The effect of ozonation time and contact time of edamame washing on color changes using the continuous type ozone washing method

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Abstract. Quality of edamame is the main requirement in the export trade, one of which is the skin color. The washing technique using ozone is expected to replace the use of chlorine in suppressing the microorganism contamination, pesticides residues and maintaining its quality. The ozonation time and ozone contact time variations were used in this study using continuous washing method. The ozonation time consisted of 0.10 and 15 minutes, while the washing contact time consisted of 0, 12, 18 and 24 minutes. Each variation was repeated 3 times to obtain an average result of changes in edamame skin color. The results of the research from the washing contact time and ozone time treatment gave identical results with the treatment without washing.

1. Introduction
Edamame (Glycine Max L. Merrill) is the district's leading agricultural commodity Jember. Edamame is grown for local, national consumption and exported to foreign countries. Edamame is exported in frozen form, one of the critical points that determine its final quality was washing step. Previously, the edamame was washed using chlorine water in a two-level washing step with different chlorine concentrations in its every step. The chlorine concentration in the first washing step was 150ppm and 175ppm in its second step [1]. Chlorine will remain in the product after washing steps as contaminants on the product. In contrast, washing was intended to reduce or remove heavy metals, pesticide residues, and pathogenic microorganisms (M.O) such as Eschericia coli, Staphylococcus aureus and Salmonella sp. Chlorine removes pathogens well, but leaves chemicals residue that are harmful to human health. Other materials that can be used for reducing pathogen M.O is ozone. Lack of ozone application in the edamame washing process was due to its high cost. The result shown in this article is one of the research series with the ultimate goal to produce a prototype of edamame washing ozone technology at a low cost [2].

Ozone has received Generally Recognize As Safe (GRAS) status as an internal assistant food processing and its compatible with the Environmental Protection Agency by Disinfection Product Rule. There are many advantages that can be obtained from the use of ozone, namely: (1) its strong oxidation properties give ozone the ability to disinfect, sterilize, and decomposition of broad spectrum agrochemicals such as oxidizing compounds bond (such as ethylene) from the product and kills the microorganisms through oxidation, so as to extend the freshness of fruit and vegetables; (2) sterilization speed ozone is higher than ultraviolet light in killing bacteria and viruses; (3) easy ozone made with air or oxygen as raw material using the electric discharge method; (4) ozone does not produce hazardous compounds because it immediately turns into oxygen (6). The use of ozone in the food sector has been approved by the FDA (Food and Drug Administration) for use as an antimicrobial in food processing.
and food storage [3]. Other ozone uses are currently being applied in various ways, such as for drinking water purification, raw material sterilization, food preservative, medical equipment sterilization, water treatment for cooling water purposes, processing industrial wastewater and oil refining, controlling odor and color, and manufacturing ultrapure water in the electronics industry [4]. The effectiveness of ozone depends on the direct and indirect reactions between ozone and materials affected by ozone concentration, ozonation method, ozonation time and contact time ozone [5]. These two parameters determine the reduction amount of organic compounds, heavy metals and m.o pathogens in the material. In previous research, research on ozonation method and ozone concentration used along with the time of contact made, but has not been studied further regarding the time of ozonation and its effect on accumulation ozone concentration during the washing process. The duration of ozone accumulation can also affect compound or composition interactions in foodstuffs [6].

Based on the description above, we can conclude the problem of how long the ozonation takes and the ozone contact time which should be used in the edamame leaching process can produce an effective and efficient ozone technology edamame washing prototype machine. The general objective of this research is to make a prototype of the edamame washing machine with ozone technology. These general objectives are broken down into three specific objectives [7]. First, assessing time ozonation and contact time of ozone in edamame leaching. Second, assessing the effect of time ozonation and contact time of ozone on the sensory properties of edamame produced. Third, compare the quality of edamame based on the microbial residue from the best treatment time ozonation and contact time of ozone with chlorine leaching and its effects on content dietary fiber and vitamin C edamame [8].

2. Literature review

Literature review that will be used for this research are: Washing functions to reduce the number of microbes that stick to the product. Therefore, the water used for washing is completely clean and flowing. Almost all vegetable commodities that have been harvested are subject to physical contamination, especially dust or soil so it needs to be washed. Washing is done in order to remove dirt and pesticide residue (insecticide or fungicide). However, this washing is not done on vegetables with soft and easy texture scuffed / damaged. Traditionally washing step were uses tap water to get good results with recommendation for using disinfectant to remove microbes more effectively. Water then was drained and vegetable were dried by open air or hot circulating air. Commonly, chlorine was used as disinfectant with concentration of 150-200 ppm [9]. In continuous uses, Chlorine has several negative effects on human health and causes some negative impacts on the environment [10]. On health, the bad effects of chlorine include causing asthma, infections bladder, eye irritation, dry skin and hair, poisoning and cancer [11]. On environment, chlorine has negative impact since in water, it can react with organic compounds such as organo chlorine which is a toxic compound and can cause several carcinogens effect for humans being. Other compounds that may be formed in the washing process by chlorinated water is substituted chloramine [12].

Ozone (O₃) is part of active oxygen in the wild. Every ozone molecule consists of 3 oxygen atoms. Ozone is a colorless and odorless. Ozone is formed naturally by ultraviolet rays from the sun and lightning. Ozone can works as sterilizer, deodoration, decoloration and degradation. Through sterilization, ozone can kill various microorganisms such as pathogenic bacteria, viruses and fungi. Through deodoration, ozone can eliminate odors caused by various aromatic compounds and microorganisms. Through decoloration, ozone can remove organic dyes. Through degradation, ozone can decompose various organic compounds and oxidize heavy metals [13].

Increased human concern for the environment, as well as the wider impact negative chlorine, research on alternative substitutes for chlorine, including ozonation technology, was conducted. Ozone (O₃) is a triatomic molecule of oxygen that is formed as a result of a fusion oxygen free radicals with molecular oxygen. Ozone was first discovered by Schonbein in 1840, it began to be used as a disinfectant in water production in France at the earliest 1900s [14]. Ozone has been used commercially for drinking water treatment since 1906 and its use is increasing in the food industry [15]. The solubility of ozone in water is affected by temperature with solubility decreasing as the temperature increases. Ozone solubility on
temperature 0 °C is 0.6401 ozone / L of water, where the temperature is 60 °C ozone is insoluble in water (4). Methods in the formation of ozone include: corona discharge, ultraviolet radiation, electrolysis and chemical radiation [16]. Every method is used to generate ozone influenced by the energy to break and hold bonds. The oxygen atom is shaped molecules that can be separated and then reformed into ozone. One of the methods, namely Corona discharge is the provision of high voltage that is passed through the air gap. In producing ozone, this high voltage is intended to break down $O_2$ molecules and form them molecule atoms $O$ (ozone) and pass air or oxygen between the electrode poles. This process is carried out by passing oxygen gas ($O_2$) to an area that has a pressure height between the two electrodes [9]. The oxygen molecule will undergo ionization, which is the process of releasing an atom or molecules from their bonds, becoming oxygen ions ($O^*$). Ionized oxygen molecules this is called the plasma state. The type of oxygen ion is $O^*$, $O_2^*$, $O^-$, $O_2^-$ and $O_3^-$ [17]. The combination of these can produce ozone. Ozone is created in this process begins with the formation of oxygen free radicals. Then the oxygen radicals react with oxygen generates ozone. Compared to chlorine, ozone has a fast disinfecting agent kill microorganisms 3250 times faster and 150% more powerful oxidative. The oxidation potential of ozone is higher when compared to chlorine 1.36 V and oxygen 1.23 V [18].

Quality is the character of a product which shows its product excellence degree or its ability to meet needs certain. The main parameter to be concern in measuring product quality is physical appearance by the sense of sight. The first factor affect the product appearance is color. Physical appearance constitutes the most important criteria for consumers in selection of food products sold in the market. Visual parameter to be concern especially grain products and products plantation products include color, shape and the size. In food color research, the analysis often used with one of the color models LAB as the international standard for color adopted from the Commission Internationale de l'Eclairage (CIE) in 1976. Development of methods capable of doing identification of physical quality of plantation products objective is required. One of the technological alternatives which can be used for visual sorting is to use image processing techniques. The new easy way, cheap and fast in operation is by using image processing technology digital. Image processing techniques usually used to perform transformations from one image to another, temporarily the task of repairing information lies in humans through compiling the algorithm [19].

Image processing for grain quality classification provides more accurate results when compared manual classification results. Image processing techniques can provide good information when combined with the system decision making who can give high accuracy. One of the tools decision making is a network method artificial nerve. The application of artificial neural networks is processing various data generated by the system visuals in a decision-making effort based on these data and their relationship each other. Combination use of image processing technology and artificial neural network allows giving optimal results, because it has a deep advantage solve problems that are non-linear. Research that applies image processing and artificial neural networks including bleaching edamame uses image processing and artificial neural network showed 81.4% accuracy; classification system for nuts green using computer vision and networking artificial nerve obtained classification truth of 90.6%; classification of the physical quality of rice by using image processing technology digital and artificial neural networks succeed with accuracy rate of 93.25%. Combined image processing technology with artificial neural networks can be used as an alternative to improve the identification process based on the quality class of nutmeg non-destructively [20].

3. Materil and methods

3.1. Research Area

Research activity was carried out from May to November 2020. The research was conducted at the Politeknik Negeri Jember Agricultural Engineering Metal and Wood Laboratory and Politeknik Negeri Jember Food Engineering Technology Laboratory, Jember, East Java.

3.2. Data Collection Method

The tools used in this research are ozone generator, washing tub, ozone meter, washing tank prototype, burette, erlenmeyer, crustang, laptop, scale, and knife. Material used in this study were fresh
edamame, KI 2%; H₂SO₄ 0.1 N; Na₂S₂O₃ 0.2N; starch 2%; Chlorine, tap water. Edamame skin color analysis based on RGB and Lab values. The control treatment (a₀b₀) which was not subjected to washing and ozonation treatment became the benchmark in determining whether there was an effect of each treatment. Color analysis using ImageJ software [21].

3.3. Analysis Method

Analysis methods for research include:

3.3.1. Research Design. The equipment consists of machine vision instruments to place objects and capture images, the inside of the instrument wall and background object coated with black is aiming for facilitate the object segmentation process besides that black color absorbs light so it is very both used as background objects because it doesn't reflect that light was arrested. Machine vision instrument equipped with a webcam image sensor the light source comes from TL lamps 2 watts of 5 watts, and a PC to store image and data processing, following monitors for display image. Data collection process image is equipped with an intensity measuring device Luxmeter light to ensure uniformity the light intensity the object gets during the process image retrieval, array of retrieval equipment image can be seen in Figure 1.

![Figure 1. Image Processing Program](image)

The research object used was fresh edamame (Glycine max L. Merrill) purchased at PT. Mitra Tani 27 Jember. The research method used is a method experimental. Statistical testing includes diversity testing with the F test and statistical tests further difference. The treatment consisted of two factors, namely the ozonation time and the ozone contact time. Time ozonation is when water is drained with ozone before it is used for leaching edamame fresh. Contact time is the time required for washing fresh edamame. The ozone concentration used is 6 ppm according to the results of previous studies. The ozonation times used were 0, 10 and 15 minutes with contact times of 0, 12, 18 and 24 minute (Table 1).

| Contact Time Ozonation Time | 0 minute (b₀) | 12 minute (b₁) | 18 minute (b₂) | 24 minute (b₃) |
|-----------------------------|--------------|---------------|---------------|---------------|
| 0 minute (a₀)               | (a₀b₀) control | (a₀b₁)        | (a₀b₂)        | (a₀b₃)        |
| 10 minute (a₁)              | (a₁b₀)       | (a₁b₁)        | (a₁b₂)        | (a₁b₃)        |
| 15 minute (a₂)              | (a₂b₀)       | (a₂b₁)        | (a₂b₂)        | (a₂b₃)        |

3.3.2 Color Analysis. Image analysis and processing are carried out with the help of software ImageJ and Borland Delphi7 with image processing program scripts contained in image processing toolbox. Color analysis stage with programs that have been created with Delphi7 software starting with: reading the file image, change the original image to gray image, convert the gray image becomes a binary image, the next binary image converted to excess image to get RGB values, the RGB vector image is then converted to HSV and Lab formats. RGB values contained in a pixel can be transformed into the CIE XYZ color space via 3x3 matrix transformation process. This transformation involves tristimulus values, ie an arrangement of the three components of light - linear which fulfill the function CIE color matching. In the XYZ color space, several a color is represented as a value that is always positive. Calculations for
the transformation of the color space RGB to XYZ (with white reference value), is through the calculation of the transformation matrix as in the following equation:

\[
\begin{bmatrix}
X \\
Y \\
Z \\
\end{bmatrix} = \begin{bmatrix}
0.412453 & 0.35758 & 0.180423 \\
0.212671 & 0.715160 & 0.072193 \\
0.019334 & 0.119193 & 0.950227 \\
\end{bmatrix} \begin{bmatrix}
R \\
G \\
B \\
\end{bmatrix}
\]

(1)

Color Space L*a*b* or what is known as CIELAB is the most complete color space established by the International Commission on illumination color (French Commission Internationale de l'eclairage, known as CIE). This color space is capable depicts all visible colors with human eye and is often used as a reference color space. Color space conversion calculation from XYZ to L*a*b* based on the following equation:

\[
L^* = 116 \left( \frac{Y}{Y_n} \right)^{1/3} - 16, \text{ for } \frac{Y}{Y_n} > 0.008856
\]

(2)

\[
L^* = 903.3 \frac{Y}{Y_n} \text{ then } a^* = 500 \left( f \left( \frac{X}{X_n} \right) - f \left( \frac{Y}{Y_n} \right) \right)
\]

(3)

\[
b^* = 200 \left( f \left( \frac{Y}{Y_n} \right) - f \left( \frac{Z}{Z_n} \right) \right) \text{ where } f \left( t \right) = t^{1/3} \text{ for } t > 0.008856
\]

(4)

\[
f \left( t \right) = 7.787t + \frac{16}{116}
\]

(5)

There are delta rates that can be used on a scale CIELAB colors. \(\Delta L^*, \Delta a^*, \Delta b^*\) indicate how much much difference between standard and sample with another. Delta price can be used as a control quality or equation setting. Tolerance values can be determined from the delta price. If the delta value is more than given the tolerance value, then there is a difference which is far between the image of the standard object with the image sample, so some type of correction is required if delta value out of set tolerance. As for example, if the price \(\Delta a^*\) is out of tolerance, then red or green color intensity requires adjustment back.

3.3.3 Statistic Analysis

Statistical analysis was performed on values Average RGB and Lab values. Statistical analysis used is the one-way variance test with a significance level of 95% and will proceed to the Duncan Multiple Range test Test (DMRT) if proven different real [22].

\[
\text{DMRT} \alpha = \frac{R \left( p; df \text{ galat} ; a \right)}{\sqrt{K \text{ Galat} \frac{r}{p}}}
\]

(6)

4. Results and discussion

The result of research as following a preference test (hedonic) components:

4.1. RGB

The resulting RGB analysis can be seen in Figure 2. Figure 2 showed various RGB levels can be seen according to the histogram.
Figure 2. RGB Color Histogram

From the histogram above can be seen for the average RGB value of R = 65.29 while for control is 58,42. The average value for parameter G was 66.05 with a control variable of 61.43, for parameter G an average of 28.49 with the control variable 29.76. The results of the calculation of RGB values can be seen in Table 2.

| No | Variation   | Score          |   |
|----|-------------|----------------|---|
|    |             | R     | G     | B   |
| 1  | a0b0 (control) | 58,42 | 61,43 | 29,76 |
| 2  | a0b1        | 52,35 | 48,31 | 24,62 |
| 3  | a0b2        | 63,76 | 61,32 | 25,54 |
| 4  | a0b3        | 70,86 | 64,81 | 29,63 |
| 5  | a1b0        | 68,3  | 68,23 | 28,72 |
| 6  | a1b1        | 64,21 | 65,82 | 26,06 |
| 7  | a1b2        | 67,28 | 77,02 | 27,56 |
| 8  | a1b3        | 63,41 | 62,36 | 26,78 |
| 9  | a2b0        | 69,58 | 63,3  | 29   |
| 10 | a2b1        | 69,24 | 76,32 | 28,49 |
| 11 | a2b2        | 63,92 | 68,94 | 30,84 |
| 12 | a2b3        | 65,36 | 70,11 | 36,22 |

RGB color analysis shows if each treatment is not too different. This means that the ozone washing treatment carried out can retain the edamame skin color as the control variable [23].

One-way variance test results on RGB data for each replication with the 95% significance level provides conclusions The significance value is above 0.05, so that H0 is accepted and H1 is rejected. Multiple Duncan test results Range Test (DMRT) in Figure 3 as well shows that the difference in ozone time and contact time does not have a significant effect on the difference in the color of the edamame skin, both with the RGB value.

Figure 3. Interpretation of the standard deviation value and the level of significance of the variation

4.2. Lab Value

The approach taken is to choose small sample area for each color and for calculate the average color of each sample area in a * b * space, use this color marker to classify each pixel. Color XYZ uses three filters X (red), Y (green), and Z (blue). L * a * b *. Lab color calculation values can be seen in Table 3.
Table 3. Lab Color Analysis

| No | Variation | L     | a     | b     |
|----|-----------|-------|-------|-------|
| 1  | a0b0 (control) | 24.92 | -7.02 | 18.99 |
| 2  | a0b1      | 19.83 | -2.35 | 18.19 |
| 3  | a0b2      | 25.34 | -4.77 | 22.13 |
| 4  | a0b3      | 27.2  | -2.96 | 22.19 |
| 5  | a1b0      | 28.08 | -6.45 | 23.63 |
| 6  | a1b1      | 26.87 | -7.27 | 23.62 |
| 7  | a1b2      | 30.87 | -12.28 | 27.42 |
| 8  | a1b3      | 25.66 | -5.46 | 21.78 |
| 9  | a2b0      | 26.63 | -2.72 | 21.78 |
| 10 | a2b1      | 30.82 | -10.85 | 26.92 |
| 11 | a2b2      | 27.94 | -8.79 | 22.14 |
| 12 | a2b3      | 28.54 | -8.14 | 19.71 |

The results of the Lab analysis, statistical analysis tests were carried out to determine whether there was an effect of ozone washing results. Statistical analysis of variance test resulted in an error value above 0.05 or 5%. This shows if H0 is accepted and H1 is rejected (Figure 4).

The results show that ozone leaching does not affect the Lab value. This shows that washing can be applied to edamame because it can maintain the fresh color of edamame skin which is one of the parameters of export sales. Edamame skin color looks still green and fresh with the same odor as the original raw material before ozone washing.

5. Conclusion
The results of the research from the washing contact time and ozone time treatment gave identical results with the treatment without washing.

6. Acknowledgments
Our thank goes to Politeknik Negeri Jember, who has funded this research via PNBP grant.

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