

Effects of ozone-washing in a series of ozonation methods for inhibition of total microbial growth in some varieties of chili (*Capsicum annum* L.)

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Abstract. A series of ozonation processes in order to inhibit decomposition, caused by decomposing microorganisms, in some varieties of chili has been done. This study was conducted using four varieties of chili; i.e. Red Chili (RC), Curly Red Chili (CRC), Hot Chili Pepper (HCP), and Thai White Chile (TWC); treated and stored using ozonation techniques. Four steps in the series of ozonation method including dissolving ozone in water, washing the products with ozone solution, drying, and storing products in cold storage dedicated ozone. Each variety of chili was divided into two treatment groups, i.e. ozone-washed and not washed. All samples were stored in a cold storage with 15 gr/m³ ozone delivered per day. TPC testing was conducted to observe the number of microbes causing decomposition. The results showed that CRC is the most durable varieties which can stay fresh until day-71. The growth rate of microbes in ozone-washed CRC is 9,94x10³ (CFU/g)/day for TPC and 0,22x10³ (CFU/g)/day for YMC, compared to 1,36x10⁵ (CFU/g)/day for TPC and 1,35x10⁴ (CFU/g)/day for YMC in not washed product. These results showed that ozone-washed treatment can suppress the growth rate of microbes in chilies.

1. Introduction

Chili pepper (*Capsicum annum* L.) is a species of the horticulture plants which belong to the members of the family Solanaceae. Indonesia is one of the great country after India, China and Pakistan in the production of chili in the world. In Indonesia, chili is one of the essential ingredients for the consumption of the people. Consumption total of chili is expected to rise at 2016-2020 to 1.70 kg/capita caused by increased consumption of red pepper with an average of 0.75 kg/capita/year and cayenne pepper 2.77 kg/capita/year. At 2018 chili consumption is expected to total up to 3.00 kg/capita, at 2019 (3.05 kg/capita) and at 2020 (3.10 kg/capita) [1]. The chili production decline will trigger a rise in inflation. Chili has become a special commodity because of its ability to trigger high inflation [2]. Based on the Central Bureau of Statistics Indonesia in 2010, chili contributes 0.32 percent to the total national inflation amounted to 6.96 per cent in Indonesia who make chili prices soaring to record highs. In 2018 estimated surplus of 95.12 thousand tons of chili and at 2019 surplus chilies of 80.77 thousand tons until 2020 became surplus decline to 65.98 thousand tons of chili [1]. This will result in the occurrence of



deflation, in which prices generally will fall in price included chili. To stabilize the price of agricultural products especially the chili then need for technologies that are able to maintain product availability of chili in the community.

Usually, chili is harvested by farmers will be directly sold to the market. This is done because the product fresh vegetables such as chilies will fast rotting if too much long stored. If the mast is the case then the price of chilies in the market will plummet because farmers cannot keep the chili too long. One way to reduce spoilage in fresh vegetables is by washing using plain water or chlorinated water to reduce microbial contamination in whole or in part surface of fruits and vegetables that cause decay [3]. The use of pesticides on fruits and vegetables sparked concerns associated with the toxicity of pesticides on health and the environment [4]. So there is considerable interest in alternative sanitation agents against other safe and effective [5].

Ozone as antimicrobial agent has broad spectrum characteristics, strong and active against bacteria, fungi, viruses, protozoa, as well as bacterial and fungal spores [6]. Inactivation of microorganism by ozone is a complex process that attacks various components including cell membrane and the cell wall (e.g. unsaturated fats) and components of cell contents (e.g. enzymes and nucleic acids). Microorganisms are killed by disturbance in cell walls or membranes which causes leakage of the contents of the cells or cell lysis. Lysis of cell is a mechanism of inactivation are faster than the workings of other disinfectants in penetrating the cell membrane so that more effective to kill microorganisms. Mechanism of ozone destroying microorganisms through lysis of the cell doesn't cause resistance to microorganisms [7]. In addition to ozone will break down quickly into the oxygen without leaving residue. This makes the ozone as a disinfectant agent is superior than other disinfecting agent.

U.S. Food and Drug Administration (FDA) formally approved the use of antimicrobial agents as ozone for the treatment, storage and processing of food in the gaseous phase and aqueous phase of ozone. The agreement was published on June 26, 2001 [8]. In many countries, research on the use of ozone to prolong the period of storage and keeping fresh product quality has been done. In Portugal, influence of ozone treatments could reduced *Listeria innocua* counts by up to 2.8 log cfu on red bell peppers and total mesophiles on strawberries [9]. In Spain, effects of ozone postharvest treatments on quality of fresh-cut red bell peppers could Reduced mesophilic bacteria counts by up to 1 log cfu, psychrotrophic bacteria counts by up to 2.3 log cfu, yeast and moulds counts by up to 1.2 log cfu [10]. In Romania, the continuous ozonation of washing water with relatively low concentration of ozone (0.5 mg/L) gave better results on bacteria reduction [11]. In Indonesia, there aren't much research that describe about ozone to extend the storage period of minimally processed in chilies. The purpose of this research was to know the effects of ozone-washed treatment in a series ozonation method for chilies.

2. Materials and methods

2.1. *Washing the chili with ozonated water*

An early stage in the process of leaching of chili is by dissolving the ozone from the ozone generator to the water in the tub of the washing. Dissolving was done during the 120 minutes in 150 L water. Furthermore, chili peppers are washed in a tub of the washing for 15 minutes. The washing is done with conventional methods, with of soaking up all the chili water leached by ozonated water. After 15 minutes of washing, chili is drained at the table for 5 minutes to remove residual water on chili.

2.2. *Storing in cold storage dedicated ozone*

Chili were washed ozone and not washed ozone kept in cold storage dedicated ozone. Cold storage was set to temperature range 2-7 °C. During the period of storage of the chili in cold storage, ozone was applied in cold storage to keep freshness of chili and stay last longer. Ozone streamed into cold storage with the density 15 g/m³ per day for the duration of the storage.

2.3. Enumeration of microorganism

Laboratory analysis to enumerate of Total Plate Count (TPC) were performed in accordance with the Indonesian National Standard 2332.3:2015. Then enumeration of Yeast and Mould Count (YMC) were performed in accordance with the Indonesian National Standard 2332.7:2015.

2.4. Proximate analysis

Laboratory analysis to proximate test were performed in accordance with the Indonesian National Standard 01.2891:1992.

3. Results and discussion

3.1. TPC

Four varieties of chili were used in this research form Red Chili (RC), Curly Red Chili (CRC), Hot Chili Pepper (HCP), and Thai white Chile (TWC). Maximum TPC on chili were not washed ozone at day 0 before storage was observed in thai white chile a lot $9,3 \times 10^4$ CFU/g then followed by curly red chili, hot chili pepper, and red chili. Maximum TPC on chili were washed ozone at day 0 before storage was founded in curly red chili a lot $3,9 \times 10^4$ CFU/g then followed by red chili whereas thai white chile and hot chile pepper at same value as minimum TPC. After chili washed ozonated water there was a decline the number of microbes. The decline in the number of microbes most occurred on the thai white chile then followed by hot chili pepper, curly red chili and red chili.

Effect of ozone treatment on fruit and vegetables reduced *Listeria innocua* counts by up to 2.8 log cfu [9]; reduced mesophilic bacteria counts by up to 1 log cfu, psychrotrophic bacteria counts by up to 2.3 log cfu [10]; reduced mesophilic bacteria counts by up to 3.27 log cfu, coliforms counts by up to 3.66 log cfu [11].

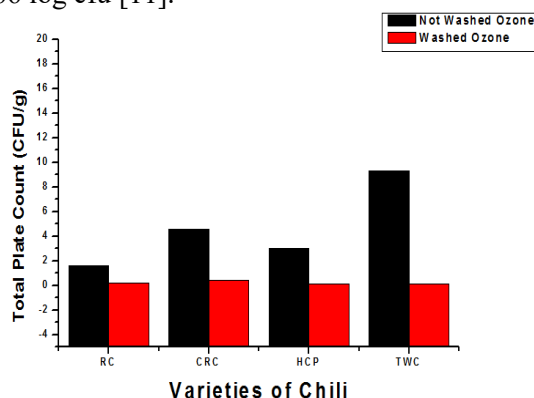


Figure 1. Total plate count on varieties of chili were not washed ozone and washed ozone at day 0 before storage; RC (red chili); CRC (curly red chili); HCP (hot chili pepper); and TWC (thai white chile); (CFU/g; $\times 10^4$).

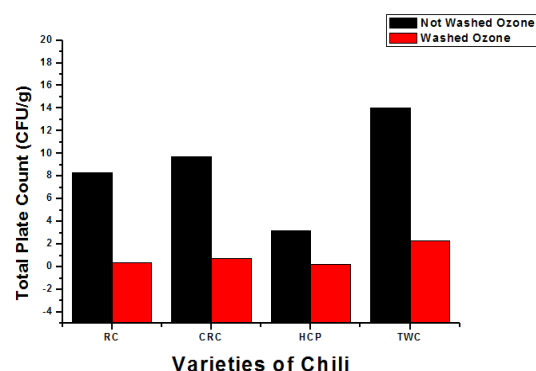


Figure 2. Total plate count on varieties of chili were not washed ozone and washed ozone at last day after storage; RC (red chili) at day 38; CRC (curly red chili) at day 71; HCP (hot chili pepper) at day 15; and TWC (thai white chile) at day 38; (CFU/g; $\times 10^6$).

3.2. YMC

Analysis of YMC showed that maximum YMC on chili were not washed ozone at day 0 before storage was observed in red chili a lot 54×10^4 CFU/g then followed by thai white chile, hot chili pepper, curly and red chili. Maximum YMC on chili were washed ozone at day 0 before storage was founded in hot chili pepper a lot 22×10^3 CFU/g then followed by red chili whereas thai white chile and curly red chili at same value as minimum YMC. After chili washed ozone there was a decline the number of microbes. The decline in the number of fungi most occurred on thai white chile then followed by red chili, hot

chili pepper, and curly red chili. Effect of ozone treatment on fruit and vegetables reduced yeast and moulds counts by up to 1.2 log cfu [10] and up to 2.02 log cfu [11].

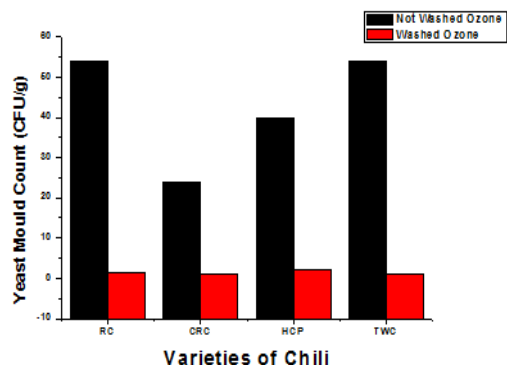


Figure 3. Yeast mould count on varieties of chili were not washed ozone and washed ozone at day 0 before storage; RC (red chili); CRC (curly red chili); HCP (hot chili pepper); and TWC (thai white chili); (CFU/g; $\times 10^2$).

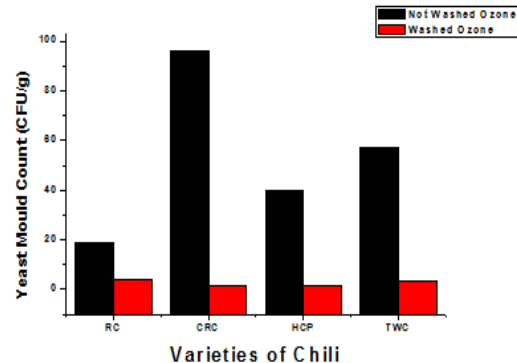


Figure 4. Yeast mould count on varieties of chili were not washed ozone and washed ozone at last day after storage; RC (red chili) at day 38; CRC (curly red chili) at day 71; HCP (hot chili pepper) at day 15; and TWC (thai white chili) at day 38; (CFU/g; $\times 10^4$).

Ozone with concentrations of 0.05 ppm [12] and, 0.115 and 0.530 ppm [13] were also tested against these fungi during cold storage. At 0.115 ppm of ozone, lesion expansion and mycelial height of both pathogens were reduced, while sclerotiums' formation was prevented. In other hands after keep cherries in cold storage from the fifth day, a 1–2 log reduction was recorded for yeasts and molds [14].

Based on TPC and YMC test, curly red chili washed ozone and kept in cold storage dedicated ozone could be stored longer than other varieties of chili that kept in same condition. Hot chili pepper washed ozone and kept in cold storage dedicated ozone had the shorter age stored more than the other of chili that kept in same condition.

3.3. Proximate analysis

During the period of storage, proxymate analysis has been done on four varieties of chili. Proxymate test includes ash content, water content, fat content, protein content, vit B content, and carbohydrate content.

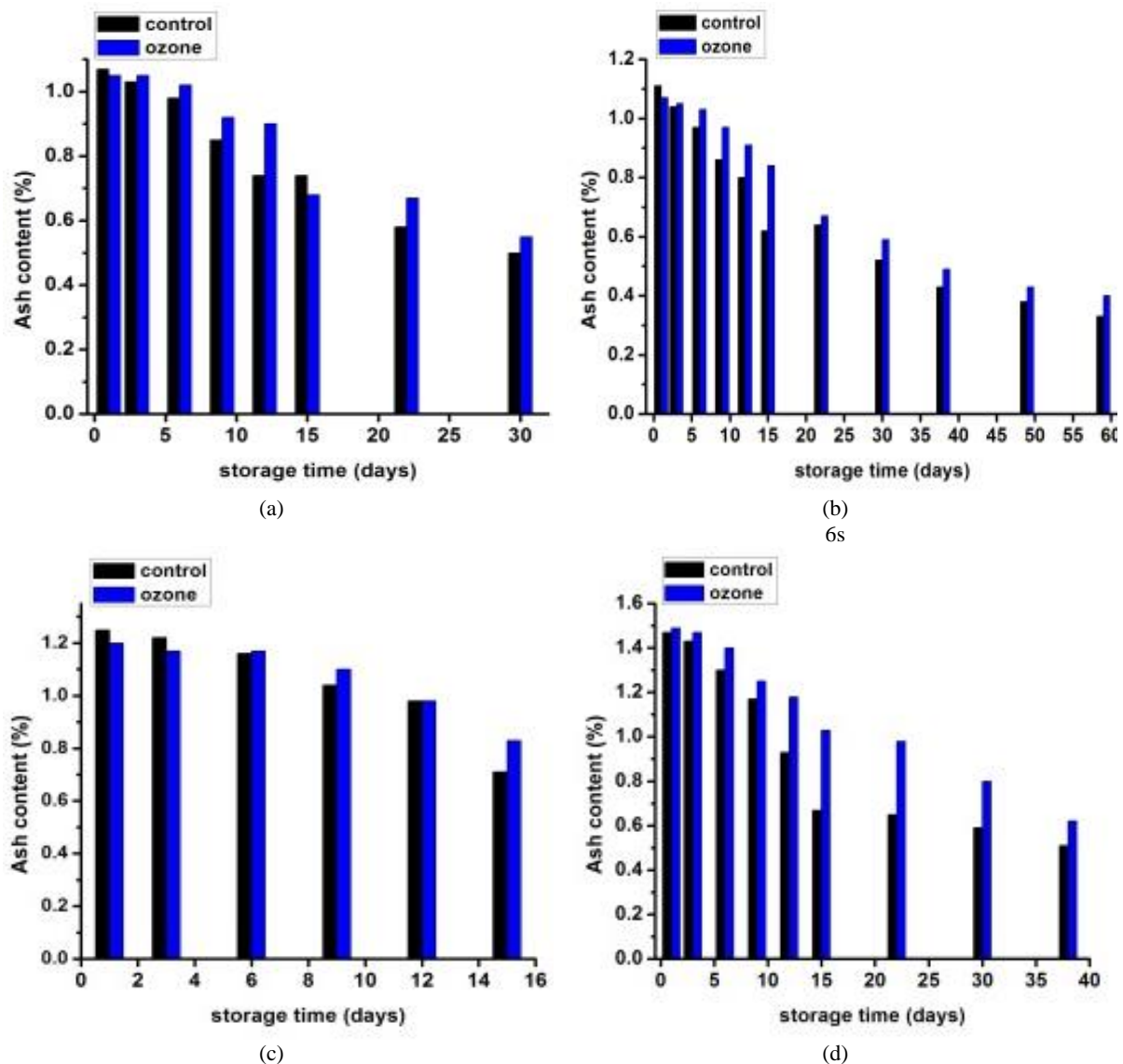


Figure 5. Ash content of chili were not washed ozone and washed ozone during the period of storage; red chili (a); curly red chili (b); hot chili pepper (c); thai white chile (d).

Maximum ash content was founded on thai white chile followed by hot chili pepper, curly red chili, and red chili at day before stored. Total mineral content is determined by the ash value. Minerals have both direct and indirect effects on human health. The direct effects of minerals focus on the consequences of their consumption on human nutrition, while the indirect effects refer to their incidence in fruit and vegetable quality and subsequent consumer acceptance [15]. In the DASH dietary pattern, vegetables contribute an average of 14.3%, 15.5%, 16.2% and 10.4% to the intakes of calcium, magnesium, potassium and zinc, respectively [16]. Graphics of ash content on chili during the period of storage can be seen in figure 5.

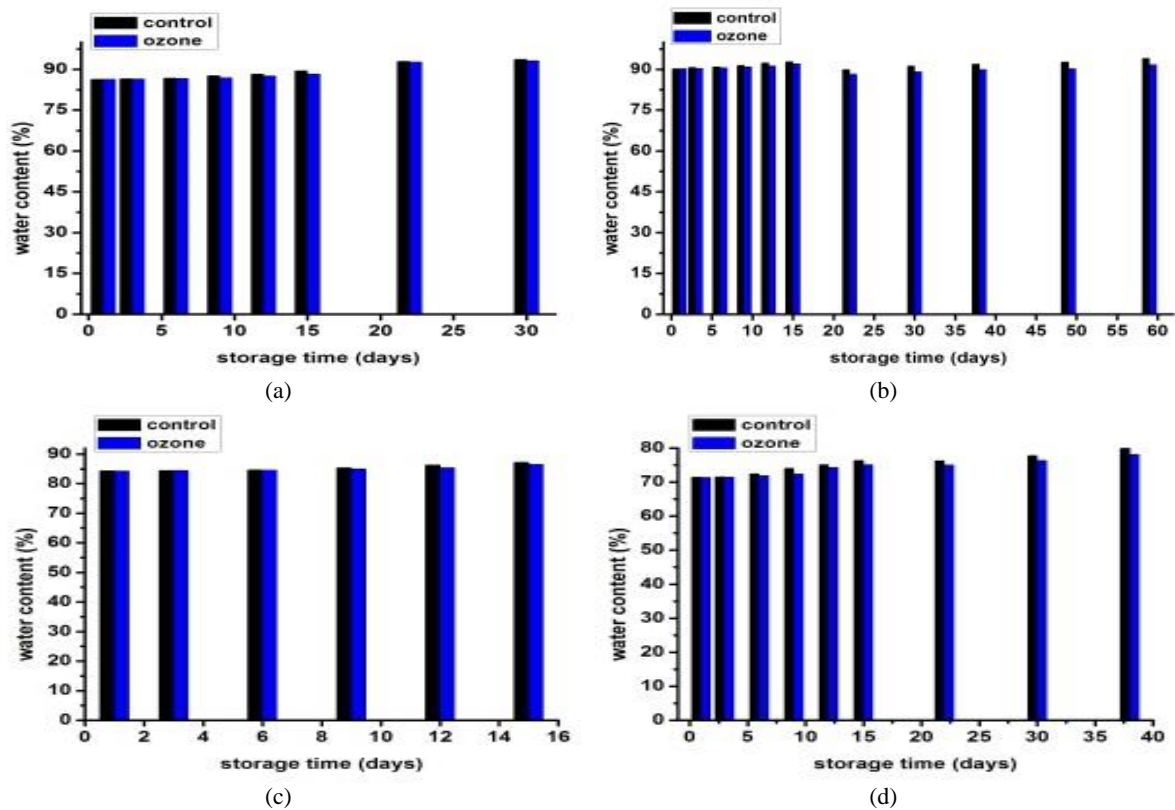


Figure 6. Water content of chili were not washed ozone and washed ozone during the period of storage; red chili (a); curly red chili (b); hot chili pepper (c); thai white chile (d).

Maximum water content was founded on curly red chili followed by hot chili pepper, red chili, and thai white chile at day before stored. The most abundant single component of fruits and vegetables is water, which may account for up to 90% of the total mass [15]. Graphics of water content on chili during the period of storage can be seen in figure 6.

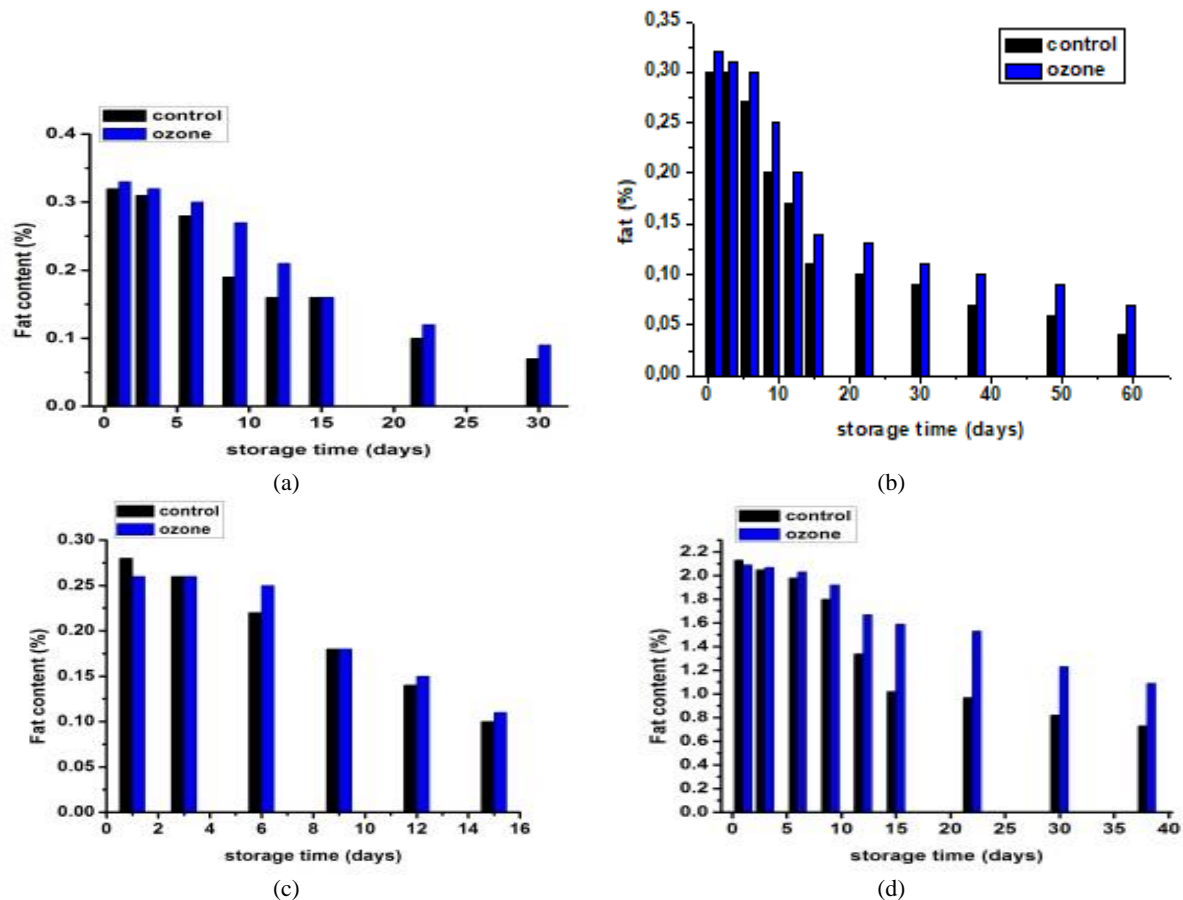


Figure 7. Fat content of chili were not washed ozone and washed ozone during the period of storage; red chili (a); curly red chili (b); hot chili pepper (c); thai white chile (d).

Maximum fat content was founded on thai white chile followed by red chili, curly red chili, and hot chili pepper at day before stored. Postharvest products are relatively low in total lipids and fat content of fruits and vegetables is usually below 1% [15]. Graphics of fat content on chili during the period of storage can be seen in figure 7.

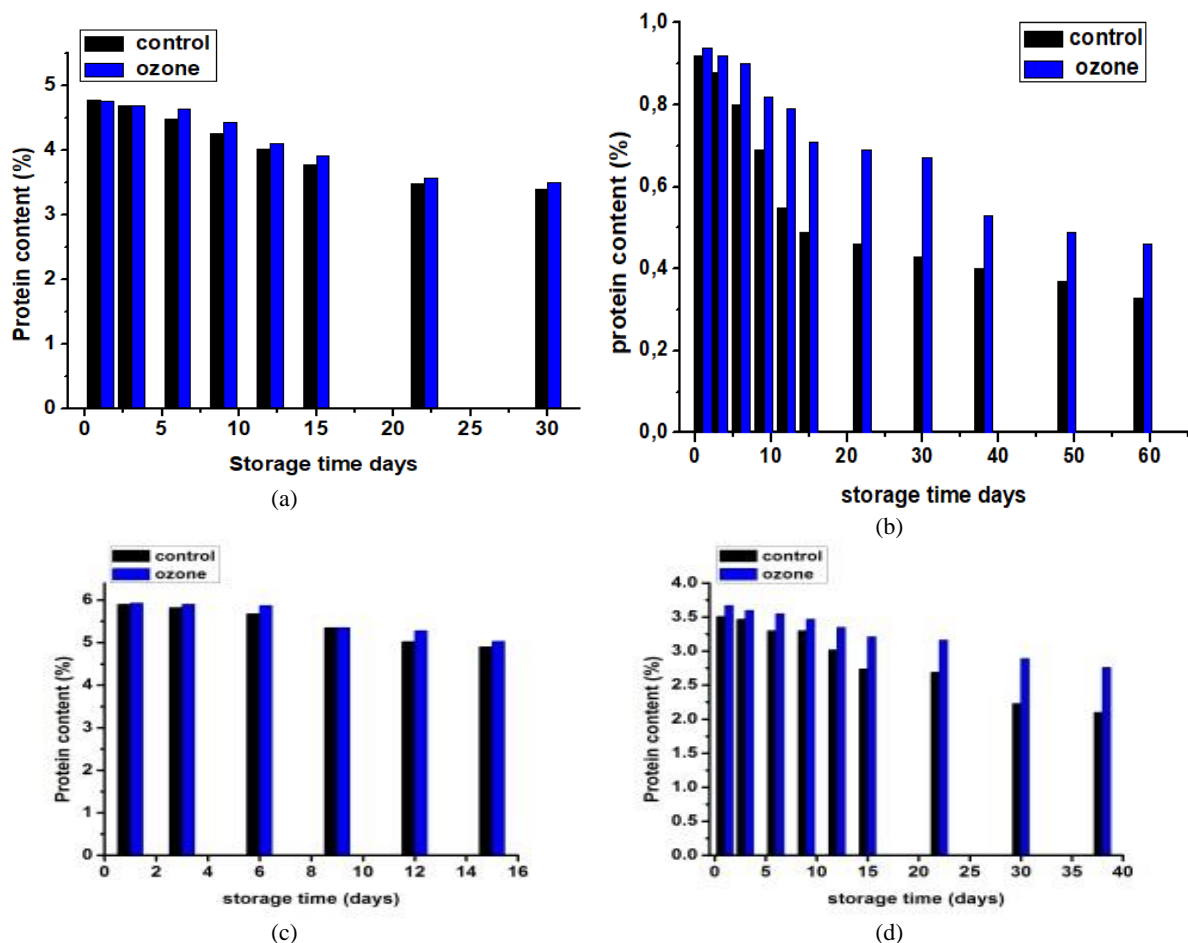


Figure 8. Protein content of chili were not washed ozone and washed ozone during the period of storage; red chili (a); curly red chili (b); hot chili pepper (c); thai white chile (d).

Maximum protein content was founded on hot chili pepper followed by red chili, thai white chile, and curly red chili at day before stored. Proteins represent less than 1% of the fresh mass of fruit and vegetable tissues [15]. Fruits and vegetables account for 1.2% and 5.5%, respectively, of the protein in the US food supply [17]. Graphics of protein content on chili during the period of storage can be seen in figure 8.

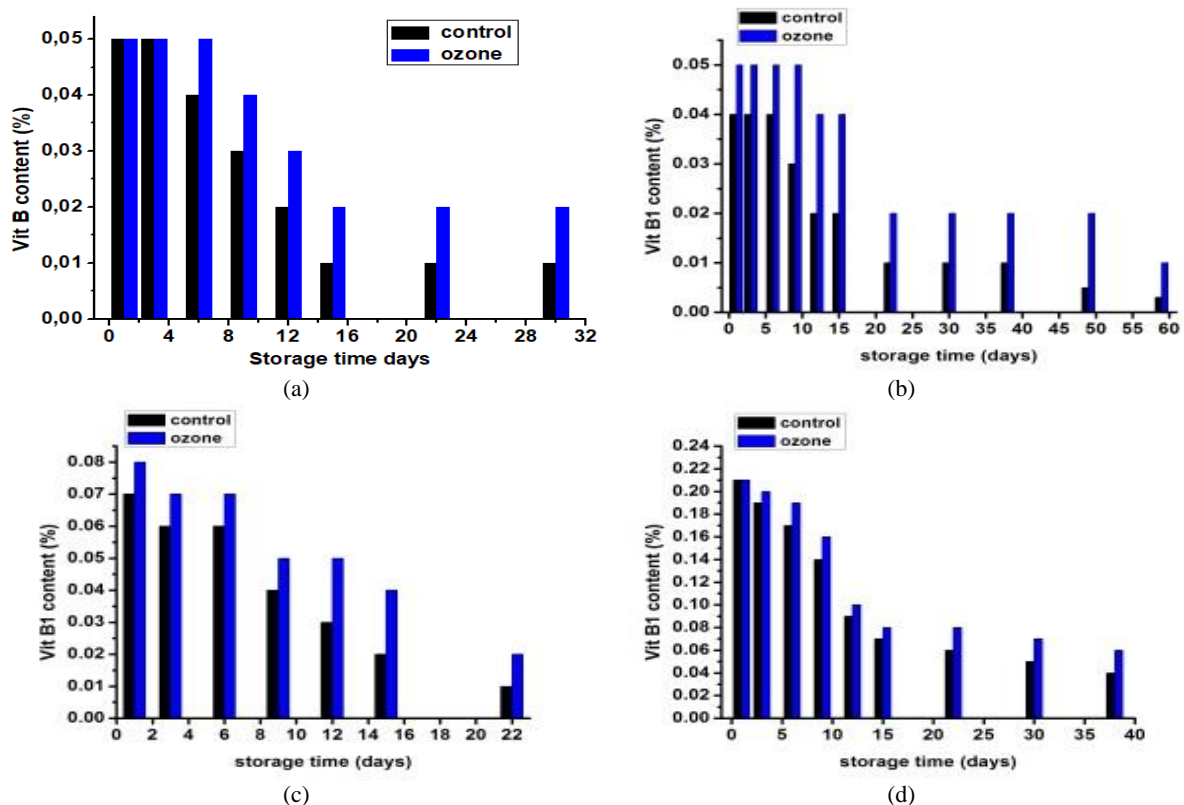


Figure 9. Vitamin B content of chili were not washed ozone and washed ozone during the period of storage; red chili (a); curly red chili (b); hot chili pepper (c); thai white chile (d).

Maximum vitamin B content was founded on thai white chile followed by hot chili pepper, red chili, and curly red chili at day before stored. Vitamins are organic molecules required in trace amounts for normal development, which cannot be synthesized in sufficient quantity by the organism and must be obtained from the diet. The vitamins present in fruits and vegetables make an important contribution to human nutrition, as they have specific functions in normal body performance. Thiamine is required in the human body for the metabolism of carbohydrates [15]. Graphics of vitamin B content on chili during the period of storage can be seen in fig. 9.

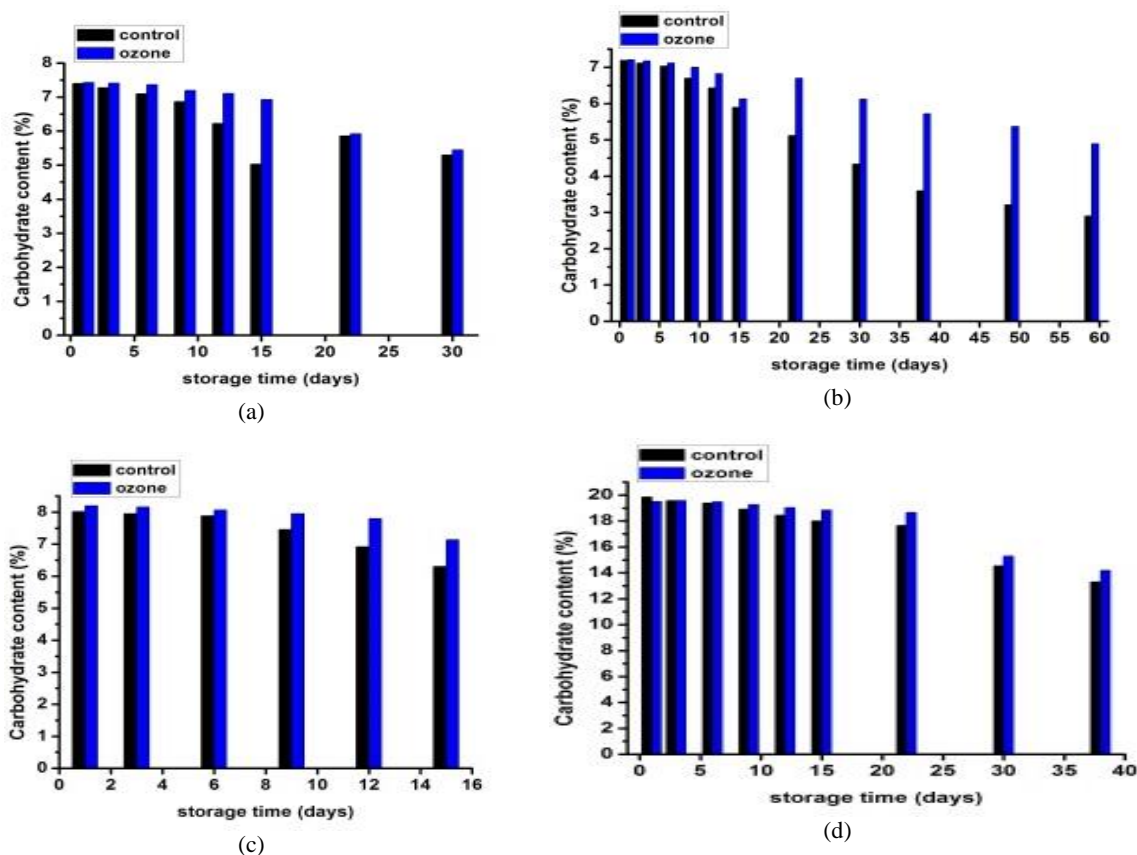


Figure 10. Carbohydrates content of chili were not washed ozone and washed ozone during the period of storage; red chili (a); curly red chili (b); hot chili pepper (c); thai white chili (d).

Maximum carbohydrates content was founded on thai white chili followed by hot chili pepper, red chili, and curly red chili at day before stored. carbohydrates are the most abundant constituents in fruits and vegetables, representing 50% to 80% of the total dry weight. Carbohydrate functions include, among others, the storage of energy reserves and the make-up of much of the structural framework of cells. Simple carbohydrates, which are also the immediate products of photosynthesis, are important components of sensorial quality attributes [15]. Graphics of carbohydrates content on chili during the period of storage can be seen in fig. 10.

Proximate test results showed that on the first treatment day before stored, the content in chili were washed ozone to lower than that in chili not washed ozone, it indicates that there were a few number of nutritional content dissolved or lost in water due to washing process. There were no much different in the nutritional content degradation between chili were washed ozone and not washed ozone during the period of storage.

4. Conclusion

Storing chili in cold storage dedicated ozone could extend the self-life of chili. Chili were washed ozone could stay last longer compared chili were not washed ozone, whereas kept in same cold storage dedicated ozone. Based on proximate analysis, there were no much different in the nutritional content degradation between chili were washed ozone and not washed ozone during the period of storage. A series of ozonation methods could inhibits the total microbial growth in chili so able to extend the self-life of chili without affect in nutritional content.

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