

## ONE MONTH ANALYSIS OF COLOR, PH, WATER CONTENT AND ANTIOXIDANT ACTIVITY OF CHILI PASTE DURING STORAGE WITH OZONE PRE-TREATMENT

Ailsa Afra Mawarid<sup>1</sup>, Ahmad Ni'matullah Al-Baarri<sup>2\*</sup>, Setya Budi Muhammad Abduh<sup>2</sup>, Anang Mohamad Legowo<sup>2</sup>, Muhammad Nur<sup>3</sup>, Mulyana Hadipernata<sup>4</sup>, Sofie Kamila Muflihani<sup>2</sup>, Fa'zun Sintha Anggriyani<sup>2</sup>, Fatma Puji Lestari<sup>5</sup>, and Widia Pangestika<sup>6</sup>

<sup>1</sup>Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Jawa Tengah, Indonesia

<sup>2</sup>Department of Food Technology, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Jawa Tengah, Indonesia

<sup>3</sup>Department of Physics, Faculty of Science and Mathematics, Universitas Diponegoro, Semarang, Jawa Tengah, Indonesia

<sup>4</sup>Agroindustry Research Center, National Research and Innovation Agency, Jakarta Pusat, DKI Jakarta, Indonesia

<sup>5</sup>Agricultural Product Technology Department, Faculty of Agricultural Technology, Universitas 17 Agustus 1945, Semarang, Jawa Tengah, Indonesia

<sup>6</sup>Food Technology Laboratory, Center of Research and Services Diponegoro University, Universitas Diponegoro, Semarang, Jawa Tengah, Indonesia

\*Corresponding : [albari@live.undip.ac.id](mailto:albari@live.undip.ac.id)



### ABSTRACT

**Background:** Chili paste are the food products that could maintain color and flavor in a semi-solid form having qualities similar to the fresh harvested ones and are suitable to use. It has observed that ozone treatment helped in strengthening quality of food products.

**Objectives:** The research was conducted to apply ozone pretreatment in the production of chili paste with and without ozone treatment, ozone washed, and ozone sprayed.

**Methods:** As much as ozone at the concentration of 0.25 ppm was used as pretreatment for 15 minutes in chili manufacture and stored for one month or 30 days. Analysis of L\*, a\*, b\* color and browning index, pH values, water content, and antioxidant activity was conducted in every three days.

**Results:** The study's results showed that the color parameters (L\*, a\*, b\*) of chili paste decreased across different storage conditions (room, warm, cold) and treatments. Chili paste without ozone treatment had L\* values of 46.24%, 53.83%, and 39.01%, while ozone-washed paste showed values of 32.83%, 39.12%, and 27.66%, and ozone-sprayed paste had values of 32.58%, 38.19%, and 27.53%. The Browning Index increased significantly in untreated chili paste, with values up to 34.43%, compared to 20.64% for ozone-washed and 20.10% for ozone-sprayed paste. The pH values and antioxidant activities both decreased, with the most notable reduction in antioxidant activity observed in untreated paste (39.87% to 33.32%), while ozone treatments resulted in higher retention of antioxidant activity (30.61% and 34.00%). Additionally, water content increased slightly during storage, with untreated paste showing higher values compared to ozone-treated samples.

**Conclusion:** Chili paste that undergoes pre-treatment with ozone can inhibit physical and chemical changes, thereby preventing damage during storage.

**Keywords:** Chili; ozone; pasta; storage

### INTRODUCTION

Chili pepper (*Capsicum* spp.) is an important horticultural crop in Indonesia, known for its elongated shape and red color when ripe<sup>1</sup>. The spicy flavor of chili peppers comes from capsaicin, a compound with the molecular formula C<sub>18</sub>H<sub>27</sub>NO<sub>3</sub><sup>2</sup>. Chili paste is often preferred for storage because it maintains a quality similar to that of fresh chili peppers<sup>3</sup>. However, without any preservation

treatment, the shelf life of chili paste in cold storage is limited to only 10 days.

During storage, chili paste undergoes several changes, including discoloration, texture softening, off-flavor development, and weight increase<sup>4,5</sup>. Therefore, special treatments such as ozone pre-treatment are necessary to extend the shelf life of chili paste. Ozone is an effective option for food preservation as it can reduce microbial growth

that causes spoilage without leaving harmful residues<sup>6,7</sup>.

Ozone can be produced using various techniques, and recent technological advancements have made its production easier<sup>8</sup>. Ozone is recognized as Generally Recognized as Safe (GRAS) because it is easily decomposed without leaving residue<sup>9</sup>, however threshold for ozone has suggested as much as 0.1 ppm for 8 hours and 0.3 ppm for exposure in 15 minute<sup>10,11</sup>. The reduction in chili paste quality can be detected through a decrease in brightness due to the oxidation of beta-carotene and carotenoids, which causes darkening<sup>12</sup>.

Changes in the pH value of chili paste during storage can also occur, allowing the growth of pathogenic microorganisms such as bacteria (pH > 4.5) and fungi (pH < 4.5)<sup>13</sup>. Storing chili paste can also increase water content due to the absorption of water vapor from the environment and microorganisms<sup>14</sup>. A decline in the quality of chili paste can also be detected through the reduction of phenolic compounds, which are related to its antioxidant content<sup>15</sup>.

Research on the application of ozone in chili paste is still rare. In this study, various methods of using ozone were evaluated to improve the quality of chili paste. The effects of ozone on quality characteristics, including color ( $L^*$ ,  $a^*$ ,  $b^*$  values), Browning Index, pH value, water content, and antioxidant activity of the chili paste, were investigated.

## METHODS

The study design was a true experimental laboratory using a completely randomized design (CRD) with three repetitions. This research was conducted from September to December 2023 at the Food Technology Laboratory and Plasma Research Center, Central Laboratory for Research and Services, Universitas Diponegoro, Semarang. Red chili peppers were sourced from a local fruit producer at Babadan Ungaran Market, Semarang Regency, Central Java, located 20 minutes from the laboratory. The study utilized various equipment, including a chopper (Oxone, Indonesia), aluminum retort pouch packaging, an ozone generator (D'ozone, Plasma Research Center, Diponegoro University), a hand sealer (Q2 Impulse Sealer PFS-8300, Japan), purified water, filter cloths, microtubes, a micropipette (Eppendorf, Germany), ethanol (Merck, Germany), a pH meter (Tekcoplus, Taiwan), a UV-Vis spectrophotometer (Shimadzu, Japan), and a colorimeter (CHNSpec C-10, China).

Chili preparation referred to previous researcher<sup>16</sup> with small modification on sorting process in chili samples with second class based on

Indonesian National Standard (SNI) 01-4480-1998 about Chili (SNI, 1998). Passed quality of chilies was then separated from the stalk and placed in a sanitized container, then cleaned using the non-chlorinated flow water before it was soaked with pure water for 15 minutes. This preparation process was done to minimize contamination in chili peppers. The chili were divided into three equal parts with 1.5 kg each, there were chili peppers a control sample (non-ozonated) and the other chili was subjected to ozonation in two different treatment, there are washed using ozone water and sprayed using ozone gas.

The application of ozone in chili peppers was carried out at the Centre for Plasma Research, UPT Integrated Laboratory using a method that referred to the previous method<sup>17</sup>, ozone was produced by a ozone generator double dielectric barrier discharge (DDBD) namely D'ozone and oxygen gas was used as an input gas. The application for the fresh chili were ozonated gaseous using the concentration 240 mg/L and flow rate 0.8L/min, and the ozone washed by the generated ozone that used by the DDBD generator was continuously into 10 L pure water for 15 minutes with ozone concentration 0,25 ppm.

The manufacture of red chili paste referred to previous research<sup>18</sup> where chili peppers were ground into pasta using chopper for 2 minutes at low speed. As much as 35 grams chili paste were packed in a 9x13 cm retort pouch, sealed by heating sealer and steamed in boiling water for 10 minutes. The chili paste sample were stored at room, warm, and cold that was used the temperature at 25°C (room temperature), 45°C (warm temperature), and 5°C (cold temperature), respectively for a period of 30 days.

Color change analysis referred to previous research<sup>19</sup> using colorimeter to value in  $L^*a^*b^*$  color.  $L^*$  was identified as lightness that reflected light with color results in of the achromatic white, gray and black and the range between 0-100<sup>20</sup>. The  $a^*$  value was identified as redness color that can expressed from  $a^*$  negative between -80 to 0 for green color and  $a^*$  positive between 0 to 100 for red color<sup>21</sup>. The  $b^*$  value was identified as the yellowness, the yellow color at the range of positive values between 0 to 70 and blue color as negative values between -70 to 0<sup>22</sup>. The test of analysis by adding information from a sensor of colorimeter to the sample. Before the measurement of chili paste, 2 gram of samples were placed on a white plastic board to maintain a dependable background. The analysis were taken at six randomly chosen locations on the surface of each sample of chili paste. Color analysis was done in the form of  $L^*$ ,  $a^*$ ,  $b^*$  value and

Browning Index (BI) value. The formula of browning index values is as follows:

$$\frac{B - ([100(x - 0.31)])}{0.17}$$

Where:

$$\frac{x - ((a + 1.75L))}{((5.645L + a - 3.012b))}$$

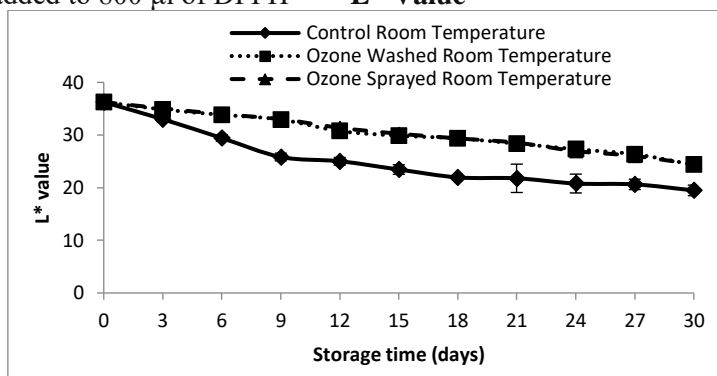
The pH value analysis was monitored according to the method of previous researcher<sup>23</sup> using 2 grams of chili paste that was diluted in 8 ml aquadest. Water content testing refers to the modified<sup>24</sup> method using 2 grams of chili paste.

Analysis of antioxidant activity using the DPPH method that refers to previous method<sup>25</sup> using 1 gram of chili paste that was diluted in 10 mL of ethanol, then mixed for 1 minute using vortex at high speed. To obtain supernatant of sample, centrifugation at 4000 rpm for 10 minute was then applied. The DPPH stock solution was made by dissolving 5 mg of DPPH with 250 mL of ethanol. The sample of 500 µl was added to 800 µl of DPPH

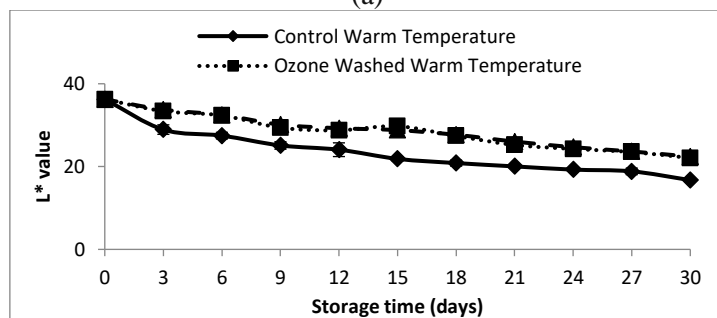
stock solution and prepared for 30 minutes, then the absorbance value was measured using a UV-Vis spectrophotometer at λ 517 nm. Antioxidant activity was represented as % inhibition.

The data were obtained in triplicate and showed as average ± standard deviation. The analysis was conducted using Microsoft Excel to provide graphs with error bars. The trendline was also created to improve the illustrate information descriptively.

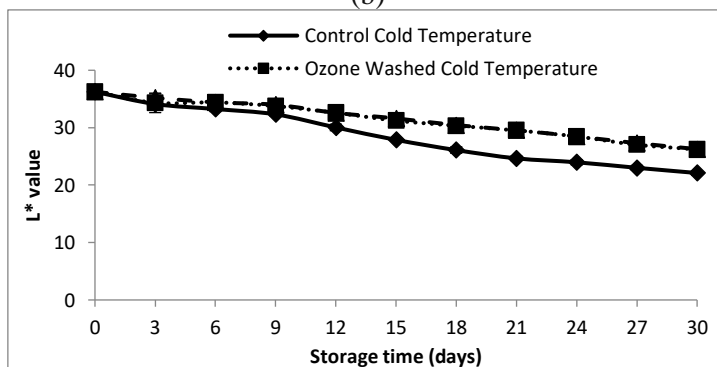
**RESULT**  
**Color Value**  
**L\* Value**



(a)



(b)



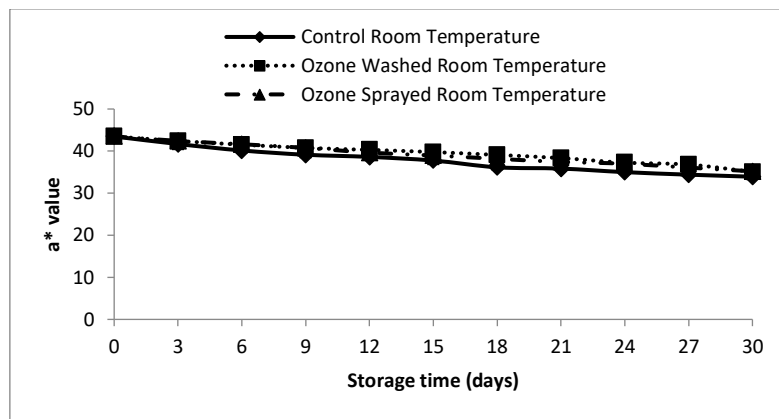
(c)

**Illustration 1. Changes In L\* Value in Chili Paste Without Treatment, with Ozone Wash and Ozone Sprayed Stored at (a) Room Temperature, (b) Warm Temperature and (c) Cold Temperature**

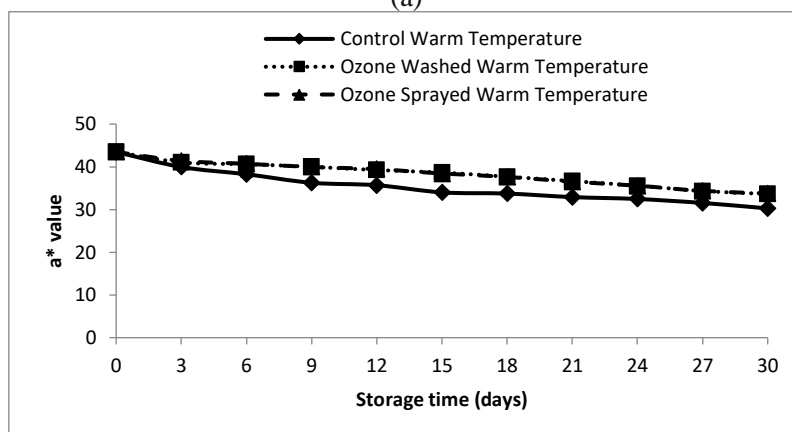
Based on Illustration 1, the value of L\* has decreased by 27.53-53.83% during 30 days of storage. Decreased L\* value in no treated-chili paste at room temperature had range value from 39.01 to

53.83%, while the ozone treated chili paste had range of value from 27.53 to 38.19% indicating the less decrease change in brightness value in ozone treated-chili paste.

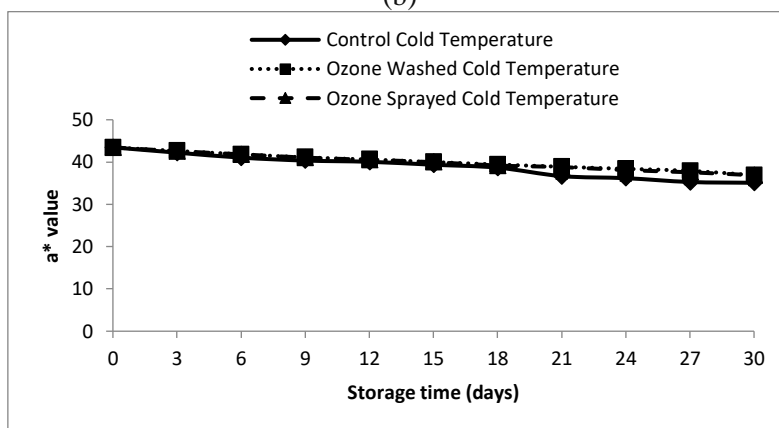
**a\* Value**



(a)



(b)



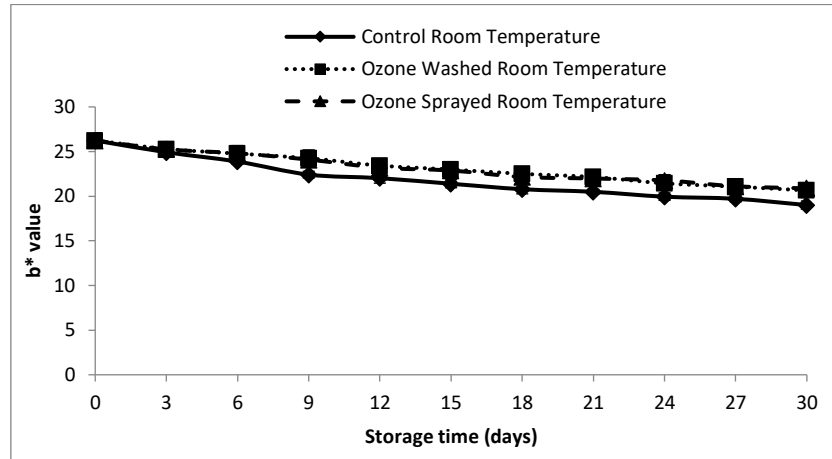
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**Illustration 2. Changes in a\* Value In Chili Paste Without Treatment, With Ozone Wash and Ozone Sprayed Stored at (a) Room Temperature, (b) Warm Temperature and (c) Cold Temperature**

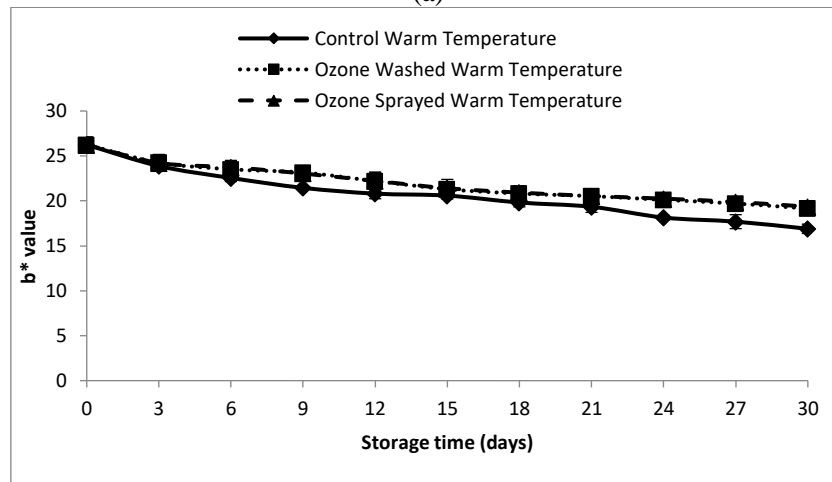
Based on Illustration 2, the value of a\* has decreased ranging from 15.17 to 30.38% during 30 days of storage. The a\* value in chili paste without ozone treatment had range of decrease from 19.27 to 30.38%, while ozone treatment hinder the change starting from 15.16-22.60% The highest decrease in

reddish color is in chili paste without ozone treatment which is 30.38% and the lowest decrease is in the treatment with ozone sprayed at cold temperatures, which is 15.16%. The decrease in redness in chili paste is caused by color degradation.

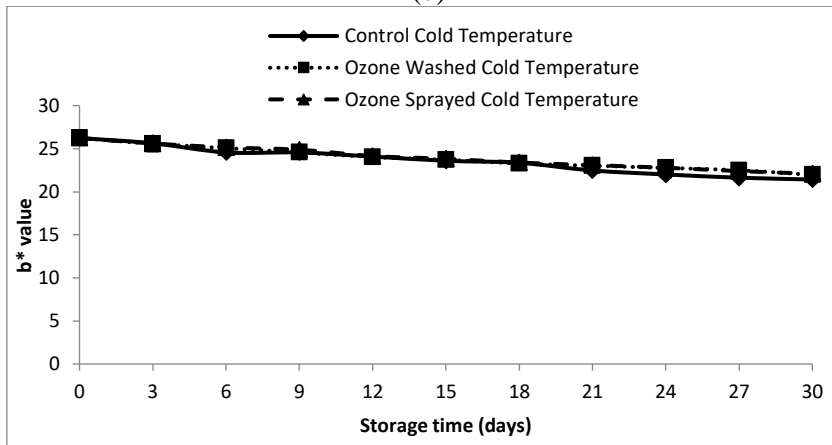
**b\* Value**



(a)



(b)



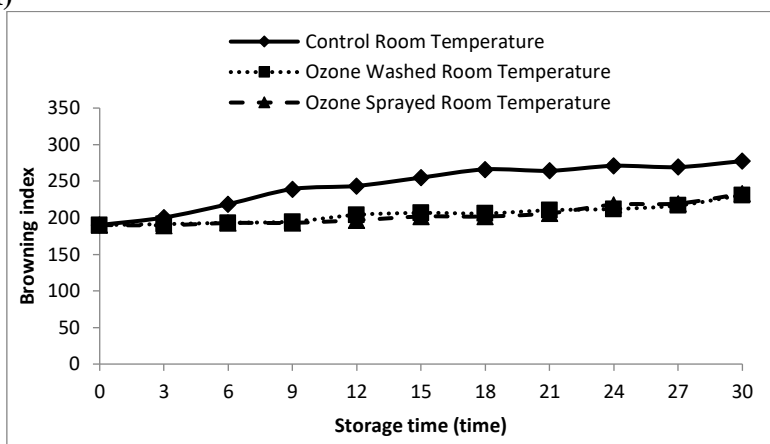
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**Illustration 3. Changes In b\* Value in Chili Paste Without Treatment, with Ozone Wash and Ozone Sprayed Stored at (a) Room Temperature, (b) Warm Temperature and (c) Cold Temperature**

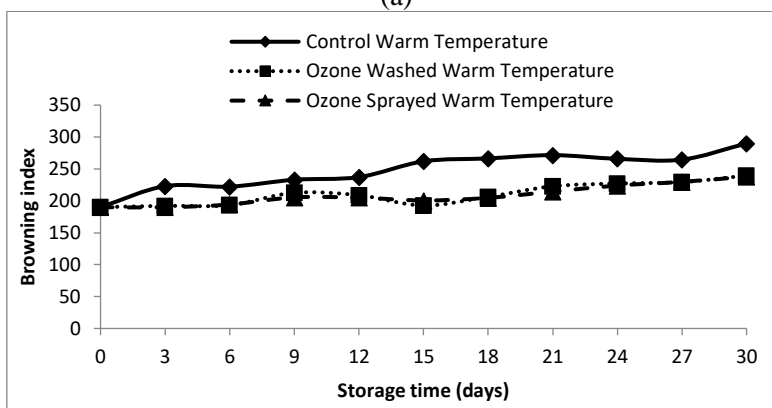
Based on Illustration 3, the value of b\* has decreased ranging from 16.33 to 35.61% during 30 days of storage. The b\* value in chili paste without ozone treatment had range of decrease from 18.35 to 35.61%, while ozone treatment hinder the change

starting from 15.82 to 27.04%. The highest decrease of greenish color was in chili paste without ozone treatment which was 35.61% and the lowest decrease was in the treatment with ozone sprayed at cold temperatures, which is 15.82%.

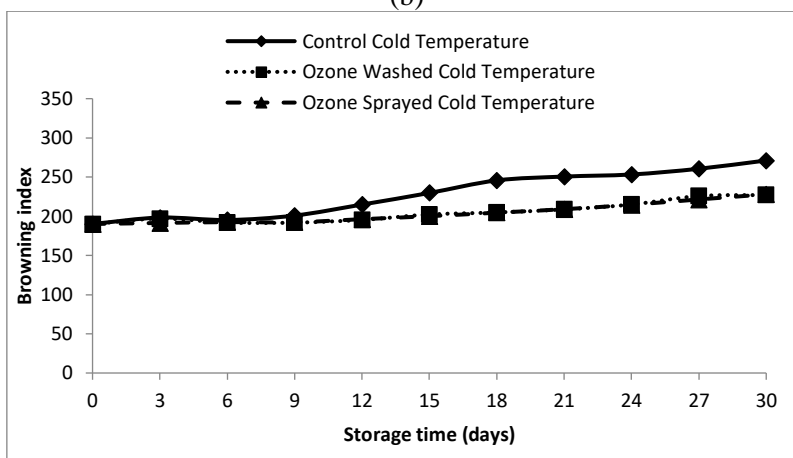
**Browning Index (BI)**



(a)



(b)



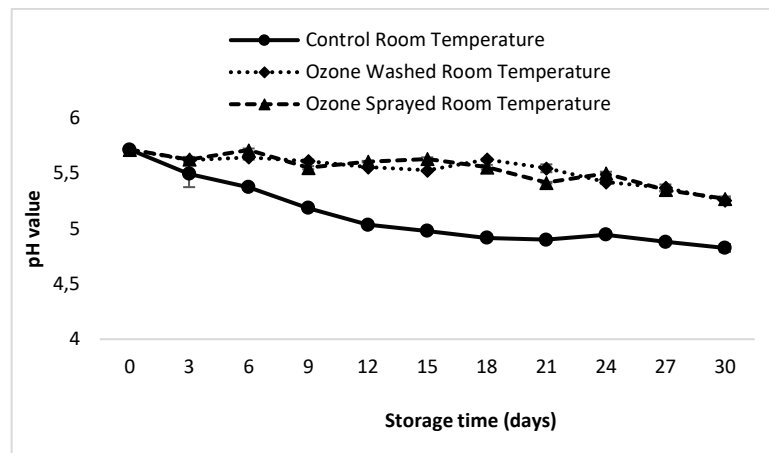
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**Illustration 4. Changes In The Browning Index Value in Chili Paste Without Treatment, with Ozone Wash and Ozone Sprayed Stored at (A) Room Temperature, (B) Warm Temperature and (C) Cold Temperature**

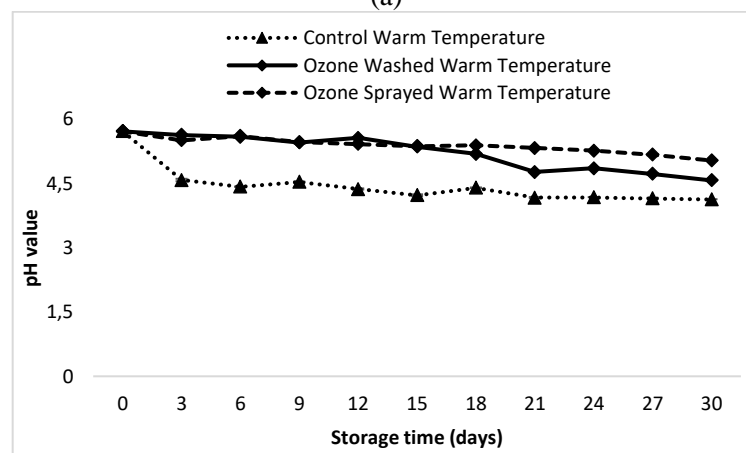
Based on Illustration 4, the browning index value has increased ranging from 16.27 to 34.43% during 30 days of storage. The browning index in chili paste without ozone treatment had range of increase from 29,90 to 34.43%, while ozone treatment hinder the change starting from 16.27 to 20.64%. The highest decrease in browning index

discoloration was chili paste without ozone treatment at 34.43% and the lowest decrease was in the treatment with ozone washing at cold temperatures, which was 16.27%. This suggests that ozone treatment may inhibit the increase in browning index in chili paste compared to chili paste without ozone treatment.

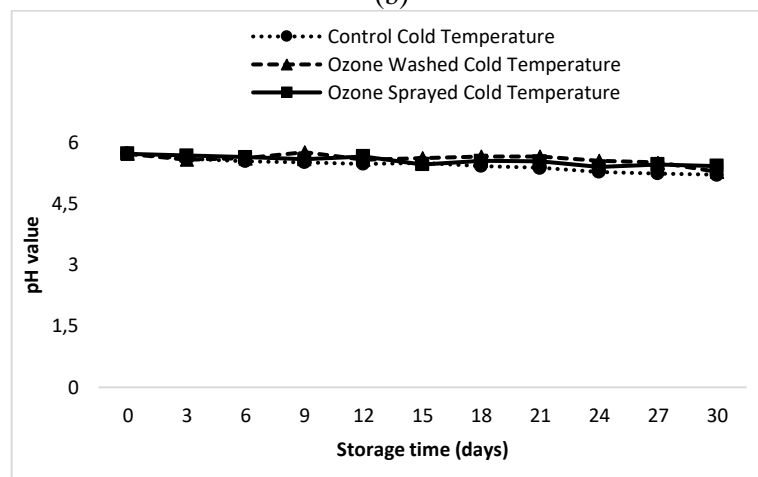
pH value



(a)



(b)



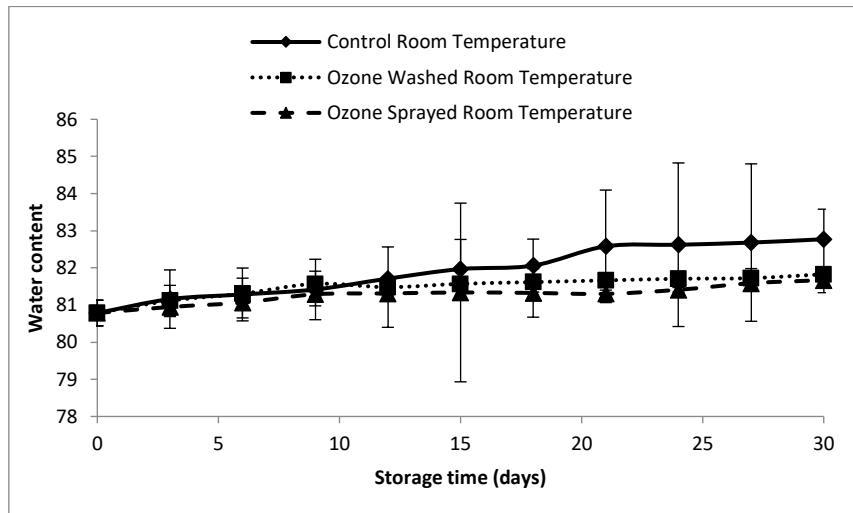
(c)

**Illustration 5. Changes in pH Values In Chili Paste Without Treatment, With Ozone Wash and Ozone Sprayed Stored at (a) Room Temperature, (b) Warm Temperature and (c) Cold Temperature**

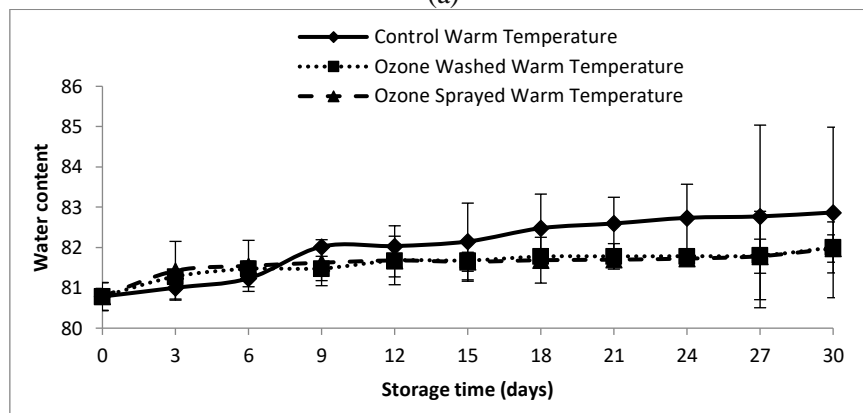
Based on Illustration 5, the pH value of chili paste has the same pattern of decline that tends to decrease during storage. The pH value has decreased ranging from 5.25 to 27.82% during 30 days of storage. The pH value in chili paste without ozone treatment had range of decrease from 8.05 to

27.82%, while ozone treatment hinder the change starting from 5.25 to 20.04%. The highest decrease in pH value of chili paste without ozone treatment which is 27.82% and the lowest decrease is in the treatment with ozone sprayed at cold temperatures, which is 5.25%.

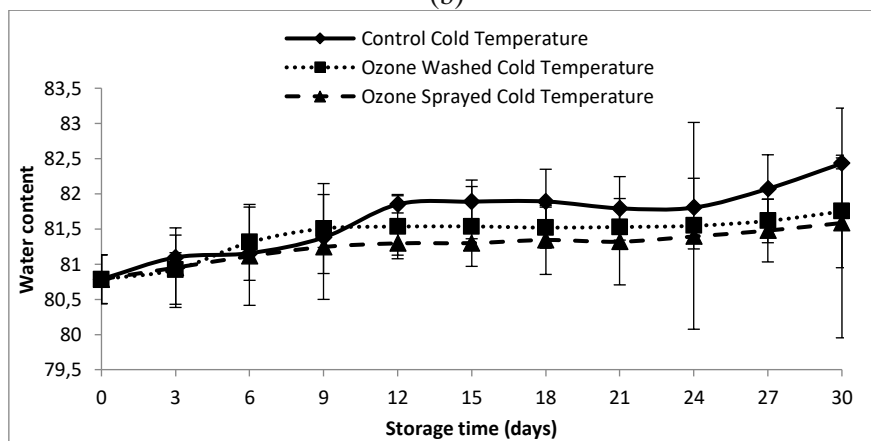
Water Content



(a)



(b)



(c)

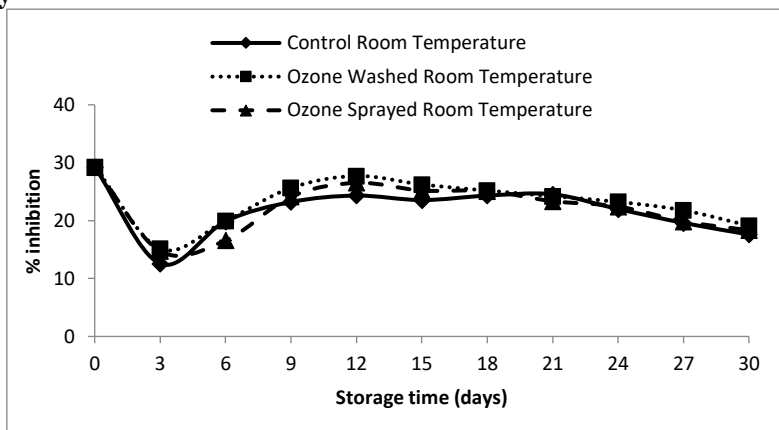
Illustration 6. Changes in Water Content in Chili Paste Without Treatment, With Ozone Wash and Ozone Sprayed Stored at (a) Room Temperature, (b) Warm Temperature and (c) Cold Temperature

Based on Illustration 6, the storage of chili paste for 30 days has increased. Water levels tend to increase during storage both with ozone treatment and without ozone treatment. However, chili paste with ozone treatment is able to inhibit changes in water content compared to chili paste without ozone. The increase in water content in chili paste during the 30-day storage process increased from 0.98 to

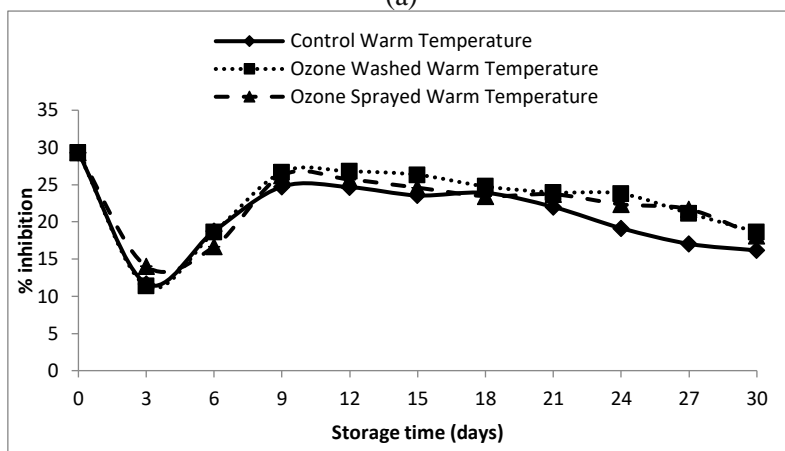
2.52%. The water content in chili paste without ozone treatment had range of increase from 2.00 to 2.52%, while ozone treatment hinder the change starting from 0.98-1.48%. The highest increase in water content is in chili paste without ozone treatment which is 2.52% and the lowest increase is in the treatment with ozone sprayed at cold temperatures, which is 0.98%.



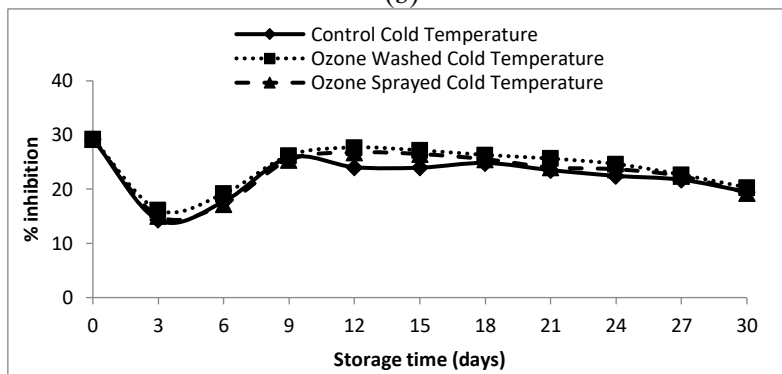
**Antioxidant Activity**



(a)



(b)



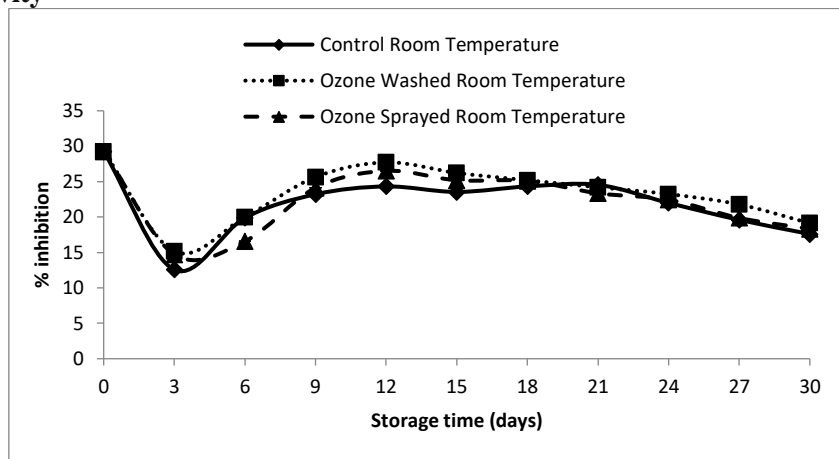
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**Illustration 7. Changes In Antioxidant Activity In Chili Paste Without Treatment, With Ozone Wash And Ozone Sprayed Stored At (a) Room Temperature, (b) Warm Temperature And (c) Cold Temperature**

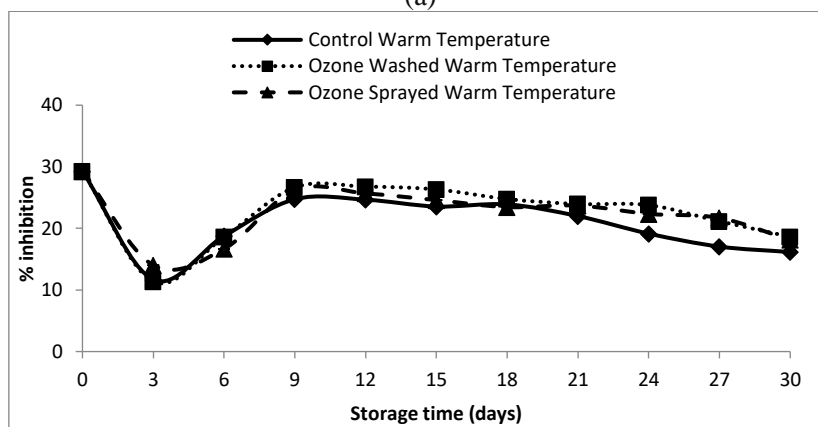
Based on data in Illustration 7, the percent inhibition in chili paste decreased from 48.26 to 60.10% on all treatments on the day 3. The highest decrease was achieved in chili paste control at warm temperatures, which was 60.10%. Percent inhibition increased again on the day 6 until and reached a more stable stage starting on the day 12 although there appeared to be a decrease that was not sharp until the day 30. The specific difference between treatments that occurred on the day 12 was considered very specific, which is seen from the wide range of values, which is 24.07 to 27.71%. The highest

percentage of insulation on the day 12 was achieved in chili paste with ozone washed treatment at room temperature, which is 27.71%. The percent inhibition of chili paste without ozone treatment had range of decrease from 33.32 to 44.83% on day 30, while ozone treatment hinder the change starting from 30.61-38.06%. The highest increased of percent inhibition in chili paste without ozone treatment which is 44.83% and the lowest increase is in the treatment with ozone washed at cold temperatures, which is 30.61%.

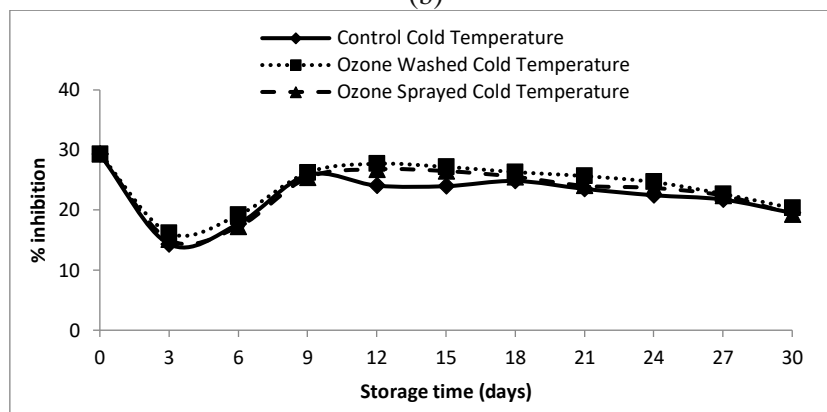
**Antioxidant Activity**



(a)



(b)



(c)

**Illustration 7. Changes In Antioxidant Activity In Chili Paste Without Treatment, With Ozone Wash And Ozone Sprayed Stored At (a) Room Temperature, (b) Warm Temperature And (c) Cold Temperature**

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values, which is 24.07 to 27.71%. The highest percentage of insulation on the day 12 was achieved in chili paste with ozone washed treatment at room temperature, which is 27.71%. The percent inhibition of chili paste without ozone treatment had range of decrease from 33.32 to 44.83% on day 30, while ozone treatment hinder the change starting from 30.61-38.06%. The highest increased of percent inhibition in chili paste without ozone treatment which is 44.83% and the lowest increase is

in the treatment with ozone washed at cold temperatures, which is 30.61%.

## DISCUSSION

### Color

#### L\* Value

Based on Illustration 1. ozone treatment in both washing and sprayed was able to inhibit the rate of discoloration in chili paste<sup>26</sup>. The brightness in chili paste tends to be dark caused because oxidation of beta-carotene may reduce carotenoid components so that the red chili pigment is damaged during storage<sup>27</sup>. The ozone-treated samples showed less decrease in brightness compared to untreated samples. This is consistent with previous studies, who found that ozone exposure reduced postharvest quality loss in chili peppers. The mechanism behind this preservation effect could be attributed to ozone's ability to inhibit enzymatic reactions responsible for color degradation<sup>28</sup>.

#### a\* Value

Based on Illustration 2. Ozone treatment is thought to inhibit the decrease in reddish color in chili paste because ozone has an inhibitory effect on enzymes and compounds responsible for color<sup>29</sup>. In addition, temperature and length of storage can cause a decrease in the content of color pigments in chili peppers and degradation of carotenoids resulting in a decrease in reddish color<sup>27,30</sup>. Ozone treatment was able to slow down the decrease in redness of the chili paste. This is particularly important for chili products, as the red color is a key characteristic. The findings align with research by previous researcher, who observed that ozone treatment helped maintain color in fresh-cut green peppers. The preservation of redness could be due to ozone's protective effect on carotenoid pigments, which are responsible for the red color in chili peppers<sup>31</sup>.

#### b\* Value

Based on Illustration 3. The greenish color in chili paste decreases due to the degradation of carotenoid pigments<sup>32</sup>. Previous research<sup>33</sup> stated that carotenoids in red peppers can be damaged by the influence of warm, light and oxygen so that there can be a decrease in yellowish color. The ozone pre-treatment in chili paste can inhibit the decline of yellowish color because ozone can inhibit non-enzymatic and enzymatic reactions<sup>34</sup>. The study found that ozone pre-treatment inhibited the decline of the b\* value, indicating better preservation of yellowish/greenish hues. This is supported by previous researcher who found that postharvest treatments can influence carotenoid biosynthesis in chili peppers. The preservation of these color

components suggests that ozone treatment may help maintain the overall visual quality of the chili paste<sup>26</sup>.

### Browning Index (BI)

Based on Illustration 4. Discoloration to browning can occur due to enzymatic and non-enzymatic reactions<sup>7</sup>. Chili paste that is given ozone has a slower metabolism than the control chili paste so that it can weaken enzymatic browning<sup>35</sup>. The largest increase in browning index value was found in chili paste without treatment in warm temperatures. This is in accordance with previous research<sup>12,36</sup> which states that warm temperatures can cause polyene components in carotenoids to oxidize and be sensitive to light. One of the most significant findings was the ability of ozone treatment to inhibit the increase in the Browning Index. This is crucial for maintaining the visual appeal of the chili paste during storage. The results are in line with studies by Sachadyn-Król and Agriopoulou (2020), who reported that ozone can act as an abiotic elicitor, improving the health-promoting properties of plant products and potentially inhibiting enzymatic browning<sup>29</sup>.

### pH Value

Based on Illustration 5. high temperatures can lead to a decrease in pH due to the conformation of carotenoid and anthocyanin structures to be unstable<sup>37,38</sup> and Degradation of carbohydrates into organic acids that can increase the value of acidity and cause pH values to decrease<sup>39</sup>. This decrease in pH value is in accordance with previous research<sup>40</sup> which states that long storage can lower the pH value in chili peppers. Respiration that takes place quickly will increase the amount of acids - organic acids that can result in a decrease in the value of pH<sup>41</sup>. The influence of high temperature causes the carotenoid structure to turn unstable and will increase acidity, causing a decrease in pH<sup>42</sup>. The study found that ozone treatment helped maintain more stable pH values in the chili paste during storage. This is a critical finding, as pH affects both the taste and microbial stability of the product. The results show that ozone-treated samples had a slower rate of pH decline compared to untreated samples. This finding is supported by previous research reported that ozone treatment can help preserve the quality of fruits and vegetables during storage. The mechanism behind this pH stabilization could be related to ozone's ability to inhibit microbial growth and slow down the production of organic acids through respiration<sup>38</sup>.

### Water Content

Based on Illustration 6. according to previous research<sup>12</sup> on chili paste states that

temperature and storage can affect the water content of chili paste, the higher the temperature and length of storage, the higher the water content of a foodstuff<sup>43</sup>. Microbial activity produces H<sub>2</sub>O and water vapor as one of the results of microbial metabolism so that during storage it affects the water content in the product<sup>44</sup>. The treatment of ozone can inhibit the increase in water content because ozone serves to reduce the total microorganisms that cause an increase in water content in chili paste<sup>45</sup>. High water content as a food material will be susceptible to damage both physically, chemically and microorganisms<sup>46</sup>. The study demonstrated that ozone treatment was effective in inhibiting the increase in water content of chili paste during storage. This is a crucial finding, as water content directly affects the texture and microbial stability of the product. The results align with research by Asgar et al. (2017), who found that ozonation could help maintain the freshness characteristics of red chili peppers during storage. The lower increase in water content in ozone-treated samples could be attributed to ozone's ability to reduce microbial activity, which is often responsible for moisture production during storage<sup>34</sup>.

#### Antioxidant Activity

Based on Illustration 7. This study successfully proved that ozone has the ability in the prevention of cell damage so that it can better maintain antioxidant activity in chili paste<sup>47</sup>, so that the antioxidant activity of chili paste that is given ozone treatment tends to be higher compared to control. The role of ozone in increasing antioxidant activity is by activating the enzyme phenylalanine ammonium lyase (PAL) and contributing to the inhibition of polyphenol oxidase (PPO) and peroxidase (POD) enzymes that cause oxidation of phenolic compounds in fresh cut fruits and vegetables<sup>48,49</sup> and decreased respiration rate<sup>50</sup>.

Long storage of chili paste affects the ability of antioxidant activity. Based on previous research<sup>51</sup> states that antioxidant activity can decrease during storage. Decreased antioxidant activity in chili paste can be affected by the presence of oxidative stress, in which case antioxidant activity can be associated with a decrease in the content of ascorbic acid and changes in the phenolic composition in chili paste<sup>28</sup>. In addition, according to<sup>52</sup> the high storage temperature of chili paste causes a decrease in the antioxidant content found in chili carp. Based on the results of study, chili paste with ozone treatment was able to slow the percent change in the percentage of the activity of antioxidants compared to chili paste that was not treated.

This study, while providing valuable insights into ozone pre-treatment for chili paste

preservation, has several limitations that offer opportunities for future research. The 30-day storage period may not fully capture long-term effects, and the fixed ozone concentration of 0.25 ppm limits optimization possibilities. The focus on a single chili variety, absence of sensory evaluation and detailed microbial analysis, and limited investigation of nutritional changes beyond antioxidant activity constrain the study's comprehensiveness. Future research should address these limitations by conducting longer-term storage studies, exploring various ozone concentrations and application methods, investigating multiple chili varieties, incorporating sensory and microbial analyses, and examining a broader range of nutritional components. Additionally, mechanism studies to understand ozone's effects on cellular structures, combination treatments with other preservation techniques, scale-up studies for industrial application, economic and environmental impact assessments, and consumer perception studies would significantly advance our understanding of ozone treatment in food preservation. These enhancements would provide a more holistic view of ozone pre-treatment's potential in maintaining chili paste quality and safety, ultimately contributing to the development of more effective food preservation strategies.

#### CONCLUSIONS

Based on research that has been done, it can be concluded that the best chili paste is chili paste that is given ozone treatment at cold temperature, especially ozone sprayed. The administration of ozone in chili peppers as an initial treatment of chili paste is able to suppress discoloration in the form of  $L^*$ ,  $a^*$ ,  $b^*$  and browning index values, pH values, water content and changes in antioxidant activity.

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