International Food Research Journal 22(1): 304-310 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Impact of assorted spices on lipid quality alteration of refrigerated fish muscle

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Article history

Received: 8 August 2013
Received in revised form: 26 June 2014
Accepted:8 July 2014

Keywords

Lipid oxidation
Malonaldehyde
Natural antioxidant
Spices
Fish muscle

Abstract

Biologically important polyunsaturated fatty acid rich fish lipid is highly susceptible to oxidative deterioration. To delay the rate of oxidation three spices - fennel, black-pepper, cinnamon and a synthetic antioxidant, butylated hydroxytoluene (BHT), were mixed separately with homogenized tilapia fish muscle. A non-spiced control was also stored along with the spiced varieties under refrigeration. Over a span of five weeks, total lipid composition and oxidative stability of fish oil was evaluated at an interval of one week on basis of concentration of cholesterol, phospholipid, fatty acid, peroxide, *p*-anisidine, thiobarbituric acid (TBA), iodine, conjugated diene-triene values. Overall increasing trend was observed in all the values in control except iodine value. Moreover the values recorded were comparatively higher in control than the spiced ones. Cinnamon spiced samples recorded lowest cholesterol and fatty acid concentration indicating successful control of autoxidation. Cinnamon could also remarkably lower phospholipid concentration in the fish samples. Peroxidation was effectively controlled by fennel and pepper on longer duration. Contrary to other spices cinnamon decreased the amount of malonaldehyde accumulation in oil. Overall cinnamon was found to be most effective antioxidant in longer duration where as pepper and fennel was effective for a shorter span.

Introduction

The prevalence of long chain polyunsaturated fatty acids is more in fish lipids as compared to beef and chicken (Calder, 2004; Woods *et al*., 2005). Hence fish lipids are usually more susceptible to attack by oxygen (Burns and Wagner, 1991). Lipids are susceptible to oxidative processes like autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms (Jadhav *et al.*, 1996). It leads to formation of off-flavors and loss of essential amino acids, fat-soluble vitamins, and other bioactive components (Vercellotti *et al*., 1992; Shahidi *et al*., 2000). Lipid oxidation is therefore one of the major phenomena attributing to quality deterioration processes that limits the shelf-life of lipid rich foods (Markley, 1961; Stansby, 1967).

The rate of this reaction can be retarded by antioxidants, which occur either naturally as an inherent constituent of foods or may be added externally to products. Synthetic antioxidants such as BHT, BHA, propyl gallate have been extensively used as antioxidants in the industry. Due to toxic effects of synthetic antioxidants (Martinez-Tome *et al*., 2001) there is an increasing industrial trend to replace synthetic antioxidants with natural compounds like spices and herbs to control the rate of lipid oxidation (Rice-Evans *et al*., 1996; Zheng and Wang, 2001). In this study because of considerable lipid availability tilapia fish was judiciously chosen as the source of lipid. Three popular Indian spices were selected to test their potential to inhibit the lipid peroxidation in fish and their efficiency was compared with a synthetic antioxidant, BHT.

Materials and Methods

Chemicals and reagents

P-anisidine and 2-thiobarbituric acid were purchased from Loba chemie (India). Phosphatidylcholine was obtained from Himedia (India). Ammonium thiocyanate, ferric chloride and all other solvents including chloroform, methanol, isooctane as well as reagents were procured from Merck (India). Cholesterol kit was purchased from Coral Clinical system, a division Crest Biosystem. All chemicals used were of analytical grade.

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Preparation of fish sample

Total amount of 3 Kg of Tilapia fish (each having an average weight of 300 gram, length 6.5 inches and age of 3 months) was procured from Chowbaga bheri located in east Kolkata wetland. Fish muscles were minced and homogenized in a blender. The mass was further divided in 26 equal portions each weighing 100 gms for mixing seperately 10 gms of each of three spices - fennel seeds *(Foeniculum vulgare)*, black pepper *(Piper nigrum)*, cinnamon *(Cinnamomum verum)*, (all grinded and sieved at 125 micron), a synthetic antioxidant BHT, and for using as non-spiced control. They were put in the press-and-lock polythene freezer bag (101 micron thickness) and stored in the freezer chamber of a refrigerator (LG-Fresh Master) at 0-4^{o}C. Samples were kept in five batches and taken for analyses at day 0, 7, 14, 21, 28 and 35th day.

Extraction of lipid

Lipids of stored tilapia fish muscle samples were extracted following the method of Bligh and Dyer (1959).

Determination of cholesterol, phospholipids and free fatty acid

Cholesterol was estimated by CHOD – PAP method. It is an enzymatic, colorimetric method with cholesterol esterase, cholesterol oxidase, and 4-aminoantipyrine. In this method the blank contained only 1 ml of working reagent. The standard contained 1ml reagent and 0.01ml cholesterol standard and the specimen contained 1ml reagent and 0.01ml test sample. After 5 minutes of incubation at 370C the absorbance of standard (Abs S) and specimen (Abs T) was measured at 505 nm and compared against reagent blank. The amount of cholesterol was calculated by using this formula

Cholesterol in mg/dl= Abs T/Abs S * 200

Phospholipid was estimated by the colorimetric method of Stewart JCM (1980) based on the formation of a complex between phospholipids and ammonium ferrothiocyanate. Phospholipid residues were dissolved in 2 ml chloroform and 1 ml of thiocyanate reagent was added. It was vortexed for 1 min and centrifuged at low speed. The lower red chloroform layer was removed with a Pasteur pipette, absorbance was recorded at 488 nm and compared with known amounts of a standard phosphatidylcholine solution (1 mg/ml).

Free fatty acid value was calculated using IUPAC method 2.201. In a conical flask, 25 ml of diethyl ether was mixed with 25 ml alcohol and 1 ml of phenolphthalein solution. The mixture was carefully neutralized with 0.1N sodium hydroxide. This is called Neutral Solvent. 0.1 grams of oil were taken and the sample was dissolved in the neutral solvent and was titrated with aqueous 0.1N sodium hydroxide, shaking vigorously until a pink colour was formed that persisted for 15 seconds. Acid Value was calculated as:

Acid Value = Volume of sodium hydroxide solution (ml.) X 5.61/ weight of the oil sample

Peroxide value

Peroxide value was calculated using IUPAC method 2.501. In a test tube, 0.2 grams of oil sample were taken and saturated solution of potassium iodide was added. A mixture of glacial acetic acid and chloroform (20 ml) was added to the mixture. This was then warmed in water bath for 30 seconds. The content was then poured into a flask containing 20 ml of 5 % potassium iodide solution. To it was added 25 ml of distilled water and 1 ml of starch solution. This was then titrated against 0.01N sodium thiosulphate solution. Peroxide Value was calculated as:

Peroxide Value = Volume (ml) X Strength of sodium thiosulphate X 1000/ Weight of the oil sample

Thiobarbituric acid value

Thiobarbituric acid value was calculated using IUPAC method 2.531. In a test tube, 200 mg of oil sample was taken and 5 ml of thiobarbituric acid reagent was added. The mixture was stoppered and warmed in water bath at 95^{O}C for 120 minutes. It was then cooled and the absorbance was measured (A_s) at 530 nm in a 10 mm cell against water. A reagent blank absorbance (Ab) was also carried out.

Thiobarbituric acid number = 50 X (A_s – A_b)/ weight of the sample

Para-anisidine value

Para-anisidine value was calculated using IUPAC method 2.504. In a 25 ml volumetric flask, 0.2 grams of oil was taken and diluted with isooctane. The absorbance (A_1) of the solution was measured at 350 nm against a blank isooctane. Sample solution (5 ml) was mixed with 1 ml of *p*–anisidine solution. After 10 minutes, the absorbance of this solution was measured (A_2).

P–anisidine values = 25 X [1.2 X (A_2 – A_1)] / weight of the oil sample

Iodine value

Iodine value was measured using IUPAC method 2.205. In a conical flask, 0.2 grams of oil sample was taken and 1 ml of carbon tetrachloride was added. About 2 ml of Wij's solution was added and allowed to stand in the dark for 30 minutes. To it was added 1.5 ml of 10% potassium iodide and 10 ml of distilled water and titrated against 0.1N sodium thiosulphate using 1 ml of starch solution as an indicator. A blank was also prepared along with oil samples. Iodine Value was calculated as:

Iodine Value = (Blank titrated value – titrated oil sample value) X 1.269/ weight of the sample

Conjugated diene and triene value

These values were calculated using IUPAC method 2.505. In a 100 ml conical flask 100 mg of oil sample was added to 75 ml purified isooctane. The flask was warmed to completely dissolve the sample, cooled to room temperature and allowed to stand for 15 minutes. The absorbance was measured at 233 nm. Conjugated dienes value was calculated as:

Conjugated dienes value = A_0/ weight of the sample X cell length in centimeters.

Absorbance measurements were repeated at 268 nm for CT determination

Statistical analysis

Completely Randomized Design (CRD) was used in this study. Data from three different experiments were subjected to analysis of variance (ANOVA) ($P<0.05$). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 16.0 for windows, SPSS Inc.).

Results and Discussion

Evaluation of variation in total lipid composition

Cholesterol

Cholesterol concentration of the fish oil extracted from refrigerated spiced fish samples and control recorded over a span of five weeks is shown in Figure 1. It is observed (Figure 1a) that in control which contain no spices, the cholesterol concentration increased significantly and almost uniformly ($P<0.05$) during this time. Cholesterol being a monounsaturated lipid is susceptible to oxidation and hence undergoes autoxidation in air to form cholesterol oxide products which increase the total cholesterol concentration (Park and Addis, 1987).

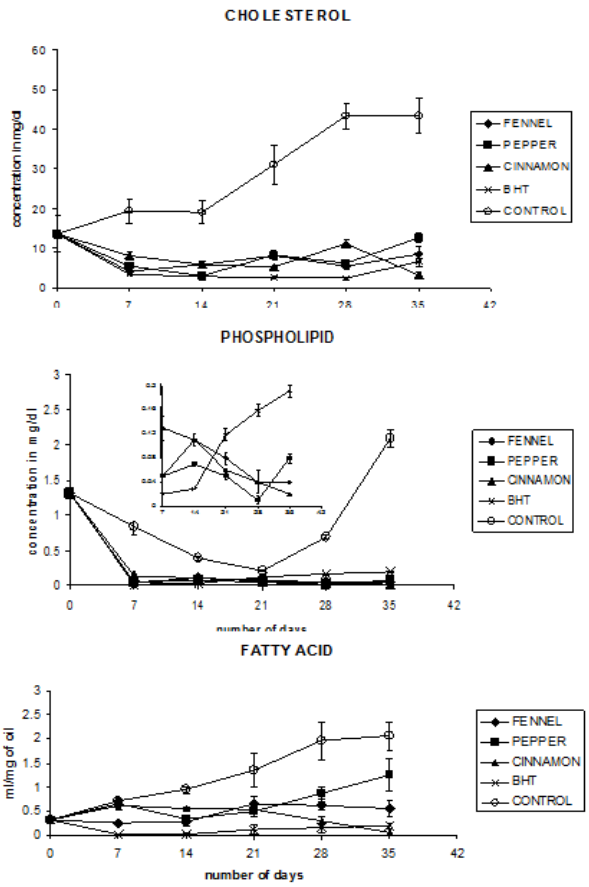

Figure 1. Variation in lipid composition in terms of changes in (a) cholesterol, (b) phospholipid and (c) fatty acid of the fish oil recorded on 0, 7, 14, 21, 28 and 35th day of storage. Fish oil extracted from the three spiced (fennel, pepper, cinnamon) fish muscles and BHT mixed sample along with a non-spiced control were evaluated in triplicate for cholesterol, phospholipid and fatty acid after refrigerated storage

The degree of oxide formation is related to heating time, storage conditions, level of activator present and packaging (Paniangvait *et al*., 1995). Therefore it is conceivable, that cholesterol oxidation should proceed in a way analogous to fatty acid oxidation. Smith (1987) also suggested that the hydroperoxides of polyunsaturated fatty acids formed during lipid oxidation initiates cholesterol oxidation.

Addition of spices recorded markedly decreased concentration of cholesterol in all the cases from initial week ($P<0.05$). Fennel and pepper recorded the lowest concentration of 4.17 mg/dl and 2.93 mg/dl on 7th and 14th day respectively whereas cinnamon exhibited the minimum of 3.19 mg/dl in the last week. Except cinnamon the other two spices and BHT showed increasing trend in the cholesterol value in the later part of the study. Though there is a significant overall decrease in cholesterol concentration due to addition of spices, only cinnamon decreased the concentration uniformly till the end ($P<0.05$).

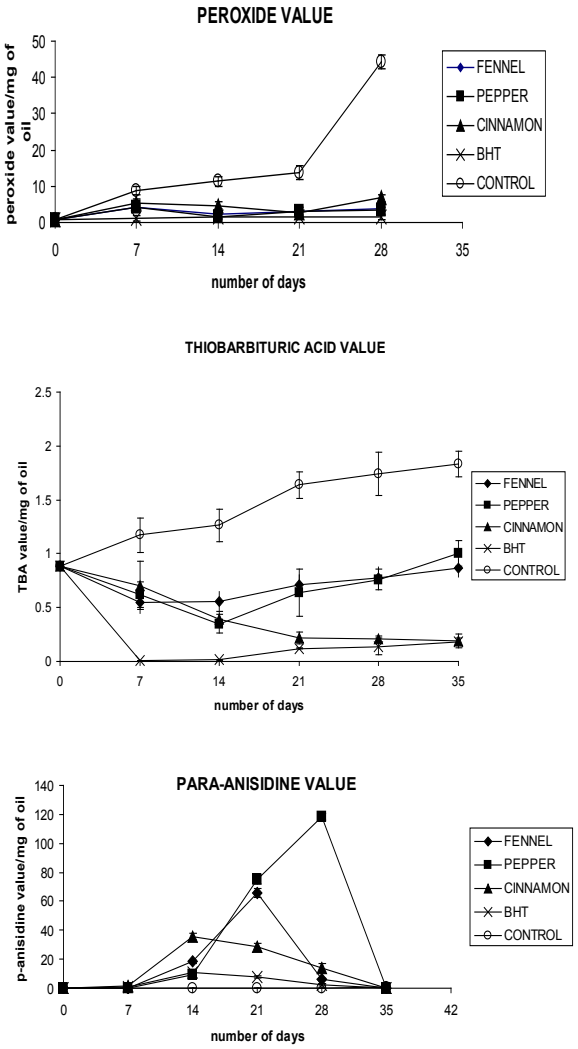

Figure 2. Estimation of lipid oxidation based on (a) peroxide value, (b) Thiobarbituric acid value and (c) *p*-anisidine value of the fish oil recorded on 0, 7, 14, 21, 28 and 35th day of storage. Fish oil extracted from the three spiced (fennel, pepper, cinnamon) fish muscles and BHT mixed sample along with a non-spiced control were evaluated in triplicate for peroxide, Thiobarbituric acid and *p*-anisidine value after refrigerated storage

This indicates that possibly cinnamon has most successfully controlled the oxidation (P<0.05) of oil while the efficiency of pepper and fennel decreased with time. Since oxidation of cholesterol in general is mediated by reactive pro-oxidant species (Osada *et al*., 1994) and proceed in a way analogous to fatty acid oxidation the formation of COPs in animal products can be minimized by the application of antioxidants addition to foods.

Phospolipid

Concentration of the phospholipid in the fish oil extracted from the spiced fish samples and the non-spiced control estimated at an interval of week for a span of five weeks have been plotted in Figure 1b. From Figure 1b it is found that the concentrations of phospholipids in the fish oils have decreased remarkably (P<0.05) in all the cases in first few weeks. When mixed with spices the phospholipid concentration of the samples decreased considerably recording lower value than the non-spiced control (P<0.05). This indicates that either the phospholipids have undergone hydrolytic reactions at a greater extent in spices or has reacted with the flavor components in spices. Both in control and BHT the phospholipid concentration increased after 21st and 7th day. The minimum values recorded in control and BHT on these days were 0.21 mg/dl and 0.02 mg/dl respectively. Concentration of phospholipids were reduced significantly (P<0.05) by the fennel, pepper and cinnamon till the end and recorded values as low as 0.04 mg/dl, 0.08 mg/dl and 0.02 mg/dl respectively. Fennel spiced sample recorded the lowest phospholipid concentration of 0.01 mg/dl on 28th day but again underwent a rise in the last week. Hence it can be concluded that cinnamon mixed sample could most successfully decrease the phospholipid content (P<0.05) till the end.

Acid value

The graph in Figure 1c delineate the free fatty acid value of the fish lipid extracted from the muscle of the spiced fish and the non-spiced control stored for a span of five weeks in refrigerated condition. The acid value measures the amount of carboxylic acid groups in free fatty acids generated in the fat due to storage. Increase in this value leads to formation of off-flavour as a result of degradation of fat (Chang *et al*., 1978; Choe and Min, 1997). A steady and significant increase in values was observed in case of control as well as in BHT sample (P<0.05), whereas in case of cinnamon there was a gradual decrease in the fatty acid content (P<0.05). Though an overall remarkable increase in the acid value occurred in fennel and pepper (P<0.05) in the duration between the initial and final week, the value reached its peak (0.664 ml/mg) at fourth week in case of fennel. Fatty acid value in black pepper underwent a depression and recorded the lowest value of 0.342 ml/mg at third week and ultimately increased from thereafter till 4th week. This decrease in the fatty acid value might be attributed to the higher antioxidant potential of these spices to control oxidation in these respective initial weeks. It is to be noted that cinnamon could lower the fatty acid value most effectively and the value recorded in the final week (0.065 ml/mg) is even lower than the synthetic antioxidant BHT.

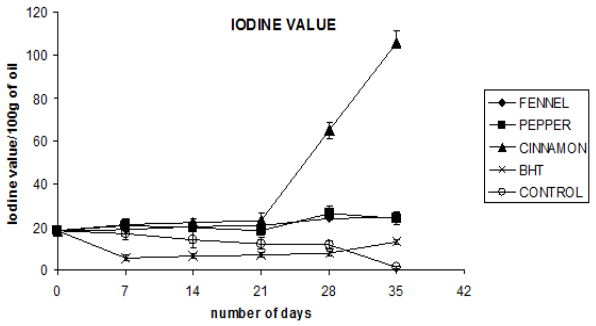

Figure 3. Change in iodine value of the fish oil recorded on 0, 7, 14, 21, 28 and 35th day of storage. Fish oil extracted from the three spiced (fennel, pepper, cinnamon) fish muscles and BHT mixed sample along with a non-spiced control were evaluated in triplicate for iodine value after refrigerated storage

Evaluation of oxidative stability:

Peroxide value

Peroxide value of the spiced fish samples and the control is expressed in Figure 2a. The control recorded higher peroxide value than the other spiced varieties which indicates remarkable reduction of peroxidation with addition of spices and BHT ($P<0.05$). The increasing peroxide value in control indicates higher rate of oxidation with time. In case of other spices the peroxide values although increased with time ($P<0.05$) they initially underwent a reduction in the consecutive one or two weeks. This was followed by an increase in value in all the cases. This trend indicates that the antioxidant activity of the spices must have been higher in the 2nd and 3rd week, which helped in controlling the rate of peroxidation. In BHT the peroxide value initially showed a gradual increase which was followed by a slight decrease in value in the later weeks. Though the peroxide value increased in the 4th week in all the cases (except BHT), the final week recorded no peroxide value. This might be due to decomposition of peroxides to secondary oxidation products on long storage (Velasco and Dobargens, 2002; Fullana *et al*., 2004). Though pepper most effectively reduced the peroxide formation in the initial level ($P<0.05$) recording the lowest value of 1.677, the most effective overall decrease ($P<0.05$) was revealed in case cinnamon since it retained a low value of 2.532 even in the 3rd week. This successful control of peroxidation in case of cinnamon might have also attributed to the effective control of cholesterol oxidation as exhibited by the lowest cholesterol concentration in Figure 1a.

Thiobarbituric acid value

Thiobarbituric acid value of the spiced fish samples along with the non-spiced control was estimated and showed in Figure 2b. It measures the rate of oxidative rancidity by the formation of oxidized lipids – malonaldehyde which is a non-volatile aldehyde (Kishida *et al*., 1993; Cesa, 2004). The TBA values delineate a gradual and significant increase in malonaldehyde concentration in case of fennel, BHT, and control ($P<0.05$). The increase in the TBA value is highest in case of control. Only in black pepper a sudden drop in value occurred at third week which was again followed by a gradual rise. This shows that these spices control the oxidation effectively for a shorter period of time. The most interesting result was observed in cinnamon where the TBA value gradually decreased ($P<0.05$) thereby arresting the formation of malonaldehyde and other TBA reactive substances. In case of cinnamon at 5th week the value dropped down to 0.191 which is comparable with BHT's antioxidant potential. However at the initial level cinnamon was not very effective in arresting the formation of thiobarbituric reactive substances.

Para – anisidine values

Figure 2c represents para-anisidine value of the samples which reflects the magnitude of aldehydic secondary oxidation products (McGinely, 1991). The graph showed lower p-anisidine values in case of control than the other spiced samples which is a marked deviation from the normal expected trend. In control the p-anisidine value though low had undergone steady and significant increase till the last week ($P<0.05$). On 14th, 21st and 28th days all the spiced fish samples recorded drastically high p-anisidine values. Cinnamon and BHT reached their peak (35.73 and 11.12 respectively) on 14th day while fennel, recorded the highest (66.09) on 21st day. Pepper showed maximum value of 118.10 in 4th week. Except for non-spiced control the values for all other samples decreased noticeably in the last week. Fennel measured the lowest 0.061 in the final week. The unusual high value observed in spices may be due to presence of volatile aldehyde compound in the spices.

Iodine value

Iodine value of the spiced fish samples and the non-spiced control is delineated in Figure 3. It measures the degree of unsaturation of oil. In case of control the iodine value decreased considerably with each week ($P<0.05$). This indicates a loss of unsaturated fatty acid due to oxidative degradation on storage. On the contrary values gradually increased at a significant rate in all the spiced samples ($P<0.05$). In case of fennel, cinnamon, BHT the values increased

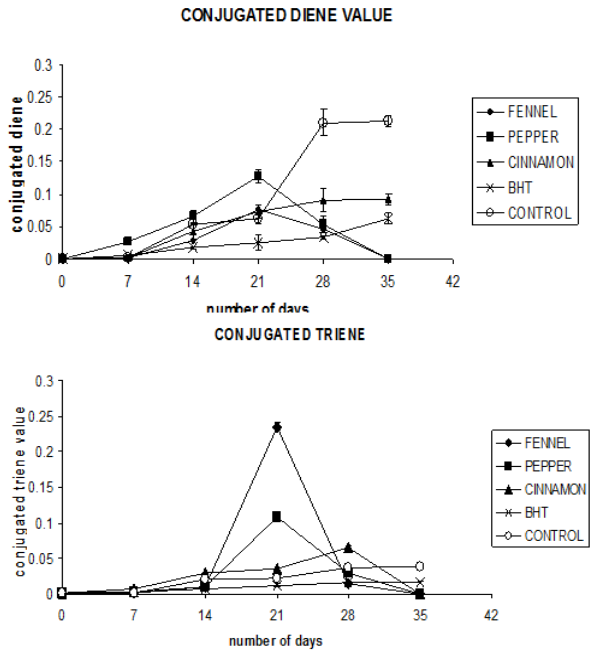

Figure 4. Change in (a) conjugated diene and (b) triene value of the fish oil recorded on 0, 7, 14, 21, 28 and 35th day of storage. Fish oil extracted from the three spiced (fennel, pepper, cinnamon) fish muscles and BHT mixed sample along with a non-spiced control were evaluated in triplicate for conjugated diene and triene value after refrigerated storage

gradually whereas in black pepper the value dropped to a minimum of 18.5 (g/gm of oil) on 21st day and then increased in the last two weeks. Among all the spices the maximum value recorded was 106 (g/gm of oil) in the last week in cinnamon spiced fish sample oil. These observations related to the spiced samples reinforce the oxidation controlling potential of the spices.

Conjugated dienes and trienes value

The UV absorbance measurement of conjugated diene and triene values at 233 nm and 268 nm are delineated in Figure 4a and 4b respectively. In case of fish samples mixed with BHT and non-spiced control these values uniformly increased with time till the final week ($P<0.05$). Formation of high contents of CD may be related to the presence of higher contents of polyunsaturated fatty acids (Liu and White, 1992). Conjugated triene may be produced by dehydration of conjugated diene hydroperoxides (Fishwick and Swoboda, 1977).The CD values of the pepper and fennel spiced fish samples increased till 3rd week and documented a maximum of 0.077 and 0.128 respectively. The CD values of these spices underwent a depression in the next two weeks. In case of cinnamon the CD values increased steadily and significantly ($P<0.05$) till the final week and attained a maximum of 0.092. The highest CD values of each of the spiced samples are still lower than the final week value of control. In samples spiced with fennel and pepper the CT values increased steadily till 3rd week ($P<0.05$) and recorded a maximum of 0.235 and 0.101 respectively which was followed by gradual decrease in subsequent weeks. In cinnamon mixed sample the CT value increased significantly till the 4th week ($P<0.05$) and reached a peak value of 0.066 which then underwent a decrease in the final week.

The maximum CT values attained in case of three spices are higher than the highest values recorded in BHT and non-spiced control. The high CT and CD values of the spiced samples recorded typically in the 3rd and 4th week may be due to other compounds present in the spices which absorb in the region of 233 and 268 nm.

Conclusion

This study revealed that all the three spices can control lipid oxidation considerably. Under domestic refrigerating condition cinnamon could act as most successful antioxidant to prevent the oxidation in tilapia fish. Cholesterol, phospholipid and free fatty acid level could be controlled noticeably and significantly by cinnamon ($P<0.05$). The interesting finding was that cinnamon could also reduce the malonaldehyde accumulation in the fish lipid. The decreasing conjugated diene-triene values of spiced samples in the later part of storage indicated increased accumulation of the antioxidants in the fish oil on long storage with spices which makes it beneficial to human body. Finally it can be concluded that cinnamon showed the maximum efficiency in controlling oxidation whereas other spices like pepper and fennel were found to be effective in short term storage.

Acknowledgement

This study was financially supported by J.D.Birla Institute, Jadavpur University, Kolkata, India.

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