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Effect of Natural Fermentation on the Chemical Composition, Mineral Content, Phytochemical Compounds, and Antioxidant Activity of *Ziziphus spina-christi* (L.) "Nabag" Seeds

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Abstract: Effects of fermentation on the chemical composition, mineral, total phenolic, total flavonoid, tannin, vitamin C, total carotenoid content, and antioxidant activity of "Nabag" *Ziziphus spina-christi* (L.) seeds were investigated. The fermentation process was carried out for 6, 12, 24, and 48 h. The fermentation significantly ($p < 0.05$) improved the chemical composition and mineral content of "Nabag" seeds, particularly the Ca, Fe, and Zn content. The phenolic, vitamin C, total carotenoid content, and antioxidant activity were significantly ($p < 0.05$) increased as a result of fermentation compared with unfermented *Ziziphus spina-christi* (L.) seeds. Fermentation of the seeds for 48 h resulted in the highest increase in crude fiber, Ca, Fe, Zn, and bioactive compounds. These results indicate the potential utilization of fermented "Nabag" seeds in the production and formulation of functional foods rich in crude fiber, essential minerals, and bioactive compounds.

Keywords: "Nabag" seeds; fermentation; chemical composition; phytochemical compounds; antioxidant activity

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1. Introduction

Ziziphus species (*Ziziphus jujube*, *Ziziphus lotus*, and *Ziziphus spina-christi* L.) fruits are considered a rich source of nutritional and phytochemical compounds [1]. Ziziphus plants have been traditionally used for the treatment of various diseases. Among the different Ziziphus species, *Ziziphus spina-christi* has been described as a rich source of several active compounds including alkaloids, sterols (β-sitosterol), flavonoids, triterpenoids, sapogenins, and saponins. These biologically active ingredients may potentially serve as antibacterial, antioxidant, antimicrobial, and anti-inflammatory agents [2].

In Sudan, *Ziziphus spina-christi* (L.) grows wildly and is popularly known as "Siddir". It provides an edible fruit, "Nabag," which is commonly used to fulfill the food and nutritional needs of the local people [3]. The fruit was found to contains high levels of total phenolic compounds and can inhibit lipid peroxidation and increase the activity of endogenous antioxidant enzymes [4]. Moreover, the pulp is a good source of B vitamins, calcium, potassium, sodium, phosphorous, copper, iron, and zinc [5]. *Ziziphus spina-christi* seeds were reported to be a rich source of carbohydrate and protein that contains a significant amount of sulfur-containing amino acids [6]. It is also reported to contain higher mineral content, phenolic content, and antioxidant activity compared with those of other fruits [7].

Fermentation is an extremely old method that subjects food to the activity of microorganisms and enzymes. This prolongs shelf life, and it also improves the palatability,

digestibility, flavor, and nutritional value of the food. Apart from nutritional improvement, fermentation can increase the functional properties of fermented food by breaking down complex substrates such phenolic compounds into simpler components [8]. Georgetti et al. [9] observed an incremental increase in phenolic acid and flavonoids in fermented soybean flour. Phenolic acid decarboxylase and esterase activities were reported in fermented cowpeas [10]. Moreover, fermentation was found to enhance the color and flavor and increase the bioactive compound levels and antioxidant activity of jujube *Zizyphus jujuba* Miller wine [11].

To date, there are no reports on the impact of fermentation on the nutritional and antioxidant properties of "Nabag" *Ziziphus spina-christi* (L.) seeds. Therefore, this study was carried out to evaluate the effect of natural fermentation on the chemical composition, mineral content, phytochemical compounds, and antioxidant activity of "Nabag" *Ziziphus spina-christi* (L.) seeds grown in Sudan.

2. Materials and Methods

2.1. Sample Collection and Preparation

The "Nabag" *Ziziphus spina-christi* (L.) fruit was purchased from the Central Market in Khartoum North, Sudan. The fruit was washed thoroughly with tap water and air-dried at room temperature (25 °C) until it reached uniform moisture content (10%) for 24 h. The fruit was stoned to separate the flesh from the seed; then, the seeds were collected, ground to a fine powder, and stored at 4 °C for further analysis.

2.2. Fermentation

Fermentation was conducted naturally, by mixing "Nabag" seed powder with distilled water 1:3 (*w*/*v*) in a beaker and incubating at 37 °C for 0, 6, 12, 24, and 48 h. The samples were mixed with a glass rod, transferred to aluminum trays (30 cm diameter), and dried at room temperature. Then, dried samples were ground into a fine powder and stored in polyethylene bags at 4 °C for analysis. Control samples of "Nabag" seed powder (unfermented) were also ground and stored under the same conditions.

2.3. Chemical Composition and Total Mineral Content

The moisture, ash, and crude fiber content of the seeds were measured according to the AOAC [12]. The crude protein of the seeds was determined using the Kjeldahl method according to the AOAC [12]. Crude oil of Nabag samples was determined using a Soxhlet apparatus according to the AOAC [12]. The total mineral content of the control and unfermented seeds was measured using atomic absorption spectrometry following the method of AOAC [12]. Samples were burned in a muffle furnace at 550 °C, and the extract was dissolved in 5 mL of 5N HCl. The extracts were stored in bottles for further analysis. Mineral content was determined using an atomic absorption spectrophotometer (Shimadzu AA-680, Shimadzu, Japan).

2.4. Vitamin C Determination

Vitamin C in the seeds was measured via a titration method using the 2,6-dichloroindo phenol indicator dye according to the AOAC method [12]. Five grams of each sample were blended with 0.4% oxalic acid and filtered, and the volume was adjusted to 100 mL with oxalic acid. A volume of 10 mL was added to 5 mL of 10% oxalic acid and titrated against 2,6-dichlorophenol indophenol dye.

2.5. Determination of Tannin Content

The tannin content of control and fermented seeds was determined using the modified vanillin–HCl method as described by [13]. Approximately 1 mL of 1% HCl in methanol extract was added to 5 mL of vanillin reagent (4% HCl in methanol and 0.5 mL vanillin in methanol) and mixed. The reaction was maintained at 30 °C, and the absorbance was read

after 20 min at 500 nm using an ultraviolet–visible (UV-VIS PD-303 UV) spectrophotometer. The tannin content was expressed as catechin equivalents (mg catechin/g).

2.6. Determination of Carotenoids

Carotenoids were measured according to the method of Jacques et al. [14]. Carotenoids were extracted with 25 mL of cold acetone. The extract was fractionated using petroleum ether (20 mL) and washed with distilled water (100 mL). The extract volume was adjusted to 25 mL using petroleum ether. The absorbance was measured at 450 nm, and the total carotenoid content was expressed as milligrams per 100 g of DM.

2.7. Preparation of Phenolic and Antioxidant Extracts

Extracts of the phenolics and antioxidants of "Nabag" seeds were prepared using the method described by Talhaoui et al. [15]. Seed flour was mixed with absolute methanol at a ratio of 1:25 (w/v) and shaken at ambient temperature for 24 h. The extraction was performed twice, and the collected extract was dried under vacuum using a rotary evaporator and kept dry for further analysis.

2.7.1. Determination of Total Phenolic Content

The method of Waterhouse [16] using Folin–Ciocalteu's reagent was used to measure the total phenolic content (TPC) of the seeds. A solution (20 µL) of dried methanolic extract solution (1:10 w/v) was mixed with 1.58 mL H_2O and 100 µL of Folin–Ciocalteu reagent. Then, 300 µL of Na_2CO_3 (20%) was added to the solution and incubated at 20 °C for 2 h. The absorbance was recorded at 765 nm relative to a blank solution. A calibration curve was prepared using different concentrations of gallic acid (R^2 = 0.9672), and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample (DW).

2.7.2. Determination of Total Flavonoid Content

The total flavonoid content (TFC) of the extracts was determined using the method described by Kim et al. [17]. A mixture of methanolic extract (1 mL), 5% $NaNO_2$ solution (300 µL), and 10% aluminum chloride (300 µL) was incubated at 25 °C for 5 min. Then, 1 mol/L sodium hydroxide (2 mL) was added. The volume was brought to 10 mL with H_2O, and the solution was thoroughly vortexed. The absorbance was recorded at 510 nm. A calibration curve was generated from different concentrations of catechin (R^2 = 0.974). TFC was expressed as milligrams of catechin equivalents (CE) per gram of sample (DW).

2.7.3. Antioxidant Activity Assays

DPPH Radical Scavenging

The DPPH radical scavenging ability of the extracts from unfermented and fermented seeds was determined using the method described by Chang et al. [18]. Tris-HCl buffer (50 mM; pH 7.4; 0.9 mL) and 0.1 mL of sample extract or deionized H_2O, which served as a control, were mixed and incubated at room temperature for 30 min. After the incubation period, the absorbance of the mixture was recorded at 517 nm. DPPH scavenging was calculated and expressed as Trolox equivalents (mg TE/g).

Ferric Reducing Antioxidant Power Assay

Freshly prepared ferric reducing antioxidant power (FRAP) working solution (2.5 mL of a 10 mmol/L TPTZ solution in 40 mmol/L HCl, 25 mL of 0.1 mol/L acetate buffer, pH 3.6, and 2.5 mL of 20 mmol/L $FeCl_3$) was incubated at 37 °C for 10 min. Methanol extract (0.5 mL, diluted 10 times) was mixed with 2 mL FRAP working solution in a 10 mL test tube and diluted to 10 mL with distilled water. The mixture was kept in the dark for 20 min. The absorbance of the remaining FRAP solution was measured at 593 nm against a blank using a UV/visible spectrophotometer [19]. The results were expressed as micromoles of Trolox equivalents per 100 g of sample (mg TE/g).

Total Reducing Power

The total reducing power (TRP) of the samples was determined by the method of Gulcin [20]. The extract (1 mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and then 2.5 mL of 1% potassium ferricyanide and incubated at 50 °C for 20 min, which was followed by the addition of 2.5 mL of 10% trichloroacetic acid and centrifugation at 1038× *g* for 10 min. Then, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled H_2O and 0.5 mL of 0.1% ferric chloride. The absorbance of the mixture was read at 700 nm. Ascorbic acid was used as a reference standard, and results were expressed as ascorbic acid equivalents (AAE) per gram of sample.

Hydrogen Peroxide Scavenging Assay

A hydrogen peroxide scavenging assay was done according to Jayaprakasha et al. [21]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). One mg/mL solution of extract was prepared, mixed with 3 mL of phosphate buffer, and 1 mL of H_2O_2 (40 mM) was added. After a 10 min incubation, the absorbance was recorded at 230 nm. The H_2O_2 scavenging ability was calculated as follows:

$$\text{The } H_2O_2 \text{ scavenging ability of samples (\%)} = [(Ab - As)/Ab] \times 100. \quad (1)$$

where Ab is the absorbance of the control and As is the absorbance of the sample.

2.8. Statistical Analysis

Data are presented as the mean of three replicates. The data were analyzed using a completely randomized block design using a one-way analysis of variance. Multiple significant differences in the means ($p < 0.05$) were determined using the least significant difference (LSD) range test. Multivariate analysis was conducted using HJ-Biplot PCA algorithms as described in the XSTAT software package. Linear partial least squares regression analysis (PLS) was used to analyze the relationships between microwave treatment and quality parameters using XLSTAT software.

3. Results and Discussion

3.1. Effect of Natural Fermentation on the Proximate Composition of "Nabag" (Ziziphus spina-christi L.*) Seeds*

Table 1 presents the effect of natural fermentation on the proximate composition (moisture, crude oil, and protein) of "Nabag" (*Ziziphus spina-christi* L.) seeds. The moisture content of the fermented seeds gradually increased as fermentation time progressed from 6 to 48 h compared to the unfermented seeds. Fermentation of the "Nabag" (*Ziziphus spina-christi* L.) seeds up to 48 h was found to cause no significant change in dry matter, protein, and ash content. On the other hand, the fermentation process significantly ($p < 0.05$) increased the oil contents, compared to unfermented seeds, but there were no significant differences among fermented seeds. It increased from 6.1% to 8.3%, 8.3%, 8.3%, and 7.9% after fermentation for 6, 12, 24, and 48 h, respectively. The results for the crude fiber change as a function of fermentation showed that the crude fiber content increased significantly after fermentation for 12, 24, and 48 h with a parallel decrease in total carbohydrate content (CHO).

The chemical composition of the "Nabag" (*Ziziphus spina-christi* L.) seeds found in this study is within the range reported in other studies. Adekunle and Adenike [22], Ahmed et al. [23], and Osman and Ahmed [24] reported that the values for the ash, protein, fat, and fiber of "Nabag" seeds were in the range of 2.9–5.8%, 4.8–31.9%, 1.24–4.29%, and 21.3–31.8%, respectively.

Table 1. Effect of natural fermentation on the proximate composition of "Nabag" (*Ziziphus spina-christi* L.) seeds.

Fermentation Time (h)	Moisture (%)	Ash (%)	Oil (%)	Protein (%)	Fiber (%)	CHO (%)
Control	94.4 ± 0.76	3.3 ± 0.07	6.1 ± 0.01 b	9.2 ± 0.76	35.5 ± 3.04 c	40.3 ± 0.93 a
6 (h)	94.0 ± 0.81	3.3 ± 0.19	8.3 ± 0.02 a	9.2 ± 0.10	40.9 ± 1.45 b	23.5 ± 0.51 b
12 (h)	93.9 ± 0.49	3.2 ± 0.10	8.3 ± 0.06 a	9.4 ± 0.65	49.5 ± 1.56 a	23.5 ± 0.57 b
24 (h)	93.8 ± 0.01	3.1 ± 0.02	8.3 ± 0.80 a	9.9 ± 0.06	49.9 ± 2.26 a	22.5 ± 0.63 b
48 (h)	93.7 ± 0.26	3.1 ± 0.29	7.9 ± 0.11 a	9.9 ± 0.06	50.4 ± 1.57 a	22.4 ± 0.46 b
F-test	**ns**	**ns**	**	**ns**	**	**
LSD 0.05	**-**	**-**	**0.642**	**-**	**3.679**	**1.156**

Values are means (±SD) of triplicate samples. Means not sharing a common letter in a column are significantly ** different at ($p < 0.05$). Means in the same column without letter are not significantly ($p < 0.05$) different, ns; no significant difference at ($p < 0.05$) as assessed by least significant difference (LSD).

Our findings showed that the fermentation process of "Nabag" seeds improved the oil content; the increase could be attributed to the enzymes produced by the healthy bacteria during fermentation that can break down seed shell, thus releasing oil from seeds, while the increase of crude fiber could be due to the utilization of simple sugars as sources of energy by the microorganisms [25]. Generally, natural fermentation improves the nutrient composition of the "Nabag" seed. Hence, the fermented seeds could be used as a source of energy and crude fiber, which are essential for human nutrition, particularly in developing countries.

3.2. Effect of Natural Fermentation on the Mineral Content of "Nabag" (Ziziphus spina-christi L.*) Seeds*

Table 2 presents the macro (Ca, Mg, Na, K, and P) and micro (Fe and Zn) elements in unfermented and fermented "Nabag" (*Ziziphus spina-christi* L.) seeds. The mineral analysis showed that unfermented Nabag seeds contain a substantial amount of Mg (40.7), K (38.1) and Fe (31.6), moderate content of Ca (24.7), Na (20.3), and Zn (18.9), and low content of P (1.1). These values were in agreement with those found in previous studies of "Nabag" seeds [22,24]. The fermentation process significantly decreased the K and Na content of the seeds, whereas there was a significant ($p < 0.05$) increase in Ca, Zn, and Fe with fermentation progress. The highest increased Ca and Fe was found after 48 h of fermentation. The increase in mineral content of fermented "Nabag" seeds could be attributed to the release of these minerals from their chelated complex compounds through the activities of microorganisms during fermentation [26]. However, the reduction of K and Na in the seeds after fermentation was probably due to their utilization by certain organisms for growth and metabolism [27].

Table 2. Effect of natural fermentation on the mineral content of "Nabag" (*Ziziphus spina-christi* L.) seeds.

Fermentation Time	Macro Element (mg/g)					Micro Element (mg/100 g)	
	Ca	Mg	K	Na	P	Zn	Fe
Control	2.47 ± 0.29 d	4.07 ± 0.12	2.03 ± 0.15 a	38.1 ± 2.14 a	1.07 ± 0.03.	17.8 ± 0.14 b	31.6 ± 0.00 b
6 h	2.67 ± 0.15 cd	4.22 ± 0.29	1.49 ± 0.33 b	26.0 ± 1.41 b	1.05 ± 0.02	18.4 ± 0.98 b	32.1 ± 0.64 b
12 h	2.87 ± 0.12 bc	4.24 ± 0.75	1.06 ± 0.15 c	26.0 ± 0.01 b	1.03 ± 0.05	20.7 ± 0.00 a	33.1 ± 2.77 ab
24 h	3.13 ± 0.12 ab	4.27 ± 0.37	0.91 ± 0.02 c	21.8 ± 1.05 c	1.02 ± 0.03	20.3 ± 1.15 a	33.5 ± 2.05 ab
48 h	3.27 ± 0.12 a	4.31 ± 0.04	0.68 ± 0.05 d	18.8 ± 1.05 d	1.01 ± 0.42	20.8 ± 1.73 a	35.2 ± 0.86 a
F-test	**	**ns**	**	**	**ns**	**	**
LSD 0.05	**0.3073**	**-**	**0.1882**	**1.922**	**-**	**1.742**	**2.247**

Values are means (±SD) of triplicate samples. Means not sharing a common letter in a column are significantly ** different at ($p < 0.05$). Means in the same column without letter are not significantly ($p < 0.05$) different, ns; no significant different at ($p < 0.05$) as assessed by least significant difference (LSD).

The present study indicates that the fermented "Nabag" seeds are a valuable source of Fe and Ca that are needed for pregnant and lactating women in under-developed countries. Further studies are needed for optimizing the fermentation processing conditions of "Nabag" seed by using different fermentation methods or using pure culture in order to enhance the mineral bioavailability.

3.3. Effect of Natural Fermentation on the Content of the Phytochemical Compounds of "Nabag" (Ziziphus spina-christi L.*) Seeds*

Table 3 shows the effect of fermentation on TPC, TFC, tannin, vitamin C, and total carotenoid content. During 48 h fermentation, there was a progressive increase in TPC, with increase of fermentation time. It increased from 59.7 mg GAE/g to 80.2 mg GAE/g. This finding agrees with that of Dueñas et al. [10], who reported that natural or lactic acid fermentation at 37 °C for 48 h increased phenolic acid derivatives in cowpeas. Similarly, Svensson et al. [28] also observed significant increase in phenolic acids in red sorghum fermented at 34 °C for 24 h. In the same way, Katina et al. [29] reported that fermentation of ray bran with baker's yeast for 14 h resulted in a favorable release of phenolic acids and enhanced phenolic acid content. Furthermore, a significant increase in phenolic content was observed during millet flour after 72 h of fermentation [30]. In contrast, Álvarez et al. [31] reported a decrease in phenolic content during the fermentation of Cupuassu beans after 6 days of fermentation. The increased phenolic content could be attributed to the ability of the fermentation process to convert conjugated forms of phenolic compounds to their free forms [32]. The increase in TPC could also be due to the production of proteolytic enzymes by the microorganisms that are responsible for hydrolyzing complexes of phenolic into soluble-free phenols [32].

Table 3. Effect of natural fermentation on the phytochemical content of "Nabag" (*Ziziphus spina-christi* L.) seeds.

Fermentation Time	TPC (mg GAE/g)	TFC(mg CE/g)	Tannins(mg/g)	Vitamin C(mg/g)	Carotenoids (mg/g)
Control	59.7 ± 0.75^{d}	42.4 ± 0.72^{e}	35.49 ± 0.71^{a}	5.47 ± 0.72^{c}	6.5 ± 0.12^{e}
6 (h)	64.7 ± 0.41^{c}	60.6 ± 0.26^{b}	16.65 ± 0.23^{b}	7.19 ± 0.54^{b}	9.6 ± 0.15^{d}
12 (h)	65.4 ± 1.89^{c}	64.9 ± 0.72^{a}	16.53 ± 0.23^{b}	8.59 ± 0.72^{a}	11.2 ± 0.29^{c}
24 (h)	71.9 ± 1.89^{b}	53.7 ± 0.71^{c}	15.79 ± 0.36^{c}	8.91 ± 0.47^{a}	13.4 ± 0.31^{b}
48 (h)	80.2 ± 1.80^{a}	47.6 ± 0.64^{d}	13.21 ± 0.36^{d}	9.06 ± 0.27^{a}	14.0 ± 0.44^{a}
F-test	**	**	**	**	**
LSD 0.05	**3.394**	**1.130**	**0.505**	**1.009**	**0.520**

Values are means (±SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly ** different at ($p < 0.05$). Means in the same column without superscript are not significantly ($p < 0.05$) different, ns; no significant different at ($p < 0.05$) as assessed by least significant difference (LSD).

Table 3 shows that the TFC of *Ziziphus spina-christi* seeds increased significantly during 48 h of fermentation compared unfermented seeds. The highest TFC value (64.9 mg CE/g) was obtained when the seeds were fermented for 12 h. Increasing the fermentation time to 24 and 48 h significantly ($p < 0.05$) reduced the TFC to 53.7 and 47.6 mg CE/g, respectively. Similar observations of an increase in TFC content have been reported by investigators. Lizardo et al. [33] found that the fermentation increased the TFC in cherry silverberry. Similarly, the fermentation of okra seeds for 24 h was found to increase the total flavonoids by 492.10% compared with those of unfermented seeds, and prolonged fermentation time gradually reduced TFC [33]. In contrast, Ehsan et al. [7] observed a decrease in TFC during the fermentation of pistachio hulls with an increase in fermentation period, which they attributed to the sample concentration of flavonoid compound or the duration of the fermentation process. The increased TFC content of the seeds during fermentation may be due to the ability of microorganisms to release the bound flavonoid components and increase their availability for extraction [34].

Data on the effect that fermentation had on the tannin content of the *Ziziphus spina-christi* seeds (Table 3) indicated that the tannins content progressively decreased as the

period of fermentation increased. Fermentation for 6, 12, 24, and 48 h resulted in a reduction of tannin content by 53.1%, 53.4%, 55.5%, and 62.8%, respectively. Several workers have observed a significant reduction of tannin by natural fermentation. Dhull et al. [35] reported decreased tannin content in fermented seeds and grains. Similarly, Osman [36] found that natural fermentation significantly decreases the tannins content of three sorghum varieties. The reduction in tannin content in the fermented product may be attributed to increased microbial enzyme activity, which results in the degradation and extractability of the tannin compounds [37].

The increase in vitamin C content during fermentation of the "Nabag" seeds is shown in Table 3. During a 48 h fermentation, vitamin C content was significantly ($p < 0.05$) increased. Generally, it increased from 5.47 mg/100 g in unfermented seeds to 9.06 mg/100 g in fermented seeds. Similarly, an increase in vitamin C during fermentation was reported by other researchers. Adetuyi and Ibrahim [34] observed that fermentation of okra (*Abelmoschus esculentus*) seeds up to 120 h resulted in an increase in vitamin C content, with the highest increase occurring after a 24 h fermentation. Similarly, Kusznierewicz et al. [38] reported an increase in vitamin C content after fermentation of cabbage.

Table 3 shows the total carotenoid content of unfermented and fermented Ziziphus spina-christi seeds. Similar to vitamin C, there was a progressive increase in total carotenoid content with increase of fermentation time. The fermentation of seeds for 48 h resulted in the highest total carotenoid content of 14.0 mg/g compared to 6.5 mg/g for unfermented "Nabag" seeds. This finding agrees with that of Oloo et al. [39], who reported a significant increase in the carotenoid content of orange flash potato fermented for 2 days. Similarly, Ortiz et al. [40] observed that the fermentation of bio-fortified maize for 24 and 72 h resulted in a 100% increase of pro-vitamin A carotenoid, while prolonging the fermentation time to 120 h or more reduced the carotenoid content of maize.

3.4. Effect of Natural Fermentation on Antioxidant Activity of "Nabag" (Ziziphus spina-christi L.*) Seeds*

Various antioxidant activity assays such as (DPPH) scavenging activity, TRP, FRAP, and hydrogen peroxide scavenging activity assays were used to evaluate the effect of fermentation on the antioxidant activity of "Nabag" (*Ziziphus spina-christi* L.) seeds. The results of the antioxidant activities of fermented "Nabag" seeds as a function of fermentation time is shown in Table 4. The antioxidant activities progressively increase in the four methods used to assess the activity. There was a significant ($p < 0.05$) increase in the DPPH (50.0 mg Trolox/g), FRAB (77.6 mg Trolox/g), TRP (4.7 mg AAE/g), and H_2O_2 (76.3%) at 48 h when compared to 25.7 mg Trolox/g, 49.6 mg Trolox/g, 2.1 mg AAE/g, and 50.0% for unfermented seeds for the four methods. Adetuyi and Ibrahim [34] reported that the antioxidant activity of fermented okra seeds was significantly higher than that of unfermented seed, while prolonging the fermentation period to 72 h reduced the antioxidant activity.

Table 4. Effect of natural fermentation on the antioxidant activity of "Nabag" (*Ziziphus spina-christi* L.) seeds.

Fermentation Time	DPPH(mg Trolox/g)	FRAP(mg Trolox/g)	TRP(mg AAE/g)	H_2O_2(%)
Control	25.7 ± 0.50^{d}	49.6 ± 0.76^{e}	2.1 ± 0.12^{e}	50.0 ± 0.08^{e}
6 (h)	36.5 ± 0.18^{c}	56.8 ± 0.38^{d}	2.4 ± 0.13^{d}	63.3 ± 0.11^{d}
12 (h)	37.1 ± 0.28^{c}	66.8 ± 0.38^{c}	$3.S3 \pm 0.15^{c}$	68.2 ± 0.09^{c}
24 (h)	39.7 ± 0.69^{b}	68.7 ± 0.50^{b}	4.3 ± 0.06^{b}	72.1 ± 0.10^{b}
48 (h)	50.0 ± 0.30^{a}	77.6 ± 0.52^{a}	4.7 ± 0.05^{a}	76.3 ± 0.11^{a}
F-test	**	**	**	**
LSD 0.05	**0.947**	**0.198**	**0.198**	**0.180**

Values are means (±SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly ** different at ($p < 0.05$). Means in the same column without a superscript are not significantly ($p < 0.05$) different, ns; no significant difference at ($p < 0.05$) as assessed by least significant difference (LSD).

The enhanced antioxidant activity resulting from fermentation could be attributed to the increase in phenolic compounds and flavonoids, due to the microbial enzymes hydrolysis of phenolic glycosides and release of aglycone, which contributes to increased antioxidant activity. Additionally, fermentation was reported to induce the structural breakdown of plant cell walls, resulting in the release of various antioxidant compounds [41].

3.5. Principal Component Analysis and Partial Least Squares Regression Analysis

PCA using the HJ-Biplot method was performed to categorize the relationships between the natural fermentation of "Nabag" (*Ziziphus spina-christi* L.) seeds and its phytochemical compound contents (TPC, TFC, tannin, vitamin C, and total carotenoid) and antioxidant activities (DPPH, FRAP, TRP, and H_2O_2). The results clearly showed the association of the fermentation process on physiochemical characteristics, phytochemical compounds, and antioxidant activities of the seeds (Figure 1). Interestingly, the contribution of the axes of the principal components, PC1 and PC2, was 81.53% and 15.52%, respectively, which resulted in a high variability (97.05%) of the plotted components. Moreover, a strong positive correlation was evident between radiation treatment and the quality parameters of spices, since an acute angle was found between the vectors of these parameters. Yan and Fregeau-Reid [42] explained that the eccentricity of characters that appear at a <90° angle is positively correlated, whereas the factors that formed > 90° angles are associated with a negative correlation. Those with a 90° angle show no correlation in the biplot. Consequently, the treatments were divided into three separate groups. The control sample (unfermented seeds) showed greater values for tannin content. The fermented seeds, particularly at 24 and 48 h, showed the highest correlation between treatment and content of phytochemical compounds and antioxidant activity. Accordingly, these findings revealed that fermenting "Nabag" (*Ziziphus spina-christi* L.) seeds improves their physicochemical properties and antioxidant activity. Hence, these seeds may be used for the production of nutritional foods after fermentation.

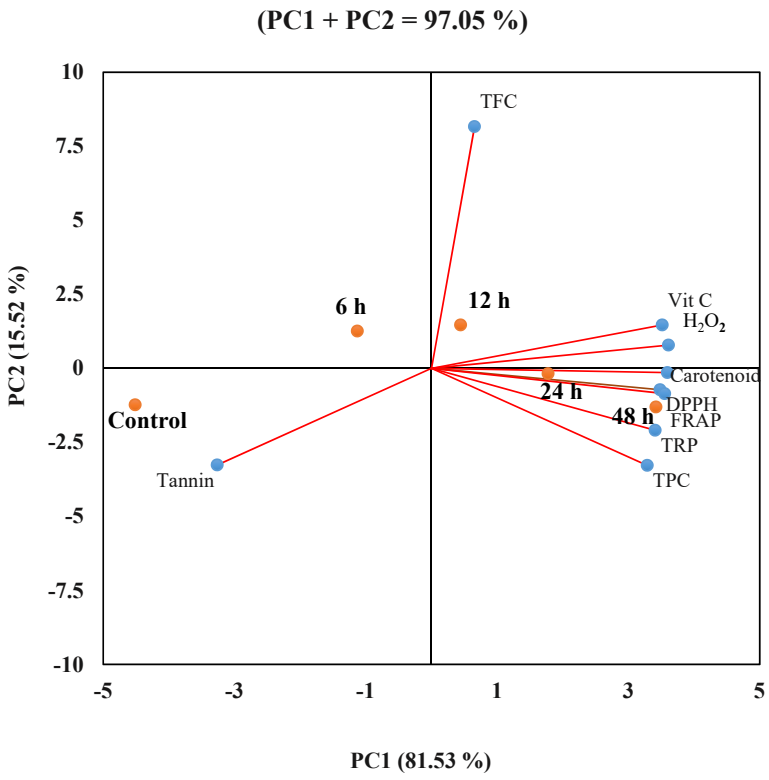

Figure 1. Principal Component Analysis (PCA) of fermented "Nabag" (*Ziziphus spina-christi* L.) seeds, phytochemical compound contents (TPC, TFC, tannin, vitamin C, and total carotenoid), and antioxidant activities (DPPH, FRAP, TRP, and H_2O_2).

A partial least squares regression analysis (PLS) was performed to validate the effects of fermentation on phytochemical compounds and antioxidant activity of "Nabag" (*Ziziphus spina-christi* L.) seeds. The interactive effects of fermentation and the fermentation time (x variables) on the measured parameters (y variables) of the seeds were clearly observed (Figure 2). The PLS revealed that the fermentation of "Nabag" (*Ziziphus spina-christi* L.) seeds, particularly those fermented for 48 h, exhibited a positive validation score for most of the studied parameters. Thus, the PLS specified that the application of fermentation for 24 to 48 h reflects the most valid treatment for functional food applications.

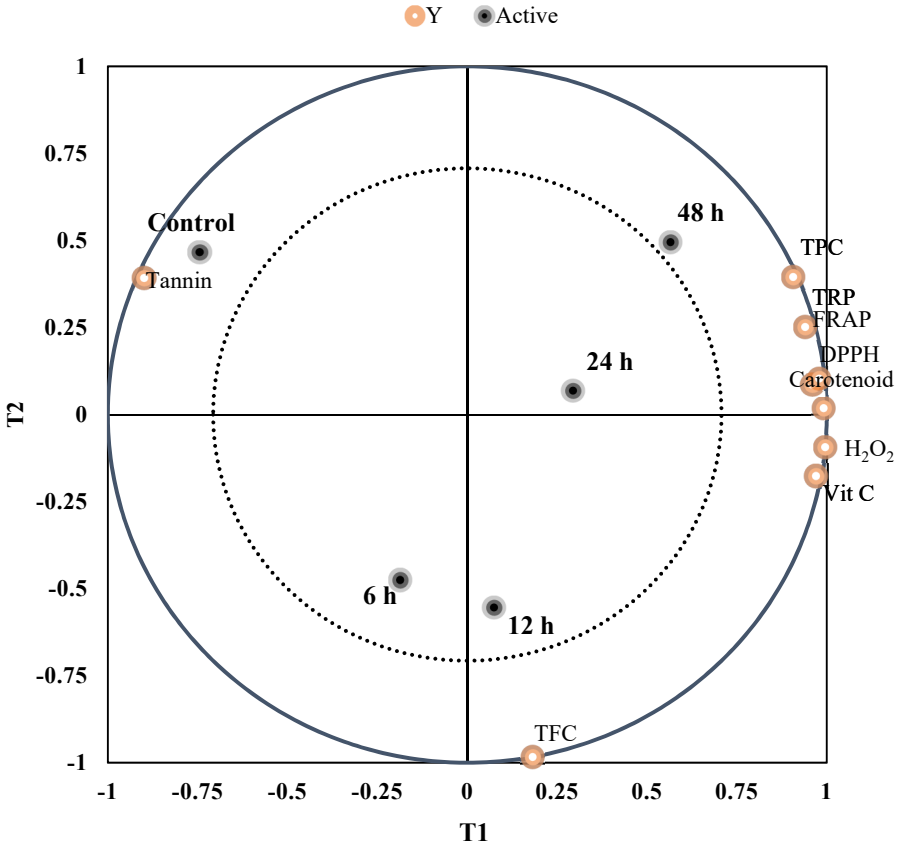

Figure 2. Partial least squares regression analysis (PLS) of fermented "Nabag" (*Ziziphus spina-christi* L.) seeds, phytochemical compound contents (TPC, TFC, tannin, vitamin C, and total carotenoid), and antioxidant activities (DPPH, FRAP, TRP, and H_2O_2).

4. Conclusions

This study evaluated the effects of natural fermentation for different periods of time (6, 12, 24, and 48 h) on the chemical composition, mineral content, phytochemical compound content, and antioxidant activity of "Nabag" *Ziziphus spina-christi* (L.) seeds. The fermentation process caused a marked increase in chemical composition, mineral (Ca, Zn, and Fe), TPC, TFC, vitamin C, and total carotenoid content of "Nabag" seeds. Fermentation also enhanced the antioxidant activity in terms of DPPH, FRAP, TRP, and H_2O_2 scavenging activity of the "Nabag" seeds. Fermentation for 48 h was the optimal time for fermentation treatment. Due to its high content of crude fiber, Ca, Fe, and the bioactive component, the fermented Nabag" seeds could be used in formulation of functional food to combat anemia in pregnant woman and as a source of Ca for lactating women in underdeveloped countries. However, more studies should be conducted with longer fermentation time to characterize the bioactive compounds of raw and fermented "Nabag" seeds to develop new supplementary foods.

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