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Antioxidant Properties of Freeze Dried Mix Fruits and Vegetables Product 'Miss Freezy' Using Lyophilization Method

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ABSTRACT

Background: The proper processing of fruits and vegetable-based food products will improve appearance, taste, bioactivities, and preservation. Freeze-dried (FD) or lyophilization of foods extends shelf life and preserves food's nutritional value and bioactivities. The objective of this study is to compare the antioxidant properties, nutritional value, and vitamins of fresh fruit-vegetables and the combined FD product. **Methods:** A combination of fruits (mango, papaya, star-fruit, guava, banana, bengkuang), and vegetables (cucumber), called "Miss Freezy" were cleaned with an ozonizer and processed using the lyophilization method. The antioxidant activities measured included 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and Hydrogen Peroxide (H_2O_2) assay. While the proximate analysis measured was the determination of water content, ash content, fat content, protein and carbohydrate content, and vitamin C. **Results:** The value of antioxidant activity in fruit-vegetable combination FD products was higher than in fresh fruit. The median inhibition concentration (IC_{50}) of DPPH, ABTS, and H_2O_2 scavenging activities were guava 48.74 µg/ml, guava 128.39 µg/ml, and banana 155.55 µg/ml, respectively. The results showed that the water content of fresh fruits, and vegetables was greater than FD products and inversely proportional to the level of ash, fat, protein, carbohydrates, and vitamin C lower than FD products. **Conclusions:** Based on the results of antioxidant activities, proximate analysis and vitamin C of the FD product was higher than fresh fruits and vegetables. The FD fruit-vegetable combination product can be used as an alternative snack product that is practical and healthy.

Introduction

The rapid development of globalization requires higher working time, and as a result, people choose fast food which is easier and more practical to obtain. Fast food consumption leads to an unhealthy lifestyle and food patterns (Wadolowska *et al.*, 2018) because it is regarded as

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an energy-dense and low-nutrient food, and tends to be rich in sugar and saturated fat. This is closely related to the high prevalence of obesity (Jiang *et al.*, 2019) which can cause type 2 diabetes, heart disease, lung disease, musculoskeletal issues, and many types of cancer (Goto *et al.*, 2018).

Based on the World Health Organization (WHO) and Food and Agriculture Organization (FAO), the daily minimum consumption of 400 g of fruits and vegetables may minimize the emergence of chronic diseases and alleviate micronutrient deficiencies. Fruits are a great source of fiber, vitamins, minerals, and bioactive compounds such as phenolic compounds, carotenoids, and betalains. The phytochemicals in these fruits function as immunomodulators, anticarcinogens, and antioxidants (Belmonte-Herrera *et al.*, 2022, Yahia *et al.*, 2019). As in kiwi fruit, guava, mango, papaya, star fruit, blueberries, strawberries, watermelon, pomegranate, jicama, cucumber, and bananas have a high antioxidant content (Misran and Jaafar, 2019).

In general, people prefer fresh fruits over processed fruit chips. However, fruits cannot be stored for a long period because they are easily spoiled. This spoilage is affected by storage temperature, as well as O_2 and CO_2 levels in the air which is caused by the destruction of the material by microbes that activate specific enzymes resulting in an unpleasant taste and aroma (Czerwiński *et al.*, 2021). Fruit spoilage can be minimized through preservation, such as drying, heating, cooling, and canning (Joardder *et al.*, 2019, Kumar *et al.*, 2022). Food processing techniques can affect the quality and bioactive content such as loss of pigment degradation, nutrients, and antioxidant compounds in the form of carotenoids and anthocyanins, especially in fruits and vegetables (Onwude *et al.*, 2022, Shonte *et al.*, 2020), total polyphenol content, and total flavonoid content during drying (Thamburaj *et al.*, 2022). The long drying times at relatively high temperatures during the falling rate periods mostly lead to the thermal degradation of some heat-sensitive fruit compounds (Ndisanze and Koca, 2022, Shonte *et al.*, 2020). Christofi *et al*. reported that the phenolic compounds in canned products, including neochlorogenic acid, chlorogenic acid, procyanidin B1, and catechin, drastically decreased during the canning process (Christofi *et al.*, 2022). The preservation of fruits through freezing and cooling will affect the microstructure of frozen products due to the formation of large ice crystals so that the fruits will be softer (Alabi *et al.*, 2020).

Freeze-dried (FD), also known as lyophilization is a high-quality food preservative (Dalmau *et al.*, 2019). FD is a process where water in the form of ice under low pressure is removed from a material through sublimation and ends by drying the product to the required humidity (Nowak and Jakubczyk, 2020). FD operates at low temperatures and in high vacuum, because it contains substances that are heat sensitive and prone to oxidation. Freeze drying method with a lack of liquid water, oxygen-free environment, and low operating temperature can maintain the bio-compound content of the fruits, vegetables, and other materials. Several studies related to the comparison of the antioxidant value of FD products with fresh fruit have been reported (Beh *et al.*, 2012, Chang *et al.*, 2006, Olivas-Aguirre *et al.*, 2017, Toobpeng *et al.*, 2017). However, no one has reported a comparison of the results of the proximate analysis and the comparison of antioxidant values in FD fruit-vegetable combination and the fresh fruit-vegetable combination. Therefore, this study aims to compare the value of antioxidant activity, nutritional value, and vitamin C of "Miss Freezy" products consisting of cucumber (*Cucumis sativus*), mango (*Mangifera indica*), papaya (*Carica papaya*), guava (*Psidium guajava*), star fruit (*Averrhoa belimbing*), jicama or bengkoang (*Pachyrhizus erosus*) and banana (*Musa* sp.) between fresh fruit-vegetable and FD product.

Materials and Methods

Sample preparation

Miss Frezzy consists a mix freeze-dried (FD) fruits and vegetables including cucumber, mango, papaya, guava, star fruit, bengkoang, and banana, all purchased at Pasar Sederhana, Bandung, West Java, Indonesia with the best quality selection. One kilogram of each fruit was put into a container

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filled with water and sterilized using an ozonizer (Superwise ARG 745) for 10-15 minutes (Kuźniar *et al.*, 2022). Furthermore, the fruits-vegetables were peeled and sliced. The fruit was be processed into chips after being weighed based on the weight before and after drying. The manufacturer employed a freeze dryer for lyophilization method of fruit chips (FD-F-CE, China) tool (Nowak and Jakubczyk, 2020). The lyophilization process using a freeze dryer was carried out for 42 hours at a temperature of 35-50 ℃. To prepare for antioxidant assay, FD fruits were pulverized using a homogenizer. Then, serial concentrations of fresh fruit-vegetable and FD samples were made by dissolving the samples in 100% DMSO, then 4 concentration series were made by dissolving the sample stock in 10% DMSO.

Miss Freezy packing

The FD products that have been produced were then mixed and weighed until it reaches a total weight of 120 grams (±17.14 grams/each fruit-vegetable); then, the products were packaged in a jar filled with silica gel and labeled with an attractive design and important information about Miss Freezy products. This product has a distribution permit certificate with SPP-IRT number: 2043273060483-27.

Proximate and vitamin C analysis

The samples (fresh and FD) were subjected to proximate analysis which consisted of tests for water, ash, fat, protein content, and total carbohydrates and analysis of vitamin C content. Proximate analysis and measurement of vitamin C levels was carried out by PT Vicma Lab Indonesia, Bogor, West Java as a testing laboratory certified by the National Accreditation Committee (KAN) which can provide nutritional fact certificates for product labels. The entire proximate analysis procedure carried out refers to the Indonesian National Standards Agency No. SNI 01-2891-1992 concerning methods of testing food and beverages (Nasional, 1992, Widyani, 2020). While the analysis of vitamin C levels refers to procedures from PT. Vicma Lab Indonesia, the mixture of samples was carried out using the HPLC method (mobile phase: 3mM KH_2PO4 in 0.35% H_3PO_4; HPLC waters e2695; column RP C-18 waters 4.6 x 150 mm 5 μm; UV-Vis detector 2489 waters; wavelength: 248 nm; column temperature: 25 ^{0}C; flow rate: 0.5 ml/min; injection volume: 20 μl) (Lewis Lujan *et al.*, 2014).

DPPH scavenging activity

To the 96-well plates, 50 μl of a sample (fresh and FD) at various concentrations (50.00; 100.00; 200.00 and 400.00 μg/ml), then 200 μl of 2,2-Diphenyl-1-picrylhydrazil (DPPH) (Sigma Aldrich, D9132) was added (0.077 mmol in methanol). In the well blank, 250 μl of sample solvent was added, and in the control well, 250 μl of DPPH solution was added. The well plate was incubated for 30 minutes in the dark, and the absorbance was measured at λ=517 nm. This method refers to methods from previous research (Widowati *et al.*, 2022).

2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid or ABTS)-reducing activity

In 96-well plate sample, 2 μl of a sample (fresh and FD) with various concentrations (12.50; 25.00; 50.00; and 100.00 μg/ml) was added and followed by 198 μl of ABTS reagent. In the well blank, 200 μl of sample solvent was added, and in the control well, 200 μl of ABTS solution was added. The well plate was incubated for 6 minutes in the dark, and the absorbance was measured at λ=745 nm .

H_2O_2 scavenging activity

In the sample and control wells, 12 μl of 1mM ferrous ammonium sulfate was added (Merck, 1.03792.1000). Then, 60 μl of a sample (fresh and FD) at various concentrations (75.00; 150.00; 300.00; and 600.00 μg/ml) and 3 μl H_2O_2 at 5mM concentration (Merck, 1.08597.1000) were added to the sample well, while to the control well, 63 μl sample solvent was added. In the well blank, 150 μl of sample solvent was added. The well plate was incubated for 5 minutes in the dark, and after that, 75 μl of phenanthroline solution was added and the absorbance was measured at λ=510 nm. This procedure followed previous studies (Prahastuti *et al.*, 2020).

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Data analysis

SPSS software Version 22.0 was used to analyze data, and the results were presented in the form of histogram (mean±SD) using GraphPad Prism Version 8.0.1 software. The normality and homogeneity of data were examined using Saphiro Wilk test and Levene test, respectively. The Kruskal-Wallis and Mann-Whitney tests were used to analyze the group differences if the data were not normally distributed. Analysis of variance (One Way ANOVA) followed by Tukey HSD Post-Hoc and independent samples test were used to determine whether the data were normally distributed. Statistically significant differences were defined as those with P-value ≤ 0.05.

Results

Percentage of weight loss during the processing row materials using freeze dryer

The fresh fruits utilized as raw materials were weighed before processing as well as after the ozonation procedure. The results of the subsequent weigh-in to calculate the percentage of weight reduction for the freeze-dried product are shown in **Table 1**.

Table 1. Percentage of weight loss of fruits after lyophilization process.

No.	Material name	Entry weight (g)	Dry weight (g)	Weight loss (%)
1.	Starfruit	750	71	90.53
2.	Jicama	477	64	86.58
3.	Mango	400	67	83.25
4.	Papaya	815	98	87.98
5.	Banana	600	177	70.50
6.	Cucumber	867	67	92.27
7.	Guava	900	217	75.89

Entry Weight: the weight of fresh fruit after peeling and before the lyophilization process; Dry eight: the weight of the fruit after the lyophilization process.

The percentage of weight loss was calculated through the equation:

$$(\%) = \frac{\text{pre-process sample weight - sample weight after processing}}{\text{pre-process sample weight}} \times 100\%$$

Table 1 showed that FD samples had lower weight than fresh fruits and vegetables. The highest percentage of weight loss of FD fruit was found in star fruit (90.53%) and cucumber (92.27%); this data showed that starfruit and cucumber contain very high water content compared to banana and guava.

Proximate and vitamin C analysis

The results of the proximate analysis (moisture, ash, fat, protein, carbohydrate content) and vitamin C from fresh fruit-vegetable and FD products can be seen in **Table 2** which shows that the water content of all FD products was lower than fresh samples. The highest water content of fresh fruit-vegetable was star fruit and cucumber, while the lowest water content was banana.

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Table 2. Results of proximate analysis and vitamin C on fresh fruits, vegetables, and FD product.

Sample	Group	Water (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Vitamin C (mg/100g)
Mixed fruits-vegetable	Fresh	82.74	2.17	1.05	0.86	13.18	5.83
	FD	9.87	2.42	7.05	7.10	73.86	45.03
Star fruit	Fresh	90.42	0.43	0.47	2.91	5.57	
	FD	13.55	0.52	3.03	16.73	66.17	
Jicama	Fresh	86.17	0.50	1.14	0.87	11.32	
	FD	7.19	0.65	4.50	9.10	78.79	
Mango	Fresh	81.25	0.41	0.87	0.87	16.60	
	FD	7.91	0.53	1.41	5.22	84.93	
Papaya	Fresh	86.77	1.51	0.71	0.85	10.16	
	FD	11.01	1.78	5.70	6.05	75.86	
Banana	Fresh	71.37	1.70	0.81	1.57	24.55	
	FD	5.92	1.83	3.70	18.90	69.65	
Cucumber	Fresh	96.48	0.56	0.78	0.79	1.39	
	FD	10.28	0.63	3.85	10.24	75.00	
Guava	Fresh	85.44	1.63	0.54	0.86	11.54	
	FD	7.70	1.70	4.97	9.42	76.21	

The ash, fat, protein, carbohydrate, and vitamin C levels, of FD samples were higher than fresh fruit-vegetable samples.

DPPH scavenging activity

The DPPH free radical scavenging activity can be seen in **Figure 1**. The highest DPPH scavenging activity of fresh samples was mango, and the FD product was guava. **Figure 1** shows the DPPH scavenging activity of fresh fruits-vegetable and FD product was at the highest concentration (400 µg/ml). In figure the data was displayed as mean±SD. Statistical differences were generated using Mann Whitney test for FD fruit (P<0.05).

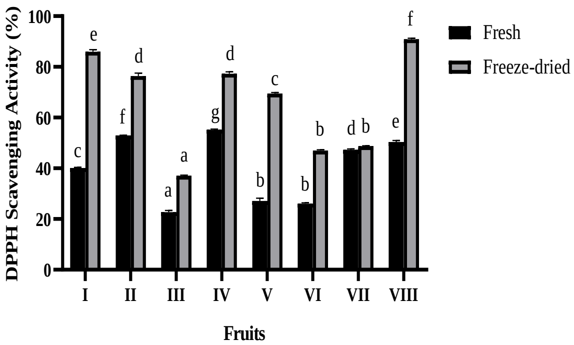

Figure 1. The DPPH scavenging activity of fresh fruits-vegetable and FD product; **I**: Mix Fruits-Vegetable.

II: Star Fruit; **III**: Jicama; **IV**: Mango; **V**: Papaya; **VI**: Banana; **VII**: Cucumber; **VIII**: Guava.

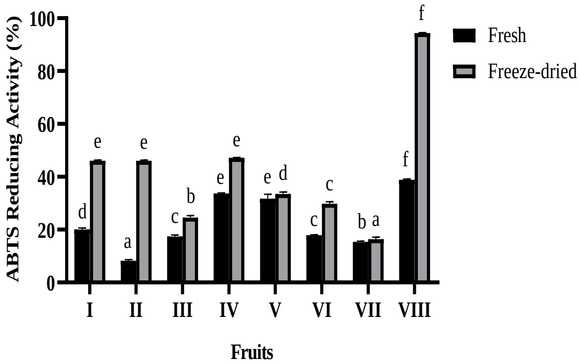

Figure 2. ABTS scavenging activity of fresh fruits-vegetable and FD product.

I: Mix fruits; **II**: Star fruit; **III**: Jicama; **IV**: Mango; **V**: Papaya; **VI**: Banana; **VII**: Cucumber; **VIII**: Guava.

ABTS-reducing activity

The ABTS scavenging activity can be seen in **Figure 2**, where FD product was higher compared to the fresh fruits-vegetable toward ABTS scavenging activity. The highest ABTS-reducing activity of fresh samples and FD products was guava. The highest ABTS-reducing activity for all fresh samples, and all FD products was from concentration of 100 µg/ml.

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Table 3. The IC_{50} value of DPPH scavenging activity on fresh fruits-vegetable and FD product .

Sample	Fresh			FD		
	Equation	R^2	IC_{50} value(µg/ml)	Equation	R^2	IC_{50} Value(µg/ml)
Mix fruits-vegetable	y = 0.0733x + 11.026	0.99	531.77±4.24	y=0.1625x + 20.141	0.99	183.81±2.02
Star fruit	y = 0.0462x + 34.727	0.99	330.75±4.73	y=0.1508x + 16.276	0.99	223.54±6.42
Jicama	y = 0.0361x + 8.3263	0.99	1156.47±53.47	y=0.0428x + 20.163	0.99	696.62±5.76
Mango	y = 0.0692x + 28.137	0.99	316.09±1.35	y=0.125x + 27.99	0.99	176.04±1.54
Papaya	y = 0.0528x + 5.562	0.99	844.09±56.06	y=0.1387x + 13.376	0.99	264.01±5.56
Banana	y = 0.0331x + 12.896	0.99	1120.74±36.35	y=0.0698x + 19.328	0.99	439.55±6.33
Cucumber	y = 0.0965x + 9.088	0.99	423.95±0.91	y=0.0715x + 20.119	0.99	418.31±1.48
Guava	y = 0.0746x + 20.201	0.99	399.21±9.81	y=0.1547x + 29.822	0.99	128.39±13.51

The data are represented in mean±SD. Each sample concentration was done in triplicate. The coefficient of regression (R^2) and the IC_{50} of the sample was calculated by linear regression; The IC_{50} value of DPPH free radical scavenging activity on fresh fruits-vegetable and FD products can be seen in Table 3. The IC_{50} value of fruits-vegetable was higher compared to the FD product.

Table 4. IC_{50} value of ABTS scavenging activity regarding fresh fruits-vegetable and FD product.

Sample	Fresh			Freeze dried		
	Equation	R^2	IC_{50}Value(µg/ml)	Equation	R^2	IC_{50} Value(µg/ml)
Mix fruits-vegetable	y = 0.1706x + 3.1101	0.99	274.95±6.99	y=0.5106x - 4.8205	1.00	107.38±0.81
Star fruit	y = 0.0875x - 0.3897	0.99	577.03±28.05	y=0.4917x - 3.8782	0.99	104.28±10.09
Jicama	y = 0.1825x - 1.0117	1.00	279.91±11.68	y=0.2611x - 1.1337	0.99	195.94±5.93
Mango	y = 0.3027x + 3.5619	1.00	153.44±2.58	y=0.4161x + 5.6973	1.00	106.48±0.74
Papaya	y = 0.3116x + 0.9489	0.99	157.65±7.16	y=0.3253x + 1.3556	1.00	149.57±2.98
Banana	y = 0.119x + 6.1077	0.99	369.35±13.95	y=0.33x - 3.7848	0.99	163.02±3.18
Cucumber	y = 0.1391x + 1.3675	1.00	349.89±9.08	y=0.1762x - 1.1337	1.00	290.65±11.72
Guava	y = 0.2x + 18.976	1.00	155.18±1.89	y=0.8806x + 7.0802	1.00	48.74±0.13

The data are presented in mean ± SD. Each sample concentration was done in triplicate. The coefficient of regression (R^2) and the IC_{50} of the sample was calculated by linear regression.

The fruit that exhibited as the most active antioxidant, based on the IC_{50} value of ABTS scavenging activity was mango for the fresh sample and guava for the FD product (**Table 4**).

H_2O_2 scavenging activity

H_2O_2 scavenging activity can be seen in **Figure 3**, the FD product was higher compared to the fresh fruits-vegetable toward H_2O_2 scavenging activity. The highest H_2O_2 scavenging activity of fresh samples was observed in papaya, and the FD product was guava. The highest H_2O_2-reducing activity for all the fresh samples and all FD products was a concentration 600 µg/ml.

The fruit that exhibited as the most active antioxidant, based on the IC_{50} value of H_2O_2 scavenging activity was papaya for the fresh sample and banana for the FD product (**Table 5**).

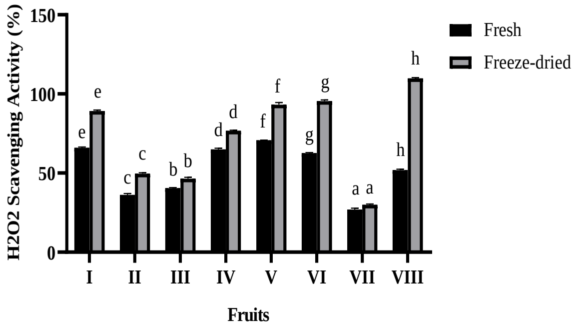

Figure 3. The H_2O_2 scavenging activity of fresh fruits-vegetable and FD product.

I: Mix Fruits; **II**: Star Fruit; **III**: Jicama; **IV**: Mango; **V**: Papaya; **VI**: Banana; **VII**: Cucumber; **VIII**: Guava.

Discussion

In this study, FD method was used to compare

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the value of antioxidant activity, nutritional value, and vitamin C content in fresh and FD fruit products. FD method was chosen because it can encapsulate bioactive compounds and micronutrients including vitamins, minerals, fibers, and phenolic compounds (Septembre-Malaterre *et al.*, 2018) in fruits and vegetables resulting in stable and maintained products in both color and compound composition (Casati *et al.*, 2019). Other studies also stated that FD products can maintain maximum food quality without reducing the content of vitamin C and carotenoids which are affected primarily by temperature and moisture content during the drying process (Oyinloye and Yoon, 2020). FD is also known to extend the shelf life of food by preventing microbial growth and slowing down lipid oxidation (Shofian *et al.*, 2011). Thus, the shape, appearance, taste, nutrition, porosity, color, taste, texture, and biological activity of the fresh samples makes this technique one of the most attractive and applicable processes for drying foodstuffs (Hariyadi, 2013, Hasbullah and Putra, 2022).

The used fruits in this study were washed through the ozonation technique; this method can maintain the purity of raw materials to keep them fresh and help extend their shelf life by preventing ethylene activation and contamination (Botondi *et al.*, 2015). The use of ozonation can degrade ozone into oxygen which leaves no residue (Sachadyn-Król and Agriopoulou, 2020); ozone easily decomposes into oxygen in water (27 °C, ozone solubility 580 mg/l) (Miller *et al.*, 2013), so it is used as a washing application that contributes to microbial reduction and extends shelf life (Piechowiak *et al.*, 2022) through cellular lysis, thereby destroying resistant microorganisms and spores (Ding *et al.*, 2019).

Based on the result, the water content of FD in both single and mixed fruits-vegetable products (Miss Freezy) had a significant shrinkage with high loss of water content **(Table 1 and Table 2)**. In lyophilization process, at the stage before sublimation drying, the previously processed raw material froze quickly, which changed its water content to a solid state. After the product was frozen (in solid form), the solid crystals sublimated into vapor, dehydrating, and drying the product by removing 90–95% of its water (Liu *et al.*, 2022). Reducing the water content concentrated the nutrients and allowed them to have a higher health value than fresh fruit or vegetables (Janowicz *et al.*, 2022). This result was supported by other studies which explained that FD tropical fruit pulps showed a considerable loss of moisture (around 70%). Due to their hygroscopic properties after drying, barbados cherry (21.44% wet basis) and guava (15.99%wet basis) pulps had relatively high residual moisture content values compared to other pulps (Marques *et al.*, 2006).

Table 5. The IC_{50} value of fresh and FD products toward H_2O_2 scavenging activity

Sample	Fresh			Freeze dried		
	Equation	R^2	IC_{50} value (µg/mL)	Equation	R^2	IC_{50} value (µg/mL)
Mix fruits-vegetable	y = 0.0941x + 10.123	1.00	423.92±2.49	y = 0.1159x + 19.159	0.99	266.04±1.97
Star fruit	y = 0.0539x + 4.0182	1.00	853.02±23.12	y = 0.0702x + 6.7612	0.99	615.78±10.00
Jicama	y = 0.0644x + 2.2007	1.00	742.73±7.22	y = 0.0807x - 2.3292	1.00	648.58±9.92
Mango	y = 0.0917x + 10.429	1.00	431.68±2.77	y = 0.1162x + 8.1381	0.99	360.35±1.69
Papaya	y = 0.0912x + 16.427	0.99	367.99±0.83	y = 0.1151x + 25.8	0.99	210.39±0.71
Banana	y = 0.0794x + 15.563	0.99	433.90±3.49	y = 0.1038x + 33.852	1.00	155.55±2.92
Cucumber	y = 0.0407x + 2.5434	1.00	1167.52±31.30	y = 0.0629x - 8.2661	1.00	926.48±12.05
Guava	y = 0.06x + 15.8	1.00	569.80±8.17	y = 0.1387x + 28.084	0.99	158.09±1.01

The data are represented in mean ± SD. Each sample concentration was done in triplicate. The coefficient of regression (R^2) and the IC_{50} of the sample was calculated by linear regression.

Other studies also reported that FD tropical fruits like pineapple, barbados cherry, guava,

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papaya, and mango not only preserve, their flavor, color, and taste but also retain high nutritive value (Marques *et al.*, 2006). Based on the proximate composition analysis of their study, FD products, banana, apple, and mango peel powder have lower moisture, but allowed the increase in fat, ash, protein, dietary fiber, and carbohydrate (Zahid *et al.*, 2021). In other studies, FD trout product reduced moisture value (2.11%) and increased fat (23.53%), protein (66.41%), and ash (5.20%) values, respectively. Thus, the increase in fat, protein, and ash contents is thought to be related to proportional water loss (Corapci and Guneri, 2019). Kowalska *et al*. reported that Freeze drying of fresh fruits had a higher vitamin C content compared to using microwave convective-vacuum drying method processing. The osmotic substance sugar may be responsible for protective impact of vitamin C and its degradation related to a decrease in water content (Kowalska *et al.*, 2018, Santos and Silva, 2008). The research supported the current results of this study that the nutritional value and vitamin C content of freeze-dried fruits tended to have higher values than fresh fruits **(Table 2)**.

Phenolic compounds in fruits had a mechanism of protection against disease which was made possible by the presence of several antioxidants. The antioxidant activity of fruits acts as a scavenger that maintains the proliferation of oxidative chains. Phenolic compounds have double bonds and a strong antioxidant capacity, but are easily degraded during long-term storage (highly affected by temperature) because of their thermolabile molecular structure, so it can induce the epimerization of a bioactive compound (Swallah *et al.*, 2020).

In this study, antioxidant assay was used to analyze antioxidant content of fresh fruit and the fruit preserved by the freeze-drying technique. Antioxidant activity was assessed using various methods, including DPPH, H_2O_2, and ABTS (Prahastuti *et al.*, 2020). In this study, a freeze-dried product of mixed fruits had the highest DPPH scavenging activity (IC_{50} value=183.81 µg/ml) compared to fresh fruits. In another study, Tsai *et a*l. also reported that the content of antioxidants in freeze-dried fruits (1.70 and 2.0 µg/g) was higher than fresh fruits (0.35 and 0.33 µg/g) (Tsai *et al.*, 2007). In another study, extracts of star fruit and mango showed excellent scavenging effects on DPPH radicals. The oxidation reaction was relatively fast compared to hydroxylation, which contributed more effectively to the degradation of phenolic content and antioxidant activity. Different types of fruit have different polyphenol oxidase (PPO) characteristics (Shofian *et al.*, 2011).

Meanwhile, based on ABTS results, mixed FD fruit products gave the lowest IC_{50} value (107.38 µg/ml) when compared to fresh fruit (274.95 µg/ml). This was also in line with the results on H_2O_2 scavenging activity, and the FD product exhibited the highest inhibition activity in H_2O_2 scavenging activity (65.92%; IC_{50} value=266.04 µg/ml). In another study, it was shown that guava *(Psidium guajava)* and mango *(Mangifera indica L.)* is a tropical fruit with a high content of phenolic compounds, carotenoids, and ascorbic acid which contribute to increasing antioxidant capacity (Chen and Yen, 2007, Rocha Ribeiro *et al.*, 2007).

In the lyophilization process, temperature, time, pressure, and pH during storage are the most crucial factors in the freeze-drying process that can affect antioxidant activity (Beh *et al.*, 2012, Walkowiak-Tomczak, 2007). In their research, Silva-Espinoza *et al*. reported that optimal FD conditions were at low pressure with high shelf temperatures. Under these conditions, Silva-Espinoza proved that the sample used in their research (orange puree) did not change in terms of color or nutrition (Silva-Espinoza *et al.*, 2019). This study demonstrated that FD products had a high quality and also showed good antioxidant activity, which may indicate that product storage and stability-related aspects are well-defined (Ciurzynska and Lenart, 2011). In encapsulated FD products, Galmarini *et al*. reported that the content of bioactive compounds remained constant when stored in a dark place at temperatures around 28 and 38 °C for 70 days of storage (Esparza *et al.*,

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2020, Galmarini *et al.*, 2013). Other studies reported that at a storage temperature of 20 °C in the presence of air, FD products pretreated with foam were stable for up to 70 days (Bhatta *et al.*, 2020, Raharitsifa and Ratti, 2010).

The variation of phytochemical components in all the samples can affect the value of the antioxidant capacity of the fruit. Therefore, mixed-fruit products can lead to high antioxidant capacity. Some studies suggested consuming at least five servings of fruit and vegetables each day. The nutrients in fruits are beneficial for maintaining weight, promoting proper health development, and satiety apart from providing a variety of tastes (Swallah *et al.*, 2020). On the other hand, the mixed-fruit drying-method can produce valuable and attractive food products for food industry due to its high nutritional composition, attractive color, and its potential as an antioxidant. Other findings from this study suggested that FD is the best approach for drying fruit while preserving its nutritional content, antioxidant activity, and sensory qualities (Orak *et al.*, 2012). Due to the operation of the freeze-drying process and its main feature, which is the removal of water bypassing the liquid phase of the solvent, this process removed almost 100% of water content from freeze-dried products. The low water content in the product facilitated long-term food preservation (Ciurzynska and Lenart, 2011, Janowicz *et al.*, 2022). This method has advantages in the method used (lyophilization method) as well as the results of antioxidant testing, proximate analysis and vitamin C which supported the fact that fresh-dried products can be used as alternatives to healthy snack products. Although several reports stated that freeze-dried products last for a long time, this research is still limited to the information related to storage stability of freeze-dried products. Therefore, for further studies, observations can be made regarding the length of storage time for several product packaging options.

Conclusion

Fruit-vegetable combination products (Miss Freezy) had a higher antioxidant activity compared with fresh fruits. Based on the proximate analysis, the moisture content of fresh vegetables was higher than that of Miss Freezy products, but the ash content, fat level, protein content, carbohydrates, and vitamin C were higher in Miss Freezy products. Based on the assays results of antioxidant activity, proximate analysis, and vitamin C obtained, it can be concluded that FD fruit-vegetable combination products can be used as an alternative to healthy and practical snack products.

Authors’ contributions

Wahyu Widowati contributed to the project’s conception, development of the overall research plan, study oversights, and had responsibility for final content; Ariesa Pandanwangi, Diah Kusumawaty, Teddy Marcus Zakaria, Teresa Liliana Wargasetia and Shiela Stefani contributed to conducting the experiments and data collection; Diah Kusumawaty assisted as a reviewer; Hanna Sari Widya Kusuma was responsible for the final content; Fadhilah Haifa Zahiroh analyzed the data, performed statistical analysis, and created the original manuscript; Ruth Meiraning Tyas ,Vini Ayuni, and Syifa Indah Suci Ati wrote, reviewed and edited the manuscript.

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Conflict of interests

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References

Alabi KP, Zhu Z & Sun D-W 2020. Transport phenomena and their effect on microstructure of frozen fruits and vegetables. *Trends in Food Science & Technology*. **101**: 63-72.

Beh LK, et al. 2012. Comparison of total phenolic content and antioxidant activities of freeze-dried commercial and fresh fruit juices. *Journal of medicinal plants research.* **6 (48)**: 5857-5862.

Belmonte-Herrera BH, et al. 2022. Lesser-Consumed Tropical Fruits and Their by-Products: Phytochemical Content and Their Antioxidant and Anti-Inflammatory Potential. *Nutrients.* **14 (17)**: 3663.

Bhatta S, Stevanovic Janezic T & Ratti C 2020. Freeze-drying of plant-based foods. *Foods.* **9 (1)**: 87.

Botondi R, et al. 2015. Ozone fumigation for safety and quality of wine grapes in postharvest dehydration. *Food chemistry.* **188**: 641-647.

Casati CB, Baeza R & Sánchez V 2019. Physicochemical properties and bioactive compounds content in encapsulated freeze-dried powders obtained from blueberry, elderberry, blackcurrant and maqui berry. *Journal of berry research.* **9 (3)**: 431-447.

Chang C-H, Lin H-Y, Chang C-Y & Liu Y-C 2006. Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. *Journal of food engineering.* **77 (3)**: 478-485.

Chen H-Y & Yen G-C 2007. Antioxidant activity and free radical-scavenging capacity of extracts from guava (Psidium guajava L.) leaves. *Food chemistry.* **101 (2)**: 686-694.

Christofi M, et al. 2022. Profiling carotenoid and phenolic compounds in fresh and canned fruit of peach cultivars: Impact of genotype and canning on their concentration. *Journal of food composition and analysis.* **114**: 104734.

Ciurzynska A & Lenart A 2011. Freeze-drying-application in food processing and biotechnology-a review. *Polish journal of food and nutrition sciences.* **61 (3)**: 165-171.

Corapci B & Guneri N 2019. Comparative assessment of nutritional composition and physicochemical properties of fresh, freeze-dried and rehydrated rainbow trout (Oncorhynchus mykiss Walbaum, 1792) mince. *Food science and technology*. **40**: 163-169.

Czerwiński K, Rydzkowski T, Wróblewska-Krepsztul J & Thakur VK 2021. Towards impact of modified atmosphere packaging (MAP) on shelf-life of polymer-film-packed food products: Challenges and sustainable developments. *Coatings.* **11 (12)**: 1504.

Dalmau ME, Eim V, Rosselló C, Cárcel JA & Simal S 2019. Effects of convective drying and freeze-drying on the release of bioactive compounds from beetroot during in vitro gastric digestion. *Food & Function.* **10 (6)**: 3209-3223.

Ding W, et al. 2019. Ozone disinfection of chlorine-resistant bacteria in drinking water. *Water research.* **160**: 339-349.

Esparza I, Cimminelli MJ, Moler JA, Jiménez-Moreno N & Ancín-Azpilicueta C 2020. Stability of phenolic compounds in grape stem extracts. *Antioxidants.* **9 (8)**: 720.

Galmarini MV, et al. 2013. Stability of individual phenolic compounds and antioxidant activity during storage of a red wine powder. *Food and bioprocess technology*. **6**: 3585-3595.

Goto T, Hirayama A, Faridi MK, Camargo Jr CA & Hasegawa K 2018. Obesity and severity of acute exacerbation of chronic obstructive pulmonary disease. *Annals of the American Thoracic Society*. **15 (2)**: 184-191.

Hariyadi P 2013. Freeze drying technology: for better quality & flavor of dried products. *Foodreview Indonesia.* **8 (2)**: 52-57.

Hasbullah R & Putra NS 2022. Study on the Vacuum Pressure and Drying Time of Freeze-drying Method to Maintain the Quality of

Strawberry (Fragaria virginiana). *Journal of agricultural engineering.* **11 (2)**: 279-291.

Janowicz M, Ciurzyńska A, Karwacka M, Kowalska J & Galus S 2022. Mathematical Estimation of the Energy, Nutritional and Health-Promoting Values of Multi-Layer Freeze-Dried Vegetable Snacks. *Applied sciences.* **12 (13)**: 6379.

Jiang Y, et al. 2019. Association between take-out food consumption and obesity among Chinese university students: a cross-sectional study. *International journal of environmental research and public health.* **16 (6)**: 1071.

Joardder MU, Hasan Masud M, Joardder MU & Masud MH 2019. Food preservation techniques in developing countries. *Food preservation in developing countries: Challenges and solutions.* 67-125.

Kowalska J, et al. 2018. Dried strawberries as a high nutritional value fruit snack. *Food science and biotechnology.* **27**: 799-807.

Kumar A, et al. 2022. Design and Fabrication of Solar Dryer System for Food Preservation of Vegetables or Fruit. *Journal of food quality.* **2022 (1)**: 6564933.

Kuźniar P, Belcar J, Zardzewiały M, Basara O & Gorzelany J 2022. Effect of Ozonation on the Mechanical, Chemical, and Microbiological Properties of Organically Grown Red Currant (Ribes rubrum L.) Fruit. *Molecules.* **27 (23)**: 8231.

Lewis Lujan LM, et al. 2014. Nutritional and Phenolic composition of Morinda citrifolia L.(Noni) fruit at different ripeness stages and seasonal patterns harvested in Nayarit, Mexico. *International journal of food sciences and nutrition.* **3 (5)**: 421-429.

Liu Y, Zhang Z & Hu L 2022. High efficient freeze-drying technology in food industry. *Critical reviews in food science and nutrition.* **62 (12)**: 3370-3388.

Marques LG, Silveira AM & Freire JT 2006. Freeze-drying characteristics of tropical fruits. *Drying technology.* **24 (4)**: 457-463.

Miller FA, Silva CL & Brandao TR 2013. A review on ozone-based treatments for fruit and vegetables preservation. *Food engineering reviews.* **5 (2)**: 77-106.

Misran A & Jaafar AH 2019. Protein. In *Postharvest Physiology and Biochemistry of Fruits and Vegetables* (ed. E. Yahia and A. Carrillo-Lopez), pp. 315-334. Elsevier.

Nasional BS 1992. SNI 01-2891-1992: Cara uji makanan dan minuman. In *Jakarta: Badan Standarisasi Nasional*.

Ndisanze MA & Koca I 2022. Dehydration and rehydration kinetics modeling in the phytochemical, aroma, and antioxidant capacity of tree tomato fruit dried with microwaves and freeze driers: a comparative study. *Processes.* **10 (8)**: 1437.

Nowak D & Jakubczyk E 2020. The freeze-drying of foods-The characteristic of the process course and the effect of its parameters on the physical properties of food materials. *Foods.* **9 (10)**: 1488.

Olivas-Aguirre FJ, et al. 2017. Radical scavenging and anti-proliferative capacity of three freeze-dried tropical fruits. *International journal of food science & technology.* **52 (7)**: 1699-1709.

Onwude D, et al. 2022. Physics-driven digital twins to quantify the impact of pre-and postharvest variability on the end quality evolution of orange fruit. *Resources, conservation and recycling.* **186**: 106585.

Orak HH, et al. 2012. Effects of hot air and freeze drying methods on antioxidant activity, colour and some nutritional characteristics of strawberry tree (Arbutus unedo L) fruit. *Food science and technology international.* **18 (4)**: 391-402.

Oyinloye TM & Yoon WB 2020. Effect of freeze-drying on quality and grinding process of food produce: A review. *Processes.* **8 (3)**: 354.

Piechowiak T, Grzelak-Błaszczyk K, Sójka M & Balawejder M 2022. One-time ozone treatment improves the postharvest quality and antioxidant activity of Actinidia arguta fruit. *Phytochemistry.* **203**: 113393.

Prahastuti S, et al. 2020. The ethanol extract of the bastard cedar (Guazuma ulmifolia L.) as antioxidants. *Pharmaciana.* **10 (1)**: 77-88.

[DOI: 10.18502/jnfs.v9i4.16901]

Raharitsifa N & Ratti C 2010. Foam- mat freeze- drying of apple juice part 2: Stability of dry products during storage. *Journal of food process engineering.* **33**: 341-364.

Rocha Ribeiro SM, Queiroz JH, Lopes Ribeiro de Queiroz ME, Campos FM & Pinheiro Sant'Ana HM 2007. Antioxidant in mango (Mangifera indica L.) pulp. *Plant foods for human nutrition.* **62**: 13-17.

Sachadyn-Król M & Agriopoulou S 2020. Ozonation as a method of abiotic elicitation improving the health-promoting properties of plant products-A review. *Molecules.* **25 (10)**: 2416.

Santos P & Silva M 2008. Retention of vitamin C in drying processes of fruits and vegetables—A review. *Drying technology.* **26 (12)**: 1421-1437.

Septembre-Malaterre A, Remize F & Poucheret P 2018. Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. *Food research international.* **104**: 86-99.

Shofian NM, et al. 2011. Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits. *International journal of molecular sciences.* **12 (7)**: 4678-4692.

Shonte TT, Duodu KG & de Kock HL 2020. Effect of drying methods on chemical composition and antioxidant activity of underutilized stinging nettle leaves. *Heliyon.* **6 (5)**: e03938.

Silva-Espinoza MA, Ayed C, Foster T, Camacho MdM & Martínez-Navarrete N 2019. The impact of freeze-drying conditions on the physico-chemical properties and bioactive compounds of a freeze-dried orange puree. *Foods.* **9 (1)**: 32.

Swallah MS, Sun H, Affoh R, Fu H & Yu H 2020. Antioxidant potential overviews of secondary metabolites (polyphenols) in fruits. *International journal of food science.* **2020**.

Thamburaj S, Rajagopal V, Palanivel R & Pugazhendhi S 2022. Effect of different drying treatments on total polyphenolics content and in-vitro biological properties of Ficus benghalensis fruit: A comparative study. *Biocatalysis and agricultural biotechnology.* **39**: 102249.

Toobpeng N, Powthong P & Suntornthiticharoen P 2017. Evaluation of antioxidant and antibacterial activities of fresh and freeze-dried selected fruit juices. *Evaluation.* **10**.

Tsai H-L, Chang SK & Chang S-J 2007. Antioxidant content and free radical scavenging ability of fresh red pummelo [Citrus grandis (L.) Osbeck] juice and freeze-dried products. *Journal of agricultural and food chemistry.* **55 (8)**: 2867-2872.

Wadolowska L, et al. 2018. Prudent-Active and Fast-Food-Sedentary Dietary-Lifestyle Patterns: The Association with Adiposity, Nutrition Knowledge and Sociodemographic Factors in Polish Teenagers-The ABC of Healthy Eating Project. *Nutrients.* **10 (12)**: 1988.

Walkowiak-Tomczak D 2007. Changes in antioxidant activity of black chokeberry juice concentrate solutions during storage. *Acta Scientiarum Polonorum Technologia Alimentaria.* **6 (2)**: 49-54.

Widowati W, et al. 2022. Antioxidant Properties of TeNan Herbal Tea Formulation "Telang (Clitoria ternatea) and Pineapple (Ananas comosus)". *Jurnal Kedokteran Brawijaya.* 87-93.

Widyani R 2020. In Vitro Studies: Potensial Use of Dyospiros kaky, Avium grafeolens, Guazuma ulmifolia, Hibiscus sabdariffa as an Agent of Anti-cholesterol. *Journal of environmental science and engineering.* **9**: 162-164.

Yahia EM, García-Solís P & Celis MEM 2019. Contribution of fruits and vegetables to human nutrition and health. In *Postharvest physiology and biochemistry of fruits and vegetables*, pp. 19-45. Elsevier.

Zahid HF, Ranadheera CS, Fang Z & Ajlouni S 2021. Utilization of mango, apple and banana fruit peels as prebiotics and functional ingredients. *Agriculture.* **11 (7)**: 584.

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