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Antioxidant Evaluation in Dessert Spices Compared with Common Food Additives. Influence of Irradiation Procedure

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The antioxidant properties of seven dessert spices (anise, cinnamon, ginger, licorice, mint, nutmeg, and vanilla) were compared with those of the common food antioxidants butylated hydroxyanisole (BHA) (E-320), butylated hydroxytoluene (BHT) (E-321), and propyl gallate (E-310). The influence of irradiation process on antioxidant activity was also evaluated. Mint and cinnamon exhibited a higher percentage of inhibition of oxidation than the other spices analyzed and the food antioxidants, as tested by the lipid peroxidation assay ($LOO^{\bullet}$). Nutmeg, anise, and licorice showed the strongest protection in the deoxyribose assay ($OH^{\bullet}$). Vanilla exhibited the highest antioxidant activity in the peroxidase-based assay (H_2O_2). Nutmeg, propyl gallate, ginger, and licorice improved the stability of oils (sunflower, corn, and olive) and fats (butter and margarine) against oxidation (110 °C Rancimat). Cinnamon was a better superoxide radical scavenger than the other analyzed spices and additives. When the Trolox equivalent antioxidant capacity (TEAC) assay was used to provide a ranking order of antioxidant activity, the result in decreasing order of antioxidant capacity was cinnamon ≅ propyl gallate > mint > anise > BHA > licorice ≅ vanilla > ginger > nutmeg > BHT. Irradiated samples did not show significant differences ($p < 0.05$) in the antioxidant activity with respect to the non-irradiated samples (1, 3, 5, and 10 kGy) in the assays used.

KEYWORDS: Anise; cinnamon; ginger; licorice; mint; nutmeg; vanilla; antioxidant; scavenging free radical; BHA; BHT; propyl gallate

INTRODUCTION

Spices and herbs have been used throughout history as natural sources of flavoring and for their preservative properties. The spices possess antioxidant activity because they contain chemicals (including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthlalids) with biological activities that may provide therapeutic effects. However, the characteristic aroma of spices limits their use (*1*).

Anise (*Pimpinella anisum* L.) exhibits antimicrobial, antimutagenic, and antipyretic activities. Furthermore, it has been shown to have anticonvulsant effects and has been used for the treatment of constipation (*2*−*6*).

Due to its delicate flavor, cinnamon (*Cinnamomum zeylanicum* L.) is widely used in foods. It is also suitable for treating the common cold, for improving glucose metabolism in diabetics, as an antimicrobial and fungitoxic agent, and for inhibiting various cancer cell lines (*7*−*10*).

Ginger (*Zingiber officinale* L.), with its pleasant aroma and pungency, is used for cooking. Its effect on the pancreatic digestive enzymes is a factor that contributes to its well-recognized digestive stimulant action. It also possesses antimicrobial, antitumoral, and antiplatelet aggregation activities and is an antioxidant (*11*).

Licorice (*Glycirrhiza glabra* L.) is widely used in the food industry as a sweetening agent and as a foaming agent in alcoholic and nonalcoholic beverages. It also has antibacterial, antiviral, antiinflammatory, antihepatotoxic, and antimutagenic effects (*12*−*17*).

Mint (*Mentha piperita* L.) is widely used as a condiment in ice creams, candies, chewing gums, cakes, and meats, although it also has been used as a spasmolytic and antibacterial agent and promoter of gastric secretion. It has been seen to possess antimutagenic properties (*18*, *19*).

Nutmeg (*Myristicia fragans* L.), too, is commonly used as a spice and flavoring agent in foods. It can also be used against diarrhea, mouth sores, and insomnia; furthermore, it has antiplatelet and antibacterial effects and is associated with the activation and detoxification of xenobiotic compounds, including chemical carcinogens and mutagens. It improves glucose and insulin metabolism (*10*, *20*−*23*).

Vanilla (*Vanilla planifolia* L.) is most valued for its flavor and imparts a delicate, rich, and mellow aroma with sweet spicy, woody, and balsamic notes. Its extracts are widely used for ice

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cream, chocolate, cakes, and cookies, but it also acts as an antimicrobial compound and has antimutagenic effects in bacteria (*24*, *25*).

However, one of the problems associated with the use of spices and herbs is their possible contamination by harmful bacterial, particularly spore-forming bacteria. Although spices and herbs are microbiologically stable products owing to their low moisture content, once they come into contact with water-rich food products such as meat or soup, microorganisms quickly develop (*26*).

Several decontamination methods exist, but the most versatile treatment among them is processing with ionizing radiation. In addition to strict hygiene in preparation, an irradiation dose of 8−10 kGy is particularly valuable as an endproduct decontamination procedure not affecting sensory, nutritional, and technical qualities (*27*).

The U.S. Code of Federal Regulations limits dry spice irradiation to 30 kGy (*28*). Recently, two new European Directives, "the framework Directive" 1999/2/EC and the "implementing Directive" 1999/3/EC, were adopted to establish a list of foodstuffs that may be treated with ionizing radiation. Included in this list are dried aromatic herbs and spices, which may be irradiated with a maximum overall average absorbed radiation dose of 10 kGy (*29*).

The aim of this paper was to characterize the antioxidant properties of dessert spices (anise, cinnamon, ginger, licorice, mint, nutmeg, and vanilla) and to compare them with the common food additives butylated hydroxyanisole (BHA, E-320), butylated hydroxytoluene (BHT, E-321), and propyl gallate (PG, E-310) for possible use as natural antioxidants. Furthermore, there is little information on the effect of irradiation on the free radical scavenging capacity of spices, and so it was considered to be important to ascertain whether the irradiation procedures for decreasing the microbiological counts affect their antioxidant activity and ability to scavenge different free radicals.

The effect of this processing technique on food antioxidant properties has not been described extensively and is a matter of controversy because of the presence of chemicals with prooxidant or antioxidant activity that may modify the food antioxidant capacity or affect physiological metabolism.

In this paper antioxidant activity is determined by six methods: (i) The lyposome system provides data concerning peroxyl radical scavenging by the samples. This radical can be generated in foods and in the human body, and its formation is the major step in lipid peroxidation, although it can also be formed in non-lipid systems. (ii) The hydroxyl radical system evaluates the food's capacity to neutralize this reactive radical generated in the human body in physiological conditions, although hydroxyl radicals can also be generated from peroxyl radicals. In this system, any hydroxyl radicals formed immediately attack the sugar. (iii) H_2O_2 is generated in vivo by activated phagocytes and by several oxidase enzymes, and it is known to play an important role in the killing of several bacterial and fungal strains. H_2O_2 is reduced either directly or indirectly to produce $OH^{\bullet}$. (iv) The Rancimat test is used to obtain information on whether the antioxidant activity resists heating. (v) The Trolox equivalent antioxidant capacity (TEAC) assay is usually accepted by the scientific community as an antioxidant indicator. (vi) The in vivo production of superoxide may be functional (by the endothelial cell for the regulation of vascular tone) or accidental (by most types of macrophages).

MATERIALS AND METHODS

Antioxidant additives (BHA, BHT, and propyl gallate) and the chemicals used were of the highest quality available and were purchased from Sigma Chemical Co. (Poole, Dorset, U.K.). The dessert spices, anise (*P. anisum* L.), cinnamon (*C. zeylanicum* L.), ginger (*Z. officinale* L.), licorice (*G. glabra* L.), mint (*M. spicata* L.), nutmeg (*M. fragans* L.), and vanilla (*V. planifolia* L.), were purchased from a supermarket.

Irradiation of the Samples. Glass tubes (150 × 15 mm) were filled with the spice samples and common food additives and irradiated by a Rhodotron (IBA, Belgium) circular electron accelerator (Ionmed, Tarancón, Spain) with an energy level of 10 MeV. The treatment was performed in a single step, taking into consideration the volumetric density of the product and characteristics of the installation. The programmed irradiation doses were 1.0, 3.0, 5.0, and 10.0 kGy. The variability of the real dose of irradiation absorbed by the samples was <1% of the programmed dose applied. The celullose acetate dosimeter (FWT 60.00) also verified the homogeneity of the dose and validated the irradiation process at 600 nm. After irradiation, the samples were immediately stored at ambient temperature in the dark.

Sample Preparation. The irradiated and control (non-irradiated) spices were powdered using a mortar and pestle. Each powdered spice (5 g) was extracted for 3 h by stirring at room temperature with 100 mL of water and centrifuged at 3000 rpm for 10 min. The spice extracts (5%) were used in the different assays. BHA, BHT, and propyl gallate were used at the permitted commercial concentration of 100 μg/g (*30*).

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. (*31*). The experiments were conducted in a physiological saline buffer (PBS) (3.4 mM $Na_2HPO_4-NaH_2PO_4$, 0.15 M NaCl), pH 7.4. The assay mixtures, in a final volume of 1 mL, were made up with PBS, 500 μL of 0.5 mg/mL phospholipid liposomes, 100 μL of 100 μM $FeCl_3$, 100 μL of the tested samples, and 100 μL of 100 μM ascorbate (added last to start the reaction). 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 to dissolve it. Incubation was carried out at 37 °C for 60 min, at the end of which 1 mL each of 1% (w/v) thiobarbituric acid (TBA) and 2.8% (w/v) trichloroacetic acid were added. 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. The percentage of peroxidation inhibition was expressed as the decrease in peroxidation obtained by adding the tested compounds (100% oxidation referred to an assay containing no added compound).

Hydroxyl Radical ($OH^{\bullet}$) Scavenging. In a final volume of 1.2 mL, the reaction mixtures contained the following reagents: 100 μL of 10 mM KH_2PO_4−KOH buffer, pH 7.4, 100 μL of 2.8 mM H_2O_2, 100 μL of 2.8 mM deoxyribose (when used), 100 μL of 50 μM $FeCl_3$ premixed with 100 μL of 100 μM ethylenediaminetetraacetic acid (EDTA) before addition to the reaction mixture, and 100 μL of the tested spices (or 100 μL of common food additives dissolved in water). One hundred microliters of ascorbate (100 μM), when used, was added to start the reaction. The tubes were incubated at 37 °C for 1 h. The products of the hydroxyl radical ($OH^{\bullet}$) attack on deoxyribose were measured as described in Aruoma et al. (*32*) at 532 nm. The results are expressed as a percentage inhibition of the deoxyribose attack, where 100% attack is defined as the absorbance levels recorded for deoxyribose without the addition of the tested compounds. A parallel assay was also made omitting ascorbate. In this form, false scavenger activity results are eliminated.

Hydrogen Peroxide (H_2O_2) Scavenging. The tested spices (100 μL) or common food additives dissolved in water (100 μL) were incubated with 200 μL of 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. The remaining H_2O_2 was measured as the formation of a chromophore recorded at 436 nm in reaction mixtures containing, in a final volume of 1 mL, 690 μL of 0.15 M KH_2PO_4−KOH buffer, pH 7.4, 50 μL of guaiacol solution (made by adding 100 μL of pure guaiacol liquid to 100 mL of water), and 10 μL of Sigma type IV horseradish peroxidase (5 mg/mL in the same phosphate buffer) (*33*).

Rancimat Test. The different spices or common food additives were macerated with 100 g of refined oils (sunflower, corn, and olive) or fats (butter or margarine) for 3 h. The composition of the margarine was vegetable oils and hydrogenated fats (61%), water, skimmed milk (11%), emulsifier (lecithin or monoglyceride fatty acids), calcium salts, salt (0.3%), acidulate (lactic acid), preservative (potassium sorbate), aroma, vitamin E, coloring (β-carotene), and vitamin D.

The Rancimat method (Metrohm model 743, Herisan, Switzerland) determines the induction period by measuring the increase in the volatile acidic byproducts released from the oxidizing oil or fat at 110 °C. The concentration of the degradation products, which are transferred into distilled water, is assessed by measuring the conductivity. Longer induction periods suggest stronger activity (*34*).

Iodine Value. Iodine values were measured according to the method described by Hanus 920.158 AOAC (*35*).

Reaction with Superoxide Radical ($O_2^{\bullet -}$). The generation of $O_2^{\bullet -}$ by hypoxanthine−xanthine oxidase was carried out essentially as described in Aruoma et al. (*32*). Reaction mixtures contained, in a final volume of 3 mL, 0.1 mL of 30 mM hypoxanthine (dissolved in minimum potassium hydroxide solution), 0.1 mL of 0.3 mM EDTA, 0.1 mL of 3 mM cytochrome *c*, and 88 mM (final concentration) KH_2PO_4−KOH buffer, pH 7.4. In each case, the reaction was started by adding 0.3 mL of xanthine oxidase (Sigma X1875), freshly diluted in the above phosphate buffer to give 1 unit of enzyme activity per 800 μL), and the rate of cytochrome *c* reduction was measured at 550 nm, at 25 °C. The effects of 5% spice extracts in aqueous medium or 100 μg/g of common food additives were tested in the system containing cytrochrome *c*.

Measurement of Total Antioxidative Activity by the TEAC Assay. The 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate) ($ABTS^{\bullet -}$) radical anion solution was generated from 2.5 mM 2,2′-azobis(2-amidinopropane) hydrochloride (ABAP) and 20 mM $ABTS^{2-}$ stock solution in 100 mL of PBS (containing 100 mM phosphate and 150 mM NaCl, pH 7.4). The radical solution was protected from light during incubation at 60 °C for 12 min and later stored at room temperature. Absorbance at 734 nm was measured to check $ABTS^{\bullet -}$ formation (the absorbance values must be between 0.35 and 0.45 absorbance unit) (*36*). The 5% spice extracts (or 100 μg/g of common food additives) were dissolved in 1 mL of deionized water. The antioxidant activity of the samples analyzed (40 μL mixed with 1960 μL of the radical solution) was measured at 734 nm for a period of 6 min. The decrease in absorption at 734 nm observed at 6 min after the addition of each compound was used to calculate the TEAC. A calibration curve was prepared with different concentrations of Trolox (a standard solution used to evaluate equivalent antioxidant capacity). By measuring the increase in absorption (ΔAbs) over 6 min (with a standard range of 0−10 μM), absorbance values could be corrected for the solvent.

$$\Delta Abs_{Trolox} = Abs_{t=6min,Trolox} - Abs_{t=6min,solvent}$$

The regression coefficient (rc) is calculated from the calibration curve.

$$\Delta Abs_{Trolox} = rc \times [Trolox]$$

To establish the TEAC values of commercial antioxidants or analyzed samples and its standard deviations, the increase in absorption was measured in the same way. The TEAC was calculated as follows:

$$TEAC_{sample} = \Delta Abs_{sample}/rc$$

The TEAC represents the concentration of a Trolox solution that has the same antioxidant capacity as the analyzed sample.

Data Analysis. Every analysis was carried out in triplicate. The results and their standard deviations were analyzed with the Statistical Package for Social Sciences Windows 9.0 and the analysis of variance (ANOVA) procedure. Fisher's least significant difference multiple-range test was used to discriminate between means.

RESULTS AND DISCUSSION

Inhibition of Phospholipid Peroxidation. The effects of dessert spices and common food additives, without or with the application of different irradiation doses, on liposomal peroxidation are shown in **Table 1**.

Table 1. Inhibition of Peroxidation in the Lipid System Using Ox Brain Phospholipids by Dessert Spices and Common Food Additives at Different Irradiation Doses[a]

added to reaction mixture[b]	irradiation (kGy)	% inhibition	added to reaction mixture[c]	irradiation (kGy)	% inhibition
anise	0	78.62 ± 1	BHA	0	64.80 ± 5
	1	75.17 ± 2		1	69.46 ± 7
	3	77.93 ± 2		3	67.89 ± 3
	5	77.24 ± 1		5	69.53 ± 6
	10	76.55 ± 2		10	68.34 ± 3
cinnamon	0	82.07 ± 1	BHT	0	13.82 ± 2
	1	79.73 ± 4		1	14.13 ± 5
	3	82.43 ± 2		3	13.87 ± 4
	5	82.43 ± 5		5	13.94 ± 3
	10	83.10 ± 0		10	13.84 ± 1
ginger	0	74.32 ± 4	propyl gallate	0	59.43 ± 1
	1	75.00 ± 6		1	60.82 ± 5
	3	74.32 ± 1		3	61.01 ± 1
	5	74.32 ± 5		5	58.80 ± 5
	10	74.32 ± 6		10	59.10 ± 2
mint	0	82.76 ± 2			
	1	84.14 ± 2			
	3	84.59 ± 2			
	5	84.23 ± 1			
	10	82.76 ± 1			
nutmeg	0	73.79 ± 3			
	1	73.79 ± 3			
	3	67.59 ± 1			
	5	73.10 ± 3			
	10	73.10 ± 6			
licorice	0	68.28 ± 0			
	1	71.04 ± 3			
	3	66.21 ± 2			
	5	69.66 ± 1			
	10	69.66 ± 5			
vanilla	0	71.72 ± 1			
	1	71.03 ± 1			
	3	69.00 ± 1			
	5	70.35 ± 4			
	10	69.00 ± 4			

[a] Statistical differences were analyzed by ANOVA ($p < 0.05$). [b] Compounds in aqueous medium at 5% concentration. [c] Compounds in aqueous medium at concentration = 100 μg/g.

Mint and cinnamon were the best antioxidants ($p < 0.05$), scavenging peroxyl radicals ($LOO^{\bullet}$), at 82.76 and 82.07%, respectively, higher than the additives PG, BHA, and BHT at the permitted concentration (100 μg/g), which showed 59.43, 13.82, and 64.80%, respectively.

In decreasing order of inhibition, the rest of the analyzed spices were anise, ginger, nutmeg, vanilla, and licorice, all of which showed better antioxidant activity than BHA, propyl gallate, and BHT. The only spice that did not show a statistically significant difference ($p < 0.05$) with the additives was licorice with respect to BHA.

Our results agree with those obtained by several authors who evaluated the antioxidant activity of different spices, such as cinnamon (*37*), licorice (*38*), vanillin (*39*), and ginger (*40*, *41*) in lipidic systems.

The antioxidant activity of ginger extract was retained even after 30 min of boiling at 100 °C, indicating that the active constituents of this spice were resistant to thermal denaturation (*42*) and suggesting that it could be used as an additive in food processes involving heating. Indeed, this is why several studies

Table 2. Deoxyribose Damage Caused by the OH• Radical in the Presence of Dessert Spices, at Different Irradiation Doses, Compared with the Activity of Common Food Additives[a]

added to reaction mixture[b]	irradiation (kGy)	RM + DR[c]	% inhibition	omit ASC[d]	added to reaction mixture[e]	irradiation (kGy)	RM + DR	% inhibition	omit ASC
none		1.33 ± 0,05		0.44					
anise	0	0.50 ± 0.01	62.41	0.22	BHA	0	1.10 ± 0.06	17.29	0.11
	1	0.47 ± 0.01	64.66	0.24		1	1.16 ± 0.02	12.72	0.11
	3	0.48 ± 0.03	63.91	0.24		3	1.15 ± 0.05	13.53	0.11
	5	0.52 ± 0.01	60.90	0.22		5	1.09 ± 0.01	18.05	0.08
	10	0.52 ± 0.05	60.90	0.24		10	1.06 ± 0.02	20.30	0.09
cinnamon	0	0.84 ± 0.03	36.84	0.69	BHT	0	1.22 ± 0.04	8.16	0.25
	1	0.83 ± 0.06	37.59	0.52		1	1.23 ± 0.05	7.51	0.27
	3	0.86 ± 0.03	35.34	0.64		3	1.21 ± 0.06	9.02	0.28
	5	0.80 ± 0.04	39.85	0.58		5	1.24 ± 0.03	6.76	0.21
	10	0.80 ± 0.05	39.85	0.68		10	1.23 ± 0.01	7.51	0.23
ginger	0	0.68 ± 0.05	48.87	0.45	propyl	0	2.11 ± 0.02		1.03
	1	0.67 ± 0.03	49.62	0.45	gallate	1	2.00 ± 0.06		1.12
	3	0.70 ± 0.05	47.37	0.47		3	2.09 ± 0.02		0.96
	5	0.70 ± 0.05	47.37	0.47		5	2.01 ± 0.05		1.14
	10	0.66 ± 0.02	50.38	0.43		10	2.05 ± 0.03		1.15
mint	0	0.61 ± 0.01	54.13	0.45					
	1	0.57 ± 0.04	57.14	0.42					
	3	0.62 ± 0.04	53.38	0.45					
	5	0.60 ± 0.06	54.89	0.46					
	10	0.66 ± 0.01	50.38	0.50					
nutmeg	0	0.45 ± 0.01	66.17	0.16					
	1	0.45 ± 0.04	66.17	0.15					
	3	0.49 ± 0.01	63.17	0.16					
	5	0.50 ± 0.01	62.41	0.16					
	10	0.48 ± 0.05	63.91	0.15					
licorice	0	0.56 ± 0.07	57.90	0.37					
	1	0.56 ± 0.01	57.90	0.36					
	3	0.58 ± 0.02	56.39	0.36					
	5	0.57 ± 0.03	57.14	0.37					
	10	0.56 ± 0.03	57.90	0.37					
vanilla	0	1.02 ± 0.05	23.30	0.70					
	1	1.05 ± 0.03	21.05	0.60					
	3	1.03 ± 0.02	22.56	0.64					
	5	1.01 ± 0.04	24.06	0.62					
	10	1.04 ± 0.05	21.81	0.60					

[a] Statistical differences were analyzed by ANOVA ($p < 0.05$). [b] Compounds in aqueous medium at 5% concentration. [c] RM, reaction mixture; DR, deoxyribose. [d] When ascorbate is omitted from the reaction mixture. [e] Compounds in aqueous medium at concentration = 100 μg/g.

have proposed adding this spice to different types of foods such as meat products (*43*), fish products (*44*), and oils (*45*) to improve conservation.

Reactions of Dessert Spices with Hydroxyl Radicals in the Deoxyribose Assay. The effects of dessert spices on deoxyribose attack by OH• radical compared with the activity of different compounds frequently used as food additives are shown in **Table 2**.

The results can be divided into several groups. The first comprises nutmeg, anise, and licorice, which show the strongest protective actions (66.17, 62.41, and 57.90% inhibition, respectively). When ascorbate was omitted from the reaction, nutmeg and anise still had very good antioxidant effect ($p < 0.05$), pink chromogen decreasing with respect to the control. Licorice had a slightly lower antioxidant effect than the other two spices. These findings confirm that the ability of the spices to protect deoxyribose is due to their direct capacity to scavenge hydroxyl radicals.

The second group comprises mint and ginger, both of which show moderate antioxidant activity. Mint does not show statistically significant differences ($p < 0.05$) from licorice. However, mint and ginger do not scavenge hydroxyl radicals, because when ascorbate was omitted, their pink chromogens were similar to those of the control.

The third group includes cinnamon and vanilla, with medium percentages of inhibition ($p < 0.05$). When ascorbate was added to the reaction, they protected deoxyribose. However, when ascorbate was omitted, the level of the pink chromogen exceeded that of the control (**Table 2**), so that they acted as prooxidants. The addition of ascorbic acid greatly increases the rate of OH• generation by reducing iron and maintaining the supply of Fe^{2+}. According to Martínez-Tomé et al. (*34*), these spices probably react with ascorbate, decreasing the amount of OH• generated, but they do not act as hydroxyl scavengers.

BHA and BHT scavenge much lower levels of OH• radicals than anise, nutmeg, and licorice. Martínez-Tomé et al. (*34*) also observed the prooxidant activity of propyl gallate, which probably has a synergistic effect with ascorbate and stimulates deoxyribose degradation.

The results obtained with ginger were in accordance with those of Aruoma et al. (*31*), although some authors (*46*) have obtained contradictory data for ginger in the presence of hydroxyl radicals.

Hydrogen Peroxide Scavenging. When the samples scavenge the hydrogen peroxide, there is a decrease in the absorption spectrum after the peroxidase test (*33*).

In our study, **Table 3** shows the hydrogen peroxide scavenging capacity of different dessert spices compared with the

Table 3. Scavenging of Hydrogen Peroxide by Different Desserts Spices Compared with the Activity of Common Food Additives in the Peroxidase-Based Assay at Different Irradiation Doses[a]

added to reaction mixture[b]	irradiation (kGy)	absorbance (A_{436nm})	added to reaction mixture[c]	irradiation (kGy)	absorbance (A_{436nm})
none		0.84 ± 0.02			
anise	0	0.58 ± 0.02	BHA	0	0.68 ± 0.01
	1	0.62 ± 0.04		1	0.69 ± 0.02
	3	0.61 ± 0.03		3	0.63 ± 0.01
	5	0.61 ± 0.01		5	0.64 ± 0.05
	10	0.61 ± 0.01		10	0.65 ± 0.03
cinnamon	0	0.39 ± 0.01	BHT	0	0.70 ± 0.02
	1	0.39 ± 0.02		1	0.72 ± 0.01
	3	0.38 ± 0.02		3	0.71 ± 0.04
	5	0.37 ± 0.00		5	0.73 ± 0.02
	10	0.38 ± 0.01		10	0.74 ± 0.01
ginger	0	0.47 ± 0.00	propyl	0	0.73 ± 0.01
	1	0.45 ± 0.01	gallate	1	0.70 ± 0.02
	3	0.48 ± 0.01		3	0.69 ± 0.05
	5	0.49± 0.04		5	0.66 ± 0.05
	10	0.47 ± 0.02		10	0.68 ± 0.03
mint	0	0.42 ± 0.02	*N*-acetyl-cysteine	0	0.07 ± 0.05
	1	0.41 ± 0.02			
	3	0.43 ± 0.02			
	5	0.42 ± 0.02			
	10	0.41 ± 0.03			
nutmeg	0	0.89 ± 0.05			
	1	0.87 ± 0.06			
	3	0.95 ± 0.07			
	5	0.89 ± 0.06			
	10	0.87 ± 0.05			
licorice	0	0.37 ± 0.07			
	1	0.41 ± 0.08			
	3	0.39 ± 0.05			
	5	0.42 ± 0.06			
	10	0.41 ± 0.05			
vanilla	0	0.27 ± 0.04			
	1	0.21 ± 0.02			
	3	0.27 ± 0.06			
	5	0.25 ± 0.01			
	10	0.23 ± 0.02			

[a] Statistical differences were analyzed by ANOVA ($p < 0.05$). [b] Compounds in aqueous medium at 5% concentration. [c] Compounds in aqueous medium at concentration = 100 μg/g.

activity of common food additives submitted to different irradiation doses. Vanilla exhibited the highest activity ($p < 0.05$) of all the spices analyzed, showing an inhibition percentage of 67.5%.

Licorice, cinnamon, mint, and ginger also showed good inhibitory capacities of about 56.4, 53.9, 50.3, and 44.3% inhibition, respectively. Anise, which exhibited 30.9% inhibition, differed significantly ($p < 0.05$) from the other spices mentioned.

However, nutmeg, BHA, BHT, and propyl gallate did not react with hydrogen peroxide at all and must be considered inefficient in this respect. In this assay, *N*-acetyl-L-cysteine, with 90.46% hydrogen peroxide scavenging ($p < 0.05$), was used as positive hydrogen peroxide scavenger control (*33*). The results agree with those obtained by Martínez-Tomé et al. (*34*) for other Mediterranean spices.

Rancimat Results and Iodine Values. Vegetable oils and lards, when heated or exposed to light in the presence of oxygen, may undergo partial autoxidation, forming hydroperoxides. Different kinds of substances may accelerate and/or inhibit the formation of these hydroperoxides (*47*), and it is possible to evaluate the protection provided by a given ingredient to a food (rich in oils or fats) that is prepared in given conditions of heat.

Table 4. Effect of Irradiated and Non-irradiated Spices and Common Food Antioxidants on the Oxidative Stability of Oils or Fats, Expressed as Induction Period (IP) Determined by Rancimat Method

added to oil or fat	irradiation (kGy)	sunflower oil IP[a] (h)	corn oil IP[a] (h)	olive oil IP[a] (h)	margarine IP[a] (h)	butter IP[a] (h)
none (control)		3.81	7.57	19.39	7.17	9.28
anise	0	4.11	6.88	19.20	6.38	10.39
	1	3.73	7.57	20.75	6.81	10.30
	3	3.96	7.79	21.91	6.81	11.14
	5	3.73	7.48	23.66	7.46	10.77
	10	3.85	7.18	19.97	6.17	10.21
cinnamon	0	4.15	7.26	19.97	7.10	10.95
	1	4.23	7.64	22.88	7.67	11.69
	3	4.23	7.26	23.46	7.67	11.14
	5	4.00	8.01	24.63	7.46	12.06
	10	4.11	8.01	21.52	7.46	12.34
ginger	0	4.46	8.69	27.34	8.46	50.20
	1	4.34	8.17	30.83	8.68	45.94
	3	4.42	8.85	26.95	8.89	47.70
	5	4.46	8.39	29.10	8.82	42.22
	10	4.34	8.54	30.44	8.96	44.73
mint	0	3.58	7.94	21.52	7.67	9.74
	1	3.77	7.41	22.11	7.67	10.12
	3	3.77	7.79	22.69	7.67	9.84
	5	3.77	7.64	21.72	7.67	10.02
	10	3.77	7.64	21.52	7.96	9.37
nutmeg	0	4.76	8.85	35.29	8.46	58.18
	1	4.53	8.69	31.99	9.03	56.79
	3	4.53	8.92	32.38	8.32	58.93
	5	4.65	9.31	37.03	8.96	59.76
	10	4.61	9.15	33.93	8.46	61.34
licorice	0	4.31	8.92	25.60	8.53	57.44
	1	4.15	8.32	22.88	8.32	56.89
	3	4.11	8.32	24.24	8.96	57.26
	5	4.11	8.69	23.46	8.25	57.63
	10	4.19	8.92	26.18	8.39	57.07
vanilla	0	4.11	8.32	24.24	8.53	30.72
	1	4.19	8.17	23.46	8.46	24.87
	3	4.19	7.71	23.28	8.39	28.77
	5	4.08	8.17	23.46	8.60	28.78
	10	4.08	7.86	23.46	8.25	30.53
BHA[b]	0	4.19	7.72	20.36	7.53	22.27
	1	4.27	7.72	19.97	7.89	24.59
	3	4.19	7.80	20.17	7.74	20.69
	5	4.19	8.02	20.55	7.67	21.34
	10	4.27	7.95	20.36	7.82	20.42
BHT[b]	0	4.27	8.25	20.94	7.31	12.99
	1	4.23	8.33	20.55	7.53	13.27
	3	4.34	8.18	20.94	7.60	13.36
	5	4.31	8.25	21.33	7.67	13.47
	10	4.27	8.33	21.52	7.53	13.55
propyl gallate[b]	0	7.10	11.81	38.97	8.60	60.13
	1	6.90	11.66	42.46	9.32	59.86
	3	6.90	12.11	43.43	8.82	60.78
	5	6.97	11.96	85.70	8.89	61.53
	10	7.01	12.04	43.63	8.82	61.43

[a] Rancimat tested at 110 °C. [b] Concentration = 100 μg/g.

When the induction periods of the different control oils and fats analyzed were compared, olive oil (19.39 h) showed the best oxidative stability followed ($p < 0.05$) in decreasing order by butter (10.65 h), corn oil (7.59 h), margarine (7.18 h), and sunflower oil (3.81 h) (**Table 4**).

Olive oil has a higher tocopherol content than the other studied samples. A protective effect and a synergistic effect with phenols and flavonoids have also been found to contribute

Table 5. Reaction of Different Dessert Spices and Common Food Additives with the Superoxide Radical and Variations with the Application of Different Irradiation Doses[a]

systems and compounds tested[b]	irradiation (kGy)	cytochrome *c* system abs (min^{-1}) ΔA (min^{-1})	% inhibition	systems and compounds tested[c]	irradiation (kGy)	cytochrome *c* system abs (min^{-1}) ΔA (min^{-1})	% inhibition
HX, XO[d]		0.67 ± 0.01					
+ SOD[d]		0.08 ± 0.01	88.1				
+ anise	0	0.32 ± 0.01	52.2	BHA	0	0.38 ± 0.01	43.3
	1	0.40 ± 0.05	40.3		1	0.44 ± 0.02	34.3
	3	0.36 ± 0.06	46.3		3	0.39 ± 0.04	41.8
	5	0.42 ± 0.01	37.3		5	0.43 ± 0.05	35.8
	10	0.39 ± 0.03	41.8		10	0.41 ± 0.05	38.8
+ cinnamon	0	0.19 ± 0.02	71.6	BHT	0	0.61 ± 0.02	9.0
	1	0.11 ± 0.01	83.6		1	0.63 ± 0.04	6.0
	3	0.12 ± 0.05	82.1		3	0.66 ± 0.01	1.5
	5	0.18 ± 0.05	73.1		5	0.62 ± 0.04	7.5
	10	0.21 ± 0.01	68.7		10	0.61 ± 0.03	9.0
+ ginger	0	0.29 ± 0.01	56.7	propyl gallate	0	0.50 ± 0.01	25.4
	1	0.35 ± 0.01	47.8		1	0.55 ± 0.04	17.9
	3	0.32 ± 0.04	52.2		3	0.56 ± 0.01	16.4
	5	0.32 ± 0.03	52.2		5	0.54 ± 0.05	19.4
	10	0.33 ± 0.04	50.7		10	0.52 ± 0.04	22.4
+ mint	0	0.37 ± 0.01	44.8				
	1	0.35 ± 0.04	47.8				
	3	0.33 ± 0.02	50.7				
	5	0.32 ± 0.01	52.2				
	10	0.34 ± 0.04	49.3				
+ nutmeg	0	0.51 ± 0.02	23.9				
	1	0.57 ± 0.05	14.9				
	3	0.58 ± 0.01	13.4				
	5	0.52 ± 0.04	22.4				
	10	0.52 ± 0.05	22.4				
+ licorice	0	0.37 ± 0.01	44.8				
	1	0.37 ± 0.02	44.8				
	3	0.38 ± 0.01	42.3				
	5	0.36 ± 0.05	46.3				
	10	0.32 ± 0.01	52.2				
+ vanilla	0	0.60 ± 0.01	10.5				
	1	0.66 ± 0.02	1.5				
	3	0.61 ± 0.05	9.0				
	5	0.66 ± 0.01	1.5				
	10	0.60 ± 0.02	10.5				

[a] Statistical differences were analyzed by ANOVA ($p < 0.05$). [b] Compounds in aqueous medium at 5% concentration. [c] Compounds in aqueous medium at concentration = 100 μg/g. [d] HX, hypoxanthine; XO, xanthine oxidase; SOD, superoxide dismutase.

significantly to the retardation of oxidation process (*48*). The polyunsaturated fatty acid content of oils is an important factor that affects their oxidative stability (*49*). The order of positive influence of fatty acids on oxidative stability is palmitic acid followed by stearic acid and oleic acid. Of the unsaturated fatty acids, linolenic acid had the most negative influence on oxidative stability, followed by linoleic acid and oleic acid (*50*).

The time required for the formation of a sufficient concentration of initiating radicals (initiation phase) in the oils or fats was slightly greater when the dessert spices or common food additives were added to oil or fat, delaying the onset time of the propagation phase of the radical chain reaction. The fact that the addition of spices to oils and fats improves their stability is probably due to the scavenging activity of free radicals that results from the antioxidant capacity of the spices (**Table 4**).

Among the analyzed spices, nutmeg presented the longest induction time with the oils and fats used ($p < 0.05$). The effect of nutmeg on corn oil does not differ significantly from the effects of ginger and licorice. In margarine, ginger exhibited a protection factor similar to that of nutmeg, whereas in butter licorice showed no significant difference from nutmeg. Among the common food additives assayed, propyl gallate showed higher protection factors in oils and fats than the other additives and spices analyzed ($p < 0.05$).

Our results agree with the data found in the bibliography for the extracts of mint (*51*) and anise (*52*) with sunflower oil, which had a better stabilizing effect than BHT.

Also, nutmeg extracts have been described to have an antioxidant effect on soybean oil and lard, because the spice is rich in eugenol, isoeugenol, and other phenolic compounds. The strong remarkable antioxidant effect of these compounds is due to the presence of a phenolic OH group, which inhibits the chain reaction by acting as hydrogen donor or free radical acceptor. The radical intermediates of the phenolic antioxidants are relatively stable due to resonance delocalization and the lack of positions suitable for attack by molecular oxygen (*53*).

In ref *54*, Gordon and An confirmed the good antioxidant activity of licorice extracts when they were assessed in lard during oxidative processes using the Rancimat method. A more recent study identified the constituents of licorice as flavonoids, isoflavonoids, and phenolic compounds that, by oxidizing into stable radicals in the reaction medium, were responsible for the antioxidant activity (*55*). The synergistic effects of flavonoid

mixtures may be responsible for the high antioxidant activity observed in mixtures (*56*).

He et al. (*57*) found that ginger had a better antioxidant effect than cinnamon in lard and in peanut oil, probably due to the presence of tocopherols, phospholipids, and phenolic compounds, which have aromatic rings, or due to the synergistic effect of these compounds (*40*). Yanishlieva and Marinova (*58*)-indicated that mint ethanol extract is very effective in delaying the autoxidation process in triacylglycerols at 100 °C.

Vanillin and vanillic acid are frequently used as flavoring agents in ice creams, breakfast cereals, gelatins, puddings, and nonalcoholic beverages; both have been seen to protect food from peroxidation (*59*).

Our findings were also confirmed by determining the iodine values in oils and fats before and after using the Rancimat method. In sunflower oil the values decreased from 107−116 to 100−106. The iodine values were 99−108 for corn oil, decreasing to 81−100 after processing with Rancimat at 110 °C. For olive oil, the values decreased from 62−69 to 58−66, for margarine from 78−90 to 74−82, and for butter from 26−30 to 21−29. These decreases in the iodine values correlated well with the decrease in unsaturated fatty acids and temperature (*60*). These data closely reflect the values found in other experiments for margarine (*61*) and olive and sunflower oils (*62*).

Although hydrogenation stabilizes oil, the sample of fat containing added lecithin was much less stable in the Rancimat test than when stored without air. This was probably due to the tendency of lecithin to make oils foam, which reduces their stability and causes an effective increase in the surface area of the oil in contact with oxygen (*63*).

Reaction with Superoxide. A mixture of hypoxanthine and xantine oxidase at pH 7.4 generates $O_2^{\bullet-}$, which can be measured by its ability to reduce ferricytochrome *c* to ferrocytochrome *c*. Any added compound that is itself able to react with the $O_2^{\bullet-}$ ion should decrease the rate of reduction of ferricytochrome *c*. Superoxide dismutase inhibits its reduction by 90% (*32*).

Table 5 shows the scavenging of superoxide radical by different dessert spices compared with the activity of the common food additives submitted to different irradiation doses. Cinnamon exhibited the highest antioxidant activity ($p < 0.05$) of all the spices and additives analyzed with a percentage of inhibition of 71.6%. Anise and ginger also showed good inhibitory effects of about 52.2 and 56.7% inhibition, respectively, both significantly different with respect to the other spices. The other spices and common food additives analyzed were less efficient as scavengers of superoxide radical and in decreasing order of inhibition were licorice ≅ mint ≅ BHA > nutmeg ≅ propyl gallate > vanilla ≅ BHT, with percentages that varied from 44.8 to 9% for the samples. Irradiated samples did not show significant differences ($p < 0.05$) from non-irradiated samples.

Others researchers have demonstrated that extracts such as green tea (*64*), garlic (*65*), or clove oil (rich in eugenol) (*66*) are good superoxide radical scavengers.

TEAC Assay. TEAC values can be assigned to all compounds able to scavenge ABTS by comparing the scavenging capacities of these compounds with that of Trolox, a water soluble vitamin E analogue. The quantitative evaluation of antioxidant capacity based on TEAC can be used to provide a ranking order of antioxidants (*26*).

Table 6 shows the TEAC of the dessert spices and common food antioxidants submitted (or not) to different irradiation doses. The best spice sample in this respect was cinnamon ($p < 0.05$), which showed a TEAC value similar to that of propyl gallate. These were followed by mint, which showed better antioxidant activity than 0.5 mM Trolox and anise (with similar antioxidant values). In decreasing order the rest of spices and additives were BHA > licorice ≅ vanilla > ginger > nutmeg > BHT. The irradiated samples did not show significant differences ($p < 0.05$) from non-irradiated samples.

Although few spices have been analyzed by TEAC, thyme has been seen to possess antioxidant activity (*67*), probably because it contains compounds such as phenolics and flavonoids, which, when analyzed with this assay, have been seen to be good antioxidants.

In summary, taking into account the different free radical assays, licorice, anise, cinnamon, and mint show high antioxi-

Table 6. Scavenging of ABTS Radical Anions by Dessert Spices and Common Food Antioxidants and Variations with the Application of Different Irradiation Doses[a]

added to reaction mixture[fb]	irradiation (kGy)	TEAC[c]	added to reaction mixture[d]	irradiation (kGy)	TEAC[c]
none (control)					
Trolox (0.05 mM)		1.00 ± 0.0	BHA	0	9.99 ± 0.1
Trolox (0.25 mM)		5.00 ± 0.0		1	9.23 ± 0.1
Trolox (0.50 mM)		10.00 ± 0.0		3	9.82 ± 0.2
				5	9.57 ± 0.3
anise	0	10.27 ± 0.1		10	9.65 ± 0.3
	1	10.12 ± 0.1			
	3	10.17 ± 0.2	BHT	0	0.16 ± 0.1
	5	10.15 ± 0.2		1	0.17 ± 0.2
	10	10.27 ± 0.1		3	0.15 ± 0.1
				5	0.19 ± 0.1
cinnamon	0	11.69 ± 0.2		10	0.18 ± 0.2
	1	11.62 ± 0.5			
	3	11.54 ± 0.5	propyl	0	11.61 ± 0.1
	5	11.60 ± 0.1	gallate	1	11.61 ± 0.1
	10	11.62 ± 0.4		3	11.60 ± 0.2
				5	11.59 ± 0.1
ginger	0	8.46 ± 0.1		10	11.59 ± 0.1
	1	8.28 ± 0.2			
	3	8.52 ± 0.1			
	5	8.28 ± 0.1			
	10	8.31 ± 0.2			
mint	0	11.17 ± 0.2			
	1	11.16 ± 0.1			
	3	11.15 ± 0.4			
	5	11.13 ± 0.1			
	10	11.14 ± 0.3			
nutmeg	0	5.13 ± 0.1			
	1	4.89 ± 0.1			
	3	4.93 ± 0.2			
	5	5.18 ± 0.1			
	10	5.02 ± 0.2			
licorice	0	8.98 ± 0.1			
	1	8.86 ± 0.1			
	3	8.87 ± 0.2			
	5	9.04 ± 0.1			
	10	9.03 ± 0.2			
vanilla	0	8.81 ± 0.1			
	1	8.66 ± 0.1			
	3	8.98 ± 0.1			
	5	8.81 ± 0.3			
	10	8.78 ± 0.3			

[a] Statistical differences were analyzed by ANOVA ($p < 0.05$). [b] Compounds in aqueous medium at concentration = 5%. [c] TEAC is the millimolar concentration of a Trolox solution having an antioxidant capacity equivalent to that of the dilution of the substance under investigation. [d] Compounds in aqueous medium at concentration = 100 μg/g.

dant and scavenging capacities, whereas nutmeg, vanilla, and ginger show slightly lower antioxidant and scavenging activities. However, the results obtained by Rancimat test point to better antioxidant properties for nutmeg, licorice, ginger, and vanilla than for mint, cinnamon, and anise. These variations may be due to the reaction medium and the composition of these spices, an "antioxidant paradox", because the antioxidant activity of phenolic compounds depends on the number of hydroxyl groups in the molecule and can be increased by steric hindrance (*68*). The more polar compounds are more protective in a continuous lipid system owing to their location at the lipid−air interface, which provides better protection than lipophilic antioxidants that remain in solution in the oil phase. On the other hand, lipophilic antioxidants are more active in an oil-in-water emulsion system, the active surface being oriented to the oil−water interface (*69*).

Influence of Irradiation Procedure on Antioxidant Activity. In **Tables 1−6**, we can observe that there were no significant differences ($p < 0.05$) between irradiated and non-irradiated samples. Radiation doses up to 10 kGy have no effect on the scavenging capacity of free radicals ($LOO^{\bullet}$, $OH^{\bullet}$, $O_2^{\bullet-}$) or reactive oxygen species such as H_2O_2 for the spices and additives analyzed. Furthermore, the antioxidant activities evaluated by TEAC were similar in both irradiated and non-irradiated samples.

Irradiation at the doses studied did not show any significant influence on the antioxidant activity of some spices such as nutmeg (*70*) and anise (*71*) evaluated against sunflower oil or lard oxidation by peroxide value.

Nevertheless, Variyar et al. (*72*) obtained a higher yield of oil from γ-irradiated (10 kGy) ginger as compared to control (this could be attributed to radiation induced disruption of the cell wall struture and consequent higher extractability from the tissues), without affecting its flavor quality. Later, Variyar et al. (*73*) detected quantitative differences in the essential nutmeg oil constituents and an increased amount of phenolic acid (probably, due to the degradation of tannins and consequent higher extractability of phenolic acids) in γ-irradiated samples (*74*). In any case, the insignificant effect of irradiation on the chemical composition of dry spices can be explained by their low water content, which limits the possibility of free radicals being formed (*75*).

In conclusion, from our results we can relate that irradiation treatment up to 10 kGy does not affect the good antioxidant properties of the studied dessert spices and so can be safely used as a preservative technique.

ABBREVIATIONS USED

ABAP, 2,2′-azobis(2-amidinopropane) hydrochloride; $ABTS^{2-}$, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate); $ABTS^{\bullet-}$, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate) radical anions; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; EDTA, ethylenediaminetetraacetic acid; $LOO^{\bullet}$, peroxyl radical; MDA, malondialdehyde; $O_2^{\bullet-}$, superoxide anion radical; $OH^{\bullet}$, hydroxyl radical; PBS, phosphate-buffered saline; PG, propyl gallate; TBA, thiobarbituric acid; TEAC, Trolox equivalent antioxidant capacity.

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