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THE EFFECT OF POST-PRODUCTION SHELF TIME ON THE ANTIOXIDANT ACTIVITY OF SOY MILK

Pengaruh Waktu Simpan Pasca Produksi terhadap Aktivitas Antioksidan Susu Kedelai

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ABSTRACT

This study was conducted to analyze the effect of post-production storage time on antioxidant activity in soy milk. Antioxidants are compounds that can ward off free radicals, so it is important to know their stability over time. The storage time used in this study was 1, 4, 7, 10, and 13 hours post-production. Antioxidant activity testing was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Qualitatively, the color change of the DPPH solution was observed from purple to light yellow, while quantitatively the absorbance value was measured using UV-Vis Spectrophotometry. The inhibition value and IC_{50} were calculated to determine the strength of antioxidant activity in soy milk. The results showed that soy milk with a storage time of 1 hour had the best antioxidant activity with an IC_{50} value of 28.19 μg/ml which was classified as very strong. At 4 hours of storage time, the IC_{50} value of 37.28 μg/ml is classified as moderate, while at 7 hours and 10 hours the IC_{50} values were 146.95 μg/ml and 119.38 μg/ml, respectively, which are also classified as moderate. At 13 hours, the antioxidant activity decreased drastically with an IC_{50} value of 228.90 μg/ml, which is classified as very weak. The antioxidant activity of soy milk decreased with increasing storage time. Factors such as temperature, light, and components of soy milk contribute to the degradation of antioxidant activity. Therefore, consumption of soy milk should be done within a shorter time after production to obtain optimal antioxidant benefits.

Keywords: *Antioxidants, DPPH, Post Production, Save Time, Soy Milk*

ABSTRAK

Penelitian ini dilakukan untuk menganalisis pengaruh waktu simpan pasca produksi terhadap aktivitas antioksidan pada susu kedelai. Antioksidan merupakan senyawa yang dapat menangkal radikal bebas, sehingga penting untuk mengetahui kestabilannya seiring waktu. Waktu simpan yang digunakan dalam penelitian ini adalah 1, 4, 7, 10, dan 13 jam pasca produksi. Pengujian aktivitas antioksidan dilakukan dengan metode DPPH (2,2-diphenyl-1-picrylhydrazyl). Secara kualitatif, perubahan warna larutan DPPH diamati dari ungu menjadi kuning muda, sedangkan secara kuantitatif diukur nilai absorbansi menggunakan Spektrofotometri UV-Vis. Nilai inhibisi dan IC_{50} dihitung untuk menentukan kekuatan aktivitas antioksidan pada susu kedelai. Hasil penelitian menunjukkan bahwa susu kedelai dengan waktu simpan 1 jam memiliki aktivitas antioksidan paling baik dengan nilai IC_{50} sebesar 28.19 μg/ml yang tergolong sangat kuat. Pada waktu simpan 4 jam, nilai IC_{50} sebesar 37.28 μg/ml tergolong sedang, sedangkan pada 7 jam dan 10 jam nilai IC_{50} masing-masing sebesar 146.95 μg/ml dan 119.38 μg/ml, yang juga tergolong sedang. Pada 13 jam, aktivitas antioksidan menurun drastis dengan nilai IC_{50} sebesar 228.90 μg/ml yang tergolong sangat

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lemah. Aktivitas antioksidan pada susu kedelai mengalami penurunan seiring bertambahnya waktu simpan. Faktor-faktor seperti suhu, cahaya, dan komponen penyusun susu kedelai berkontribusi terhadap degradasi aktivitas antioksidan. Oleh karena itu, konsumsi susu kedelai sebaiknya dilakukan dalam waktu yang lebih singkat setelah produksi untuk mendapatkan manfaat antioksidan yang optimal.

Kata Kunci: *Antioksidan, DPPH, Pasca Produksi, Waktu Simpan, Susu Kedelai*

INTRODUCTION

Free radicals have unpaired electrons, which makes them incherently and highly reactive. Due to this instability, they tend to steal electrons from stable molecules such as DNA, lipids, proteins, and carbohydrates, in an effort to achieve stability. This process can cause damage to biological molecules and contribute to oxidative stress (Phaniendra et al. 2015). Oxidative stress reduces the ability of blood to transport oxygen and can trigger cellular apoptosis. If exposed to oxidative stress continuously, it will cause pathological conditions such as cardiovascular disorders, as well as more specific degenerative diseases, namely hypertension, heart disease, including hypertension, heart disease and diabetes mellitus (Berawi & Marini 2018). To mitigate the harmful effects of free radicals, the body relies on antioxidant compounds that inhibit the autooxidation process (Kesuma and Yenrina 2015).

Antioxidants are compounds that inhibit the oxidation of other molecules by neutralizing free radicals, thus preventing cell damage. They play a vital role in protecting the body against oxidative stress caused by free radicals. Oxidative stress is implicated in the pathogenesis of various diseases such as cancer, cardiovascular diseases, diabetes, and neurodegenerative disordes (Lobo et al. 2010) . These compounds play an important role in reducing the impact of oxidative stress caused by various factors, such as stress, radiation, UV exposure, and air and environmental pollution (Berawi and Marini 2018). Plants are rich sources of natural antioxidants, which can be found in various parts including roots, stems, bark, leaves, flowers, fruits and seeds (Sumiwi Sri et al. 2011). One significant source of natural antioxidants is soybeans (Glycine max), which are known to contain various secondary metabolites, including flavonoids, phenols, tannins, steroids, and terpenoids (Hasanah Uswatun et al. 2019). Isoflavones found in soybeans act as natural antioxidants (Saija et al. 1995). Soybeans can be processed into various products that retain their antioxidant properties, one of which is soy milk. The production of soy milk in many communities is still traditional, involving steps such as selection, washing, soaking, grinding or refining, and filtering. Previous research has demonstrated that different gemination durations of black soybeans significantly influence the antioxidant activity of black bean sprout milk (Pertiwi et al. 2013). Additionally, storage temperature and duration have been shown to affect the microbial content in homemade soy milk (Harlita et al. 2023).

Despite these findings, there is limited research on how post-production storage time affects the antioxidant activity of soy milk. Interviews with soy milk sellers, it can be concluded that soy milk is marketed from post-production starting from 7 am to 11 am. Meanwhile, according to Harlita et al. (2023), soy milk should not be consumed if it is more than 2 hours at room temperature to meet SNI requirements. Therefore, this study aims to evaluate the relationship between post-production storage time and the antioxidant activity of soy milk. This study explores the post-production storage time range of soy milk, namely 1,4,7,10 and 13 hours.

MATERIALS AND METHOD

Place and Time of Research

This research will be conducted in November 2024 at the Chemistry Education Laboratory, Faculty of Teacher Training and Education, Sriwijaya University Indralaya.

Materials

Soy Milk

The making of soy milk is based on Sorga et al. (2013), done tradionally at home through seed selection, washing, soaking, peeling the skin, grinding, and filtering. The soy milk was made using a 1:10 ratio of soybeans to water. Speciafically, 100 grams of Dena variety soybeans *(Glycine max)* and 1 liter of water were used.

DPPH Reagent and Equippment

In the process of testing antioxidant activity using tools and materials in the form of analytical scales, beakers, measuring cups, glass funnels, stirrers, droppers, UV-Vis spectrophotometry, aluminum foil, DPPH powder (1.9 mg), distilled water (Aquadest), Methanol p.a (100 ml), filter paper and labels.

Method

Experimental Design

This study used an experimental Completely Randomized Design (CRD) with one factor, the post-production storage time of soy milk. The factor had five levels (1,4,7,10, and 13 hours) with five treatments and three repetitions to ensure sample accuracy.

Storage Conditions

The homemade soy milk samples, without additives and using local soybeans of the Dena variety (*Glycine Max*) characterized by white, slightly dull, and uneven seed sizes, were kept in transparent containers exposed to indirect sunlight from 7 am to 8 pm throughout the experiment.

DPPH Antioxidant Assay

The antioxidant activity of soy milk was determined by the DPPH method. a 200 µL aliquot of soy milk samples was mixed with 3 mL of 0.1 mM DPPH solution in methanol. The mixture was incubated in the dark at room temperature for 30 minutes. The absorbance was then measured at 517 nm using the UV-Vis spectrophotometer.

RESULT AND DISCUSSION

Soybean Extraction Results

Soybean seeds were extracted to produce soy extract, commonly referred to as soy milk. The soybean extraction process included seed selection, washing, soaking, grinding, filtering, cooking, and subsequent storage. The cooked soy milk was divided into five portions and stored in labeled glass jars. This allowed for easy observation and testing of antioxidant activity based on post-production storage time. An illustration of the final soy milk product is presented in Figure 1.

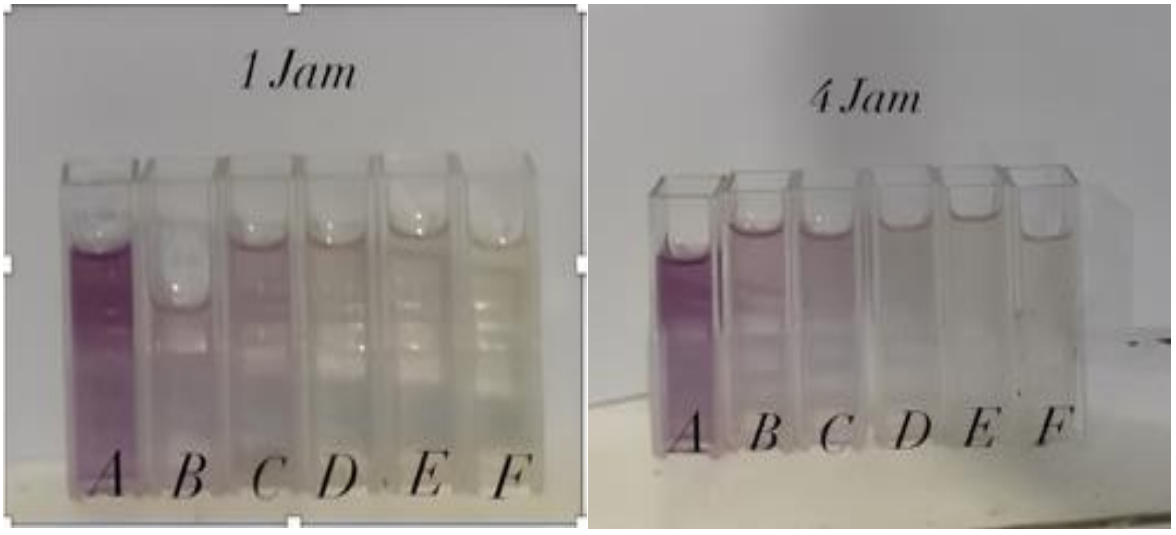
Figure 1. Soy Milk

Antioxidant Activity Test Using the DPPH (2,2 -diphenyl-1-picryhydrazyl) Method

The antioxidant activity test using the DPPH method begins with wavelength measurement. Qualitative analysis is carried out based on changes in sample color compared to negative controls, while quantitative analysis includes calculating absorbance, inhibition, and IC_{50}. Wavelength measurement aims to observe changes in absorbance based on concentration for maximum analysis results (Leo and Daulay 2022). Before the antioxidant activity test, the maximum wavelength of the DPPH solution was determined using spectrophotometry at 19 ppm DPPH. As shown in Figure 2, the peak absorbance accured at 517 nm, confirming this as the appropriate wavelength for subsequent measurements. Qualitative analysis was conducted by observing changes in the color of the samples compared to the negative control. Quantitative analysis involved calculating absorbance, inhibition percentage, and IC_{50} values.

Figure 2. DPPH Wavelength

After determining the wavelength, soy milk was diluted with a ratio of 1:4 to obtain a concentration of 50–500 ppm. Each sample (2 ml) was mixed with 8 ml of DPPH solution, then incubated for 30 minutes in a dark place, according to Luo's study (2011), which states that the ideal DPPH reaction time is 30–40 minutes. Antioxidant activity tests were carried out qualitatively and quantitatively. Qualitatively, Figure 3 shows the results of a 30-minute incubation.



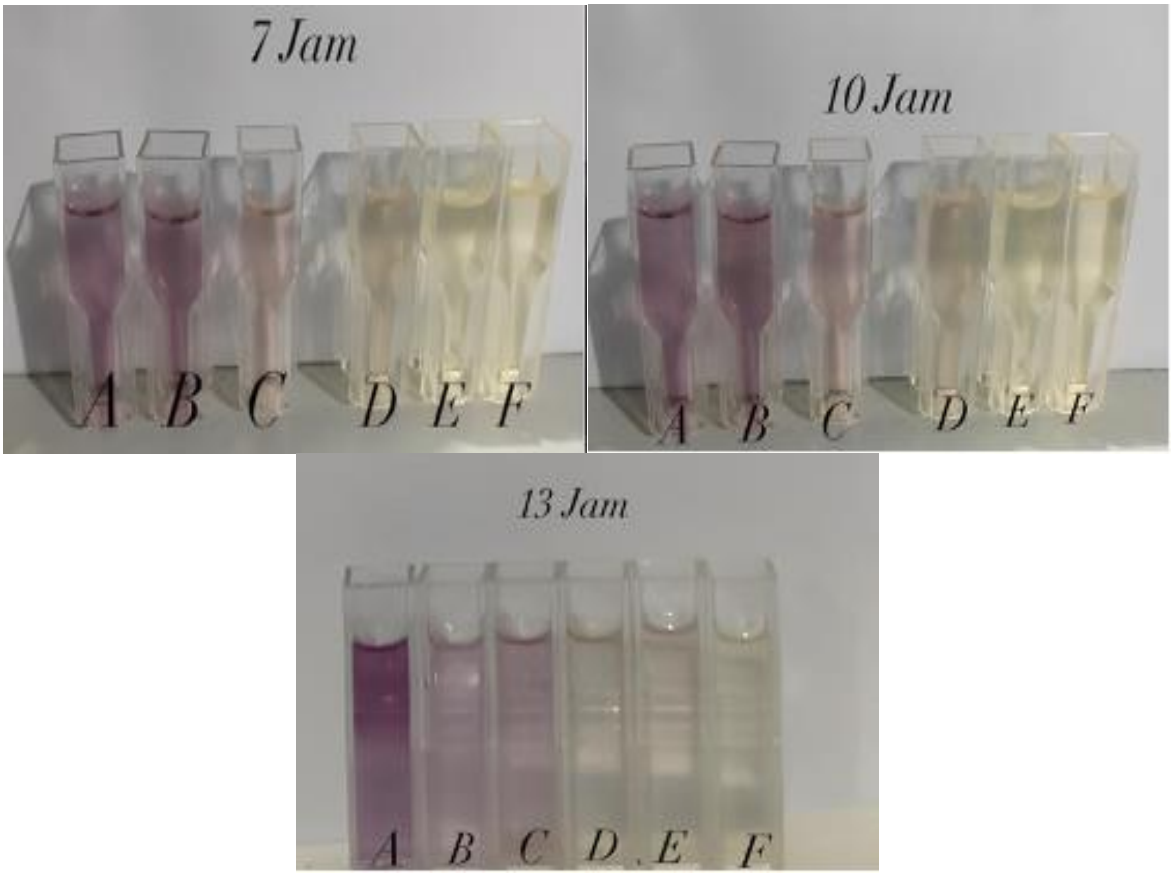


Figure 3. Color change of the solution that has been incubated for 30 minutes

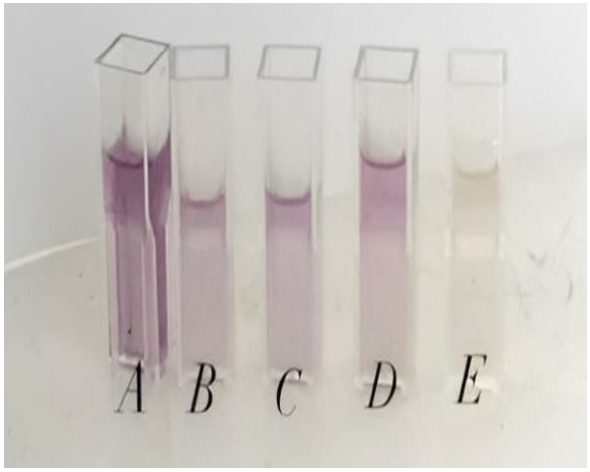


Figure 4. Positive Control Test of Vitamin C (Ascorbic Acid)

Antioxidant activity testing in soy milk using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method is based on the principle that antioxindant compounds act as electron or hydrogen atom donors, reducing DPPH radicals and causing a color change from purple to yellow. This color shift indicates the presence of antioxidant activity, as antioxidants neutralize free radicals by donating hydrogen atoms.

Ascorbic acid is commonly used as a standard antioxidant due to its efficient electron-donating ability (Akbari et al. 2016). It reacts with DPPH through a reduction mechanism where DPPH accepts a hydrogen atom and becomes DPPH-H. the resulting ascorbate radicals can further react with other DPPH molecules, forming ascorbyl and dehydroascorbate radicals (Muliasari et al. 2023). This cascade of

reactions lead to a reduction in free radicals, which is visually detectable by a color change and quantitatively measured at 517 nm using UV-Vis spectrophotometry. However, ascorbic acid is known to be sensitive to environmental factors such as light and temperature, which may affect its stability and antioxidant activity (Purnama Sari and Sartika Daulay 2022)To assess antioxidant activity, quantitative parameters such as absorbance, percent inhibition, and IC_{50} values were calculated (Muhtadi et al. 2016 ; (Sirivibulkovit et al. 2018).

Absorbance Measurement

Absorbance measurement can be done after incubation of the sample for 30 minutes. This absorbance measurement is obtained from the value that comes out of UV-vis spectrophotometry. Absorbance measurement is done 3 times and the average is obtained as in tables 1 and 2.

Table 1. Results of Absorbance Measurement of Samples and Negative Controls

No.	Sample	Concentration (ppm)	Average Absorbance
1.	Soy Milk 1 Hour	50	0.352
		100	0.19833333
		200	0.18033333
		300	0.066
		400	0.05066667
		500	0.014
	Negative Control		0.59566667
2	Soy Milk 4 Hour	50	0.40366667
		100	0.374
		200	0.274
		300	0.20166667
		400	0.105
		500	0.07866667
	Negative Control		0.66533333
3	Soy Milk 7 Hour	50	0.3143333
		100	0.26033333
		200	0.18233333
		300	0.227
		400	0.151
		500	0.04833333
	Negative Control		0.48733333
4	Soy Milk 10 Hour	50	0.39233333
		100	0.333
		200	0.22466667
		300	0.13833333
		400	0.06066667
		500	0.19366667
	Negative Control		0.63133333
5	Soy Milk 13 Hour	50	0.368
		100	0.266
		200	0.27833333
		300	0.17633333
		400	0.10366667
		500	0.109
	Negative Control		0.46733333

Table 2. Positive Control Absorbance Measurement Results

Sample	Concentration (ppm)	Average Absorbance
Vitamin C	15	0.484
	20	0.31866667
	25	0.34966667
	30	0.26766667
	35	0.13166667

The absorbance measurements showed a clear decrease in DPPH absorbance as the concentration of the soy milk sample increased. This trend indicates successful antioxidant activity, where higherconcentrations of antioxidant compounds more effectively neutralize free radicals. According to the Lambert-Beer Law, absorbance is directly proportional to concentration however, in this assay, a decrease in absorbance reflects a stronger antioxidant effect, as more DPPH radicals are reduced by the antioxidants (Fadhilah et al. 2022). These findings are consistent with previous studies (Hasti and Makbul 2022 ; Masrifah et al. 2017)., which observed a reduction in DPPH absorbance and a visible color change from purple to a lighter shade as antioxidant concentration increased both indicators of antioxidant activity.

Absorbance data (Appendix tabel 1) also revealed fluctuations across repeated measurements, even when the same soy milk sample was used. While some values remained stable, with a coefficient of variation below 5%, others showed higher variability. Possible contributing factors include differences in cuvette cleanliness, slight changes in solution oxidantion, or temperature variation during the assay (Yoyok 2017). These variations fall within acceptable experimental limits and are considered part of normal laboratory variability.

Inhibition Measurement

Inhibition measurements were obtained from the results of blank absorbance (negative control) and sample absorbance and the results were obtained as in tables 3 and 4.

Table 3. Effect of Time on Percentage of Inhibition During Post-Production Shelf Life

ppm	**Percentage (%) Inhibition**				
	1 Jam	**4 Jam**	**7 Jam**	**10 Jam**	**13 Jam**
50	40.91	39.33	35.50	37.86	21.26
100	66.70	43.79	46.58	47.25	43.08
200	69.73	58.82	62.59	64.41	40.44
300	88.92	69.69	53.42	78.09	62.27
400	91.49	84.22	69.02	90.39	77.82
500	97.65	88.18	90.08	69.32	76.68

Table 4. Percentage (%) Inhibition of vitamin C as a positive control

Sampel	**Konsentrasi (ppm)**	**Inhibisi**
Vitamin C	15	31.15
	20	54.67
	25	50.26
	30	61.93
	35	81.27

The percentage of inhibition. calculated from the absorbance values of each soy milk sample increased with higher concentrations. This indicates a stronger ability of the antioxidants in soy milk to neutralize free radicals. as reflected in the reduction of DPPH molecules (Rozi et al. 2023). However. at a fixed concentration. the inhibition percentage decreased as the post production storage time increased. This suggests that antioxidant compounds in soy milk degrade over time. reducing their effectiveness in scavenging free radicals. The decline in antioxidant activitu is likely due to antioxidation or degradation of bioactive compounds such as isoflavones and phenolics. which are sensitive to environmental factors like temperature. light. and air exposure. Overall. prolonged storage negatively impacts the antioxidant potential of soy milk. supporting the need for timely consumption to retain its functional benefits.

IC_{50} Value Determination

The IC_{50} value or the inhibitory concentration at which 50% of free radical activity suppresses. was determined using the linear regression equation (Y = a + bx). This value serves as an indicator of antioxidant potency. with lower IC_{50} values reflecting higher antioxidant activity (Mailandri Mely 2012). According to Blois (1958). antioxidants are categorized as very strong (IC50 $<$ 50 ppm). strong (50–100 ppm). moderate (100–500 ppm). and weak ($>$ 500 ppm). The results of the study are in Table 5.

Table 5. IC50 Value of Antioxidant Activity of Soy Milk Based on Post-Production Shelf Life. Antioxidant Category According to (Blois 1958).

Sample	IC_{50} Value (µg/ml)	Antioxidant Activity Description
Vitamin C (Positive Control)	22.2764908	Very Strong
Soy Milk (1 Hour)	28.1866667	Very Strong
Soy Milk (4 Hour)	137.283737	Weak
Soy Milk (7 Hour)	146.954023	Weak
Soy Milk (10 Hour)	119.38	Weak
Soy Milk (13 Hour)	228.900738	Very Weak

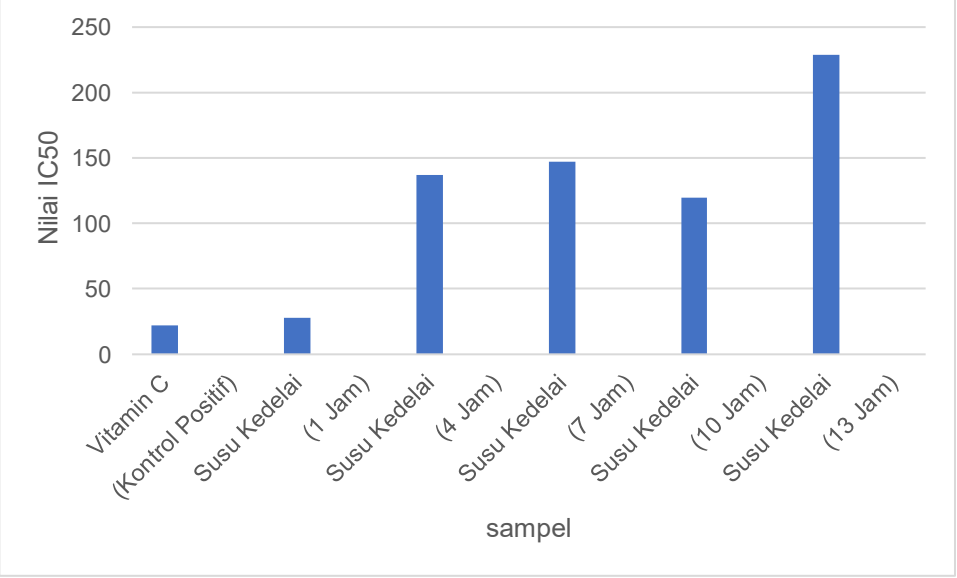


Figure 5. IC50 Value Diagram of Samples Compared to Positive Control

The 1-hour soy milk sample demonstrated the lowest IC_{50} value at 28.19 ppm. indicating very strong antioxidant activity. while the 13-hour sample showed a markedly weaker activity with an IC_{50} of 228.90 ppm. These findings suggest a decline in antioxidant effectiveness with prolonged storage. likely due to oxidative degradation of bioactive compounds. For comparison. the positive control. vitamin C (ascorbic acid). had an IC_{50} of 22.28 ppm. Among all samples. the 1-hour soy milk sample most closely approximated the antioxidant strength of vitamin C. confirming that shorter post-production storage time enhances the bioactivity of soy milk.

Statistical Data Analysis Results

The antioxidant activity data were analyzed using SPSS® 23. To begin. a normality test was performed using the Shapiro–Wilk method. as the sample size was fewer than 50. The results indicated that all groups were normally distributed. with p-values greater than 0.05 (Table 3).The results of the SPSS data test on the five soy milk samples based on the duration of storage after production can be seen in table 6.

Table 6. Soy Milk Antioxidant Data Analysis Results

Sample	Nomality (Shapiro-Wilk)	Homogenity of Variences	Annova One-Way Sig. Value
A	.635		
B	.985		
C	.238	.187	.000
D	.347		
E	.135		

A homogeneity of variance test followed. confirming that the data were homogeneous ($p > 0.05$). allowing for a one-way ANOVA to be conducted. The ANOVA test. used to identify significant differences in antioxidant activity (IC_{50} values) across different storage durations. yielded a significant result ($p = 0.000$; $p < 0.05$). indicating that storage time significantly influenced antioxidant activity. The results of the Duncan post hoc test can be found in table 7.

Table 7. Duncan's Post Hoc Test Method on Soy Milk Post-Production Time

Sample	IC_{50} value	Standar Devisiasi	Duncan Test
A	28.0937	12.79467	A
D	96.3890	15.67436	B
B	135.0667	27.92588	C
C	163.9200	.60225	C
E	229.0133	11.62216	D

Subsequently. a Duncan post hoc test was conducted to determine which groups differed significantly. The results (Table 13) showed that the IC_{50} values varied significantly based on post-production storage time. with samples grouped into categories A to E. The 1-hour storage sample (Group A) had the lowest IC_{50} value. indicating the highest antioxidant activity. while the 13-hour storage sample (Group E) showed the highest IC_{50}. indicating the weakest antioxidant activity. Intermediate samples such as those stored for 4 and 7 hours were grouped together and did not differ significantly. These findings support the rejection of the null hypothesis (H_0). which posited no effect of storage time. and confirm that prolonged storage leads to decreased antioxidant activity in soy milk.

Finally. the observed variation in standard deviation values among samples suggests that other experimental factors. such as temperature fluctuations or sample handling. may have contributed to measurement variability.

CONCLUSION

The results of this study demonstrate that post-production storage time significantly influences the antioxidant activity of soy milk. Antioxidant effectiveness. as indicated by IC_{50} values. decreased with longer storage durations. Soy milk tested one hour after production exhibited very strong antioxidant activity. while samples stored for 4. 7. and 10 hours showed weakened activity. and the 13-hour sample was classified as very weak. These findings support previous research suggesting that antioxidant compounds degrade over time during storage. Therefore. it is recommended to consume soy milk shortly after production to maximize its antioxidant benefits. Further studies may explore preservation methods to maintain antioxidant stability during storage.

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