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Oxidative Stability of Fish Oil And Impact of Oregano Essential Oil on The Prevention of Lipid Oxidation-Study By FTIR-Spectroscopy

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Abstrakt

The thermal stability and fatty acid composition of oregano fish oil (FIO) was studied by comparing; pure fish oil, fish oil with 1% oregano oil, fish oil with 2% oregano oil, applying FTIR (Fourier - Transform Infrared) - spectroscopy and GC /MS (gas chromatography - mass spectrometry). The physico-chemical parameters analyzed (value of peroxides, value of iodine (g) and value of acids (mg/g)) have resulted in significant changes in different components of the composition of the year of origin fish, the value of peroxide (mmol/ kg) for pure fish oils, oils with 1% oregano oil, and with 2% oregano oil. Then the iodine value (g) for pure fish oils, oils with 1% oregano oil, and with 2% oregano oil as well as the acidity value for pure fish oils, oils with 1% oregano oil, and with 2% oil oregon. FTIR spectroscopy is an analytical device (method) which clarifies the basic principles of the mechanism of stereo-chemical changes in the analyzed substances, as well as to understand which of the chemical components are responsible for increasing the thermal stability of fish oil. In this study, in addition to the above-mentioned parameters, it was found that temperature (°C) is adequate for increasing the stability of origin fish oil, because this phenomenon corresponds to the processes when oxidation reactions and Maillard reactions form compounds with maximum antioxidant activity (lipid stability).

***Keywords*:** Fish oregano oil; Ratio frequency; Maillard reaction; FTIR-Spectroscopy; GC/MS.

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1. Introduction

Most of the oils contain triglicerides as a mayor components and usually if they contain two different fatty acids such as palmitic and oleic, oil may contain six different triglycerides and in this case between them has different physico-chemical characteristics. Different ways of fatty acids distribution in triglyceride molecules as a result will have completely different physical characteristics and especially different chemical interactions of each triglyceride molecules [1].

Fish oil contain high level of polynunsaturated fatty acids especially icosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and icosatetraenoic acid (ETA), which have human health benefits and important physiological functions.

Nevertheless, unsaturated fatty acids of the triglycerides they aeasy oxidized because of their many double bonds in their chemical structure. The first step of the lipid oxidation is the formation of the unstable toxic hydroperoxyde compounds known as primary oxidized compounds based on interaction with molecular oxygen, which by further easy decomposition, they are converted into secondary oxidized compounds such as aldehyde, ketones, alcohols, esters etc [2].

Fish oil sensitivity causes the significant problem in the incorporating of the fish oil in other food products, which exposed to light and air at a high temperature, can cause lipid oxidation and, as a result, could be formed off-flavour compounds in food products. Usually this could be happened during the storage conditions and especially during the microencapsulation process, especially by spray-drying, which is the commonly applied technique in the industry [3, 4].

Oregano essential oil were used and before as an antioxidant addition in restrain the lipid oxidation in different food sample such as chicken and turkey meat [5, 6].

However, there are still no published reports regarding the protective effect of oregano essential oil on the lipid stability of fish oil. For this purpose, the main goal research is applying low percent of natural oils such as oregano esential oil, to prolong triglyceride oxidation in commercial fish oil samples during the thermal treatment.

The aim of this research consists of studying the influence of natural essential oils such as oregano oil on fish oil. Thermal oxidative stability especially polyunsaturated fatty acids such as EPA, DHA and ETA and the possibility of using vibrational spectroscopy as an alternative suitable method in comparison with conventional methods such as physico-chemical parameters as well as more advanced ones such as GC/FID.

2. Materials and methods

2.1. Sample preparation

For testing were prepared three different follows mixture:

- Pure natural fish oil
- Fish oil and added 1% essential oregano oil
- Fish oil and added 2% essential oregano oil

Pure sample of commercial Fish oil purchased in local pharmacy in Kosovo. Thermal oxidative stability of two blended mixtures compared with natural pure fish oil (control) were placed in a 500 mL beaker and subjected to heating in a conventional oven (Memmert Laboratory, Memmert, Germany) at three different temperatures starting from 100˚C, 120 ˚C and 140 ˚C for a period of 60 minute in order to accelerate the lipid oxidation process. These oils kept at room temperature and further subjected to analytical measurements.

The oxidative stability of three samples evaluated by determining follows parameters: physicochemical parameters (iodine value, peroxide and acidic number) fatty acid profile by GC/FID and FTIR Spectroscopy evaluating.

2.2. Physicochemical Meauserements

For prepared fish oil samples were analyzed follows parameter, determination of peroxide number according to regulation ISO 3960:2017, determination of iodine value according to regulation ISO 3961:2019 and determination of acidic number according to regulation ISO 660:2010.

2.3. Reagents

Sodium hydroxide (NaOH), Hydrochloric acid (HCl), iodobromine (IBr), sodium thiosulphate ($Na_2S_2O_3$), potassium hydroxide (KOH), potassium iodide (KI), and acetic acid (CH_3COOH) were of Analytical Grade and purchased from Merck Darmstadt Germany.

2.4. Fatty acid profile determination by GC-FID

The prepared samples were analyzed by gas chromatography equipped with a flame ionization detector, (GC Agilent Technologies 7890BA GC System, USA) using the column of SP2560 size 100 mx 0.25 mm with 0.25 μm film, Agilent Technologies, USA). Carrier gas used Helium by flow rate 1.4 ml.

The type of injector was splited ratio 200:1 with the temperature of 250 ˚C. Flame Ionization Detector (FID) with the temperature of 300 ˚C applied. The applied temperatures of GC/FID oven system was gave in Table 1. The results were expressed as a percentage (%) of total fatty acid.

Table 1: Temperature applied in GC-FID.

Parameters	**Rate°C/min**	**Value°C**	**Hold time min**	**Run time min**
Initial	/	80	2	2
Ramp 1	5	120	2	10
Ramp 2	15	185	3	20
Ramp 3	2	210	5	40

2.5. FTIR spectroscopy analysis

The IR spectra recorded with a Fourier transform spectrometer, IRAffinity-1 (Shimadzu Fourier IR Spectrophotometer, Shimadzu Corporation Kyoto Japan) fitted with a Michelson interferometer and a DLaTGS detector. Spectra were acquired (64 scans/sample or background) in the range of 4000–1000 cm^{-1} at a resolution of 4 cm^{-1}, using IRsolution software. Oil aliquots placed between two CaF_2 windows, using a 0.025 mm Teflon spacer. Before recording each spectrum, the CaF_2 was cleaned acetone and than with a cellulose tissue soaked.

3. Results and Discussion

Fish oil prepared sample heated from 100 -140˚C and mauserements was did for all samples before heating and after each cycle of heating process.

3.1. Physicochemical Parameters

Peroxide value of edible oil is common parameter to determine the content of hydroperoxide in lipids, which usually formed during the lipid oxidation. This parameter was monitored show rapidly increased in pure fish oil samples and in the case of hydroperoxide value decrease, this could be explain because unstability of hydroperoxide and their conversion to other secondary oxidized coimpounds. Same trend of changes can monitored and for iodine value because this parameter presents the number of double bonds and high iodine value number is equal with high number of double bonds and decreases of this parameter explain the decrease of the doule bond in fatty acid of the lipid structure. Iodine value rapidly decreases in the pure fish oil samples compare with other samples, which contain oregano essential oil in low concentration level.

Acidic value in general could increased in the case when occur triglyceride decomposition in free fatty acids and if this is not happened acidic value do not change in signicant level, which occur in monitored sample in our case.

Table 1: Peroxide value, iodine value and acidic value for fish oils without and with oregano oil during heating.

Peroxide value (mmol/kg)	Pure fish oil	Fish oil with 1 % oregano oil	Fish oil with 2% oregano oil
Fresh oil	15.6	15.7	15.7
100˚C	15.9	15.2	15.00
120˚C	16.7	16.4	15.48
140˚C	16.2	15.9	14.8
Iodine value (g)			
Fresh oil	1.23	1.25	1.25
100	1.07	1.25	1.25
120	1.06	1.22	1.24
140	0.98	1.19	1.18
Acidic value (mg/g)			
Fresh oil	0.35	0.35	0.35
100	0.37	0.36	0.34
120	0.36	0.37	0.36
140	0.36	0.36	0.37

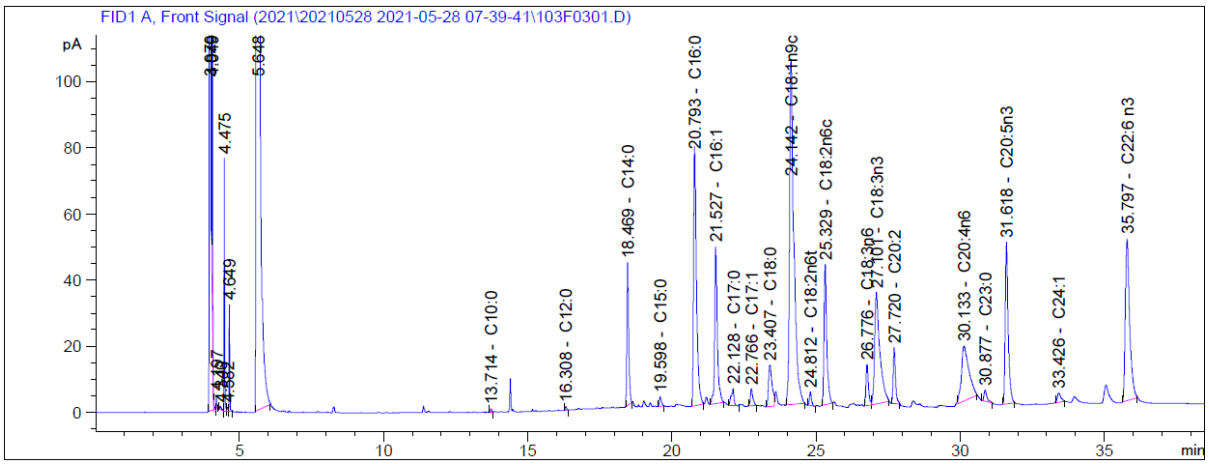

Figure 1: Chromatograms obtained by GC/FID for of the examined samples.

3.2. Fatty acid profile

Fatty acid profile of lipids in fish oil measured by GC-FID was done for all fatty acids but in general there wasn't significant changes before and after treatment except the polyunsaturated fatty acid (20:4n6), (20:5n3) and (22:6n3) which it was excpected to be sensitively in oxidation. For this purpose, we present only trend of changes for three fatty acids.

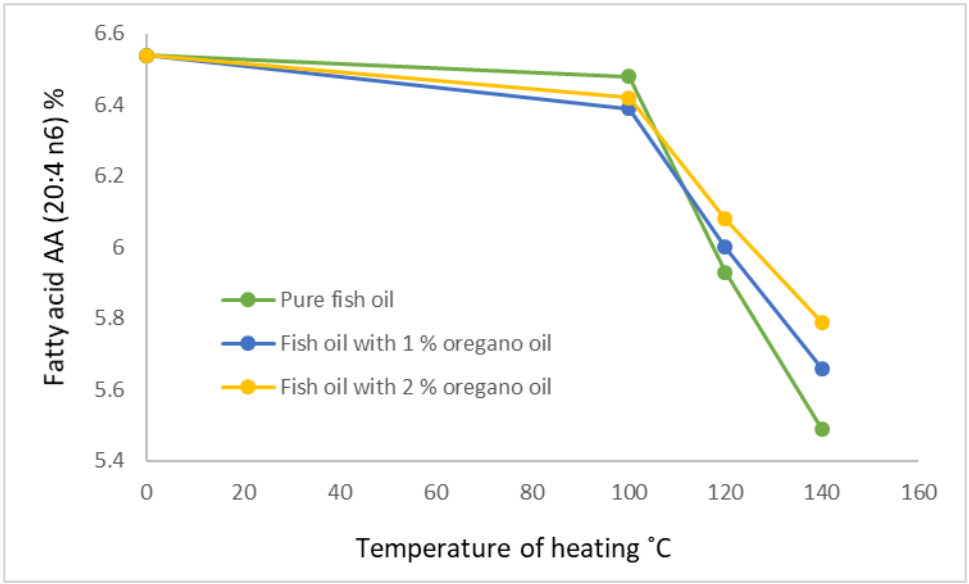

Figure 2: Monitoring Fatty acid AA (20:4 n6) of fish oil and with addition of oregano oil.

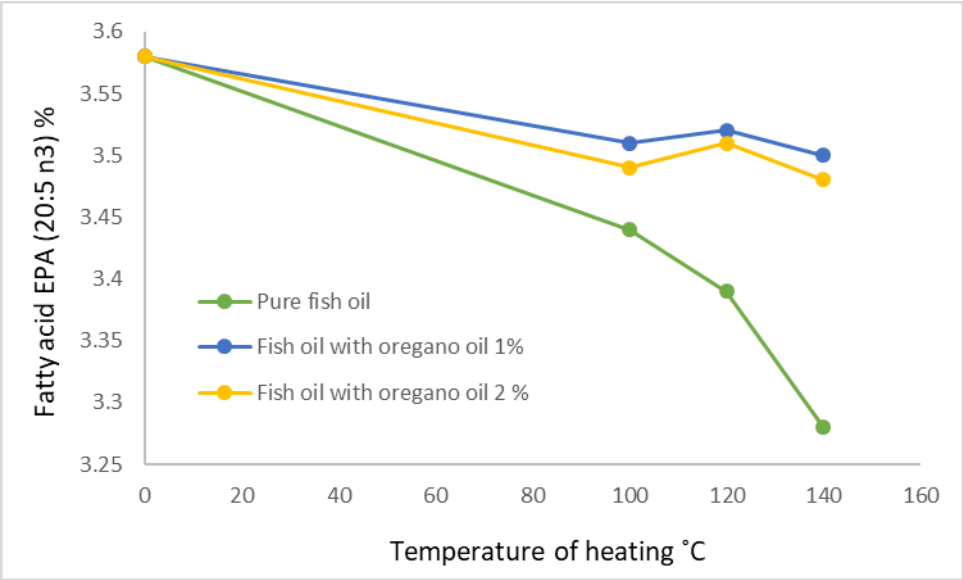

Figure 3: Monitoring Fatty acid EPA (20:5 n3) of fish oil and with addition of oregano oil.

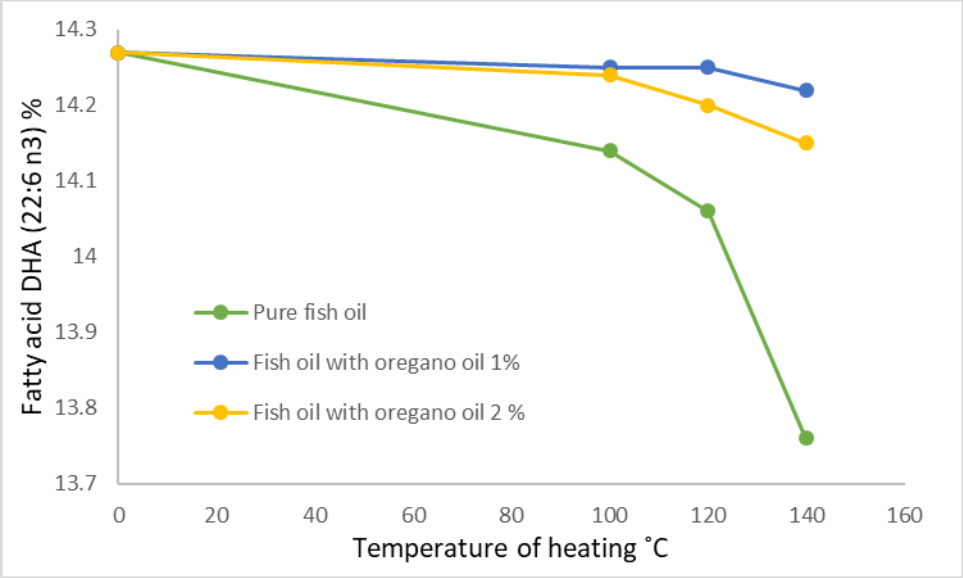

Figure 4: Monitoring Fatty acid DHA (22:6 n3) of fish oil and with addition of oregano oil.

3.3. FTIR Spectroscopy

FTIR spectra peaks are common for the majority of vegetable (edible) oils. Intesity of peak at 3010 cm^{-1} can be interpreted as the unsaturation level. Analysis of FTIR spectra based on carbonyl group at 1745cm^{-1} indicate triglycerides as mayor compounds in anlysed oil`s samples [7, 8].

FTIR characterization of analysed samples of oils during the first heating cycle they presented in Figure 5 and 6.

Infrared vibrational spectroscopy is one possibility to detect the stages of oxidation in the final and initial stages of oxidation of edible oils intensity ratio. Peak observed in 3444 cm-1 present the vibrational of the

hydroperoxides formed during the first step of the triglyceride oxidation known as unstable compounds. However, for the better presentation we will use the ratio of the bands. Figure 5 present the increasing of the hydroperoxides compounds in the pure oil samples and it is not the same changes in the oil samples with oregano extract and high protective activity has with 2 % composition of the oregano extract.

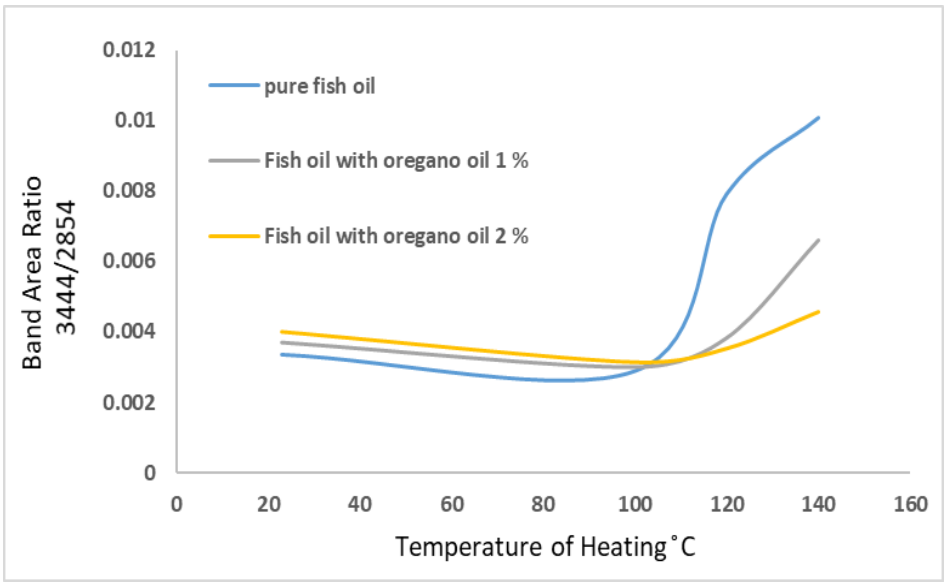

Figure 5: Ratio values of the band area changes.

In figure 6 present the triglyceride oxidative stability and for the pure fish oil without any aditives shows higher unstability because of the rapid decrease this ratio during the oil heating. Completely different pathways show the same oil with composition of the oregano extract, which is strongly confirmation of the increasing of the triglyceride stability as a major constituents in the edible oils.

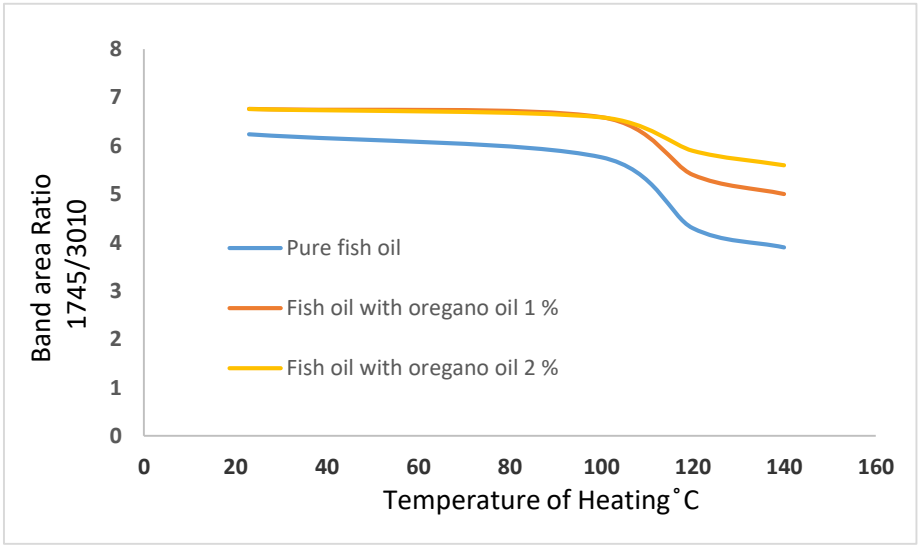

Figure 6: Ratio values of the band area changes.

4. Conclusion

The results obtained in this study oregano oil is a promising addition as natural antioxidants to vegetable (edible) oils. It can protect oils from oxidation processes, even at high temperatures for a certain time. From the results obtained, oregano oil was the most effective in delaying the oxidation of fish oil. Fish oil samples with Oregon oil have shown improved thermal stability compared to pure fish oil. In general, oregano oil in fish oil effectively protects its triglyceride structure (with minor structural changes).

Fatty acid profile determined by GC method confirms our fundings, which are in correlation with the investigation of the same samples by vibrational spectroscopy.

The FTIR spectroscopy used in this study proven as a suitable method for the analysis of structural changes of vegetable (edible) oil during heating. This methodology brings advantages over conventional laboratory procedures and allows rapid, specific, non-invasive analyses, independent of the number of chemicals and without the requirement of special sample preparation.

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