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Rapid decline in antioxidant activity of freshly prepared strawberry (*Fragaria x ananassa*) juice during short-term cold storage: a DPPH study

Penurunan cepat aktivitas antioksidan sari buah stroberi (Fragaria x ananassa) yang baru dibuat selama penyimpanan dingin jangka pendek: studi DPPH

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Abstract

Strawberries (Fragaria × ananassa) are rich in phenolic antioxidants, particularly anthocyanins, which degrade during processing and storage. However, the rate of antioxidant loss in freshly prepared strawberry juice during the first few days of typical home refrigeration—the exact period a consumer would drink it—remains insufficiently quantified. This study evaluated the antioxidant activity of freshly prepared strawberry juice stored at 4°C (chiller) for 0, 1, 3, and 5 days using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. Sample concentrations of 100–500 ppm (w/v) were tested in triplicates. The presence of flavonoids was qualitatively confirmed using the Shinoda test. IC50 values were derived from the linear regression of the percentage of inhibition versus concentration. Fresh juice exhibited the highest antioxidant capacity (IC50 = 140 ppm; moderate activity). After only one day of chiller storage, the IC50 increased substantially to 225 ppm, a ~61% increase, indicating a significant loss of potency. Further increases to 257 ppm (day 3) and 272 ppm (day 5) placed the samples in the weak activity category (IC50 > 150 ppm per Yuslianti, 2018). An anomalous decrease in the% inhibition at 500 ppm in the fresh sample was noted and discussed as a likely measurement artifact. In conclusion, these findings demonstrate that strawberry juice loses a substantial proportion of its antioxidant potency within 24 h of preparation. Strawberry juice should ideally be consumed within one day of preparation for meaningful antioxidant benefit.

Keywords: Antioxidant activity, Strawberry juice, DPPH, Cold storage, Phenolic degradation, Anthocyanin

Abstrak

Stroberi (Fragaria x ananassa) kaya akan antioksidan fenolik, terutama antosianin, yang diketahui mudah terdegradasi akibat proses pengolahan dan penyimpanan. Namun, laju kehilangan antioksidan pada sari buah stroberi segar selama beberapa hari pertama penyimpanan di lemari pendingin rumah tangga—periode yang relevan bagi konsumen—belum cukup terdokumentasi. Penelitian ini mengevaluasi aktivitas antioksidan sari buah stroberi segar yang disimpan pada suhu 4°C selama 0, 1, 3, dan 5 hari menggunakan metode DPPH (1,1-difenil-2-pikrilhidrazil). Konsentrasi sampel 100–500 ppm diuji secara triplikat. Keberadaan flavonoid dikonfirmasi secara kualitatif dengan uji Shinoda. Nilai IC50 diperoleh dari regresi linier antara % inhibisi dan konsentrasi. Sari buah segar menunjukkan kapasitas antioksidan tertinggi (IC50 = 140 ppm; aktivitas sedang). Setelah satu hari penyimpanan, IC50 meningkat signifikan menjadi 225 ppm (~61%), menunjukkan penurunan potensi

yang bermakna. Peningkatan lebih lanjut menjadi 257 ppm (hari ke-3) dan 272 ppm (hari ke-5) menempatkan sampel dalam kategori aktivitas lemah. Kesimpulan, bahwa sari buah stroberi kehilangan sebagian besar potensi antioksidannya dalam 24 jam pertama. Oleh karena itu, sari buah stroberi sebaiknya dikonsumsi dalam satu hari setelah pembuatan.

Kata Kunci: Aktivitas antioksidan, Sari buah stroberi, DPPH, Penyimpanan dingin, Degradasi fenolik, Antosianin

Introduction

Strawberries (Fragaria x ananassa) are among the most widely consumed berry fruits globally, valued for their distinctive flavor and rich content of bioactive phytochemicals, including phenolic acids, flavonoids, particularly anthocyanins, and vitamin C (Salas-Arias et al., 2023). These compounds are well-documented for their capacity to neutralize free radicals and reactive oxygen species (ROS), which, when present in excess, drive oxidative stress implicated in cardiovascular disease, type 2 diabetes, premature aging, and cancer (Rani et al., 2024; Pizzino et al., 2017). Oxidative stress arises when cellular antioxidant defences, including enzymatic systems such as superoxide dismutase, catalase, and glutathione peroxidase, are overwhelmed by free radical exposure from both endogenous metabolic processes and exogenous sources such as pollution, cigarette smoke, and ultraviolet radiation (Pratama & Busman, 2020). Dietary antioxidants from plant foods can supplement the body's endogenous defense mechanisms (Widiasrini et al., 2024).

This is a particular concern in developing countries such as Indonesia, where the population-wide intake of fruits and vegetables remains critically low. Data from the 2023 Indonesian National Health Survey (SKI 2023) confirm that the majority of Indonesians across all age groups fail to meet the daily recommended intake of fruits and vegetables. This low consumption is attributed to food preferences, limited nutritional literacy, busy lifestyles, and the convenience of processed and fast foods (Irwan & Kadir, 2024). Processing fruit into juice is widely promoted as a practical alternative that preserves nutritional value while improving ease of consumption and acceptability (Soo et al., 2025). However, the bioactive compounds responsible for the antioxidant activity in fruit juices are chemically labile and susceptible to degradation during storage (Zhang et al., 2022).

Long-term polyphenol degradation in processed and commercially pasteurized fruit juices has been relatively well established (Zhang et al., 2022; Xue et al., 2024).

However, the rate of antioxidant decay in freshly prepared, unprocessed strawberry juice during the first five days of domestic chiller storage, which is the period when a household consumer is most likely to drink it, has not been sufficiently quantified. This gap is significant because pasteurized commercial products differ substantially from fresh hand-blended juice in terms of oxygen exposure, cell disruption, and the activity of endogenous oxidative enzymes. The novelty of this study lies in its consumer-relevant time points (1, 3, and 5 days of chiller storage), its use of simple freshly prepared juice (rather than ethanol extract or pasteurized product), and the application of the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, which is one of the most widely used and standardized methods for quantifying free radical scavenging capacity in natural samples (Kedare & Singh, 2023).

This study aimed to evaluate the antioxidant activity of freshly prepared strawberry (Fragaria × ananassa) juice stored in a chiller at 4°C for 1, 3, and 5 days, as measured by the DPPH radical scavenging method.

Methods

Study Design and Setting

This study employed a longitudinal experimental design with repeated measures to compare the antioxidant activity of strawberry juice at four time points (fresh, day 1, day 3, and day 5). All laboratory procedures will be conducted at the Pharmaceutical Laboratory of Universitas Sumatera Utara from September to October 2025. Measurements were performed in triplicates for each sample and time point.

Sample Preparation

Fresh strawberries (Fragaria × ananassa) in good visual condition were purchased from a

local supermarket in Medan, Indonesia. It should be noted that the specific cultivar and ripeness stage of the strawberries were not controlled, which represents a study limitation, as the antioxidant content is known to vary substantially across strawberry cultivars and maturation stages (Yongzhi et al., 2022).

The strawberries were washed thoroughly under running water, drained, and 200 g was weighed and homogenized using a blender. The freshly prepared juice was divided into aliquots, placed in sealed plastic bottles, and stored in a chiller at approximately 4°C. Samples were collected for analysis immediately after preparation (fresh) and after 1, 3, and 5 days of storage. Prior to analysis, the juice was filtered through filter paper, partially evaporated, reconstituted in 10 mL p.a. ethanol, evaporated again, and transferred to a 25 mL volumetric flask and homogenized to produce the concentrated test sample.

Tools and Materials

The equipment used in this study included a blender, stirring rod, glass beakers, suction bulb, glass funnel, measuring cups, filter paper, volumetric flasks, pipette mats, analytical balance, dropper pipettes, spatula, evaporating dish, hotplate, Branson ultrasonic device, UV-Vis spectrophotometer, digital scale, plastic storage bottles, and a refrigerator maintained at 4°C.

The materials used included freshly prepared strawberry juice, DPPH (1,1-diphenyl-2-picrylhydrazyl), p.a. methanol, p.a. ethanol, concentrated HCl, amyl alcohol, and magnesium powder.

Phytochemical Screening (Shinoda Test)

The qualitative detection of flavonoids was performed using the Shinoda test. Fifteen milliliters of strawberry juice was combined with 0.2 g of magnesium powder, two drops of concentrated HCl, three drops of amyl alcohol, and mixed. The development of an orange color in the amyl alcohol layer indicates a positive result, attributed to the reduction of flavone ring structures to anthocyanidins by magnesium and HCl (Dubale et al., 2023; Masfria et al., 2024).

This qualitative approach is a standard, cost-effective preliminary method for identifying major flavonoid classes in plant materials (Jaicharoensub et al., 2023).

Antioxidant Activity Testing by DPPH

The DPPH radical scavenging assay was selected for its simplicity, sensitivity, and established use in evaluating the antioxidant capacity of plant extracts and fruit juices (Kedare & Singh, 2023). A DPPH stock solution (SS I, 200 ppm) was prepared by dissolving 20 mg of DPPH in methanol in a 100 mL volumetric flask. A working solution (SS II, 40 ppm) was prepared by diluting 5.0 mL of SS I with 25 mL of methanol. The maximum absorption wavelength of the DPPH solution was determined by scanning 3 mL of the 40 ppm solution in a cuvette in the range of 400–800 nm.

For each measurement, the test sample was added to a 5 mL volumetric flask containing the DPPH working solution and incubated for 30 min in a sealed, dark container to prevent photodegradation. The absorbance was measured at the identified maximum wavelength using a UV-Vis spectrophotometer. A blank control (DPPH + methanol without sample) was prepared and measured alongside all test samples to obtain the control absorbance (control) required for calculating the % inhibition. All measurements were performed in triplicates.

Data Analysis

The percentage of DPPH inhibition was calculated using the following equation:

$$\%\ \text{DPPH inhibition} : \frac{Control\ absorbance\text{-}Sample\ absorbance}{Control\ absorbance} x100\% \quad (1)$$

The IC50 value (concentration required to inhibit 50% of DPPH radicals) was determined by linear regression analysis, plotting the% inhibition (Y-axis) against the concentration in ppm (X-axis), using the 100–400 ppm data points. The anomalous value at 500 ppm for fresh juice (see Results) was excluded from the regression analysis for that sample set, and this exclusion is disclosed and justified in the Results section. The goodness of fit of the linear model was assessed using the coefficient of determination (R^2). Antioxidant activity was categorized according to Yuslianti (2018) as follows: very strong < 50 ppm; strong 50–100 ppm; moderate 100–150 ppm; weak > 150 ppm.

This study was descriptive and did not include a formal statistical comparison (e.g.,

ANOVA) of the IC50 values across time points because all measurements represented the mean values of triplicate absorbance readings rather than independent biological replicates. This is acknowledged as a limitation; future studies should employ independent replication with statistical testing (one-way ANOVA with Tukey's post-hoc test) to confirm whether the differences between storage days are statistically significant.

Ethical Considerations

This study did not involve animal or human participants. Confirmation of exemption from the full ethical review was obtained from the Ethics Committee of the Faculty of Medicine, Universitas HKBP Nommensen.

Result and Discussion

Phytochemical Screening

Qualitative phytochemical screening of strawberry juice via the Shinoda test produced a positive result for flavonoids, evidenced by a color change to orange in the amyl alcohol layer (Table 1; Figure 1). This is consistent with the findings of Haikal et al. (2025), who confirmed the presence of flavonoids in Fragaria x ananassa, including anthocyanins, detectable by thin-layer chromatography. Flavonoids, including anthocyanins, quercetin, and kaempferol, donate hydrogen atoms to free radicals and terminate oxidative chain reactions, thereby contributing to the observed antioxidant activity (Widiasrini et al., 2024; Ansar et al., 2024).

Table 1. Phytochemical Screening Results Data

Compound Name	Test Results
Flavonoid	+

*+: Contains secondary metabolite compounds

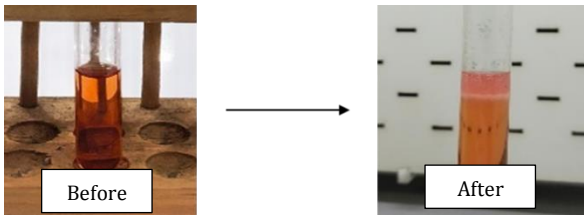

Figure 1. Flavonoid Test Results

Antioxidant Activity: % DPPH Inhibition

Table 2 presents the DPPH inhibition data for all four sample conditions across the 100–500 ppm concentration range. Fresh juice consistently exhibited the highest % inhibition at every concentration tested, followed in decreasing order by day-1, day-3, and day-5 stored juices, respectively. At 400 ppm, the highest concentration within the linear range, fresh juice inhibition was 90.667%, compared to 80.661% (day 1), 76.144% (day 3), and 75.923% (day 5). This progressive decline is consistent with the oxidative degradation of polyphenolic compounds during chiller storage (Yongzhi et al., 2022).

Important note on the 500 ppm fresh juice data point. The fresh juice at 500 ppm recorded an absorbance of 0.078 AU and % inhibition of 79.58%, which was lower than the 400 ppm value (0.097 AU; 90.67%). This reversal violates the expected dose-response relationship, in which inhibition should increase monotonically with concentration. Possible explanations include (1) turbidity or precipitation of juice solids at high concentrations interfering with spectrophotometric absorbance readings, (2) an analytical pipetting error, or (3) exhaustion of available DPPH radicals causing measurement instability. This data point was flagged as an artifact and excluded from the linear regression used to calculate the IC50 for fresh juice. This exclusion is consistent with the standard analytical practice for outlier identification in regression-based assays.

Table 2. Antioxidant Activity Data of Strawberry Juice

Test Solutions	Conc. (ppm)	Absorbance (AU)	% Inhibition
Fresh	100	0,382	63.248
	200	0,262	74,793
	300	0,174	83,259
	400	0,097	90,667
	500*	0,078*	79,581*
Day 1 (chiller)	100	0,616	40,735
	200	0,498	52,087
	300	0,323	68,924
	400	0,201	80,661
	500	0,123	80,032
Day 3 (chiller)	100	0,677	34,866
	200	0,545	47,565
	300	0,374	64,017
	400	0,295	71,618
	500	0,172	76,144
Day 5 (chiller)	100	0,721	30,633
	200	0,583	43,909
	300	0,436	58,052

400	0,299	71,233
500	0,163	75,923

*The 500ppm fresh juice value is anomalous (lower inhibition than 400 ppm) and was excluded from the IC50 regression. All other values represent the means of triplicate measurements.

IC50 Values and Antioxidant Classification

Table 3 summarizes the IC50 values calculated using linear regression for each storage condition. Based on the classification criteria of Yuslianti (2018)—very strong < 50 ppm; strong 50–100 ppm; moderate 100–150 ppm; weak > 150 ppm—fresh juice (IC50 = 140 ppm) falls at the upper boundary of the moderate category. However, all three stored samples exceeded 150 ppm and were classified as weak.

Table 3. Results Data IC_{50} Value of Strawberry Juice

Test Solution	IC50 Value (ppm)	Activity Category*	Change from Fresh
Fresh (0 days)	140	Moderate	—
Day 1 (chiller)	225	Weak	+61% ↑ IC50
Day 3 (chiller)	257	Weak	+84% ↑ IC50
Day 5 (chiller)	272	Weak	+94% ↑ IC50

* Classification according to Yuslianti (2018): very strong < 50 ppm; strong 50–100 ppm; moderate 100–150 ppm; weak > 150 ppm. Values represent the means of triplicate measurements.

The most critical finding was the substantial loss of antioxidant potency within just one day of storage. A jump from an IC50 of 140 ppm to 225 ppm represents a ∼61% increase in the concentration required to achieve 50% radical inhibition, which is a substantial decline in potency, not a marginal change. This means that a consumer drinking day-1 juice must consume nearly twice as much juice to obtain the same antioxidant effect as that obtained from fresh juice. The continued rise to 257 ppm (day 3) and 272 ppm (day 5) confirmed progressive degradation, although the rate of increase slowed after day 1, suggesting an initial rapid oxidative burst followed by a slower degradation phase.

The fresh juice IC50 of 140 ppm in this study is lower (more potent) than the ethanol extract IC50 of 173.73 ppm reported by Maulana et al. (2023), although a direct comparison is methodologically limited because the extraction method, solvent, and sample concentration differ markedly. The substantially lower IC50 of 31.45 ppm reported by Hartono et al. (2019) is likely attributable to their use of a concentrated methanol extract rather than diluted juice, and possibly to differences in strawberry cultivar or ripeness of the fruit. These comparisons highlight the importance of standardizing sample preparation when comparing antioxidant assay results across different studies.

Mechanisms of Antioxidant Degradation During Storage

The rapid decline in antioxidant activity observed in this study is best explained by the established chemistry of phenolic compound degradation, particularly anthocyanins, which are the primary contributors to the antioxidant activity in strawberries (Xue et al., 2024). Several mechanisms operate concurrently.

1. Enzymatic oxidation by polyphenol oxidase (PPO):

Blending strawberries to prepare fresh juice disrupts cell compartmentalization and releases polyphenol oxidase (PPO) from cellular organelles. PPO catalyzes the rapid oxidation of phenolic substrates, particularly hydroxycinnamic acids and anthocyanins, in the presence of oxygen, leading to the formation of brown quinone products and a simultaneous loss of antioxidant capacity (Yongzhi et al., 2022). This enzymatic activity is only partially suppressed at refrigeration temperatures (4°C), which accounts for the large IC50 increase observed even after just one day. PPO activity is a key reason that freshly blended juice degrades more rapidly than whole fruit.

2. Non-enzymatic oxidation of anthocyanins

Anthocyanins, responsible for the red pigment of strawberries, are highly sensitive to oxygen, light, and temperature. Even at 4°C, residual dissolved oxygen in the juice drives non-enzymatic oxidative degradation of anthocyanin chromophores, progressively reducing the radical scavenging capacity (Xue et al., 2024; Wagner et al., 2023). Zhang et al. (2022) demonstrated that anthocyanin degradation in red berry juices follows first-order kinetics and proceeds even during cold storage, consistent with the monotonic IC50 increases observed in this study.

3. pH-dependent structural changes:

Anthocyanin stability is highly pH-dependent, with maximum stability at low pH values. Progressive changes in juice pH during storage, driven by organic acid metabolism or microbial activity, can shift anthocyanin equilibrium toward colorless hemiketal or yellow chalcone forms, reducing both color and antioxidant potency (Susanty & Sampepana, 2017). Although pH was not monitored in this study (a limitation), this mechanism likely contributed to the observed degradation.

It should be noted that microbiological contamination, although a plausible factor during extended storage, was not assessed in this study. Any conclusions regarding the microbial contribution to antioxidant loss remain speculative and would require microbiological analysis for confirmation.

Study Limitations

Several limitations of this study should be acknowledged. First, the strawberry cultivar, ripeness stage, and exact geographic origin of the fruit were not recorded or controlled, which limits reproducibility and generalizability, given the well-known variation in polyphenol content across cultivars and maturity stages (Yongzhi et al., 2022).

Second, the IC50 values were reported as means of triplicate absorbance measurements rather than as independent biological replicates with statistical comparison; thus, the observed differences between time points cannot be confirmed as statistically significant without further ANOVA-based analysis.

Third, pH monitoring, microbial load assessment, and anthocyanin quantification were not performed, which limited the mechanistic interpretation. Fourth, the study did not include a room-temperature control or a comparison with commercially processed or pasteurized strawberry juice samples. Future studies should address these limitations by using certified cultivars, performing statistical tests, and including mechanistic biomarkers.

Conclusion

This study demonstrates that freshly prepared strawberry (Fragaria x ananassa) juice contains flavonoids (confirmed qualitatively) and exhibits moderate antioxidant activity when freshly prepared (IC50 = 140 ppm by DPPH assay). However, the antioxidant potency declines substantially and rapidly during chiller storage at 4°C. After just one day of storage, the IC50 increased by approximately 61% (to 225 ppm), indicating that consumers would need to drink nearly twice as much day-1 juice to obtain the same antioxidant effect as that of fresh juice. Storage until day 3 (IC50 = 257 ppm) and day 5 (IC50 = 272 ppm) further places the product in the weak activity category.

The primary drivers of this degradation are enzymatic oxidation by polyphenol oxidase released during blending and the non-enzymatic oxidation of anthocyanins by dissolved oxygen, even at refrigeration temperatures. Based on these findings, strawberry juice should be consumed within 24 h of preparation to retain clinically meaningful antioxidant capacity. Future research should employ controlled cultivar selection, independent biological replication with statistical analysis, and mechanistic measurements (anthocyanin quantification, PPO activity, and pH monitoring) to confirm and extend these findings.

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