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Antioxidant effect of essential oils of Thymus, Salvia and Rosemarinus on the stability to auxidation of refined oils

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ABSTRACT

The main idea of this work was to study the stability to autoxidation of refined oils with the addition of three essential oils. First, antioxidative activity of the essential oils which are isolated by water distillation from three plants (Thymus Capitatus Hoffmanns, Salvia officinalis L and Rosemarinus officinalis L.), was evaluated by using a β-carotene/linoleic acid system. Peroxidation of the linoleic acid was effectively inhibited by the three essential oils. In fact, they showed strong antioxidative properties when compared to BHT and BHA. Second, the stability to auto-oxidation of refined oils is evaluated using the Rancimat method. This method provides the induction time for decomposition of hydroperoxydes, produced by fat oxidation. This stability was also evaluated by the determination of the Peroxides Value (PV). As a result, it can be suggested that the stability of refined oils was improved with the addition of the essential oils, which possess antioxidant activities. These findings suggest a potential use of these essential oils as natural preservative ingredient in food industry.

Keywords: Antioxidant ; Lipid peroxidation ; *Salvia ; Thyme ; Rosemarinus* ; β-Carotene/linoleic acid test ; Rancimat method ; Peroxides Value.

INTRODUCTION

Preservation of the lipidic fraction of foods from oxidative deterioration represents an important aim from the point of view of quality and shelf-life. It is indeed well known that the products derived from lipid oxidation reduce organoleptic quality and safety, besides reducing nutritional properties because of destruction of oxygen-sensitive vitamins.

Oils and fats are susceptible to oxidation. Traditionally, chemically synthesized compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ter-butyl hydroquinone (TBHQ), are widely used as antioxidants in oil products [1]. However, these synthetic antioxidants (BHT, BHA and TBHQ) are known to have toxic and carcinogenic effects on human health. For example, BHT causes liver expansion [2]. Therefore, exploration of natural antioxidants are gaining importance day-by-day. Currently, a very promising way to overcome this is to use vegetable antioxidants for nutritional, therapeutic or food quality preservation purposes [3]. In recent years, spice extracts have appeared on the market as antioxidants for food industry use. The antioxidant capacity of some of these compounds has been proved to be comparable to, and sometimes higher than, that of

synthetic antioxidants [4, 5]. In particular, the Lamiaceae family includes a large number of plants that are well known for their antioxidant properties. Among these, rosemary and sage have been widely used and most of their antioxidant components have been identified. It has been established that the antioxidant effects are mainly due to phenolic compounds [6].

This investigation attempts to study the antioxidant activity of essential oils of three Tunisian aromatic plants and their efficacy in preventing the oxidative changes on storage of refined oils.

MATERIALS AND METHODS

2.1. Plant material

Three Tunisian aromatic plants were used for this study: *Thymus Capitatus Hoffmanns, Salvia officinalis L and Rosemarinus officinalis L*. These plants were collected from different regions of north Tunisia at the flowering stage. Collected plant materials were dried in the shade, and then the leaves of plants were separated from the stem until used.

2.2. Oil samples

Refined olive, corn and soybean oils were purchased from the local market. The oil samples were stored in accordance with the experimental conditions.

2.3. Isolation of the essential oils

According to the literature [7], the essential oil chemical composition depends on the isolation method. In this study, we used the water-distillation method. Both, leaves and water are putted in a 10 l balloon flask. After heating, the steam produced is condensed by refrigeration. The essential oil is obtained through simple decanting. The duration of water-distillation was 3 h; the time was measured from the falling of the first drop of distillate. The isolation oil of the three plants on about 500 g of composite sample leaves at atmospheric pressure, as well as the harvest or drying process, were carried out using the same protocol. The essential oil was dried over Na_2SO_4 and stored at + 4° C until tested and used.

2.4. β-carotene-linoleic acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [8, 9]. A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade) and 25 µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water saturated with oxygen (30 min 100 ml/min) were added with vigorous shaking. Two thousand five hundred microlitres of this reaction mixture were dispensed into test tubes and 350 µl portions of the extracts, prepared at 2 g/l concentrations, were added and the emulsion system was incubated for 48 h at room temperature. The same procedure was repeated with synthetic antioxidants, BHT and BHA, as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT, BHA and blank consisting of only 350 µl ethanol. The antioxidant activity was expressed as inhibition percentage with reference to the control after 48 h using the following equation: $AA = 100(DR_C - DR_S)/DR_C$, where AA = antioxidant activity; DR_C = degradation rate of the control = [ln(a/b)/ 48]; DR_S = degradation rate in presence of the sample = [ln(a/b)/48]; a = absorbance at time 0; b = absorbance at 48 h.

2.5. Oxidative stability

2.5.1. Rancimat method

The three essential oils were added to 2 g of pure lard oil, giving a final concentration of 0.01% (w/w). Oxidation induction time was evaluated by the Rancimat method. Stability was expressed as the oxidation induction time (h), measured with the Rancimat apparatus (Metrohm AG, Herison, Switzerland) using an oil sample of 2 g, warmed to 120° C and a purified air flow rate of 20 l/h. In the Rancimat method, the volatile degradation products were trapped in distilled water and determined conductometrically. The induction time (IT) was defined as the time necessary to reach the inflection point of the conductivity curve [10].

2.5.2. Measurement of Peroxide Value (PV)

The samples were also analyzed for peroxide value (PV) for the determination of the oxidative stability [11].

2.6. Statistical analyses

The experiments results were expressed as mean ± standard deviation. Analysis of variance was determined by Multifactor ANOVA using STATGRAPHICS Centurion XV Version 15.2.11. Differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

3.1. Antioxidant activity

In the β-carotene linoleic acid system, oxidation of linoleic acid was effectively inhibited by the two synthetic antioxidants BHT and BHA (Fig. 1). They showed respectively 97.64 % and 94.64 % inhibition (Fig. 2). It can also be noticed that, the three essential oils showed strong antioxidant property when compared to BHT and BHA. Indeed, thyme, rosmarinus and sage showed respectively 88 %, 78.8 % and 73.87 % inhibition (Fig. 2). Antioxidant activity of the sample decreased in the following order: BHT > BHA > thyme > rosmarinus > sage.

The three essential oils used in this study could be used in food as a natural antioxidant to replace the artificial antioxidant. Natural extracts are regarded safe and could be added to food, resulting in an antioxidant activity comparable to that of the synthetic antioxidants. The study of Ozcan and Arslan (2011) [12] showed that the essential oils of rosemary have stronger antioxidant effect on poppy oil. In another study, these authors reported the antioxidant effects of the same spice volatile oils in sun flower oil in their study with an order like: summer savory > rosemary > sage > marjoram > oregano > anise > tarragon [12].

3.2. Oxidative stability

The refined vegetable oils selected for the study were olive oil, corn oil and soybean oil. The selection of corn and soybean oils was made owing to their common use as cooking media and also high relative reaction rates of their unsaturated fatty acids with oxygen [13]. Indeed, Relative reaction rates of unsaturated fatty acids with O_2 and inherent stability of the oils, calculated by List and Erickson [13], have been summarized in Table 1. Oxidation, which consists of a complex series of chemical reactions, is characterized by a decrease in the total unsaturated content of the oil due to abstraction of hydrogen adjacent to double bond and the formation of free radicals [14].

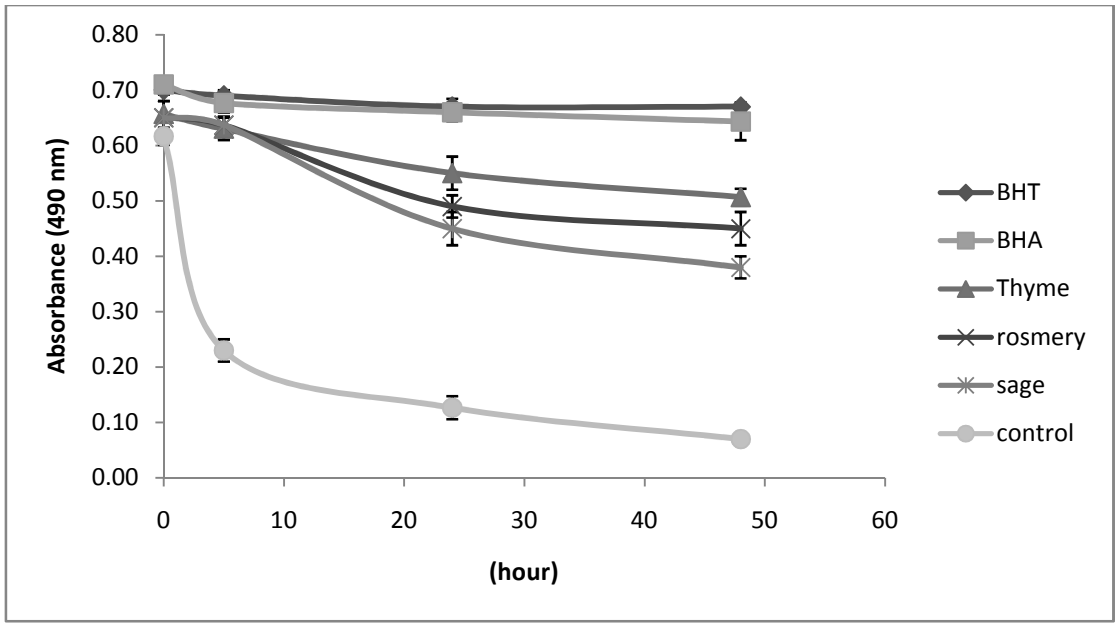

Fig 1 Absorbance change of β-carotene at 490 nm in the presence of the three essential oils

To follow the oxidation rate in each oil sample, the samples were analyzed periodically for IT (Fig 3) and PV (Fig.4). Results showed that IT of oil samples was improved with the addition of essential oils. In addition, it should be noted that, in the case of control sample, IT increased from 67 h for the virgin olive oil to 26.17 h for the refined olive oil (Fig. 3). This difference could be explained by the fact that virgin olive oil contained natural antioxidative components such as tocopherols and phenols which assure a natural stability. Tocopherols act as antioxidants by

trapping the hydroperoxide intermediates and stopping the autoxidation chain reaction [15-16]. Based on these results, it can be assumed that, the higher the phenol content, the longer the IT of hydroperoxide decomposition.

Concerning the second stability test, PV of different oil samples decrease with the addition of the three essential oils (Fig.4).These findings confirmed previous results. It can be assumed that, the improvement of stability to auto-oxidation is due to the presence of some components in essential oils, which have antioxidant activity such as thymol, carvacrol, γ-terpinene, α-pinene, eucalyptol and camphor [17].

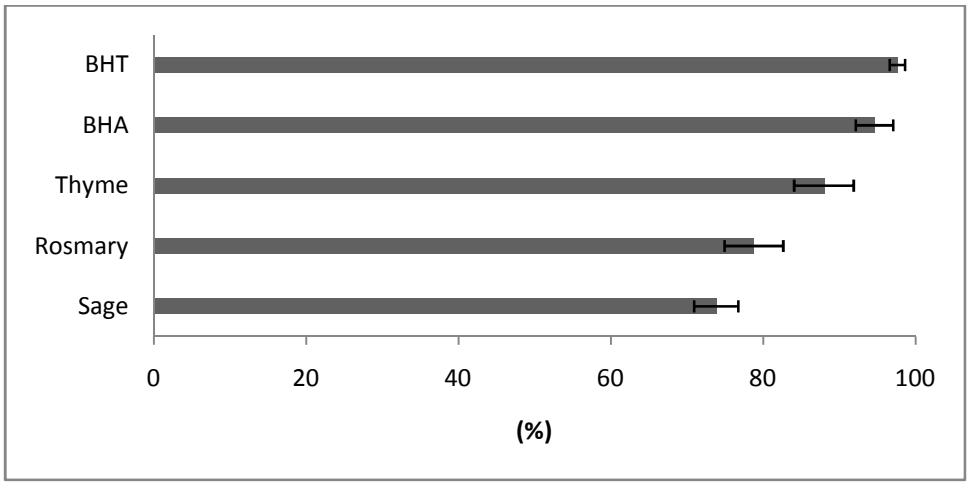

Fig2 Inhibition percentage measured in β-carotene-linoleic acid assay

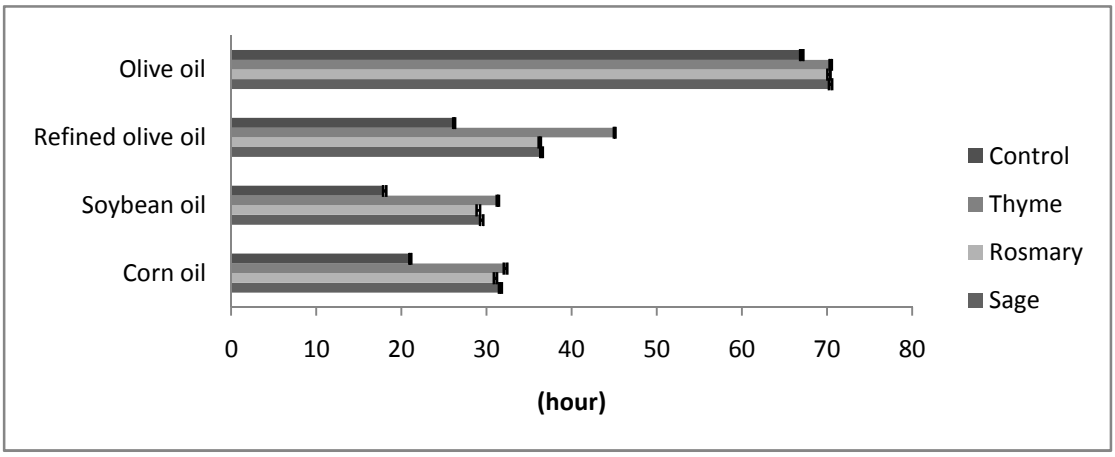

Fig 3. Effect of adding essential oils on Induction time (IT) of the olive oil, refined olive oil, soybean oil and corn oil

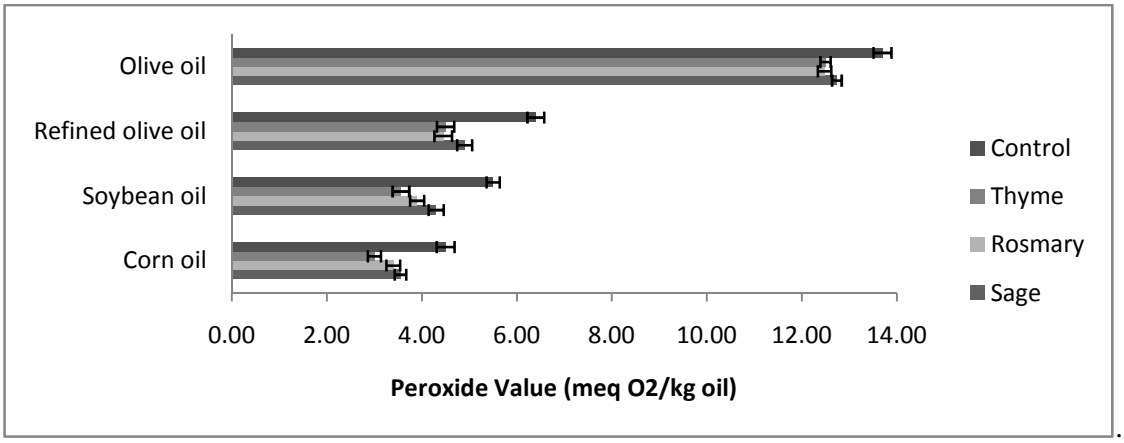

Fig4. Effect of adding essential oils on Peroxide value (PV) of the virgin olive oil, refined olive oil, corn oil and soybean oil

Table 1. Relative reaction rates of unsaturated fatty acids with oxygen and inherent stability of the oils

Fatty acids	Content (%)	Relative reaction rates	Reaction product with O_2
Soybean oil			
Oleic acid	22.0	1	0.228
Linoleic acid	51.0	10	5.100
Linolenic acid	6.80	25	1.700
Sum of reaction rates (inherent stability) = 7.028			
Corn oil			
Oleic acid	24.2	1	0.242
Linoleic acid	58.2	10	5.820
Linolenic acid	0.70	25	0.175
Sum of reaction rates (inherent stability) = 6.237			
Olive oil			
Oleic acid	76.2	1	0.762
Linoleic acid	6.80	10	0.680
Linolenic acid	0.70	25	0.175
Sum of reaction rates (inherent stability) = 1.617			

Source: List and Erickson [13]

CONCLUSION

It might be possible to keep perishable fat-containing food longer by a direct addition of essential oils of aromatic plants due to the measured antioxidative effects of these essential oils. They could be used as a food additive to replace artificial antioxidants. Further research is needed on the determination of the correlation between the antioxidant capacity and the chemical composition of the spice essential oils.

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