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Antioxidant Properties of Mediterranean Spices Compared with Common Food Additives

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ABSTRACT

In this study, the antioxidant properties of Mediterranean food spices (annatto, cumin, oregano, sweet and hot paprika, rosemary, and saffron) at 5% concentration and of common food additives (butylated hydroxyanisole [BHA], butylated hydroxytoluene [BHT], and propyl gallate) at 100 μg/g are compared. The ability of these compounds to inhibit lipid peroxidation was, in decreasing order, rosemary > oregano > propyl gallate > annatto > BHA > sweet paprika > cumin > hot paprika > saffron > BHT. Deoxyribose damage is partially inhibited in the presence of cumin extract that exhibits the strongest protective action. The rest of the spices also protect deoxyribose better than the BHA and BHT used in the assay. Finally, the results obtained in the assay point to the prooxidant effect of propyl gallate. Hydrogen peroxide scavenging activity is measured by using peroxidase-based assay systems. In aqueous medium, the spice extracts show lower antioxidant activity than propyl gallate, the decreasing order being cumin > oregano > annatto > rosemary > hot paprika > sweet paprika. BHA and BHT did not scavenge H_2O_2. Spices are able to scavenge HOCl and protect α_1-antiproteinase. The results indicate that rosemary and oregano are more effective HOCl scavengers than the other substances analyzed, which, in decreasing order, were propyl gallate, annatto, sweet and hot paprika, saffron, and cumin. The effect of Mediterranean food spices on the oxidative stability of refined olive oil tested by the Rancimat method was compared with common food additives during storage (72 h, 2, 4, and 6 months) at room temperature. The results showed that the spice extracts analyzed have significant stabilizing effects ($P < 0.05$).

In the preparation and commercialization of foodstuffs or prepared foods, the aim is always to obtain a product of maximal quality that is as well preserved as possible before reaching the consumer. Normal practice is to add substances that prevent degradation (stabilizers, antioxidants), improve flavor (sweeteners), increase firmness (thickeners, agglutinants), color maintenance, or antimicrobials *(36)*.

Despite the widespread use of antioxidants, lipid oxidation is still a major cause of food quality deterioration during the storage of oils, fats, and fat-containing foods. The most widely used synthetic antioxidants, butylated hydroxyanisole (BHA; E-320), butylated hydroxytoluene (BHT; E-321), and propyl gallate (E-310), are chain-breaking inhibitors of lipid peroxidation, acting through a mechanism that produces a reaction between the radicals and proteins like albumin or fatty-acid side chains *(24)*. However, BHA and BHT are quite volatile and easily decompose at high temperatures *(1)*. The use of spices as antioxidants in processed foods is a promising alternative to the use of synthetic antioxidants, especially because of increasing consumer interest in natural food additives *(30)*.

Traditionally, spices have been added to different types of food to improve the flavor *(28)*. Currently, there is a growing awareness that spices also improve the oxidative stability of processed products and, as a consequence, spice extracts are being marketed as antioxidants for use in the food industry *(29)*.

Leaves and extract of rosemary (*Rosmarinus officinalis* L.) have an agreeable odor and a slightly camphoraceous taste. The volatile oil is used in medicine and as food flavoring, especially in foods containing animal fats (meats, sausages), soups, sauces, vegetable oils, and other food products *(23, 39)*.

Oregano (*Origanum vulgare* L.) is widely used in medicine and food flavoring, mainly in stews and Italian foods like pizza, macaroni, and spaghetti. The essential oils of oregano have expectorant, antispasmodic, tonic, antiseptic, analgesic, antimicrobial, antifungicidal, and germicidal properties *(46)*.

Rosemary and oregano show high antioxidant activity in their ground form and as extracts, in which a number of phenolic compounds with strong antioxidant activity have been identified. Flavonoids and other phenolics are thought to play a preventive role in the development of cancer and heart disease *(25)*.

There is increasing interest in the spice paprika (*Capsicum annuum* L.), which is an excellent source of natural color *(33)*, due to carotenoid pigments *(34)*. Carotenoids act as a natural antioxidant that quenches singlet oxygen, acting as electron donors. Certain carotenoids have been considered as anticancer agents, antiulcer agents through

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lators of the immune response *(11)*. They also contain vital micronutrients, such as vitamins C and E, that have been confirmed by many epidemiological studies as reducing the risk of cancer and cardiovascular disease when taken daily in adequate amounts *(31)*.

Annatto (*Bixa orellana* L.) extracts are listed among the colors that may be used singly or in combination with additives like emulsifying salts (sodium citrate and disodium phosphate) *(44)*, in the cream and butter industries *(43)* and in certain foods like cheese, where a pinkish-brown or salmon color develops during manufacture *(45)*.

From ancient times to the present, saffron (*Crocus sativus* L.) has had diverse uses in medicine in preventing cardiovascular disease *(15)*. As a spice it has been used in food recipes, including rice, bread, buns, cakes, and soups *(15)* in all oriental civilizations and in the Mediterranean region, where it is widely used in typical dishes like paella in Spain. Saffron is valued both for its coloring and flavoring properties *(38)*.

The seeds of *Cuminum cyminum* L. are known as cumin, a product of the Mediterranean Basin. It has been used as a food spice flavoring foods, especially bakery products for sprinkling on bread and cheese *(26)*. Recently, many medical properties have been attributed to the cumin seed and/or its oil, including antineoplasic, antibacterial, antifungal, and antihelminthic effects *(48)*.

Since 1993 it has been accepted in the United States, that certain foods (legumes, vegetables, fruits, spices) reduce the risk of disease. The Food and Drug Administration has assessed scientifically the correlation between the presence of these compounds, nutrients, or foods in the diet and the level of risk to certain diseases. Both the Food and Drug Administration and the Scientific Committee on Food for Novel Foods and Novel Foods Processes in Europe, on the basis of scientific information available and on the basis of agreement between experts, provide health claims as a consequence of available scientific evidence *(12, 40)* for use in the labeling of these special foods called functional food, a type of Novel Foods, clarified by Diplock et al. *(18)*, emphasizing the presence of components, nutrient or nonnutrient, that help to maintain the health claims and to reduce disease risks.

The aim of this study is to characterize some spices frequently used in the Mediterranean diet, in several systems, in order to assess their antioxidant activity compared with common food additives (BHA, BHT, and propyl gallate). In addition, the activity of these spices is studied during storage at room temperature for their use as natural additives and for the design of new functional foods with high antioxidant properties.

MATERIALS AND METHODS

Sample preparation. Propyl gallate, BHA, BHT, and the chemicals used were of the highest quality available and were purchased from Sigma Chemical Co. (Poole, Dorset, UK). The Mediterranean food spices like annatto (*B. orellana* L.), cumin (*C. cyminum* L.), oregano (*O. vulgare* L.), sweet and hot paprika (*C. annuum* L.), rosemary (*R. officinalis* L.), and saffron (*C. sativus* L.) were purchased from a supermarket. The dried spices were powdered using a mortar and pestle. Every powdered spice (5 g) was extracted for 1 h by stirring at room temperature with 100 ml water or ethanol and centrifuged at 3,000 rpm for 10 min. The 5% spice extracts were used in the different assays. BHA, BHT, and propyl gallate were used at the permitted commercial concentration of 100 μg/g *(19)*. Propyl gallate was also tested at 5% concentration, except for the Rancimat test.

Peroxidation of phospholipid liposomes. The ability of compounds to inhibit lipid peroxidation at pH 7.4 was tested using ox brain phospholipid liposomes, as described in Aruoma et al. *(10)*. The experiments were conducted in phosphate-buffered saline (3.4 mM Na_2HPO_4-NaH_2PO_4, 0.15 M NaCl), pH 7.4. In a final volume of 1 ml, the assay mixtures were made up with phosphate-buffered saline, 0.5 mg/ml phospholipid liposomes, 100 μM $FeCl_3$, and 100 μl of the tested samples (5% extract) dissolved in either water or ethanol, and 100 μM ascorbate (added last to start the reaction). Ethanol does not affect the outcome of the lipid peroxidation assay, while BHT is not fully soluble in aqueous solution, and its emulsion is not homogeneous. In order to dissolve it, deionized water, with a conductivity of not more than 4 μS/cm was used. Incubations were at 37°C for 60 min. At the end of this incubation period, 0.1 ml of 2% (wt/vol) BHT was added to each mixture followed by 1 ml each of 1% (wt/vol) thiobarbituric acid (TBA) and 2.8% (wt/vol) trichloroacetic acid. The solutions were heated in a water bath at 80°C for 20 min to develop the malondialdehyde thiobarbituric adduct ($[TBA]_2$-MDA). The $(TBA)_2$-MDA chromogen was extracted into 2 ml of butan-1-ol, and the extent of peroxidation was measured in the organic layer as absorbance at 532 nm.

This TBA test measures not only peroxidation occurring in the experiment itself but also the peroxidation taking place during the acid-heating stage of the assay. In order to avoid any interference, the TBA test was performed in the presence of the antioxidant BHT to inhibit peroxidation during the assay itself.

Hydroxyl radical scavenging. In a final volume of 1.2 ml, the reaction mixtures contained the following reagents: 10 mM KH_2PO_4-KOH buffer (pH 7.4), 2.8 mM H_2O_2, 2.8 mM deoxyribose (where used), 50 μM $FeCl_3$ premixed with 100 μM EDTA before addition to the reaction mixture, and 100 μl of the tested samples (5% spice extracts) and additives dissolved in water. Ascorbate (100 μM), where used, was added to start the reaction. The tubes were incubated at 37°C for 1 h. The products of the hydroxyl radical ($OH^{\cdot}$) attack on deoxyribose were measured as described in Aruoma et al. *(9)*.

Scavenging of hydrogen peroxide. The samples to be tested with H_2O_2 (dissolved in either water or ethanol) were incubated with 0.84 mM H_2O_2 for 10 min at 25°C. Aliquots of these compounds were then taken and assayed for remaining H_2O_2 by using the peroxidase system *(8)*. The remaining H_2O_2 was measured as the formation of a chromophor recorded at 436 nm in reaction mixtures containing, in a final volume of 1 ml, 0.15 M KH_2PO_4-KOH buffer, pH 7.4, 50 μl guaiacol solution (made by adding 100 μl of pure guaiacol liquid to 100 ml water), and 10 μl of Sigma type IV horseradish peroxidase (5 mg/ml in the same phosphate buffer).

Reactions with hypochlorous acid. The hypochlorous acid (HOCl) reaction was studied using the elastase assay, essentially as described by Aruoma et al. *(9)*. For the assay, 68 μM HOCl (produced immediately before use by adjusting NaOCl to pH 6.2 with dilute H_2SO_4) and the tested samples were incubated for 20 min in a final volume of 1.0 ml in phosphate-buffered saline, pH 7.4, containing 140 mM NaCl, 2.7 mM KCl, 16 mM Na_2HPO_4,

and 2.9 mM KH_2PO_4. α_1-Antiproteinase (2 mg/ml) was added to the reaction mixture. This allows any HOCl remaining to inactivate α_1-antiproteinase. After a further 20-min incubation, 0.05 ml of 2.5 mg/ml elastase was added. This mixture was allowed to stand 30 min more before the 2 ml of phosphate-buffered saline was added. The remaining elastase activity was then measured by adding elastase substrate (5 mg/ml, *N*-succinyltriala-*p*-nitroanilide), which is hydrolyzed by elastase, resulting in an increase in A_{410}.

Rancimat test for oxidative stability. Sample preparation in the Rancimat test consisted of maceration of the different spices and antioxidants with 100 g refined olive oil (provided by the manufacturing company and free of added antioxidants or preservatives). The spices were used at 5% (wt/wt) concentration while the widely used antioxidant additives, BHA, BHT, and propyl gallate, were used at the permitted commercial concentration of 100 μg/g *(19)*.

All oxidative stability measurements were performed with a Rancimat apparatus (Metrohm model 743; Herisan, Switzerland) by measuring the induction period of refined olive oil with or without the addition of the tested compounds, using the Automated Swift Test. Determination of the induction period, at 110°C, was based on the detection of volatile acids. The induction period is the time elapsed until the inflection point of the conductivity versus time curve recorded by the Rancimat *(14)*.

All samples were prepared on the same day (day 0) and divided into four batches before being stored at room temperature. These were analyzed after 72 h and after 2, 4, and 6 months of maceration using 5 g of sample and an air flow rate set of 20 liters/h. The relative activity of the antioxidants is expressed by the protection factor, oxidative stability, or antioxidant index, which is calculated by dividing the induction period of oil with added antioxidants by the induction period of the control (olive oil alone) *(10)*.

This technique has been questioned by some authors *(20)*, but, in agreement with Bonilla et al. *(13)*, we decided to apply it in this work because it is a commonly used procedure in the food industry and governmental analytic laboratories.

Data analysis. The data were analyzed using the Statistical Package for Social Sciences Windows 9.0. The analyses of variance were carried out after triplicate experiments, calculating the significance level by using the least significant difference multiple range test.

RESULTS AND DISCUSSION

Inhibition of phospholipid peroxidation. Lipid peroxidation is sometimes a major mechanism of cell injury in organisms subjected to oxidative stress, although it is by no means the only mechanism of injury *(8)*. Ox brain phospholipid liposomes undergo rapid nonenzymatic peroxidation when incubated with $FeCl_3$ and ascorbic acid at pH 7.4 *(2)*. At low concentrations, ascorbate accelerates lipid peroxidation through its ability to reduce iron into the active ferrous state, while, at high concentrations, ascorbic inhibits lipid oxidation by inactivating free radicals *(17)*.

Table 1 shows the inhibition of lipid peroxidation in the presence of some Mediterranean food spices and common food additives. Rosemary, oregano, and propyl gallate

TABLE 1. *Inhibition of peroxidation in the lipid system using ox brain phospholipids by Mediterranean food spices compared with the activity of different compounds frequently used as food additives*[a]

Added to reaction mixtures[b]	% inhibition
None (control)	—[e]
Annatto[c]	78.46
Cumin[c]	63.08
Oregano[c]	93.09
Paprika, hot[c]	59.26
Paprika, sweet[c]	65.78
Rosemary[c]	94.81
Saffron[c]	55.13
Propyl gallate[c]	83.07
Propyl gallate[d]	51.69
BHA[d]	71.05
BHT[d]	22.96

[a] Statistical differences were analyzed by analysis of variance ($P < 0.05$).
[b] Compounds in aqueous medium.
[c] Concentration 5%.
[d] Concentration 100 μg/g.
[e] No % inhibition detected.

inhibition than BHA at 100 μg/g but lower antioxidant activity with respect to rosemary, oregano, and propyl gallate.

Sweet paprika, cumin, hot paprika, and saffron, although less effective scavengers than propyl gallate at 5% concentration, showed inhibition percentages higher than 50%. With the exception of saffron, all the tested spices showed a better antioxidant capacity than propyl gallate when this synthetic additive was analyzed at the permitted concentration (100 μg/g) *(19)*. At this concentration, propyl gallate did not show significant differences with respect to saffron. BHT showed the lowest peroxyl radical scavenging capacity of all the compounds tested.

Some of the synthetic compounds tested in this study are organic structures, and some food additives like α-tocopherol *(41, 49)* have been reported to be incorporated in model membranes and biomembranes. Thus, the experiment was carried out with the compounds dissolved in ethanol. When this was done, spices and food additives showed similar results ($P < 0.05$) to those observed in an aqueous medium. In the ethanol medium, there were no significant differences between rosemary, oregano, propyl gallate, and annatto extracts at 5% concentration, the results showing 90, 82, 80, and 79% inhibition, respectively. Again, rosemary and oregano exhibited higher antioxidant activity than propyl gallate, while the rest of the spices showed lower efficacy.

When the inhibition of lipid peroxidation by commercial food additives, BHA, BHT, and propyl gallate (100 μg/g) was analyzed and compared with cumin, hot paprika, and sweet paprika (5% extracts), the resulting percent in-

increase in inhibitory activity when analyzed in ethanol medium.

The antioxidant activity shown by oregano and rosemary in this study was as expected according to previous reports *(10)*. These authors analyzed a spice cocktail that included oregano, rosemary, thyme, and sage and concluded that the plant extracts were capable of minimizing liposomal lipid peroxidation.

Rosemary reportedly reduces lipid peroxidation in meat products *(23)*, in vegetable oil, and fish oil *(21)*, and its antioxidant effects have also been in peanut oil *(16)*. Similar studies with oregano have found that when added to potato chips *(27)*, French dressing, and mayonnaise, it retards oxidative deterioration *(30)*. On the other hand, Abdalla and Roozen *(1)* observed that the antioxidant activity of oregano extracts was more effective in sunflower oil than in a 20% oil-in-water emulsion.

Also, the results obtained with sweet and hot paprika were in accordance with those of Aguirrezábal et al. *(3)*, who, by measuring the TBA reactive substances content, established that these spices are effective in inhibiting lipid oxidation in dry sausages. Matsufuji et al. *(33)* studied the antioxidant ability of paprika components by measuring the free-radical oxidation of methyl linoleate and found effective antioxidant activity. Other authors have detected good levels of antioxidant vitamins like ascorbic acid and tocopherols in paprika *(31)*.

Assessing the antioxidant action of Mediterranean food spices by the deoxyribose assay. The deoxyribose method evaluates the ability to damage carbohydrates. Hydroxyl radicals damage sugar deoxyribose. Highly reactive hydroxyl radicals ($OH^{\cdot}$) are generated by a mixture of ascorbate and $FeCl_3$-EDTA at pH 7.4 *(2)*. The deoxyribose is broken down into fragments and, on heating with TBA at low pH, generates a pink chromogen. The addition of ascorbic acid greatly increases the rate of $OH^{\cdot}$ generation by reducing iron and maintaining the supply of Fe^{2+} *(4)*.

Table 2 shows the results of the deoxyribose damage by $OH^{\cdot}$ radical, in the presence of Mediterranean food spices and common food additives, studied in aqueous medium. The results are expressed as a percent inhibition of deoxyribose attack, where 100% attack is defined as the absorbance levels resulting for deoxyribose without the addition of the tested compounds (control).

This attack is partially inhibited in the presence of the compounds tested. Cumin exhibited the strongest protective action (more than 60% inhibition) ($P < 0.05$), while the rest of the spices exhibited decreasing degrees of protection: annatto and hot paprika more than 50% inhibition ($P < 0.05$); sweet paprika, saffron, rosemary, and oregano about 30 to 40% inhibition. There were no significant differences between oregano, rosemary, and saffron inhibition ($P < 0.05$).

These findings show that the spices scavenge $OH^{\cdot}$ radicals and protect deoxyribose better than BHA and BHT. The results also show the prooxidant effect of propyl gal-

TABLE 2. *Deoxyribose damage by OH· radical in the presence of Mediterranean food spices compared with the activity of different compounds frequently used as food additives*[a]

Added to reaction mixtures[b]	Damage to deoxyribose (A_{532})[c]		
	RM + DR[d]	% inhibition	Omit ASC[d]
None (control)	2.466	—[g]	0.855
Annatto[e]	1.118	54.66	0.530
Cumin[e]	0.828	66.42	0.299
Oregano[e]	1.712	30.58	1.687
Paprika, hot[e]	1.218	50.61	0.626
Paprika, sweet[e]	1.519	38.40	1.152
Rosemary[e]	1.603	35.00	1.511
Saffron[e]	1.570	36.33	1.679
Propyl gallate[e]	2.723	—[g]	2.767
Propyl gallate[f]	2.902	—[g]	1.867
BHA[f]	1.839	25.42	0.201
BHT[f]	2.245	8.95	0.559

[a] Statistical differences were analyzed by analysis of variance ($P < 0.05$).
[b] Compounds in aqueous medium.
[c] A_{532}, absorbance values recorded at 532 nm; when deoxyribose was omitted the values ranged from 0.001 to 0.006 absorbance units.
[d] RM, reaction mixtures; DR, deoxyribose; ASC, ascorbate.
[e] Concentration 5%.
[f] Concentration 100 μg/g.
[g] No % inhibition detected.

a synergistic effect with ascorbate and stimulates deoxyribose degradation.

Madsen et al. *(29)*, using an electron spin resonance spin trapping method, have shown that phenolic-rich extracts, such as oregano and dittany (a member of the Labiatae family like rosemary and oregano), are able to scavenge the hydroxyl radicals generated in the Fenton reaction.

Table 2 also shows that when ascorbate was omitted, oregano, saffron, rosemary, and sweet paprika ($P < 0.05$) exhibited prooxidant activity because the level of the pink chromogen exceeded that of the control. These spices showed poor antioxidant activity in the full assay (RM + DR), probably because they do not act as hydroxyl scavengers but react with ascorbate to decrease the amount of $OH^{\cdot}$ generated *(35)*. In this sense, other investigators observed that extracts from *Cassia tora* L. (Chinese herb Jueming-zi) inhibited the oxidation of deoxyribose induced by Fe^{3+}-EDTA/H_2O_2/ascorbic acid but acted as prooxidant in the absence of ascorbate *(50)*.

This assay was only developed with the compounds dissolved in water because organic solvents, such as ethanol, may themselves act as good scavengers of $OH^{\cdot}$ *(5)*.

Hydrogen peroxide scavenging. The generation of hydrogen peroxide by activated phagocytes is known to play an important role in the killing of several bacterial and fungal strains. Additionally, hydrogen peroxide is generated in vivo by several oxidase enzymes *(24)*. There is increas-

TABLE 3. *Scavenging of hydrogen peroxide by different Mediterranean food spices compared with the activity of different compounds frequently used as food additives using peroxidase-based assays*[a]

Added to reaction mixtures[b]	Absorbance ($A_{436\ nm}$)
None (control)	0.723
Annatto[c]	0.401
Cumin[c]	0.227
Oregano[c]	0.305
Paprika, hot[c]	0.529
Paprika, sweet[c]	0.614
Rosemary[c]	0.478
Saffron[c,d]	—[f]
Propyl gallate[e]	0.073
Propyl gallate[c]	0.705
BHA[c]	0.896
BHT[c]	0.814

[a] Statistical differences were analyzed by analysis of variance ($P < 0.05$).
[b] Compounds in aqueous medium.
[c] Concentration 5%.
[d] This compound shows intense color, from 390 to 490 nm, that interfered with the hydrogen peroxide generated signal.
[e] Concentration 100 μg/g.
[f] No % inhibition detected.

the synthesis and activation of several inflammatory mediators *(47)*.

The scavenging of hydrogen peroxide activity is easily and sensitively measured by using peroxidase-based assay systems, when one looks for a decrease in the absorption spectrum after the compound is added to peroxidase-H_2O_2 mixtures.

Table 3 shows the scavenging of hydrogen peroxide by the Mediterranean food spices and food additives studied.

In aqueous medium, the spice extracts show lower antioxidant activity ($P < 0.05$) than propyl gallate (5%). Cumin and oregano, with 69 and 58% inhibition, respectively, did not show significant differences ($P < 0.05$) and exhibited higher antioxidant activity than annatto and rosemary, with 44 and 34% inhibition, respectively, hot paprika and sweet paprika showed a moderate inhibitory effect of about 20% inhibition at this concentration. On the other hand, the intensive color of saffron extract in aqueous medium interfered with the scavenging activity signal of hydrogen peroxide. BHA and BHT did not react with H_2O_2 and must be considered inefficient.

When dissolved in ethanol medium, annatto, sweet and hot paprika, and saffron extracts showed greater absorbance than the control, perhaps because interfering materials have maximum absorption wavelengths near 410 nm. Propyl gallate (5%), which exhibited similar results to those obtained in aqueous medium, showed significant differences ($P < 0.05$) from the rest of the spices tested. However, propyl gallate did not show this scavenging capacity at the permitted concentration in foods (100 μg/g). BHA and BHT dissolved in ethanol medium are inefficient catalysts of any subsequent oxidation because they did not react with H_2O_2. Oregano and rosemary ethanol extracts were the only two

TABLE 4. *Scavenging of HOCl by Mediterranean food spices using the elastase assay compared with the activity of different compounds frequently used as food additives*[a]

Added to reaction mixtures[b]	Absorbance ($A_{410\ nm}$)
None (control)	0.802
Annatto[c]	0.130
Cumin[c]	0.253
Oregano[c]	0.052
Paprika, hot[c]	0.187
Paprika, sweet[c]	0.185
Rosemary[c]	0.020
Saffron[c]	0.214
Propyl gallate[c]	0.084
Propyl gallate[d]	0.116
BHA[d]	1.202
BHT[d]	1.016

[a] Statistical differences were analyzed by analysis of variance ($P < 0.05$).
[b] Compounds in aqueous medium.
[c] Concentration 5%.
[d] Concentration 100 μg/g.

spices to show inhibition (48 and 24% inhibition, respectively), though both lower than the corresponding results in the aqueous medium described above.

These spices show high levels of phenolic compounds *(25)* that can react with hydrogen peroxide and act as substrates for peroxidases *(7)*.

Scavenging of HOCl. HOCl is produced by the neutrophil-derived enzyme, myeloperoxidase, at inflammation sites and when activated neutrophils infiltrate reoxygenated tissues. One of the most important targets attacked by HOCl in vivo is α_1-antiproteinase, the major circulating inhibitor of proteolytic enzymes such as elastase *(7)*. Thus, a good test for physiologically relevant HOCl scavenging activity by a compound is to analyze whether that compound, at the concentrations achieved in vivo, can protect α_1-antiproteinase against inactivation by HOCl *(37)*.

In Table 4 the scavenging of HOCl by Mediterranean food spices is compared with the capacity of different compounds frequently used as food additives. After incubation of HOCl with α_1-antiproteinase, which is very rapidly inactivated by HOCl, α_1-antiproteinase loses its elastase-inhibitory capacity.

Hence, when compounds are dissolved in aqueous medium, spices are able to scavenge HOCl and protect α_1-antiproteinase. Our results indicate that rosemary and oregano extracts were more effective HOCl scavengers ($P < 0.05$) (97 and 93%, respectively) than the rest of samples analyzed, which, in decreasing order, were propyl gallate, annatto, sweet and hot paprika, saffron, and cumin (90, 84, 77, 77, 73, and 68% inhibition, respectively) ($P < 0.05$). The antioxidant activity exhibited by propyl gallate did not vary significatly ($P < 0.05$) when comparing the concentrations used in this assay, 100 μg/g, which is the permitted commercial concentration *(19)* and at 5%, i.e., at the same concentration as the spices. This last concentration was too high and it guarantees the importance of the effect produced

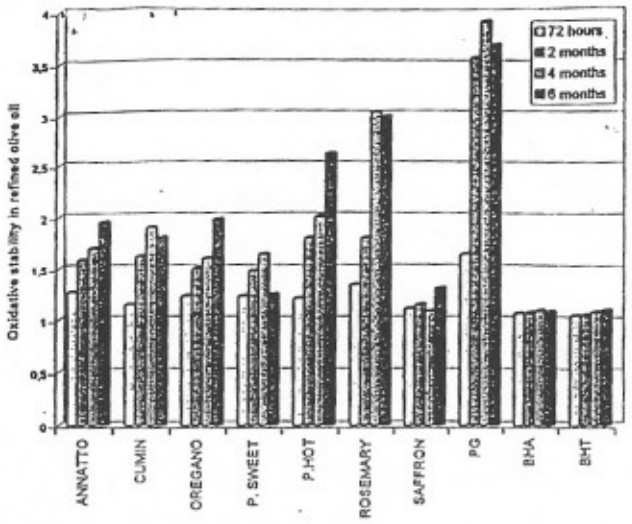

FIGURE 1. *Oxidative stability during storage at room temperature for Mediterranean food spices, compared with common food additives. BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; P, paprika; PG, propyl gallate.*

by rosemary and oregano and their traditional uses as medicinal herbs *(23, 39, 46)*. BHA and BHT were unable to scavenge HOCl in aqueous medium.

When the compounds were dissolved in ethanol medium, saffron and cumin extracts exhibited stronger antioxidant activity than in aqueous medium, with 90 and 72% inhibition. The rest of the spices, and propyl gallate, showed lower antioxidant activity than in aqueous medium, as described above. In decreasing order, propyl gallate (5%), annatto, propyl gallate (100 μg/g), hot paprika, sweet paprika and oregano, exhibited 81, 72, 62, 61, 59, and 54% inhibition, respectively. Finally, BHA and BHT showed the lowest antioxidant activity in ethanol medium with percents inhibition of 51 and 48%, respectively.

According to Aruoma et al. *(9)*, propyl gallate effectively protects α_1-antiproteinase activity against HOCl. Other compounds, too, have been considered as scavengers of HOCl, including synthetic antioxidants like BHT (in organic solvent) *(32)*.

Rancimat results. Refined olive oil alone (control) starts the radical chain reactions of the propagation phase of autoxidation after 8.9 h. The time required for the formation of a sufficient concentration of initiating radicals (initiation phase) was slightly greater when the spices or food additives were added, delaying the time of onset of the propagation phase of the radical chain reaction.

Figure 1 shows the effect of the food spices and additives on the oxidative stability of refined olive oil tested by the Rancimat method during storage at room temperature. After 72 h, the following order of stability was obtained as a result of the addition of the different samples: propyl gallate > rosemary > annatto > oregano ≅ sweet paprika > hot paprika > cumin > saffron > BHA ≅ BHT. All the spices were used at 5% concentration, while the food additives were tested at the permitted commercial concentration (100 μg/g oil) by European law for BHA, BHT, and propyl gallate *(20)*.

Propyl gallate was a significantly ($P < 0.05$) more effective antioxidant than all the other compounds tested. Among the spices tested, rosemary conferred a significantly ($P < 0.05$) greater protection, followed by annatto. The difference between oregano and sweet paprika was too small to be significant statistically, although the level of protection was higher than that obtained with the widely used BHA and BHT. Gordon and Kourimská *(22)*, who used the Rancimat test, observed that BHA and BHT had no antioxidant activity, probably reflecting the volatility of these additives.

After 2 months' storage with or without added spices or food additives, oil rancidity was evaluated in order to ascertain their effectiveness in retarding the development of rancidity. The short induction time of the control olive oil sample (7.9 h) is apparently due to the increased formation of chain-initiating radical forms *(10)*, while longer induction periods point to the strong activity of the added antioxidants *(42)*. All the compounds tested showed antioxidant activity (Fig. 1) in the following decreasing order: propyl gallate, rosemary, hot paprika, cumin, annatto, oregano, sweet paprika, and saffron. Propyl gallate exhibited a much longer induction period despite its much lower concentration. After 2 months' maceration, all the tested spices showed a higher antioxidant index than after 72 h maceration.

After 4 months' maceration, the Rancimat test revealed that prolonged storage increases the susceptibility of refined olive oil to oxidation (5.8 h). Figure 1 shows the increased oxidative stability of refined olive oil macerated with spices compared with the results for 2 months. Also at this time (4 months) rosemary was seen to have increased its protection factor more than the other spices ($P < 0.05$).

After 6 months' maceration, the olive oil exhibited a shorter induction time (4.7 h), and the antioxidant activity of the tested compounds were propyl gallate > rosemary > paprika hot > oregano > annatto > cumin > paprika sweet > saffron > BHT ≅ BHA (Fig. 1), with rosemary showing a similar stabilizing effect to that obtained after 4 months. However, annatto, oregano, and saffron showed higher stabilizing effects than after 4 months.

The increased induction time after more prolonged maceration with the spices (2, 4, and 6 months) can be explained by a time-dependent increase in the extraction of active components from the spices *(10)*.

These results are in agreement with those obtained by Schwarz et al. *(42)* for samples of rosemary, using the Rancimat test. Also, Aruoma and Halliwell *(6)* and Aruoma et al. *(10)*, again working with Rancimat, reported good antioxidant activity for oregano and rosemary in different types of fats. These compounds can act as chain-breaking antioxidants and can react with peroxyl radicals, introducing a lag period into the peroxidation process that corresponds with the time taken for the antioxidant to be consumed.

In conclusion, the above results indicate that the Mediterranean spices, like annatto, cumin, oregano, sweet and

hot paprika, rosemary, and saffron, that are traditionally used for their aromatic properties in the preparation of Mediterranean food, exhibit good antioxidant activity as scavengers of several reactive oxygen species. This fact supports the replacement of the synthetic antioxidant by such natural spice extracts that because of their contribution of characteristic colors and flavors would prompt the use of these spices in the design of new functional foods.

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