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Status of Free Radicals in Indian Monsooned Coffee Beans γ-Irradiated for Disinfestation

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Free radicals in two cultivars of Indian monsooned coffee beans, γ-irradiated for hygienic and quarantine purposes, were examined by entrapping the small amount of samples in potassium chloride powder in ESR quartz tubes. In contrast to a prominent free radical signal at $g = 2.002$, observed in spermoderm (silver skin) and cotyledon (whole seed without skin) parts of normal coffee beans, the same was not discernible in monsooned coffee bean parts of both cultivars. The ESR signal was found to be more prominent in the spermoderm than in the whole seed portion of the normal coffee beans. Common practices of roasting and powdering were found to generate quantitatively more free radicals in coffee beans than γ-irradiation alone. Phenols, contributing maximally to observed free radical signals in coffee beans, were significantly different in normal and monsooned coffee beans. These observations on insignificant free radical population in irradiated monsooned coffee beans may be attributed to their inherent possession of high water activity, favoring decay of free radicals produced. Textural studies with monsooned coffee beans, before and after mild heat treatments, supported these findings.

KEYWORDS: Specialty coffee; monsooning; radiation processing; hygienic and quarantine treatments

INTRODUCTION

Monsooned coffee is a "specialty coffee" of India, which has gained worldwide recognition for its unique taste, flavor, and cup quality. This coffee is in great demand and is preferred by consumers, particularly in the Scandinavian countries, Italy, France, Japan, and, now, the United States as a base for espresso coffee.

Monsooned coffee is prepared according to an unscientific but traditional method adapted during the past 50 years at the coffee-curing works situated in the coastal towns of Mangalore and Tellicherry in Karnataka and Kerala states of India, respectively. Monsooning is a process wherein the dry processed "A" grade (6−7 mm size grade) coffee beans belonging to Arabica (*Coffea arabica* Linnaeus) and Robusta (*Coffea canephora* Pierre ex Froehner) coffee varieties are exposed to the moist winds of the monsoon season in open warehouses (godowns), situated at the coastal belt for a period of 6−7 weeks. During such processing, coffee beans, being highly hygroscopic, are allowed to absorb moisture from an initial level of 9−10% up to 18−22% (*1*). Due to this absorption of atmospheric moisture, the coffee beans bloat to double their original size accompanied by the bleaching of natural color. The beans acquire a totally different taste and flavor similar to that of an aged coffee. The coffee beans thus processed are marketed as "Indian Monsooned Malabar Coffee" (*2*).

Radiation processing is increasingly being accepted as one of the most effective and economic methods available today to treat agricultural and horticultural commodities for hygienic purposes and for overcoming quarantine barriers in international trade (*3*, *4*). No other method of food processing has undergone such a thorough assessment of safety as the method of radiation processing, and the pertinent details have been provided in many excellent reviews (*5*, *6*). Of late, a number of studies have been carried out to identify the chemical changes, mainly related to the generation of free radicals and radiolytic products, in different foods and food components after exposure to ionizing radiation (*7*−*10*). Although a few studies have been reported recently, until now no scientific information has been available on the status of free radicals in monsooned coffee beans (*1*, *2*, *11*). Because Indian Monsooned Malabar Coffee has good scope for export in better markets under the new WTO regime, the present studies were undertaken to assess the status of free radicals in monsooned coffee beans, exposed to quarantine doses of γ-irradiation, and to evaluate their quality parameters. Such information is required not only to remove misconceptions from consumers' minds but also to provide suitable information to law enforcement authorities responsible for regulating the trade in food commodities.

MATERIALS AND METHODS

Coffee Beans. Arabica and Robusta monsooned coffee beans, conforming to the standards set by the Coffee Board, Government of

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India, were collected from the local coffee-curing works situated at Mangalore (Aspinwall and Co., Mangalore Karnataka, India). Only the healthy beans from the same batch with no apparent physical damage or insect infestations were used in the present investigations. Normal Arabica and Robusta coffee beans were also used for comparative studies.

Irradiation. Coffee beans packed in biaxially oriented polypropylene (BOPP, 25 μm) bags were subjected to γ-radiation doses of 0.25, 0.50, 0.75, and 1.0 kGy in a Gamma cell-220, ^{60}CO source (AECL, Ottawa, ON, Canada), having a dose rate of 15 Gy min^{-1}. Fricke dosimetry was carried out periodically for measuring the absorbed dose (*12*). The irradiated samples were stored in desiccators kept in a deep freezer maintained at −20 °C until further analysis. The non-irradiated samples stored similarly served as control.

Sample Preparation. Whole seed and silver skin parts of coffee beans were cut into small pieces, and these comminuted parts were used as such or after powdering in a glass pestle and mortar as reported earlier (*13*).

Washing with Methyl Cellosolve. Powdered portions of coffee beans were washed with methyl Cellosolve (ethylene glycol mono methyl ether; Sigma, St. Louis, MO) to remove the naturally occurring phenolic compounds (*14*). Sample powder was stirred with a ~20 times volume of solvent for 15 min and centrifuged at 1240*g* for 10 min using a Sorvall clinical centrifuge. The residue was washed twice more and vacuum-dried for irradiation and ESR scanning.

Annealing. Irradiated and control coffee beans were separately placed in Borosil Petri plates and heated in a Thermostat vacuum oven (Townson and Mercer Ltd., Croydon, U.K.) set at 50 ± 0.5 °C for 68 h.

Roasting of Coffee Beans. Whole coffee beans were roasted individually using a gas-operated kitchen hot plate with constant stirring until the typical aroma accompanied with a brownish surface color was noticed. Roasted beans, after attaining room temperature, were transferred to Borosil Petri plates and stored in a desiccator until further use.

Texture Analysis. An Instron Universal Texture Testing Machine TM model (Instron Engineering Corp., Canton, MA) equipped with a fabricated cylindrical brass metal probe (30 × 8 mm) was used for texture analysis (*15*). The instrument was calibrated before use, and kilograms of force exerted on 1.56 mm compression of coffee beans was recorded using automode selection for the movement of crosshead (speed = 0.5 cm/min) and chart recorder (20 cm/min).

Electron Spin Resonance (ESR) Measurements. An X-band Bruker EMX 6/1 spectrometer (Bruker Analytische Messtechnik, GmbH, Karlsruhe, Germany) was used for recording the ESR spectra of coffee bean samples packed in quartz sample tubes (Bruker, 3.5 mm i.d.) fixed at the same position in the ESR instrument cavity. The calibration and sensitivity of the instrument were checked routinely by employing 1,1-diphenyl-2-picrylhydrazyl (DPPH, Bruker No. 9702 D 153) as standard for *G* value determinations.

For visualizing the natural abundance of free radicals, 2−3 mm sized cut pieces (10−30 mg) were weighed in a Sartorius BA-61 analytical balance (Gottingen, Germany) and entrapped in a 25 mm deep column of KCl powder packed in sample tubes for ESR scanning. The first-derivative absorption spectra were recorded between 342 and 352 mT scan field at settings of 9.731 GHz microwave frequency of 1 mW power, 0.4 mT modulation amplitude, 163.84 m•s time constant, and 41.94 s sweep time. Receiver gain and microwave power were varied to confirm fine structures and optimization of signals. The signal height was computed as peak-to-peak amplitude of the first-derivative spectrum, and free radicals were quantitated as signal intensity, expressed as arbitrary units per unit sample weight (AU/mg).

Moisture Content and Water Activity. The moisture contents of the coffee beans were measured gravimetrically as per the standard procedure (*16*). Percent water activity (a_w) was measured by using an Aqua Lab CX 2T water activity meter (Decagon Devices, Inc.).

Total Phenols and Chlorogenic Acid Content. Total phenols and chlorogenic acid (CGA) content of the coffee beans were determined by using the standard procedures (*17*).

Statistical Analysis. The statistical significance of data was carried out by analysis of variance (ANOVA), using Origin version 4.10 software (Microcal Software Inc., Northampton, MA).

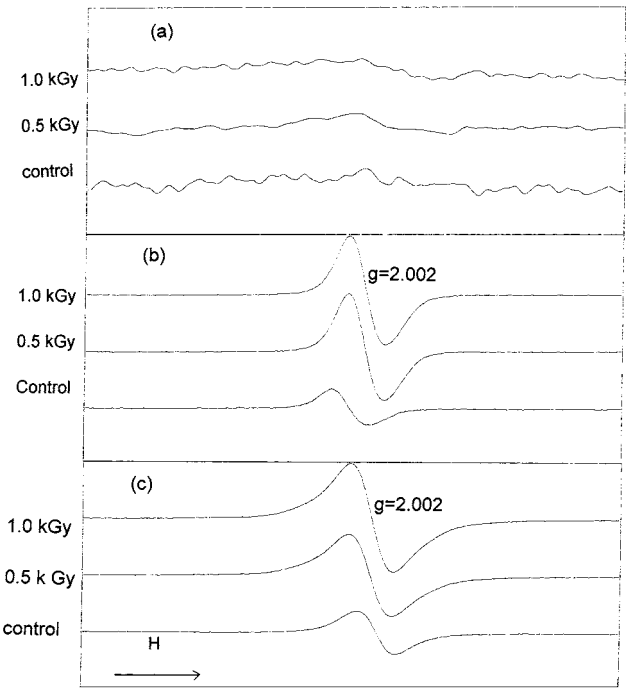

Figure 1. Effect of powdering, roasting, and γ-irradiation on ESR signal intensity of Arabica monsooned coffee beans: (a) green coffee, cut pieces (comminuted); (b) green coffee, comminuted and powdered; (c) roasted coffee, cut pieces. The weighed samples were entrapped in KCl powder in sample tubes and scanned for ESR signals as per details provided under Materials and Methods. Signal height data obtained at a receiver gain setting of 1×10^5 were normalized for 100 mg of sample weight and stack plotted as shown using computer software.

RESULTS AND DISCUSSION

Monsooned coffee beans showed insignificant natural abundance of free radicals measured in cut pieces of whole seed. The stack plot of normalized ESR signals for 100 mg of sample weight in **Figure 1** depicts (a) sole effect of γ-irradiation, (b) irradiation and powdering effect, and (c) roasting followed by irradiation of whole seed (cotyledons) of monsooned coffee beans. It can be seen that irradiation up to a quarantine dose of 1 kGy failed to give any measurable signal of free radicals in the majority of green Arabica monsooned coffee beans. Powdering and roasting generated free radical signals as observed on γ-irradiation alone. **Figure 1** also shows ESR signal enhancement by the combined effect of radiation and powdering or roasting. **Table 1** provides data on moisture and phenolics content of nonmonsooned and monsooned coffee beans. Monsooned coffee beans of both cultivars were found to have a statistically significant higher moisture content and water activity than nonmonsooned coffee beans. Similarly, chlorogenic acid, recognized as a more potent antioxidant than tocopherol or ascorbic acid (*18*), was found to be more abundant in monsooned varieties of both the cultivars. They have been shown to act as free radical scavengers in vivo (*19*). As reported earlier, the spermoderm (silver skin), a thin papery layer, remains embedded in the central groove of cured coffee beans (*13*). It showed total phenolics contents of 4.12 and 3.16 mg/g for nonmonsooned and monsooned varieties of cv. Robusta, whereas values for Arabica were merely 0.91 and 0.75 mg/g, respectively (data not given in **Table 2**). The plant kingdom is rich in quinone-like products that not only attribute pale yellow to dark brown color to the product but also contribute a strong ESR signal close to $g = 2.002$ (*13*, *20*). The histogram in **Figure 2**

Table 1. Total Phenols, Chlorogenic Acid, Moisture Content, and Water Activity in Non-Monsooned (NM) and Monsooned (M) Coffee Bean Cultivars[a]

coffee cv.	variety	total phenols (mg/g)	total chlorogenic acid (%)	moisture content (%)	water activity (%)
Arabica	NM	2.23 ± 0.02	6.35 ± 0.04	12.00 ± 0.03	58.46 ± 0.02
	M	3.83 ± 1.40*	7.08 ± 0.02*	18.98 ± 0.09*	84.24 ± 0.01*
Robusta	NM	4.76 ± 0.12	7.95 ± 0.16	10.94 ± 0.18	56.90 ± 0.00
	M	3.91 ± 0.04*	9.72 ± 0.04*	18.62 ± 0.16*	84.02 ± 0.02*

[a] Values are mean ± SD of five determinations. *, significantly different from non-monsooned variety data at $p < 0.05$.

Table 2. Free Radicals in Arabica Monsooned Coffee Beans after Annealing and γ-Irradiation[a]

	signal intensity (AU/mg)			
	Arabica		Robusta	
radiation dose (kGy)	untreated	annealed (50 °C, 68 h)	untreated	annealed (50 °C, 68 h)
0	7.5 ± 3.0a	75.0 ± 24.0	14.7 ± 4.7a	83.4 ± 7.0
0.25	9.3 ± 1.5a	442.8 ± 16.9	10.1 ± 1.8b	493.9 ± 66.6
0.50	7.5 ± 0.5a	756.5 ± 53.1	13.1 ± 2.6a	816.1 ± 235.3
0.75	7.8 ± 1.7a	1162.0 ± 152.4	11.1 ± 3.2a	1055.0 ± 42.7
1.00	8.3 ± 2.0a	1282.4 ± 56.5	10.7 ± 2.5a	1304.4 ± 133.2

[a] Values are mean ± SD of five independent observations. For untreated monsooned coffee beans, values with the same letter are not significantly different ($p > 0.05$). ESR instrumental settings, with the exception of receiver gain value of 1×10^5, were the same as described under Materials and Methods.

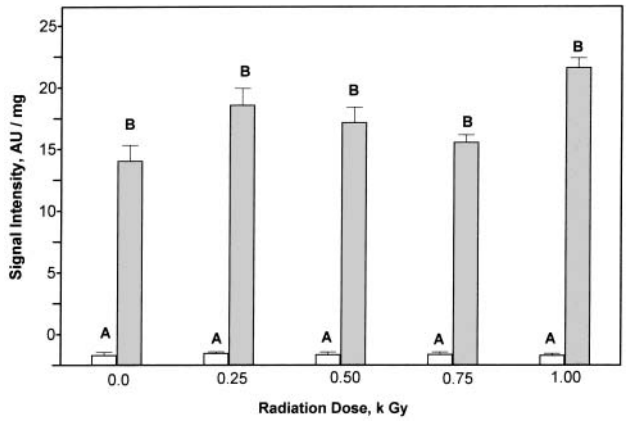

Figure 2. ESR signal intensity in parts of Arabica monsooned coffee beans. Comminuted whole seed (cotyledon, white bars) and silver skin (spermoderm, shaded bars) were entrapped individually in KCl powder and scanned for ESR spectra. Each column represents the mean ± SD of three independent observations. Columns marked with the same letter are not statistically significant at $p > 0.05$.

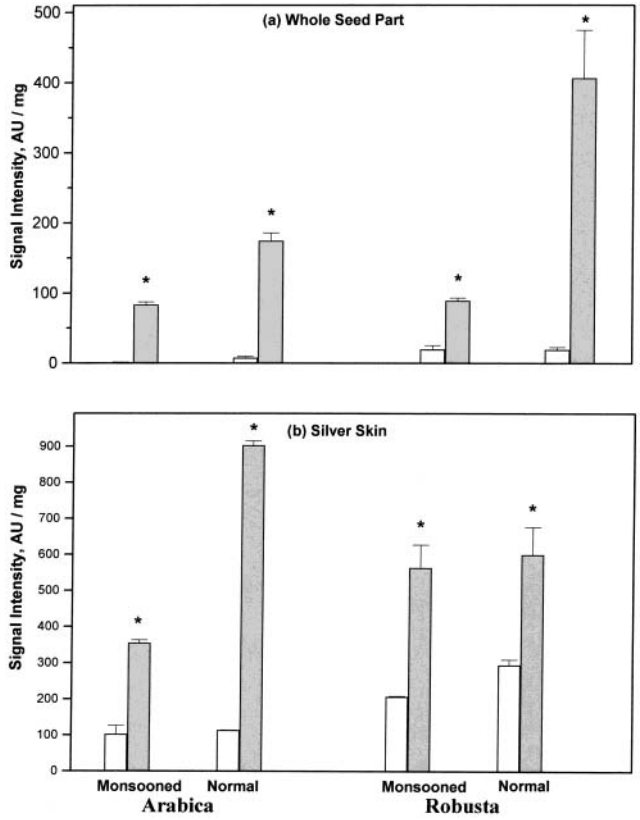

Figure 3. Effect of powdering on free radicals in monsooned and normal coffee bean cultivars. Whole seed and silver skin were comminuted and used as such (white bars) or after powdering (shaded bars) in a glass pestle and mortar. Data of powdered samples in columns marked with an asterisk are significantly different from corresponding cut pieces of both monsooned and normal coffee bean samples at $p < 0.05$.

shows the natural occurrence of free radicals in cotyledons of monsooned coffee beans as insignificant but ~20 times more significant in silver skin. γ-Irradiation up to a 1.0 kGy dose failed to cause any significant rise in free radical profiles of monsooned coffee bean parts. The data in **Figure 3** compare normal and monsooned coffee beans of the two cultivars in terms of powdering. This common kitchen practice escalated the free radical signals manyfold in both cultivars, but the observed increase was greater in nonmonsooned than in monsooned coffee beans. In general, the natural occurrence of free radicals in Robusta was greater than in Arabica. Similarly, the silver skin of Robusta, already rich in free radical population, seems to favor both generation and decay of free radicals due to radical−radical interaction on powdering (**Figure 3b**). Fine powdering in a glass pestle and mortar involves pounding and vigorous grinding, resulting in mild heating of the sample. Therefore, studies were undertaken to examine the status of free radicals following mild heat treatment in an oven (50 °C, 68 h). Heating resulted in 8.07 and 8.57% losses of moisture content in monsooned and nonmonsooned coffee beans, respectively. The moisture content and particularly bound water play crucial roles in controlling the net radiochemical changes on irradiation and storage (*21*, *22*). Annealed coffee beans were subsequently irradiated with various doses of γ-radiation and examined for ESR signals. The quantitative data on free radicals in Arabica and Robusta monsooned coffee beans failed to show any significant increase following irradiation but gave a statistically significant linear dose−response relationship following irradiation after annealing (**Table 2**). Our observations on the status of free radicals in normal and heat-treated monsooned coffee beans seem to suggest that the annealing process partially reversed the monsooning phenomenon. This hypothesis was examined in terms of textural index of coffee beans, affected by heating and radiation processing. The compression test tracings in **Figure 4a** reveal a low textural property of monsooned coffee beans being augmented by the process of mild oven heating. Similarly, the histogram in **Figure 4b** reveals

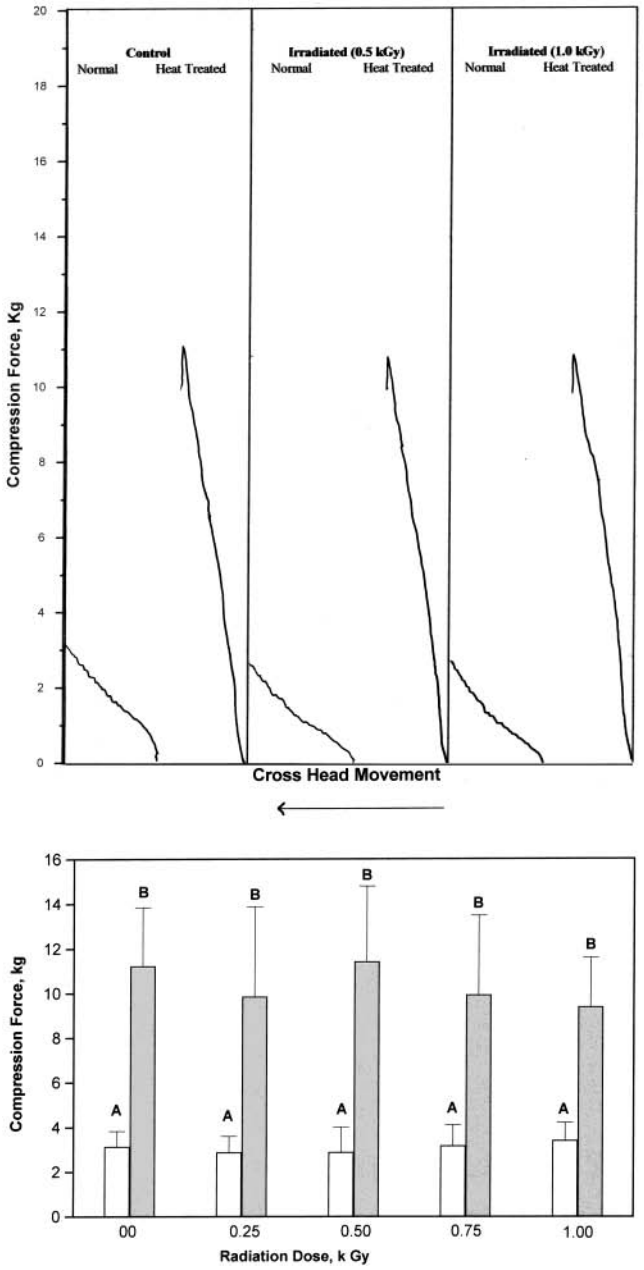

Figure 4. Compression test of Arabica monsooned coffee beans treated with mild heat and γ-irradiation: (a, top) force–distance compression curves of monsooned Arabica coffee beans irradiated before and after heating in a hot air oven (50 °C, 68 h); (b, bottom) histogram showing compression force data for monsooned coffee beans before (white bars) and after annealing (shaded bars). Data of columns marked with a "B" are statistically significant from "A" at $p < 0.05$.

that a compression force of 3.2 ± 0.7 kg exerted by the cotyledon part of monsooned coffee beans remained unaffected to γ-radiation exposure but increased significantly to 11.2 ± 2.6 following exposure to heat. The acquired new value was close to 7.8 ±1.9, observed for normal coffee beans of the same cultivar (data not shown in **Figure 4**). On physical examination, the monsooned coffee beans were found to be soft and amenable to knife cutting, whereas heat-treated monsooned beans became very hard, requiring a nail cutter for comminuting them into pieces. Moreover, on compression testing with the Instron Texture Testing Machine the latter gave a cracking sound, whereas nonheated monsooned coffee beans just compressed without exerting any sound or much force.

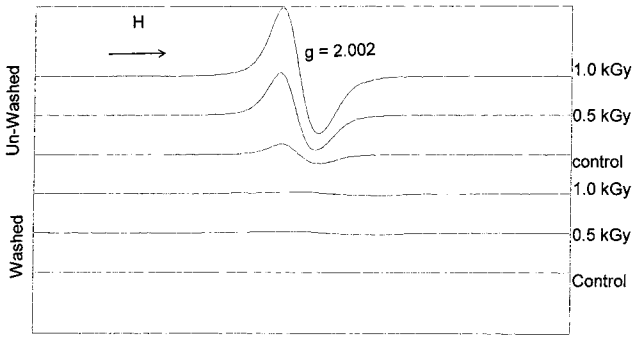

Figure 5. Free radicals profile in Arabica monsooned coffee powder exposed to γ-radiation doses before and after methyl cellosolve washings.

In biological samples, the nature and numbers of free radical species depend on the radiation dose, dose rate, and external conditions such as temperature, water content, and microenvironment around the molecules changed (*23*). Phenols are ubiquitously present in the plant kingdom and are major contributors of ESR signals due to their high *G* value, that is, number of molecules destroyed or products formed per 100 eV of energy absorbed (*24*). The validation of the statement was examined by ESR scanning of nonmonsooned Arabica coffee beans irradiated as such or after phenolic washings. ESR scanning in **Figure 5** show a dose-dependent increase in the signal of free radicals in the endosperm part of normal Arabica coffee beans, which was eliminated following methyl Cellosolve washings. Washed coffee powder on subsequent irradiation failed to show any substantial ESR signal, indicating the role played by the polyphenols. In γ-irradiated coffee beans, whereas phenols play a major role in contributing for free radicals, the spatial distribution of water, absorbed during monsooned curing, seems to play a crucial role in making them inactive and thus clearing the way for consumer's acceptance of radiation hygienized monsooned coffee beans.

From the consumer's acceptance point of view and for finding a better market for international trade in high-priced commodities, Indian Monsooned Malabar Coffee was found to be most suitable for processing by γ-irradiation for hygienic and quarantine purposes.

Irradiated monsooned coffee beans can safely be labeled with the irradiation logo and a message saying "free from fumigants".

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