〈총 설〉
KOREAN J. POST-HARVEST SCI. TECHNOL. AGRI. PRODUCTS
Vol. 2. No. 1, pp. 209~223(1995)

Study on Preservation of Vegetables by Ozone Treatment

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Introduction

Ozone has a potency for sterilization, deodorization, decolorization and dexcomposing of organic matters by strong oxidation. Sterilization of water supply was examined, and its effects were confirmed from ancient France. Ozone is effective for microorganisms and virus, it becomes to use not only sterilization and deodorization of water supply and water in pool but also sterilization of rooms in hospital.

Regarding food field. ozonation is found that it is useful way instead of food additives, and many reports were published recently. Technically, it is also used for sterilization, deodorization and decolorization.

As the point of issue, control of ozone concentration is difficult, the sterilization efficiency of low moisture foods or air is inferior, and human body or food materials are influenced by ozone.

Sterilization power and conditions for utilization of foods were investigated in my laboratory. So, here with I will explain to you one of them today.

The utilization pattern of ozone for foods is divided into two ways, namely exposing with ozone gas, and soaking or showering with ozonated water. Ozone is unstable, it is known that the half life of the ozone gas is about 1 hour, ozonated city water needs 20 minutes, ozonated distilled water needs 165 minutes. Fig. 1 showed the utilization pattern of ozone for foods. As the ozone gas, it is utilized for sterilization of materials, processing and production of water, and sterilization of air in factory or refrigerator rooms. Ozonated water, it is utilized for sterilization of defrozing water, washing water and processing water. Both ozone gas and water are able to be enclosed in packing films.

The ozonated water treatment is proved more effective than gas treatment in keeping freshness of fruits or vegetables, so, in this opportunity some results of ozonated water will be introduced.

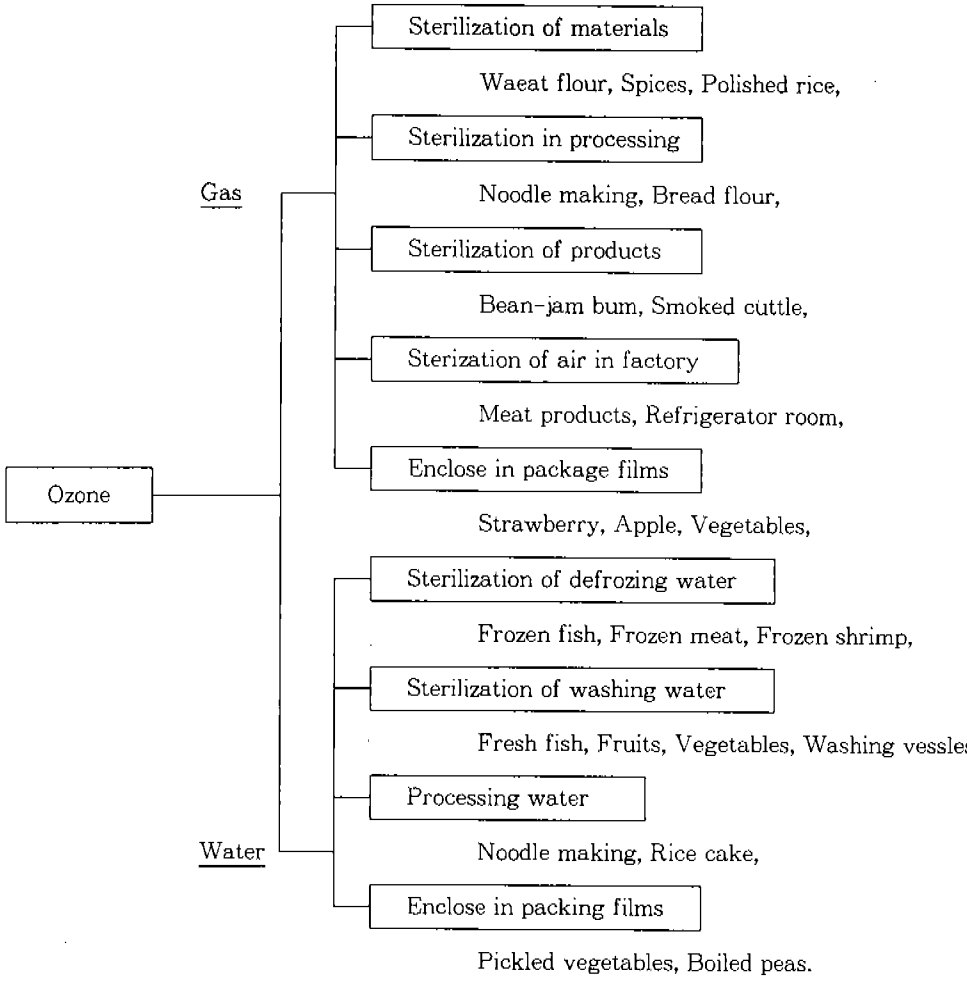


Fig. 1. System of ozone utilization for food industry

1. Apparatus for ozone treatment and measurement of ozone content

Apparatus for ozone treatment is shown in Photo 1. The gas material is air, it is flowed to ozonizer by compressor to ozone gas preparation stage. The concentration of ozone gas is chcked by ozone monitor, and carried to the water tank. Then oznated water is prepared by aeration in it. Samples were soaked there or showered in another tank. Ozonated water is recovered, and used for some experiments.

Photo 2 is apparatus for treatment in large quantities. Ozonated water is carried to water tank and circulated, and ozone concentration in water tank keeped constant. This water tank volume is 200 litters, it is possible to be use in big scale test.

Concentration of Ozone in water measured by DPD method. It is shown in Fig. 2. The principle of this method is to measure free and chloramine chlorine in water. Althogh there are so many measurement method of ozone in water, we decided the DPD method because of its accuracy and easily.

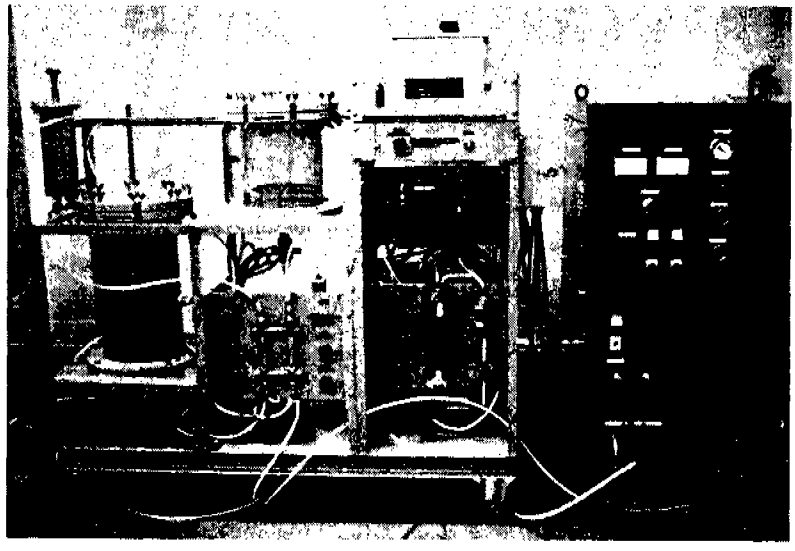


Photo 1

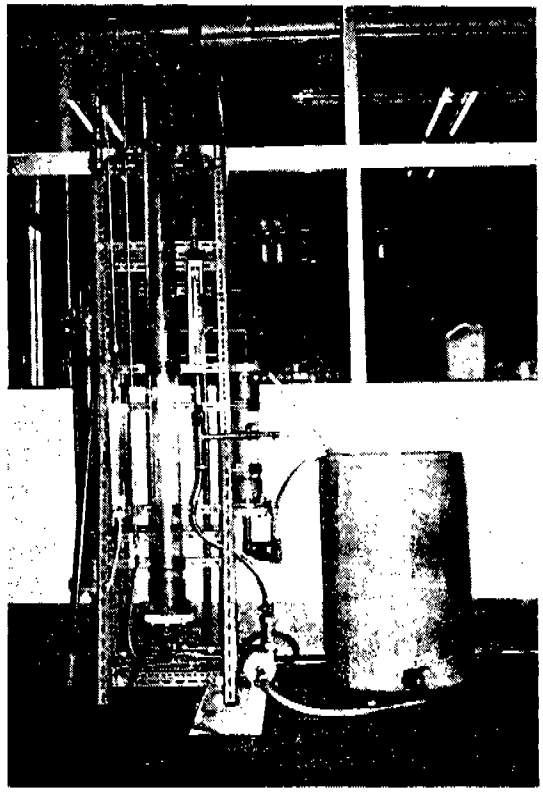


Photo 2

300ml △Flask
— KI
— 5ml of phosphate buffer
— 5ml of DPD solution[1)]
— 100ml of ozonated water
Titration with FAS solution*[2)]

Fig. 2. Determination of ozonated water concentration by DPD method

Concentration of ozonated water(ppm) = Titration value × 48/71
***1) Diethyl-p-phenylene diamine solution**
***2) Ferrous ammonium sulphate solution**

2. Effects for microorganisms

Sterilization power against mainly microorganisms were investigated. The suspension of preinculbated microorganisms was contacted with ozonated water for 10 minutes and viable cell count was investigated.

The results was shown in Table 1. *L. casei* and *Ps. fluorescens* died completely after contacted with 0.1 ppm ozonated water for 3 minutes. *E. coli* died completely after contacted with 1.0ppm ozonated water for 3 minutes or more, *St. aureus* died at 1.0ppm for 6 mimutes or more, *S. cerevisiae* died at 0.5ppm 3 minutes or more. But *B. subtilis* has the resistancy against ozone, it does not died completely eventhogh after contacted with 3 ppm ozonated water for 10 minutes.

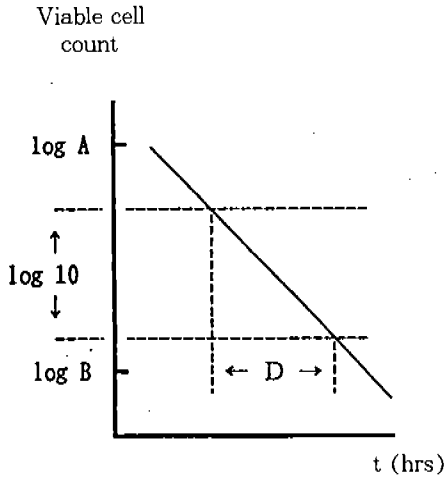
Table 1. Sterilization power of ozonated water against microorganisms

Species (Initial)	Concentration of ozonated water (ppm)	Treating time(min.) 3	6	10
L. casei (1.1×10^5)	0.1	0	0	0
	0.5	0	0	0
	1.0	0	0	0
	2.0	0	0	0
	3.0	0	0	0
Ps. fouorescens (3.4×10^4)	0.1	0	0	0
	0.5	0	0	0
	1.0	0	0	0
	2.0	0	0	0
	3.0	0	0	0
E. coli (2.4×10^5)	0.1	1.5×10^3(0.6)	1.4×10^3(0.6)	1.3×10^3(0.5)
	0.5	2.0×10^2(0.1)	1.9×10^2(0.1)	1.6×10^2(0.1)
	1.0	0	0	0
	2.0	0	0	0
	3.0	0	0	0
St. aureus (2.9×10^5)	0.1	2.4×10^5(82.8)	2.3×10^5(79.3)	2.2×10^5(75.9)
	0.5	4.1×10^4(14.1)	8.8×10^3(3.0)	8.6×10^3(3.0)
	1.0	6.0×10^4(20.7)	0	0
	2.0	4.6×10^4(15.9)	0	0
	3.0	8.6×10^2(0.3)	0	0
B. subtilis (1.5×10^5)	0.1	1.2×10^5(80.0)	1.2×10^5(80.0)	1.1×10^5(73.3)
	0.5	1.1×10^5(73.3)	1.0×10^5(66.7)	1.0×10^5(66.7)
	1.0	8.6×10^4(57.3)	8.2×10^4(54.7)	8.1×10^4(54.0)
	2.0	5.0×10^4(33.3)	4.8×10^4(32.0)	4.8×10^4(32.0)
	3.0	6.2×10^3(4.1)	5.0×10^3(3.3)	3.2×10^3(2.1)
S. cerevisiae (1.6×10^5)	0.1	6.7×10^3(4.2)	6.6×10^3(4.1)	6.1×10^3(3.8)
	0.5	0	0	0
	1.0	0	0	0
	2.0	0	0	0
	3.0	0	0	0

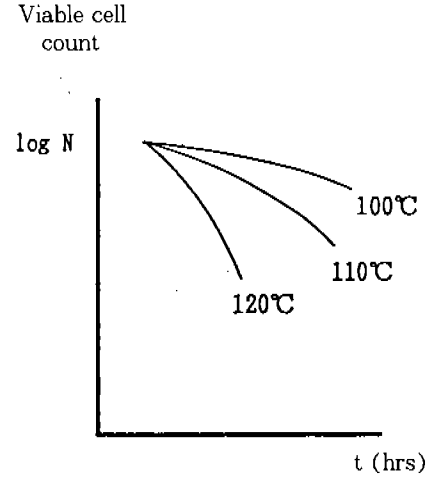
() Survival per cent

Hence, the resistancy against ozone of microorganisms was different among species.

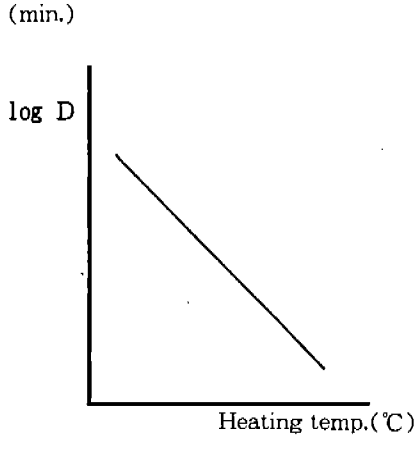
Then the sterilization of ozone was tried to be expressed similar with theory of heat sterilization. Theory of heat sterilization was showed in Fig. 3. Relationship between heating time and viable cell count at constant temperature changes linearly. D value is the time for log 10 changes of viable cell count.



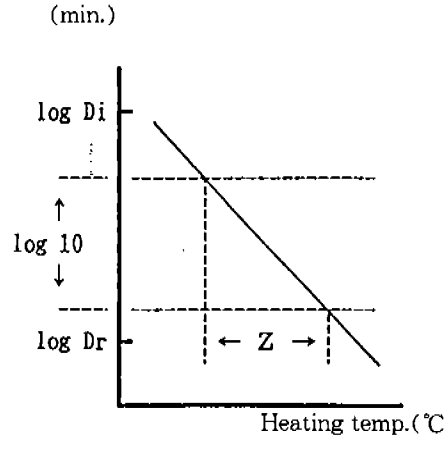
Death of microorganisms in constant temperature



Effect of heating temp.



Thermal reduction time curve(TRT curve)



Relationship between D value and heating temperature

Fig. 3. Theory of heat sterilization for microorganisms

But the death of microorganisms in different temperature is shorter in higher temperature. Relationship between heating temperature and D value changes linearly. Z value is the heating temperature for log 10 changes of D value. So, in the same way, D value and Z value were obtained to replace heating temperature with concentration of ozonated water.

The microorganisms shown in Table 2 were low the D value, they were influenced by ozone concentration more than contact time. So, it suggested that these microorganisms were died immediately by ozone treatment.

Table 2. D and Z value of microorganisms by ozone treatment

	D value(mim.)			Z value
	0.2ppm	0.5ppm	0.7ppm	(ppm)
E. coli	5.5	4.9		1.92
St. aureus	5.0	4.0		2.98
L. casei	3.3	3.3	2.3	2.01
S. cerevisiae	7.5	6.0	4.8	2.50

Table 3 was shown the results of *Bacilus species*. Their Z value was high, especially B. subtilis and *B. coagulans*. So, it is necessary to prolong contact time with oznated water.

It is well known that sterilization power of ozone decrease by contamination with organic matter. Sterilization power in case of contamination with food materials were investigated.

Table 3. D and Z value of microorganisms by ozone treatment

	D value(min.)				Z value
	0.5ppm	1.0ppm	1.5ppm	2.0ppm	(ppm)
B. natto		20.2		9.2	2.57
B. subtilis		13.5		12.5	6.60
B. coagulans	6.0	5.5	4.7		7.10
B. cereus	6.8	6.3	5.5		4.45

The changes of viable cell count contaminated with 1% of casein, starch or skim milk were shown in Table 4. Sterilization power was different among microorganisms and food materials. The general trend of sterilization power was lower in casein than in starch.

Sterilization power in case of contaminated with some saccharides or amino acids were investigated. Residual ozone contents with them were shown in Table 5. Concentration of ozone decreased immediately just after contact with them, it was also lower in amino acids than in saccharides.

Table 4. Sterilization power of ozonated water in the existercec of food materials.

Microorganisms (Initial)	Concentration of ozone (ppm)	Food materials(1%) Control	Casein	Starch	Skim milk
L. casei (1.1×10^5)	0.1	0	3.2×10^4(29.1)	4.9×10^3(4.5)	9.4×10^3(8.5)
	0.5	0	1.9×10^4(17.3)	4.8×10^3(4.4)	7.2×10^3(6.5)
	1.0	0	1.3×10^4(11.8)	2.0×10^3(1.8)	5.6×10^3(5.1)
	2.0	0	7.1×10^3(6.5)	2.1×10^2(0.2)	3.1×10^3(2.8)
	3.0	0	5.3×10^3(4.8)	0	1.1×10^3(1.0)
Ps. fouorescens (3.4×10^4)	0.1	0	9.9×10^4(29.1)	5.4×10^3(15.9)	7.7×10^3(22.6)
	0.5	0	7.4×10^3(21.8)	1.1×10^3(3.2)	4.6×10^3(13.5)
	1.0	0	3.2×10^3(9.4)	0	3.9×10^2(13.5)
	2.0	0	2.0×10^3(5.9)	0	2.4×10^2(0.7)
	3.0	0	9.0×10^2(2.6)	0	5.9×10 (0.2)
E. coli (2.4×10^5)	0.1	1.3×10^3(0.5)	2.2×10^5(91.7)	8.8×10^4(36.7)	1.1×10^5(45.8)
	0.5	1.6×10^2(0.1)	2.1×10^5(87.5)	8.4×10^3(3.5)	8.7×10^3(3.6)
	1.0	0	1.8×10^5(75.0)	3.3×10^3(1.4)	9.1×10^3(3.8)
	2.0	0	1.7×10^5(70.8)	1.5×10^3(0.6)	4.5×10^3(1.9)
	3.0	0	1.5×10^5(62.5)	0	3.3×10^3(1.4)
St. aureus (2.9×10^5)	0.1	2.2×10^5(75.9)	2.7×10^5(93.1)	2.3×10^5(79.3)	2.6×10^5(89.7)
	0.5	8.6×10^3(3.0)	9.9×10^4(34.1)	4.5×10^4(15.5)	4.9×10^4(16.9)
	1.0	0	6.2×10^4(21.4)	8.2×10^2(0.3)	2.3×10^4(7.9)
	2.0	0	4.1×10^4(14.1)	1.1×10^2(0.1)	1.5×10^4(5.2)
	3.0	0	2.0×10^4(6.9)	0	1.0×10^4(3.4)
B. subtilis (1.5×10^5)	0.1	1.1×10^5(73.3)	1.4×10^5(93.3)	1.2×10^5(80.0)	1.3×10^5(86.7)
	0.5	1.0×10^5(66.7)	1.3×10^5(86.7)	1.1×10^5(73.3)	1.2×10^5(80.0)
	1.0	8.1×10^4(54.0)	1.1×10^5(73.3)	8.7×10^4(58.0)	9.5×10^4(63.3)
	2.0	4.8×10^4(32.0)	6.1×10^4(40.0)	5.2×10^4(34.7)	5.4×10^4(36.0)
	3.0	3.2×10^3(2.1)	1.4×10^4(9.3)	6.9×10^3(4.6)	7.8×10^3(5.2)
S. cerevisiae (1.6×10^5)	0.1	6.1×10^3(3.8)	2.6×10^4(16.3)	1.3×10^4(8.1)	8.1×10^4(10.0)
	0.5	0	1.9×10^4(11.9)	6.1×10^3(3.8)	9.2×10^3(5.8)
	1.0	0	7.9×10^3(4.9)	3.8×10^3(2.4)	5.1×10^3(3.2)
	2.0	0	5.8×10^3(3.6)	1.1×10^3(0.7)	2.8×10^2(1.8)
	3.0	0	1.6×10^3(1.0)	0	6.8×10^3(0.4)

() Survival per cent

Table 5. Survived concentration of ozonated water in case of containing food materials (%)

cMaterials (1.0%)	Treating time(min.)			
	1	5	10	20
Control	77.0	63.5	54.2	44.0
Glucose	4.2	1.2	0	0
Fructose	8.7	1.8	0	0
Sucrose	5.7	1.3	0	0
Dextrin	4.0	2.0	1.0	0
Starch	4.2	1.2	0.5	0
Triptophane	0	0	0	0
Glutamic acid	0	0	0	0
Alanine	1.5	0	0	0
Cystine	3.0	1.5	0	0
Valine	1.0	0	0	0
Methionine	0	0	0	0
Casein	1.0	0	0	0

Concentration of ozonated water : 1.0ppm

4. Preservation for vegetables

The keeping freshness of vegetables were investigated. Many cutted vegetables are sell recently at supermarket in Japan. The way of keeping freshness is only but washing with hypochlorite. So, ozone treatment is expected as its improvement.

Changes of viable cell count of shredded cabbage in case of treated with 2 ppm ozonated water for 10 minutes and stored at 10℃ were shown in Fig. 4.

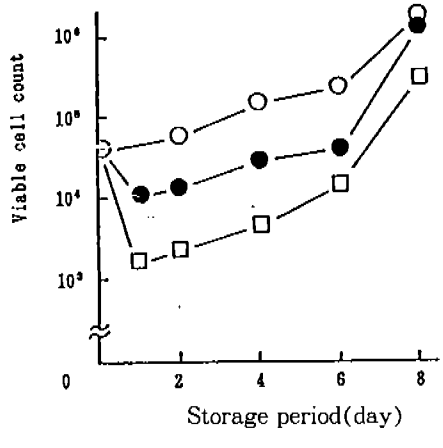


Fig. 4. Changes of viable cell count of ozonated water treated shredded cabbage during storage
○—○ Non-treatment, ●—● water treatment, ■—■ 2ppm.

Viable cell count of ozonated water treated cabbage was lower than the others, it was found that ozone treatment was effective way for inhibition of microorganisms growth. The color of shredded cabbage was shown in Table 6.

Table 6. Color defferences of shredded cabbage during storage.

	Storage period	L	a	b	W	YI	⊿E
Control	0(day)	84.29	0.62	7.45	82.61	16.68	—
Water treatment	0	85.43	0.29	6.21	84.19	13.79	1.71
	2	84.65	0.68	8.60	82.39	19.42	1.20
	4	81.34	0.78	10.07	78.78	23.69	3.94
	6	81.59	0.89	9.68	79.18	22.56	3.51
	8	77.89	1.27	11.49	75.06	28.58	7.59
2ppm ozonated water treatment	0	85.59	0.42	6.99	83.98	15.52	1.39
	2	81.98	0.49	8.22	89.19	18.57	2.43
	4	83.00	0.80	9.28	80.62	21.31	2.24
	6	81.88	0.78	8.88	79.81	20.72	2.80
	8	80.44	1.05	10.05	77.99	23.72	4.68

Alcohol insoluble solid prepared from sample was measured the color of them by color difference meter. The titles meant are as follws; L; lightness, a:red, b:yellow, W:whiteness, YI:yellow index, ⊿E:color defferences to non-treatment sample. ⊿E value of stored sample for 8 days, with water treatment showed 7.59 as with ozone treatment showed 4.66, the color changes were keeped by ozone treatment.

Photo 3 showed the real shredded cabbage. Non treatment is showed left, 2ppm ozone treatment showed right. Thty were stored at 10℃ for 5 days. Ozone treated sample was found good in keeping freshness.

The photos of ozone treated cabbage were showed as follows. Photo 4 showed ozone treatment with 0.5ppm for 30minutes. There was no difference compared with non-treatment. Photo 5 showed ozone treatment with 2ppm for 5minutes. There was also no difference. Photo 6 showed treatment with 2ppm for 40minutes. It was found that surface cell was damaged. So, high concentration and long time treatment exposure was not suitable way for cabbage preservation. The microscope appearance of no treated surface cells were shown in Photo 7. It was observed that cells were formed orderly. The microscope appearance of surface cells treated by 2ppm for 40minutes was shown in Photo 8. It was clearly observed that cells were damaged.

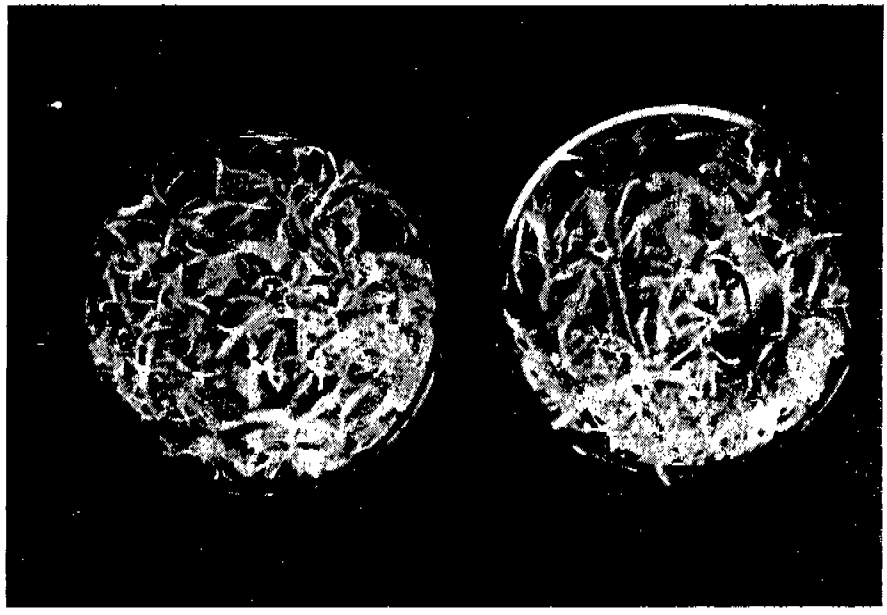


Photo 3

Photo 4

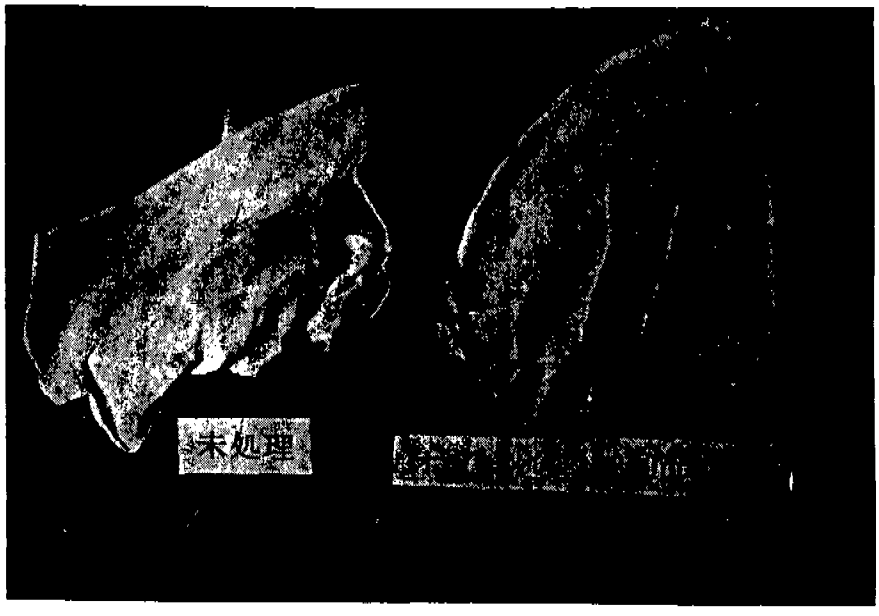


Photo 5

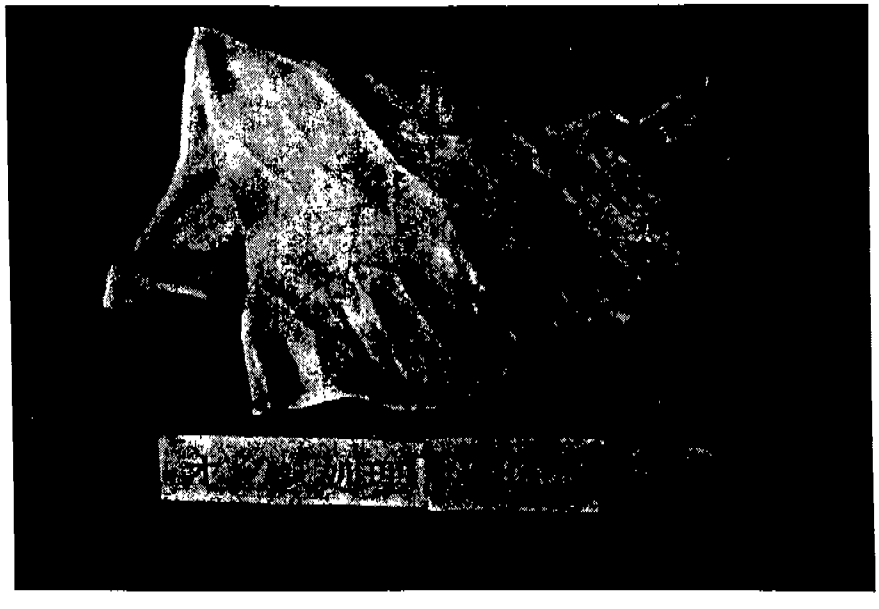


Photo 6

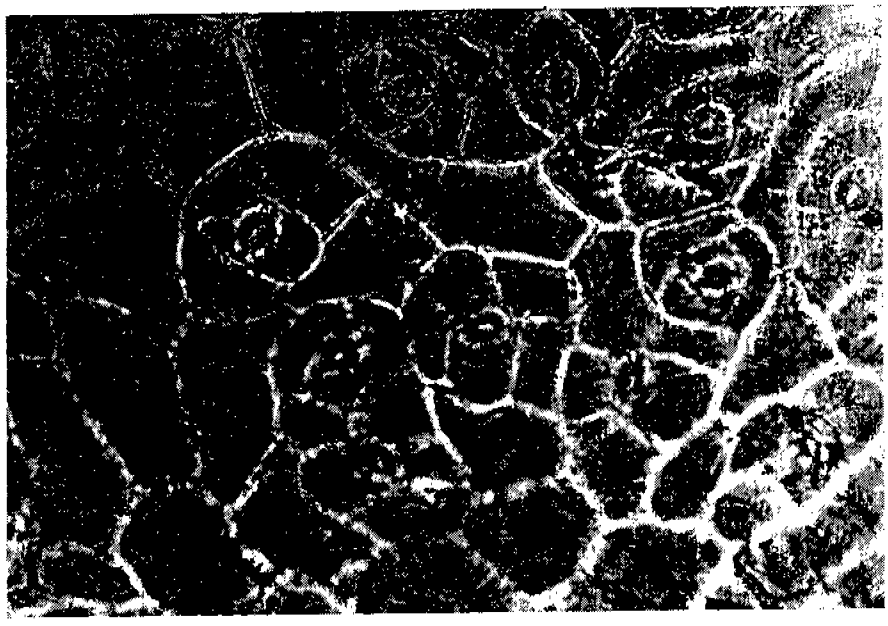


Photo 7

Photo 8

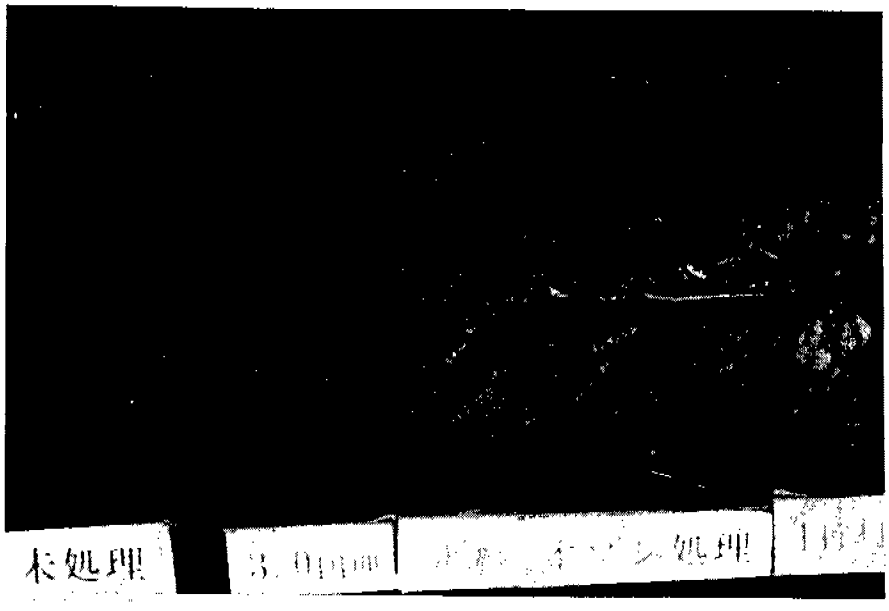


Photo 9

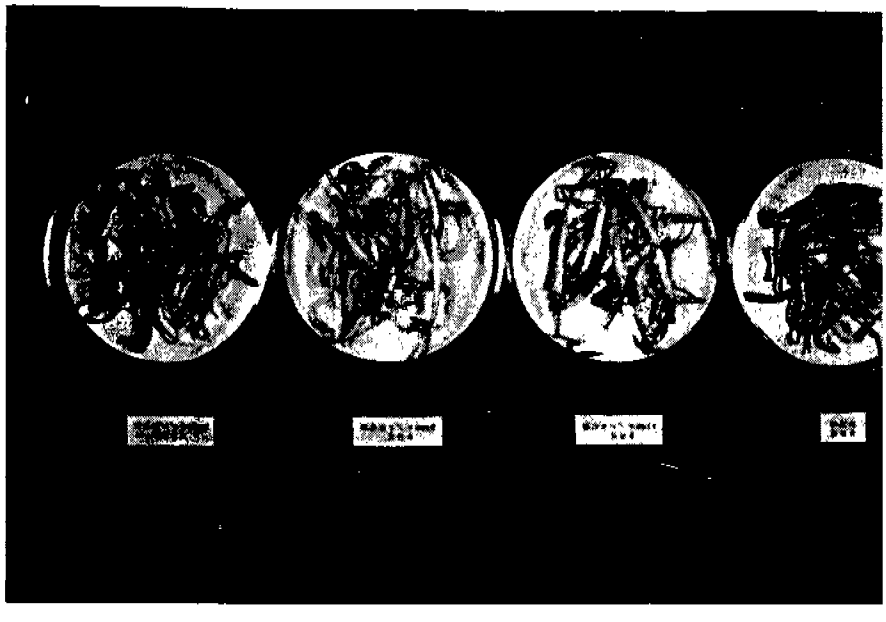


Photo 10

Photo 11

Then pection and reduced sugar eluted in ozonated water were investigated. The results were showed in Table 7. The eluted materials incereased proportionately with ozone concentration and treating time. It is expected that the reason was cell damage.

Table 7. Eluted pectin and reducing sugar in ozone treated water (μg /100 g of fresh sample)

Concentration of ozonated water (ppm)	Pectin			Reducing sugar	
	5min.	30min.	60min.	30min.	60min.
0	9.85	10.49	11.49		
0.2	13.32	16.02	25.43	3.86	15.93
0.5	15.61	20.01	25.95	3.46	21.00
1.0	24.17	32.56	34.21	3.07	21.10
2.0	29.78	32.92	34.92	11.15	48.56
3.0	23.68	37.11		49.52	

Other vegetable results were shown in some photos. Spinach was shwon in Photo 9. It was treated with 3ppm for 15minutes. There was no change the surface cells treated high concentration ozonated wear for long time. Hence, the ozone tolerance of cells were different among vegetables.

The next sample, bean sprouts were examined. Changes of viable cell count of bean sprouts treated with 1~3ppm for 10minutes and stored at 10℃ were shown in Fig. 5. The microorganisms growth was inhibited by ozone treatment. The color of bean sprouts was shown in Table 7. ⊿E value of stored sample for 8 days, with water

treatment showed 1.89 as with ozone treatment showed 1.06, the color changes were retained slightly by ozone treatment. Photo 10 showed the real bean sprouts. It showed in order from left to right side, non-treatment, 1ppm, 2ppm and 3ppm treatment and stored at 10℃ for 8 days. 2ppm ozone treated sample was the best in keeping freshness than the others. 3ppm ozone terated sample was not so good. It expected that bean sprouts cell was damaged by high concentration of ozone.

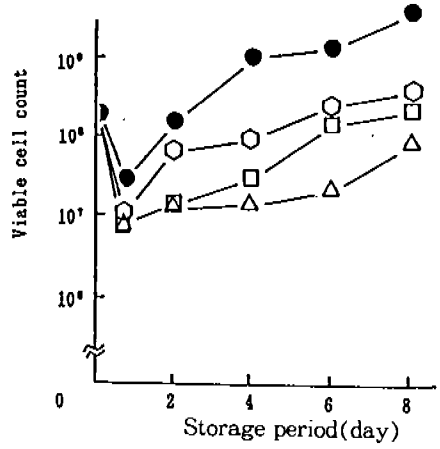


Fig. 5. Changes of viable cell count of ozonated water treated bean sprouts during storage
●—● Water treatment, ◇—◇ 1ppm, □—□ 2ppm, △—△ 3ppm.

Photo 11 showed lettuce treated with 2ppm ozonated water for 10minutes and stored at 10℃ for 3 days. Ozone treated sample was shown at left side, the browing of cut end was inhibited completely. Ozone treatment was effective way not only sterilization for microorganisms but also inhibition for enzyme activities.

5. Future development

Unilization of ozone for keeping freshness of vegetables were introduced. Ozone treatment is efferective way but it is poisonous for human body, the concentration of ozone in workroom must be keep less than 1ppm.

Ozone is unstable and effedt for sterilization is different among microorganisms. So, it is necessary to test for each sample in the suitable conditions. Ozonation also influences enzyme activities, utilization of ozone will be larger. So, it is expected that ozone treatment will be widely utilized for food industry.