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The effect of differences in ozonation time and storage temperature on physical, chemical, and sensory characteristics of Japanese spinach (*Spinacia oleracea* L.)

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Abstract

Japanese spinach (*Spinacia oleracea* L.) is a vegetable commodity with high economic value in Indonesia. Japanese spinach contains a lot of nutrients such as magnesium, iron, folic acid, calcium, potassium, sodium, vitamins A, B, C, and vitamin K, which are very good for health. In reducing the risk of decreased quality of postharvest Japanese spinach, the right ozonization technology and storage temperature can lead to quality maintenance of Japanese spinach. The ozonization treatment and the right storage temperature can maintain the nutritional value and some characteristics of Japanese spinach, such as its physics, chemicals, and sensory characteristics. Ozonization is used for the disinfection of spinach before storage. This study aimed to determine the effect of ozonization immersion time and storage temperature on the physical, chemical, and sensory characteristics of Japanese spinach during storage. The ozonization immersion time was 5, 10, and 15 mins. The storage temperature variations were room temperature (25°C) and cold temperature (10°C). This study was conducted using a Factorial Complete Randomized Design (CRD). The results showed that ozonization and cold temperature treatments could maintain the physical quality (weight loss and texture), chemical quality (water content and total phenolic), and sensory quality (appearance and hardness) of Japanese spinach during storage. A total of 5 min of ozonization immersion time and cold temperature storage is the most effective treatment of its water content, total phenolic, weight loss, texture, appearance, and hardness.

1. Introduction

Japanese spinach (*Spinacia oleracea* L.) is a vegetable commodity with high economic value, easy cultivation, and has a relatively large market opportunity (Arianti *et al*., 2015). Japanese spinach originated from Central Asia and became famous in Europe in the 15th century. The high nutritional value of Japanese spinach makes it a rich source of antioxidants, especially when eaten fresh, steamed or heated. Spinach has become a significant vegetable crop in most regions around the world, and major increases in output volumes have arisen in recent decades due to increased demand in many countries (Sabaghnia *et al*., 2014). It is nutrient-rich and low in price (Ren *et al*., 2018).

A leafy vegetable belonging to the goosefoot family is spinach (*Spinacia oleracea* L.). Various pharmacological activities of *Spinacia oleracea* have been documented, such as antioxidant, anti-inflammatory, antiproliferative, CNS depressant, antihistamine, gamma radiation protection, and hepatoprotective. Different secondary metabolites have been recorded from this plant, such as flavonoids, carotenoids, and phenolic compounds (Deven and Steesh, 2014). But like other horticultural products, Japanese spinach is a vegetable that is easily damaged and has a relatively short shelf life. Japanese spinach has high respiration, reaching 40-70 mL of CO_2/kg-h. Thus, it is very susceptible to quality degradation. Post-harvest handling of spinach must be done properly so that the quality of spinach can be maintained and it can be stored longer, because the quality of spinach will continue to decline due to respiration, which can remodel components in vegetables (Hakiki *et al*., 2019).

Fresh-cut fruits and vegetables have attracted much more attention worldwide over the past few decades, because of the enhanced market understanding of sensory and nutritional characteristics as well as the severe public health issues triggered by foodborne pathogenic outbreaks due to improper storage or processing. The fresh-cut industry is in desperate need of new and advanced shelf life extension technologies (Ma *et al*., 2017). Several experiments have found that only

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low ozone amounts (less than 0.5 mg/L) can destroy microorganisms in water, and even ozone can sterilize water. Ozone is a strong disinfectant, concentrations of 0.02 mg/L can be toxic to *Escherichia coli* and *Streptococcus facealis*. Ozone will react with cell protoplasm by acting as an oxidizing agent. Preservation of vegetables with ozone will not change the nutritional content because of the content of hydroxyl radicals (-OH), a free radical that has a very high oxidation potential (2.8 V), more than ozone (1.7 V) and chlorine (1.36 V). To extend the shelf-life of the vegetables, water containing ozone gas may be used to wash fruits and vegetables until they are sterile without eliminating colour, fragrance, or decaying organic compounds in food. The application of ozonization technology to tomatoes has been proven to extend the freshness of tomatoes by up to three weeks (Asgar *et al*., 2015). Ozonated-water washes are more effective at reducing microbial loads of the studied fruits and vegetables compared to simple water dipping. Washings of both deionized water and ozonated water are less susceptible to total watercress coliforms. In explaining the reduction of microbial loads, a Weibull-based model was adequate and could lead to developing more efficient sanitizing processes (Alexandre *et al*., 2011).

In addition to the ozonization treatment, it is also important to note that postharvest vegetable storage is effective in maintaining its quality. One of the factors that damages the quality of postharvest food is storage temperature. There is a real effect caused by temperature on the shelf life of agricultural products. The FAO (Food and Agriculture Organization) states that each agricultural product is vulnerable to damage or decay whenever it is exposed to high temperatures due to increased respiration rates (Babaremu *et al*., 2019). In maintaining spinach, which is a vegetable that is easy to rot, spinach is stored at a temperature of 10−15°C to keep it durable (Anakottapary, 2014). Storage temperature can affect natural maturation, injury, water loss, microorganism attack, and physical damage to decay. For a given temperature range, each increase in storage temperature can double the rate of chemical reactions (Babaremu *et al*., 2019). Therefore, the aim of this study was to determine the effect of ozonization immersion time and storage temperature on the physical characteristics such as weight loss and texture, chemical characteristics such as moisture content, phenolic content, pH, and chlorophyll, sensory characteristics such as colour, hardness, and appearance of Japanese spinach during storage.

Ozone and IR treatment completely lowered the artificial bioburden (1×10^6 CFU/mL) by < 20 min, whereas UV treatment was < 40 min. Combination therapy showed a slightly improved order of UV and IR treatment, followed by ozone, followed by UV and IR, followed by ozone, and also showed a higher efficacy of UV and IR combination than individual therapies alone. These systems can conveniently be retrofitted through the food processing line to ensure that the product is safely decontaminated prior to shipment (Watson *et al*., 2020). Studies using 1.5 ppm of aqueous ozone have found an improved activity of antimicrobials against SCFA buffers (acetic, propionic, and butyric acid) in *Staphylococcus aureus*, *Enterica*, and *Klebsiella pneumonia* (Britton *et al*., 2020). The treatments are for aqueous and gaseous ozone. It has been shown to be successful in inactivating microorganisms such as both *Escherichia coli* and *Listeria innocuous*. Therefore, inactivating micro-organisms in microbiologically high-risk products such as fresh-cut green vegetables may be recommended for these applications. In ozone applications, however, sufficient steps should be taken to ensure product safety. In addition, extensive studies are needed to clarify the complex interactions and mechanisms of the antioxidant components of ozone and leafy vegetables (Karaca and Velioglu, 2014).

UV irradiation can be considered an important method to reduce *Salmonella* exposure in eggs. The advantage of this method is that it does not have a bad effect on eggs, is easy to apply and requires lower costs than ozone treatment (Mattioli *et al*., 2020). Combination treatment greatly decreased nitrate content and preserved a higher level of storage in terms of overall soluble solids (TSS) and ascorbic acid content relative to independent or untreated therapies. Combination treatment for chlorophyll content was slightly higher than control and ClO treatments but lower than ultrasonic treatment. The findings showed that tUS and ClO together are promising alternatives for reducing nitrate content and maintaining the consistency of leafy vegetables stored (Mu *et al*., 2020). As a post-harvest action step to inactivate microbial pathogens on goods, irradiation of fresh fruits and vegetables was used. *Pseudomonas fluorescens* (Pf) had high irradiation susceptibility and spinach and Romaine lettuce differed in their populations. These findings suggest that low irradiation values, resulting in low bacterial survival, are necessary to inactivate Pf in development (Olanya *et al*., 2015). On the surface of the spinach leaves, the antimicrobial effectiveness of the blend of X-ray irradiation and citric acid (CA) against *E. coli* O157:H7 and *Listeria monocytogenes* has been studied, and the mechanisms underlying their synergistic interaction have been elucidated. Cell counts of *E. coli* and *L. monocytogenes* as treated with 0.3 kGy X-ray irradiation and 1% CA mixture were also recorded. On O157:H7 and on spinach leaves, respectively, decreased by 4.23 and 3.69 log

CFU/mL. The synergistic decrease of *E. coli* O157: H7 and *L. monocytogenes* cell counts, the dual treatments for which were 0.95 and 1.14 log units, respectively (Jeon and Ha, 2020).

Spinach leaves inoculated with three pathogens were handled separately or simultaneously with UV-A light and AA. 3.50-, 3.29-, and 4.30-log CFU mL reductions in *E. coli* O157: H7, *Salmonella enterica* serovar Typhimurium, and *L. monocytogenes*, respectively, resulted in a 90-min concurrent application of UVA and AA. which included a reduction of 2.44-, 2.21-, and 3.42 -log in CFUs, respectively, due to the synergistic effect. Four mechanistic investigations were conducted to explain the process of this synergistic bactericidal impact (Jeong and Ha, 2019). Especially, these results imply that the deposition of these two pathogen strains on spinach epicuticle layers increases significantly when cells are grown under nutrient-restricted conditions, indicating that food safety research that only includes well-nourished cells can underestimate the attachment of surface production. This disparity in adhesion can be partly attributable to increasing heterogeneity of the cell surface charge, as defined by changes in the structure of the EPS and minor changes in the total charge of the cell surface for both *E. coli* O157: H7 and *S. enterica* serovar Typhimurium (Mayton *et al*., 2019). Based on our previous research, ozone treatment and freezing temperature of chicken meat were effective in maintaining pH and inhibiting the rate of increase of TBARS and TVB-N (Prabawa *et al*., 2019). The purpose of this study was to determine the effect of variations in ozonation time and storage temperature on the physical, chemical, and sensory analysis properties of Japanese spinach (*Spinacia oleracea* L.).

2. Materials and methods

2.1 Material

The main ingredient used in this study is Japanese spinach (*Spinacia oleracea* L.), obtained from the Mutiara Organik Farmers Group, Ngablak, Magelang, Central Java, Indonesia. A Japanese spinach is selected that has fulfilled the harvest age of 35 days, there are no injuries to the leaves or stems, and the average size is uniform (about 30 cm).

2.2 Experimental design and treatments

This study used 2 factors, ozonization immersion time and storage temperature. The variations of ozonization soaking time used in this study are 5, 10, and 15 mins. The storage temperature variations used in this study are room temperature (25°C) and cold temperature (10°C). Ozonised Japanese spinach is packaged using a 10-micron plastic wrap and mica measuring 17 × 30 cm as a container and then stored for 2 days. Physical, chemical, and sensory characteristics of Japanese spinach were analyzed on the 0 and 2nd day.

2.3 Ozonization

As much as 1 kg of Japanese spinach is soaked in a reservoir filled with water with an ozone mixture using a D'ozone ozone generator. D'ozone ozone generators use a total of 6 ozone reactor types of Dielectric Barrier Discharge Plasma (DBDP), which can produce ozone at a rate of 150−500 grams/hour. Soaking is done for 5, 10, and 15 mins.

2.4 Moisture content measurement

Moisture content measurement used a gravimetric method (Liman *et al*., 2014). Japanese spinach leaves were crushed and weighed as much as 5 g. Empty porcelain plates were weighed and recorded as W0. Porcelain plates with specimens (leaves) were weighed and recorded as W1. Porcelain plates containing specimens were heated in an oven at 105°C for 24 hrs. Dry samples are cooled for 20 – 30 min under controlled conditions. The cooled sample is then weighed and recorded as W2. The moisture value is calculated using the formula.

$$\text{Moisture Content (\%)} = \frac{W1-W2}{W1-W0} \times 100$$

Where W0 = empty porcelain plate, W1 = empty porcelain plate + fresh sample and W2 = empty porcelain plate + dried sample.

2.5 Total phenolic content measurement

Total phenolic measurement was performed using the Folin-Ciocalteu method (Bajčan *et al*., 2013). A total of 0.2 mL of sample extract was dropped on a 50 mL volumetric flask and then diluted with water. Then, 2.5 mL of Folin-Ciocalteu reagent was added to the extract, which had been diluted. After 3 min, 7.5 mL of Na_2CO_3 solution was added to the mixture. Then, the sample was added with distilled water to a volume of 50 mL, homogenized, and then allowed to stand at room temperature for 2 hrs. The solution was homogenized and the absorbance was measured using a UV-visible spectrophotometer at 765 nm. The total phenolic sample is expressed as gallic acid in mg/kg of fresh sample weight.

2.6 pH measurement

The pH measurement in this study uses a pH meter. As much as 5 g of Japanese spinach sheets are crushed and mixed with 20 mL of distilled water, then homogenized using a vortex for 5 mins. The pH is calculated at room temperature (25°C) using a pH meter (PH-009-A pen-type pH meter) (Ramos *et al*., 2020).

2.7 Chlorophyll content measurement

Chlorophyll content testing used a spectrophotometric method (Asimovic *et al.*, 2016). As much as 1 g of leaves are crushed using a mortar and then extracted using 80% acetone, 100 mL, and stirred until the chlorophyll dissolves. The filtrate was measured for absorbance at wavelengths of 662 nm and 644 nm.

2.8 Weight loss measurement

Weight loss testing using weight comparison methods (Jung *et al.*, 2012). Samples were weighed on day 0 and after storage for two days. After weighing, the weight loss value is calculated using the formula:

$$\text{Weight loss (\%)} = \frac{W0 - W1}{W0} \times 100$$

Where W0 = sample weight before storage and W1 = sample weight after storage

2.9 Texture measurement

Texture measurement used Universal Testing Machine (UTM) (Borowski *et al.*, 2015). The sample was put on the UTM penetrator needle. One sheet of Japanese spinach was placed in a container penetrated to test the level of hardness of Japanese spinach leaves.

2.10 Sensory analysis

A descriptive test was used for sensory analysis based on the method of Kim *et al.* (2019). A total of 30 panellists will be asked to rate 4 sensory attributes to match the characteristics of Japanese spinach, including colour, texture, appearance, and overall. The preference rating uses a score of 1 – 5. The scale for each sensory parameter is as follows: colour = 1: reddish, 2: pale reddish, 3: reddish white, 4: pale white, 5: yellowish. Texture = 1: mushy, 2: slightly mushy, 3: slightly chewy, 4: chewy, 5: very chewy. Aroma = 1: very rotten, 2: rotten, 3: fishy, 4: slightly fishy, 5: not fishy. Overall = 1: dislike very much, 2: dislike, 3: neutral, 4: like, 5: like very much.

2.11 Statistical analysis

This study used a completely randomized factorial design (RALF) with 2 treatment factors. SPSS version 22 and One-Way ANOVA were used to analyze the collected data.

3. Results and discussion

3.1 Moisture content

The amount of water contained in a material expressed as a percentage is referred to as its water content. Moisture content can be used as a product quality measure, as it is specifically based on product storage. The moisture content is calculated by subtracting the amount of humidity lost after 24 hrs at 105±3°C (Cristina *et al.*, 2018). On the first and second days, the water content of Japanese spinach is calculated. The moisture content of Japanese spinach stored at a cold temperature is lower than the room temperature (Table 1). An increase in storage temperature can increase the water content relatively higher than a low temperature. Moisture content in the treatment of low storage temperatures and packaging can maintain freshness because the process of respiration rate is inhibited (Singh and Sagar, 2010). Respiration can increase water content due to the fact that the process of respiration can activate enzymes in material cells. Enzyme activity can increase hydrolysis to produce CO_2 and H_2O, and it can increase water content (Khan et al., 2017).

Table 1. Moisture content (%) of Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	90.796±0.6^d	89.581±0.1^d
	5	88.281±0.2^a	87.050±0.2^a
	10	88.977±0.3^b	87.691±0.2^b
	15	89.541±0.2^c	88.306±0.0^c
2	0	95.059±0.1^d	93.767±0.2^d
	5	92.348±0.6^a	89.867±0.1^a
	10	92.999±0.2^b	90.984±0.3^b
	15	93.997±0.3^c	91.731±0.6^c

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

Japanese spinach treated with ozonation results in lower water content than without ozonation, but the longer the time of ozonization can increase the water content. Ozone can slow down the metabolism rate, close stomata, and cause ultrastructural changes in epicuticular wax so that it can inhibit the rate at which it increases water level (Lin *et al.*, 2019). The longer the immersion time, the more the water content rises. This increase was caused by the oxidation of food reserves as a result of damage to fruit cell walls by ozone. Ozone reacts with easily oxidized cell components, especially cell components containing double bonds, sulfhydryl groups, and phenolic rings, so the ozone reaction with these cell components causes cells to become damaged and cell components to break down further into simpler compounds (Yasa *et al.*, 2013).

3.2 Total phenolic content

A lot of polyphenols (200 mg gallic acid equivalent/100 g spinach) are known to be produced by spinach, which is a green vegetable (Derrien *et al.*, 2018). Spinach contains types of polyphenols such as pcomic acid, ferulic acid, and ortho-comic acid (Subhash *et al*., 2010). Phenolic compounds can inhibit free radicals, such as peroxide decomposition, metal inactivation, or capture oxygen in biological systems and prevent oxidative disease (Aryal *et al.,* 2019). Changes in the chemical composition of ozone-treated foods are found to be marginal at amounts below 1 ppm (1 ppm corresponds to 1.96 mg/m^3) (Brodowska *et al*., 2018).

Japanese spinach carried out by ozonation produces a higher total phenolic content than without ozonation. The use of ozone can inhibit the activity of the polyphenol oxidase enzyme and increase the antioxidant defence system through increased peroxidase activity (Lin *et al*., 2019). Phenylalanine ammonia lyase (PAL) activation resulting from the presence of ozone gas can result in increased phenolic content. PAL is indeed one of the key enzymes associated with the plant synthesis of phenolic compounds. Treatment with ozone can inhibit enzymes such as polyphenol oxidase and peroxidase that can cause phenolic compounds in fruits and vegetables to oxidize. Increased phenolic content may also be due to modification of the cell wall during exposure to ozone. This modification could have increased the extraction power and released some phenolic compounds in the cell wall (Onopiuk *et al*., 2017). The longer ozonation decreased the total phenolic value of Japanese spinach. Soaking in an ozone solution for too long will reduce the phenolic content due to ozone decomposition accompanied by the production of many free radicals such as hydroperoxyl (% H_2O), hydroxyl (% OH), and superoxide radicals (% O_2). Giving the right ozone dose can induce antioxidant defence reactions (Lv *et al*., 2019). Thus, the effective time for soaking Japanese spinach in maintaining total phenolics during storage is 5 min.

The total phenolic content of Japanese spinach stored at room temperature is lower than that of cold-stored spinach. That is because, at room temperature (25°C), the Japanese spinach phenolic content has been degraded. Storage with lower temperatures can reduce biochemical processes in vegetables such as ethylene production, respiration, or enzyme activity, although the phenolic content remains low due to degradation by polyphenol oxidase and peroxide. Storage of spinach in cold temperatures is more effective in maintaining phenolic content (Serea *et al*., 2014). An increase in temperature from 2-22°C does not cause degradation of the bioactive components of cherries (Moldovan *et al*., 2016). Hence, the most effective storage temperature for maintaining phenolic content in Japanese spinach is at 10°C. In this study, the total content of phenolic compounds on day 0 was 65.81-66.17 mg eqv gallic acid/100 g (Table 2), while according to Zikalala *et al*. (2017), the total content of phenol compounds is 3.07 mg/g (Table 3). This difference can be caused by differences in the samples used.

Table 2. Total phenolic compound (mg eqv gallic acid/ 100 g) of Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	66.17±0.3^a	81.840±0.3^a
	5	77.70±0.1^d	93.125±0.5d
	10	70.62±0.3^{bc}	90.170±0.4^b
	15	70.84±0.4^c	92.248±2.5^c
2	0	65.81±0.5^b	80.660±0.4^b
	5	70.42±0.5^c	87.965±0.2^c
	10	64.695±0.7^b	80.620±0.5^b
	15	62.285±0.7^a	81.045±0.0^a

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

Table 3. The nutritional content of baby spinach leaves per 100 g of spinach (Zikalala *et al*., 2017)

	Japanese Spinach
Iron	0.91 ppm
Zinc	1.123 ppm
Selenium	0.001 ppm
Total Phenols	3.07 mg/g
Total Carotenoids	0.89 mg/g
Flavonoid content	4.00 mg/g
Antioxidant	0.49 mg/g
Protein	8.3%
Nitrate	0.02%

3.3 pH Value

The value of the acidity (pH) states the acidity or basicity of a solution (Susanty and Sampepana, 2017). A pH value is calculated on the zero and second-day of storage. Japanese spinach stored at room temperature can reduce the pH more rapidly than at cold temperatures (Table 4). During storage, the pH value of seaweed decreases with the decrease in chlorophyll content. Hydrolysis occurs during storage, which lowers the pH value. The effect of increasing the storage temperature is that respiration takes place faster, which causes the number of organic acids to increase and the pH value to decrease. The decreased pH value is caused by a product interacting with CO_2 in the air, resulting in the breakdown of chlorophyll in lettuce, which is assisted by the chlorophyllase enzyme that the pH goes down (Rohmat *et al*., 2014).

Storage at cold temperatures is more effective in maintaining and reducing the level of pH reduction in Japanese spinach. Ozonation does not affect the pH value of Japanese spinach on either the 0 or the second day. The pH value of Japanese spinach was not significantly affected by ozonation treatment. According to Miller *et al*. (2013), there was no substantial change in the pH of fruits and vegetables handled with ozonation, indicating that the presence of ozonation did not affect the pH of fruits and vegetables. During storage, the slightly lower albumen pH observed in ozone-treated eggs indicated that ozone concentrations and exposure periods were successful in reducing the rate of albumen liquefaction, thus helping to preserve albumen consistency by monitoring the pH of the albumen. While yolk pH rose during storage, for all ozone concentrations, the rise was lower than the original pH for up to 4 weeks (Yüceer *et al*., 2016).

Table 4. pH Value of Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	6.050±0.1[a]	6.350±0.1[a]
	5	6.275±0.1[c]	6.525±0.1[c]
	10	6.150±0.1[b]	6.450±0.1[b]
	15	6.250±0.1[b]	6.475±0.1[b]
2	0	5.350±0.1[a]	5.750±0.1[a]
	5	5.650±0.1[c]	6.100±0.0[c]
	10	5.500±0.0[b]	5.950±0.1[b]
	15	5.550±0.1[b]	5.800±0.1[b]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

3.4 Chlorophyll

Chlorophyll is one of the most important bioorganic molecules. It is the main pigment in photosynthesis (Milenković *et al*., 2012). There are positive effects of chlorophyll on inflammation, oxidation, and wound healing as it is a high nutrient source for antioxidants (İnanç, 2011). However, chlorophyll degradation is caused by non-enzymatic reactions, including heat, light, oxygen, and pH (Wang *et al*., 2013). It can be seen in Table 5, the chlorophyll content of Japanese spinach stored at room temperature is relatively lower than Japanese spinach stored at cold temperatures. High storage temperatures can decrease the chlorophyll due to chlorophyll rupture caused by the chlorophyllase enzyme. The chlorophyllase enzyme hydrolyzed the phytol chain from chlorophyll to form chlorophyllide, and phytol causes the decrease in chlorophyll content. The initial process of chlorophyll degradation is the loss of magnesium from the central molecule or the loss of the phytol tail chain that causes the CH_3 group on C-7 atoms to be separated and the chlorophyll bond is broken (Rohmat *et al*., 2014).

Based on the results, it is known that washing with ozonation treatment can increase the chlorophyll content in Japanese spinach. Washing with ozone can reduce the rate of discolouration in broccoli by preventing the activity of the enzyme chlorophyllase, which can damage chlorophyll, and the induction of antioxidants by ozone to protect chlorophyll. However, excessive ozone exposure can cause discolouration of lettuce leaves due to the decreased chlorophyll content (Miller *et al*., 2013). Ozonation treatment is effective in maintaining Japanese spinach chlorophyll during storage and the effective soaking time is 5 mins. The increased content of chlorophyll in lettuce may be due to several reasons. One problem may be that a large quantity of sewage sludge nutrients may provide useful trace elements required for plant chlorophyll syntheses, such as calcium (Ca), magnesium (Mg), and Zn (Chekli *et al*., 2017). Another consideration was that, by promoting plant growth and metabolism, the trace elements accelerated the transport of each other. This was the advantage of using sludge hydroponic solution, as without adding any additives, the sludge solution could be used immediately after dilution. Meanwhile, based on the third theory, when lettuce is exposed to ozone, it causes an increase in the concentration of chlorophyll in the plant by filtering ions and avoiding external osmotic pressure (Yang *et al.,* 2018).

Table 5. Chlorophyll content (mg/100 g) on Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	285.92±2.0[a]	304.67±0.5[a]
	5	312.62±0.8[c]	335.54±2.4[c]
	10	292.75±0.4[a]	298.57±2.2[ab]
	15	304.67±0.5[b]	293.77±2.9[b]
2	0	245.08±2.7[b]	280.44±0.1[a]
	5	284.51±1.4[c]	324.33±1.5[c]
	10	240.35±0.9[b]	301.18±1.3[b]
	15	230.98±0.5[a]	297.52±2.0[a]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

3.5 Weight loss

Weight loss illustrates the loss of water contained in vegetables and fruits (Fan *et al*., 2019). The critical limit of weight loss in vegetables is 10%; if there is a loss of weight above 10% of the original weight, then the fresh vegetables are not worth selling (Asgar *et al*., 2011). Based on Table 6, Japanese spinach treated with ozonation shows a lower weight loss than without ozonisation. Ozonation treatment can effectively maintain water content in vegetables to reduce weight loss (Lin *et al*., 2019). Weight loss during storage tends to increase, ozone molecules are thought to enter the flower cabbage tissue through the stomata, then contact with cell walls, which are then oxidized by ozone and can cause lysis. The ozone will make inactive enzymes in the membrane and cell nucleus, and it will inhibit respiration (Asgar *et al*., 2011). The ozonization treatment for 5 min is effective in reducing the increase in Japanese spinach weight loss during storage. Japanese spinach's weight loss when stored at high temperatures is relatively greater than when stored at low temperatures. Lower temperatures can depress respiration rates, thereby reducing the risk of weight loss (Asgar *et al*., 2015). When spinach leaves were blended with fine, whole leaves inside the same box, the propensity of all the spinach leaves within the bag to deteriorate more easily increased. The spinach production line industry must be very careful in choosing the right leaves before packaging (Ariffin *et al*., 2017).

Table 6. Weight loss (%) of Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	3.160±0.3[c]	1.770±0.1[c]
	5	2.128±0.1[a]	1.020±0.1[a]
	10	2.873±0.2[b]	1.585±0.1[b]
	15	3.025±0.0[c]	1.865±0.7[c]
2	0	6.403±0.4[c]	4.115±0.4[c]
	5	3.835±0.1[a]	1.255±0.2[a]
	10	4.968±0.2[b]	3.205±0.3[b]
	15	4.865±0.2[b]	3.193±0.4[b]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

3.6 Texture

Texture is an important quality attribute that can help producers and consumers in determining food viability. Fresh fruits and vegetables that have a firm texture are highly desired by consumers (Ma *et al*., 2017). As it can be seen in Table 7, Japanese spinach stored at low temperatures can better maintain its texture value than high-temperature storage. At room temperature, transpiration and respiration processes occur faster, and greater evaporation of water causes turgor pressure in the material to decrease. This loss of texture is due to a decline in cell wall turgor (Benítez *et al*., 2012). Japanese spinach with ozonation treatment shows a better texture value than without ozonation because ozonization can reduce the rate of respiration in Japanese spinach that the texture seems fresher. The texture of post-harvest agricultural products is influenced by respiration and the storage time of the metabolism (Babaremu *et al*., 2019). Ozonation can reduce the rate of respiration to maintain the quality and texture of the food. According to (Lin *et al*., 2019), ozone can slow down the digestion of tissue, close stomata, and induce ulfastructural epicuticular wax changes such that breathing rate can be inhibited.

Table 7. Texture of Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	0.073±0.002[a]	0.084±0.001[a]
	5	0.083±0.001[c]	0.090±0.001[c]
	10	0.080±0.004[b]	0.086±0.003[b]
	15	0.078±0.004[b]	0.086±0.001[b]
2	0	0.064±0.002[a]	0.078±0.000[a]
	5	0.075±0.001[c]	0.088±0.001[c]
	10	0.071±0.000[bc]	0.082±0.000[bc]
	15	0.072±0.001[c]	0.080±0.001[c]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

3.7 Colour

Colour is one of the most important components in determining the quality of a material. Colour is an indicator of consumer preferences in choosing spinach (Asgar *et al*., 2017). In this study, a descriptive attribute test with 5 values was used to describe the colour of the Japanese spinach sample. The higher value indicates the best colour of Japanese spinach. The details of the colour assessment in the descriptive text of the smallest values include yellow, yellowish-green, yellow spot green, green, and dark green. The study's findings (Table 8) revealed that the organoleptic value of spinach color did not differ significantly with storage temperature or ozonation time. Ozone does not reduce the original quality of vegetable colour characteristics. The ozonation treatment maintains the colour of the material during storage to produce a better shelf life (Lin *et al*., 2019). In addition, panellists preferred ozonation samples of Japanese spinach. That is because ozone has disinfectant properties that can sterilize water and clean vegetables, giving the impression that they look cleaner than those

Table 8. Colour of Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	4.47±0.5[a]	4.60±0.6[a]
	5	4.50±0.5[a]	4.70±0.5[a]
	10	4.47±0.6[a]	4.57±0.5[a]
	15	4.53±0.5[a]	4.63±0.5[a]
2	0	3.40±0.5[a]	4.37±0.6[a]
	5	4.07±0.5[b]	4.13±0.6[ab]
	10	3.83±0.6[ab]	4.43±0.5[a]
	15	3.67±0.5[ab]	4.40±0.5[ab]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

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washed without ozone, but washing with ozone does not eliminate the original colour characteristics of vegetables (Asgar *et al*., 2011). Changes in colour are caused by changes in natural pigments, such as chlorophyll, carotenoids, and anthocyanins, or by other enzymatic browning and non-enzymatic pigments (Miller *et al*., 2013). In general, ozone does not change the colour of the product.

3.8 Appearance

Appearance is one of the important quality factors for food ingredients as it is a visual assessment carried out on several criteria such as size, shape, colour, condition, freshness, and disability or injury (Asgar *et al*., 2011). Leaf vegetables, including spinach, are one type of vegetable that withers quickly (Rahayu *et al.,* 2013). In this study, a descriptive test of the appearance attributes with 5 scales describes the appearance of Japanese spinach. The higher value represents the best appearance of Japanese spinach. The results of the study (Table 9) show that ozonization affects the value of the appearance of Japanese spinach during storage. Ozonated Japanese spinach has a higher appearance value than without ozonation. Ozonization treatment can reduce the signs of spoilage and successfully preserve the consistency of the material's appearance during storage (Lin *et al*., 2019). Fruits and vegetables treated with ozonation and stored in cold storage have a better physical appearance compared to controls. Storage for three days showed a significant difference where the control showed decay while the fruits and vegetables that were treated with cold storage were still in fresh conditions (Prasetyaningrum, 2017). The hue angle values of the 6-mins UV-B studies showed the yellowing of fresh-cut spinach leaves was minimized and ultraviolet irradiation therapy improved the optical consistency of the samples (Ufuk Kasım and Kasım, 2017).

Table 9. Appearance of Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	3.40±0.5[a]	3.93±0.6[a]
	5	3.67±0.5[b]	4.37±0.5[b]
	10	3.60±0.5[ab]	4.17±0.4[ab]
	15	3.53±0.5[ab]	4.03±0.6[ab]
2	0	2.73±0.5[a]	3.50±0.5[a]
	5	3.07±0.4[c]	4.23±0.4[c]
	10	2.93±2.5[b]	3.87±0.4[b]
	15	2.87±0.4[ab]	3.70±0.5[ab]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

3.9 Hardness

Texture or hardness is one of the essential consistency characteristics for the viability of food for both suppliers and customers. Fresh vegetables with good qualities in toughness, hardness, and crunchy texture are preferred by consumers. Consumers or panellists tend to be more sensitive to texture differences than to taste (Ma *et al.,* 2017). In this study, a descriptive attribute texture test with 5 values of red spinach was described. Similarly, the higher value shows the best Japanese spinach texture. The details of the texture assessment in the descriptive test of the smallest value include very soft, soft, somewhat soft, hard, and very hard. Texture testing was carried out on the leaves and stems of Japanese spinach. The results of the study (Table 10) show that ozonation treatment and storage temperature affect the value of Japanese spinach hardness. Japanese spinach stored at low temperatures has a higher hardness value than at room temperature. Storage at low temperatures can inhibit respiration, thereby delaying softening, discolouration, quality changes, and other chemical processes. The hardness of red chilli during storage has decreased because of the cell wall texture changes due to chemical changes during the metabolic process (Asgar *et al*., 2017). Ozonation treatment can prevent wilting and maintain the texture of Japanese spinach during softening. This is because ozonation can suppress the rate of respiration, which can damage the texture or hardness of Japanese spinach (Lin *et al*., 2019).

Table 10. The hardness of Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	3.50±0.5[a]	4.00±0.5[a]
	5	3.87±0.4[b]	4.40±0.5[b]
	10	3.80±0.4[b]	4.17±0.4[b]
	15	3.77±0.6[ab]	4.13±0.6[ab]
2	0	2.73±0.5[a]	3.80±0.4[a]
	5	3.53±0.5[c]	4.33±0.5[c]
	10	3.20±0.4[b]	4.00±0.5[b]
	15	3.23±0.6[b]	3.97±0.6[b]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

3.10 Overall

In this attribute, panellists were asked to rate the Japanese spinach as a whole based on their respective ratings. This assessment consists of 5 scales where the greater the value, the preferred Japanese spinach. Japanese spinach was used for sensory analysis using whole spinach as shown in Figure 1. There is a significant difference between cold and room temperature storage. The overall value of Japanese spinach at cold temperatures is higher than at room temperature (Table 11). The ozonation treatment showed a significant difference in the overall value of Japanese spinach on the second day. Japanese spinach treated with ozonation received a higher overall value than at room temperature. Table 11 shows that 5 mins of ozonization immersion time displayed the best results. Thus, the most effective treatment for maintaining the sensory quality of Japanese spinach during storage is ozonation with an immersion time of 5 mins and kept at cold temperatures.

Figure 1. Japanese Spinach (Suwarto, 2014)

Table 11. Organoleptic overall of Japanese Spinach with different ozone treatment and storage temperature

Day	Ozonization Time (mins)	Storage Temperature (°C)	
		25	10
0	0	3.57±0.5[a]	4.10±0.4[a]
	5	3.83±0.4[b]	4.50±0.5[b]
	10	3.70±0.5[ab]	4.23±0.4[ab]
	15	3.67±0.5[a]	4.20±0.4[a]
2	0	2.40±0.5[a]	3.70±0.5[a]
	5	3.17±0.4[c]	4.33±0.5[c]
	10	2.73±0.5[b]	3.93±0.3[b]
	15	2.87±0.7[b]	3.90±0.3[b]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different according to the ANOVA test at the 5% significance level.

4. Conclusion

Most of the postharvest Japanese spinach damage is caused by respiration rate. The ozonation process and storage temperature affect the physical, chemical, and sensory properties of Japanese spinach. Ozonation treatment for 5 minutes and storing in cold temperatures can reduce water content increase (87.050% and 89.867%), total phenolic (93.125 and 87.965 mg eqv gallic acid/100 g), chlorophyll content (335.54 and 324.33 mg/100 g), weight loss (1.020% and 1.255%), texture (0.090 and 0.088), and sensory qualities such as ozone exposure for too long will damage the quality of Japanese spinach. According to the findings of this study, ozonation treatment for 5 minutes followed by storage at cold temperatures is effective in preserving the physical, chemical, and sensory qualities of Japanese spinach during storage.

Conflict of interest

The authors declare no conflict of interest.

References

Alexandre, E.M.C., Brandão, T.R.S. and Silva, C.L.M. (2011). Modelling Microbial Load Reduction in Foods Due to Ozone Impact. *Procedia Food Science*, 1, 836–841. https://doi.org/10.1016/j.profoo.2011.09.126

Anakottapary, D.S. (2014). Modifikasi Alat Refrigerator Dengan Penambahan Proses Pengkabutan Air Untuk Penyimpanan Sayur Bayam. *Jurnal Logic*, 14(2), 65–70. [In Bahasa Indonesia].

Arianti, Y.S. and Utami, B.W. (2015) Strategi Pengembangan Agribisnis Bayam Jepang Organil di Desa Batur, Kecamatan Getasan, Kabupaten Semarang. *Agrista*, 3(3), 387–399. [In Bahasa Indonesia].

Ariffin, S.H., Gkatzionis, K. and Bakalis, S. (2017). Leaf injury and its effect towards shelf-life and quality of ready-to-eat (RTE) spinach. *Energy Procedia*, 123, 105–112. https://doi.org/10.1016/j.egypro.2017.07.265

Aryal, S., Manoj, K.B., Krisha, D., Puspa, K., Roshani, G. and Niranjan, K. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants*, 8(4), 96. https://doi.org/10.3390/plants8040096

Asgar, A., Musaddad, D., Setyabudi, D.A. and Hasan, Z.H. (2015). Teknologi Ozonisasi Untuk Mempertahankan Kesegaran Cabai Cultivar Kencana Selama Penyimpanan. *Jurnal Penelitian Pascapanen Pertanian*, 12(1), 20-26. https://doi.org/10.21082/jpasca.v12n1.2015.20-26 [In Bahasa Indonesia].

Asgar, A., Musaddad, D. and Sutarya, R. (2017). Pengaruh Ozonisasi dan Kemasan untuk Mereduksi Residu Pestisida dan Mempertahankan Karakteristik Kesegaran Cabai Merah dalam Penyimpana. *Jurnal Hortikultura*, 27(2), 241–252. https://doi.org/10.21082/jhort.v27n2.2017.p241-252 [In Bahasa Indonesia].

Asgar, A., Sugiarto, A.T., Sumartini. and Ariani, D. (2011). Kajian Ozonisasi (O_3) Terhadap Karakteristik Kubis Bunga (*Brassica oleracea* var. botrytis) Segar selama Penyimpanan Suhu Dingin. *Berita Biologi*, 10(6), 787–795. https://doi.org/10.1017/CBO9781107415324.004 [In Bahasa Indonesia].

FULL PAPER

Asimovic, Z., Sarajevo, F.S., Cengic, L. and Murtic, S. (2016). Spectrophotometric determination of total chlorophyll content in fresh vegetables. *Work Faculty Agriculture Food Science Unversity Sarajev*, 66(1), 104–107.

Babaremu, K.O., Adekanye, T.A., Okokpujie, I.P., Fayomi, J. and Atiba, O.E. (2019). The significance of active evaporative cooling system in the shelf life enhancement of vegetables (red and green tomatoes) for minimizing post-harvest losses. *Procedia Manufacturing*, 35, 1256–1261. https://doi.org/10.1016/j.promfg.2019.06.084

Bajčan, D., Tomáš, J., Uhlířová, G., Árvay, J., Trebichalský, P., Stanovič, R. and Šimanský, V. (2013). Antioxidant potential of spinach, peas, and sweetcorn in relation to freezing period. *Czech Journal of Food Sciences*, 31(6), 613–618. https://doi.org/10.17221/529/2012-CJFS

Benítez, S., Chiumenti, M., Sepulcre, F., Achaerandio, I. and Pujolá, M. (2012). Modeling the effect of storage temperature on the respiration rate and texture of fresh cut pineapple. *Journal of Food Engineering*, 113(4), 527–533. https://doi.org/10.1016/j.jfoodeng.2012.07.022

Borowski, J., Narwojsz, A., Borowska, E.J. and Majewska, K. (2015). The effect of thermal processing on sensory properties, texture attributes and pectic changes in broccoli. *Czech Journal of Food Sciences*, 33(3), 254–260. https://doi.org/10.17221/207/2014-CJFS

Britton, H.C., Draper, M. and Talmadge, J.E. (2020). Antimicrobial efficacy of aqueous ozone in combination with short chain fatty acid buffers. *Infection Prevention in Practice*, 2(1), 100032. https://doi.org/10.1016/j.infpip.2019.100032

Brodowska, A.J., Nowak, A. and Śmigielski, K. (2018). Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. *Critical Reviews in Food Science and Nutrition*, 58(13), 2176–2201. https://doi.org/10.1080/10408398.2017.1308313

Cristina, V., Suzane, M., Ferreira, M. and Silva, V. (2018). Measuring dry extract in dairy products: optimizing the methodology. *Ciencia Rural*, 48(7), e20170883. https://doi.org/10.1590/0103-8478cr20170883

Derrien, M., Aghabararnejad, M., Gosselin, A., Desjardins, Y. and Angers, P. (2018). Optimization of supercritical carbon dioxide extraction of lutein and chlorophyll from spinach by-products using response surface methodology. *LWT - Food Science and Technology*, 93, 79–87. https://doi.org/10.1016/j.lwt.2018.03.016

Deven, M. and Steesh, B. (2014). Pharmacological Activity of *Spinacia Oleracea* Linn. A Complete Overview. *Asian Journal Pharmaceutical Research and Development*, 2(1), 83–93.

Fan, K., Zhanga, M., Bhandarid, B. and Jiang, F. (2019). A Combination Treatment of Ultrasound and ε-Polylysine to Improve Microorganisms and Storage Quality of Fresh-cut Lettuce. *LWT - Food Science and Technology*, 113, 108315. https://doi.org/10.1016/j.lwt.2019.108315

Hakiki, D.N., Darmawati, E., Purwanto, A. and Hideto, U. (2019). Perubahan Kualitas Pasca Panen Bayam Organik selama Penyimpanan setelah Perlakuan Heat Shock dan Hydrocooling. *Journal of Chemical Information and Modeling*, 53(9), 1689–1699. https://doi.org/10.1017/CBO9781107415324.004 [In Bahasa Indonesia].

İnanç, A.L. (2011). Chlorophyll: Structural Properties, Health Benefits and Its Occurrence in Virgin Olive Oils. *Academic Food Journal*, 9(2), 26–32.

Jeon, M.J. and Ha, J.W. (2020). Synergistic bactericidal effect and mechanism of X-ray irradiation and citric acid combination against food-borne pathogens on spinach leaves. *Food Microbiology*, 91, 103543. https://doi.org/10.1016/j.fm.2020.103543

Jeong, Y.J. and Ha, J.W. (2019). Combined treatment of UV-A radiation and acetic acid to control foodborne pathogens on spinach and characterization of their synergistic bactericidal mechanisms. *Food Control*, 106, 106698. https://doi.org/10.1016/j.foodcont.2019.06.024

Jung, Y.J., Padmanabahn, Hong, J.H., Lim, J. and Kim, K.O. (2012). Consumer Freshness Perception of Spinach Samples Exposed to Different Storage Condition. *Postharvest Biology and Technology*, 73, 115–121. https://doi.org/10.1016/j.postharvbio.2012.06.005

Karaca, H. and Velioglu, Y.S. (2014). Effects of ozone treatments on microbial quality and some chemical properties of lettuce, spinach, and parsley. *Postharvest Biology and Technology*, 88, 46–53. https://doi.org/10.1016/j.postharvbio.2013.09.003

Khan, W., Singh, V., Sagar, A. and Singh, S.N. (2017). Response of phosphorus application on growth and yield attributes of sweet corn (*Zea mays L. saccharate*) varieties. *Journal of Pharmacognosy and Phytochemistry*, 6(5), 2144–2146.

Kim, M.A., van Hout, D., Zandstra, E.H. and Lee, H.S. (2019). Consumer acceptance measurement focusing on a specified sensory attribute of products: Can the attribute-specified degree of satisfaction-difference (DOSD) method replace hedonic scaling? *Food*

Quality and Preference, 75, 198–208. https://doi.org/10.1016/j.foodqual.2019.03.009

Chekli, L., Kim, J.E., Saliby, I.E., Kim, Y., Phuntsho, S., Li, S., Ghaffour, N., Leiknes, T. and Shon, H.K. (2017). Fertilizer drawn forward osmosis process for sustainable water reuse to grow hydroponic lettuce using commercial nutrient solution. *Separation and Purification Technology*, 181, 18–28. https://doi.org/10.1016/j.seppur.2017.03.008

Liman, M.G., Abdullahi, A.S., Lawal Maigoro, A. and Umar, K.J. (2014). Effects of Three Drying Techniques on Mineral Composition of Some Leafy Garden Vegetables. *IOSR Journal of Applied Chemistry*, 7(1), 38–42. https://doi.org/10.9790/5736-07123842

Lin, S., Chen, C., Luo, H., Xu, W., Zhang, H., Tian, J.J., Ju, R. and Wang, L. (2019). The combined effect of ozone treatment and polyethylene packaging on postharvest quality and biodiversity of *Toona sinensis* (A.Juss.) M.Roem. *Postharvest Biology and Technology*, 154(5), 1–10. https://doi.org/10.1016/j.postharvbio.2019.04.010

Lv, Y., Tahir, I.I. and Olsson, M.E. (2019). Effect of ozone application on bioactive compounds of apple fruit during short-term cold storage. *Scientia Horticulturae*, 253, 49–60. https://doi.org/10.1016/j.scienta.2019.04.021

Ma, L., Zhang, M., Bhandari, B. and Gao, Z. (2017). Trends in Food Science and Technology Recent developments in novel shelf life extension technologies of fresh-cut fruits and vegetables. *Trends in Food Science and Technology*, 64, 23–38. https://doi.org/10.1016/j.tifs.2017.03.005

Mattioli, S., Ortenzi, R., Scuota, S., Cartoni Mancinelli, A., Dal Bosco, A., Cotozzolo, E. and Castellini, C. (2020). Impact of ozone and UV irradiation sanitation treatments on the survival of *Salmonella* and the physical–chemical characteristics of hen eggs. *Journal of Applied Poultry Research*, 29(2), 409–419. https://doi.org/10.1016/j.japr.2020.01.004

Mayton, H.M., Marcus, I.M. and Walker, S.L. (2019). *Escherichia coli* O157:H7 and *Salmonella Typhimurium* adhesion to spinach leaf surfaces: Sensitivity to water chemistry and nutrient availability. *Food Microbiology*, 78, 134–142. https://doi.org/10.1016/j.fm.2018.10.002

Milenković, S.M., Zvezdanović, J.B., Andelković, T.D. and Marković, D.Z. (2012). The identification of chlorophyll and its derivatives in the pigment mixtures: HPLC-chromatography, visible and mass spectroscopy studies. *Advanced Technologies*, 1(1), 16–24. http://alfa.tf.ni.ac.rs/casopis/sveska1/c2.pdf

Miller, F.A., Silva, C.L.M. and Brandão, T.R.S. (2013). A Review on Ozone-Based Treatments for Fruit and Vegetables Preservation. *Food Engineering Reviews*, 5(2), 77–106. https://doi.org/10.1007/s12393-013-9064-5

Moldovan, B., Popa, A. and David, L. (2016). Effects of storage temperature on the total phenolic content of Cornelian Cherry (*Cornus mas* L.) fruits extracts. *Journal of Applied Botany and Food Quality*, 89, 208–211. https://doi.org/10.5073/JABFQ.2016.089.026

Mu, Y., Feng, Y., Wei, L., Li, C., Cai, G. and Zhu, T. (2020). Combined effects of ultrasound and aqueous chlorine dioxide treatments on nitrate content during storage and postharvest storage quality of spinach (*Spinacia oleracea* L.). *Food Chemistry*, 333, 127500. https://doi.org/10.1016/j.foodchem.2020.127500

Olanya, O.M., Niemira, B.A. and Phillips, J.G. (2015). Effects of gamma irradiation on the survival of *Pseudomonas fluorescens* inoculated on romaine lettuce and baby spinach. *LWT - Food Science and Technology*, 62(1), 55–61. https://doi.org/10.1016/j.lwt.2014.12.031

Onopiuk, A., Półtorak, A., Moczkowska, M., Szpicer, A. and Wierzbicka, A. (2017). The impact of ozone on health-promoting, microbiological, and colour properties of *Rubus ideaus* raspberries. *CYTA - Journal of Food*, 15(4), 563–573. https://doi.org/10.1080/19476337.2017.1317669

Prabawa, S., Putri, D.K.R., Kawiji, Yudhistira, B. (2021). Pengaruh Variasi Waktu Ozonisasi dan Suhu Penyimpanan Terhadap Karakteristik Fisika, Kimia, dan Sensoris pada Daging Ayam Broiler (*Gallus Domesticus*). *Jurnal Ilmiah Rekayasa Pertanian dan Biosistem*, 9(2), 168-184. https://doi.org/10.29303/jrpb.v9i2.277 [In Bahasa Indonesia].

Prasetyaningrum, A. (2017). Prototype Penyimpanan Buah Dan Sayur Menggunakan Ozon Dan Metode Evaporative Cooling Sebagai Sistem Pendingin. *Jurnal Aplikasi Teknologi Pangan*, 6(1), 31–35. https://doi.org/10.17728/jatp.213 [In Bahasa Indonesia].

Rahayu, S., Asgar, A., Hidayat, I., Kusmana, K. and Djuariah, D. (2013). Quality Evaluation of Some Genotype of Spinach (*Amaranthus* sp.) Cultivated in West Java. *Berita Biologi*, 12(2), 153–160.

Ramos, B., Brandão, T.R.S., Teixeira, P. and Silva, C.L.M. (2020). Biopreservation approaches to reduce *Listeria monocytogenes* in fresh vegetables. *Food Microbiology*, 85, 103282. https://doi.org/10.1016/j.fm.2019.103282

FULL PAPER

Rohmat, N., Ibrahim, R. and Riyadi, P. (2014). Pengaruh Perbedaan Suhu Dan Lama Penyimpanan Rumput Laut *Sargassum polycystum* Terhadap Stabilitas Ekstrak Kasar Pigmen Klorofil. *Jurnal Pengolahan Dan Bioteknologi Hasil Perikanan*, 3(1), 118–126. [In Bahasa Indonesia].

Sabaghnia, N., Asadi-Gharneh, H.A. and Janmohammadi, M. (2014). Genetic diversity of spinach (*Spinacia oleracea* L.) landraces collected in Iran using some morphological traits. *Acta Agriculturae Slovenica*, 103(1), 101–111. https://doi.org/10.14720/aas.2014.103.1.11

Serea, C., Barna, O., Manley, M. and Kidd, M. (2014). Effect of storage temperature on the ascorbic acid content, total phenolic content and antioxidant activity in lettuce (*Lactuca sativa* L.). *Journal of Animal and Plant Sciences*, 24(4), 1173–1177.

Singh, U. and Sagar, V.R. (2010). Quality characteristics of dehydrated leafy vegetables influenced by packaging materials and storage temperature. *Journal of Scientific and Industrial Research*, 69 (10), 785–789.

Subhash, G.P., Virbhadrappa, S.R. and Vasant, O.K. (2010). *Spinacia oleracea* Linn: a Pharmacognostic and Pharmacological Overview. *International Journal of Research in Ayurveda and Pharmacy*, 1 (1), 78–84.

Susanty, A. and Sampepana, E. (2017). Pengaruh Masa Simpan Buah terhadap Kualitas Sari Buah Naga Merah (*Hylocereus polyrhizus*). *Jurnal Riset Teknologi Industri*, 11(2), 76–82. https://doi.org/10.26578/jrti.v11i2.3011 [In Bahasa Indonesia].

Suwarto, R. (2014). *Budidaya* Horenzo Alias Bayam Jepang. Retrieved from website: http://pertaniandanpeternakanku.blogspot.com [In Bahasa Indonesia].

Ufuk Kasım, M. and Kasım, R. (2017). Yellowing of fresh-cut spinach (*Spinacia oleracea* L.) leaves delayed by UV-B applications. *Information Processing in Agriculture*, 4(3), 214–219. https://doi.org/10.1016/j.inpa.2017.05.006

Wang, R., Xu, Q., Yao, J., Zhang, Y., Liao, X., Hu, X., Wu, J. and Zhang, Y. (2013). Post-effects of high hydrostatic pressure on green color retention and related properties of spinach puree during storage. *Innovative Food Science and Emerging Technologies*, 17, 63–71. https://doi.org/10.1016/j.ifset.2012.11.007

Watson, I., Kamble, P., Shanks, C., Khan, Z. and El Darra, N. (2020). Decontamination of chilli flakes in a fluidized bed using combined technologies: Infrared, UV and ozone. *Innovative Food Science and Emerging Technologies*, 59, 102248. https://doi.org/10.1016/j.ifset.2019.102248

Yang, P., Gou, Y. and Qiu, L. (2018). Effects of ozone-treated domestic sludge on hydroponic lettuce growth and nutrition. *Journal of Integrative Agriculture*, 17(3), 593–602. https://doi.org/10.1016/S2095-3119(17)61868-9

Yasa, I.W.S., Zainuri and Zaini, A. (2013). Keefektifan Teknologi Ozon Dalam Perbaikan Mutu Buah Mangga Gedong Gincu Lokal Lombok presented at the Prosiding Seminar Nasional Hasil-Hasil Penelitian, p. 529–534. Denpasar, Indonesia: UNMAS Press [In Bahasa Indonesia].

Yüceer, M., Aday, M.S. and Caner, C. (2016). Ozone treatment of shell eggs to preserve functional quality and enhance shelf life during storage. *Journal of the Science of Food and Agriculture*, 96(8), 2755–2763. https://doi.org/10.1002/jsfa.7440

Ren, Z-Q., Rao, Z.-H. and Ji, H.-Y. (2018). Identification of Different Concentrations Pesticide Residues of Dimethoate on Spinach Leaves by Hyperspectral Image Technology. *IFAC-PapersOnLine*, 51(17), 758–763. https://doi.org/10.1016/j.ifacol.2018.08.104

Zikalala, B.O., Nkomo, M., Araya, H., Ngezimana, W. and Mudau, F.N. (2017). Nutritional quality of baby spinach (*Spinacia oleracea* L.) as affected by nitrogen, phosphorus and potassium fertilisation. *South African Journal of Plant and Soil*, 34(2), 79–86. https://doi.org/10.1080/02571862.2016.1225231

