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Evaluation of the Antioxidant Properties of Mediterranean and Tropical Fruits Compared with Common Food Additives

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ABSTRACT

Several Mediterranean and tropical fruits have been analyzed in order to assess their antioxidant activity compared with that of common food additives (butylated hydroxyanisole [BHA], butylated hydroxytoluene [BHT] and propyl gallate). Among Mediterranean fruits, red grape and plum were more effective ($P < 0.05$) scavengers of peroxyl radicals than BHA, BHT, and propyl gallate. Of the tropical fruits, banana was the most effective scavenger of peroxyl radicals. Mediterranean and tropical fruits showed very good scavenger activity against hydroxy radicals ($OH^{\cdot}$), protecting deoxyribose better than BHA and BHT. The HOCl scavenging ability of Mediterranean fruits tested was, in decreasing order, lemon > plum > apricot > white grape > melon > red grape > mandarin > watermelon > peach > medlar > apple > orange > cherry > strawberry. However, the four varieties of pear were poor scavengers ($P < 0.05$). Among tropical fruits, the order of efficiency as HOCl scavengers was passion fruit > lime > passiflora > kumquat > avocado > pineapple > physalis > papaya fruit > carambola > mango > banana. All Mediterranean fruits showed an effect on hydrogen peroxide except peach. Tropical fruits also had a strong effect on hydrogen peroxide except avocado, which had no effect. The effect of Mediterranean and tropical fruits on the protection factor of refined olive oil, analyzed by the Rancimat method and compared with common food additives, was clear. Watermelon conferred a significantly ($P < 0.05$) greater protection than the other Mediterranean fruits. Among tropical fruits, physalis had the most stabilizing effect.

We are in the middle of a revolution that is changing the concept of food and our way of eating. Some foods might provide an optimal mix of phytochemicals, such as natural antioxidants, fibers, and other bioactive compounds. Food research and the food industry in general has reacted to these data by improving traditional processes or by providing new technological solutions to create products (e.g., "light" and functional products) that help consumers keep in line with these nutritional issues *(33)*.

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions *(27)*. They seek out electrons that set off chain reactions that damage cells, lipids, proteins, and DNA until they are quenched and return to a stable state *(14)*.

In the body, the normal metabolism of oxygen in living cells, environmental pollutants, radiation, pesticides, various medications, and contaminated water cause the unavoidable production of oxygen-derived free radicals (hydroxyl, peroxyl, hydrogen peroxide, hypochlorous acid), which have been implicated in more than a hundred disease conditions in humans. It is postulated that an upset oxidative balance could be a contributing factor in a broad spectrum of diseases *(9, 27)*.

The characterization of antioxidants can be carried out using a variety of assays. A direct test of antioxidant ability is to examine whether a substance inhibits the peroxidation of artificial lipid systems, such as brain phospholipid liposomes incubated with $FeCl_3$ and ascorbic acid, by scavenging peroxyl radicals *(2)*. The deoxyribose assay evaluates whether a compound is a scavenger of hydroxyl radicals ($OH^{\cdot}$), in which case, it will compete with deoxyribose for the $OH^{\cdot}$ and inhibit deoxyribose degradation. Highly reactive radicals are generated by a mixture of ascorbate and $FeCl_3$-EDTA *(1)*. Furthermore, compounds can be tested for their potential to interfere with HOCl, which 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. Thus, a good test for physiologically relevant HOCl scavenging activity by a given compound is to analyze whether that compound, at the concentrations achieved in vivo, can protect α_1-antiproteinase against inactivation by HOCl *(5)*.

Hydrogen peroxide is generated in vivo by several oxidase enzymes and by activated phagocytes and it is known to play an important role in the killing of several bacterial and fungal strains *(19)*. There is increasing evidence that H_2O_2, either directly or indirectly via its reduction product $OH^{\cdot}$, can act as a messenger molecule in the synthesis and activation of several inflammatory mediators *(39)*. Thus, if a putative scavenger is incubated with H_2O_2 using a peroxidase-based assay system, any loss of H_2O_2 can be measured. To assess oxidative stability in the food industry, the Rancimat test, in which the scavenger to be tested is added

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to a lipidic food and the degree of protection is evaluated *(38)*, is performed. If the constituents of the tested fruits act as antioxidants, they might prevent lipid peroxidation not only in the foods to which they are added (increasing the shelf life), but also in the body by scavenging free radicals (e.g., circulating as the consequence of metabolic processes and alterations caused by diseases), contributing to consumer well-being and health.

The levels of essential antioxidant vitamins, in contrast to other antioxidative defences, are due mainly to their presence in the diet. Fruit and vegetables are the main sources of antioxidant vitamins (vitamin E, vitamin C, β-carotene), which act as $OH^{\cdot}$ free radical scavengers, making these foods essential to human health *(14)*. However, more than 80% of the total antioxidant capacity in fruits and vegetables comes from ingredients other than vitamins. Indicating the presence of other potentially important antioxidants in these foods *(30)*. The antioxidant ability of these nutrients is responsible for the role of these foods in protection against disease. The literature shows a negative association between the intake of fruits and vegetables and heart disease mortality, cancer, and reduced blood pressure *(23)*.

Many types of flavonoids (flavones, flavonols, flavanols, flavanones, anthocyanins, flavanols, flavanone glycosides, chalcones, lignins) have been described, and the number of characterized substances is continually increasing *(36)*. Many of these flavonoids exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, vasodilatory actions, and various actions against hormone-dependent cancers *(13)*.

Flavonoids are natural antioxidants and show a much stronger antioxidant activity against peroxyl radicals than vitamin E, vitamin C and glutathione *(10)*. Rice-Evans et al. *(35)* studied the chemical properties of bioflavonoids in terms of the availability of the phenolic hydrogens as hydrogen-donating radical scavengers and singlet oxygen quenchers. Flavonoids have also been recognized as hydroxyl radical scavengers *(22)*, lipid peroxidation inhibitors *(24)*, modulators of the activity of enzyme systems, including cyclooxygenase and lipooxygenase *(24)*, and chelators of toxic heavy metals *(41)*. Most flavonoids came from vegetables and fruits. The most common flavonoids in food are quercetin and catechin.

Other phenolic compounds, such as cinnamic acids (caffeic, chlorogenic, ferulic, sinapic and *p*-coumaric acids) appear to be more active antioxidants than phenolic (*p*-hydroxybenzoic), vanillic, and syringic acids *(36)*. They retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food *(25)*.

Flavonoids and other phenolics are thought to play a preventive role in the development of cancer (antipromotor activity, anti-invasive effect, and inhibition of enzymes like protein tyrosine kinase), thrombosis (inhibition of platelet aggregation, influence on the metabolism of arachidonic acid, reductions in tromboxane and calcium levels, and increases in levels of prostacyclin), and heart disease *(11)*.

Thus, the World Health Organization recommends the consumption of at least five portions of fruit and/or vegetables per day. However, our knowledge of the antioxidant activity of fruits is limited, mainly because few varieties of fruits have been studied. For this reason, the aim of this study is to characterize some Mediterranean and tropical fruits in order to assess their antioxidant activity compared with that of common food additives (butylated hydroxyanisole [BHA], butylated hydroxytoluene [BHT], and propyl gallate). Such a study, it is hoped, will contribute to our understanding of the beneficial effects of fruits in the diet, thus avoiding the need for dietary supplements of antioxidant properties.

MATERIAL AND METHODS

Material. Propyl gallate, BHA, BHT, and all other chemicals were of the highest quality available and were purchased from Sigma Chemical Co. (Poole, Dorset, UK).

Twenty-two Mediterranean fruits (traditionally associated with the Mediterranean diet and grown in temperate climates) and eleven tropical fruits (grown in tropical and equatorial zones) were analyzed. The fruits were purchased in a local supermarket and had the maximum quality and optimal ripeness for commercial distribution. Both qualities provided for high flavor and taste. Different batches for every variety of fruit were collected and then divided into five aliquots immediately before being submitted to the different antioxidant assays.

Sample preparation. All of the fruits were peeled and cored (if necessary), except cherries and white and red grapes (which were not peeled), physalis (outer membrane removed), and strawberries (leaves removed). In general, every fruit was prepared as normally used by the consumer at home. The fruits were then homogenized for 30 s with a household mixer (Moulinex Turbo Blender) before assay. The widely used antioxidant additives, BHA, BHT, and propyl gallate, were analyzed at the concentration of 100 μg/g *(15)*.

Peroxidation of phospholipid liposomes. The ability of samples to inhibit lipid peroxidation at pH 7.4 was tested using ox brain phospholipid liposomes, essentially as described in Aruoma et al. *(6)*. The experiments were conducted in a physiological saline buffer (phosphate-buffered saline, pH 7.4) (3.4 mM Na_2HPO_4–NaH_2PO_4, 0.15 M NaCl). 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 $FeCl_3$, 100 mg of test fruits (or 100 μl of common food additives dissolved in water), and 100 μM ascorbate (added last to start the reaction). Ascorbic acid is known to stimulate lipid peroxidation and the formation of reactive oxygen species such as $OH^{\cdot}$. Because BHT is not fully soluble in aqueous solution and its emulsion is not homogeneous, deionized water with a conductivity of not more than 4 μS/cm was used *(37)*. The samples were incubated at 37°C for 30 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. Peroxidation inhibition was expressed as the decrease in peroxidation obatained by adding the tested compounds (100% peroxidation referred to an assay containing no added compound [control]).

This TBA test measures not only the peroxidation occurring in the experiment itself, but also that which takes place during the acid heating stage. 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 *(3)*.

Hydroxyl radical scavenging. In a final volume of 1.2 ml, the reaction mixtures contained 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 mg of the tested fruits (or 100 μl of the common food additives dissolved in water). Ascorbate (100 μM) was added to start the reaction. The tubes were incubated at 37°C for 1 h. The products of the $OH^{\cdot}$ attack on deoxyribose were measured as described in Aruoma et al. *(4)* at 532 nm. The results are expressed as percent inhibition of the deoxyribose attack, where 100% attack is defined as the absorbance levels recorded for deoxyribose without the addition of the tested compounds (control).

A parallel assay was also made omitting ascorbate. The addition of ascorbic acid greatly increases the rate of $OH^{\cdot}$ generation by reducing iron and maintaining the supply of Fe^{2+} *(5)*. At the same time, by omitting ascorbate, false scavenger activity results are eliminated, probably because the compounds react with ascorbate but do not act as hydroxyl scavengers.

Reactions with hypochlorous acid. The hypochlorous acid (HOCl) reaction was studied using the elastase assay, essentially as described by Aruoma et al. *(4)*. For the assay, 68 μM HOCl (produced immediately before use by adjusting NaOCl to pH 6.2 with dilute H_2SO_4) and 100 mg of the test fruits (or 100 μl of common food additive dissolved in water) 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. In the present assay, fruits were filtered through 0.2-μm pore size filters. α_1-Antiproteinase (7 mg/ml) was added to the reaction mixture so that it would be inactivated by any remaining HOCl. 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 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}.

Scavenging of hydrogen peroxide. The fruits (100 mg) (or 100 μl of food common additives dissolved in water) were incubated with 0.84 mM H_2O_2 for 10 min at 25°C. Aliquots of these compounds were then assayed for remaining H_2O_2 by using the peroxidase system *(3)*. The remaining H_2O_2 was measured as the formation of a cromophor recorded at 436 nm in reaction mixtures containing, in a final volume of 1 ml, 0.150 M KH_2PO_4–KOH buffer, pH 7.4; 50 μl guaiacol solution (made by adding 100 μl of pure guaiacol to 100 ml water); and 10 μl of Sigma type IV horseradish peroxidase (5 mg/ml in the same phosphate buffer).

Rancimat test to evaluate protection factor. Sample preparation in the Rancimat test consisted of macerating 100 mg of the tested fruits (or 100 μg/g common food additives) in 3 g refined olive oil (provided by the manufacturing company and free of added antioxidants or preservatives). The mixtures were then incubated for 1 h at room temperature before analysis.

All measurements to evaluate the protection factor 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 *(18)*. Determination of the induction period was based on the detection of volatile acids at 110°C with an air flow rate of 20 liter/h *(8)*. The relative activity of the antioxidants is expressed by the protection factor (PF) 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) *(2, 38)*.

This technique has been questioned by some authors *(16)*, but in agreement with Bonilla et al *(7)*, 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. Every sample was made in quintuplicate and the results obtained are the mean of three replicates. The data were analyzed using the Statistical Package for Social Sciences Windows 9.0 (SPSS Inc., Chicago, Ill.). Analyses of variance were determined, and the significance level using Fisher's multiple range least significant difference test was calculated *(32)*.

RESULTS AND DISCUSSION

Inhibition of phospholipid peroxidation. Table 1 shows the inhibition of lipid peroxidation in the presence of some Mediterranean and tropical fruits compared with common food additives. Among Mediterranean fruits, red grape and plum were more effective ($P < 0.05$) scavengers of peroxyl radicals than BHA, BHT, and propyl gallate at the permitted concentrations. A second group of Mediterranean fruits showed inhibition percentages >50% ($P < 0.05$), showing less effective scavenger activity than BHA and more antioxidant activity than propyl gallate and BHT. A third group showed inhibition percentages of about 30 to 40% ($P < 0.05$), but with less capacity than propyl gallate and BHA. The rest of the Mediterranean fruits showed a lower inhibition percentage, which did not differ significantly ($P < 0.05$) from BHT activity. Mandarin showed the lowest antioxidant activity.

Among tropical fruits, banana was the most effective scavenger of peroxyl radicals and was even better than BHA, BHT, and propyl gallate. A second group showed 69 and 62% inhibition ($P < 0.05$), and a third group showed percentages of inhibition of between 30 and 40% ($P < 0.05$). The rest of the tropical fruits were worse scavengers. The results showed that kumquat and papaya fruit did not act as peroxyl radical scavengers.

Mediterranean and tropical fruits have a high vitamin content. However, other nonvitamin compounds, including cinnamic acid derivatives such as chlorogenic acid, which have an antioxidant capacity and are found in grape, have also been seen to act as powerful inhibitors of lipid oxidation *(34)*. Still other compounds also show antioxidant activity, including phenolic acids, cinnamic acid as coumaric and ferulic derivatives and gallic esterols, flavonols, epicatechin, and anthocyanins *(27)*. Neochlorogenic acid (3′-caffeoylquinic acid), a cinnamic acid derivative which predominates in, for example, plums *(20)*, was one of the best scavengers of peroxyl radicals of all the antioxidants studied.

Other fruits such as pear, apple, plum, and peach also possess hydroxycinnamic acids (caffeic acid, *p*-coumaric acid, ferulic acid) *(29)*. Caffeic acid and its esterified derivatives, may work mainly as radical scavengers and may also be powerful metal chelators *(35)*.

The presence in apple of cinnamic acids (chlorogenic acid), flavanols (epicatechin), anthocyanins, flavonols, and

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

Sample	% inhibition
Control	—[b]
Mediterranean fruits	
Apple *Malus communis* Poir var. *Golden delicious*	29.4 ± 2 CD
Apple *Malus communis* Poir var. *Royal Gala*	30.7 ± 2 CD
Apple *Malus communis* Poir var. *Starking*	13.7 ± 1 D
Apricot *Prunus armeniaca* L.P. var. *Gala rojo*	31.3 ± 2 CD
Cherry *Prunus avium* L. var. *Stark*	60.4 ± 3 B
Red grape *Vitis vinifera* L. var. *Napoleon negra*	73.3 ± 4 A
White grape *Vitis vinifera* L. var. *Aledo*	60.9 ± 3 B
Lemon *Citrus limon* (L.) Burm. F. var. *Verna*	53.8 ± 3 B
Mandarin *Citrus reticulata* Blanco var. *Fortuna*	3.1 ± 1 E
Medlar *Eriobotryae japonica* (Thunb.) Lindl. var. *Algar*	23.8 ± 2 D
Melon *Cucumis melo cantalupensis* Naud var. *Aitana*	66.8 ± 3 AB
Melon *Cucumis melo saccharinus* Naud var. *Sancho*	61.8 ± 3 B
Orange *Citrus sinensis* (L.) Osbeck var. *Navelina*	22.2 ± 2 D
Orange *Citrus sinensis* (L.) Osbeck var. *Salutiana*	15.1 ± 1 D
Peach *Prunus persica* (L.) Batsch var. *Queen nest*	36.5 ± 2 C
Pear *Pyrus communis* (L.) var. *Blanquilla*	32.7 ± 2 CD
Pear *Pyrus communis* (L.) var. *Conferencia*	29.6 ± 2 CD
Pear *Pyrus communis* (L.) var. *Flor de Invierno*	44.1 ± 2 C
Pear *Pyrus communis* (L.) var. *Passacrana*	34.4 ± 2 C
Plum *Prunus domestica* (L.) var. *Red-Beauti*	73.3 ± 4 A
Strawberry *Fragaria vesco* L. var. *Camarosa*	40.2 ± 3 C
Watermelon *Citrullus vulgaris* Schrad var. *Sugar baby*	39.5 ± 2 C
Tropical fruits	
Avocado *Persea americana* Mill var. *Bacon*	69.2 ± 3 AB
Banana *Musa acuminata* Colla var. *Canarias*	81.8 ± 4 A
Carambola *Carica pentagona* var. *Averrhoa*	36.7 ± 2 C
Kumquats *Fortunella margarita* (Lour.) Swingle var. *Nagami*	— F
Lime *Citrus aurantifolia* (Chris) Siwng var. *Persea*	5.2 ± 1 E
Mango *Mangifera indica* (L.) var. *Tommy*	35.3 ± 2 C
Passion fruit *Passiflora eduliss* (Sims.) var. *Flavicarpa*	39.2 ± 2 C
Papaya fruit *Carica papaya* (L.) var. *Pentagona*	— F
Passifora *Passiflora eduliss* (Sims.) var. *Eduliss*	62.2 ± 3 B
Physalis (cape gooseberry) *Physalis peruviana* L. var. *Goldenberry*	9.0 ± 1 DE
Pineapple *Ananas comosus* (L.) Merrill var. *Cayenne lisa*	41.9 ± 3 C
Propyl gallate[c]	51.7 ± 2 B
BHA[c]	71.0 ± 3 AB
BHT[c]	23.0 ± 1 D

[a] Statistical differences were analyzed by ANOVA ($P < 0.05$). Values with the same letter are not significantly different ($P > 0.05$).
[b] —, no inhibition detected.
[c] 100 μg/g concentration. Compounds dissolved in aqueous medium.

chalcones has been determined in the linoleic acid system *(26)*.

The strawberry, rich in phenolic compounds shows itself as the most potent scavenger of peroxyl radicals, relying on the contribution of vitamin C (less than 30%) for its total antioxidant activity. This fruit was a more powerful scavenger than other fruits like plum, orange, red grape, white grape, banana, apple, and pear *(22, 42)*. Meltzer and Malterud *(28)* have also detected alkoxyl and peroxyl radical scavenging activities in orange, which they attributed to the presence of flavonols.

The results described above agree with those of Miyake et al. *(31)*, who studied the antioxidant properties of lemon using a linoleic acid autoxidation system and who attributed them to the content in flavonoids such as eriocitrin, hesperidin, narirutin, diosmin, 6,8-di-C-β-glucosyldiosmin, neoeriocitrin, naringin, and neohesperidin.

Some researchers attribute the antioxidant activity of mango to its dietary fibres and to its high carotenoid content *(27)*.

Assessment of the antioxidant activity of Mediterranean and tropical fruits by the deoxyribose assay. Table 2 shows the results of the deoxyribose damage caused by $OH^{\cdot}$ in the presence of Mediterranean and tropical fruits or the common food additives (BHA, BHT, propyl gallate) added at permitted concentrations.

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

Sample	Damage to deoxyribose (A_{532}, nm)[b]		
	RM+DR	% inhibition	Omit ASC
Control	1.947	0	0.604
Mediterranean fruits			
Apple *Malus communis* Poir var. *Golden delicious*	0.265 ± 0.03 D	86.4	0.085
Apple *Malus communis* Poir var. *Royal Gala*	0.191 ± 0.02 CD	90.2	0.054
Apple *Malus communis* Poir var. *Starking*	0.158 ± 0.02 CD	91.9	0.064
Apricot *Prunus armeniaca* L.P. var. *Gala rojo*	0.119 ± 0.02 C	93.8	0.045
Cherry *Prunus avium* L. var. *Stark*	0.064 ± 0.01 B	96.7	0.095
Red grape *Vitis vinifera* L. var. *Napoleon negra*	0.348 ± 0.03 E	82.1	0.098
White grape *Vitis vinifera* L. var. *Aledo*	0.137 ± 0.02 CD	92.9	0.056
Lemon *Citrus limon* (L.) Burm. F. var. *Verna*	0.085 ± 0.01 B	95.6	0.084
Mandarin *Citrus reticulata* Blanco var. *Fortuna*	0.059 ± 0.01 B	96.9	0.026
Medlar *Eriobotryae japonica* (Thunb.) Lindl. var. *Algar*	0.060 ± 0.01 B	96.9	0.078
Melon *Cucumis melo cantalupensis* Naud var. *Aitana*	0.174 ± 0.02 CD	91.0	0.057
Melon *Cucumis melo saccharinus* Naud var. *Sancho*	0.170 ± 0.02 CD	91.2	0.069
Orange *Citrus sinensis* (L.) Osbeck var. *Navelina*	0.260 ± 0.03 D	86.6	0.087
Orange *Citrus sinensis* (L.) Osbeck var. *Salutiana*	0.249 ± 0.02 D	87.2	0.091
Peach *Prunus persica* (L.) Batsch var. *Queen nest*	0.251 ± 0.02 D	87.1	0.089
Pear *Pyrus communis* (L.) var. *Blanquilla*	0.23 ± 0.025 D	87.9	0.081
Pear *Pyrus communis* (L.) var. *Conferencia*	0.116 ± 0.01 C	94.0	0.069
Pear *Pyrus communis* (L.) var. *Flor de Invierno*	0.153 ± 0.01 CD	92.1	0.087
Pear *Pyrus communis* (L.) var. *Passacrana*	0.096 ± 0.01 B	95.1	0.057
Plum *Prunus domestica* (L.) var. *Red-Beauti*	0.057 ± 0.01 AB	97.1	0.060
Strawberry *Fragaria vesca* L. var. *Camarosa*	1.182 ± 0.03 G	39.3	0.068
Watermelon *Citrullus vulgaris* Schrad var. *Sugar baby*	0.176 ± 0.02 CD	90.9	0.061
Tropical fruits			
Avocado *Persea americana* Mill var. *Bacon*	0.100 ± 0.01 BC	94.9	0.056
Banana *Musa acuminata* Colla var. *Canarias*	0.081 ± 0.01 B	95.8	0.068
Carambola *Carica pentagona* var. *Averrhoa*	0.436 ± 0.03 F	77.6	0.051
Kumquats *Fortunella margarita* (Lour.) Swingle var. *Nagami*	0.102 ± 0.02 BC	94.7	0.048
Lime *Citrus aurantifolia* (Chris) Siwng var. *Persea*	0.045 ± 0.01 B	97.7	0.064
Mango *Mangifera indica* (L.) var. *Tommy*	0.116 ± 0.01 C	94.1	0.089
Passion fruit *Passiflora eduliss* (Sims.) var. *Flavicarpa*	0.090 ± 0.01 B	95.3	0.076
Papaya fruit *Carica papaya* (L.) var. *Pentagona*	0.039 ± 0.01 AB	98.0	0.071
Passifora *Passiflora eduliss* (Sims.) var. *Eduliss*	0.020 ± 0.01 A	98.9	0.043
Physalis (cape gooseberry) *Physalis peruviana* L. var. *Goldenberry*	0.219 ± 0.03 D	88.7	0.051
Pineapple *Ananas comosus* (L.) Merrill var. *Cayenne lisa*	0.078 ± 0.01 B	95.9	0.103
Propyl gallate[c]	2.291 ± 0.05 J	—[d]	1.319
BHA[c]	1.452 ± 0.04 H	25.4	0.142
BHT[c]	1.772 ± 0.05 I	8.9	0.395

[a] Statistical differences were analyzed by ANOVA ($P < 0.05$). Values with the same letter are not significantly different ($P > 0.05$).
[b] A_{532}, absorbance values recorded at 532 nm. When deoxyribose was omitted, the values ranged from 0.001 to 0.004 absorbance units. RM, reaction mixtures; DR, deoxyribose; ASC, ascorbate.
[c] 100 μg/g concentration. Compounds dissolved in aqueous medium.
[d] —, no inhibition detected.

The Mediterranean fruits showed themselves to be very good scavengers of OH·, protecting deoxyribose better than BHA and BHT. There were no significant differences between the two varieties of oranges analyzed and peach ($P < 0.05$) or between *Royal Gala* and *Starking* apples ($P < 0.05$). Among varieties of pear, there were no significant differences between *Conferencia, Flor de invierno,* and *Passacrana* ($P < 0.05$). Strawberry showed the lowest percent inhibition (<50%).

The tropical fruits were also very good scavengers of OH·. There were no significant differences between passiflora and papaya fruit ($P < 0.05$) or between papaya fruit and lime, pineapple, banana, passion fruit, or avocado ($P < 0.05$), all showing >75% inhibition.

The assay for BHA and BHT were in accordance with those described by Murcia and Martínez-Tomé *(32).* In both cases, the percentages of inhibition were no greater than 25%. The results also showed the prooxidant effect of pro-

TABLE 3. *Inactivation of α_1-antiproteinase by hypochlorous acid. Effect of Mediterranean and tropical fruits compared with the activity of different compounds frequently used as food additives*[a]

Sample	Absorbance (A_{410}, nm)
Control	1.054
Mediterranean fruits	
Apple *Malus communis* Poir var. *Golden delicious*	0.832 ± 0.04 E
Apple *Malus communis* Poir var. *Royal Gala*	0.751 ± 0.03 E
Apple *Malus communis* Poir var. *Starking*	0.787 ± 0.04 E
Apricot *Prunus armeniaca* L.P. var. *Gala rojo*	0.398 ± 0.02 C
Cherry *Prunus avium* L. var. *Stark*	0.891 ± 0.04 EF
Red grape *Vitis vinifera* L. var. *Napoleon negra*	0.552 ± 0.03 D
White grape *Vitis vinifera* L. var. *Aledo*	0.400 ± 0.02 C
Lemon *Citrus limon* (L.) Burm. F. var. *Verna*	0.045 ± 0.01 A
Mandarin *Citrus reticulata* Blanco var. *Fortuna*	0.553 ± 0.03 D
Medlar *Eriobotryae japonica* (Thunb.) Lindl. var. *Algar*	0.685 ± 0.03 D
Melon *Cucumis melo cantalupensis* Naud var. *Aitana*	0.549 ± 0.03 D
Melon *Cucumis melo saccharinus* Naud var. *Sancho*	0.537 ± 0.03 D
Orange *Citrus sinensis* (L.) Osbeck var. *Navelina*	0.830 ± 0.04 E
Orange *Citrus sinensis* (L.) Osbeck var. *Salustiana*	0.802 ± 0.03 E
Peach *Prunus persica* (L.) Batsch var. *Queen nest*	0.615 ± 0.04 D
Pear *Pyrus communis* (L.) var. *Blanquilla*	1.008 ± 0.05 G
Pear *Pyrus communis* (L.) var. *Conferencia*	1.041 ± 0.05 G
Pear *Pyrus communis* (L.) var. *Flor de Invierno*	1.042 ± 0.05 G
Pear *Pyrus communis* (L.) var. *Passacrana*	1.043 ± 0.05 G
Plum *Prunus domestica* (L.) var. *Red-Beauti*	0.332 ± 0.02 C
Strawberry *Fragaria vesca* L. var. *Camarosa*	0.990 ± 0.03 F
Watermelon *Citrullus vulgaris* Schrad var. *Sugar baby*	0.563 ± 0.03 D
Tropical fruits	
Avocado *Persea americana* Mill var. *Bacon*	0.335 ± 0.02 C
Banana *Musa acuminata* Colla var. *Canarias*	0.865 ± 0.03 E
Carambola *Carica pentagona* var. *Averrhoa*	0.781 ± 0.03 E
Kumquats *Fortunella margarita* (Lour.) Swingle var. *Nagami*	0.221 ± 0.01 BC
Lime *Citrus aurantifolia* (Chris) Siwng var. *Persea*	0.056 ± 0.01 A
Mango *Mangifera indica* (L.) var. *Tommy*	0.862 ± 0.04 E
Passion fruit *Passiflora eduliss* (Sims.) var. *Flavicarpa*	0.043 ± 0.01 A
Papaya fruit *Carica papaya* (L.) var. *Pentagona*	0.758 ± 0.03 E
Passifora *Passiflora eduliss* (Sims.) var. *Eduliss*	0.073 ± 0.01 A
Physalis (cape gooseberry) *Physalis peruviana* L. var. *Goldenberry*	0.752 ± 0.03 E
Pineapple *Ananas comosus* (L.) Merrill var. *Cayenne lisa*	0.340 ± 0.02 C
Propyl gallate[b]	0.152 ± 0.02 B
BHA[b]	1.580 ± 0.05 I
BHT[b]	1.335 ± 0.05 H

[a] Statistical differences were analyzed by ANOVA ($P < 0.05$). Values with the same letter are not significantly different ($P > 0.05$).
[b] 100 μg/g concentration. Compounds dissolved in aqueous medium.

pyl gallate, which exhibits a synergistic effect with ascorbate and stimulates deoxyribose degradation, as observed by Aruoma et al. *(4)*.

Table 2 shows that Mediterranean and tropical fruits also exhibited strong antioxidant activity when ascorbate was omitted because they were able to scavenge any $OH^{\cdot}$ generated and protect deoxyribose sugar (although the level of $OH^{\cdot}$ generated was lower). In all samples, it can be observed that the pink chromogen was much lower than that of the control.

Our results are in agreement with the data shown by Chambers et al. *(12)* on hydroxyl scavengers in orange, pineapple, papaya, and citrus fruits. Plumb et al. *(34)*, who established that flavanone (naringin) and cinnamic acid (chlorogenic acid) are responsible for hydroxyl radical scavenging activity, obtained percentages very similar to our results in grapefruit, apple, pear, and peach. Subsequently, in 1997 Miller and Rice-Evans *(30)* identified the flavanones hesperidin and narirutin, which, together with carotenoids and antioxidant vitamins, conferred good antioxidant activity.

Scavenging of hypochlorous acid. Table 3 shows the scavenging of HOCl by Mediterranean and tropical fruits compared with the activity of different compounds frequently used as common 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.

Of the Mediterranean fruits, lemon was the most effective HOCl scavenger, even more than propyl gallate, whereas BHA and BHT were unable to scavenge HOCl in an aqueous medium (Table 3).

Plum, apricot, and white grape exhibited >60% ($P < 0.05$) inhibition, which was lower than propyl gallate at the permitted concentrations. The following group of fruits had effective scavenging capacity, with percentages of inhibition between 35 and 50% ($P < 0.05$). Similar activities (all ≈25%) were shown by the three apple varieties (*Golden, Royal Gala, Starking*; $P < 0.05$) and the two orange varieties (Navelina and Salutiana; $P < 0.05$) studied here. Cherry and strawberry exhibited the lowest HOCl scavenging activity. However, the four varieties of pear were poor scavengers ($P < 0.05$).

Among tropical fruits, passion fruit, lime, and passiflora showed the highest inhibition percentage, which was higher than that shown by propyl gallate. Kumquat, avocado, and pineapple were also very good scavengers of HOCl, with about 70% inhibition ($P < 0.05$). However, the rest of the tropical fruits exhibited low efficiency.

According to Murcia and Martínez-Tomé *(32)*, propyl gallate effectively protects α_1-antiproteinase activity against HOCl. Several phenolic compounds (such as vanillin, ferulic acid, catechins, carnosic acid, carnosol, and propyl gallate) react quickly with HOCl and can protect α_1-antiproteinase and other susceptible targets against damage in vitro. This may have physiological significance, given interest in the use of natural phenolic antioxidants as therapeutic agents *(11)*.

Activated neutrophils contain and secrete the enzyme myeloperoxidase, which uses H_2O_2 to oxidize chloride ions to the powerful oxidant HOCl *(5)*. Vitamin C is a powerful HOCl scavenger and an alternative substrate for myeloperoxidase (slowing HOCl formation). Thus, Mediterranean and tropical fruits with their high vitamin C content are able to scavenge HOCl, as was the case with lemon and lime. According to the USDA handbook *(40)*, lemon, melon, papaya, strawberry, lime, mango, orange, watermelon, grapefruit, pineapple, avocado, apricot, banana, and plum possess high levels of vitamin C. Although strawberry and papaya present good levels of vitamin C, the percentage by which they inhibit HOCl is not very high, which strengthens the hypothesis that total antioxidant activity depends on the constituents of the fruit.

Hydrogen peroxide scavenging. Table 4 shows the effect on hydrogen peroxide by the Mediterranean and tropical fruits compared with the activity of different compounds frequently used as food additives. The scavenging of hydrogen peroxide activity is easily and sensitively measured by using peroxidase-based assay systems and looking for a decrease in the absorption spectrum after the compound is added to peroxidase-H_2O_2 mixtures.

Among Mediterranean fruits, four have very good antioxidant activity: melon *saccharinus*, watermelon, strawberry, and melon *cantalupensis*. A second group showed a percent inhibition of around 50% ($P < 0.05$), and a third group had a moderate inhibitory effect of >25% inhibition ($P < 0.05$). Apricot showed much lower antioxidant activity than the rest of the fruits, except peach, which was the only Mediterranean fruit analyzed that did not show antioxidant activity. All the Mediterranean fruits mentioned above showed better antioxidant activity than propyl gallate, which did not show this capacity when analyzed at the permitted concentration in foods.

All the tropical fruits also performed well on hydrogen peroxide, except avocado, which did not react with H_2O_2 (Table 4). All the other tropical fruits exhibited a higher antioxidant capacity than propyl gallate at the permitted concentration in foods.

Passiflora exhibited the highest H_2O_2 scavenging efficiency with 96% inhibition, followed by papaya fruit. A second group also showed very good antioxidant activity, >75% ($P < 0.05$), whereas the rest of the fruits exhibited percent inhibitions of between 60 and 35%.

BHA and BHT did not react with H_2O_2.

All fruits analyzed showed high levels of phenolic compounds *(21)*, which can react with hydrogen peroxide and act as substrates for peroxidases *(5)*. Different hydrogen peroxide concentrations were evaluated (from 6.72 to 0.84 mM) to detect possible interference of the phenol compounds contained in fruits using *N*-acetyl-L-cysteine as a positive control of hydrogen peroxide scavenging *(3)*. When the hydrogen peroxide concentration was increased, the absorbance values also increased.

Rancimat results. Refined olive oil alone (control) starts the radical chain reactions of the propagation phase of autoxidation after 8.8 h. The time required for the formation of a sufficient concentration of initiating radicals (initiation phase) was slightly greater when the fruits or food additives were added, delaying the time of onset of the propagation phase of the radical chain reaction and showing the protection factor of these products.

Figure 1 shows the protection factor obtained by the Rancimat method for refined olive oil with Mediterranean fruits compared with common food additives. Watermelon conferred a significantly ($P < 0.05$) greater protection than the rest of Mediterranean fruits. However, propyl gallate was the most effective ($P < 0.05$) compound tested, followed by pear *Conferencia* > orange Salutiana ≅ apricot ($P < 0.05$). The rest of the Mediterranean fruits showed lower oxidative stability.

Figure 2 shows the protection factor by the Rancimat method obtained for refined olive oil with tropical fruits compared with common food additives. Physalis had the strongest stabilizing effect except for, once again, propyl gallate which led to a longer induction period. Papaya fruit and carambola ($P < 0.05$) also increased the protection factor of olive oil to a greater extent than the rest of the tropical fruits analyzed.

The results are in agreement with Gordon and Kourimska *(17)*, who used the Rancimat test and observed that BHA and BHT had no antioxidant activity, probably reflecting the volatility of these additives.

Aruoma et al. *(6)*, working with Rancimat, reported the

TABLE 4. *Effect on hydrogen peroxide by Mediterranean and tropical fruits compared with the activity of different compounds quently used as food additives using peroxidase-based assay*[a]

Sample	Absorbance (A_{436}, nm)
Control	0.661
Mediterranean fruits	
Apple *Malus communis* Poir var. *Golden delicious*	0.472 ± 0.03 CD
Apple *Malus communis* Poir var. *Royal Gala*	0.419 ± 0.03 C
Apple *Malus communis* Poir var. *Starking*	0.247 ± 0.01 B
Apricot *Prunus armeniaca* L.P. var. *Gala rojo*	0.613 ± 0.04 D
Cherry *Prunus avium* L. var. *Stark*	0.322 ± 0.02 B
Red grape *Vitis vinifera* L. var. *Napoleon negra*	0.422 ± 0.03 C
White grape *Vitis vinifera* L. var. *Aledo*	0.484 ± 0.03 CD
Lemon *Citrus limon* (L.) Burm. F. var. *Verna*	0.321 ± 0.02 B
Mandarin *Citrus reticulata* Blanco var. *Fortuna*	0.313 ± 0.02 B
Medlar *Eriobotryae japonica* (Thunb.) Lindl. var. *Algar*	0.341 ± 0.03 BC
Melon *Cucumis melo cantalupensis* Naud var. *Aitana*	0.145 ± 0.01 AB
Melon *Cucumis melo saccharinus* Naud var. *Sancho*	0.015 ± 0.01 A
Orange *Citrus sinensis* (L.) Osbeck var. *Navelina*	0.255 ± 0.02 B
Orange *Citrus sinensis* (L.) Osbeck var. *Salutiana*	0.316 ± 0.02 B
Peach *Prunus persica* (L.) Batsch var. *Queen nest*	0.667 ± 0.04 DE
Pear *Pyrus communis* (L.) var. *Blanquilla*	0.409 ± 0.03 C
Pear *Pyrus communis* (L.) var. *Conferencia*	0.207 ± 0.01 B
Pear *Pyrus communis* (L.) var. *Flor de Invierno*	0.311 ± 0.02 B
Pear *Pyrus communis* (L.) var. *Passacrana*	0.375 ± 0.03 BC
Plum *Prunus domestica* (L.) var. *Red-Beauti*	02.52 ± 0.02 B
Strawberry *Fragaria vesca* L. var. *Camarosa*	0.127 ± 0.02 AB
Watermelon *Citrullus vulgaris* Schrad var. *Sugar baby*	0.116 ± 0.01 AB
Tropical fruits	
Avocado *Persea americana* Mill var. *Bacon*	0.809 ± 0.04 F
Banana *Musa acuminata* Colla var. *Canarias*	0.141 ± 0.01 AB
Carambola *Carica pentagona* var. *Averrhoa*	0.140 ± 0.01 AB
Kumquats *Fortunella margarita* (Lour.) Swingle var. *Nagami*	0.345 ± 0.02 BC
Lime *Citrus aurantifolia* (Chris) Siwng var. *Persea*	01.24 ± 0.01 AB
Mango *Mangifera indica* (L.) var. *Tommy*	0.135 ± 0.01 AB
Passion fruit *Passiflora eduliss* (Sims.) var. *Flavicarpa*	0.422 ± 0.04 C
Papaya fruit *Carica papaya* (L.) var. *Pentagona*	0.081 ± 0.01 A
Passifora *Passiflora eduliss* (Sims.) var. *Eduliss*	0.024 ± 0.01 A
Physalis (cape gooseberry) *Physalis peruviana* L. var. *Goldenberry*	0.235 ± 0.03 B
Pineapple *Ananas comosus* (L.) Merrill var. *Cayenne lisa*	0.341 ± 0.03 C
Propyl gallate[b]	0.644 ± 0.05 D
BHA[b]	0.819 ± 0.05 F
BHT[b]	0.74 ± 0.05 E

[a] Statistical differences were analyzed by ANOVA ($P < 0.05$). Values with the same letter are not significantly different ($P > 0.05$).
[b] 100 μg/g concentration. Compounds dissolved in aqueous medium.

good antioxidant activity for oregano and rosemary, which contain high levels of phenolic structures, in different types of fat. These compounds can act as chain-breaking antioxidants and react with peroxyl radicals, introducing a lag period into the peroxidation process that corresponds with the time taken for the antioxidant to be consumed. However, no studies concerning the protection factor of oil used the Rancimat test with fruits. This also could be the mechanism in the case of fruits.

Our results show that fruits may have differing capacities to scavenge different reactive oxygen species, which means that antioxidants must be selected according to the radical to be scavenged or to the compositional structure of the food to be protected. Although there are several assays to evaluate the antioxidant capacity of foods, the results obtained in this paper indicate that Mediterranean and tropical fruits exhibit very good antioxidant activity as scavengers of several reactive oxygen species, which confirms the desirability of including several servings of these products in the daily diet.

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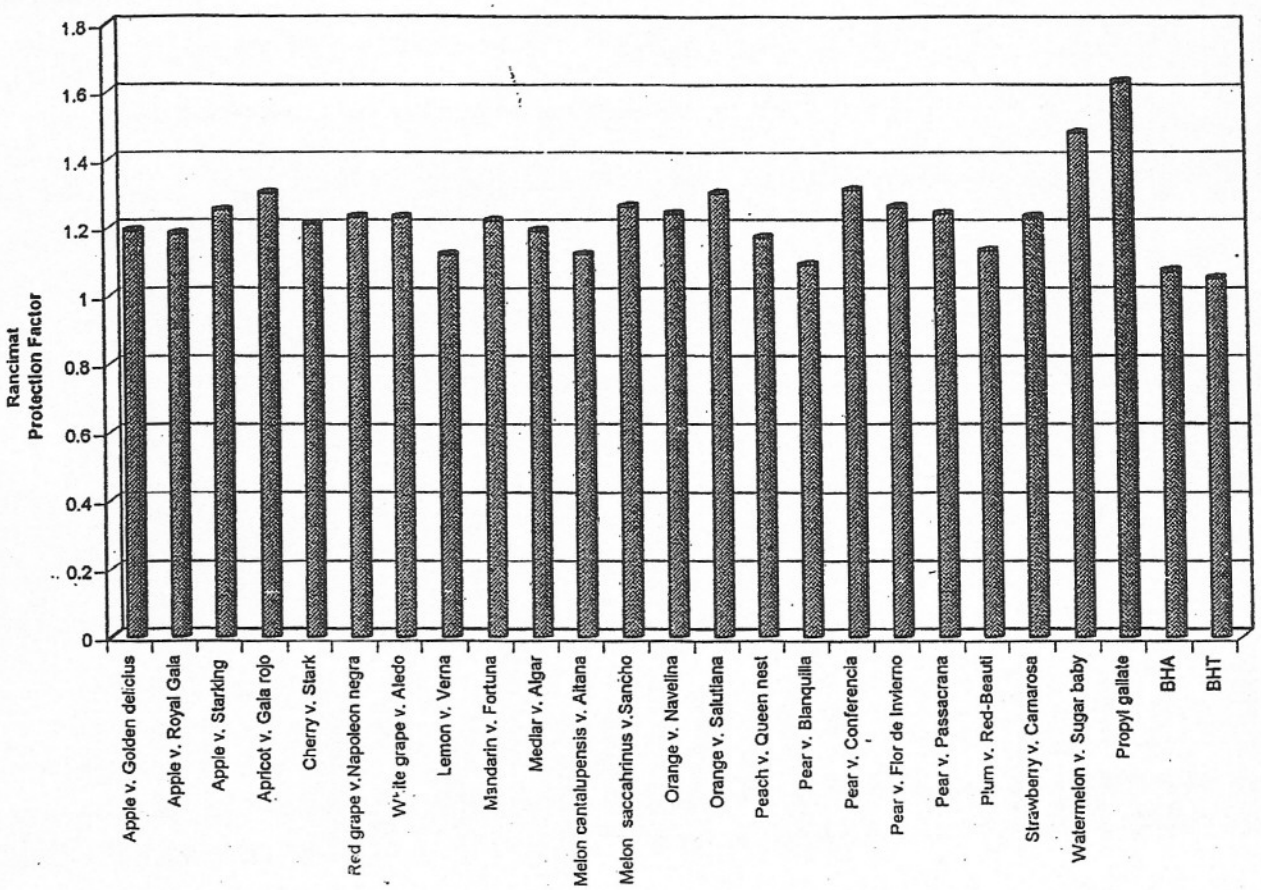

FIGURE 1. *Protection factor obtained for refined olive oil with Mediterranean fruits compared with common food additives by Rancimat test at 110°C.*

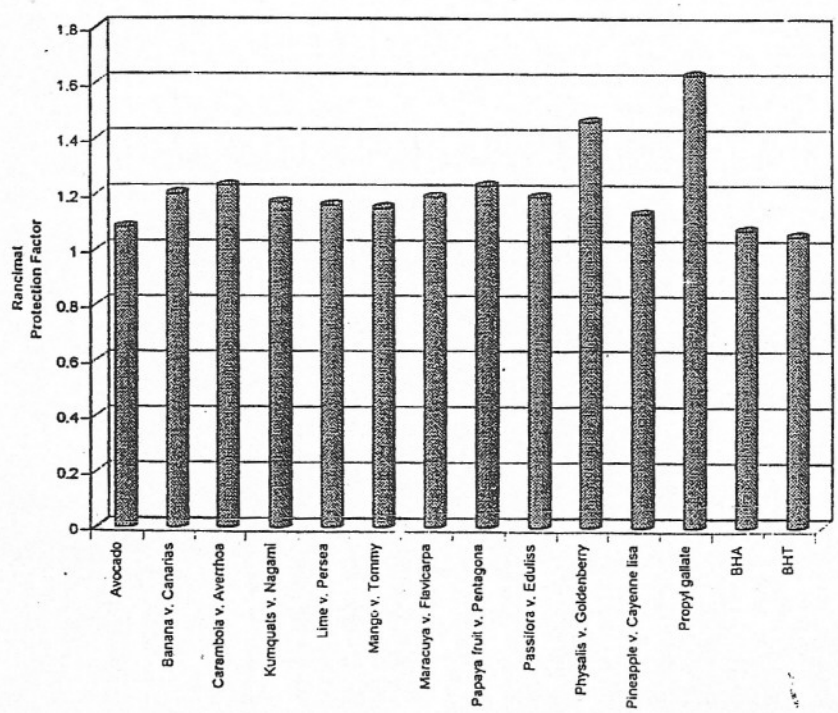

FIGURE 2. *Protection factor obtained for refined olive oil with tropical fruits compared with common food additives by Rancimat test at 110°C.*

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