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RESEARCH ARTICLE

THE EFFECTS OF GASEOUS OZONE TREATMENT ON THE SAFETY AND SHELF LIFE OF BERRIES.

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Abstract

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Introduction:-

Berries are a perishable food and can be consumed fresh or minimally processed. Berries can also be a frozen ingredient in many food products and preparations. Because berries are pulpy fruits with a soft skin and high moisture and sugar contents, they are susceptible to physical damage that accelerates their deterioration by increasing water loss, which can increase microbial contamination (EFSA 2014). These fruits are generally not washed or blanched before selling so there is a microbiological safety risk; several recent cases of infections and poisonings occurred as a result of microbial contamination (Palumbo *et al.* 2013; ECDC 2014; EFSA 2014). Moreover, berries also have a short shelf-life due to fungal growth. Hence, there is a need for effective methods to prevent spoilage and microbial contamination of fresh berries. Ozone gas could be a potential treatment. This gas is a potent sanitizer that is effective against a wide range of microorganisms (Khadre *et al.* 2001). Moreover, ozone gas is an unstable compound that decomposes rapidly into oxygen without leaving residues, as may occur with chlorine derivatives. Chlorine can form toxic or carcinogenic chlorinated organic compounds that requires that the product be rinsed, in addition to proper wastewater disposal (Kim *et al.* 2003; Xu Liangji 1999; Karaka H. and Velioglu Y.S. 2007, Concha-Meyer *et al.* 2014).

The objective of the present study was to evaluate the effects of a high concentration of gaseous ozone on the microbiological quality of blackberries and blueberries and on the survival of pathogenic microorganisms, such as *Listeria innocua* (non-pathogenic indicator for *L. monocytogenes*) and *Escherichia coli* that can contaminate these berries. Moreover, the effect of low concentrations of gaseous ozone to increase the shelf life of refrigerated berries was also tested.

Materials and methods:-

Berries:-

Fresh blueberries and blackberries were purchased from local markets.

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Gaseous ozonation systems:-

Ozone gas was generated using an ozone generator (Model Water Proof - MET s.r.l., Bologna, Italy) that produces ozone (16 g/h) from purified and concentrated ambient air (98% O₂). The ozone concentration was checked using an ultraviolet ozone analyser (Model GM6000RTI, Anseros Company, Tübingen, Germany). The ozone gas that was produced was delivered at a flow rate of 7 L/min.

Tests at high ozone gas concentrations were carried out in a Plexiglas chamber equipped with a fan to homogeneously diffuse gaseous ozone. The ozone gas concentration was monitored and controlled using a chemical detector (Model Regulator 100 ppm, MET Company s.r.l., Bologna, Italy).

Tests at low ozone gas concentrations were carried out in a refrigerator equipped with an ozone gas generator (Model 200 mg/h, Mosquito, MET Company s.r.l. Bologna Italy). For the control condition, an identical refrigerator without an ozone gas generator was used. Trials were carried out in high humidity (RH>90%) that was checked using a hygrometer (Model 174H, Testo Spa, Milano, Italy).

Microorganisms and sample inoculation:-

The following microorganisms were used for berry contamination:

- ❖ *Escherichia coli* SSICA E21 from fresh meat
- ❖ *Escherichia coli* ATCC 25922 from clinical isolate
- ❖ *Listeria innocua* ATCC 33090 from cow brain
- ❖ *Listeria innocua* SSICA F1 from frozen mushrooms
- ❖ *Listeria innocua* SSICA 8 from frozen peas

The microorganisms were cultured at 37°C for 24 h in Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK).

A mixture of *L. innocua* or *E. coli* strains was prepared by combining 2 ml of each culture, washing by centrifugation at 4000 rpm for 10 minutes (Model Megafuge 11R, AhsiS.p.a., Milano, Italy) and suspending the mixture in a saline solution.

To inoculate the berry samples (30 g), 150 µl of the microorganism suspensions were deposited as a droplet on the skin of the berries. The berries were left in a laminar flow cabinet for 1-2 hours in order to allow the microorganisms to attach. The concentration of both bacterial species ranged from 10⁴ to 10⁵ cfu/g.

Ozone gas treatments:-**Gaseous ozone treatments at room temperature (100 ppm):-**

To evaluate the effects of 100 ppm ozone gas treatment on native microflora, samples of blackberries and blueberries (50 g) were transferred to aluminium-perforated baskets and placed in a Plexiglas chamber equipped with an ozone gas generator. The exposure times were 15, 30, 60 and 90 minutes. Tests were performed in triplicates at room temperature.

In the evaluation test on pathogen survival, the same ozone gas concentration was tested against *L. innocua* and *E. coli* mixtures that were used to contaminate the 30 g berry samples. The samples were treated for 15 and 30 minutes.

Gaseous ozone treatments at low temperatures (0.3 ppm and 1 ppm):-

Each sample of blackberries and blueberries (50 g) was placed in an open plastic basket and stored in the refrigerator with continuous ozone gas exposure. For comparison, other fruit samples were placed in an identical refrigerator without ozone gas exposure. At increasing exposure times, samples were collected from the refrigerators and analysed. Tests were carried out in triplicate under two different conditions: 0.3 ppm at 4°C for up to 20 days and 1 ppm at 8°C for up to 7 days.

For the test on pathogen survival, treatments with 1 ppm ozone gas at 8°C were also carried out on 30 g berry samples that were inoculated with *L. innocua* and *E. coli* mixtures. After 24, 48 and 72 h exposure times, three samples from each refrigerator were collected and counted.

Microbiological analysis:-

Treated berries (30 g) were placed in a sterile-filtered stomacher bag and diluted to 1:10 in buffered peptone water (ISO – APT). Microbial counts were performed using a plate count technique on the *Listeria* Oxford Agar Base

(MOX) for *L. innocua* (37°C for 48 h), TBX medium for *E. coli* (44°C for 24 h), and Malt Extract Agar (MEA acidified to pH = 4.0 with citric acid 50% w/w) for yeast and mould (25°C for 5 days). All media were purchased from Oxoid (Basingstoke, England).

Statistical analysis:-

Microbial counts were transformed to logarithms before means and standard deviations were computed, and counts were reported as Log cfu/g. The statistical elaboration of the results was performed using SPSS 13.0.

Results and Discussion:-

The microbial population on berries mainly consists of yeast and mould, so the effects of gaseous ozone on these microorganisms were tested.

Yeast and mould populations resulted in floating, especially on blackberries. Sometimes a high mould concentration (10^5 cfu/g) together with a low yeast concentration (it was not possible to enumerate the yeasts because of overgrowth of MEA plates by moulds) was found. At other times, the latter microorganisms were predominantly expressed. In blueberries, both fungi were detected at concentrations between 10^3 and 10^4 cfu/g.

Microbial concentrations were correlated with the ripeness and integrity of the fruit. In fact, lower consistency, bruising and mechanical damage increase microbial proliferation (EFSA 2014).

Gaseous ozone treatments at room temperature (100 ppm):-

The results of these trials were carried out with a high ozone gas concentration, as shown in Table 1. Treatment with a 100 ppm ozone gas concentration for 15 minutes resulted in less than 1 Log reduction for yeast and mould, on both blueberries and blackberries. When the treatment time was increased to up to 30 minutes, a 1.5 Log reduction was observed for mould on both berries and for yeast on blueberries.

A further increase in treatment time (60 min) did not significantly affect microbial population reduction. A 90-minute treatment time resulted in a 2 Log reduction in mould on both berries, while no significant differences were observed for yeast on blueberries compared to the 30 minutes treatments.

The results indicated that a 30-minute treatment improves the microbiological quality of fresh blueberries and blackberries, a treatment time suitable for production requirements.

Gaseous ozone treatments at low temperature (0.3 ppm and 1 ppm):-

The results of trials with low ozone gas concentrations are shown in Figures 1 and 2.

For the trials carried out at 4°C with 0.3 ppm of ozone gas, the continuous ozone gas exposure did not significantly affect fungi concentration within twenty days of storage in both berries. A slight increase in the mould concentration in blueberries stored without ozone gas was observed, while the yeast concentration did not significantly change.

Barth *et al.* (1995) reported that a continuous exposure of blackberries to 0.3-ppm ozone at 2°C for 12 days inhibited fungal development, while 20% of control fruits showed decay. Palou *et al.* (2002) observed that continuous exposure to ozone at a concentration of 0.3 ppm for 4 weeks at 5°C, inhibited the growth of mould on "Elegant Lady" peaches; however, aerial growth and sporulation resumed in ambient atmospheres.

In the present study, the trials carried out at 8°C with 1 ppm ozone gas exposure to both berries, did not change the fungi concentration after 7 days of storage.

The slight variations in fungi population observed during storage were probably due to different contamination rates of fruits, as seen by the high standard deviation values.

Pathogen inactivation:-

Trials were carried out using a mixture of three *L. innocua* strains (gram-positive bacteria) and another mixture of two *E. coli* strains (gram-negative bacteria);

L. innocua was chosen as a non-pathogenic indicator of *L. monocytogenes* due to its phylogenetic proximity (Fan *et al.* 2007; Fairchild and Foegeding 1993).

According to the Regulation EC No. 2073/2005 on microbiological criteria for foodstuffs, berries are considered 'ready-to-eat' do not allow the growth of *L. monocytogenes* (due to their low pH). Thus, its concentration during the shelf life of berries must be less than 100 cfu/g.

E. coli is commonly present in faecal material and is widely used as a hygiene indicator. The hygienic microbial criteria for *E. coli* in 'ready-to-eat' pre-cut fruits and vegetables are between 100 and 1000 cfu/g (Regulation EC No. 2073/2005).

100ppm ozone gas treatment:-

The efficacy of 100ppm gaseous ozone treatment on reducing contamination from the *L. innocua* and *E. coli* mixture was comparable to that observed on the native microflora in berries.

A 15-minutes treatment resulted in a slight reduction of both *L. innocua* and *E. coli* inoculation on blackberries and blueberries. A 30-minutes treatment resulted in more than one logarithmic decrease (ranged from 1.22 to 1.85 log reductions) on both fruits (Table 2).

In a previous study (Previdi *et al.* 2011) it was observed that ozone gas dissolved in sanitizing water was more effective against inoculated cells than native microorganisms.

Native microbiota are mainly comprised of fungi and eukaryotic cells that provide defence mechanisms, such as the production of waxy cuticles, biofilm formation. These cells can be protected by cracks or cuts on fruits. The artificially inoculated bacteria, (prokaryotic and much more simple cells), that underwent to ozone gas exposure for 1-2 hours after inoculation, could presumably have lower chances to stick to a substrate; in the present study, gaseous ozone treatment resulted in the same degree of microbial inactivation.

The decimal reductions achieved in the present study were lower than those reported by Bermúdez-Aguirre *et al.* (2013), where more than 2 Log reductions were obtained on tomatoes artificially inoculated with *E. coli* and treated for 3 minutes with 5 ppm of ozone gas. However, the same treatment on carrots and lettuce achieved less than 1 Log reduction compared to the aforementioned microorganism.

Alwiet *et al.* (2014) treated chili peppers inoculated with *E. coli* O157: H7, *L. monocytogenes* and *Salmonella enterica* Typhimurium with five ozone gas concentrations (1, 3, 5, 7 and 9 ppm) and increasing exposure times (from 30 minutes to 24 h). The highest death rates were obtained with 9 ppm ozone gas treatment for 6 h (2.89, 2.56 and 3.06 logarithmic reductions for *E. coli* O157: H7, *Salmonella* Typhimurium and *L. monocytogenes* population, respectively).

In literature it was reported that the higher resistance observed in Gram-negative bacteria could be due to a high phospholipid content, which results in increased cell wall rigidity, and provides extra protection for the cell membrane against ozone gas. Despite the presence of a peptidoglycan layer in *L. monocytogenes* cell walls, this Gram-positive bacterium proved to be very susceptible to ozone gas treatment (Alwiet *et al.* 2014).

1 ppm ozone gas treatments:-

The efficacy of 1ppm gaseous ozone in reducing the population of *L. innocua* and *E. coli* inoculated on berries that were stored at 8°C are shown in Figures 3 and 4.

Within three days of storage, ozone gas treatment reduced *L. innocua* population by 3 logarithms in blackberries and 1.88 logarithms in blueberries compared to time zero. Additionally, 1.88 and 1.29 logarithm reductions in blackberries and blueberries, respectively, were observed, compared to the inoculated, untreated samples.

Concha-Meyer *et al.* (2014) studied the survival of *L. monocytogenes* in fresh blueberries stored at 4°C with 4ppm ozone gas and at 12°C with 2.5-ppm of ozone gas: the ozone gas treatments achieved 3 and 2 logarithm reductions, respectively, when compared with air treatment.

In the present study, we observed that storage for several days in the refrigerated condition in the presence of 1 ppm of ozone gas, maintained good sanitizing action against *Listeria*.

Exposure to 1 ppm of gaseous ozone at 8°C on the inoculated fruits with *E. coli* resulted in no significant differences between treated and untreated fruits after three days of storage (Figure 4).

Table 1:-Log reductions of yeasts and moldson blackberries and blueberries after treatment with 100-ppm gaseous ozone (experiments were replicated three timeswith triplicate determination per experiment; data are expressed as Log cfu/g ± standard deviation).

Exposure time (minutes)	Blackberries		Blueberries	
	Yeasts	Molds	Yeasts	Molds
15	0.91 ± 0.21	0.76 ± 0.21	0.78 ± 0.17	0.92 ± 0.18
30	1.04 ± 0.15	1.56 ± 0.63	1.43 ± 0.73	1.46 ± 0.56
60	0.78 ± 0.36	1.67 ± 0.48	1.20 ± 0.54	1.92 ± 0.94
90	nd	1.81 ± 0.61	1.46 ± 0.85	2.12 ± 0.52

nd= not detected.

Table 2:- Log reductions of *L.innocua* and *E. coli* on blackberries and blueberries after treatment with 100-ppm gaseous ozone (experiments were replicated three timeswith triplicate determination per experiment; data are expressed as Log cfu/g ± standard deviation).

Exposure time (minutes)	Blackberries		Blueberries	
	<i>L.innocua</i>	<i>E. coli</i>	<i>L.innocua</i>	<i>E. coli</i>
15	0.71 ± 0.40	0.54 ± 0.52	0.26 ± 0.16	0.47 ± 0.31
30	1.85 ± 0.74	1.22 ± 0.36	1.38 ± 0.31	1.25 ± 0.39

Figure 1: Effect of gaseous ozone (0.3 ppm) on populationsof yeasts and molds in blackberries and blueberries during twenty days storage at 4°C. Error bars represent 95% confidence limits (n = 3).

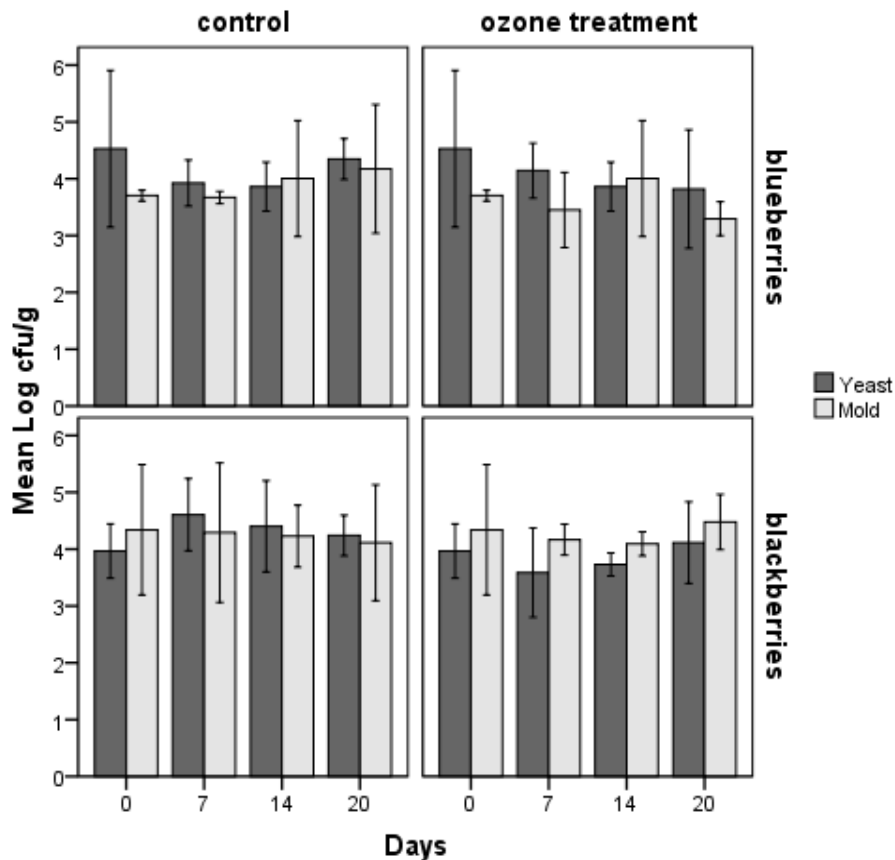


Figure 2:-Effect of gaseous ozone (1 ppm) on populations of yeasts and mold on blackberries and blueberries during seven days storage at 8°C. Error bars represent 95% confidence limits (n = 3).

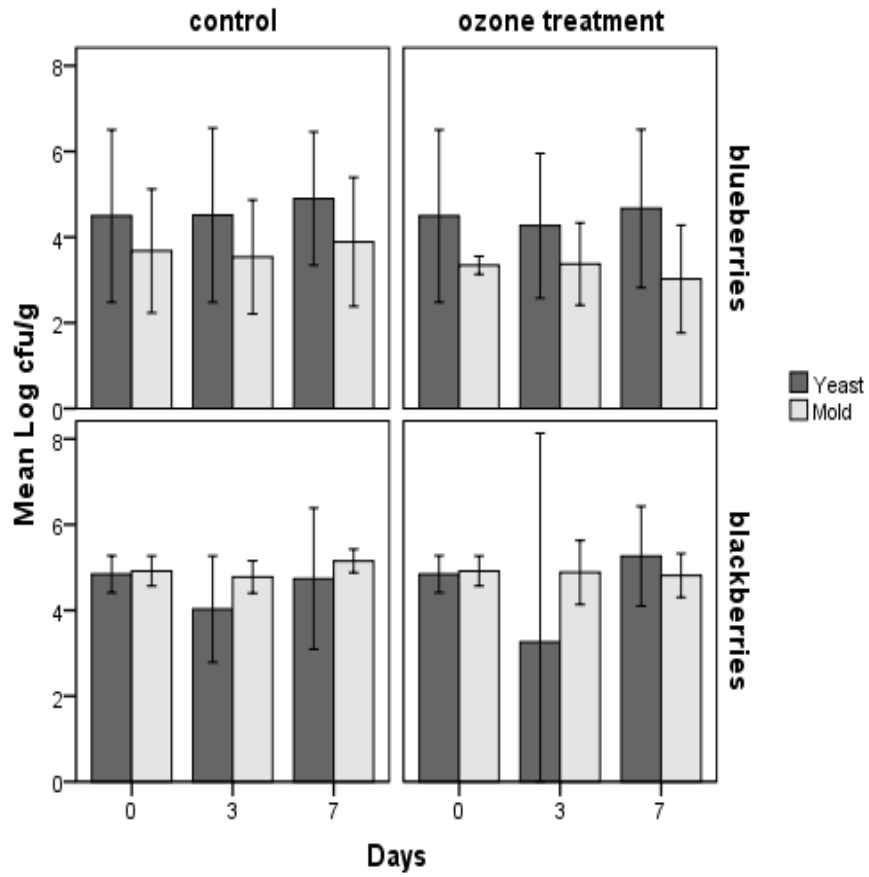


Figure 3:-Effect of gaseous ozone (1 ppm) on *Listeria innocua* mixture inoculated on blackberries and blueberries during three days storage at 8°C. Error bars represent 95% confidence limits (n = 3).

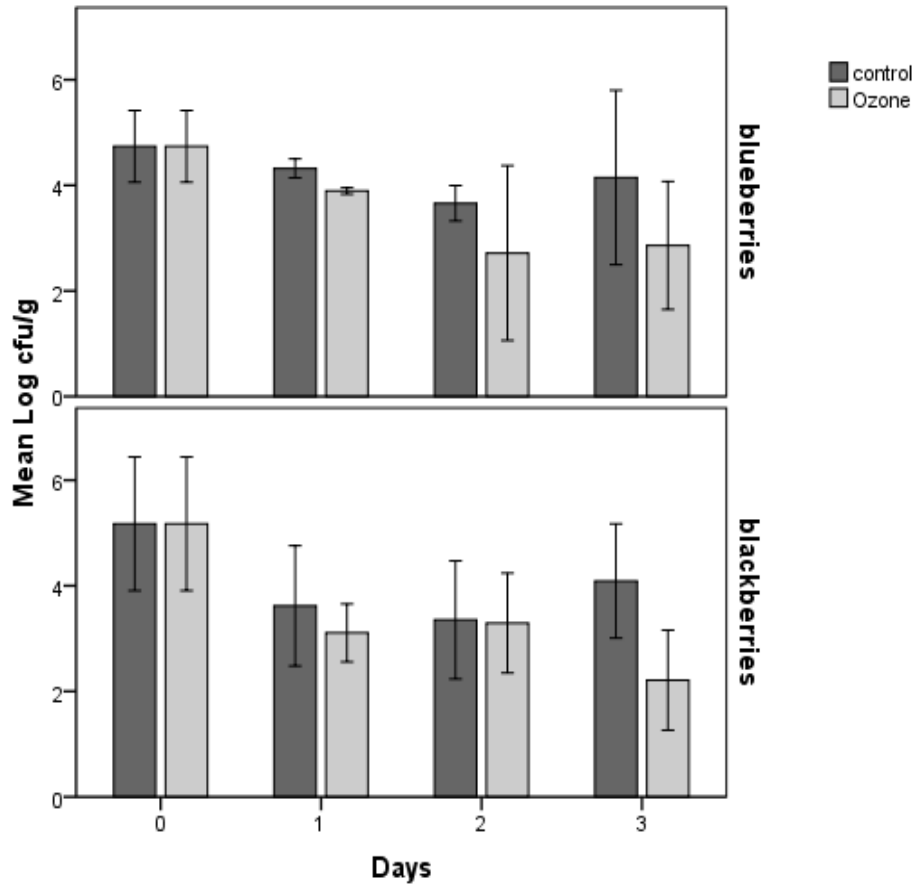
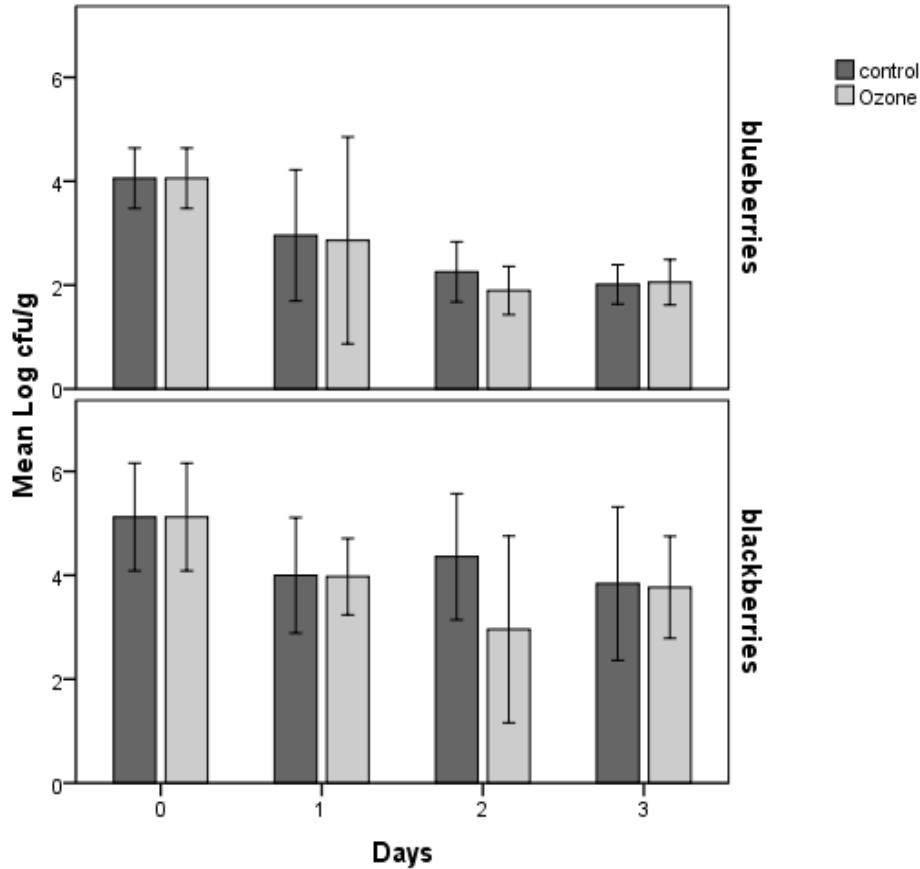


Figure 4:- Effect of gaseous ozone (1 ppm) on *E. coli* mixture inoculated on blackberries and blueberries during three days storage at 8°C. Error bars represent 95% confidence limits ($n = 3$).



Conclusions:-

A critical point for the microbiological safety in the processing line of ready-to-eat vegetables is the washing step. Several studies have investigated the antimicrobial activity of different compounds and have indicated that a log reduction of more than 2.0 of naturally present microorganisms is rarely achieved (Tomasoni *et al.* 2001; Guerzoni *et al.* 1996; Gras *et al.* 1994).

Therefore, the observations from the present study are in agreement with the previous studies that used gaseous ozone as a sanitizer: a 100 ppm ozone concentration for 30 minutes resulted in a 1.5 Log reduction in mould on both types of berries and in yeast on blueberries. Similar results were observed for *L. innocua* and *E. coli* inoculation of both types of berries.

Storage for three days in refrigerated condition in the presence of 1 ppm of ozone gas resulted in the sanitization of *L. innocua* (3 Log reduction in blackberries and 1.88 Log reduction in blueberries).

Furthermore, because berries are generally not washed and are stored and transported under refrigerated conditions, ozone gas treatment could improve the microbiological quality of fresh blueberries and blackberries.

References:-

1. Alwi N.A., Ali A., 2014. Reduction of *Escherichia coli* O157, *Listeria monocytogenes* and *Salmonella enterica* s.v. Typhimurium population in fresh-cut bell pepper using gaseous ozone. *Food Control* 46, 304-311.
2. Barth M.M., Zhou C., Mercier J., Payne F.A., 1995. Ozone Storage Effects on Anthocyanin Content and Fungal Growth in Blackberries. *J. Food Sci.* 60, 1286–1288.
3. Bermúdez-Aguirre D., Barbosa-Cánovas G.V., 2013. Disinfection of selected vegetables under nonthermal treatments: chlorine, acid citric, ultraviolet light and ozone. *Food Contr.* 29, 82-89.
4. Concha-Meyer A., Eifert J., Williams R., Marcy J., Welbaum G., 2014. Survival of *Listeria Monocytogenes* on fresh blueberry (*Vaccinium corymbosum*) Stored under Controlled Atmosphere and Ozone. *J. Food Protect.* 77, 832-836.
5. ECDC, EFSA (EFSA supporting publication– Second update) 2014. Outbreak of hepatitis A in EU/EEU countries. EN-581, 14 pp.
6. EFSA, 2014. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in berries). 12(6), 3706.
7. Fairchild T.M., Foegeding P.M., 1993. A proposed nonpathogenic biological indicator for thermal inactivation of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 59, 1247-1250.
8. Fan L., Song J., McRae K.B., Walker B.A., Sharpe D., 2007. Gaseous ozone treatment inactivates *Listeria innocua* in vitro. *J. Appl. Microbiol.* 103, 2657-2663.
9. Gras M., Druet-Michaud C., Cerf O., 1994. La flore bactérienne des feuilles de salade fraîche. *Sci. Alim.* 14, 173-188.
10. Guerzoni M.E., Gianotti A., Corbo M.R., Sinigaglia M., 1996. Shelf life modelling for fresh-cut vegetables. *Posthar. Biolo. Technol.* 9, 195-207.
11. Karaka H., Velioglu Y.S., 2007. Ozone applications in fruit and vegetable processing. *Food Rev. Int.* 23, 91-106.
12. Khadre M.A., Yousef A.E. and Kim J.G., 2001. Microbiological Aspects of Ozone Applications in Food: A Review. *J. Food Sci.* 66, 1242-1252.
13. Kim J.G., Yousef A.E. and Khandre M.A., 2003. Ozone and its current and future application in food industry. *Advan. Food Nutr. Res.* 45, 168-218.
14. Mukherjee A., Speh D., Jones AT., Buesing KM and Diez-Gonzalez F., 2006. Longitudinal microbiological survey of fresh produce grown by farmers in the upper Midwest. *J. Food Prot.* 69, 1928-1936.
15. Palou L., Crisosto C.H., Smilanick J.L., Adaskaveg J.E., Zoffoli J.P., 2002. Effect of continuous 0.3 ppm ozone exposure on decay development and physiological response on peaches and table grapes in cold storage. *Posth. Biol. Technol.* 24, 34-48.
16. Palumbo M., Harris L.J., Danyluck M.D., 2013. Survival of foodborne pathogens on berries. *Univ. Florida Ins. Food Agric. Sci.* 1-11.
17. Previdi M.P., Franceschini B., Fortini G., 2011. Impiego dell’ozono nel lavaggio e nella sanitizzazione di vegetali freschi destinati alla IV gamma. *Ind. Cons.* 1, 1-17.
18. Tomasoni L., Previdi M.P., Sansebastiano G., Chiuri A., 2001. Lavaggio e sanitizzazione di verdure destinate alle IV gamma. *Ind. Cons.* 76, 21-31.
19. Xu Liangji., 1999. Use of Ozone to improve the Safety of Fresh fruits and Vegetables. *Food Technol.* 53, 58-62.