COMPARATIVE ANALYSIS OF THE ANTIOXIDANT CAPACITY OF SOME NATURAL AND SYNTHETIC ANTIOXIDANTS ADDED TO PALM OIL

ORIGINAL SCIENTIFIC PAPER

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DOI: 10.51558/2232-7568.2022.15.2.19

RECEIVED 2023-01-16 **ACCEPTED** 2023-02-27

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ABSTRACT:

Various synthetic and natural antioxidants are used to reduce oxidation and its negative impact on the oil during the food frying process. Considering that some studies show the negative impact of synthetic antioxidants on the health of consumers, natural alternatives are being used more and more. BHA and BHT are synthetic antioxidants that are widely used in the food industry and a large number of natural compounds such as phenols, anthocyanins, flavonoids, vitamins, etc. show antioxidant properties. In this study, the antioxidant capacity of oregano and rosemary essential oil was tested and compared with the antioxidant capacity of BHA and BHT. The antioxidant capacity was evaluated by the removal of DPPH radicals and by iron reduction (FRAP). The results of this study showed that the studied essential oils exhibited antioxidant capacity. These oils have a high antioxidant capacity, however, compared to synthetic antioxidants, they show a significantly lower antioxidant capacity but they can be used as natural antioxidants during food processing.

KEYWORDS: antioxidant capacity; essential oils; synthetic antioxidants; lipid oxidation

INTRODUCTION

Lipid oxidation and oxidative stress occur due to various influences in the processes of food production, processing and preparation. This is recognized as a major problem in the use of edible oils during frying, as well as other methods of food preparation. Oxidation as well as oxidative stress cause negative changes in the chemical, sensory and nutritional properties of food.Various synthetic and natural antioxidants are used to reduce oxidation and its negative impact on the oil during the food frying process. Synthetic antioxidants are cheaper than natural ones, but it is generally accepted that natural antioxidants have a stronger, more efficient and health-safe effect than synthetic ones.

Antioxidants are chemical compounds that can be used to improve the oxidative stability of oils and fats by interrupting the free-radical mechanism or autooxidation [1].A large number of compounds of plant origin have antioxidant properties and can neutralize free radicals. Such compounds are most often phenols, carotenoids, anthocyanins, flavonoids, unsaturated fatty acids, vitamins, enzymes, etc. This has stimulated interest in using them as oxidants during food processing [2].

Antioxidants are compounds capable of donating hydrogen radicals to free radicals available to prevent oxidative damage [3]. Free radicals are highly reactive molecules with unpaired electrons that can cause various oxidative stresses. Oxidative stress involves the generation of reactive oxygen and nitrogen species. Such species have been implicated in aging and various pathological processes because they damage the structures of cells, lipids, membranes, proteins, and DNA [4].

People's eating habits are changing very quickly today, and fast food, usually fried, is often consumed. Although the common trend is to reduce or limit the consumption of fried foods, their consumption level is gradually increasing owing to their properties such as being tasty, easily prepared and micro-biologically safe. In order to minimize the negative effects and/or to maximize the positive health effects and to maintain the quality of the fried products it is necessary to addition of antioxidant additives into oil [1].

Lipid oxidation can be prevented by using various chemical additives. However, several questions about safety of these chemicals used for food preservation were raised. In fact, they were suspected to have negative effects on consumer's health. Thus, natural

antioxidants have become highly demanded as alternatives and better choice to the chemical additives [5].Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used widely in the food industry to prevent lipid oxidation. BHA and BHT have been reported to have toxic and carcinogenic effects[6]. Therefore, natural antioxidants have attracted increased interest because natural ingredients may be safer than synthetic ingredients. As natural antioxidants, essential oils with high antioxidant capacity, such as oregano, rosemary, and other plant species rich in phenolic components, are increasingly being used. Essential oils are natural, volatile complex compounds characterized by the odor of their corresponding aromatic plants, which synthesize them as secondary metabolites [4].

In this paper focus is on oregano and rosemary essential oil. The compounds responsible for antioxidant activity of oregano include caffeic, coumaric and rosamarinic acids, carvacrol, thymol, and flavonoids. The compounds responsible for antioxidant activity of rosemary include phenolic acids (caffeic, ferulic, and rosmarinic acid) and phenolic diterpenes (carnosic acid and carnosol) [3].
The aim of this study is to compare the antioxidant capacity of natural antioxidants such as oregano and rosemary essential oil with synthetic antioxidants such as BHA and BHT, which are among the most commonly used in the food industry.

EXPERIMENTAL

MATERIALS

Natural and synthetic antioxidants were used in this research. As natural antioxidants were used oregano androsemary oil and as synthetic were used butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

Oregano oil (*Origanum vulgare*) - according to the declaration, it is 100% essential oil of wild oregano. It contains 85% carvacrol and 5% thymol. Country of origin is India.

Rosemary oil (*Rosmarinus officinalis, Lamiiaceae*) as cineol-type essential oil in a vegetable carrier, colorless to pale yellow in color, with a characteristic, refreshing scent of conifers was used. Rosemary oil is obtained by steam distillation from fresh rosemary flower tops. Country of origin is Bosnia and Herzegovina. According to the manufacturer's declaration, rosemary essential oil contains: 1,8-cineole, monoterpenes: alpha and beta pinene, camphene and limonene, alcohols, monoterpenols (linalool, alpha-terpineol and borneol), and iso-bornylacetate ester, and contains ketones: camphor, verbenone and thujone.

Butylated hydroxyanisole - (2-*tert*-butyl-4-methoxyphenol) – BHA, serial number: 1001176424, manufacturer: MERCK, Germany.

Butylated hydroxytoluene - (2,6-Di-*tert*-butyl-4-methylphenol) – BHT, serial number: 10112785, manufacturer: MERCK, Germany.

METHODS

PREPARATION OF SAMPLES

Extraction of active substances from the oil: Weigh about 1 g of oregano and rosemary oil into a 100 mL erlenmayer flask and add 9 mL of methanol. Samples were placed on a shaker for 1 hour (400 beats per minute). After that, the samples were transferred to cuvettes and centrifuged for 10 minutes at 3000 rpm. Then, the supernatant was transferred to a 10 mL flask and filled with methanol to the mark. For oregano oil, a dilution of 1000 was taken.

Synthetic antioxidants: Weighed 10 mg in a 10 mL flask, dissolved in methanol and filled to the mark. Dilutions were made from this, 1 mL in 10 mL.

The antioxidant activity was determined spectrophotometrically on a UV-VIS spectrophotometer (Shimadzu UV-1800) using two methods: the DPPH method, which uses 2,2-diphenyl-1-picrylhydrazyl radical as the radical, and the FRAP method.

DPPH RADICAL SCAVENGING ACTIVITY ASSAY

The ability of the extract to act as a free synthetic DPPH radical scavenger was measured by the decrease in absorbance at a wavelength of 517 nm after the addition of the extract [7]. Determination of the antioxidant capacity was determined by the DPPH method, i.e. neutralization of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical according to the method described in the work by Matejić et al. (2013) with minimal changes [8].

A specified sample volume (10-100 μL) is taken, add methanol to 4 mL and 1 mL of 0.5 mM DPPH solution in methanol. After 30 minutes of reaction at room temperature in the dark, the absorbance value was measured at wavelenght 517 nm using a UV-VIS spectrophotometer (Shimadzu UV-1800) against a blank. The blank composed of 4 mL of methanol without the extract mixed with 1 mL of 0.5 mM DPPH solution in methanol. The radical scavenging activity of each solution was calculated as the inhibition percentage according to the following equation:

$$\% \text{ Inhibition} = [(A_{blank}-A_{sample})/A_{blank}]\times 100 \quad (1)$$

where A_{blank}: the blank absorbance at 517 nm, A_{sample}: the sample absorbance at 517 nm. The percentage inhibition of DPPH was expressed as IC50. The IC50 value defined as the concentration of antioxidant that could reduce the initial concentration of free radicals to 50%[9].

FERRIC ION REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

The FRAP method is based on the ability of the extract to reduce F^{3+} ions to Fe^{2+} ions. The resulting Fe^{2+} ions with the TPTZ reagent (2,4,6-tri(2-pyridyl)-s-triazine) form a blue-colored complex, which reaches an absorption maximum at 593 nm [10].

The FRAP reagent was prepared by mixing 25 mL of 0.3 M acetate buffer (pH 3.6), 2.5 mL TPTZ (10 mM, in 40 mM HCl) and 2.5 mL $FeSO_4 \times 6H_2O$ (20 mM). The FRAP reagent is thermostated at 37°C. All solutions were prepared on the day of use. 3 mL of FRAP reagent freshly was added to 100 µL of the extract and incubated in a water bath for 30 minutes at 37 °C. After 30 minutes, the absorbance value was measured at wavelenght 593 nm using a UV-VIS spectrophotometer (Shimadzu UV-1800) against a blank. The blank composed of 3 mL of FRAP solution mixed with 100 µL of methanol.

Series of standard $FeSO_4 \times 7H_2O$ solutions in concentrations of 200-1000 µmol/L was prepared to create a standard curve. FRAP values were calculated according to the calibration curve for $FeSO_4 \times 7H_2O$:

$y=0.0008x+0.0681$

where y is the absorbance at 593 nm and x is the concentration of $FeSO_4 \times 7H_2O$ in µmol/L; $R^2=0.9952$. FRAP values were expressed as µmol Fe^{2+}/g of sample.

RESULTS AND DISCUSSION

The results of the antioxidant capacity estimated by DPPH and FRAP assay are shown in Table 1. As stated in the experimental part, the percentage of DPPH radical inhibition is expressed as IC50. The IC50 value is defined as the concentration of antioxidants that can reduce the initial concentration of free radicals to 50%. The lower the IC50 value, the greater the antioxidant capacity of that sample, i.e. a lower concentration of antioxidants is needed to inhibit the DPPH radical.

Table 1. Antioxidant activity of BHA, BHT, oregano and rosemary oil determined by DPPH and FRAP assay.

Sample	IC50 (mg/mL)	FRAP(µmol Fe^{2+}/1 g of sample)
BHA	0.0052	12341
BHT	0.011	9928
Oregano oil	0.474	4304
Rosemary oil	56.61	3584

The IC50 value for synthetic antioxidants (BHA and BHT) is 0.0052 and 0.011mg/mL respectively. According to Ceylan et al. [11], the IC50 value for BHAis 0.035± 0.007mg/mL and for BHT0.020± 0.001mg/mL. The FRAP value for BHA is 12341µmol Fe^{2+}/g of sample and BHT 9928µmol Fe^{2+}/g of sample. According to Ceylan et al., the FRAP value is higher for BHA than for BHT, and according to Kasote et al. [12] it is the other way around, although the values are approximately the same. The FRAP value for BHA is 8333± 7.44, and for BHT 8666± 7.22µmol Fe^{2+}/g of sample according to Kasote et al. According to the results in this work, the antioxidant capacity is higher for BHA.

When it comes to natural antioxidants, it can be noted that oregano oil has a significantly better IC50 value than rosemary oil, while the results for the FRAP value don't show such a difference. The IC50 value for natural antioxidants (oregano and rosemary oil) is 0.474mg/mL and 56.61 mg/mL, respectively. The FRAP value for oregano oil is 4304µmol Fe^{2+}/1 g, and for rosemary 3584 µmol Fe^{2+}/1 g of sample. Figure 2 shows the results of FRAP values graphically. Wang et al. [13] investigated the antioxidant capacity of rosemary essential oil from China and the Mediterranean region, and obtained IC50 values of 21.56mg/mL, 23.8mg/mL and 16.45 mg/mL.They also determined the antioxidant capacity using other methods, and came to the conclusion that the antioxidant activity of rosemary showed that samples grown in China perform better than those of the Mediterranean region [13]. It can be concluded that the concentration of essential oil compounds varies depending on the time of harvest, growing conditions, geographical location, altitude, climate, genetic diversity and phenological age of the plant at the time of cutting [14]. According to Almeida et al. [15]the IC50 value for oregano oil is 0.5 mg/mL, which is almost the same value as in this study.

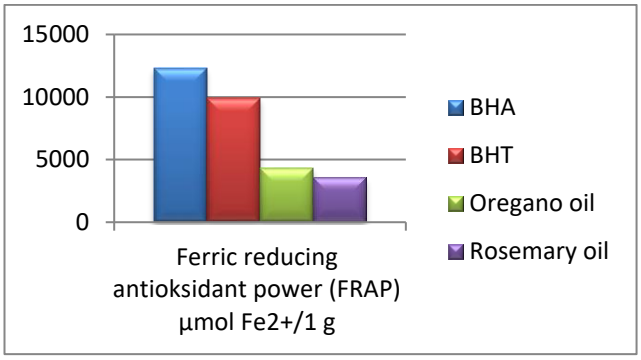

Figure 1. Graphically representation of FRAP values.

From the presented results, it can be seen that synthetic antioxidants have a higher antioxidant capacity than natural ones. Butylated hydroxyanisole (BHA, E320) has the highest antioxidative capacity, followed by butylated hydroxytoluene (BHT, E321), oregano essential oil, and finally rosemary essential oil. The antioxidant capacity values determined by both methods were generally parallel to each other.

This following fact should also be taken into account that BHA and BHT are very effective during the storage and transport of oils and fats, but they are less effective at frying temperatures, due to their volatility. Also, rosemary extracts are particularly active as antioxidants at the high temperatures in frying fats. They protect the oils during frying and their antioxidant activity is carried over into the fried foods[6].

CONCLUSION

In recent years, studies have focused on natural antioxidants such as essential oils because of the toxicity and carcinogenicity of synthetic antioxidants. The results of this study showed that the studied essential oils exhibited antioxidant capacity. These oils have a high antioxidant capacity, however, compared to synthetic antioxidants, they show a significantly lower antioxidant capacity. However, looking at the long term and taking into account the health aspect and food safety, it is better to use natural ones.According to these results, essential oils can be used as natural antioxidants during food processing, to prevent lipid oxidation. Especially when compared to synthetic antioxidants, natural antioxidants are easily acceptable by consumers. They are considered safe, the legislation does not require any safety tests. People have used such additives for hundreds of years before and they not only prolong the shelf life, but also increase the nutritional value of foods.

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