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Oxidative stability and discoloration of frozen tilapia fillet dipped in alkali-aided protein hydrolysates from tilapia byproducts

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Abstract

The alkali-treated protein hydrolysate (APH) prepared from tilapia byproducts was applied to frozen tilapia fillet to monitor quality changes under accelerated freeze/thaw conditions. Tilapia fillets were dipped in various solutions for 10 min i.e. 0.5% sodium tripolyphosphate (STPP), and 0.5% & 1.0% APH with or without 0.05% citric acid (CA) before being subjected to repeated freezing/thawing (FT) (0, 5 and 10 cycles). The APH solution demonstrated a remarkable 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity (1,360 -1,561 µg trolox/mL). The fillets dipped with 1%APH with CA had the highest protein solubility, the lowest change of TBARS and FTIR lipid peak intensities, as well as the lowest whiteness reduction after 10 FT cycles ($P<0.05$). Therefore, the synergistic effect of APH derived from fish byproducts and citric acid was able to extend the oxidative shelf-life and preserve qualities (drip loss, color, and protein solubility) of tilapia fillet during accelerated conditions of frozen storage. The results of this study suggest that hydrolysates of protein recovered by alkaline extraction from tilapia byproducts can be applied as a natural antioxidant to prevent oxidation reactions in food products.

Keywords: alkali-treated protein hydrolysate, tilapia byproducts, frozen tilapia fillets, dipping solution, lipid oxidation

1. Introduction

Quality deterioration of frozen fillets i.e. poor texture, off-flavors development and yellow discoloration causes the product to be unacceptable. These are important quality parameters that have a great impact on consumer acceptance and the market value of aquatic food products [1]. Lipid oxidation is one of the primary causes resulting in discoloration, drip loss, hardened texture, and off-flavor development in fish products [2]. Hamre et al. [3] reported the yellow appearance of frozen Norwegian spring-spawning herring, which was related to an increase in TBARS and peroxide values.

Oxidative deterioration of muscle food during processing or storage can be prevented by antioxidants [4]. One of the applications to fish products is to add antioxidant to the muscle while glazing [5]. The most commonly used synthetic antioxidants in the food industry are butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate. However, the consumption of synthetic antioxidants is restricted in many countries since they have harmful effects on consumer health [6]. Consequently, the alternative natural antioxidants and their potential use in food products have been paid increasing attention. Byproducts from fish processing waste have attracted considerable interest especially for protein hydrolysate production. As the waste still consists of proteins of a quality as good as those in muscle, they should be efficiently utilized for human consumption. Antioxidant peptides derived from hydrolysates (the scales of silver carp; *Hypophthalmichthys molitrix*) catalyzed by Flavourzyme at 5 min significantly reduced conjugated dienes of mince and improved whiteness in surimi gels [7]. Mahi mahi red muscle dipped in tilapia protein (pH-shift) hydrolysates showed a significantly decreased

TBARS over 90 h storage time [8]. Protein hydrolysate solution prepared by hydrolysis of shrimp waste (5 mg/ml) lowered thiobarbituric acid (TBA) values of croaker fish fillet and maintained yellowness of skin colour compared to the untreated control sample during 10 days at 4°C [9].

In our previous study, protein hydrolysates produced from alkali-aided extracted proteins (APH) from tilapia byproducts exhibited significant antioxidant activities and may have been capable of inhibiting lipid peroxidation in frozen fillet. Thus, this research aimed to evaluate the effect of alkali-aided extracted protein hydrolysates (APH) on the physicochemical changes and stability of lipid oxidation in tilapia fillet under accelerated storage conditions of temperature abuse by alternate freezing and thawing.

2. Materials and Methods

2.1 Materials

Frozen Nile tilapia byproducts (TB) including head and frame, fin, belly flap meat and trimmed meat as well as fillet purchased from a frozen tilapia fillet manufacturer (Grobest Thailand, Co., Ltd., Nakhonphanom province, Thailand) were packed in a polyethylene bag placed between layers of ice in a polystyrene box. Samples were transferred by a temperature controlled truck (-12 ± 2˚C) to the Department of Food Technology, Khon Kaen University, Thailand within 5-6 h. All the chemicals and reagents used were of analytical grade. A commercial enzyme, Protease G6 (EC 3.4.21.62) (DuPont™Genencor® Science, USA) with an activity of 580,000 DU g^{-1}) was purchased from Siam Victory Chemicals Co., Ltd., Thailand.

After thawing at an ambient temperature for ~ 1 h, each TB type was ground by a meat grinder (BIRO 8-22 E97, The BIRO MFG. Co., USA). Each ground sample was spread on an aluminum tray with a 3 cm thickness before being freeze dried for ~ 72 h (Freeze Dryer, GAMMA 2-16 LSC, Martin Christ, Germany). APH exhibited the highest antioxidant activity which was produced according to the conditions selected from the previous study [10]. The APH was prepared from a mixture of freeze dried head and frame, fin, belly flap meat and trimmed meat at 1:1:1:1 ratio using alkaline treatment as described in Hultin et al. [11] with slight modifications followed by proteolysis. In brief, one part of TB was mixed with 9 parts of cold deionized water and homogenized at 8,000 rpm for 1 min (at 4 ˚C). The pH of the homogenized sample was adjusted to 11.0 using 2 N NaOH to solubilize proteins from the TB, and the mixture was centrifuged at 10,000 x g for 20 min at 4 ˚C. The supernatant was filtered using a double-layered cheese cloth. The pH and temperature of the filtrate were adjusted to 9.5 (using 2 N HCl) and 65˚C, respectively, in a reactor (Fermenter, Biostat B, B. Braun Biotech International, Germany). The reaction was started by adding Protease G6 at a level of 1.5 % v/w of proteins and hydrolysis time of 60 min. The reaction was inactivated using a microwave oven (EMS 3067x, Electrolux, PRC) for 5 min (final temperature ~100˚C using power level at 900 watt). The suspension was cooled and centrifuged, then the supernatant was collected and lyophilized. The protein hydrolysate powder (APH) was vacuum packed and kept in an aluminum foil at -30˚C until used.

Five dipping solutions including 0.5% sodium tripolyphosphate (STPP), 0.5% APH solution with and without addition of 0.05% citric acid (CA) (0.5APH & 0.5APH+CA) and 1% APH solution with/without addition of 0.05% (1APH & 1APH+CA) were prepared. The pH, protein content [12], ABTS radical scavenging [13] and metal chelating [14] activities of the dipping solutions were determined.

2.2 Sample preparation

Frozen Nile tilapia fillets (TF) were thawed at ambient temperature for ~ 1 h and then were cut manually into 100 ± 10 g each and divided into 6 portions (~18 pieces for each portion). Each portion was individually dipped in one of the five solutions for 10 min (~ 4˚C). Then the fillets were removed and allowed to drain for 10 min. The fillets were then sealed individually in resealable plastic bags (17 x 20.3 cm, Tesco, Ek-Chai Distribution System Co. Ltd., Bangkok, Thailand) and frozen in air-blast freezer at -35˚C for 3-4 h (Air Blast freezer, Rivacold Ice 13-10, Tewkesbury, Gloucestershire, UK). The frozen fillets were alternately stored at -20±2˚C in a freezer (SF-C995, Sanyo, Bangkok, Thailand) and at 5±2 ˚C in a cold room (KS 35-4, Searle Manufacturing Company, UK) for the freezing/thawing cycle. Samples were taken to be analyzed for changes of physico-chemical properties, as well as lipid oxidation at 0, 5, and 10 cycles (1 cycle: thaw 5˚C for 6 h, freeze at -18˚C for 18 h).

2.3 pH value and color

Approximately 3 g of minced dipped fillet were homogenized (ACE, Nihonseiki Kaisha Co., Japan) with 27 ml of distilled water at 10,000 rpm for 1 min (in an ice bath). The pH of the homogenized sample was measured using a pH meter (FiveEasy FE20-1, Mettler-Toledo AG, Schwerzenbach, Switzerland).

The color of tilapia fillets dipped in antioxidant mixtures was determined at regular intervals using a Hunter Lab (Hunter Lab, UltraScan XE, USA). The *L** (lightness), *a** (redness) and *b** (yellowness) values were recorded at 6 different locations on both surfaces of the treated fillets. Whiteness was calculated as described by Lanier et al. [15].

2.4 Drip loss

The frozen samples were removed from the freezer and thawed at 5 ± 2 ˚C for 24 h, removed from the plastic bag and placed on a paper towel to release liquid drip, and then the fillet pieces were weighed again. Calculation for drip loss is as follows (1):

$$\text{Drip loss (\%)} = \frac{\text{weight of frozen sample - weight of thawed sample}}{\text{weight of frozen sample}} \text{x} 100 \quad (1)$$

2.5 Protein solubility

Samples (3 g) were minced with a chopper (AT711161-Multi Moulinette, Groupe S E B (Thailand) Co. Ltd., Bangkok) and homogenized in 27 mL of 0.6 M NaCl in 20 mM phosphate buffer (pH 7.0) for 1 min. The homogenate was centrifuged at 10,000 x g for 20 min at 4 ˚C. The supernatant was diluted with 0.6 M NaCl in 20 mM phosphate buffer and protein determination was performed using the Biuret method [16] with bovine serum albumin (BSA) as a standard. Protein solubility was reported as a percentage of the solubilized protein in a phosphate buffer based on the weight of the sample. Changes in protein solubility during storage were expressed as a percentage of the initial protein solubility at 0 freeze/thaw cycle.

2.6 Lipid oxidation (TBARS)

Lipid oxidation of the fillet samples was measured by the 2-thiobarbituric acid distillation method as modified by Ahn et al. [17]. TBARS are expressed as mg malonaldehyde (MDA)/kg muscle. Ten grams of the samples were mixed with 50 mL of distilled water using an Ace homogenizer at 10,000 rpm for 1 min (ice bath), then transferred to a distillation tube. 2.5 mL of 4 N HCl and a few drops of Dow antifoam B were added to the mixture. After distillation (Distillation Unit, Kjeltec^{TM}8100, Foss, Sweden) the 2.5 mL of distilled mixture were poured into a test tube and then 2.5 mL of 0.02 M 2-thiobarbituric acid in 90% acetic acid were added. The test tubes were covered with an aluminum foil and heated in a boiling water bath for 30 min to develop the chromogen. After cooling at room temperature, the absorbance was measured at 538 nm using a UV-visible spectrophotometer (Lambda 25, Perkin Elmer, Shelton, CT) and using 1,1,3,3-tetra-ethoxypropane (Sigma) as the standard.

2.7 Changes of lipid structures in frozen fillet by ATR-FTIR spectroscopy

All fillet treatments were freeze dried (Gamma 2-16 LSC, Christ, UK) to remove water content prior to performing an FTIR analysis. Prior to analysis, the crystal cell was cleaned with acetone, wiped dry with soft tissue and the background scan was run. For spectra analysis, a freeze dried sample was applied directly onto the crystal cell and the cell was clamped into the mount of the FTIR spectrometer. To determine the structural information of tilapia fillet dipped in various solutions, the FTIR spectra were recorded using an attenuated total reflectance- Fourier transform infrared (ATR- FTIR) spectrometer equipped with deuterated glycine sulfate detector (DTGS) (Bruker, TENSOR 27 System, Germany). ATR-FTIR was conducted at 4 cm^{-1} resolutions with 16 scans. Analysis of spectral data was carried out using the OPUS 7.0 data collection software program (Bruker Co.).

2.8 Statistical analysis

Data were analyzed using the SPSS statistical program, version 19. Analysis of variance (ANOVA) was performed with the General Linear Models (GLM) at the level of 95% ($P<0.05$). The differences among means were analyzed by Duncan's multiple range test (DMRT). All experiments were carried out in triplicate.

3. Results and Discussion

3.1 Antioxidant properties of dipping solutions

The pH of STPP and the APH containing solutions were higher than 9.0 (Table 1). Adding citric acid significantly reduced the pH. Protein content results confirmed that 1% APH solution contained higher protein

than 0.5% APH (P<0.05). Regarding their antioxidant activities, STPP (0.5% w/v) had a higher ability of metal chelation (203.2 µg EDTA/mL) than all the APH solutions with and without citric acid) (44.2-53.1 µg EDTA/mL). It has been known that STPP can inhibit lipid oxidation in cooked meat products by forming a tridentate complex with metal ion or "chelating of pro-oxidant" through its phosphoryl oxygens [18]. Contrarily, STPP exhibited nearly no ABTS radical scavenging activity (8.6 µg trolox/mL) while all solutions containing APH showed much higher ABTS values (1,360-1,561 µg trolox/mL) (P<0.05). The APH consisted of proteins and peptides, which have been known to possess antioxidant potency. Our previous study demonstrated that the APH contained antioxidant peptides through the reactions of metal chelating ability, ABTS radical scavenging ability, and oxygen radical absorbance capacity (ORAC-hydrophilic & lipophilic) [10]. Peptides can act as electron donors and scavenge free radicals and terminate the radical chain reactions depending on their amino acid composition and sequence structure [19]. Therefore, the APH may inhibit free radicals and pro-oxidants to prevent oxidation and maintain color quality in dipped fillets during freezing/thawing.

Table 1 pH, antioxidant activities, and protein content of the prepared dipping solutions

Dipping solutions [1]	pH	ABTS radical scavenging activity (µg trolox/mL)	Metal chelating ability (µg EDTA/mL)	Protein content (mg BSA/mL)
DW	7.09 ^{f} ±0.01	-	-	-
STPP	9.13 ^{c} ±0.03	8.60 ^{b} ±0.84	257.76 ^{a} ±9.90	-
0.5APH	9.20 ^{b} ±0.02	1359.91 ^{a} ±76.24	52.53 ^{b} ±0.08	6.14 ^{c} ±0.40
0.5APH+CA	7.24 ^{e} ±0.03	1411.11 ^{a} ±82.67	44.19 ^{b} ±5.56	5.88 ^{c} ±1.09
1APH	9.44 ^{a} ±0.01	1500.96 ^{a} ±265.22	53.13 ^{b} ±0.91	12.92 ^{b} ±0.64
1APH+CA	8.07 ^{d} ±0.01	1560.60 ^{a} ±209.58	51.50 ^{b} ±0.85	14.56 ^{a} ±0.09

[1] DW = distilled water, STPP = 0.5% sodium tripolyphosphate, 0.5APH = 0.5% alkali-aided extracted protein hydrolysates, 1APH = 1% alkali-aided extracted protein hydrolysates, CA = 0.05% citric acid)
[2] Different letters in the same column indicate statistically significant differences (P<0.05).

3.2 pH value and drip loss of frozen dipped fillets

Discoloration of dark muscle developed if the pH values were about ≤ 6.3 [20-21]. Changes in pH of dipped fillets were measured at 0, 5 and 10 freeze/thaw (FT) cycles (Figure 1). The initial pH of all samples was around 6.0-6.3. After 10 FT cycles, the pH of all treatments tended to increase and the pH of all dipped fillets (6.4-6.46) was higher than that of fillets without dipping (6.33). This might be due to the high pH of the dipping solutions (Table 1). An increased pH of fish muscle during a long storage or under unsuitable storage conditions is due to the decomposition of nitrogen compounds in fish muscle [22]. However, the increasing rate of the pH during temperature abuse of the dipped samples (2.02-3.86%) was lower than that of the fillets without dipping (4.57%) indicating slower biochemical changes by the dipping solutions. The lowest pH increase was observed in the 0.5APH+CA (2.02%).

There was an interaction effect of freeze/thaw cycles and dipping treatments on drip loss (P<0.05). Myosin aggregation during frozen storage of fish fillets can cause muscle toughening and drip loss during thawing [23]. The highest drip loss was noted in fillets with no dipping at both 0 and 10 FT cycles. After repeated freezing-thawing storage, the lowest drip loss was seen in 0.5APH (15.35%) and 0.5 APH+CA while 1APH (15.25%) revealed the highest drip loss (18.56%) (Figure 2). Interestingly, an increase in APH from 0.5% to 1% regardless of citric acid did not reduce drip loss. The assumption of this phenomenon is that the fillets dipped in 0.5APH with and without citric acid underwent a higher degree of lipid oxidation as evidenced by the TBARS values (Figure 4) and the FTIR (Figure 5) results. Oxidation of lipids and proteins in fish muscle yields protein crosslinking thus reducing drip loss. Taheri [24] investigated quality changes of black promfet fillets by glazing with rainbow sardine protein hydrolysate during 6 months frozen storage and reported that the drip losses of the non-glazed fillets was lower than glazed fillets. However, an improved yield, thaw yield and cook yield in all glazed fillets were observed.

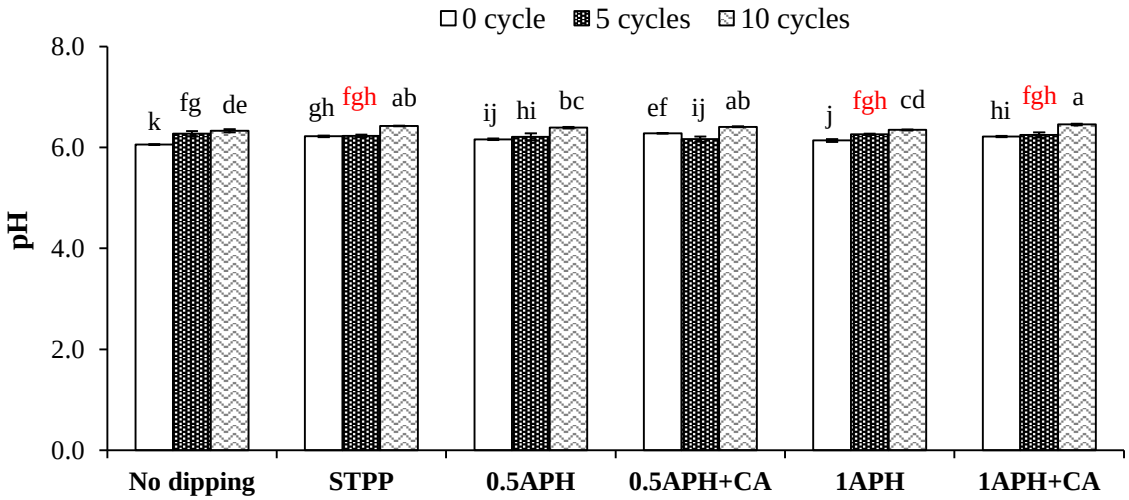

Figure 1 pH values of tilapia fillets treated with various dipping solutions at 0, 5 and 10 freeze/thaw cycles (No dipping = fillet without dipping, STPP = 0.5% sodium tripolyphosphate, 0.5APH = 0.5% alkali-aided extracted protein hydrolysates, 1APH = 1% alkali-aided extracted protein hydrolysates, CA = 0.05% citric acid). Different letters on each bar indicate significant differences ($P<0.05$) among treatments and freeze/thaw cycles.

3.3 Color

The color changes of dipped fillets are shown in Table 2. In general, whiteness of the fillets decreased with increased FT cycles. Frozen storage resulted in color changes that are due to changes in light absorption and light scattering caused by freeze denaturation [25]. Whiteness values were in the range of 87.5-88.1% and 85.8-87.3% at 0 and 10 FT cycles, respectively (data not shown). After 10 cycles, L*, a* and b* values increased in different rates with increasing FT cycles as influenced by different dipping solutions. The a* value maximally increased in the fillets without dipping. STPP and APH helped to reduce an increase of redness with a great enhancement of citric acid for the latter case. An increase in b* values possibly results from lipid oxidation and also protein degradation or denaturation. Only 1APH and 1APH+CA can lessen an increasing rate of yellowness (b*) of the fillets. When considering changes in whiteness values, the 1APH+CA was revealed as the most effective dipping solution to prevent discoloration of tilapia fillet during frozen storage (Table 2**)**. This might be due to the antioxidant ability of peptides in APH plus citric acid enhancement. The delaying of myoglobin oxidation in meat can be attained by an addition of antioxidants, such as ascorbic acid, citric acid, and tocopherol, thus possibly extending color shelf life [26].

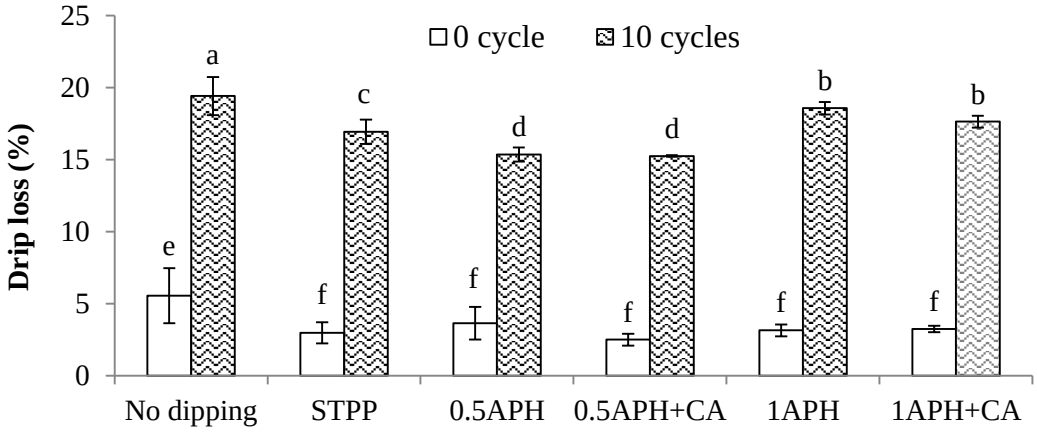

Figure 2 Drip loss in tilapia fillets treated with various dipping solutions at 0, 5 and 10 freeze/thaw (FT) cycles and reduction (%) at 0 and 10 FT cycles (No dipping = fillets without dipping, STPP = 0.5% sodium tripolyphosphate, 0.5APH = 0.5% alkali-aided extracted protein hydrolysates, 1APH = 1% alkali-aided extracted protein hydrolysates, CA = 0.05% citric acid). Different letters on each bar indicate a significant difference ($P<0.05$) among treatments and freeze/thaw cycles.

Table 2 Changes of L* a* b* and whiteness values of tilapia fillets treated with various dipping solutions compared between 0 and 10 freeze/thaw (FT) cycles.

	Change of color values (%) [2,3]			
Treatment [1]	L*	a*	b*	Whiteness
No dipping	7.21	256.26	19.82	-1.99
STPP	3.89	72.82	21.75	-2.51
0.5APH	5.29	53.99	19.48	-2.04
0.5APH+CA	5.57	19.58	19.42	-2.25
1APH	6.66	77.25	11.49	-1.09
1APH+CA	10.09	22.62	5.39	-0.24

[1] No dipping = frozen fillets without dipping, STPP = 0.5% sodium tripolyphosphate, 0.5APH = 0.5% alkali-aided extracted protein hydrolysates, 1APH = 1% alkali-aided extracted protein hydrolysates, CA = 0.05% citric acid.

3.4 Protein solubility

Changes in protein extractability can be a useful tool to evaluate the extent to which fish protein denaturation and aggregation occur during frozen storage or as a result of temperature abuse situations such as freeze–thawing cycles [27]. There was an interaction effect of freeze/thaw cycles and dipping treatment on protein solubility ($P<0.05$). Protein solubility of all treatments decreased after subjected to freeze/thaw cycles ($P<0.05$) (Figure 3). This occurrence indicates that protein denaturation had occurred due to the freezing and frozen storage, followed by hydrophobic interactions, disulfide bonding and ionic interactions [22]. The dipping treatments, particularly STPP and 1APH+CA, tended to maintain a protein solubility better than other treatments. The 1% APH with citric acid revealed the highest protein solubility ($P<0.05$), and its reduction of protein solubility was comparable to the STPP treatment (8.48 and 9.40%). This might imply the ability of APH to reduce protein denaturation during frozen storage resulting in the retention of protein solubility. It has been found that inappropriate freezing processes and frozen storage may cause cell damage due to ice crystal formation leading to a release of pro-oxidants (e.g. metals and heme pigments). Consequently, chemical reactions such as protein denaturation and lipid oxidation are promoted [28]. Zhang et al. [29] reported the cryoprotective effect of the hydrolysate from Antarctic krill which may be active by binding with water molecules surrounding the myofibrils excluded by the hydrophobic residues of the protein molecules during freezing. Moreover, hydrolysates from various aquaculture sources have been proven to reduce lipid oxidation in animal products during chilled or frozen storage [7,8,9] thus lessening protein crosslinking resulting in the retention of protein solubility. The APH dipping solution exhibited ABTS radical scavenging activity (Table 1), metal chelating ability, and ORAC assays [10]. Thus, protein hydrolysates from tilapia byproducts was able to retard denaturation of fish protein by maintaining protein extractability and preventing the lipid oxidation during freezing or thawing processes.

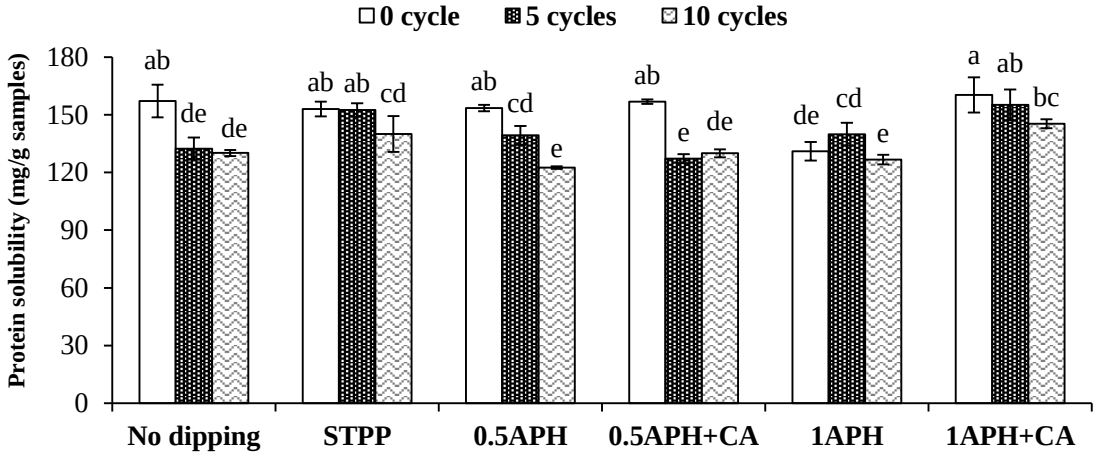

Figure 3 Protein solubility of tilapia fillets treated with various dipping solutions at 0, 5 and 10 FT cycles (No dipping = fillets without dipping, STPP = 0.5% sodium tripolyphosphate, 0.5APH = 0.5% alkali-aided extracted protein hydrolysates, 1APH = 1% alkali-aided extracted protein hydrolysates, CA = 0.05% citric acid). Different letters on each bar indicate significant differences ($P<0.05$) among treatments and freeze/thaw cycles.

3.5 Thiobarbituric acid reactive substances (TBARS)

TBARS is a secondary oxidation product and widely used as an indicator of the degree of lipid oxidation by measuring malondialdehyde (MDA) content. MDA is formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen [30]. In Figure 4, the TBARS values of tilapia fillets subjected to dipping treatments during freeze/thaw cycles (0 & 10) are presented. At initial storage, the TBARS of No dipping, 1APH, and 1APH+CA were significantly higher than those of STPP and 0.5APH with and without CA. The higher TBARS of 1APH at 0 FT cycle probably related to the TBA reactive substances contained in the APH. Tsikas [31] stated that biological substances also contain MDA in free or covalently bound form with proteins, nucleic acids, lipoproteins and certain amino acids. For all treatments, increases in TBARS values were noted when freeze/thaw cycles increased. It should be noted that fillets dipped in 0.5 APH and 0.5APH+CA revealed an increase in TBARS values after 5 FT cycles and then a decrease after 10 FT cycles. During storage, MDA can be further oxidized to other substances which do not interact with TBA [32] resulting in decreasing TBARS values. The 1APH treatment exhibited an ability to reduce MDA formation when working with CA, which corresponded to its high protein solubility (Figure 3) at the end of the storage. Therefore, the dipping of fillets at the optimum concentration of APH with citric acid had a significantly positive effect on decreasing fat deterioration. This is supported by the results in Table 1 revealing strong APH antioxidant functions via scavenging free radicals. In addition, the tilapia protein hydrolysates [8], and Pollock skin hydrolysates [33] were reported for their prevention of lipid oxidation in fish muscles.

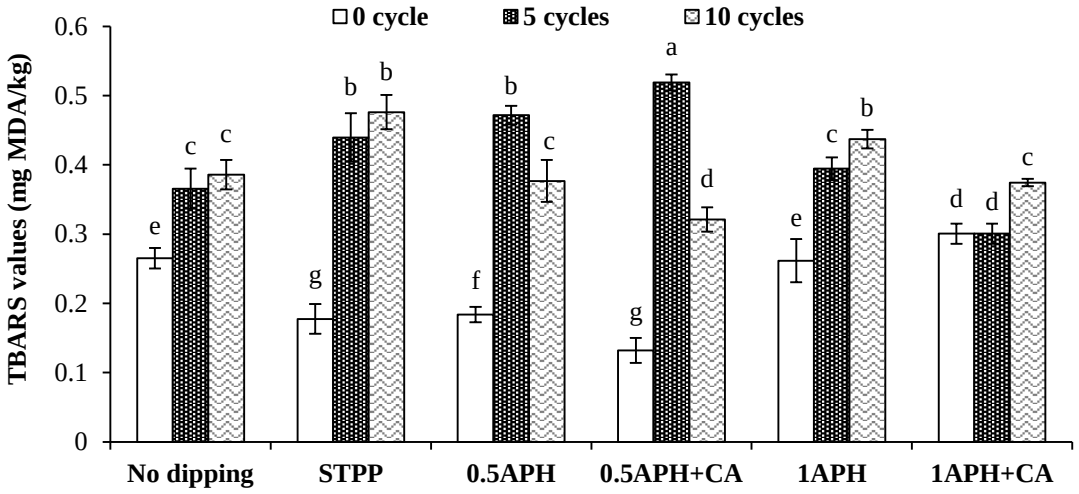

Figure 4 TBARS values of tilapia fillets treated with various dipping solutions at 0 and 10 FT cycles (No dipping = fillets without dipping, STPP = 0.5% sodium tripolyphosphate, 0.5APH = 0.5% alkali-aided extracted protein hydrolysates, 1APH = 1% alkali-aided extracted protein hydrolysates, CA = 0.05% citric acid). Different letters on each bar indicate significant differences ($P<0.05$) among treatments and freeze/thaw cycles.

3.6 Lipid structure changes by FTIR spectroscopy

Changes of lipids in food during processing and/or storage can be monitored using FTIR spectroscopy by determining frequency changes in specific bands. This technique allows one to differentiate the oxidation stages of analyzed lipid samples [34]. Figure 5 represents the FTIR spectrum of freeze dried tilapia fillets obtained between wavenumbers around 3500 and 800 cm^{-1}.

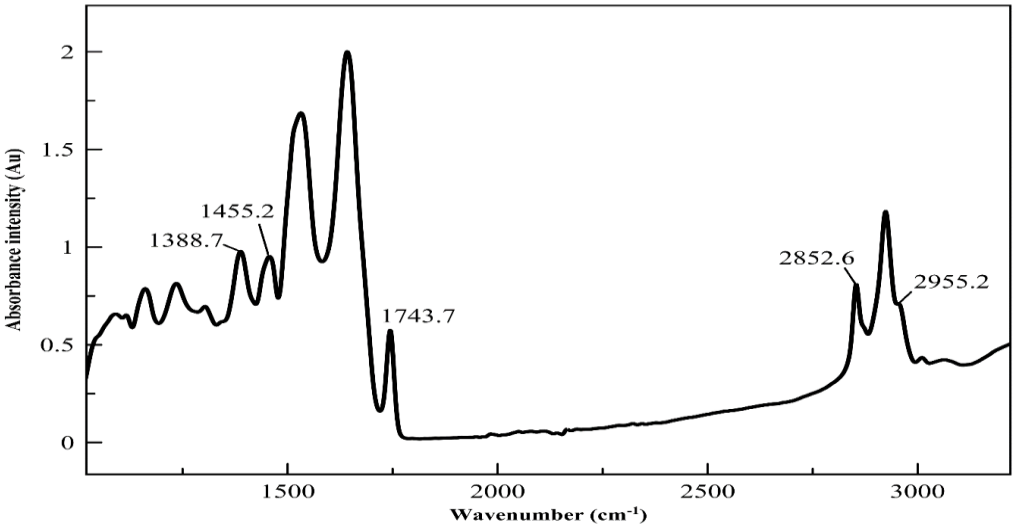

Figure 5 Representative FTIR spectrum of freeze dried tilapia fillets in 3500-800 cm^{-1} region.

The important peaks involving lipid functional groups include 2926 and 2854 (symmetric and asymmetric stretching vibrations of CH_2, respectively), and 1745 (ester C=O stretching vibrations) [35]. Figures 6A, 6B, and 6C show peak intensities at 2924, 2854, and 1744 cm^{-1}, respectively, of various freeze dried fillet treatments at 0, 5, and 10 FT cycles. The results obtained show similar changes of peak intensities during storage. Peaks of No dipping at all wavenumbers demonstrate an increased intensity when FT cycles are increased. Whereas, a decreased trend can be seen in all dipping treatments, except for the 1APH+CA which has a constant intensity. Giménez et al. [35] also reports that bands in wave numbers 2925 and 2854 cm^{-1} detected in oils containing polyunsaturated fatty acids decrease with oxidation. The peak at around 1750 cm^{-1} typically responds to the carbonyl absorption of triglycerides ester linkage, which is a significant FTIR spectra denoting lipid oxidation. Tenyang et al. [36] disclosed that boiled catfish oil had a stronger band at ~1750 cm^{-1}, meaning higher carbonyl groups of ester linkage of triglycerides. In addition, changes in these three FTIR peaks especially for the 1APH+CA were rather concomitant with the changes in the TBARS values (Figure 4). Nevertheless, no significant difference was discernible among dipping treatments and FT cycles (data not shown). From these results, it can be stated that the 1APH+CA dipping solution showed the most effective prevention of lipid oxidation in fish fillets.

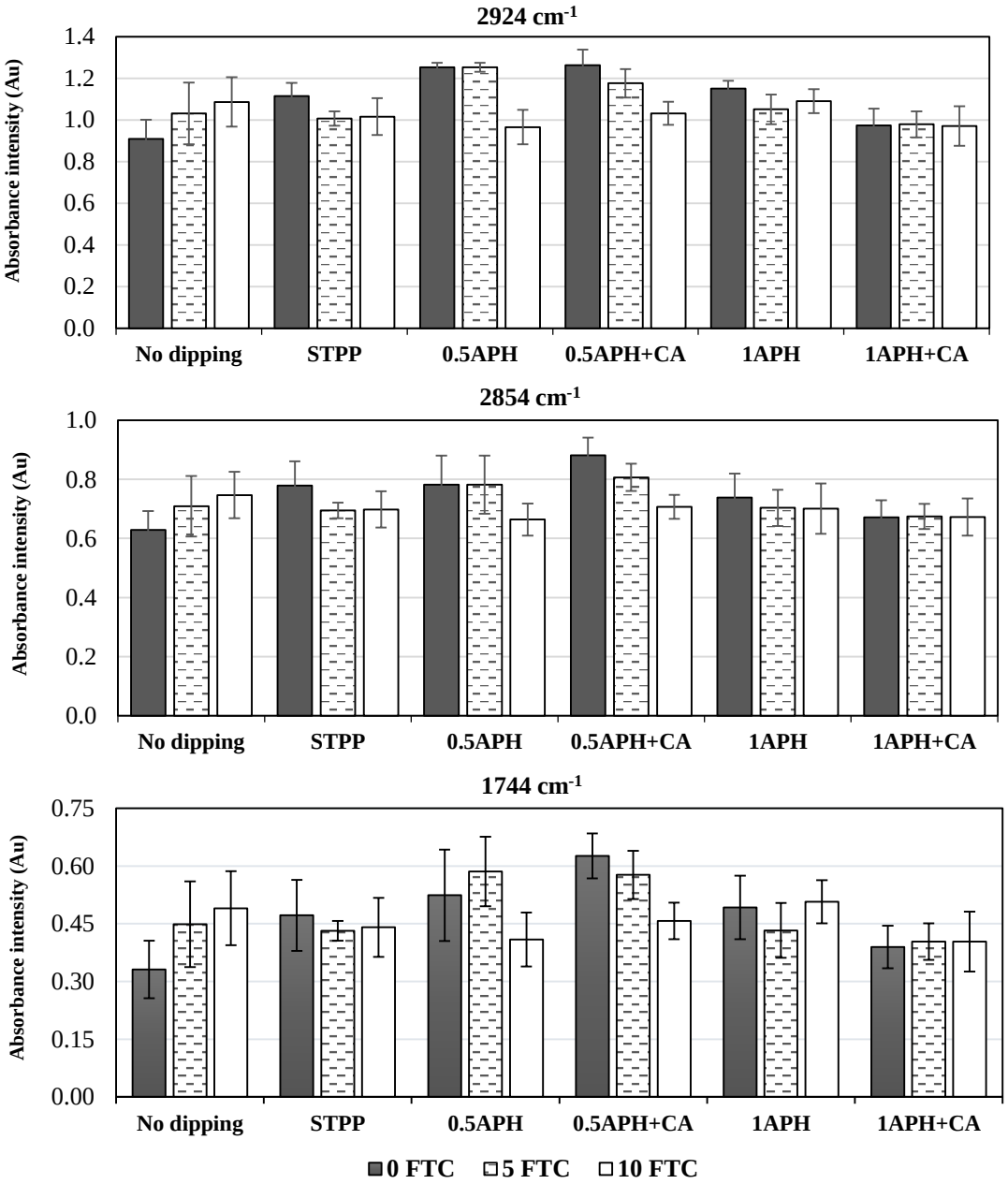

Figure 6 FT-IR intensity at wavenumbers 2924, 2854, and 1744 cm^{-1} of fillets treated with various dipping solutions at 0, 5 and 10 FT cycles. (No dipping = fillets without dipping, STPP = 0.5% sodium tripolyphosphate, 0.5APH = 0.5% alkali-aided extracted protein hydrolysates, 1APH = 1% alkali-aided extracted protein hydrolysates, CA = 0.05% citric acid).

4. Conclusion

The alkali-treated protein hydrolysates (APH) dipping solution demonstrated high ABTS radical scavenging activity rather than metal chelating ability. The solution containing 1% APH plus 0.05% citric acid can retain protein solubility and whiteness as well as slow down lipid oxidation of the fillets. Therefore, the synergistic effect of APH derived from tilapia byproducts and citric acid was able to extend the oxidative shelf-life and preserve qualities (drip loss, color, and protein solubility) of tilapia fillets during accelerated conditions of frozen storage. The results of this study suggest that hydrolysates of protein recovered from tilapia byproducts can be applied as a natural antioxidant to prevent oxidation reactions in fish products i.e. frozen glazed fillets.

5. Acknowledgements

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