UNIVERSITI PUTRA MALAYSIA

DISINFECTION OF RICE USING OZONE TREATMENT

NOR NADIAH ABDUL KARIM SHAH

DISINFECTION OF RICE USING OZONE TREATMENT

By

NOR NADIAH ABDUL KARIM SHAH

Thesis Submitted to the School of Graduate Studies, University Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

November 2009

Dedicated to my beloved
husband Mohd Khair
son Muhammad Iman
daughter Mia Sarah
and parents

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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November 2009

Chairman : Russly Abdul Rahman, PhD

Faculty : Engineering

The main objectives of this study are 1) to determine the effect of different ozone concentrations and exposure times for *Sitophilus oryzae* in milled rice in terms of mortality and survivality rates, 2) to investigate the effect of different ozone concentrations and exposure times for reducing pathogenic *Bacillus cereus* in rice and 3) to evaluate the changes in physicochemical characteristics of rice exposed to different ozone treatments (pH, color, moisture content, cooking quality, total solids, hardness of uncooked and cooked rice, adhesiveness of cooked rice and sensory evaluation).

Ozonation treatment was done in various concentrations (0.1, 0.2, 0.3 and 0.4 ppm) and exposure times (0, 60, 120, 180, 240, 300, 360 and 420 minutes). One hundred gram of white milled rice samples were ozonated in a gas tight lieberg condenser (56 x 2.5 cm diameter) placed in an air conditioned room of 20°C ± 3°C, and 50% relative humidity. The glass condenser was connected to an ozone generator (Model OM-1, Top Ozone, Malaysia) in which ozone was produced from the ambient air. In order to generate

commercial levels of ozone, corona discharge method is used in this type of ozonator. After sample was subjected to ozone treatment, it was removed and tested before being stored in polyethylene bag at room temperature before sensory evaluation tests take place.

From the results obtained for the mortality rate of *Sitophilus oryzae* L., there is a significant difference ($P<0.05$) between the maximum exposure times and control, non-ozonated rice samples. It was shown that at 360 minutes of 0.3 ppm ozone exposure to be the minimum lethal limit of *Sitophilus oryzae* L. This result also applies to the survival rate of *Sitophilus oryzae* L., where it was found to decrease sharply even when the lowest dosage (0.1 ppm) of ozone level was hosed to the rice samples ($P < 0.05$). At ozone concentration of 0.3 ppm and 360 minutes, no rice weevils emerged after a month. These results proved that ozone is lethal to *Sitophilus oryzae* L. at or above 0.3 ppm of ozone concentrations which would be potent to eliminate all rice weevils in rice.

Ozonation treatments on *Bacillus cereus* have shown positive results, where all of ozone concentrations gave a significant difference ($P < 0.05$) on *Bacillus cereus* counts. Significant trends were observed in comparison with the non-ozonated rice samples. Non-ozonated rice samples were found with an average of 5.50 ± 0.28 log count ($cfug^{-1}$). At 0.1 ppm, the minimum value of *Bacillus cereus* were found with 5.20 ± 0.02 log count ($cfug^{-1}$) at 420 minutes of ozone exposure. Meanwhile, 4.84 ± 0.03 log count of *Bacillus cereus* were found in 0.2 ppm at 420 minutes. Up to 1.63 log reductions of *Bacillus cereus* counts were observed above 0.3 ppm ozone concentration at the end of 420 minutes of treatment. *Bacillus cereus* counts were shown to decrease to 3.62 ± 0.38

log count ($cfug^{-1}$) at 0.4 ppm ozone concentration after 420 minutes of ozone treatment. These results shown a maximum reduction of 31% of Bacillus cereus count when ozone of 0.4 ppm was exposed to white milled rice.

Physicochemical tests were done to see the effect of ozonation treatment towards pH, color, moisture content, cooking quality, total solids, hardness of uncooked and cooked rice and adhesiveness of cooked rice. Results have shown that moisture content, adhesiveness of cooked rice and hardness of uncooked rice have no significant changes ($P > 0.05$) in comparison with non - ozonated rice. Meanwhile, color, pH, total solids and cooking quality results have shown significant changes ($P < 0.05$) than non - ozonated rice samples. These analyses proved that there are limitations to how much ozone concentration and exposure times that can be exposed to rice without causing any detrimental effects on the physicochemical properties of rice.

As a conclusion, ozone has effectively shown its anti-microbial and fumigation characteristics that are invaluable in food industry, where effective applications to ensure safer food products are highly prioritize. Based on this study, sound advantages of ozone applications can be seen on rice. Even though, it does not leave any residue due to quick decomposition of its structure, restrictions should be applied through limitation of ozone concentrations or exposure times.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat keperluan untuk Ijazah Master Sains

NYAHINFEKSI BERAS MENGGUNAKAN RAWATAN OZON

Oleh

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Objektif utama penyelidikan ini adalah untuk 1) mengenalpasti kesan kepekatan ozon dan sorotan masa yang berlainan terhadap kebolehidupan dan bilangan maut *Sitophilus oryzae* L. dalam beras yang telah diproses, 2) menyelidik kesan kepekatan ozon dan sorotan masa yang berlainan untuk mengurangkan bilangan mikrobial *Bacillus cereus* di dalam beras dan 3) menentukan kesan terhadap sifat fizikal dan kimia yang dikenakan rawatan ozon yang berlainan (pH, warna, kadar kelembapan, kualiti memasak, jumlah pepejal, kekerasan beras sebelum dan sesudah dimasak, daya rekat beras masak dan penentuan sensori).

Rawatan ozon dilakukan dalam kepekatan yang berbeza (0.1, 0.2, 0.3, 0.4 ppm) dan sorotan masa (0, 60, 120, 180, 240, 300, 360 dan 420 minit). Seratus gram beras yang telah diproses diozonkan di dalam kondenser lieberg yang kedap udara (56 x 56 x 2.5 cm diameter) yang diletakkan di dalam bilik berhawa dingin yang bersuhu 20 ± 3ºC dan

mempunyai 50% kelembapan relatif. Kondenser gelas tersebut disambungkan kepada penjana ozon (Model OM-1, Top Ozone, Malaysia) dan ozon dihasilkan dari udara persekitaraan. Untuk menghasilkan ozon pada kadar komersil, pengeluaran lingkaran sinar digunakan. Apabila sampel telah dirawat dengan ozon, sampel beras tersebut dikeluarkan dan diujikaji sebelum disimpan di dalam beg poliethelene pada suhu bilik sebelum ujikaji penentuan sensori dijalankan.

Keputusan yang diperoleh untuk kadar bilangan maut *Sitophilus oryzae* L. menunjukkan kesan yang signifikan ($P < 0.05$) di antara sampel sorotan masa maksimum dan kawalan. Ia juga menunjukkan pada 360 minit dan kepekatan ozon 0.3 ppm adalah had maut minimum untuk *Sitophilus oryzae* L. Keputusan ini juga boleh digunakan kepada kadar kebolehidupan *Sitophilus oryzae* L., di mana ia didapati menurun dengan drastik walaupun kepekatan ozon terendah dikenakan pada sampel beras ($P < 0.05$). Pada kepekatan ozon 0.3 ppm dan sorotan masa 360 minit, tiada langsung kutu beras yang hidup selepas sebulan rawatan ozon. Keputusan ini membuktikan bahawa ozon adalah penyebab maut terhadap *Sitophilus oryzae* L. apabila kepekatan ozon pada atau melebihi 0.3 ppm.

Rawatan ozon kepada *Bacillus cereus* telah menunjukkan hasil yang memberangsangkan, di mana semua kadar kepekatan ozon memberikan kesan yang signifikan ($P > 0.05$) kepada bilangan *Bacillus cereus*. Hala signifikan boleh dilihat apabila dibandingkan dengan keputusan bilangan *Bacillus cereus* di dalam beras yang tidak diozonkan. Beras yang tidak diozonkan didapati mengandungi 5.50 ± 0.28 log bilangan ($cfug^{-1}$) secara purata. Pada kadar kepekatan ozon 0.1 ppm, jumlah *Bacillus* *cereus* minimum yang didapati adalah 5.20 ± 0.02 log bilangan ($cfug^{-1}$) pada 420 minit sorotan masa. Manakala, 4.84 ± 0.03 log bilangan dijumpai pada kadar kepekatan 0.2 ppm dan 420 minit. Kadar pengurangan sehingga 1.63 log didapati pada kadar kepekatan ozon melebihi 0.3 ppm pada penghujung 420 minit sorotan masa. *Bacillus cereus* juga menunjukkan pengurangan kepada 3.62 ± 0.38 log bilangan pada kadar kepekatan 0.4 ppm, 420 minit. Keputusan ini menunjukkan pengurangan maksimum sebanyak 31% kepada bilangan mikrobial *Bacillus cereus* apabila kadar kepekatan ozon 0.4 ppm dikenakan kepada beras yang telah diproses.

Ujikaji terhadap sifat fizikal dan kimia beras dilakukan untuk menganalisis kesan rawatan ozon terhadap pH, kadar kelembapan, warna, daya rekat beras masak, kekerasan beras masak dan belum dimasak, kualiti memasak dan jumlah pepejal. Keputusan yang diperoleh menunjukkan kadar kelembapan, daya rekat beras masak dan kekerasan beras belum dimasak tidak melalui sebarang kesan signifikan ($P > 0.05$) jika dibandingkan dengan keputusan yang diperoleh dari beras yang tidak diozonkan. Manakala, warna, pH, jumlah pepejal dan kualiti memasak menunjukkan kesan signifikan ($P < 0.05$) dibandingkan dengan beras yang tidak menjalani proses ozonasi. Analisis ini membuktikan terdapat had kepada kadar kepekatan ozon dan sorotan masa yang boleh dikenakan terhadap beras tanpa menyebabkan sebarang kesan yang merosakkan terhadap sifat fizikal dan kimia beras.

Kesimpulannya, ozon telah menunjukkan sifat anti – mikrobial dan fumigasi yang sangat berharga kepada industri makanan, di mana dengan aplikasi efektif untuk menjamin mutu makanan yang selamat sebagai keutamaan. Berdasarkan kajian ini, kesan kelebihan ozon dapat diperlihat kepada beras. Walaupun ozon tidak meninggalkan sebarang residu terhadap makanan, kegunaanya hendaklah dihadkan dilandaskan kepada kadar kepekatan ozon dan sorotan masa.

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I certify that a Thesis Examination Committee has met on **18th of November, 2009** to conduct the final examination of Nor Nadiah Abdul Karim Shah on her thesis entitled **"Disinfection of Rice using Ozone Treatment"** in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science (Food Engineering) degree.

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declared that it has not been previously, and is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

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NOR NADIAH ABDUL KARIM SHAH

Date:

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LIST OF ABBREVIATION

ANOVA	analysis of variance
AVE	average
BERNAS	Padiberas Nasional Berhad
DG	Degree of gelatinization
DSC	Differential Scanning Calorimetry
GBSS	Granule-bound starch synthase
GC	Gas Chromatography
GLM	General Linear Model
GRAS	Generally recognized as safe
NIR	Near Infra-Red
PEMBA	polymyxin pyruvate-egg yolk-manitol-bromothymol blue agar
ppm	parts per million
RVA	Rapid Visco Analysis
SAS	Statistical Analysis System
SPSS	Statistical Package for Social Sciences
STD DEV	standard deviation
STD ERR	standard error
TPA	Texture analysis profile

NOMENCLATURE

L^*	lightness (+) or darkness (-)
a^*	redness (+) or greenness (-)
b^*	yellowness (+) or blueness (-)

CHAPTER 1

1 INTRODUCTION

1.1 An Overview on Rice and Ozone in Malaysia

Rice is arguably the most important staple food for people in the world, much more to Malaysians, where rice has been the traditional foods for more than a century. More than 600 million tones of rice are stored every year in Padiberas Nasional Berhad (BERNAS) factories all over Malaysia (Anonymous, 2009a).

However, insects and fungi create serious quality issues in stored rice and annual storage losses are estimated at a range of 30 – 42% annually (Anonymous, 2009a). The only way to eliminate pests completely from a food grain without leaving pesticide residues is fumigation. Currently, there are only two registered fumigants for stored food; methyl bromide and phosphine. However, under Montreal Protocol, developing countries, including Malaysia, has to phase out methyl bromide usage by the year 2015 (Anonymous, 2009b). Meanwhile, phosphine is currently being reviewed by Crop Protection & Plant Quarantine Division, Ministry of Agricultural, Malaysia. Assuming that phosphine has been legalized, it would be the only licensed fumigant for stored food grain particularly, rice. With only one fumigant remaining, insect and microorganisms resistance becomes a greater risk. It has been shown that some stored product insects already exhibiting some levels of phosphine resistance and some have shown resistance to methyl bromide (Zeetler *et al*., 1989; Zettler and Cuperus, 1990; Kells *et al*, 2001). Loss of fumigants, resistance to remaining fumigants and a trend by consumers to move away from residual chemicals, necessitates the development of additional control strategies.

Bacillus cereus, has created its own pandemic in health related issue. *Bacillus cereus* is a gram positive facultative anaerobic spore-forming rod shaped bacterium. It is known to produce many types of toxins, two (diaarhoegenic and emetic) of which are known to cause foodborne illness (Johnson, 1984; Kramer and Gilbert, 1989). This bacteria is also responsible for spoilage of a variety of food products, particularly pasteurized milk and cream.

Uncooked rice grains are frequently contaminated with *Bacillus cereus* spores, which are resistant to heat and can survive boiling. If cooked rice is subsequently stored at room temperatures, the heat resistant spores will germinate, proliferate, and may produce emetic toxin (Gilbert *et al*., 1974; Parry and Gilbert, 1980; Johnson *et al*., 1984; Ueda, 1994). When a large numbers of *Bacillus cereus* are present in raw rice and when toxin is produced in boiled rice, *Bacillus cereus* becomes a hazard. Therefore, rice to be used for food processing should be decontaminated in order to prevent spoilage and foodborne diseases (Hirata, 1996).

Bacillus cereus emetic food poisoning is associated mainly with the consumption of rice-based products and farinaceous foods such as pasta and noodles of which is frequently contaminated. Levels of *Bacillus cereus* greater than 10^3 cfug^{-1} has been found both in cooked and uncooked rice (Rusul and Yaacob, 1995). When foods containing *Bacillus cereus* spores are cooked, the spores often survived and heatshocked into germination. If these foods are then left to cool at ambient temperature, germination and vegetative growth may begin, leading to production of the emetic toxin.

Ozone, meanwhile, has been reported to have adverse effects towards insects, fungi and its anti-microbial effect has been proven to effectively kill 90% of microbial populations (Ishizaki, 1986). Moreover, ozone has been deemed as a safe fumigation alternatives and a good anti microbial agents that this material now, is much sought after in food handling and hygiene industry.

Ozone can be generated by electrical discharges in air and is currently used in the medical industry to disinfect against microorganisms and viruses, as a means of reducing color, for removing taste and environmental pollutants in industrial applications (Kim et al., 1999). The attractive fact of ozone is that it has a very short half life (20 – 50 min in water) compared to other fumigation chemical and does not leave a residue. Currently, ozone is being classified as generally recognized as safe (GRAS) (USFDA, 1982).

Electrical generation of ozone eliminates the handling, storage, and disposal problems of conventionally used post-harvest pesticides. This attributes makes ozone an attractive candidate for controlling insects and fungi in stored grains; however, few studies have been published on its efficacy as an insecticide.

It has been reported that ozone concentration of 50 ppm for 30 days resulted in 100% mortality of adult flour beetles and maize weevils, *Sitophilus zeamais* and greatly reduced emergence of larval Indian meal moths, *Plodia interpunctella* (Kells *et al*, 2001). A study has been carried out by Strait (1998), reported that ozone, following fumigation of small-scale grain storage bins (18kg) containing yellow maize, dispersed throughput the grain mass and was toxic to insects within that mass.

1.2 Objectives

Rice infestations, micro or macro have shown a world-wide problem that resulted with more than 20% economic loss. Ozone meanwhile, with its positive adversity could be an attractive possibility in disinfecting rice without any damaging effects.

Therefore the following objectives of the study are:

1. To determine the effect of different ozone concentrations and exposure times on the mortality and survivality rates for *Sitophilus oryzae* in milled rice.
2. To investigate the effect of different ozone concentrations and exposure times for reducing pathogenic *Bacillus cereus* in rice.
3. To evaluate the changes in physicochemical characteristics of rice exposed to different ozone treatments (pH, color, moisture content, cooking quality, total solids, hardness of uncooked and cooked rice and adhesiveness of cooked rice).
4. To evaluate consumer preferences and noticeable changes of ozonated rice in sensory evaluation tests.

1.3 Scopes of Research

The introductory chapter briefly reviews the importance of rice in Malaysia together with the factors that influences the growing demand for rice. The problem statement, the objectives of research and its significance that supported the contributions of this thesis are also presented in this chapter.

Chapter 2 reviews previous studies in ozonation works, focusing on raw food materials and food products. Ozone generation method, its direct effects towards food products and its antimicrobial properties are elaborately discussed. Technical aspects and ozone advantages are also discussed in this chapter. This study focuses on rice macro and micro infestation that are the main objectives of this study.

Chapter 3 describes the experimental design and methods used in performing this research. The methods in obtaining all the responses, *Bacillus cereus* counts, *Sitophilus oryzae* L. counts, physicochemical and sensory evaluation on cooked ozonated rice samples are presented.

Chapter 4 reports the investigations on the main objectives of this research; *Bacillus cereus* counts after ozonation, *Sitophilus oryzae* L. counts after ozonation in terms of mortality and survivality rates, and the physicochemical properties of ozonated white milled rice. Sensory evaluations of ozonated rice are explored.

A brief summary on all work and findings are presented in Chapter 5. The recommendations for future work are given in the final chapter.

1.4 Contributions of Thesis

The contributions of this study are an endless venture, where, rice is undoubtly the cheapest basis of carbohydrates to human worldwide. This study if proven successful could provide the alternative fumigation to rice against *Sitophilus oryzae* L. without giving away the normal properties and nutritions of rice. Ozone being the cheapest and the easiest fumigation to handle could save BERNAS's allocation for fumigation in a long run. Consumer preference is almost at an advantage where, the risk of deadly chemicalized fumigation could be eliminated. Antimicrobial properties of ozone can be an ultimate advantage where, *Bacillus cereus* would be killed in raw rice prior to cooking lowering the risks of food poisoning and ultimately prolonging the shelf life of uncooked and cooked rice.

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