

Effects of NaCl Solution on the Physico-Chemical Changes of 'Surimi' From Anchovy (*Clupeonella* spp.) During the Frozen Storage

ABSTRACT

Aims: In this study the effect of washing process on shelf-life of surimi of *Clupeonella* spp. with cold-water, NaCl solution of 1.5 and 3 percent strength was investigated.

Study design: Data collected were analyzed by one-way analysis of variance (ANOVA).

Place and Duration of Study: Coastal line of Caspian sea near the Bandar Anzali, Gilan province of Iran.

Methodology: A total of 100 kg fish were captured in the Caspian sea near the Bandar Anzali coast. The data collected from the surimi during the storage time.

Results: 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 100g⁻¹, respectively. Washing with NaCl solution 3 percent sample showed the high amount of extracted protein. 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

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

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 proximate 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]. 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. This fish is 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].

The addition low molecular weight carbohydrate, such as sucrose and sorbitol, in the surimi to stabilize actomyosin, which is highly unstable during the frozen storage. Washing cycles is an essential step in removing water-soluble proteins, primarily sarcoplasmic proteins, which is thought to impede the gel-forming ability of surimi and reduce the product quality [1]. In addition, Lin and Park [9] evaluated the effects of washing cycles and salt concentrations on the proteins extraction. Sarcoplasmic proteins were readily soluble in water and removed in the initial washing steps. Myofibrillar proteins became relatively soluble and were lost during extensive washing. Control of water/meat ratio, washing time, and washing cycles was critical in reducing the loss of myofibrillar proteins [1,9]. Therefore, the objective of this study was to investigate the effects of washing (NaCl solution, 1.5 and 3%) and common cryoprotectants (comprising 4% sucrose and 4% sorbitol) on the physico- chemical changes of anchovy surimi during long-term frozen storage at -20°C.

2. MATERIALS AND METHODS

2.1. Materials

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

2.2. Methods

2.2.1. Sample preparation

Fresh anchovy (*Clupeonella* spp.) fish (100 kg; weight range: 6-10 g) were captured in the Caspian sea near the Bandar Anzali coast, transported to the central market of Bandar Anzali (Iran) and finally carried out to National Fish Processing Research Center. Throughout this process (10 h), the fish were maintained in ice flake. Upon arrival to National Fish Processing Research Center, ten fishes were separated and analyzed as starting raw fish, while the remaining fish were cleaned and minced with blander machine. The whole minced fish were divided into three batches (3×25 kg). To removed the excess fat from minced fish before treated for surimi process was washed with 0.2% sodium bicarbonate [1]. To prepare surimi by the conventional washing process, fish minced was washed with cold (4°C) water (control) and cold NaCl solution (1.5% (T₁) and 3% (T₂)) using a washing media/mince ratio of 3:1 (v/w) for 45 min. The washed mince was subjected to dewatering by covering with cheesecloth and squeezing in screw until the water to come out [1,10,11]. A cryoprotectant consisting of 4% (w/w) sucrose (ADM Food Additives Division, Decatur, Ill., U.S.A.) and 4% (w/w) Neosorb sorbitol (Roquette America, Keokuk, Iowa, U.S.A.) was mixed with both T₁ and T₂ treatments and control without cryoprotectants. Stabilized minces were then packed (1 kg per bag) in 0.090-mm nylon, freezer bags (China) and frozen at -20°C in a double-contact plate freezer. All mince samples were transported for 4 h to the food processing Pilot Plant, University of Tehran, Karaj, Iran. Upon arrival, sample bags were stored at -20°C in chest freezer. Periodically, two bags of each type of mince were randomly evaluated for quality every 15 days until 80 days of storage.

2.2.2. Proximate composition

Moisture, protein (N×6.25), fat, and ash contents of the minced fish were determined for each group in duplicate following AOAC methods [12].

75 **2.2.3. Determination of Total volatile basic nitrogen (TVB-N)**

76 Total volatile basic nitrogen (TVB-N) value was estimated by the method Goulas and
77 Kontominas [13]. A 10 g sample of fish flesh was mixed with 50 ml of distilled water using a
78 Moulinex mixer. The mixture was quantitatively transferred with 200 ml of distilled water into a
79 500 ml round bottom flask and was distilled after the addition of 2 g of MgO and one drop of
80 silicone to prevent foaming. Distillation process was continued until a final volume of 125 ml of
81 distillate was obtained and the final volume was titrated with an aqueous 0.1 N hydrochloric acid
82 solution. The quantity of TVB-N in mg 100g⁻¹ of fish flesh was calculated:

83
$$\text{TVB - N (mg N/100g)} = \frac{V \times C \times 14}{W} \times 100$$

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

86 **2.2.4. Measurement of expressible moisture**

87 Mince cylinders (1×1 cm) were weighed (A), placed between 2 pieces of weighed Whatman No.
88 42 filter paper, and put on a Pyrex watch glass. Initial load of 500 g was applied on the top of
89 the sample for 5 min, followed with another 500 g loading for an additional 20 min. After
90 pressing with the loads, samples were weighed (B). The drip under pressure was determined as
91 (A) - (B) and calculated against sample weight (A) as a percentage [14].

92 **2.2.5. Determination of pH**

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

96 **2.2.6. Peroxide value (PV)**

97 According to IUPAC [14], peroxide value is expressed in terms of milliequivalents (meq) of
98 active oxygen per kilogram of sample that oxidizes potassium iodide under condition of the test.
99 It is determined by titrating iodine liberated from potassium iodide with sodium thiosulphate
100 solution. About 0.3 g of sample was weighed into a 250 mL flask with stopper and dissolved in
101 10 ml chloroform. Then 25 mL acetic acid added to the mixture. Then 1 mL of saturated KI
102 solution was added and immediately stopper was put and kept in dark place for 5 min. Finally,
103 75 mL distilled water was added and then shaken. Sample was titrated with 0.01 N, Na₂S₂O₃
104 solution until yellow color roughly disappeared. After that, 1mL of starch solution (1.0 %) was
105 added and titration continued until dark blue color was disappeared. A blank test was also done,
106 without lipid with the same condition.

107
$$\text{Peroxide value (meq/kg)} = \frac{1000 (V_1 - V_2) N}{W}$$

108 Where V₁ is the volume in milliliters of the sodium thiosulphate solution of normality N used for
109 the determination, V₂ is the volume of the sodium thiosulphate solution used for the blank test,
110 W is the weight (g) of the test sample, N is the exact normality of the sodium thiosulphate
111 solution.

112 **2.2.7. Total plate count (TPC)**

113 Twenty-five grams of fish slices was aseptically weighed and homogenized in the stomacher
114 bags (BAGMIXER® 400, Model P) with 225 ml sterile peptone water for 1 min. The
115 homogenized sample was serially diluted using 9 ml peptone water. Further serial dilutions were
116 made and 0.1 ml of each dilution was pipette onto the surface of the plate count agar (Merck,
117 Germany) in triplicates, after which they were incubated for 2 days at 30 °C [12].

2.3. Statistical analysis

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

3. RESULTS AND DISCUSSION

Proximate composition of the minced fish was calculated are given in table 1. The moisture content was found to be 74.20 %. The fat content was estimated to 5.19% whereas the protein content was 19.40 g 100g⁻¹ minced fish. The total ash content was estimated and found to be 1.19 %.

Table 1. Proximate compositions of fish minced

Characteristics	Anchovy (<i>Clupeonella</i> spp.)	132
Protein (%)	19.40 ± 1.03	
Fat (%)	5.19 ± 0.87	
Moisture (%)	74.20 ± 2.52	
Ash (%)	1.19 ± 0.08	

Total volatile basic nitrogen (TVB-N) values for anchovy are presented in Fig. 1. The initial TVB-N value of anchovy samples on the first day (8.8 mg N 100g⁻¹) is indicated of freshness of raw material. This value is in good agreement with that of Fan et al. [15], who reported that the initial TVB-N content in raw chub mackerel was 9.96 mg N 100g⁻¹. Similar TVB-N value was reported for fresh hake: 10.44 mg N 100g⁻¹ [16]. As results show, the TVB-N level increased gradually with time of storage, for control, T₁ and T₂ treatments. The increases of TVB-N is expected because it is related to bacterial spoilage [17]. This increase was significantly higher ($P \leq 0.05$) in the control and T₁ rather than T₂ treatments and can be attributed to the preservative effect of salt.

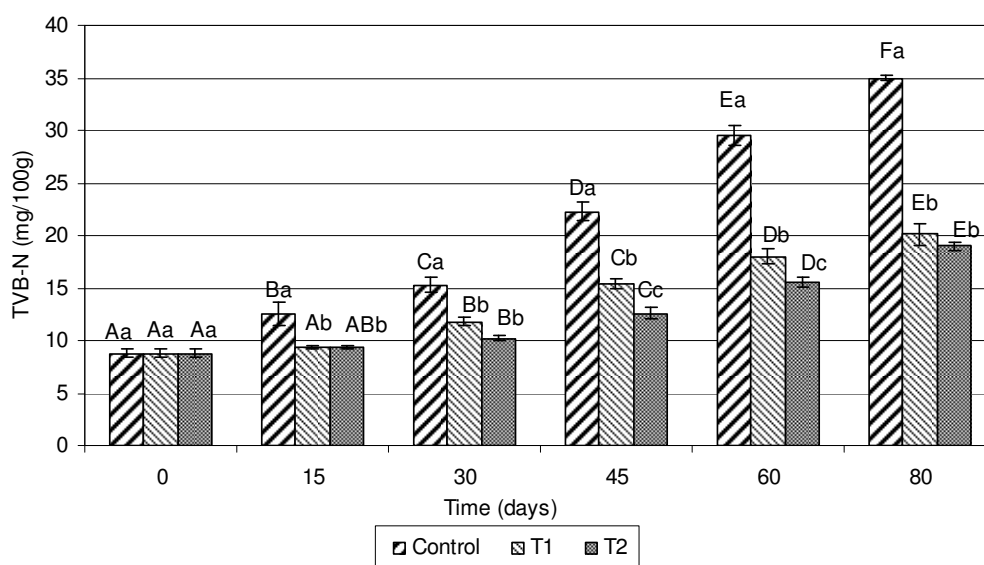


Fig. 1. Effects of washing and cryoprotectants on the total volatile base-nitrogen (TVB-N) of surimi during the storage at -20°C

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

A level of 35-40 mg TVB-N/100 g of fish muscle is usually regarded as spoiled [18]. However, various authors have reported different acceptability levels for different fish species, specific treatments, and processing conditions for TVB-N value: 35–40 mg 100g⁻¹ [17] and 25–30 mg 100g⁻¹ [19].

The TVBN content of NaCl solution washing samples (for both washing methods) remained significantly lower than the acceptability limit of 35 mg N 100g⁻¹ of muscle set by the Connell [17]. The corresponding TVBN levels of control samples exceeded the above limit of 35 mg N 100g⁻¹ after approximately 60 days of storage (control, 36.18 mg N 100g⁻¹), while the T₁ and T₂ treatments 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, T₁ and T₂ treatments, respectively (Fig. 2). It was reported that, the pH limit for consumption is about 7.0 [20]. 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 [21]. An important intrinsic factor related to fish flesh is the very high post-mortem pH (> 6.0). Generally, pH profiles of both T₁ and T₂ of anchovy surimi were very similar, almost with no significant changes ($P \geq 0.05$) occurring during storage at -20°C, while there is significant differences ($P \leq 0.05$) with control (Fig. 2). Most fish contain very little carbohydrate (< 0.5 percentage) 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 [21].

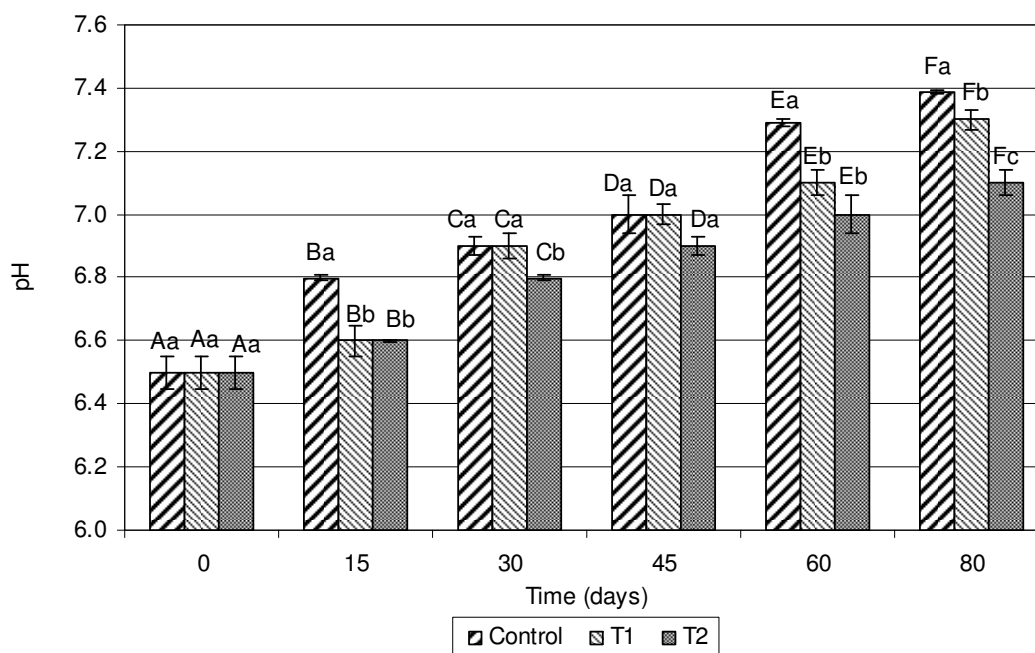
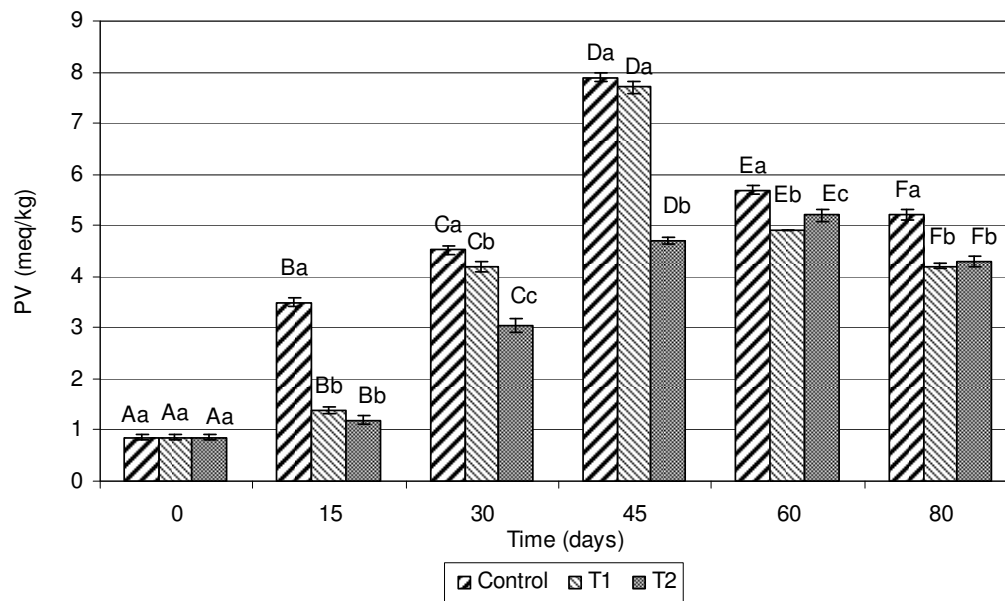


Fig. 2. Effects of washing and cryoprotectants on the pH value of surimi during the frozen storage at -20 °C

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

Oxidative abuse of the oil is determined by peroxide value (PV). The Peroxide value of an oil or fat is used as a measurement of the extent to which rancidity reactions have occurred during storage. PV of crude fish lipid ranges between 3 to 20 meq O_2 Kg^{-1} [22]. In order to assess the oxidative changes in surimi during frozen storage peroxide value was determined and has been presented in Fig. 3. Significant ($P \leq 0.05$) differences were observed in peroxide value at different time intervals. Peroxide value increased from 0.85 meq/kg in control, T₁ and T₂ treatments to 7.9 meq kg^{-1} , 7.7 meq kg^{-1} and 5.2 meq kg^{-1} in 45 days, respectively. After that, the value decreased to 5.2 meq kg^{-1} , 4.2 meq kg^{-1} and 4.3 meq kg^{-1} , respectively. The decrease in peroxide value after 45 days of storage may be attributed to the instability of peroxides and started the secondary oxidation of products [23].



188

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

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

193 The initial total plate count (TPC) of fish sample was 2.72 log CFU/g, and the low initial TPC
 194 indicated very good fish quality. Changes in TPC of surimi during the frozen storage are shown
 195 in Fig. 4. TPC of sample washed with T₁ solution was found to be the same as that of the
 196 sample with T₂ solution during the first 15 days of the frozen storage, but later, was observed to
 197 be increasing more slowly than T₁ treatment and reached 7.5 log CFU/g on the 80th day of the
 198 frozen storage. It did exceed the maximal permissible limit of 7.0 log CFU/g for the bacterial
 199 count in fish International Commission on Microbiological Specifications for Foods [24], while
 200 the TPC of control sample reached about 7.5 log CFU/g on the 45th day during the frozen
 201 storage. Our findings are in agreement with the report of Fan et al. [15] on the effect of chitosan
 202 coating on quality and shelf life of silver carp during frozen storage.

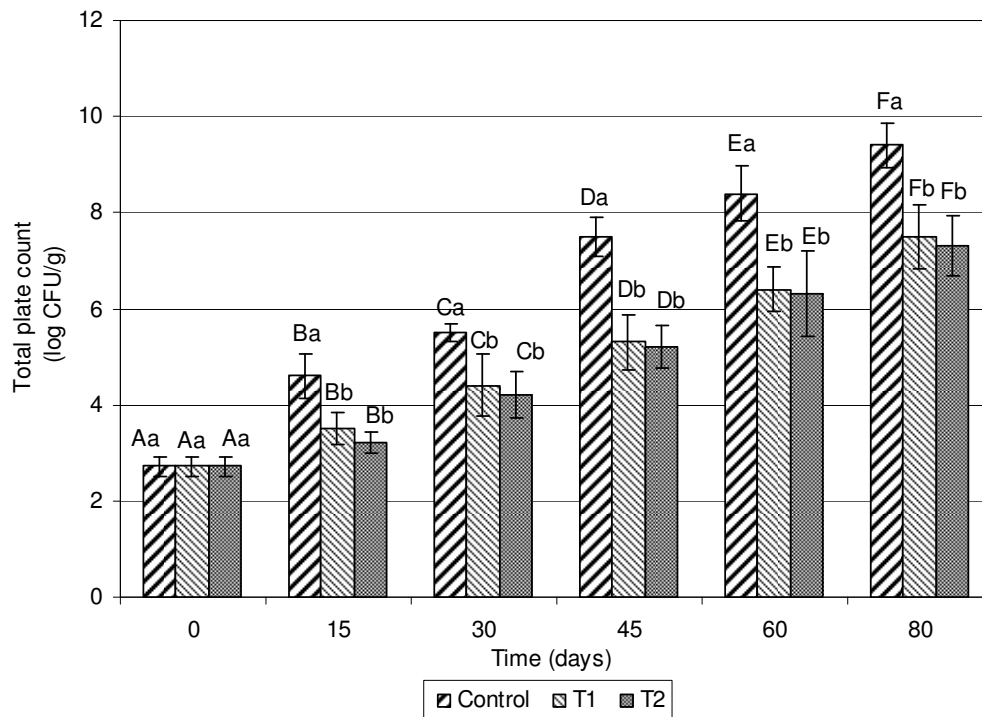


Fig. 4: Effects of washing and cryoprotectants on the total plate counts of surimi during the frozen storage at -20°C

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

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

The kinetic growth of TPC in T_2 and T_1 at the each storage time was significantly ($P \leq 0.05$) lower than control; this could be due to the inhibitory effect of salt and cryoprotectants on spoilage bacteria. The result of the treatment also indicated that T_2 treatment was equally effective in inhibiting spoilage bacteria growth and extending the storage life of surimi to 80 days compared to 45 days for treatment with cold water (control).

Moisture content is a determinative indicator of surimi quality [25] and the moisture content of high quality of commercial surimi is about 72 to 77% [1,9]. The moisture content of each treatments of surimi is shown in Fig. 5. Although dewatering by squeezing in screw removed excess water from the washed minced meat, the raw surimi showed the highest moisture content (81.3%) was significantly ($P \leq 0.05$) higher than the initial moisture content of T_1 (78.8%) and T_2 (78.7%) (Fig. 5). The decrease in the moisture content of surimi during the frozen storage was due to dehydration process [1]. Park, Lin and Park, Park and Lanier [1,2,9] suggested that the dehydration of protein molecules is caused by the migration of water molecules to form ice crystals. The denaturation of myofibrillar proteins through aggregation and unfolding is started by this phenomenon. Washing would increase hydration of the mince meat by removal of fat and water-soluble constituents, such as blood, pigments, proteins, and salts [25]. The moisture content of sample washed with cold water (control) was significantly ($P \leq 0.05$) higher than two other treatments. There were no significant differences ($P \geq 0.05$) in moisture content of the T_1 and T_2 treatments with CP during storage at -20°C (Fig. 5).

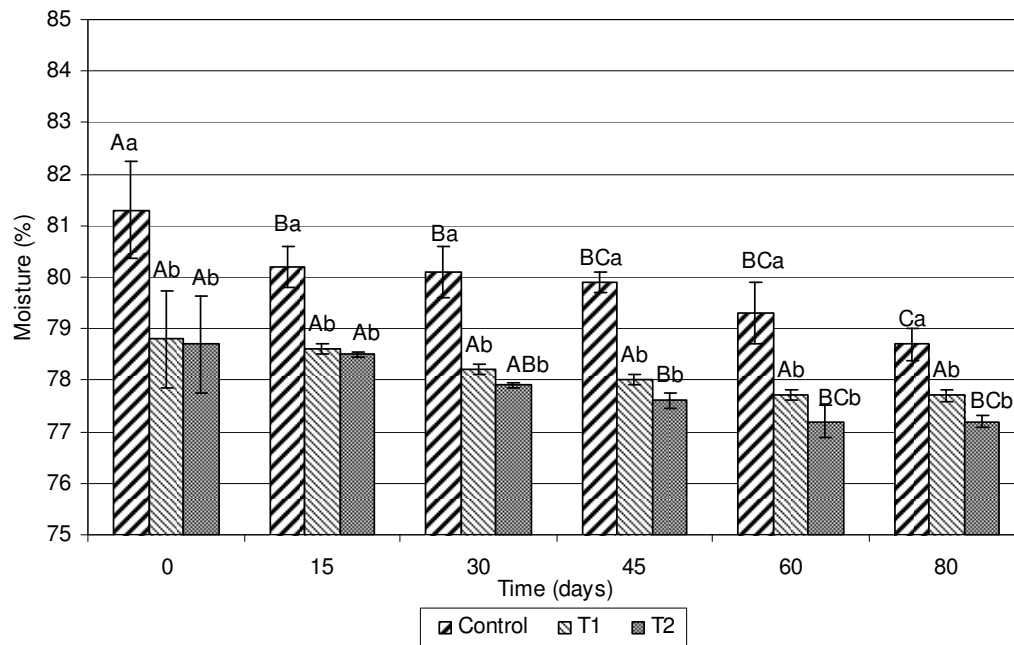


Fig. 5. Effects of washing and cryoprotectants on the moisture of surimi during the frozen storage at -20 °C

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

Expressible moisture was higher ($P \leq 0.05$) in control, T_1 and T_2 treatment 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 [26]. During the storage time the expressible moisture in T_1 and T_2 was significantly ($P \leq 0.05$) lower than control. However, there was no significant ($P \geq 0.05$) difference was observed in T_1 and T_2 in the most storage time. Cryoprotectants lessened the amount of expressible moisture of the mince in storage [10]. The effect of cryoprotectants was greater in T_1 treatment ($P \leq 0.05$) compared with T_2 . 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 [10].

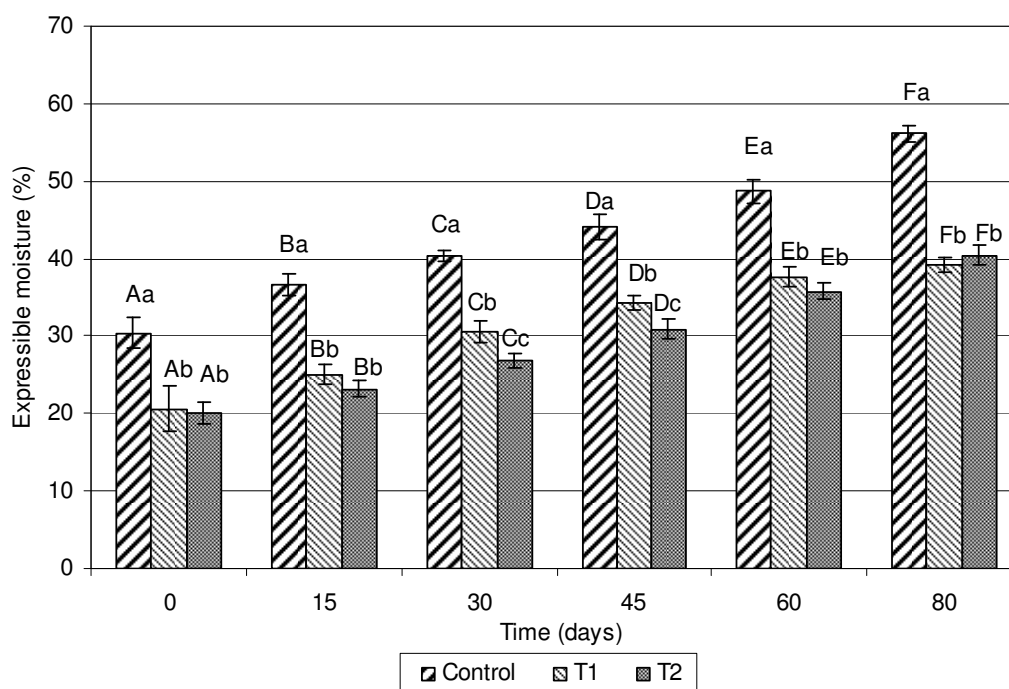


Fig. 6. Effects of washing and cryoprotectants on the expressible moisture of surimi during the frozen storage at -20°C

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

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.

257 REFERENCES

- 258 1. Park JW. Surimi and Surimi Seafood. Second Edi. Park JW, editor. CRC Press; 2005. p.
259 960.
- 260 2. Park JW, Lanier TC. Processing of surimi and surimi seafoods. In: Martin RE, Carter EP,
261 Flick Jr. GJ, Davis LM, editors. Marine and freshwater products handbook. Technomic
262 Publishing Co. Lancaster; 2000. p. 417–43.
- 263 3. Chen H-H, Huang YC. Rheological properties of HPMC enhanced surimi analyzed by
264 small- and large-strain tests—II: Effect of water content and ingredients. Food Hydrocoll.
265 2008;22(2):313 – 322.
- 266 4. Park JW, Korhonen RW, Lanier TC. Effects of Rigor Mortis on Gel-forming Properties of
267 Surimi and Unwashed Mince Prepared from Tilapia. J Food Sci. 1990;55(2):353–5.
- 268 5. Yong JN. Surimi : a significant protein food of the future? Information Group Services
269 (Market Information Section), Laboratories of the British Food Manufacturing Industries
270 Research Association. Surrey, UK; 1988.
- 271 6. Daskalov GM, Mamedov E V. Integrated fisheries assessment and possible causes for
272 the collapse of anchovy kilka in the Caspian Sea. ICES J Mar Sci. 2007;64(3):503–11.
- 273 7. Hashimoto Y. Taste-producing substances in marine products. In: F. Shahidi & J.R.
274 Botta, editor. The technology and quality. Blackie academic and professional. London,
275 Newyork , Tokyo; 1965. p. 263–5.
- 276 8. Fuke S. Taste-Active components of sea foods with special reference to umami
277 substances. In: Shahidi F, Botta JR, editors. Seafoods: Chemistry, Processing
278 Technology and Quality. Boston, MA: Springer US; 1994.
- 279 9. Lin TM, Park JW. Extraction of Proteins from Pacific Whiting Mince at Various Washing
280 Conditions. J Food Sci. 1996;61(2):432–8.
- 281 10. Reddy GVS, Srikar LN. Preprocessing Ice Storage Effects on Functional Properties of
282 Fish Mince Protein. J Food Sci. 1991;56(4):965–8.
- 283 11. Ensoy U, Kolsarici N, Candogan K. Quality characteristics of spent layer surimi during
284 frozen storage. Eur Food Res Technol. 2004;219(1):14–9.
- 285 12. AOAC. Official methods of analysis of AOAC International. Horwitz W, editor.
286 Gaithersburg Md.: Association of Official Analytical Chemists; 2000.
- 287 13. Goulas AE, Kontominas MG. Effect of salting and smoking-method on the keeping
288 quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. Food
289 Chem. 2005;93(3):511–20.
- 290 14. IUPAC. Standard methods for the analysis of oils, fats and derivatives. determination of
291 peroxide value. Method 2.504. Oxford: Alden Press; 1979. p. 138.
- 292 15. Fan W, Sun J, Chen Y, Qiu J, Zhang Y, Chi Y. Effects of chitosan coating on quality and
293 shelf life of silver carp during frozen storage. Food Chem. 2009;115(1):66–70.

- 294 16. Ruiz-Capillas C, Moral A. Sensory and biochemical aspects of quality of whole bigeye
295 tuna (*Thunnus obesus*) during bulk storage in controlled atmospheres. Food Chem.
296 2005;89(3):347–54.
- 297 17. Connell JJ. Methods of assessing and selecting for quality. Control of Fish Quality. 3rd
298 ed. Springer, Berlin; 1990.
- 299 18. Lakshmanan PT. Quality assurance in seafood processing, Society of Fisheries
300 Technologists (India). In: TSG Iyer M, Kandoran, Mary Thomas PM, editors. Fish
301 spoilage and quality assessment. Cochin; 2000. p. 26–40.
- 302 19. López-Caballero ME, Pérez-Mateos M, Montero P, Borderías AJ. Oyster Preservation by
303 High-Pressure Treatment. J Food Prot. International Association for Food Protection;
304 2000;63(2):6.
- 305 20. Ruiz-Capillas C, Moral A. Correlation between biochemical and sensory quality indices in
306 hake stored in ice. Food Res Int. 2001;34(5):441–7.
- 307 21. Gram L, Huss HH. Microbiological spoilage of fish and fish products. Int J Food
308 Microbiol. 1996;33(1):121–37.
- 309 22. Yong F.V.K. The refining and hydrogenation of fish oil. fish oil bulletin 17. International
310 Association of Fish Meal Manufacturers. Hertfordshire,UK; 1986.
- 311 23. Manral M, Pandey MC, Jayathilakan K, Radhakrishna K, Bawa AS. Effect of fish (*Catla*
312 *catla*) frying on the quality characteristics of sunflower oil. Food Chem. 2008;106(2):634–
313 9.
- 314 24. ICMSF. International Commission on Microbiological Specifications for Foods.
315 Microorganisms in Foods 7. Microbiological Testing in Food Safety Management. Kluwer
316 Academic/Plenum Publishers, Springer, London; 2002.
- 317 25. Suvanich V, Jahncke ML, Marshall DL. Changes in Selected Chemical Quality
318 Characteristics of Channel Catfish Frame Mince During Chill and Frozen Storage. J
319 Food Sci. 2000;65(1):24–9.
- 320 26. Samson AD, Regestein JM. Textural changes in frozen cod frame minces stored at
321 various temperatures. J Food Biochem. 1986;10(4):259–73.
- 322
- 323 }
- 324