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*Research Article*

# Effect of Preservation Methods on Physicochemical Quality, Phenolic Content, and Antioxidant Activity of Stevia Leaves

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The effect of freeze-drying and gamma irradiation at 0.5, 1, and 2 kGy on the physicochemical composition (moisture, fat, ash, mineral, and chlorophyll contents), microstructure, total phenolic content, and antioxidant capacity of stevia leaves was investigated in the present study. The results obtained indicated that freeze-drying and gamma irradiation treatments caused significant reduction ($p \leq 0.05$) of moisture and fat contents in comparison with those of commercial leaves, while ash content was not significantly affected. Mineral composition was analysed. Among the analysed elements, potassium and iron levels were higher in the freeze-dried and irradiated samples, respectively. The microstructure was analysed using a scanning electron microscope. Micrographs revealed that a higher porous size structure was obtained by freeze-drying, and degradation of cell wall structure was more clearly visualized by irradiation at 2 kGy. However, the main functional groups were stable as confirmed by Fourier transform infrared spectroscopy analysis. The effects on chlorophyll content, phenolic profile, and antioxidant properties were evaluated before and after ten months of storage. In terms of chlorophyll contents, the freeze-dried leaves exhibited the highest content. Chlorophylls a and b decreased when storage progressed for freeze-dried leaves as well as for gamma-irradiated leaves. Both preservation methods gave significant advantages in increasing the total phenolic content and DPPH scavenging activity. Moreover, a significant increase of bioactive compounds and antioxidant activity was observed as the gamma irradiation dose increased. In addition, the storage time increased the amounts of polyphenols and DPPH scavenging activity. After 10 months of storage, gamma-irradiated leaves had the highest total phenolic content as well as the DPPH scavenging activity followed by freeze-dried leaves. The results indicate that freeze-drying and gamma irradiation at the studied doses could be effective postharvest methods for preservation of stevia leaf quality.

## 1. Introduction

Stevia (*Stevia rebaudiana* Bertoni), native to Brazil and Paraguay, is a genus of appropriately 200 species of perennial herbs belonging to the Asteraceae family [1, 2]. The leaves of stevia contain sweetening compounds called steviol glycosides, and the most abundant are stevioside and rebaudioside A which are nearly 300 times sweeter than sugar; their intense sweetness has made them significant scientific and commercial interest worldwide [3, 4]. In Morocco, the culture of stevia was officially introduced for the first time in 2008. Since then, this plant was grown by smallholders or at an experimental level by several public or private institutions, within research programs [5].

Stevia leaves as a natural sweetener are used as sugar and artificial sweetener substitutes resulting in the increasing harmful health impacts of sugars such as obesity and diabetes [1]. Some of the artificial sweeteners such as aspartame, saccharin, acesulfame-K, neotame, and sucralose with their extensive utilization lead to fatal maladies such as cancer and phenylketonuria [2].

Assessment of the microbiological quality of stevia from local markets in Jordan revealed its contamination with aerobic mesophilic bacteria and fungi [6]. For the prevention of microbial contamination and the production of high quality of medicinal and aromatic plants (MAPs), several technologies exist for the preservation of MAPs, among which are freeze-drying and gamma irradiation. Freeze-drying produces the highest quality food product obtainable by any drying method. Despite unmatched advantages, freeze-drying has always been considered the most expensive operation for manufacturing a dehydrated product owing to high energy consumption and high costs of both operation and maintenance [7]. It is well known that freeze-drying can retain the original color, aroma, taste, shape, and nutrients of fruits and vegetables to the utmost extent. However, the shortcomings of its long drying time and high energy consumption limit its wide application [8].

Radiation is a physical treatment for the preservation of many different types of food commodities. Being a cold process, irradiation can ensure shelf-life extension and eliminate microbial contamination while retaining the color, flavor, taste, and aroma of the food product [9]. A joint expert committee convened by the FAO/IAEA/WHO stated that irradiation of any food commodity up to 10 kGy presents no toxicological hazard [10].

For preserving stevia leaves, some drying methods are applied. Lemus-Mondaca et al. [11] evaluated the influence of different drying techniques (convective drying and vacuum-, microwave-, infrared-, sun-, shade-, and freeze-drying) on proximal analysis, vitamins C and E, fatty acid and amino acid profiles, and steviosides from stevia leaves. Gasmalla et al. [12] determined the effect of three drying methods (sunlight for about 5 days, oven at 60°C for 16 h, and microwave). Periche et al. [13] applied different drying conditions (hot-air-drying at 100°C and 180°C, freeze-drying, and shade-drying). Hidar et al. [14] investigated the effect of solar convective drying on kinetics and quality attributes of stevia leaves grown in Morocco. In addition, the far-infrared-drying technique was used to dry *Stevia rebaudiana* leaves [15].

Aromatic and medicinal herbs are among the products submitted to decontamination assays based on irradiation treatment [16]. *Aloysia citrodora* P., *Melissa officinalis* L. and *Melittis melissophyllum* L. [16], *Mentha piperita* L. [17], *Thymus vulgaris* and *Mentha pulegium* [18], *Rosmarinus officinalis* [19], and *Thymus satureioides* [20] are among the studied plants, namely, submitted to gamma radiation. To date, there is no study available on the effects of gamma irradiation on stevia leaves. Thus, the aim of this study was to determine the effects of different processing methods (freeze-drying and gamma irradiation at low doses) on physicochemical quality and antioxidant activity of stevia leaves.

## 2. Material and Methods

*2.1. Raw Material.* Fresh and commercial dried *Stevia rebaudiana* Bertoni leaves used in this study were obtained from MOGADOR Cooperative (Essaouira, Morocco). Commercial samples were available as dried leaves for herbal infusion preparation.

*2.2. Methods*

*2.2.1. Sample Preparation Procedure.* In this study, freeze-drying and gamma irradiation processes were applied on fresh and commercial stevia leaves, respectively.

*(1) Freeze-Drying Process.* Fresh stevia leaves were first frozen overnight and then freeze-dried for 24 h at 0.100 mbar and −50°C in a freeze-dryer (Christ ALPHA 1-2 LD plus, France).

*(2) Gamma Irradiation Treatment.* Gamma irradiation was carried out in a cobalt-60-based gamma chamber in the BOUKHALEF ionization station of the National Institute for Agricultural Research (Tangier, Morocco) at a dose rate of 5.14 kGy/min. The samples were packed, sealed in polythene bags, and irradiated with low doses of 0.5, 1, and 2 kGy.

Following the preservation treatment, commercial (control), freeze-dried, and irradiated leaves were stored at room temperature, and all samples were evaluated at day 0 (treatment day) and 10 months after treatment.

*2.2.2. Determination of Proximal Composition.* Moisture, fat, and ash contents were determined according to the AOAC standard method [21].

*2.2.3. Analysis of Chlorophyll a and Chlorophyll b.* Stevia chlorophyll was extracted by soaking 200 mg of leaf sample in 4 mL of acetone-water mixture (90%) for 5 min according to the method described by Hidar et al. [14]. The absorbance of the extracts at 630, 645, and 665 nm was measured with a spectrophotometer (UV-2550, Shimadzu Corporation, Kyoto, Japan), and the chlorophyll contents were calculated using the following equations [22]:

(1) $\text{Chlorophyll a (mg/g)} = 11.6 * \text{A665} - 1.31 * \text{A645} - 0.14 * \text{A630}$

(2) $\text{Chlorophyll b (mg/g)} = -4.34 * \text{A665} + 20.7 * \text{A645} - 4.42 * \text{A630}$

where A665, A645, and A630 are the absorbance of the extracts at 665, 645, and 630 nm, respectively.

*2.2.4. Analysis of Mineral Composition.* The mineral composition of the samples was measured by X-ray diffraction using a portable XRF analyser (Olympus NDT, Waltham, USA) [23].

*2.2.5. Determination of Total Phenolic Compounds*

*(1) Preparation of Phenolic Extract*. The extracts used for the determination of total phenolic content were prepared according to the Hidar et al. [14] method.

*(2) Determination of Total Phenolic Content*. 50 $\mu$l of the extract of the stevia leaves was mixed with 0.25 ml of Folin–Ciocalteu reagent and 1.25 ml of deionized water. The mixture was vortexed and kept at room temperature for 3 min, and then 0.5 ml of 20% sodium carbonate was added. The mixture was incubated at 40°C for 30 min. The absorbance was measured at 750 nm using a spectrophotometer (UV-2550, Shimadzu Corporation, Kyoto, Japan). The content of total phenols was expressed in mg of gallic acid equivalent (GAE) per 1 g of stevia plant.

*2.2.6. Determination of DPPH Radical Scavenging Activity.* The free-radical scavenging effect was estimated according to the method of Periche et al. [13].

*2.2.7. FTIR Spectroscopy for Quantitative Analysis.* Fourier transform infrared (FTIR) spectra were plotted using VERTEX 70 (Bruker, VERTEX 70 DTGS, Germany) operating in the range of 4000–400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. The FTIR spectra were taken in the transmittance mode. The characterized discs consisted of dried stevia leaves previously ground and mixed thoroughly with potassium bromide in 1 : 99 (sample: KBr) ratio, respectively.

*2.2.8. Microstructure Analysis Using Scanning Electron Microscopy (SEM).* In order to investigate the effect of preservation methods on the microscopic structure, the stevia leaves were analysed with a VEGA3 TESCAN scanning electron microscope (Tescan, Brno, Czech Republic). To stabilize the surface structure, the samples were carbon-coated using a Cressington 108 carbon/A carbon coater. The acceleration voltage was set at 15 kV in order to be sensitive to the extreme surface morphology.

*2.2.9. Statistical Analysis.* All analyses were performed at least in triplicate, and mean values were reported. IBM SPSS 20.0 software was used to perform the statistical analyses. When significant differences were observed ($p \leq 0.05$), Tukey's test was used to determine the differences among the mean values.

## 3. Results and Discussion

*3.1. Proximate Composition Determination.* The results of proximal properties of stevia are presented in Table 1. The results showed that there were no significant differences ($p > 0.05$) in ash content; however, the moisture and fat contents decreased after freeze-drying and gamma irradiation. The fat content was lower at the end of the preservation processes. For gamma irradiation, the reduction in fat levels could have been caused by a reduced activity of the enzymes involved in the de novo synthesis of fatty acids induced by the irradiation treatment [24]. Besides, irradiation can cause fat oxidation [25].

In other study, Lemus-Mondaca et al. [11] found values of 10.5 and 9.86 g/100 g for fat and ash contents for freeze-dried stevia, respectively. The results obtained are in agreement with those of previous work done by Khattak [26] who reported that the ash content was found to be the same for control and irradiated samples of *Emblica officinalis* up to the dose levels of 9 kGy. Additionally, Pinela et al. [24] found that irradiation of *Rumex induratus* leaves with 1, 2, and 6 kGy had a significant effect on the fat content.

Regarding the effect of gamma irradiation, the different doses induced no significant changes ($p > 0.05$) in the moisture, fat, and ash contents. Our findings revealed that the effect of gamma irradiation on proximate composition is not dose-specific. This finding is generally in agreement with that of the previous study on ash content of chyawanprash [27].

*3.2. Mineral Content.* As reported in the literature, stevia is a good source of minerals (K, Ca, Na, Mg, Cu, Mn, etc.) [28]. Table 1 shows the results of the effect of freeze-drying and gamma irradiation on the mineral elements, i.e., calcium, potassium, zinc, iron, phosphorus, and manganese, in stevia leaves. The results showed that stevia (commercial, freeze-dried, and irradiated) contains potassium and calcium as highlighted elements, which are in agreement with the findings of Lemus-mondaca et al. [11]. Potassium aids in the maintenance of normal fluid and mineral balance in the control of blood pressure. Calcium aids in the formation of strong bones and teeth [29].

The results indicated that freeze-drying and gamma irradiation treatments caused no significant changes ($p > 0.05$) in Ca, P, and Zn contents, while they caused fluctuations in the Mn, Fe, and K contents ($p \leq 0.05$) (Table 1). Potassium and iron concentration levels were higher in this study for freeze-dried and irradiated stevia, respectively. Moreover, the manganese levels in the freeze-dried and irradiated samples were significantly higher. Similarly, Sanni et al. [30] found that gamma irradiation increased the presence of magnesium, calcium, iron, and manganese in the treated samples of sorrel seeds (*Hibiscus sabdariffa*) and decreased the presence of sodium, potassium, lead, and copper when the increase in the dose of irradiation was observed. However, Bamidele and Akanbi [31] reported that gamma irradiation has no significant effect on mineral content of the pigeon pea flour. For all mineral elements, the values had no observable trends with increasing irradiation doses. In contrast, Hassan et al. [32] studied the effect of gamma irradiation on the levels of calcium, phosphorus, and iron in peanut at doses of 1.0, 1.5, and 2.0 kGy and reported that there was a gradual increase in the levels of these minerals with increasing irradiation doses.

Table 1: Proximate composition (g/100 g) and mineral elements (mg/kg dm) of commercial, freeze-dried, and gamma-irradiated stevia.

| | Commercial (C) | Freeze-dried (FD) | Gamma-irradiated (GI) | | |
|---|---|---|---|---|---|
| | | | 0.5 kGy | 1 kGy | 2 kGy |
| Moisture content | $9.93 \pm 0.9^{a}$ | $6.86 \pm 0.2^{c}$ | $8.99 \pm 0.9^{b}$ | $9.22 \pm 0.9^{b}$ | $8.89 \pm 1^{b}$ |
| Fat content | $3.41 \pm 0.04^{a}$ | $2.34 \pm 0.03^{b}$ | $2.13 \pm 0.03^{c}$ | $1.96 \pm 0.02^{c}$ | $1.63 \pm 0.02^{c}$ |
| Ash content | $8.72 \pm 1^{a}$ | $9.12 \pm 0.9^{a}$ | $8.49 \pm 0.8^{a}$ | $8.58 \pm 0.8^{a}$ | $9.36 \pm 1^{a}$ |
| Mineral content (ppm) K | $4458 \pm 38^{c}$ | $9000 \pm 50^{a}$ | $7819 \pm 53^{b}$ | $7796 \pm 37^{b}$ | $7633 \pm 56^{b}$ |
| Ca | $8360 \pm 48^{a}$ | $9104 \pm 55^{a}$ | $9614 \pm 55^{a}$ | $8943 \pm 52^{a}$ | $8940 \pm 53^{a}$ |
| P | $1031 \pm 41^{a}$ | $1091 \pm 43^{a}$ | $1041 \pm 41^{a}$ | $1024 \pm 42^{a}$ | $1038 \pm 46^{a}$ |
| Mn | $304 \pm 18^{b}$ | $402 \pm 21^{a}$ | $483 \pm 21^{a}$ | $443 \pm 19^{a}$ | $431 \pm 25^{a}$ |
| Fe | $541 \pm 17^{b}$ | $515 \pm 15^{b}$ | $954 \pm 22^{a}$ | $950 \pm 17^{a}$ | $945 \pm 24^{a}$ |
| Zn | $36 \pm 2^{a}$ | $34 \pm 2^{a}$ | $39 \pm 2^{a}$ | $37 \pm 2^{a}$ | $32 \pm 2^{a}$ |

The results are presented as the mean ± standard deviation (SD; $n = 3$). Different letters in the same row indicate significant differences at $p \leq 0.05$.

The significant differences in mineral content found between the samples were most probably not an effect of the preservation method, but this might be due to a heterogeneous distribution of the minerals in the analysed sample. It could also mean that the minerals are not evenly distributed in the different plant tissues of stevia [33].

*3.3. FTIR Analysis.* The presence of functional groups in commercial and processed stevia samples was analysed by FTIR spectroscopy. In general, FTIR profiles from the different stevia leaves (Figure 1) indicated the presence of the peak near 3417 $cm^{-1}$ (3200–3550 $cm^{-1}$), mainly due to the C-O-H bending vibrations, which is associated with the presence of the hydrogen bond [34]. The stevia FTIR spectrum also showed asymmetric and symmetric stretching vibrations of $sp^3$-CH appearing at 2926 and 2870 $cm^{-1}$ [35, 36]. The low intensity band, which was recorded at 1730 $cm^{-1}$, is assigned to the stretching vibration of the $^{-}$C=O bond ($^{-}$C=O stretching), and this is due to the presence of steviol glycosides, which also have the characteristic band [34]. The band around 1643 $cm^{-1}$ is also assigned to the stretching vibration of the $^{-}$C=O bond (-C=O stretching). Furthermore, the bands at 1441 $cm^{-1}$ and 1359 $cm^{-1}$ correspond to the bending vibration of the CH bond (1470–1350 $cm^{-1}$). Finally, the FTIR spectra of the samples showed bands at 1154, 1072, and 1024 $cm^{-1}$, which are characteristic absorption bands of the steviol glycosides [34].

Based on the obtained FTIR spectra, the applied preservation methods led to a similar band profile for the studied stevia samples.

*3.4. Microstructure Examination.* The effects of different preservation methods on the microstructure of stevia samples were observed under scanning electron microscopy.

From the obtained images (Figure 2), the treatment effects on tissue structure can be observed by comparing all treated leaves. During freeze-drying, the ice sublimation creates pores, the walls of which may shrink due to surface forces. The porous structure, an important symbol for the freeze-dried products, allows for a fast rehydration process because water easily reoccupies the empty spaces [7, 36]. A similar observation was previously reported for freeze-dried bananas and lemon balm, respectively [36, 37].

Regarding gamma irradiation impact, no significant effect with doses of 0.5 and 1 kGy was observed on the microstructure when compared with those in commercial samples [38]. However, irradiation at 2 kGy caused a shape deformation with the breakage of the structure.

A similar result was found for mango irradiated in the 0.5 kGy to 0.75 kGy irradiation dose range; more intact cells were shown [39].

*3.5. Chlorophyll Analysis.* Color is a key quality factor that influences consumer acceptance and the market value of products [40]. Table 2 shows the obtained values of chlorophyll content of treated and untreated stevia. Our results suggested that freeze-dried leaves showed significantly higher chlorophyll contents before and after 10 months of storage ($p \leq 0.05$). It was confirmed that freeze-drying led to the maximum retention of chlorophylls (Chl a, Chl b) in *Hibiscus sabdariffa* leaves [41]. Our results are in agreement with other findings; Branisa et al. [42] reported that freeze-drying has less degradation effect on chlorophyll content compared to other thermal methods used for drying of *Melissa officinalis* and *Urtica dioica*. Yu et al. [43] had shown that freeze-dried asparagus has high contents of Chl a and Chl b compared to vacuum-dried, infrared-dried, or hot-air-dried samples.

It has been suggested that the freeze-drying process resulted in the formation of ice crystals and caused rupturing of cell structures leading to better solvent access and extraction [42].

There are few studies analysing the behaviour of gamma radiation on chlorophyll in plants and the levels of these compounds during storage. In this study, the effects of irradiation and storage on chlorophyll content are also indicated. Significant decreases in chlorophylls a and b were observed at 0.5, 1, and 2 kGy. Our data are in agreement with the results of previous work where the concentrations of chlorophylls a and b were lower in irradiated leaves [44]. It has been reported that the chlorophyll content in plants gradually decreases after irradiation, which may result from the release of chlorophyll from its protein complex, followed by dephytinization and possibly pheophytinization [45]. The

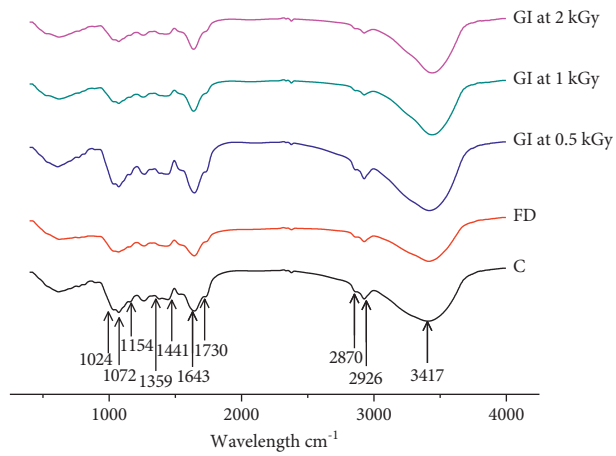


Figure 1: FTIR spectra of stevia leaves. C: commercial; FD: freeze-dried; GI: gamma-irradiated.

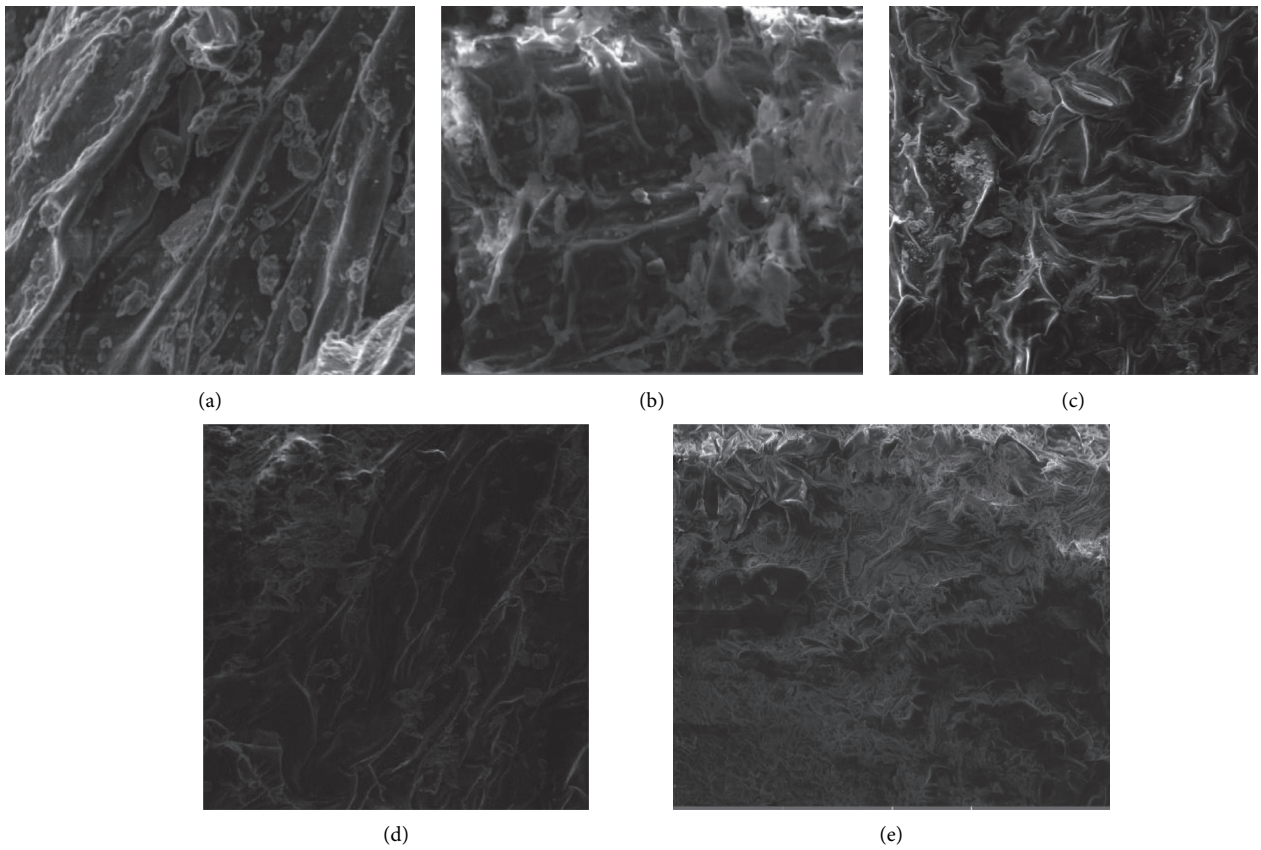


Figure 2: Surface microstructure of stevia leaves: (a) commercial; (b) freeze-dried; (c) gamma-irradiated at 0.5 kGy; (d) gamma-irradiated at 1 kGy; (e) gamma-irradiated at 2 kGy.

results also suggested that storage had a major impact on chlorophyll content. Chlorophylls a and b decreased when storage progressed; this decrease is probably related to the action of chlorophyllase. Similarly, it was confirmed that total chlorophyll and chlorophyll a decreased as storage progressed [46].

Some studies reported the effect of drying methods on the color and pigment content of stevia leaves. Abou-arab et al. [47] indicated values of 10.1, 6.6, 3.9, and 20.1 g $g^{-1}$ of chlorophylls a, b, carotenoids, and total pigments, respectively. Additionally, in the same study, after sun-drying at temperatures between 25 and 30°C for 24 to 48 h, final values of 4.7, 2.7, 0.76, and 7.5 g $g^{-1}$ of chlorophylls a, b, carotenoids, and total pigments were found.

*3.6. Determination of Total Phenolic Content (TPC).* Phenolics are aromatic secondary plant metabolites widely spread throughout the plant kingdom and associated with

Table 2: Influence of different preservation treatments and storage period on chlorophyll content of stevia leaves.

| | Chl a (mg/g) | Chl b (mg/g) |
|---|---|---|
| *Before storage* | | |
| C | $13.72 \pm 0.6^{b}$ | $5.94 \pm 0.5^{b}$ |
| FD | $19.51 \pm 0.6^{a}$ | $6.07 \pm 0.17^{a}$ |
| GI at 0.5 kGy | $10.69 \pm 0.5^{c}$ | $4.55 \pm 0.2^{ac}$ |
| GI at 1 kGy | $9.2 \pm 0.2^{c}$ | $3.88 \pm 0.2^{c}$ |
| GI at 2 kGy | $7.17 \pm 0.2^{d}$ | $4.76 \pm 0.1^{c}$ |
| *After 10 months of storage* | | |
| C | $6.38 \pm 0.3^{e}$ | $2.39 \pm 0.09^{d}$ |
| FD | $10.01 \pm 0.5^{c}$ | $4.83 \pm 0.4^{c}$ |
| GI at 0.5 kGy | $2.77 \pm 0.2^{f}$ | $1.76 \pm 0.01^{d}$ |
| GI at 1 kGy | $2.00 \pm 0.2^{f}$ | $1.34 \pm 0.01^{d}$ |
| GI at 2 kGy | $2.50 \pm 0.2^{f}$ | $1.21 \pm 0.01^{d}$ |

The results are expressed as mean ± standard deviation. Different letters in the same column indicate significant statistical differences. The significant differences at a level of 5% were performed by Tukey's test. C: commercial; FD: freeze-dried; GI: gamma-irradiated.

color, sensory qualities, and nutritional and antioxidant properties of foods [41]. The TPC as affected by preservation methods and storage time is shown in Figure 3. As can be seen, the content of total polyphenols varied with the method of preservation and the storage time.

On day 0 of storage (before storage), the highest content of phenolic compounds is found for freeze-dried leaves. Other studies have also documented that freeze-drying increases the extraction of bioactive compounds compared to hot-air-drying [42, 48]. It has been suggested that lyophilization resulted in the formation of ice crystals and caused the breakdown of cellular structures leading to better solvent access and better extraction [49].

It is speculated that the temperature of freeze-drying, as low as −50°C in the present study, is beneficial for maintaining the stability of polyphenols in the sample, which resulted in a high total polyphenol content in the freeze-dried leaves even after 10 months of storage [50].

Considerably variable amounts of TPC in stevia leaves were previously reported: 25.18 mg gallic acid equivalent (GAE)/g [51]; 56.74 mg GAE/g, obtained with air-drying [52]; 130.67 mg catechin/g, for air-drying at 40°C/12 h [53]; 0.709 mg GAE/g of dry sample [54]; and 20.85 and 22.25 mg GAE/g for water extract and methanol-water, respectively [55].

Storage time had significant effects on the TPC (Figure 3). Similarly, gamma irradiation exhibited a profound influence on TPC content, and an increase of irradiation doses has been reported to significantly increase the amounts of TPC for irradiated stevia leaves. This increase may be related to the effect of irradiation, which affects chemical bonds and consequently induces the release of low-molecular-weight fragments [19, 23]. Likewise, other studies have correlated this change with the activation of the biosynthesis of phenylalanine ammonia-lyase (PAL) as a regulatory enzyme in the biosynthesis of phenolic compounds [23]. There was a significant difference between TPC levels before and after storage for both preservation processes ($p \leq 0.05$), compared with control (commercial). Depolymerization of the cell wall polysaccharides, alteration of the membrane integrity, and decomposing the insoluble phenolics as a result of irradiation could also be the reasons for the increase of soluble phenolic compounds [56].

*3.7. Antioxidant Properties.* The results of antioxidant activity of stevia leaves are presented in Figure 4. For the DPPH radical scavenging activity in the commercial, freeze-dried, and gamma-irradiated stevia leaves, significant differences were observed, regardless of whether before and after 10 months of storage. Additionally, a significant increase for stored samples was observed.

Regarding the preservation method, DPPH radical scavenging activity was significantly increased in irradiated leaves. Indeed, whether it be before or after storage, gamma-irradiated samples had the highest DPPH scavenging activity, followed by the freeze-dried one which was higher than the commercial one. A significant increase was observed as the irradiation dose increased.

In this study, gamma-irradiated leaves had the strongest antioxidant capacity, which was related to the retention of more phenolic compounds. Our results agree with those obtained by Variyar et al. [57] whom found that the radical scavenging ability of gamma-irradiated soybean, between 0.5 and 5 kGy on DPPH radicals, increased dose-dependently. On the contrary, they differ from what Woon Lee et al. [58] had demonstrated that, in fresh ready-to-use tamarind juice, a non-significant increase in DPPH scavenging activity was observed as the irradiation dose increased, and a significant increase with stored samples was revealed. Among drying methods, freeze-drying has already been reported to retain the maximum antioxidant potential referenced to polyphenol compounds in leafy extracts of dried herbs [42].

Several studies reported that stevia leaves have a good DPPH radical scavenging activity. Shukla et al. [59] studied the antioxidant activity of ethanolic extracts from stevia leaves which exert a higher antioxidant activity when compared to ascorbic acid and against DPPH ($IC_{50} = 93.46\ \mu g/mL$). The DPPH radical scavenging activity as affected by air-drying methods was studied, and the

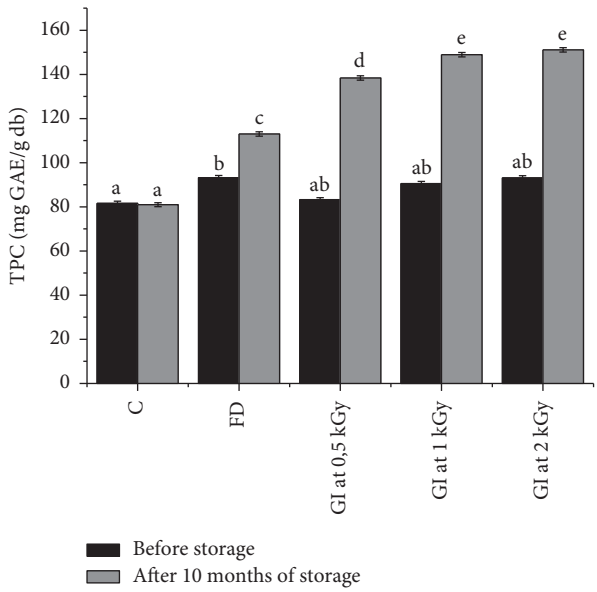


Figure 3: Total phenolic content of stevia leaves subjected to different preservation treatments and storage period. Values are the mean ± SD of three replicates and significant at $p \leq 0.05$. C: commercial; FD: freeze-dried; GI: gamma-irradiated.

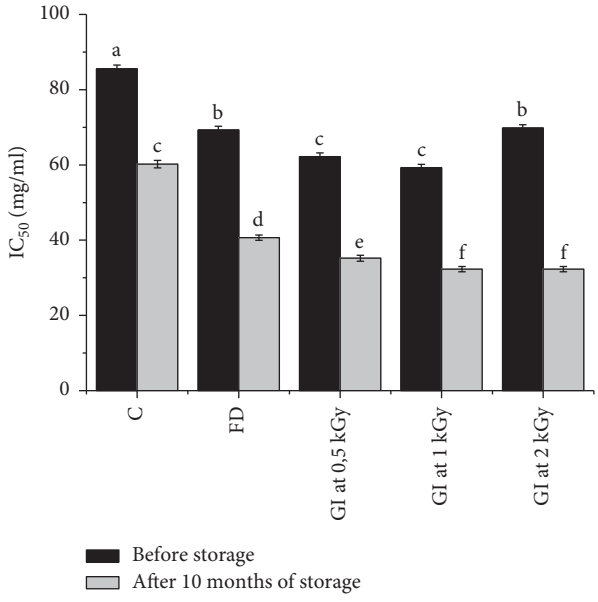


Figure 4: DPPH free-radical scavenging activity of differently preserved stevia leaves as influenced by storage time. Values are the mean ± SD of three replicates and significant at $p \leq 0.05$. C: commercial; FD: freeze-dried; GI: gamma-irradiated.

amount of antioxidants found for hot-air-drying at 180°C, shade-drying, hot-air-drying at 100°C, and freeze-drying was 126 mg Trolox equivalent/g, 75.9 mg Trolox equivalent/g, 64.9 mg Trolox equivalent/g, and 48.5 mg Trolox equivalent/g stevia [13]. The radical scavenging effect of the water extract and methanol-water extract determined by the DPPH test was 5.00 and 2.90 (mg of vitamin C/mL), respectively [55].

One should bear in mind that the presented results of antioxidant capacity are related to the DPPH assay and the resulting antioxidant potential can be influenced by the nature of solvent as well as the extraction method [42].

## 4. Conclusion

Freeze-drying and gamma irradiation are conventional methods for preserving medicinal and aromatic plants. The purpose of this study was to investigate the effects of these preservation methods on the physicochemical and surface properties of stevia leaves. Generally, gamma irradiation has been documented in the literature as a processing technique to improve the microbiological quality and safety of medicinal and aromatic plants.

In this study, stevia leaves showed better quality after gamma irradiation at low doses in terms of the content of total polyphenols and antioxidants. Therefore, gamma irradiation can be applied to alleviate any problem related to stevia postharvest contamination.

Moreover, our results allow us to consider lyophilization, despite its higher cost, to be a more effective drying method for reasons of preserving chlorophylls and polyphenol compounds as well as in terms of antioxidant capacity. Thus, both freeze-drying and gamma irradiation could be potential postharvest methods for obtaining high-quality stevia leaves; these processes positively affect bioactive compounds and antioxidant activity even after ten months of storage.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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