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Investigate lipid and protein oxidation in herring (*Sardinella gibbosa*) by sodium acetate during preservative time

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ABSTRACT

The study was carried out to investigate lipid and protein oxidation of herring (*Sardinella gibbosa*) that was treated with (2.5% w/v) sodium acetate at 4°C. After 3, 6, 9, and 12 days, lipid and protein oxidation were evaluated by indications such as pH value, peroxide (PV), thiobarbituric acid-reactive substances (TBARS), protease enzyme activity and sulfhydryl. The results showed that, pH was from 6.33 ± 0.005 to 7.42 ± 0.005 ($p < 0.05$); PV was 1.73 ± 0.31 to 7.13 ± 0.5 mEq/kg ($p < 0.05$), and TBA was from 53.3 ± 0.28 µmol MDA/kg to 207.4 ± 0.47 µmol MDA/kg ($p < 0.05$). Protease enzyme activity reduced from 0.323–0.175 U/mg protein ($p < 0.05$), and sulfhydryl was from 14.39–7.198 µmol/g protein ($p < 0.05$). Consequently, sodium acetate effectively prevents lipid and protein oxidation, extending herring meat's life.

Keywords: Herring, natri acetate, lipid oxidation, protein oxidation.

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INTRODUCTION

Fish is a nutrient source that is necessary and beneficial for human health because of its high protein content and high unsaturated fatty acids in lipid composition [1]. However, the high protein and lipid content is the cause of impulsing spoilage and toxicity from fish after death, leading to physico-chemical changes that are the main cause of quality loss. Fish meat is easily oxidized due to its high content of unsaturated fatty acids [2] and damaged by enzymatic hydrolysis and microbial growth [3]. After death, lipids in fish are changed, leading to lipolysis and auto-oxidation [4]. Lipid and protein oxidation in fish preservation needs special attention because this process loses nutrients and creates unpleasant odors.

Lipid oxidation is a complex process in which unsaturated fatty acids react with molecular oxygen. It is often through a free radical mechanism forming hydroperoxides and the primary oxidation product [5]. In parallel with lipid oxidation, protein oxidation is also influenced by many factors, including proteolytic enzyme (protease) activity. The protease enzyme catalyzes the hydrolysis of peptide bonds $(–CO–NH–)_n$ in protein molecules into peptones, polypeptides, peptides, amino acids, and final volatiles with unpleasant odors such as ammonia, hydrogen sulfide, indole, scatole, and phenol. Protease enzymes can be endogenous or bacterial.

Many studies have used various preservation methods to prolong the shelf life while demonstrating the safety of fresh foods, including fishery products [6]. Sodium salts of organic acids such as acetic, lactic, and citric acids, which contain anti-microbial, antioxidant and flavor-enhancing have been proposed to extend the shelf life of rainbow trout [7], Persian sturgeon [8], as well as rainbow trout fillets [9].

Herring is rich in nutrients with high protein, lipids, and fatty acids, especially polyunsaturated fatty acids. Therefore, it is necessary to pay attention to quality improvement and extend the shelf life of this valuable commodity during storage. This study investigates sodium acetate's antioxidative properties in prolonging herring's storage duration.

MATERIALS AND METHODS

Preparation and treatment of fish sample

Herring (*Sardinella gibbosa*), with an average weight of 55–60 g, was bought from Cua Be port, Nha Trang city, Khanh Hoa province, Vietnam in June 2022. Whole fish were immediately washed and kept in ice with a fish/ice ratio of 1/2 (w/w) and transported to the Marine Biochemistry laboratory, Institute of Oceanography.

The fish were removed from the head, gutted, washed, and drained. Before treatment with sodium acetate, pH, moister, lipid, protein, peroxide, TBARS, protease activity, and SH group were assessed in the fresh sample. The schematic diagram of the experiment is described in Figure 1. Sodium acetate solutions were prepared at a concentration of 2.5%.

Figure 1. Herring (*Sardinella gibbosa*) is using in the research

The fish was dipped in pre-chilled (4°C) solutions of sodium acetate for up to 10 minutes. The ratio of the fish to each solution was 1:2 (g:mL). The control sample was not dipped in sodium acetate solution. The treatments were placed in Styrofoam boxes separately and packaged by over-wrapping them with polyvinylidene film. The samples were then stored under refrigerated conditions (4°C) for up to 12 days. After 3; 6; 9, and 12 days of storage, fish samples were determined for parameters including pH, peroxide and TBARS, protease enzyme activity, and sulfhydryl index (–SH).

Preparation of chemicals

All of the chemicals used in this study were of analytical grade obtained from Sigma-Aldrich (Steinheim, Germany).

Physicochemical analysis

The fish were analyzed in triplicate for moisture content according to the standard methods of AOAC (2016) [10]. Lipid was extracted according to Bligh and Dyer's method (1959) [11], protein was measured using Bradford assay (1976) [12].

Measuring pH: 20 g of fish muscle was well mixed with 20 mL of KCl 0.15 M, and then the pH values were recorded with a pH meter [13].

Lipid oxidation measurement

Measurement of peroxide (PV)

The peroxide value was determined using the standard AOAC method (2016) [10]. Minced fish (5 g) was thoroughly homogenized with 20 mL of chloroform: methanol solution (2:1) and shaken for one hour. The mixture was centrifuged at 700g at 25°C for 5 minutes. The obtained liquid (10 mL) was added to 25 mL of acetic acid: chloroform solution (3:2) into a 250 mL elernmeyer flask, and then added 1 mL of potassium iodide (KI) solution. The mixture was shaken for 1 min and incubated in the dark for about 5 min for a complete reaction. After incubation, the solution was added 75 mL of distilled water shaken well, and 1 mL of 1% starch indicator was added. The mixture was titrated with 0.01 N $Na_2S_2O_3$ until the solution turned colorless, and the volume of titrated solution was recorded to calculate the result. The results were expressed as milliequivalents of peroxide per kg of lipid (mEq/kg).

Measurement of TBARS

The TBARS value was determined according to Lemon (1975) [14] with slight modifications. 5 g of minced fish was homogenized in a 10 mL trichloroacetic acid (TCA) 7.5% solution, extracted for 15 minutes, and filtered through filter paper. The obtained liquid was added to 0.02 M thiobarbituric acid (TBA) in equal volumes to obtain a total volume of 10 mL. The mixture was heated and kept at 90°C for 40 minutes. It was then cooled under running water to room temperature before measuring the absorbance at 532 nm. The results were expressed as μmol malondialdehyde per kg fish sample (μmol MDA/kg).

Protein oxidation measurement

Determination of protease enzyme activity (U/mg protein)

The activity of the protease enzyme was determined according to the Anson method (1938) [15] through the amount of tyrosine formed from 1% casein hydrolysis in 30 minutes at room temperature (28 ± 2°C). Determination of formed tyrosine was based on the chromogenic reaction with folin, using 1 mL of filtrate, 2 mL of 0.5 N NaOH, 0.5 mL of folin, reaction time 10 minutes, and measuring the absorbance at 660 nm. Stop the reaction using 2 mL trichloroacetic acid (TCA).

Measurement of sulfhydryl

Sulphydryl groups (thiol content) were measured using the method of Ellman (1959) [16] with a slight modification. 0.5 g of minced herring muscle was homogenized in 10 mL of

0.05 M Tris-HCl buffer (pH 8). 1 mL of the homogenate was mixed well with 9 mL of Ellman buffer (pH 8) (containing 0.6 M NaCl, 6 mM ethylenediaminetetraacetic acid (EDTA), 8 M Urea, 2% sodium dodecyl sulfate (SDS). The mixture was vortexed and centrifuged (1,400 g; 15 minutes, 5°C) to remove the residue. 3 mL of the centrifuge solution was added to 40 µL of 0.01 M DTNB (2-nitrobenzoic acid) reconstituted in 0.05 M sodium acetate. All samples were vortexed and incubated at 40°C for 15 minutes. The mixture was absorbance at 412 nm to determine the sulfhydryl group content. The sulphydryl content was determined using a molar extinction coefficient of 13,600 M^{-1} cm^{-1} and expressed as (µmol/g protein).

Data analysis

The obtained data were calculated using Microsoft Excel 2010, and the graphs were generated using R software. Means were compared by using ANOVA and the Turkey test. The significance of differences was defined at the 5% level ($p < 0.05$).

RESULTES AND DISCUSSION

Proximate Composition

The proximate composition of herring averaged: 73.1 ± 0.62% moisture, 22.14 ± 0.63% crude protein, and 5.62 ± 0.63% crude lipid. These results are similar to the values reported by Chrisolite et al., (2016) [17] in herring from the Thoothukudi coast. This protein's lipid and moisture content varied from 15.43% to 22.76%, 1.25% to 6.77%, and 70.79% to 78.16%, respectively. The proximate composition of fish mainly depends on several factors like diet, size, sex, physiological state of fish and ecological conditions [18].

Changes in pH values

The changes in pH values of herring during the 12 days are presented in Figure 2. During the storage period, the pH values increased, and there was a difference between the control and experimental samples ($p < 0.05$) from the sixth day. Haghparast et al., (2010) [7] reported that the pH in rainbow trout (*Chorhynchus mykiss*) treated with sodium acetate was also significantly lower than the control sample under cold storage conditions.

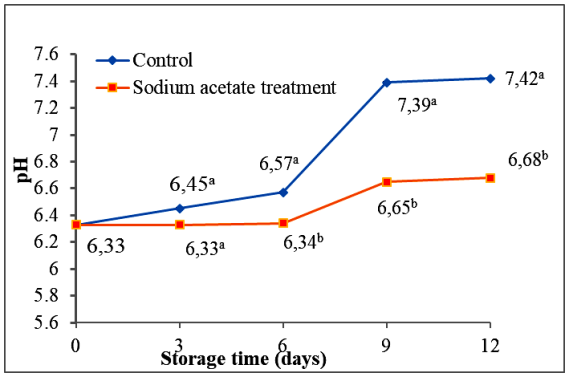

Figure 2. Change of PH during storage at 4°C of herring muscle (Mean with different letters significantly differ ($p \leq 0.05$); the error bars show the standard deviation)

The pH change in fish muscle during storage is mainly due to the breakdown of ATP and the release of glycogen to form H^+. In addition, after a period of storage, amino acids, and organic compounds create NH_3, which changes the pH of fish muscle [13, 19].

Peroxide value assessment

Peroxide value is a primary indicator of oxidation of fat [20]. Changes in the mean PV content of the samples are depicted in the Figure 3. The initial PV in herring analyzed was ranged from 1.73 mEq/kg to 7.13 mEq/kg. In general, there is a clear change in PV value between storage days. The control samples had a faster increase in PV than the sodium acetate treated samples ($p < 0.05$), especially at day 9. Peroxide is an unstable compound that will eventually become malonaldehyde, and this material can be stablished with the amino acid crosslinking and the result is production of amine bonds [21]. The maximum PV was found to be 7.13 mEq/kg on the day of 9 and decreased by the end of storage period. This

result is similar to the study of Chaijan et al., (2006) [22] on frozen herring. They reported that the PV value increased to day 9 then gradually decreased to day 15 of the storage process. According to Alghazeer et al., (2008) [23], PV is the primary product of lipid oxidation, which easily oxidized to form secondary products such as aldehydes and ketones and other non-radical compounds. The PV value of the sodium acetate treated samples was smaller than the control samples, indicating that the sodium acetate solution can inhibit the formation of primary lipid oxidation in herring during cold storage.

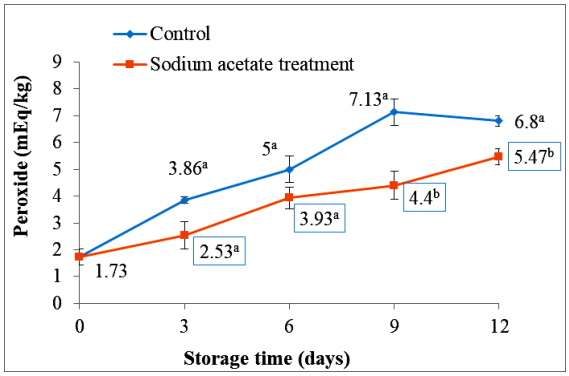

Figure 3. Change of PV during storage at 4°C of herring muscle (Mean with different letter significantly differ ($p \leq 0.05$), the error bars show the standard deviation)

TBARS assessment

Secondary oxidation product accumulation was were measured by determining the TBARS [24]. The study results in Figure 4 show that the TBARS index has a marked change between storage days. The control sample had a faster increase in TBARS value than the sodium acetate-treated samples ($p < 0.05$). TBARS value in the treated samples in the first three days of storage increased slowly (from 53.3–85.5 µmolMDA/kg) and increased rapidly after day 6 (from 85.5–157.3 µmolMDA/ kg); however, this value is still lower than the control samples. The TBARS value in the control samples from day 3 to day 9 gradually increased and then decrease again on day 12. This result is similar to the study of Tran Minh Phu et al., (2018) [25] on the snakehead (*Channa striata*) fillet under ice storage in combination with acetic acid treatment. The decrease in TBARS is because secondary oxidation products continue to be converted into other products under the influence of enzymes and microorganisms [26]; at point the oxidation products have been completely decomposed. The TBARS value of the treated samples was always smaller than that of the control samples, indicating that sodium acetate can inhibit the formation of secondary lipid oxidation in herring after cold storage.

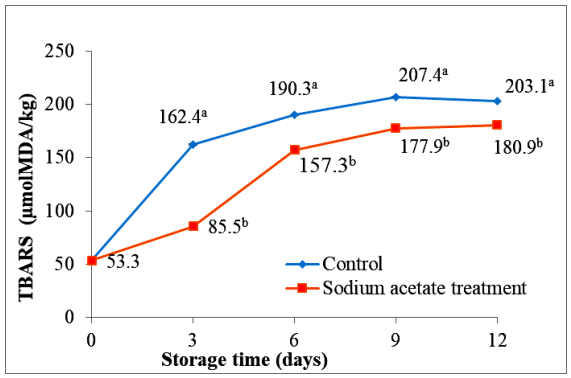

Figure 4. Change of TBARS during storage at 4°C of herring muscle (Mean with different letter significantly differ ($p \leq 0.05$), the error bars show the standard deviation)

Changes in protease enzyme activity

In general, after 12 days of storage, the protease activity gradually decreased (Fig. 5), and there was a significant difference in protease activity among treatments during storage ($p > 0.05$). The enzyme activity in the sodium acetate-treated samples decreased faster than in the control samples (from 0.323–0.175 U/mg protein), which means that soaking in sodium acetate helps prevent the growth of bacteria, inhibits enzyme activity, thereby slowing down the oxidation of protein in fish during storage. This result is similar to the result of Nguyen Van Muoi et al., (2019) [27], demonstrating that dipping snakehead (*Channa striata*) in NaCl, protease activity also decreased during 15 days of refrigerated storage. Protein oxidation leads to structural

changes of different amino acids, reducing the number of carbonyl groups and sulfhydryl groups [28, 29], thereby changing water holding capacity, the meat's elasticity, and nutritional value [30].

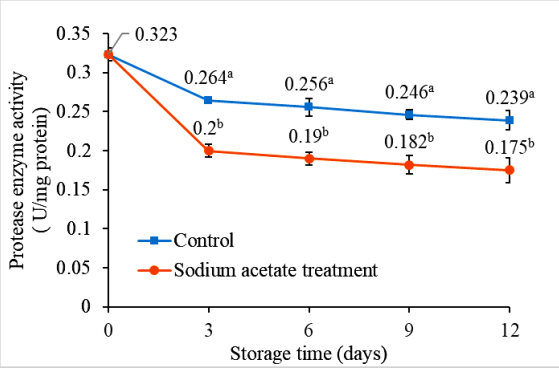

Figure 5. Change of protease enzyme activity during storage at 4°C of herring muscle (Mean with different letter significantly differ ($p \leq 0.05$), the error bars show the standard deviation)

Sunfhydryl (SH) assessment

The sulfhydryl group (–SH) measures protein oxidation, specifically cysteine oxidation. Oxidation of cysteine causes and forms a disulfide bridge; therefore high sulfhydryl group shows low oxidation and vice versa [31]. The results of sulfhydryl group determinations are shown in Figure 6. The sulfhydryl group showed a significant ($p < 0.05$) decrease in storage in all samples, indicating that part of the sulphydryl group is oxidized to disulfides. A decrease in total sulphydryl group content was reported due to the formation of disulfide bonds through the oxidation of sulphydryl groups or disulfide interchanges [32, 33].

The sulfhydryl content in the sodium acetate-treated samples, decreased more strongly than in the control samples. Thus, samples treated with sodium acetate before storage will reduce cysteine oxidation, meaning protein oxidation takes place more slowly than samples without sodium acetate treatment. After the 6th day of storage, the sulfhydryl content in all samples decreased strongly, showing that the protein oxidation process was intense at this time, and the fish showed signs of spoilage. Nguyen Van Muoi et al., (2019) [27] reported that when using NaCl in preserving snakehead (*Channa striata*) at cold temperatures, the sulfhydryl content in the fish also decreased gradually. Besides, Berna et al., (2017), and Aria et al., (2015) [34, 35] also had similar records in the preservation of mackerel (*Scomber scombrus*).

Consequently, the reduction of protease enzyme activity will limit the oxidation of cysteine, which is shown by the reduction of the sulfhydryl content. The decrease in protease enzyme activity, the lower the sulfhydryl content is, and vice versa.

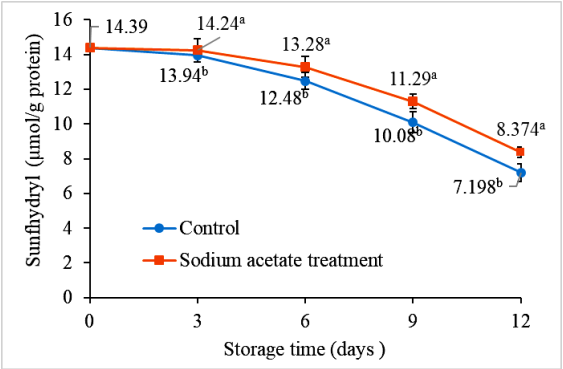

Figure 6. Change of sunfhydryl during storage at 4°C of herring muscle (Mean with different letter significantly differ ($p \leq 0.05$), the error bars show the standard deviation)

In general, lipid and protein oxidation occurred in parallel during cold storage. However, the reduction of protease enzyme activity due to the action of sodium acetate salt and especially the storage conditions at low temperatures had a positive effect in limiting lipid oxidation and protein oxidation in herring muscle.

CONCLUSION

This study demonstrates that a sodium acetate solution of 2.5% can reduce the rate of lipid oxidation and protein oxidation in herring, so that they can be employed as valuable

antioxidants. Therefore, sodium acetate can be a safe organic preservative for refrigerated fish.

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REFERENCES

[1] Hu, F. B., Bronner, L., Willett, W. C., Stampfer, M. J., Rexrode, K. M., Albert, C. M., Hunter, D., and Manson, J. E., 2002. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *Jama*, *287*(14), 1815–1821. doi:10.1001/jama.287.14.1815

[2] Sullivan, J. C., and Budge, S. M., 2012. Fish oil sensory properties can be predicted using key oxidative volatiles. *European Journal of Lipid Science and Technology*, *114*(5), 496–503. https://doi.org/10.1002/ejlt.201100330

[3] Uçak, İ., Özogul, Y., and Durmuş, M., 2011. The effects of rosemary extract combination with vacuum packing on the quality changes of Atlantic mackerel fish burgers. *International Journal of Food Science & Technology*, *46*(6), 1157–1163. https://doi.org/10.1111/j.1365-2621.2011.02610.x

[4] Hardy, R., 1980. Fish lipids. Part 2. In Connell, J. J., (ed.). *Advances in Fish science and Technology. Oxford: Fishing News*. 103 p.

[5] Simic, M. G., and Taylor, K. A., 1987. Free radical mechanisms of oxidation reactions. *Warmed-over flavor of meat*, 69–72.

[6] Sallam, K. I., 2007. Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. *Food Control*, *18*(5), 566–575. https://doi.org/10.1016/j.foodcont.2006.02.002

[7] Haghparast, S., Kashiri, H., Shabanpour, B., and Pahlavani, M. H., 2010. Antioxidant properties of sodium acetate, sodium citrate and sodium lactate on lipid oxidation in rainbow trout (*Onchorhynchus mykiss*) sticks during refrigerated storage (4°C). *Iranian Journal of Fisheries Sciences*, *9*(1), 73–86. doi: 10.22092/IJFS.2018.114084

[8] Kashiri, H., Haghparast, S., and Shabanpour, B., 2011. Effects of sodium salt solutions (sodium acetate, lactate and citrate) on physicochemical and sensory characteristics of Persian sturgeon (*Acipenser persicus*) fillets under refrigerated storage. *Journal of Agricultural Science and Technology*, *13*(1), 89–98.

[9] Yağin, C., and Büyükyörük, S., 2017. The effects of chitosan, sodium lactate and sodium diacetate on the shelf life of hot smoked and vacuum packed rainbow trout fillets. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *64*(1), 1–6. doi: 10.1501/Vetfak_0000002765

[10] Latimer, G. W., 2016. Official methods of Analysis of AOAC International. 20th Edition. *AOAC International, Suite 300, 2275 Research BLVD, Rockville, Maryland 20850–3250, USA*

[11] Bligh, E. G., and Dyer, W. J., 1959. A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology*, *37*(8), 911–917. https://doi.org/10.1139/o59-099

[12] Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

[13] Hultmann, L., Phu, T. M., Tobiassen, T., Aas-Hansen, Ø., and Rustad, T., 2012. Effects of pre-slaughter stress on proteolytic enzyme activities and muscle quality of farmed Atlantic cod (*Gadus morhua*). *Food chemistry*, *134*(3), 1399–1408. https://doi.org/10.1016/j.foodchem.2012.03.038

[14] Lemon, D. W., 1975. An improved TBA test for rancidity new series circular, No: 51. *Halifax, NS, Canada: Halifax-Laboratory*.

[15] Anson, M. L., 1938. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. *The Journal of General Physiology*, *22*(1), 79–89. doi: 10.1085/jgp.22.1.79

[16] Ellman, G. L., 1959. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, *82*(1), 70–77. https://doi.org/10.1016/0003-9861(59)90090-6

[17] Kannaiyan, K., 2016. Seasonal variation in the proximate composition of sardine (Sardinella gibbosa) from Thoothukudi coast. *Indian Journal of Geo-Marine Science*, *45*(6), 800–806.

[18] Conell, J. J., 1975. Advances in Fish Sciences and Technology. *Fishing news books, Surrey, England*. pp. 88–92.

[19] Duun, A. S., and Rustad, T., 2007. Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. *Food chemistry*, *105*(3), 1067–1075. https://doi.org/10.1016/j.foodchem.2007.05.020

[20] Adeyemi, O. T., Osilesi, O. O., Onajobi, F., Adebawo, O., and Afolayan, A. J., 2013. Stability study of smoked fish, horse mackerel (*Trachurus trachurus*) by different methods and storage at room temperature. *African Journal of Biochemistry Research*, *7*(6), 98–106. doi: 10.5897/AJBR2013.0672

[21] Cakli, S., Kilinc, B. E. R. N. A., Cadun, A., Dincer, T., and Tolasa, S., 2007. Quality differences of whole ungutted sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) while stored in ice. *Food Control*, *18*(5), 391–397. https://doi.org/10.1016/j.foodcont.2005.11.005

[22] Chaijan, M., Benjakul, S., Visessanguan, W., and Faustman, C., 2006. Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. *Food Chemistry*, *99*(1), 83–91. https://doi.org/10.1016/j.foodchem.2005.07.022

[23] Alghazeer, R., Saeed, S., and Howell, N. K., 2008. Aldehyde formation in frozen mackerel (*Scomber scombrus*) in the presence and absence of instant green tea. *Food Chemistry*, *108*(3), 801–810. https://doi.org/10.1016/j.foodchem.2007.08.067

[24] Pegg, R. B., 2001. Spectrophotometric measurement of secondary lipid oxidation products. *Current Protocols in Food Analytical Chemistry*, *1*(1), D2–4. https://doi.org/10.1002/0471142913.fad0204s01

[25] Phu, T. M., Trinh, D. T. M., and Thuy, L. T. M., 2018. Ice storage of snakehead fillet (*Channa striata*) combined with acetic acid treatment. *Can Tho University Journal of Science*, *54*, 147–155. (in Vietnamese).

[26] Nirmal, N. P., and Benjakul, S., 2009. Melanosis and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) treated with catechin during iced storage. *Journal of Agricultural and Food Chemistry*, *57*(9), 3578–3586. https://doi.org/10.1021/jf900051e

[27] Muoi, N. V., Truc, T. T., and Long, B. T., 2019. Effect of cold storage in combination with salting on lipid and protein oxidation of snakehead (*Channa striata*) muscle. *Can Tho University Journal of Science*, *55*(2), 258–266. (in Vietnamese).

[28] Martinaud, A., Mercier, Y., Marinova, P., Tassy, C., Gatellier, P., and Renerre, M., 1997. Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *Journal of Agricultural and Food Chemistry*, *45*(7), 2481–2487. doi: 10.1021/jf960977g

[29] Xiong, H., Zeng, Y. C., Lewis, T., Zheng, J., Persidsky, Y., and Gendelman, H. E., 2000. HIV-1 infected mononuclear phagocyte secretory products affect neuronal physiology leading to cellular demise: Relevance for HIV-1-associated dementia. *Journal of Neurovirology*, *6*(SUPPL. 1), S14–S23.

[30] Rowe, L. J., Maddock, K. R., Lonergan, S. M., and Huff-Lonergan, E., 2004. Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. *Journal of Animal Science*, *82*(11), 3254–3266. https://doi.org/10.2527/2004.82113254x

[31] Lara, M. S., Gutierrez, J. I., Timón, M., and Andrés, A. I., 2011. Evaluation of two natural extracts (*Rosmarinus officinalis* L. and *Melissa officinalis* L.) as antioxidants in cooked pork patties packed in MAP. *Meat Science*, *88*(3), 481–488. https://doi.org/10.1016/j.meatsci.2011.01.030

[32] Shigeru, H., and Shuryo, N., 1985. Contribution of hydrophobicity, net charge and sulfhydryl groups to thermal properties of ovalbumin. *Canadian Institute of Food Science and Technology Journal*, *18*(4), 290–295. https://doi.org/10.1016/S0315-5463(85)71960-8

[33] Lanier T. C., 2000. Surimi gelation chemistry. In: Park J. W. (ed.), *Surimi and Surimi Seafood. New York: Marcel Dekker Inc.*, pp. 237–265.

[34] Özalp Özen, B., and Soyer, A., 2018. Effect of plant extracts on lipid and protein oxidation of mackerel (*Scomber scombrus*) mince during frozen storage. *Journal of food science and technology*, *55*, 120–127. https://doi.org/10.1007/s13197-017-2847-6

[35] Babakhani, A., Farvin, K. S., and Jacobsen, C., 2016. Antioxidative effect of seaweed extracts in chilled storage of minced Atlantic mackerel (*Scomber scombrus*): effect on lipid and protein oxidation. *Food and bioprocess technology*, *9*, 352–364. https://doi.org/10.1007/s11947-015-1630-9