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Original Research Article

The comparison of antioxidant properties between frozen and freeze-dried strawberries *(Fragaria x ananassa)*

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ABSTRACT

Strawberries (*Fragaria x ananassa*) are a delectable fruit renowned for their numerous health benefits, due to their high concentrations of active compounds, including antioxidants, vitamin C, phenolic acids, and flavonoids. However, the phytochemical composition of strawberries is vulnerable to degradation during post-harvest storage and food preparation. Both freezing and freeze-drying are commonly used techniques in strawberry processing, but their effects on nutritional quality and antioxidant activity have not been thoroughly studied. This study aims to investigate the nutritional compounds of fresh strawberries and compare the physical quality and antioxidant activity of frozen and freeze-dried strawberries. An observational analytical design was used. Chemical profiling using LC-MS was conducted to identify major antioxidant compounds, while also comparing changes in colour, texture, and weight between frozen and freeze-dried strawberries. Additionally, antioxidant activity was measured using the DPPH free radical scavenging assay, and the levels of total tannins, flavonoids, and polyphenols were quantified in both frozen and freeze-dried strawberries. The results showed that 88 phytochemical compounds were found in fresh strawberries, with kaempferol and quercetin being the dominant antioxidants. Freeze-dried strawberries had better physical qualities, including a brighter colour and crunchier texture, but they lost more weight compared to frozen strawberries. The freeze-drying technique also improved antioxidant capacity, as shown by lower IC_{50} values and higher levels of tannins, flavonoids, and polyphenols compared to frozen technique. This study implies that the freeze-drying technique is a promising method for preserving the nutritional and health-promoting properties of strawberries in post-harvest periods.

Keywords: frozen, freeze-drying, strawberry, antioxidant capacity, physical quality, phytochemical profile

Introduction

Strawberries (*Fragaria x ananassa*) are among the world's most popular berries, recognized for their exquisite flavor, unique fragrance, and health benefits. For that reason, the global demand for strawberries is steadily increasing, with the fruit being consumed in both fresh and processed forms, such as jams, juices, and jellies.[1] In Indonesia, Strawberries (*Fragaria x ananassa*) are widely cultivated particularly in the highlands of Java, Sumatra, and Bali, with a total national production of 27.721 ton in 2023.[2]

Strawberries are commonly linked to various health benefits due to their major source of bioactive compounds, which have nutraceutical properties. The vibrant hue of strawberries is mostly attributed to polyphenols, specifically anthocyanins, which are one of the active compounds present in the fruit. Research shows that strawberries rank among the top 100 richest sources of dietary polyphenols worldwide.[3]

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As a major source of bioactive compounds, the strawberry (*Fragaria x ananassa*) is valued for its high levels of flavonoids, phenolic acids, lignans, and tannins, as well as its high levels of vitamins, especially ascorbic acid, and folate.[4] The results of several studies clearly show that strawberries can help fight inflammation,[5] high glucose, high cholesterol,[6] and cancer.[7] They can also help reduce oxidative stress and improve the health of people with cardiovascular disease (CVD), cancer, type 2 diabetes, obesity, and neurodegeneration.[3]

However, despite all its benefits, strawberries are a non-climacteric, very perishable fruit that can get physically damaged while being stored and transported.[1] The storage duration and temperature can influence physical quality and the micronutrient and phytochemical profiles of strawberries. According to several previous studies, anthocyanins, which are known as the most important antioxidants inside strawberries, are less stable at higher temperatures.[4,8–11] Therefore, selecting the appropriate processing method for strawberries is crucial to maintain the effectiveness of strawberries in delivering the expected health benefits. Currently, two common methods for processing fresh fruit using low temperatures are freezing and freeze-drying. Both freezing and freeze-drying offer effective ways to preserve strawberries, each with its own set of advantages and limitations. Freezing is simpler, more cost-effective, and better for maintaining texture, making it suitable for storage. However, it requires refrigeration and can cause texture degradation upon thawing. Freeze-drying, on the other hand, offers superior nutrient retention and extended shelf life, without the need for

refrigeration, but comes at a higher cost and may alter the fruit's texture and flavour. Freeze-drying is particularly effective at retaining the nutrient content of strawberries, particularly the levels of polyphenols.[4,12–14] However, to the best of our knowledge, no research has yet compared the effects of freezing versus freeze-drying on strawberry processing to preserve the natural compounds of the fresh fruit, particularly its polyphenol content, in order to determine the optimal method.

This study aims to evaluate the chemical composition of strawberry fruits and compare the physical quality and antioxidant activity of frozen versus freeze-dried strawberries. Additionally, the research compares the antioxidant properties of the strawberries, as indicated by the concentrations of total tannins, total flavonoids, and total polyphenols, in both frozen and freeze-dried forms.

Materials and Methods

Materials

Fresh strawberries (Fragaria × ananassa) were obtained from Lumbung Stroberi, Desa Wisata Pandanrejo, Batu City, East Java, Indonesia, in January 2023. It was harvested at the full ripeness stage. This study used Mencir variety strawberries, which it was identified by Herbal Materia Medica Laboratory, Batu, Malang, East Java (000.9.3/ 2633/ 102.20/ 2023). The fresh strawberries were thoroughly cleaned under running water. The leaves and damaged parts of strawberries were removed.

Methods

Sample Preparation

Frozen Strawberries

After the cleaning process, the strawberries were cut into cubic of 50 x 50 x 50 mm^3. All the cut strawberries were stored in a closed and sealed opaque plastic storage container. The fruits inside the container were stored in the freezer at -18 ± 2°C for 7 days until the assay began.

Freeze-Dried Strawberries

After the cleaning process, the cut strawberries inside the opaque plastic storage container were stored in the freezer at -18 ± 2°C for 3 days before the freeze-drying process Freeze-drier using LabFreez FD-12-MR (SN: FD2019090711). The freeze-drier was operated at -50°C, five pa, for 72 hours. After the process, the freeze-dried strawberries were stored in the freezer at -18 ± 2°C for 6 hours until the assay began.

Identifying chemical components

The fresh strawberries were thoroughly cleaned and then crushed using a Philips Fruit Juicer Blender (HR-1866). To identify the chemical components in the strawberry juice, a Shimadzu LCMS-8040 LC/MS system was employed. A 1 µL sample of the juice was injected into the liquid chromatography (LC) system, which was equipped with a Shim Pack FC-ODS column (150 × 2 mm, 3 µm particle size) maintained at a temperature of 35°C. Phenolic compounds were separated using isocratic elution, with acetonitrile as the mobile phase at a flow rate of 0.5 mL/min. The analysis was conducted in negative ion mode with the following parameters: sampling cone voltage set to 23 V, capillary voltage of 3.0 kV, source temperature at 100°C, desolvation temperature of 350°C, and a gas flow rate of 60 mL/min. Mass spectra were recorded in electrospray ionization (ESI) negative ion mode, covering a mass range of m/z 10–1000. The scan duration was 0.6 seconds per scan, and the total analysis time was 80 minutes.

The LC-MS analysis produced a chromatogram with distinct peaks, providing detailed information about the composition, retention times, and mass spectral data of the identified chemical components in the strawberry juice.

Identifying physical quality

This study evaluated the differences in physical quality between frozen and freeze-dried strawberries, focusing on color, hardness, and weight changes during food processing. To assess the color and hardness of each fruit sample, sensory evaluation was performed. Fruit weight was measured using a digital scale with 0.01-gram precision (Oxone®). For the analysis, 500 milligrams of each sample were used, and the changes in fruit weight before and after processing were calculated using the following equation:[15]

The changes of fruit weight = (Wf – Wd) / Wd x 100%

Note: Wf = mass of the sample before processing procedure (mg), Wd = mass of the sample after processing procedure (mg).

Determination of DPPH radical scavenging activity

Frozen and Freeze-Dried Strawberry Solution

Each 100 milligrams of frozen and freeze-dried strawberry was dissolved in 10 mL of ethanol. From each solution, 0.05, 0.10, 0.15, and 0.20 mL were transferred into separate tubes, and each was then diluted with ethanol to a final volume of 1.0 mL.

DPPH solution

To prepare the DPPH stock solution, 7 milligrams of DPPH were dissolved in 50 mL of ethanol. The solution was then filtered using ethanol, resulting in a mixture with an absorbance of approximately 0.973 at 517 nm. A control or standard specimen typically consisted of 3 mL of the DPPH solution in 1000 µL of ethanol. For assessing the antioxidant activity of the strawberries, 3 mL of the DPPH solution was combined with the strawberry solution (separately applied to both frozen and freeze-dried strawberries).

The percentage of DPPH scavenging activity

Both the control and test specimen tubes were kept in complete darkness for 30 minutes. After the incubation period, the absorbance was measured at 517 nm. To calculate the percentage of antioxidant activity, the following formula was used:[16]

% of antioxidant activity= [(Ac−As) ÷Ac] × 100%

Note: Ac—Control reaction absorbance; As—Testing specimen absorbance.

The concentrations of the frozen and freeze-dried strawberries which were able to decrease 50% from DPPH free radical initial activity were determined as IC_{50} (mg/ mL).

Determination of total tannin content

The total tannin content was determined using the Folin-Ciocalteu method. First, 60 milligrams of frozen and freeze-dried strawberries were dissolved in 10 mL of water. A 1.0 mL aliquot of the solution was then transferred into a 10 mL volumetric flask, and the volume was adjusted by adding 0.5 mL of Folin-Ciocalteu phenol reagent. The mixture was shaken thoroughly and allowed to sit for 5 minutes at room temperature. Next, 2 mL of a 10% sodium carbonate solution was added, and the mixture was well mixed. The solution was left at room temperature for 10 minutes and then filtered to collect the residue.

For both test and standard solutions, the absorbance was measured against a blank at 770 nm using a UV/visible spectrophotometer. The tannin content was determined in duplicate. The final tannin content values were calculated as the mean of the duplicate measurements ± standard deviation and expressed as grams of tannic acid equivalents (TAE) per 100 grams of sample (% w/w TAE).

Determination of total flavonoid content

Sample Preparation

One gram of each frozen and freeze-dried strawberry was treated with 20.0 mL of acetone R, 1.0 mL of 0.5% (w/v) hexamethylenetetramine, and 2.0 mL of 25% (w/v) hydrochloric acid R in a 100 mL round-bottom flask. The mixture was refluxed for 2 hours in a water bath and then filtered through cotton wool. The plant residue and filter were washed with 20.0 mL of acetone R, and the washings were refluxed for an additional 10 minutes. After the solution cooled, it was filtered again and made up to 100 mL with acetone.

Twenty milliliters of this solution were transferred into a funnel, and 20 mL of water was added. Then, 15 mL of ethyl acetate R was added, and the mixture was shaken thoroughly for 10 minutes. This extraction procedure was repeated three times, using 10.0 mL of ethyl acetate each time. The combined organic phases were washed with 50 mL of water

and made up to 50 mL with ethyl acetate to obtain the Stock Solution (SS).

Determination Procedure

The total flavonoid content (TFC) was determined using the aluminum chloride ($AlCl_3$) method. To prepare the probe solution (PS), 1.0 mL of 2% (w/v) $AlCl_3$ in methanol was added to 10.0 mL of the stock solution (SS), and the mixture was diluted to 25.0 mL using a 0.5% (v/v) methanolic solution of acetic acid. Simultaneously, the contrast solution (CS) was prepared by diluting 10.0 mL of SS to 25.0 mL with a methanol/acetic acid solution. The absorbance of PS was measured against CS at 425 nm after 30 minutes. A standard calibration curve with quercetin was used for the measurements. The flavonoid content was expressed as a percentage of the total flavonoid content (TFC) and calculated as the mean of duplicate determinations ± standard deviation. The analysis was conducted on the same day for both frozen and freeze-dried strawberries. The percentage of total flavonoid content was determined using the following formula:[17]

Total Flavonoid Content (%) = A x DF / gram of sample

Note: A = measured absorbance; DF = dilution factor (1.25)

Determination of total polyphenol content

Gallic Acid Solution Preparation

The total polyphenol content in both frozen and freeze-dried strawberries was determined using a spectrophotometric method described by Kang *et al.*[18] Gallic acid, with a purity of 99.75%, was used as the standard solution. It was prepared in concentrations ranging from 5 to 25 ppm using water as the solvent. For each concentration, 1 mL of the standard solution was transferred into a 96-well microplate. Next, 0.5 mL of Folin-Ciocalteu reagent was added, and the mixture was incubated for 5 minutes. Subsequently, 2 mL of 10% sodium carbonate was added, and the solution was incubated for an additional 10 minutes at room temperature in a dark environment. The absorbance was measured using a spectrophotometric microplate reader (ELISA reader) at a wavelength of 770 nm.

Sample Preparation

A total of 60 milligrams of both frozen and freeze-dried strawberries was dissolved in 10 mL of water (H_2O). From each sample, a 1.0 mL aliquot was transferred into a 10 mL volumetric flask. Then, 0.5 mL of Folin-Ciocalteu phenol reagent was added, and the mixture was shaken thoroughly. The solution was allowed to stand at room temperature for 5 minutes. Next, 2 mL of 10% sodium carbonate solution was added, and the mixture was stirred well. The solution was then left at room temperature for 10 minutes before being filtered to separate the residue. The absorbance of both the test and standard solutions was measured against a blank at a wavelength of 770 nm using a spectrophotometer. The polyphenol content was determined in duplicate, with results expressed as the mean ± standard deviation. The polyphenol content was reported as milligrams of Gallic Acid Equivalent (GAE) per gram of sample.

Results and Discussion

Strawberries' Chemical Components

Strawberries are generally abundant in antioxidant compounds. It possesses high concentrations of either vitamin C or phenolic compounds. Strawberries provide as a valuable source of dietary fiber. Strawberries contain significant polyphenolic phytochemicals, including flavonoids, phenolic acids, tannins, and lignans.[4] According to Souza *et al.*,[19] the chemical and physical features of fruits are influenced by cultivar, environmental factors (such as weather, humidity, soil conditions, and sun exposure), harvest timing, maturity, ripening stage, and post-harvest management.

This research used Mencir variety strawberries at full ripeness, sourced from Lumbung Stroberi in Desa Wisata Pandanrejo, Batu City, East Java, Indonesia. The strawberries were identified by the Herbal Materia Medica Laboratory in Batu, Malang, East Java (000.9.3/2633/102.20/2023). Based on the peak areas in the LC-MS chromatogram, 88 phytochemical compounds were identified in strawberries, as presented in Table 1 and Figure 1. Among these, five major compounds were identified: Kaempferol-3-*O*-rhamnoside, Kaempferol 3-*O*-*β*-*D*-(6"-coumaroyl)-glucopyranoside, Quercetin, Quercetin-3-*O*-rhamnoside, and Quercetin-3,7,4'-triglucoside. Their respective compositions in the strawberries were 2.87%, 2.86%, 2.57%, 2.52%, and 2.52%. The LC-MS spectrum validated the existence of five major compounds, with retention durations of 21.43, 33.62, 11.4, 22.6, and 49.9 minutes, respectively. Five distinct fragmentation spectra were generated for each molecule. The spectra indicated potential mass-to-charge ratios (m/z) of 432.4, 594.5, 302.2, 448.4, and 788.7. In contrast, anthocyanins, such as pelargonidin and cyanidin, were identified as minor constituents. Figure 1. The LC - MS Chromatogram of Strawberries Fruit Table 1. Phytochemical compounds identified in strawberries by LC-MS analysis. This finding aligns with previous research, which reported quercetin and kaempferol as the main flavanols in strawberries, while pelargonidin and cyanidin are recognized as the primary anthocyanin compounds. Despite the use of different strawberry varieties, no significant differences in chemical components were observed between the strawberries in this study and those examined in the previous study.[20]

Physical Quality

Different food processing methods can significantly impact the physical quality of fruits. Among these, color, as critical parameter for antioxidant capacity in some fruits, is one of the most frequently changed throughout processing. This study found significant differences in color quality between frozen and freeze-dried strawberries. As shown in Figure 2, freeze-dried strawberries exhibited higher lightness and redness compared to their frozen counterparts, indicating superior color retention in the freeze-dried samples.

Figure 2. Physical Quality difference between frozen (A) and freeze-dried strawberries (B)

The strawberries used in this study were fully ripe, meaning that 100% of the fruits had achieved their red color. The red coloration of strawberries is primarily attributed to anthocyanins, with pelargonidin 3-glucoside as the main component.[21] The study found that freeze-dried strawberries displayed a more vibrant red hue compared to frozen strawberries, suggesting that freeze-dried strawberries may contain higher concentrations of anthocyanins.

Freeze-drying is a process that removes water from a material through sublimation, preserving polyphenol content more effectively than other fruit processing methods. This finding aligns with previous research, which showed that dehydrated fruits often have higher antioxidant levels than fresh or frozen fruits.[9] In contrast, the freezing process may affect the chemical properties of plant products, potentially leading to the formation of brown compounds or changes in other natural pigments due to enzymatic and oxidative reactions.[22] This could explain why the frozen strawberries had a darker hue than the freeze-dried ones.

A comparison was also made between the texture hardness of freeze-dried and frozen strawberries in this study. This research utilized a subjective assessment based on tactile sense. The frozen strawberries exhibited a softer, wrinkled appearance, whereas the freeze-dried strawberries displayed a distinctly crunchier feel.

The hardness of fruits can be preserved through an osmotic dehydration process. As the water content in the fruit samples decreases, the hardness of the fruit increases.[21] Previous studies have shown that the proportion of freezable water in a product affects its moisture content, which, in turn, influences the softness of the cellular tissue. Higher moisture content generally leads to a softer texture, while lower moisture content results in a firmer or crunchier texture.[23] The freeze-drying process removes water content from strawberries, which may explain why freeze-dried strawberries have a crunchier texture compared to frozen strawberries.

The change in fruit weight was also assessed before and after processing. As shown in Table 2, the weight of frozen strawberries showed no significant change during processing, with a weight loss of only 5%. In contrast, freeze-dried strawberries experienced a substantial weight reduction, losing 90% of their original weight.

Strawberries consist of approximately 90% water; hence their weight is predominantly determined by their water and moisture content.Water

content plays a critical role in both the size and structure of the fruit. During frozen storage, samples with higher water content tend to form

Table 1. Phytochemical compounds identified in strawberries by LC-MS analysis

Peak	Identification	Observed m/z	Composition (%)	Peak	Identification	Observed m/z	Composition (%)
1	Acetic acid	60.0211	0.85498	45	Kaempferol-3-*O*-rhamnoside	432.3810	2.86651
2	Methyl thiopropionate	104.0296	0.35684	46	Fragarin	433.3885	0.34020
3	Niacin	123.0320	0.08501	47	Quercetin-3-arabinoside	434.3530	2.29735
4	Ethyl thiobutyrate	132.0609	0.28415	48	Taxifolin 3-*α*-*L*-arabinofuranoside	436.3690	1.71660
5	Ethyl (methylthio)acetate	134.0402	0.40949	49	Quercetin-3-*O*-rhamnoside	448.3800	2.52386
6	Methyl 3 (methylthio) propionate	148.2200	0.41467	50	Kaempferol-3-*O*-*D*-glucoside	448.3800	1.94233
7	Malic acid	134.0870	0.76470	51	Cyanidin-3-glucoside	449.3875	0.88765
8	p-hydroxybenzoic acid	138.1220	0.96005	52	Quercetin-3-glucoside	463.3715	2.29730
9	Arabinose	150.1300	1.92484	53	Pelagornidin 3 (6”- acetylglucoside)	475.4255	0.52769
Peak	**Identification**	**Observed m/z**	**Composition (%)**	**Peak**	**Identification**	**Observed m/z**	**Composition (%)**
10	Methyl thiohexanoate	146.2480	0.24582	54	Querciturone	478.3620	1.92473
11	p-Coumaric acid	164.1600	0.68177	55	Malvidin-3-glucoside	493.4405	0.67790
12	Rhamnose	164.1570	2.02710	56	Cyanidin 3-*O*-(6-*O*-acetyl-*β*-*D*-glucoside)	491.4245	0.88773
13	Cinnamic acid	148.1610	1.26651	57	5-carboxypyranopelagornidin 3-*O*-*β*-glucopyranoside	501.4195	0.79285
14	Ethyl 3-(methylthio) propionate	148.2200	0.41063	58	Raffinose	504.4380	1.70625
15	Methyl 2-(methylthio) butyrate	148.0558	0.33393	59	Pelagornidin 3-(6”-malonylglucoside)	519.4345	0.90031
16	Methyl (methylthio) acetate	120.1660	0.19318	60	Cyanidin 3-(3”-malonylglucoside)	535.1082	0.78504
17	Gallic acid	170.1200	2.13063	61	*α*-carotene	536.8880	0.52762
18	Methyl thiooctanoate	174.3020	0.33910	62	*β*-carotene	536.8880	0.67257
19	Asrobic acid	176.1240	1.37514	63	Lutein	568.8860	0.29656
20	Caffeic acid	180.1590	1.93519	64	Grayanoside A	476.4780	0.41293
21	Fructose	180.1560	2.14735	65	Pelagornidin-3-*O*-rutinoside	579.5305	1.70625
22	Glucose	180.1560	1.96174	66	Procyanidin B1	578.5260	2.28811
23	Citric acid	192.1230	1.53864	67	Procyanidin B3	578.5260	1.93524
24	Ferulic acid	194.1860	1.92137	68	Procyanidin B6	578.5260	1.74688
25	Pantothenic acid	219.2370	0.39006	69	Pelagornidin 3-rhamnoside 5-glucoside	579.5305	1.49016
26	Resveratrol	228.2470	0.77506	70	Kaempferol 3-*O*-*β*-*D*-(6”-coumaroyl)-glucopyranoside	594.5250	2.85622
27	Melatonin	232.2930	0.29654	71	Cyanidin 3-(6-*p*-coumaroylglucoside)	595.5325	0.68293
28	Thiamin	265.3545	0.45518	72	Cyanidin-3-*O*-rutinoside	595.5295	0.62082
29	Imperatorin	270.2840	0.28722	73	Quercetin 3xyloside-7-glucoside	596.4940	1.83173
30	Kaempferol	286.2390	2.41120	74	Rutin	610.5210	2.05813
31	Ellagic acid	302.1940	1.75817	75	Cyanidin 3.5-diglucoside	611.5285	1.25329
32	Quercetin	302.2380	2.57490	76	Stachyose	666.5790	0.90021
Peak	**Identification**	**Observed m/z**	**Composition (%)**	**Peak**	**Identification**	**Observed m/z**	**Composition (%)**
33	Myricetin	318.2370	1.43516	77	Afzelechin (4*α*à8) pelagornidin 3-*O*-*β*-glucopyranoside	705.6445	1.09267
34	1-p-coumaroyl-*β*-*D*-glucose	327.3071	1.60394	78	Epiafzelechin(4*α*à8) pelargonidin 3-*O*-*β*-glucopyranoside	705.6445	1.25318
35	Sucrose	342.2970	1.00261	79	Catechin (4*α*à8) pelargonidin 3-*O*-*β*-glucopyranoside	721.6435	0.68293
36	Chlorogenic acid	354.3110	1.48936	80	Cyanidin 3-*O*-(4-coumaroyl)-rutinoside	739.6595	0.55877
37	Riboflavin	376.3690	0.45524	81	Pelargonidin 3-glucosyl-rutinoside	741.6715	0.88562
38	*β*-tocopherol	416.6900	0.22527	82	Pelargonidin 3-rutinoside-7-glucoside	741.6715	0.68285
39	Cyanidin 3-O-arabinoside	419.3615	0.68302	83	Cyanidin 3-*O*-(6-*O*-glucosyl-2-*O*-xylosylgalactoside)	743.6435	0.45526

40	Pelagonidin 3-arabinoside	403.3625	1.20039	84	Pelargonidin 3-*O*-(6-*O*-caffeoyl-*β*-*D*-glucoside) 5-*O*-*β*-*D*-glucoside	757.6735	0.41280
41	*β*-sitosterol	414.7180	0.67251	85	Quercetin-3.7.4'-triglucoside	788.6610	2.51993
42	*α*-tocopherol	430.7170	0.29677	86	Cyanidin 3-(6"-ferulylglucoside) 5-glucoside	787.6995	0.29678
43	Pelargonidin 3-O-*β*-*D*-glucopyranoside	433.38885	1.82112	87	Verbascose	624.5920	0.79677
44	Cyanidin 3-rhamnoside	433.3885	0.61980	88	Pelargonidin 3-(6-ferulyl-2-glucosylglucoside)-5-glucoside	933.8415	0.99220

Table 2. Sample weight loss and antioxidant activity capacity (IC_{50}) with examination of strawberries' chemical components.

Sample	**Frozen Strawberry (mg)**	**Freeze-Dried Strawberry (mg)**
The weight loss before and after the fruit processing procedure		
Average Before (n=5)	500	500
Average After (n=5)	475	50
Average Weight Loss (%) ± SD	5 ± 0.45	90 ± 0,14
The capacity of antioxidant activity (IC_{50}) on repeated evaluation		
Replicate 1 (% w/w)	0.1	1.3
Replicate 2 (% w/w)	0.1	1.3
Average ± SD (% w/w)	0.1 ± 0.1	1.3 ± 0.7
Total tannin content on repeated evaluation		
Replicate 1 (% w/w)	0.003	0.01
Replicate 2 (% w/w)	0.003	0.01
Average ± SD (% w/w)	0.003 ± 2.32	0.01 ± 0.86
Total polyphenol content on repeated evaluation		
Replicate 1	0.1	1.2
Replicate 2	0.1	1.2
Average ± SD	0.1 ± 0.2	1.2 ± 0.7

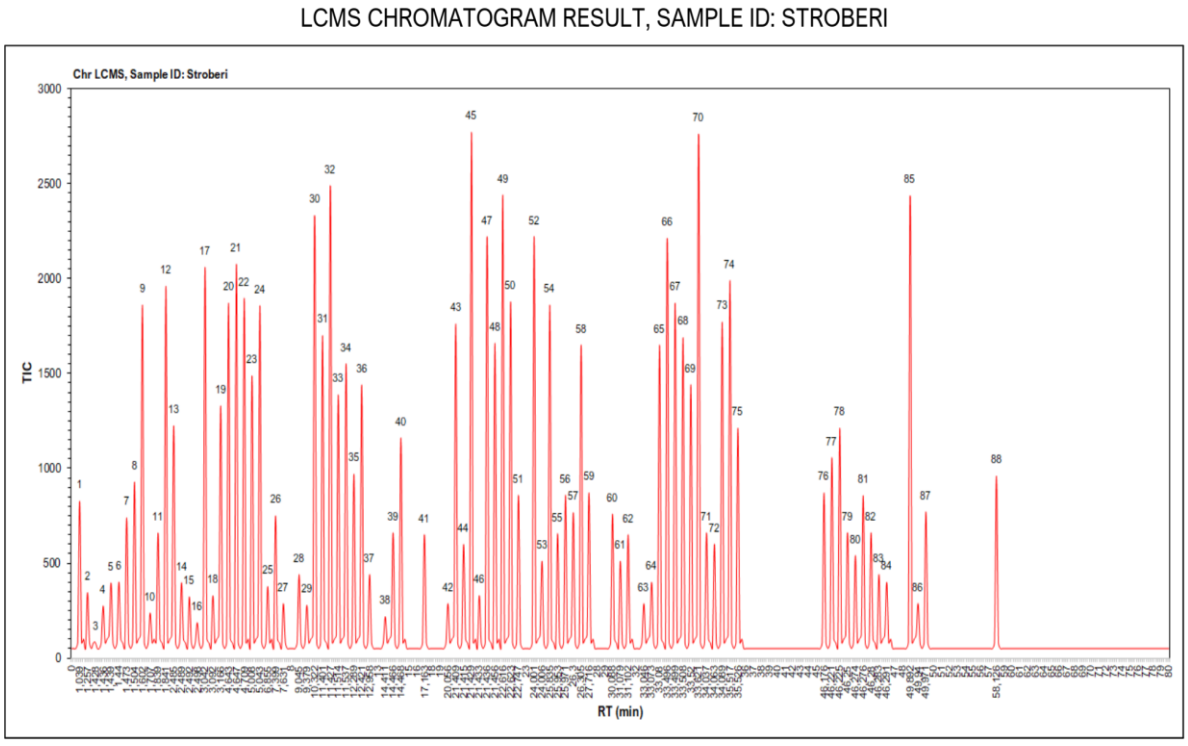

Figure 1. The LC-MS Chromatogram of Strawberry Fruit

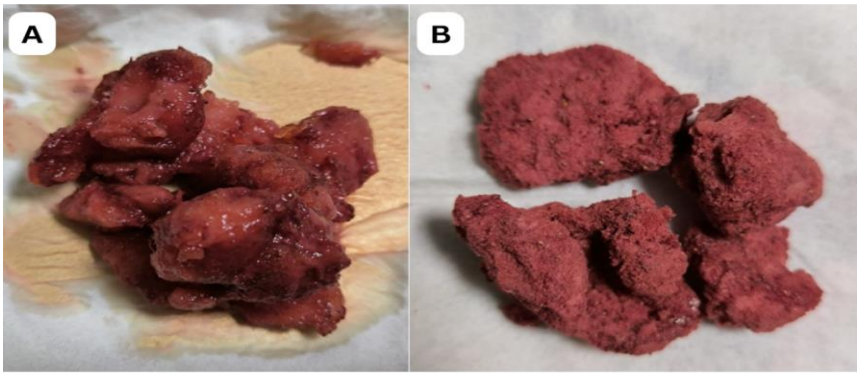

Figure 2. Physical Quality difference between frozen (A) and freeze-dried strawberries (B)

more ice crystals within the flesh of the fruit.[23,24] In contrast, during the drying process, organic compounds in the fruit decompose, reducing the water content. According to Ansar *et al.*,[15] the longer the drying process, the greater the reduction in fruit mass. This could help to explain why frozen strawberries did not significantly reduce weight whereas freeze-dried strawberries did.

DPPH Radical Scavenging Activity

The antioxidant activity of frozen and freeze-dried strawberries was evaluated using the DPPH assay. This method employs 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a stable free radical, which exhibits its highest absorption band at 517 nm. The DPPH radical scavenging activity of both samples was assessed in duplicate on the same day. The antioxidant capacity was expressed as the average IC_{50} value (the concentration required to scavenge 50% of DPPH radicals) from duplicate tests ± standard deviation (refer to Table 2). The results showed that the IC_{50} value for frozen strawberries was 15.89 ± 0.3 mg/mL, whereas freeze-dried strawberries demonstrated a significantly lower IC_{50} value of 1.38 ± 0.7 mg/mL, indicating stronger antioxidant activity.

The antioxidant activity of frozen and freeze-dried strawberries was assessed using the DPPH assay.[25,26] The assay determines the concentration at which DPPH free radical activity is reduced by 50%, known as the IC_{50} value. Greater antioxidant activity in the samples is indicated by a lower IC_{50} value.[27,28] The study revealed that both frozen and freeze-dried strawberries exhibited very strong antioxidant properties, as shown by their IC_{50} values in Table 2, with both values falling below 50 mg/mL. However, freeze-dried strawberries demonstrated a stronger antioxidant capacity than frozen strawberries, as indicated by their lower IC_{50} values. The freeze-drying process, which removes water from the material under low pressure, helps preserve the nutrient content of strawberries, particularly polyphenols. Additionally, the condensation step during freeze-drying helps retain slightly higher levels of total phenolic content compared to freezing. Furthermore, the lower processing temperature and reduced oxygen availability under vacuum conditions during freeze-drying contribute to better preservation of vitamin C. [4,12–14] This may elucidate why the freeze-drying method retained a greater antioxidant content compared to frozen strawberries.

Total Tannins Profile

The total tannin content in both frozen and freeze-dried strawberries was determined using the Folin-Ciocalteu colorimetric method described by Ahad *et al*.[29] Tannin content was expressed as milligrams of tannic acid equivalents (TAE) per gram of sample (% w/w TAE).[30]
The analysis was performed in duplicate on the same day for each sample, and the total tannin content was reported as the mean ± standard deviation. Table 2 indicates that the total tannin level in frozen strawberries was 0.1 ± 0.1% w/w, much lower than the 1.3 ± 0.7% w/w found in freeze-dried strawberries.

The antioxidant activity of plants and fruits is often correlated with the amount of their phenolic compounds. Tannins, a type of phenolic compound, have a complex structure with two aromatic rings connected by three carbon atoms. Tannins are active secondary metabolites containing phenolic compounds that are difficult to separate and crystallize. As a result, tannins can play an important role as antioxidants in fruits.[30]

According to Table 2, frozen strawberries had lower total tannin content than freeze-dried strawberries. This difference can be attributed to the dehydration treatments used in the freeze-drying process, which help release phytochemical components from the fruit. As a result, the components of freeze-dried strawberries become more accessible compared to those in frozen strawberries. Additionally, freeze-drying is often considered the most effective method for preserving temperature-sensitive compounds. During the freeze-drying process, ice crystals form within the plant matrix, rupturing the cell structure of the fruit and facilitating the release of plant components.[31] This elucidates why freeze-dried strawberries possess a greater total tannin content compared to frozen strawberries.

Total Flavonoid Profile

The flavonoid content was determined using the aluminum trichloride method. The total flavonoid content was expressed as milligrams of quercetin equivalents (QE) per gram of sample (w/w QE). The analysis was conducted in duplicate on the same day for both frozen and freeze-dried strawberries, and the results were reported as the mean ± standard deviation. As shown in Table 2, the total flavonoid content of frozen strawberries was 0.003 ± 2.32% w/w, which was lower than that of freeze-dried strawberries, measured at 0.01 ± 0.86% w/w.

Flavonoids are secondary metabolites primarily composed of a benzopyrone ring, with phenolic or polyphenolic groups attached at various positions. Flavonoids play an important role in plant coloration and have been shown to exhibit several beneficial activities for humans, including anti-inflammatory, antioxidant, anti-allergic, and anti-cancer properties.[32,33]

According to Table 2, frozen strawberries had lower total flavonoid content than freeze-dried strawberries. This finding is consistent with the study by Gregorio *et al*.,[34] which demonstrated that freeze-drying increased the total flavonoid content in red onions by altering tissue structure. The freeze-drying procedure modified the strawberries' structure, facilitating the extraction of flavonoids, especially those with higher non-polar characteristics.[34] Conversely, Rababah *et al*.[35] found that the freezing process could reduce flavonoid content due to glycosylation reactions caused by glucosidase activity during frozen storage. This reaction affects the solubility, reactivity, and stability of flavonoids in the fruit.[35] This may explain why frozen strawberries lower total flavonoid content had compared to freeze-dried strawberries.

Total Polyphenol Profile

The total polyphenol content was determined using the method proposed by Kang *et al*.[18] Gallic acid was used as the standard, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample (% w/w GAE). The analysis was performed in duplicate on the same day for each sample, with the total polyphenol content reported as the mean ± standard deviation. As shown in Table 2, the polyphenol content of frozen strawberries was 0.1 ± 0.2% w/w,

which was lower than that of freeze-dried strawberries, measured at 1.2 ± 0.7% w/w.

"Polyphenols" or "phenolic compounds" refer to substances that contain a benzene ring with one or more hydroxy groups. These compounds are classified into several categories based on their chemical structures, including: (1) phenolic acids, (2) cinnamic acids, (3) lignins, (4) lignans, (5) tannins, (6) flavonoids, (7) quinones, (8) stilbenes, and (9) betacyanins. Polyphenols are important antioxidants that attenuate oxidative stress and exhibit potent anti-inflammatory and anti-aging effects.[14]

According to Table 2, frozen strawberries had lower total polyphenol content than freeze-dried strawberries. Some studies suggest that freezing can cause the release of bioactive compounds, such as phenolic acids and anthocyanins, from the fruit.[22] In contrast, the drying process in freeze-drying disrupts the plant matrix, which facilitates the release of phenolic compounds. Freeze-drying is also a more time-consuming process that involves both freezing and dehydration. This extended exposure duration of active chemicals to the solvent most likely improves phenolic component extraction and measurement, resulting in higher values in freeze-dried strawberries than frozen ones.[36,37]

Table 2. Sample weight loss and antioxidant activity capacity (IC_{50}) with examination of strawberries' chemical content.

Conclusion

This study demonstrates that strawberries are rich in essential antioxidants, which may help prevent and improve various chronic degenerative diseases. There were no significant differences in chemical components of the fresh strawberries in this study and those in the previous study. Processing techniques, such as freezing and freeze-drying, effectively preserve both the physical quality and nutritional content of the fruit. However, freeze-dried strawberries exhibited a more vibrant color, a crispier texture, and greater weight reduction compared to frozen strawberries. The antioxidant activity, measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, revealed that freeze-dried strawberries had stronger antioxidant activity than frozen strawberries. This finding was further supported by higher levels of total tannins, total flavonoids, and total polyphenols in freeze-dried strawberries compared to their frozen counterparts.

Conflict-of-Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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