The improvement of white pepper quality using ozone application

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Abstract. The processing of white pepper is still done in the traditional way so that it has low quality. To improve the quality of white pepper has been tried using the ozone application. This research aimed to study characteristics of white pepper using ozone application. The experiment designed Randomized Complete Design (CRD) with two factors and three replications. The first factor was water soaking duration (4 and 6 days). The second factor was an ozone application for 0, 20, 40 and 60 minutes using an ozone generator. The analysis performed on physical, chemical, microbiological and flavor parameters. The results showed that the best treatment was soaked for 6 days and combined with soaking in 0.25 ppm ozonated water for 20 minutes. This condition produced white pepper according to the SNI standards with characteristics: yield of 28.55 ± 0.6%, bulk density of 622.77 ± 17.8 g/l, moisture of 11.45 ± 0.5%, dark pepper berries of 0.94 ± 0.2%, foreign matter of 1.03 ± 0.20%, piperine content of 4.89 ± 0.12%, essential oil of 2.60 ± 0.02%, lightness of 35.10 ± 4.21, TPC of 1.25 x 10² CFU/g, and butanoic acid as an off-flavor were not detected.

1. Introduction

Indonesian pepper is quite famous in the world market because it has a very distinctive spicy taste. Based on the processing method, there are two types of pepper, namely white pepper and black pepper. White pepper from Indonesia is known as Muntok white pepper in the main producing area at Bangka-Belitung province, while black pepper is known as Lampung Black Pepper in the main producing area in Lampung province. Muntok white pepper has a strong distinctive aroma with a hot and soft taste, while Lampung black pepper has an aroma, taste and spiciness that bite the tip of the tongue [1].

The processing of white pepper is still traditional, with stages, including soaking, peeling, washing, and drying. At the farm level, the soaking, peeling and washing processes are generally carried out with limited and dirty water. The soaking process of peppers takes 12–14 days; prolonged soaking creates the risk of contamination by microbes resulting in off-flavor if there is no water flow into the soaking area [2].

The quality of white pepper at the farm level generally does not fulfilled export quality requirements. Off-flavor of white pepper is caused by the presence of 3-methyl indole (skatol) and 4-methyl phenol (p-cresol) compounds, in addition to 3-methyl phenol, butanoic acid, and acids 2/3-methyl butanoate. These compounds are not from the pepper, but from accumulation during the soaking process. The formation of 3-methyl indole and 4-methyl phenol compounds was still relatively low until the third day of the soaking process. These compounds began to form on the fourth day of the soaking process [3].

Efforts to improve the quality of white pepper which are reducing off-flavor have been carried out, including by replacing some of the soaking water for the pepper [4], short soaking using a peeler [5],
using the pectinase enzyme [6]. However, the results are still inefficient and ineffective both from the colour and the high price of enzymes.

Ozone application is an alternative for removing off-flavor and reducing microbial contamination in white pepper. At present, the use of ozone as an antimicrobial has been widely used in various fields of food because it does not cause changes in the chemical composition of essential oils, but it has efficiently reduced the number of contaminated microbes [7]. The relatively low ozone concentration and short contact time are sufficient to inactivate bacteria, fungi, yeast, parasites, and viruses [8]. The E. coli population in black pepper was reduced from 7.5-1.0 log CFU/g after 360 minutes at 0.1 and 0.5 ppm ozone concentrations, whereas the same microbial reduction was obtained after 240 minutes at 1.0 ppm ozone concentration [9]. The maximum allowable exposure limit is 0.1 ppm.

2. Materials and methods

1.1. Materials
The raw material used in this research was berries pepper of Natar 1 variety obtained from Sukamulya Experimental Garden, Sukabumi, West Java-Indonesia. The main equipment’s used were ozone generator, chromameter, digital balance, cabinet dryer, and other analytical equipment.

1.2. Ozon application on berries pepper
The pepper harvested with a harvesting time of 8 - 9 months, then thresed to separate berries and stalk. The pepper berries were weighed for 2 kg, for every treatment. Then, the berries were put in container and 10 L tap water, then soaked container placed at room temperature. The experiment designed Randomized Complete Design (CRD) with two factors and three replications. The first factor was soaking duration (4 and 6 days). The second factor was ozone application for 0, 20, 40- and 60-minutes soaking in ozonated water using ozone generator. After that, pepper berries were decorticated, washed, then dried at a temperature of 50-60°C for 10-12 hours.

2.3. Analysis
White pepper analysed their performance, such as moisture content [10], bulk density [10], dark colour berries [10], foreign matter [10], colour [11], a piperine content [10], an essential oil content [10], total plate count [12] [13], and off-flavor compound were carried out by GC-MS through identification of off-flavor causative compounds [14]. All data were subjected to the analysis of variance (ANOVA) using Minitab 14 version. Differences between mean values were established using Duncan’s multiple range tests at a confidence level of 95%. All experiments were performed in duplicates.

3. Results and discussion

3.1. Physical characteristics
Statistical analysis showed that the combination of soaking duration and application of ozone were significantly different for moisture content, dark pepper berries, and foreign matter, but insignificantly in yield, bulk density, and lightness (Table 1). White pepper produced from this research had a moisture content ranging from 9.85 to 11.85%, which is fulfilled of standards requirements i.e. 13%. The moisture content of white pepper is not much different from white pepper treated with a pectinase enzyme in the soaking process, ranging from 8.76 to11.82% [6]. Soaking for 6 days produced greater moisture than soaking for 4 days. The longer of soaking process will cause more water absorption in pepper berries. The dark colour berries are in accordance with the standards required for quality I, while foreign matters are at quality II. The longer the soaking, the percentage of dark colour berries also increases. In this study, during the soaking process, there was no replacement for water. Besides that, the blackish colour is also caused by the skin of the pepper, which is still hard; it means that skin not peeled properly. In the 6 days soaking, there was a tendency that the longer the ozone was soaked, the lower the dark colour berries pepper.
The foreign matter in white pepper may come from the part of the skin that is carried away with the drying process. The bulk density showed between 592.08 to 627.26 g/l, several treatments have been according to the minimum standard is 600 g/l. The low of bulk density is due to the variation in the aging of the berries pepper used. In one stalk of the pepper fruit, there is often a non-uniform level of aging or abnormal fruit growth, so that when dried it has a light weight.

| Duration of ozonated water soaking (minutes) | Yield (%) | Bulk Density (g/l) | Moisture (%) | Dark colour berries (%) | Foreign matter (%) | Lightness |
|---------------------------------------------|-----------|--------------------|--------------|------------------------|-------------------|-----------|
| Soaking for 4 days                          |           |                    |              |                        |                   |           |
| 0                                           | 30.58±0.6a| 593.85±32.1a       | 10.05±1.1a   | 0.36±0.4ab             | 1.31±0.0b         | 28.77±2.91a |
| 20                                          | 32.23±1.1a| 583.04±0.2a        | 10.10±1.0a   | 0.32±0.0a              | 1.40±0.3bc        | 29.97±4.43a |
| 40                                          | 30.72±0.1a| 592.08±12.3a       | 11.20±0.3ab  | 0.21±0.2a              | 2.18±0.5c         | 35.87±5.48a |
| 60                                          | 28.42±1.2a| 627.14±0.2a        | 9.85±0.4a    | 0.37±0.2ab             | 0.37±0.0a         | 41.75±5.37a |
| Soaking for 6 days                          |           |                    |              |                        |                   |           |
| 0                                           | 32.97±3.4a| 598.70±21.6a       | 10.10±0.4ab  | 1.05±0.3b              | 0.65±0.0ab        | 35.92±2.55a |
| 20                                          | 28.55±0.6a| 622.77±17.8a       | 11.45±0.5ab  | 0.94±0.2ab             | 1.03±0.20bc       | 35.10±4.21a |
| 40                                          | 30.30±2.2a| 627.26±16.9a       | 11.80±0.4b   | 0.58±0.2ab             | 1.01±0.22ab       | 33.35±8.76a |
| 60                                          | 26.24±1.2a| 597.04±38.4a       | 11.85±0.4b   | 0.39±0.1ab             | 1.83±0.25bc       | 24.56±14.6a |

Remark: numbers followed by the same letter on the same column are not significantly different based on Duncan's test 5%.

The difference in soaking duration between 4 and 6 days didn’t cause a significant difference in yield. Naturally, it takes 12 to 14 days of soaking duration to make the skin of the pepper soft so that is peeling off easily. Pepper skin has a tough and thick texture because it is composed of cellulose. Berries pepper skin consists of three layers of pericarp, exocarp (outermost layer), mesocarp, the middle layer that connects exocarp and endocarp which is the inner most layer [15]. The yield of white pepper produced in this study ranged from 28.42 to 32.97%. These results were not much different from the results of the research by Gophanatan et al [16] which produced white pepper yields of about 27-32% through enzymatic processes.

The difference of two days soaking durations also didn’t cause a difference in the lightness of white pepper. Based on the results of Nurdjannah's [4], the longer soaking (up to 12 days) by replacing part of the water (1/4 to 1 part) during the soaking process resulted in lighter white pepper. This is because during the soaking process of pepper berries, the skin will damaged, so the compound that causes browning will be carried away in the soaking water. Berries pepper contains tannin compounds that dissolve easily in water and cause the peppercorns to turn brown to black when in contact with oxygen. Meanwhile, the measured ozone concentration from the ozone generator was almost the same between treatments which resulted in an insignificant colour. For more details, the visual colour resulting from the processing of white pepper is illustrated in Figure 1.
3.2. Total Plate Count (TPC)

Many studies have been conducted regarding ozone as an anti-microbial. Ozone can function as an active sanitizer that can destroy all forms of microorganisms at relatively low concentrations [17]. Ozone can affect the function and activity of organelles and cellular components. Ozone produces free radicals and will influence cellular respiration by destroying the dehydrogenation enzymes in microorganisms [18].

The mechanism of inactivation of vegetative bacteria by ozone first attacks the cell surface which is the main target of the ozonation process, resulting in the degradation of unsaturated lipids in the cell membrane. When most of the cell membranes have been destroyed, there will be cell disruption which causes the cell to leak and finally cell lysis. When this reaction is not sufficient to destroy bacterial cells, ozone can penetrate into cells and oxidize other important components such as enzymes, proteins, and nucleic acids [19]. Contamination by microbes and the formation of off-flavor in the final product can occur if the soaking process is carried out for a long time in stagnant water conditions [6].

The results of this study indicate that the ozonation process tends to reduce microbial growth, from the initial amount of $2.2 \times 10^8$ to $6.1 \times 10^2$ CFU/g after 60 minutes of ozone application combined with 6 days soaking of berries pepper (Figure 2). Meanwhile, for the 4-day soaking treatment, the TPC value decreased slightly and tended to slope due to the low initial TPC value of pepper. This TPC value is low level, the desired level of microbial decontamination of spices is below 3 log CFU/g [20]. The TPC value of white pepper at the farmer level in Bangka is $2.4 \times 10^4$ [21] and $4.4 \times 10^7$ CFU/g of white pepper in East Kalimantan [7]. The TPC value is not required according to SNI, only the level of contamination/mold is required to be a maximum of 1%. The ozone measured in this study is at a concentration of 0.20 to 0.43 ppm. According to Emer et al [9], the use of ozone at a concentration of 0.1 ppm for 360 minutes is sufficient to inactivate *E. coli* in black pepper powder without changing its organoleptic properties.
3.3 Piperin and essential oils

The chemical quality of white pepper is determined by piperine and essential oil content. The piperine content of white pepper is 3.35 to 5.35%. In the 6 days soaking treatment produced higher piperine than the 4 days soaking. Increasing the soaking duration has no cause a decrease in piperine. Piperine is the main compound that gives pepper a distinctive spicy taste. This compound is a yellowish-white crystalline form and is an alkaloid from the piperidine group. It is almost insoluble in water; however, it is easily soluble in alcohol and ether [22]. The application of ozone was not changes in piperine content, but at 6 days of soaking, the use of ozone tended to reduce piperine, at 40 minutes of soaking. Meanwhile, at 60 minutes of ozone soaking, there was no decrease in piperine levels. Piperine has analgesic and antipyretic activity in mice, and shows comparable results to indomethacin as a standard drug [23], also resulting in beneficial therapeutic effects [24].

The white pepper from this research contains 2 to 2.6% essential oil. The soaking treatment and ozone application was not any increasing or decreasing the essential oil. This is because of the drying process for pepper berries uses the same method. The essential oil from pepper is a mixture of a large number of volatile chemical compounds [25]. The variation in composition is influenced by the variety, land, agro-climate, and quality of raw materials [26]. Essential oils are volatile components that contribute to flavour. The components of the essential oils of white pepper and black pepper are dominated by sesquiterpenes, including α-caryophyllene, β-caryophyllene, and β-farnesene. Dried green pepper has a better aroma than white and black pepper. This product has a high monoterpene content so that it can produce the maximum flavor. In general, the monoterpenes give a top-peppery note flavor, the sesquiterpenes give a pepper flavour, while the sesquiterpene compounds are oxygenated. Piperin and essential oil content on the variation of treatment soaking and ozone application were presented in Figure 3.

![Figure 2. TPC value from variation of treatment soaking and ozone application](image)

![Figure 3. Piperin and essential oil contents on the variation of treatment soaking and ozone application](image)
Determination of the best treatment through the ranking test showed Table 2. Based on Table 2, white pepper with 6 days of soaking, combined with 20 minutes of ozone application, is the best treatment. There are five parameters that are included in the test, they are bulk density, lightness, piperine, essential oils, and total microbial content.

**Table 2.** Ranking test of white pepper on the variation of treatment soaking and ozone application

| Duration of ozonated water soaking (minutes) | Bulk density | Lightness | Piperine | Essential oil | TPC | Total |
|--------------------------------------------|--------------|-----------|----------|---------------|-----|-------|
| Soaking for 4 days                         |              |           |          |               |     |       |
| 0                                          | 6            | 7         | 5        | 5             | 2   | 25    |
| 20                                         | 8            | 6         | 8        | 4             | 4   | 30    |
| 40                                         | 7            | 3         | 7        | 2             | 2   | 21    |
| 60                                         | 2            | 1         | 6        | 3             | 3   | 15    |
| Soaking for 6 days                         |              |           |          |               |     |       |
| 0                                          | 4            | 2         | 1        | 8             | 4   | 19    |
| 20                                         | 3            | 4         | 2        | 1             | 1   | 11    |
| 40                                         | 1            | 5         | 4        | 7             | 3   | 20    |
| 60                                         | 5            | 8         | 3        | 6             | 3   | 25    |

3.4. Off flavor Substances

Based on the results of identification with GCMS, processing the pepper without the application of ozone causes the accumulation of odor-causing compounds (butanoic acid) of 1.01% in the final product (Figure 4a). Through the application of ozone for 20 minutes was able to remove these compounds and was not detected through GCMS (Figure 4b).

Several odor-causing compounds in white pepper have been reported in Thailand, which has been identified as 3 methyl indole, 4 methyl phenol, 3 methyl phenol, and butanoic acid as the main sources of fecal odor. Odors will appear due to anaerobic degradation [3]. Pepper Berries soaked in water for a long time can result in a build-up of the above chemicals, causing an unpleasant odor [27]. The odor problem in white pepper has until now become a major problem in trade and as a result, there has been the frequent rejection by consumers.
4. Conclusions

Application of ozone on white pepper production is expected to generate high quality by reducing the number of microbes and eliminating off-flavour compounds. The best treatment was soaked for 6 days combined with 20 minutes ozone application. This condition process has fulfilled the requirement of SNI standards with a yield of 28.55 ± 0.6%, bulk density of 622.77 ± 17, g/l moisture of 11.45 ± 0.5%, dark colour berries of 0.94 ± 0.2%, foreign matter of 1.03 ± 0.20%, piperine content of 4.89 ± 0.12%, essential oil of 2.60 ± 0.02%, lightness of 35.10 ± 4.21, TPC of $1.25 \times 10^2$ CFU/g. In addition, the odor-causing compound (butanoic acid) was not detected at the end of the product.

References

[1] Anon 2019 Lampung pepper from indonesia [Online]. Available: https://www.akospice.com/pepper-from-indonesia-lampung-pepper/ [Accessed 7-Jan-2019]
[2] Winarti C, Nurdjannah N 2010 Teknologi Pengolahan Lada Putih dan Hitam Pedoman teknis. Bogor: Balai Besar Litbang Pascapanen Pertanian [In Bahasa Indonesia]
[3] Steinhaus M, Schieberle P 2007 Off-flavours in pepper production - Molecular background, formation and prevention J of the Pepper Industry. 3 1 23-33
[4] Nurdjannah N 2001 Pengaruh lama perendaman dan penggantian air terhadap mutu lada putih yang dihasilkan Prosiding Simposium Nasional II Tumbuhan Obat dan Aromatik Bogor: Pusat Penelitian dan Pengembangan Biologi-LIPI dan UNESCO 8 Oktober 2001 [In Bahasa Indonesia]
[5] Hidayat T, Risfaheri, Nurdjannah N 2002 Pengaruh perlakuan buah lada sebelum pengupasan dan kecepatan putaran piringan terhadap kinerja alat pengupas lada yang dimodifikasi Buletin Penelitian Tanaman Rempah dan Obat. 13 1 19-28
[6] Usmiati S, Nurdjannah N 2006 Pengupasan kulit buah lada dengan enzim pektinase. Jurnal Littrit 12 2 80-86
[7] Brodowska A, Smigiels K 2014 Comparison of methods of herbs and spices decontamination CHEMIK. 68 2 97–102
[8] Kim G, Yousef A, Dave S 1999 Application of ozone for enhancing the microbiological safety and quality of foods; a review J. Food Prot. 62 9 1071–1085
[9] Emer Z, Akbas M Y, Ozdemir M 2008 Bactericidal activity of ozone against Escherichia coli in whole and ground black peppers J Food Prot. 71 5 914-917
[10] SNI (Standar Nasional Indonesia) 2013 SNI 0004:2013: Lada putih Jakarta: Badan Standarisasi Nasional [In Bahasa Indonesia]
[11] Kawachi S, Suzuki Y, Uosaki Y, Tamura K 2015 Microbial reduction and quality changes in powdered white and black pepper by treatment with compressed oxygen or carbon dioxide gas Food Sci. and Technology Res. 21 1 51–57
[12] Waje C K, Kim H K, Kim K S, Todoriki S, Kwon J H 2008 Physicochemical and microbiological qualities of steamed and irradiated ground black pepper (Piper nigrum L.) J. Agric. Food Chem. 56 4592–4596

[13] Calvo L, Torres E 2010 Microbial inactivation of paprika using high-pressure CO$_2$ J. Supercrit. Fluids. 52 134–141

[14] 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. Appl. Microbiol. 116 890–902

[15] Aziz N S, Seng N S S, Razali N S M, Lim S J, Mustapha W A W 2019 A review on conventional and biotechnological approaches in white pepper production J. Sci. Food Agric. 99 6 2665–2676

[16] Gopinathan K M, Manilal M V B 2004 Pectinolytic decortication of pepper (Piper nigrum L.). Abstract J. of Food Science and Technology-Mysore. 41 1:74–77

[17] Khadre M A, Yousef A E, Kim J G 2001 Microbiological aspects of ozone applications in food: a review. J. Food Sci. 66 9 1242-1252

[18] Prabha V, Barma R D, Singh R, Madan A 2015 Ozone Technology in food processing: a review Trends in Biosciences. 8 16 4031-4047

[19] Miller F A, Silva C L M, Branda T R S 2013 A Review on Ozone-Based Treatments for Fruit and Vegetables Preservation Food Eng Rev. 5 77–106

[20] Ferrentino G, Spilimberger S 2011 High pressure carbon dioxide pasteurization of solid foods: Current knowledge and future outlooks Trends Food Sci. Technol. 22: 427–441

[21] Syakir M, Hidayat T, Maya R 2017 Karakteristik mutu lada putih butiran dan bubuk yang dihasilkan melalui pengolahan semi mekanis di tingkat petani J. Pascapanen 14 3134–143

[22] Vasavirama K, Upender M 2014 Review Article Piperine: A valuable alkaloid from piper species Int J Pharm Sci. 6 4 34-38

[23] Sabina E P, Nasreen A, Vedi M, Rasool M 2013 Analgesic, Antipyretic and Ulcerogenic Effects of Piperine: An Active Ingredient of Pepper J. of Pharm. Scie & Res. 5 10 203-206

[24] Gorgani L, Mohammadi M, Najafpour G D, Nikzad M 2017 Piperine-the bioactive compound of black pepper: From isolation to medicinal formulations Comprehensive Reviews in Food Science and Food Safety. 16 1 124-140

[25] CBI [Centre for the Promotion of Imports from developing countries] 2018 Exporting oleoresins for food to Europe. Netherlands Enterprise Agency, Netherlands Ministry of Foreign Affairs [online]. Available:https://www.cbi.eu/market-information/ natural-food-additives/oleoresins[2 August 2019]

[26] Heartwin PAD, Korikanthimath V S 2003 Processing and quality of black pepper-a review J. Spices and Aromatic Crops. 12 1 1-13.

[27] Sreekala G S, Meenakumari K S, Vigi S 2019 Microbial isolate for the production of quality white pepper (Piper nigrum L.) J. Trop. Agric. 57 2 114-121