Effect of chlorine dioxide in reduction of harmful microbes on fermented hot pepper paste

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Abstract In this study, the in vitro effects of chlorine dioxide (ClO₂) in growth reduction against *Candida glabrata*, *Zygosaccharomyces bisporus*, *Saccharomycopsis capsularis* and *Pichia pastoris* involving in deterioration of fermented hot pepper paste were studied to assess the applicability of chlorine dioxide to preparation of fermented hot pepper paste, and the concentration of ClO₂ required for destruction of harmful microorganisms through the fumigation of fermented hot pepper paste was evaluated. ClO₂ was treated by using ClO₂ generator for 15 min. *C. glabrata*, *Z. bisporus* and *S. capsularis* were reduced by ClO₂ concentration dependent and not detected by ClO₂ over 10 ppmV, whereas the *P. pastoris* was significantly perished by the treatment of ClO₂ over 30 ppmV. We suggest that the ClO₂ fumigation in stages of the preparation, disintegration, and fermentation of the paste

made of fermented hot pepper might be useful for control of harmful microbes therein.

Keywords Chlorine dioxide · Growth inhibition · Fermented hot pepper paste · Harmful microbes

Introduction

Fermented hot-pepper paste is an essential fermented flavor for Korean foods wherein the sweet taste of sugar created by an amylolysis, the savory taste of amino acid and nucleic acid originated from the protein of fermented soybeans, the hot taste of red-pepper, the saline taste of salt, and the sour taste created by organic acid created by fermentation and metabolism of microorganisms are integrated in harmony (Park et al., 2017). In general, the fermented hot-pepper paste is basically made of soybeans fermented by diverse microbes such as mold or bacteria; the inoculation of various strains into soybeans during the process of fermentation would require difficult technical control to restrict unnecessary kinds of bacterial strains (Jang et al., 2000).

Since fermented foods are the results of fermentation triggered by microbes introduced from nature, an introduction of useful microbes for fermentation is desirable, whereas the introduction of harmful microbes would affect the quality of fermented foods. The biggest difficulty in producing fermented hot pepper paste would be the process of fermentation that progresses continuously to the stage of products released to market and being stored on shelves. The progression of unnecessary fermentation process will be followed by the formation of pellicle by yeast, the creation of gases, and continuous proliferation of *Lactobacillus* resulting in rapid increase in acidity (Park et al.,

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2000). Currently, the heat sterilization at 70 °C is commercially employed to remove unnecessary microorganisms in raw materials or in the process of manufacturing fermented hot pepper paste (Kwon and Byun, 1996), whereas the unheated physicochemical methods using treatment with preservatives such as sorbate or benzoate, and with pH or ozone are reported (Kwon, 2017). Besides, the approaches using gamma rays or electron beams are also reported (Kim et al., 2001; Kwon and Byun, 1996).

Customers have revealed a higher level of preference to unheated sterilization methods with demands for safe foods containing no residuals resulted from such treatment. Sealed containers are typically used for fermented hot-pepper paste products to be stored in a low temperature environment. However, the pressure of carbon dioxide gases to be generated by microorganisms as time goes by would swell the containers. Thus, the packaging methods using absorbent of carbon dioxide gases resulted from microorganisms or using permeable pores of packaging materials are commercially used in the supply chain of the packaging and delivery of fermented hot pepper paste products (Park et al., 2017).

Chlorine dioxide is characterized by its solubility in water approximately 10 times higher than that of sodium hypochlorite which is commercialized as a disinfectant for foods with a lower level of reactivity to organic materials leaving less byproduct from that place. It does not create the trihalomethane, the carcinogenic substance, and it also does not create the chloramine from a reaction with ammonia (Moore et al., 1978). Its oxidation capacity is approximately three times higher than that of sodium hypochlorite and is a disinfectant for foods due to its characteristics that leaves no residual harmful substance to foods by its post-treatment decomposition in the air (Kim, 2001). The bactericidal effect of chlorine dioxide depends on the following conditions: the types of objects, level of concentration, humidity, the time required for treatment, and light conditions, etc. The bactericidal effects obtained from diverse fresh agricultural products including blueberries (Wu and Kim, 2007; Xu et al., 2016), paprika (Kim and Song, 2017), etc., have been reported. It has been registered as an additive for sterilizing and bleaching of flours for processed foods, and now is started to be enrolled in the applications for removal of biofilms and spatial sterilization.

In the experiment conducted in the present study, the in vitro effects of chlorine dioxide over destruction or growth inhibition of microbes involving in deterioration or spoilage of fermented hot pepper paste were identified to assess the applicability of chlorine dioxide to preparation of fermented hot pepper paste, and the concentration of chlorine dioxide required for destruction of harmful

microorganisms through the fumigation of fermented hot pepper paste was identified.

Materials and methods

Preparation of yeast strains

The four kinds of representative strains, registered as the yeasts causing deterioration of fermented hot pepper paste products, were purchased from Korean Culture Center of Microorganisms (KCCM) and cultured in respective media fit for each strain (Table 1). *Candida glabrata* is known as the yeast of deterioration, whereas the *Zygosaccharomyces bisporus* and *Pichia pastoris* create a sour taste of fermented hot pepper paste; *Saccharomycopsis capsularis* is characterized by its characteristics of pellicle formation. The media for culture of each yeasts strain were selected and prepared; the terminal mycelia of stock strains were then collected, plated, and cultured thereon under temperatures suitable for each strain. The cultured colonies were diluted with 0.85% NaCl and adjusted into 0.5 of McFarland turbidity, which could produce about 10^8 colony forming units (CFU/mL). The adjusted yeasts were grown in Tryptic Soy Agar plates (TSA, Difco, USA) and used for all subsequent experiments. The suspension was then diluted again by applying additional sterilized distilled water up to respective levels of 1/100 (10^6 CFU/mL), 1/1000 (10^5 CFU/mL), and 1/10,000 (10^4 CFU/mL). The suspensions prepared thereby were taken by 100 µL and smeared evenly on surfaces of respective media by using sterilized cotton swabs. The petri dishes were overturned in a way the smeared surfaces of media facing downward.

Treatment of chlorine dioxide

The various concentrations (0, 3, 10, 20, 30, and 60 ppmV) of chlorine dioxide were tested for evaluate of the effective yeastcidal concentration for 15 min through the gas supplying equipment while the lid of petri dish was opened. ClO_2 was generated by a chlorine dioxide generator (CA-300, PurgoFarm Co. Ltd., Hwasung, Korea) using an electrochemical method. Briefly, aqueous NaClO_2 was electrolyzed and the cleaved sodium ion was migrated to the cathode through patented multi-porous membrane electrode assembly leaving a high pure ClO_2 (> 99%) in the anode chamber. The evaporating ClO_2 was blown out through a vent into a fumigation chamber ($54 \times 44 \times 46$ cm) by controlled depending on the preset concentration of ClO_2 , which was monitored by ClO_2 concentration controller (PurgoFarm Co. Ltd) (Fig. 1A). When the ClO_2 fumigation were over 30 ppmV, all microbes were completely destroyed (Fig. 1B), the four

Table 1 Four kinds of food spoilage yeast strains related in fermented hot pepper paste and their characteristics

Name	Characteristics	No. of KCCM	Culture conditions
<i>Candida glabrosa</i>	Deterioration	50,576	24–26 °C, YM media
<i>Zygosaccharomyces bisporus</i>	Sourness	50,168	20–25 °C, YM media
<i>Saccharomycopsis capsularis</i>	Film formation	7818	24–26 °C, PDA media
<i>Pichia pastoris</i>	Sourness	7190	24–26 °C, YM media

Fig. 1 Chlorine dioxide (ClO_2) generator (A) and microbial death effects on four kinds of food spoilage strain from fermented hot pepper paste by chlorine dioxide treatment when treated over 30 ppmV chlorine dioxide concentration (B)

concentrations of ClO_2 (0, 3, 10, 20, and 30 ppmV) were subsequently treated to all yeast strains.

Measurement of microbial reduction effect of ClO_2

The plates treated with chlorine dioxide distinguished by each concentration were cultured for 3 days under temperature of 25 °C. Numbers of cultured colonies were counted on completion of culture, and the final numbers of bacteria therein were calculated by multiplying counted numbers of bacteria by dilution rate and then the natural logarithm thereof were taken.

Statistical analysis

For all experiments, each treatment repeated for three times; the results of collected from experiments were analyzed by using the Sigma Plot program to obtain values of mean and standard error (SE). The values were represented as numeric number or plotted as error bars on a graph.

Results and discussion

In the results, the strain #1 (*C. glabrosa*), and the strains of #2 (*Z. bisporus*), #3 (*S. capsularis*), and #4 (*P. pastoris*) rendered respective concentrations of diluted suspension of 10^5 CFU/mL and 10^4 CFU/mL that formed colonies granting the count at reasonable level in the districts not treated with chlorine dioxide (data not shown). Therefore, the experiments to be conducted thereafter were determined to be executed with these levels of dilution for the smearing and treatment with chlorine dioxide for respective concentrations of each strain.

The cell growths of all tested strains were reduced by dependent on the treated concentration of chlorine dioxide and completely inhibited over 30 ppmV (Fig. 1B). The baseline colony-density of *C. glabrosa* was 6.97 Log before ClO_2 fumigation. After exposure to ClO_2 at 3 ppmV, the total yeast colony density was 6.70 Log, respectively. Furthermore, complete growth inhibition was detected on the concentration of 10 ppmV of ClO_2 . The *Z. bisporus* strain which is a yeast creating a sour taste of fermented hot pepper paste was gradually reduced colony densities with of 5.94 Log (3 ppmV), and 4.85 Log (10 ppmV), finally it was shown that complete cell death

by treatment with the concentration of 20 ppmV of ClO_2 . *S. capsularis* strain which is known as a yeast triggering the formation of pellicle on fermented hot pepper paste has showed colonial densities of 5.39 Log (untreated) and 5.44 Log CFU/mL (3 ppmV), respectively; it was found completely destroyed by treatment with the concentration of 10 ppmV of ClO_2 . *P. pastoris* strain which is a yeast inducing sour taste of fermented hot pepper paste showed of 7.98 Log (untreated) and 6.34 Log (3 ppmV), 5.51 Log (10 ppmV), and 3.48 Log (20 ppmV), respectively; it was shown completely growth inhibition by treatment of 30 ppmV of ClO_2 (Fig. 2A) (Table 2). The rate of reducing of strains of *C. glabrosa* and *S. capsularis*, corresponded to

Fig. 2 Effect of ClO_2 treatment on the inactivation of *C. glabrosa*, *Z. bisporus*, *S. capsularis* and *P. pastoris* inoculated on each media (A) and effect of inactivation and death of 4 kinds of food spoilage yeast strain related in fermented hot pepper paste according to treated ClO_2 concentration (B). For all experiments, each treatment repeated for three times; the results of collected from experiments were analyzed by using the Sigma Plot program to obtain values of mean and standard error (SE). The values were represented as a numeric number or plotted as error bars on a graph

each level of concentration of chlorine dioxide appeared on an inverted “S” shaped curve plotted on a graph (Fig. 2B); this suggested the destruction of strains quite sensitively responded to a slight increase in the concentration of chlorine dioxide. Whereas, the strains of *Z. bisporus* and *P. pastoris* appeared on the slightly decreasing linear line corresponded to the destruction of strains decreased accordingly. This suggests the slower rate of destruction might be attributable to the comparatively higher level of viscosity and cohesiveness of the colonies of these strains that potentially disabled an infiltration of chlorine dioxide into the inside of suspension, or possibly prevented the contact of chlorine dioxide with the surface of smeared suspension. It might also be attributable to strong resistance of the strain of *P. pastoris* against chlorine dioxide that let the *P. pastoris* undestroyed and be suppressed during pre-treatment process and then started to grow again with the resume of post-culture.

There have been diverse studies explored various fresh agricultural products and reported effects of chlorine dioxide on destruction or inhibition of growth of microorganisms therein. The fresh and convenience foods were employed for studies conducted with liquid chlorine dioxide solutions applied for the control of harmful microorganisms such as *Listeria monocytogenes*, *Salmonella*, *Erwinia carotovora*, etc., whereas the gaseous chlorine dioxide was employed for the identification of bactericidal effects and residual tendency over raw materials of the fresh convenience foods or whole fruits (Gomez-Lopez et al., 2009). For the case of the mold of *Alternaria alternata* that infects and corrupts agricultural products, the application of chlorine dioxide of 0.075 ppmV and resulted in destruction and suppression of growth of the mold have been reported (Morino et al., 2007). Bacteria, yeast, and mold were inoculated on surface of blueberry to identify optimal concentration and time for treatment with the chlorine dioxide from which, the *Pseudomonas aeruginosa* was found with an optimal concentration of 15 ppmV for the treatment with chlorine dioxide for 15 min, whereas the *Salmonella typhimurium* was found with the effectiveness of chlorine dioxide rather dependent on treatment time than its concentration; the shorter duration of treatment time rendered bigger effects regardless of the level of concentration. The *Staphylococcus aureus*, *Yersinia enterocolitica*, and natural yeast and mold were reported with respective optima concentrations and treatment durations of 15 ppmV for 30 min, 5 ppmV for 2 h, and 15 ppmV for 1 h suggesting different conditions required for the treatment with chlorine dioxide (Wu and Kim, 2007).

It has been reported the minimum inhibitory concentration (MIC) of 10 ppmV of *Bacillus subtilis*, 50 ppmV of *Leuconostoc mesenteroides*, 75 ppmV of *Lactobacillus*

Table 2 Log reduction of food spoilage microbes related in fermented hot pepper paste according to treated ClO₂ concentration

Concentration of treated ClO ₂ (ppmV)	Density of food spoilage microbes related in fermented hot pepper paste (CFU/mL)			
	<i>C. glabrosa</i>	<i>Z. bisporus</i>	<i>S. capsularis</i>	<i>P. pastoris</i>
0	6.97 ± 0.87	6.03 ± 0.14	5.93 ± 0.15	7.98 ± 0.67
3	6.70 ± 0.59	5.94 ± 0.08	5.44 ± 0.16	6.34 ± 0.40
10	0	4.85 ± 0.02	0	5.51 ± 0.26
20	0	0	0	3.48 ± 0.00
30	0	0	0	0

fermentum, or 125 ppmV of *L. plantarum* is necessary to reduce the number of bacteria (*B. subtilis*, *L. plantarum*, *L. fermentum*, and *L. mesenteroides* etc.) to be infected in the process of alcohol fermentation creating ethanol (Meneghin et al., 2008). Paprika was reported that the treatment with chlorine dioxide of varied phases. The *Escherichia coli* O157:H7 and *S. typhimurium* were inoculated on paprika and put into experiments conducted to identify proper conditions for treatment with chlorine dioxide from which the treatment for 30 min with 50 ppmV of chlorine dioxide was found effective commonly over both *E. coli* O157 and *Salmonella*. The treatment of 5 min with liquid chlorine dioxide solution of 50 ppmV was also reported effective; and the treatment with the combination of chlorine dioxide and solution was reported with even bigger effect (Morino et al., 2007).

Three major kinds of microorganisms, residing in fermented hot pepper paste such as *Bacillus*, acidogenic bacteria, and yeast, have been reported. *Bacillus* is characterized by its heat resistance and chemical resistance thereby sterilization thereof is difficult, whereas most of the yeast and acidogenic bacteria are halotolerant microorganisms growing in an environment of hypersalinity. Thus, effective control of these microorganisms has also been known difficult (Kim et al., 2001). The *B. subtilis*, found contaminating the alcohol fermentation process in the study conducted in Brazil, also affects the fermentation of fermented hot pepper paste for which the application of chlorine dioxide of 10 ppmV was found effective for the reduction thereof (Meneghin et al., 2008) suggesting it can be employed as an effective means to reduce the *Bacillus subtilis* in the preparation of fermented hot pepper paste.

As a strong antioxidant destroying the mold, microbes, and even viruses, the chlorine dioxide was employed for the present study conducted to identify the conditions required for the application thereof in the treatment introduced for the destruction of the yeast. The effective concentrations of the chlorine dioxide, to be applicable to the treatment for different microorganisms residing in fermented hot pepper paste, need to be clarified further. The

availability of chlorine dioxide in both gaseous and liquid phases also requires the identification of respective conditions for effective and optimal treatment with chlorine dioxide corresponding to different processes of preparation of fermented hot pepper paste. The conditions identified for the application of chlorine dioxide seem they could be exploited as useful tools to reduce the level of unnecessary microorganisms spoiling fermented hot pepper paste products comprehensively and effectively.

In conclusion, the four kinds of yeast involved in the deterioration of fermented hot pepper paste product were found completely growth inhibited by the treatment with chlorine dioxide of concentrations distributed in the range 10–30 ppmV. The identified conditions are expected to be effectively employed for the fumigation in diverse processes comprising preparation of raw materials, the disintegration of raw materials, and maturation of fermented hot pepper paste products.

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Compliance with ethical standards

Conflict of interest These authors declare that they have no conflict of interest.

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