CHAPTER 4

EFFECT OF SOME ADDITIVES ON THE INHIBITION OF LIZARDFISH TRIMETHYLAMINE-*N*-OXIDE DEMTHYLASE AND FROZEN STORAGE STABILITY OF MINCED FLESH

4.1 Abstract

Effects of some additives on the inhibition of trimethylamine-*N*-oxide demethylase (TMAOase) from lizardfish (*Saurida tumbil*) muscle were investigated. Sodium citrate and pyrophosphate could inhibit TMAOase activity in a concentration dependent manner, most likely due to their chelating property. Sodium alginate was the hydrocolloid possessing the inhibitory activity towards TMAOase ($P < 0.05$). During the storage of lizardfish mince at $-20^{\circ}C$ for 24 weeks, the addition of 0.5% sodium alginate and 0.3% pyrophosphate in combination with 4% sucrose and 4% sorbitol as the cryoprotectants resulted in the retarded increase in TMAOase activities with the coincidental lowered formation of dimethylamine (DMA) and formaldehyde (FA), compared with the control (without additives) ($P < 0.05$). The loss in solubility of muscle proteins was also impeded with the addition of those compounds, suggesting their role in the inhibition of TMAOase as well as the retardation of protein denaturation induced by FA.

4.2 Introduction

Frozen storage is considered very important for the preservation of fish. Depending on intrinsic factors including species and season as well as technological factors such as handling practices prior to freezing, freezing rate, storage temperature, and the presence of protective barriers against oxidation, the practical storage life of frozen fish may vary substantially (Careche *et al*., 1999). During frozen storage of some fish species, formaldehyde (FA) and dimethylamine (DMA) are formed stoichiometrically from trimethylamine oxide (TMAO) by demethylation, catalyzed mainly by trimethylamine-*N*-oxide demethylase (TMAOase) (Amano *et al*., 1963; Yamada and Amano, 1965). The produced FA can react with a number of chemical groups including a number of amino acid

residues, terminal amino groups, and various low-molecular-weight compounds, leading to the denaturation and cross-linking of proteins (Nielsen and Jorgensen, 2004). This is accompanied by the losses in textural property and functional properties (Careche *et al*., 1999). To retard the freeze-toughening of fish, numerous chemical additives have been used (DaPonte *et al*., 1986). Both sodium citrate and H_2O_2 were found to slow the rate of DMA formation in frozen gadoid mince (Parkin and Hultin, 1982b). Sodium pyruvate and H_2O_2 were shown to decrease the extent of toughening in fillets of cod and minced red hake during frozen storage (Racicot *et al*., 1984). Additionally, the protective effects of sugars, phosphates and several carbohydrates on the denaturation of fish muscle during frozen storage were also examined (Noguchi, 1984).

Lizardfish (*Saurida* spp.) have been considered as a potential raw material for high-grade surimi in Japan, due to their high yield, white colour, good flavour, and high gel-forming ability (Morrissey and Tan, 2000). Lizardfish are generally considered as the low-market-value fish in Southeast Asia due to their appearance and susceptibility to spoilage. As a consequence, the large quantities are landed as trawl by-catch. In Thailand, lizardfish have been used to produce the low-grade surimi, which is mainly served for low-priced fishcake products (Benjakul *et al*., 2003b). The gel-forming ability of this fish decreased rapidly during post-harvested handling (Yasui and Lim, 1987). This was associated with proteolysis as well as the formation of FA (Benjakul *et al*., 2003b). FA production is considered as one of the major causes of the liability of lizardfish in the frozen state (Benjakul *et al*., 2005). Therefore, the addition of some compounds capable of inhibiting TMAOase as well as stabilizing the protein structure during the extended storage should be the promising approach to retard the quality deterioration of this species. This study aimed to examine the influence of various additives on TMAOase activity and the physicochemical and biochemical changes of lizardfish mince during frozen storage at -20°C.

4.3 Materials and Methods

Chemicals

All chemicals for TMAOase activity assay were purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan). TMAO was obtained from Aldrich Chemical Company, Inc (Milwaukee, WI, USA). Acetylacetone, Triton X-100, maltodextrin DE

20, sodium pyruvate, sodium tripolyphosphate, and sodium pyrophosphate were purchased from Fluka (Buchs, Switzerland). Tris (hydroxymethyl) aminomethane, hydrogen peroxide, and chlorine were obtained from Merck (Darmstadt, Germany). L-ascorbic acid was procured from Sigma Chemical Co. (St. Louis, MO, USA).

Fish sample

Lizardfish (*Saurida tumbil*) of the average weight of 200-250 g/fish, caught off the Songkhla Coast along the Gulf of Thailand and off-loaded approximately 36-48 h after catching, were purchased from the dock in Songkhla. Fish kept in ice with fish/ice ratio of 1:2 (w/w) were transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, fish were headed, gutted and washed with water. The flesh was separated from skin and ground in a meat grinder (MX-T2G National, Tokyo, Japan).

Preparation of TMAOase crude extract from lizardfish muscle

The TMAOase extract was prepared according to method of Benjakul *et al*. (2004) with some modifications. To prepare the crude extract of TMAOase, finely chopped muscle of lizardfish was added with 2 volumes of chilled 20 mM Tris-acetate buffer, pH 7.0 containing 0.1 M NaCl and 0.1% Triton X-100. The mixture was homogenized for 3 min using a homogenizer (IKA Labortechnik, Selangor, Malaysia) at a speed of 12,000 rpm. The homogenate was centrifuged at 19,400 × g for 1 h at 4°C using a Sorvall Model RC-B Plus centrifuge (Newtown, CT, USA). The supernatant obtained was referred to as "TMAOase crude extract".

TMAOase activity assay

TMAOase activity was assayed using TMAO as a substrate in the presence of selected cofactors (Benjakul *et al*., 2004). To 2.5 ml of assay mixture (24 mM Tris-acetate containing 24 mM TMAO, 2.4 mM cysteine, 2.4 mM ascorbate and 0.24 mM $FeCl_2$, pH 7.0), 0.5 ml of enzyme solution was added to initiate the reaction. The reaction was conducted at 25°C for 80 min and 1 ml of 10% (w/v) trichloroacetic acid was added

to terminate the reaction. The reaction mixture was then centrifuged at 8,000 × g for 15 min and the supernatant was subjected to DMA determination. One unit of TMAOase is defined as the activity, which releases 1 nmol DMA per min.

Effects of some additives on TMAOase activity

To study the effect of various additives on TMAOase activity, TMAOase crude extract was mixed with an equal volume of stock solutions of additives to obtain the final concentrations designated. The mixture was allowed to stand at room temperature (26-28°C) for 10 min. The residual activity was then determined. The additives exhibiting the highest inhibitory activity towards TMAOase were chosen for further study.

Effects of selected additives on physicochemical and biochemical changes of lizardfish mince during frozen storage

Fish sample preparation

The lizardfish mince (0.5 kg) containing cryoprotectants (4% (w/w) sucrose and 4% (w/w) sorbitol) were mixed with the selected additives showing the highest inhibitory effects on TMAOase using a mixer (KitchenAid, Michigan, USA). Additionally, the mince with no cryoprotectants and additive was used as the control. All samples were packaged in a polyethylene bag and sealed tightly in air. All samples were kept at -20°C for 6 months. At definite time intervals (0, 2, 4, 6, 8, 12, 16, 20 and 24 weeks), samples were removed, thawed with running water (26-28°C) to obtain the core temperature of 0-2°C and subjected to TMAOase activity determination and chemical analyses.

Determination of trimethylamine oxide (TMAO)

The sample (2.5 g) was added with 10 ml of 5% (w/v) trichloroacetic acid and homogenized with a homogenizer (IKA Labortechnik, Selangor, Malaysia) at a speed of 12,000 rpm for 2 min. The homogenate was centrifuged at 3,000 × g for 15

min. The supernatant containing TMAO was used for analysis. TMAO was determined after reduction to TMA by the method of Benjakul *et al.* (2004). The supernatant (2 ml) was added to 2 ml of 1% $TiCl_3$ and incubated at 80°C for 90 sec, followed by cooling with a running water. TMA was determined according to the method of Conway and Byrne (1936). TMAO was then calculated after subtracting the indigenous TMA content in the samples and expressed as micromoles per gram.

Determination of dimethylamine (DMA) and free formaldehyde (FA)

The sample (2.5 g) was added with 10 ml of 5% (w/v) trichloroacetic acid and homogenized with a homogenizer at a speed of 12,000 rpm for 2 min. The homogenate was centrifuged at 3,000 × g for 15 min and the supernatant was removed. To the pellet, 5 ml of 5% (w/v) trichloroacetic acid were added and homogenized as previously described. The supernatants were combined and neutralized to pH 6.0-6.5 and the final volume was made up to 25 ml using distilled water. The supernatant was then used for DMA and FA determinations as described by Benjakul *et al.* (2004).

Protein solubility

Protein solubility was determined as described by Benjakul and Bauer (2000) with a slight modification. To 2.5 g sample, 22.5 ml of 0.6 M KCl were added and the mixture was homogenized for 30 s. The homogenate was stirred at room temperature (25-27°C) for 4 h, followed by centrifuging at 12,000×*g* for 30 min at 4°C. To 10 ml of supernatant, 2.5 ml of cold 50% (w/v) trichloroacetic acid were added to obtain the final concentration of 10%. The precipitate was washed with 10% trichloroacetic acid and then solubilized in 0.5 M NaOH. Protein content was determined using the Biuret method (Robinson and Hodgen, 1940) using bovine serum albumin as a standard. The solubility was expressed as the percentage of the soluble protein, compared with total protein in the sample.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out using Duncan's multiple range Test (DMRT) (Steel and Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 10.0 for windows, SPSS Inc., Chicago, IL).

4.4 Results and Discussion

Effect of some additives on lizardfish muscle TMAOase activity

The effects of various additives on TMAOase activity from lizardfish muscle are shown in Table 4. TMAOase activity was strongly inhibited by sodium pyrophosphate ($P < 0.05$). In the presence of 0.2% and 0.4% sodium pyrophosphate, the activities of 68.3% and 76.4% were inhibited, respectively. In addition, sodium citrate at a concentration of 0.4% also showed the high inhibitory effect on TMAOase activity (61.3% inhibition). Pyrophosphate and citrate might function as the chelating agents, which can sequester the ions located in the active site or those required for TMAOase activity. Ferrous or ferric ions are found in TMAOase active site and determine the activity of this enzyme (Parkin and Hultin, 1986a). Krueger and Fennema (1989) reported that sodium citrate significantly lessened DMA production of Alaska pollack fillets throughout the storage at -10°C. Parkin and Hultin (1982b) found the inhibition effect of 1 mM EDTA on DMA production induced by a microsomal fraction from the skeletal muscle of red hake (*Urophycis chuss*). However, EDTA showed no inhibitory activity against TMAOase from lizardfish muscle. This was possibly a result of insufficient concentration of EDTA used for the inhibition of TMAOase activity. Additionally, Spinelli and Koury (1981) found that addition of $Fe^{2+/3+}$ and EDTA to muscle homogenates of Pacific whiting (*Merluccius productus*), rockfish (*Sebastes rubesimus*) and dover sole (*Microstomus pacificus*) induced DMA formation during storage at -5°C. Some metal chelators including potassium cyanide and EDTA were shown to inhibit mackerel lipoxygenase, a non-heme iron containing enzyme (Banerjee, 2006).

For oxidizing agents, only H_2O_2 showed the inhibitory activity towards TMAOase and the greater inhibition was noticeable as the concentrations increased ($P <$

0.05). Krueger and Fennema (1989) found the inhibition effect of 0.85% (v/v) H_2O_2 on DMA formation of Alaska pollack (*Theragra chalcogramma*) fillets during frozen storage at -10°C. Racicot *et al*. (1984) also reported that the presence of H_2O_2 in red hake mince at concentration as low as 0.05% (w/w) reduced DMA production during 7 weeks of frozen storage at -12°C. H_2O_2 is thought to create oxidizing conditions in the fish muscle and affects the reducing conditions necessary for TMAOase activity (Parkin and Hultin, 1982b; Racicot *et al*., 1984). Nevertheless, the negligible inhibition towards TMAOase from lizardfish muscle was found when chlorine was used (Table 4). General inhibitors of redox reactions such as H_2O_2 were strong oxidants and normally promote the inhibition of enzymatic TMAO breakdown (Phillippy and Hultin, 1993). Additionally, TMAO demethylation can be stimulated or inhibited by compounds involved in redox reactions with an electron transfer mechanism (Sikorski and Kostuch, 1982).

For hydrocolloids, sodium alginate at a level of 0.5% showed the highest inhibition (30.3%), whereas nonionic (locust bean gum) and ionic (xanthan gum, carboxymethylcellulose) gums had a slight inhibitory effect on TMAOase activity. Sodium alginate is a polysaccharide consisting of guluronic acid and mannuronic acid residues and complexes with metal ions via its carboxyl groups on polyguluronate units (Gupta *et al*., 2002). Carageenan was found to inhibit the TMAOase activity in a concentration-dependent manner. Carageenan is a polysaccharide chain comprising galactose units and 3,6-anhydro galactose, both sulfated and nonsulfated, joined by alternating α-1,3, β-1,4 glycosidic linkages (Wong, 1989). Sulfate groups might form the salt bridge with ions needed for TMAOase activity or chelate with ferrous or ferric ions in the active site of enzyme. From the result, carboxymethylcellulose, locust bean gum and xanthan gum also showed the inhibitory effect on TMAOase activity, depending on the concentration used. Carboxymethylcellulose contains a hydrophilic carboxyl group (Wong, 1989), which can interact with mono and divalent cations (Whistler and Daniel, 1990). Therefore, the inhibitory effect of anionic and ionic gums might be a result of the interaction of ionic groups of gum with the ions required for TMAOase activity.

The addition of sodium pyruvate and maltodextrin also resulted in the decreases in TMAOase activity. High inhibition was noticeable with increasing concentrations ($P < 0.05$). However, Krueger and Fennema (1989) reported that treatment of Alaska pollack fillets with 0.85 M sodium pyruvate had no significant effect on DMA formation. Maltodextrins were strong inhibitors of FA production at -10°C

showing the inhibition levels greater than 30% over the entire storage period (Herrera *et al.*, 1999). Herrera *et al.* (2000) found that adding maltodextrins to minced blue whiting muscle inhibited FA production during storage at −10 and −20°C.

Due to the highest inhibitory effect on TMAOase activity of sodium pyrophosphate (0.3%) and sodium alginate (0.5%) among all additives tested, they were chosen for further use in combination with cryoprotectants (4% sucrose and 4% sorbitol) to lower the protein alterations taking place in frozen-stored lizardfish mince induced by TMAOase activity.

Table 4. Effect of various additives on the inhibition of TMAOase activity from lizardfish muscle[a,b]

Additives	Final concentration	% Inhibition
Chelator		
EDTA	1 mM	0a
	2 mM	0a
Sodium citrate	0.2%	31.58±0.12l
	0.4%	61.32±0.24m
Anionic compounds		
Sodium tripolyphosphate	0.2%	0a
	0.4%	5.57±0.19c
Sodium pyrophosphate	0.2%	68.30±1.60n
	0.4%	76.42±1.46o
Oxidizing agent		
H_2O_2	10 ppm	16.73±1.38i
	30 ppm	24.17±1.79j
Chlorine	10 ppm	0a
	30 ppm	0a
Hydrocolloids		
Carageenan	0.25%	5.35±0.14bc
	0.5%	11.18±0.75fgh
Carboxymethylcellulose	0.25%	3.38±0.21b
	0.5%	12.48±0.10gh
Locust bean gum	0.25%	5.15±0.09bc
	0.5%	6.78±0.07cd
Sodium alginate	0.25%	9.32±0.28ef
	0.5%	30.28±0.51kl
Xanthan gum	0.25%	10.08±0.05ef
	0.5%	ND
Others		
Sodium pyruvate	0.2%	8.49±0.74de
	0.4%	12.84±1.58h
Maltodextrin DE 20	4%	10.77±0.22fg
	8%	28.58±0.01j

[a]Values are given as mean ± SD from triplicate determinations.

[b]Different letters in the same column indicate significant differences ($P < 0.05$).

ND: not determined. Xanthan gum at 0.5% was not completely soluble and was not used for inhibition study.

Changes in TMAOase activity in lizardfish mince during frozen storage

TMAOase activity in lizardfish muscle was monitored during frozen storage at -20°C. A marked increase in activity was found in all samples within 4 weeks of storage ($P < 0.05$) (Figure 21). Thereafter, TMAOase activity continuously decreased up to 24 weeks. An increase in TMAOase activity within the first six weeks might result from the disruption of cell membrane during frozen storage. The formation and accretion of ice crystals, dehydration, and increase in unfrozen phase led to the changes in muscle tissues as well as cell damage (Shenouda, 1980). Therefore, freezing can disrupt muscle cells, resulting in the release of mitochondrial and lysosomal enzymes into sarcoplasm, especially with increasing frozen storage time (Hamm, 1979). The subsequent decrease in activities was possibly caused by the denaturation of TMAOase and the lower extraction efficacy of TMAOase from the muscle, owing to the protein aggregation, particularly with increasing storage time. Generally, the control (without cryoprotectants and additives) had higher TMAOase activity, compared with samples containing cryoprotectants and additives throughout the storage ($P < 0.05$). When mince was mixed with cryoprotectants prior to frozen storage, those compounds were able to retard the denaturation of protein or disruption of cells as evidenced by the lower TMAOase released. From the result, the differences in TMAOase activity among all samples were noticeable. In the presence of cryoprotectants, the samples with a higher level of pyrophosphate comprised the lower TMAOase activity. The lowest TMAOase activity was found in the frozen-stored lizardfish mince containing sodium pyrophosphate (0.3%) and sodium alginate (0.5%) throughout the storage. The lowered activity of TMAOase in mince added with pyrophosphate and alginate was postulated to be due to the inhibition of TMAOase by those compounds. Both pyrophosphate and alginate with anionic groups, phosphate and carboxyl groups, most likely chelated the ions localized in the active site of enzyme or required for its activity.

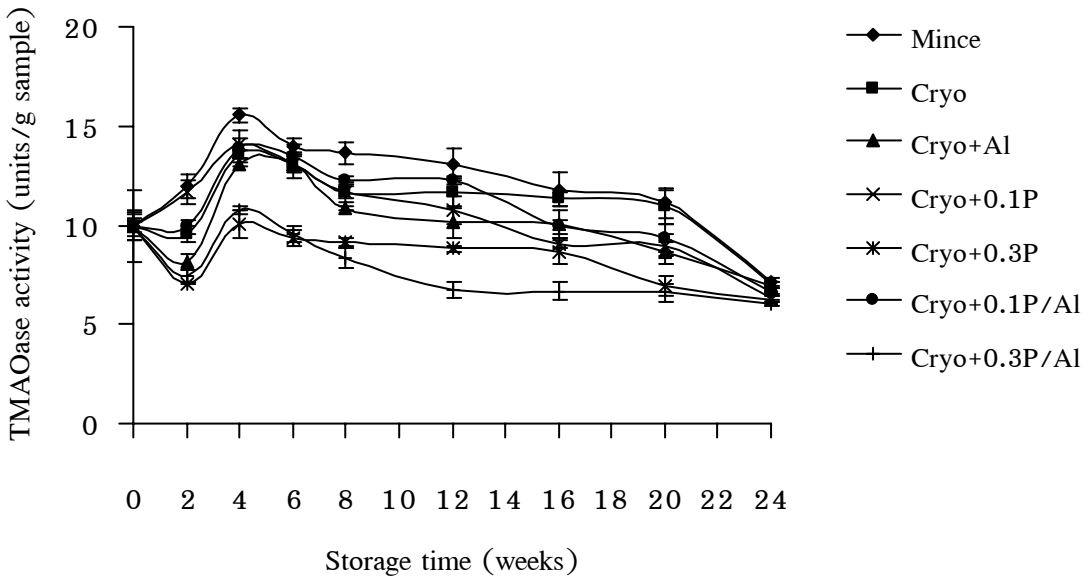

Figure 21. Changes in TMAOase acitivity in lizardfish mince without and with various additives during frozen storage at −20°C for 24 weeks. Cryo: 4% sucrose and 4% sorbitol; Cryo+Al: cryoprotectants with 0.5% sodium alginate; Cryo+0.1P: cryoprotectants with 0.1% pyrophosphate; Cryo+0.3P: cryoprotectants with 0.3% pyrophosphate; Cryo+0.1P/Al: cryoprotectants with 0.1% pyrophosphate and 0.5% sodium alginate; Cryo+0.3P/Al: cryoprotectants with 0.3% pyrophosphate and 0.5% sodium alginate; Mince: without cryoprotectants and additives. Bars represent standard deviation from triplicate determinations.

Changes in TMAO, DMA and FA contents of lizardfish mince during frozen storage

Formation of DMA and FA in lizardfish mince is depicted in Figures 22A and 22B. In general, DMA and FA contents increased continuously as the storage time increased up to 24 weeks of storage ($P < 0.05$). The greatest changes were observed in the control sample (without cryoprotectants and additives) where DMA and FA contents were 0.92 μmol/g and 0.57 μmol/g sample after 24 weeks of storage. Tejada *et al.* (2002) also found the formation of DMA and FA during frozen storage of hake mince. It has been suggested that the action of the TMAOase system in several species of the family Gadidae is responsible for rapid textural deterioration (Amano *et al.*, 1963). Equimolar quantities of DMA and FA are formed from TMAO (Dingle *et al.*, 1977). On the other

hand, the sample containing 0.3% sodium pyrophosphate and 0.5% sodium alginate showed the lowest changes in DMA and FA formation throughout the storage time. However, no marked differences in DMA and FA contents were observed among other samples. Ayyad and Aboel-Niel (1991) reported that frozen mince of mackerel added with 0.5% alginate showed the lower DMA and FA contents, compared with the control sample. From the results, the lower amount of FA measured was noticeable, compared with that of DMA produced. FA is reactive with free amino acids and proteins (Castell and Smith, 1973; Dingle and Hines, 1975). This most likely caused the loss in FA extractability from the muscle. As a consequence, the lower content of free FA was found, compared with total FA content, which is equivalent to DMA amount. The deterioration in quality of frozen stored fish due to the breakdown of TMAO is basically attributed to the formation of FA rather than DMA (Gill *et al*., 1979). From the result, it was noticeable that FA and DMA contents of lizardfish mince in this study were much lower than that found in previous experiment. This was probably due to the different level of TMAOase in lizardfish caught from different fishing grounds. Factors including gender, maturation stage, temperature of the habitat, and feeding status, or size can influence the actual level of TMAOase activity (Sotelo and Rehbein, 2000).

TMAO content gradually decreased throughout the storage time (Figure 22C) with concomitant increases in DMA and FA (Figures 22A and 22B). Increases in DMA and FA contents indicated the breakdown of TMAO in lizardfish mince. The highest decreasing rate of TMAO was also found in the control sample, compared with other samples. Parkin and Hultin (1982b) suggested that the relative low approximate K_m for TMAO of the system, indicating that TMAOase could function at a relatively high activity until most of TMAO is converted. Although lizardfish mince was kept under frozen storage, TMAOase activity still maintained and played an important role in textural changes of frozen lizardfish mince due to the low activation energy needed of this enzyme. Benjakul *et al*. (2003a) reported that the activation energy of TMAOase from lizardfish kidney was 30.5 kJ mol^{-1} K^{-1}. From the results, the lowest changes in DMA, FA and TMAO contents were found in sample containing both sodium alginate and pyrophosphate. Lowered TMAOase activity caused by those two compounds might be associated with the retarded formation of FA and DMA in the lizardfish mince.

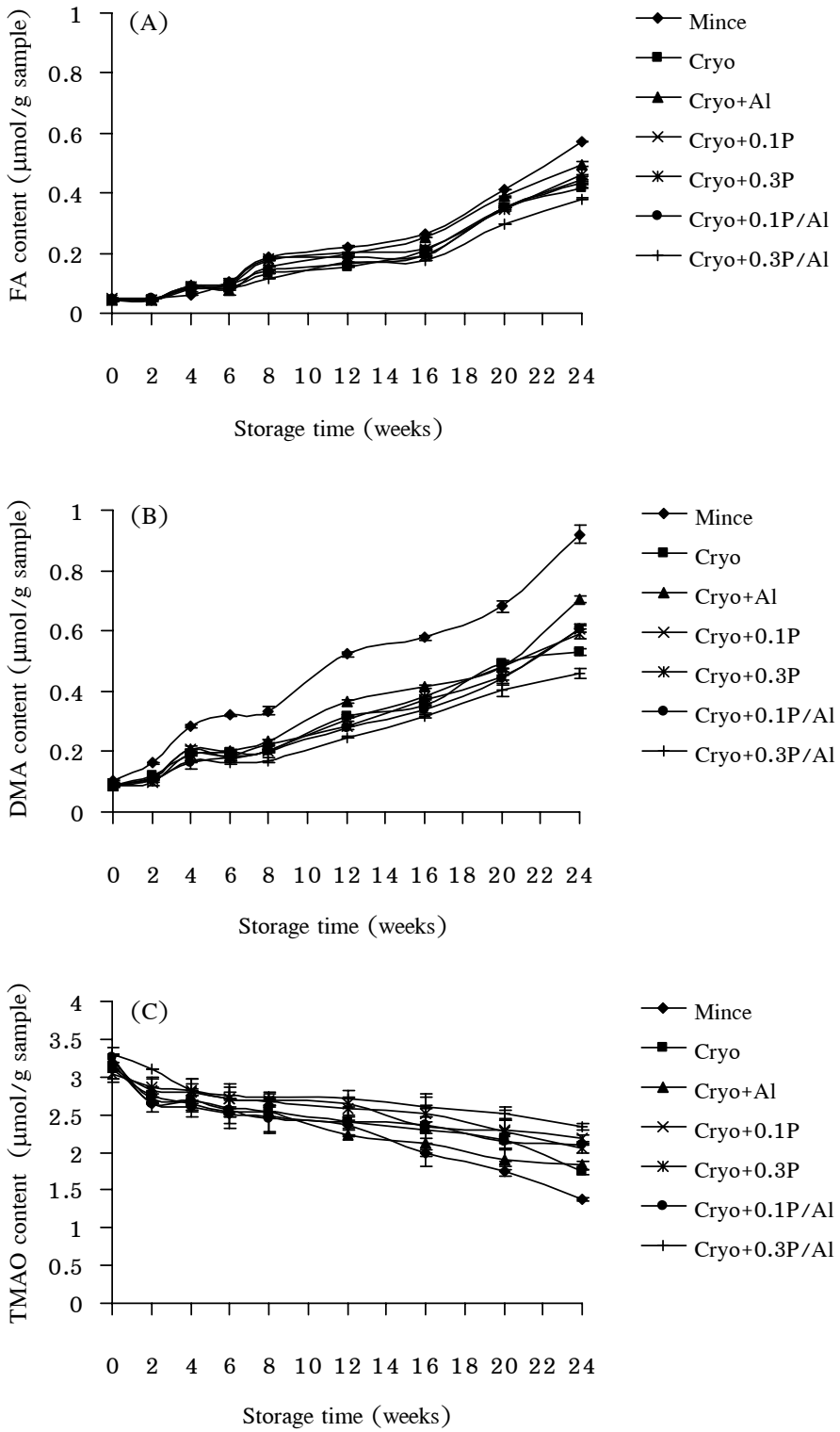

Figure 22. Changes in FA (A), DMA (B) and TMAO (C) contents in lizardfish mince without and with various additives during frozen storage at −20°C for 24 weeks. Bars represent standard deviation from triplicate determinations. Key: see Fig. 1 caption.

Changes in protein solubility of lizardfish mince during frozen storage

Solubility of muscle protein in lizardfish mince in 0.6 M KCl decreased continuously during the prolonged storage at -20°C ($P < 0.05$) (Figure 23). The sharp decrease was noticeable within the first 2 weeks. Among all samples, the control without cryoprotectants and additives had the lowest protein solubility. With the addition of cryoprotectants, higher solubility was observed regardless of additive used. However, sample added with cryoprotectants and 0.5% alginate had no differences in solubility, compared with the sample mixed with only cryoprotectants. Cryoprotectants increase the surface tension of water as well as the binding energy, preventing withdrawal of water molecules from the protein and thus stabilizing the protein (Rahman, 1999). As the result, higher solubility was observed in the samples containing cryoprotectants. From the result, the addition of sodium pyrophosphate and alginate in combination with cryoprotectants (4% sucrose and 4% sorbitol) lowered the decrease in protein solubility. Decreases in protein solubility during frozen storage were noticeable in minced trout (Moral *et al*., 1986), minced cod (Tejada *et al*., 1996) and hake actomyosin (Del Mazo *et al*., 1999). Tejada *et al*. (2003) also found the decrease in protein solubility of hake muscle during storage at -20°C for 365 days. Generally, less protein could be extracted with salt solutions where the greater amount of FA was produced (Sotelo *et al*., 1995b).

From the result, the inhibitory activity of 0.5% sodium alginate and 0.3% pyrophosphate towards TMAOase (Table 4) might be associated with the decreased FA production in lizardfish mince (Figure 22A). As a result, the loss of protein solubility of the sample containing both compounds was arrested. Herrera *et al*. (1999) postulated that FA molecules would initially bind easily accessible reactive groups, and it would cause some chemical modifications in the native structure. Apart from the inactivation of TMAOase by pyrophosphate and alginate, the restriction of molecular diffusion caused by those compounds and cryoprotectants added could lessen the movement of reactants for the reactions, both enzymatic reactions and protein denaturation. Additionally, cryostabilizers presumably play an important part in the inhibition of the reactivity of FA by reducing the exposure and availability of reactive groups, as well as by restricting the diffusion of the FA molecules toward these groups (Herrera *et al*., 1999).

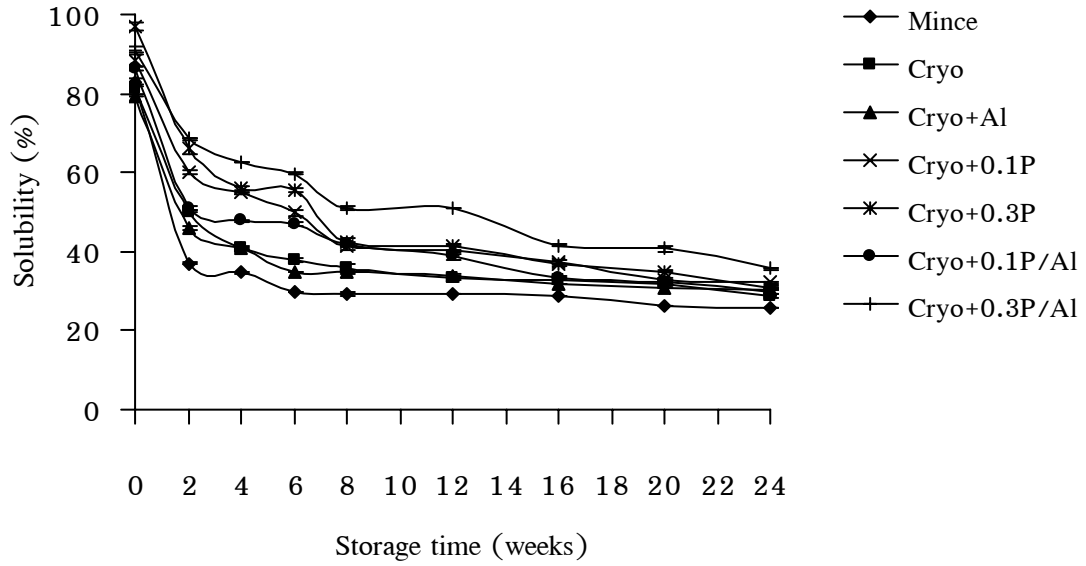

Figure 23. Changes in protein solubility of lizardfish mince without and with various additives during storage at $-20^{\circ}C$ for 24 weeks. Bars represent standard deviation from triplicate determinations. The solubility was expressed as the percentage of soluble protein relative to total protein content in the sample. Key: see Fig. 1 caption.

4.5 Conclusion

Sodium pyrophosphate and sodium citrate showed the high inhibitory effect on TMAOase activity from lizardfish muscle. Sodium alginate also inhibited TMAOase to some extent. The addition of 0.3% pyrophosphate together with 0.5% alginate in combination with cryoprotectants could lower TMAOase activity as well as retard protein denaturation induced by FA in lizardfish mince during frozen storage.