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Effects of γ-Irradiation on the Free Radical and Antioxidant Contents in Nine Aromatic Herbs and Spices

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Nine spice and aromatic herb samples (i.e., basil, bird pepper, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage) were γ-irradiated at a dose of 10 kGy according to commercial practices. The effects of the disinfection treatment on the content of organic radicals and some nutrients (namely, vitamin C and carotenoids) in the samples were investigated by chromatographic and spectroscopic techniques. Irradiation resulted in a general increase of quinone radical content in all of the investigated samples, as revealed by electron paramagnetic resonance spectroscopy. The fate of these radicals after storage for 3 months was also investigated. The cellulose radical was clearly observed in a few samples. Significant losses of total ascorbate were found for black pepper, cinnamon, nutmeg, oregano, and sage, whereas a significant decrease of carotenoids content was observed for cinnamon, oregano, parsley, rosemary, bird pepper, and sage.

KEYWORDS: Spices; aromatic herbs; antioxidants; ascorbate; carotenoids; front-surface spectroscopy; γ-ray irradiation; EPR; free radicals

INTRODUCTION

There is at present increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their antioxidant and antimicrobial properties. These properties are due to many active phytochemicals, including vitamins, flavonoids, terpenoids, carotenoids, cumarins, curcumins, etc. Thanks to these nutrients, spices and aromatic herbs are considered to be essential in diets or medical therapies for delaying aging and biological tissue deterioration due to free radicals. Moreover, their antioxidant properties render spices and aromatic herbs very important as preservative agents in food, and antimicrobial properties are attributed to their essential oils. This characteristic is of great interest for the food industry because it offers the possibility to substitute natural for synthetic preservatives. In addition, some herbs such as rosemary and sage are used to produce drugs classified as phytopharmaceuticals, representing a significant part of the world pharmaceutical market (*1*−*8* and references cited therein).

The use of spices and aromatic herbs presupposes that their properties (organoleptic characteristics, content of antioxidants, etc.) will be maintained during their manufacture, from the harvest to sale, and that their microbiological status will not cause final product contamination.

The final microbial status of herbs and spices is determined by the natural content of microorganisms in plants and by the harvesting, drying, transporting, and packaging processes (*9*). To inactivate microorganisms, spices and aromatic herbs are usually subjected to disinfectant treatments. The most employed treatments are essentially three (*10*): fumigation with ethylene oxide, thermal treatment with steam, and irradiation with γ-rays or high-energy electrons. The use of irradiation instead of ethylene oxide to ensure hygienic quality of spices and herbs has increased in the past 10 years, especially because of the phasing out of ethylene oxide in many countries due to possible toxic residues and occupational health hazard for workers in the fumigation plants. Moreover, irradiation offers a broader spectrum for application for sanitizing dry ingredients than thermal treatments, often at a more competitive cost (*11*).

Numerous international studies performed by both research scientists and government agencies have also established that food irradiation can be considered a radiologically, microbiologically, and toxicologically safe technology (*12*, *13*). Nevertheless, questions focusing on nutrient loss and free radicals and radiolytic byproduct formation during irradiation, as well as on the organoleptic quality of irradiated food, are still being debated in the scientific literature (*13*).

The present work aimed to study the effects of γ-ray irradiation, performed according to commercial practices, on

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the content of nutrients and free radicals in spices and aromatic herbs. To this purpose, nine widely used spices and aromatic herbs, that is, bird pepper (*Capsicum frutescens*), black pepper (*Piper nigrum*), cinnamon (*Cinnamomum zeylanicum*), nutmeg (*Myristica fragrans*), oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), and parsley (*Petroselinum sativum*), were irradiated in an industrial plant with a dose ensuring complete disinfection of samples (10 kGy, the maximum used for commercial applications), and changes in their content of free radicals and some antioxidants were investigated by means of chromatographic and spectroscopic techniques. We mainly focused on vitamin C, that is, on ascorbic (AsA) and dehydroascorbic (DHA) acids, determined spectrophotometrically following Wang et al. (*14*). The interest in vitamin C is due to its antioxidant properties, which strongly determine the biological functions of this molecule in both plant and animal metabolism. In fact, AsA can enzymatically and nonenzymatically interact with damaging oxygen free radicals and their derivatives, thus protecting plants from oxidative stress and mammals from oxidative stress-related diseases such as cancer, cardiovascular diseases, and aging. Humans are unable to synthesize vitamin C and therefore require AsA as an essential component of their diet (*15*, *16*). Spices and herbs can be considered a source of this nutrient as well as of other important antioxidants such as carotenoids; these, too, are not biosynthesized by humans. Carotenoids are indeed able to scavenge oxygen free radicals (*17*, *18*). In the human body some of them can be converted into vitamin A, an essential nutrient for humans. Moreover, they are colored pigments (yellow, orange, red) widely used by the food industry (*19*, *20*). In this work, the content of carotenoids was qualitatively investigated by applying a front-surface absorbance technique set up in our laboratories (*21*), a technique that also allows color changes associated with the treatment to be monitored. Moreover, a quantitative determination of lutein and zeaxanthin, two of the most widespread carotenoids in plants (*22*), was carried out by HPLC.

The determination of all the nutrients was performed on both un-irradiated and irradiated samples and was accompanied by measurements of the free radical content by electron paramagnetic resonance (EPR) spectroscopy. The production of either primary or secondary free radicals is indeed one of the main effects of γ-irradiation due to electron release from molecules by high-energy ionizing radiations. Whereas in aqueous environments radicals readily undergo termination reactions and, hence, have short lifetimes, in dry samples radiolytically produced radicals can be trapped within a solid matrix and they can have much longer (even many months) lifetimes (*23*−*31*). This allows radicals to be detected by EPR spectroscopy in dry samples and renders this technique a practical detection method of spice and herb irradiation nowadays included in international protocols (*25*−*27*, *32*−*36*). However, radicals can be present in spices and herbs also before irradiation because of either the natural aging and decomposition of some components, such as oils, or the presence of transition metals, and they can undergo further modifications upon treatment with ionizing radiations. The quality and quantity of radicals can vary during the storage of samples for long times, depending on temperature, humidity, and other environmental factors (*30*, *31*, 37). Thus, the application of EPR spectroscopy to our un-irradiated and irradiated samples at two different times (namely, within 48 h following irradiation and after 3 months of storage) could contribute to enlarge the information present in the literature on the stability of natural and radiation-induced radicals. Moreover, due to the implication of radical reactions in the processes causing food spoilage, a correlation could exist between losses of vitamins and antioxidants and free radical content.

MATERIALS AND METHODS

Preparation and Storage of Samples. Spices and aromatic herbs were provided by Enrico Webb James S.n.c. (Livorno, Italy). Nutmeg, cinnamon, black pepper, and bird pepper were whole, whereas basil, parsley, rosemary, and sage were minced. Each sample was divided in two lots of ~200 g, one of which was irradiated and the other was kept as control. All samples were enclosed in polyethylene bags, sealed under air. The irradiation was performed in an industrial irradiation plant (Gammarad Italia S.p.a., Bologna, Italy) using a ^{60}Co source. The samples, in their own package, were enclosed in a cardboard box and exposed to γ-radiation at room temperature to an absorbed dose of 10 kGy. The conveyor system of the plant ensured a treatment with a highly precise and accurate dose. Nevertheless, the average absorbed dose was determined using an alanine dosimeter (*38*) in each sample bag; it was found to be 10 ± 1 kGy. All, un-irradiated and irradiated, samples were kept under the same conditions, that is, at room temperature (23 ± 2 °C) and a relative humidity of 60−80%. Before measurements, both control and irradiated samples were ground using a mixer mill (Retsch MM200), sieved with a Retsch AS 200 basic analytical sieve shaker (ASTM 63 μ), and stored in glass containers.

Extraction and Determination of Ascorbate. The ascorbate content was determined on spice and herb extracts by employing the method by Wang et al. (*14*). This method is based on the reduction of Fe^{3+} to Fe^{2+} by ascorbic acid in an acidic medium followed by the formation of a red chelate between Fe^{2+} and 4,7-diphenyl-1,10-phenanthroline showing an absorption band centered at 534 nm. For total ascorbate (AsA + DHA) determination, extracts were treated with dithiothreitol to reduce DHA to AsA. Thus, absorbance was measured both on the untreated and on the reduced extracts for AsA and AsA + DHA determination, respectively. At least three measurements were performed for each sample, and the results were expressed as the mean value ± the standard error (SE). Absorbance was measured with a Unicam PU8600 spectrophotometer. A standard curve in the range of 3−20 nmol of ascorbate was used. Extractions were performed at 4 °C with pestle and mortar, adding 2 g of sand to the powder and using 6 mL of 5% (w/v) trichloroacetic acid. The extracts were thus centrifuged at 8000*g* and 4°C for 5 min, the supernatants were filtered, and their volumes were measured. The quantity of ground herbs and spices used in the extractions was conveniently chosen to obtain absorbance values within the calibration curve.

Extraction and Determination of Carotenoids. Carotenoids were extracted from spice and herb powders following the procedure of Pinzino et al. (*39*). For each sample, 50 mg of powder was repeatedly extracted with 2 mL of methanol until the extraction medium did not show any detectable absorbance in the spectral region between 400 and 500 nm. In most cases, four extractions were sufficient. The extracts were pooled, reduced to 1 mL under vacuum, and then filtered and stored at −20 °C. Carotenoids were analyzed by isocratic RP-HPLC using a Jasco 880 pump equipped with a Shimadzu SPD-10-A spectrophotometric detector and a Nucleosil 300-7 C-18 column (4.6 × 250 mm). Extracts were eluted at 30 °C using 96% methanol as mobile phase at a flow rate of 0.5 mL/min and detected at 445 nm. In these conditions, the retention time was 15 min for lutein and 15 min and 45 s for zeaxanthin. The lutein and zeaxanthin contents were calculated by comparing the elongations of the corresponding peaks with that of a standard solution of pure lutein in methanol, the concentration of which was determined by measuring the absorbance at 445 nm (ϵ_{445} = 133000 M^{-1} cm^{-1}). Three measurements were performed for each sample (injected volumes of 15−20 μL); the results are reported as the mean value ± the SE. In the case of bird pepper, the chromatographic peak of capsanthin was also identified (*40*); it had a retention time of 12 min and 12 s.

Determination of Bird Pepper Capsaicinoids. Methanol extracts of bird pepper were also analyzed by HPLC to determine capsaicinoids. The analyses were performed using the same HPLC apparatus above-described, methanol/water 70:30 v/v as mobile phase, and an observa-

Table 1. Content of AsA and AsA + DHA (Mean ± SE, Micromoles per Gram)

	AsA			AsA + DHA		
sample	untreated	γ-irradiated	significance test[a]	untreated	γ-irradiated	significance test[a]
basil	13.8 ± 0.2	11.4 ± 0.2	+	23.0 ± 0.6	22.1 ± 0.4	−
bird pepper	6.0 ± 0.2	5.6 ± 0.3	−	9.4 ± 0.7	10.1 ± 0.3	−
black pepper	6.6 ± 0.5	6.0 ± 0.2	−	14.7 ± 0.9	12.6 ± 0.4	+
cinnamon	15.3 ± 0.4	10.5 ± 0.6	+	20.4 ± 0.7	18.3 ± 0.9	+
nutmeg	17.1 ± 0.6	8.8 ± 0.2	+	24.5 ± 0.7	16.2 ± 0.5	+
oregano	26.4 ± 3	25.5 ± 0.2	−	43.9 ± 0.2	39 ± 1	+
parsley	7.8 ± 0.1	6.8 ± 0.2	+	12.5 ± 0.4	11.7 ± 0.5	−
rosemary	25.4 ± 0.7	25.6 ± 0.1	−	46 ± 4	54 ± 4	−
sage	41.6 ± 0.5	36 ± 6	−	52 ± 2	47 ± 2	+

[a] + and − indicate significant and nonsignificant differences between values determined on untreated and γ-irradiated samples, respectively.

tion wavelength of 280 nm. In these conditions, capsaicin (retention time of 23 min), dihydrocapsaicin (retention time of 32 min), homodihydrocapsaicin (retention time of 35 min and 30 s),and nordihydrocapsaicin (retention time of 21 min and 50 s) were identified in the methanol extracts by comparing the chromatograms with that of a standard solution of capsaicin and with those reported in the literature for HPLC analyses performed in similar conditions (*41*).

Front-Surface Absorbance Measurements. Front-surface absorbance measurements in the vis−UV spectral region (250−650 nm) were performed on powder samples of spices and aromatic herbs using the method of Zandomeneghi et al. (*21*). Experiments were performed on a conventional spectrofluorometer (Jasco FP770) suitably set to detect light scattered from powder samples. The samples, ∼0.6 mm thick, were enclosed in a homemade cell, suitably designed to avoid specular reflections in measurements on powder (*42*). The cell windows were 30° tilted with respect to the incident beam. Powdered $BaSO_4$ was used as a reference light scatterer (*43*). It is worth noting that the nutmeg sample was unsuitable for measurements in these conditions.

EPR Measurements. EPR measurements were performed using a Varian (Palo Alto, CA) E112 (X-band) spectrometer. The spectrometer was interfaced to a 100 MHz personal computer by means of a homemade data acquisition system consisting of an acquisition board (*44*) and a software package especially designed for EPR and ENDOR experiments (*45*). The content of free radicals was measured on herb and spice powders (∼100 mg) inserted in a quartz tube with an internal diameter of 4 mm. Spectra were recorded using a standard EPR cavity, a microwave power of 1 mW, a time constant of 0.125 s, and a modulation amplitude of 1.25 G. Measurements were made at room temperature within 48 h following irradiation and after 3 months of storage at room temperature and 60−80% relative humidity. Quantification of organic radicals was performed by comparison of the double integral of the signal at $g = 2.005 \pm 0.001$ with that of the standard Varian weak pitch (10^{13} spins per centimeter of length) measured under identical instrumental conditions (*46*). Results were expressed as the mean value ± the SE.

Significance Tests. Significance tests were performed on mean values determined for AsA, AsA + DHA, lutein, zeaxanthin, and free radicals. Means drawn from two differently treated samples ($\bar{X}_a$ and $\bar{X}_b$, respectively) were considered to be significantly different to a level of 5% when the condition $|t| \geq z(0.975)$ was fulfilled (*47*), where

$$t = \frac{\bar{X}_a - \bar{X}_b}{\left(\frac{s_a^2}{n_a} + \frac{s_b^2}{n_b}\right)^{1/2}} \quad (1)$$

and $z(0.975) = 1.96$. In eq 1, s_a^2 and s_b^2 are the standard deviations and n_a and n_b are the numbers of measurements performed on the two samples, respectively.

RESULTS

Ascorbate Content. AsA and AsA + DHA were spectrophotometrically determined in spice and herb extracts following the method of Wang et al. (*14*). As shown in **Table 1**, the nine herbs and spices investigated have different contents of vitamin C, oregano, sage, and rosemary being those with the highest contents. In particular, AsA ranged from 6 to 42 μmol/g, whereas contents of total ascorbate were within 9−52 μmol/g. AsA represented 60−70% of total ascorbate in all samples except black pepper (45%) and sage (80%). After irradiation, a general decrease of vitamin C content was observed for all of the samples (see **Table 1**). On the basis of significance tests described under Materials and Methods, the decrease of AsA could be considered significant for basil (18%), cinnamon (32%), nutmeg (48%), and parsley (13%), whereas the total ascorbate content decreased significantly in black pepper (14%), cinnamon (10%), nutmeg (34%), oregano (11%), and sage (10%).

Analysis of Carotenoids. HPLC analyses were performed on methanol extracts of control and γ-irradiated spices and herbs. The column effluents were spectrophotometrically analyzed at 445 nm. Several chromatographic peaks could be assigned to carotenoids on the basis of the vis−UV spectra recorded on the corresponding effluents. Lutein and zeaxanthin were identified in the chromatograms of all samples and quantified as described under Materials and Methods; the results are reported in **Table 2**. The highest lutein and zeaxanthin contents were found for basil, oregano, parsley, rosemary, and sage. On the other hand, lutein could not be detected in nutmeg extracts and was quite low in cinnamon and bird pepper, whereas zeaxanthin content was not detectable in either cinnamon and nutmeg. As far as bird pepper is concerned, a low content of lutein was found (∼1 μg/g), whereas zeaxanthin (∼6 μg/g) and capsanthin (∼14 μg/g) were more abundant.

γ-Irradiation resulted in a generally decreased lutein and zeaxanthin content (see **Table 2**). However, on the basis of the tests described under Materials and Methods (eq 1), significant decreases of lutein were found only in sage, oregano, parsley, and rosemary, where after irradiation the content of lutein was reduced by 12, 13, 16, and 38%, respectively. As far as zeaxanthin is concerned, significant losses were found for bird pepper (61%), oregano (11%), parsley (16%), and rosemary (37%). In the case of bird pepper, a significant decrease (∼40%) of capsanthin was also observed. Moreover, it was found that the contents of capsaicin, dihydrocapsaicin, homodihydrocapsaicin, and nordihydrocapsaicin in bird pepper remained substantially unchanged after irradiation.

Front-Surface Absorbance Measurements. Front-surface absorbance (A) spectra obtained for powdered samples of spices and herbs using the method of Zandomeneghi et al. (*21*) are shown in **Figure 1**. All of the leaf samples (see **Figure 1a**) showed an absorption band at ∼290 nm, where also the aromatic

Table 2. Lutein and Zeaxanthin Contents (Mean ± SE, Micrograms per Gram) in Untreated and γ-Irradiated Samples

	lutein			zeaxanthin		
sample	untreated	γ-irradiated	significance test[a]	untreated	γ-irradiated	significance test[a]
basil	240 ± 7	221 ± 7	–	18.0 ± 0.5	18.0 ± 0.5	–
bird pepper	0.90 ± 0.04	1.00 ± 0.04	–	5.6 ± 0.2	2.2 ± 0.2	+
black pepper	6.6 ± 0.2	6.5 ± 0.2	–	6.2 ± 0.2	5.8 ± 0.2	–
cinnamon	1.00 ± 0.03	nd[b]		nd	nd	
nutmeg	nd	nd		nd	nd	
oregano	206 ± 6	180 ± 5	+	44 ± 1	39 ± 1	+
parsley	123 ± 4	103 ± 3	+	12.2 ± 0.4	10.2 ± 0.4	+
rosemary	114 ± 3	71 ± 2	+	33 ± 1	20.8 ± 0.7	+
sage	58 ± 2	51 ± 2	+	6 ± 1	6 ± 1	–

[a] + and – indicate significant and nonsignificant differences between values determined on untreated and γ-irradiated samples, respectively. [b] Not detectable.

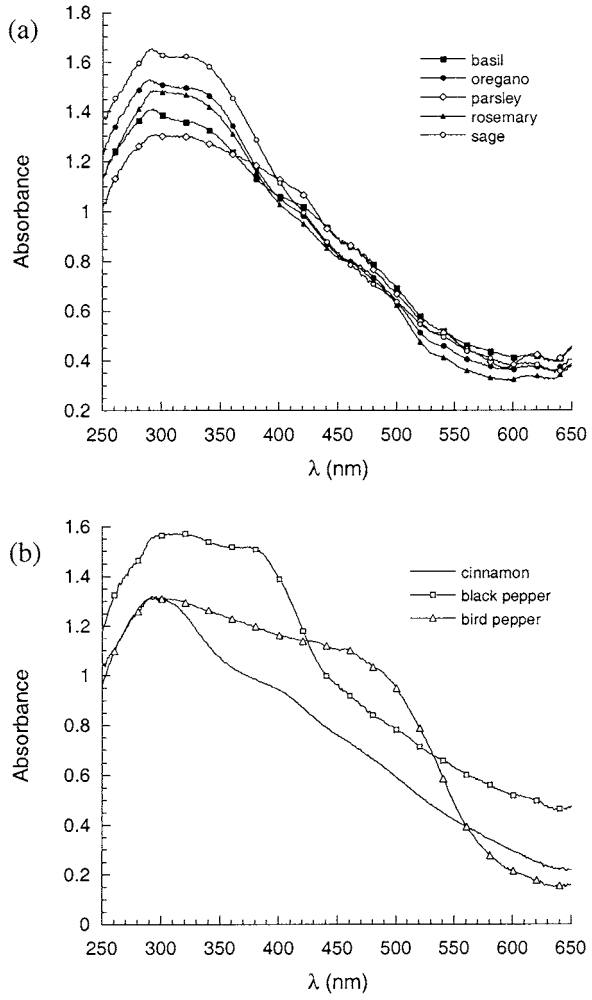

Figure 1. Front-surface absorbance spectra.

amino acids present in the proteins of spices and herbs absorb. However, it has been demonstrated that this high-intensity band is not proportional to the content of chromophores present in the sample and, for this reason, cannot be utilized to compare data from different samples (*21*). On the other hand, by analyzing the bands occurring in the spectral region from 400 to 650 nm, useful information on the chromophore groups and pigments contained in the samples can be achieved. In particular, bands due to carotenoid absorptions are to be expected in the spectral region between 400 and 530 nm (*21*, *48*, *49*). Shoulders were observed at ~417 nm for basil, oregano, parsley, and rosemary, that is, leaves with high lutein contents (see **Table 2**). Moreover, for all leaf samples a shoulder at ~465 nm was observed. All of these features can be assigned to carotenoids in leaves. A weak absorption band was also observed at ~620 nm for all leaf samples, which can be attributed to chlorophyll *b* (*50*). Black pepper showed absorption bands at ~298 and ~375 nm, whereas cinnamon had an absorption band at ~295 nm and a shoulder at ~385 nm; both of these spices show broad absorption bands in the carotenoid spectral region. As far as bird pepper is concerned, absorption was observed over all the investigated spectral region, with an intense band centered at 450−460 nm. This band is due to peculiar carotenoids of bird pepper (capsanthin, capsorubin, β-carotene, zeaxanthin, β-cryptoxanthin, and, in minor amount, lutein) and to their esterified derivatives, whereas capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin, and homodihydrocapsaicin) show a band centered at 280 nm (*51*, *52*).

Comparison between the absorbance trends of untreated and γ-irradiated samples in the 320−650 nm spectral region might provide qualitative information on the changes induced by irradiation on spice and herb color and, in turn, on compounds bearing chromophores. To this purpose, a more direct comparison can be made by calculating the absorbance of irradiated samples with respect to the untreated ones as

$$A(\lambda) = -\log \frac{I_i(\lambda)}{I_u(\lambda)} \qquad (2)$$

where I_i and I_u are the reflectance values of the irradiated and untreated samples at wavelength λ, respectively. In this way, absorbance values different from 0 indicate changes in light absorption of the irradiated samples with respect to the control ones. Appreciable differences were found for basil, bird pepper, black pepper, oregano, parsley, and rosemary (see **Figure 2**). In particular, irradiation resulted in a stronger absorption in the spectral region between 300 and 500 nm for basil and rosemary and between 300 and 600 nm for oregano. On the contrary, a decreased absorbance was found between 350 and 650 nm for black pepper and between 380 and 650 nm for bird pepper.

Free Radical Determination. EPR measurements were performed on both untreated and γ-irradiated samples; a selection of the obtained spectra is shown in **Figures 3** and **4**. In un-irradiated samples, EPR signals due to transition metal ions, such as Fe^{3+} and Mn^{2+} (see **Figure 3**), and a line centered at $g = 2.005 \pm 0.001$ (see **Figures 3** and **4**) due to organic radicals (*23*, *24*, *28*, *30*, *31*), attributed to quinone radicals (*53*), were observed. The six-line signal of Mn^{2+} (*26*, *34*, *35*, *54*) centered at $g = 2.006$ was easily distinguishable in the spectra of cinnamon and black pepper. Signals ascribable to Fe^{3+} ions, centered at $g = 2.10 \pm 0.05$, were observed for all of the samples; they appeared to be remarkably strong for oregano,

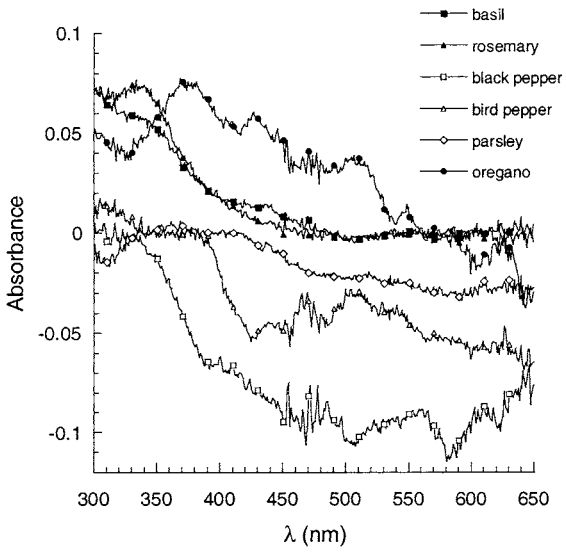

Figure 2. Front-surface absorbance calculated using eq 2.

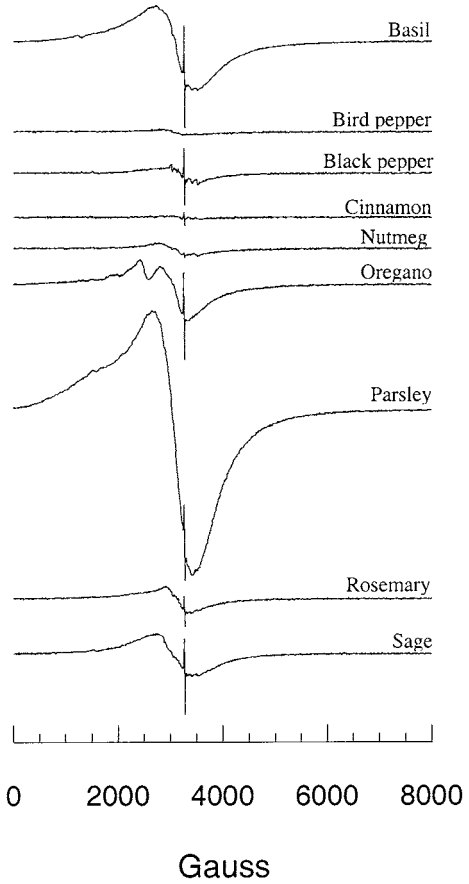

Figure 3. EPR spectra of untreated samples.

sage, basil, and parsley. The amplitude and the line width (4.5−8.5 G) of the signal due to organic radicals were found to be dependent on the kind of spice or aromatic herb investigated (see **Figure 4**), indicating that different vegetable materials can have different contents of organic radicals, with a different distribution. As can be observed in **Table 3**, basil had the highest content of free radicals (83.5×10^{15} spins/g); a slightly smaller content was found in oregano, sage, parsley, and rosemary, whereas cinnamon, nutmeg, bird pepper, and black pepper show quite small radical contents.

After the treatment with ionizing radiations, an increase of the intensity of the organic radical signal ($g = 2.005 \pm 0.001$) was generally observed (see **Figure 4** and **Table 3**); in many cases a line broadening was also found, due to the fact that different kinds of radicals could be formed during the irradiation. For some samples (nutmeg, cinnamon, bird pepper, and black pepper), shoulders of this signal were also observed, due to signals separated by 14.5 G; in some cases weak signals at $g =$ 2.022 and 1.994 separated by ~45 G were also revealed. These signals are reported in the literature for some fruits irradiated with γ-rays (*23*, *24*, *55*). Finally, weak signals due to the cellulose radicals (*37*) (signals centered at $g = 2.005$ with a hyperfine splitting of ~60 G) could be clearly distinguished in the spectra of nutmeg, oregano, basil, and parsley. These signals are considered as a certain sign of irradiation in many protocols (*25*−*27*, *32*−*36*, 56, 57).

To monitor the trend of the organic radical content with time, EPR measurements were repeated after storage of spices and aromatic herbs at room temperature (23 ± 2 °C) and 60−80% relative humidity for 3 months, a period corresponding to the shelf life of these products. Changes in the intensity of the spectral signals were found (see **Figure 4** and **Table 3**): whereas a decrease of intensity was always observed for the cellulose radical signal in irradiated samples, which could bring this signal below the detection limit (*28*, *30*, *31*), both increases and decreases were found for the signal at $g = 2.005 \pm 0.001$, either in the control or in the irradiated samples, depending on the spice or aromatic herb investigated.

DISCUSSION

The effects of irradiation with γ-rays (10 kGy, performed according to commercial practices) were investigated on commercial samples of dry basil, bird pepper, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage. To this aim the contents of ascorbate, main carotenoids, and free radicals were measured in control and treated samples by means of chromatographic and spectroscopic techniques. Moreover, changes induced by the irradiation on the color of the nine spices and herbs were monitored by front-surface absorption spectroscopy with particular attention to the fate of carotenoids.

As far as control samples are concerned, higher vitamin C and carotenoid contents were found in leaf samples (see **Table 1** and **2**), as expected on the basis of the biological functions of these antioxidants in these plant organs (*15*, *17*, *18*). Values of total ascorbate ranged from ~9 μmol/g of dry matter in bird pepper to ~52 μmol/g in sage; except for black pepper, AsA was the predominant form (60−80%). In herbs, lutein ranged from ~60 μg/g of dry matter in sage to 240 μg/g in basil, whereas it was ~1 μg/g in bird pepper and cinnamon. Zeaxanthin ranged from 6 μg (in sage) to 44 μg (in oregano) per gram of dry matter in herbs; it was ~6 μg/g in black and bird pepper and was below the detection limit in cinnamon and nutmeg. Moreover, bird pepper contains many other orange and yellow carotenoids, as shown by front-surface absorption spectroscopy (see **Figure 1b**) and HPLC analysis. Among them, capsanthin was revealed to be the most abundant.

Leaf samples had higher radical contents, too (see **Table 3**). This result could be due not only to the higher oxidative stress exposition of leaves with respect to other plant organs but also to the possible radical production during herb processing. In fact, herbs were furnished as minced leaves, whereas spices were furnished as entire seeds, fruits, or stems.

Irradiation with γ-rays generally causes a loss of nutrients (see **Tables 1** and **2**) and, as expected, an increase of radiolytic products (see **Table 3**) in the investigated samples, in agreement with the results reported in the literature for other foods (*12*,

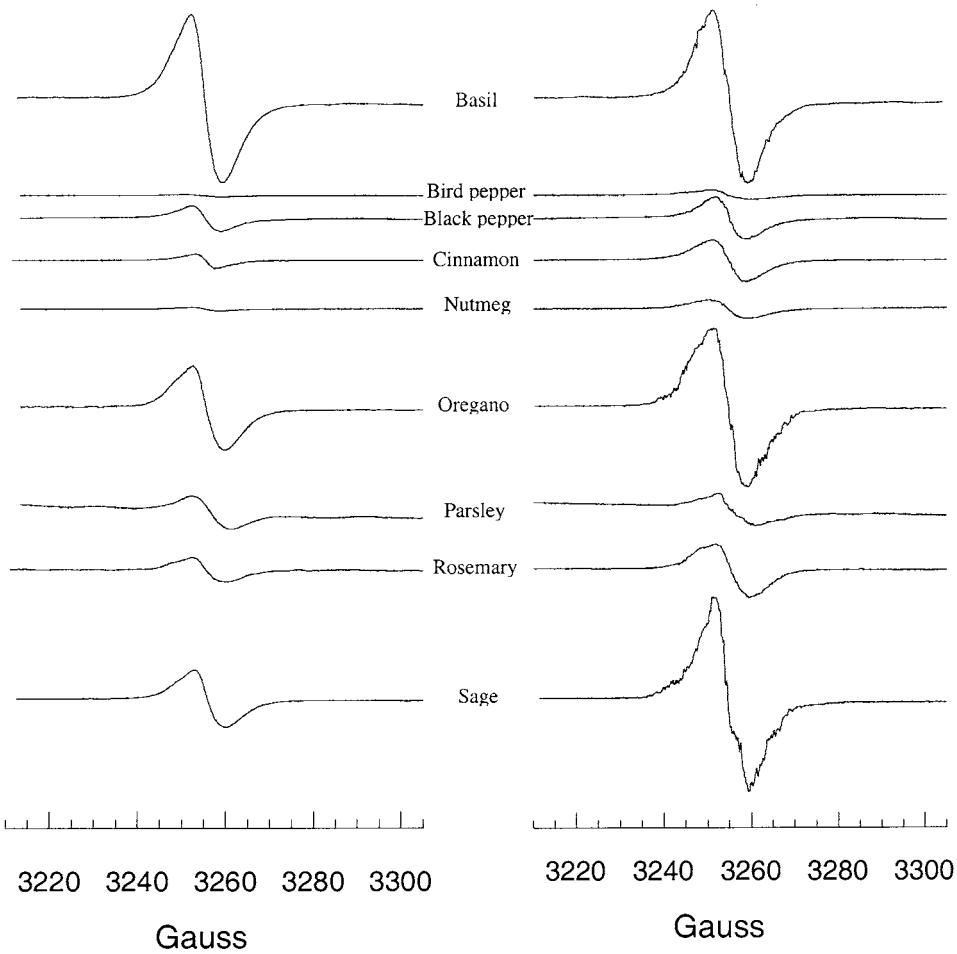

Figure 4. EPR spectra of organic radicals in untreated (left) and irradiated (right) samples.

Table 3. Contents (Mean ± SE, Spins per Gram × 10^{-15}) of Organic Radicals in Untreated and γ-Irradiated Samples

sample	untreated (U)	γ-irradiated (I)	untreated after 3 months (U_3)	γ-irradiated after 3 months (I_3)	significance tests[a]					
					U/I	U/U_3	U_3/I	U/I_3	I/I_3	U_3/I_3
basil	83.5 ± 4.2	83.8 ± 4.2	104.0 ± 5.2	90.2 ± 4.5	−	+	+	-	−	+
bird pepper	2.5 ± 0.1	6.3 ± 0.3	1.6 ± 0.1	5.9 ± 0.3	+	+	+	+	−	+
black pepper	9.9 ± 0.5	23.6 ± 1.2	15.0 ± 0.7	10.0 ± 0.5	+	+	+	-	+	+
cinnamon	3.6 ± 0.2	19.8 ± 1.0	2.9 ± 0.1	15.3 ± 0.8	+	+	+	+	+	+
nutmeg	1.35 ± 0.07	8.9 ± 0.4	1.12 ± 0.05	2.2 ± 0.1	+	+	+	+	+	+
oregano	38.4 ± 1.9	89.3 ± 4.5	31.8 ± 1.6	57.8 ± 2.9	+	+	+	+	+	+
parsley	21.5 ± 1.1	61.1 ± 3.1	23.9 ± 1.2	55.8 ± 2.8	+	−	+	+	−	+
rosemary	10.2 ± 0.5	25.8 ± 1.3	6.6 ± 0.3	27.6 ± 1.4	+	+	+	+	−	+
sage	29.1 ± 1.5	90.2 ± 4.5	33.1 ± 1.7	47.2 ± 2.4	+	−	+	+	+	+

[a] + and − indicate significant and nonsignificant differences between values determined on samples indicated by column headings.

13, 58−60). In particular, the quinone radical content more than doubled in parsley, rosemary, oregano, sage, black pepper, and bird pepper, increased >5 times in cinnamon and almost 7 times in nutmeg, but remained substantially unchanged in basil.

Storage of samples for a period of the order of spice and herb shelf life (3 months) resulted in a marked intensity decrease of the cellulose radical signal in irradiated samples; in many cases this signal completely disappeared. On the other hand, the signal at $g = 2.005 \pm 0.001$, due to quinone radicals, showed both intensity increases and decreases in control samples (see **Table 3**). In fact, both radical formation and recombination could occur during the storage period, depending on the sample and the storage conditions. In particular, untreated basil showed a significant increase of quinone radicals (+20%). The same behavior was observed for black pepper (+34%), whereas significantly decreased free radical contents were found in bird pepper (−36%), cinnamon (−19%), nutmeg (−17%), oregano (−17%), and rosemary (−35%); no significant changes were found for the remaining untreated samples. Storage resulted in a decrease of quinone radicals in all irradiated samples except for basil and rosemary, in which, however, the radical content cannot be considered to be significantly increased. Losses of radicals after storage were generally higher for irradiated samples than for untreated ones; particularly marked losses were found for sage (−48%), black pepper (−58%), and nutmeg (−75%). As a result, basil and black pepper irradiated and stored for 3 months showed a lower radical content than the respective control samples. This behavior could be attributed to a lower stability of radiolytically produced radicals with respect to those naturally occurring in spices and herbs. On the basis of these results, EPR detection seems not to be completely adequate for the identification of irradiated spices and herbs, except when

the cellulose radicals are clearly detected or the method by Yordanov and Gancheva (*30*) is employed.

A significant loss of total ascorbate was observed for black pepper (−14%), cinnamon and sage (−10%), oregano (−11%), and, in particular, nutmeg (−34%) (see **Table 1**), in agreement with what was reported in the literature for the fate of vitamin C in irradiated food (*12*, *58*−*61*). Nevertheless, these losses are quite small when compared to those observed for some spices and herbs during blanching and drying treatments (*62*, *63*), that is, treatments requiring relatively high temperatues (*64*). Moreover, the treatment resulted in many cases in a decrease of the reduced form of ascorbate (AsA) with respect to the oxidized one (DHA). The largest effects were observed for cinnamon and nutmeg. However, exchanges between reduced and oxidized forms of ascorbate do not result in a loss of nutrients because both AsA and DHA have vitaminic value (*15*).

Irradiation caused front-surface absorption changes for basil, bird pepper, black pepper, oregano, parsley, and rosemary (see **Figure 2**). In the case of bird pepper, a particularly sharp decrease of absorbance was found in the spectral region where carotenoids absorb, clearly indicating a general decrease in the concentration of these pigments upon irradiation, in agreement with the HPLC results on zeaxanthin (−61%) and capsanthin (−40%). A significant decrease of zeaxanthin was also found in oregano, parsley, and rosemary (see **Table 2**), whereas the content of lutein significantly decreased in cinnamon, oregano, parsley, rosemary, and sage. Both lutein and zeaxanthin remained substantially unchanged in basil and black pepper. Chatterjee et al. reported that γ-irradiation (1−10 kGy) did not cause any significant change in the color power (ASTA color measured at 460 nm) of red chilli varieties (*65*). Similar results were obtained by Vajdi and Pereira on γ-irradiated paprika powder (*66*). The same authors (*66*) also did not observe any noticeable difference in CIELAB color between untreated and γ-irradiated samples. These findings disagree with data obtained in our work. Moreover, qualitative color losses observed by us on ground irradiated bird pepper can be considered smaller than those reported by Almela et al. for paprika sterilized by a high-temperature short-time treatment (*67*) and by Vajdi and Pereira for paprika sterilized by fumigation with ethylene oxide. In fact, these authors found $\Delta E^* \geq 3$ in CIELAB color measurements, that is, a color difference that can be visually distinguished. However, a comparison between results obtained on different plant materials and, above all, by quite different analytical methods is not straightforward.

Finally, our HPLC analyses showed that irradiation does not alter capsaicinoid content in bird pepper, thus preserving the characteristic pungency of this spice.

ABBREVIATIONS USED

AsA, ascorbic acid; DHA, dehydroascorbic acid; EPR, electron paramagnetic resonance; HPLC, high-performance liquid chromatography.

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