Conference Paper

Characteristics of Guava (*Psidium guajava* L.) Treated With Ozonation During Ambient Storage

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Abstract

Crystal guava (*Psidiumguajava* L.) is a kind of fruit which easily damaged due to various factors. Without proper treatment, crystal guava will had physical damages. The aims of this study were to determine the differences in physical, chemical, microbiological characteristics, and pesticide residue between crystal guava treated and untreated in ozone. Ozonation process was conducted by using ozonizer TIP-01 at the concentration of 1.1 ppm for 5 minutes, and stored at room temperature (25± 2 °C) observed for 6 days with 2 replicates. The explanatory methods was employed followed by t-test. The characteristics observed were color lightness, hardness, vitamin C content, moisture content, totally microorganism, and pesticide residue. The results showed that after storage for 6 days at room temperature, the ozone treatment reduced the lightness, hardness, vitamin C content, totally microorganism, and pesticide residue, while moisture content increased.

Keywords: guava; ozone; ambient storage.

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1. Introduction

Crystal guava (*Psidium guajava* L.) is one of the Indonesian horticultural products that have good market potential. The fruit is popular because it has not much seed and has a crunchy texture. The fruit is good for health because it contain higher vitamin C compared to sweet orange and lemon. Vitamin C content in crystal guava is about 87 mg/100 g, in sweet orange is 49 mg/100 g, and in lemon is 10.5 mg/100 g. Crystal guava also contain mineral such as iron, phosphate and lime.

Naturally agricultural product brought along a number of spoilage microorganisms, as well as in the crystal guava. Without proper treatment, the microorganisms can cause damage to color and texture of crystal guava. Generally microorganisms in fruits consisting of gram-negative bacteria, molds and yeasts. One of crystal guava damage due to microorganisms activities rot anthracnose due to the activiy of *Colleto*

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trichumgloeo sporoides which causes a dark patches slightly concave. The spots will expand causing decay and other physical damage. Until now the use of fungicides and synthetic pesticides are often used to prevent such damage. However, the use of fungicides and pesticides intensively will have a negative impact because it will leave chemical residues on products. Therefore it is necessary to do post-harvest handling that can reduce microorganisms and chemical residues. O'Donnel *et al.* [1] stated that post-harvest handling such as ozonation is able to reduce the number of spoilage microorganisms and can reduce chemical residues present in fruits.

Ozone (O_3) has strong oxidizing properties which able to kill microorganisms and eliminate hazardous materials, so it has good prospects as an environmentally friendly cleaning materials. Ozone can be applied during handling, storage and processing of fresh food and ingredients that have been minimally processed, either in the form of gas or solution [2]. According to O'Donnel *et al.* [1], the use of ozone in the handling of agricultural products are based on its ability to eliminate mycotoxins and pesticide residues.

Research on ozonation of cauliflower minimum processed (CMP) has been done since 2010-2013 by Setiasih *et al*. [17]. Results of the study in 2010 showed that CMP soaked in ozone water at 2 ppm concentration and packed in plastic PEDR without perforations have the longest shelf life, which was about 55 days. Results of the study in 2012 showed that the effectiveness of ozone to the reduction of pesticide residues and reduction of microorganisms increases with the addition of concentration and soaking time. The design of tool ozonation (Ozonizer TIP-01) which technically can be used for washing vegetables with minimal process in industrial packing houses scale has been done in 2013. The results showed that washing CMP by using TIP- ozonizer 01 at the ozone concentration of 1.9 ppm and 5 minutes soaking time could reduce 59.93% of pesticide residues and reduce 46.30% of total number of microoganisms.

Technically ozonizer TIP-01 can be used for washing vegetables minimally processed at packing house scale. In order to optimize the use of ozonizer TIP-01, so in 2015/2015 the tools tested on crystal guava assisted farmers cultivated at Faculty of Agriculture, Padjadjaran University.

The aim of this study was to determine the influence of ozonation on the brightness of color, hardness, vitamin C content, water content, total microorganisms, and pesticide residues crystal guava ozone treated using ozonizer TIP-01 and stored for 6 days at room temperature (25 ± 2°C).

2. Materials and Method

The research was conducted in the laboratory of Department of Food Industrial Technology, Faculty of Agro-industrial Technology, Padjadjaran University. The main material was crystal guava obtained from assisted farmers cultivated at Faculty of Agriculture, Padjadjaran University in Cipinang village Banjaran, Bandung regency, with fruits aged 3 months and hadaverage weight of 250 ± 25 g. Other material were chemicals for analyses.

The explanatory methods was employed followed by t-test to test the difference between crystal guava without ozonation (CGUO) and crystal guavatreated by ozone (CGTO). Ozone concentrations used were 1.1 ppm and 5 minutes soaking time (the result of prelimenary research). The characteristics observed were color lightness, hardness, vitamin C content, moisture content, total number of microorganism, and pesticide residue [3].

The research conducted in several stages are as follows: First step was preparation and selection of crystal guava corresponding to the desired specifications. Second step was grouping of crystal guava into two groups. One group of crystal guava put into the washing bath ozonizer TIP-01, and then passed through an ozone water at 1.1 ppm concentration until all the crystal guava submerged and left for 5 minutes. After 5 minutes, crystal guava drained with a spinner for 2 minutes. Crystal guava which have ozone treatment (CGTO) and did not have ozone treatment (CGUO) placed separately on trays and stored at room temperature (25 ± 2°C) to be observed for 6 days with the observation interval every 2 days.

3. Result and Discussion

3.1. Brightness of Color

Color is one of the quality index of foodstuffs to be considered as a first quality parameter noted by the consumer material [4]. Value L * (Lightness) is a value that states the level of brightness on a product. Brightness values ranging from 0 (dark/black) to 100 (white/light). The data observed of CGUO and CGTO brightness during 6 days of storage at room temperature (25 ± 2°C) can be seen in Figure 1.

There were no significant different of CGUO and CGTO color brightness after 0 and 2 days of storage. After storage on 4 and 6 days, they were significantly different in brightness color where CGUO brighter than CGTO. The color of CGTO skin surface becomes brownish caused by dark spotts patches slightly concave. It showed that the surface of the fruit had tissue damage due to oxidation reaction by ozone. The results was similar with that of Reference [5] whom reported that papaya fruit treated

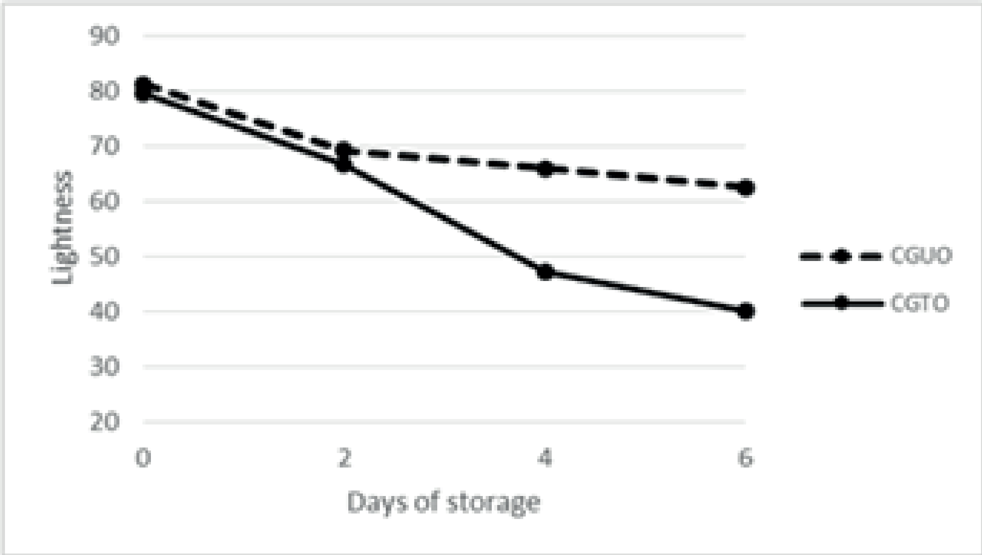

Figure 1: Brightness fruit surface during ambient storage.

with ozone at 3.5 and 5.0 μLL-1 concentrations showed brownish on surface damage, because the fruit had physiological stress due to ozone treatment.

According to Pratt and Goeschl [6], the physical and chemical stress on fresh food commodities can lead to increased ethylene production. The opinion was in line with Forney, C. F [7] which states that the broccoli treated with ozone at 700 μgL-1 concentration could increased ethylene production and browning occurs on the surface of the broccoli.

3.2. Hardness

Crystal guava stored at room temperature will have maturation process followed by decay process. The process was always accompanied by hardness decrease. Hardness measurement in this research was conducted at three different points which were top, middle and bottom of the fruit by using penetrometer. The data observed can be seen in Figure 2.

The results showed that there were not significantly different between CGUO and CGTO hardness after storage of 0, 2, 4 and 6 days. These indicated that ozonation treatment did not give dominant influence on crystal guava hardness. Nevertheless the CGUO and CGTO actually had hardness decreased. It was caused by proto pectin that were insoluble will have hydrolysis into pectin and pectinic acid that were water-soluble during fruit ripening process. As a result the cell wall weakening and decline in cohesion between cells. Protopectin changes into pectin during the ripening process of fruit will cause the fruit becomes soft [8].

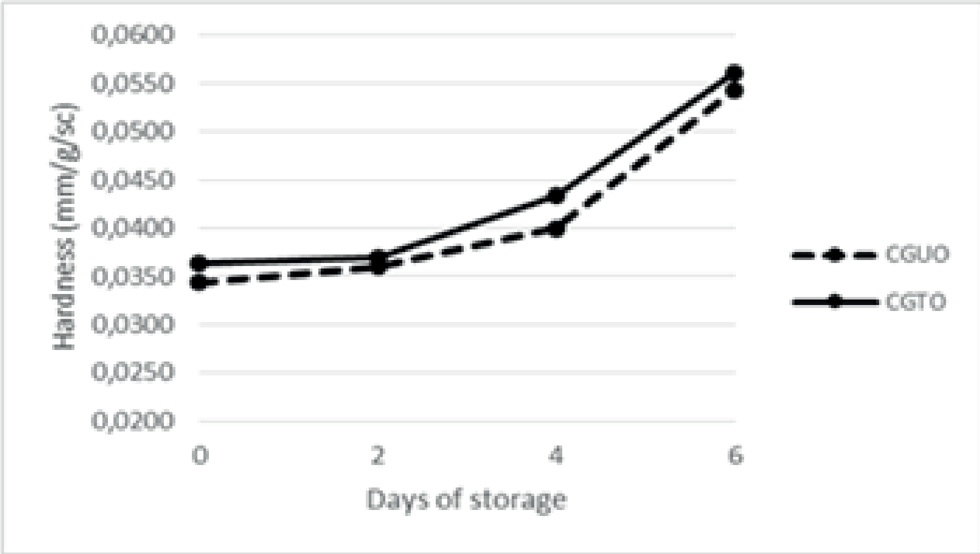

Figure 2: Hardness of fruit during ambient storage.

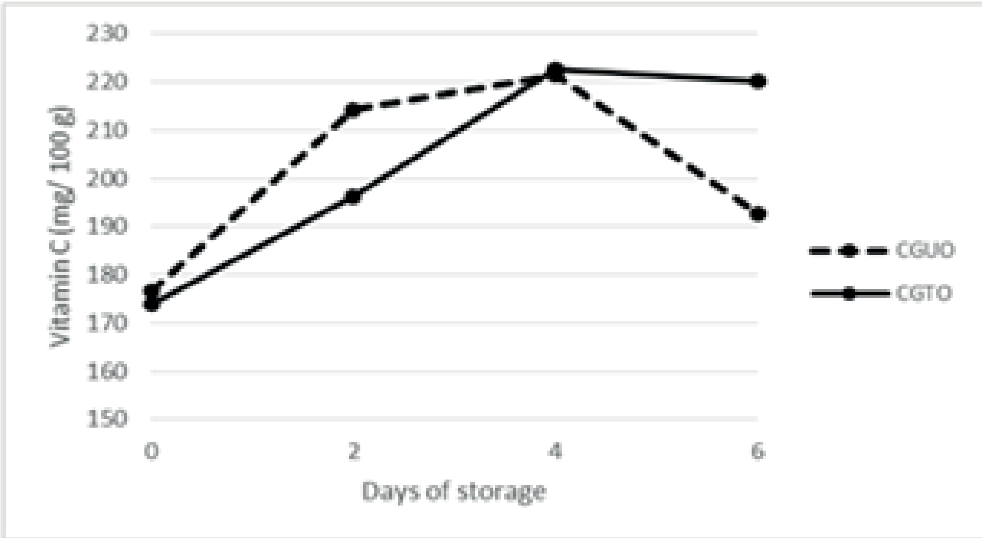

Figure 3: Vitamin C Content of fruit during ambient storage.

3.3. Vitamin C Content

The vitamin C content observed of CGUO after storage 0, 2, 4 and 6 days did not show significantly different (Figure 3). The vitamin C content of CGUO higher than CGTO after 2 days of storage. This phenomena caused by respiration process occurs normally in CGUO, whereas in CGTO the process inhibited by ozone layer water on the fruit's surface. The vitamin C content of both treatment were the same level after 4 day storage, and decrease after 6day storage. The decrease of vitamin C content of CGUO higher than CGTO.

These results were likely due to ozone only affects the exposed surface of crystal guava directly. The immersion for 5 minutes did not cause ozone penetrates into the

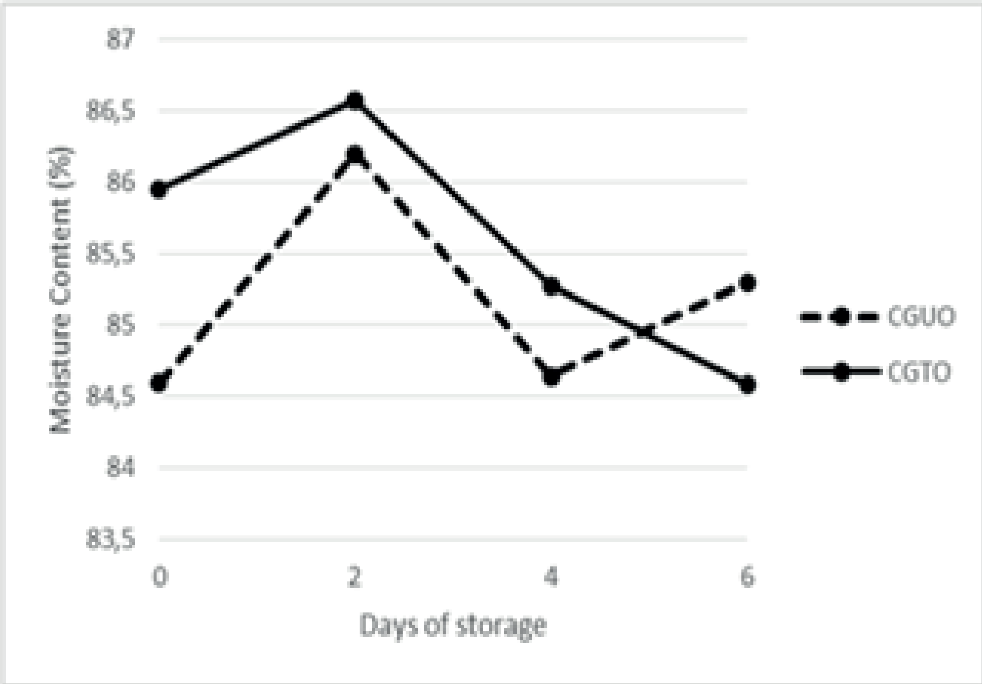

Figure 4: Water Content of CGUO and CGTO.

inside of crystal guava fruit, so the effect only on the surface of the material [1]. The amount of vitamin C content on the surface of fruit were not as much as in the inside of fruit, so only slightly vitamin C decreased caused by the ozonation. After 6 day of storage the ozonation treatment was only able to reduce 1.52% of vitamin C content.

Another factor that influence to the vitamin C content might be shape of crystal guava treated with ozone were intact, and the fruit surface did not have physical injuries, which causes vitamin C inside the fruit has not changed and was not oxidized by ozone. The results of this study were similar to the results conducted by Tzortzakis *et al*. [9] who apply ozone in gaseous form into the tomatoes at 1 ppm and 0.05 ppm concentration and stored at temperature of 13°C and 95% RH. The results showed that there were not significant difference between the vitamin C content of tomatoes treated with ozonation and that of control.

4. Water Content

Water content of CGUO and CGTO after storage 0, 2, 4 and 6days did not show significantly different (Figure 4). However on storage days 0, 2 and 4, the water content of CGUG lower than CGTO. This assumed because some ozone water could penetrates through the fruit skin during immersion of 5 minutes, thereby affecting the moisture content of the fruit.

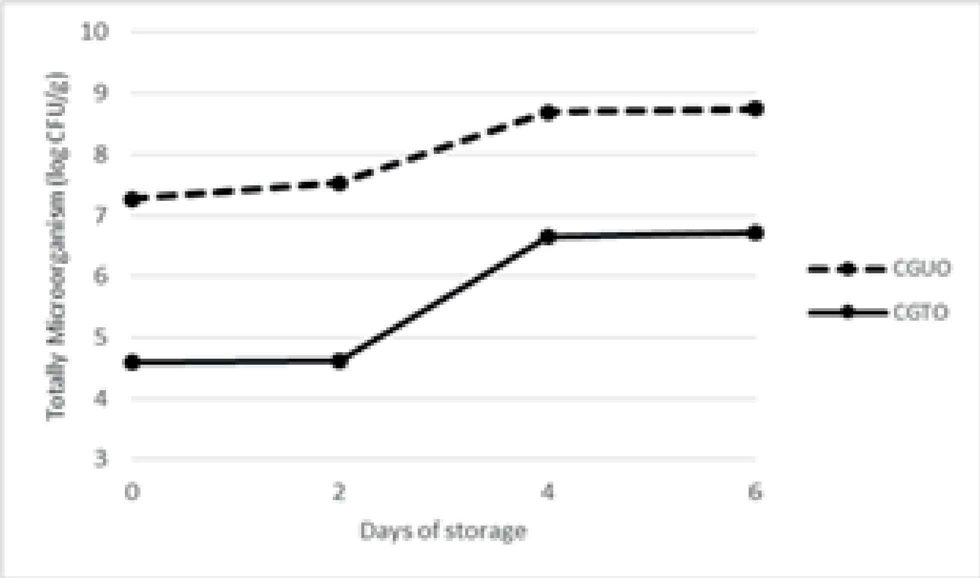

Figure 5: Total Number of Microorganism in CGUO and CGTO.

In addition it may be related to the process of transpiration occurs. On CGUO, the surface of fruit were still covered by the ozone layer thus inhibiting transpiration process. After 6 day of storage, the water content of CGUO were higher than that CGTO (increase of 1.61%).

4.1. Total Number of Microorganisms

Total number of microorganisms in CGUO and CGTO during 6 days of storage at room temperature (25 ± 2°C) can be seen in Figure 5. The data showed that total number of microorganisms of both CGUO and CGTO significantly different after storage 0, 2, 4 and 6 days.It shows that the ozonation influence to the microorganisms growth incrystal guava. Total number of microorganisms CGTO lower than CGUO during 6 days storage. Ozonation process was able to reduce the total number of microorganisms on 0-day by 2.68 log CFU/g (36.85%), and on 6-day of storage by 2.03 log CFU/g (23.17%). According to Tzortzakis *et al.* [9], the microorganism contaminants found in fruits generally were Gram-positive bacteria group such as *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus*; and Gram-negative bacteria such as *Pseudomonas aeruginosa*; class of yeasts such as *Candida albicans* and *Aspergillusniger*. Restiano *et al*. [11] also states that ozone water effectively kill various types of microorganisms such as Gram-positive bacteria, Gram-negative, yeasts and molds.

The reduction of total number of microorganisms in CGTO related to nature of ozone that can kill microorganisms through oxidation reaction on some important components in the microorganism cells. According to Miller *et al.* [12] ozone was more effective against vegetative bacteria cells than against spore-former bacteria. This was because spore-former bacteria had thick protective layer of cells, thus making the bacteria more resistant to ozone. Furthermore the mechanism of inactivation of vegetative bacteria by ozone were as follows: first, the surface of bacterial cells that become primary target attached by ozone, then degradation occurs on unsaturated lipids in the cell membrane. When most of the cell membrane had been destroyed, disruption resulting cells undergo cell leakage and eventual cell lysis. When this reaction was not sufficient to destroy the bacterial cell, ozone can penetrate into the bacteria cells and oxidize other critical components such as enzymes, proteins and nucleic acids. The initial target of ozone on *E. coli* bacterial cell was a cell membrane which in turn affects lipid and protein components.

Gram-negative bacteria were more sensitive to ozone than gram-positive bacteria. This was apparently due to gram-negative bacteria which had lipopolisacaride and lipoprotein layer that were most susceptible to damage resulting increased permeability of cells and ultimately undergo cell lysis [13]. The cell wall of Gram-positive bacteria contain 90% of the peptidoglycan layer, whereas in Gram-negative bacteria only 5-20%. Furthermore Rey *et al.* [14] states that the peptidoglycan layer containing N-acetyl Glucosamine were resistant to ozone reaction in solution phase at pH 3 to 7.

The death of bacterial cell can be caused by the damage of nucleic acids. According to Waluyo et al. [15], thymine is more sensitive to ozone than cytosine and uracil. Ozone is also able to destroy the viral RNA and change in the polypeptide chain of intracellular enzymes, furthermore ozone can oxidize proteins in cells leading to more rapid cell death. Besides being able to destroy the bacterial cell, ozone also can inactivate fungi. It might related to the integrity of cell membranes that is the target of ozonation [12].

4.2. Pesticide Residues

Pesticides sprayed on crystal guava by farmers had several active components. The active component in the pesticide were *chlorpyrifos, profenofos and tebukonazol*. Pesticides containing the active components *chlorpyrifos* and *profenofos* known as class of *organophosphate* pesticides that has usefulness as an insecticide. Tebukonazol a pesticide for rice crop and has utility as a fungicide [15]. Table 1 presented the testing results of the of pesticide residues found in CGUO and CGTO, with maximum residue limits allowed for fruit based on ISO 7313: 2008.

Active components of *chlorpyrifos* and *profenofos* not detected either in CGUO and CGTO, but tebukonazol was detected. *Chlorpyrifos* and *profenofos* were group of *organophosphate* pesticides that has usefulness as an insecticide. *Tebukonazol* is an active component of a pesticide commonly used for rice crop and has utility as a fungicide [15].

Table 1: Pesticide Residu(mg/kg) in CGUO and CGTO.

Active Component	CGUO	CGTO	Maximum Residue Limits (SNI 7313:2008)	Reduction of Pesticide Residue
Klorpirifos	ND	ND	1	0%
Profenofos	ND	ND	1	0%
Tebukonazol	0,03	0,02	0,2	33,33%
ND: not detected				

The presence of pesticide residues on fruits associated with spraying, the effectiveness and the mode of action of the active component. In general, crystal guava grown by crop farmers and sprayed with some kind of pesticide since the plants small to the plant mature and enter the productive period. Spraying conducted once a month, and focus on the leaves. Although spraying was only focused on the leaves, but the active component can also be spread throughout the whole plant. Another thing that enables to detect an active components *chlorpyrifos* and *profenofos*in crystal guava fruit because farmers usually wrap the fruit since the fruit was small by perforated plastic and spongenet until the fruit was ready to be harvested. Another purpose of wrapping the fruit that was not attacked by pests and falls due to high wind

The active component *tebukonazol* were found in CGUO at 0.03 mg/kg and in CGTO at 0.02 mg/kg. The decrease of pesticide residues with ozonation treatment was 33,33%. Ozone reactswith molecules of chemical pesticides cause chemical molecules break down into simpler and non-toxiccompounds, so the carcinogenic effects of pesticides can be reduced [16]. The results of this experiment in line with results of Khadre, *et al.* [2] which found that ozone treatment at 1.4 ppm concentration for 15 minutes at the vegetables were able to lower pesticide residues of 27-34%.

5. Conclusion

The brightness color, hardness, vitamin C content, water content, total number of microorganisms and pesticide residues of crystal guava were treated with ozone changes after 6 days of stored at room temperature (25 ± 2°C). After 6 day of storage, ozonation treatment (concentration of 1.1 ppm for 5 minutes) was able to reduce 19.48% of the brightness, 3.50% of hardness, 1.52% of vitamin C content, 23.17% of total number of microorganisms, and 33.33% of pesticide residues of crystal guava, while the water content increased by 1.61%.

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