

Stability of the Antioxidant Activity of Flavonoids after Gamma Irradiation

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ABSTRACT

Food irradiation has the purpose of destroying insects or microorganisms, thereby increasing the safety and shelf life of foods. Flavonoids are ubiquitous plant secondary products with radical scavenger ability. In the present study their antioxidant stability after gamma irradiation was evaluated. The flavonoids showed fast scavenger ability measured with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) after high doses of radiation. The low damage caused by irradiation on antioxidant capacity of the flavonoids shows their potential use in combating chemical oxidation of biomolecules in irradiated foods.

Keywords: Flavonoid, Food Irradiation, Scavenger Capacity, Gamma Irradiation

1. Introduction

Radiation treatments of biological materials have been applied for various processes: sterilization of materials, reduction of microbial contamination of food, and increasing the safety and shelf life of foods. Gamma irradiation can damage biomolecules directly by rupturing covalent bonds as a result of transfer of photon energy, and indirectly, by producing free radicals and other non-radical reactive oxygen species [1]. Investigations have shown that free radicals cause chemical oxidation [2] and oxidative stress [3] which makes the recovery of biologically active materials unlikely.

Flavonoids are among the most ubiquitous of plant secondary metabolites and aid in the reproduction and protection of plants [4]. Their low toxicity and high antioxidant activity [5] have been documented. The evaluation of the radioprotective effects of flavonoids on organoleptic properties, sensory and cytological has been described in strawberries [6]. Recently, some studies showed the radioprotective effects of flavonoids in gamma-irradiated mice [7-9]. Irradiated flavonoid solutions produce chemical reactions between the solute and reactive species, which are the result of the radiolyzed solvent operating to quickly repair the chemical modifications to biomolecules [10,11].

One of the most important probes for evaluating the fast repair of antioxidant capacity is the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), a stable nitrogen synthetic radical [12]. Therefore, the aim of our study was to evaluate the free radical scavenging ability and radiostability of flavonoids isolated from *S. jacobinensis* bark after a high dose of γ -radiation.

2. Materials and Methods

2.1. Flavonoid Isolation

S. jacobinensis bark was collected from trees in the semi-arid region of the state of Pernambuco, Brazil. *S. jacobinensis* dry bark was mixed in a beaker with 250 ml of ethanol/acetic acid/water (70:4:26, v:v:v) for 2 h at 4°C. The extract containing flavonoids was chromatographed in an anion-exchange DEAE-Cellulose (Sigma) column (40 × 2.5 cm) equilibrated with ethanol-water (70:30, v:v). Elution was performed with ethanol-acetic acid-water (70:4:26, v:v:v) at a flow rate of 2 ml/min. Each fraction collected was checked by thin layer chromatography (TLC) to determine their compositions using ethyl acetate/formic acid/acetic acid/water (100:11:11:26, v:v:v:v). Vanillin dipping in hydrochloric acid was used for detection under UV (254 nm). TLC conditions were

optimized using (+)-catechin standard. The obtained flavonoid was denominated SejaBF.

2.2. LC-ESI-MS

The analysis of SejaBF was revealed by LC analyses (Waters) with a diode-array detector, in a Deltapak C₁₈ column (2.0 mm × 150 mm; 3.5 μm size; 60 Å; Walters) at a flow rate of 0.4 ml/min, keeping the column at a constant temperature (30°C) and using 0.1% TFA/H₂O (solvent A) and 90% ACN/0.1 % TFA/H₂O (solvent B) as solvents. A linear gradient elution was used with 5% - 95% B in A for 30 min. The molecular mass was determined by electrospray ionization ion-trap mass spectrometry using a Waters LC/ESI+/MS scanning at 200 - 2500 m/z.

2.3. Gamma Irradiation

The SejaBF aliquots (1 mg/ml) in 10 mM phosphate buffer (pH 7.0) in borosilicate glass vials (16 - 125 mm) were frozen and irradiated in atmospheric O₂ using a Gammacell 220 Excel ⁶⁰Co gamma ray irradiator (Ontario, Canada) at doses of 0.020, 0.8, 3.0 and 35 kGy at a rate of 8.8 kGy/h.

2.4. Antioxidant Activity Determination

Spectrophotometric analysis was used to determine the inhibition concentration (IC₅₀) and the inhibition percentage (IP). IC₅₀ is the amount of antioxidant necessary to decrease the initial concentration of DPPH radical (75 μM) by 50%. IP is the percentage of total DPPH radical (75 μM) which reacted with antioxidant at the steady

state (20 min). The experiments were performed using the SmartSpec 3000 spectrophotometer (Bio-Rad) according to the methods of Soler-Rivas [13] and Moure [14]. Pure methanol was used as a negative control. (+)-Catechin and ascorbate in methanol (1 mg/ml) were used as positive controls. The disappearance of the DPPH radical was monitored by the decrease in absorbance at 515 nm. The DPPH radical concentration in the reaction mixture was calculated by the calibration curve according to the following nonlinear regression equation ($R = 0.997$): $A_{515\text{ nm}} = 0.01029 [\text{DPPH}] - 0.0177$, where [DPPH] is expressed in mg/ml.

2.5. Statistical Analysis

Data are presented as mean ± S.E.M of five independent experiments. The curve, student's t test and its 95% confidence intervals (CI 95%) were obtained by nonlinear regression using the GraphPrism® program (GraphPad Software Inc., San Diego, CA, USA).

3. Results and Discussion

Flavonoids, denominated *S. jacobinensis* bark flavonoids (SejaBF), were isolated and partially characterized by a combination of anion-exchange DEAE-Cellulose and high performance liquid chromatography. The LC/MS protocol showed positive molecular ion m/z 307 (M+1)—epigallocatechin and m/z 613 (2M+1), which could be an adduct of epigallocatechin (**Figure 1**). Adduct formation with sodium and a protonated water adduct have been observed in positive ion mode for most compounds

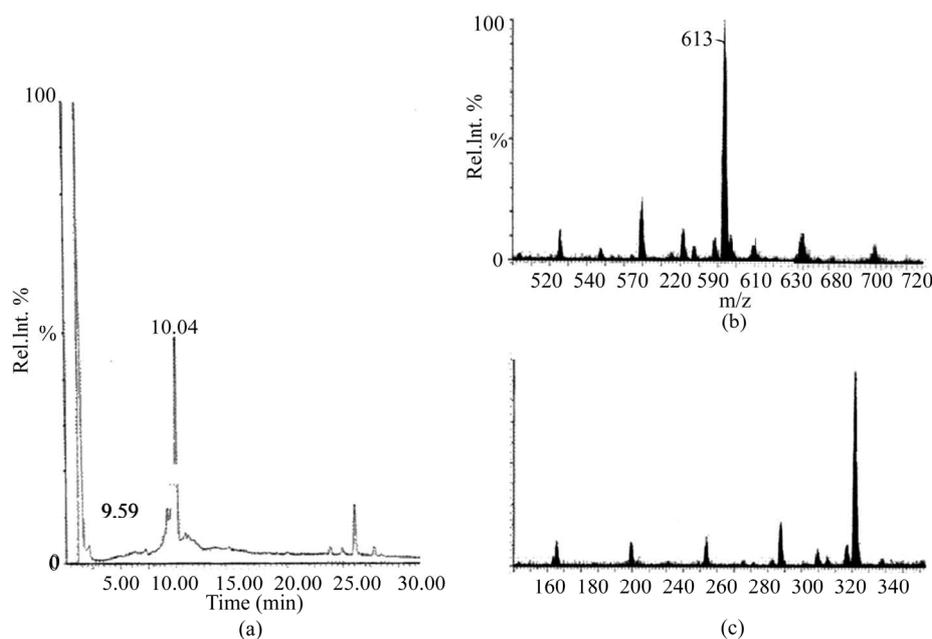


Figure 1. *S. jacobinensis* flavonoids (SejaBF) ESI-MS spectra. SejaBF reverse phase chromatography in C₁₈ column by HPLC system (a). MS with retention time of 10.04 min (b) and 9.59 min (c).

[15]. The identification of heterogeneous adducts by antioxidant capacity and HPLC/MS protocol was demonstrated in order to perform an analysis of the procyanidins [16]. However, UV/VIS spectrum and a fragmentation pattern (MS/MS) should be used in future studies for complete characterization.

SejaBF exhibited high antioxidant capacity by the DPPH method when compared to ascorbate, an antioxidant widely used as a standard. However, its antioxidant activity decreased after irradiation, in relation to the IC_{50} and IP (Table 1). IP reduction (19.2% at high dose) was significant although the IC_{50} reduction was not.

DPPH radical scavenger capacity (%DPPH_{REM}) was significantly reduced only in the initial 5 min after a high dose of radiation (Figure 2). The DPPH radical is scavenged by antioxidants through donation of hydrogen to form a stable, reduced DPPH molecule. The radical scavenger is reduced after high doses of gamma radiation due to displacement from the B-ring to the 2,3-double bond, indicating a relatively small reactivity (because of the absence of high density spin on a given site) for these intermediate species [17,18]. The existence of such intermediate species after irradiation apparently does not abolish the antioxidant properties and can maintain the radioprotective effects of flavonoids. A feature already observed in gamma-irradiated strawberry [6]. Our results are in good agreement with the presence of stabilized quinones, which have been observed after H-abstraction from the B-ring of flavonoids in radiolytic solutions [19]. Irradiation can cause oxidative damage and impair flavor in food. However, the action of effective and radiostable natural antioxidants may prevent chemical oxidation of biomolecules in irradiated food.

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Table 1. Radical scavenging performance.

Samples	IP (%)	IC_{50} (mg/g DPPH)
SejaBF 20 Gy	89.13 ^a	8.11 ± 0.14 ^a
SejaBF 800 Gy	7.90 ± 0.14 ^a	88.30 ^a
SejaBF 3 kGy	79.63 ^{bf}	12.70 ± 0.14 ^{bf}
SejaBF 35 kGy	70.88 ^{cf}	21.17 ± 0.28 ^{cf}
SejaBF non-irradiated	90.11 ^a	8.89 ± 0.28 ^a
(+)-catechin	90.85 ^a	14.53 ± 0.01 ^d
Ascorbate	39.22 ^b	92.94 ± 0.01 ^c

Values with the same superscript letter are not significantly different at $p < 0.05$ (Student's T-test). IC_{50} and IP (0.5 mg/ml) of SejaBF were calculated at the steady state (20 min). [#]Values of IP (%) are significantly different at $p < 0.05$ in relation to non-irradiated SejaBF.

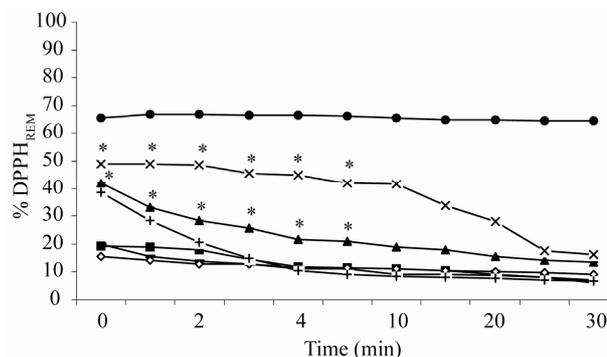


Figure 2. Kinetic behaviour of irradiated antioxidants. The error in the determination of %DPPH_{REM} to relative values of different doses is approximately ± 1%, smaller than the size of the symbols. *Values significantly different at $p < 0.05$ (Student's T-test) in relation to (○) non-irradiated *S. jacobinensis* flavonoids (SejaBF). SejaBF after irradiation: (x) 35 kGy; (▲) 3 kGy; (■) 800 Gy; (□) 20 Gy; (●) ascorbate and (+)-catechin.

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REFERENCES

- [1] E. S. Kempner, "Effects of High-Energy Electrons and Gamma Rays Directly on Protein Molecules," *Journal of Pharmaceutical Sciences*, Vol. 90, No. 10, 2001, pp. 1637-1646. doi:10.1002/jps.1114
- [2] J. Terao, M. Piskula and Q. Yao, "Protective Effect of Epicatechin, Epicatechin Gallate and Quercetin on Lipid Peroxidation in Phospholipid Bilayers," *Archives of Biochemistry and Biophysics*, Vol. 308, No. 1, 1994, pp. 278-284. doi:10.1006/abbi.1994.1039
- [3] O. I. Aruoma, "Free Radicals, Oxidative Stress, and Antioxidants in Human Health and Disease," *Journal of the American Oil Chemists Society*, Vol. 75, No. 2, 1998, pp. 199-212. doi:10.1007/s11746-998-0032-9
- [4] B. Winkel-Shirley, "Biosynthesis of Flavonoids and Effects of Stress," *Current Opinion in Plant Biology*, Vol. 5, No. 3, 2002, pp. 218-223. doi:10.1016/S1369-5266(02)00256-X
- [5] M. Garcia-Alonso, G. Rimbach, M. Sasai, M. Nakahara, S. Matsugo, Y. Uchida, J. C. Rivas-Gonzalo and S. de Pascual-Teresa, "Electron Spin Resonance Spectroscopy Studies on the Free Radical Scavenging Activity of Wine Anthocyanins and Pyranoanthocyanins," *Molecular Nutrition & Food Research*, Vol. 49, No. 12, 2005, pp. 1112-1119. doi:10.1002/mnfr.200500100
- [6] F. Breitfellner, S. Solar and G. Sontag, "Effect of Gamma Irradiation on Flavonoids in Strawberries," *European Food Research & Technology*, Vol. 215, No. 1, 2002, pp. 28-31. doi:10.1007/s00217-002-0536-8
- [7] N. Orsolić, V. Benković, A. Horvat-Knezević, N. Kopjar, I. Kosalec, M. Bakmaz, Z. Mihaljević, K. Bendelja and I. Basić, "Assessment by Survival Analysis of the Radio-

- protective Properties of Propolis and its Polyphenolic Compounds,” *Biological & Pharmaceutical Bulletin*, Vol. 30, No. 5, 2007, pp. 946-951. [doi:10.1248/bpb.30.946](https://doi.org/10.1248/bpb.30.946)
- [8] V. Benković, N. Orsolić, A. H. Knezević, S. Ramić, D. Dikić, I. Basić and N. Kopjar, “Evaluation of the Radioprotective Effects of Propolis and Flavonoids in Gamma-Irradiated Mice: The Alkaline Comet Assay Study,” *Biological & Pharmaceutical Bulletin*, Vol. 31, No. 1, 2008, pp. 167-172. [doi:10.1248/bpb.31.167](https://doi.org/10.1248/bpb.31.167)
- [9] V. Benković, A. H. Knezević, D. Dikić, D. Lisicić, N. Orsolić, I. Basić and N. Kopjar, “Radioprotective Effects of Quercetin and Ethanolic Extract of Propolis in Gamma-Irradiated Mice,” *Archives of Industrial Hygiene & Toxicology*, Vol. 60, No. 2, 2009, pp. 129-138. [doi:10.2478/10004-1254-60-2009-1908](https://doi.org/10.2478/10004-1254-60-2009-1908)
- [10] Y. Hanasaki, S. Ogawa and S. Fukui, “The Correlation between Active Oxygens Scavenging and Antioxidative Effects of Flavonoids,” *Free Radical Biology & Medicine*, Vol. 16, No. 6, 1994, pp. 845-850. [doi:10.1016/0891-5849\(94\)90202-X](https://doi.org/10.1016/0891-5849(94)90202-X)
- [11] D. Kozłowski, P. Marsal, M. Steel, R. Mokrini, J. L. Duroux, R. Lazzaroni and P. Trouillasa, “Theoretical Investigation of the Formation of a New Series of Antioxidant Depsides from the Radiolysis of Flavonoid Compounds,” *Radiation Research*, Vol. 168, No. 2, 2007, pp. 243-252. [doi:10.1667/RR0824.1](https://doi.org/10.1667/RR0824.1)
- [12] W. Brand-Williams, M. E. Cuvelier and C. Berset, “Use of a Free Radical Method to Evaluate Antioxidant Activity,” *LWT-Food Science and Technology*, Vol. 28, No. 1, 1995, pp. 25-30.
- [13] C. Soler-Rivas, J. C. Espin and H. J. Wichers, “An Easy and Fast Test to Compare Total Free Radical Scavenger Capacity of Foodstuffs,” *Phytochemistry Analysis*, Vol. 11, No. 5, 2000, pp. 1-9. [doi:10.1002/1099-1565\(200009/10\)11:5<330::AID-PCA534>3.0.CO;2-G](https://doi.org/10.1002/1099-1565(200009/10)11:5<330::AID-PCA534>3.0.CO;2-G)
- [14] A. Moure, D. Franco, J. Sineiro, H. Dominguez, M. J. Núñez and J. M. Lema, “Antioxidant Activity of Extracts from Gevuina Avellana and Rosa Rubiginosa Defatted Seeds,” *Food Research International*, Vol. 34, No. 2-3, 2001, pp. 103-109. [doi:10.1016/S0963-9969\(00\)00136-8](https://doi.org/10.1016/S0963-9969(00)00136-8)
- [15] P. Swatsitang, G. Tucker, K. Robards and D. Jardine, “Isolation and Identification of Phenolic Compounds in Citrus Sinensis,” *Analytica Chimica Acta*, Vol. 417, No. 2, 2000, pp. 231-240. [doi:10.1016/S0003-2670\(00\)00937-5](https://doi.org/10.1016/S0003-2670(00)00937-5)
- [16] D. Ferreira and D. Slade, “Oligomeric Proanthocyanidins: Naturally Occurring O-Heterocycles,” *Natural Product Reports*, Vol. 19, No. 5, 2002, pp. 517-541. [doi:10.1039/b008741f](https://doi.org/10.1039/b008741f)
- [17] C. Zhao, Y. Shi, W. Wang, W. Lin, B. Fan, Z. Jia, S. Yao and R. Zheng, “Fast Repair Activities of Quercetin and Rutin toward dGMP Hydroxyl Radical Adducts,” *Radiation Physics and Chemistry*, Vol. 63, No. 2, 2002, pp. 137-142. [doi:10.1016/S0969-806X\(01\)00224-9](https://doi.org/10.1016/S0969-806X(01)00224-9)
- [18] A. Marfak, P. Trouillas, D. P. Allais, Y. Champavier, C. A. Calliste and J. L. Duroux, “Radiolysis of Kaempferol in Water/Methanol Mixtures. Evaluation of Antioxidant Activity of Kaempferol and Products Formed,” *Journal of Agricultural and Food Chemistry*, Vol. 51, No. 5, 2003, pp. 1270-1277. [doi:10.1021/jf020836g](https://doi.org/10.1021/jf020836g)
- [19] A. S. Pannala, T. S. Chan, P. J. O’Brien and C. A. Rice-Evans, “Flavonoid B-Ring Chemistry and Antioxidant Activity: Fast Reaction Kinetics,” *Biochemical and Biophysical Research Communications*, Vol. 282, No. 5, 2001, pp. 1161-1168. [doi:10.1006/bbrc.2001.4705](https://doi.org/10.1006/bbrc.2001.4705)