



RESEARCH ARTICLE

Effect of freeze drying on the nutritional quality and antioxidant activity of selected underutilized fruit seed powders

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ABSTRACT

This study examines the effect of freeze-drying on the chemical composition, minerals and antioxidant activity of underutilized Jamun (*Syzygium cumini*), Bael (*Aegle marmelos*) and Custard apple (*Annona squamosa*) seeds. Fresh seeds were dehydrated by freeze drying and results showed significant increase in ash, crude protein, crude fat, crude fibre and minerals but a slight decrease in total phenolics, vitamin C and antioxidant activity. Significant ($p \leq 0.05$) differences for the vitamin C and total phenolic content were found between the fresh and freeze-dried seed powders except for Bael seed powder. Further, there was no significant ($p \geq 0.05$) difference observed in the antioxidant activity of the fresh and freeze-dried seeds. However, the results of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay revealed that fresh seeds of Jamun followed by Custard apple had relatively higher antioxidant activity. In case of minerals, a significant ($p \leq 0.05$) variation was recorded among the fresh and freeze-dried seeds. Overall, based on nutritional analysis performed in *in natura* and freeze-dried seeds, freeze drying resulted in better nutrient retention and thus, can be opted as technological method when proximate composition is under consideration.

Keywords: Wild fruit seeds; drying; Bioactive compounds; Nutritional analysis; nutraceutical potential

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INTRODUCTION

Underutilized fruits, also known as minor, wild or neglected fruits are neither cultivated commercially nor traded extensively (Chandra et al., 2017). Several underutilized fruits such as Jamun (*Syzygium cumini*), Bael (*Aegle marmelos*) and Custard apple (*Annona squamosa*) produce edible and highly flavored fruits and have excellent nutritional and therapeutic properties (Manandhar et al., 2018 ; Jagtap and Bapat, 2008; Hameed et al., 2020).

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The proximate composition of these fruits had been widely studied. The fruits were shown to contain vitamin C, fibers, amino-acids, saccharides and large amounts of minerals (Baliga et al .,2011; Brandao and Santos, 2016). Additionally, these fruits were shown to contain polyphenols, flavonoids, anthocyanins and carotenoids and therefore, act as a source of supplementary nutrition (Sagrawat et al ., 2006; Maity et al .,2009; Mainasara et al .,2018) . Numerous studies have demonstrated the potential use of these fruits in preventing cellular compounds such as membranes, nucleic acid, proteins and lipids from oxidative disorders like diabetes, obesity, various forms of cancer, neurodegenerative diseases and cardiovascular diseases (Baliga et al ., 2011; Baliga et al.,2011 ; Mainasara et al ., 2018).

Processing generally divides these fruit into edible pulp and inedible seed and skin portion (Ettalibi et al., 2020). The pulp alone represents about 75% of these fruits and is widely used in the manufacturing of juices, alcoholic beverages, confections etc (Kaur et al., 2018). On the other hand, seeds and skin, the promising fruit by-products constituting about 20-25% are discarded as waste (Kaur et al., 2018). The valorization of these by-products on one hand could reduce waste disposal problems and on the other hand serve as a valuable source of bioactive compounds such as sugars, minerals, organic acids, dietary fibre and phenolics (Satari and Karimi , 2019). Nowadays, more investigations have attracted attention on the by-products generated after the fruit processing. Research studies have demonstrated the potential use of these by-products as antioxidant, anti-diabetic, anti-inflammatory, anti-cancer, anti-scorbutic, antimicrobial, anti-allergenic etc. (Aqil et al., 2012; Mwihia et al ., 2017). Mainly, studies of underutilized fruit seed by-product have focused on the extraction and characterization of bioactive compounds. However, seeds obtained after processing have high moisture content that limits their storage life and exploitation. Therefore, the aims of this study were to characterize nutritional parameters of fresh fruit seeds of three underutilized fruits namely Jamun, Bael and Custard apple and to study of the freeze drying impact on the quality criteria of the dried seed powders.

MATERIALS AND METHODS

Fresh fruits of Jamun and Custard apple were procured from the Narwal Fruit Mandi, Jammu while as Bael fruit was collected from the Rainfed Research Sub-Station for Sub Tropical Fruits, Raya of SKUAST-Jammu. The fruits of Bael were stored in the pilot plant of Division of Food Science and Technology, SKUAST-Jammu till proper ripening. The fruits were washed under running tap water to remove the adhered dirt followed by pulping and separation of seeds manually. The seeds were washed immediately and freeze-dried at a temperature of -60 °C with the vacuum pump operating at a pressure of 410^{-4} mbar. The dried seeds obtained were ground to a fine powder using a grinder, packed in laminated pouches and stored at ambient temperature until analyzed.

Proximate Analysis

Moisture content was determined by using an electronic moisture analyser at 105 °C by taking about 2g sample in aluminum sample holder and the evaporative moisture losses were automatically expressed as percentage moisture content. Ash content of fresh as well as dried samples was performed at 550 °C using muffle furnace

(AOAC, 2005). Crude protein was expressed as $N \times 6.25$ as determined according to the Micro Kjeldahl procedure. The crude fat was extracted using petroleum ether as a solvent for about 16 hours (boiling point 60-80 °C). Determination of crude fibre content in the samples was done using acid/ alkali digestion method (Dangoggo et al., 2011).

Determination of Ascorbic Acid

About 50 mL of 3% metaphosphoric acid was mixed with 10 gram sample. Volume make up was done to 100 mL with 3% metaphosphoric acid. The mixture obtained was then filtered. 10 mL of filtrate obtained was the mixed with 1.0 mL of 40% formaldehyde and 0.1mL of HCL and then allowed to stand for 10 minutes. The mixture thus was titrated with 2,6-dichlorophenol-indophenol dye to an end point of pink colour. For estimation of dye factor titration of about 10 mL standard ascorbic acid solution (mg/100mL) was done (Kulkarni and Karadbhajne, 2015). The amount of ascorbic acid (mg/100g) was calculated as

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titrate} \times \text{dye factor} \times \text{volume made up} \times 100}{\text{Aliquot of extract taken} \times \text{weight or volume of the sample}}$$

Free Radical Scavenging Activity

Extraction of 100mg of sample was done with 1 mL of methanol. The mixture was centrifuged and the supernatant was recovered after filtration. 1ml of the supernatant was mixed with 3.9 mL of 0.1mMol DPPH solution. The reaction mixture was incubated in dark for half an hour. Absorbance was taken at 515 against a blank²¹. The percentage scavenging activity was determined using the formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{absorbance sample}}{\text{absorbance control}} \times 100$$

Total Phenolic Content

Total phenolic content of samples was estimated spectrophotometrically at 765 nm by Folin-Ciocalteu method (Singleton, 1999). Gallic acid was used as a standard. Results were expressed as gallic acid equivalents in milligrams per 100 g dry matter.

Mineral analysis

5 grams of fresh and dried samples were incinerated at 600 °C for 24 hours. The ash content thus obtained was dissolved in diluted HCL (1:3 ratio of HCL: water) and then few drops of concentrated nitric acid were added to the mixture. The mixture thus obtained was heated on a hot plate. After proper heating the contents were cooled and distilled water was used to make up volume up to 50 mL. The minerals like Ca, Mg, K, were estimated using atomic absorption spectrophotometer (Ekpete et al., 2013).

Statistical analysis

One-way ANOVA followed by Tukey's test ($p \leq 0.05$) was performed to determine the significant differences among the fresh and freeze-dried samples whereas paired t-test was conducted to determine the significant differences between the fresh and freeze-dried samples using SPSS 16.0. All experiments were performed in triplicates, and values were reported as the mean \pm standard deviation (SD).

RESULTS AND DISCUSSION

Effect of freeze-drying on the proximate composition of some underutilized fruit seeds

The results of the proximate composition (moisture, ash, crude protein, crude fat, crude fibre) of fresh and freeze dried fruit seeds are presented in Table 1. Significant ($p \leq 0.05$) differences were observed between the proximate parameters of the selected underutilized fruit seeds. The results from the study showed a significant moisture loss of approximately 65-70% among the selected fruit seeds. However, the value of residual moisture content for Jamun seed powder (14.39 % w.b.) and Custard apple seed powder (13.00 % w.b.) due to their hygroscopic characteristics after drying was relatively high when compared with Bael fruit seed powder. The increased leftover moisture can be explained by structural changes in the soluble saccharides with a high level of amorphousness, which makes the powder highly hygroscopic and sensitive to physico-chemical as well as microbial changes. The molecular networks though found in the physical state significantly affect the moisture retaining properties in food products. This rubbery-glassy physical state in turn relies on the processing operations like drying and freezing, which lead to differences in the isotherms of freeze-dried products (Marques et al., 2006).

The ash content in fresh samples was lower than those of freeze dried samples. The statistical analysis revealed that the ash content of freeze-dried Custard Apple seed powder (6.32%) was significantly ($p \leq 0.05$) higher when compared to Jamun (3.22%) and Bael (3.66%) seed powders, respectively. Ash content for both Jamun and Bael seeds were not significantly ($p \geq 0.05$) different from one another. The freeze dried seed powders showed a significant increase in the ash content due to removal of water, thereby increasing the nutrient concentration (Wijewardana et al., 2016) reported the higher values of ash in freeze dried Bael seed powder (4.10%). The high ash content of the fruit seeds explains their higher concentration of minerals (calcium, magnesium, potassium etc.) (Shonte et al., 2020). However, the aforementioned value of ash content for freeze-dried jamun seed powder is in accordance with that of 3.23 % reported by Prasad et al. (2010) but lower than (6.7%) reported by (Shahnawaz et al., 2009).

Table 1. Proximate composition of fresh and freeze-dried seed powders of selected underutilized fruits

Analysis	Seed	Composition (%)		
		Jamun	Bael	Custard apple
Moisture (% w.b.)	<i>in natura</i> \pm SD	51.16 \pm 2.55 ^{aa}	47.51 \pm 5.66 ^{aa}	43.62 \pm 1.37 ^{aa}
	FD \pm SD	14.39 \pm 2.10 ^{bβ}	10.76 \pm 0.72 ^{aβ}	13.00 \pm 0.53 ^{abβ}
Ash	<i>in natura</i> \pm SD	2.32 \pm 0.81 ^{aa}	2.86 \pm 1.23 ^{aa}	4.26 \pm 1.30 ^{aa}
	FD \pm SD	3.22 \pm 0.60 ^{aa}	3.66 \pm 1.04 ^{aβ}	6.32 \pm 0.16 ^{bα}
Crude Protein	<i>in natura</i> \pm SD	5.37 \pm 0.90 ^{bα}	1.63 \pm 0.46 ^{aa}	17.93 \pm 0.51 ^{cα}
	FD \pm SD	7.43 \pm 0.48 ^{bα}	2.54 \pm 0.48 ^{aa}	18.27 \pm 0.39 ^{cα}

Crude Fat	<i>in natura</i> ±SD	3.60 ± 1.17 ^{aa}	13.67 ± 1.09 ^{ba}	27.33 ± 1.85 ^{ca}
	FD±SD	4.52 ± 0.17 ^{aa}	14.45 ± 1.30 ^{ba}	31.76 ± 0.65 ^{cb}
Crude Fibre	<i>in natura</i> ±SD	1.86 ± 0.27 ^{aa}	5.07 ± 0.96 ^{aa}	29.89 ± 2.27 ^{ba}
	FD±SD	4.17 ± 0.05 ^{ab}	7.13 ± 0.61 ^{ba}	37.17 ± 1.72 ^{cb}

FD=Freeze-dried

SD=Standard Deviation

Results are expressed as mean ± standard deviation of triplicate analysis and values denoted with the same small letter within the same row are not significantly ($p \geq 0.05$) different among fruit seeds tested by Tukey's test. Values with the same mathematical symbol within the same column are not significantly ($p \geq 0.05$) different between the fresh and freeze-dried fruit seeds by paired t-test.

Crude protein and fat content for all the studied fresh seed powders were quite variable. The statistical analysis revealed that crude protein and fat content of fresh and freeze-dried seed powders tested varied significantly ($p \leq 0.05$) ranging from 1.60 to 18.30% and 3.00 to 32.00%, respectively. Fresh and freeze dried Custard apple seed powder was found to have highest crude protein (17.93 and 18.27%) and fat content (27.33 and 31.76%) followed by Bael seed powder (13.67 and 14.45%) in terms of crude fat content and Jamun seed powder in terms of crude protein content (5.37 and 7.43%). Freeze-drying resulted in slight non-significant ($p \geq 0.05$) increase in crude protein and fat content of the studied samples which might be due to increased nutrient density with moisture evaporation (Umuhozariho et al., 2020). The observations in the present investigation were comparable to those reported by (Fedha et al., 2010). However, Shahnawaz et al. (2009) reported lower values of crude protein (5.12%) and crude fat (0.94%) content in freeze dried Jamun seed powder.

The crude fibre content of the fresh and freeze dried seed powders are presented in Table 1. Significant differences ($p \leq 0.05$) were observed between fruit seed powders and between fresh and freeze dried samples, with the fresh Jamun seed powder containing the lowest crude fibre content (1.86%). From the statistical analysis, it was found that the crude fibre content of freeze-dried Custard Apple seed powder (37.17%) was significantly ($p \leq 0.05$) higher when compared to Jamun (4.17%) and Bael (7.13%) seed powders, respectively. Shahnawaz et al. (2009) recorded 12.42% of the crude fibre in freeze-dried Jamun seed powder which is three times higher the value obtained in the present study. The results of the proximate analysis revealed that the freeze-drying influenced the proximate composition of the selected underutilized fruit seed powders. Also, freeze drying resulted in better nutrient retention in terms of ash, protein, fat and fibre content. Thus, freeze drying can be opted as technological method when proximate composition is under consideration.

Effect of freeze drying on the bioactive composition of selected underutilized fruit seeds

The bioactive composition namely vitamin C, antioxidant activity and total phenolic content of the fresh and freeze-dried seeds are presented in Table 2-4. Vitamins (like vitamin C and E) or mineral activity is notably responsible for the antioxidant properties of fruits and vegetables. Collectively, they are termed as "phytonutrients" (Rafiq et al., 2019). The role of vitamin C is not restricted to its antioxidant characteristics but can also be used as "quality index" of dried products. The retention of this nutrient guarantees the preservation of

other important nutrients also (Marques et al., 2006). Significant differences ($p \leq 0.05$) were observed between fruit seed powders and between fresh and freeze dried samples. The vitamin C content of fresh and freeze-dried extracts is within a range of concentration from 1.64 to 11.76 mg/100g and 1.22 to 9.17 mg/100g (Table 2). Drying results in the reduction of ascorbic acid content for all the seed powders which may be attributed to oxidative degradation. The maximum loss of vitamin C occurred in the freeze-dried Custard apple seed powder. Our experimental results for freeze-dried Jamun seed powder are in accordance with the findings of (Shanawaz et al., 2009). Significant reduction of vitamin C also did not occur in freeze-dried kinnow peel according to (Rafiq et al., 2019).

Table 2. Vitamin C content of fresh and freeze-dried seed powders of selected underutilized fruits

Seed	Vitamin C (mg/100g)	
	<i>in natura</i> ±SD	FD±SD
Jamun	1.64 ± 0.19 ^{aα}	1.22 ± 0.07 ^{aβ}
Bael	3.03 ± 0.30 ^{ba}	2.67 ± 0.73 ^{ba}
Custard Apple	11.76 ± 0.10 ^{ca}	9.17 ± 0.72 ^{cβ}

FD=Freeze-dried

SD=Standard Deviation

Results are expressed as mean ± standard deviation of triplicate analysis and values denoted with the same small letter within the same column are not significantly ($p \geq 0.05$) different among fruit seeds tested by Tukey's test. Values with the same mathematical symbol within the same row are not significantly ($p \geq 0.05$) different between the fresh and freeze-dried fruit seeds by paired t-test.

Table 3. DPPH radical scavenging activity of fresh and freeze-dried seed powders of selected underutilized fruits

Seed	DPPH radical scavenging activity (%)	
	<i>in natura</i> ±SD	FD±SD
Jamun	92.01 ± 1.33 ^{ba}	90.59 ± 1.37 ^{ca}
Bael	18.19 ± 0.39 ^{aα}	15.03 ± 0.42 ^{aβ}
Custard Apple	89.27 ± 2.03 ^{ba}	86.38 ± 0.71 ^{ba}

DPPH=2,2-diphenyl-1-picrylhydrazyl

FD=Freeze-dried

SD=Standard Deviation

Results are expressed as mean ± standard deviation of triplicate analysis and values denoted with the same small letter within the same column are not significantly ($p \geq 0.05$) different among fruit seeds tested by Tukey's test. Values with the same mathematical symbol within the same row are not significantly ($p \geq 0.05$) different between the fresh and freeze-dried fruit seeds by paired t-test.

Antioxidant activity of natural compounds has been shown to be involved in termination of free radical reactions (Fang et al., 2002). Table 3 showed the antioxidant activity of the methanolic extracts from selected underutilized

fruit seed powders as evaluated by free radical scavenging assay. Compared to Bael seed (18%), fresh extracts of Jamun seed and Custard apple seed exhibited excellent scavenging effects on DPPH radicals in the range of 87-93%. No significant ($p \geq 0.05$) difference was observed for free radical scavenging activity between fresh and freeze-dried seed powders, except for freeze-dried Bael seed powder which showed non-significant ($p \leq 0.05$) lower scavenging activity compared to fresh sample. Chang et al. (2006) also reported no significant differences in the scavenging activity of fresh and freeze-dried tomatoes. Our experimental results are in parallel with the findings of Shofian et al. (2011) and Ironi et al. (2013). Changes in the structure of phenolic compounds during drying would result in the formation of low molecular weight compounds which might affect the antioxidative activity. Similar findings were presented by Jeong et al. (2004) and Chen et al. (2011).

Table 4 shows the amount of total phenolics in fresh and freeze dried seed powders of the selected fruit seeds. The fresh seeds contained significantly ($p \leq 0.05$) higher amount of phenolics compared to the freeze dried samples except the Bael seeds, in which the phenolic content of fresh and freeze dried form varied non-significantly ($p > 0.05$). The results from the study also indicates that the total phenolic content of fresh and freeze dried fruit seeds varied significantly ($p \leq 0.05$) from 4.69 to 1605.57 mg GAE/100g). Fresh Jamun seeds (1605.57 GAE/ 100g) showed highest total phenolic content followed by fresh Custard apple seeds (43.75 GAE/ 100g) and fresh Bael seeds (5.32 GAE/ 100g). The slight reduction in the total phenolic content of the selected fruit seeds upon freeze drying might be attributed to the destruction of phenolic acids by oxidation or by thermal degradation (Adiletta et al., 2016). Our results were parallel with the findings of Coklar and Akbulut. (2017) and (Izli et al., 2018). The technique of freeze drying results in higher retention of bioactives as compared to other drying methods which in-turn confirms the smallest loss of bioactive compounds including the total phenolic content.

Table 4. Total phenolic content of fresh and freeze-dried seed powders of selected underutilized fruits

Seed	TPC (mgGAE/100g)	
	<i>in natura</i> ±SD	FD±SD
Jamun	1605.57 ± 145.34 ^{ba}	1522.20 ± 123.83 ^{bβ}
Bael	5.32 ± 0.27 ^{aa}	4.69 ± 0.19 ^{aa}
Custard Apple	43.75 ± 1.59 ^{aa}	41.18 ± 2.10 ^{aβ}

TPC=Total Phenolic Content

FD=Freeze-dried

SD=Standard Deviation

Results are expressed as mean ± standard deviation of triplicate analysis and values denoted with the same small letter within the same column are not significantly ($p \geq 0.05$) different among fruit seeds tested by Tukey's test. Values with the same mathematical symbol within the same row are not significantly ($p \geq 0.05$) different between the fresh and freeze-dried fruit seeds by paired t-test.

Effect of freeze-drying on the mineral content of selected underutilized fruit seeds

Table 5 gives the values of the mineral content such as calcium, magnesium and potassium of the fresh and freeze-dried seed powders. Compared to fresh samples, freeze drying resulted in higher mineral content which might be due to increased dry matter concentration. The calcium content of all the selected fresh and freeze-dried seed powders was quite variable, ranging from 1.68 to 236.48 mg/100g. The difference in the calcium content of the fresh samples might be due to the different stages of ripeness in the fruits analyzed and experimental errors. Significant differences ($p \leq 0.05$) were observed between fresh fruit seed powders, with the Bael seed powder containing the lowest calcium content (1.68%). Calcium content of fresh Custard apple seed powder varied significantly ($p \leq 0.05$) from the freeze-dried samples while no significant ($p \geq 0.05$) differences were observed for other fruit seed powders. The potassium content varied significantly ($p \leq 0.05$) between the fresh and freeze-dried seed samples, with Jamun seed powder containing the maximum potassium content (606.16 and 621.56 mg/100g) followed by Custard apple seed powder (362.65 and 379.44 mg/100g) and Bael seed powder (107.21 and 117.77 mg/100g), respectively. However, no significant ($p \geq 0.05$) difference was observed for potassium content between fresh and freeze dried Jamun and Custard apple seed powder, except for fresh Bael seed which showed significantly ($p \leq 0.05$) lower potassium content in comparison to the freeze-dried sample. The observations in the present investigation were comparable to those reported by (Rafiq et al., 2019). However, the findings of the Sehwal and Das.(2018) showed quite different results compared to our findings for fresh Jamun seed powder. The researchers reported a much lower calcium (31.90 mg/100g) and potassium (79.77 mg/100g) content while as the magnesium (115.08 mg/100g) content was slightly higher than the present study.

Table 5. Mineral content of fresh and freeze-dried seed powders of selected underutilized fruits

Minerals Seed	Composition (mg/100g)			
		Jamun	Bael	Custard apple
Ca	<i>in natura</i> ±SD	136.60 ± 24.09 ^{ba}	1.68±0.57 ^{aa}	201.33 ± 12.98 ^{ca}
	FD±SD	147.95 ± 20.43 ^{ba}	2.64±0.26 ^{aa}	236.48 ± 22.17 ^{cβ}
K	<i>in natura</i> ±SD	606.16±43.25 ^{ca}	107.21±0.99 ^{aa}	362.65 ± 6.67 ^{ba}
	FD±SD	621.56±43.02 ^{ca}	117.77±3.03 ^{aβ}	379.44 ± 12.64 ^{ba}
Mg	<i>in natura</i> ±SD	112.50±16.27 ^{ca}	0.92±0.08 ^{aa}	42.92 ± 1.41 ^{ba}
	FD±SD	116.09±24.26 ^{ca}	1.13±0.09 ^{aβ}	50.35 ± 1.47 ^{bβ}

FD=Freeze-dried

SD=Standard Deviation

Results are expressed as mean ± standard deviation of triplicate analysis and values denoted with the same small letter within the same row are not significantly ($p \geq 0.05$) different among fruit seeds tested by Tukey's test. Values with the same mathematical symbol within the same column are not significantly ($p \geq 0.05$) different between the fresh and freeze-dried fruit seeds by paired t-test.

A significant ($p \leq 0.05$) variation was found in the magnesium content of the fresh and freeze dried samples except the Jamun seed powder in which there was a non-significant ($p \geq 0.05$) difference between the fresh and freeze dried samples. The highest magnesium content was observed in freeze-dried Jamun seed powder (116.09

mg/100g) and the lowest was found in fresh Bael seed (0.92 mg/100g). Magnesium content varied significantly between the seeds in both fresh and freeze dried form. Among the seeds taken for estimation, Jamun seed was found to possess higher amounts of magnesium 112.50 and 116.09 mg/100g in fresh and freeze dried samples, respectively. The lowest values of 0.92 mg/100g (fresh) and 1.13 mg/100g (freeze dried) were found in Bael seeds. These differences among the magnesium content of the different fruit seeds may be due to the differences in the structure as well as composition of the individual fruit seeds (Bharati et al., 2010). The higher amount of magnesium in the freeze dried samples as compared to the fresh ones could be due to the concentration effect resulting by reduction of moisture content and the subsequent increase in the dry matter levels (Ukegbu et al., 2013).

CONCLUSION

The research was conducted to present an overview of the richness of nutrients and bioactive compounds in selected underutilized fruit seeds. Results obtained from the study suggest that the seeds produced as a waste from fruit processing could be used as a source of bioactive compounds in both fresh as well as dried forms. Also, freeze drying provides promising results in the preservation of both physicochemical as well as bioactive constituents and thus can be considered as the best drying method to maintain the nutrients, bioactive components and antioxidant activity of the dried seed samples. Thus, freeze drying can be used to stabilize the fruit wastes produced without affecting the nutritional as well as phytochemical composition to a greater extent.

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