoprotectants on the total volatile base-nitrogen (TVB-N) of surimi during the storage at -20°C

Different lower case letters (for the same days) and different uppercase letters (for different days) indicated significant difference ($P < 0.05$)

A level of 35-40 mg TVB-N/100 g of fish muscle is usually regarded as spoiled [17]. However, various authors have reported different acceptability levels for different fish species, specific treatments, and processing conditions for TVB-N value: 35–40 mg/100 g [16] and 25–30 mg/100 g [18].

The TVBN content of NaCl solution washing samples (for both washing methods) remained significantly lower than the acceptability limit of 35 mg N/100 g of muscle set by the Connell [16]. The corresponding TVBN levels of control samples exceeded the above limit of 35 mg N/100 g after approximately 60 days of storage (control samples, 36.18 mg N/100 g), while the 1.5 and 3% NaCl solution samples were not approached this limit until 80 days of storage. The results showed the potential benefit of the NaCl solution washing and keep the TVBN levels below normal during long storage.

pH values for surimi stored at -20°C increased progressively throughout the experiment and reached to 7.39, 7.30 and 7.1 after 80 days for control, sample washed with 1.5% and 3% NaCl solution groups, respectively (Fig. 2). It was reported that, the pH limit for consumption is about 7.0 [19]. Where as, levels of pH 7.0 were exceeded after 60 days of storage for all of our samples. However, post-mortem pH varies from 6.0 to 7.1, depending on season, species and other factors. An important intrinsic factor related to fish flesh is the very high post-mortem pH (> 6.0). Generally, pH profiles of both washed with 1.5 and 3% NaCl solution of anchovy surimi were very similar, almost with no significant changes ($P > 0.05$) occurring during storage at -20 °C, while there is significant differences ($P < 0.05$) with control (Fig. 2). Most fish contain very little carbohydrate (< 0.5%) in the muscle tissue and only small amounts of lactic acid are produced post mortem. This has important consequences for the microbiology of fish as amongst other factors it allows the pH sensitive spoilage bacteria to grow [20].



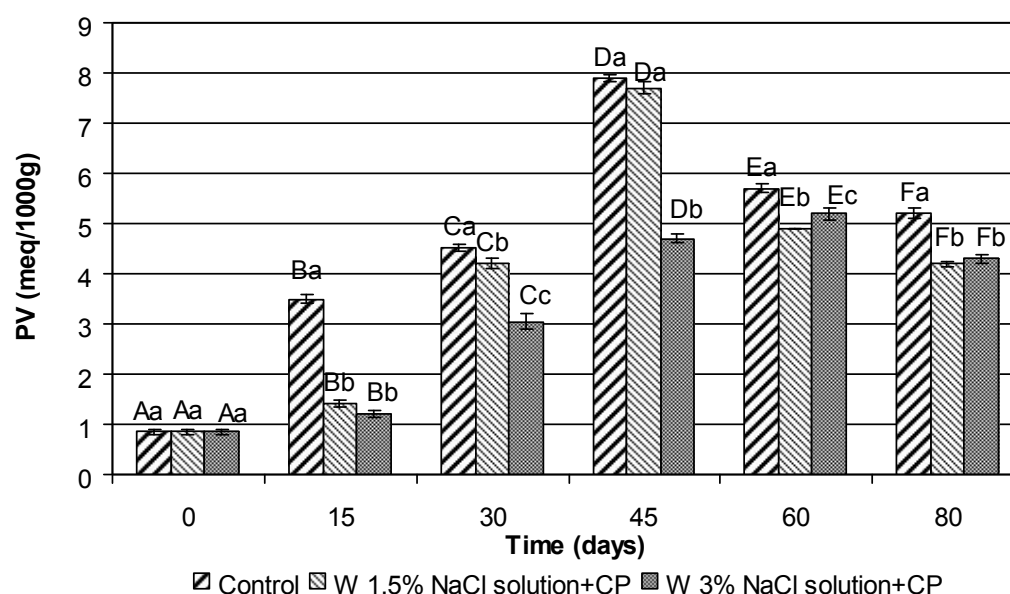
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173 **Fig. 2. Effects of washing and cryoprotectants on the pH value of surimi during the**
 174 **frozen storage at -20 °C**

175 *Different lower case letters (for the same days) and different uppercase letters (for different days) indicated*
 176 *significant difference ($P < 0.05$)*

177

178 Oxidative abuse of the oil is determined by peroxide value (PV). The Peroxide value of an oil or
 179 fat is used as a measurement of the extent to which rancidity reactions have occurred during
 180 storage. PV of crude fish lipid ranges between 3 to 20 meq O_2 / Kg [21]. In order to assess the
 181 oxidative changes in surimi during frozen storage peroxide value was determined and has been
 182 presented in Fig. 3. Significant ($P < 0.05$) differences were observed in peroxide value at
 183 different time intervals. Peroxide value increased from 0.85 meq/kg in control, washed with 1.5
 184 and 3% NaCl solution to 7.9 meq/kg, 7.7 meq/kg and 5.2 meq/kg in 45 days, respectively. After
 185 that, the value decreased to 5.2 meq/kg, 4.2 meq/kg and 4.3 meq/kg, respectively. The
 186 decrease in peroxide value after 45 days of storage may be attributed to the instability of
 187 peroxides and started the secondary oxidation of products [22].



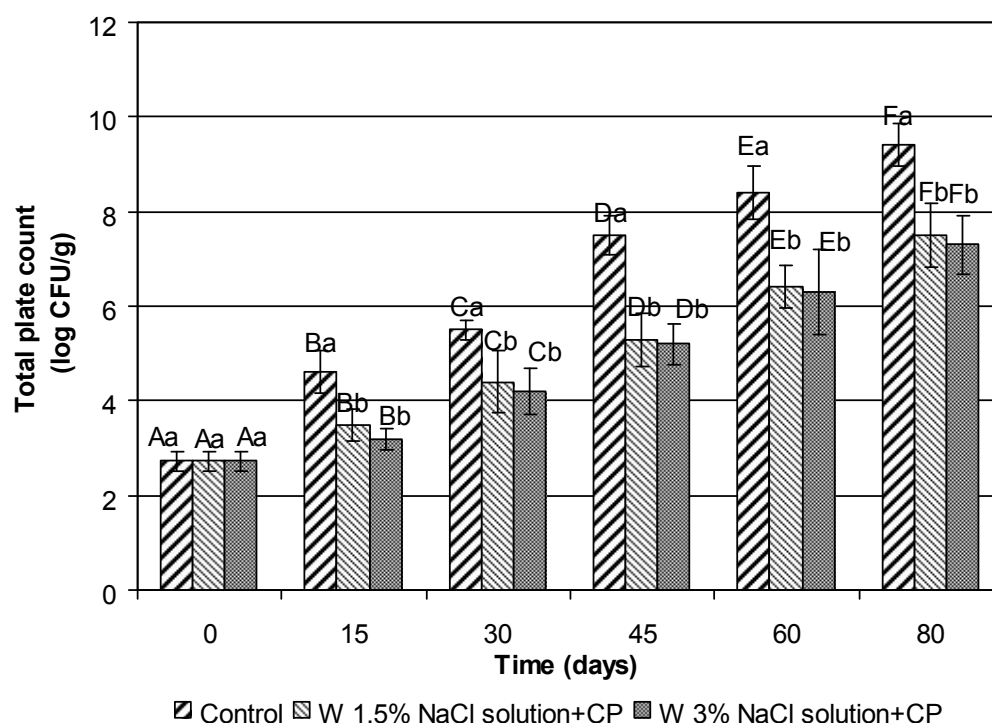
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189 **Fig. 3. Effects of washing and cryoprotectants on the peroxide value (meq/1000g) of**
 190 **surimi during the frozen storage at -20 °C**

191 *Different lower case letters (for the same days) and different uppercase letters (for different days) indicated*
 192 *significant difference ($P < 0.05$)*

193

194 The initial total plate count (TPC) of fish sample was 2.72 log CFU/g, and the low initial TPC
 195 indicated very good fish quality. Changes in TPC of surimi during the frozen storage are shown
 196 in Fig. 4. TPC of sample washed with 1.5% NaCl solution was found to be the same as that of
 197 the sample with 3% NaCl solution during the first 15 days of the frozen storage, but later, was
 198 observed to be increasing more slowly than that in sample wash with 1.5%NaCl solution and
 199 reached 7.5 log CFU/g on the 80th day of the frozen storage. It did exceed the maximal
 200 permissible limit of 7.0 log CFU/g for the bacterial count in fish International Commission on
 201 Microbiological Specifications for Foods [23], while the TPC of control sample reached about
 202 7.5 log CFU/g on the 30th day during the frozen storage. Our findings are in agreement with the
 203 report of Fan et al. [14] on the effect of chitosan coating on quality and shelf life of silver carp
 204 during frozen storage.



205

206 **Fig. 4: Effects of washing and cryoprotectants on the total plate counts of surimi during**
 207 **the frozen storage at -20 °C**

208 *Points represent mean values of six determinations $\pm$ standard deviation ($n = 2 \times 3$)*

209 *Different lower case letters (for the same days) and different uppercase letters (for different days) indicated*
 210 *significant difference ($P < 0.05$)*

211

212 The significant reduction in TPC observed in samples washed with 3%, 1.5% NaCl solution and
 213 control could be attributed to the inhibitory effect of salt on spoilage bacteria. The result of the
 214 treatment also indicated that washing with 3% NaCl solution was equally effective in inhibiting
 215 spoilage bacteria growth and extending the storage life of surimi to 80 days compared to 30
 216 days for treatment with cold water (control).

217 Effects of washing and cryoprotectants (CP) on the sensory scores of surimi are presented in
 218 Table 2. The sensory scores were high in all fresh minces (0 day storage) with or without added
 219 CP. In frozen storage, sensory scores decreased in all washed mince with or without added CP.
 220 In washed mince with 1.5 and 3% NaCl solution with CP, the value was not significant
 221 difference ($P > 0.05$) until 45 and 60 days of storage, respectively. While the sensory score of
 222 control group (without CP) showed significant ($P < 0.05$) difference after 30 days of storage,
 223 indicating a protecting effect of CP in such mince. This result was agreed with finding of
 224 Suvanich et al. [11], who found a significant increase of sensory scores in the surimi of channel
 225 cat fish frame mine during the frozen storage with sucrose and sorbitol added.

226

227

228 **Table 2. Effects of washing and cryoprotectants on sensory scores¹ (overall**
 229 **acceptability) of surimi during the frozen storage**

Time (days)	Control	Washed with 1.5% NaCl solution+CP	Washed with 3% NaCl solution+CP
0	7.7 ± 0.58Da	8.0 ± 1.00Da	7.8 ± 0.29Ba
15	6.3 ± 0.58CDa	7.8 ± 0.29Db	7.7 ± 0.58Bb
30	5.5 ± 0.87Ca	7.2 ± 0.29CDb	7.2 ± 0.28Bb
45	4.8 ± 0.76BCa	6.3 ± 0.29BCb	6.8 ± 0.29ABb
60	3.8 ± 0.29ABa	5.5 ± 0.50ABb	6.5 ± 0.50ABc
80	2.5 ± 0.50Aa	4.2 ± 0.29Ab	5.5 ± 0.87Ab

230 ¹ Means reported are the results of triplicate.

231 ^a Means within each row with different lower case are significantly ($P < 0.05$) different

232 ^A Means within each column with different upper case are significantly ($P < 0.05$) different. CP =
 233 cryoprotectants

234

235 Moisture content is a determinative indicator of surimi quality [11]. Although screw press
 236 dewatering removed excess water from the washed minced meat, its moisture content (81.3%)
 237 was still higher ($P < 0.05$) than that of the initial moisture content (74.2%) (Fig. 5 and Table 1).
 238 Washing would increase hydration of the mince meat by removal of fat and water-soluble
 239 constituents, such as blood, pigments, proteins, and salts [11]. The moisture content of sample
 240 washed with cold water (control) due to addition of the cryoprotectant was higher but no
 241 significant ($P > 0.05$) difference than two other samples. There were no significant difference (P
 242 > 0.05) in moisture content of the washed mince with 1.5 and 3% NaCl solution with CP during
 243 storage at -20 °C (Fig. 5).

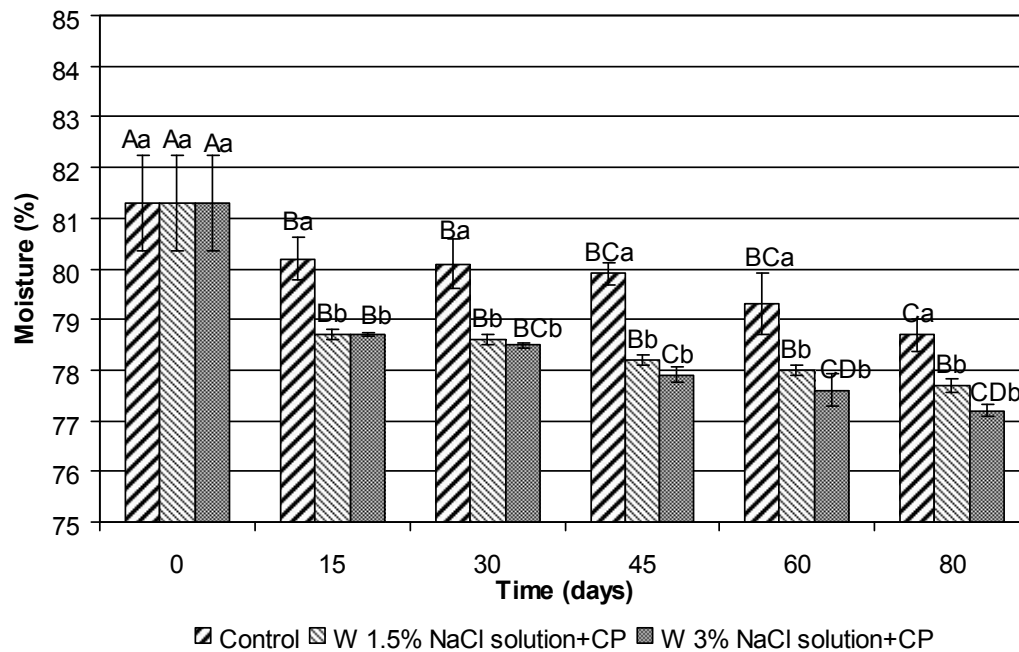


Fig. 5. Effects of washing and cryoprotectants on the moisture of surimi during the frozen storage at -20 °C

Different lower case letters (for the same days) and different uppercase letters (for different days) indicated significant difference ($P < 0.05$)

Expressible moisture was higher ($P < 0.05$) in control mince than in washed mince with 1.5 and 3% NaCl solution with cryoprotectants (Fig. 6). The expressible moisture progressively increased at -20 °C, which was expected, because the texture deteriorates with the increment of free water during frozen storage [24]. Increase of expressible moisture in washed mince with 1.5 and 3% NaCl solution was significant ($P < 0.05$) at 45 days but was not significant ($P > 0.05$) thereafter. Cryoprotectants lessened the amount of expressible moisture of the mince in storage [13]. The effect of cryoprotectants was greater in washed mince with 1.5% NaCl solution ($P < 0.05$) compared with washed mince with 3% NaCl solution. Expressible moisture is indices of water-holding properties of muscle foods. Increased water holding in the cryoprotectant-incorporated minced might be due to huge functional OH groups of sucrose and sorbitol, through which they could bind to the proteins, resulting in increased protein hydration [13].

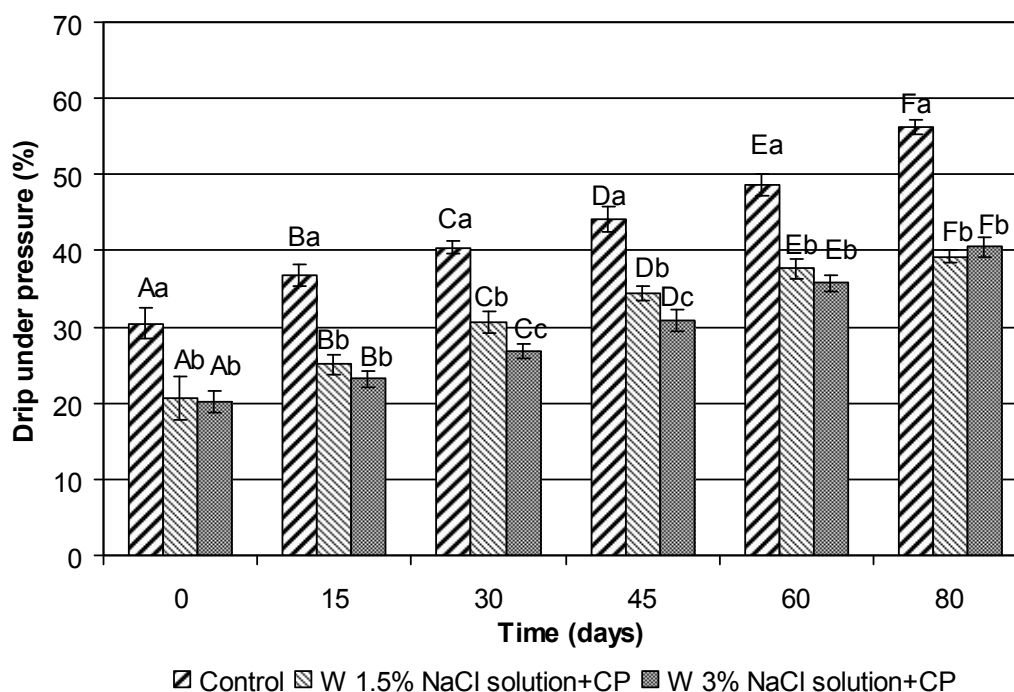


Fig. 6. Effects of washing and cryoprotectants on the expressible moisture of surimi during the frozen storage at -20 °C

Different lower case letters (for the same days) and different uppercase letters (for different days) indicated significant difference ($P < 0.05$)

4 CONCLUSION

On the basis of the present results, washing with 3 % NaCl solution treatment on anchovy (*Clupeonella spp.*) leads to a retention of the good quality characteristics for longer and an extension of the shelf life during frozen storage. The conclusion was supported by the results of microbiological (total plate count), chemical (pH, TVB-N, PV), and sensory assessment analyses.

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