Journal of Food Quality

Modification and Valorisation of Food Ingredients with Non-thermal Technologies

Lead Guest Editor: Muhammad K. Khan
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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 μl 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 ± 0.9^{a}	6.86 ± 0.2^{c}	8.99 ± 0.9^{b}	9.22 ± 0.9^{b}	8.89 ± 1^{b}
Fat content	3.41 ± 0.04^{a}	2.34 ± 0.03^{b}	2.13 ± 0.03^{c}	1.96 ± 0.02^{c}	1.63 ± 0.02^{c}
Ash content	8.72 ± 1^{a}	9.12 ± 0.9^{a}	8.49 ± 0.8^{a}	8.58 ± 0.8^{a}	9.36 ± 1^{a}
Mineral content (ppm) K	4458 ± 38^{c}	9000 ± 50^{a}	7819 ± 53^{b}	7796 ± 37^{b}	7633 ± 56^{b}
Ca	8360 ± 48^{a}	9104 ± 55^{a}	9614 ± 55^{a}	8943 ± 52^{a}	8940 ± 53^{a}
P	1031 ± 41^{a}	1091 ± 43^{a}	1041 ± 41^{a}	1024 ± 42^{a}	1038 ± 46^{a}
Mn	304 ± 18^{b}	402 ± 21^{a}	483 ± 21^{a}	443 ± 19^{a}	431 ± 25^{a}
Fe	541 ± 17^{b}	515 ± 15^{b}	954 ± 22^{a}	950 ± 17^{a}	945 ± 24^{a}
Zn	36 ± 2^{a}	34 ± 2^{a}	39 ± 2^{a}	37 ± 2^{a}	32 ± 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

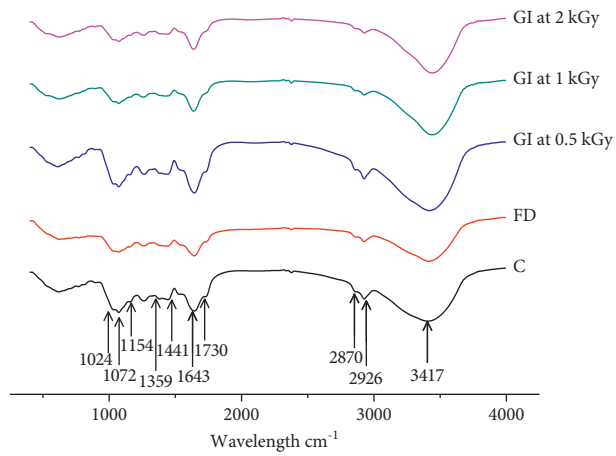

Figure 1: FTIR spectra of stevia leaves. C: commercial; FD: freeze-dried; GI: gamma-irradiated.

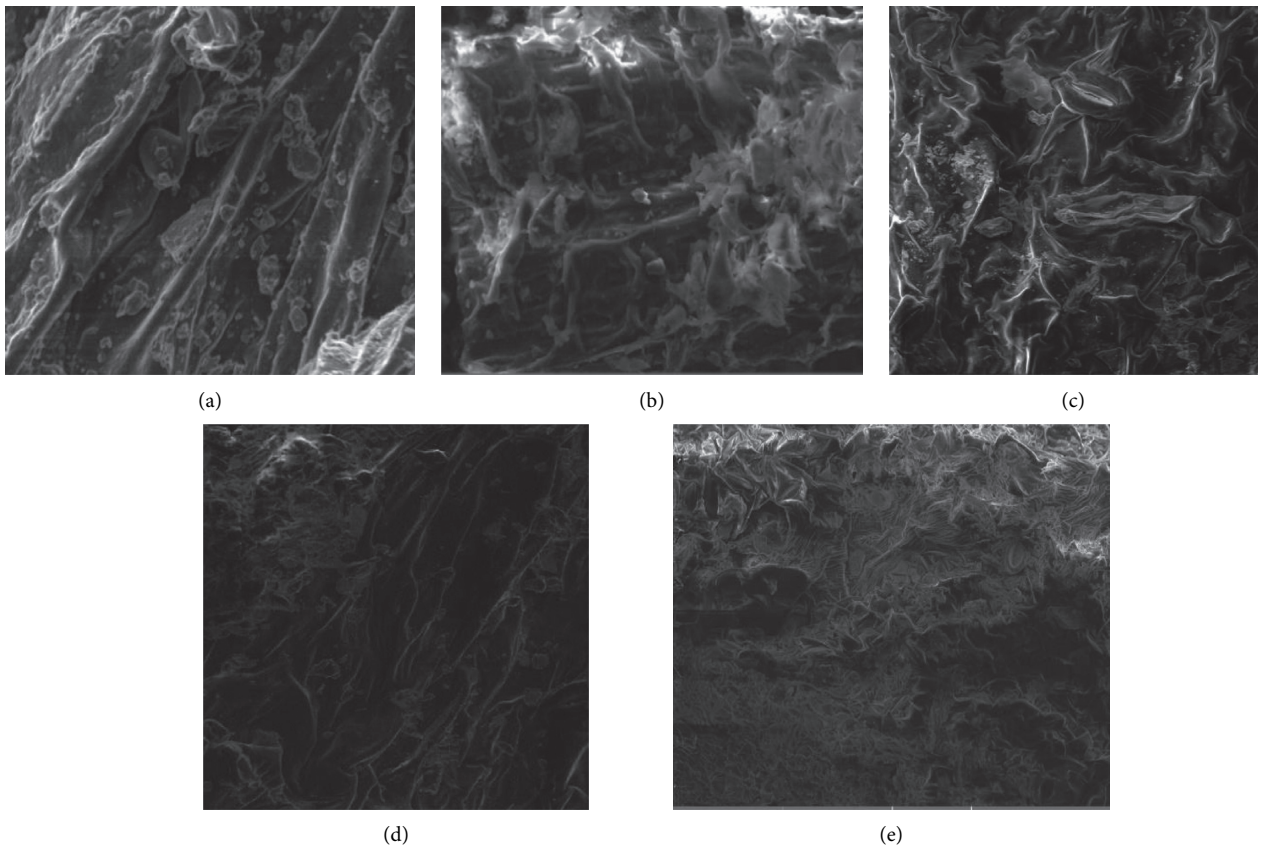

Figure 2: Surface microstructure of stevia leaves: (a) commercial; (b) freeze-dried; (c) gamma-irradiated at 0.5 kGy; (d) gamma-irradiated at 1 kGy; (e) gamma-irradiated at 2 kGy.

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 ± 0.6^{b}	5.94 ± 0.5^{b}
FD	19.51 ± 0.6^{a}	6.07 ± 0.17^{a}
GI at 0.5 kGy	10.69 ± 0.5^{c}	4.55 ± 0.2^{ac}
GI at 1 kGy	9.2 ± 0.2^{c}	3.88 ± 0.2^{c}
GI at 2 kGy	7.17 ± 0.2^{d}	4.76 ± 0.1^{c}
After 10 months of storage		
C	6.38 ± 0.3^{e}	2.39 ± 0.09^{d}
FD	10.01 ± 0.5^{c}	4.83 ± 0.4^{c}
GI at 0.5 kGy	2.77 ± 0.2^{f}	1.76 ± 0.01^{d}
GI at 1 kGy	2.00 ± 0.2^{f}	1.34 ± 0.01^{d}
GI at 2 kGy	2.50 ± 0.2^{f}	1.21 ± 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

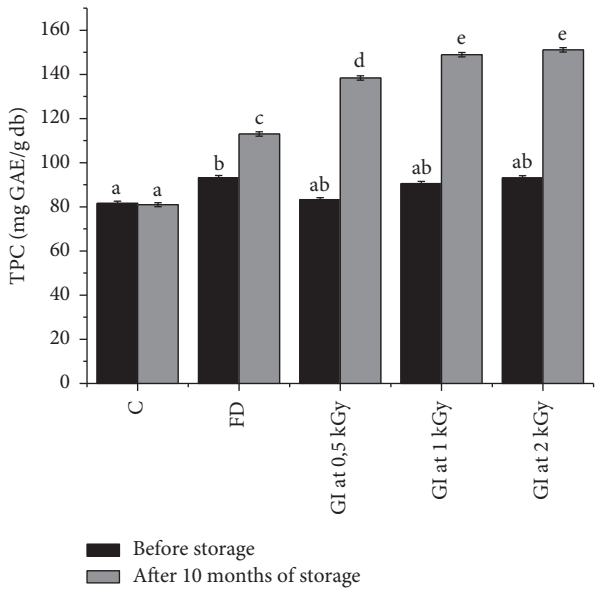

Figure 3: Total phenolic content of stevia leaves subjected to different preservation treatments and storage period. Values are the mean ± SD of three replicates and significant at $p \leq 0.05$. C: commercial; FD: freeze-dried; GI: gamma-irradiated.

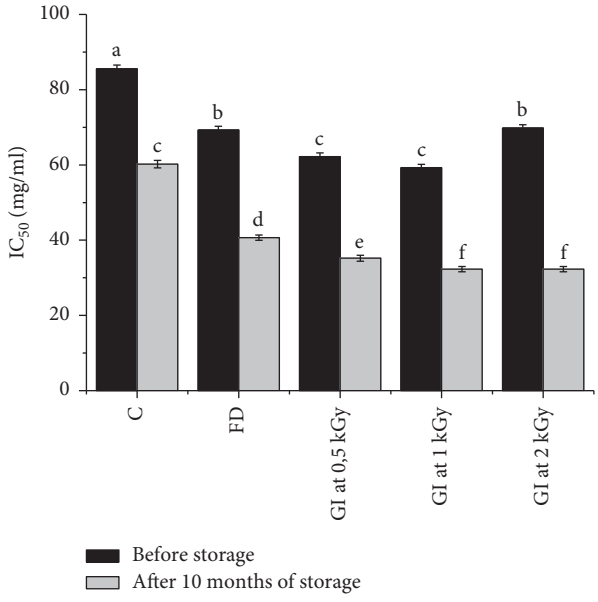

Figure 4: DPPH free-radical scavenging activity of differently preserved stevia leaves as influenced by storage time. Values are the mean ± SD of three replicates and significant at $p \leq 0.05$. C: commercial; FD: freeze-dried; GI: gamma-irradiated.

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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Research Article

Ultrasound-Assisted Modification of Insoluble Dietary Fiber from Chia (*Salvia hispanica* L.) Seeds

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Modification of insoluble dietary fiber (IDF) to soluble dietary fiber (SDF) improves not only the various health benefits but also the functional properties for improved product development. This research aimed to examine the effects of sonication treatment on the functional and physicochemical properties with possible structural changes in chia seeds dietary fiber. Central composite design was applied to optimize the sonication treatment process (amplitude 55%, time 20 min, and temperature 40°C) based on the oil holding capacity (OHC) and water holding capacity (WHC) as responses. Under these optimum conditions, ultrasound-treated IDF exhibited better functional and physicochemical properties such as OHC, WHC, glucose adsorption capacity (GAC), and water retention capacity (WRC) than untreated IDF. Fourier-transform infrared spectroscopy further confirmed the structural changes in treated and untreated IDF to explain the changes in the studied parameters.

1. Introduction

Dietary fiber (DF) has intrigued researchers' interest due to its numerous health benefits [1]. Some of the potential health benefits are reducing the blood lipid and sugar level, controlling body weight, and lowering the risk of cardiovascular and colorectal cancer [2]. These effects are due to DF's physicochemical properties such as oil holding capacity, water holding capacity, water retention capacity, cation exchanges, and fermentability [3]. In humans, the activities of some bacterial enzymes (e.g., β-D-glucuronidase, β-D-glucosidase, ureases, and mucinase) in the hindgut and faeces are linked with damaged intestinal barrier function [4]. Because these bacterial enzymes are active components of intestinal bacteria that can release active metabolites, they may impair gastrointestinal function and increase the risk of colon carcinogenesis. It has been demonstrated in numerous studies that dietary fiber can successfully reduce these bacterial enzymes, while also maintaining intestinal function and health [5, 6]. In 1953, Hipsley coined the term "dietary fiber" to describe the plant cell wall as nondigestible components [7]. After several versions, the most consistent definition that is now accepted is from Trowell et al. "Dietary fiber consists of remnants of plant cells resistant to hydrolysis (digestion) by the alimentary enzymes of man [8]." Based on water solubility, dietary fiber is typically classified into two types: insoluble dietary fiber (IDF), such as cellulose, hemicellulose, and lignin, and soluble dietary fiber (SDF) such as pentosans, pectin, gums, and mucilage [9].

The contents of insoluble and soluble dietary fibers in foods, primarily in cereals, are 65–80 percent and 20–35 percent, respectively, of the total dietary fiber [10]. In terms of physiological importance, soluble dietary fibers are more significant than insoluble dietary fibers in both groups [11]. Soluble dietary fiber has better versatility than insoluble fractions due to its rapid fermentation and breakdown of short-chain fatty acids and higher intake through probiotics. Soluble dietary fiber has a hypocholesterolemic effect since it binds to cholesterol and sugar, lowering their absorption and transport in the blood [12]. Furthermore, soluble dietary fibers move with no trouble through the gastrointestinal tract due to the softness of the stools; meanwhile insoluble dietary fibers do not solubilize in water and pass quickly

through the gastrointestinal tract by adding bulk to the waste and avoiding constipation and hemorrhoids [13].

The health effects of IDF are often not as significant as those of SDF because of their distinct physicochemical and functional qualities, thus also limiting the applications in the food processing industries [14]. As a consequence, developing a modification approach that improves the uniformity and other functional qualities of IDF is highly needed [15]. DF from various food sources is currently modified using a variety of biological, chemical, and physical methods. The processing conditions alter the composition and surface morphology of DF, improving its structural and biochemical properties [16]. Biological techniques are very expensive because they require the use of isolated enzymes and different bacterial strains [17]. Chemical processes that use more quantities of strong acids and alkalis can generate a lot of waste and are bad for the environment [18]. Certain physical methods, including pressurized hot water extraction, require controlled temperature and pressure conditions [19]. Ultrasound is an innovative technique that has been very common to use in different scientific and food processing laboratories. In fact, the application of ultrasonic waves can break polysaccharide chemical bonds to improve the functional and physicochemical qualities of food by changing the surface hydrophilicity [20]. In this work, the ultrasound technique was selected instead of other techniques for the modification of IDF into SDF from chia seeds because of its advantages of low environmental impact, protection, and high performance [21]. Cavitation is the basic principle upon which ultrasound works. Ultrasound is a kind of energy generated by sound waves with frequencies that are inaudible to human ears [22]. When sound waves travel through any product, a large amount of energy is produced due to the compression and rarefaction of the medium particles [23]. Ultrasound produces various effects in the solid-fluid system that can affect resistance (internal and external) to mass transfer between solid and fluid [24]. In recent years, many researchers have used ultrasound for the modification of IDF from different fiber enriched plant sources. For instance, modification of insoluble dietary fiber using ultrasound was investigated in garlic straw [25, 26], rose pomace [27], and okara fiber [28]. Generally, the DF modification indicators include oil holding capacity (OHC) and water holding capacity (WHC), which play an important role in the food preparation process because they influence other functional and sensory characteristics. OHC values of DF reflect its polysaccharide structure, density, and surface properties [29], while WHC is important for determining storage conditions and calculating cost-effectiveness for food applications [30]. It has also been shown in several studies that DF can significantly control postprandial blood glucose levels by reducing the utilization of sugars [31], which could be analyzed through glucose adsorption capacity.

Considering all the above points, there is an imminent need to significantly transform the insoluble dietary fiber into soluble dietary fiber and, thus, to produce soluble dietary fiber enriched value-added items. The current study aimed to chemically extract dietary fiber and assess the effect of ultrasound on the modification of insoluble dietary fiber into soluble dietary fiber in chia seeds.

2. Materials and Methods

2.1. Procurement of Raw Material and Chemicals. Chia seeds (*Salvia hispanica* L.) were purchased from a local market. Megazyme International Ltd. provides ready-to-use Megazyme assay kit for carbohydrate and protein degradation (Bray, Ireland). All other chemicals were also of analytical grade and were purchased from Sigma-Aldrich Ltd. (Burlington, MA, USA).

2.2. Extraction of IDF. Chia seeds were ground through Quadrumate Senior Mill (C. W. Brabender, Duisburg, Germany) and passed through a 0.25 mm sieve and stored at 4°C. Fat was removed from ground chia powder before fiber extraction and the sample was dried overnight at 105°C in a hot air oven (Universal UF75, Memmert GmbH + Co. KG, Germany) to a constant weight. A Modified Method 991.43 of the Association of Official Analytical Chemists (AOAC) has been used for the extraction of insoluble dietary fiber [32]. Briefly, 900 ml of water was taken into a 1000 ml beaker with 30 g of chia powder, followed by adding a-amylase in the mixture. The beaker was placed into the water bath for 35 min for the incubation process of the enzyme. The incubation period started when the mixture reached 85–95°C. Once the incubation period was completed, the sample was then removed from the water bath. The temperature of the hydrolysate was cooled to 60–65°C, and then 50 mg/ml of pepsin was dissolved in the solution to hydrolyze the protein content. After that, pH was adjusted to 4.5 by adding 3 mol/l of acetic acid, and 3 ml of amyloglucosidase was added for further hydrolysis and the temperature of the solution was maintained at 60°C for 30 min. For enzyme deactivation, the solution was heated for 10 min at a high temperature and then centrifuged at $4500 \times g$ for 15 minutes (Heraeus Megafuge 8R, USA). The precipitate was collected and washed with 78 and 95 percent ethanol. The remaining portion (IDF) was dehydrated in a hot air oven overnight at 60°C and was kept safe for further use.

2.3. Ultrasonic Process. A modified method adopted by Huang et al. was used for the modification of IDF [20]. 10 g of the IDF was taken in a glass beaker and mixed in 300 ml of distilled water. A homogenizer (HQ-2475, MXBAOHENG, China) was used for obtaining a homogeneous mixture and placed in a water bath at 20–45°C for 10 min, selecting 8000 rpm. After pretreatment, the solution was placed in a sonication apparatus (VCX750, Sonics & Materials, Inc., USA) at different temperatures (20–60°C) and time intervals (10–30 min). The magnitude range of ultrasound was used between 250 and 500 W in the form of amplitude between 20 and 80%. Ultrasound treatment was run in pulse mode with 5 sec on-time and 5 sec off-time. The solution was removed from the sonicator and was stored in a freezer at −20°C for further use.

2.4. Experimental Design and Data Analysis. During this study, three independent variables have been chosen as ultrasonic process responses: ultrasonic amplitude (A), ultrasonic time (B), and temperature (C). Three levels for each factor were chosen to examine their effect and interaction of the factors. The assessment indicators were oil holding capacity (OHC) (Y_1) and water holding capacity (WHC) (Y_2). Fifteen runs were performed to improve the accuracy of the process; a complete experimental design is presented in Table 1 about the actual values of the independent response of variables. The following equation expressed the quadratic model which is commonly used.

fx1

2.5. Determination of Functional Properties

2.5.1. Oil Holding Capacity. A modified method [33] was used to measure the oil holding capacity of IDF. 1.0 g of IDF sample and 30 ml of soybean oil were taken in a centrifuge tube and the solution was mixed thoroughly and left at 25°C for 16 h. After that, the mixture was centrifuged at 4000 × g for 10 min, and oil was separated as supernatant from the solution. The following equation expressed the weight of IDF after oil absorption:

$$\mathrm{OHC\,(g/g)} = \frac{M2 - M1}{M1}, \tag{1}$$

where M_2 is the weight after oil (g) absorption by IDF and M_1 indicates the initial weight before oil absorption (g) by IDF.

2.5.2. Glucose Adsorption Capacity (GAC). The GAC was determined by Peerajit et al. with little modification [16]. Initially, a glucose solution (100 mmol/L) was prepared and was mixed with 1 g of IDF in 100 ml solution; the mixture was placed in an incubator for six hours at 37°C. After glucose adsorption achieved equilibrium, the sample was centrifuged at 4,500 × g for 20 min. The absorbed amount of glucose by IDF was calculated by using anthrone colorimetry to measure the glucose content of the supernatant.

The following equation expressed the GAC:

$$\mathrm{GAC\,(mmol/g)} = \frac{(C_0 - C_1)}{M}, \tag{2}$$

where M represents the weight of IDF in grams, C_0 shows the glucose concentration (mmol/L) in original solution, and C_1 indicates concentration of glucose (mmol/L) in supernatant when adsorption reached equilibrium position. Volume (L) of the solution is represented by V.

2.6. Determination of Physicochemical Properties

2.6.1. Water Holding Capacity. The mixture was prepared in a 100 ml beaker by taking 70 ml of distilled water and 1 g of ultrasound-treated IDF. The mixture has been properly stirred till homogenized and was stored at room temperature for 24 hours for further experiment. After that, the mixture was centrifuged for 10 min at 4000 × g; supernatant and residue were separated, and the residues were weighted. The following equation was used for the calculation of WHC as g/g water to dry sample [34]:

$$\mathrm{WHC\,(g/g)} = \frac{M2 - M1}{M1}, \tag{3}$$

where M_1 represents the weight of IDF before water absorption and M_2 shows the weight after absorption of water.

2.6.2. Water Swelling Capacity. A total of 1.0 g of the sample was hydrated in a graduated test tube with 25 mL of deionized water. After that, the solution was mixed thoroughly to eliminate any entrapped air bubbles until it was kept at ambient temperature for 4 hours and the bed volume was recorded. WSC was measured as the volume of the mixture per gram of sample weight (g) [34].

2.7. Fourier-Transform Infrared Spectroscopy. The secondary structures of protein concentrates were characterized using Fourier-transform infrared (FT-IR) spectra. The dried IDF samples spectra were recorded using a spectrophotometer with a diamond ATR (attenuated total reflectance). A Nicolet 6700 spectrophotometer was used to record the FT-IR spectra (Bruker Alpha ATIR, FT-IR, USA). Before being pelletized, KBr was used to mix properly with IDF (1 : 250, wt./wt). The spectra of FT-IR were analyzed at 2 cm resolution with 32 times of scan in the wave range 400–4,000 cm [35].

2.8. Statistical Analysis. All experiments were carried out in triplicate. Data obtained were subjected to analysis of variance (ANOVA). Each treatment of ultrasound modified fiber (UMF) was statistically analyzed for its significant values using software package (MATLAB 9.2) as described by Montgomery [36]. Optimized run was performed in triplicate and the average mean values were reported with standard deviation. Moreover, the sample analysis was done, and the significant variation was determined among means at a probability level of 5%.

3. Results and Discussion

3.1. Design Interpretation. A central composite design (CCD) was used to establish the ideal levels of each variable of ultrasonic parameter for higher response. The selected trails have been shown to approximate the entire set of experimental parameters. The ultrasonic action efficiency was demonstrated by the OHC (Y1) and WHC (Y2) of US-treated IDF reported in Table 1.

Matrix Laboratory (MATLAB) software was used to apply statistical analysis and regression line on the basis of an experimental design. For various responses, the processing conditions were analyzed and optimized. Some insignificant terms ($p > .05$) were dropped from the model (Figure 1). Regression model was designed by using the stepwise regression method, and the experimental findings were used to create the equations as shown in Table 2.

Table 1: Central composite design representing the experimental trials along with oil holding capacity (OHC) and water holding capacity (WHC) as responses.

Run	Independent variables			Dependent variables			
	Amplitude	Time	Temperature	OHC		WHC	
				Actual value	Predicted value	Actual value	Predicted value
1	−1(20)	0(20)	1(60)	2.61	2.42	4.94	4.86
2(C. P)	0(50)	0(20)	0(40)	5.36	5.21	6.91	6.89
3	0(50)	−1(10)	1(60)	3.36	3.23	5.87	5.85
4	0(50)	−1(10)	−1(20)	3.12	3.12	5.42	5.47
5(C. P)	0(50)	0(20)	0(40)	5.33	5.25	6.93	6.84
6(C. P)	0(50)	0(20)	0(40)	5.34	5.27	6.90	6.91
7	1(80)	−1(10)	0(40)	4.11	3.92	6.26	6.13
8	0(50)	1(30)	1(60)	3.42	3.42	5.93	5.88
9	−1(20)	1(30)	0(40)	3.25	3.44	5.72	5.89
10	1(80)	0(20)	1(60)	2.88	3.07	5.12	5.27
11	1(80)	0(20)	−1(20)	2.57	2.76	4.89	4.97
12	1(80)	1(30)	0(40)	4.21	4.02	6.31	6.21
13	0(50)	1(30)	−1(20)	3.22	3.23	5.65	5.67
14	−1(20)	−1(10)	0(40)	3.18	3.37	5.61	5.71
15	−1(20)	0(20)	−1(20)	2.47	2.28	4.73	4.58

Run nos. 2, 5, and 6 are the central points of US treatment.

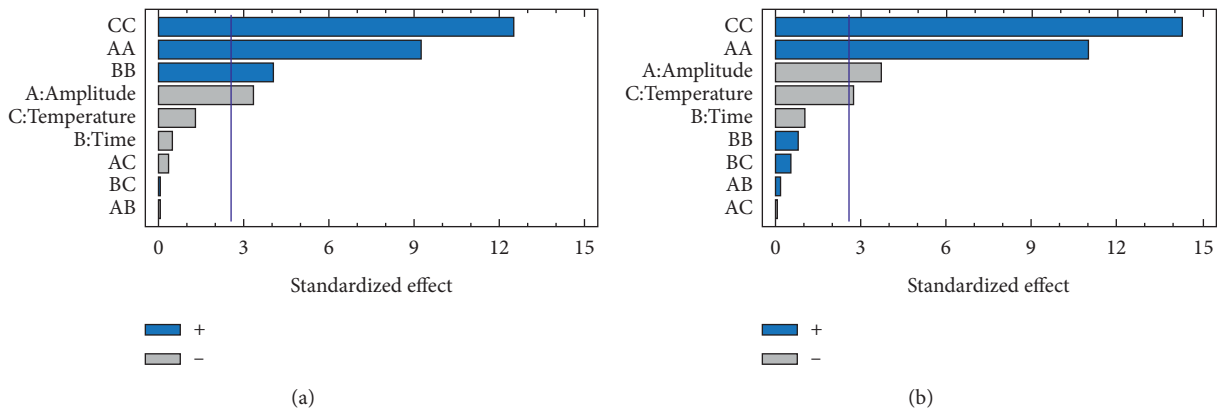

Figure 1: Pareto charts representing significant and nonsignificant factors for OHC (a) and WHC (b).

According to the statistical analysis, the proposed models were found to describe the observed significant values ($p < .05$) and ($R^2 > 98.483$), which are a satisfactory value for the response. The adjusted-R^2 value was close to R^2, indicating that the experiential and predicted data from the regression model were highly correlated. The best ultrasonic process parameters for maximum value of each response were evaluated by using equations (1) and (2) and their findings are reported in Table 2. Both responses were observed on different ultrasonic conditions and were presented as processes one and two for optimum value, as presented in Table 3. Table 4 shows the analysis of the variance representing the significant and insignificant factors. The effect of different parameters can be seen in the response surface plots showing the trend of effect in combination on OHC (Figures 2(a)–2(c)) and WHC (Figures 2(d)–2(f)). Processes one and two were considered to be the optimum ultrasonic processes for modification of IDF with higher OHC and WHC, respectively.

3.2. Optimum Ultrasonic Process. The OHC obtained using processes one and two was 5.37 g/g and 5.35 g/g, respectively, as shown in Table 4. Meanwhile, the influence coefficient of OHC is greater than WHC, as calculated by (5.37–5.35)/5.35. As a result, process one can be selected as the best ultrasonic process. Response surface plots created with MATLAB software were used to show the interaction impacts of the independent variables [37]. The optimum level of OHC occurred in the ranges of the tested variables at amplitude of 53.69%, time of 20.41 min, and 40.74°C, which were rounded to 55%, 20 min, and 40°C, respectively, according to equipment specification and feasibility of working.

3.3. Functional and Physiochemical Properties of Modified IDF. The ability to hold oil and water by chia seeds fiber is very important and plays a vital role in human health food applications. Several items are now made from or fortified

Table 2: The regression equations of the fitted model for OHC and WHC.

Response	Regression form	Regression equation
OHC (equation (1))	Actual	Oil holding capacity = 5.35 + 0.2825*amplitude + 0.04125*time + 0.11125*temperature − 1.155*amplitude^2 + 0.0075*amplitude*time + 0.0425*amplitude*temperature − 0.5075*time^2 − 0.01*time*temperature − 1.5625*temperature^2
WHC (equation (2))	Actual	Water holding capacity = 6.9 + 0.1975*amplitude + 0.05625*time + 0.14625*temperature − 0.86125*amplitude^2 − 0.015*amplitude*time + 0.005*amplitude*temperature − 0.06375*time^2 − 0.0425*time*temperature − 1.11875*temperature^2

OHC = oil holding capacity; WHC= water holding capacity; A = amplitude; B= time; C= temperature.

Table 3: Influence coefficient of ultrasonic processes based on OHC and WHC.

Ultrasonic process	OHC, Y1 (g/g)	WHC, Y2 (g/g)
Process 1 ($A = 53.69\%$, $B = 20.41$ min, $C = 40.74^{\circ}C$)	5.37	6.92
Process 2 ($A = 53.34\%$, $B = 24.09$ min, $C = 41.16^{\circ}C$)	5.35	6.9
Influence coefficient	0.0038	0.0028

Table 4: Analysis of variance (ANOVA) of the predicted quadratic model for studied parameters.

Source	DF	Oil holding capacity		Water holding capacity	
		MS	p value	MS	p value
A: amplitude	1	0.63845*	0.0209	0.31205*	0.0140
B: time	1	0.0136125^{NS}	0.6479	0.0253125^{NS}	0.3400
C: temperature	1	0.0990125^{NS}	0.2474	0.171112*	0.0407
AA	1	4.92563**	0.0003	2.73878**	0.0001
AB	1	0.000225^{NS}	0.9527	0.0009^{NS}	0.8503
AC	1	0.007225^{NS}	0.7380	0.0001^{NS}	0.9497
BB	1	0.950977**	0.0098	0.0150058^{NS}	0.4539
BC	1	0.0004^{NS}	0.9369	0.007225^{NS}	0.5976
CC	1	9.01442**	0.0001	4.6213**	0.0001
Total error	5	0.057765		0.022775	
Total (corr.)	14				
R^2		97.9951		98.483	
R^2 (adjusted for d. f.)		94.3864		95.7523	

* Significant, ** highly significant, significant at 0.05.

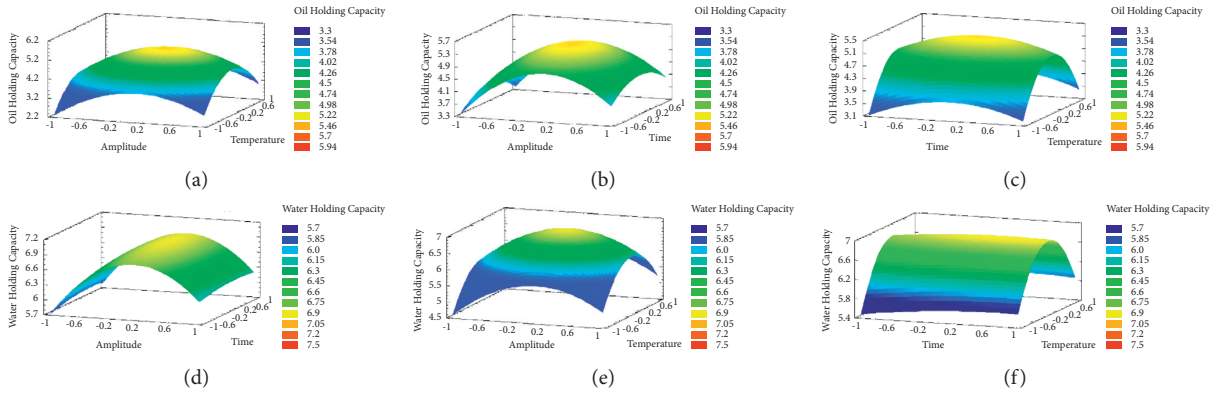

Figure 2: Response surface plots along with contour on curve showing effect of parameters on: OHC (a–c) and WHC (d–f).

with chia seeds in the food industry in various regions across the globe. These include breakfast cereals, cakes, pasta, and cookies [38]. According to Oliviera et al., flour made from chia seeds can be used to make pasta as an alternative to wheat flour. During this study, they observed that pasta made with chia flour had higher nutritive value than control pasta [39]. It contained statistically significantly higher levels of protein, minerals, and dietary fiber. Furthermore, introduction of chia to frankfurters provided a product enriched with dietary fiber, minerals, and amino acids [40]. OHC and WHC have been measured and found to be depending on the structure of chia seeds fiber. Table 5 shows the results of OHC and WHC of chia seeds fiber before and after ultrasound treatments. The best results for OHC and WHC responses (5.35 g/g and 6.92 g/g) were found in US-treated runs 2, 5, and 6, in which independent variables (amp. 50%, time 20 min, and temp. 40°C) were used to examine the response. The US-treated run no. 15 (amp. 20%, time 20 mints, and temp. 20°C) produced the lowest OHC and WHC response values of 2.47 g/g and 4.73 g/g, respectively. The results showed that the low amplitude of US resulted in little to no change in the particle size of chia seeds fiber. The reason behind this mechanism is that the large surface area of the particle size absorbed the low amplitude of US [41]. The average particle size decreased, and chia seeds fiber homogeneity increased as ultrasonic amplitude increased. This was a result that caused more cavitation and mechanical impacts due to the high amplitude of ultrasounds [42]. So, when ultrasound amplitude was 80%, there was no further reduction in the mean particle size.

The interaction between particles of fiber induced by the suspension of high viscosity was probably the reason for this mechanism [43, 44]. Temperature is another variable that affects the modification of fiber. When the temperature

Table 5: Correlation indicators of the quadratic polynomial regression analysis.

Response	R-value	Adjusted R-value	p value	Durbin–Watson	Optimum process conditions
OHC, Y_1 (g/g)	97.9951	94.3864	0.2033	1.75659	Process 1: $A = 53.69\%$, $B = 20.41$ min, $C = 40.74^{\circ}C$
WHC Y_2 (g/g)	98.483	95.7523	0.1179	1.61786	Process 2: $A = 53.34\%$, $B = 24.09$ min, $C = 41.16^{\circ}C$

A = amplitude; B = time; C=temperature; OHC = oil holding capacity; WHC = water holding capacity.

Table 6: Comparison of physicochemical properties of ultrasound-treated and untreated IDF.

IDF	OHC (g/g)	GAC (mmol/g)	WSC (ml/g)	WHC (g/g)
Untreated	2.61	2.96	5.6	3.2
Ultrasound-treated	5.35	3.72	8.4	6.92

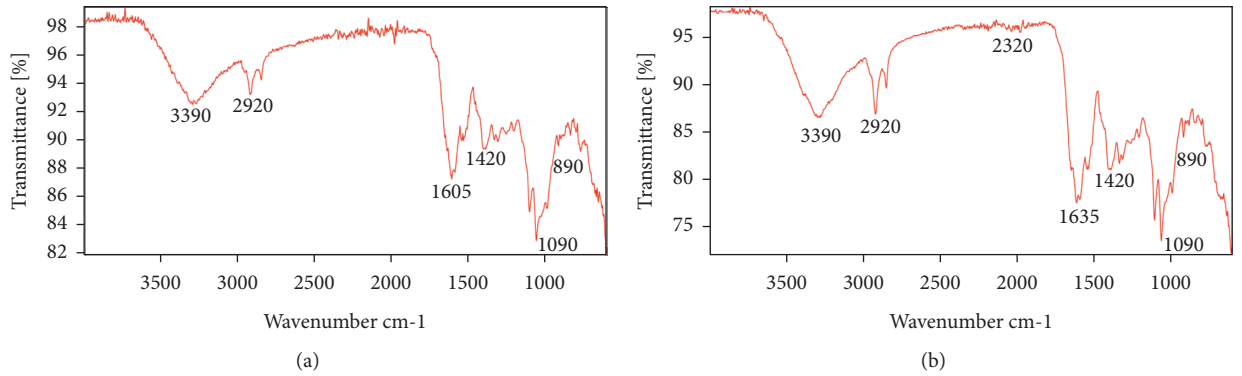

Figure 3: FT-IR spectra of chia seed IDF: untreated IDF (a) and sonicated IDF (b).

reached 40°C, the maximum change has occurred, resulting in the maximum yield. Similarly, when the temperature was at its highest (60°C), this resulted in the reduction of the yield, as shown in pictures 1 and 2.

GAC and WSC are also the functional and physicochemical properties, respectively; therefore the GAC and WSC were also examined and are shown in Table 6. This means that the values against US-treated GAC and WSC (3.72 mmol/g, 8.4 ml/g) increased as compared to the untreated ones (2.96 mmol/g and 5.6 ml/g), respectively. The improvements in functional properties and alteration of structural chemistry because of the destruction of C-H and the asymmetric vibrations of COOH and stretching of carboxyl group might also be responsible for higher GAC and WRC in ultrasound-treated IDF [45]. These results show that the IDF of chia seeds pretreated with ultrasound had a good functional and physicochemical profile, making it a useful ingredient for food products.

3.4. Structural Characterizations of US-Treated and Untreated IDF by Ultrasound. The FT-IR spectra could help to explain the functional groups and bonding information of the samples [46]. Figure 3 shows the spectrum of IDF from chia seeds, which ranges from 400 to 4,000 cm. The absorption bands about 3,390 cm^1 were caused by O-H bond stretching to hydrogen and hydroxyl groups which formed by hemicelluloses and cellulose, with absorption peaks at 3,386.44, 3,396.09, and 3,386.44 cm^1, were found in the spectra of untreated and US-treated IDF [47, 48]. The absorption peaks in different DFs were practically identical, and the band at 2,920 cm^1 was relevant to the C-H stretching of methylene and methyl group. Furthermore, asymmetric stretching vibrations of the carboxyl group COOH created the rather intense absorbance peaks at 1,633.44, 1,637.29, and 1,635.37 cm^1 in US-treated and untreated fiber. The untreated fiber sample showed a smaller peak at 775 cm^1 inside the fingerprint area. However, this peak was shifted to 833 cm^1 in the ultrasound-treated samples, showing that the long chain of $-CH_2$ groups was broken after the modification and the oligosaccharide content was boosted [49]. It seems to be that the untreated IDF has a distinctive absorption peak at 1,603 cm, which is most likely due to C5C sequence of aromatic rings [50]. In US-treated IDF, a change from 1,603 to 1,635 cm was found when compared to the two spectra, which might be attributed to the breakdown of organic molecules. Furthermore, in US-treated IDF, a new peak at 2,320 cm was observed, which is typically triple bond compounds, indicating that dehydrogenation was triggered by ultrasound. After ultrasonic treatment, the positions of the peaks changed. Ultrasound may be concluded by breaking the intramolecular chemical bonds, resulting in the demolition of organic compounds, the significant increase of carboxyl and hydrophilic groups, water binding, and the reduction of particle size, contributing to maximum OHC, WRC, and WSC [51]. After the modification, the WHC increased, and the OHC and WRC of the SDFs were significantly higher than those of the IDFs [52]. The

improvement of these properties indicated a good application prospect of modified fiber in food processing [53]. This could be due to the ultrasound treatment leading to a comparatively looser texture [54].

4. Conclusion

This study aimed to extract DF from Chia seeds (*Salvia hispanica* L.) and partially modify IDF into SDF through ultrasound, which is a nonthermal and innovative technique. The current study revealed an effective modification method to improve the compositional, structural, and functional properties of DFs. This study revealed that the physicochemical and functional properties of the IDF were increased by the pretreatment of ultrasound at amplitude 55%, time 20 min, and temperature 40°C. FT-IR results demonstrated that ultrasound treatment improved the specific surface area of IDF, correlating improved functional and physicochemical properties of modified IDF. Furthermore, chia seeds fiber has been used already in food processing of different breakfast items like pasta, cookies, and cakes. Also, this study aims to promote the use of chia seeds fiber in health and food applications by exploring its physicochemical properties.

Abbreviations

DF: Dietary fiber
IDF: Insoluble dietary fiber
SDF: Soluble dietary fiber
US: Ultrasound
PHWE: Pressurized hot water extraction
OHC: Oil holding capacity
WHC: Water holding capacity
GAC: Glucose adsorption capacity
WSC: Water swelling capacity
UD: Uniform design
FT-IR: Fourier-transform infrared spectroscopy
AOAC: Association of Official Analytical Chemists
UMF: Ultrasound modified fiber
DPS: Data processing system.

Data Availability

All types of data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

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