Iranian Journal of Fisheries Sciences 18(4) 699-715 2019
DOI: 10.22092/ijfs.2019.118535

# Oxidative stability, biochemical indices and characteristics of the fatty acid composition of fish protein isolated from tuna (*Thunnus albacares*) canning by-product stored at -24°C for 6 months

**Shaviklo A.R.[1]*; Mozafari H.[2]; Motamedzadegan A.[3]; Damavandi-Kamali N.[4]**

Received: January 2017 Accepted: May 2017

**Abstract**

Tuna processing by-product is estimated at more than 100,000 metric tonnes annually in Iran, which could be a potential source of edible protein in human food. Hence, recovering proteins from tuna by-products is a big achievement in the seafood industry. In this work tuna protein isolates (TPI) were extracted from dark/ red meat using the isoelectric solubilization/ precipitation method. Oxidative stability, biochemical indices and characteristics of the fatty acid composition of TPI (pH 6.5) containing 16.2% protein, 1.2% fat and mixed with a blend of salt and sucrose – as a cryostabilizer - and stored 6 months at -24°C were studied. The levels of peroxide (PV), TBARS, FFA and total volatile basic nitrogen (TVB-N) were significantly increased in TPI without cryostabilizers during the storage time. The results revealed that 22 fatty acids were identified in the light and dark tuna meat and TPI. The fatty acid composition of light and dark muscle of tuna and TPI containing salt and sucrose was the same. However a significant change was observed only in the TPI free from cryostabilizers during frozen storage. The results confirm inhibiting of lipid oxidation due to the incorporation of salt and sucrose to TPI.

**Keywords**: Tuna processing by-product, Protein isolates, Frozen storage, Fatty acid profile, Cryostabilizer

1-Department of Animal Processing, Animal Science Research Institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.
2-Department of Food Science, Islamic Azad University-Sari branch, Mazandaran, Sari, Iran.
3-Department of Food Science and Technology, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.
4-Department of Seafood Processing, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran.
*Corresponding author's Email: shaviklo@gmail.com

## Introduction

It is well documented that the pH-shift process/ isoelecteric solubilization and precipitation method can convert any type of fish raw materials into a functional fishery ingredient which is called fish protein isolate/ FPI (Hultin *et al.,* 2005). Developing FPI from whole fish, fish fillets and fish processing by-products have been already reported (Nolsøe and Undeland 2009), but isolating protein from complicated raw materials like tuna dark muscle, which is a canning by-product has not been studied. Tuna flesh consists of 2 types of muscles *i.e* white/light and dark/ red muscles. The tuna dark meat is removed from the fish flesh during the canning process. A high level of polyunsaturated fatty acids (PUFAs), and greater concentrations of heme and sarcoplasmic proteins are found in dark muscle (Sánchez-Zapata *et al.,* 2011). These attributes make it difficult to use tuna dark meat for human food preparation. Therefore, annually a large quantity of tuna canning by-products are wasted/ converted to nonhuman products like animal feed or used for pet food production (Klomklao *et al.,* 2013). Hence, finding new processes to change tuna processing by-products (mainly dark muscle) into valuable ingredients is a major global challenge facing the tuna canning industry (Shaviklo *et al.,* 2016). Therefore, recovering proteins from dark meat of tuna for food application can be an innovative way for the utilization of such raw materials (Shaviklo *et al.,* 2017).

Operational units in the pH-shift technology make it possible to remove lipids and insoluble impurities, such as bone or skin from the extracted proteins (Hultin *et al.,* 2005). Although, FPI can be used like fish mince or surimi for food product development (Shaviklo et al., 2010a; Shaviklo *et al.,* 2016); it may lose its functionality and stability during freezing and frozen storage. Therefore, to ensure long-term frozen stability of FPI, applying additives/ ingredients (cryostabiliser) is required (Thawornchinsombut and Park, 2006; Shaviklo *et al.,* 2010b).

Many ingredients/ cryostabilizers have been introduced to the surimi (washed minced fish) industry. However the most important cryostabilizers in surimi and fish mince are salt, sugar (sucrose, glucose and sorbitol) and polyphosphates (Tanikawa, 1985). Common salt/ sodium chloride (NaCl) is one of the most widely used additive in food products due to its low cost and versatile functions (Albarracin *et al.,* 2011). Although salt is used in the seafood industry as a preservative in fish curing (Belitz *et al.,* 2009) and/or as a cryoprostabilizer in the surimi industry (Tanikawa, 1985); there are some questionable reports about its functions in different food systems. Several authors reported that salt can perform as a pro-oxidant which can accelerate lipid oxidation in muscle foods, including fish and fishery products (Sakai *et al.,* 2006). While others noted that salt can be applied as an antioxidant in seafood processing and preservation (Mozuraityte *et al.,*

2006). On the other hand, the antioxidant activity of sucrose in different food products was noted (Faraji and Lindsay, 2004; Shaviklo *et al.,* 2010c). Sugar has been used to stabilize frozen surimi as a cryostabilizer (Tanikawa, 1985), and inhibit chemical changes (Zhang *et al.,* 2015; Qin *et al.,* 2017) and decrease biogenic amine accumulate in fish flesh (Bover-Cid *et al.,* 2001). Using salt and sugar together may improve the preservative activity of each. For centuries a mixture of salt and sucrose has been used for preserving *"kaen"* (salt added) surimi by the Japanese (Tanikawa, 1985) and for *gravid* (a traditional fish product) by the Scandinavians (Lyhs *et al.,* 2001). Salt-added surimi consists of 2.5% salt and 10% sugar (sucrose, glucose and sorbitol); while salt-free surimi contains 0.2-0.3% of polyphosphate and 5-8% sugar (Tanikawa, 1985).

There are few published documents reporting the storage stability of frozen fish protein isolates (FPI). The stability of FPI in different conditions was studied by Thawornchinsombut and Park (2006). Using a cryostabilizer in FPI to prevent freeze-induced aggregation during frozen storage was recommended by them. However, using a mixture of trehalose and polyphosphate for frozen preservation of protein isolated from squid was also suggested by Campo-Deano *et al.,* (2010). The results of our previous work (Shaviklo *et al.,* 2010b, 2012) recommended that isolated proteins processed by the pH-shift method should be protected during frozen storage. Therefore, adding salt and sucrose as cryostabilisers to FPI was noted (Shaviklo *et al.,* 2010b). The overall objective of this study was to study the oxidative stability, biochemical indices and characteristics of the fatty acid composition of fish protein isolated from tuna (*Thunnus albacares*) dark muscle incorporated with a blend of salt and sucrose during 6 months storage at -24˚C.

## Materials and methods

*Tuna protein isolates (TPI)*

Frozen yellow tail tuna (50 kg) was obtained and transferred from a tuna canning company in Bandar Anzali (Guilan, Iran) to the processing lab under frozen conditions (-18˚C). Thawed tuna fish was filleted manually and the dark meat was removed from the light meat. An alkali aided process was applied to extract proteins from the tuna dark meat (Shaviklo *et al.,* 2017). TPIs were squeezed manually to decrease the water content to 75% by using cheese cloth. TPI and a mixture of salt (1.3%) and sucrose (5%) (Shaviklo *et al.,* 2010b) were weighed separately and mixed completely for 3 minutes using a high speed vertical cutter (VCB-62, HILDE® Sweden). TPIs with/ without cryostabilizer were air packed immediately after mixing. Each polyethylene bag containing 1 kg sample, was blast frozen and stored at a temperature of -24˚C except samples that were evaluated at week 0 and were stored at +2˚C.

*Physico-chemical analysis*

*Proximate analysis*

The Soxhlet method (Soxtec System-Texator, Sweden) and the Kjeldahl method (Kjeltex System-Texator, Hagonas, Sweden) were used for crude lipid and crude protein determination (AOAC, 2006), respectively. The moisture content was determined by drying samples for 4 h at 105°C until constant weight was achieved. Ash content was determined by charring samples overnight at 550°C (AOAC, 2006). Carbohydrate was calculated by the difference.

Peroxide value (PV) was determined according to the AOAC method (AOAC, 2006) and reported as milliequivalents of oxygen / kilograms of lipid. Thiobarbituric acid reactive substances (TBARS) were determined by a slightly modified steam distillation method (Tarladgis *et al.*, 1960), where the sample size was reduced to 5 g and antioxidants (5 ml of 0.5% propyl gallate and 0.5% ethylene diamine tetra acetic acid in water) were added to the sample during blending. Malondialdehyd-bis- (diethyl acetate) was used as a standard. Free fatty acids (FFA) contents were determined by titration (0.1 M, NaOH) of the TL extracts (10 g) after adding ethanol) 15 ml) and using phenolphthalein as an indicator. The FFA content was calculated as % oleic acid (AOAC, 2006). Total volatile basic nitrogen (TVB-N) was determined applying steam distillation followed by the titration method (Malle and Poumeyrol 1989). The pH of the samples was determined using a digital pH meter (Knick-Portamess 913 pH, Berlin, Germany). All samples were analyzed at room temperature. The pH value was the average value of the two readings.

*Fatty acid determination*

Lipid of the light and the dark tuna meat and TPI samples was extracted. Then, it was saponified with 0.5 N methanolic NaOH and further transesterified with BF3 in methanol (AOAC, 2006). The fatty acid methyl esters were analyzed by gas chromatography using a GC Hewlett Packard, Agilent 6890 with 120 m long ×0.25 mm internal diameter silica capillary column (BPX – 70 SGE, HP, USA) equipped with a flame ionization detector and split injector. Nitrogen was applied as the carrier gas at 20 $cm^3$ $min^{-1}$, the temperature program was: an initial column temperature of 140 °C held for 5 min, then increased at 4 °C $min^{-1}$ until it reached 170 °C and held for 3 min and then increased again at 2 °C $min^{-1}$ until 200 °C and then maintained at 250 °C. Fatty acid peaks in the test sample were determined by comparing the retention times of the test sample with that of the standard mixture of FAME (Supleco TM, 37 component FAME MIX) which contained from C4:0 to C22:6n-3.

*Statistical Analyses*

Analysis of variance (ANOVA) was carried out using the statistical program NCSS 2007 (NCSS, Statistical Software, Kaysville, UT) for the statistical analysis of physicochemical and fatty acid determination. Student's t-test was used to determine whether

there was a difference in the sample groups (TPIs with/ without cryostabiliser). The results were given as a mean±standard deviation. Significance of difference was defined at the 5% level.

## Results

*Proximate composition and pH*

TPI consisted of 16.20±0.95% protein, 79.91±1.15% moisture, 1.17±0.13% fat, 1.11±0.21% ash and 1.71±0.51% carbohydrate. The initial pH value of the TPI was 6.2. Changes in pH values of control isolates were significant, unlike the salt-sucrose added TPI. The pH of control TPI reached 6.81 at the end of the frozen storage (Table 1).

**Table 1: pH changes in TPI during frozen storage.**

| Storage time (month) | TPI without salt and sucrose (control) | TPI with salt and sucrose |
|---|---|---|
| M0 | 6.21±0.05 [b] | 6.05±0.06 [a] |
| M2 | 6.33±0.02 [b] | 6.11±0.05 [a] |
| M4 | 6.51±0.04 [a] | 6.15±0.03 [a] |
| M6 | 6.81±0.01 [a] | 6.19±0.01 [a] |

Values are means of 3 analyses. Different small letters show significant difference within a column ($p<0.05$).

*Lipid Oxidation (PV and TBARS values)*

In the control TPI, the variation of PV was more significant than in the salt-sucrose added TPI. The PV was not different in the first month of storage (Fig. 1). However, a linear increase of the PV was detected up to month 6 of the frozen storage. Peroxide development during frozen storage was not significant in a salt-sucrose added TPI.

Secondary lipid oxidation in TPI prototypes was studied by the TBARS measurement. The higher level of TBARS value was obtained significantly from the control TPI samples. The TBARS values indicated a significant increased rate of lipid oxidation (from 0.31 to 2.96 mg of MDA $kg^{-1}$ fish flesh) in the control TPI during the frozen storage (Fig. 2).

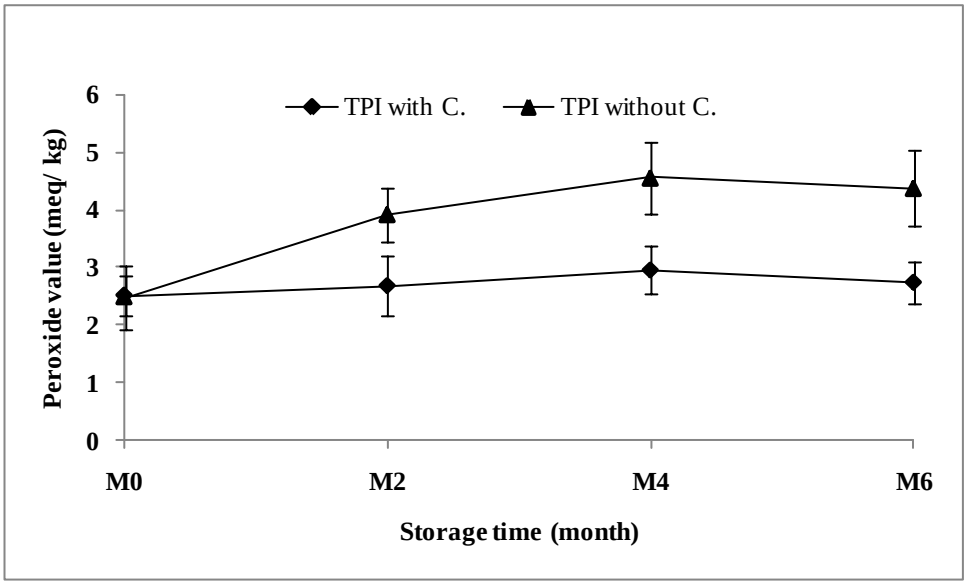


**Figure 1: Peroxide values of tuna protein isolates with and without cryostabilizer (C).**

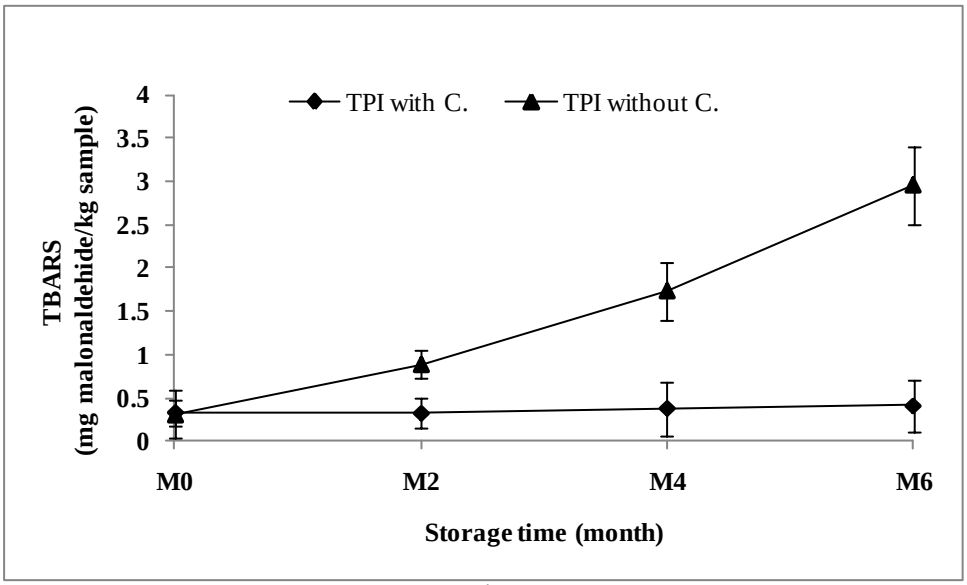


**Figure 2: TBARS (mg malonaldehide $kg^{-1}$ sample) values of tuna protein isolates with and without cryostabilizer (C).**

*FFA*

FFA in both samples at month 0 were very low due to the removal of free fatty acid content during the dewatering process; while it was higher in the control TPI for the other months (Fig. 3). There were significant differences between the control and salt-sucrose added TPI during frozen storage. The salt-sucrose added TPI had a lower FFA content compared to the control sample. Storage time had no effect on the FFA of the salt-sucrose added TPI, while the FFA values of the control TPI were significantly increased during 6 months storage. The FFA content was increased significantly from 0.97% to 7.21% in the control TPI.

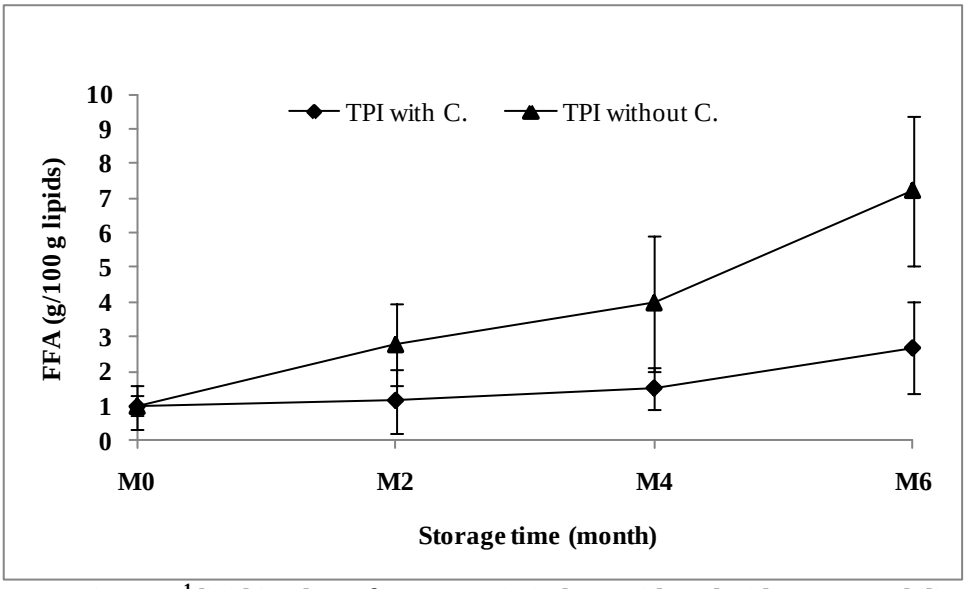


**Figure 3: FFA (g 100 g$^{-1}$ lipids) values of tuna protein isolates with and without cryostabilizer (C).**

*Total volatile basic nitrogen (TVB-N)*

The influence of salt and sucrose on TVB-N production of the TPI stored 6 months in -24 ℃ is shown in Figure 4. The initial TVB-N level in the fresh TPI was 6.78 mg 100g$^{-1}$. During the frozen storage TVB-N levels were increased significantly. The highest significant changes were reported for the control TPI. Compared to the control, sucrose-salted TPI delayed the increase rate of the TVB-N after the 6 months frozen storage.

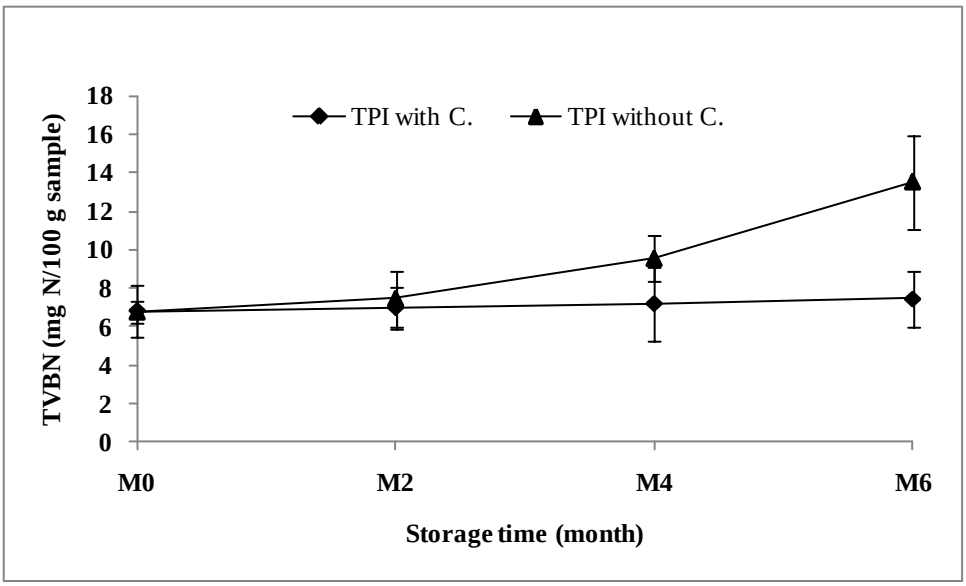


**Figure 4: TVBN (mg N 100g$^{-1}$ sample) values of tuna protein isolates with and without cryostabilizer (C).**

*Fatty acid compositions*

The fatty acid compositions of the lipid extracted from the tuna meat and TPI is shown in Table 2. Altogether 22 fatty acids were identified in all samples. Four major fatty acids in the tuna meat and the TPI samples were palmitic acid (C16:0), stearic acid (C18:0), oleic acid

(C18:1) and docosahexaenoic acid (DHA:C22:6n-3). Among the saturated fatty acids (SFAs) the higher fatty acids were palmitic acid and stearic acid respectively. There were no significant differences between the palmetic acid (23.86-24.24%) and the stearic acid (15.65-16.37%) of the tuna light/ and dark meat and the fresh TPI. The palmetic acid was expanded significantly during the 6 months frozen storage (26.21% in the salt-sucrose added TPI and 30.78% in the control TPI). Adversely, the stearic acid was lowered significantly only in the control TPI (12.31%) during the frozen storage. The oleic acid was the higher monounsaturated fatty acids (MUFAs) in the tuna meat and the TPI samples. It was not changed in all samples during the frozen storage.

**Table 2: Fatty acid profiles of tuna light and dark meats and tuna protein isolates.**

| Fatty acid | Tuna light meat | Tuna dark meat | Fresh TPI, Month 0 | Frozen TPI with salt and sucrose, Month 6 | Frozen TPI, without salt and sucrose, Month 6 |
|---|---|---|---|---|---|
| C14:0 | 1.24±0.09$^{b}$ | 1.19±0.05$^{b}$ | 1.28±0.15$^{a}$ | 2.21±0.11$^{a}$ | 1.81±0.07$^{b}$ |
| C15:0 | 0.69±0.06$^{b}$ | 0.71±0.05$^{b}$ | 0.63±0.07$^{b}$ | 1.64±0.14$^{a}$ | 0.72±0.07$^{b}$ |
| C16:0 | 23.86±0.89$^{c}$ | 23.41±0.95$^{c}$ | 24.24±0.19$^{c}$ | 30.78±1.16$^{a}$ | 26.21±0.18$^{b}$ |
| C17:0 | 1.17±0.09$^{b}$ | 1.29±0.08$^{b}$ | 1.15±0.11$^{b}$ | 2.16±0.18$^{a}$ | 0.92±0.08$^{c}$ |
| C18:0 | 15.65±1.18$^{a}$ | 16.25±1.75$^{a}$ | 16.37±1.95$^{a}$ | 12.31±1.15$^{b}$ | 16.41±1.09$^{a}$ |
| C20:0 | 0.49±0.09$^{b}$ | 0.47±0.07$^{b}$ | 0.53±0.09$^{b}$ | 1.05±0.05$^{a}$ | 0.49±0.08$^{b}$ |
| C22:0 | 0.25±0.07$^{d}$ | 0.44±0.09$^{c}$ | 0.42±0.06$^{c}$ | 0.75±0.08$^{a}$ | 0.55±0.05$^{b}$ |
| C24:0 | 0.14±0.08 | 0.12±0.05 | 0.15±0.07 | 0.14±0.09 | 0.13±0.05 |
| Σ SFA | 43.49 | 43.84 | 44.77 | 51.04 | 47.24 |
| C16:1 | 3.57±0.56$^{b}$ | 3.49±0.45$^{b}$ | 3.51±0.75$^{b}$ | 4.81±0.61$^{a}$ | 3.21±0.53$^{b}$ |
| C17:1 | 0.82±0.08$^{a}$ | 0.76±0.07$^{b}$ | 0.83±0.09$^{a}$ | 0.84±0.07$^{a}$ | 0.81±0.06$^{a}$ |
| C18:1, n-9 | 19.62±1.25 | 19.72±1.53 | 19.06±1.47 | 19.41±1.85 | 18.7±1.61 |
| C20:1, n-9 | 1.31±0.18 | 1.26±0.11 | 1.31±0.15 | 1.43±0.19 | 1.28±0.13 |
| C22:1, n-9 | 0.54±0.08$^{b}$ | 0.53±0.07$^{b}$ | 0.57±0.04$^{b}$ | 0.74±0.07$^{a}$ | 0.53±0.08$^{b}$ |
| C24:1, n-9 | 0.56±0.04 | 0.62±0.08 | 0.52±0.09 | 0.55±0.06 | 0.49±0.07 |
| ΣMUFA | 26.42 | 26.38 | 25.79 | 27.78 | 25 |
| C18:2, n-6 | 3.51±0.14$^{a}$ | 3.37±0.56$^{a}$ | 3.4±0.71$^{a}$ | 2.4±0.18$^{b}$ | 3.21±0.26$^{a}$ |
| C18:3, n-3 | 0.16±0.08$^{b}$ | 0.21±0.06$^{a}$ | 0.19±0.10$^{a}$ | 0.11±0.07$^{b}$ | 0.17±0.09$^{a}$ |
| C20:2, n-6 | 0.48±0.06$^{a}$ | 0.51±0.08$^{a}$ | 0.46±0.05$^{a}$ | 0.29±0.05$^{b}$ | 0.41±0.04$^{a}$ |
| C20-3, n-3 | 1.11±0.15 | 1.25±0.17 | 1.29±0.23 | 1.19±0.19 | 1.31±0.81 |
| C20:4, n-6 | 3.44±0.14$^{b}$ | 3.26±0.21$^{b}$ | 3.51±0.18$^{b}$ | 4.81±0.31$^{a}$ | 3.89±0.52$^{b}$ |
| C20:5, n-3 | 4.88±0.21$^{a}$ | 4.41±0.35$^{a}$ | 4.77±0.98$^{a}$ | 2.12±0.74$^{b}$ | 4.19±0.54$^{a}$ |
| C22:5, n-3 | 1.36±0.09 | 1.35±0.18 | 1.26±0.08 | 1.15±0.18 | 1.21±0.14 |
| C22:6, n-6 | 15.16±0.91$^{a}$ | 15.42±0.85$^{a}$ | 14.57±0.71$^{a}$ | 9.11±0.91$^{c}$ | 13.37±0.82$^{b}$ |
| ΣPUFA | 30.09 | 29.78 | 29.44 | 21.18 | 27.76 |
| Sum | 100 | 100 | 100 | 100 | 100 |
| Polyene Index | 0.84 | 0.85 | 0.80 | 0.36 | 0.67 |
| EPA+DHA | 20.04 | 19.83 | 19.34 | 11.23 | 17.56 |
| Σ n-3 | 7.5 | 7.22 | 7.51 | 4.57 | 6.88 |

**Table 2 continued:**

| | | | | | |
|---|---|---|---|---|---|
| Σ n-6 | 22.59 | 22.56 | 21.93 | 16.61 | 20.88 |
| n-3/n-6 | 0.33 | 0.32 | 0.34 | 0.28 | 0.33 |
| n-6/n-3 | 3.01 | 3.12 | 2.92 | 3.03 | 3.63 |

Values are means of 3 analyses. Different small letters show significant difference within a row ($p<0.05$). SFA: saturated fatty acid; MUFA: monosaturated fatty acid; PUFA: polyunsaturated fatty acid



Actually, there was no significant decrease in the fatty acid composition of the light and dark tuna meat and the TPI made from the dark meat. Only the fatty acid profile in the control TPI was changed significantly during the frozen storage period. The greater proportion of the polyunsaturated fatty acids (PUFAs) in the tuna meat and the fresh TPI was for Docosahexaenoic Acid (DHA) making up from 14.57 to 15.16% of the total fatty acids. The DHA was significantly decreased in the salt-sucrose added TPI (13.37%) and the control TPI (9.11%) after the 6 months frozen storage. The proportion of Eicosapentaenoic Acid (EPA) remained unchanged (4.19-4.88%) in the tuna meat, fresh and the frozen TPI containing salt and sucrose and stored for 6 months in freezer. The storage time adversely changed the EPA content in control TPI (2.12%).

The PUFAs made up about 30% of the total fatty acids of the tuna light and dark meat and the fresh TPI, but it decreased significantly during the frozen storage of the TPI samples. The PUFAs were decreased to 28% and 21% in the salt-sucrose added TPI and the control TPI after 6 months frozen storage respectively. The results indicated that the highest significant change in fatty acids was found for the control TPI. Compared to the fresh TPI, 62% of DHA and 44% of EPA were lost in the control TPI after the 6 months storage. However during the same storage time the SFA and MUFA of the control TPI were increased significantly.

## Discussion

*Proximate composition and pH*

Types of the raw materials, processing techniques and dewatering method are the critical factors influencing the proximate compositions of FPI samples (Hultin *et al.*, 2005). The initial pH value of the TPI was 6.2, similar to the value (6.4) of FPI haddock (*Melanogrammus aeglefinus*) cut-offs reported by Shaviklo *et al*. (2010b). Although the initial pH value of fish depends on the fish species, seasonality, diet, level of activity, and so on (Belitz *et al*., 2009), it is due to precipitation pH when isolating proteins by the pH-shift method or additives used for pH adjustment (Shaviklo *et al.*, 2010b). The pH of control TPI reached 6.81 at the end of the frozen storage possibly due to the protein degradation by either endogenous or microbial enzymes accordingly or the development of alkaline objects (Liu *et al.*, 2010). It was reported that the addition of salt to fish meat can decrease pH in the range of 0.1 to 0.2 units, possibly due to replacing $Na^+$ at the surface of proteins and releasing $H^+$ (Shaviklo *et al*., 2010b).

*Lipid oxidation (PV and TBARS values)*

A notable development of the hydroperoxide was observed during frozen storage of TPI prototypes. This may be demonstrated by the increasing of rancidity during the frozen storage. In comparison with the surimi, FPI is linked to the more accelerated oxidative degradation of lipids (Hultin *et al.*, 2005; Shaviklo *et al.*, 2010b). This may confirm inhibiting of lipid oxidation, due to adding salt and sucrose to TPI.

As expected, by increasing the storage time the TBARS and PV expanded in the control TPI due to the greater lipid oxidation. Because of the higher content of fat in FPI than surimi, more oxidation products such as peroxides and malonaldehyde were developed during frozen storage of the samples.

High peroxide values are a definite measure of rancidity in fats. Increasing oxidation and proteolysis rates can increase secondary products, such as malondialdehyde and biogenic amine formation (Belitz *et al.*, 2009). The effects of the salt and sucrose on lipid oxidation were in agreement with the other works (Tokur *et al.*, 2006; Shaviklo *et al.*, 2010c). The significant differences between the peroxide and TBARS values of the TPI samples compared to the surimi were due to the higher fat content of the TPI. Surimi contains less than 0.1% fat content while the TPI sample had 1.2% fat content.

Increasing TBARS values has been reported during frozen storage of fishery products (Liu *et al.*, 2010). It was reported that the TBARS value cannot suggest the real rate of lipid oxidation, due to the interaction of malonaldehyde with other compounds in the fish flesh, such as nucleosides and nucleic acid, amines, proteins, phospholipids or other aldehydes that are end-products of lipid oxidation. This interaction relies extremely on the fish species (Huss, 1988). Development of TBARS level in the control TPI during frozen storage was lower than that recommended for fish flesh.

Sugar (sucrose) has been reported to display antioxidant properties due to decreasing the availability of water, which is required to solubilise potent oxidants (Clarke, 1995). Sucrose can reduce rancidity and discoloration and prevent the deterioration of sensory properties (Faraji and Lindsay, 2004). Furthermore, the hydrolysis products of sucrose, *i.e* glucose and fructose, have the ability to block the reactive sites of ions such as copper and iron and, to a lesser extent, cobalt acting as food preservatives by impeding catalytic oxidation reactions (Clarke, 1995).

*FFA*

The development of lipid hydrolysis was observed during frozen storage for the control TPI. This higher content of FFA in the control TPI could be associated with the enzymatic activities (Bakar *et al.*, 2008). A significant increase in FFA during the frozen storage in different fish products was already reported (Huss, 1988). The FFA index, which demonstrates the level of lipid hydrolysis (hydrolytic rancidity), is one of the most important indexes for the quality assessment of the fish lipid. Therefore, the lower FFA content of the

salt-sucrose added TPI suggests the higher quality and lowers further oxidation. The FFA content of the control TPI passed the maximum limit for FFA content.

There is a direct correlation between lipolysis and lipid oxidation of the seafood products (Bakar *et al.,* 2008). The high content of the FFAs has a negative effect on sensory attributes and developing an oily taste, bitterness and a metallic taste in the fish products (Belitz *et al.,* 2009). Similar findings were obtained when studying lipid hydrolysis in the flesh of Chinese fresh water fish (Kaneniwa *et al.,* 2000). The level of the FFAs during storage of the fishery products relates to the hydrolytic activity of lipase as well as oxidative reactions which change the FFAs released in lipolysis (Sahari *et al.,* 2009). The lipids and phospholipids are hydrolyzed by lipase and phospholipase, yielding FFAs, which are oxidized into peroxides (Belitz *et al.,* 2009). The FFA is oxidized faster than that found in triglycerides and phospholipids (Kaneniwa *et al*., 2000). Therefore, it can significantly influence the stability, functionality and nutritional quality of the aquatic food products.

*Total volatile basic nitrogen (TVB-N)*

The significant increase of the TVB-N during the frozen storage is caused by the activity of endogenous enzymes and spoilage bacteria (Huss, 1988). This could be related to the preservative function of the salt and sucrose. Advanced TVB-N development in the control TPI during frozen storage was lower than the acceptable level of TVB-N (25 mg 100 $g^{-1}$) reported for fish flesh (IVO, 2010).

Acting as an indicator of fish freshness TVB-N is commonly carried out to evaluate spoilage of the fish and fishery products. The TVB-N levels differ from species to species and in each species, it depends on age, gender, environment and season. In frozen conditions in which the bacterial count and activity are gradually decreased, factors like texture enzymes are effective to produce TVB-N. It is also reported that an increase in ammonia released from amination of adenosine mono phosphate or histamine can increase TVB-N levels during storage (Nazemroaya *et al.,* 2011). The levels of 30 to 35 mg $100g^{-1}$ fish meat are reported as the limit of acceptance of the fish (IVO, 2010). Therfore, the TVB-N content in the TPI prototypes was well within the limit of acceptability. This depends on the freezing effect, as it inhibits the bacterial activity.

Salt (Zhang *et al.,* 2015; Qin *et al.,* 2017) and sucrose (Fan *et al.,* 2014) were found to inhibit the increase of TVB-N in fish products. The sugar treatment can significantly cut down pH value and lower TVB-N accumulation, though it promoted bacterial growth to some extent and decreased the sensory quality of fish at later stages of the storage (Fan *et al.,* 2014). The salt and sucrose inhibit microbial growth due to reduction of $a_w$ and interfere with the enzymatic activity of the microbe and weaken the molecular structure of its DNA (Albarracin *et al.,* 2011). Fan *et*

*al*. (2014) reported that a mixture of 1.5% salt and 1.2% sugar could lower chemical changes in fish muscle and expand the shelf life of black carp fillets during storage at 4°C for 16 days.

*Fatty acid compositions*

Although the pH-shift process reduced fat content in the TPI significantly, the overall percentage of the fatty acids and their compositions did not change. Similar findings were reported for surimi made from Channel Catfish frames (Hoke *et al.*, 2000). They also reported that leaching did not have an effect on the fatty-acid composition of neutral and phospholipids in the surimi.

The higher content of the SFAs (43-47%), followed by the PUFAs (28-30%) and the MUFAs (25-26%) were observed in the tuna light and dark meat, the fresh TPI and salt-sucrose added TPI that is in agreement with other related works (Khoddami *et al.*, 2009; Karunarathna and Attygalle 2010). However, this ratio for control TPI stored 6 months in the freezer was SFA>MUFA>PUFA.

The fatty acid composition of the tuna meat and the fresh and frozen TPI with/ without salt and sucrose indicated high content of the palmitic acid, oleic acid and the DHA. These results were in accordance with those reported for fatty and lean fishes (Khoddami *et al.*, 2009). The composition of fatty acids depends on fish species, the seasonal changes and environmental effects of tropical fish species and also in the post-spawning period (Huss, 1988).

The DHA content of the tuna light and dark meat and the fresh TPI (14.57-15.16%) was greater than that reported for *Sardina pilchardus* (11.30%) (Beltran and Moral, 1991), *Sardinella lemuru* liver (12.97%) (Khoddami *et al.*, 2009) and salmon by-product 12.9% (Wu and Bechtel, 2008) and was lower than that found for *Clupea pilchardus* (16.92%) (Castrillon *et al.*, 1997). However, the n-3 PUFA content of tuna meat and TPI was within the range of some other fish such as sardine head, sardine liver, tilapia, African catfish, (Khoddami *et al.*, 2009).

The frozen storage just influenced the n-3 PUFA content of the TPI without salt and sucrose (control). The sum of eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) for the fresh TPI and 6-months stored salt-sucrose added TPI was 19 and 18% respectively indicating cryostabilizing of salt and sucrose during frozen storage. This was decreased dramatically after 6 months of frozen storage of control TPI (EPA+DHA= 11%).

The relative nutritional value of fish lipid is evaluated by the n-6/n3 proportion (Bakar *et al.*, 2008). The n-6/n-3 ratio of the tuna meat and the TPI samples was (3.0-3.6) which was in the range recommended by the World Health Organization (less than 5.0 in the total human diet) (Vujkovic *et al.*, 1999). The ratio of n-3/n-6 was 0.33 for the tuna meat and the fresh and frozen salt-sucrose added TPI and this ratio decreased to 0.28 after the 6 months of frozen storage for the control TPI. The PUFA content of fish flesh is very susceptible to lipid oxidation (Belitz *et al.*, 2009). The formation of volatiles

related to rancidity occurred due to the decomposition of the PUFA during the lipid oxidation process within storage time (Rasoarahona *et al.,* 2005). Fish tissues are highly susceptible to lipid oxidation and rapid deterioration owing to the high degree of unsaturated lipids. The sensory attributes of the fish are strictly influenced by oxidative changes. Changes in color and nutritional value are noted in the later development of lipid peroxidation, (Belitz *et al.,* 2009).

The EPA and DHA contents of the control TPI were significantly decreased at the end of storage time, which is in a line with the other related works. The fatty acid changes during frozen storage of red tilapia fillets and accordingly the formation of hydroperoxide has been reported (Rasoarahona *et al.,* 2005). Lipid deterioration is the main cause of fatty acid decomposition and enzymatic hydrolysis of unsaturated fatty acids (Huss, 1988).

A good index to analyze lipid oxidation in fishery products is the Polyene Index (EPA+DHA/ C16:0 ratios) (Taheri *et al.,* 2012). In this study, this ratio decreased from 0.80 (for fresh TPI) to 0.36 (for control TPI) significantly. The reduction of polyene index was also noted in Mackerel (Sahari *et al.,* 2009) and Cobia fish (Taheri *et al.,* 2012). This study demonstrated that the fatty acid composition of the frozen stored TPI was markedly influenced by the absence of salt and sucrose.

The blend of salt and sucrose could inhibit lipid oxidation (PV and TBARS values), and prevent the increase of FFA in the TPI sample. The highest PV and TBARS values were observed in the TPI without salt and sucrose during 6 months frozen storage. No significant change was reported for the fatty acids of TPI containing salt and sucrose during frozen storage. Saturated fatty acids significantly increased, whereas the unsaturated fatty acids significantly decreased in the control sample during storage. Therefore, the function of salt and sucrose on inhibiting lipid oxidation and fatty acid decomposition was confirmed in this study. A shelf life of more than 6 months is expected for frozen salt-sucrose added TPI.

**Acknowledgments**

Financial support from the Iranian National Science Foundation is truly acknowledged.

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