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Effect of Freeze-Drying of Prickly Pear (Opuntia Ficus-Indica) on Flavonoids and Antioxidants

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Abstract: Prickly pear fruits (PPF) play a high-quality role in health due to its rich content of antioxidant and phenolic materials. This research is a cross sectional descriptive study, the efficacy of the drying method on the chemical attributes of PPF pulp on quality and safety during the storage period. The sample was divided into control and treatment by drying (oven-drying (OD) and freeze-drying (FD)). The flavonoid concentration in the pulp methanolic extracts was measured using spectrophotometry; the diphenylpicrylhydrazyl (DPPH) capacity was assessed using an adapted DPPH free radical-scavenging method, values of total phenolic content (TPC) and total flavonoid content (TFC) of fresh and dried PPF. The drying method had a significant effect on TFC; values were 39.88 and 18.05 µg/g CE fresh weight (FW) for the OD and FD, respectively. The FD exhibited about 77.56 ± 8.4 for TPC whereas the TFC estimated to 26.8 ± 11.4 mg GAE/100 gm FW. The DPPH for FD samples was significantly higher (249.8 ± 20.6) than that of the OD (75.2 ± 7.2). Mean values of Cupric Ion Antioxidant Reducing Capacity (CUPRAC) were 1005, 973 and 553 μM_{TE} for fresh, FD and OD samples respectively. Meanwhile, the values were in significant differences in comparison between FD and OD of 973.5 ± 87.1 and 553.15 ± 16.6, respectively. The chemical composition, antioxidant capacity (DPPH, FARAP & CUPRAC) of PPF, and TFC of fruits were changed by drying. FD had the best results for the antioxidant capacity values as were the closest to the values of the fresh sample. Therefore, FD for PPF storage is proven to be recommended.

Keywords: freeze-drying, prickly pear, Opuntia ficus-indica, antioxidants.

刺梨(仙人掌)冷冻干燥对类黄酮和抗氧化剂的影响

摘要：仙人掌果(PPF)因其含有丰富的抗氧化物质和酚类物质而发挥着优质的保健作用。本研究是一项横断面描述性研究，研究了干燥方法对 PPF 纸浆化学属性的影响，以及储存期间的质量和安全性。通过干燥（烘箱干燥（外径）和冷冻干燥（FD））将样品分为对照和处理。使用分光光度法测量果肉甲醇提取物中的类黄酮浓度；使用经过调整的 DPPH 自由基清除方法、新鲜和干燥 PPF 的总酚含量(台积电)和总黄酮含量(三氟化碳)值评估二苯基苦基肼(DPPH)能力。干燥方式对三氟化碳有显着影响；外径和 FD 的 CE 鲜重(固件)值分别为 39.88 和 18.05 微克/克。台积电的 FD 约为 77.56 ± 8.4，而三氟化碳估计为 26.8 ± 11.4 毫克通用电气工程师协会/100 克固件。FD 样品的 DPPH (249.8 ± 20.6) 明显高于外径(75.2 ±7.2)。新

鲜样品、FD 样品和外径样品的铜离子抗氧化还原能力(CUPRAC)的平均值分别为 1005、973 和 553 μMTE。同时，FD 和外径之间的值分别为 973.5 ± 87.1 和 553.15 ± 16.6，具有显着差异。PPF 的化学成分、抗氧化能力（DPPH、法拉普和 CUPRAC）和水果的三氟化碳因干燥而发生变化。FD 的抗氧化能力值最好，因为它最接近新鲜样品的值。 因此，事实证明，推荐使用用于 PPF 存储的 FD。

关键词：冷冻干燥，刺梨，仙人掌，抗氧化剂。

1. Introduction

PPF harvested from various species of the cactus, genus Opuntia of the cactus family Cactaceae, it is rich in nutritive values, including minerals, ascorbic acid, phenolic substances, amino acids, and antioxidants. Additionally, it plays a high-quality role in health due to the richness of high antioxidant activity and phenolic materials [1]. Due to its antioxidant activity, phenol content, and ascorbic acid content, PPF has essential functional effects [2]. Antioxidants in PPF display anti-inflammation, anticancer, hypocholesterolemic, hypoglycemic, and hypolipidemic effects [3]. The antioxidant activity of this fruit was shown to be double that of tomato, apple, pear, and grape. It was also discovered to be comparable to orange, red grape raisin, and grapefruit [4].

The composition of PPF and cladodes depends especially on the species, the horticultural practices, and the level of ripening. Cactus stems, as other vegetables, have low proteins and fats, but the crude fiber is higher than that in most other vegetables [5]. Flavonoids (found in phenolic compounds) are another important constituent in Opuntia pulp fruits. Several flavonoids have an antioxidative effect [6]. Opuntia ficus-indica (OFI) has traditionally marked the folk medicine, owing to its therapeutic properties due to excess of bioactive molecules, involving organic acids, phenolic acids, flavonoids, betalains, carotenoids, vitamins, biothiols, taurine, saponins, fatty acids, and phytosterols. The content of these bioactive molecules varies within cladodes, fruits or prickly pears, peels, seeds, and flowers [7].

[8] investigated the nutritional composition, anti-nutritional factors, phytochemical and sensory attributes of cactus pear seeds collected from Ethiopia. The sample provided 392.84 kcal/100 g energy on a dry weight basis. The dietary Ca, K, P, Fe, and Zn contents of the sample accounted 390.14 mg, 446.46 mg, 206.18 mg, 4.37 mg, and 2.01 mg per 100 g, respectively. Despite the high phytate content (259.20 mg/100 g), the sample had an appreciable amount of antioxidant capacity (43 to 95% of inhibition). [9] determined the phenolic and flavonoid contents of two Opuntia species (OFI and Opuntia streptacantha (OS)) and evaluated the antioxidant activity of the fruit pulp. The study found that OS fruit extract contained more phenolic and flavonoids than OFI. Using the DPPH method, the reducing power, the highest activities were found for extracting OS fruit. The amount of phenolic and flavonoid compounds implied as possible factor that influenced the antioxidant power of the OFI and OS fruit extract. [10] referred to the properties of fruits, great sources of antioxidants (betalains, total phenolic content, vitamin C, and β-carotene). Following the DPPH method, the antioxidant activity was recorded, and the results reviled that OFI has 526.238 mg of AAE/100 g and 513.764 mg of AAE/100 g antioxidant activity for methanol and distilled water, respectively.

This study investigates the content of antioxidant and phenolic compounds in OFI after the FD process.

2. Materials and Methods

The chemical content of PPF, moisture, ash content, and nutritional properties allow evaluating the antioxidant capacity and bioactive contents. The research hypothesis is that freeze- and oven-drying processes affect the PPF chemical properties, phenolic and flavonoid components, and antioxidant capacity.

2.1. Preparation of the Samples

The whole fresh fruits with a maturation level were selected from freshly harvested fruits, manually peeled, and the pulp of the selected fruits was divided into two groups. The pulp of the first group were sliced into 1 and 1.5 cm thickness pieces, placed in aluminum trays (14 cm^2), and kept at -20°C for at least 24 hours before the OD technique. The other group was chopped into 1×1 cm pieces, placed in the aluminum trays, frozen in liquid nitrogen, and were kept at -80°C. The frozen pulps (200 g) were freeze-dried and stored at -20°C using a Labconco FreeZone 4.5 freeze-dry system (Labconco, Kansas City, MO, USA) with the condensing temperature of -55°C and vacuum pressure of 7 Par. Half of the freeze-dried pulp samples were packed into polyethylene bags, and the other half was put in glass jars, both of them kept in a refrigerator at 4±1°C until analysis. For the extraction procedure, the pulps were pressed by hand. Five grams of squeezed pulps were added to 50 ml of 50% methanol for each fruit sample and extracted for 24 h at room temperature

with magnetic stirring. The supernatant was filtered through a cotton plug followed by 0.45 µm microbiological filters after centrifugation at 4500 g for 10 min. The extract was then kept at 4°C in a dark bottle for further study.

2.2. Chemicals and Reagents

Table 1 The reagents and chemicals used in this work were of analytical grade

Item	Supplier
Copper (II) chloride dehydrate, Ammonium acetate, Sodium nitrate, Neocuproine, Sodium acetate trihydrate, Iron (III) Chloride, Sodium carbonate, Potassium Persulfate, Aluminum chloride, Gallic acid, Sodium hydroxide, Rutin stander	AppliChem-Germany
2,2-diphenyl-1-picrylhydrazyl (DPPH)	Biological Industries-Germany
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)	
Folin-Ciocalteu reagent	
Ferric-reducing power antioxidant activity (FRAP) reagent	
Methanol	Sigma Aldrich - USA
Hydrochloric	
De-ionized water	Prepared in the lab

2.3. Determination of TPC

The Folin-Ciocalteu method [11] was applied to determine TPC. In brief, the methanolic extracts (100 µl) were mixed with 900 µL of Folin-Ciocalteu reagent (diluted 1:10 with water). After 5 min, 7% sodium bicarbonate solution (0.75 ml) was added to the mixture and vortexed for 30 seconds. The previously mentioned solution was maintained at room temperature for 90 minutes. The absorbance of the solution was read by a spectrophotometer (Sica type) at 765 nm. Compared to a gallic acid calibration curve, TPC was reported as gallic acid equivalents (GAE). All readings were expressed as the mean of three replications (mg of gallic acid equivalents/100 g of FW) ± standard deviation (SD).

2.4. Determination of TFC

The generation of complex flavonoid-aluminium complexes was used to determine the flavonoid concentration in the pulp methanolic extracts using spectrophotometry [12]. 1 ml of pulp extract was mixed with 1 ml of 2% aluminum chloride solution (w/v). After a 15-minute incubation period at room temperature, the absorbance of the reaction mixtures was quantified at 430 nm against a blank. The standard used to create the calibration curve was rutin. All measurements were repeated for three times and the results were represented as rutin equivalents (mg RE/100 g dry FW).

2.5. Determination of DPPH Radical-Scavenging Activity

The DPPH capacity of PPF pulp was assessed using an adapted DPPH free radical-scavenging method, and the absorbance was estimated at 515 nm, with concentrations represented as micromolar of Trolox equivalents (a gallic acid calibration curve); TPC was reported as gallic acid equivalents (GAE). All readings were expressed as the mean of three replications (mg of gallic acid equivalents/100 g of FW) ± standard deviation (SD).MTE; dry weight [13].

2.6. Determination of CUPRAC

The CUPRAC of PPF pulp extracts was assessed based on the Apak team method [14]. Absorbance was measured at 450 nm and expressed as micromolar of Trolox equivalents (µMTE; dry weight) [13].

2.7. Determination of FRAP

The FRAP assay was carried out using the method described by [15]. Every 200 ml of the test tube extract received an aliquot of 3.0 mL of FRAP reagent. The mixture was stored at 37°C in the dark. Absorbance was read at 593 nm after 30 min. Micromole Trolox Equivalent per gram fresh weight (µmol TE/g FW) was used to calculate the recoded values.

2.8. Statistical Analysis

The gathered data were analyzed using the Statistical Package for the Social Sciences (SPSS vr. 16). Using analysis of variance, the statistically significant difference between fresh and freeze-dried fruits according to antioxidant compounds and capacity was determined at $p \leq 0.05$ and $p \leq 0.01$.

3. Results and Discussion

3.1. Chemical Attributes of PPF

The chemical composition parameters are listed in Table 2. Pulp recorded a high amount of water (90.32%). These results were in the same trend as those obtained in previous studies [16]. Ash and total carbohydrates in the pulp amount to 0.06 and 9.42%, respectively. Results obtained by [16] showed a high amount of water in the pulp (84.14%).

Table 2 Chemical composition of PPF (%, w/w, FW)

Item	The Pulp
Moisture content %	90.32 ± 3.14*
Dry Matter %	9.68 ± 1.02
Organic Matter %	9.62 ± 0.89

Continuation of Table 2	
Ash %	0.06 ± 0.002
Carbohydrates %	9.42 ± 0.06

Notes: All values are means of three replicates ± SE; * Statistically significant difference ($p < 0.01$)

3.2. Efficacy of the Dry Method on the Chemical Attributes of Fruit Pulp

Other than the moisture content, ash, and crude fiber, there are no significant differences in the rest of the measurements shown in Table 3. Across the two methods of drying, the average value of moisture content removed from PPF pulp was between 87.7 ± 1.79 and 89.21 ± 0.13%. This suggests that the freeze-dryer caused more moisture loss than OD. FD or OD at the temperature lower than 50°C and for short times is recommended by [17] and [18]. The FD technique minimizes the effect on the nutritional values of foodstuffs. Also, the use of FD for moisture removal was more effective compared to other drying methods.

Table 3 Efficacy of FD and OD on the chemical composition of PPF pulp (%, W/W)

%, Item	%, Chemical Composition		
	Fresh	**OD**	**FD**
Moisture content	90.32 ± 1.55	3.25 ± 0.15**	1.11 ± 0.01**$^{++}$
Dry Matter	9.68 ± 0.42	96.75 ± 4.12**	98.89 ± 3.44**
Protein (N × 6.25)	0.13 ± 0.002	1.38 ± 0.11*	1.39 ± 0.06*
Crude Fats	0.07 ± 0.005	0.72 ± 0.009*	0.70 ± 0.01*
Crude Fiber	0.12 ± 0.005	1.26 ± 0.003*	3.72 ± 0.26**$^{++}$
Ash	0.06 ± 0.001	0.59 ± 0.003*	4.36 ± 0.15**$^{++}$
Carbohydrates	9.42 ± 0.76	89.35 ± 3.18**	92.44 ± 5.03**

Notes: All values are means of three replicates ± SE; * indicates statistical significant difference (*$p < 0.05$; **$p < 0.01$) compared to fresh PPF pulp; + indicates statistical significant difference (+$p < 0.05$; ++$p < 0.01$) between OD and FD samples.

3.3. Efficacy of the Drying Method on TPC and TFC of PPF Pulp

The data presented in Table 4 clearly indicate a statistically significant difference ($p < 0.05$) in values of TPC and TFC of fresh and dried PPF pulp belonging to the two drying methods. The freeze-drying method of PPF pulp exhibited 77.56 ± 8.4 mg GAE/100 gm FW for TPC, whereas TFC was estimated to 26.81 ± 11.4 mg GAE/100 gm FW for the same sample. These values are significantly higher than those reported by [19], who found that TPC was 45.2 ± 7.4 mg GAE/100 gm. The impact of the drying method on the TPC of fruits has been investigated. The drying process was reported to induce a drop in TPC by [20, 21]. The study by [22], however, indicated that the drying process increased TPC, and this increase may be because heat treatment converts some phenolic compounds, and some are released to the medium. In this respect, it can be said that the drying method does not have the same effect on the TPC of different products [23]. A previous study by [24] has shown that the content of phenols in fruit extracts affects the activity of antioxidants. Concerning the effect of different drying methods on the TFC of PPF pulp, the results in Table 4 clearly show that the drying method had a significant effect on the TFC, as the recorded values were 39.88 and 18.05 µg/g CE FW; for the OD and FD method, respectively. The data presented in Table 4 indicate that our study may support this hypothesis. In a study by [25], they found that TFC in PPF was 1.91 ± 0.29 mg QE/g DM. TFC may decrease because of some interactions in the structure of fresh vegetables and fruits that are subjected to preservation techniques such as freeze or dry [26]. Generally, TPC and TFC levels in fresh and frozen PPF pulp were higher than those in oven-dried fruits in this research. These findings may support the hypothesis that freezing is the optimal strategy for preserving PPF.

Table 4 Effect of drying methods (FD and OD) on TPC and TFC of PPF pulp

Parameters	Fresh sample (control)	Drying method FD	OD
TPC	103.05 ± 7.11	77.56 ± 8.4*$^{+}$	26.81 ± 11.4**
TFC	56.32 ± 3.48	39.88 ± 5.1*$^{+}$	18.05 ± 1.7*

Notes: All values are means of three replicates ± SE; * indicates statistical significant difference (*$p < 0.05$; **$p < 0.01$) compared to fresh PPF pulp; $^{+}$ indicates statistical significant difference ($^{+}p < 0.05$; $^{++}p < 0.01$) between OD and FD samples.

3.4. Efficacy of the Drying Method on the Antioxidant Capacity of PPF Pulp

3.4.1. The DPPH Radical Scavenging Activity

Table 5 indicates significant differences ($p < 0.05$) in free radical scavenging activity between fresh, freeze-dried, and oven-dried PPF pulp. The DPPH scavenging activity for freeze-dried samples was significantly ($p < 0.05$) higher (249.80 ± 20.6) than that of the oven-dried sample (75.21 ± 7.2). The scavenging activity of the PPF extract on DPPH radicals were also demonstrated by [27].

3.4.2. CUPRAC

The CUPRAC analysis of the three various samples tested OFI fruit pulp indicated variation in values between types: fresh, freeze-dried, and oven-dried (Table 5). The mean values of CUPRAC were 1005, 973, and 553 µMTE for fresh, freeze-dried, and oven-dried samples, respectively. There were no significant differences ($p > 0.05$) in values between fresh and freeze-dried samples. Meanwhile, the values showed significant differences ($p < 0.05$) in comparison between the two drying methods (973.52 ± 87.1 and 553.15 ± 16.6 for freeze-drying and oven drying,

respectively). Reducers work by breaking the free radical chain by donating a hydrogen atom, converting free radicals to more stable molecules and so reducing oxidative damage [28, 29].

3.4.3. FRAP Assay

The FRAP assay determines a compound's overall reducing capacity by determining its ability to decrease the Fe3+/tri pyridyl triazine complex to its blue-colored ferrous state. Table 5 lists the reducing power of the PPF pulp. Our results showed that the fresh PPF pulp exhibited a higher FRAP value of 510.12 ± 13.5 µmol TE/g FW compared to the two drying methods used in this study. Also, the freeze-dried PPF pulp exhibited higher FRAP values (413.47 ± 17.1 µmol TE/g FW) compared to that of the oven-dried samples (200.57 ± 10.1 µmol TE/g FW) and there was a significant ($p < 0.05$) difference between the means. There was no significant change in FRAP rates between the fresh and freeze-dried samples ($p > 0.05$).

The obtained results in Table 5 clearly indicate that the two drying methods used in this study had the same effect of both the DPPH assay and FRAP assay. The FRAP method was developed to be better suited for evaluating the antioxidant activity. These results are in agreement confirmed the findings of [30], who developed the FRAP method for better evaluations of the antioxidant activity of water and lipid soluble components.

Table 5 Effect of drying methods (FD and OD) on the antioxidant activity of PPF pulp

Parameters	Fresh sample (control)	Drying method FD	OD
DPPH	318.00 ± 11.7	249.80 ± 20.6	75.21 ± 7.2*+
CUPRAC	1005.80 ± 36.1	973.52 ± 87.1	553.15 ± 16.6*+
FARAP	510.12 ± 13.5	413.47 ± 17.1	200.57 ± 10.1*+

Notes: All values are means of three replicates ± SE; * indicates statistical significant difference (*$p < 0.05$; **$p < 0.01$) compared to fresh PPF pulp; $^{+}$ indicates statistical significant difference ($^{+}p < 0.05$; $^{++}p < 0.01$) between OD and FD samples.

4. Conclusion

In this study, we have examined the efficacy of two drying methods (oven drying and freeze-drying) on the chemical composition and antioxidant capacity (DPPH, FARAP & CUPRAC) of PPF pulp. Freeze-drying had the best results for the antioxidant capacity values as closest to the values of the fresh sample. Overall, this study indicated that freeze-drying could improve the overall quality of the dried PPF pulp samples in terms of antioxidant capacity. Comparing the two drying methods, the average value of moisture content removed from PPF pulp was higher with 87.7 ± 1.79 and 89.21 ± 0.13%. The study also recorded values of TPC and TFC of fresh and dried PPF pulp, belonging to the two drying methods. The freeze-drying method of PPF pulp exhibited about 77.56 ± 8.4 mg GAE/100 gm FW. These findings may support the hypothesis that freezing is the optimal strategy for preserving PPF. Finally, considering the results of this study, we recommend freeze-drying method for drying PPF pulp.

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