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Effect of methanol extracts of rosemary and olive vegetable water on the stability of olive oil and sunflower oil

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RESUMEN

Efecto de extractos metanólicos de romero y del agua de vegetación de la aceituna sobre la estabilidad del aceite de oliva y del aceite de girasol.

Extractos metanólicos de fenoles de hojas secas de romero y filtrados de agua de vegetación de la aceituna, en combinación con BHA, se añadieron al aceite de oliva (mezcla de aceite de oliva refinado y virgen, 3 a 1) y al aceite de girasol, evaluándose sus efectos antioxidantes usando condiciones aceleradas. Estas condiciones incluyeron el test del horno de oxidación (a 63 °C) y el método de conductividad (Rancimat a 120 °C). También se aplicó al proceso de fritura a 180 °C. Los extractos metanólicos de fenoles y el BHA se añadieron a cada aceite en las siguientes concentraciones: 200 ppm de extracto de romero, 200 ppm de extracto de agua de vegetación de la aceituna, 100 ppm de extracto de romero + 100 ppm de BHA, 100 ppm de extracto de agua de vegetación + 100 ppm de BHA y 200 ppm de BHA. En general, el efecto antioxidante de los aditivos fenólicos de romero y de BHA tuvo el siguiente orden: 200 ppm de extracto de romero > 100 ppm de extracto de romero + 100 ppm de BHA > y 200 ppm de BHA. La adición de 200 ppm de extracto de agua de vegetación y 100 ppm de extracto de agua de vegetación + 100 ppm de BHA mostró un efecto antioxidante similar al de 200 ppm de BHA.

PALABRAS-CLAVE: Aceite de girasol - Aceite de oliva - Agua de vegetación de la aceituna - Efecto antioxidante - Estabilidad - Romero.

SUMMARY

Effect of methanol extracts of rosemary and olive vegetable water on the stability of olive oil and sunflower oil.

Methanol phenolic extracts of dry rosemary leaves and olive vegetable water filtrate, in combination with BHA, were added to olive oil (blend of refined and virgin olive oil, 3 to 1) and to sunflower oil and their antioxidant effects under accelerated conditions were evaluated. Accelerated conditions included the oven test (at 63 °C) and the conductivity method (Rancimat at 120 °C). Frying process at 180 °C was also applied. The methanol phenolic extracts and the BHA were added to each oil at the following concentrations: 200 ppm rosemary extract; 200 ppm olive vegetable water extract; 100 ppm rosemary extract + 100 ppm BHA; 100 ppm vegetable water extract + 100 ppm BHA and 200 ppm BHA. In general, antioxidant effect of phenolic additives of rosemary and of BHA was in the following order: 200 ppm rosemary extract > 100 ppm rosemary extract + 100 ppm BHA > and 200 ppm BHA. The addition of 200 ppm vegetable water extract and 100 ppm vegetable water extract + 100 ppm BHA exhibited similar antioxidant effect to that of 200 ppm BHA.

KEY-WORDS: Antioxidant effect - Olive oil - Olive vegetable water - Rosemary - Stability - Sunflower oil.

1. INTRODUCTION

Natural and synthetic antioxidants are used to delay or to retard the oxidative deterioration of fats and oils. The major food antioxidants are phenolic compounds and are generally referred to as phenolic antioxidants (Sherwin, 1990). Recently, consumers show a growing tendency to reject all synthetic additives to foods, including synthetic antioxidants. Thus, there has been an increased interest on the use of natural antioxidants, especially those extracted from herbs, spices and other plant materials (Manganari y Oreopoulou, 1991; Tsuda *et al.*, 1993; Frankel *et al.*, 1997; Chen *et al.*, 1998; Hall *et al.*, 1998). Several substances including alcoholic extracts of different spices, paprika and rosemary, show good antioxidant activity. Rosemary extracts exhibited higher antioxidant effect compared to other natural extracts (Houlihan *et al.*, 1984; Houlihan *et al.*, 1985). According to Satue *et al.*, (1995) antioxidant compounds extracted from virgin olive oil increased the oxidative stability of refined olive oil. Antioxidants present in virgin olive oil, contribute to its oxidative stability (Kiritsakis, 1998; Blekas y Boskou, 1998). Vegetable water, a main by-product of olive fruit processing, contains sufficient amount of polyphenols (18-125 mg/g), which may act as antioxidants and prevent lipid oxidation (Unal, 1994).

The present study was undertaken to gain knowledge on the effect of rosemary leaves and of vegetable water extracts on olive oil (blend of refined and virgin olive oil, 3 to 1) and sunflower oil, under accelerated oxidative conditions.

2. EXPERIMENTAL PART

2.1. Oils - Rosemary Leaves and Vegetable Water

Refined olive oil and sunflower oil, obtained from a local market, was used in this study. Virgin olive oil was provided by a local producer. Parts three to one (w/w) of refined and virgin olive oil were mixed and blend olive oil was formed. Rosemary leaves were collected from the garden of the Mediterranean Agronomic Institute of Chania (MAICh). The leaves were left to dry at room temperature. Vegetable water from a double phase decanter, from the olive oil mill of the Subtropical and Olive Trees Institute of Chania, was used.

2.2. Preparation of Samples

2.2.1. *Methanol Extracts of Rosemary Leaves*

The methanol phenolic extracts of rosemary were prepared according to the method proposed by Wu *et al.* (1982). 500 g of dried rosemary leaves were ground to pass through 0.5 mm sieve, and phenols were extracted with 3 L of methanol (95%) at 60 °C for 2 h. The mixture was filtered and the residue was extracted again with 2 L of methanol. The combined filtrate was bleached with 100 g of active carbon for 15 min, and then filtered to yield a light-brown filtrate. The methanol solution was concentrated to 300 ml by a rotary evaporator and then filtered to remove any precipitates.

2.2.2. *Vegetable Water Extracts*

The vegetable water extracts were prepared according to the method proposed by Balice and Cera (1984). 50 ml of vegetable water, were filtered through a Watman 41 filter paper and then acidified with HCl (pH= 2), and saturated with NaCl. Then, 50 ml of ethyl acetate were added and the mixture was stirred for 10 min. Ethyl acetate solution was dried over anhydrous sodium sulphate.

2.2.3. *Addition of Antioxidants to Oil Samples*

100 g of each oil (blend olive oil and sunflower oil) was transferred to 250 mL flask and the exact amount of the phenol extract or the synthetic antioxidant was added to the oil. Methanol phenolic extracts and the synthetic antioxidant were added alone and in combinations as follows: 200 ppm rosemary extract, 200 ppm vegetable water extract, 100 ppm rosemary extract + 100 ppm BHA, 100 ppm vegetable water extract + 100 ppm BHA and 200 ppm BHA. After complete mixing, the solvent was evaporated at 55 °C under vacuum, in a rotary evaporator. The volume was completed with oil and the mixture was left to equilibrate.

2.3. Oven Test

Oil samples (50 g each) containing the additives were transferred into a series of capped transparent glasses and put in an oven at 63 °C. The peroxide value (PV) of the oil samples was periodically determined by the AOCS (1989) method.

2.4. Conductivity Method

The antioxidant activity of the tested additives was also determined using a Rancimat apparatus (Metrohm AG, CH-9100 Herisau, Switzerland) (Laubli and Bruttel, 1986). 3 g of oil samples were used. Temperature was set at 120 °C and the flow rate at 20 L/h. The induction period was recorded automatically.

2.5. Frying Process

The frying process started 10 days after the additives were added to the oils. During the 10 days period all treated samples and the control were stirred occasionally for 20 min. 350 g of potatoes were fried (French fries) in 900 g of oil at 180 °C for 15 min. The frying procedure was repeated eight times. The total frying lasted 120 min. 30 g of oil sample was weighted after each frying and stored in the refrigerator until the time of analysis. Peroxide value and specific absorbance (K_{232}, K_{270} and ΔK) measurements as described by Kiritsakis (1998) were conducted to measure the degree of oxidation. The phenol content of the oil samples was also determined during the study, by the Gutfinger (1981) method.

2.6. Statistical Analysis

The Statistical analysis was carried out using the LSD test and DUNCAN's Multiple Range test to evaluate the significant differences between the samples.

3. RESULTS AND DISCUSSION

3.1. Evaluation of Additives by the Oven Test

Table I shows the effect of methanol phenolic extracts on peroxide formation in oil samples when

the oven test was used. In the control samples of blend olive oil, the PV was 77 meq/kg oil at 120 days, while the control samples of sunflower oil reached 85 meq/kg oil in 12 days. This was expected due to the faster oxidation (Chan *et al.*, 1982) of the polyunsaturated sunflower oil and to the presence of natural antioxidants in blend olive oil, which act as potent antioxidants (Papadopoulos and Boskou, 1991). The relative inhibitory effect of the different additives in blend olive oil was in the following order: 200 ppm rosemary extract > 100 ppm rosemary extract + 100 ppm BHA > 200 ppm BHA. Neither 200 ppm vegetable water extract nor 100 ppm vegetable water extract + 100 ppm BHA improved the oxidative stability of the samples. Samples containing 200 ppm rosemary extract exhibited the highest oxidative stability for both oils. Manganari and Oreopoulou (1991) also found high antioxidant effect of rosemary extracts when tested in lard and in corn oil.

Table I

The effect of phenolic additives on peroxide formation in blend olive oil and in sunflower oil (Oven test at 63 °C)

Samples	Number of days												
	0	3	6	9	12	15	30	45	60	75	90	105	120
BO Control	3a					8b	16c	23d	33e	42f	50h	62j	77y
BO + 200 ppm BHA	3a					6ab	12.5c	17d	27e	36f	47h	59j	69y
BO + 100 ppm Vw + 100 ppm BHA	3a					6a	10c	16d	27e	36f	48h	60j	73y
BO + 200 ppm Vw	3a					6b	12c	20d	31e	41f	50h	62j	77y
BO + 100 ppm Ros + 100 ppm BHA	3a					6ab	8b	12c	19d	26e	35f	46h	57j
BO + 200 ppm Ros	3a					5ab	7b	12c	17d	24e	31e	37f	44h
So Control	6a	18b	46d	56e	85f	119j							
SO + 200 ppm BHA	6a	11b	28c	36c	48d	62e							
SO + 100 ppm Vw + 100 ppm BHA	6a	14b	33c	49d	71f	102j							
SO + 200 ppm Vw	6a	15b	40cd	52de	79f	111j							
SO + 100 ppm Ros + 100 ppm BHA	6a	9a	11ab	16b	23bc	33c							
SO + 200 ppm Ros	6a	7a	8a	12ab	18b	25bc							

Values with the same letter are not significantly different with Duncan's Multiple Range Test.

BO = Blend olive oil (mixture of refined olive oil with virgin olive oil, 3/1).
SO = Sunflower oil.
Vw = Vegetable water extract.
Ros = Rosemary extract.

3.2. Evaluation of Additives by the Conductivity Method

Table II shows that the results recorded by the Rancimat apparatus were consistent with those obtained by the oven test (Tables I). The induction period for the control samples of blend olive oil and those of sunflower oil was 5.55 and 2.03 h, respectively. Under these conditions, phenolic extracts increased the resistance of oil samples to oxidation. Vegetable water extract seemed to show similar antioxidant effect to BHA. Both oil samples containing 100 ppm rosemary extract + 100 ppm BHA, or 200 ppm rosemary extract showed longer induction period than oil samples containing the other additives (Table II). Blend olive oil containing 200 ppm rosemary extract exhibited longer induction time (11.2 h) than sunflower oil samples (3.25 h) as compared to their respective controls.

Table II
The effect of phenolic additives on the oxidative stability of blend olive oil and sunflower oil as determined by the Rancimat apparatus at 120 °C

Sample	Induction time (h)
BO Control	5.55
BO + 200 ppm BHA	6.85
BO + 100 ppm Vw + 100 ppm BHA	6.65
BO + 200 ppm Vw	6.37
BO + 100 ppm Ros + 100 ppm BHA	8.78
BO + 200 ppm Ros	11.2
SO Control	2.03
SO + 200 ppm BHA	2.12
SO + 100 ppm Vw + 100 ppm BHA	2.08
SO + 200 ppm Vw	2.15
SO + 100 ppm Ros + 100 ppm BHA	2.73
SO + 200 ppm Ros	3.25

See Table I for abbreviations.

3.3. Evaluation of Additives during the Frying Process

Table III shows that K_{232} values for both oils increased with the frying time indicating the formation of conjugated hydroperoxides. At the end of the frying process the K_{232} values for blend olive oil containing no additives were lower than those of the control sunflower oil. The blend olive oil had initial K_{232} value 2.64 which increased to 4.30 by the end of frying. In contrast, sunflower oil had an initial value of 3.72 which increased to 10.11 (Table III). Blend olive oil samples containing 200 ppm rosemary extract showed the lowest K_{232} value (3.92) by the end of frying. Our results agree with others (Manganari y Oreopoulou, 1991; Houlihan *et al.*, 1984).

Table III
Absorbance Values (K_{232}) of blend olive oil and sunflower oil treated with phenolic additives and subjected to frying process

Samples	Number of Fryings				
	0	2	4	6	8
BO Control	2.64a	3.19c	3.65d	3.94e	4.3f
BO + 100 ppm Vw + 100 ppm BHA	2.64a	3.30c	3.34c	3.78d	4.12f
BO + 200 ppm Vw	2.9b	2.9b	3.42c	3.56c	4.04ef
BO + 100 ppm Ros + 100 ppm BHA	2.62a	3.18bc	3.64d	3.9e	4.18f
BO + 200 ppm Ros	2.70a	3.13bc	3.40c	3.7d	3.92e
SO Control	3.72a	6.51b	8.26c	9.22d	10.1e
SO + 100 ppm Vw + 100 ppm BHA	3.77a	6.76b	8.36c	9.28d	10.2e
SO + 200 ppm Vw	3.75a	6.35b	7.73b	8.42c	9.38d
SO + 100 ppm Ros + 100 ppm BHA	3.72a	5.51b	6.57b	7.02b	7.51c
SO + 200 ppm Ros	3.74a	5.41b	6.26b	6.98b	7.34c

See Table I for abbreviations.
Values with the same letter are not significantly different with Duncan's Multiple Range Test.

The K_{270} values increased significantly in both oils with frying time (Table IV), indicating a continuous formation of secondary oxidation products (Hamilton, 1989; Kiritsakis,1998). The initial K_{270} value for control samples of blend olive oil was 0.81 and increased to 1.19 by the end of frying. However, the control samples of the sunflower oil had initial value 2.43 which increased to 3.55, indicating the formation of more decomposition oxidation products in sunflower oil than in blend olive oil. The latter contains more oleic and less linoleic (Kiritsakis, 1998) and according to Nawar (1985) thermal oxidation of linoleic gives higher amount of decomposition products than oleic. However, samples containing 100 ppm rosemary extract + 100 ppm BHA, or 200 ppm rosemary extract showed the highest absorbance at 270 nm at the end of frying. These results were inconsistent with K_{232} values and indicate that the measurement of UV absorbance is not the best parameter to evaluate oil alterations during frying. Frying temperature and food humidity may affect the values. ΔK values did not provide additional information since values increased but not significantly in both oils with frying time (Table V).

Table IV
Absorbance Values (K_{270}) of blend olive oil and sunflower oil treated with phenolic additives and subjected to frying process

Samples	Number of Fryings				
	0	2	4	6	8
BO Control	0.81a	1.00a	1.18c	1.18c	1.19c
BO + 100 ppm Vw + 100 ppm BHA	0.89a	1.00b	1.09c	1.16c	1.20d
BO + 200 ppm Vw	0.90a	0.96b	1.08c	1.11c	1.19c
BO + 100 ppm Ros + 100 ppm BHA	0.97a	1.07c	1.27d	1.30d	1.33d
BO + 200 ppm Ros	0.99a	1.07c	1.25d	1.45e	1.54f
SO Control	2.43a	2.91b	3.42c	3.47c	3.55d
SO + 100 ppm Vw + 100 ppm BHA	2.67a	2.95b	3.45c	3.48c	3.53d
SO + 200 ppm Vw	2.71a	2.92b	3.41c	3.44c	3.46c
SO + 100 ppm Ros + 100 ppm BHA	2.43a	2.90b	3.44c	3.46c	3.59d
SO + 200 ppm Ros	2.45a	2.96b	3.45c	3.47c	3.65e

See Table I for abbreviations.
Values with the same letter are not significantly different with Duncan's Multiple Range Test.

Table V
Absorbance Values (ΔK) of blend olive oil and sunflower oil treated with phenolic additives and subjected to the frying process

Samples	Number of Fryings				
	0	2	4	6	8
BO Control	0.10a	0.10a	0.12a	0.11a	0.10a
BO + 100 ppm Vw + 100 ppm BHA	0.09a	0.10a	0.12a	0.11a	0.11a
BO + 200 ppm Vw	0.09a	0.11a	0.11a	0.11a	0.10a
BO + 100 ppm Ros + 100 ppm BHA	0.09a	0.11a	0.13a	0.13a	0.13a
BO + 200 ppm Ros	0.10a	0.11a	0.13b	0.13a	0.14a
SO Control	0.41a	0.42a	0.48a	0.43a	0.44a
SO + 100 ppm Vw + 100 ppm BHA	0.42a	0.43a	0.47b	0.45a	0.44a
SO + 200 ppm Vw	0.42a	0.45a	0.44a	0.48b	0.44a
SO + 100 ppm Ros + 100 ppm BHA	0.43a	0.42a	0.46a	0.47a	0.47a
SO + 200 ppm Ros	0.43a	0.43a	0.49a	0.51b	0.54b

See Table I for abbreviations.
Values with the same letter are not significantly different with Duncan's Multiple Range Test.

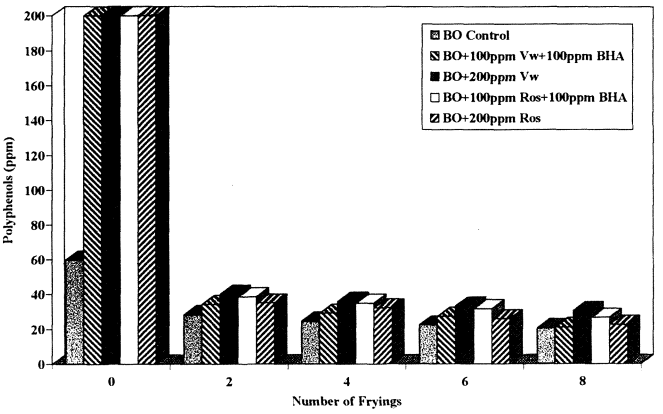

Figure 1
Changes in the polyphenol content of blend olive oil during the frying process
(Abbreviations as in Table I)

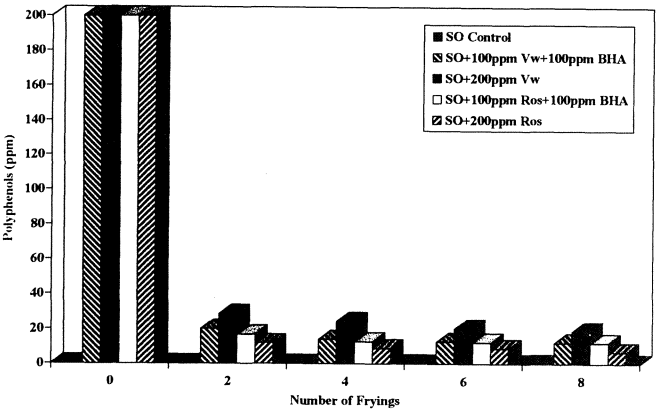

Figure 2
Changes in the polyphenol content of sunflower oil during the frying process
(Abbreviations as in Table I)

In both (blend olive oil and sunflower) oils a significant decrease in polyphenol content with the time was observed (Figs. 1, 2), due to the effect of frying temperature (Loliger, 1989). Natural polyphenols (60 ppm) present in the control samples of blend olive oil (control samples of sunflower oil contained no polyphenols), were more resistant to high temperature than polyphenols from the antioxidants extracts. Among the added extracts, those from vegetable water showed higher resistance. Samples to which 200 ppm polyphenols from the vegetable water were added, contained at the end of the experiment, the higher amount of polyphenols, which was 30.5 ppm and 18.6 ppm for blend olive oil

and sunflower oil respectively. The presence of lower amount of polyphenols, at the end of the experiment, in samples to which 200 ppm of rosemary extract extract was added compared to those containing 200 ppm vegetable water extract (Figs. 1, 2) may explains the higher antioxidant effect of rosemary extracts. Polyphenols were probably used and the oxidation was retarded.

In conclusion, under accelerated oxidation conditions (63 °C in oven and 120 °C in Rancimat), methanol phenolic extracts of rosemary leaves and vegetable water exhibited antioxidant activity. Better results were obtained from rosemary than vegetable water.

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