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Effects of NaCl Solution on the Physicochemical Changes of 'Surimi' from Anchovies (*Clupeonella* spp.) During the Frozen Storage

Ali Yassoralipour[1*], Ali Khoddami[2], Sohrab Moini[3], M. C. Sabu[4], Mohd Jahir Khan[1] and Mehrdad Hassangholipour Yasouri[5]

[1]School of Chemical Sciences and Food Technology, Faculty of Science and Technology Universiti Kebangsaan Malaysia (UKM), Bangi 43600, Malaysia.
[2]Department of Plant and Food Sciences, Faculty of Agriculture and Environment, University of Sydney, NSW, 2006, Australia.
[3]Department of Food Science, Faculty of Agriculture, University of Tehran, Karaj, Iran.
[4]Faculty of Pharmacy, UCSI University, South Wing, Cheras, Malaysia.
[5]Department of Fishery, Islamic Azad University of Bandar Anzali, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Author AY designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors AK, SM, MCS, MJK and MHY managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: In this study, the effect of washing process on shelf-life of surimi of *Clupeonella* spp. with cold-water, 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: Costal line of Caspian sea near the Bandar Anzali, Gillan 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-

**Corresponding author: E-mail: ali.yassoralipour@ukm.my;*

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. 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: Based on the present results, washing with 1.5 and 3% NaCl solution treatment on Anchovies (*Clupeonella* spp.) leads to a retention of the good quality characteristics for longer and an extension of the shelf life during frozen storage.

Keywords: Clupeonella spp.; surimi; minced meat; NaCl solution; Anchovies.

1. INTRODUCTION

Due to the high surimi demand for the production of a large variety of minced fish analogues and the recent decline in 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 bacteria count and sensory test helps to identify the shelf life of surimi prepared using anchovies (*Clupeonella* spp.). Surimi is a Japanese word ("suru" meaning "to process or mash", and "mi" meaning "meat"); in short, surimi can be described as minced meat, processed and stabilized 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 primarily made from white-fleshed fish such as Alaska Pollock (*Theragra chalcograma*) or Pacific Whiting (*Merluccius productus*) [1]. There are a number of species that are now utilized for commercial surimi processing, with functional and compositional properties varying depending on the species used [1,2,4]. Fish like cod, hake, whiting, Atlantic menhaden, croaker, Chilean mackerel and New Zealand hoki have been found to be suitable [5].

Anchovies (*Clupeonella* spp.) are the most abundant species of fish in the Caspian Sea. This fish is important commercially in the Caspian Sea, accounting for > 80% of the total catch in the past decade, as well as being a crucial part of the food chain [6]. Due to its special taste, Anchovies meat is not universally popular. Other researchers have shown that water-soluble protein and non-protein nitrogen (NPN) have a significant effect on the taste of this fish [7,8]. Low molecular weight carbohydrates, such as sucrose and sorbitol, are added tothe surimi to stabilize actomyosin, which is highly unstable during frozen storage. Washing cycles is an essential step in removing water-soluble proteins and primarily sarcoplasmic proteins, which are 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 protein 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 the water/meat ratio, the 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 physicochemical changes of Anchovies surimi during long-term frozen storage at -20°C.

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 Anchovies (*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 taken to the National Fish Processing Research Centre. Throughout this process (10 h), the fish were maintained in ice flake. Upon arrival at the National Fish Processing Research Centre, ten fishes were separated and analyzed as raw fish, while the remaining fish were cleaned and minced with a Waring blender (Model 32BL79, USA). The minced fish were divided into three batches (3×25 kg). To removed the excess fat from the minced fish before being treated for the surimi process, they were washed with 0.2% sodium bicarbonate [1]. To prepare surimi using the conventional washing process, the minced fish were washed with cold (4°C) water (control) and cold NaCl solution (1.5% (T_1) and 3% (T_2)) using a washing media/mince ratio of 3:1 (v/w) for 45 min. The washed mince was subjected to dewatering by covering with a cheesecloth and squeezing manually until the water was removed [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 T_1 and T_2 treatments and the control was without cryoprotectants. The stabilized minces were then packed (1 kg per bag) in 0.090-mm nylon freezer bags (China) and frozen at -40°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, the sample bags were stored at -20°C in a 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 the AOAC methods [12].

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

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

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

where (V) is the volume of hydrochloric acid added, (C) is its concentration and (W) is the weight of the sample in grams.

2.2.4 Measurement of expressible moisture

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

2.2.5 Determination of pH

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

2.2.6 Peroxide value (PV)

According to IUPAC [14], peroxide value is expressed in terms of milliequivalents (meq) of active oxygen per kilogram of sample that oxidizes potassium iodide under the conditions of the test. It is determined by titrating iodine liberated from potassium iodide with sodium thiosulphate solution. About 0.3 g of sample was weighed into a 250 ml flask with a stopper and dissolved in 10 ml of chloroform. Following this, 25 ml of acetic acid added to the mixture. Then 1 ml of saturated KI solution was added and immediately stopper was put and kept in dark place for 5 min. Finally, 75 ml of distilled water was added and then shaken. The sample was titrated with 0.01 N, $Na_2S_2O_3$ solution until the yellow colour had roughly disappeared. After that, 1ml of starch solution (1.0 %) was added and titration continued until the dark blue colour disappeared. A blank test was also done, without lipid and with the same conditions.

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

V_1 is the volume in millilitres of the sodium thiosulphate solution of normality N used for the determination, V_2 is the volume of the sodium thiosulphate solution used for the blank test, W is the weight (g) of the test sample, and N is the exact normality of the sodium thiosulphate solution.

2.2.7 Total plate count (TPC)

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

2.3 Statistical Analysis

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

3. RESULTS AND DISCUSSION

The proximate composition of the minced fish was calculated and is given in Table 1.

Table 1. Proximate composition of fish minced

Characteristics	Anchovies (*Clupeonella* spp.)
Protein (%)	19.40 ± 1.03
Fat (%)	5.19 ± 0.87
Moisture (%)	74.20 ± 2.52
Ash (%)	1.19 ± 0.08

The total volatile basic nitrogen (TVB-N) values for anchovies are presented in Fig. 1. The initial TVB-N value of Anchovies samples on the first day (8.8 mg N $100g^{-1}$) indicates the freshness of the 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 $100g^{-1}$. A 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 the time of storage for control, T_1 and T_2 treatments. The increase in 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 T_1 treatments rather than the T_2 treatment and can be attributed to the preservative effect of salt.

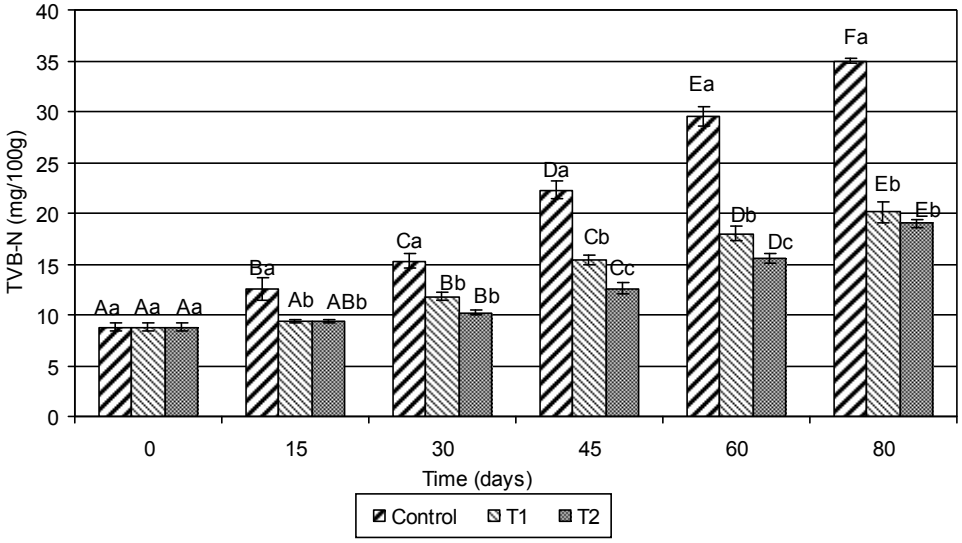

Fig. 1. Effects of washing and cryoprotectants on the total volatile base-nitrogen (TVB-N) of surimi during storage at -20°C

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, such as 35–40 mg $100g^{-1}$ [17] and 25–30 mg $100g^{-1}$ [19].

The TVBN content of the 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 Connell [17]. The corresponding TVBN levels of the control samples exceeded the above limit of 35 mg N $100g^{-1}$ after approximately 60 days of storage (36.18 mg N $100g^{-1}$), while the T_1 and T_2 treatments did not approach this limit until 80 days of storage. The results showed the potential benefits of NaCl solution washing in terms of keeping the TVBN levels below normal during long storage.

The pH values for surimi stored at -20°C increased progressively throughout the experiment and reached 7.39, 7.30 and 7.1 after 80 days for the control, T_1 and T_2 treatments respectively (Fig. 2). It has been reported that the pH limit for consumption is about 7.0 [20], whereas a level of pH 7.0 was 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, the pH profiles of both T_1 and T_2 Anchovies surimi were very similar with almost no significant changes ($P \geq 0.05$) occurring during storage at -20°C, while there were 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 pH sensitive spoilage bacteria to grow [21].

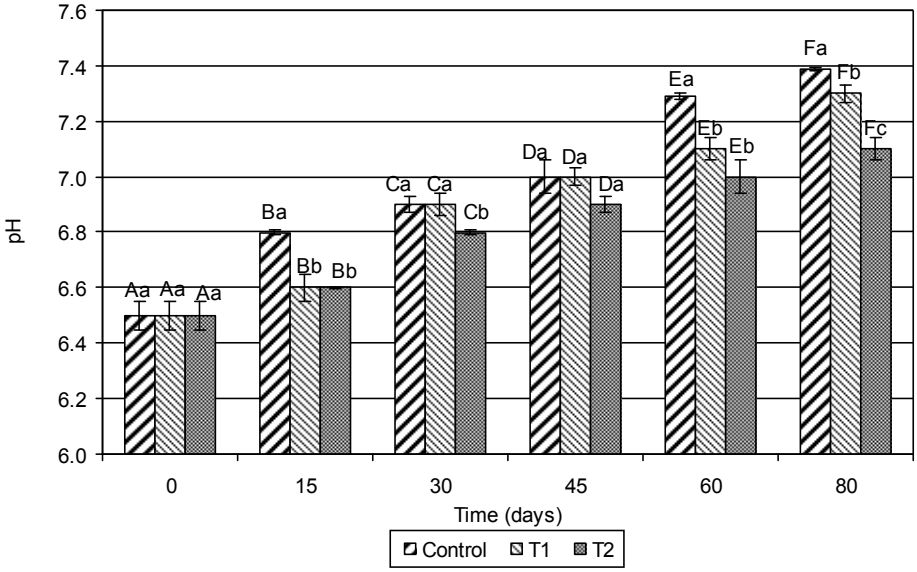

Fig. 2. Effects of washing and cryoprotectants on the pH value of surimi during frozen storage at -20°C

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

Oxidative abuse of the oil is determined by the peroxide value (PV). The Peroxide value of oil or fat is used as a measurement of the extent to which rancidity reactions have occurred during storage. The PV of crude fish lipid ranges from between 3 to 20 meq O_2 Kg^{-1} [22]. In order to assess the oxidative changes in surimi during frozen storage, the 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. The peroxide value increased from 0.85 meq/kg in the control, T_1 and T_2 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 values 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 starting the secondary oxidation of products [23].

The initial fish sample total plate count (TPC) was 2.72 log CFU/g, and the low initial TPC indicated very good fish quality. Changes in the TPC of surimi during the frozen storage are shown in Fig. 4. The TPC of sample washed with T_1 solution was found to be the same as that of the sample with T_2 solution during the first 15 days of frozen storage, but later was observed to be increasing more slowly than the T_1 treatment and reached 7.5 log CFU/g on the 80^{th} day of frozen storage. It did exceed the maximal permissible limit of 7.0 log CFU/g for the bacterial count in fish set by the International Commission on Microbiological Specifications for Foods [24], while the TPC of the control sample reached about 7.5 log CFU/g on the 45^{th} day during frozen storage. Our findings are in agreement with the report of Fan et al. [15] regarding the effects of chitosan coating on the quality and shelf life of silver carp during frozen storage.

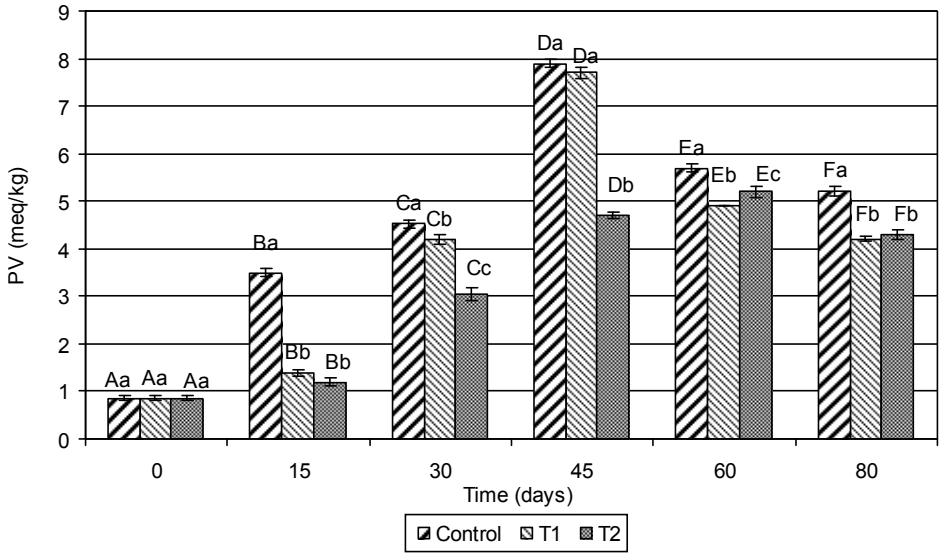

Fig. 3. Effects of washing and cryoprotectants on the peroxide value (meq/kg) of surimi during frozen storage at -20°C

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

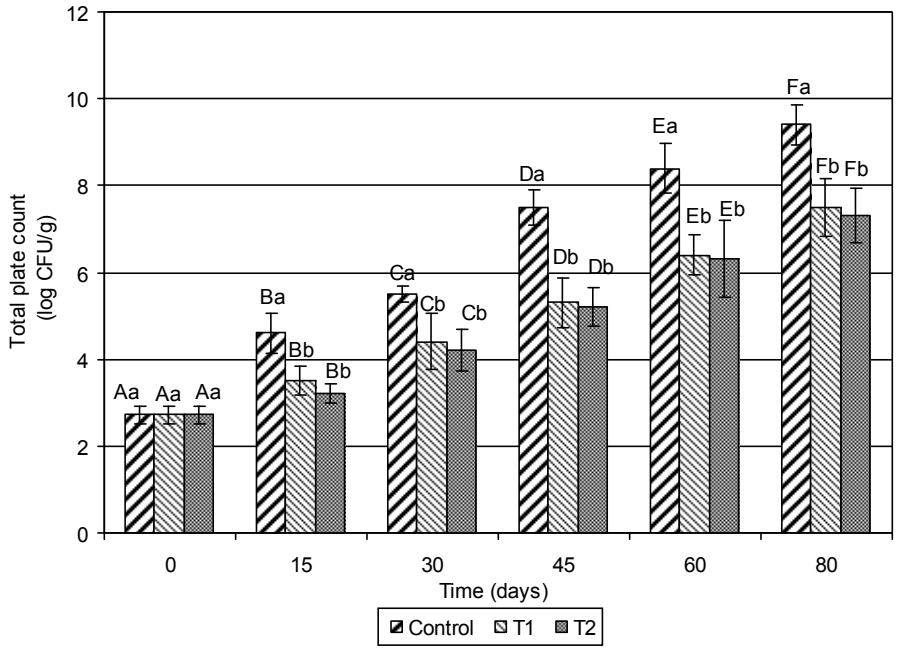

Fig. 4. Effects of washing and cryoprotectants on the total plate counts of surimi during frozen storage at -20°C

Points represent mean values of six determinations ± standard deviation (n= 2 × 3)

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

The kinetic growth of TPC in T_2 and T_1 at each storage time was significantly ($P \leq 0.05$) lower than control; this could be due to the inhibitory effects of salt and cryoprotectants on spoilage bacteria. The results of the treatment also indicated that the 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 commercial surimi is about 72 to 77% [1,9]. The moisture content of each surimi treatment 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%), which was significantly ($P \leq 0.05$) higher 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 frozen storage was due to the dehydration process [1]. Park, Lin and Park along with 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 initiated by this phenomenon. Washing can increase the hydration of the mince meat by removing fat and water-soluble constituents such as blood, pigments, proteins, and salts [25]. The moisture content of the sample washed with cold water (control) was significantly ($P \leq 0.05$) higher than the two other treatments. There were no significant differences ($P \geq 0.05$) in the moisture content of the T_1 and T_2 treatments with CP during storage at -20°C (Fig. 5).

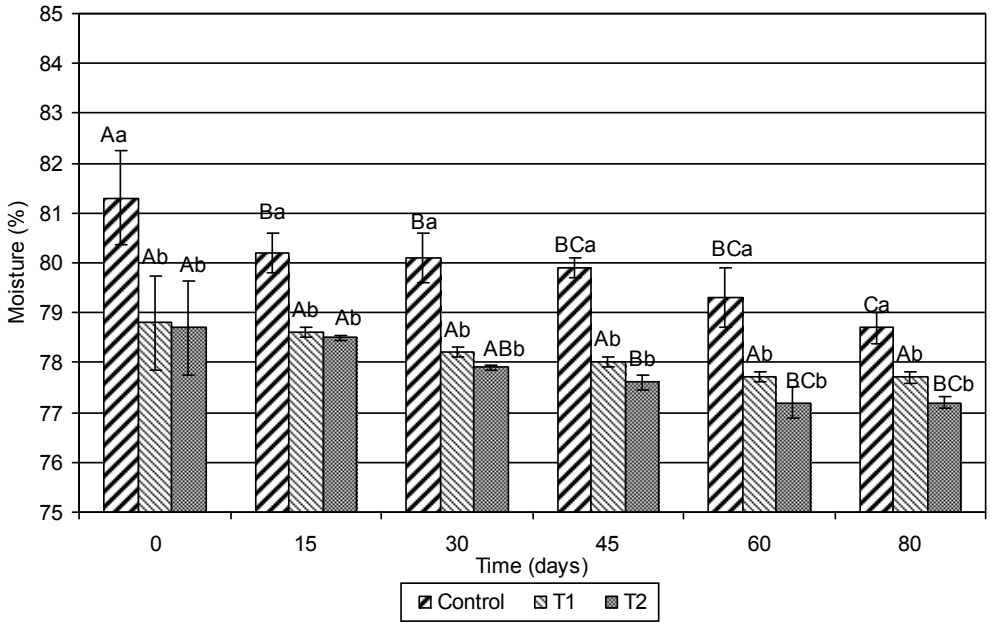

Fig. 5. Effects of washing and cryoprotectants on the moisture of surimi during frozen storage at -20°C

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

Expressible moisture was higher ($P \leq 0.05$) in the control, T_1 and T_2 treatments 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, no significant ($P \geq 0.05$) difference was observed in T_1 and T_2 during the majority of the storage time. Cryoprotectants lessened the amount of expressible moisture in the mince during storage [10]. The effect of cryoprotectants was greater in the T_1 treatment ($P \leq 0.05$) compared with T_2. Expressible moisture is indices of the water-holding properties of muscle foods. Increased water holding in the cryoprotectant-incorporated mince 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].

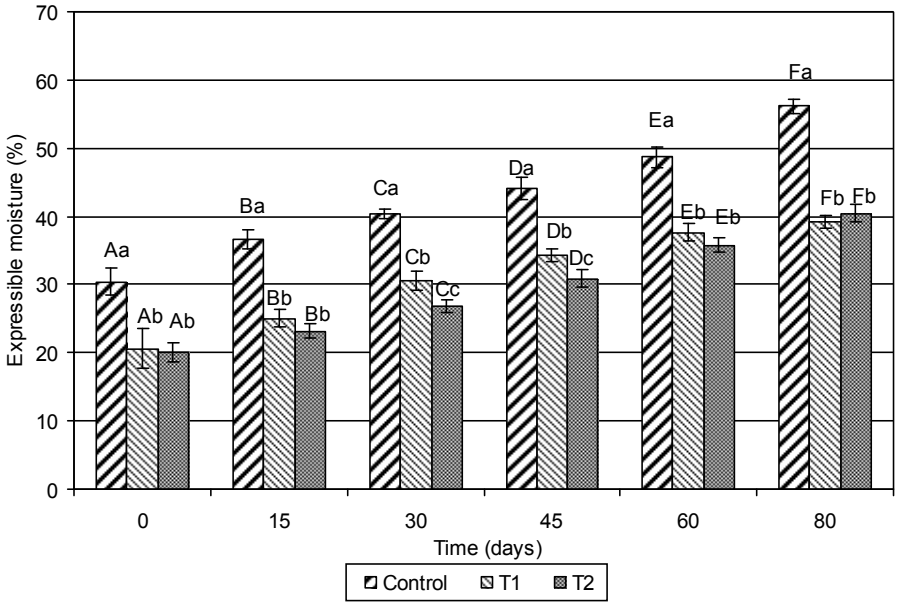

Fig. 6. Effects of washing and cryoprotectants on the expressible moisture of surimi during frozen storage at -20°C

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

4. CONCLUSION

On the basis of the present results, washing with 1.5% and 3% NaCl solution treatment on anchovies (*Clupeonella* spp.) leads to a retention of the good quality characteristics for longer and an extension of the shelf life during frozen storage. This conclusion was supported by the results of microbiological (total plate count) and physicochemical (pH, TVB-N, PV) analyses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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