

The Investigation of Decontamination Effects of Ozone Gas on Microbial Load and Essential Oil of Several Medicinal Plants

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Abstract

Today, Ozone as a disinfectant method, without putting on the harmful effects on human and plant products, it is alternative common methods for disinfection of plant material. The research as a factorial experiment was conducted on the basis of randomized complete block design with three replications and the effects of Ozone gas on decreasing the microbial load of some important medicinal plants include: peppermint (*Mentha piperita*), summer savory (*Satureja hortensis*), Indian valerian (*Valeriana wallichii*), meliss (*Melissa officinalis*) and Iranian thyme (*Zataria multiflora*) were investigated. Medicinal plants leaves were treated with Ozone gas concentration 0.3, 0.6 and 0.9 ml/L at times of 10 and 30 then total count, coliform and mold and yeast of the samples were studied. The result showed that Ozone gas decreases microbial load of medicinal plants samples, but Ozone gas and Ozone gas in medicinal plants interaction had no effect on essential oil content. The lowest and the highest of microbial load were detected in samples treated with concentration of 0.9 ml/L of Ozone gas and control respectively. The highest and the lowest of microbial load were observed in Iranian thyme and Indian valerian respectively. Also result showed that Ozone gas treatment for 30 min had the greatest of effect in reducing the microbial load and 0.9 ml/L Ozone gas concentration had the lowest of microbial load. Results of this survey reflect that the use of Ozone as a method of disinfection for medicinal plants is a decontamination.

Keywords: decontamination, essential oil, medicinal plants, microbial load, Ozone gas

Introduction

Decontamination of microorganisms in herbal plant, usually performed with decontamination method includes decontamination with methyl bromide, ethylene oxide, heat treatment, irradiation with gamma rays or electron beam and Ozone (Leistriz, 1997).

The use of ethylene oxide and methyl bromide due to the release of toxic compounds, persistent and destructive effects for the environment, has been limited. Heat treatment is cause of changing in the organoleptic properties and color of medicinal plants (Inan *et al.*, 2007; Juri *et al.*, 1986).

Therefore, selecting a suitable method for disinfection medicinal plants is very important. The use of Ozone by the United States Department of Agriculture in 1997 for the recovery of water was approved for poultry industry. One year after the original study information, on the Ozone in the world by a special committee, in 1997, voted to GRAS (general recognized as safe) of Ozone, as a disinfection and decontamination methods for foods. EPA (Environmental protection agency) researchers have confirmed that Ozone is safer than chlorine and other disinfectants (Kahfourshan *et al.*, 2005).

Due to the high oxidizing properties of Ozone (Perry and Yousef, 2011), and without having unfavorable effects on products, in comparison with other oxidizing agents, it became replacing of chlorine (due to dangerous for human and environment and not significant effect on microbes, Rice *et al.*, 1982). The Ozone as a treatment of postharvest in vegetables and fruits are used as gas and water (Barth *et al.*, 1995; Karac *et al.*, 2007; Tzortzakis *et al.*, 2007).

The important point about the use of Ozone as a disinfectant, half-life of Ozone is only 20 min in water at room temperature and when it decomposes into simple molecules of oxygen, which is not harmful for consumers (Graham *et al.*, 1997).

Information of little about effect of Ozone on microbial load of medicinal plants was reported. Inan *et al.* (2007) were evaluated the effect of different concentrations of Ozone on the red pepper. The results showed that the amount of Aflatoxin B1 in red pepper decreased after ozonation.

Effect of Ozone on microbial inactivation and sensory characteristics of red pepper was examined in 2008 by Akbas and Ozdemir. Results showed that Ozone treatments with concentration of 1 mg/L for 360 min can reduce the number of *Escherichia coli* and *Bacillus cereus* but Ozone concentrations greater than 5 mg/L can be used for the re-

duction of *B. cereus* spores. The aim of this study is the investigation of concentrations effect of 0.3, 0.6 and 0.9 ppm of Ozone gas as an important decontamination method on the microbial load of medicinal plants such as summer savory, Iranian thyme, peppermint, meliss and Indian valerian.

Materials and methods

Samples and sampling

In order to prepare of medicinal plants samples; peppermint and Iranian thyme were collected from the farm of Faculty of Agriculture, Ferdowsi University of Mashhad. Other samples such as summer savory and Indian valerian were purchased from traditional shops, and they were transferred to the Plant Science Institute of Ferdowsi University of Mashhad, and spices of medicinal plants were confirmed.

Study of microbial load

The 10 gram samples of medicinal plants were weighed and diluted with 90 ml buffered peptone saline (peptone, 1%, NaCl, 8.5 g in 1 L water, Liofilchem Co., Italia). And it was homogenated for 2 min. The homogenate was used for determination of microbial load. Enterobacteriaceae and coliform were determined using with 0.1 ml of each dilution in Violet Red Bile lactose (VRBL) agar (Liofilchem Co., Italia), over-layered with VRBL agar and the plates were incubated at 37°C for 24 h (according to the method of ISO4832). According ISO 7954 for determination of mold and yeast used from yeast extract glucose chloramphenicol agar (Liofilchem Co., Italia). The total count (plate count agar, Liofilchem Co., Italia) was determined by method of ISO 4833. Each sample was analyzed in 3 replicates (each replicate was tested with duplicate pour plates).

Ozonation of medicinal plants

Herbal samples for ozonation with Ozone gas were transferred to laboratory of Ferdowsi University of Mash-



Fig. 1. Trial steps

had. Ozone gas was produced by corona discharge Ozone generator with production capacity 10 g/h of Ozone gas (OZONEAB co. A.S10. Iran) using Oxygen. Oxygen produced (the source of Oxygen was water of by LANGFEL Co. model, LFY-I-5A-W) with capacity of maximum production 10 L/min. Finally, Ozone gas was transferred to special barrel of medicinal plants samples. Then, by the sensor of Ozone measure (OZONEAB Co) the amount of Ozone entering to the barrel was measured. (Fig. 1 and 2).

Essential oils analysis

The essential oils of the treated Medicinal plants samples with Ozone gas were extracted by water distillation using Clevenger type apparatus.

Statistical analysis

All tests were performed in three replication. Analysis of variance and means comparison were calculated, by using SAS 9.1 (Cary, NC, USA, 2002-2003). Means square comparisons were different at the 5% significance level by the least significant difference test.

Results

Tab. 1 provides data for the reduction of total count, mold and yeast, coliform and essential oil on medicinal plants following Ozone gas treatments. It is evident that microbial load of medicinal plants affected by Ozone gas had different concentrations ($p < 0.01$) (Tab. 1). But Ozone

Tab. 1. Mean squares of microbial load of medicinal plants at different Ozone gas concentration

SOV	df	Total count	Mold and Yeast	Coliform	Essential oil
Ozone(O)	6	8.931**	12.715**	19.197**	0.022ns
Medicinal plants (MP)	4	7.642**	52.194**	41.855**	32.277**
O×MP	24	1.166**	1.883**	1.493**	0.021ns
Error	70	0.078	0.112	0.191	0.064

** significant at $p < 0.01$; NS= Non-Significant

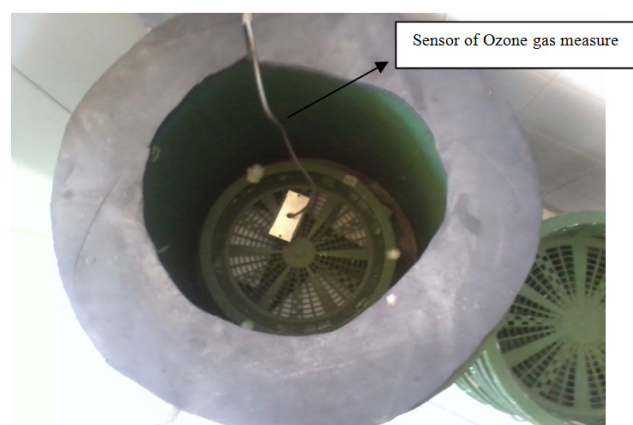


Fig. 2. The barrel of medicinal plants samples

gas and interaction effect had no effect on essential oil content. Ozone gas had a significant decrease on total count, mold and yeast and coliform (Tab. 2). It was found that by increasing the Ozone gas concentrations from 0.3 ppm to 0.9 ppm, microbial load reduction significantly increased ($p < 0.05$). The highest and lowest level of contamination was observed in the control and in the 0.9 ppm concentration of Ozone gas for 30 min, respectively (Tab. 2). The medicinal plants samples which were treated with Ozone gas are shown in Tab. 3. The result indicated that the highest of total count, mold and yeast, coliform and essential oil were observed in Indian valerian, summer savory, and Iranian thyme and the lowest of total count, mold and yeast, coliform and essential oil were observed in meliss, Iranian thyme and meliss respectively.

The decontamination curves for total count mould and yeast and coliform in peppermint, summer savory, Iranian thyme, Indian valerian and meliss after ozonation treatment are shown in Fig. 3, 4 and 5. The results showed that the 0.9 ppm concentration for 30 min of Ozone gas was the effective concentration for reduction 1.12 and 1.79 log cycles of total count contamination of peppermint and summer savory. The microbial analysis after ozonation with concentration of 0.9 ppm for 30 min showed that no total count was observed in Iranian thyme comparing with control samples (Fig. 3). And After ozonation with 0.9 ppm concentration for 30 min of Ozone gas the total count were decreased at 3.5 log cycle in meliss, also decrease in the total count with an increase of the Ozone gas concentration (0.3 ppm for 10 min by 0.9 ppm for 30 min). Decrease in the total count with an increase of the Ozone gas concentration (0.6 ppm for 30 min) in sum-

Tab. 2. Mean squares of microbial load at different Ozone gas concentration

Ozone	Total count	Mold and Yeast	Coliform
Control	4.48 a	3.65 a	3.62 a
0.3 ppm, 10 min	4.37 a	3.27 b	3.37 ab
0.3 ppm, 30 min	4.29 ab	2.81 c	3.23 bc
0.6 ppm, 10 min	4.09 b	2.45 d	3.01 c
0.6 ppm, 30 min	3.86 c	2.05 e	2.53 d
0.9 ppm, 10 min	3.51 d	1.81 f	1.64 e
0.9 ppm, 30 min	2.26 e	0.94 g	0.46 f

Means followed by the same letter in a column are not significantly different using LSD test at $p=0.05$

Tab. 3. Mean squares of microbial load of medicinal plants

Medicinal plants	Total count	Mold and Yeast	Coliform	Essential oil
Peppermint	4.24 b	3.59 b	3.42 b	2.23 b
Summer savory	4.15 b	1.41 d	3.81 a	2.02 c
Iranian thyme	3.30 c	0.32 e	0.20 e	2.76 a
Indian valerian	4.17 a	4.18 a	2.44 d	0.23 d
Meliss	3.08 d	2.64 c	2.89 c	0.04 e

Means followed by the same letter in a column are not significantly different using LSD test at $p=0.05$

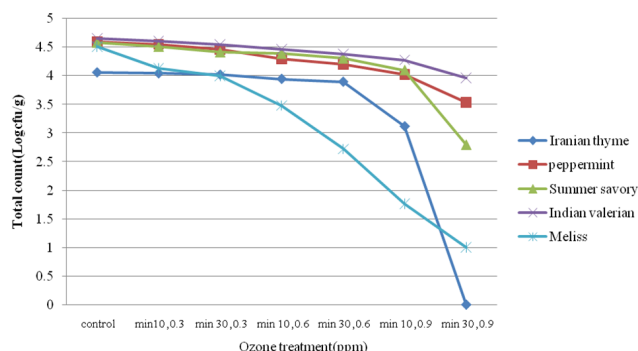


Fig. 3. The effect of treatment of Ozone gas on the total count in medicinal plants

mer savory, peppermint and Iranian thyme. The results in the present study showed that total count decreased in all the medicinal plants samples due to using Ozone gas concentration 0.9 ppm for 30 min. Also peppermint, summer savory and Iranian thyme samples that were treated with concentration of 0.9 ppm for 30 and 10 min had lower total count in comparison to control and other treatments. No significant difference was observed among control and other treatments of Ozone gas concentration.

The data in Fig. 4 show the mold and yeast count of medicinal plants in response to different concentration of Ozone gas. Non-ozonation sample showed maximum level of mold and yeast count. Mold and yeast count levels decreased gradually (from concentration of 0.6 ppm for 10 min to 0.9 ppm for 30 min) with increase in Ozone gas concentration in meliss and peppermint. The highest and the lowest level of decontamination effect of Ozone gas observed in meliss and peppermint, respectively. No mold and yeast contamination was detected after ozonation with concentration of 0.3 ppm for 30min in Iranian thyme, 0.6 ppm for 30 min in summer savory and 0.9 ppm for 30 min in meliss. Otherwise, ozonation treatment resulted in complete sterilization of the Iranian thyme, summer savory and Meliss. The rate of reduction was little for doses up to 0.9 ppm for 30 min summer savory comparative with 0.3 ppm for 10 min. no significant differences observed on decreasing other medicinal plants samples that treated by Ozone gas.

The effects of the Ozone gas treatment on the coliform of medicinal plants are shown in Fig. 5. According to data

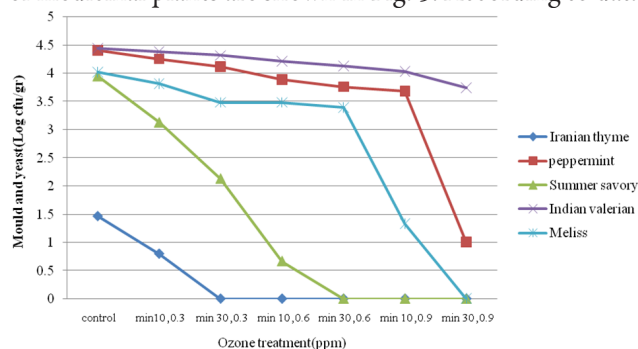


Fig. 4. The effect of treatment of Ozone gas on the mold and yeast in medicinal plants

of this figure, independent of the Ozone gas concentration, there was significant decrease on the coliform in medicinal plants. Coliform were eliminated by Ozone gas concentration of 0.6 ppm for 10 min up to 0.9 pp for 30 min in Iranian thyme samples. Also coliform were eliminated with Ozone gas concentration of 0.9 ppm for 30 min in meliss and Indian valerian. There was 4.02 and 2.45 log reduction of coliform at a concentration of 0.9 ppm for 30 min Ozone gas in peppermint and summer savory, respectively. The highest levels of coliform contamination were found in control and the lowest levels of coliform contamination were found in ozonation with concentration of 0.9 ppm for 30 min in all medicinal plants

By end all, the results of this study indicated that Ozone gas treatment had significantly effects had on microbial load of medicinal plants samples under investigation, Moreover, The total count, Molds and Yeasts and coliform was found 4.66, 4.44 and 4.45 in the control, respectively, not found in the samples of medicinal plants affective the Ozone. Tzortzakís *et al.* (2007) and Naitoh *et al.* (1987) were indicated that ozonation was affected on decreasing of fungal decay of strawberries, and microbial load of cereal grain powders, peas, beans, and whole spices. Scholars reported that Ozonation of onions, barley grain, black pepper, cereals, grains, peas, beans, spices, apple, orange, blackberries and grapes resulted to the reduction of microbial load (Allen *et al.*, 2003; Beucha *et al.*, 1992; Khadre, 2001; Kim *et al.*, 1999; Naitoh *et al.*, 1988; Song *et al.*, 2000; Zhao and Cranston, 1995). In this study, it was observed that, in the concentrations of Ozone utilized for decontamination of medicinal plants, the concentrations of 0.9 ppm for 30 min had the highest effect on total count, mold and yeast and coliform in summer savory, peppermint, Indian valerian and Iranian thyme. Han *et al.* (2002) and Proctor *et al.* (2004) observed that decreased the count of *Escherichia coli* O157:H7 increased ozone gas concentration and exposure time. Then, concentration of 0.9 ppm Ozone for 10 min had the lower contamination in plant samples was investigated. The highest of the total count and mold and yeast was observed in the Indian valerian and the lowest of total count was observed in meliss and Iranian thyme. Also, the highest and the lowest of

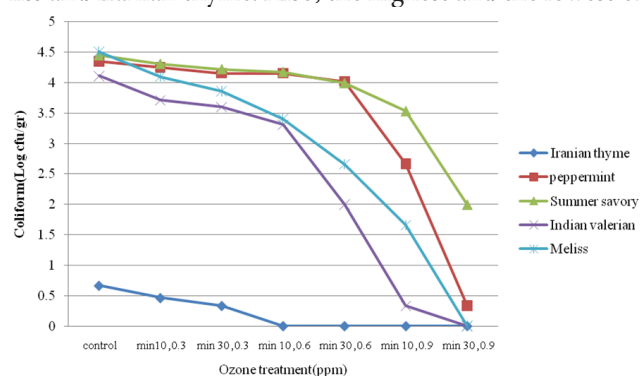


Fig. 5. The effect of treatment of Ozone gas on the coliform count in medicinal plants

coliform were found in summer savory and Iranian thyme. Control had the highest of microbial load and concentration of 0.9 Ozone gas had the lowest of microbial load. Researchers suggest that the use of Ozone, cause of the control or reduce the microbial load. This research results in studies of Inan *et al.* (2007) and Akbas *et al.* (2007) was reflected. Ozone effects on *Escherichia coli* and *Bacillus cereus* were evaluated in Pistachio. Results showed that Ozone gas was affected by increasing concentration and increases of time increase, and in physical and chemical properties (free fatty acids, color, peroxide value and fatty acid composition) of the peroxide value did not change in pistachios (Akbas and Ozdemir, 2006). Ozone effects on *Escherichia coli*, *Bacillus cereus* bacteria and *Bacillus cereus* spores in dried fig samples were analyzed. Results indicated that Ozone reduced these bacteria and *Bacillus cereus* spores and Ozone had no negative effect on the sensory properties of samples were investigated. Akbas and Ozdemir (2008), Perez *et al.* (1999) studied effect of Ozone on postharvest quality of strawberry fruit; they showed that Ozone is effective in preventing fungal spoilage. Also ozonation was decreased *Bacillus cereus* and *Micrococcus* by 6 log cycles in spice.

Conclusions

The research conducted that Ozone had a great impact on reducing the microbial load of peppermint, summer savory, Iranian thyme, Indian valerian and meliss. The Ozone concentration of 0.9 ppm for 30 min was the most effective in reducing the microbial load of medicinal plants. Results of this survey and research results of scholars other indicat that the use of Ozone as a method of disinfection for medicinal plants decontamination is a suitable method of decontamination.

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