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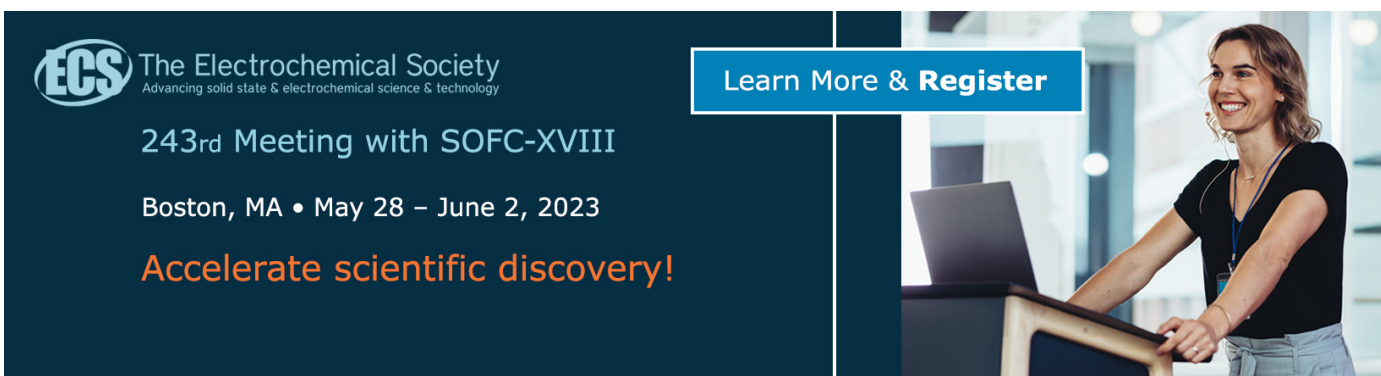
Preserving snapper spinach (*Amaranthus hybridus*) using ozone technology and cold temperature

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Preserving snapper spinach (*Amaranthus hybridus*) using ozone technology and cold temperature

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Abstract. Indonesia has abundant natural resources, one of which is agricultural products. One of the abundant agricultural products in Indonesia is snapper spinach (*Amaranthus hybridus*) which contains various kinds of nutrients. Snapper spinach can contain harmful contaminants, one of which is *E. coli* bacteria. These bacteria can cause snapper spinach to rot easily, so it needs to be removed or reduced. This study aims to determine the effect of ozone and/or cold temperatures on the shelf-life quality of snapper spinach (*Amaranthus hybridus*). The research methods include the preparation process, the washing process, the storage process (using ozone, cold temperatures, and cold ozone). The results of the study were tested using the TPC test. The best research result is that the process of adding cold temperatures after the ozone method treatment is very influential both in terms of physical and in terms of microbial contamination, seen in samples treated with ozone washed using water and stored at cold temperatures looks better than those stored at room temperature.

1. Introduction

Indonesia has abundant natural resources, one of which is agricultural products. One of the abundant agricultural products in Indonesia is spinach snapper (*Amaranthus hybridus*) which contains various kinds of nutrients. Spinach snapper can contain harmful contaminants, one of which is *E. coli* bacteria. These bacteria can cause snapper spinach to rot easily, so it needs to be removed or reduced.

Spinach snapper (*Amaranthus hybridus*) is a food that is favored by all levels of society in Indonesia, because it can provide a cold feeling in the stomach, can facilitate digestion, and contain lots of nutrients [1]. Various kinds of content in snapper spinach (*Amaranthus hybridus*) namely ascorbic acid, protein, and mineral nutrients such as Mg, Ca, Fe, K, P, and Na, which are considered as nutritional values in vegetables [2]. The content of spinach snapper (*Amaranthus hybridus*) must be maintained even though it has been stored for a long period of time.

In spinach snapper (*Amaranthus hybridus*) there are many vitamins, minerals needed by the human body. Spinach leaves contain iron that is needed by humans, and there is a high fiber content so it is very good. Fiber has a function that is not replaced by other substances in triggering physiological and metabolic conditions that can provide protection to the digestive tract [3]. Adequacy of fiber intake is now recommended higher, given the many benefits that are beneficial to the health of the body. Adequate Intake (AI) for dietary fiber for adults is 20 - 35 g/day. Spinach contains 0.8 mg/100 g of fiber [4].



In addition to the high nutritional content of spinach snapper (*Amaranthus hybridus*), there are also harmful contaminants contained in spinach vegetables traded in the market. The quality of vegetables in Indonesia is still inconsistent because the level of vegetable contaminants is still quite high. Several studies have also shown that microbial contamination in fruits and vegetables is at 10⁶-10⁷ cells/g sample, while the conditions required are 10³ cells/g sample [5].

Microbial contamination of vegetables usually comes from irrigation water that is contaminated with sewage, soil, and animal manure where the irrigation water contains a lot of *Salmonella sp*, *Escherichia Coli*, and *shigella sp*. Contamination will be higher on plant parts that are in the ground or close to the ground such as spinach [6]. Microbial contamination contained in spinach when consumed by humans can cause digestive disorders, because *Escherichia Coli* (E-Coli) is a bacterium that causes acute diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome [7]. There are several ways to deal with bacterial contaminants in snapper spinach, namely ozonation, cold temperatures, a combination of ozonation-cold temperatures.

Ozone (O₃) as a strong oxidizing agent which has a chemical potential of 2.07 eV has the potential as a disinfectant which is known to be able to kill pathogenic microorganisms such as viruses, bacteria and fungi [8]. Water containing ozone can be used to wash vegetables so that the microorganisms contained in vegetables can be reduced, without losing color, aroma, and not reducing organic compounds contained in foodstuffs and leaving no residue on vegetables so as to prolong the freshness life. In the treatment of red chilies using a cold storage temperature of 10°C and an ozone concentration of 1 ppm can make red chilies last for 14 days of storage with the color, freshness and appearance of red chilies are still good [9]. Ozonation treatment on tomatoes has been shown to extend the shelf life of freshness up to three weeks [10]. According to Rahayu [3] spinach has a shelf life of one day at room temperature (21±2°C). In the study [11] it was stated that there was a decrease in the number of *Escherichia coli* bacteria on CCA media after exposure to ozone gas (O₃) and the most effective time in killing *Escherichia coli* bacteria was 20 minutes because there were no bacterial colonies growing on 20 minutes.

In addition to preserving vegetables using ozone technology, cold temperatures can also affect preservation. At lower temperatures, the metabolism will run less than perfect or even stop because the temperature is too low. Storage at low temperatures can also prolong the life of the tissues in these foodstuffs because their respiratory activity decreases and will inhibit the activity of microorganisms. However, cold storage does not kill microbes, but only inhibits their activity, therefore, any food that is to be refrigerated must still be cleaned first [12].

In this study, ozone technology and cold temperatures will be applied for the preservation of spinach snapper (*Amaranthus hybridus*). The results of the study will be tested using the TPC test to determine the contaminants of *E. coli* bacteria contained in snapper spinach.

2. Methodology/Experimental

The materials used in this study were spinach snapper (*Amaranthus hybridus*) and aquadest. The ozone generator used in this study has an ozone gas output concentration of 400 mg/h, power consumption was 15W, and electrical requirement was AC220V±10%50Hz. The cold temperature (refrigerator) used in this study was 4 – 6 °C. The treatment of spinach samples includes Table 1 below.

Samples of research results were tested using TPC test to determine the content of *E. coli* bacteria found in spinach snapper (*Amaranthus hybridus*) before treatment and after treatment until the 7th day. The samples tested by TPC were spinach snapper (*Amaranthus hybridus*) before treatment (control) and spinach snapper (*Amaranthus hybridus*) after treatment on the 3rd day, 5th day and 7th day according to Table 1.

Table 1. Sample Treatment

Sample	Treatment
Bowl 1	Room temperature (Washed in plain water and stored at room temperature)
Bowl 2	Cold temperature (Washed using plain water and stored in cold temperature/refrigerator)
Bowl 3	Ozone (Soaked in water then drained by an ozonator with an output of 400 mg/h for 20 minutes and stored at room temperature)
Bowl 4	Ozone-cold temperature (Soaked with water then flowed by an ozonator with an output of 400 mg/h for 20 minutes and stored in a cold temperature/refrigerator)

3. Results and Discussion

In this study, samples of spinach snapper (*Amaranthus hybridus*) were tested using the TPC test, namely spinach snapper before treatment (control), days 3, 5, and 7 with the treatment in Table 1. The results can be seen in Figure 1.

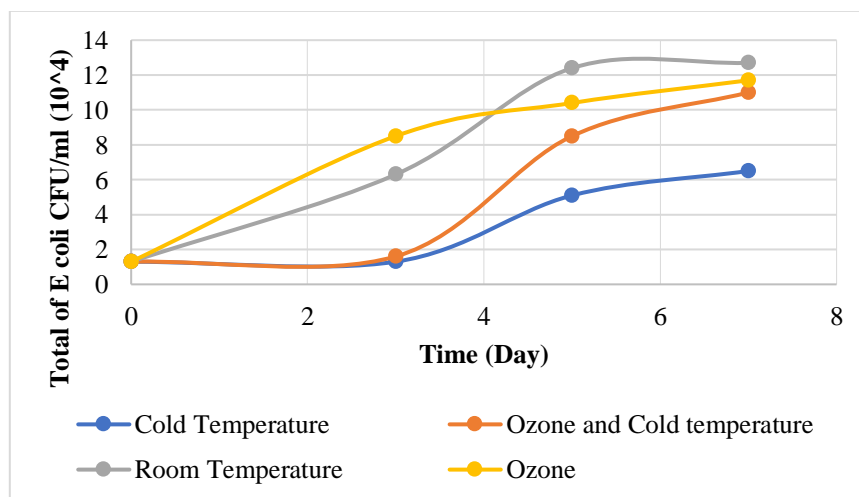


Figure 1. Number of *E. coli* bacteria in snapper spinach vegetables

In Figure 1 it can be seen that the least *E. coli* contamination was found in the spinach treatment using cold temperatures. Next is treatment using ozone and cold temperatures then treatment using ozone. The most *E. coli* contamination was at room temperature treatment.

Based on the results of the study, ozone works at room temperature effectively on days 5 and 7. This is in accordance with the statement of Ambarsari [13] that washing using ozone is quite effective in suppressing microorganisms such as fungi and bacteria carried from the land. after harvest. In other research, ozone can also kill *E. coli* in vegetable [14]. However, these microorganisms cannot be completely eliminated so there is still the possibility that microorganisms will survive and thrive.

Therefore, cold storage treatment is needed as an effort to inhibit the growth of microorganisms after the washing process.

According to the Food and Drug Supervisory Regulation No. 13 of 2019 concerning the Maximum Limit of Microbial Contamination in Processed Food, it is stated that the E-Coli bacterial contamination limit in spinach is 100 colonies/gram. So based on the results of the TPC test that has been carried out, spinach that is still fit for consumption is spinach that is washed using plain water and stored in cold temperatures on the 3rd day, and spinach that is washed using ozone water and stored in cold temperatures on the 3rd day.

Based on Figure 1, the number of *E coli* bacteria at room temperature treatment (washed with plain water and stored at room temperature) increased. This is because bacteria can penetrate the inside of spinach leaves, where cleaning only on the surface cannot overcome the problem of bacteria after washing using plain water [15]. Meanwhile, in the cold temperature treatment (washed with plain water and stored at cold temperatures), the number of *E coli* bacteria in spinach was less than the room temperature treatment. This is in accordance with research of Siburian [16] that the decay process will be faster at high temperatures and can be inhibited by storage at low temperatures. Cold storage can reduce or even destroy spoilage microbes.

Furthermore, in the treatment with ozone (washed with ozone water and stored at room temperature) the number of *E coli* bacteria increased. This is because the storage of spinach vegetables at room temperature can trigger bacterial growth because the temperature is above 30°C. Temperature can affect microorganisms in two ways, namely when the temperature rises, the metabolic rate increases and growth is accelerated, and vice versa if the temperature decreases the metabolic rate also decreases and growth is slowed [17].

Based on Figure 1, in the ozone-cold temperature treatment (washed with ozone water and stored at a cold temperature), the number of *E coli* bacteria increased. The increase in bacteria in this treatment was triggered because when placing bowl 4 to a cold temperature just below the freezer, the spinach had time to freeze. Thus, according to the statement, if food is removed from frozen storage and allowed to thaw again, the growth of spoilage microorganisms will run rapidly [18]. However, the bacteria detected in this treatment were not too many due to storage at cold temperatures which greatly affected the growth process of microorganisms.

4. Conclusion

According to the Food and Drug Supervisory Regulation No. 13 of 2019 concerning the Maximum Limit of Microbial Contamination in Processed Food, it is stated that the *E. coli* bacterial contamination limit in spinach is 100 colonies/gram. Based on these regulations, the consumption of spinach is the cold temperature treatment and the cold temperature treatment using ozone. The best research result is that the process of adding cold temperatures after the ozone method treatment is very influential both in terms of physical and in terms of microbial contamination, seen in samples treated with ozone washed using water and stored at cold temperatures looks better than those stored at room temperature.

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