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# Preventive effect of herb extracts on lipid oxidation in fish oil

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***Abstract***

*Oxidative decay of oil is causing changes in its chemical, sensory and nutritional properties and is considered a major problem effecting loses in quality and nutritional value. This paper investigated the influence of the Lamiaceae plant extracts addition on oxidative stability of fish oil. The test was performed under accelerated oxidation by Rancimat method where different concentrations of herb extracts (basil, thyme and oregano) as well as effect of different temperatures were studied. Fatty acid profile of oil was analysed using gas chromatography. As expected, the applied temperature had significant impact on fish oil stability and the induction periods were prolonged after the addition of herb extracts. The best results at 80°C were obtained after addition of basil extract what resulted with induction period of 4.60 h. The addition of higher concentrations also resulted with prolonged induction periods; for oregano extract the results were dose-dependent, after addition of 50 and 100 µL of thyme extract the induction period remained the same (0.32 h), while the highest concentration of basil extract, probably due to presence of some components that acts as a prooxidants, resulted with lower IP value.*

***Keywords:*** *fish oil oxidation, fatty acids, Rancimat test, herb extracts, induction period*

## Introduction

Lipid oxidation is a complex process of oxidative deterioration in various lipid containing foods that causes its rancidity and production of off-flavours. Other changes such as loss of nutritional value and compromising food safety, biological damages, functional property changes, etc. affect the shelf life of different foods (Ladikos and Lougovois, 1990; Ramanathan and Das, 1992; Hamilton et al, 1997; Jacobsen, 1999). Lipid oxidation is enhanced by the presence of metal ions that act as prooxidants and facilitate the transfer of electrons leading to increased formation of free radicals (Ladikos and Lougovois, 1990). Beside hydroperoxides, which are the primary products, lipid oxidation results in production of other low molecular weight volatile compounds that are responsible for the changes in the aroma and flavour properties of food. Therefore, the result of lipid oxidation is usually a complex mixture of compounds; aldehydes, ketones, alcohols, hydrocarbons, esters, furans, lactones epoxides, hydroxy compounds, hexanal or malondialdehyde. The chemical composition and quantity of formed compounds depends primary on the fat composition but also on other factors like processing technology or/and storage conditions, type and concentration of proteins and/or antioxidants/prooxidants present in the food matrix, physicochemical characteristics, other present ingredients, etc. (Gray, 1978; Ladikos and Lougovois, 1990; Jacobsen, 1999).

The complexity of lipid oxidation process and its dependence on different factors makes the measurement of lipid oxidation a challenging task. A number of different chemical and physical methods have been developed to describe the oxidation process in a lipid media. The most common methods are peroxide value, thiobarbituric acid reactive substances (TBARS) test, Kries test, oxirane determination, conjugated diene method, gas chromatography, chemiluminescence, fluorescence emission, Raman spectroscopy, infrared spectroscopy or magnetic resonance, polarography, refractometry, etc. (Gray, 1978; Ladikos and Lougovois, 1990; Farhoosh, 2007; Barriuso et al, 2013).

At the ambient conditions, oxidation occurs slowly thus, these methods are usually time-consuming, costly and generally require reasonable analytical expertise. Several accelerated methods employing high temperatures and air-flow supply have been developed to estimate the oxidative stability and shelf life of oil-containing products in a relatively short time. In these methods, the rate of hydroperoxides formation and their breakdown into volatile products usually takes place simultaneously (Matthäus, 1996; Farhoosh, 2007; Farhoosh and Moosavi, 2007). Rancimat test is inexpensive, easy to use and reproducible method most commonly used for assessment of the oxidative stability of fats and oils. The test is based on the conductivity changes experienced by deionised water after collecting the volatile organic compounds, produced in the accelerated oil oxidation process (Farhoosh, 2007; Farhoosh and Moosavi, 2007). The induction period (IP), a measure of stability or shelf-life of edible fats and oils, is defined as time required to reach the end-point of oxidation which is detected as a sudden increase of water conductivity (Mendez et al, 1996).

Fish oils, containing high level of polyunsaturated fatty acids (PUFAs), have very important role in human diet. This characteristic and high content of metal ions makes fish oil

more susceptive to lipid oxidation than other fats and oils. The choice of antioxidants which are used to stabilize fish oil for human consumption is restricted to a few substances, with tocopherols being the most frequently used; however their activity is not always effective, especially in the presence of high concentrations of metals (García-Moreno et al, 2010).

In recent times, there is a great tendency to use natural compounds as food additives instead of synthetic ones. However, the number of reports on the use of plant extracts for the control of lipid oxidation of fish oil is scarce. Thus the aim of this study was to investigate the influence of the herb extract additions on prevention/delay of lipid oxidation in fish oil. For this purpose, the effects of three Lamiaceae spices extracts in different concentrations and under the different oxidation parameters on the fish oil oxidative stability were investigated using Rancimat apparatus.

## Materials and Methods

**Fish oil**

Commercially available fish oil, produced by Kemig d.o.o. (Donja Zelina, Croatia), was used as a lipid medium in this study. According to the product specification it was produced from different species of codfish - *Gadus* spp. (*oleum jecoris aselli*), and the content of unsaturated fatty acids in it was 85%. Furthermore, it contained vitamin A (data from declaration sheet, 1190 International Units per g). The fish oil, before the experiments, was stored at +4ºC.

**Herb extracts preparation and analysis**

Dry plan materials; basil (*Ocimum basilicum* L.), thyme (*Thymus serpyllum* L.) and oregano (*Origanum vulgare* L.), produced and distributed by Suban (Samobor, Croatia), were purchased from a local herbal pharmacy. Dry homogenized plant material (5 g) was extracted with 250 ml of ethanol : water mixture (80 : 20, v/v) at 60°C during 60 minutes. After the cooling, samples were filtered and the residual tissue was washed with solvent (3×10 mL). The extractions were performed in triplicate for each plant, and after the filtration, the three sample extracts were combined into final extract and evaporated to the volume of 150 mL.

**Fatty acid composition analysis**

Fatty acid profile of codfish oil was analysed using gas chromatography (GC). Fatty acid methyl esters were prepared by transmethylation using 2 mol/L solution of potassium hydroxide in methanol and heptane and analysed by GC, model Varian 3900 equipped with FID detector and a Restek capillary column (100 m × 0.25 mm ID; 0.2 μm film thickness). The oven temperature was programmed from 140°C (held for 5 min) to 240°C (held for 20 min) at the rate 4°C/min, while the injector temperature was 225°C and detector temperature 240°C. The fatty acid methyl esters were identified by comparing the retention time of each component to a standard (Supelco 37 Component FAME Mix, Sigma-Aldrich).

**Total phenolic content**

The total phenolic content in plant extracts was estimated using the Folin-Ciocalteu method (Katalinić et al, 2013). Spectrophotometric measurements were performed on a SPECORD 200 Plus, Edition 2010 (Analytik Jena AG, Jena, Germany). The measurements were performed in triplicate for each sample and the results are expressed as gallic acid equivalents, in grams per litre of extract (g/L).

***Rosmarinic acid content***

For quantification and identification of the rosmarinic acid high-performance liquid chromatography (HPLC) was used. The HPLC system used was HP 1090 Series II, equipped with UV/Vis photodiode array detector (Agilent Technologies, Palo Alto, California, USA). The compound was separated on a Nucleosil 100-C18 column using solvent A (water : acetic acid, 98 : 2, v/v) and solvent B (acetonitrile : acetic acid, 98 : 2, v/v) and with the following gradient program: 0 min 92% A and 8% B; 18 min 80% A and 20% B, 25 min 60% A and 40% B, 30 min 55% A and 45% B, 40 min 35% A and 65% B, 50 min 20% A and 80% B maintaining at 20 % A for 4 min (54 min), then from 20 to 90 % A at 57 min, and maintaining at 90 % for 3 min (60 min). The applied flow rate was 1.0 mL/min. The rosmarinic acid was confirmed by its spectral characteristics and retention time, and it was quantified using external standard calibration curve (Generalić Mekinić et al, 2014).

**Rancimat method**

The resistance of fish oil (with and without addition of herb extracts) to auto-oxidation was determined using Rancimat model 743 (Metrohm, Switzerland). In order to investigate the influence of the concentration of the added antioxidants, different volumes of the herb extracts were added (25 μL, 50 μL and 100 μL) to the fish oil (3.0 g). The effect of antioxidant concentration on oil stability was studied at temperature of 120°C. Furthermore, the oxidative stability of fish oil after the addition of 25 μL of extract was tested at three different temperatures (80°C, 100°C and 120°C), while the airflow in all experiments was constant (20 L/h). The oxidative stability was expressed as induction period (IP) in hours and oxidative stability index (OSI) which was calculated as in Kulišić et al (2005):

OSI = Induction time of oil with antioxidant / Induction time of pure oil

**Statistical analysis**

The obtained data were analyzed by performing the analysis of variance (one-way ANOVA), followed by a least significant difference test at 95% confidence level. These analyses were performed using Statgraphics® Plus v. 5.1 Professional (Manugistics, Inc., Rockvill, MD, SAD) software package. All measurements were performed in triplicate and the results are expressed as mean value ± standard deviation (SD).

## Results and Discussion

In this study, the effects of the herb extract addition on fish oil oxidation were studied. For that purpose, three ethanolic extracts from the Lamiaceae plants were prepared, basil, thyme and oregano, respectively. The phenolic content in plant extracts was determinated by Folin-Ciocalteu method, and the results are present in Fig. 1. The concentrations of phenolics in

basil and oregano extracts were extremely high, 7.08 and 7.44 g/L, while in thyme it was significantly lower (5.20 g/L). Rosmarinic acid is one of the most abundant phenolic acids occurring in plants and it is usually used as chemotaxonomic marker of Lamiaceae plant family (Zgórka and Głowniak, 2001; Kivilompolo and Hyötyläinen, 2007; Wojdyło et al, 2007). The full HPLC phenolic profile of extracts from plants belonging to Lamiaceae family, also for thyme and oregano extracts that are included in this study, is reported in papers we have previously published (Generalić et al, 2011; Generalić et al, 2012). These results are presented in order to potentially find the connection between the amounts of added phenolics/rosmarinic acid on oxidative stability of fish oil. The concentrations of rosmarinic acid in basil and oregano extracts were 0.61 g/L and 0.58 g/L, while almost three-fold higher amount was detected in thyme (Fig. 1). The impact of rosmarinic acid content in Lamiaceae extracts on their antioxidant properties, detected using a spectrum of *in vitro* antioxidant assays was confirmed in Generalić Mekinić et al (2014).

Food lipids are components that are very susceptible to oxidation processes, which result in rancidity, degradation of sensory properties and nutritional quality of food, so lipid oxidation reactions are one of the major causes of lipid-containing foods deterioration (Mendez et al, 1996; Farhoosh, 2007). These processes could occur during manufacturing, storage, distribution and even final preparation of food. Fish oil is a great source of PUFAs (which makes it the most popular dietary supplements worldwide), but presence of heme pigments and metalic ions, makes it highly prone to oxidation and one of the most labile supplements sold to consumers (Kaitaranta, 1992; Maqsood and Benjakul, 2010; Cameron-Smith et al, 2015). Tab. 1 shows fatty acid composition of codfish oil. Among detected saturated fatty acids (SFA) in analysed oil palmitic acid was present in highest concentration (16.61%). The dominant monounsaturated fatty acids (MUFAs) were oleic and erucic acid, and the most important PUFAs were eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). A high ratio of dietary n-3/n-6 polyunsaturated fatty acids was also confirmed (63.5). The n-6/n-3 ratio has proven to be good index for comparing relative nutritional value of fish oil, with a higher ratio being of great importance in the prevention of coronary hearth diseases, plasma lipid levels and cancer risks (Kinsella et al, 1990). The profile and content of fatty acids in various fish species is different and oxidative degradation of PUFAs has been reported even at low temperatures such as -18, 0 and 4°C (Boran et al, 2006; Šimat et al, 2015; Čagalj et al, 2016). Čagalj (2016) found no significant PUFAs damage in cod oil (containing vitamin D as antioxidant) cooked at 95°C over a period of five hours. The use of antioxidants as inhibitors of free radical autoxidation has a major importance in preserving PUFAs from oxidative deterioration at high temperatures (Frankel et al, 1996).

Lipid oxidation at ambient conditions occurs slowly, so to estimate the oxidative stability of products in a relatively short period, usually accelerated methods for determinations of oxidative stability of fats and oils are employed (Farhoosh, 2007). Oxidation of food components in food systems could be prevented, delayed or avoided by antioxidants (Kivilompolo and Hyötyläinen, 2007), and plants are well known as rich sources of compounds with good antioxidant activity. Among diverse group of phytochemicals present in plants, their antioxidant activity is usually attributed to the presence of phenolics (Generalić Mekinić et al, 2014; Katalinić et al, 2006; Shan et al, 2005). The use of plants in food preservation was a common practice since the ancient times, and among different plant species Lamiaceae plants were the most used and commercialized. These plants are well known because of high content of phenolics and good antioxidant properties which exceeded the effect of many currently used synthetic preservatives (Katalinić et al, 2006; Bonanni et al, 2007; Wojdyło et al, 2007; Generalić et al, 2012; Roby et al, 2013; Generalić Mekinić et al, 2014).

Tab. 2. presents the results for oxidative stability of fish oil, without and with the addition of 25 μL of herb extracts, tested at different temperatures. According to the results obtained for pure fish oil it can be concluded that the applied temperature has significant impact on its stability and IP. The IP of fish oil at 80°C was 4.40 h, while at higher temperatures it was significantly shorter, 0.50 h at 100°C and 0.13 h at 120°C. Rancimat test has been mainly used for vegetable oils, where high temperatures are required to obtain reasonable IP values, but Méndez et al (1996) investigated use of the Rancimat test for assessment of the relative stability of fish oil. In their study, anchovy, hake liver and sardine oils were investigated, and their IPs ranged from 1.4 to 3.2 h. The best results were obtained for anchovy oil at all studied temperatures (from 55 to 90°C) and the authors concluded that the IP depends primarily on oil fatty acid composition. In their study anchovy and sardine oil had similar content of SFAs and MUFAs, while hake liver oil contained lower amount of SFA and higher content of MUFAs. The content of total PUFAs in all investigated oils was similar, but anchovy and sardine oils were rich in EPA and DHA. Therefore, the authors confirmed that PUFA/SFA can be used as a measure of oil tendency to undergo autoxidation.

The samples of codfish oil that were used in our study contained about 85% of unsaturated fatty acids but also vitamins A and D what certainly supported the prolonged stability of oil. The addition of 25 μL of all extracts prolonged the IP of fish oil (Tab. 2). At 80°C the IP for fish oil with basil extract was 4.60 h and oxidative stability index (OSI) was 1.05, while other two extracts showed lower IPs, 4.45 h for oil with thyme and 4.52 h for oil with oregano, respectively. The antioxidant effect of the herb extract addition could be described by "polar paradox", the observation that polar compounds are more effective in non-polar lipids, whereas non-polar antioxidants act better in polar lipid emulsions (Shahidi and Zhong, 2011). There are few reports on the use of plant extracts, phenolic compounds and other antioxidative substances for the control of lipid oxidation of fats and oils (Kaitaranta, 1992; Ramanathan and Das, 1992; Nieto et al, 1993; Frankel et al, 1996; Maqsood and Benjakul, 2010). Among them Ramanathan and Das (1992) and Nieto et al (1993) investigated the effect of several phenolics and other commonly used synthetic and natural antioxidants on lipid oxidation in fish and fish oils using TBARS assay. The results obtained in both studies indicate that several phenolics have better antioxidant effect than synthetic antioxidants. In study of Nieto et al (1993) synergistic effect was obtained when mixtures of antioxidants were tested. The differences between the results obtained in our study after addition of 25 μL of extracts at 100°C were not significant, and they ranged from 0.55 to 0.56 h. At 120°C, same volumes of basil and oregano

extract provided more than two-fold longer IPs, while the IP value for the fish oil with 25 μL of thyme extract was only 0.17 h (IP 1.31) probably due to lowest concentration of phenolics in this extract. The results obtained at higher temperatures impart that rosmarinic acid which is present in thyme extract in more than two-fold higher concentration than in other extracts, doesn't have good antioxidant properties in lipid medium such is fish oil. Furthermore, the presence off rosmarinic acid in such high concentration could potentially be the reason for low oxidative stability of oil sample that contains thyme extract especially since Murakami et al (2007) and Muñoz-Muñoz et al (2013) proved that rosmarinic acid has prooxidant activity and produce reactive oxygen species in the presence of metal ions. According to the presented results, it can be concluded that in our study rosmarinic acid also acts as a prooxidant but the overall effect of extracts is probably result of interactions of other present phenolics. Also, as Rancimat test use flow of air and high temperatures to accelerate oxidation, the obtained results should be interpreted with care because it is well known that the mechanism of oxidation changes with temperature. Furthermore, the tested samples develop excessive levels of rancidity, which are not relevant to normal stage (ambient) conditions (Farhoosh, 2007).

According to the results obtained at 120°C for 25 μL, the addition of basil extract did not significantly prolong the stability of oil (from 0.13 h to 0.17 h), but more than two-fold longer inhibition period were obtained for other two extracts. As measurements of the extract addition to oil heated at temperature of 120°C provided the greatest differences between the samples, we decided to test the addition of higher extract concentrations (volumes) on oil oxidative stability. In Tab. 3 are presented the results obtained at 120°C after the addition of higher concentrations of extracts (50 and 100 μL). As can be seen, the addition of extracts in all cases prolonged the IP value but there were again some differences between the extracts. The addition of 50 μL of basil extract provided IP value of 0.36 h, while higher concentration of 100 μL resulted with IP value of 0.33 h. The reason for this probably lies in the fact that higher concentrations of some antioxidants present in basil extract could cause the opposite effect and induce their prooxidative activity. Similar conclusions could be brought for thyme extract where the IP value of oil after the thyme extract addition, both in concentration of 50 and 100 μL, resulted with same IP values (0.32 h). These results once more confirmed the lowest activity of thyme extract among all tested in the prevention of oil oxidative degradation. Thyme extract had the lowest content of total phenols but the highest concentration of rosmarinic acid. On the other hand oregano extract addition in higher concentrations affected the stability of fish oil and prolonged its IPs, to 0.34 h when 50 μL was added, and to 0.37 h when 100 μL was added. This extract contained the lowest share of rosmarinic acid, but the highest content of total phenols.

## Conclusions

This study aimed to investigate the effect of Lamiaceae plant extract addition of the oxidative stability of lipid mediums. Therefore fish oil, which is a rich source of polyunsaturated fatty acids and therefore highly prone to oxidation, was used. Results showed that despite the usage of relatively high temperatures (80, 100 and 120 °C) for this food system, the addition of phenolic extracts, even in small amounts, prolonged the IP of the oil. Moreover, addition of thyme extract, especially at higher concentrations, impart that the extracts or some of their components potentially have prooxidant activity. The reasons for that could be in a fact that mechanism of oxidation changes with temperature and concentration of the added antioxidant, as well with the proved fact that rosmarinic acid act as a prooxidant when metal ions are present in higher concentrations. Therefore, further studies are needed to test the major phenolics from these plants or the extract fractions in wide range of concentrations, but also in other mediums such as vegetable oils, where high temperatures are required to obtain reasonable IP.

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***Table 1.*** *Fatty acid profile of codfish oil.*

| Fatty acid | | | Composition [%] |
|---|---|---|---|
| Saturated fatty acids (SFA) | Lauric acid | C12:0 | 0.02 ± 0.02 |
| | Tridecanoic acid | C13:0 | 0.01 ± 0.01 |
| | Myristic acid | C14:0 | 0.36 ± 0.02 |
| | Pentadecanoic acid | C15:0 | 0.13 ± 0.01 |
| | Palmitic acid | C16:0 | 16.61 ± 0.18 |
| | Heptadecanoic acid | C17:0 | 0.42 ± 0.02 |
| | Stearic acid | C18:0 | 4.07 ± 0.34 |
| | Arachidic acid | C20:0 | 0.43 ± 0.01 |
| | Heneicosanoic acid | C21:0 | 1.30 ± 0.02 |
| | Behenic acid | C22:0 | 0.18 ± 0.0 |
| | Tricosanoic acid | C23:0 | 0.71 ± 0.01 |
| | Lignoceric acid | C24:0 | 1.10 ± 0.04 |
| | | ∑ SFA | 25.35 ± 0.44 |
| Monounsaturated fatty acid (MUFA) | Myristoleic acid | C14:1 | 0.50 ± 0.03 |
| | Palmitoleic acid | C16:1 | 0.79 ± 0.01 |
| | *Cis*-10-heptadecenoic acid | C17:1 | 0.47 ± 0.02 |
| | Elaidic acid | C18:1*n*-9t | 0.20 ± 0.0 |
| | Oleic acid | C18:1*n*-9c | 23.66 ± 0.1 |
| | *Cis*-11-eicosenoic acid | C20:1 | 3.11 ± 0.04 |
| | Erucic acid | C22:1*n*-9 | 13.98 ± 0.12 |
| | Nervonic acid | C24:1 | 0.65 ± 0.0 |
| | | ∑ MUFA | 43.35 ± 0.11 |
| Polyunsaturated Fatty Acid (PUFA) | Linolelaidic acid | C18:2*n*-6t | 0.12 ± 0.0 |
| | Linoleic acid | C18:2*n*-6c | 0.10 ± 0.02 |
| | γ-linolenic acid | C18:3*n*-6 | 0.06 ± 0.0 |
| | Linolenic acid | C18:3*n*-3 | 0.64 ± 0.01 |
| | *Cis*-11,14-eicosadienoic acid | C20:2 | 0.02 ± 0.02 |
| | *Cis*-8,11,14-eicosatrienoic acid | C20:3*n*-6 | 0 |
| | *Cis*-11,14,17-eicosatrienoic acid | C20:3*n*-3 | 0.11 ± 0.0 |
| | Arachidonic acid | C20:4*n*-6 | 0.20 ± 0.01 |
| | *Cis*-13,16-docosadienoic acid | C22:2 | 0.07 ± 0.04 |
| | Eicosapentaenoic acid (EPA) | C20:5*n*-3 | 13.63 ± 0.06 |
| | Docosahexaenoic acid (DHA) | C22:6*n*-3 | 16.35 ± 0.26 |
| | | ∑ PUFA | 31.30 ± 0.33 |

***Table 2.*** *Influence of the temperature on oxidative stability of fish oil before and after addition of herb extracts (25 µL)*

| Sample | TP (mg GAE) | IP (h) | | | OSI | | |
|---|---|---|---|---|---|---|---|
| | | 80°C | 100°C | 120°C | 80°C | 100°C | 120°C |
| **Fish oil (control)** | - | 4.40 ± 0.00 a | 0.50 ± 0.00 a | 0.13 ± 0.00 a | - | - | - |
| **Fish oil + basil extract** | 0.18 | 4.60 ± 0.06 b | 0.56 ± 0.00 b | 0.30 ± 0.00 c | 1.05 | 1.12 | 2.31 |
| **Fish oil + thyme extract** | 0.13 | 4.45 ± 0.01 a | 0.55 ± 0.01 b | 0.17 ± 0.00 b | 1.01 | 1.10 | 1.31 |
| **Fish oil + oregano extract** | 0.19 | 4.52 ± 0.04 ab | 0.55 ± 0.01 b | 0.31± 0.01 d | 1.03 | 1.10 | 2.38 |

***TP- total*** *phenols in miligrams of gallic acid equivalents (GAE), in 25 µL of extract; IP- induction period; OSI. Oxidative stability index*
***Numbers following*** *the different letters (a, b, c, d) in the column show the significant differences using the Multiple Range Tests, at $p < 0.05$.*

***Table 3.*** *Effect of the addition of herb extracts in different concentrations into fish oils on its oxidative stability at 120ºC*

| Added volume | 50 µL | | | 100 µL | | |
|---|---|---|---|---|---|---|
| Sample | TP* (mg GAE) | IP (h) | OSI | TP** (mg GAE) | IP (h) | OSI |
| **Fish oil (control)** | - | 0.13 ± 0.00 a | - | - | 0.13 ± 0.00 a | - |
| **Fish oil + basil extract** | 0.35 | 0.36 ± 0.01 a | 2.77 | 0.71 | 0.33 ± 0.01 a | 2.54 |
| **Fish oil + thyme extract** | 0.26 | 0.32 ± 0.00 b | 2.46 | 0.52 | 0.32 ± 0.01 b | 2.46 |
| **Fish oil + oregano extract** | 0.37 | 0.34 ± 0.03 b | 2.61 | 0.74 | 0.37 ± 0.01 c | 2.85 |

***TP- total*** *phenols in miligrams of gallic acid equivalents (GAE), the amount in *50 µL of extract, and in 100 µL of extract; IP- induction period; OSI. Oxidative stability index*
***Numbers following*** *the different letters (a, b, c, d) in the column show the significant differences using the Multiple Range Tests, at $p < 0.05$.*

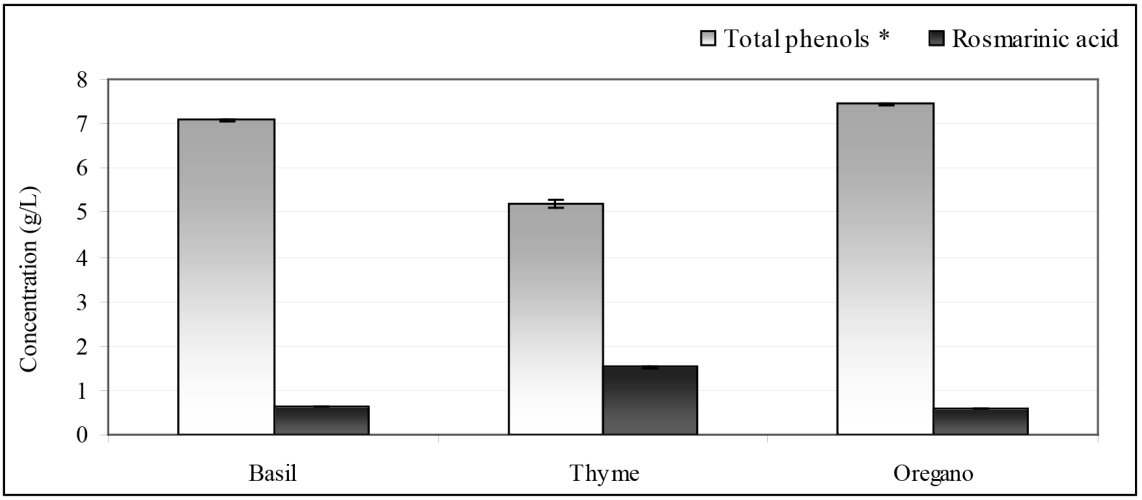


***Figure 1.*** *Concentrations of total phenols and rosmarinic acid in extracts of basil, thyme and oregano.*

* Total phenols are expressed in grams of gallic acid equivalents per litre