EVALUATION OF THE ANTIOXIDANT EFFECT OF *THYMUS VULGARIS* EXTRACT SUNFLOWER OIL USED IN FOOD THERMAL APPLICATIONS

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ABSTRACT
This study was performed to investigate the retarding lipid oxidation of sunflower oil using thyme extract (TE) compared with butylated hydroxytoluene (BHT). The sunflower oil was subjected to convection heating for 1, 4, 8, 12, and 16 hours. The analyses which were made in order to watch the progress of lipid oxidation are the peroxid value (PV), p-anisidine value (p-AV) and TOTOX value. The antioxidant characteristics of thyme was measured determining the ferric reducing antioxidant power (FRAP) value and total phenolics value. The results of this study highlight that thyme extract (TE) showed a significant inhibitory effect on lipid oxidation during heat treatment. Thyme extract in doses of 200 and 600 ppm inhibited the lipid oxidation in a similar manner to buylated hydroxytoluen, and in a dose of 1000 ppm resulted a significant decrease of investigated indices such as peroxid value, p-anisidine value and TOTOX value. Thyme natural extract can be used for improving oxidative stability of sunflower oil replacing successfully butylated hydroxytoluen according to the results.

Keywords: thyme extract, sunflower oil, convective heating antioxidant oxidative stability

INTRODUCTION

Sunflower oil is commonly used in food as a frying oil and is the fourth largest oil source in the world, after soybean, palm and canola oil. In some countries is the most popular vegetable oil and it is used as a source of essential linoleic acid. The main deterioration process that occurs during thermal processing is lipid oxidation and the decomposition of oxidation products which result in decreased nutritional value and sensory quality (SHWETA ET AL., 2012; POIANA M. 2012)

A method of protection against oxidation is to use specific additives which inhibit oxidation. Synthetic antioxidants are added for improving oxidative stability of vegetable oils but because of their toxicity and carcinogenicity this addition is discouraged and the interest for sources of natural antioxidants increased (ZHANG ET AL., 2010).

Thymus vulgaris or common thyme is a low growing herbaceous plant, sometimes becoming somewhat woody. It is native to Southern Europe, where it is often cultivated as a culinary herb. Thyme contains a variety of flavonoids, including apigenin, naringenin, luteolin, and thymonin. These flavonoids increase thyme's antioxidant capacity, and combined with its status as a very good source of manganese, give thyme a high standing on the list of anti-oxidant foods. The mechanism of antioxidative activity of phenolic compounds consist in their capability of radical scavenging, metal chelating and synergism with other antioxidants (KAYOKO ET AL., 2002; NADA ET AL., 2010).

MATERIAL AND METHOD

Materials

The sun flower oil without addition of any antioxidants used was "Tip" oil which is produced exclusively for Real Hipermarket.
Thyme extract was obtained from dried leaves originated from Romania (Fares). All other chemicals and solvents used were purchased from Merck (Darmstadt, Germany) and were of analytical grade.
Rotary evaporator: Heidolph Laborata 4000
Convection oven: Esmach, Italy, 1200W
Spectrophotometer: Analytic Jena Specord 205

Antioxidant Activity (FRAP Assay)

The antioxidant activity of thyme extract was measured using the ferric reducing antioxidant power (FRAP) assay (BENZIE AND STRAIN, 1996). In order to evaluate antioxidant activity, 0,1 g TE, respectively BHT were mixed with 20 mL ethanol/water (70:30, v/v) for 10 min, then the solution was filtered and used for analysis. FRAP values were obtained reading the absorbance changes at 595 nm using a UV-VIS spectrophotometer. Results were expressed as mM Fe^{3+} equivalents/g TE, respectively BHT.

Total Phenols Assay

Total phenolic content of TE was determined using the Folin-Ciocalteu colorimetric method (SINGLETON ET AL., 1999). A calibration curve using gallic acid was prepared and the absorbance of the standards and samples were measured at 750 nm. Results were expressed as mM gallic acid equivalents/g.

Peroxid Value (PV)

Oil sample of 2.00 g was weighed into a 250 mL Erlenmeyer flask then 10 mL of chloroform and 15 mL of glacial acetic acid were added. The flask was swirled until the sample was dissolved then 1 mL of saturated potassium iodine (KI) solution was added. The flask was closed and let for 1 min in dark and after 5 more minutes to stand. 75 mL of distilled water was added then in the presence of starch it was slowly titrate with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) (AOCS, 1998).

The p-anisidine value (p-AV)

The p-anisidine value is a measurement of carbonyl content in the oils and was determined by the standard method according to AOCS (AOCS, 1998)]. It is based on 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.
Two grams of the sunflower oil samples were dissolved in 25 mL isooctane and absorbance (A_1) of this fat solution was measured at 350 nm against a blank of isooctane. An aliquot (5 mL) of this solution, respectively 5 mL of isooctane was transferred to each of two test tubes of 10 mL and 1 mL of anisidine solution (0.25% g/v glacial acetic acid) was added to each. After 10 min the absorbance (A_2) was measured at 350 nm against isooctane containing p-anisidine. p-AV was calculated according to the formula:

$$\text{p-AV} = 25 \times 1.2 \times A_2 - A_1 / w$$

TOTOX value

The total oxidation value (TOTOX) was used to estimate the oxidative deterioration of lipids. TOTOX value is defined as the sum of both values (PV and p-AV) to total oxidation and was calculated according to the formula:

$$\text{TOTOX value} = 2 \times PV + p\text{-}AV$$

RESULTS

Antioxidant characteristics of thyme extract (TE) and butylated hydroxytoluene (BHT) are presented in *Table 1*.

Table 1. Antioxidant characteristics of TE and BHT

Sample	FRAP value (mM Fe^{3+}/g)	Total phenolics (mM GAE/g)
Thyme extract (TE)	76.4	120.2
BHT	1.36	-

Acording to results FRAP value is significantly higher for thyme extract and Total phenolics was determined only for thyme extract because BHT it is a synthetic antioxidant.

Peroxid value was used as indicator for the primary oxidation of sunflower oil. The primary products of lipid oxidation are hydroperoxides and determination of peroxides can be used as oxidation index for the early stages of lipid oxidation. The results of PV are presented in *Table 2*.

Table 2. Effect of TE and BHT on peroxid value during sunflower heating in convection oven

Time (hours)	Control	BHT 200 ppm	TE 200 ppm	TE 600 ppm	TE 1000 ppm
1	2.87	2.60	2.74	2.63	2.55
4	3.52	3.28	3.36	3.12	3.03
8	5.13	4.02	3.82	3.36	3.14
12	5.65	5.52	5.38	4.08	4.00
16	7.02	6.83	6.30	6.27	6.03

p-AV value is a mesurement of the secondary oxidation products (alcohols, ketones, aliphatic aldehydes and acids). Results regarding p-AV value are presented in *Table 3*.

Table 3. Effect of TE and BHT on p-AV during sunflower oil heating in convection oven

Time (hours)	Control	BHT 200 ppm	TE 200 ppm	TE 600 ppm	TE 1000 ppm
1	17.27	15.02	14.85	14.34	14.07
4	18.00	15.48	15.60	14.92	14.27
8	19.44	16.72	16.93	15.65	15.29
12	26.12	23.46	23.41	22.18	22.10
16	28.16	25.44	25.83	25.11	24.85

CONCLUSIONS

The present research was carried out in refined sunflower oil free of additives, supplemented by three concentration levels of thyme extract (200, 600, 1000 ppm) and one level of BHT (200 ppm). The samples were thermal treated in convective oven for 1, 4, 8, 12 and 16 hours.
According to the results it can be observed that because of thermal treatments oxidation of sunflower oil increased. Supplementation with thyme extract and BHT reduced the lipid oxidation resulting significant differences. The inhibitory effect of TE against primary oxidation of lipids was concentration-dependent.
Significant differences were also observed at p-AV results and it can be seen that phenolic compounds from thyme had a strong inhibitory effect on the secondary lipid oxidation.
The efficiency of thyme extract regarding oxidative stability of sunflower oil during thermal applications increased with increasing concentration of the natural extract and prove that thyme extract is a very efficient inhibitor of lipid oxidation.

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