Optimization of gaseous ozone application in reducing total amount of microorganism in Muntok white pepper

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Abstract. Muntok white pepper with export quality has a high economic value. One of the standard quality required for white pepper is total microorganism (TPC). However, Muntok white pepper produced from traditional postharvest method contains high number of total microorganism, thus not fulfilled the standard quality required. Ozone is a potent oxidant that is trusted to be an effective antimicrobial sanitizer for water, food, and food processing surfaces. In the current research, Response Surface Modelling (RSM) was employed to investigate the optimum condition of ozonation, consist of: ozone flowrate (1 – 4 L/minute) and exposure time (10 – 40 minutes) with 5 minutes of ozone input in each treatment. The response obtained is the maximum reduction of total microorganism (TPC) followed by the validation of the optimum condition of ozonation. The use of low flowrate with long ozone exposure time will deliver the lowest TPC value. The application of ozone at the condition of 1 L/min flowrate in 10.85 minutes of exposure time has resulted the TPC value of $4.6 \times 10^2 - 4.95 \times 10^2$ CFU/g with the highest validity level of 85.11%

1. Introduction

Indonesia is the second biggest pepper exporter in the world, right after Vietnam. Over 25.000 metric tons of white pepper produced in Indonesia. Around 20,000 metric tons of white pepper has exported from Indonesia on 2019 to some important destination including USA, India, Singapore, Netherlands and Germany [1]. One of the best quality and well-known white pepper comes from Muntok, BangkaBelitung, which is then called Muntok white pepper. This high opportunity should be followed by the quality standards required.

The quality standard requires some parameters for white pepper, including total microorganism (TPC). The USFDA [4] expects the total microorganism in spices should be less than $10^6$ CFU/gram. On the other hand, it is observed that total microorganism (TPC) of Muntok white pepper is $12 - 70 \times 10^6$ CFU/gram [2]. The high value of TPC can be affected by the traditional postharvest process which is not pay attention to sanitation and hygiene. To peel off the pepper berries fruit, it is usually macerated in the water for 7 – 15 days which causes serious water pollution that affecting the quality of pepper [3]. After peeling, the pepper is sundried that conducted in the open, leaving it prone to the influence of microorganisms from surrounding. Since fumigation using ethylene oxide and propylene oxide for sanitation on food is not approved [5] and irradiation in spices might produce radiolysis
products that can migrates into food [6], it is necessary to find the possible technology for reducing the high number of total microorganism in white pepper.

In 1997, the U.S. expert panel has confirmed ozone as a GRAS (Generally recognize as safe) substances [7], also regarded by Food and Drug Administration in United States as an antimicrobial agent in foods [4]. Ozone is mainly used as disinfectant on food industry due to the high oxidizing properties without any residue and unfavorable effects [8]. The effect of gaseous ozone on reducing some types of microorganism has studied and applied to other dried food product, such as oregano, figs, peanut grains, pistachios [6, 9, 10, 11].

There are few literature explaining the effect of gaseous ozone to total amount of microorganism in white pepper. It is reported that the gaseous ozone concentration of 6.7 ppm for 60 minutes effectively reduced 3 log numbers CFU/g of the microbial population to ground black pepper [12]. The use of gaseous ozone with low concentration and longer duration of ozonation was found to be effective to inactivate E. Coli in whole and ground black peppers without alteration of the organoleptic properties [13]. Therefore, it is necessary to optimize the gaseous ozone dosage applied to whole white pepper for the maximum reduction of total amount of microorganism.

Response Surface Modelling (RSM) was employed to investigate the optimum condition of ozonation. The RSM will suggest the modelling ozone gas treatment effectively as the other previous studies about gas ozone treatment [14]. The objective of this study were (a) to determine the effects of ozone gas flowrate and exposure time on reducing the total amount of microorganism on Muntok white pepper; (b) to develop a mathematical predictive model for optimizing the ozone treatment for Muntok white pepper using RSM.

2. Materials and methods

2.1. Experimental design, data analysis and response surface modelling (RSM)

The effects of 2 factors (ozone flowrate and exposure time) on the reducing total amount of microorganism were studied using RSM and software of Design Expert (10.0.0) was employed. Based on the technical factor in the laboratory and the literature studied, the ranges of 2 factors being studied were determined to be: 1 to 4 L/minute ozone flowrate and 10 to 40 minutes exposure time. A 2-factor Central Composite Design experimental plan (13 experiments) was used (table 1). The measured responses was the value of Total microorganism. The 13 experiments were run in a random order.

2.2. Preparation of sample and ozone concentration analysis

The Muntok white pepper were harvested from Bangka (Bangka Belitung). The Muntok white pepper then double-washed and produced by PT Cinquer Agro Nusantara (Tegalluar, Kabupaten Bandung, Indonesia). The ozone treatment were performed in a gas-tight stainless steel cylinder chamber (60 cm of height and 40 cm of diameters). The ozonizer used wascorona discharge ozone generator (LUSO OZ-5G). Output of ozone was controlled and adjusted by microcontroller with interface through computer, designed by Physics Instrumentation Laboratory, Padjadjaran University (Sumedang, Indonesia). The data input was flowrate (L/minute) and time (second). The ozone concentration was analysed with iodometric method [15]. The concentration of ozone recorded after 5 minutes treatment were 34.27 mg/L on 1 L/min ozone flowrate; 24.67 mg/L on 2 L/min ozone flowrate; 19.97 mg/L on 3 L/min ozone flowrate; and 16.27 mg/L on 4 L/min ozone flowrate.

2.3. Application of ozone to white pepper

The sample of Muntok white pepper weighed for 25 ± 0.05 grams and then put into the sterilized chamber. The other 25 ± 0.05 grams white pepper sample was analysed with total microorganism method to know the TPC value of sample before treated by ozone (control). The chamber was sterilized by alcohol 75% and ozone treatment for 1 minute. The effectiveness of ozonation treatment
on inactivation of microorganisms in foods can be affected by ozone concentration and exposure time [9]. In this study, ozone treatment was performed at ambient laboratory condition (28 ± 1 °C).

| Run | Flowrate (L/min) | Time (Minute) | TPC value (CFU/g) |
|-----|------------------|---------------|-----------------|
| 0   | Without gaseous ozone |               | 7.61 x 10^7     |
| 1   | 2.5              | 25            | 1.08 x 10^3     |
| 2   | 2.5              | 25            | 5.00 x 10^2     |
| 3   | 1                | 10            | 4.00 x 10^2     |
| 4   | 4                | 10            | 3.00 x 10^3     |
| 5   | 2.5              | 46.7          | 3.23 x 10^3     |
| 6   | 4                | 40            | 2.60 x 10^3     |
| 7   | 2.5              | 25            | 6.23 x 10^2     |
| 8   | 0.38             | 25            | 1.50 x 10^2     |

2.4. Microbiology analysis

The microbial analysis were enumerated by the standard plate count procedure. Nonozonized white pepper and ozonized white pepper samples (25 ± 0.05 grams each) were diluted with 225 mL of sterile peptone water (0.1%, w/v) in erlenmeyer glass and homogenized with shaker at medium speed for 2 minutes. Decimal dilutions were prepared in peptone water solution, and they were plated out by the pour plate technique using standard plate count agar (PCA). The plates than incubated at 37 °C for 42 hours.

3. Result and discussion

3.1. Model fitting

The effects of gaseous ozone flowrate (1 to 4 L/min) and time exposure (min) in reducing the total amount of microorganism were studied using Response Surface Modelling. A mathematical model was developed to predict the number of TPC as a function of ozone flowrate and time exposure. The analysis of interaction between the factors was done by considering the response results. Table 1 showed the combination of both factor variables based on Design Expert 10.0.0 with the response. The result of TPC analysis for white pepper before ozoned (control) was 7.61 x 10^7 CFU/g. Based on the USFDA standard, this TPC value was not required to be imported for consumption in USA. Based on Fit Summary analysis on table 2, both of linear model and quadratic model were significant from the Sequential p-value (p < 0.05) to fit the data of response (TPC value) to the factors and had no significant lack of fit (p > 0.05). Comparing the lack of fit p-values, the quadratic model had a better significant number. Therefore, the quadratic model adequately represented the true relationship.
Table 1. Run of experimental design and data.

| Run | Flowrate (L/min) | Time (Minute) | TPC value (CFU/g) |
|-----|-----------------|---------------|------------------|
| 9   | 2.5             | 25            | 1.15 x 10^3     |
| 10  | 2.5             | 3.79          | 2.55 x 10^3     |
| 11  | 1               | 40            | 3.00 x 10^2     |
| 12  | 4.6             | 25            | 2.88 x 10^3     |
| 13  | 2.5             | 25            | 1.60 x 10^3     |

Table 2. Model summary statistics.

| Source        | Sequential p-value | Lack of Fit p-value | Adjusted R-squared | Predicted R-squared |
|---------------|--------------------|---------------------|--------------------|---------------------|
| Linear        | 0.0100^a           | 0.0762^b            | 0.5222             | 0.2703              |
| 2FI           | 0.8621^b           | 0.0583^b            | 0.4710             | 0.2301              |
| Quadratic     | 0.0167^a           | 0.2520^b            | 0.7887             | 0.3945              |
| Cubic         | 0.5652^b           | 0.1149^b            | 0.7646             | -2.2291             |

3.2. Model analysis

As seen in Table 3, the ozone flowrate and exposure time are all statistically significant at p < 0.05, suggesting that the 2 factors will significantly affect the reduction of total amount of microorganism. The lack of fit p-value was not significant, showed that there was no discrepancies found in the model. The R-squared showed that 87.68% of data was enough to describe the interaction between both factors could affect the response significantly. The Adj R-squared showed that 78.87% of the model could describe the real condition about interaction between both factors so the model could predict the response result in the certain factor conditions.

Table 3. Analysis of variance (ANOVA)’s model of reduction TPC value by gaseous ozone.

| Source        | Sum of Squares | df | Mean Square | F Value | p-value Prob > F (95%) |
|---------------|----------------|----|-------------|---------|------------------------|
| Model         | 1.402 x 10^7   | 5  | 2.803 x 10^6| 9.96    | 0.0044^a *significant  |
| A (Flowrate)  | 9.594 x 10^6   | 1  | 9.594 x 10^6| 34.09   | 0.0006^a              |
| B (Time)      | 26641.85       | 1  | 26641.85    | 0.095   | 0.7673                 |
| AB            | 22500.00       | 1  | 22500.00    | 0.080   | 0.7856                 |
| A^2           | 77171.17       | 1  | 77171.17    | 0.27    | 0.6167                 |
| B^2           | 4.373 x 10^6   | 1  | 4.373 x 10^6| 15.54   | 0.0056^a              |
| Residual      | 1.970 x 10^6   | 7  | 2.814 x 10^5| 2.03    | 0.2520^b not significant|
| Lack of Fit   | 1.190 x 10^6   | 3  | 3.965 x 10^5| 2.03    | 0.2520^b not significant|

The factors interaction on Table 3 presented 5 variables with each of them has 1 degree of freedom (df). Those variables has 2 linear effects, 1 interaction effect, and 2 quadratic effects. The variable with Prob > F value less than 0.05 showed that the variable affected significantly to the response, the
variables were flowrate (A) and time\(^2\) (B\(^2\)). Therefore, both flowrate and time had a significant effect to the response. Ozonation process can be affected by the exposure time of ozone in contact with ingredients. The exposure time could increase the ozone solubility [17]. Exposure time of ozone could also determine the ozone concentration. The lower ozone flowrate might lead to the higher concentration, and vice versa [16].

3.3. Optimization model
The equation of optimization was known subsequently based on the analysis of variance (ANOVA). The equation of optimization (equation 1) was obtained in the form of actual values to predict the TPC value as the response from the desired flowrate and exposure time factors. The letter A described the ozone flowrate factor (L/min) and B described the ozone exposure time (min).

\[ y = 1355.78 + 579.35(A) - 164.00(B) - 3.33(AB) + 46.81(A^2) + 3.52(B^2) \] (1)

Based on the equation (1), the TPC value response was affected by all of the factors. Each factors affected the response according to the constant value in the equation. A positive constant value indicated that the value was directly proportional to the response, while the negative constant has an inverse effect on the response. Based on the equation (1), the TPC value will increase with the increase of the ozone flowrate. The TPC value will also increase with the lower duration of ozone exposure time. Therefore, the maximum TPC value reduction can be achieved with a low ozone flowrate and the longer exposure time.

These results were in accordance with previous studies that performed on different dried food products similar to white pepper. The treatment of gaseous ozone to whole and ground blackpepper was significantly affected by the different ozone concentration and exposure time [13]. The influence of gaseous ozone to reduce the microbial population maximally on dried oregano was on the highest concentration of ozone on the experiment treatments [9]. The usage of high concentration of gaseous ozone on dried figs were significantly reduced the number of \textit{B. cereus} spores for the longest time exposure in experimented, that is 360 minutes [6].

3.4. Model validation
The solution provided can also be used to validate the model produced by RSM. The model validation aims to confirm that the optimal conditions of ozonation by re-testing the prediction of optimal conditions. The RSM model was considered adequate if the optimal condition predictive value approached validation values [18]. This also determined the validity of a model in predicting response. Based on table 4, it described that the criteria of the observed factors had the same level of importance, that was 3. The value of the level of interest showed that the highest desirability value is expected to provide the best solution. The highest d value is 94.2% and provided a solution to produce the lowest response (TPC value).

| Table 4. Optimization criteria and the solution of ozonation process to reduce TPC value. |
|---|
| Factor | Target | Minimum limit | Maximum limit | Level of importance | Solution of RSM model | Desirability (%) |
|---|---|---|---|---|---|---|
| Flowrate (L/min) | Minimize | 1 | 4 | +++ | 1 | 94.2 |
| Exposure time (minute) | Minimize | 10 | 40 | +++ | 10.85 | |
| TPC (CFU/g) | Minimize | 150 | 3230 | +++ | 581.48 | |

This optimation value needed to be validated to see if the actual conditions will get the same results with the calculation using the optimal equation model. This validation was done by reconducted the experiment using the result of the RSM solution factor and reanalysed the response, as described in table 5. The percentages of validity were high, there were 85.13% and 79.11%. All the experimental
results were fit in the predicted result ranges, verifying that the model was valid. These results suggested that ozone gas is a potential effective sanitizer for dried food materials and spices and resulted in requiring the quality standard for USFDA recommendation.

**Table 5. Validation trials of RSM.**

| Trial | Ozone flowrate (L/min) | Exposure time (min) | TPC (CFU/g) | Validity (%) |
|-------|------------------------|---------------------|-------------|--------------|
| 1     | 1                      | 10.85               | 495         | 85.13        |
|       |                        |                     | 460         | 79.11        |

4. Conclusions

Using RSM, a predictive model was successfully developed for reduction of total amount of microorganism denoted in TPC value on Muntok white pepper by gaseous ozone treatment. Statistical analysis showed that ozone gas flowrate and exposure time were all significant factors for affecting the total amount of microorganism. The maximum TPC value reduction can be achieved with a low ozone flowrate and the longer exposure time, since the lower ozone flowrate will lead to the higher ozone concentration. However, the studies related to the effect and quality changes of ozone treatment on white pepper need to be studied in the future. These results suggested that ozone gas is a potential effective sanitizer for dried food materials or spices and requiring the quality standard for USFDA recommendations.

References

[1] [IPC] International Pepper Community 2017 Country Profile: Indonesia–Pepper in Indonesia Available at: www.ipcnet.org Accesed on July 15th 2019
[2] Nurdjannah N and Hidayat T 2006 Mechanical pepper processing and its Application in East Kalimantan. Focus on Pepper (Piper nigrum L.). Journal of Pepper Industry. Focus on Pepper (Piper nigrum L.). International Pepper Community. Vol II, No.23–60 ISSN:1829–6858 Crop. 2(1)
[3] Vinod V, Kumar A, Zachariah T 2014 Isolation, characterization and identification of pericarp-degrading bacteria for the production of off-odour-free white pepper from fresh berries of Piper nigrum L. J. Applied Microbiol. 116(4) 890–902
[4] U.S. Food and Drug Administration 2001 Secondary direct food additives permitted in food for human consumption, final rule Fed. Regist. 66 33829–30
[5] ASTA, American Spice Trade Association 2011 Clean, Safe Spice: Guidance from the American Spice Trade Association (Washington DC)
[6] Akbas MY, Ozdemir M 2008 Effect Of Gaseous Ozone On Microbial Inactivation And Sensory Of Flaked Red Peppers. Int. J. Food Sci. Technol. 43 1657–62
[7] Graham D M 1997 Use of ozone for food processing Food Technol. 51(6) 72–5
[8] Novak J, Demirci A, Han Y 2008 Gas novel chemical processes: ozone, upercritical CO,
electrolyzed oxidizing water, and chlorine dioxide Food Sci. Technol. Int. 14 437–41
[9] Torlak E, Durmus S and Ulca P 2013 Efficacy Of Gaseous Ozone Against Salmonella And Microbial Population On Dried Oregano Int. J. Food Microbiol. 165 276–80
[10] Ernandes R D S, Faroni L R D, Martins, A M, da Costa A R and Cecon P R 2011 Decomposition Kinetics of Gaseous Ozone in Peanuts Eng. Agri. Jaboticabal 31(5) 930–9
[11] Akbas M Y and Ozdemir M 2006 Effectivenessof ozone for inactivation of E. coli and B. cereus in pistachios Int. J. Food Sci. Technol. 41 513–9
[12] Zhao J and Cranston P M 1995 Microbial decontamination of black pepper by ozone and the effect of the treatment on volatile oil constituents of the spice J. Sci. Food Agri. 68 11–8
[13] Emer Z, Akbas M Y and Ozdemir M 2008 Bactericidal Activity of Ozone Against Eschericia coli In Whole and Ground Black Pepper J. Food Protect. 71(5) 914–7
[14] Han Y, Floros J D, Linton R H, Nielsen S S and Nelson P E 2002 Response surface modeling for the inactivation of \textit{Eschericia coli} O 15:H 7 on green peppers (\textit{Capsicum annuum}) by ozone gas treatment \textit{J. Food Sci.} \textbf{67}(3) 1188–93

[15] Masschelein W J 1998 Measurement of HighOzone Concentrations in Gases by KITitration and Monitoring by UV-Absorption (Also: on the Design of Idometric WashingFlasks) \textit{Ozone: Science and Engineering} \textbf{20}(6) 489–93

[16] Syafrudin A and Novia 2013 Produksi OzondenganBahan Baku Oksigen Menggunakan AlatOzon Generator \textit{Jurnal Teknik Kimia} \textbf{19}(2) Available at: http://jtk.unsri.ac.id/index.php/jtk/article/view/132

[17] Cavalcante D A, Leite Júnior B R C, Tribst A A L and Cristianini M 2013 Improvement of the Raw Milk Microbiological Quality by Ozone Treatment \textit{Int. Food Res. J.} \textbf{20}(4) 2017–21

[18] Montgomery D C 2001 \textit{Design and Analysis of Experimental 5 th Edition.} (New York, US: John Wiley & Son)

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