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ORIGINAL ARTICLE

The effect of ozonated water on changes in the quality of blanched edamame during cold storage

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Abstract

Vegetable soybean (*Glycine max* (L.) Merr.) is a seasonal vegetable and has limited shelf life. The present study aimed to determine the use of ozonated water as a sanitizer in order to extend the shelf life of blanched edamame and also to study the effect of ozonation on the quality indicators of packaged soybeans. Raw unwashed beans were used as control to test treatments beans washed and blanched in water bath at 93 ± 2 °C for 2 minutes; part of them was cooled in ice cold distilled water and part was cooled in ozonated ice cold distilled water. Chilled edamame was packed in a polypropylene bag, hermetically sealed and stored at the temperature of +4.0 °C for 21 days. The use of ozonated water for cooling the blanched edamame effectively inhibited the development of harmful microorganisms without significant color changes of pods and beans. The data obtained from the analytical hierarchy process revealed that the ozonated water used to cool blanched edamame compared to pure water did not significantly affect the change in phenolic content in 11 days of storage.

Keywords: Vegetable soybean; Antimicrobial agent; Chemical compounds; Microbiology.

Highlights

- The effect of ozonated water for cooling of blanched edamame was studied for the first time.
- The ozonated water could prolong the shelf life of blanched edamame and suppress microorganisms.
- Phenolic compounds of edamame beans were not detrimentally affected by ozonated water treatment.
- Ozonated water did not affect the color of the edamame pod during storage.

1 Introduction

Vegetable soybean (*Glycine max* (L.) Merr.) is widely used for preparing dish “edamame”, seeing that it is directly called “edamame”, therefore both terms are often used to denote this vegetable (Landim et al., 2016; Carneiro et al., 2020). Vegetable soybean belongs to the same species as the traditional grain soybean. This type of soybeans at the consumption stage have a green color, a soft texture, a large seed size, a sweet taste and light bean aroma (Saldivar et al., 2010). For the first time, vegetable soybeans were recorded in China, as early as second century BC (Mentreddy et al., 2002). In addition, vegetable soybean is very popular in Japan, where it is known for more than 400 years. Nowadays, vegetable soybean is consumed as a popular food in Asia and the United States of America (USA) (Battistini et al., 2018). In the recent years, vegetable soybean becomes more popular in countries of The Baltic Sea region as well. These soybeans are harvested when they reach about 80% of maturity (Carneiro et al., 2020). Vegetable soybean regarding vegetation period in most cases is between 75 and 100 days. Such vegetation period length is common for the majority of agricultural crops in Latvia. This leads to assumption that vegetable soybean can be successfully grown in Latvia as well (Howard et al., 1999). After five years of experiments, as suitable for growing in Latvia agro-ecological conditions, it could be found the Japanese cultivars ‘Chiba Green’ and ‘Midori Giant’. These cultivars provide sufficient yield of good quality pods. Quality characteristics depend on genotype, harvest time, growth and environmental conditions. In fact, not only environmental factors, but also genotype can strongly affect the seed biochemical composition (Mentreddy et al., 2002). Generally, vegetable soybeans contain significant amounts of vitamins, calcium, protein, isoflavones and also have higher ascorbic acid and β-carotene contents, and lower indigestible oligosaccharides and anti-nutritional substances content which might offer numerous health benefits (Saldivar et al., 2010). The soybean taste depends on sucrose, glutamine acid and alanine content in beans where sucrose promotes sweetness; on the other hand, saponin, isoflavonoids and L-arginine promote bitterness. Indeed, shortly cooked or blanched edamame beans are a good source of ascorbic acid, vitamin E and dietary fiber (Mozzoni et al., 2009). The rise in interest of edamame is probably due to its nutritional value and taste. Edamame provides not only essential amino acids but can be considered as a complete source of protein. The average edamame's nutritional content, based on a portion amount of 155 g (one cup), is related to 189 calories, 8 g of fat, 16 g of carbohydrate, 3 g of sugar and 17 g of protein (Lara et al., 2019).

However, it is well known that edamame is perishable and can be stored with a short shelf life, so it is a challenge to maintain its shelf life and quality after harvest (Xu et al., 2012). Under the right storage conditions, edamame can retain its flavor and good appearance for up to two weeks. Typically, edamame is blanched before freezing and storage. Usually hot water (70-100 °C) is used for 2-5 minutes for edamame blanching (Lara et al., 2019). It is important to highlight, that improper blanching of vegetable soybeans can affect the product quality by softening the texture, browning the color and reducing the amount of nutrients in the content (Saldivar et al., 2010). Moreover, heat treatment for edamame prevents deterioration caused by microorganisms, reduces nutritional factors and inactivates lipoxygenase enzymes that promote flavoring and influence color and texture (Czaikoski et al., 2012).

The application of ozone improves the microbiological safety of different food products, and prolongs their shelf life without substantially changing their nutritional, chemical, and physical properties (Brodowska et al., 2018). Additionally, it can be used in vegetable, fruit, meat, fish, as well as herb, spice and beverage industries. Several studies indicated a positive effect of ozonated water on extending the shelf life of fresh or sliced salads. This treatment method is able to reduce the microbial contamination of the product without adversely affecting its visual, textural and nutritional quality when stored at low temperatures, i.e., on average at +4 °C (Glowacz et al., 2015; Sengun & Kendirci, 2018). Studies of the use of ozonated water for the treatment of various fresh leafy lettuces, spinach and parsley did not show any negative effect on changes in the content of biologically active substances (phenols, vitamin C, chlorophyll) in the samples compared to unwashed leaves (Karaca &

Velioglu, 2014). Nowadays, eating habits indicate a tendency to eat fresher or minimally cooked products. This category most often includes peeled, steamed or blanched vegetables which can be used in the preparation of various salads without further processing, where edamame is a popular ingredient. No studies have been found in the scientific literature on the use of ozone in the treatment of blanched vegetable soybeans (edamame) to extend shelf life in refrigerated form. Therefore, this study aimed to determine the effect of cooling with ozonated water of blanched edamame on changes in the quality of edamame beans during storage. The objective of this work aimed to investigate the change in color, soluble solids, ascorbic acid, chlorophyll, as well as total phenolic compounds, tannins, carotenoids and microbial indicators of fresh vegetable soybean during the 21- days of shelf life.

2 Material and methods

The research has been carried out at the Unit of Processing and Biochemistry and Unit of Agronomic Research and Variety Testing of the Institute of Horticulture according to the scheme (Figure 1).

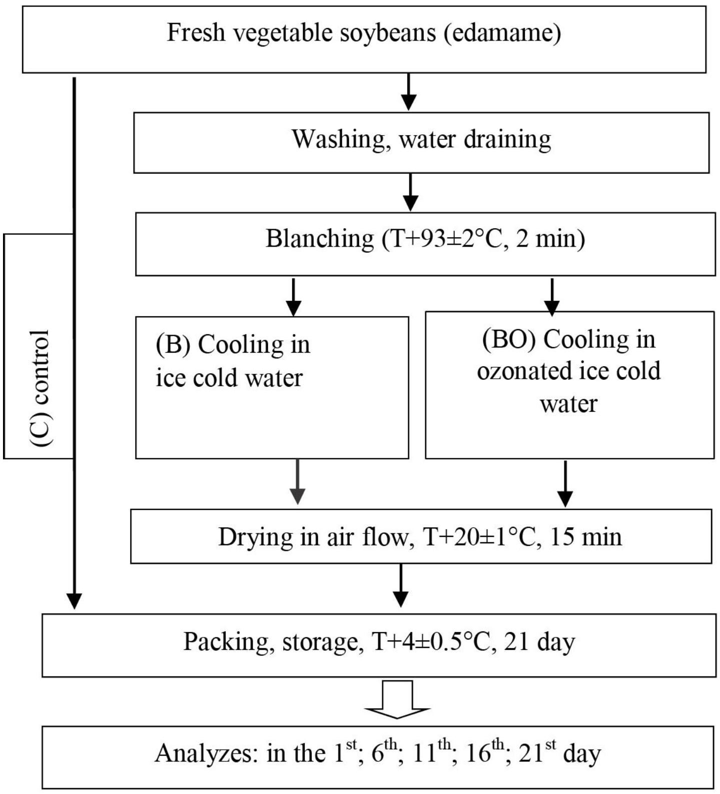

Figure 1. Research scheme.

2.1 Plant material

Field trials were located 90 km to the west from Rīga (Pūre) (57°37'N, 22°921'E, 57 m altitude). Vegetable soybean cultivar 'Midori Giant' was grown from planting seedlings. Planting seedlings were grown in trays (cell volume 140 cm^3), planted on the field on 22 May. Precipitation sum of vegetation season (May – September) was 152 mm. The average air temperature was 6.8-20.5 °C. The soil type was a sandy loam, pHKCl 6.2, P205 – 224 mg kg^{-1}, K20 – 300 mg kg^{-1}, and organic matter 3.5%. During the growing period, plant fertilization and chemical plant protection was not applied.

Yield was harvested in 100 days after planting seedlings on the field, at maturity stage 77-79 according to BBCH81.

2.2 Sample preparation

For the study, the beans were sorted, and pods with 2-3 beans were used. 1st sample (C): Control – raw, unwashed edamame beans. 2nd sample (B): beans washed, drained of excess water, blanched in water bath at 93 ± 2 °C temperature for 2 minutes, cooled in ice cold distilled water. 3rd sample (BO): beans washed, drained of excess water, blanched in water bath at 93 ± 2 °C temperature for 2 minutes, cooled in ice cold distilled water enriched with ozone using A2Z Ozone systems (INC, USA) (oxygen was used as a feed gas from oxygen cylinder: 5 l min^{-1}, concentration of ozone 40 pp; feeding time: 2 min). After cooling both treated samples were dried using the equipment "ORAKAS" (Marlemi, Finland) with forced air circulation at 20.0 ± 1.0 °C temperature for 15 min. All samples were packed in polypropylene bags of 100 ± 5.0 g edamame pods in each, hermetically sealed and prepared in three identical replicates. Samples were stored in a commercial freezer/cooler camera for 21 days at the temperature of +4.0 ± 0.5 °C, controlled by MINILog Gresinger electronic.

2.3 Physical, chemical microbiological analysis

The following physical and chemical properties were determined for edamame samples as following: for pods and beans – color; and for beans - soluble solids, total phenolic content, tannins, vitamin C, chlorophyll a and b and total carotenoid content. The content of microorganisms – mesophilic aerobic and facultative anaerobic (total bacteria count), yeasts and molds were determined in packaged edamame. The presence of *Escherichia coli* at the first day of packed samples was determined. At each time of measurement, three identical packages for each packaging mode were randomly selected on the first sampling day (1st) and at 6th, 11th, 16th, and 21st days of storage.

Microbial analyses (colony forming units (log CFU g^{-1})). Examination methods of Total Aerobic Microbial Count (TAMC) was based on ISO- 4832-1:2013 - Microbiology of food chain- Horizontal methods of microorganisms counting. Determination of yeasts and molds was performed according to the standard ISO 21527-1:2008 - Microbiology of food and animal feeding stuffs.

Soluble Solids Content (SSC) regarding the determination in fresh edamame beans was done at room temperature (range from 18 to 22 °C) by using handheld digital refractometer PAL-1 (ATAGO, Tokyo, Japan) and expressed as percent soluble solids (Brix °).

The changes of **color** of edamame pods and beans surface during storage were measured by CIE L*a*b* color system (standard illuminant D65, 45°/0° angle) using spectrophotometer CM-2500c (Japan). Color values were recorded as L* (brightness) – L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) and b* (-b, blueness, +b, yellowness). The color measurements were performed in 15 replications for each sample and expressed as intensity of green color (-a/b) and total color difference Δ*E of pods and beans surface, calculated by the formula as reported by Patras et al. (2011).

The content of **vitamin C** (ascorbic acid) was determined by standard method EN 14130:2003 "Foodstuffs – Determination of vitamin C by HLPC", and expressed in mg 100 g^{-1} ascorbic acid on Fresh Weight (FW).

The Total Phenolic (TP) content was determined by the photometric method using Folin- Ciocalteu reagent described by Singleton et al. (1999) and expressed as Gallic Acid Equivalents (GAE) (mg GAE 100g^{-1} FW).

The **Total Tannin** (TT) content was determined by the Folin- Ciocalteu procedure, after removing the tannins by their adsorption on insoluble polyvinylpolypyrrolidone (PVPP) (Malenčić et al., 2012).

Calculated values were subtracted from TP contents, and TT contents were expressed as milligrams of GAE per $100g^{-1}$ of FW.

Determination of chlorophylls (mg g^{-1}) and **carotenoids** (µg g^{-1}). The edamame was crushed, weighed at 2.5-gram homogeneous sample, and 30 mL of 80% acetone was added, vortexed for three minutes, centrifuged at 5000 rpm for ten minutes. The clear part of the extract was poured onto filter paper, and then filtered. In fact, 30 mL of 80% acetone was again added to the precipitate and the extraction was repeated, the clear extract was supplemented and made up to 60 mL with acetone. The samples were kept in the dark in an ice bath until the absorbance was read by the spectrophotometer. Absorption was measured as previously described by Lichtenthaler & Wellburn (1983). It was recorded that chlorophyll *a* showed the maximum absorbance at 662 nm, whereas chlorophyll *b* at 646 nm and total carotenoids showed this absorbance at 470 nm and the amount of these pigments was calculated by the Formulas 1:

$$C_{ha} = 11.75\,A662 - 2.350\,A645 \tag{1a}$$

$$Chb = 18.61A645 - 3.960A662 \tag{1b}$$

$$C_{x+c} = 1000A470 - 2.270\text{Ca} - 81.4 \times \frac{Cb}{227} \tag{1c}$$

where: C_{ha} = Chlorophyll a, C_{hb} = Chlorophyll b, C_{x+c} = Total carotene.

2.4 Statistical analysis

Experimental data processing was carried out by Multivariate General Linear Model procedure SPSS 23 software package. The mean arithmetic value and standard deviation were calculated for the obtained results using Microsoft Office Excel 2007. Correlation between the parameters was determined by analysis of Pearson correlation coefficient, Tukey's multiple range test was used for the clarification of significant differences ($p < 0.050$) among the studied samples.

Analytic Hierarchy Process (AHP) was used to evaluate the changes of vegetable soybean quality parameters: Total chlorophyll (Tch), TP, intensity of green color (-a/b) of pods and beans surface during storage time with descriptive method. The main quality properties of edamame were TP, Tch, (-a/b) pods, (-a/b) beans and vitamin C content, which were accepted as basic criteria for evaluation by relative significance scale for intensity of importance 1-9. To estimate the uppermost criteria of edamame quality by importance, the highest value was assigned TP – 9 and next Tch - 7, (-a/b) pods - 5, (-a/b) beans - 3, vitamin C - 1. The priority coordinates' vectors were calculated according to AHP, suggested in literature (Saaty, 2008).

The dynamics regarding the determined quality parameters of edamame green beans quality changes during storage (TAMC, TP intensity of green color (-a/b) pods and beans and vitamin C content) was calculated by Formula 2:

$$\frac{Xbs - Xas}{Xas} \cdot 100 \tag{2}$$

where: X_{bs} – values of parameters before storage; X_{as}– values of parameters following 11 days of storage.

3 Results and discussion

The green **color** of the pods is one of the main characteristics of edamame quality, which plays a dominant role and an effective feature in attracting customers to purchase it in the market (Kasim & Kasim, 2015). The results of surface color L* and intensity of green color (-a/b) values of analyzed edamame pods and beans for all samples are summarized in Table 1.

Table 1. Dynamics of L* value and intensity of the green color (-a/b) during edamame.

Storage time, days	Samples	L*		(-a/b)	
		Pods	Beans	Pods	Beans
1	C	50.87 ± 3.5^{ba}	54.82 ± 3.9^{bc}	-0.38 ± 0.04^{a}	-0.40 ± 0.09^{a}
	B	51.47 ± 3.0^{ab}	54.64 ± 2.5^{bc}	-0.36 ± 0.08^{a}	-0.39 ± 0.07^{a}
	BO	45.49 ± 3.2^{dc}	47.31 ± 3.1^{ed}	-0.37 ± 0.05^{a}	-0.39 ± 0.10^{a}
6	C	56.37 ± 3.1^{a}	60.29 ± 3.5^{a}	-0.17 ± 0.04^{b}	-0.24 ± 0.02^{b}
	B	47.09 ± 2.9b^{c}	54.94 ± 2.5b^{c}	-0.09 ± 0.02^{c}	-0.30 ± 0.06^{ba}
	BO	47.20 ± 1.9^{c}	56.27 ± 3.1b^{a}	-0.14 ± 0.03^{cb}	-0.36 ± 0.12^{ab}
11	C	49.87 ± 2.9^{b}	53.00 ± 2.8^{cb}	-0.07 ± 0.01^{dc}	-0.22 ± 0.13^{ba}
	B	43.79 ± 2.5^{ed}	52.35 ± 3.2^{cd}	-0.10 ± 0.01^{c}	-0.26 ± 0.10^{ba}
	BO	45.93 ± 1.7^{dc}	46.16 ± 3.9^{ed}	-0.01 ± 0.01^{f}	-0.26 ± 0.09^{b}
16	C	54.39 ± 2.8^{a}	56.44 ± 3.0^{ba}	-0.03 ± 0.04^{ef}	-0.16 ±0.10^{b}
	B	45.16 ± 2.0^{dc}	50.74 ± 3.3^{cd}	-0.04 ± 0.01^{e}	-0.21 ± 0.07^{b}
	BO	43.91 ± 2.9^{ed}	48.57 ± 3.1^{d}	-0.00 ± 0.01^{f}	-0.24 ± 0.08^{b}
21	C	46.02 ± 3.0^{dc}	59.21 ± 3.7^{a}	-0.03 ± 0.01^{e}	-0.16 ± 0.09^{b}
	B	43.68 ± 2.4^{ed}	53.0 ± 4.2^{cb}	-0.04 ± 0.02^{e}	-0.21 ± 0.08^{b}
	BO	44.57 ± 1.4^{d}	49.72 ± 3.4^{d}	-0.00 ± 0.01^{f}	-0.24 ± 0.09^{b}

The means data are indicated by standard deviations (made from 15 replicates) followed by different small letters within the same column and indices, are significantly different at the $p < 0.05$ level. C - control, raw, unwashed edamame beans; B - washed, blanched, cooled in ice cold distilled water; BO - washed, blanched, cooled in ice cold distilled water enriched with ozone.

Comparing the L* value of edamame pods between C and blanched samples B and BO, it could be noted that results were significantly different ($p = 0.002$), whereas there were no significant differences found in B and BO samples ($p = 0.095$). The L* value of edamame beans also showed a significant difference between C, B, and BO samples ($p = 0.001$), moreover, it was determined that the L* value increased slightly in the first days of storage for all samples. The calculated intensity of green index (-a/b) for edamame pods during the study differed significantly between the analyzed samples ($p < 0.035$) and storage days ($p = 0.00$). However, between bean colors in all samples the index did not differ significantly ($p = 0.325$), but a difference was observed between the storage days ($p = 0.012$). After blanching, the intensity of the green color of the pods and beans was higher in the B and BO samples compared to the control sample. The study concluded that the ozonated water used to cool blanched beans did not cause significant differences in L * value and intensity of green color (-a/b) between B and BO samples compared to the pure water. The total color difference Δ*E of the edamame samples were calculated between L*, a*, b* values before storage and following twenty-one days of storage. Smallest Δ*E were determined for the pods (accordingly, 10.50; 13.23) and beans (accordingly, 11.42; 13.92) of the BO and control samples.

The studies described a slight increase in the L* value for beans at the beginning of storage, as well as a decrease in the brightness (L*) due to blanching associated with changes in green color after heat treatment (Santana et al., 2012). The L*, a*, b* values of bean pods mainly depend on the maturity at harvesting. When prolonging the storage time and raising the temperature of the decomposition of chlorophyll, it could be observed that these procedures may result in the release of carotenoids and the green color of the beans changes to yellow (Proulx et al., 2010). Several studies have shown that the blanching time of soybeans up to 5 minutes provided a higher intensity of green color (Xu et al., 2012), as well as good results were obtained with 2 minutes blanching of edamame in a steam jacket-cattle where intensity of green color of shelled beans was 0.46 and for pods 0.44 (Mozzoni et al., 2009). Cold ozonated water treatments did not affect the color properties of lettuce samples during storage period at +4 °C for 14 days, it was found that between both treated and untreated samples there was no difference ($p > 0.05$) (Sengun & Kendirci, 2018).

Soluble Solids Content (SSC). At the beginning of the study, the SSC of fresh edamame sample was 12.47 ºBrix, but after blanching the SSC slightly increased (on average 12, 5 ºBrix).

Table 2. The dynamics of total soluble solids, vitamin C, Total Phenolic (TP) and Total Tannin (TT) contents of the edamame during storage.

Storage time, days	Samples	Soluble solids, ºBrix	Vitamin C, mg 100 g^{-1}	Total Phenolic (TP), mg GAE 100 g^{-1}	Total Tannin (TT), mg GAE 100 g^{-1}
1	C	12.43 ± 0.2^{b}	65.61 ± 1.1^{a}	85.10 ± 0.9^{a}	50.40 ± 0.2^{a}
	B	12.50 ± 0.1^{ba}	56.00 ± 1.6^{c}	78.94 ± 1.9^{cb}	45.30 ± 3.4^{cb}
	BO	12.52 ± 0.2^{ba}	52.00 ± 2.1^{d}	79.11 ± 1.9^{cb}	43.30 ± 2.0^{dc}
6	C	12.67 ± 0.2^{a}	58.42 ± 2.0^{b}	82.56 ± 2.5^{ab}	46.21 ± 1.5^{c}
	B	12.57 ± 0.3^{ba}	41.64 ± 0.3^{f}	78.62 ± 1.7^{cb}	48.23 ± 0.4^{b}
	BO	12.58 ± 0.3^{ab}	40.85 ± 1.4^{ef}	79.02 ± 1.1^{c}	50.76 ± 1.5^{a}
11	C	12.71 ± 0.3^{ab}	48.09 ± 3.3^{e}	80.52 ± 0.9^{bc}	26.88 ± 2.4^{ef}
	B	12.40 ± 0.2^{b}	32.57 ± 0.2^{g}	74.05 ± 1.7^{e}	17.92 ± 1.4^{h}
	BO	12.42 ± 0.3^{ba}	25.65 ± 0.4^{j}	76.39 ± 1.9^{d}	23.88 ± 2.3^{ef}
16	C	12.20 ± 0.3^{ba}	41.04 ± 2.9^{f}	70.76 ± 1.5^{f}	17.88 ± 2.4^{h}
	B	12.13 ± 0.2^{cb}	27.86 ± 0.2^{ih}	66.92 ± 1.8^{g}	10.59 ± 1.2^{j}
	BO	12.11 ± 0.2^{cb}	20.89 ± 1.3^{k}	66.39 ± 1.9^{e}	20.55 ± 0.2^{g}
21	C	11.0 ± 0.3^{d}	30.33 ± 2.6^{hg}	57.41 ± 4.3^{h}	17.88 ± 2.4^{h}
	B	11.70 ± 0.4^{c}	17.61 ± 0.4^{l}	45.25 ± 3.1^{i}	15.26 ± 1.7^{i}
	BO	11.69 ± 0.5^{cd}	16.02 ± 0.5^{m}	44.06 ± 4.1^{ji}	14.51 ± 1.1^{ij}

The means data are indicated by standard deviations (made from 3 replicates) followed by different small letters within the same column and indices, are significantly different at the $p < 0.05$ level. C - control, raw, unwashed edamame beans; B - washed, blanched, cooled in ice cold distilled water; BO - washed, blanched, cooled in ice cold distilled water enriched with ozone.

Various factors, including the place of cultivation and harvesting time can affect the SSC content, for example, in vegetable soybeans harvested in Japan when bean pods are green, the SS content could vary from 8.5 to 12.0 °Brix. This result and also ours one were contradicted with Karapanos et al. (2017) by analyzing 36 fresh beans pods harvested in Greece at a suitable stage for consumption when compared with vegetables where soluble solids content ranged between 5.07 and 7.57 ºBrix. During the first days of storage, the SSC in the beans slightly increased, seeing that after the first day it decreased, which may be due to faster respiration rate under stress conditions, which promoted the ripening of the beans (John et al., 2018). In addition, it could be noted that hermetically sealed polypropylene bags promoted anaerobic respiration in beans. We observed that at the end of storage, the SSC of the control samples decreased by 11.5%, however, in the B and BO samples the decrease was significantly lower (6.4 and 6.6%, respectively). It was concluded that during the 21-days of storage, the values of SSC between all analyzed samples did not differ significantly ($p = 0.95$).

The content of **vitamin C** of fresh edamame beans was 65.61 mg 100 g^{-1} (Table 2). The treatment process significantly affected the content of vitamin C in the beans ($p < 0.000$). Blanching and cooling decreased the amount of vitamin C for 14.6% when cooled in ice water and for 20% if cooled in ozonated water. It was concluded that compared to the first day, at the end of the study, the vitamin C content decreased in all samples, seeing that it dropped to 54% (C), 73% (B) and 73% for BO sample. Kumar with colleagues reported that significant genotypic variations in vitamin C content have been identified by analyzing 16 vegetable soybean green pods 34.8-88.7 mg 100 g^{-1} (Kumar et al., 2014). Czaikoski et al. (2012) identified a 19.6% reduction in vitamin C after four days of storage of blanched soybeans at 5 °C. After washing lettuce, concerning spinach and parsley with ozonated water, it could be found a loss of 40.1% of ascorbic acid (Karaca & Velioglu, 2014). In contrast, such vegetables as red bell peppers, cucumbers and zucchini after ozone gas treatment stored for 14 days did not show a significant decrease in vitamin C compared to control samples (Glowacz et al., 2015). Ascorbic acid is a water-soluble vitamin, and its loss can be observed after treatment with distilled water. In addition, ascorbic acid could also be oxidized by agents such as ozone. Decomposition of ascorbic acid can occur by both oxidation and non-oxidation mechanisms (Karaca & Velioglu, 2014).

Molecular ozone reactions are selective and limited to unsaturated aromatic and aliphatic compounds, as the ozone reaction with ascorbate is faster than with double bond compounds (fatty acids) (Kumar et al., 2014; Glowacz et al., 2015).

The **Total Phenolic** (TP) content of the fresh edamame sample was 85.1 mg GAE per 100 g^{-1} (Table 2). After heat treatment – blanching, the phenolic content in B and BO samples decreased and was on average 7% lower than the control. Significant changes in TP content were observed throughout the storage time of packed edamame samples ($p = 0.016$). After six (6) days, TP decreased by 3% in the control sample, but less than 1% in the B and BO samples. In turn, it could be observed a decrease of 5% regarding the TP sample after 11 days, but after 16 days it reached an average of 16%. At the end of the study, TP content of edamame had decreased in control sample by 32.5%, whereas B and BO samples had decreased by 42.6% and 44.3%, respectively. The study demonstrated that cooling with ozonated water did not cause significant changes ($p = 0.775$) in the TP content of blanched edamame samples. It was determined that as a result of green beans blanching at 95 °C and cooling in cold distilled water for 2-3 minutes, the TP content decreased on average by 16.8% (Patras et al., 2011). In turn, washing fresh herbs in ozonated water reduced the TP content by 14% (Karaca & Velioglu, 2014). Beltrán et al. (2005) reported that after fresh-cut lettuce washing with various procedures (including ozone treatments), there was no effect on the final phenolic content. While Glowacz et al. (2015) observed that the TP content was increased in red bell peppers exposed to ozone at 0.1 µmol mol^{-1}, but after treatment with ozone at 0.3 µmol mol^{-1}, the values decreased.

The presence of **tannins** in vegetable soybeans affects their taste properties. The TT content of the fresh edamame sample was not high (50.4 mg GAE 100 g^{-1}). When comparing to control sample, in the blanched samples of B and BO, the TT decreased by 10% and 14%, respectively (Table 2). During storage, the TT content decreased significantly in all samples – from 64.5% in the control sample to an average of 66.4% in the B and BO samples.

Chlorophylls are an important pigment in green vegetables and fruits, since it also plays an important role in our diet. These pigments are accompanied by carotenoids, whose color is frequently masked by green chlorophylls. Fresh edamame beans contain a significant amount of chlorophylls (*a* - 33.9 and *b* - 17.9 mg 100 g^{-1}), in addition to consuming stage 3.3 mg 100 g^{-1} of **carotenoids** in beans (Table 3).

Table 3. The dynamics of chlorophyll *a*, chlorophyll *b*, vitamin C and total carotenoids content of the edamame during storage.

Storage time, days	Samples	Chlorophyll *a*, mg 100 g^{-1}	Chlorophyll *b*, mg 100 g^{-1}	Total carotenoids, µg 100 g^{-1}
0	C	33.85 ± 0.7a	17.89 ± 0.2c	3.25 ± 0.1a
	B	22.60 ± 0.9^{e}	16.50 ± 0.2^{d}	2.26 ± 0.1^{g}
	BO	20.80 ± 0.2^{f}	16.56 ± 0.5^{ed}	2.74 ± 0.1^{e}
6	C	26.77 ± 0.9^{c}	16.32 ± 0.1^{ed}	3.13 ± 0.1^{b}
	B	18.50 ± 0.4^{h}	16.17 ± 0.2^{fe}	3.00 ± 0.0^{c}
	BO	17.06 ± 0.7^{i}	13.95 ± 0.9^{h}	2.21 ± 0.1^{g}
11	C	28.90 ± 0.5^{b}	16.39 ± 0.8^{e}	2.99 ±0.1^{c}
	B	15.36 ±0.1^{k}	22.19 ± 0.2^{a}	2.97 ± 0.1^{c}
	BO	19.80 ± 0.6^{g}	15.00 ± 0.1^{g}	3.31 ± 0.1^{a}
16	C	25.46 ± 0.4^{d}	16.11 ±0.2^{f}	2.81 ± 0.0^{d}
	B	15.07 ± 0.2^{l}	20.07 ± 0.3^{b}	2.90 ±0.1^{c}
	BO	16.80 ± 0.2^{j}	13.60 ± 0.2^{i}	2.90 ± 0.2^{c}
21	C	19.63 ± 0.6^{g}	16.77 ± 0.3^{d}	2.72 ± 0.1^{ed}
	B	13.33 ± 0.4^{n}	16.43 ± 0.4^{e}	2.37 ± 0.1^{f}
	BO	13.83 ± 0.4^{m}	12.80 ± 0.3^{j}	2.25 ± 0.1^{g}

The means data are indicated by standard deviations (made from 3 replicates) followed by different small letters within the same column and indices, are significantly different at the $p < 0.05$ level. C - control, raw, unwashed edamame beans; B - washed, blanched, cooled in ice cold distilled water; BO - washed, blanched, cooled in ice cold distilled water enriched with ozone.

Blanching and cooling in different water treatments affected the amount of chlorophylls. It decreased by 33.2% and 38.6% of chlorophyll *a* for B and BO samples, respectively, while decreased of chlorophyll *b* in both samples was found smaller (on average 7.6%). There was no significant difference in the chlorophyll content between B and BO treatment ($p > 0.05$). Heat treatment also negatively affected the carotenoid content in edamame, in which a reduction of 15-30% was found. During storage, the largest changes were observed in chlorophyll *a* content for all edamame samples, whereas chlorophyll *b* and carotenoid content decreased less. Thermal degradation of chlorophylls (both *a* and *b*) in broccoli juice at temperatures 60 °C was reported by Weemaes et al. (1999). They stated that chlorophyll degradation followed the first-order kinetics - chlorophyll *a* was found to be more heat sensitive than chlorophyll *b*. In other studies, it could be reported that blanching significantly reduced the chlorophyll content of edamame (17.6-27.3%) compared to the fresh sample (Song et al., 2003; Cheng et al., 2014), as well as steam blanching slightly decreased the β-carotene content of green beans (Howard et al., 1999). A study on ozone exposure found that gas did not cause significant changes in the chlorophyll content of fresh parsley (Karaca &Velioglu, 2014).

The Total Aerobic Microbial Count (TAMC) for edamame at the beginning of the study was 3.9 log CFU g^{-1}, but after blanching in B and BO samples it decreased significantly ($p < 0.05$) by 50.1% and 72.2%, respectively (Figure 2).

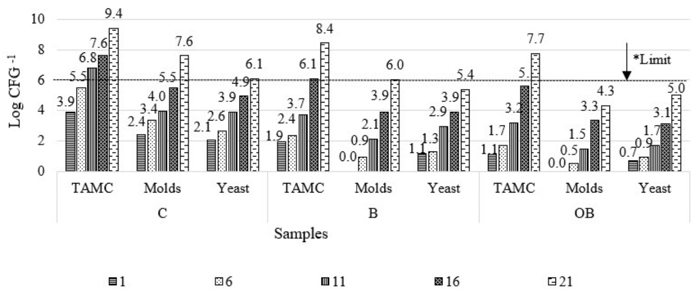

Figure 2. Total Aerobic Microbial Count, molds and yeasts count of edamame samples during storage. The means data are indicated by standard deviations (made from 3 replicates). TAMC - Total Aerobic Microbial Count. C – control, raw, unwashed edamame beans; B - washed, blanched, cooled in ice cold distilled water; BO - washed, blanched, cooled in ice cold distilled water enriched with ozone. * Limit - TAMC permissible dose $\leq 10^6$ log CFU g^{-1} according to the Latvian guidelines for the microbiological quality of some ready-to-eat foods.

Control sample contained low levels of *E. coli* (<100 CFU g^{-1}), because without washing they could contain levels of *E.coli* brought from the soil.

This indicator follows Regulation EC No 2073/2005 (European Union, 2005). In both blanched samples (B and BO), *E. coli* was not found. According to the Latvian guidelines for the microbiological quality of some ready-to-eat foods (acceptable $\leq 10^6$ CFU g^{-1}), the blanched samples were satisfactory, and TAMC contained less than limited values. In the control sample, the TAMC reached the critical limit after six (6) days of storage. Cooling blanched edamame with ozonated water prevented the development of TAMC for up to 16 days (5.6 log CFU g^{-1}), while 6.1 log CFU g^{-1} was observed in sample B. The development of yeasts and molds differed significantly between samples and positive effect of ozonated water was stated. Our findings agree with the results obtained by Xu et al. (2012), who observed that after edamame blanching, there was a significant reduction in total yeasts and molds counts. A study of the treatment of fresh salads with ozonated water at 4 °C and 15 °C showed that the treatment reduced TAMC by 45.4% and the number of yeasts-molds by 54% (Sengun & Kendirci, 2018).

Analytic Hierarchy Process (AHP) was used to assess the impact of ozonated water use for soybean cooling after blanching on soybean edamame pods shelf life. For the AHP analysis, the significance vectors were set based on the vegetable soybean quality as following: TP; Tch (*a*+*b*); vitamin C content; intensity of green color (-a/b) pods and beans. Results of AHP analysis assessment are summarized in Figure 3.

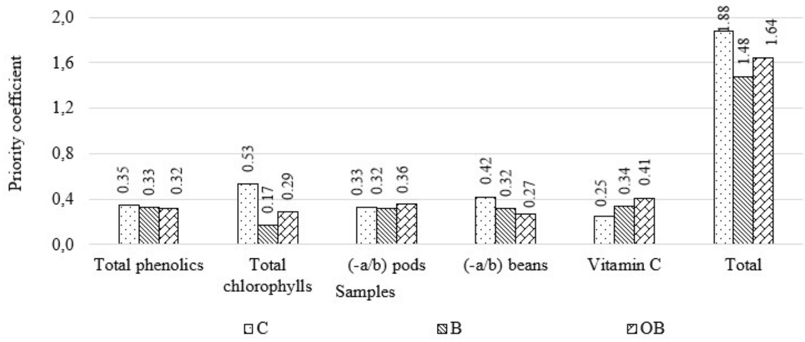

Figure 3. Results of Analytic Hierarchy Process analysis assessment. C – control, raw, unwashed edamame beans; B - washed, blanched, cooled in ice cold distilled water; BO - washed, blanched, cooled in ice cold distilled water enriched with ozone.

The most important criterion is the lowest value. The vectors presented the following values: TP = 0.10; Tch = 0.11; pods (-a/b) = 0.13; beans (-a/b) = 0.19; and vitamin C = 0.47. The data analysis did not include the absolute values of the indicators, but their changes during the eleven days of edamame storage were added. The results of our research are comparable with study, John et al. (2018) who concluded that for commercial edamame production, most cultivars were able to be stored between 10 and 14-days without significant loss in quality. When evaluate the results of AHP according to the criteria of significance, it was found that ozonated water treatment did not negatively affect the content of TP in the beans – thus being confirmed by the lowest priority coefficient found for the beans of BO treatment. The phenolic compounds are significant part of nutritional value of edamame beans; therefore, the highest important quality indicator vector was shown in this parameter. In all AHP for tested traits, it could be noted that the lowest priority coefficient was found for Tch and green color intensity (-a/b) in B treatment (pods), and for green color intensity (-a/b) in BO treatment (beans). The green color intensity is associated with quality to determine the consumer's preference. Ozonated water treatment reduced the content of vitamin C, pods color, and content of Tch.

4 Conclusions

The use of ozonated water for cooling of blanched edamame inhibited microorganisms, thus extending the shelf life to 16 days. The amount of TP of edamame beans were not detrimentally affected by ozonated water treatment, but vitamin C decreased slightly. Ozonated water did not cause significant differences of the edamame pod in color changes (chlorophyll, L* values color and the green intensity (-a/b)) between the B and BO samples during storage, which is important for consumer's preference. The findings of this study suggested that the use of ozonated water to cool blanched edamame is a promising method to extend their shelf life. Further research would be needed to evaluate the sensory properties of edamame.

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