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Antioxidative Activity of *Thymbra spicata* in Palm and Corn Oils during Storage

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ABSTRACT

Two types of oils (corn and palm), having different fatty acid composition, were investigated for their oxidative stability in the presence of *Thymbra spicata* oil (1.39 to 5.49 mg mL^{-1}) and BHT (0.014 to 2.400 mg mL^{-1}) at three storage temperatures (15, 25 and 35^{o}C), as well as control samples with no addition of antioxidant. *Thymbra spicata* oil was significantly effective on oxidation of both corn and palm oils at each studied concentrations and temperatures. Increases in concentration of *Thymbra spicata* resulted in an increase in its antioxidative activity. However, BHT was more effective against oxidation of oils than *Thymbra spicata*. The results obtained in the present study indicate that *Thymbra spicata* extract is a potential source of natural antioxidant for use in fats and oils during storage.

Key Words: BHT, lipid peroxidation, natural antioxidant, palm oil, *Thymbra spicata*

INTRODUCTION

Autoxidation is one of the major deterioration processes affecting quality parameters of fats and oils during the storage and processing. Oxidative deterioration can result in alterations of organoleptic characteristics, e.g. taste and aroma, and also impaired nutritional value in the finished products, making them unacceptable to the consumer [1]. From a nutritional and technological point of view, it is desirable to control oxidation processes by addition of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). However, the use of synthetic antioxidants in food products has been falling off due to their instability as well as due to a suspected action as promoters of carcinogenesis. Natural antioxidants have recently gained increased interest because of the belief that natural food ingredients are better and safer than synthetic ones [2]. Herbs and spices are harmLess sources for obtaining natural antioxidants [3]. Many herbs and spices are reported to exhibit antioxidant activity in food lipids [1-3].

Thymbra spicata (Labiatae) grows wild in some eastern Mediterranean countries and the dried leaves are used as a spice and as herbal tea. The major essential oils derived from *Thymbra spicata* have been reported as carvacrol, thymol, E-3-caren-2-ol, and γ-terpinene which have antioxidant and antibacterial activities [4]. Tomaino *et al*. [1] indicated that essential oils of thyme (45.3% thymol and 48.9% carvacrol) exhibited properties as free radical-scavengers/antioxidants and could be used to control lipid oxidation during food processing. It has been observed that antioxidant activity of 0.6 % thyme extract (*Thymus vulgaris* L.) was as effective as that of 0.1 % BHT when compared in reducing oxidative stability of sunflower seed oil [5]. Similarly, Yanishlieva *et al*. [3] showed that thymol had the highest antioxidant effectiveness and activity during triacylglycerols sunflower oil oxidation.

Thymbra spicata which used as a spice and as herbal tea, have strong antioxidant activity. Essential oils from *Thymbra spicata* could offer a cheap and healthy alternative to synthetic antioxidants that prolong the shelf life of lipids. This study is aimed to investigate if the *Thymbra spicata* extract have potential as antioxidants compared to synthetic ones. The objective of this study was to evaluate the effect of increasing levels of added *Thymbra spicata* oil and changing temperature on the oxidative stability of both corn and palm oils during normal storage conditions. In addition, the objective was to compare the antioxidant activity of *Thymbra spicata* oil with that of BHT.

MATERIALS AND METHODS

Corn oil used in the present study was bought from the market. Palm oil was obtained from the Marsa (Adana, Turkey) after rafination before hydrogenation and addition of any chemicals. *Thymbra spicata* was collected in Nizip, Gaziantep (South-Eastern Turkey). The leaves were separated from the branches and the air-dried leaves were stored in a polyethylene bag. HPLC grade methanol, chloroform, acetic acid, sodium thiocyanate, potassium iodide, Butylated hydroxytoluene (BHT) was purchased from Merck (Darmstad, Germany) and starch was purchased from Pancreac (Pancreac, Spain).

The separation of the essential oils from air dried *Thymbra spicata* was conducted by steam distillation in a Clavenger apparatus for 3 h. The oils were dried over anhydrous sodium sulphate, and stored in dark glass bottles until analysis. Various concentrations of *Thymbra spicata* oil (1.39, 2.33, 4.29 and 5.49 mg mL^{-1}) and BHT (0.014, 0.092, 1.400 and 2.400 mg mL^{-1}) were added to weighed corn and palm oils, respectively. Then, corn and palm oils were placed in glass bottles and stored in incubators at three selected temperatures, 15^{o}C, 25^{o}C and 35^{o}C to observe shelf-lives at normal storage conditions. BHT was used as a comparison synthetic antioxidant. Control test for each oil (with no additives) was included and subjected to the same experimental conditions.

Oxidation of oil samples was performed in the dark and the oxidation was followed by withdrawing samples (5 g) at definite time intervals and subjecting them to iodometric determination of the peroxide value. 5 g of oil samples from both corn and palm oils were weighed and 1 mL of saturated potassium iodide solution was added. It was stirred vigorously for about 1 min. Then 25 mL of acetic acid-chloroform mixture (3:2, v/v) was added and solution was left to stay for 5 min in dark place. Then, 75 mL of distilled water was poured into solution, followed by the addition of 1 mL of indicator starch solution (1%). Finally the solution was titrated with standard sodium thiocyanate solution (0.01 N) and results were expressed as milliequivalents of peroxide per kilogram of sample. The antioxidative effectiveness of *Thymbra spicata* oil and BHT in oil samples was estimated on the basis of the induction period. The induction time was determined by the method of the tangents to the two parts of the curve of peroxide value vs. time [6].

The time (in day) taken to reach a specific peroxide value, corresponding to the flex point of the curve, was considered as the induction time. The longer the induction times, the greater were the antioxidant potencies of the compounds.

Statistical analysis of obtained data was made by using SPSS (version 11.0) package program at 95 % confidence interval. Bivariate correlation's of Pearson's two tailed tests was used to compare experimental data.

RESULTS AND DISCUSSIONS

In this study, the extent of oxidation in oils was evaluated by measuring peroxide value which can be used as oxidative index during the early stage of lipid oxidation. Two types of oils (corn and palm), having different fatty acid composition, were investigated for their oxidative stability in the presence of *Thymbra spicata* oil and BHT. Different concentrations of *Thymbra spicata* (1.39 to 5.49 mg mL^{-1}) and BHT (0.014 to 2.400 mg mL^{-1}) were studied for corn and palm oils at each storage temperature (15, 25 and 35^{o}C), as well as control samples with no addition of antioxidant. Stability of any oil depends on many factors; among them firstly fatty acid composition must be considered. Corn oil has high amount of unsaturated fatty acid mainly linoleic acid of up to about 62%. In addition to linoleic acid corn oil has oleic acid in its composition, completing the unsaturated fatty acid composition of corn oil up to 82-88%. The remaining part was saturated fatty acids. Palm oil contains up to 61% total unsaturated fatty acid composition and highest amount is oleic acid which is mono-unsaturated fatty acid. The remaining part of the palm oil composition was saturated fatty acids. Oleic acid compared to linoleic acid having two double bonds is more resistant to oxidation [7].

Data presented in this study showed that *Thymbra spicata* extract was able to decrease the formation of lipid hydroperoxides compared to the control. Figs. 1-6 show the kinetic curves of peroxide accumulation during oxidation of corn and palm oils in the presence of various concentrations of *Thymbra spi cata* at different storage temperatures. For comparison, the kinetic curve of oxidation in the presence of 2.4 mg mL^{-1} BHT is also given in the present figures. All essential oil concentrations showed antioxidant effect in varying degrees in oil samples. On the basis of peroxide value, the oxidative stability both corn and palm oils varied significantly ($P<0.05$) with different concentrations of *Thymbra spicata* added and oils enriched with the highest levels of *Thymbra spicata* (5.44 mg mL^{-1}) being most stable (Figs 1-6). However, BHT was more efficient than *Thymbra spicata* at decreasing the formation of hydroperoxide in both corn and palm oils. The main components of essential oil sample used in our study were the thymol and carvacrol which have been reported to be high antioxidant activity [1, 3, 5]. The antioxidant activity of thymol and carvacrol is ascribed to hydrogen donation and its ability to scavenge peroxyl radicals [8].

Özkan *et al* . [9] observed that essential oils of *Satureja cilicica* (mainly thymol and carvacrol) showed significant efficiency to reduce the oxidation of butter during storage time at 4 and 20°C. They stated that the antioxidant properties could be attributed to diterpenes and phenolics of *Satureja cil icica*, especially thymol and carvacrol. The antioxidative effects of thyme are confirmed by Tomaino *et al*. [1] in olive oil, they indicated that essential oils of thyme exhibited properties as free radical-scavengers/antioxidants and could be used to control lipid oxidation during food processing. The results of previous studies on thyme [5, 10] are consistent with the results of our study.

Induction times of corn and palm oils which are determined from the kinetics curve of peroxide accumulation, in the presence of various concentrations of *Thymbra spicata* and BHT are presented in Table 1 and 2, respectively. At all storage temperatures induction times obtained for corn oil were lower than that of palm oil (Table 1). This was expected result, since corn oil having higher amount of unsaturated fatty acid composition may naturally oxidize more readily than the palm oil. Generally it is accepted that the higher the degree of unsaturation of an oil, the more susceptible it is to oxidative deterioration [2]. As it can be observed in Table 1 and 2, there was up to 1.2 and 1.4 fold increase in induction time of corn oil with addition of the *Thymbra spicata* and BHT, respectively. On the other hand, the induction times of palm oil augmented up to about 1.1 and 1.3 in the presence of *Thymbra spicata* and BHT depending on concentration and temperature, respectively. In earlier study, it was observed that rosemary extract and BHT significantly reduced the formation of peroxides in corn oil stored at 30°C [11]. Similarly, the addition of essential oils from *Origanum vulgare L. ssp. hirtum* increased the induction time of lard to about 85 days. In the same study the addition of thymol increased the induction time of the lard to about 15 days [12]. The BHT was more effective than *Thymbra spicata* in increasing oxidative resistance of both oil types at studied concentrations. But it may be useful to incorporate *Thymbra spicata* oil at higher levels into the oil samples. Since, the effectiveness of BHT was no more than the natural counterparts as indicated by the closer induction times in the Table 1 and 2. Bonilla *et al*. [13] interpreted that maximum lawful level for synthetic food additives are established from various toxicological parameters that need not be applicable to naturally-occurring compounds. So, natural antioxidants could be used at higher levels than the synthetic antioxidants, thereby increasing their antioxidant effectiveness.

The effectiveness of *Thymbra spicata* in corn oil was increased linearly at increased concentrations (Figs. 1-3). Similar behaviors were observed for the palm oil, the increasing concentration of *Thymbra spicata* extended the storage times of palm oil at each studied temperature (Figs. 4-6). As well, effect of increasing concentration of *Thymbra spicata* was not high compared to BHT (Table 2), but its effect could not be neglected. The results obtained in this study suggested that the antioxidant activity of *Thymbra spicata* was concentration dependent.

Özkan *et al*. [9] showed that antiradical and antioxidant activity of essential oil may depend on not only the essential oil content but also different concentrations as in agreement with the results of our study.

Change in induction times of both corn and palm oils, with no antioxidant added, showed that the temperature increase reduced the storage stability of the oil samples (Table 1). It was reported that temperature accelerates both the chain propagation reactions and peroxide decompositions [7].

Table 1. Induction times of corn and palm oils with different concentrations of T*hymbra spicata* oil at various storage temperatures

Oil Type	Temperature (°C)	Induction Times (days)				
		Control	1.39 TSO	2.33 TSO	4.29 TSO	5.49 TSO
Corn Oil	15	218	226	234	243	254
	25	137	140	147	152	161
	35	96	104	108	110	113
Palm Oil	15	224	239	244	246	250
	25	147	149	154	156	161
	35	106	111	116	120	124

TSO: Concentration of *Thymbra spicata* oil in mg ml^{-1}

Table 2. Induction times of corn and palm oils with different concentrations of bht at various storage temperatures

Oil Type	Temperature (°C)	Induction Times (days)				
		Control	0.014 BHT	0.092 BHT	1.400 BHT	2.400 BHT
Corn Oil	15	218	256	262	269	275
	25	137	167	171	180	186
	35	96	115	120	124	129
Palm Oil	15	224	254	258	264	270
	25	147	163	169	175	183
	35	106	126	131	135	143

BHT: Concentration BHT in mg ml^{-1}

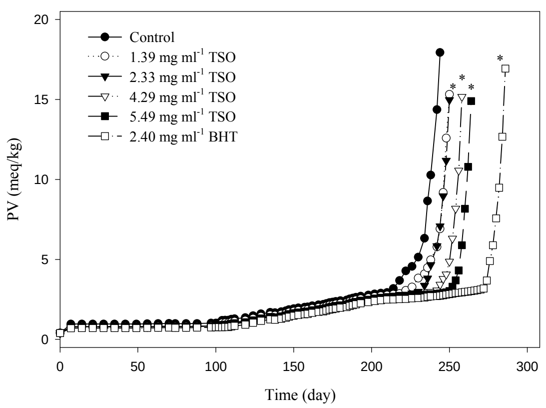

Figure 1. Kinetic curves of peroxide accumulation during storage of corn oil at 15oC with Thymbra spicata as antioxidant (*, significantly different from the control at P<0.05; TSO, Thymbra spicata oil; BHT, butylated hydroxytoluene)

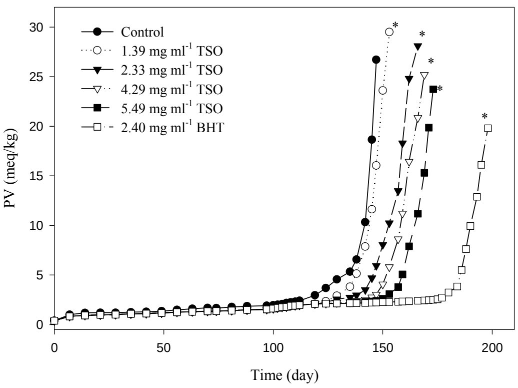

Figure 2. Kinetic curves of peroxide accumulation during storage of corn oil at 25oC with Thymbra spicata as antioxidant (*, significantly different from the control at P<0.05; TSO, Thymbra spicata oil; BHT, butylated hydroxytoluene)

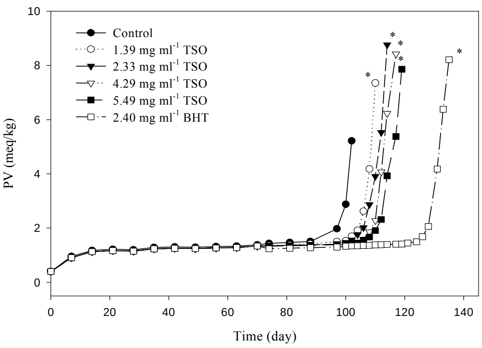

Figure 3 . Kinetic curves of peroxide accumulation during storage of corn oil at 35^{o}C with *Thymbra spicata* as antioxidant (*, significantly different from the control at P<0.05; TSO, *Thymbra spicata* oil; BHT, butylated hydroxytoluene)

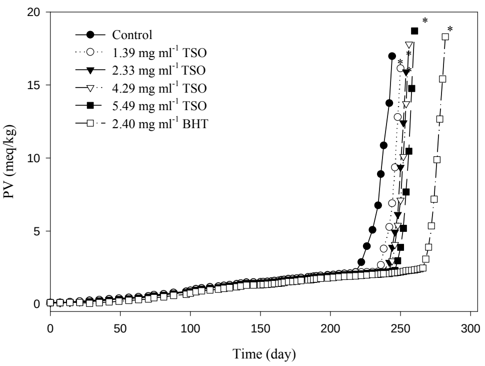

Figure 4 . Kinetic curves of peroxide accumulation during storage of palm oil at 15^{o}C with *Thymbra spicata* as antioxidant (*, significantly different from the control at P<0.05; TSO, *Thymbra spicata* oil; BHT, butylated hydroxytoluene)

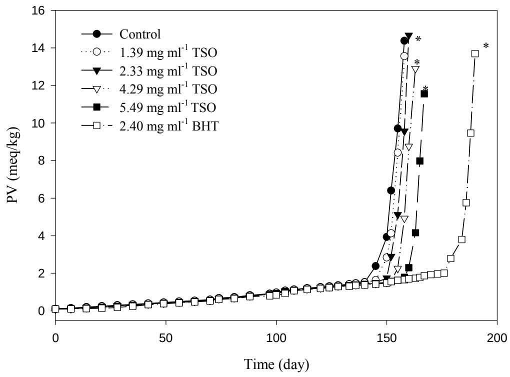

Figure 5. Kinetic curves of peroxide accumulation during storage of palm oil at 25oC with Thymbra spicata as antioxidant (*, significantly different from the control at P<0.05; TSO, Thymbra spicata oil; BHT, butylated hydroxytoluene)

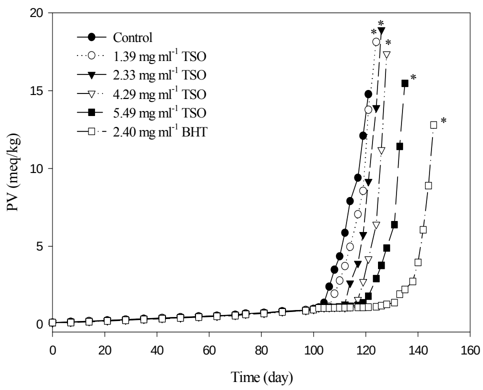

Figure 6. Kinetic curves of peroxide accumulation during storage of palm oil at 35oC with Thymbra spicata as antioxidant (*, significantly different from the control at P<0.05; TSO, Thymbra spicata oil; BHT, butylated hydroxytoluene)

CONCLUSION

Results obtained from this study showed that *Thymbra spicata* oil was significantly effective on oxidation of corn and palm oils during storage. However, BHT is more effective against oxidation of oils than *Thymbra spicata*. The effectiveness of *Thymbra spicata* can be increased by addition at higher levels. In conclusion, the possibility of substituting synthetic antioxidants with natural sources such as *Thymbra spicata* is suggested.

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