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# Effect of Chitosan Coating Farmed Trout (*Oncorhynchus mykiss)* that Enriched with α-Tocopherol on Lipid Damages during Refrigerated Storage

**Tolouie, H[1]. Mohtadi Nia, J[2]. Shakibi, A[3]. Jalaliani, H[*.1]**

[1]M.Sc of Food Science, Tabriz University of Medical Sciences, Iran
[2]Associated Professor of Food Science and Technology, Tabriz University of Medical Sciences, Iran
[3]Msc of Marine ecology, Department of Marine Science and Technology
Tehran Science and Research Branch, Islamic Azad University, Iran
[4]M.Sc of Food Technology, Department of Food Technology, Amol Science and Research Ayatollah Amoli Branch, Islamic Azad University, Iran

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## ABSTRACT

Omega-3 fatty acids as an increasingly important part of diet for health and prevention of disease would be applied. In this study in order to reduce lipid oxidation in the farmed trout, α-tocopherol in chitosan coating, was used. Chitosan solution (2%) and chitosan incorporated α-tocopherol in two concentrations (0.2% and 0.4%) were prepared for coating. The control and the coated samples were analyzed periodically at 7 days intervals to determine peroxide value (PV), anisidine value (AV), totox value (TV) and free fatty acid content (FFA). Fatty acid profile also determined by gas chromatography assay. As compared with the control samples, less changes were observed in PV, AV, TV, FFA, proportion of n6/n3 polyunsaturated fatty acids and $C22:6n_3/C16:0$ ratio between chitosan and α-tocopherol-chitosan treated samples during 28 day refrigeration. Consequently, the samples coated by chitosan enriched with α-tocopherol at any level exhibited less rapidly lipid damages than all the other samples ($p<0.05$).
**KEYWORDS:** Chitosan, Trout, α -Tocopherol, Oxidation.

## 1. INTRODUCTION

Lipid oxidation is a degradative free radical reaction that causes loss of shelf life, palatability, functionality and nutritional quality of oils, fats and foods containing unsaturated lipids. The nutritional importance of fish consumption is in great extent associated with its advantageous fatty acid profile (Sidhu 2003; Prato and Biandolino 2012). Fish oil is the most direct and complete source of long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs), which likely provide much of the health benefits. Both epidemiological and interventional studies have demonstrated that marine n-3 PUFAs, play an important role in the prevention and treatment of coronary artery disease (Lamm, Dehaven and Riggs 2000; Uauy and Valenzuela 2000), hypertension (Riggs et al. 2002), diabetes (Markl et al. 2001), arthritis and other inflammatory and autoimmune disorders (Simopoulos, 2002).

Lipid peroxidation in fish and seafood affects their nutritive value and may lead to disease following the consumption of products that could potentially cause a toxic reaction (Je et al. 2007; Jung et al. 2007). Hence, efficient method to minimize lipid oxidation in fish oil-enriched foods are necessary in order to make such kind of foods successful in the marketplace (Horn, Nielsen and Jacobsen 2009; Jacobsen and Let 2006).

Interest in chitosan, a biodegradable, non-toxic, non-antigenic and biocompatible biopolymer, arisen from the fact that chitosan are reported to exhibit numerous health-related beneficial effects, including antitumor, hemostatic and antimicrobial activities in foods (No et al. 2002; Chung and Chen 2008; Friedman and Juneja 2010). previous researchers have reported chitosan antimicrobial activity against a variety of pathogenic and spoilage microorganisms, including fungi and Gram-positive and Gram negative bacteria (Shahidi, Arachchi and Jeon 1999; Zheng and Zhu 2003; Sayas-Barberá et al. 2011). However, chitosan does not have significant antioxidant Activity (Kanatt, Chander and Sharma 2008).

Numerous studies have indicated that lipid oxidation in meat and meat products may be controlled or minimized through the use of antioxidants (Gray, Gomaa and Buckley 1996; Nissen et al. 2004; Georgantelis et al. 2007). As compared with other bio-based food packaging materials, chitosan has the advantage of being able to incorporate functional substances such as nutraceuticals and antioxidants (Chen et al. 2002; Jeon, Kamil and Shahidi, 2002; Möller et al. 2004; Dutta et al. 2009), due to the presence of a high density of amino groups and hydroxyl groups in the chitosan polymer structure (Park, Daeschel and Zhao 2004). It seems that The incorporation of natural antioxidant into chitosan coating can improve functionality, also fish lipids stability by controlling the

***Corresponding author:** Amide Jalaliani, M.Sc of Food Technology, Department of Food Technology, Amol Science and Research Ayatollah Amoli Branch, Islamic Azad University, Iran. e-mail: h_j460@yahoo.com,

oxidation of fatty components and pigments. α-tocopherol is highly potent and most common natural antioxidant (Frankel 1998; Georgantelis et al. 2007). Some of the studies revealed that α-tocopherol was more effective in animal fats than in vegetable oils, as they contain nearly no natural antioxidants also reported as more stable in PUFA rich oils (Yuki and Ishikawa 1976; Zuta et al. 2007).

In this study, Four different treatments were used: uncoated samples (C), chitosan 2% (Ch), chitosan 2% incorporated 0.2% α-tocopherol (Ch+Toc 1) and chitosan 2% incorporated 0.4% α-tocopherol (Ch+Toc 2). The aim of this study was to evaluate the effects of these treatments on the oxidation of trout lipids during 4 weeks refrigerated storage (4±1ºC) and thereby select the most appropriate treatment, which could minimize lipid damages and surpass PUFAs oxidation in treated fish during storage time.

## 2. MATERIAL AND METHODS

### 2.1. Materials

Commercial food grade chitosan with a low molecular weight supplied by Sigma-Aldrich, Tween 80 and α-tocopherol with 99% purity was purchased from Merk Chemical Company. All other chemicals and reagents used were analytical grade.

### 2.2. Coating preparation

Chitosan coating was prepared by dissolving 2% w/v chitosan in a 1% v/v acetic acid solution. For complete dissolution of chitosan, this solution was left for 4 h under agitation using a hotplate magnetic stirrer at 40ºC. Then glycerol was added to chitosan at 0.75 ml/g concentration and stirred for more 15 min. The resultant chitosan coating solution was filtrated through a Whatman No. 2 filter paper to remove any undissolved particles.

Both coating containing α-tocopherol were prepared exactly the same as the control chitosan coating, after dissolving α-tocopherol in 0.2% w/v Tween 80 as a surfactant, α-tocopherol was added in two different concentration (0.2% or 0.4% w/v). The final coating forming solution was homogenised under aseptic conditions at 21600 rpm for 1 min (Polytron Kinamatica Inc., Cincinnati, USA).

### 2.3. Preparation of fish samples and storage conditions

Aquacultured freshwater trout (O. mykiss), varying from 600 g to 700 g in weight was purchase from an aquaculture farm and transferred to Tabriz university of medical sciences in Iran. After being gutted and washed, fish samples were randomly assigned into four treatment lots consisting of one control lot (uncoated) and three lots treated with the coating solutions. Fish samples were given a dip treatment in each coating solution for 3 min and then well drained in room temperature. After that, they were individually packed in polyethylene trays. All fish packs were stored in a refrigerator at 4±1 ºC for 28 days.

### 2.4. Lipid extraction

The lipids were extracted by the method described by Bligh and Dyer (1959). Approximately 50g minced fish sample were homogenized with chloroform/methanol at 2:1 v/v ratio, for 2 min, then 50 ml chloroform and 50 ml of water were added to the mixture, then it shaked on electric shaker. The homogenate was filtered through a Buchner funnel with Whatman no 1 paper, then the leachate was transferred to a graduated cylinder and allowed to stand for 24 h. the lower phase was separated and the solvent recovered by rotational vacuum evaporation.

### 2.5. Determination of peroxide value (PV)

Peroxide values of extracted oils were measured by titration of liberated iodine with standardized sodium thiosulphate solution in presence of 1.0% soluble starch as indicator, according to the AOAC official method (AOAC 1998).

### 2.6. Determination of anisidine value (AV)

Anisidine values (AV) was measured by AOCS Cd 18–90 standard method. For *p*-anisidine values, 0.5-4 g of extracted Fish oil was dissolved in 25 ml iso-octane as a solvent. The absorbance ($A_1$) was measured by spectrophotometer at 350 nm against a blank of iso-octane. An aliquot (5 ml) of the solution of fish oil or 5 ml of iso-octane (as blank) was transferred to each of two test tubes of 10 ml and 1 ml *p*-anisidine solution (0.25% g/v glacial acetic acid) was added to each. The test tubes were shaken and allowed to stand in dark place for 10 min. The absorbance ($A_2$) was measured at 350 nm against iso-octane containing *p*-anisidine. AV was calculated from the formula: $AV = 25\ (1.2\ A_2 - A_1)/\text{sample weight}$.

### 2.7. Determination of totox value (TV)

The totox value (TV) was used to estimate the oxidative deterioration of lipids. TV is defined as the sum of both values (peroxide and anisidine) to total oxidation: totox = 2PV + AV

### 2.8. Measurement of free fatty acid (FFA)

Free fatty acids, as oleic acid percentages in oil samples, were determined using an alkali titration method according to AOCS Official Method (AOCS 1993).

### 2.9. Determination of fatty acid profile

Fatty acid profile was determined in the fresh fish in first day and all groups of trout samples in day 21.

The extracted fish oil converted to methylesters and then were analyzed on gas chromatograph (equipped to Dony-1000, flame ionisation detector, Italy) by using DB-WAX column (0.25 mm i.d., 30 m length, 0.2 µm film thickness). The peaks were identified by using an external FAME standard (Supelco; Sigma–Aldrich). The initial temperature of the column was set at 100 ºC and held for 1 min. The temperature was then raised to 200 ºC which was held for 15 min followed by an increase at 230 ºC where it was held for 13 min, and then increased to 250 ºC temperature. Fatty acids were quantified by comparing their peaks with the relevant peak areas of the corresponding standard fatty acids where. Each fatty acid was reported a percentage of the total fatty acids and quantified subsequently.

### 2.10. Statistical analysis

The statistical significance of observed differences among treatment means was evaluated by Analysis of Variance (ANOVA) using $SPSS_{17}$. A confidence interval at the 95% level ($p<0.05$) was considered in all cases. The statistical significance of the differences was checked using the Turkey's student zed range test.

## 3. RESULTS AND DISCUSSION

Lipid oxidation was assessed through determination of primary (hydroperoxides), formation of secondary oxidation products was measured by *p*-anisidine value, and total Oxidation by totox value.

### 3.1. Peroxide value (PV)

Peroxide value (PV) is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Peroxide value is one of the most widely-used tests for the measurement of oxidative rancidity in oils and fats (Zhang et al. 2010). In general, the PV increased significantly during storage ($p<0.05$), significant differences ($p<0.05$) were observed in the PV between the control and each of coated samples. Jeon, Kamil and Shahidi (2002) found lower primary lipid oxidation in chitosan-coated herring and Atlantic cod samples throughout a 12 day storage at 4±1ºC. the similar result reported by Ojagh et al. (2010), that chitosan coatings is effective in retarding lipid oxidation in rainbow trout fillets at the same temperature. The mechanism by which chitosan inhibit lipid oxidation was thought to be the ability of sequester metal ions. Particularly those of iron in ferritin, hemoglobin, and myoglobin present in fish (Guzman et al. 2003; Feng et al. 2008).

As it was seen in **fig. 1** after four weeks of storage at 4°C, PV in fresh trout increased from 0.07 to 3.89 meq/1000g in the control sample and the 2.67, 1.82 and 0.97 in samples treated with ch, Ch-Toc 1 and Ch-Toc 2 respectively.

Modest increase in the PV of α-tocopherol-chitosan was observed in the coated samples. Both coating containing antioxidants have lower PV than in the control and chitosan coated samples in all experimental days (except the beginning). More efficiency inhibitory effect on primary oxidation observed as the content of α-tocopherol increased. It happens due to tocopherols work as antioxidants by donating the hydrogen of the hydroxyl group to the lipid peroxyl radical. Peroxy radicals react with tocopherols many times faster than with acyl lipids. Therefore, one tocopherol molecule can protect many magnitude greater numbers of polyunsaturated fatty acid molecules (Kamal-Eldin and Appelqvist 1996). tocopherols can also function as inhibitors of lipid oxidation by scavenging singlet oxygen molecules (Bradley and Min 1992)

Our result was in agreement with findings of Martins, Cerqueira and Vicente (2012) who found that chitosan Films with α-tocopherol exhibited a higher level of antioxidant activity. In contrast with these results Duan, Cherian and Zhao (2010) failed to detect any significant changes in antioxidant activity of oil incorporated chitosan coatings In presence of vitamin E during 3 weeks of cold storage of lingcod. It might happen duo to The Vitamin E (dl-tocopheryl acetate) that used in this study which is in a non-oxidizable form (Traber 1999; Han et al. 2004).

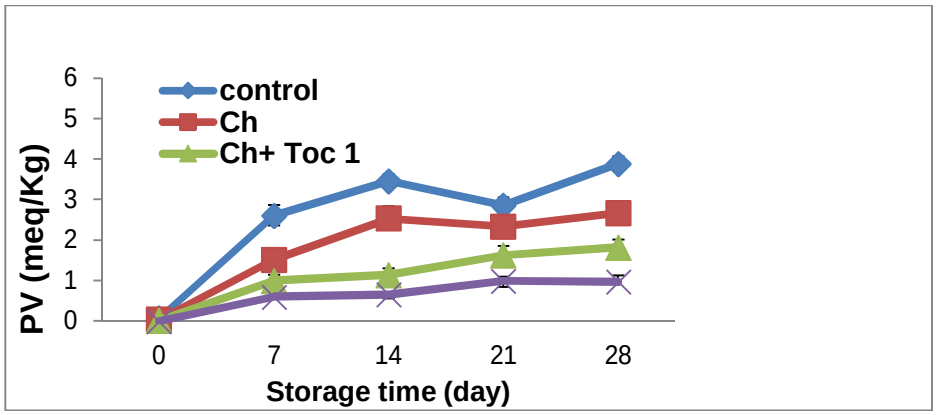


**Figure. 1** Various levels of peroxide values in the different treatments during refrigerated storage.

### 3.2. Anisidine value (AV)

The peroxide contents present in the oxidized lipid are transitory components that decompose into a variety of carbonylic and other compounds, this finding is very important as secondary oxidation products cause unpleasant changes in fish muscle. According to previous reports (Auburg 1993; Mexis, Chouliara and Kontominas 2009), TBA values may not reflect the actual rate of lipid oxidation since malondialdehyde can interact with components of fish muscle. So in this study the anisidine value was used as a measure of secondary oxidation products. The reactiveness of the aldehyde carbonyl bond on the *p*-anisidine amine group, leading to the formation of a Schiff base that absorbs at 350 nm (Laguerre, Lecomte and Villeneuve 2007). AV can indicate the oxidative history of the fat or oil, as the aldehydes normally originate from the oxidation of unsaturated fatty acids (O´ Sullivan et al. 2005).

**Fig. 2** shows the anisidine values of trout samples. AV increased significantly through out the storage time ($p<0.05$), increase in the control sample occurred at a high rate, while the increase in the AV of chitosan and Ch+Toc coated samples was less pronounced. Ch+Toc coated samples reached significantly ($p<0.05$) lower AV in comparison with the controls or Ch-coated samples. Georgantelis reported α-tocopherol has been associated with retarding the decomposition of hydroperoxides (Frankel 1998, Georgantelis et al. 2007).

In the current study the same results achieved for TBA in coated samples (data not shown). Sathivel et al. (2007) reported that chitosan coatings reduced the lipid oxidation in pink salmon fillets during the frozen storage. also Duan et al. (2010) found lower Secondary lipid oxidation products include malondialdehyde in chitosan-coated fresh lingcod fillets throughout a 3 weeks cold storage.

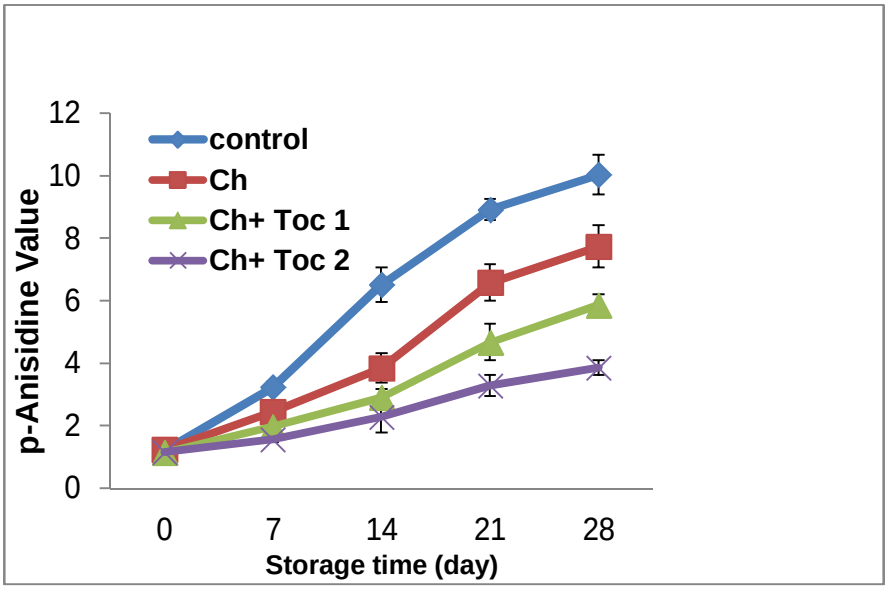


**Figure. 2** Various levels of anisidine values in the different treatments during refrigerated storage.

### 3.3. Totox value (TV)

The totox value was used as an indication of total oxidation. This parameter could depict a better picture of the overall quality status of the oil and fats (Wai, Saad and Lim 2009).

The effect of coating on the changes of TV of fish lipids is shown in **fig. 3** The rate of total oxidation, with respect to totox index of trouts was significantly decreased ($p<0.05$) by coating samples as indicated by changes in both peroxide and anisidine value during storage at 4ºC. calculated totox value of fresh trout was 1.36. The TV in uncoated samples increased to 13.44 in control samples after 2 weeks and ranged from 5.29 to 14.65 over the 4 weeks storage. The Ch+Toc coated samples showed lower oxidation throughout the refrigerated storage ($p<0.05$). Zuta et al., have shown that low concentrations of α-tocopherol minimized autooxidation of unrefined mackerel oil, over a period of 66 days at 4°C. In this study The decrease in TV of trout was antioxidant concentration depended; After 28 days of storage, as TV in the control sample was 1.36-fold higher than that of the sample treated with chitosan 2%, and 1.87-fold and 3.06-fold higher than that of the sample treated with Ch+Toc 1 and Ch+Toc 2, respectively.

These results point out that combinations of primary antioxidant such as α-tocopherol shows the synergistic effect on chitosan antioxidant properties. Gergantelis et al. (2007) exhibited more intense antioxidative activity of chitosan in combination with α-tocopherol in beef and pork burgers during frozen storage (-18ºC) for 180 days.

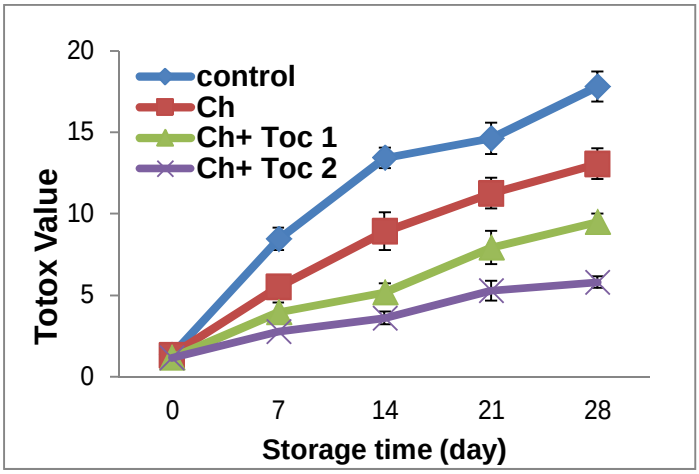


**Figure. 3** Values of totox in farmed trout in different treatments during refrigerated storage

### 3.4. Free Fatty Acids (FFAs)

Production of free fatty acids (FFAs) occurs due to hydrolysis of acylglycerols or ethyl esters during storage time. The levels of FFAs in marine lipids might reflect the degree of quality deterioration of the product, it may also affect lipid oxidation due to the fact that liberated free fatty acids are more susceptible to oxidation than are their equivalent esters, when lipoxygenase is involved (Hamilton 1989; Aubourg 2001).

As shown in **fig. 4** coating caused significant reductions in FFAs of trout fishes during 28 days'storage at 4ºC ($p<0.05$), the most free fatty acid values belonged to control one, and the least FFAs observed in chitosan incorporated 0.4% α-tocopherol, in agreement with the present findings Pereira de Abreu et al. (2010) reported that lipid hydrolysis occurred in frozen Atlantic salmon stored at 20ºC, could control by adding barley husks antioxidants in polyethylene packaging film.

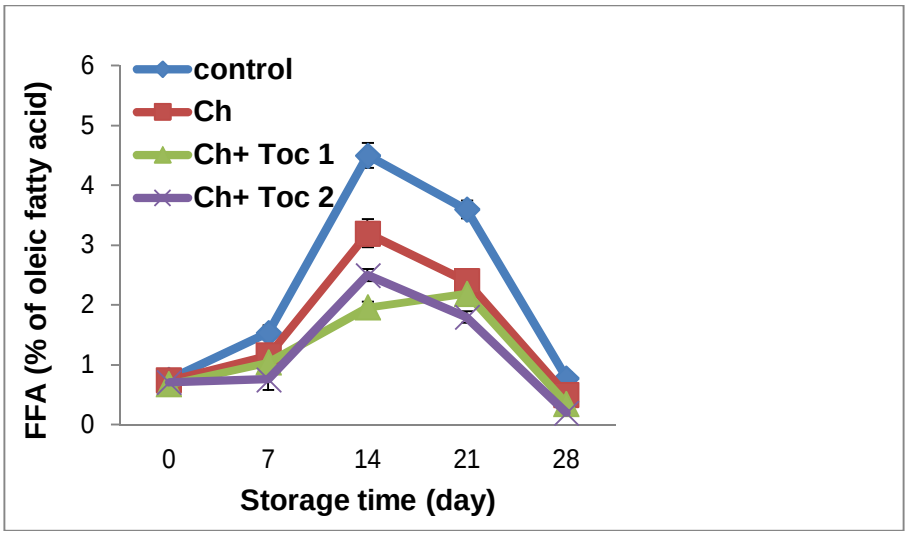


**Figure. 4** Different amounts of free fatty acids in different treatments during refrigerated storage.

### 3.5. Fatty acid profile

Increasing attention has been focused on the significance of PUFAs in human nutrition, as in particular eicoesapentaenoic acid (EPA) and docoesahexaenoic acid (DHA) display several beneficial properties for human health as if fish oil is one of the best sources of dietary supply of these fatty acids. But they are undergoing several deteriorative reactions during processing and storage, e.g., hydrolysis and oxidation (Connor 2000; Das 2008). During the oxidation of polyenoic fatty acids, the methylene group adjacent to two double Bonds, as present in as EPA and DHA, is the primary site of oxygen attack (Porter, Caldwell and Mills 1995).

UK Department of Health recommended the ratios of n6/n3 should be 4.0 at maximum (HMSO 1994). As a matter of fact the lower ratio, the better. a remarkably different value from this found in the study area, which varies from 30:1 to 40:1.

The calculated n6/n3 FA ratio in fresh trout was 0.36 (**table 1**), Similar results for n6/n3 ratio were obtained by Kinsela (1988) for cultured trout. In this study The storage time significantly affected the n6/n3 FA ratio, which showed a decreasing trend, due to storage time, at the third week of storage ($p<0.05$). Similar to n6/n3 FA ratio results, the sum of EPA and DHA content was higher in coated samples ($p<0.05$). Among the treatment groups, samples containing α-Toc had significantly higher ($p<0.05$) sum of EPA+DHA compared to samples treated only by chitosan coating or the control.

The lipid oxidation rate as measured using C22:6 ($n_3$)/C16:0 ratio decreased to 0.134 in control samples after 21 days of storage **(table1)**. These results indicate an increasing rate of lipid oxidation, which was followed by the degradation of polyunsaturated fatty acids (Siskos et al. 2007). the same order of α-tocopherol efficiency was observed as the content of tocopherol increased, inhibitory effects on lipid oxidation also increased considerably ($p<0.05$).

**Table 1** Percentage of fatty acids (g per 100 g) in fresh (day zero), untreated and treated trout after 21 days storage

| | EPA+ DHA | n6/n3 | C22:6 (n3)/C16:0 |
|---|---|---|---|
| **Fresh fish** | 16.45 ± 0.18 | 0.35 ± 0.54 | 0.584 ± 0.78 |
| **Control** | 4.62 ± 0.32 | 0.98 ± 0.93 | 0.134 ± 1.32 |
| **Chitosan** | 7.59 ± 0.24 | 0.71 ± 0.89 | 0.237 ± 1.26 |
| **Chitosan-tocopherol 0.2%** | 9.10 ± 0.28 | 0.51± 0.82 | 0.290 ± 1.12 |
| **Chitosan-tocopherol 0.4%** | 9.94 ± 0.26 | 0.44 ± 0.95 | 0.426 ± 1.11 |

## 4. CONCLUSION

The results demonstrated adding α-tocopherol, as a natural antioxidant, in chitosan coating slowing down lipid hydrolysis and oxidation in treated trout samples stored under refrigeration conditions. In this study coating with higher concentration of α-tocopherol (0.4%) exhibited more efficiency in retarding oxidation. Therefore, this coating could be used as an alternative to preserve valuable fish oil PUFAs specially, omega-3 fatty acid content, against damages during 28 days storage time.

## REFERENCES

[1] AOCS (1993) American Oil Chemists' Society Official Method Ca5a-40. Free Fatty Acid. Champaign,Ill.

[2] AOCS (1996). Official methods and recommended practices of the American oil chemists' society Method Cd 18-90. In F. Gunstone (Ed.), *p*-Anisidine value (4th ed.) Champaign, IL: AOCS Press.

[3] AOCS (1998) Official methods and recommended practices of the American oil chemists society, official methods and recommended practices. Champaign, Illinois, USA: AOCS Press.

[4] Aubourg SP (2001) Fluorescence study of the pro-oxidant effect of free fatty acids on marine lipids. J Sci Food Agric 81: 385–390.

[5] Auburg SP (1993) Review: interaction of malondialdehyde with biological molecules– new trends about reactivity and significance. Int J Food Sci Technol 28(4): 323–335.

[6] Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917.

[7] Bradley DG, Min DB (1992) Singlet oxygen oxidation of foods. Crit Rev Food Sci 31: 211.

Chen XG, Zheng L, Wang Z, Lee CY, Park HJ (2002) Molecular affinity and permeability of different molecular weight chitosan membranes. J Agr Food Chem 50(21): 5915–5918.

[8] Chung YC, Chen CY (2008) Antibacterial characteristics and activity of acid-soluble chitosan. Bioresource Technol 99: 2806–2814.

[9] Connor WE (2000) Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 71(1): 171S–175S.

Das UN (2008) Can essential fatty acids reduce the burden of disease(s)? Lipids Health Dis 7:9.

[10] Duan J, Cherian G, Zhao Y (2010) Quality enhancement in fresh and frozen lingcod (Ophiodon elongates) fillets by employment of fish oil incorporated chitosan coatings. Food Chem 119(2): 524-532.

[11] Dutta PK, Tripathi S, Mehrotra GK, Dutta J (2009) Perspectives for chitosan based antimicrobial films in food applications: a Review. Food Chem 114: 1173–1182

[12] Feng T, Du Y, Li J, Hu Y, Kennedy JF (2008) Enhancement of antioxidant activity of chitosan by irradiation. Carbohyd Polym 73(1): 126-132.

[13] Frankel EN (1998) Lipid oxidation. The Oily Press Ltd, Scotland.

[14] Friedman M, Juneja VK (2010) Review of Antimicrobial and Antioxidative Activities of Chitosans in Food. J Food Prot 73: 1737-1761.

[15] Georgantelis D, Ambrosiadis I, Katikou P, Blekas G, Georgakis SA (2007) Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4°C. Meat Sci 76(1): 172-181.

[16] Georgantelis D, Blekas G, Katikou P, Ambrosiadis I, Fletouris DJ (2007) Effect of rosemary extract, chitosan and α-tocopherol on lipid oxidation and colour stability during frozen storage of beef burgers. Meat Sci 75(2): 256-264.

[17] Gray JI, Gomaa EA, Buckley DJ (1996) Oxidative quality and shelf life of meats. Meat Sci 43: 111-123.

[18] Guzman J, Saucedo I, Revilla J, Navarro R, Guibal E (2003) Copper sorption by chitosan in the presence of citrate ions: influence of metal speciation on sorption mechanism and uptake capacities. Int J Biol Macromol 33(1-3): 57-65.

[19] Hamilton RJ (1989) The chemistry of rancidity in foods, Rancidity in Foods. Hamilton, eds. Elsevier Applied Science, New York, pp 1.

[20] Han C, Zhao Y, Leonard SW, Traber MG (2004) Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria×ananassa) and raspberries (Rubus ideaus). Postharvest Biol Tec 33(1): 67-78.

[21] HMSO, UK (1994) Nutritional aspects of cardiovascular disease (report on health and social subjects No. 46). London: HMSO.

[22] Horn AF, Nielsen NS, Jacobsen C (2009) Additions of caffeic acid, ascorbyl palmitate or y-tocopherol to fish oil-enriched energy bars affect lipid oxidation differently. Food Chem 112: 412–420.

[23] Jacobsen C, Let MB (2006) Using polyunsaturated fatty acids (PUFAs) as functional ingredients. In C. Williams & J. Buttriss (Eds.), Improving the fat content of foods. Cambridge, England. Woodhead Publishing Ltd.

[24] Je JY, Oian ZJ, Byung HG, Kim SK (2007) Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. Process Biochem 42 (5): 840-884.

[25] Jeon YJ, Kamil JYZA, Shahidi F (2002) Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. J Agr Food Chem 50(18): 5167–5178.

[26] Jung WK, Oian ZJ, Lee SH, Choi SY, Sung NJ, Byung HG, Kim SK (2007) Free radical scavenging activity of a novel antioxidative peptide isolated from in vitro gastrointestinal digests of Mytilus coruscus. J Med Food 10(1): 197-202.

[27] Kamal-Eldin A, Appelqvist L (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31: 671–701.

[28] Kanatt SR, Chander R, Sharma A (2008) Chitosan and mint mixture: A new preservative for meat and meat products. Food Chem 107(2): 845-852.

[29] Kinsella JE (1988) Fish and seafoods: Nutritional implication and quality issues. Food Technol 15: 146-150.

[30] Laguerre M, Lecomte J, Villeneuve P (2007) Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. Prog Lipid Res 46: 244-282.

[31] Lamm DL, Dehaven JI, Riggs DR (2000) Keyhole limpet hemocyanin immunotherapy of bladder cancer: laboratory and clinical studies. Eur Urol 37 (3): 41–44.

[32] Markl J, Lieb B, Gebauer W, Altenhein B, Meissner U, Harris JR (2001) Marine tumor vaccine carriers: structure of the molluscan hemocyanins KLH and HtH. J Cancer Res Clin Oncol 127: R3-R9.

[33] Martins JT, Cerqueira MA, Vicente AA (2012) Influence of α-tocopherol on physicochemical properties of chitosan-based films. Food Hydrocolloids 27(1): 220-227.

[34] Mexis SF, Chouliara E, Kontominas MG (2009) Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4°C. Food Microbiol 26(6): 598-605.

[35] Möller H, Grelier S, Pardon P, Coma V (2004) Antimicrobial and physicochemical properties of chitosan-HPMC-based films. J Agr Food Chem 52(21): 6585–6591.

[36] Nissen LR, Byrne DV, Bertelsen G, Skibsted LH (2004) The antioxidative activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. Meat Sci 68: 485–495.

[37] No HK, Park NY, Lee SH, Meyers SP (2002) Antibacterial activity of chitosan and chitosan oligomers with different molecular weights. Int J Food Microbiol 74: 65–72.

[38] O´Sullivan A, Mayr A, Shaw NB, Murphy SC, Kerry JP (2005) Use of natural antioxidants to stabilize fish oil systems. J Aquat Food Prod Technol 14: 75–94.

[39] Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH (2010) Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chem 120(1):193-198.

[40] Park SI, Daeschel MA, Zhao Y (2004) Functional properties of antimicrobial lysozyme–chitosan composite films. J Food Sci 69(8): 215–221.

[41] Pereira de Abreu DA, Losada PP, Maroto J, Cruz JM (2010). Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (Salmo salar L.). Food Res Int 43(5): 1277-1282.

[42] Porter NA, Caldwell SE, Mills KA (1995) Mechanism of free radical oxidation of unsaturated lipids, Lipids 30: 277.

[43] Prato E, Biandolino F (2012) Total lipid content and fatty acid composition of commercially important fish species from the Mediterranean, Mar Grande Sea. Food Chem 131(4): 1233-1239.

[44] Riggs DR, Jackson B, Vona-Davis L, McFadden D (2002) In vitro anticancer effects of a novel immunostimulant: keyhole limpet hemocyanin. J Surg Res 108: 279–284.

[45] Sathivel S, Liu Q, Huang J, Prinyawiwatkul W (2007) The influence of chitosan glazing on the quality of skinless pink salmon (Oncorhynchus gorbuscha) fillets during frozen storage. J Food Eng 83(3): 366-373.

[46] Sayas-Barbera B, Quesada J, Sánchez-Zapata E, et al (2011) Effect of the molecular weight and concentration of chitosan in pork model burgers. Meat Sci 88: 740-749.

[47] Shahidi F, Arachchi JKM, Jeon Y (1999) Food applications of chitin and chitosans. Trends Sci Technol 10(2): 37−51.

[48] Sidhu KS (2003) Health benefits and potential risks related to consumption of fish or fish oil. Regul Toxicol Pharm 38(3): 336–344.

[49] Simopoulos AP (2002) Omega-3 fatty acids in inflammation and autoimmune diseases. J Am Coll Nutr 21: 495–505.

[50] Siskos I, Zotos A, Melidou S, Tsikritzi R (2007) The effect of liquid smoking of fillets of trout (Salmo gairdnerii) on sensory, microbiological and chemical changes during chilled storage. Food Chem 101: 458-464.

[51] Traber MG (1999) Vitamin E. In: Modern Nutrition in Health and Disease, vol. 9. Williams & Wilkins, Baltimore, pp 347–362.

[52] Uauy R, Valenzuela A (2000) Marine oils: the health benefits of n-3 fatty acids. Nutrition 16: 680-684.

[53] Wai WT, Saad B, Lim BP (2009) Determination of TOTOX value in palm oleins using a FI-potentiometric analyzer. Food Chem 113(1): 285-290.

[54] Yuki E, Ishikawa Y (1976) Tocopherol content of nine vegetable frying oils and their changes under stimulated deep-frying conditions. J AOCS 53(11): 673-676.

[55] Zhang Y, Yang L, Zu Y, Chen X, Wang F, Liu F (2010) Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. Food Chem 118(3): 656-662.

[56] Zheng LY, Zhu JF (2003) Study on antimicrobial activity of chitosan with different molecular weights. Carbohyd Polym 54(4): 527−530.

[57] Zuta PC, Simpson BK, Zhao X, Leclerc L (2007) The effect of α-tocopherol on the oxidation of mackerel oil. Food Chem 100(2): 800-807.