Article

Red chili pepper (*Capsicum annuum* L.) quality after washing with chlorine disinfectant under different storage conditions: room temperature, refrigerator, and Zero Energy Cooling Chamber (ZECC)

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Abstract

Red chili peppers (*Capsicum annuum* L.) are a horticultural commodity that is widely cultivated in Indonesia because of their high economic value. However, storage can easily damage red chili peppers, leading to significant production losses. While cold storage (5–10°C) effectively reduces damage, it is not suitable for areas without electricity; therefore, the ZECC (Zero Energy Cool Chamber) can serve as an alternative. However, since ZECC storage tends to have higher microbial growth, pretreatment with chlorine disinfectant can be applied to suppress microorganisms before storage. This study aims to determine the optimal chlorine concentration for washing and to evaluate the changes in quality and shelf life of large red chili pepper stored at room temperature, in a refrigerator, and in a Zero Energy Cool Chamber (ZECC). The research was conducted in two stages: stage I to determine the optimal chlorine concentration and stage II to assess the weight loss, vitamin C, total acid, total soluble solids, total chlorine, and total microbes. The results showed washing with 50 ppm of chlorine yielded the best results with the lowest weight loss (17.56%). Based on weight loss, vitamin C, and chlorine analyses, ZECC storage provided the best results, whereas total acidity and microbial parameters were best maintained under refrigeration. The shelf life of large red chili pepper washed with chlorine reached up to 15 days in both the refrigerator and ZECC, and 12 days at room temperature.

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Fruit, Storage Conditions, Post-harvest Treatment.

1. Introduction

Improper post-harvest handling can cause damage to chili peppers during storage, leading to production losses of 20-30% (1,2). This damage can result from various factors, including physical, biological, or mechanical influences (3). One solution to prevent damage during storage is cold storage at a temperature range of 5-10^{o}C (4). However, cold storage is not suitable for production centers that do not have access to electricity, so the application of the ZECC (Zero Energy Cool Chamber) storage method can be used as an alternative option (5).

Although effective in preventing damage during storage, agricultural products stored using ZECC show higher levels of microorganisms compared to storage in refrigerators and at room temperature (6). To suppress the growth of microorganisms, pre-handling treatment, such as washing with disinfectants, can be carried out before storage (7). One type of disinfectant that can be used for foodstuffs is chlorine. According to the Badan Pengawas Obat dan Makanan (BPOM) No. 28 of 2019 concerning Auxiliary Materials in Food

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Processing, the use of chlorine is classified as a washing agent with a maximum residue limit of 1 mg/kg.

Several researchers, such as Wulandari have conducted research related to the ZECC storage method (6), who compared storage treatments at room temperature, in a refrigerator, and in a Zero Energy Cool Chamber (ZECC); Rajput, who examines the quality of fresh agricultural products using the ZECC storage method (8); Shil, who examines the quality of four types of chili peppers in ZECC storage (9); Shafiqa, who examine the combination of edible coatings and ZECC storage for guava fruit (10); and Dirpan, who studied the combination of washing and packaging of mangoes in ZECC storage (11). However, no research has been conducted before on the effect of washing red chili pepper with chlorine on their quality in ZECC storage. Therefore, a study was conducted with the title "Red Chili Pepper (*Capsicum annuum* L.) Quality After Washing with Chlorine Disinfectant under Different Storage Conditions: Room Temperature, Refrigerator, and Zero Energy Cooling Chamber (ZECC)'

2. Materials and Methods

2.1. Materials and Tools

The tools and instruments used in this research are a Zero Energy Cool Chamber (ZECC), fruit rack, temperature and RH sensors, moisture analyzer, penetrometer, digital hand refractometer, hose, scales, analytical scales, pH meter, stirring rod, beaker, Erlenmeyer flask, horn spoon, volumetric pipette, spoon, water bath container knife blender coarse cloth and fine cloth. The materials used in this study are red chili pepper, aluminum foil, distilled water, detergent, PP indicator, NaOH, iodine, pH 7 buffer, alcohol, cotton, and labels.

2.2. Methods

2.2.1. Stage one

The first stage was conducted to determine the best chlorine disinfectant treatment based on weight loss parameters and visual observation. The chlorine disinfectant treatment (A) consisted of three levels, namely:

A0: Washing with water (control)
A1: Washing with 50 ppm chlorine
A2: Washing with 100 ppm chlorine

The treated red chili pepper was then stored at room temperature (29.3^{o}C) and a relative humidity (RH) of 82.3%.

2.2.2. Stage two

The second stage of the study was storing the samples using three storage methods, followed by quality analysis (moisture content, weight loss, vitamin C, total acid, total soluble solids, microbiological analyses, and chlorine analyses every 3 days until the red chili pepper deteriorated (12-15 days). The storage treatment (B) consisted of 3 levels, namely:

B0: Room temperature (temperature ±27 ^{o}C)
B1: Refrigerator (temperature ± 10 ^{o}C)
B2: Zero Energy Cool Chamber (temperature ± 26 ^{o}C)

2.2.2.1. Weight loss

Weight loss was determined by measuring the initial weight (W_0) of red chili pepper before storage and the final weight (W_1) after storage. The percentage of weight loss was calculated using the following formula (12):

$$W(\%) = \frac{(mi - mt)}{mi} \times 100\% \quad (1)$$

Information:
W= Weight loss (%)
mi= Initial mass (gr)
mi= Final mass (gr)

2.2.2.2. Vitamin C

Vitamin C content was measured using a UV–VIS spectrophotometer (13). Red chili samples were blended, and 0.1 g of the homogenate was transferred into a 100 mL volumetric flask and soluble with distilled water. The solution was then filtered through filter paper, and the absorbance of the filtrate was measured using the spectrophotometer.

2.2.2.3. Total Acid

Titratable acidity was determined by diluting 5 g of the sample with 100 mL distilled water. The mixture was then filtered through filter paper. 25 mL of the filtrate was taken, and phenolphthalein (pp) indicator was added. The sample was titrated with 0.1 N NaOH to the endpoint (persistent pink color appeared). The total titratable acidity was calculated using the following formula (14):

$$\text{Total Acid} = \frac{ml\ NaOH \times N \times Fp \times Mr\ NaOH}{weight\ of\ sample} \times 100\% \quad (2)$$

Information:
Ml NaOH = Volume of NaOH used during titration
N = Normality of NaOH (0.1 N)
Fp = Dilution or dilution factor
Mr NaOH = Molar mass of NaOH

2.2.2.4. Total Soluble Solids

Total soluble solids can be measured using a digital refractometer. The sample was first blended, and 1-2 drops of the sample were pipetted onto the refractometer prism. The total soluble solids content was then read directly from the device display and expressed as %Brix (15).

2.2.2.5. Chlorine Analyses

Qualitative analysis of chlorine was performed by diluting 0.5 g sample with 50 ml of distilled water, followed by homogenization and filtration through filter paper. Then, 10 ml of the filtrate is placed in a test tube, and 1 sachet of dipropyl-p-phenylene diamine (DPD) reagent is added. A change in the color of the sample to reddish purple indicates the presence of chlorine, so quantitative testing is continued using a spectrophotometer. The test results are calculated using the following equation (16,17):

$$y = 0.1638x + 0.0601 \quad (3)$$

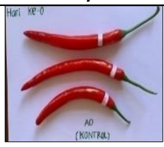
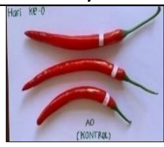
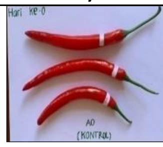
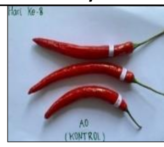
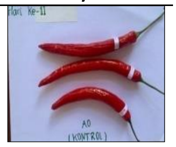
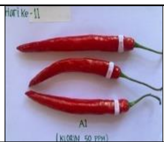
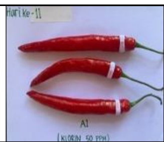
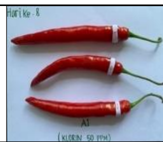
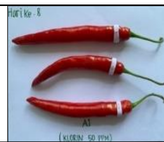
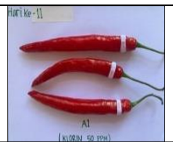
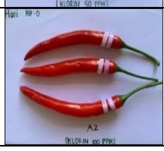
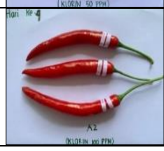
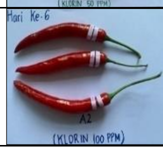
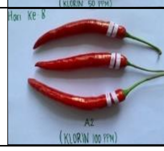
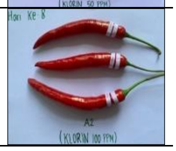
2.2.2.6. Microbiological Analyses (18,19)

The total microbial count was determined using the Total Plate Count (TPC) method. One gram of the sample was mixed thoroughly with 9 mL sterile saline diluent for 1 min using a vortex and serially diluted (10^{-1}-10^{-8}). Subsequently, 1 mL aliquots of each dilution were dispensed on appropriate plates using the standard spread plate method. The plates were incubated at 37°C for 48 hours.

3. Results and Discussion

Preliminary research was conducted to determine the optimal chlorine concentration based on visual parameters and weight loss. The chlorine concentrations used were control (A0), 50 ppm chlorine (A1), and 100 ppm chlorine (A2). The treated chili peppers were then stored at room temperature (29.3^{o}C) and relative humidity (RH) of 82.3%. Based on visual observations, the red chili pepper showed texture changes, becoming soft and wrinkled. The appearance of treated red chili pepper can be seen in Table 1.

Table 1. Visual Deterioration of Physical Quality of Red chili pepper.

Sample	Storage Duration				
	Day 0	Day 4	Day 6	Day 8	Day 12
Control					
50 ppm Chlorine					
100 ppm Chlorine					

Based on visual observations in Table 1, chili peppers washed with 50 ppm (A1) and 100 ppm (A2) showed better conditions than those without chlorine washing. The red chili pepper without chlorine treatment became wrinkled and softer due to respiration and transpiration processes during storage, causing the peppers to change in texture. Complex components in large red peppers will decompose into simpler components such as water and energy, leading to softening and wrinkling of the fruit (20). In addition to visual observations, weight loss during storage was also used as a parameter to evaluate the most effective treatment. The percentage of weight loss during storage is presented in Figure 1.

Based on Figure 1, the weight loss of the samples in all treatments increased during storage. Control samples showed the highest average weight loss (20.53%). The samples washed with 50 ppm chlorine exhibited the lowest average weight loss (17.56%). Meanwhile, washing with 100 ppm chlorine showed an average weight loss (17.69%). Based on the results of visual and weight loss parameters, washing with 50 ppm chlorine was identified as the most effective treatment and was therefore used in the next stage of the study.

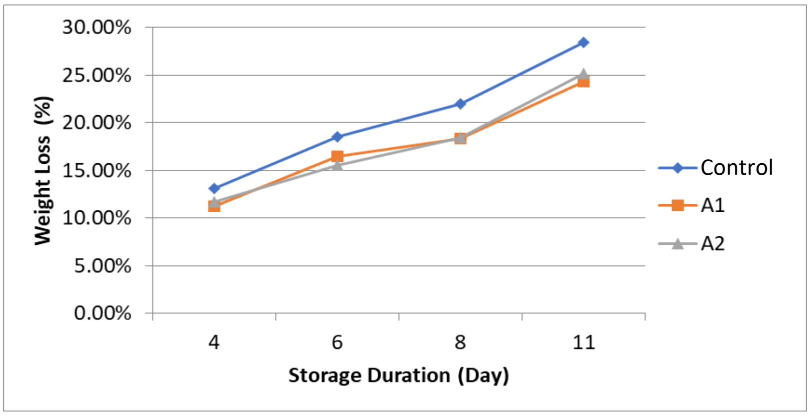


Figure 1. The effect of chlorine and storage duration on the weight loss of red chili pepper

3.1. Weight loss

During the respiration process, water, gas, and energy evaporate, causing the fruit to lose weight (20). The results of the analysis of variance showed that storage type, storage duration, and the interaction between storage type and storage duration significantly influenced (sig. 0.000) at a level of 5% ($p<0.05$) on the weight loss of red chili pepper during storage. Therefore, a further DMRT test was conducted.

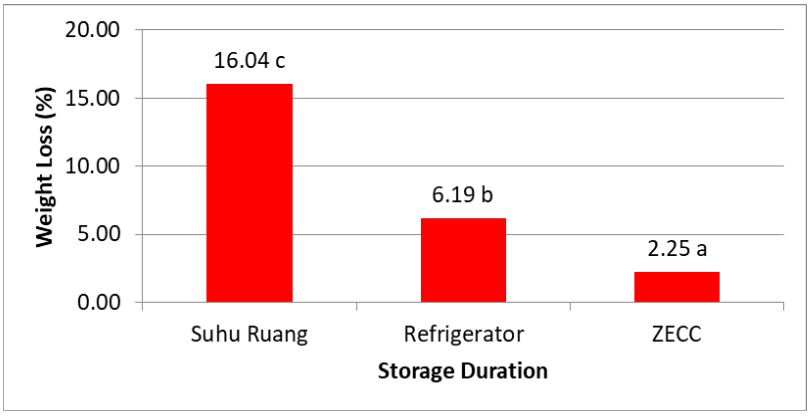


Figure 2. The relationship between storage type and weight loss of red chili pepper; values followed by different letters indicate significantly different results ($p< .05$).

The weight loss differences between each storage method can be seen in Figure 2. Red chili pepper washed with chlorine and stored at room temperature had the highest weight loss (16.04%), due to the high temperatures causing faster transpiration and respiration processes (21). The breakdown of organic compounds produces carbon dioxide (CO_2), which is volatile, and water, which evaporates during transpiration dan respiration (22). Meanwhile, the refrigerator and ZECC storage showed lower weight loss of 6.19% and 2.25% respectively, as the low temperatures and high humidity slowed the rates of respiration and transpiration (23).

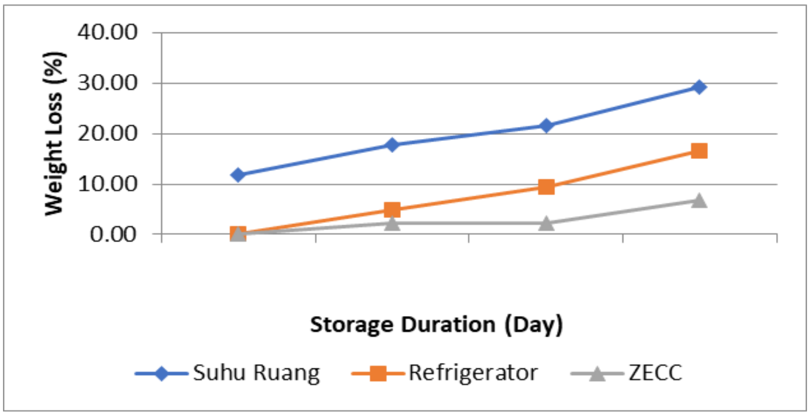


Figure 3. The effect of storage type and storage duration on the weight loss of red chili pepper.

The effect of storage duration and type on weight loss can be seen in Figure 3, showing that weight loss increases with storage time. During the 12-day storage period, the weight loss of chili peppers continued to rise. This increase is attributed to water loss and the decomposition of starch and organic acids during respiration and transpiration (24). As storage duration extends, the weight loss of the product correspondingly increases (7). Based on the weight loss parameter, ZECC storage was the best treatment, as its low temperature and relatively high air humidity suppress the rates of respiration and transpiration in red chili peppers (12).

3.2. Vitamin C

Vitamin C, or ascorbic acid, is a simple vitamin found in fruits and vegetables. It is unstable because it easily degrades into dehydroascorbic acid when it reacts with oxygen (25). The analysis of variance results showed that the treatment of storage type, storage duration, and the interaction between storage type and storage duration had a significant effect (sig. 0.000) at the 5% level ($p<0.05$) on the vitamin C content of red chili pepper during storage. Therefore, a further DMRT test was conducted. The results of the further test showed significant differences between ZECC storage, refrigerator storage, and room temperature storage. The vitamin C content of chili peppers under different storage conditions can be seen in Figure 4.

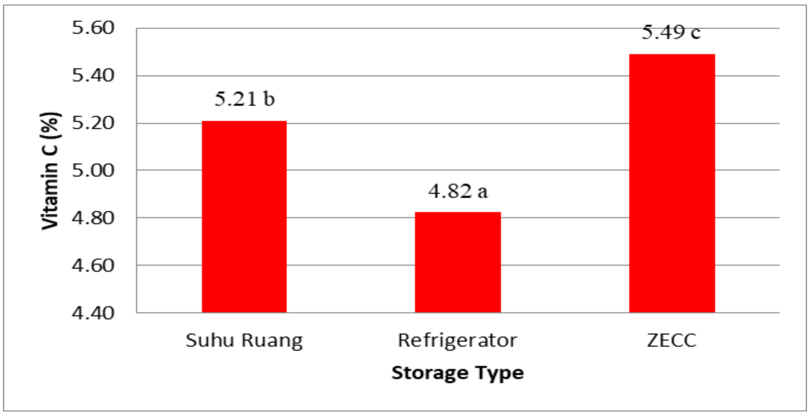


Figure 4. Relationship between storage type and vitamin C content of red chili pepper; values followed by different letters indicate significantly different results ($p <0.05$).

Red chili peppers stored in ZECC had the highest vitamin C content, with an average of 5.49% and differed significantly from room temperature (5.21%) and refrigerator (4.82%). The higher vitamin C content in ZECC storage is due to the low temperature and high relative humidity (RH), which slow down respiration and reduce the degradation of ascorbic acid (26). Conversely, the vitamin C content in refrigerator storage was lower than that in room temperature storage due to tissue damage at low temperatures. Prolonged exposure to cold conditions can cause significant cell membrane damage during storage, leading to a reduction in vitamin C content (21).

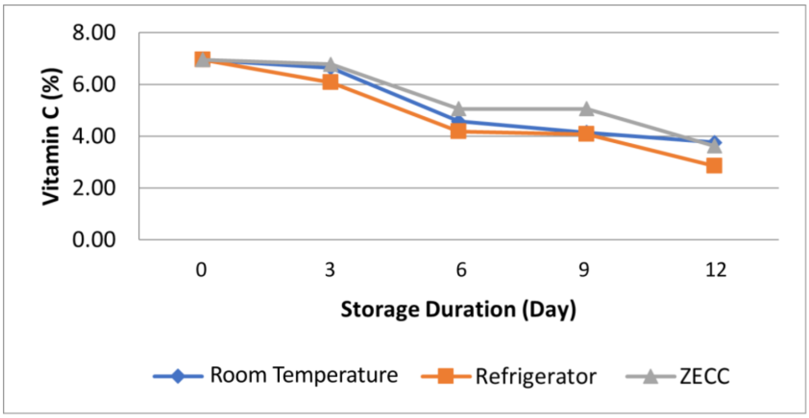


Figure 5. The effect of storage type and storage duration on the vitamin C content of red chili peppers.

Based on Figure 5, the vitamin C content of the samples decreased over time across all methods. At the end of the storage period, the vitamin C content at room temperature, in the refrigerator, and in ZECC was 3.76%, 2.85%, and 3.62%, respectively. The decrease in vitamin C content was due to the unstable nature of the vitamin, which makes it prone to oxidation and degradation (27). Vitamin C possesses a reactive hydroxyl (OH-) functional group, which makes it prone to oxidation when exposed to oxygen and accelerated by heat (28). In addition, the decrease in vitamin C may also result from the activity of the ascorbic acid oxidase enzyme, which oxidizes the ascorbic acid into L-dehydroascorbic acid. Furthermore, L-dehydroascorbic acid will further change into 2,3-diketo-L-gulonic acid, which no longer exhibits vitamin C activity (29).

3.3. Total Acid

Fruits and vegetables undergoing metabolism through the Krebs cycle produce malic acid, oxalic acid, citric acid, and other organic acids (30). The analysis of variance results showed that the treatment of storage type, storage duration, and the interaction between storage type and storage duration had a significant effect (sig. 0.000) at the 5% level ($p<0.05$) on the total acid of red chili pepper during storage. Therefore, a further DMRT test was conducted. The results of the further test showed significant differences between ZECC storage, refrigerator storage, and room temperature storage.

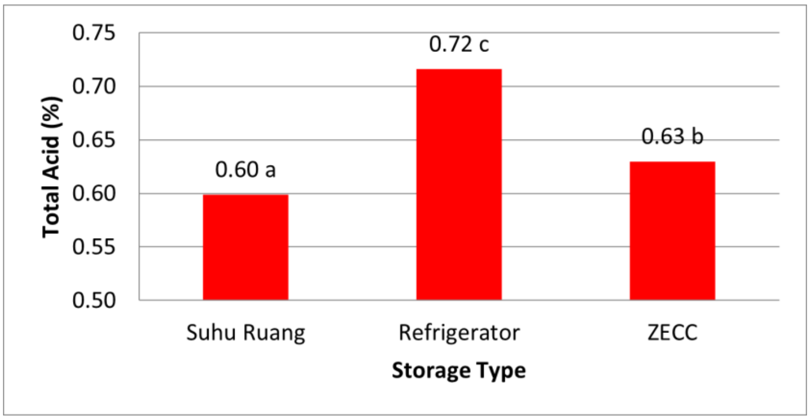


Figure 6. Relationship between storage type and total acid of red chili; values followed by different letters indicate significantly different results (p<.05).

The total acid of red chili peppers under different storage conditions is shown in Figure 6. Refrigerator storage exhibited the highest total acidity, with an average value of 0.72%, and differed significantly from room temperature storage (0.60%) and ZECC storage (0.63%). The higher total acidity in refrigerator storage due the low temperature, which inhibits the respiration process during storage and helps maintain the total acid content in the fruit (31). Low temperatures can also inhibit enzymatic reactions, including those involved in the degradation of organic acids (32). The analysis of variance results showed that the treatment of storage type, storage duration, and the interaction between storage type and storage duration had a significant effect (sig. .000) at the 5% level (p<0.05) on the total acid of red chili pepper during storage. Therefore, a further DMRT test was conducted. The results of the further test showed significant differences between ZECC storage, refrigerator storage, and room temperature storage.

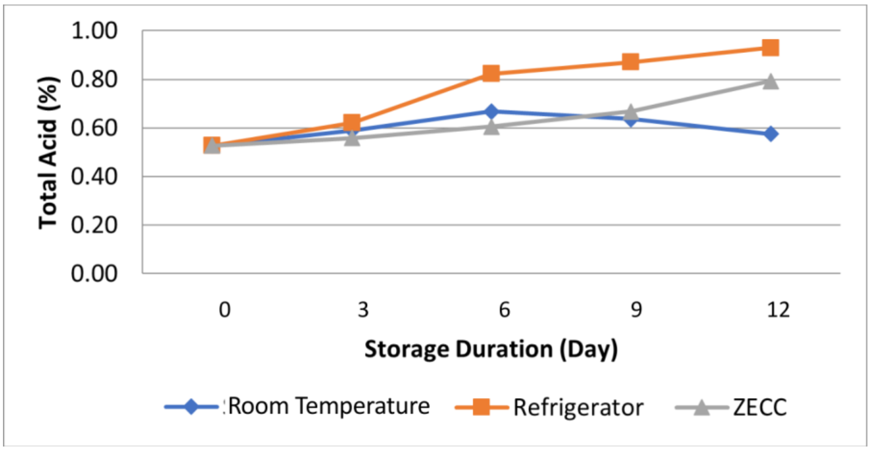


Figure 7. The effect of storage type and duration on total acid of red chili peppers.

The total acid of red chili peppers stored at room temperature, in the refrigerator, and in ZECC can be seen in Figure 7, which shows an increase in total acidity in samples stored in the refrigerator and ZECC. The total acid increase during storage occurs due to the formation of acids resulting from the decomposing of carbohydrates such as starch, cellulose, and pectin through the Krebs cycle (33). Meanwhile, storage at room temperature shows fluctuating total acid due to the aerobic conversion of pyruvic acid into organic acids, which are subsequently converted into CH_2O and energy, or used as substrates in the respiration process (34). According to previous research the decrease in the concentration of organic acids in fruits occurs because these acids are utilized as substrates during respiration (35).

3.4. Total Soluble Solids

Total soluble solids is a parameter that indicates the concentration of soluble substances in a material, consisting of simple sugars such as glucose and fructose, as well as pectin and other chemical components, expressed in %Brix units (36). The analysis of variance results showed that the treatment of storage type, storage duration, and the interaction between storage type and storage duration had a significant effect (sig. .000) at the 5% level (p<.05) on the total soluble solid of red chili pepper during storage. Therefore, a further DMRT test was conducted. The results of the further test showed significant differences between ZECC storage, refrigerator storage, and room temperature storage.

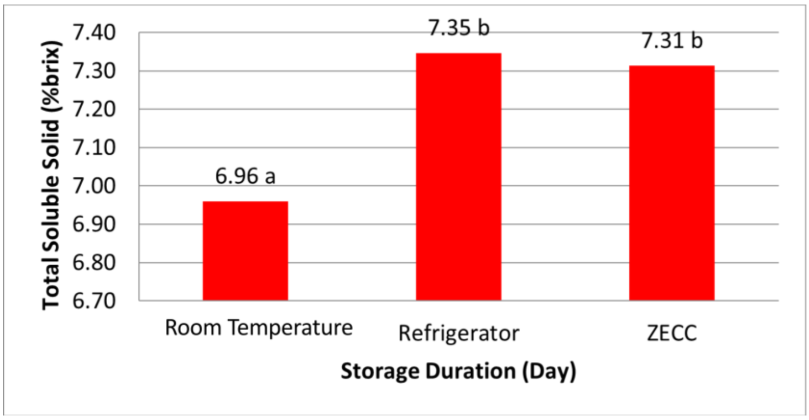


Figure 8. Relationship between storage type and total soluble solids of red chili pepper; values followed by different letters indicate significantly different results (p<0.05).

The total soluble solids of red chili pepper in each type of storage can be seen in Figure 8, where refrigerator storage shows the highest total soluble solids (7.35 %Brix), which is significantly different from ZECC (7.31 %Brix) and room temperature (6.96 %Brix). Red chili peppers stored at room temperature have lower total soluble solids due to differences in temperature and relative humidity (RH) among the storage methods (37). Room temperature accelerates respiration and transpiration, leading to a decrease in total soluble solids as simple sugars in the fruit are utilized as substrates in metabolic processes. Meanwhile, in the refrigerator and ZECC storage, there is no significant decrease due to low temperatures and relatively high RH suppresses respiration and transpiration processes, resulting in high sugar content (33).

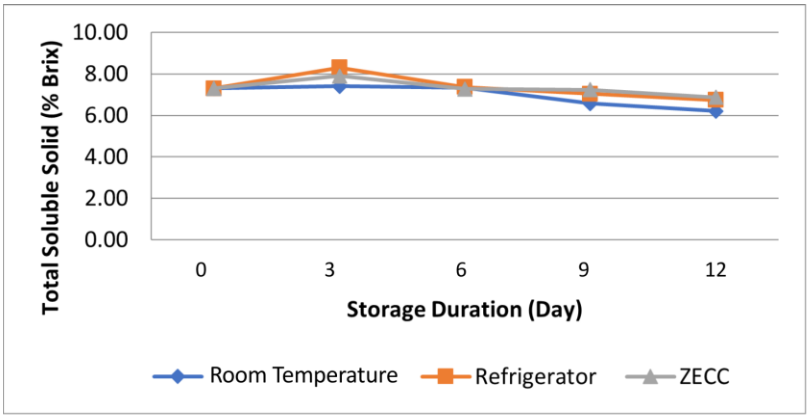


Figure 9. The effect of storage type and duration on total soluble solids of red chili.

The total soluble solids of red chili peppers stored at room temperature, in the refrigerator, and in ZECC can be seen in Figure 9, which shows the fluctuating total soluble solids during storage. TSS increased on the third day, particularly in refrigerator storage, due to the formation of simple sugars during respiration, as complex components such as starch were broken down into sugars (37). From the sixth to the twelfth day, TSS decreased as the fruit became overripe, resulting in a reduction of sugar content during storage. Storage in the refrigerator and ZECC effectively minimized the decrease in TSS compared to room temperature storage, as the low temperature and high humidity slowed respiration and transpiration, thereby reducing the loss of sugar components in the fruit (33).

3.5. Chlorine Analysis

Chlorine is a compound that is commonly used as a disinfectant due to its ability to inhibit microorganism growth. According to the Badan Pengawas Obat dan Makanan (BPOM) No. 28 of 2019 concerning Auxiliary Materials concerning Food Processing Auxiliary Materials, chlorine is classified as a cleaning agent with a maximum residue limit of 1 mg/kg. Chlorine analysis was conducted qualitatively to determine the presence of chlorine. Samples that tested positive were subsequently analyzed quantitatively to determine chlorine content. Table 2 showed that only samples from days 0 and 3 in all types of storage tested positive for chlorine. Chlorine levels decreased during storage due to the reduction of chlorine to chloride. In addition, the decrease was also attributed to the absorption of disinfectants by microorganisms and the evaporation of chlorine gas during storage (38). Quantitative analysis revealed that on day 0 of storage, the total chlorine in all samples exceeded the residue limit set by the Badan Pengawas Obat dan Makanan (BPOM). According to BPOM Regulation No. 28 of 2019 concerning Food Processing Auxiliary Materials, chlorine is classified as a cleaning agent with a maximum residue limit of 1 mg/kg. However, chlorine residues on day 0 can be reduced through a washing process, as chlorine is soluble in water (39).

Table 2. Results of qualitative and quantitative tests for total chlorine

Storage Method	Storage Duration	Qualitative Observation	Quantitative Observation (ppm)
Room Temperature	Day 0	Positive (+)	1.690
	Day 3	Positive (+)	0.585
	Day 6	Negative (-)	-
	Day 9	Negative (-)	-
	Day 12	Negative (-)	-
Refrigerator	Day 0	Positive (+)	1.684
	Day 3	Positive (+)	0.710
	Day 6	Negative (-)	-
	Day 9	Negative (-)	-
	Day 12	Negative (-)	-
ZECC	Day 0	Positive (+)	1.684
	Day 3	Positive (+)	0.415
	Day 6	Negative (-)	-
	Day 9	Negative (-)	-
	Day 12	Negative (-)	-

3.6. Microbiology Analysis

The Total Plate Count (TPC) test is a parameter used to determine the microbial load in food samples by counting the bacterial colonies that grow on a suitable agar medium (18). According to SNI-7388-2009, the microbial standard for vegetables is $5x10^5$ colonies/g. Total microbes exceeding the SNI standard indicate a decline in quality, which can lead to spoilage.

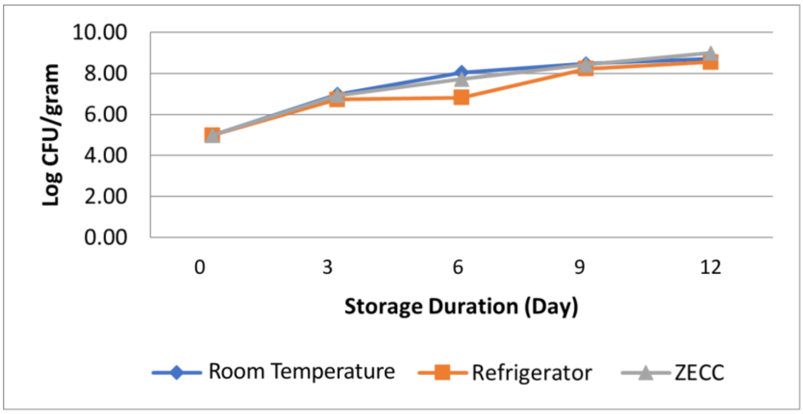


Figure 10. The effect of storage type and duration on total microbial load of red chili pepper.

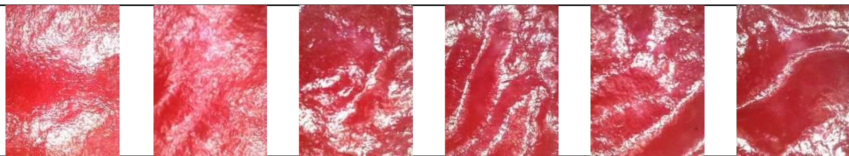
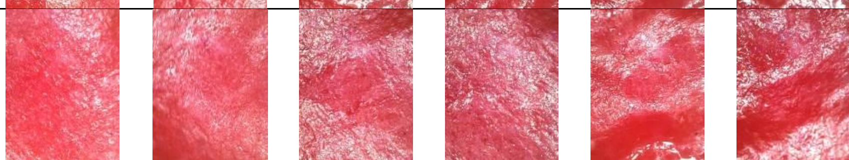
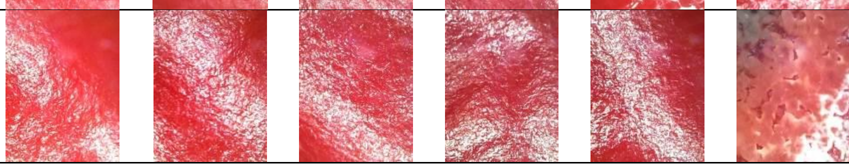
Figure 10 shows that the total microbial count increased in all storage types during the storage period. Total microbial counts increased across all storage treatments during the 12-day period. Microbial levels at room temperature rose from 4.98 log CFU/g on day 0 to 8.71 log CFU/g on day 12. Refrigerated storage exhibited a slower increase, reaching 8.54 log

CFU/g by day 12. In contrast, ZECC showed the highest final microbial load, increasing from 4.98 to 8.99 log CFU/g over the same period. Samples stored at room temperature had the highest microbial load, reaching 8.99 CFU/g, compared to 8.54 CFU/g in refrigerator storage and 8.71 CFU/g in ZECC storage. The high microbial growth at room temperature (28–29°C) occurs because this temperature range is optimal for the growth of *Colletotrichum* sp., the causative agent of anthracnose (40). Meanwhile, microbial growth in ZECC storage is attributed to the high humidity levels. The previous research stated that microbial growth is strongly influenced by humidity and water content (41).

3.7. Observations of Red chili pepper During Storage

Based on the observations results on Table 3, red chili pepper stored at room temperature began to deteriorate on the sixth day, as indicated by wrinkling of the surface. On the ninth day, the red chili pepper became softer. In contrast, chili peppers stored in the refrigerator showed signs of softening and blackened tips, presumably due to infection by the pathogenic fungus *Colletotrichum* spp., which caused anthracnose disease (42). A decline in the quality of the refrigerated red chili was observed on the twelfth day, marked by increased wrinkling. This deterioration was due to metabolic processes during storage, in which decomposing of complex compounds decompose into simpler compounds such as water and energy, which then evaporate, resulting in tissue shrinkage (41). Refrigerator storage can maintain the quality of chili peppers for up to 15 days, as the low temperature (5-10°C) and high humidity (90-95%) can maintain the freshness for 2 weeks (43). Red chili peppers stored in ZECC begin to deteriorate on the 12th day of storage, characterized by changes in texture, becoming wrinkled, and microbial growth on the stems. On the 15th day of storage, red chili pepper stored in ZECC experienced damage due to infection by the microbe *C. gloesporiodes*, which causes anthracnose disease (40). ZECC storage has a high relative humidity, which creates favorable conditions for microbial contamination of large red chili peppers (6).

Table 3. The microscopic appearance of red chili peppers after chlorine washing during storage at room temperature, in a refrigerator, and in ZECC.

<table>
<tr><th rowspan="2">Storage Method</th><th colspan="6">Storage Duration (Day)</th></tr>
<tr><th>0</th><th>3</th><th>6</th><th>9</th><th>12</th><th>15</th></tr>
<tr><td>Room Temperature</td><td colspan="6"></td></tr>
<tr><td>Refrigerator</td><td colspan="6"></td></tr>
<tr><td>ZECC</td><td colspan="6"></td></tr>
</table>

The visual observation results of red chili pepper stored at room temperature, in the refrigerator, and in ZECC are presented in Figure 11.

(a)

(b)

(c)

Figure 11. Results of visual observation on red chili peppers after washing with chlorine disinfectant during storage. (a) Room Temperature; (b) Refrigerator; (c) ZECC

4. Conclusions

Washing with 50 ppm chlorine can help maintain chili quality, as assessed by visual parameters and weight loss. Chili stored in ZECC storage was identified as the best method, with a 6.82% weight loss, 3.62% vitamin C, 0.79% total acid, 6.87% Brix total soluble solids, and a total of microbes. Refrigerator storage yielded results that were not significantly different in terms of total soluble solids, aroma, texture, and shelf life; however, it was superior in terms of total acidity and total microbes. Based on visual and microscopic observations, ZECC and refrigerator storage were able to maintain chili quality for up to 15 days.

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Author Contributions

C.A.S. conceived and designed the experiments; C.A.S. performed the experiments; C.A.S. analyzed the data; C.A.S. wrote the paper; C.A.S. monitored the planning and execution of the study

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Data Availability Statement

Available data are presented in the manuscript.

Conflicts of Interest

The author declares no conflict of interest.

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