EUGÉNIO DA PIEDADE EDMUNDO SITOE

OZONATION AT LOW-PRESSURES: RELATIONSHIP BETWEEN REACTION KINETICS AND PHYSICAL PROPERTIES OF GRAINS, CONTROL OF EGG AND LARVAE PHASES OF *Zabrotes subfasciatus*, INACTIVATION OF *Aspergillus flavus*, AND QUALITY ANALYSIS

Thesis submitted to the Agricultural Engineering Graduate Program of the Universidade Federal de Viçosa as a partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Advisor: Lêda Rita D'Antonino Faroni

Co-advisers: Ernandes Rodrigues de Alencar
Franklin Jackson Machado

VIÇOSA - MINAS GERAIS
2023

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

S623o
2023

Sitoe, Eugénio da Piedade Edmundo, 1990-

Ozonation at low-pressures: relationship between reaction kinetics and physical properties of grains, control of egg and larvae phases of *Zabrotes subfasciatus*, inactivation of *Aspergillus flavus*, and quality analysis / Eugénio da Piedade Edmundo Sitoe. – Viçosa, MG, 2023.

1 tese eletrônica (114 f.): il. (algumas color.).

Texto em inglês.

Orientador: Lêda Rita D'Antonino Faroni.

Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Engenharia Agrícola, 2023.

Inclui bibliografia.

DOI: https://doi.org/10.47328/ufvbbt.2023.728

Modo de acesso: World Wide Web.

1. Feijão - Doenças e pragas - Controle. 2. Grãos - Efeito do ozônio. 3. Ozonização. 4. Feijão - Qualidade. I. Faroni, Lêda Rita D'Antonino, 1953-. II. Universidade Federal de Viçosa. Departamento de Engenharia Agrícola. Programa de Pós-Graduação em Engenharia Agrícola. III. Título.

CDD 22. ed. 635.6529

Bibliotecário(a) responsável: Bruna Silva CRB-6/2552

EUGÉNIO DA PIEDADE EDMUNDO SITOE

OZONATION AT LOW-PRESSURES: RELATIONSHIP BETWEEN REACTION KINETICS AND PHYSICAL PROPERTIES OF GRAINS, CONTROL OF EGG AND LARVAE PHASES OF *Zabrotes subfasciatus*, INACTIVATION OF *Aspergillus flavus*, AND QUALITY ANALYSIS

Thesis submitted to the Agricultural Engineering Graduate Program of the Universidade Federal de Viçosa as a partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

APPROVED: October 18, 2023.

Assent:

Documento assinado digitalmente
gov.br
EUGENIO DA PIEDADE EDMUNDO SITOE
Data: 21/11/2023 13:44:42-0300
Verifique em https://validar.iti.gov.br

Eugénio da Piedade Edmundo Sitoe
Author

Documento assinado digitalmente
gov.br
LEDA RITA DANTONINO FARONI
Data: 21/11/2023 11:28:42-0300
Verifique em https://validar.iti.gov.br

Lêda Rita D'Antonino Faroni
Advisor

ACKNOWLEDGEMENTS

I would like to thank the countless people who have worked tirelessly daily and helped me make this endeavor possible. My heartfelt gratitude to all of them.

I thank God for bestowing me health and enlightening my life during my time and journey in Brazil.

To my family, especially my beloved mother, Lina Henrique Mucavele, for all the love and trust, for understanding my absence, and for her valuable prays. On many occasions, she has sacrificed much of her time to be present in my life, even remotely. Her support was of great importance for accomplishing this phase of my life, and I hope to repay her for the many reasons I can be proud of.

To my father, Edmundo Francisco Sitoe, for his friendship and collaboration. He has been by my side whenever I needed a lap to cry on, complain, or scream. At times of distress, wise and beautiful words came from him. "My son, brown sugar is sweet, but it is not soft," as he used to say whenever I was in pain, "The agony you are experiencing now because of work and absence from the family will go down in history, and your steps will be an example for your brothers who are looking for inspiration to overcome the difficulties of life." Thank you so much for all of your kind advice and gestures.

To my aunt, Odete Langa, for her friendship and companionship, even from afar. She spent countless long nights talking to me, making me company while I was setting up my trials. Her calls and conversations were the driving force to make me believe that every effort was worth it.

To my grandmother, Isaura Eugénio Mathe, and my brothers for their constant willingness to help and prayers.

To my advisor, Prof. Lêda Rita D'Antonino Faroni, for all the knowledge she handed on to me, for the affection in our dialogues, for the zeal in the orientation and the research follow-up. We have known each other for a short time, but it has already been enough to form a bond and see her as a role model of dedication, which I will follow for life.

To my Co-advisors, Prof. Ernandes Rodrigues de Alencar and Franklin Jackson Machado, for his friendship and guidance during the accomplishment of this work.

To Prof. Paulo Roberto Cecon, who assisted with the statistics and data analysis.

To Prof. Edimar Aparecida Filomeno Fontes for her assistance.

To all Professors of the Department of Agricultural Engineering (DEA - UFV) storage area.

To my colleagues at the Agricultural Engineering graduate program, with whom I shared good times, especially Marcus Vinicius de Assis Silva, for his friendship throughout the course.

To the Universidade Federal de Viçosa (UFV) for making this dream come true, and especially to the DEA for its welcoming and the education and good experiences it provided.

To the National Council for Scientific and Technological Development (CNPq) [grant number: 309843/2020-0 and 405894/2021-0], the Research Support Foundation of the State of Minas Gerais (FAPEMIG) [Grant Number: APQ-01701-22], and the Coordination for the Improvement of Higher Education Personnel (CAPES) [Finance Code 001], for the fellowship provided.

BIOGRAPHY

EUGENIO DA PIEDADE EDMUNDO SITOE, son of Edmundo Francisco Sitoe and Lina Herinques Mucavele, was born on May 22, 1990, in the district of Manjacaze, province of Gaza-Mozambique.

In 2011, I graduated as an Agricultural Technician at Chókwè's College of Agriculture in the Gaza-Mozambique province.

In 2016, I obtained a B.Sc. Hons degree in Agricultural Engineering at the High Polytechnic Institute of Gaza-ISPG (Instituto Superior Politécnico de Gaza – Mozambique).

In 2019, I got a Master's degree in Agroecology with a focus on the conservation of agricultural ecosystems at the High Polytechnic Institute of Gaza-ISPG, under the supervision of Dr. António Elísio José.

In March 2020, I joined the doctoral course in the Agricultural Engineering Graduate Program at the Federal University of Viçosa (UFV). I focused my studies on the field of Storage and Processing of Agricultural Products, under the supervision of Prof. Lêda Rita D'Antonino Faroni. I submitted my dissertation for defense on October 19, 2023.

ABSTRACT

SITOE, Eugénio da Piedade Edmundo, D.Sc., Universidade Federal de Viçosa, October 2023. **Ozonation at low-pressures: relationship between reaction kinetics and physical properties of grains, control of egg and larvae phases of *Zabrotes subfasciatus*, inactivation of *aspergillus flavus*, and quality analysis.** Advisor: Lêda Rita D'Antonino Faroni. Co-advisers: Ernandes Rodrigues de Alencar and Franklin Jackson Machado.

The use of ozone gas (O_3) has achieved satisfactory results in controlling insect pests, inactivating fungi, and maintaining the quality of stored grains. The ozonation of grains in a closed system at low-pressures is also a promising strategy for packaged products. The objectives of this study were the following: (i) to evaluate the effect of the low-pressure ozone injection system, establishing the relationship between the reaction kinetics and physical properties of the grains; (ii) to evaluate the effect of ozone gas injections on the control of egg and larval phases of *Zabrotes subfasciatus* (Boh.) and inactivation of *Aspergillus flavus* in bean grains; and (iii) to evaluate the quality of beans exposed to ozone or oxygen and stored for 180 days. The study was carried out in three stages. First, the low-pressure ozone injection system was evaluated to determine the physical properties of the grains and to determine possible quality changes in common beans, cowpeas, popcorn, corn, paddy rice and polished rice. For this purpose, 5.0-kilogram samples of grains were exposed to ozone or oxygen (control) at low pressures in a 70-liter hypobaric chamber. The ozone exposure was preceded by the reduction of the internal chamber pressure to 500 hPa. Then, ozone was injected until the pressure inside the chamber reached 1000 hPa. In the study, an ozone concentration of 32.10 mg L^{-1} was applied at a flow rate of 1 L min^{-1}. The second step evaluated the effect of the ozone gas injections on the control of new-insect emergence from the egg and larval stages of *Z. subfasciatus* (Boh.) and on the inactivation of *A. flavus* in bean grains. Ozone was applied to 5.0 kg of bean grains at 61.37 mg L^{-1} and a flow rate of 1 L min^{-1} in 1, 4, 7, and 10 injections. Oxygen was used as the control, injected at a flow rate of 1 L min^{-1}. The injection escalation occurred at regular intervals of 2 h and 15 min. The third stage assessed the effect of ozone (treatment) or oxygen (negative control) administered in 10 injections on the technological properties, phenolic compounds, and antioxidant capacity of beans stored for 180 days. Five samples of 1.0 kg of bean grains were packaged in low-density polypropylene bags to evaluate the effect of the treatments during storage. The packages were stored in a chamber at 25°C and 60±5% relative humidity. The quality of the beans treated with ozone or oxygen and the control (untreated grain) was determined after 180 days of storage by analyzing the technological properties, phenolic compounds, and antioxidant capacity. The results of the first stage made it clear that the physical properties of the grains were affected by the reaction kinetics of the ozone gas during the low-pressure injection process. Ozone depletion was faster in polished rice and slower in common beans. The half-life of ozone gas in different grains ranged from 17.8 to 52.9 and 16.4 to 52.9 minutes outside and inside the package, respectively. Low-pressure ozone gas injection had no effect on the grain quality characteristics. The low-pressure ozone concentration of 32.10 mg L^{-1} caused mortality of 99.45 and 73.06 of *S. zeamais* in popcorn and corn, 92.10% of *C. maculatus* in cowpea and 100% of *Z. subfasciatus* in common bean. In the second phase, it was observed that the ozonation of beans at low-pressures in 10 injections provided the creation of a modified atmosphere inside the packaging that was efficient in controlling 100% of egg and larval phases of *Z. subfasciatus*, implying in less mass loss, and inactivation of 70% of A. flavus. In the third step, it was found that the application of ozone in 10 injections did not change the chemical composition, color, cooking properties, and level of antioxidant capacity of the grains. All treatments showed a decrease in the phenolic compound content and antioxidant capacity during storage. This study concludes that the application of ozone at low pressure is a promising technology for insect-pest control and fungal inactivation without affecting the commercial quality of the grains.

Keywords: Hypobaric chamber. Ozonation. Pest insects. Bean Quality.

RESUMO

SITOE, Eugénio da Piedade Edmundo, D.Sc., Universidade Federal de Viçosa, outubro de 2023. **Ozonização á baixas pressões: relação entre a cinética de reação e propriedades físicas dos grãos, controle de fases de ovo e de larva de *Zabrotes subfasciatus*, inativação de *Aspergillus flavus* e análise de qualidade**. Orientadora: Lêda Rita D'Antonino Faroni. Coorientadores: Ernandes Rodrigues de Alencar e Franklin Jackson Machado.

A aplicação do gás ozônio (O_3) tem atingido resultados satisfatórios no controle de insetos-praga, inativação fúngica e na manutenção da qualidade em grãos armazenados. A ozonização dos grãos em sistema fechado a baixa pressão é uma estratégia com potencial para produtos já embalados. Os objetivos deste estudo foram: (i) Avaliar o sistema de injeção de ozônio a baixa pressão: relação entre a cinética de reação e propriedades físicas dos grãos; (ii) avaliar o efeito das injeções do gás ozônio no controle de fases de ovo e de larva de *Zabrotes subfasciatus* (Boh.) e na inativação de *Aspergillus flavus* em grãos de feijão e (iii) avaliar a qualidade do feijão exposto ao ozônio e oxigênio e armazenado por 180 dias. Os estudos foram conduzidos em 3 etapas. Na primeira etapa, foi avaliado o sistema de injeção do ozônio a baixa pressão em relação às propriedades físicas dos grãos e determinaram-se as possíveis alterações na qualidade de feijão comum, feijão caupi, milho de pipoca, milho, arroz com casca e arroz polido. Para tal, amostras de 5.0 kg de grãos foram expostas ao gás O_3 e ao oxigênio (controle) aplicado a baixas pressões em câmara hipobárica de volume interno igual a 70 L. A aplicação do ozônio foi antecedida pela redução da pressão interna da câmara para 500 hPa. Em seguida, o ozônio foi injetado até que a pressão interna da câmara atingisse o valor de 1000 hPa. No estudo foi aplicada uma concentração de ozônio de 32.10 mg L^{-1} na vazão volumétrica de 1 L min^{-1}. Na segunda etapa, avaliou-se o efeito das injeções do gás ozônio no controle de emergência de novos insetos a partir de fases de ovo e de larva de *Z. subfasciatus* (Boh.) e na inativação de *A. flavus* em grãos de feijão. Aplicou-se uma concentração de ozônio de 61.37 mg L^{-1} na vazão volumétrica de 1 L min^{-1} em 1, 4, 7 e 10 injeções em 5 kg de grãos de feijão. O oxigênio foi usado como controle e injetado na vazão volumétrica de 1 L min^{-1}. O incremento das injeções foi feito em intervalos regulares de tempo de 2 h e 15 min. Na terceira etapa, avaliou-se o efeito do ozônio (tratamento) e oxigênio (controle negativo) aplicado em 10 injeções nas propriedades tecnológicas, nos compostos fenólicos e na capacidade antioxidante de feijão armazenado por 180 dias. Para avaliação do efeito dos tratamentos durante o armazenamento, cinco amostras de 1.0 kg de grãos de feijão foram embaladas em sacos de polipropileno de baixa densidade. As embalagens foram armazenadas em câmara a 25 ºC e umidade relativa de 60±5%. A qualidade do feijão tratado com ozônio, oxigênio e testemunha (grãos não tratados) e armazenada durante 180 dias, foi determinada a partir da análise das propriedades tecnológicas, compostos fenólicos e capacidade antioxidante. Os resultados da primeira etapa sugerem que as propriedades físicas do grão influenciaram a cinética de reação do gás ozônio durante o processo de injeção a baixa pressão. A decomposição do ozônio foi mais rápida no arroz polido e mais lenta no feijão comum. A meia-vida do gás ozônio em diferentes grãos variou de 17.8 a 52.9 e 16.4 a 52.9 min, fora e dentro da embalagem, respectivamente. A injeção de gás ozônio a baixas pressões não alterou os atributos de qualidade dos grãos. A concentração de ozônio a baixa pressão (32,10 mg L^{-1}) causou a mortalidade de 99.45 e 73.06 de *S. zeamais* no milho pipoca e milho, 92.10% de *C. maculatus* no feijão-caupi e 100% de *Z. subfasciatus* no feijão comum. Na segunda etapa, observou-se que a ozonização do feijão a baixas pressões em 10 injeções proporcionou a criação de uma atmosfera modificada no interior da embalagem que foi eficiente no controle de 100% de fases de ovo e de larva de *Z. subfasciatus,* implicando em menor perda de massa, e na inativação de 70% de *A. flavus*. Na terceira etapa, foi constatado que a aplicação do ozônio em 10 injeções não alterou a composição centesimal, cor, propriedades de cozimento e medida de capacidade antioxidante. Ao longo do armazenamento registrou-se decréscimo no teor de compostos fenólicos e capacidade antioxidante para todos os tratamentos. Foi possível concluir com este estudo que a aplicação de ozônio a baixas pressões é uma tecnologia promissora para o controle de insetos-praga e inativação fúngica e não afeta a qualidade comercial dos grãos.

Palavras-chave: Câmara hipobárica. Ozonização. Insetos-pragas. Qualidade de Feijão.

SUMMARY

GENERAL INTRODUCTION

Grains are a fundamental part of the food system and a source of nutrients in the human diet. Consumer acceptance of grains is directly related to the physical and chemical qualities of the product. However, grain availability and quality are affected by many factors, remarkably the contamination by insect pests, fungi, and mycotoxins (Ogecha et al., 2019; Rodrguez-Gonzlez et al., 2020; Fulano et al., 2021).

Insect pests cause mass loss, alter the nutritional value, and promote commercial devaluation of stored grains (Costa et al., 2014; Silva et al., 2022a). Fungi accelerate grain decay and promote organoleptic changes (Mohapatra et al., 2017; Tsehaye et al., 2017; Ropelewska, 2019). Around 25-50% of the losses in stored grains are caused by insect pests and fungi (Kumar & Kalita, 2017).

Various control methods are used to ensure grain protection during storage. Usually, insect pest control is done with phosphine gas (PH_3) (Alzahrani & Ebert, 2023). However, this method is no longer feasible for application in cereals, partly due to the development of resistance in some insect-pest species, but also due to the presence of pesticide residues in food (Konemann et al., 2017; Nayak et al., 2020; Wakil et al., 2021). In addition, fewer pesticides are available for crop protection due to environmental concerns and consumer safety (Phillips & Throne, 2010).

Given the limited control options and the considerable potential for insecticide resistance, new methods are needed for controlling insect pests and inactivating fungi in stored produce (Germinara et al., 2010; Licciardello et al., 2013; Ribeiro et al., 2020). One of the promising alternatives is ozone gas, which has proved effective in controlling a variety of insect pests and fungi in several stored products (Silva et al., 2016; Subramanyam et al., 2017; Brodowska et al., 2018; Zhu, 2018). Ozone can diffuse and permeate large quantities of grains. After reacting with the product, ozone quickly decomposes into oxygen without leaving any residue (Isikber & Athanassiou, 2015; Kaur et al., 2022). These properties make ozone an ecologically attractive alternative for managing insects and fungi in stored grain (Kells et al., 2001, Savi et al., 2020).

Ozone in its gaseous form causes mortality of insects plaguing several commodities, and its toxicity has been thoroughly studied by researchers under both laboratory and field conditions, using different application techniques (Erdman, 1980; Kells et al., 2001; Pimentel et al., 2009; McDonough et al., 2011; Silva et al., 2019; Silva et al., 2022a; Sitoe et al., 2023). The effect of ozone on insect pests is related to interference with respiration and oxidative stress caused to cell membranes (Hollingsworth & Armstrong, 2005). In addition, ozone acts on the sulfhydryl groups of enzymes, deactivating some cellular activities and altering the bases of nucleic acids, purines, and pyrimidines of living organisms (Silva et al., 2022a).

The application of ozone gas to grains with recirculation systems (Hardin et al., 2010), in a continuous flow, employing helical conveyors (McDonough et al., 2011), and during the grain drying process has also been studied for fixed beds (Granella et al., 2018). Flow-through ozone efficiently prevented new-insect eclosion from the eggs. It also controlled larvae, pupae, and adults of *Rhyzopertha dominica* Fabricius, 1792 (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae) (Mishra et al., 2019; Amoah & Mahroof, 2019), as well inactivated fungi in corn (Ribeiro et al., 2021) and chestnut (Ferreira et al., 2021). However, there are few reports on the use of low-pressure ozone to control insect pests and inactivate fungi in packaged grains. Therefore, it is essential to conduct research evaluating the usage of the method in different crops.

The reaction kinetics of ozone applied at low pressures to different grains need to be studied to verify changes that the gas may cause to the products. These studies would make it possible to design the application of ozone systems and establish appropriate ozone concentrations for large-scale operations (Silva et al., 2022; Sitoe et al., 2023). In this context, the objectives of this work were the following: (i) to evaluate the effect of a low-pressure ozone injection system, establishing the relationship between the reaction kinetics and physical properties of the grains (beans, cowpeas, corn, popcorn kernels, paddy rice, and polished rice); (ii) to evaluate the effect of ozone-gas injections for insect control and fungal inactivation in beans; and (iii) to evaluate the quality of the beans exposed to ozone at various storage times.

REFERENCES

Amoah, B. A., & Mahroof, R. M. (2019). Ozone as a potential fumigant alternative for the management of *Sitophilus oryzae* (Coleoptera: Curculionidae) in wheat. *Journal of economic entomology*, *112*(4), 1953-1963.

Bernardes, W. A., Silva, E. O., Crotti, A. E., & Baldin, E. L. (2018). Bioactivity of selected plant-derived essential oils against *Zabrotes subfasciatus* (Coleoptera: Bruchidae). *Journal of Stored Products Research*, 77, 16-19.

Brodowska, A. J., Nowak, A., & Śmigielski, K. (2018). Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. *Critical reviews in food science and nutrition*, *58*(13), 2176-2201.

Costa, J. T., Forim, M. R., Costa, E. S., De Souza, J. R., Mondego, J. M., & Junior, A. L. B. (2014). Effects of different formulations of neem oil-based products on control *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Bruchidae) on beans. *Journal of Stored Products Research*, 56, 49-53.

Erdman, H. E. (1980). Ozone toxicity during ontogeny of two species of flour beetles, *Tribolium confusum* and *T. castaneum*. *Environmental Entomology*, *9*(1), 16-17.

Ferreira, W. F. D. S., de Alencar, E. R., Blum, L. E. B., Ferreira, M. D. A., Mendonca, M. A., Racanicci, A. M. C., & Urruchi, W. M. I. (2021). Ozonation of Brazil nuts in aqueous media at different ph levels: Ozone decomposition, *Aspergillus flavus* inactivation, and effects on nut color and crude oil lipid profile. *Ozone: Science & Engineering*, *43*(4), 351-362.

Fulano, A. M., Lengai, G. M., & Muthomi, J. W. (2021). Phytosanitary and technical quality challenges in export fresh vegetables and strategies to compliance with market requirements: case of smallholder snap beans in Kenya. *Sustainability*, *13*(3), 1546.

Germinara, G. S., Conte, A., Lecce, L., Di Palma, A. N. T. O. N. E. L. L. A., & Del Nobile, M. A. (2010). Propionic acid in bio-based packaging to prevent Sitophilus granarius (L.) (Coleoptera, Dryophthoridae) infestation in cereal products. *Innovative Food Science & Emerging Technologies*, *11*(3), 498-502.

Granella, S. J., Christ, D., Werncke, I., Bechlin, T. R., & Coelho, S. R. M. (2018). Effect of drying and ozonation process on naturally contaminated wheat seeds. *Journal of Cereal Science*, *80*, 205-211.

Hardin, J.A., Jones, C.L., Bonjour, E.L., Noyes, R.T., Beeby, R.L., Eltiste, D.A., Decker, S. Ozone Fumigation of Stored Grain; Closed-loop Recirculation and Rate of Ozone Consumption. *Journal of stored products research*, v. 46, n. 3, p. 149-154, 2010.

Hollingsworth, R. G., & Armstrong, J. W. (2005). Potential of temperature, controlled atmospheres, and ozone fumigation to control thrips and mealybugs on ornamental plants for export. *Journal of economic entomology*, *98*(2), 289-298.

Isikber, A. A., & Athanassiou, C. G. (2015). The use of ozone gas for the control of insects and micro-organisms in stored products. *Journal of Stored Products Research*, *64*, 139-145.

Kaur, K., Pandiselvam, R., Kothakota, A., Ishwarya, S. P., Zalpouri, R., & Mahanti, N. K. (2022). Impact of ozone treatment on food polyphenols–A comprehensive review. *Food Control*, 109207.

Kells, S. A., Mason, L. J., Maier, D. E., & Woloshuk, C. P. (2001). Efficacy and fumigation characteristics of ozone in stored maize. *Journal of stored products research*, *37*(4), 371-382.

Konemann, C. E., Hubhachen, Z., Opit, G. P., Gautam, S., & Bajracharya, N. S. (2017). Phosphine resistance in *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) collected from grain storage facilities in Oklahoma, USA. *Journal of Economic Entomology*, *110*(3), 1377-1383.

Kumar, D., & Kalita, P. (2017). Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. *Foods*, *6*(1), 8.

Licciardello, F., Muratore, G., Suma, P., Russo, A., & Nerín, C. (2013). Effectiveness of a novel insect-repellent food packaging incorporating essential oils against the red flour beetle (*Tribolium castaneum*). *Innovative Food Science & Emerging Technologies*, *19*, 173-180.

McDonough, M. X., Campabadal, C. A., Mason, L. J., Maier, D. E., Denvir, A., & Woloshuk, C. (2011). Ozone application in a modified screw conveyor to treat grain for insect pests, fungal contaminants, and mycotoxins. *Journal of Stored Products Research*, *47*(3), 249-254.

Mishra, G., Palle, A. A., Srivastava, S., & Mishra, H. N. (2019). Disinfestation of stored wheat grain infested with *Rhyzopertha dominica* by ozone treatment: process optimization and impact on grain properties. *Journal of the Science of Food and Agriculture*, *99*(11), 5008-5018.

Mohapatra, D., Kumar, S., Kotwaliwale, N., & Singh, K. K. (2017). Critical factors responsible for fungi growth in stored food grains and non-chemical approaches for their control. *Industrial Crops and Products*, *108*, 162-182.

Nayak, M. K., Daglish, G. J., Phillips, T. W., & Ebert, P. R. (2020). Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. *Annual Review of Entomology, 65*, 333-350.

Ogecha, J. O., Arinaitwe, W., Muthomi, J. W., Aritua, V., & Obanyi, J. N. (2019). Incidence and Severity of common bean (*Phaseolus vulgaris* L.) pests in agro-ecological zones and farming systems of Western Kenya. *East African Agricultural and Forestry Journal*, *83*(3), 191-205.

Phillips, T. W., & Throne, J. E. (2010). Biorational approaches to managing stored-product insects. *Annual review of entomology, 55*, 375-397.

Pimentel, M. A. G., LRD'A, F., Guedes, R. N. C., Sousa, A. H., & Tótola, M. R. (2009). Phosphine resistance in Brazilian populations of *Sitophilus zeamais* motschulsky (Coleoptera: Curculionidae). *Journal of Stored Products Research, 45*(1), 71-74.

Ribeiro, D. F., Faroni, L. R. D. A., Pimentel, M. A., Prates, L. H. F., Heleno, F. F., & De Alencar, E. R. (2021). Ozone as a Fungicidal and Detoxifying Agent to Maize Contaminated with Fumonisins. *Ozone: Science & Engineering*, 1-12.

Ribeiro, L. P., Domingues, V. C., Gonçalves, G. L., Fernandes, J. B., Glória, E. M., & Vendramim, J. D. (2020). Essential oil from Duguetia lanceolata St.-Hil. (Annonaceae): Suppression of spoilers of stored-grain. *Food Bioscience*, 100653.

Rodríguez-González, Á., Campelo, M. P., Lorenzana, A., Mayo-Prieto, S., González-López, Ó., Álvarez-García, S., & Casquero, P. A. (2020). Spores of Trichoderma strains sprayed over *Acanthoscelides obtectus* and *Phaseolus vulgaris* L. beans: Effects in the biology of the bean weevil. *Journal of Stored Products Research*, *88*, 101666.

Ropelewska, E. (2019). Post-harvest assessment of wheat and barley kernel infections with fungi of the genus Fusarium using thermal analysis. *Journal of Stored Products Research*, *83*, 61-65.

Savi, G. D., Gomes, T., Canever, S. B., Feltrin, A. C., Piacentini, K. C., Scussel, R., & Angioletto, E. (2020). Application of ozone on rice storage: A mathematical modeling of the ozone spread, effects in the decontamination of filamentous fungi and quality attributes. *Journal of Stored Products Research*, *87*, 101605.

Silva, G. N., Faroni, A., Cecon, P. R., de Sousa, A. H., & Heleno, F. F. (2016). Ozone to control *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat grains. *Journal of Stored Products and Postharvest Research*, *7*(4), 37-44.

Silva, M. V. A., Faroni, L. R. A., Sousa, A. H., Prates, L. H. F., & Abreu, A. O. (2019). Kinetics of the ozone gas reaction in popcorn kernels. *Journal of Stored Products Research*, 83, 168-175.

Silva, M. V. D. A., Faroni, L. R. D. A., de Alencar, E. R., de Sousa, A. H., Cecon, P. R., Nogueira, J. V. F., & Mason Filho, V. (2022). Ozone injection at low pressure: decomposition kinetics, control of *Sitophilus zeamais*, and popcorn kernel quality. *Ozone: Science & Engineering*, *44*(1), 66-78.

Silva, Y. N. M., Silva, P. R. R., de França, S. M., Silva, Y. D. M., Brito, R. D. C., Barbosa, D. R. E. S., & Silva, G. N. (2022a). Ozonation of Lima Bean (*Phaseolus Lunatus* L.): Control of *Zabrotes Subfasciatus* (Boheman, 1833)(Coleoptera: Chrysomelidae: Bruchinae) and Maintenance of Grain Quality. *Ozone: Science & Engineering*, *44*(4), 351-362.

Sitoe, E. D. P. E., Faroni, L. R. D. A., de Alencar, E. R., Silva, M. V. D. A., & Salvador, D. V. (2023). Low-pressure ozone injection system: relationship between reaction kinetics and physical properties of grains. *Journal of the Science of Food and Agriculture*, *103*(3), 1183-1193.

Subramanyam, B., Xinyi, E., Savoldelli, S., & Sehgal, B. (2017). Efficacy of ozone against *Rhyzopertha dominica* adults in wheat. *Journal of Stored Products Research*, *70*, 53-59.

Tsehaye, H., Brurberg, M. B., Sundheim, L., Assefa, D., Tronsmo, A., & Tronsmo, A. M. (2017). Natural occurrence of Fusarium species and fumonisin on maize grains in Ethiopia. *European journal of plant pathology*, *147*(1), 141-155.

Wakil, W., Kavallieratos, N. G., Usman, M., Gulzar, S., & El-Shafie, H. A. (2021). Detection of phosphine resistance in field populations of four key stored-grain insect pests in Pakistan. *Insects*, *12*(4), 288.

Xinyi, E., Li, B., & Subramanyam, B. (2019). Efficacy of ozone against adults and immature stages of phosphine susceptible and resistant strains of *Rhyzopertha dominica*. *Journal of Stored Products Research*, *83*, 110-116.

Zhu, F. (2018). Effect of ozone treatment on the quality of grain products. *Food chemistry*, *264*, 358-366.

CHAPTER 1

Low-pressure ozone injection system: relationship between reaction kinetics and physical properties of grains

The ozonation of grains in a closed system at low-pressure is a strategy with the potential for treating packaged products. Research is necessary to characterize the reaction kinetics of ozone in this type of injection system so that it is possible to design chambers and determine the ozone concentrations suitable for commercial-scale applications. The objective of this study was therefore to characterize the low-pressure ozone injection system in relation to the physical properties of the grains and determine possible changes in the grain quality-defining characteristics. Samples (5.0 kg each) of common beans, cowpea beans, corn, popcorn kernels, paddy rice, and polished rice were exposed to ozone in a 70 L hypobaric chamber. Initially, the internal pressure of the chamber was reduced to 500 hPa. Then, ozone was injected at a concentration of 32.10 mg L^{-1} at a volumetric flow rate of 1 L min^{-1} until reaching a pressure of 1000 hPa. To relate the decomposition of ozone to the grains that were being evaluated, different physical properties were determined, and quality analysis was conducted. Ozone gas half-life outside and inside the package depended on the grain type. Ozone decomposition was quickest in polished rice and slowest in common beans. The half-life of the different grains ranged from 17.8 to 52.9 and 16.4 to 52.9 min, outside and inside the package, respectively. Considering the physical properties, the surface area (AS) showed a strong and negative linear association ($p < 0.05$) with the k values outside (-0.85) and inside the packaging (-0.80). A positive linear association ($p < 0.01$) was observed between the specific surface and the k values outside (0.92) and inside (0.90) of the packaging. Regarding sphericity, a strong and negative linear association ($p < 0.05$) was observed only in the k values within the package, with a correlation coefficient of -0.87. In this context, the physical properties of grain influenced the reaction kinetics of ozone gas during the low-pressure injection process. Ozone gas injection at low-pressures did not alter the quality attributes of the grains under study.

Keywords: Hypobaric chamber. Ozonation. *Zabrotes subfasciatus*. *Callosobruchus maculatus*. *Sitophilus zeamais*.

1. INTRODUCTION

Research in developing countries has primarily aimed to boost food production rather than to examine the reduction of postharvest losses (Kumar et al., 2017). Insect pests stand out among the factors damaging grains and their by-products during the post-harvest period (Singh & Kaur, 2018). They compromise stored goods quantitatively and qualitatively, leading to reductions in dry matter, germination capacity, and nutritional quality, in addition to being vectors for fungi and other organisms (Baldin & Lara, 2008; Nesci et al., 2011; Hou et al., 2016). Annual losses due to insects are estimated at 9% in developed countries and 20% in developing nations (Huang et al., 2015; Hou et al., 2016).

Methods to curb stored-grain insect infestation may be physical, biological, or chemical (Karuppuchamy & Venugopal, 2016). Physical methods include moisture content reduction (drying), temperature control (aeration/cooling), grain cleaning, adequate packaging, and application of inert powders (Karuppuchamy & Venugopal, 2016; Daglish et al., 2018; Paponja et al., 2020). Biological control employs living organisms, such as *Amblyseius swirskii* Ath.-Henr (Acari, Gamasida, Phytoseiidae), for controlling eggs of *Zabrotes subfasciatus* Bohemann, 1833 (Coleoptera: Chrysomelidae) (Iturralde-García et al., 2020a), the larval parasitoids *Anisopteromalus calandrae* Howard (Hymenoptera: Pteromalidae) and *Lariophagus distinguishandus* Föster, 1841 (Hymenoptera: Pteromalidae), against *Callosobruchus chinensis* Linnaeus, 1758 (Coleoptera: Chrysomelidae) (Iturralde-García et al., 2020), and nematodes like *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) and *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae), against *Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae) (Mbata et al., 2018). Chemical control comprises the application of pesticides, and it can be preventive (with contact insecticides) or curative (phosphine fumigation, for example) (Daglish et al., 2018).

Phosphine (PH_3) is currently the most commonly used fumigant to control insect pests in stored grains (Kumar et al., 2017). However, the continuous use of this gas has led to increasing resistance in pest populations (Chadda, 2016; Tay et al., 2016), environmental contamination (Singh et al., 2018; Saini & Kaushik, 2021), and poses risks to the health of operators. It is therefore essential to search for other methods to tackle insect-infestation issues. Ozonation has been proposed to control insect pests in stored grains. Several authors reported that ozone gas applied in flow is efficient in managing various pest species (Sousa et al., 2016; Pandiselvam et al., 2017; Mishra et al., 2019; Paul et al., 2020; Boopathy et al., 2022). As for packaged products, the application of ozone gas with low-pressure injection systems has proven effective against *S. zeamais* in popcorn kernels (Silva et al., 2022).

Several authors investigated the reaction kinetics of ozone when applied in flow to agricultural products in bulk, such as corn, nuts, beans, and rice (Brito et al., 2018; Pandiselvam et al., 2019; Ferreira et al., 2021; Abreu et al., 2022; Assis et al., 2019; Dubey et al., 2021). Studies indicated that ozone decay depends on the chemical composition and physical properties of the grains (Afsah-Hejri et al., 2020; Souza et al., 2020). However, few reports approached the application of ozone in low-pressure injection systems (Isikber et al., 2007; Silva et al., 2022). Studies must therefore be carried out on applying ozone gas in low-pressure injection systems to control insect pests in different grains.

Knowing the ozone decomposition kinetics in this system in different grains makes it possible to dimension a treatment chamber, estimate the ozone concentration over time, and establish an adequate quantity of products to be treated. The objectives of this research, therefore, were the following: (i) to characterize the low-pressure ozone injection system in relation to grain's physical properties and (ii) to determine possible changes in the quality of ozonized grains.

2. MATERIAL AND METHODS

2.1. Grains used in the experiment

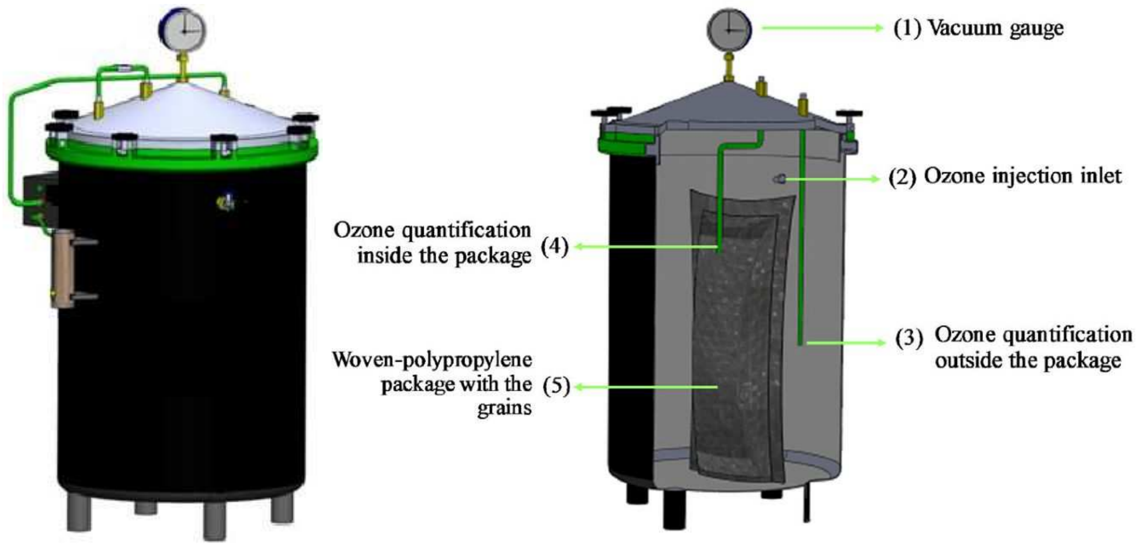
In the experiment common beans, cowpea beans, corn, popcorn kernels, paddy rice, and polished rice were used. The moisture contents (wet basis - w.b.) of the grains were: 13.94±0.05 g 100 g^{-1} in common beans, 10.61±0.05 g 100 g^{-1} in cowpea beans, 12.32±0.23 g 100 g^{-1} in corn, 12.95±0.19 g 100 g^{-1} in popcorn kernels, 13.04±0.09 g 100 g^{-1} in paddy rice, and 12.60±0.13 g 100 g^{-1} in polished rice. The grains were acquired from the local market (Viçosa, Minas Gerais, Brazil) and stored in climatic chambers (Marconi Ltda, Piracicaba, São Paulo, Brazil) at 5 °C until the testing.

2.2. Grain ozonation process

The ozonation of packed grains in a low-pressure system employed the methodology described by Silva et al. (2021). Fig. 1 presents the schematics of the hypobaric chamber with a vacuum gauge (myOZONE, Jaguariuna, São Paulo, Brazil) for pressure monitoring (1), ozone gas injection inlet (2), systems for quantification of ozone concentration outside (3) and inside the package (4), and the woven-polypropylene bag containing 5.0 kg of grains (5).

Ozone was generated from oxygen produced by a concentrator model *EverFlo* (Philips Respironics, Murrysville, PA, USA) at a volumetric flow rate of 1 L min^{-1}. Grain ozonation was carried out in a hypobaric chamber with a 70 L internal capacity, model CV10 (myOZONE, Jaguariúna, São Paulo, Brazil). The ozone gas injection was preceded by the reduction of the internal pressure of the chamber to 500 hPa with a vacuum pump model VP 260ND (TIPI – Professional Refrigeration, Rio Grande do Sul, Caxias, Brazil).

Figure 1 - Frontal and cross-sectional view of the hypobaric chamber.



Source: Sitoe (2023)

2.3. Reaction kinetics of ozone gas in a low-pressure system

To characterize the reaction kinetics, the ozone concentration was monitored inside the chamber, either empty or containing the different types of packed grains, both during and after the gas injection. The ozone input concentration was 32.10 mg L^{-1}, and the control treatment used oxygen gas instead, at a volumetric flow rate of 1 L min^{-1}. For the treatment of grains with oxygen (control), the methodology used for the injection of ozone gas was adopted. During the ozone injection, the concentration was assessed at intervals of 100 hPa, from 500 hPa to 1000 hPa internal pressure. That way, it was possible to relate the ozone concentration to the pressure inside the chamber in each grain type.

The ozone decomposition kinetics was established in the presence and absence of grains. Samples of 5.0 kg of grains packed in woven-polypropylene bags were placed inside the hypobaric chamber. After the chamber was closed, the internal pressure was reduced to 500 hPa, and ozone gas was injected. The same procedure was followed to evaluate the concentration of ozone gas in the empty chamber (without grains). Ozone injection was cut off when the pressure inside the chamber reached 1000 hPa. Once the ozone injection was interrupted, the concentrations outside and inside the package were monitored, applying the iodometric method, until they could no longer be quantified (Rakness et al., 1996). Ozone was collected outside and inside the package using a suction pump with flow control. The gas was directed to an *Erlenmeyer* containing a potassium iodide solution (KI 2%) and subsequently quantified using the iodometric method. The same process of quantification of ozone concentration was adopted by Silva et al. (2022).

For the decay of ozone concentration as a function of time, an exponential equation was fitted (Eqn 1), where a and b are the constants of the equation. To determine the decomposition rate, zero-, first-, and second-order kinetic models were fitted to the data on ozone concentration as a function of time outside and inside the package (Wright, 2004) (Table 1). From the best-fitted model and the decomposition rate constant (k), the ozone half-life (*t1/2*) was calculated.

$$C = ae^{-bt} \quad \text{(Eq.1)}$$

Table 1 - Reaction kinetics models and respective linearized equations used to determine the decomposition rate of ozone gas with the corresponding half-life times.

Order	Differential equations	Integrated and linearized equation	Half-life (min)
0	$\frac{dC}{dt} = -k$	$C = C_0 - kt$	$t_{1/2} = \frac{C_0}{2k}$
1	$\frac{dC}{dt} = -kC$	$lnC = ln\, C_0 - kt$	$t_{1/2} = \frac{\ln(2)}{k}$
2	$\frac{dC}{dt} = -kC^2$	$\frac{1}{C} = \frac{1}{C_0} + kt$	$t_{1/2} = \frac{1}{kC_0}$

C – Ozone concentration (mg L^{-1}); k – Constant of the decomposition rate (s^{-1}); t – time (s); C_0 – Initial ozone concentration (mg L^{-1}).
Source: Wright (2004).

2.4. Physical properties of the grains

The geometric parameters of different types of grains: orthogonal-axis dimensions (*A*, *B*, and *C*), volume (*V*), surface area (*SA*), sphericity (φ), specific surface (*Ss*), permeability (*K*), porosity (ε), and specific mass (ρ) were evaluated. The analysis of the physical properties used 30 randomly-selected grains of common beans, cowpea beans, corn, popcorn, paddy rice, and polished rice.

An analog caliper with 0.05 mm precision was used to gauge the three main dimensions of the grains: length (*A*), width (*B*), and thickness (*C*). These measurements were then used to calculate the *SA* (m^2) and *V* (m^3) of the grains (Mohsenin, 1970; Mohsenin, 1974;

Mohesenin, 1980; Mohsenin 1986; Jain, & Bal, 1997). The ratio between these two parameters gave the Ss (m^2 m^{-3}). The porous medium K was determined through Eq. (2) (Ergun, 1952; Massarani, 1997) and grain ε (%) was estimated by the method described by Mohsenin, (1980). Finally, the ρ of the grains (kg m^{-3}) was determined in three repetitions, with the aid of a hectoliter weight apparatus model 40 (Dallemolle, Caxias do Sul, Rio Grande do Sul, Brazil) (BRASIL, 2009).

$$K = \frac{\left(\overline{D_p}\varphi\right)^2 \varepsilon^3}{150(1-\varepsilon)^2} \qquad \text{(Eq. 2)}$$

Where: K – permeability (m^2); Dp – average particle diameter (m); φ – sphericity; ε – porosity.

2.5. Quality of the grains

The quality of the grains treated with either oxygen or ozone gas was appraised by determining their moisture content and color. The cooking time of the beans and expansion volume and flake volume of the popcorn kernels were also assessed. The percentage of surviving insects was also appraised.

2.5.1. Moisture content

Moisture content was measured according to the standards of the ASABE (*American Society of Agricultural and Biological Engineers*, 2013). Samples of common beans, cowpea beans, popcorn kernels, and corn grains were put inside a forced air convection oven at 103 °C for 72 h. In turn, paddy rice and polished rice samples were subjected to 105 °C for the same amount of time (Chen, 2003).

2.5.2. Color

Grain color was evaluated by directly reading the reflectance of the coordinates L^*, a^*, and b^* with a colorimeter model CR410 (*Konica Minolta*, Osaka, Japan). From the values obtained, it became possible to calculate the color tone or hue angle (h^*, Eq. (3)), color saturation or chroma (C^*, Eq. (4)), and total color difference (ΔE^*, Eq. (5)) (Francis, 1975; Little, 1975; Maskan, 2001; Mclellan et al., 1995).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad \text{(Eq. 3)}$$

$$h^* = arctang\left(\frac{b^*}{a^*}\right) \quad \text{(Eq. 4)}$$

$$\Delta E^* = \sqrt{(L^2 - L_0^*) + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad \text{(Eq. 5)}$$

Where L_0^*, a_0^* and b_0^* are the coordinates before the treatment with ozone or oxygen (control).

2.5.3. Cooking time

The cooking time of common beans and cowpea beans was determined using Mattson cooking apparatus (Estanhof Ltda, Lavras, Minas Gerais, Brazil) as described by Wang & Daun (2005). This device contains 25 plungers, each 21.6 cm long and weighing 82.0 g. These structures end in a tapered tip with a 0.2 mm diameter and 0.9 mm length, which rests perpendicularly to the grain during the test. Prior to the testing, the samples were soaked in distilled water at 25 °C for 16 h. After that, whole grains with the husk adhered to the cotyledons were selected to establish the cooking time. The apparatus was placed in a metallic container with boiling distilled water kept at a constant volume. The cooking time was determined when the thirteenth plunger transfixed the grain.

2.5.4. Popcorn expansion volume and flake volume

The expansion volume was assessed using the methodology by Silva et al., (2021) and Lokeswari & Mahendran (2022), considering three samples of 250 g of popcorn kernels. The popcorn expansion was performed in a Cretors Metric Weight Volume Tester (MWVT) device model CR13101 (Cretors & Company, Chicago, IL, USA). The results were expressed in mL g^{-1}. The flake volume determination followed the methodology described by García-Pinilla et al. (2021). According to this, this variable is defined as the ratio of the product of the sample mass and expansion volume to the number of popped kernels.

2.5.5. Percentage of surviving insects

Populations of *Z. subfasciatus*, *C. maculatus*, and *S. zeamais* were reared in 0.8 L glass containers at 28±2 °C, 75±5% relative humidity, and 24 h scotophase (Iturralde-García et al., 2020; Sousa et al., 2016). Common beans and cowpea beans were used as the food substrate for *Z. subfasciatus* and *C. maculatus*, respectively, while popcorn kernels and corn grains were fed to *S. zeamais*. In each assay, 180 insects were used to determine the ozonation effect. The insects were distributed in cages covered with organza fabric (100% polyester, 35 g m^{-2}, Fabtex, New Delhi, India). Three cages with 20 insects each were placed inside the bags, at the base, middle, and top. The percentage of surviving insects was evaluated 24 h after exposure to oxygen (control) or ozone gas (treatment).

2.6. Statistical analysis

The ozone gas concentration data on the reaction kinetics with the grains during and after the low-pressure injection process were submitted to regression analysis with the software *Sigma Plot* version 12.5. Pearson's correlation estimate was used to determine the relationship between the physical properties of the grains and the decomposition rate constant (*k*) outside and inside the packaging. The quality data were tested for normality and homogeneity with the Shapiro–Wilk test and Levene's test. The quality results of grains exposed to oxygen or ozone were subjected to analysis of variance (ANOVA), adopting a significance level of 5%.

3. RESULTS

3.1. Ozone reaction kinetics

Table 2 shows the mathematical models with the respective determination coefficients (r^2), describing the reaction kinetics of ozone gas applied at low-pressure outside and inside the packages with the different types of grains, at the input concentration of 32.10 mg L^{-1}. Under the conditions studied, the concentration of ozone inside the chamber and the injection time increased with the increase in pressure (500 to 1000 hPa) during the gas injection process. The total ozone injection time was 37 min in each experiment. The type of grain influenced the reaction kinetics of ozone gas applied at low pressure inside the chamber. Inside the package, at a pressure of 600 hPa, the ozone concentration values were as follows: 1.10 mg L^{-1} in common beans and 0.21 mg L^{-1} in cowpea beans, 0.34 mg L^{-1} in popcorn kernels and 0.30 mg L^{-1} in corn, and 0.25 mg L^{-1} in paddy rice and 0.00 mg L^{-1} in polished rice.

At 1000 hPa, the highest differences between the ozone concentrations inside the package were verified when comparing the values obtained for common beans (6.0 mg L^{-1}) with those for paddy rice (0.43 mg L^{-1}) and for polished rice (0.21 mg L^{-1}). A similar trend was noticed inside the chamber, in which the lowest values of gas concentration were observed when paddy rice and polished rice were evaluated. Table 3 contains the mathematical models describing the ozone concentration decay and the respective determination coefficients (r^2) of the different types of grains. In all adopted conditions, the ozone levels showed an exponential decline as a function of time.

Table 2 - Regression equations fitted to the data on ozone gas concentration applied at low pressure into the empty chamber, outside and inside the package, in grains of common beans, cowpea beans, popcorn, corn, paddy rice, and polished rice.

Empty chamber	Ozone gas reaction	
	Model	r^2
Ozone concentration as a function of pressure	$\hat{C}$ = 0.0693p - 35.5067	0.99
Injection time as a function of pressure	$\hat{T}$ = 0.0185p - 9.0585	0.99
Grains	Outside the package	
Common beans	$\hat{C}$ = 0.0174p - 8.9041	0.98
Cowpea beans	$\hat{C}$ = 0.0145p - 7.7877	0.94
Popcorn kernels	$\hat{C}$ = 0.0171p - 8.6563	0.99
Corn	$\hat{C}$ = 0.0137p - 6.8057	0.99
Paddy rice	$\hat{C}$ = 0.0110p - 5.5879	0.98
Polished rice	$\hat{C}$ = 0.0102p - 5.4411	0.96
Grains	Inside the package	
Common beans	$\hat{C}$ = 0.0116p - 6.1899	0.93
Cowpea beans	$\hat{C}$ = 0.0062p - 3.5415	0.79
Popcorn kernels	$\hat{C}$ = 0.0035p - 1.8328	0.88
Corn	$\hat{C}$ = 0.0025p - 1.3104	0.86
Paddy rice	$\hat{C}$ = 0.0007p - 0.2654	0.80
Polished rice	$\hat{C}$ = 0.0004p - 0.2456	0.70

*$\hat{C}$ – estimated ozone gas concentration (mg L^{-1}); p – pressure inside the hypobaric chamber (hPa); $\hat{T}$– estimated injection time (min). The estimated parameters were significant at a 5% level ($P < 0.05$), according to the t-test.

Source: Sitoe (2023)

Table 3 - Regression equations fitted to the concentration decay data of ozone gas applied at low-pressure into the empty chamber, outside and inside the package, after the injection process in common beans, cowpea beans, popcorn kernels, corn, paddy rice, and polished rice.

	Fitted model	r^2	Fitted model	r^2
Empty chamber	$\hat{C} = 10.1986e^{-0.0136t}$	0.98		
Grains	Outside the package		Inside the package	
Common beans	$\hat{C} = 6.1843e^{-0.0112t}$	0.99	$\hat{C} = 9.0965e^{-0.0116t}$	0.99
Cowpea beans	$\hat{C} = 3.6714e^{-0.0170t}$	0.99	$\hat{C} = 7.0643e^{-0.0202t}$	0.99
Popcorn kernels	$\hat{C} = 2.4380e^{-0.0122t}$	0.97	$\hat{C} = 8.4724e^{-0.0158t}$	0.99
Corn	$\hat{C} = 1.5079e^{-0.0148t}$	0.98	$\hat{C} = 7.0143e^{-0.0208t}$	0.99
Paddy rice	$\hat{C} = 0.4366e^{-0.0290t}$	0.98	$\hat{C} = 5.6412e^{-0.0361t}$	0.99
Polished rice	$\hat{C} = 0.2426e^{-0.0679t}$	0.95	$\hat{C} = 5.6412e^{-0.0361t}$	1.0

*$\hat{C}$ – estimated concentration of ozone gas (mg L^{-1}); t – time (min). The estimated parameters were significant at a 5% level ($P < 0.05$), according to the t-test.
Source: Sitoe (2023)

The first-order kinetic model delivered the best fit to the data on the ozone level decay over time inside the chamber, both outside and inside the package containing the grains. The models are presented in Table 4, along with the respective ozone half-life (t1/2) and determination coefficient (r^2). The zero- and second-order fitted models are found in Tables 5 and 6.

In the empty chamber, ozone gas half-life was 57.76 min. Outside the package, the half-lives were as follows: 52.91 min in common beans, 29.25 min in cowpea beans, 40.53 min in popcorn kernels, 29.75 min in corn, 17.24 min in paddy rice, and 16.43 min in polished rice. With regards to the half-lives inside the package, the values obtained were equal to 52.91 min in common beans, 29.37 min in cowpea beans, 38.30 min in popcorn kernels, 34.48 min in corn, 21.53 min in paddy rice, and 17.86 min in polished rice.

Table 4 - First-order models of decomposition kinetics of ozone gas applied at low pressure into the empty chamber over time (*t*), outside and inside the package with grains of common beans, cowpea beans, popcorn, corn, paddy rice, and polished rice.

	Fitted model	$t_{1/2}$ (min)	r^2
Empty chamber	$ln\ (\hat{C}) = 2.2014 - 0.012t$	57.76	0.99
Grains	Outside the package		
Common beans	$ln\ (\hat{C}) = 2.3545 - 0.0131t$	52.91	0.99
Cowpea beans	$ln\ (\hat{C}) = 2.1747 - 0.0237t$	29.25	0.97
Popcorn kernels	$ln\ (\hat{C}) = 2.1966 - 0.0171t$	40.53	0.96
Corn	$ln\ (\hat{C}) = 2.1274 - 0.0233t$	29.75	0.98
Paddy rice	$ln\ (\hat{C}) = 1.8812 - 0.0402t$	17.24	0.99
Polished rice	$ln\ (\hat{C}) = 1.8635 - 0.0422t$	16.43	0.99
Grains	Inside the package		
Common beans	$ln\ (\hat{C}) = 2.0081 - 0.0131t$	52.91	0.99
Cowpea beans	$ln\ (\hat{C}) = 1.6755 - 0.0236t$	29.37	0.95
Popcorn kernels	$ln\ (\hat{C}) = 1.2863 - 0.0181t$	38.30	0.97
Corn	$ln\ (\hat{C}) = 0.6847 - 0.0201t$	34.48	0.95
Paddy rice	$ln\ (\hat{C}) = -0.7744 - 0.0322t$	21.53	0.97
Polished rice	$ln\ (\hat{C}) = -1.7474 - 0.0388t$	17.86	0.91

*$\hat{C}$ – estimated ozone concentration (mg L^{-1}); $t_{1/2}$ – ozone gas half-life. The estimated parameters were significant at a 5% level, according to the *t*-test.

Source: Sitoe (2023)

Table 5 - Zero-order models of decomposition kinetics of ozone gas applied at low pressure into the empty chamber over time (*t*), outside and inside the package containing grains of common beans, cowpea beans, popcorn, corn, paddy rice, and polished rice.

	Fitted model	r^2
Empty chamber	$\hat{C}$ = -0.0155*t* + 5.303	0.63
Grains	Outside the package	
Common beans	$\hat{C}$ = -0.0196*t* + 5.8128	0.77
Cowpea beans	$\hat{C}$ = -0.0278*t* + 4.6986	0.78
Popcorn kernels	$\hat{C}$ = -0.0257*t* + 5.5470	0.77
Corn	$\hat{C}$ = -0.0249*t* + 4.4344	0.74
Paddy rice	$\hat{C}$ = -0.0344*t* + 3.6617	0.72
Polished rice	$\hat{C}$ = -0.033*t* + 3.4934	0.72
Grains	Inside the package	
Common beans	$\hat{C}$ = -0.0136*t* + 4.0418	0.79
Cowpea beans	$\hat{C}$ = -0.0154*t* + 2.6566	0.88
Popcorn kernels	$\hat{C}$ = -0.0083*t* + 1.8302	0.88
Corn	$\hat{C}$ = -0.0077*t* + 1.2287	0.93
Paddy rice	$\hat{C}$ = -0.0061*t* + 0.4014	0.95
Polished rice	$\hat{C}$ = -0.0033*t* + 0.1801	0.67

*$\hat{C}$– estimated ozone concentration (mg L^{-1}).

Source: Sitoe (2023)

Table 6 - Second-order models of decomposition kinetics of ozone gas applied at low pressure into the empty chamber over time (*t*), outside and inside the package containing grains of common beans, cowpea beans, popcorn, corn, paddy rice, and polished rice.

	Fitted model	r^2
Empty chamber	$\frac{1}{\hat{C}} = 0.0448t – 4.9567$	0.58
Grains	Outside the package	
Common beans	$\frac{1}{\hat{C}} = 0.0306t -2.7219$	0.52
Cowpea beans	$\frac{1}{\hat{C}} = 0.0847t - 4.4200$	0.61
Popcorn kernels	$\frac{1}{\hat{C}} = 0.0321t - 1.6796$	0.67
Corn	$\frac{1}{\hat{C}} = 0.0965t - 5.3304$	0.59
Paddy rice	$\frac{1}{\hat{C}} = 0.2429t - 7.7915$	0.59
Polished rice	$\frac{1}{\hat{C}} = 0.3077t - 9.9228$	0.63
Grains	Inside the package	
Common beans	$\frac{1}{\hat{C}} = 0.0451t - 4.0642$	0.79
Cowpea beans	$\frac{1}{\hat{C}} = 0.0451t - 4.0642$	0.58
Popcorn kernels	$\frac{1}{\hat{C}} = 0.1345t - 8.7492$	0.62
Corn	$\frac{1}{\hat{C}} = 0.1183t - 4.3557$	0.60
Paddy rice	$\frac{1}{\hat{C}} = 0.218t + 0.6903$	0.9
Polished rice	$\frac{1}{\hat{C}} = 0.7539t + 2.136$	0.90

* $\frac{1}{\hat{C}}$ – estimated ozone concentration (mg L^{-1}).

Source: Sitoe (2023)

3.2. Physical properties of the grains

Table 7 shows the geometric parameters of the different types of grain: orthogonal-axis dimensions (A, B, and C), volume (V), surface area (SA), and sphericity (φ), used for estimating the specific surface (Ss) and permeability (K). Table 7 also presents the porosity (ε) and specific mass (ρ) of the grains. Polished rice and paddy rice had the highest specific surface values (2803.71±106.21 m^2 m^{-3} e 2313.78±96.06 m^2 m^{-3}, respectively), followed by cowpea beans, popcorn kernels, common beans, and corn grains (Table 7). Regarding porous medium permeability, the highest values were observed in corn grains (2.72 x 10^{-8}±5.17 x 10^{-9} m^2), while the porous medium formed by polished rice grains had the lowest ones (2.25 x 10^{-9} ± 2.04 x 10^{-10} m^2) (Table 7). Porosity was the highest in paddy rice (56.33±0.58%) and the lowest in cowpea beans (32.53±0.58%) (Table 7). The average values of grain density were as follows: 790.00±3.49 kgm^{-3} in common beans; 834.20±1.00 kgm^{-3} in cowpea beans; 760.67±2.05 kg m^{-3} in popcorn kernels; 725.07±0.70 kg m^{-3} in corn; 542.00±4.45 kg m^{-3} in paddy rice; and 816.40± 1.20 kg m^{-3} in polished rice (Table 7).

Table 8 shows the correlation between the physical properties of the grains and the decomposition rate constant (*k*) outside and inside the package. As for surface area (AS), a strong and negative linear association ($p<0.05$) was found with *k* values outside (-0.85) and inside the packaging (-0.80). A positive linear association ($p<0.01$) was observed between the specific surface and the *k* values outside (0.92) and inside (0.90) of the packaging. Regarding sphericity, a strong and negative linear association ($p<0.05$) was observed only in the *k* values within the package, with a correlation coefficient of -0.87. The variables volume (V), permeability (K), porosity (ε) and specific mass (ρ) did not show a significant linear association ($p>0.05$) with the *k* values.

Table 7 - Average values of the orthogonal-axis dimensions (*A*, *B*, and *C*), volume (*V*), surface area (*SA*), sphericity (*φ*), specific surface (*Ss*), permeability (*K*), porosity (*ε*), and specific mass (*ρ*) of common beans, cowpea beans, popcorn kernels, corn, paddy rice, and polished rice. [SD] – Standard deviation.

Grains	Geometric parameters of the grains						*Se* (m^2 m^{-3}) ±SD	*K* (m^2) ±SD	*ε* (%)±SD	*ρ* (kg m^{-3}) ±SD
	Orthogonal axes (mm)			*V* (mm^3) ±SD	*AS* (mm^2) ±SD	*φ* (%) ±SD				
	A±SD	*B*±SD	*C*±SD							
Common beans	10.90± 0.55	6.54± 0.24	5.17± 0.32	192.86± 17.15	214.36± 16.99	65.82± 2.74	1114.26± 66.13	1.57×10^{-8} ± 1.61×10^{-9}	35.33± 1.15	790.00± 3.49
Cowpea beans	8.48± 0.31	6.28± 0.23	5.21± 0.22	109.77± 9.99	133.58± 7.60	76.90± 2.16	1220.79± 47.21	1.03×10^{-8} ± 9.90×10^{-10}	32.33± 0.58	834.20± 1.00
Popcorn kernels	8.43± 0.58	6.38± 0.34	4.36± 0.57	89.54± 14.86	101.55± 10.22	73.18± 5.23	1146.20± 78.61	2.28×10^{-8} ± 5.32×10^{-9}	41.33± 1.15	760.67± 2.05
Corn	12.74± 0.79	8.25± 0.93	4.81± 0.73	263.78± 47.18	214.10± 24.94	62.32± 3.40	822.10 ± 62.71	2.72×10^{-8} ± 5.17×10^{-9}	39.33± 0.58	725.07± 0.70

Paddy rice	9.70± 0.52	2.27± 0.11	1.92± 0.11	22,11± 2.33	50.96± 3.68	35.93± 1.40	2313.71± 96.06	$9.79 \times 10^{-9} \pm 1.06 \times 10^{-9}$	56.33± 0.58	542.00± 4.45
Polished rice	6.67± 0.45	1.93± 0.12	1.57± 0.07	10.58± 1.35	29.62± 2.77	40.89± 1.58	2803,78± 106.21	$2.25 \times 10^{-9} \pm 2.04 \times 10^{-10}$	44.00± 1.00	816.40± 1.20

Source: Sitoe (2023)

Table 8 - Pearson´s correlation estimates between physical properties – volume (V), surface area (SA), sphericity (φ), specific surface area (Ss), permeability (K), porosity (ε) and specific mass (ρ) – of beans common, cowpea, popcorn, maize, paddy rice and polished rice with corresponding k values outside and inside the package.

	V (mm^3)	SA (mm^2)	φ (%)	Ss (m^2 m^{-3})	K (m^2)	ε (%)	ρ (kg m^{-3})
k- outside the package	-0.76^{ns}	-0.85^{a}	-0.78^{ns}	0.92^{b}	0.42^{ns}	0.59^{ns}	-0.22^{ns}
k- inside the package	-0.70^{ns}	-0.80^{a}	-0.87^{a}	0.90^{b}	0.58^{ns}	0.71^{ns}	-0.40^{ns}

[a] Significant at the 5% probability level ($p < 0.05$).

[b] Significant at the 1% probability level ($p < 0.01$).

[ns]- not significant at the 5% significance level ($p > 0.05$).

Source: Sitoe (2023)

3.4. Quality of the ozonized grains

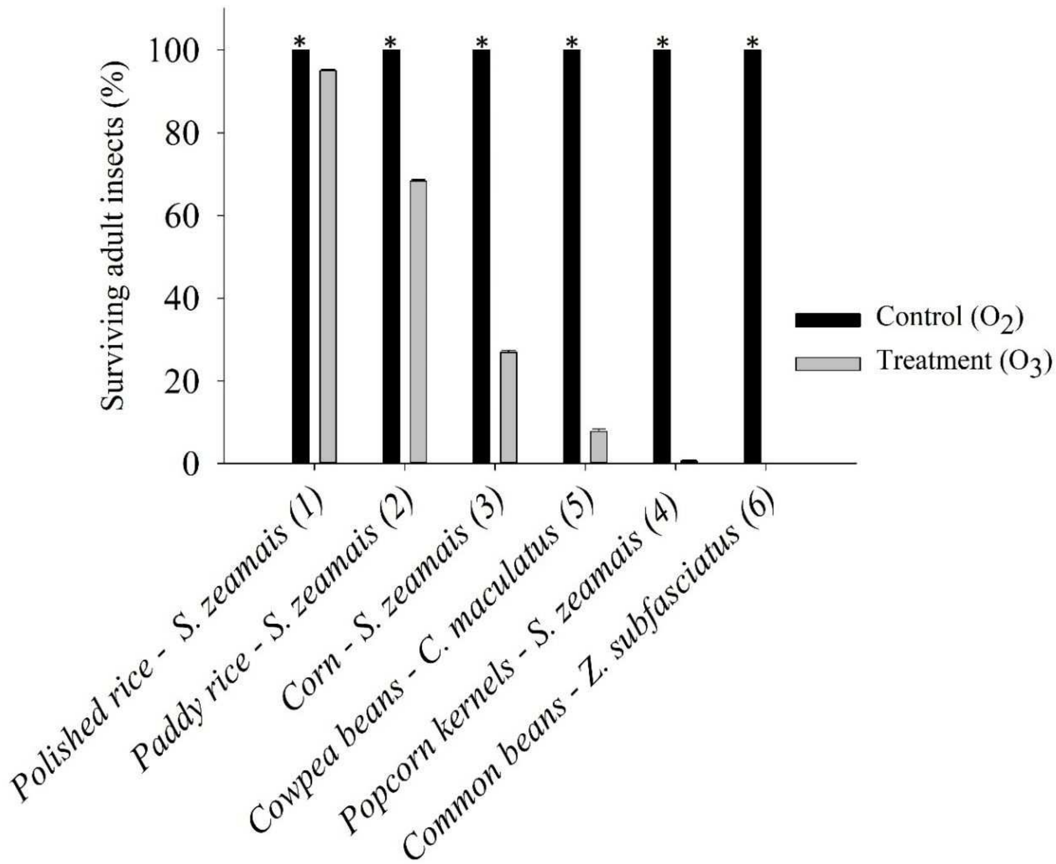
Ozonation at low-pressures of different types of grains in the hypobaric chamber did not cause a significant difference (p>0.05) when the grain quality variables were analyzed. In the grains subjected to oxygen treatment (control), water content values of 13.99, 10.63, 12.22, 12.95, 13.09 and 12.60% were observed for grains of common beans, cowpeas, popcorn corn, corn, paddy rice and polished rice, respectively. The water content values of common beans, cowpeas, popcorn, maize, paddy rice and polished rice subjected to ozone treatment were 13.94, 10.63, 12.22, 12.95, 13.09 and 12.45%, respectively. Regarding color difference (ΔE^*), values of 2.74, 1.80, 3.79, 3.02, 7.26 and 2.74 were observed for grains of common beans, cowpeas, popcorn, corn, paddy rice and polished rice, respectively. In grains treated with ozone, ΔE^* values of 2.58, 1.30, 4.22, 3.19, 6.35 and 2.58 were observed in common beans, cowpeas, popcorn, corn, paddy rice and polished rice, respectively. The color tone or hue angle (h^*) in common beans, cowpeas, popcorn, corn, paddy rice and polished rice subjected to oxygen treatment (control) were 14.39, 81.82, 79.08, 81.64, 76.35 and 93.96, respectively. For grains treated with ozone, h^* values of 16.78, 82.74, 81.39, 80.18, 75.59 and 93.44 were observed, corresponding to grains of common beans, cowpeas, popcorn corn, corn, paddy rice and polished rice, respectively. In relation to color saturation (C^*), the grains treated with oxygen (control) presented values of 10.48, 14.18, 24.42, 26.78, 18.04 and 8.99 in common beans, cowpeas, popcorn corn, corn, paddy rice and polished rice, respectively. In ozonated grains, *C** values were around 9.63, 13.48, 22.53, 26.00, 19.41 and 8.86 in common beans, cowpeas, popcorn corn, corn, paddy rice and polished rice, respectively.

The cooking time in bean grains, the expansion volume and the volume of flakes in popcorn grains also did not show significant changes ($p>0.05$) as a result of ozonation at low pressures. The cooking time values for common bean grains treated with oxygen (control) and ozone were 32.60 and 32.50 min, respectively. In cowpea, cooking time values were recorded as 26.5 and 26.6 min in grains treated with oxygen (control) and ozone, respectively. The popcorn expansion volume values were 35.70 and 35.60 mL g^{-1} in the oxygen (control) and ozone treatment, respectively. However, the volume of popcorn flakes treated with oxygen (control) and ozone was 6.60 and 6.7 cm3, respectively.

3.5. Percentage of surviving insects

No significant differences ($p < 0.05$) were observed in the percentage of surviving insects in the hypobaric chamber regarding cage position (base, middle, or top of the package) in the different types of grains. On the other hand, significant differences were noticed in all cages regarding the percentage of surviving insects exposed to ozone gas (treatment) or oxygen (control). Adults of *S. zeamais* in ozonated grains had different survival rates, being equal to 98.00% for polished rice, 65.00% for paddy rice, 26.94% for corn, and 0.55% for popcorn. In cowpea beans infested with adult *C. maculatus* individuals, survival was equal to 7.81%, whereas in common beans, there were no surviving adults of *Z. subfasciatus* (Fig. 2).

Figure 2 - Percentage (%) of surviving adult insects of *S. zeamais*, *C. maculatus*, and *Z. subfasciatus* 24 h after exposure to ozone gas applied at low pressure into the hypobaric chamber, at the input concentration of 32.10 mg L^{-1}. *The estimated variables were significant at a 5% level ($p < 0.05$), according to the t-test.



Source: Sitoe (2023)

4. DISCUSSION

4.1. Ozone gas reaction kinetics

A difference in variation in the levels of ozone gas applied at low-pressure was spotted during the injection, considering the empty chamber and the chamber containing grains. Under the conditions that were studied, ozone concentration was directly proportional to the internal pressure of the chamber and injection time. As expected, the gas concentration was low at the beginning of the process due to the low volume injected and the intense ozone reaction with the grains (Pandiselvam et al., 2017; Silva et al., 2019). With time, the internal pressure and ozone concentration both outside and inside the package augmented, making the gas more available in the intergranular space.

The ozone gas half-life differed in each type of grain investigated in this study. Both outside and inside the package, the longest half-life was registered in common beans, whereas the lowest values were found in paddy rice and polished rice. The behavior of ozone-level decay in different grains may be associated with their physical properties and chemical composition (Pandiselvam et al., 2017). Grain reactivity affects ozone half-life inside the hypobaric chamber. The concentration decay behavior of ozone gas applied at low-pressure inside the hypobaric chamber to different types of grains followed the first-order reaction kinetics, corroborating previous findings of other authors (Pandiselvam et al., 2015; Silva et al., 2022).

Under the conditions in this study, ozone half-life times in the hypobaric chamber were greater than those obtained by other authors when different types of grains were ozonated in flow. Silva et al. (2019) verified that the half-life of ozone applied in flow to popcorn kernels was 5.20, 6.22, and 5.56 min, at input concentrations of 500, 2200, and 4500 $\mu g\ L^{-1}$. In research by Pandiselvam et al. (2015) the half-lives were 11.93 and 9.24 min in rice grains with initial moisture contents of 11.2 and 14.3% (w.b.), respectively. Ravi et al. (2015) evaluated the effect of ozone gas on rice grains with 11.4 and 14.2% moisture contents, finding ozone half-lives of 13.80 and 11.61 min, respectively. It is noteworthy that, in the absence of biological materials, ozone gas half-life varies from 20 to 50 min (Souza et al., 2018; Chee et al., 2020).

In the present study, the half-life inside the package was expected to be shorter, as the ozone reaction rates in the presence of grains are higher (Silva et al., 2020; Savi et al., 2020). However, it should be noted that the reaction rates of ozone gas applied in low-pressure injection systems are influenced by the gas-flow dynamics (Silva et al., 2022). In this ozone application system, the pressure difference associated with the concentration gradient is responsible for the gas flowing into the package. As reported by Silva et al. (2022) the ozone transport into the package occurs first by forced convection and then by diffusion. During injection, the pressure gradient outside and inside the chamber is responsible for transporting ozone gas into the package. As the injection proceeds, the pressure inside the chamber increases, reducing the gradient until the internal pressure equals the atmospheric pressure. From that moment on, the injection is cut off, and ozone flows only due to the difference in concentration. Then, ozone is continuously transported into the package, either by forced convection or diffusion. These factors resulted in relatively close half-life values inside and outside the package in each grain type.

The difference in ozone concentration outside and inside the package and the reaction kinetics were affected by the physical properties of the grains. The lowest ozone concentrations both outside and inside the package were found in polished rice, whereas the highest levels were observed in common bean grains. Among the physical properties of the grains, surface area (*SA*), specific surface (*Ss*), and sphericity (φ) may explain the low ozone concentrations outside and inside the package in paddy and polished rice. In general, reaction rates are affected by the specific surface (Wright, 2004; Pandiselvam et al., 2017). In the present study, this fact became evident due to the strong correlation between the decomposition rate constant and specific surface values in the different grains. High specific surface values implied high reaction rates of ozone with the grain mass (Incropera et al., 1996; Pedersen & Meyer, 2009; Bhattarai et al., 2015).

In this study, the solid fractions of the porous media constituted by paddy rice or polished rice grains had the highest specific surface and porosity values. This justifies the high reaction rate constants and, consequently, the lowest half-life of ozone gas in these grains. On the other hand, paddy rice and polished rice exhibited the lowest permeability figures, which justifies the low ozone concentration values inside the package.

Corn grains had the lowest specific surface value, so it was expected that they would have the lowest k value. However, they were more reactive and had a k value higher than common beans and popcorn kernels. This particular behavior can be explicated by corn grains having a more fragile pericarp (35 to 200 μm thickness) in the germ region (Brewbaker et al., 1996; García-Lara et al., 2019). This site is more exposed to the action of ozone than popcorn kernels, whose thicker pericarp has more mechanical resistance (1.4 times greater) (Hoseney et al., 1983).

The chemical composition of the grains also likely influenced the reaction rate of ozone applied in a low-pressure system (Jian et al., 2013; Chadda, 2016). Corn has about 60–75% starch content, (Veljković et al., 2018) second only to rice grains. As starch is ozone reactive, (Chan et al., 2011; Zhu, 2018) this may be why corn grains showed a higher reaction rate constant, despite having a larger specific surface area than common beans and popcorn kernels.

In paddy rice grains, the husk corresponds to approximately 20% (w/w) of the total composition (Kim et al., 2004). Rice grain husk is high in fiber content, with 33% cellulose, 26% hemicellulose, and 7% lignin (Islam et al., 2018). The high ozone reaction rate might also be associated with rice composition, as high reaction rates imply low ozone concentrations in the intergranular space (Silva et al., 2020; Savi et al., 2020).

Polished rice is 90% starch (Patindol et al., 2015; Bhat & Riar, 2019). When reacting with ozone, the gas breaks down the starch granules by oxidation (Vanier et al., 2017; Zhu, 2018). An & King. (2009) applied ozone gas for 30 min and verified a reaction with rice starch, resulting in granules with greater swelling power. The starch in polished rice may explain the elevated ozone decomposition rate constants (k^{-1}) found in this study.

Common beans, cowpea beans, popcorn kernels, and corn grains were less reactive with ozone than paddy rice and polished rice. In this context, the physical properties and chemical composition of the grains, ozone input concentration, and chamber pressure are variables that influence the ozone gas reaction kinetics and, therefore, must be taken into account when designing low-pressure injection systems.

Ozone injection at low pressure is a strategy indicated for packaged goods. In this method, all gas reacts with the product, (Silva et al., 2022) which differs from what happens when ozone is applied in flow (Silva et al., 2019; Oliveira et al., 2020). Ozone injection at low pressure increases the efficiency of use of the ozone generated. All the input oxygen volume was converted into ozone, which effectively reacted with the product inside the hypobaric chamber. On the hand, in flow applications, some ozone reacts with the product, while the remaining flows freely through the grain mass, resulting in residual ozone, which must be either degraded or recirculated (Abreu et al., 2022). In the study by Silva et al. (2022) the authors compare the mass of ozone used per kilogram of popcorn kernels to control *S. zeamais*, and they found that the hypobaric chamber method required less ozone than the flowing-gas application system. In addition, the low-pressure injection system provided better operational safety, as the applications necessarily take place in hermetic chambers, and no residual ozone is generated.

4.2.Quality of the ozonized grains

The low-pressure ozone injection system did not change ($p > 0.05$) the moisture content, color difference (ΔE^*), color hue (h^*), and color saturation (C^*) of the grains. These results corroborate previous studies that showed that ozone gas did not change the moisture content of packaged grains of corn (Freitas et al., 2017), rice (Ávila et al., 2017), and linseed (*Linum usitatissimum* L.) (Bechlin et al., 2019).

Previous scientific investigations demonstrated that the process of injecting ozone gas in flow could lead to grain drying (Mendez et al., 2003; Freitas et al., 2017) and color alterations (Sivaranjani et al., 2021). The mixture of ozone and other gases has less relative humidity due to the ozone generation process, which must be input with moisture-free atmospheric air or oxygen, thus causing treated products to dry out (Freitas et al., 2017). Reducing the moisture content of popcorn kernels diminishes their expansion volume, as observed by Gökmen (2004).

Color is a crucial aspect for agricultural-product processing industries. Researchers who observed differences in the color patterns of ozonated wheat flour (Mei et al., 2016) and wheat grain (Wang et al., 2016) reported that, in an ozone treatment in flow, the values of brightness (L^*) increased whereas (b^*) declined due to the oxidation of conjugated double bonds in carotenoid pigments. Nonetheless, under the conditions of this study, no changes in the color variables were observed in the grains that were evaluated, reinforcing the potential of ozone gas application by the food industry.

Cooking time is one of the most important quality variables in beans, as the grains must be cooked either conventionally or under pressure (Freitas et al., 2016; Wood, 2017). Abreu et al. (2022) found that ozone applied in flow at inlet concentrations of 0.5, 1.5, 2.5 and 3.5 mg L^{-1} and exposure times of 1698.00, 331.20, 157.07 and 84.40 min, did not affect the cooking time and water content of cowpea beans. In this study, ozone applied at low-pressures did not change ($p > 0.05$) the cooking time of common beans and cowpea beans. Neither did it alter ($p > 0.05$) the expansion volume and flake volume of popcorn kernels subjected to low-pressure treatment. These results agree with Silva et al. (2022). They verified that different concentrations (5.0, 6.7, 8.5 and 13.0 mg L^{-1}) of ozone injected at low pressures into popcorn kernels did not alter the expansion volume and flake volume.

The present research treated paddy rice and polished rice with ozone at 0.433kg_{O3} m^{-3}_{arroz} arroz (433 g_{O3} m^{-3}_{arroz}) and 0,705 kg_{O3} m^{-3}_{arroz}, (705 g_{O3} m^{-3}_{arroz}), respectively. Savi et al. (2020) demonstrated that the application of ozone at doses higher (2261 kg_{O3} m^{-3}_{arroz}) than the ones used in this study produced no significant changes in starch content. Quality alterations in ozonated grains depend on the gas concentration, exposure time, and water content of the product (Wang et al., 2016; Afsah-Hejri et al., 2020). Thus, the results of this study as well as those by Silva et al. (2022) proved that ozone gas applied at low pressures might be a promising ozonation alternative that does not affect the nutritional aspects of grains.

4.3. Percentage of surviving insects

The ozone input concentrations adopted in this study were efficient in curbing adults of *S. zeamais* in popcorn kernels and corn grains, *C. maculatus* in cowpea beans, and *Z. subfasciatus* in common beans. Pandiselvam & Thirupathi, (2015) assessed the ozone gas reaction kinetics in Vigna radiate and concluded that the ozonation process can be an alternative to chemical compounds for insect control in storage units. In research by Gad et al. (2021) the ozone gas treatment at 2.0 mg L^{-1} for 45 days controlled *C. maculatus*. Silva et al. (2022) evaluated the ozone injection at low-pressure into packaged popcorn kernels to manage *S. zeamais*, proving the effectiveness of the method at the input concentration of 13 mg L^{-1}.

In paddy rice and polished rice grains, at the ozone input concentration of 32.10 mg L^{-1}, the survival rate of *S. zeamais* was 65.00 and 98.00%, respectively. This elevated rate is associated with the high values of the reaction rate constant of ozone gas with rice grains (Pandiselvam et al., 2015). Grain reactivity determines the efficiency of ozone gas on insect-pest mortality (Pandiselvam et al., 2017; Afsah-Hejri et al., 2020; Sivaranjani et al., 2021; Srivastava et al., 2021). Little grain reactivity and long half-life imply high concentrations of ozone in the intergranular space and, consequently, good efficiency in controlling insect pests (Sunisha, 2019).

Although the treatments in paddy rice and polished rice were only a little effective, the exposure time in this study was 172 min. Sunisha (2019) evaluated the efficiency of ozone gas at 0.92 mg L^{-1}, 1.85 mg L^{-1}, and 2.78 mg L^{-1} on the mortality of *Rhyzopertha dominica* in rice grains with 12.40% moisture content (w.b.). The author achieved 100% mortality at the exposure times of 540, 360, and 270 min. In rice grains with 14.20% water content (w.b.) treated at 0.92 mg L^{-1}, 1.85 mg L^{-1}, and 2.78 mg L^{-1}, total mortality of *R. dominica* was attained after 610, 390, and 300 min, respectively. Thus, the control efficiency of insects in rice might be enhanced by extending the exposure time. When it comes to grain ozonation in a hypobaric chamber, longer exposure to the gas can be obtained by adopting more than one treatment cycle.

In addition to the exposure time, fumigation efficiency for controlling insect pests in stored grains depends on the concentration of the fumigant, the insect species and its life stage, and the moisture content and type of grain (Pandiselvam et al., 2017). The results of this research indicated that, to assure insect mortality when treating packaged grains with ozone gas at low pressure, it is paramount to consider the grain reactivity for choosing the concentration and number of applications.

5. CONCLUSION

This study presents a new strategy for applying ozone gas to different packaged grains. Physical properties of the grains influence the reaction kinetics of ozone gas during the low-pressure injection process. Ozone decomposition was faster in polished rice and slower in common beans. The half-life of ozone in the different grains ranged from 17.8 to 52.9 and 16.4 to 52.9 min, outside and inside the package. Ozone gas injection at low pressures does not alter the quality attributes of the grains, and it was efficient in controlling the insect pests *S. zeamais* in popcorn kernels and corn grains, *C. maculatus* in cowpea beans, and *Z. subfasciatus* in common beans.

REFERENCES

Abreu, A. O., Faroni, L. R. D. A., de Assis Silva, M. V., de Sousa, A. H., de Alencar, E. R., & Silva, G. N. (2022). Ozone as an alternative fumigant for controlling *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in cowpea beans. *Journal of Stored Products Research, 97*, 101969.

Afsah-Hejri, L., Hajeb, P., & Ehsani, R. J. (2020). Application of ozone for degradation of mycotoxins in food: A review. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 1777-1808.

An, H. J., & King, J. M. (2009). Using ozonation and amino acids to change pasting properties of rice starch. *Journal of Food Science*, *74*(3), C278-C283.

ASABE- American Society of Agricultural and Biological Engineers. (2013). Moisture measurement-unground grain and seeds. *ASABE Standards*. St. Joseph, Michigan. ASAE S352.2 APR1988 (R2017).

Assis Silva, M. V., Martins, M. A., Faroni, L. R. D. A., Vanegas, J. D. B., & de Sousa, A. H. (2019). CFD modelling of diffusive-reactive transport of ozone gas in rice grains. *Biosystems Engineering*, *179*, 49-58.

Ávila, M. B., Faroni, L. R. A., Heleno, F. F., de Queiroz, M. E. L., & Costa, L. P. (2017). Ozone as degradation agent of pesticide residues in stored rice grains. *Journal of Food Science And Technology*, *54*(12), 4092-4099.

Baldin, E. L., & Lara, F. M. (2008). Resistance of stored bean varieties to *Acanthoscelides obtectus* (Coleoptera: Bruchidae). *Insect Science*, *15*(4), 317-326.

Bechlin, T. R., Granella, S. J., Christ, D., Coelho, S. R. M., & Viecelli, C. A. (2019). Evaluation of grain and oil quality of packaged and ozonized flaxseed. *Journal of Stored Products Research*, *83*, 311-316.

Bhat, F. M., & Riar, C. S. (2019). Effect of chemical composition, granule structure and crystalline form of pigmented rice starches on their functional characteristics. *Food Chemistry*, *297*, 124984.

Bhattarai, S., Bottenus, D., Ivory, C. F., Gao, A. H., Bule, M., Garcia-Perez, M., & Chen, S. (2015). Simulation of the ozone pretreatment of wheat straw. *Bioresource technology*, *196*, 78-87.

Boopathy, B., Rajan, A., & Radhakrishnan, M. (2022). Ozone: an alternative fumigant in controlling the stored product insects and pests: a status report. *Ozone: Science & Engineering*, *44*(1), 79-95.

BRASIL. (2009). Ministério da Agricultura, Pecuária e Abastecimento, *in Regras para análise de sementes*. Mapa/ACS, Brasília, p. 395.

Brewbaker, J. L., Larish, L. B., & Zan, G. H. (1996). Pericarp thickness of the indigenous American races of maize. *Maydica*, *41*(2), 105-111.

Brito, J. G. D., Faroni, L. R. D. A., Cecon, P. R., Benevenuto, W. C. A. D. N., Benevenuto, A. A., & Heleno, F. F. (2018). Efficacy of ozone in the microbiological disinfection of maize grains. *Brazilian Journal of Food Technology*, *21*, e2017022.

Chadda, I. C. (2016). Fumigation with phosphine-a perspective. *Indian Journal of Entomology*, 78(special), 39-44.

Chan, H. T., Leh, C. P., Bhat, R., Senan, C., Williams, P. A., & Karim, A. A. (2011). Molecular structure, rheological and thermal characteristics of ozone-oxidized starch. *Food Chemistry*, *126*(3), 1019-1024.

Chee, F. P., Zainuddin, N. S., Ha, H. L., & Dayou, J. (2020). Analysis and characterization of enhanced kinetic reaction on ozone generation using negative corona discharge. *Journal of Physics Communications, 4*(7), 075022.

Chen, C. (2003). Evaluation of air oven moisture content determination methods for rough rice. *Biosystems engineering*, *86*(4), 447-457.

Cie. (2004). CIE technical report. Colorimetry (3rd ed.). 552. Colorimetry (pp. 24–). ISBN 3-901-906-33-9.

Daglish, G. J., Nayak, M. K., Arthur, F. H., & Athanassiou, C. G. (2018). Insect pest management in stored grain. *Recent advances in stored product protection*, 45-63.

Dubey, S., Joshi, A., Trivedi, R., Pal, D., & Prajapati, A. K. (2021). Electro-peroxone treatment of rice grain-based distillery biodigester effluent: COD and color removal. *Water Resources and Industry*, *25*, 100142.

Ergun, S. (1952). Fluid flow through packed columns. *Chem Eng Prog,* 48:89–94.

Ferreira, W. F. D. S., de Alencar, E. R., Blum, L. E. B., Ferreira, M. D. A., Mendonca, M. A., Racanicci, A. M. C., & Urruchi, W. M. I. (2021). Ozonation of Brazil nuts in aqueous media at different ph levels: ozone decomposition, *Aspergillus flavus* inactivation, and effects on nut color and crude oil lipid profile. *Ozone: Science & Engineering, 43*(4), 351-362.

Freitas RS, Faroni LRA and Sousa AH. (2016). Hermetic storage for control of common bean weevil, *Acanthoscelides obtectus* (Say). *Journal of Stored Products Research, 66*, 1– 5.

Freitas, R. D. S., Faroni, L. R. D. A., de Queiroz, M. E. L. R., Heleno, F. F., & Prates, L. H. F. (2017). Degradation kinetics of pirimiphos-methyl residues in maize grains exposed to ozone gas. *Journal of Stored Products Research*, *74*, 1-5.

Gad, H. A., Laban, G. F. A., Metwaly, K. H., Al-Anany, F. S., & Abdelgaleil, S. A. (2021). Efficacy of ozone for *Callosobruchus maculatus* and *Callosobruchus chinensis* control in cowpea seeds and its impact on seed quality. *Journal of Stored Products Research*, *92*, 101786.

García-Lara, S., Chuck-Hernandez, C., & Serna-Saldivar, S. O. (2019). Development and structure of the corn kernel, in Corn: Chemistry and Technology, ed. by Serna-Saldivar SO. *Elsevier Science Publishers*, pp. 147–163.

García-Pinilla, S., Gutiérrez-López, G. F., Hernández-Sánchez, H., Cáez-Ramírez, G., García-Armenta, E., & Alamilla-Beltrán, L. (2021). Quality parameters and morphometric characterization of hot-air popcorn as related to moisture content. *Drying Technology*, 39(1), 77-89.

Gökmen, S. (2004). Effects of moisture content and popping method on popping characteristics of popcorn. *Journal of Food Engineering*, *65*(3), 357-362.

Hoseney, R. C., Zeleznak, K., & Abdelrahman, A. (1983). Mechanism of popcorn popping. *Journal of cereal Science*, *1*(1), 43-52.

Hou, L., Johnson, J. A., & Wang, S. (2016). Radio frequency heating for postharvest control of pests in agricultural products: A review. *Postharvest Biology and Technology*, *113*, 106-118.

Huang, Z., Zhu, H., Yan, R., & Wang, S. (2015). Simulation and prediction of radio frequency heating in dry soybeans. *Biosystems Engineering, 129*, 34-47.

Incropera, F. P., DeWitt, D. P., Bergman, T. L., & Lavine, A. S. (1996). *Fundamentals of Heat and Mass Transfer*, Vol. 6. Wiley, New York, p. 25.

Isikber, A. A., Oztekin, S., Ulusoy, R., Ozsoy, S., & Karci, A. (2007). Effectiveness of gaseous ozone alone and in combination with low pressure or carbon dioxide against *Ephestia kuehniella* (Zell.) (Lepidoptera: Pyralidae) at short exposure time. *IOBC WPRS BULLETIN*, *30*(2), 205.

Islam, M. S., Kao, N., Bhattacharya, S. N., Gupta, R., & Choi, H. J. (2018). Potential aspect of rice husk biomass in Australia for nanocrystalline cellulose production. *Chinese Journal of Chemical Engineering, 26*, 465–476.

Iturralde-García, R. D., Castañé, C., Wong-Corral, F. J., & Riudavets, J. (2020a). Biological control of *Acanthoscelides obtectus* and *Zabrotes subfasciatus* in stored dried beans. *BioControl*, *65*(6), 693-701.

Iturralde-García, R. D., Riudavets, J., & Castañé, C. (2020). Biological control of *Callosobruchus chinensis* (Coleoptera: Chrysomelidae) in stored chickpeas through the release of natural enemies. *Biological Control*, 149, 104322.

Jain, R. K., & Bal, S. (1997). Properties of pearl millet. *Journal of Agricultural Engineering Research*, *66*:85–91.

Jian, F., Jayas, D. S., & White, N. D. (2013). Can ozone be a new control strategy for pests of stored grain?. *Agricultural Research*, *2*(1), 1-8.

Karuppuchamy, P., & Venugopal, S. (2016). Integrated pest management, in *Ecofriendly Pest Management for Food Security*, ed. by Omkar. Academic Press, London, pp. 651–684.

Kim, H. S., Yang, H. S., Kim, H. J., & Park, H. J. (2004). Analysis of rice husk flour filled thermoplastic polymer composites. *Journal of Thermal Analysis and Calorimetry*, *76*(2), 395-404.

Kumar, S., Mohapatra, D., Kotwaliwale, N., & Singh, K. K. (2017). Vacuum hermetic fumigation: A review. *Journal of stored products research*, 71, 47-56.

Lokeswari, R., & Mahendran, R. (2022). Effect of plasma bubbling on textural and engineering properties of ready-to-eat pearl millet flakes and puffs. *IEEE Transactions on Plasma Science*, *50*(6), 1423-1429.

Massarani, G. (1997). Fluidodinâmica em sistemas particulados. Editora e-paper.

Mbata, G. N., Ivey, C., & Shapiro-Ilan, D. (2018). The potential for using entomopathogenic nematodes and fungi in the management of the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). *Biological control, 125*, 39-43.

Mei, J., Liu, G., Huang, X., & Ding, W. (2016). Effects of ozone treatment on medium hard wheat (*Triticum aestivum* L.) flour quality and performance in steamed bread making. *CyTA-Journal of Food*, *14*(3), 449-456.

Mendez, F., Maier, D. E., Mason, L. J., & Woloshuk, C. P. (2003). Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. *Journal of Stored Products Research*, *39*(1), 33-44.

Mishra, G., Palle, A. A., Srivastava, S., & Mishra, H. N. (2019). Disinfestation of stored wheat grain infested with *Rhyzopertha dominica* by ozone treatment: process optimization and impact on grain properties. *Journal of the Science of Food and Agriculture*, *99*(11), 5008-5018.

Mohsenin, N. N. (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers, New York.

Mohsenin, N. N. (1974). Physical Properties of Plant and Animal Materials. Gordan and Breach, New York.

Mohsenin, N. N. (1986). Physical Properties of Plant and Animal Materials. Gordon and Breach science publishers Inc., New York, p. 734.

Mohsenin, N.N. (1980). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers Inc, New York, pp. 51–87.

Nesci, A., Barra, P., & Etcheverry, M. (2011). Integrated management of insect vectors of *Aspergillus flavus* in stored maize, using synthetic antioxidants and natural phytochemicals. *Journal of stored products research, 47*(3), 231-237.

Oliveira, J. M., de Alencar, E. R., Blum, L. E. B., de Souza Ferreira, W. F., Botelho, S. D. C. C., Racanicci, A. M. C., & da Silva, C. R. (2020). Ozonation of Brazil nuts: Decomposition kinetics, control of *Aspergillus flavus* and the effect on color and on raw oil quality. *LWT*, *123*, 109106.

Pandiselvam, R., & Thirupathi, V. (2015). Reaction kinetics of Ozone gas in Green gram (*Vigna radiate*). *Ozone: Science & Engineering*, *37*(4), 309-315.

Pandiselvam, R., Subhashini, S., Banuu Priya, E. P., Kothakota, A., Ramesh, S. V., & Shahir, S. (2019). Ozone based food preservation: A promising green technology for enhanced food safety. *Ozone: Science & Engineering, 41*(1), 17-34.

Pandiselvam, R., Sunoj, S., Manikantan, M. R., Kothakota, A., & Hebbar, K. B. (2017). Application and kinetics of ozone in food preservation. *Ozone: Science & Engineering, 39*(2), 115-126.

Pandiselvam, R., Thirupathi, V., & Anandakumar, S. (2015). Reaction kinetics of ozone gas in paddy grains. *Journal of Food Process Engineering, 38*(6), 594-600.

Paponja, I., Rozman, V., & Liška, A. (2020). Natural formulation based on diatomaceous earth and botanicals against stored product insects. *Insects*, *11*(9), 613.

Patindol, J. A., Siebenmorgen, T. J., & Wang, Y. J. (2015). Impact of environmental factors on rice starch structure: a review. *Starch-Stärke*, *67*(1-2), 42-54.

Paul, A., Radhakrishnan, M., Anandakumar, S., Shanmugasundaram, S., & Anandharamakrishnan, C. (2020). Disinfestation techniques for major cereals: A status report. *Comprehensive Reviews in Food Science and Food Safety*, *19*(3), 1125-1155.

Pedersen, M., & Meyer, A. S. (2009). Influence of substrate particle size and wet oxidation on physical surface structures and enzymatic hydrolysis of wheat straw. *Biotechnology progress*, *25*(2), 399-408.

Rakness, K., Gordon, G., Langlais, B., Masschelein, W., Matsumoto, N., Richard, Y., & Somiya, I. (1996). Guideline for measurement of ozone concentration in the process gas from an ozone generator. *Ozone: Science & Engineering, 18*:209–229.

Ravi, P., Venkatachalam, T., & Rajamani, M. (2015). Decay rate kinetics of ozone gas in rice grains. *Ozone: Science & Engineering, 37*(5), 450-455.

Saini, K., & Kaushik, R. D. (2021). Phosphine: risk assessment, environmental, and health hazard, in *Hazardous Gases*, ed. by Singh J, Kaushik RD and Chawla M. Academic Press, London, pp. 327–340.

Savi, G. D., Gomes, T., Canever, S. B., Feltrin, A. C., Piacentini, K. C., Scussel, R., ... & Angioletto, E. (2020). Application of ozone on rice storage: A mathematical modeling of the ozone spread, effects in the decontamination of filamentous fungi and quality attributes. *Journal of Stored Products Research*, *87*, 101605.

Silva, M. V. A., Faroni, L. R. A., Martins, M. A., Sousa, A. H., & Bustos-Vanegas, J. D. (2020). CFD simulation of ozone gas flow for controlling *Sitophilus zeamais* in rice grains. *Journal of Stored Products Research, 88*, 101675.

Silva, M. V. A., Faroni, L. R. A., Sousa, A. H., Prates, L. H. F., & Abreu, A. O. (2019). Kinetics of the ozone gas reaction in popcorn kernels. *Journal of Stored Products Research*, *83*, 168-175.

Silva, M. V. D. A., Faroni, L. R. D. A., de Alencar, E. R., de Sousa, A. H., Cecon, P. R., Nogueira, J. V. F., & Mason Filho, V. (2022). Ozone injection at low pressure: decomposition kinetics, control of *Sitophilus zeamais*, and popcorn kernel quality. *Ozone: Science & Engineering*, *44*(1), 66-78.

Singh, B., & Kaur, A. (2018). Control of insect pests in crop plants and stored food grains using plant saponins: A review. *Lwt*, 87, 93-101.

Singh, N. S., Sharma, R., Parween, T., & Patanjali, P. K. (2018). Pesticide contamination and human health risk factor. *Modern age environmental problems and their remediation*, Springer, Cham, pp. 49-68.

Sivaranjani, S., Prasath, V. A., Pandiselvam, R., Kothakota, A., & Khaneghah, A. M. (2021). Recent advances in applications of ozone in the cereal industry. *LWT*, 146, 111412.

Sousa, A. H., LRD'A, F., Pimentel, M. A. G., Silva, G. N., & Guedes, R. N. C. (2016). Ozone toxicity to *Sitophilus zeamais* (Coleoptera: Curculionidae) populations under selection pressure from ozone. *Journal of Stored Products Research*, 65, 1-5.

Souza, J. V. D. S., Alencar, E. R. D., Junqueira, A. M., & Oliveira, G. P. D. (2018). Ozone saturation and decomposition kinetics in porous medium containing different hybrids of maize. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *22*, 286-291.

Srivastava S, Mishra G., & Mishra HN. (2021). Vulnerability of different life stages of Sitophilus oryzae insects in stored rice grain to ozone treatment and its effect on physico-chemical properties in rice grain. *Food Front 2*, 494– 507.

Sunisha, K. (2019). Ozone fumigation in stored paddy: Changes in moisture content upon storage. *Journal of Entomology and Zoology Studies*, *7*(3), 1137-1140.

Tay, W. T., Beckett, S. J., & De Barro, P. J. (2016). Phosphine resistance in Australian *Cryptolestes species* (Coleoptera: Laemophloeidae): perspectives from mitochondrial DNA cytochrome oxidase I analysis. *Pest Management Science*, *72*(6), 1250-1259.

Vanier, N. L., El Halal, S. L. M., Dias, A. R. G., & da Rosa Zavareze, E. (2017). Molecular structure, functionality and applications of oxidized starches: A review. *Food Chemistry*, *221*, 1546-1559.

Veljković, V. B., Biberdžić, M. O., Banković-Ilić, I. B., Djalović, I. G., Tasić, M. B., Nježić, Z. B., & Stamenković, O. S. (2018). Biodiesel production from corn oil: A review. *Renewable and Sustainable Energy Reviews*, *91*, 531-548.

Wang, L., Shao, H., Luo, X., Wang, R., Li, Y., Li, Y., & Chen, Z. (2016). Effect of ozone treatment on deoxynivalenol and wheat quality. *PloS one*, *11*(1), e 0147613.

Wang, N., & Daun, J. K. (2005). Determination of cooking times of pulses using an automated Mattson cooker apparatus. *Journal of the Science of Food and Agriculture*, *85*(10), 1631-1635.

Wood, J. A. (2017). Evaluation of cooking time in pulses: a review. *Cereal Chem, 94*, 32–48.

Wright, M. R. (2004). An Introduction to Chemical Kinetics, 1ª Edição edn. Editora *John Wiley & Sons*, Ltd., New York, p. 441.

Zhu, F. (2018). Effect of ozone treatment on the quality of grain products. *Food Chemistry*, *264*, 358-366.

CHAPTER 2

Application of ozone at low-pressure: Control of egg and larval phases of *Zabrotes subfasciatus* (Boh.), inactivation of *Aspergillus flavus* and qualitative changes in grains.

Ozone applied in a low-pressure system has been studied in packed grains. In the present study, we evaluated the effect of ozone applied at low-pressures for the control of egg and larval phases of *Zabrotes subfasciatus* (Boh.) and inactivation of *Aspergillus flavus* in beans and possible changes in product quality. Samples of 5.0 kg of beans packed in woven polypropylene bags were exposed to ozone in the hypobaric chamber of internal volume equal to 70 L. The application of ozone was preceded by the reduction of internal chamber pressure to 500 hPa. The ozone was then injected until the chamber's internal pressure reached 1000 hPa. An inlet ozone concentration of 61.37 mg L^{-1} was applied in 1, 4, 7, and 10 injections and a flow rate of 1.0 L min^{-1}. In the control treatment, oxygen was used. The ozonation of beans with 10 injections was efficient in controlling the egg and larval phases of *Z. subfasciatus*, reducing mass loss and inactivation of *A. flavus* in the grains. The increase in the number of injections increased the electrical conductivity of the beans. However, ozone did not affect the thousand-grain mass, percentage of whole grains after cooking, total soluble solids in the cooking broth, dimensions of the orthogonal axes of the grains, volume and length-width ratio of the cooked beans. The results suggest that the number of injections in the system at low-pressure is a determining factor for the concentration of ozone inside grain packages. In this system, the application of ozone using 10 injections caused 100% mortality of *Z. subfasciatus* eggs and larvae and was efficient in inactivating 70% of the *A. flavus* fungus in beans, without affecting the quality of the grains.

Keywords: Ozonation. Hypobaric chamber. Insect control. Fungi control. Quality.

1. INTRODUCTION

Beans (*Phaseolus vulgaris* L.) is a basic food that contributes to the nutrition, economic and social well-being of humans (Priya et al., 2020). The grain is a good source of proteins, minerals, carbohydrates (Priya et al., 2020; Minuye & Bajo, 2021) and tryptophan (Viscarra-Torrico et al., 2021), which helps in the formation of serotonin, a substance that relieves anxiety symptoms (Jenkins et al., 2016; Szeitz & Bandiera, 2018).

The availability of beans for human consumption is affected by several factors, especially pest insects (Tigist et al., 2018; Tigist et al., 2020) and storage fungi (Rodríguez-González et al., 2020). Insect pests reduce grain dry matter, nutritional quality and are vectors of fungi and other microorganisms (Nesci et al., 2011; Hou et al., 2016). Fungi are responsible for accelerating grain deterioration, reducing mass loss, mycotoxin production and organoleptic changes (Tsehaye et al., 2017; Ropelewska, 2019). It should be noted that, on average, 20-30% of agricultural commodities are held due to the attack of insect pests and fungi during handling and storage (FDA, 2017).

Zabrotes subfasciatus Boheman, 1833 (Coleoptera: Bruchidae) is one of the most important insect pests in bean storage (Silva et al., 2021; Tigist et al., 2021). Its life cycle is divided into four phases: egg, larva, pupa, and adult. The eggs are strongly adhered to the surface of the grains, by a sticky liquid secreted by the female under the laying. After hatching, the larvae penetrate the grain and feed until they reach adulthood (Howe & Currie, 1964; Tigist et al., 2018). When they reach this stage, adults do not feed, only reproduce (Souza et al., 2008; Teixeira & Zucoloto, 2012). In this context, the development of an effective and efficient method for the control of eggs and larvae of *Z. subfasciatus* can inhibit their reproduction and their damage to beans during storage.

As regards fungi, the species *Aspergillus flavus* stands out, capable of deteriorating the grains and producing aflatoxins, which are secondary metabolites of high toxicity (Silva et al., 2018; Qin et al., 2022). These substances are teratogenic, immunotoxic, mutagenic, and carcinogenic, being associated with an increased risk of development of hepatocellular carcinoma in humans (Abdel-Kareem et al., 2019). The toxic effects of these aflatoxins in humans depend on the degree of contamination of the food, time of exposure, amount of food ingested and its nutritional status (Mallmann et al., 2001; Gazzotti et al., 2015).

Regulatory agencies around the world have established phytosanitary and quarantine protocols to reduce and prevent the movement of exotic insect pests through agricultural commodity trading channels (Hou et al., 2016). The food industry relies heavily on contact insecticides, such as bifenthrin, for the protection of beans during storage. However, conventional insecticides leave residues in food (Singh & Kaur, 2018; Devi et al., 2021). In order to comply with phytosanitary regulations for international trade in agricultural commodities, there is a need to develop alternative methods for pest control of stored products (Huang et al., 2015). Therefore, new methods of pest control are increasingly studied and currently, the search for environmentally acceptable alternatives has deserved greater prominence in the protection of grains. In this scenario, ozonation has been studied as an alternative technology to traditional methods of food conservation. The application of ozone gas in the control of insect pests and the inactivation of microorganisms, including fungi, have been one of the most studied alternatives (Dasan et al., 2016; Sivaranjani et al., 2021; Ingegno & Tavella, 2022).

Several reports dealing with ozonation for the control of insects and microorganisms in products of plant origin are found in the literature. However, in general, research is restricted to the application of ozone in flow. Results on the effect of ozone applied in flow have already been reported for the control of eggs and larvae of *Rhyzopertha dominica* Fabricius, 1792 (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae) in wheat (Mishra et al., 2019; Amoah & Mahroof, 2019), of fungi in peanut grains (Alencar et al., 2012), corn (Ribeiro et al., 2022) and Brazil nut (Ferreira et al., 2021). Regarding the application of ozone in a low-pressure system, there are only two reports in which the authors used only one gas injection (Silva et al., 2022; Sitoe et al., 2023). Thus, it is necessary that new studies are performed, with the adoption of multiple injections, in such a way to increase the efficiency of ozone in pest control and microorganisms. The objective of this work was to determine the effect of ozone gas applied at low-pressures: (i) in the control of egg and larval phases of *Z. subfasciatus*: (ii) in the inactivation of *A. flavus* and: (iii) in the quality of bean grains.

2. MATERIAL AND METHODS

2.1. Raw-material

The experiment was carried out with red common beans (*Phaseolus vulgaris*), with initial water content of 11.7 g 100 g^{-1} (w.b.). The beans were acquired in a local property (Viçosa, Minas Gerais, Brazil), homogenized and stored in climatic chambers (Marconi Ltda, Piracicaba, São Paulo, Brazil) at 5 ºC, until the beginning of the experiments.

2.2. Experimental procedure

2.2.1. Treatment of beans

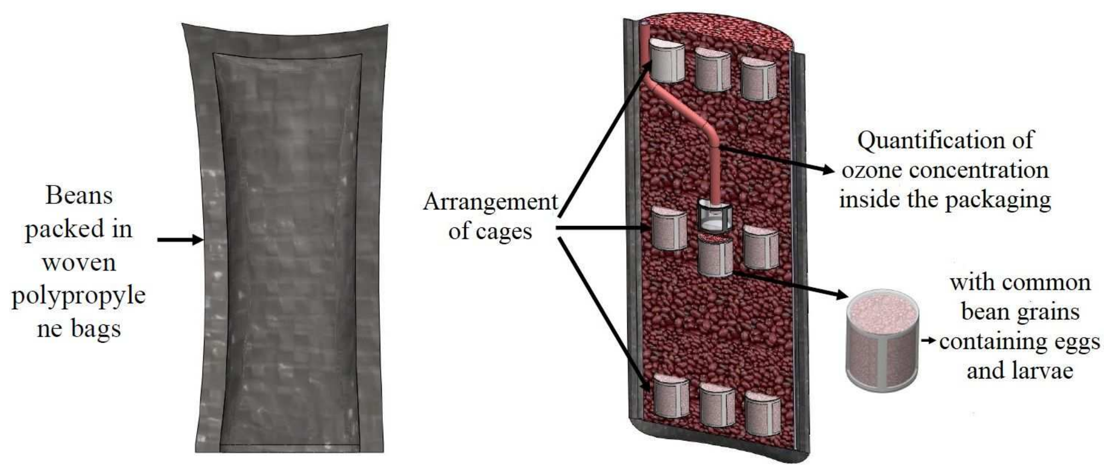
Ozone was generated using the Corona discharge technique Garamoon et al. (2009). The treatment of beans was performed in a hypobaric chamber of internal volume equal to 70 L, model CV10 (myOZONE, Jaguariúna, São Paulo, Brazil). Injections of ozone gas into the hypobaric chamber were performed according to the methodology adopted by Silva et al. (2022) and Sitoe et al. (2023). Subsequently, the internal pressure of the chamber was reduced to 500 hPa. Then, the ozone gas was injected until the internal pressure of the chamber reached the atmospheric pressure value (1000 hPa). The inlet ozone concentration of 61.37 mg L^{-1} was applied in the flow rate of 1 L min^{-1}, up to 10 injections. The injections were made at regular time intervals of 2 h and 15 min. This time corresponds to the sum of the time required to reach atmospheric pressure and the equivalent of two times the half-life of ozone for beans in low-pressure system (53 min), according to Sitoe et al. (2023). For each injection, the residual ozone concentrations were monitored outside and inside the package by the iodometric method for 150 min (Rakness et al., 1996). The inlet ozone concentration and the number of injections were determined through pre-established preliminary tests. Oxygen was used as a control treatment. The experiment was performed in triplicate for each condition.

2.2.2. Effect of ozone on egg and larval phases of *Z. subfasciatus* on beans

Initially, adult insects of *Z. subfasciatus* were reared in glass containers (0.8 L) and kept in a climatic chamber at 30±2 ºC, relative humidity of 75±5% (Iturralde- García et al., 2020). Non-infected beans were used as food substrate to obtain grains containing eggs and larvae of *Z. subfasciatus*. For this, 100 adult insects with known age were added in each petri dish (9.0 mm diameter) containing 20 g of beans. The insects remained in contact with the grains for five days. This period was defined in a preliminary test. Immediately after this period, the grains containing eggs of *Z. subfasciatus* were used in the experiment. To obtain the larval phase, the grains remained stored for ten days.

To evaluate the effect of ozone on egg and larval phases of *Z. subfasciatus*, samples of beans containing these phases were placed in organza cages (100% polyester, 35 g m^{-2}, Fabtex, New Delhi, India) and inserted into the woven polypropylene packaging containing 5.0 kg of beans (Figure 1). The grains were treated with ozone or oxygen, adopting 1, 4, 7 and 10 injections, as described in item 2.2. After the treatment of the grains, the samples containing the egg phase or larvae of *Z. subfasciatus* and previously weighed were arranged in Petri dishes and remained for 45 days of storage in a climatic chamber at 28±2 ºC, relative humidity of 75±5%. After this period, the number of adults which emerged from the grains with eggs or larvae of *Z. subfasciatus* and exposed to ozone or oxygen was quantified. The final mass of the grain samples was also obtained. Thus, it was possible to obtain the mass loss in the beans (in percentage), according to Parkin (1956) knowing the initial mass (immediately after the grain treatment) and the final mass (after 45 days) of each treatment**.**

Figure 1 - Arrangement of the cages inside the package.



Source: Sitoe (2023)

2.2.3. Effect of ozone on *A. flavus* in beans

The beans were previously inoculated with *A. flavus* strain (CCUB1405). The sequence of the strain used was deposited in the GenBank database. Initially, the inoculation of *A. flavus* was performed in Petri dishes containing the culture medium *Aspergillus flavus* and *parasiticus* Agar (AFPA) (Pitt & Hocking, 2009) remaining at 32 ºC, for seven days. To obtain suspension with spores of *A. flavus*, 10 mL of sterilized distilled water were added in each petri dish to release the conidia. Then the conidia concentration was adjusted to 10^5 spores mL^{-1}. Then, the grains were immersed in the spore suspension for 10 min (Michel & Radcliffe, 1995). After inoculation, the grains remained at room temperature (25 ºC) for seven days.

In the treatment of grains inoculated with *A. flavus*, the same conditions described in item 2.3 were adopted for grains containing eggs and larvae of *Z. subfasciatus*. Similarly, samples of 20 g of grains inoculated with *A. flavus* were placed in cages coated with organza, and later inserted in the woven polypropylene packaging containing 5.0 kg of beans. After the treatment of the grains with ozone or oxygen (control), direct plating was performed without surface disinfection, using Petri dish containing culture medium *A. flavus* and *parasiticus* Agar (AFPA), at 30 ºC, for 42 h (Pitt & Hocking, 2009). The results were expressed in percentage.

2.2.4. Analysis of bean grain quality

In the evaluation of the quality of the beans treated with ozone or oxygen, the following variables were considered: electrical conductivity, thousand-grain mass, percentage of whole grains after cooking, total soluble solids in the cooking broth, volume and width of the cooked grains. Electrical conductivity was determined according to Vieira et al. (1999). Samples of 50 grains were previously weighed, placed in a 250 mL plastic container, immersed in 75 mL of distilled water and maintained at 25 ºC for 24 h. The electrical conductivity of the solution containing the grains was measured in a conductivity meter model MCA-150 (Tecnopon, Piracicaba, São Paulo, Brazil) and the results were expressed in $\mu s\ cm^{-1}\ g^{-1}$. The thousand-grain mass (TGM) was determined according to the methodology adopted by Oyedeji et al. (2021). Samples of 500 grains were counted manually and weighed on an analytical scale (Auy220, Shimadzu, São Paulo, Brazil). The result was multiplied by two (2).

The percentage of whole grains after cooking (GIAC) was obtained from a sample of 30 g of cooked beans. The cooking time of the beans of 27 min was determined using a Mattson cooker according to Wang & Daun (2005). After cooking, the grains were weighed and then separated into two categories: i) whole grains (WG) and ii) broken grains (BG). The percentage of whole grains was determined using Eq. 1 (Garcia-Vela & Stanley, 1989).

$$GIAC = \left[\frac{(WG-BG)}{WG}\right] x\ 100 \qquad \text{(Eq. 1)}$$

The total soluble solids content in the cooking broth was determined according to Sartori (1982). Samples of 30 grams of beans were soaked in 100 mL of distilled water for 13 h and then subjected to cooking. Then, the broths obtained were filtered in a qualitative paper (unifl, weight of 80 gm^2 and thickness of 0.16 mm), arranged in containers of known mass and kept at 60 ºC, up to constant weight. The total soluble solids content in the broth was determined using Eq. 2.

$$SSC\ (\%) = \frac{MA}{MB} x100 \qquad \text{(Eq.2)}$$

In which: SSC - total soluble solids content in the cooking broth; MA - mass of bean broth after drying; and MB - mass of grains before soaking in water.

To analyze the volume and length-width ratio of the beans, samples were used from 30 previously cooked grains. An analog caliper with an accuracy of 0.05 mm was used to measure the three dimensions of the orthogonal axes of the cooked grains: length (``A```), width (``B``) and thickness (``C``). The volume of the cooked grains was calculated according to Mohsenin (1970). The length-width ratio of cooked beans was determined according to Wani et al. (2017).

2.3. Statistical analysis

The residual ozone concentration data were submitted to regression analysis using the software *Sigma Plot* version 12.5. Data on the effect of ozone on the control of eggs and larvae of *Z. subfasciatus*, percentage of mass loss, and inactivation of *A. flavus* and grain quality variables were previously submitted to normality and homogeneity analysis by the Shapiro-testWilk and Levene. Then, the data were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% probability. In the multivariate analysis, the Principal Component Analysis (PCA) was performed using the *SPSS software*, version 29.0.0.0 (IBM Corporation, USA).

3. RESULTS

3.1. Residual ozone concentration for different numbers of injections

In Table 1 are presented the regression equations, with the respective coefficients of determination (r^2), for ozone concentration outside and inside the package. The coefficients of determination (r^2) ranged from 0.96 to 0.99. Under the conditions adopted in this study, the estimated initial and final gas concentrations increased as the number of injections increased. Outside the package, estimated initial concentrations of 17.0 mg L^{-1} and 27.9 mg L^{-1} were obtained for 1 injection and 10 injections, respectively. Similarly, when the initial ozone concentrations inside the package were estimated, values equal to 11.9 mg L^{-1} and 21.2 mg L^{-1} were obtained for 1 injection and 10 injections, respectively. It appears that the initial concentration of the gas inside the package for 10 injections was approximately 1.8 times that obtained when only 1 injection was used. Similar trends were obtained when estimating the final ozone concentrations outside and inside the packages. The estimated final gas concentrations outside the bean packaging were 1.6 mg L^{-1} and 4.1 mg L^{-1} for 1 injection and 10 injections, respectively. As for the final concentrations inside the packages, the estimated values were 1.3 mg L^{-1} and 3.1 mg L^{-1}, for 1 injection and 10 injections, respectively.

Table 1 - Regression equations adjusted to the residual ozone concentration data as a function of time, outside and inside the bean packaging, for different numbers of injections and inlet concentration of 61.37 mg L^{-1}.

Number of injections	Outside the package		Inside the package	
	Fitted model	r^2	Fitted model	r^2
1 injection	$\hat{C} = 17.0308e^{-0.0159t}$	0.99	$\hat{C} = 11.9083e^{-0.0148t}$	0.99
2 injections	$\hat{C} = 18.0137e^{-0.0129t}$	0.99	$\hat{C} = 14.2936e^{-0.0129t}$	0.98
3 injections	$\hat{C} = 19.3621e^{-0.0132t}$	0.99	$\hat{C} = 14.7168e^{-0.0122t}$	0.98
4 injections	$\hat{C} = 19.8609e^{-0.0127t}$	0.99	$\hat{C} = 15.4959e^{-0.0119t}$	0.98
5 injections	$\hat{C} = 20.9950e^{-0.0119t}$	0.98	$\hat{C} = 16.5601e^{-0.0120t}$	0.97
6 injections	$\hat{C} = 22.4707e^{-0.0120t}$	0.99	$\hat{C} = 17.3278e^{-0.0112t}$	0.99
7 injections	$\hat{C} = 23.9837e^{-0.0127t}$	0.99	$\hat{C} = 18.6118e^{-0.0113t}$	0.98
8 injections	$\hat{C} = 25.3828e^{-0.0152t}$	0.99	$\hat{C} = 18.1881e^{-0.0139t}$	0.99
9 injections	$\hat{C} = 25.3138e^{-0.0128t}$	0.99	$\hat{C} = 19.6258e^{-0.0129t}$	0.99
10 injections	$\hat{C} = 27.9333e^{-0.0105t}$	0.96	$\hat{C} = 21.1937e^{-0.0116t}$	0.97

$\hat{C}$- Estimated concentration of ozone gas (mg L^{-1}); t - time (min). The estimated parameters were significant at a level of 5% ($p < 0.05$), according to the t-test. Source: Sitoe (2023)

3.2. Effect of ozone on egg and larval phases of *Z. subfasciatus* on beans

Table 2 shows the average values for the number of emerged insects after 45 days, from eggs or larvae of *Z. subfasciatus*, in grains exposed to oxygen and ozone. When oxygen was used, both in the egg or larval phase, the number of insects immersed in the grains remained between 190 and 196 (Table 2). The ozone applied at low-pressure, adopting 1 injection, did not cause a significant reduction ($p > 0.05$) in the number of emerged insects, when compared with oxygen exposure. On the other hand, as the number of injections increased, from 4 injections, partial or total control was verified (Table 2). For the egg phase, total control was verified when 10 injections of ozone were adopted. On the other hand, when 7 or 10 ozone injections were adopted, total control of the larval phase was observed.

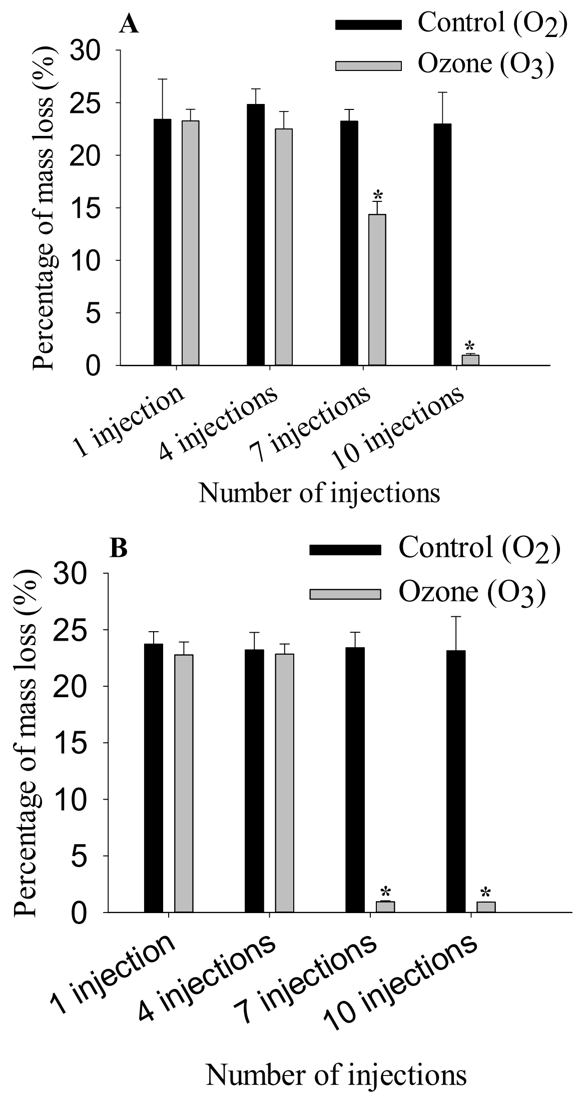
Table 2 - Number of insects emerged from the egg and larval phases of Z. *subfasciatus*, 45 days after exposure to oxygen (control) and ozone, for different numbers of injections.

Number of injections	Phase of *Z. subfasciatus*			
	Egg phase		Larval phase	
	Control (O_2)	Ozone (O_3)	Control (O_2)	Ozone (O_3)
1 injection	196.00 a	194.00 a	193.00 a	192.00 a
4 injections	195.00 b	177.00 b	193.00 a	165.00 b
7 injections	193.00 a	55.00 c	192.00 a	0.00 c
10 injections	194.00 a	0.00 d	190.00 a	0.00 d

* Averages followed by the same letter in the column, statically do not differ from each other at the level of 5% probability by the Tukey test.
Source: Sitoe (2023)

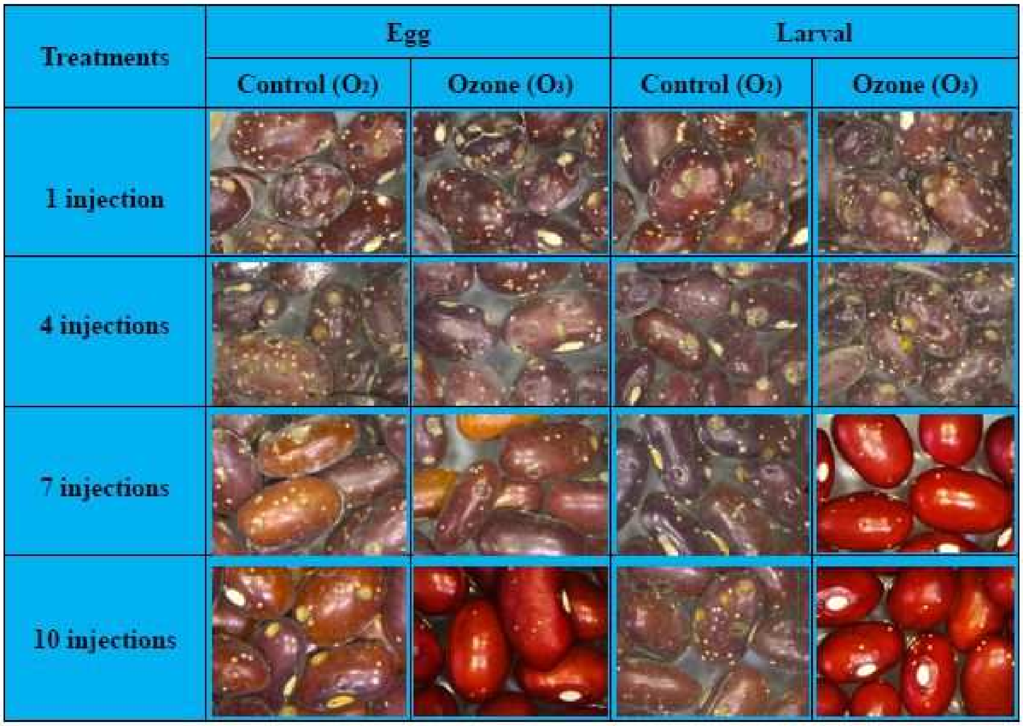
Figure 2 shows the effect of ozone on mass loss in grains exposed to oxygen and ozone, after 45 days of treatment. In the grains exposed to oxygen, the mass loss varied between 22.97±3.02 and 24.83±1.49% and 23.14±3.03 and 23.73±1.75%, for the egg and larval phases of *Z. subfasciatus*, respectively. On the other hand, the application of ozone caused a significant reduction in mass loss, and this trend was more pronounced as the number of injections increased. Regarding the egg phase, there was a significant difference ($p<0.05$) when comparing the results obtained between the treatment with ozone and oxygen when adopting 7 and 10 injections (Figure 2A). The percentages of mass loss in the ozonated grains were equal to 14.36±1.26% and 0.97±0.15%, for 7 and 10 injections, respectively. Regarding the larval phase, the lowest mass losses were also observed when 7 and 10 injections were used in the treatment with ozone, being equivalent to 0.94±0.09% and 0.92±0.01%, respectively (Figure 2B). In Figure 3, it is possible to observe the appearance of grains exposed to oxygen and ozone and containing the egg and larval phases of *Z. subfasciatus*, after 45 days.

Figure 2 - Percentage of mass loss after 45 days after bean grain treatment with oxygen (control) and ozone, for different numbers of injections, for the control of egg (A) and larval (B) phases of *Z. subfasciatus*. The asterisk indicates a significant difference between each treatment and its corresponding control according to the t-test at a significance level of 5%



Source: Sitoe (2023)

Figure 3 – Appearance of beans exposed to oxygen (control) and ozone and containing the egg and larval phases of *Z. subfasciatus*, after 45 days

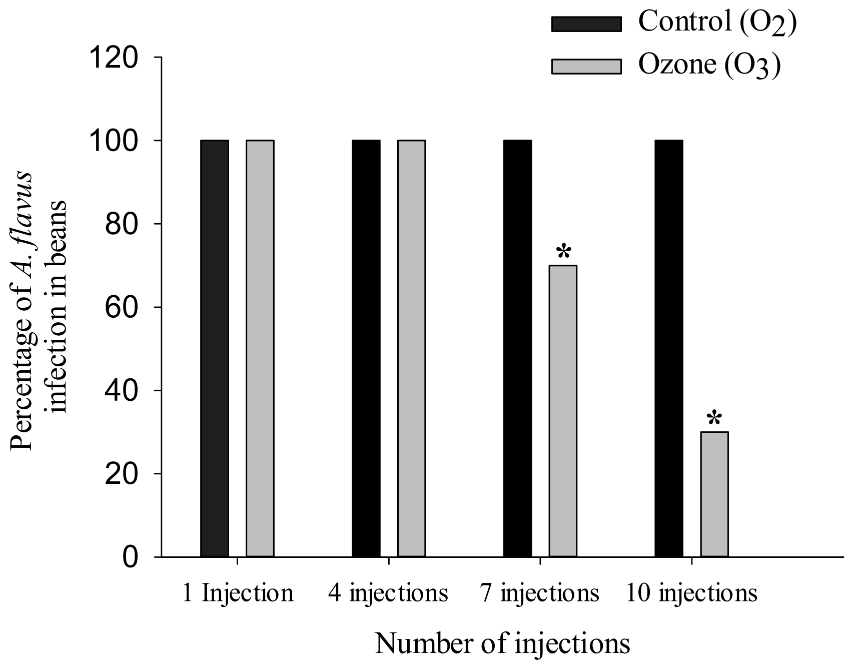


Source: Sitoe (2023)

3.4. Effect of ozone on *A. flavus* in beans

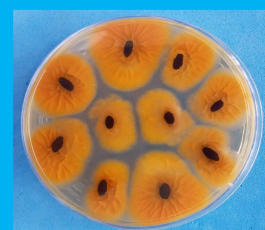
In Figures 4 and 5, there is the effect of ozone on the percentage of grain infection by *A. flavus*. It was found that ozone is capable of inactivating *A. flavus* when adopting 7 and 10 injections. In the grains exposed to oxygen, the percentage of infection was 100%. On the other hand, when adopted 7 or 10 injections of ozone, there was a significant difference ($p < 0.05$) in relation to the treatment with oxygen. When 7 and 10 injections of ozone were adopted, the percentages of infection were equivalent to 70 and 30%, respectively.

Figure 4. Percentage of *A. flavus* infection in beans exposed to oxygen (control) and ozone for different numbers of injections. The asterisk indicates a significant difference between each treatment and its corresponding control according to the t-test at a significance level of 5%



Source: Sitoe (2023)

Figure 5 - Visual representation of the infestation caused by *A. flavus* in beans (*Phaseolus vulgaris*) treated with oxygen (control) and ozone, under different numbers of gas injections.

Treatments	Percentage of *A. flavus* infection in beans	
	Control (O_2)	Ozone (O_3)
1 injection		
4 injections		
7 injections		
10 injections		

Source: Sitoe (2023)

3.5. Quality of beans

In Table 3, there are regression equations, with the respective determination coefficients (r^2), which describe the tendency of electrical conductivity as a function of the number of injections. As expected, oxygen did not alter the electrical conductivity in thec grains. On the other hand, it was found that as the number of ozone injections increases, there is an increase in electrical conductivity.

Table 3. Regression equations adjusted to the electrical conductivity data of beans (*Phaseolus vulgaris*) treated with oxygen (control) and ozone at an inlet concentration of 61.37 mg L^{-1}, depending on the number of injections

Treatment	Fitted model	r^2
Control (O_2)	$\hat{y}_i = -0.0576i + 64.7343$	0.42
Ozone (O_3)	$\hat{y}_i = 1.9332i + 61.7565$	0.96

i - number of injections.

Source: Sitoe (2023)

The thousand-grain mass, percentage of whole beans after cooking and total soluble solids in the bean cooking broth were not significantly altered ($p > 0.05$) by ozone treatment (Table 4). The average mass values of one thousand grains remained in the range between 364.6 and 380.3 g (Table 4). The percentage of whole beans after cooking ranged from 61.4 to 63.1% (Table 4). For total soluble solids in bean cooking broth, values between 8.0 and 8.8% were obtained (Table 4).

The variables of the dimensions of the orthogonal axes (length (A), width (B) and thickness (C), grain volume and length-width ratio in the cooked grains did not change due to ozonation, even after 10 injections (Table 5). The variables A, B and C remained around 15.5, 9.5 and 8.1 mm, respectively (Table 5). The volume of cooked grains ranged from 622.9 to 659.0 mm^3 (Table 5). As for the length-width ratio of cooked beans, values ranging from 1.5 to 1.7 mm were obtained (Table 5).

Table 4 - Average mass values of thousand grains, percentage of whole grains after cooking and total soluble solids in bean cooking broth, after treatment with oxygen (control) and ozone, for different numbers of injections

Number of injections	Treatments	Bean quality variables		
		Thousand-grain mass (g) ±SD	Percentage of whole grains after cooking (%) ±SD	Total soluble solids in bean cooking broth (%) ±SD
1 injection	Control (O_2)	368.26±0.12a	62.85±0.93a	8.43±0.83a
	Ozone (O_3)	374.98±2.28a	62.33±2.24a	8.75±0.68a
4 injections	Control (O_2)	373.27±1.49a	61.44±3.69a	8.22±0.45a
	Ozone (O_3)	380.29±1.26a	62.53±3.18a	8.33±2.18a
7 injections	Control (O_2)	374.28±1.83a	62.24±4.96a	8.52±2.11a
	Ozone (O_3)	364.69±2.45a	63.08±4.14a	8.03±1.10a
10 injections	Control (O_2)	371.13±1.40a	62.25±3.18a	8.57±2.06a
	Ozone (O_3)	370.44±2.47a	61.58±3.24a	8.53±1.15a

Averages followed by the same letter in the column, statically do not differ from each other at the level of 5% probability by the Tukey test.
±SD - Standard Deviation.
Source: Sitoe (2023)

Table 5. Average values of the dimensions of the orthogonal axes (length (``A``), width (``B``) and thickness (``C``)), dry bean volume and cooking and length-width ratio of beans baked beans.

Number of injections	Treatments	Orthogonal Axes (mm) of dried beans				Orthogonal Axes (mm) of cooked beans				Length-width ratio of cooked beans (mm) ±SD
		A ±SD	*B* ±SD	*C* ±SD	Volume of dry beans (m m^3) ±SD	*A* ±SD	*B* ±SD	*C* ±SD	Volume of cooked beans (m m^3) ±SD	
1 injection	Control (O_2)	10.85± 0.87a	7.35± 0.49a	6.68± 0.53a	280.25± 1.63a	15.49± 1.70a	9.87± 0.49a	8.22± 0.55a	658.96± 3.26a	1.57± 0.17a
	Ozone (O_3)	10.71± 0.60a	7.54± 0.50a	6.68± 0.43a	284.13± 1.53a	15.54± 0.90a	9.73± 0.55a	8.12± 0.76a	647.02± 2.87a	1.60± 0.14a
4 injections	Control (O_2)	10.78± 0.83a	7.46± 0.60a	6.53± 0.51a	277.86± 1.84a	15.35± 0.67a	9.62± 0.56a	8.32± 0.54a	642.34± 2.06a	1.60± 0.10a
	Ozone (O_3)	10.72 ± 0.79a	7.57± 0.52a	6.70± 0.32a	285.82± 1.43a	15.46± 1.29a	9.38± 0.79a	8.37± 0.84a	633.78± 4.09a	1.65± 0.15a
7 injections	Control (O_2)	11.08± 0.63a	7.43± 0.55a	6.54± 0.45a	282.64± 1.34a	15.42± 1.60a	9.60± 0.89a	7.95± 0.65a	622.90± 4.63a	1.61± 0.12a
	Ozone (O_3)	11.01± 0.91a	7.48± 0.60a	6.61± 0.64a	287.48± 2.03a	15.77± 1.33a	9.42± 0.66a	7.92± 0.67a	623.19± 3.96a	1.68± 0.16a
10	Control	11.17±	7.57±	6.67±	297.38±	15.55±	9.72±	8.18±	651.15±	1.60±

injections	(O_2)	1.14a	0.52a	0.45a	1.86a	1.72a	0.63a	0.45a	3.82a	0.16a
	Ozone (O_3)	10.86± 1.04a	7.47± 0.65a	6.69± 0.54a	286.55± 1.93a	15.66± 1.26a	9.68± 0.70a	8.12± 0.76a	643.36± 3.85a	1.62± 0.15a

Averages followed by the same letter in the column, statically do not differ from each other at the level of 5% probability by the Tukey test.

±SD - Standard Deviation.

Source: Sitoe (2023)

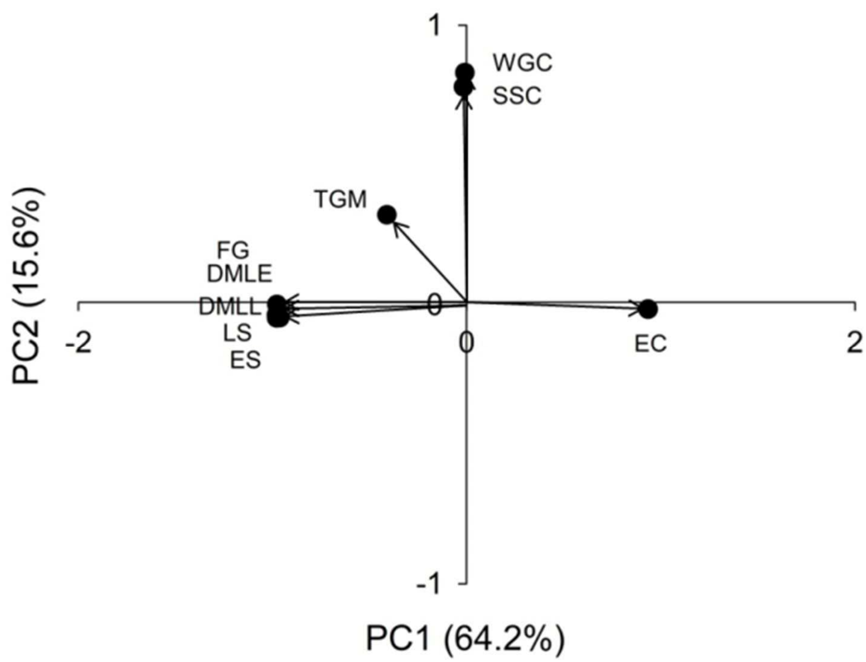
3.6. Multivariate Analysis

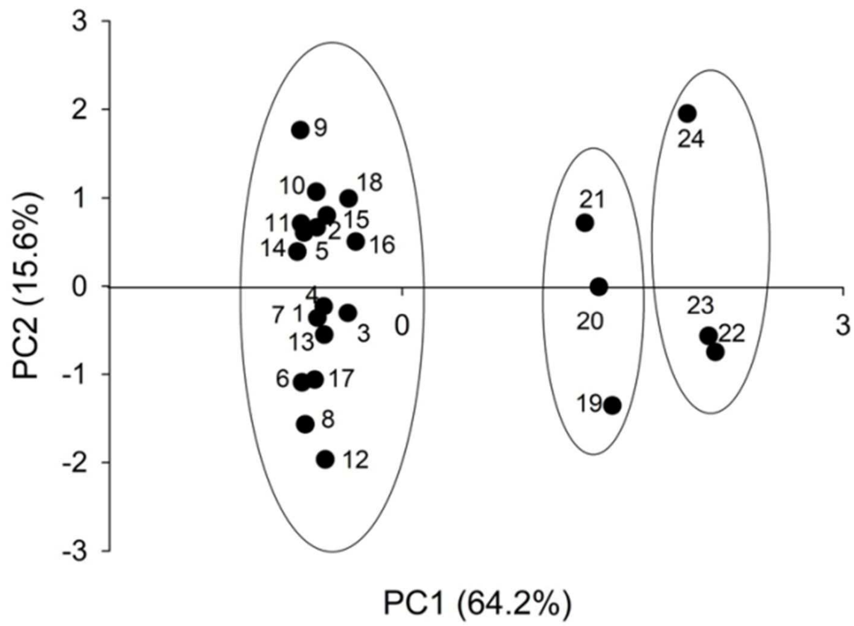
In Figure 6, the Principal Component Analysis is presented for beans submitted or not to ozonation in a low-pressure system. The variance accumulated by the first two axes of the main components PC1 and PC2 was equal to 79.8%. The ordering of the vectors of each of the variables considered is presented in Figure 6A. The variables weight of grains after cooking (WGC), total soluble solids after cooking (SSC) and thousand grain mass (TGM) are located in the second quadrant. On the other hand, percentage of grains infected by *A. flavus* (FG), number of emerged insects/egg phase of *Z. subfasciatus* (ES), number of emerged insects/larvae phase of *Z. subfasciatus* (LS), Mass loss/egg phase of *Z. subfasciatus* (DMLE) and mass loss/larval phase of *Z. subfasciatus* (DMLL) remained in the third quadrant and electrical conductivity (EC) in the fourth quadrant.

Table 6 shows the loadings or coefficients of the PC1 and PC2 equations. In the PC1 component, the variables percentage of grains infected by *A. flavus* (FG) (-0.980), number of emerged insects/egg phase of *Z. subfasciatus* (ES) (-0.979), number of emerged insects/larvae phase of *Z. subfasciatus* (LS) (-0.963), Mass loss/egg phase of *Z. subfasciatus* (DMLE) (-0.966) and mass loss/larval phase of *Z. subfasciatus* (DMLL) (-0.978) stood out (Table 6). On the other hand, electrical conductivity (EC) contributed positively to the PC1 component, with the equation coefficient equal to 0.934. Regarding the main component 2 (PC2), the variables that most contributed were weight of grains after cooking (WGC) and total soluble solids after cooking (SSC), with coefficients of the equation positive and equal to 0.830 and 0.779, respectively (Table 6).

In Figure 6B, it is possible to verify the formation of three well-defined groups, indicating that there was a difference between the treatments, due to the exposure of bean grains to ozone in low-pressure system. The first group was related to grains treated with oxygen (1, 4, 7 and 10 injections) and ozone (1 and 4 injections). The treatment of grains with ozone, when 7 injections were used, was part of the second group. On the other hand, the third group included the treatment of grains with ozone when 10 injections were adopted.

Figure 6 - Principal Component analysis of beans submitted or not to ozonation in low-pressure system, with different number of injections. A: PCA of the variables; B: PCA of the treatments. WGC - weight of grains after cooking; SSC – total soluble solids after cooking; TGM - thousand grain mass; EC - electrical conductivity; FG - percentage of grains infected by *A. flavus*; ES - number of emerged insects/egg phase of *Z. subfasciatus*; LS - number of emerged insects/larvae phase of *Z. subfasciatus*; DMLE - Mass loss/egg phase of *Z. subfasciatus*; DMLL - mass loss/larval phase of *Z. subfasciatus*. Oxygen/1 injection – (1, 2 e 3); Oxygen/4 injections – (4, 5 e 6); Oxygen/7 injections – (7, 8 e 9); Oxygen/10 injections – (10, 11 e 12); Ozone/1 injection – (13, 14 e 15); Ozone/4 injections – (16, 17 e 18); Ozone/7 injections – (19, 20 e 21); e Ozone/10 injections – (22, 23 e 24).





Source: Sitoe (2023)

Table 6 - Equations of components 1 (PC 1) and 2 (PC 2) of the Principal Component Analysis (PCA) for bean grains submitted or not to ozonation in a low-pressure system

Equações
PC1 = -0.010WGC- 0.017SSC - 0.411TGM + 0.934EC - 0.980FG - 0.979ES - 0.963LS - 0.966DMLE - 0.978DMLL
PC2 = 0.830WGC + 0.779SSC+ 0.322TGM -0.015EC -0.036FG -0.047ES -0.046LS - 0.044DMLE -0.001DMLL

WGC - weight of grains after cooking; SSC – total soluble solids after cooking; TGM - thousand grain mass; EC - electrical conductivity; FG - percentage of grains infected by *A. flavus*; ES - number of emerged insects/egg phase of *Z. subfasciatus*; LS - number of emerged insects/larvae phase of *Z. subfasciatus*; DMLE - Mass loss/egg phase of *Z. subfasciatus*; DMLL - mass loss/larval phase of *Z. subfasciatus*.

Source: Sitoe (2023)

4. DISCUSSION

In the present study, it was found that the concentration of ozone in the system at low-pressure, both outside and inside the package, is dependent on the number of injections. As the number of injections increases, gas concentration increases. This trend can be explained in a similar way to what occurs when ozone in flow is applied, as indicated by Kells et al. (2001) and verified for several agricultural products such as corn, popcorn, peanuts, Brazilnuts (Alencar et al., 2011; Sanchez et al., 2016; Souza et al., 2018; Oliveira et al., 2020). Initially, the ozone reacted with active sites located on the surface of the beans, thus occurring rapid decomposition of ozone. After the elimination of these active sites and as the number of injections increased, it was possible to verify a higher concentration of ozone, both outside and inside the packages.

Regarding the control of egg phases of larvae of *Z. subfasciatus* in packed beans, the ozone applied in low-pressure system was efficient, especially when 7 or 10 injections were adopted. It is important to mention that in these conditions, higher concentrations of ozone were found inside the packages. For the control of eggs or larvae that develop inside the grain, it is essential to apply high concentrations of ozone or to adopt longer times of exposure of grains to gas (Keivanloo et al., 2014; Goldberg et al., 2015; Mishra et al., 2019). The efficacy of ozone in the control of *Z. subfasciatus* was confirmed when the mass loss in grains was analyzed after 45 days of treatment (Figure 2 and 3). Lower mass loss was observed when the number of injections increased. For bean grains, it was shown appropriate to adopt 10 ozone injections to minimize mass loss by Z. *subfasciatus*, considering the egg and larval phases.

It is noteworthy, however, that when adopted 7 injections, total control of the egg phase was not verified. On the other hand, in this condition, total control of the larval phase was observed. This result can be explained by the difference in tolerance between the egg phase and the other immature phases of insect development. The egg phase is more tolerant to fumigants than the larval phase (Bell et al., 1977; Price & Mills, 1988; Ramya et al., 2023), due to its low respiratory activity (Zhang et al., 2014; Campbell et al., 2016). However, it is important to mention that the mechanism of action of ozone on insects has not yet been elucidated.

The ozone applied in low-pressure system was also efficient in controlling *A. flavus* in beans, especially when adopted 10 injections. Several authors have demonstrated that ozone is efficient in controlling microorganisms, including fungi (Alencar et al., 2012; Guzel-Seydim et al., 2016; Oliveira et al., 2020). However, in the research already carried out, ozone was applied in flow. Thus, the present study is the first report in which it was established a condition in which ozone is efficient in controlling fungi in grains, specifically *A. flavus*, when applied in packaged product and in low-pressure system.

The mechanism of action of ozone on microorganisms has already been elucidated. Due to its high oxidative potential (2,07 mV) (Guzel-Seydim et al., 2004) this gas can break the cell envelope and cause dispersion of cytoplasmic constituents, and consequently cell lysis (Cullen et al., 2009). This gas oxidizes vital cellular constituents such as unsaturated fatty acids from the cell wall and membrane, as well as other elements of cell content such as enzymes and nucleic acids (Cullen et al., 2009; Pandiselvam et al., 2017; Brodowska et al., 2018; Sivaranjani et al., 2021). It should be mentioned that the control of *A. flavus* in stored grains is extremely important, considering the potential of this species to produce aflatoxins (Pitt, 2000; Jackson & Dobson, 2016). Aflatoxins stand out for being carcinogenic, teratogenic, mutagenic and immunosuppressive (IARC, 1993; Trucksess & Diaz-Amigo, 2011).

Regarding the effect of ozone on bean grain quality, when applied in a system at low-pressure, there was a change when considering the variable electrical conductivity. As the number of injections increased, there was an increase in electrical conductivity. A similar trend was reported by other authors in cowpea grains (Abreu et al., 2022) and popcorn (Silva et al., 2019; Silva et al., 2022). The authors reported increased electrical conductivity in the grains as ozone concentration and exposure time increased. The increase in ozone concentration potentiates damage to grain cell membranes, resulting in increased release of ions, sugars and other metabolites (Silva et al., 2019; Abreu et al., 2022). In this context, it can be stated that the increase in the number of injections, associated with the increase in gas concentration inside the packages, justifies the trend observed in the variable electrical conductivity in the beans.

Although an increase in the electrical conductivity variable was observed, ozone applied in a low-pressure system, adopting up to 10 injections, did not cause changes in the mass of one thousand grains, in the percentages of whole grains and total soluble solids in the broth after cooking, grain size. The mass values of one thousand grains, which remained in the range between 364.6 and 380.3 g, are in agreement with the results obtained by other authors, who obtained values between 164-451 g/1.000 grains (Oomah et al., 2010; Silva et al., 2016).

The results regarding the percentage of whole grains after cooking, which ranged from 61.4 to 63.1%, are in agreement with those reported by Perina et al. (2014). It should be noted that the percentage of whole grains after cooking is an important quality variable in commercial terms since the consumer tends to opt for beans that do not lose their integrity after cooking (Perina et al., 2014). Regarding total soluble solids in the broth, the fact that ozone did not cause alteration, even when adopted 10 injections, is extremely relevant. The total soluble solids in bean broth is related to the percentage of whole grains after cooking and by the transfer of the constituents of the beans to the broth (Silva et al., 2016). The greater the number of grains broken during cooking, the easier it is to pass the starch from the grains to the cooking medium. Starch influences texture and suspended solids, and serves as a thickener (Smith, 1982). When in solution, in the process of cooking beans, starch gelatinizes, and passes its components (amylose and amylopectin) to the cooking medium. At rest, amylose molecules can associate and form precipitates, increasing the viscosity of the cooking broth (Wang et al., 2010). The dimensions of the orthogonal axes, volume and length-width ratio of cooked beans are important parameters and play a fundamental role in bean cooking. These variables are related to the water absorption capacity of grains and determine the quality of bean cooking (Reyes-Moreno et al., 1993; Dahiya et al., 2015; López-Morales et al., 2020).

It is important to note that multivariate analysis confirmed that the number of injections is fundamental in the process of grain ozonation in a low-pressure system, with the formation of three well-defined groups. There was a differentiation between grain treatments with 7 and 10 injections, remaining in different groups. Further research must be carried out to confirm the viability of the application of ozone in a system at low-pressure for bean grains and contribute to the adoption of this technology on an industrial scale. In future research, other variables can be considered in the evaluation of the effect of ozone on bean grains, such as sensory properties, amino acid profile and antinutritional constituents.

5. CONCLUSION

From the results obtained in the present study, it is possible to conclude that: i) the number of injections in the system at low-pressure is a determining factor for the concentration of ozone inside grain packages; ii) the ozonation of beans in a low-pressure system with 10 injections is efficient in controlling egg and larval phases of *Z. subfasciatus*, implying less mass loss, and inactivation of *A. flavus*; and iii) under conditions similar to those adopted in the present study, ozone does not change the grain quality, except when considering electrical conductivity.

REFERENCES

Abdel-Kareem, M. M., Rasmey, A. M., & Zohri, A. A. (2019). The action mechanism and biocontrol potentiality of novel isolates of Saccharomyces cerevisiae against the aflatoxigenic *Aspergillus flavus*. *Letters in applied microbiology, 68*(2), 104-111.

Abreu, A.O., Faroni, L. R. D. A., Assis-Silva, M.V., Sousa, A. H., Alencar, E.R., & Silva, G. N. (2022). Ozone as an alternative fumigant for controlling *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in cowpea beans. *Journal of Stored Products Research, 97*, 101969.

Alencar, E. R. D., Faroni, L. R. D. A., Martins, M. A., Costa, A. R. D., & Cecon, P. R. (2011). Decomposition kinetics of gaseous ozone in peanuts. *Engenharia agrícola, 31*, 930-939.

Alencar, E. R., Faroni, L. R. D. A., Soares, N. D. F. F., Silva, W. A., & Silva-Carvalho, M. C. (2012). Efficacy of ozone as a fungicidal and detoxifying agent of aflatoxins in peanuts. *Journal of the Science of Food and Agriculture, 92*(4), 899-905.

Amoah, B. A., & Mahroof, R. M. (2019). Ozone as a potential fumigant alternative for the management of *Sitophilus oryzae* (Coleoptera: Curculionidae) in wheat. *Journal of Economic Entomology, 112*(4), 1953-1963.

Bell, C. H., Hole, B. D., & Evans, P. H. (1977). The occurrence of resistance to phosphine in adult and egg stages of strains of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). *Journal of Stored Products Research,13*(2), 91-94.

Brodowska, A. J., Nowak, A., & Śmigielski, K. (2018). Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. *Critical reviews in food science and nutrition*, *58*(13), 2176-2201.

Campbell, B. E., Pereira, R. M., Koehler, P. G., & Trdan, S. (2016). Complications with controlling insect eggs. In: Trdan, S. (Ed.), *Insecticides Resistance*. IntechOpen, Rijeka, Croatia, 83–96.

Cullen, P. J., Tiwari, B. K., O'Donnell, C. P., & Muthukumarappan, K. (2009). Modelling approaches to ozone processing of liquid foods. *Trends in Food Science & Technology, 20*(3-4), 125-136.

Dahiya, P. K., Linnemann, A. R., Van Boekel, M. A. J. S., Khetarpaul, N., Grewal, R. B., & Nout, M. J. R. (2015). Mung bean: Technological and nutritional potential. *Critical reviews in food science and nutrition, 55*(5), 670-688.

Dasan, B. G., Boyaci, I. H., & Mutlu, M. (2016). Inactivation of aflatoxigenic fungi (Aspergillus spp.) on granular food model, maize, in an atmospheric pressure fluidized bed plasma system. *Food Control*, *70*, 1-8.

Devi, T. B., Raina, V., Sahoo, D., & Rajashekar, Y. (2021). Chemical composition and fumigant toxicity of the essential oil from *Tithoniadiversifolia* (Hemsl.) A. Grey against two major stored grain insect pests. *Journal of Plant Diseases and Protection, 128*(2), 607-615.

FDA. (2017). Food S. Drug Administration (FDA). FDA Food Code 2013.

Ferreira, W. F. D. S., Alencar, E. R., Blum, L. E. B., Ferreira, M. D. A., Mendonca, M. A., Racanicci, A. M. C., & Urruchi, W. M. I. (2021). Ozonation of Brazil nuts in aqueous media at different ph levels: Ozone decomposition, *Aspergillus flavus* inactivation, and effects on nut color and crude oil lipid profile. *Ozone: Science & Engineering*, *43*(4), 351-362.

Garamoon, A. A., Elakshar, F. F., & Elsawah, M. (2009). Optimizations of ozone generator at low resonance frequency. *The European Physical Journal-Applied Physics, 48*(2), 21002.

Garcia-Vela, L. A., & Stanley, D. W. (1989). Water-holding capacity in hard-to-cook beans (*Phaseolus vulgaris*): Effect of pH and ionic strength. *Journal of Food Science, 54*(4), 1080-1081.

Gazzotti, T., Biagi, G., Pagliuca, G., Pinna, C., Scardilli, M., Grandi, M., & Zaghini, G. (2015). Occurrence of mycotoxins in extruded commercial dog food. *Animal Feed Science and Technology, 202*, 81-89.

Goldberg, J., Bresseel, J., Constant, J., Kneubühler, B., Leubner, F., Michalik, P., & Bradler, S. (2015). Extreme convergence in egg-laying strategy across insect orders. *Scientific Reports, 5*(1), 1-7.

Guzel-Seydim, Z. B., Greene, A. K., & Seydim, A. C. (2004). Use of ozone in the food industry. *LWT-Food Science and Technology, 37*(4), 453-460.

Hou, L., Johnson, J. A., & Wang, S. (2016). Radio frequency heating for postharvest control of pests in agricultural products: A review. *Postharvest Biology and Technology, 113*, 106-118.

Howe, R. E., & Currie, J. E. (1964). Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. *Bulletin of Entomological Research, 55*(3) 437-477.

Huang, Z., Zhu, H., Yan, R., & Wang, S. (2015). Simulation and prediction of radio frequency heating in dry soybeans. *Biosystems Engineering, 129*, 34-47.

IARC. (1993). International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans: some naturally occurring substances, food items and constituents, heterocyclic aromatic amines and mycotoxins. *IARC: Lion*, *56*, 26-32.

Ingegno, B. L., & Tavella, L. (2022). Ozone gas treatment against three main pests of stored products by combination of different application parameters. *Journal of Stored Products Research, 95*, 101902.

Iturralde-García, R. D., Castañé, C., Wong-Corral, F. J., & Riudavets, J. (2020). Biological control of *Acanthoscelides obtectus* and *Zabrotes subfasciatus* in stored dried beans. *BioControl*, *65*(6), 693-701.

Jackson, S. A., & Dobson, A. D. W. (2016). Yeasts and Molds: *Aspergillus flavus*. *Reference Module in Food Science,* 785–791.

Jenkins, T. A., Nguyen, J. C., Polglaze, K. E., & Bertrand, P. P. (2016). Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients, 8*(1), 56.

Keivanloo, E., Namaghi, H. S., & Khodaparast, M. H. H. (2014). Effects of low-ozone concentrations and short exposure times on the mortality of immature stages of the Indian meal moth, *Plodiainterpunctella* (Lepidoptera: Pyralidae). *Journal of Plant Protection Research, 54*(3), 268-271.

Kells, S. A., Mason, L. J., Maier, D. E., & Woloshuk, C. P. (2001). Efficacy and fumigation characteristics of ozone in stored maize. *Journal of stored products research, 37*(4), 371-382.

López-Morales, D., La Cruz-Lazaro, E., Sánchez-Chávez, E., Preciado-Rangel, P., Márquez-Quiroz, C., & Osorio-Osorio, R. (2020). Impact of agronomic biofortification with zinc on the nutrient content, bioactive compounds, and antioxidant capacity of cowpea bean (*Vigna unguiculata* L. Walpers). *Agronomy*, *10*(10), 1460.

Mallmann, C. A., Santurio, J. M., Almeida, C. A. A., & Dilkin, P. (2001). Fumonisin B1 levels in cereals and feeds from southern Brazil. *Arquivos do Instituto Biológico, 68*(1), 41-45.

Michel, B. E., & Radcliffe, D. (1995). A computer program relating solute potential to solution composition for five solutes. *Agronomy Journal, 87*(1), 126-130.

Minuye, M., & Bajo, W. (2021). Common beans variability on physical, canning quality, nutritional, mineral, and phytate contents. *Cogent Food & Agriculture, 7*(1), 1914376.

Mishra, G., Palle, A. A., Srivastava, S., & Mishra, H. N. (2019). Disinfestation of stored wheat grain infested with *Rhyzopertha dominica* by ozone treatment: process optimization and impact on grain properties. *Journal of the Science of Food and Agriculture, 99*(11), 5008-5018.

Mohsenin, N. N. (1970). Physical properties of plant and animal materials. *New York*: *Gordon and Breach Science Publishers*.

Nesci, A., Barra, P., & Etcheverry, M. (2011). Integrated management of insect vectors of *Aspergillus flavus* in stored maize, using synthetic antioxidants and natural phytochemicals. *Journal of stored products research, 47*(3), 231-237.

Oliveira, J. M., Alencar, E. R., Blum, L. E. B., Souza-Ferreira, W. F., Botelho, S. D. C. C., Racanicci, A. M. C., & Silva, C. R. (2020). Ozonation of Brazil nuts: Decomposition kinetics, control of *Aspergillus flavus* and the effect on color and on raw oil quality. *LWT, 123*, 109106.

Oomah, B. D., Ward, S., & Balasubramanian, P. (2010). Dehulling and selected physical characteristics of Canadian dry bean (*Phaseolus vulgaris* L.) cultivars. *Food Research International, 43*(5), 1410-1415.

Oyedeji, A. B., Sobukola, O. P., Green, E., & Adebo, O.A. (2021). Physical properties and water absorption kinetics of three varieties of Mucuna beans. *Scientific Reports, 11*(1), 1-8.

Pandiselvam, R., Sunoj, S., Manikantan, M. R., Kothakota, A., & Hebbar, K. B. (2017). Application and kinetics of ozone in food preservation. *Ozone: Science & Engineering*, *39*(2), 115-126.

Parkin, E. A. (1956). Stored product entomology (the assessment and reduction of losses caused by insects to stored foodstuffs). *Annual Review of Entomology, 1*(1), 223-240.

Perina, E. F., Carvalho, C. R. L., Chiorato, A. F., Lopes, R. L. T., Gonçalves, J. G. R., & Carbonell, S. A. M. (2014). Technological quality of common bean grains obtained in different growing seasons. *Bragantia*, *73*(1), 14-22.

Pitt, J. I. (2000). Toxigenic fungi: which are important? *Med Mycol, 38*(Supplement_1), 17-22.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage*. *New York*: Springer 519, 388.

Price, L. A., & Mills, K. A. (1988). The toxicity of phosphine to the immature stages of resistant and susceptible strains of some common stored product beetles, and implications for their control. *Journal of Stored Products Research, 24*(1), 51-59.

Priya, T. R., & Manickavasagan, A. (2020). Common bean. In: Manickavasagan, A., Thirunathan, P. (eds). Pulses: *Processing and Product Development*, 77-97.

Qin, Y. L., Zhang, S. B., Lv, Y. Y., Zhai, H. C., Hu, Y. S, & Cai, J. P. (2022). Transcriptomics analyses and biochemical characterization of *Aspergillus flavus* spores exposed to 1-nonanol. *Applied Microbiology and Biotechnology, 106*(5), 2091-2106.

Rakness, K., Gordon, G., Langlais, B., Masschelein, W., Matsumoto, N., Richard, Y., & Somiya, I. (1996). Guideline for measurement of ozone concentration in the process gas from an ozone generator. *Ozone: Science & Engineering, 18*(3), 209-229.

Ramya, R. S., Srivastava, C., Subramanian, S., & Ranjith, M. (2023). Inheritance pattern and expression of resistance to phosphine in larval stage of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Asia-Pacific Entomology, 26*(1), 102040.

Reyes-Moreno, C., Paredes-López, O., & Gonzalez, E. (1993). Hard-to-cook phenomenon in common beans—A review. *Critical Reviews in Food Science & Nutrition, 33*(3), 227-286.

Ribeiro, D. F., Faroni, L. R. D. A., Pimentel, M. A., Prates, L. H. F., Heleno, F. F., & Alencar, E. R. (2022). Ozone as a Fungicidal and Detoxifying Agent to Maize Contaminated with Fumonisins. Ozone*: Science & Engineering*, *44*(1), 38-49.

Rodríguez-González, Á., Campelo, M. P., Lorenzana, A., Mayo-Prieto, S., González-López, Ó., Álvarez-García, S., & Casquero, P. A. (2020). Spores of *Trichoderma strains* sprayed over *Acanthoscelides obtectus* and *Phaseolus vulgaris* L. beans: Effects in the biology of the bean weevil. *Journal of Stored Products Research, 88*, 101666.

Ropelewska, E. (2019). Post-harvest assessment of wheat and barley kernel infections with fungi of the genus Fusarium using thermal analysis. *Journal of Stored Products Research, 83*, 61-65.

Sanchez, B. A. O., Alencar, E. R., Oliveira Pineli, L. D. L., Ferreira, W. F. S., & Almeida-Roberto, M. (2016). Tracing interactions among column height, exposure time and gas concentration to dimension peanut antifungal ozonation. *LWT-Food Science and Technology, 65*, 668-675.

Santos, R. R., Faroni, L. R., Cecon, P. R., Ferreira, A. P, & Pereira, O. L. (2016). Ozone as fungicide in rice grains. *Revista Brasileira de Engenharia Agrícola e Ambiental, 20*(3), 230-235.

Sartori, M. R. (1982). Technological quality of dry beans *(Phaseolus vulgaris L.)* stored under nitrogen. *Kansas State University*.

Silva, J., Pereira, M. N., & Scussel, V. M. (2018). Ozone gas antifungal effect on extruded dog food contaminated with *Aspergillus flavus*. *Ozone: Science & Engineering, 40*(6), 487-493.

Silva, M. B. D. O., Carvalho, A. J. D., Carneiro, J. E. D. S., Aspiazú, I., Alves, É. E., David, A. M. S. D. S., & Alves, P. F. S. (2016). Technological quality of grains of common beans selected genotypes from the carioca group. *Semina. Ciências Agrárias, 37*(4), 1721-1732.

Silva, M. V. A., Faroni, L. R. A., Sousa, A. H., Prates, L. H. F., & Abreu, A. O. (2019). Kinetics of the ozone gas reaction in popcorn kernels. *Journal of Stored Products Research*, *83*, 168-175.

Silva, M. V. D. A., Faroni, L. R. D. A., Alencar, E. R., Sousa, A. H., Cecon, P. R., Nogueira, J. V. F., & Mason-Filho, V. (2022). Ozone Injection at Low-Pressure: Decomposition Kinetics, Control of *Sitophilus zeamais*, and Popcorn Kernel Quality. *Ozone: Science & Engineering, 44*(1), 66-78.

Silva, Y. N. M., Silva, P. R. R., França, S. M., Silva, Y. D. M., Brito, R. D. C., Barbosa, D. R. E. S., & Silva, G. N. (2021). Ozonation of Lima Bean (*Phaseolus Lunatus* L.): Control of *Zabrotes Subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae: Bruchinae) and Maintenance of Grain Quality. *Ozone: Science & Engineering, 44*, 1-12.

Singh, B., & Kaur, A. (2018). Control of insect pests in crop plants and stored food grains using plant saponins: A review. *LWT*, *87*, 93-101.

Sitoe, E. D. P. E., Faroni, L. R. D. A., Alencar, E. R., Silva, M. V. D. A., & Salvador, D. V. (2023). Low-pressure ozone injection system: relationship between reaction kinetics and physical properties of grains. *Journal of the Science of Food and Agriculture, 103*(3), 1183-1193.

Sivaranjani, S., Prasath, V. A., Pandiselvam, R., Kothakota, A., & Khaneghah, A. M. (2021). Recent advances in applications of ozone in the cereal industry. *LWT*, *146*, 111412.

Sivaranjani, S., Prasath, V. A., Pandiselvam, R., Kothakota, A., & Khaneghah, A. M. (2021). Recent advances in applications of ozone in the cereal industry. *LWT*, *146*, 111412.

Smith, P. S. (1982). Starch derivatives and their use in foods. In: Lineback, D. R.; Inglett, G. E. (Ed.). *Food carbohydrates*. Westport: AVI, 237-269.

Souza, G. A. D., Carvalho, M. R. D. O., Martins, E. R., Guedes, R. N. C., & Oliveira, L. O. D. (2008). Genetic diversity estimated through ISSR markers in populations of *Zabrotes subfasciatus*. *Pesquisa Agropecuária Brasileira, 43*(7), 843-849.

Souza, J. V. D. S., Alencar, E. R. D., Junqueira, A. M., & Oliveira, G. P. D. (2018). Ozone saturation and decomposition kinetics in porous medium containing different hybrids of maize. *Revista Brasileira de Engenharia Agrícola e Ambiental, 22*, 286-291.

Szeitz. A., & Bandiera, S. M. (2018). Analysis and measurement of serotonin. *Biomedical Chromatography, 32*(1), e4135.

Teixeira, I. R., & Zucoloto, F. S. (2012). Intraspecific competition in *Zabrotes subfasciatus*: physiological and behavioral adaptations to different amounts of host. *Insect Science, 19*(1), 102-111.

Tigist, S. G., Melis, R., Sibiya, J., & Keneni, G. (2018). Evaluation of different Ethiopian common bean, *Phaseolus vulgaris* (Fabaceae) genotypes for host resistance to the Mexican bean weevil, *Zabrotes subfasciatus* (Coleoptera: Bruchidae). *International Journal of Tropical Insect Science, 38*(1), 1-15.

Tigist, S. G., Melis, R., Sibiya, J., Amelework, B. A., Keneni, G., & Tegene, A. (2020). Genetic diversity analysis of common bean (*Phaseolus vulgaris* L.) genotypes for resistance to Mexican bean weevil (*Zabrotes subfasciatus*), using single nucleotide polymorphism and phenotypic markers. *Acta Agriculturae Scandinavica, Seção B—Soil & Plant Science, 70*(6), 495-506.

Tigist, S. G., Raatz, B., Assefa, A., Melis, R., Sibiya, J., Keneni, G., & Tsegaye, D. (2021). Introgression of bruchid (*Zabrotes subfasciatus*) resistance into small red common bean (*Phaseolus vulgaris*) background and validation of the BRU_00261 (snpPV0007) resistance marker. *Plant Breeding, 140*(6) 1081-1089.

Trucksess, M. W., & Diaz-Amigo, C. (2011). Mycotoxins in Foods. *Encyclopedia of Environmental Health, 40*(3), 888-897.

Tsehaye, H., Brurberg, M. B., Sundheim, L., Assefa, D., Tronsmo, A., & Tronsmo, A. M. (2017). Natural occurrence of Fusarium species and fumonisin on maize grains in Ethiopia. *European journal of plant pathology, 147*(1), 141-155.

Vieira, R. D., Paiva, J. A., Perecin, A., & Perecin, D. (1999). Electrical conductivity and field performance of soybean seeds. *Seed Technology*, *21*(1), 15-24.

Viscarra-Torrico, R. C., Pajak, A., Garzón, A. S., Zhang, B., Pandurangan, S., Diapari, M., & Marsolais, F. (2021). Common bean (*Phaseolus vulgaris* L.) with increased cysteine and methionine concentration. *Legume Science, 3*(3), e103.

Wang, N., & Daun, J. K. (2005). Determination of cooking times of pulses using an automated Mattson cooker apparatus. *Journal of the Science of Food and Agriculture, 85*(10), 1631-1635.

Wang, N., Hatcher, D.W., Tyler, R. T., Toews, R., & Gawalko, E. J. (2010). Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicerarietinum* L.). *Food Research International*, *43*(2), 589-594.

Wani, I. A., Sogi, D. S., Wani, A. A., & Gill, B. S. (2017). Physical and cooking characteristics of some Indian kidney bean (*Phaseolus vulgaris* L.) cultivars. *Journal of the Saudi Society of Agricultural Sciences, 16*(1), 7-15.

Zhang, L., Shi, W. B., & Feng, M. G. (2014). Histopathological and molecular insights into the ovicidal activities of two entomopathogenic fungi against two-spotted spider mite. *Journal of invertebrate pathology, 117*, 73-78.

CHAPTER 3

Application of ozone at low-pressure: Effect on the technological properties, phenolic compounds, and antioxidant capacity of common beans during storage.

Common beans (*Phaseolus vulgaris* L.) are used in human nutrition and are susceptible to damage from insect pests and fungi. Ozonation has proven to be a safe technology to control insect pests and inactivate microorganisms. The aim of this study was to determine the effect of ozone (61.37 mg L^{-1}) applied at low pressure on the technological properties, phenolic compounds and antioxidant capacity of grains. Samples of 5.0 kg of beans packed in woven polypropylene bags were exposed to ozone in the hypobaric chamber of internal volume equal to 70 L. Ozone and oxygen (control) was applied in 10 injections and a flow rate of 1 L min^{-1}. The effect of the treatments on grain quality was assessed over 180 days of storage. Ozone did not significantly affect ($p > 0.05$) the percentage composition of the beans, which exhibited 18.76% of protein, 11.66% of water, 3.44% of minerals, 1.32% of lipids, and 64.51% of carbohydrates. A significant difference ($p < 0.05$) was noticed between the treatments and the control for total titratable acidity. The color variables luminosity (L^*), color saturation or chroma (C^*) and hue (h^*) of the grains were not significantly altered ($p < 0.05$) by ozone. There was an isolated effect ($p < 0.05$) of ozone on the quantification of phenolic compounds in the bean samples, but no significant impact ($p > 0.05$) of ozone on the antioxidant capacity in beans. However, the phenolic compounds content and antioxidant capacity decreased over storage in all treatments. Ozone application at low-pressures proved a promising technology to control insect pests and promote fungal decontamination without significantly altering commercial bean quality throughout storage.

Keywords: Food quality. Phenolic compounds. Antioxidant capacity. Stored product insect.

1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most cultivated and consumed legumes worldwide, occupying a prominent position in the human diet (Wani et al., 2017; Thompson, 2019). It has been used in dietary formulations for treating and preventing diabetes, cardiovascular diseases, colon cancer, and lowering blood cholesterol levels (Wani et al., 2017). In addition, common bean are a source of proteins, carbohydrates, dietary fiber, vitamins and minerals (Los et al., 2018; Bernardes et al., 2018).

Like other cereal and legume grains, beans are susceptible to damage by insect pests and fungi (Ogecha et al., 2019). The action of insect pests reduces the dry matter content, compromises the nutritional quality, and contributes to a higher incidence of fungi and other microorganisms (Nesci et al., 2011; Hou et al., 2016). Scientific investigations on alternative methods for insect-pest control and fungal inactivation have pointed to ozonation as a promising technology (Abreu et al., 2022; Ribeiro et al., 2022; Sitoe et al., 2023).

Ozonation is a chemical process generally recognized as safe (GRAS) and regulated by the *United States Federal Drug Administration* (FDA) for usage in fresh agricultural products (FDA, 2001). It is a safe and environmentally-friendly technology for insect-pest management and microorganism inactivation, and it also acts by degrading mycotoxins and pesticide residues in stored grains and their by-products (Sousa et al., 2008; Pandiselvam et al., 2019; Sivaranjani et al., 2021).

Although ozone gas can protect grains and their by-products, its oxidative properties might cause undesirable quality changes. Information on the effect of ozone gas on grain quality is essential for the food-processing industry (Pandiselvam et al., 2022; Nickhil et al., 2022). Previous studies investigated the impact of ozone gas on the quality of chickpeas (Nickhil et al., 2022) and beans (Alexandre et al., 2018; Gad et al., 2021). However, there is still limited data on the latent effect of low-pressure ozone application on the quality of stored beans. Although studies are still incipient, knowing the characteristics and outcomes of this process is of utmost importance for adopting ozone as a new, viable technology.

On that account, this study aimed to determine the technological properties of the ozonation process. It also sought to assess the phenolic compounds content and antioxidant capacity in stored beans after exposure to ozone gas at a level effective for controlling *Zabrotes subfasciatus* and *Aspergillus flavus*.

2. MATERIAL AND METHODS

2.1. Ozonation of beans

Common beans were purchased from a local supermarket in Viçosa, Minas Gerais, Brazil. They were homogenized and stored in a cold chamber at 5 ºC until the beginning of the experiment. Ozone was generated from the oxygen produced by a concentrator model EverFlo (Philips Respironics, Murrysville, Pennsylvania, USA). The grains were treated inside a 70-liter hypobaric chamber model CV10 (*myOZONE*, Jaguariúna, São Paulo, Brazil). Prior to ozone injection, the internal pressure of the chamber was reduced to 500 hPa with a vacuum pump model VP 260ND (TIPI Professional Refrigeration, Caxias, Rio Grande do Sul, Brazil), and a vacuum gauge was used to monitor it. Then, the hypobaric chamber was filled with ozone gas until the internal pressure reached 1000 hPa. The duration of each injection was 37 minutes, with a 135-minute gap between consecutive ones, as determined by Sitoe et al, (2023). According to the tests conducted by those authors, ten ozone injections at 61.37 mg L^{-1} and a volumetric flow rate of 1 L min^{-1} created a modified atmosphere inside the packaging with an ozone concentration of 21.20 mg L^{-1}. By achieving this ozone level within the packaging, it was possible to prevent the emergence of *Z. subfasciatus* from eggs and larvae and inactivate the fungus *A. flavus* in beans.

Ozone gas was applied to 5.0 kg bean samples packed in woven-polypropylene bags. The negative control corresponded to the injection of only oxygen at a volumetric flow rate of 1 L min^{-1}, whereas the positive control comprised samples treated with neither ozone nor oxygen.

2.2. Bean quality

One-kilogram bean samples packed in low-density polypropylene bags were used to investigate the effect of the treatments during storage. They were stored in a climatized chamber at 25 ºC and 60±5% relative humidity for 180 days.

The technological properties, concentration of phenolic compounds, and antioxidant capacity of beans were determined to appraise the grain quality after exposure to ozone gas. At the beginning of storage, the percentage composition and total titratable acidity were measured only after ozonation. Analyses of color, electrical conductivity, water absorption capacity, hydration coefficient, hydration capacity, percentage of tough-skin grains, cooking time, phenolic compounds content, and antioxidant capacity were carried out immediately after the treatments and then every 45 days for 180 days of storage.

2.2.1. Technological properties of beans during storage

Bean samples were ground with a BENE-CASA grain grinder (Vila Santa Cruz, Franca, São Paulo, Brazil) to determine the percentage composition, total titratable acidity, phenolic compounds content, and antioxidant capacity. The resulting bean flour was transferred to Falcon tubes (50 mL) and stored in a freezer (-20 ºC) until the analyses.

Mineral content was determined by the method 945.45 (AOAC, 2005). The water content of the grains was measured according to ASABE (American Society of Agricultural and Biological Engineers, 2013). Protein content was assessed by the Kjeldahl method 991.22 (AOAC, 2005) following the experimental procedure described by Oktavianawati et al. (2020) using 6.25 as the conversion factor. Lipid content was determined with the Soxhlet method 920.39 (AOAC, 2005). Carbohydrate content was established according to method 986.25 (AOAC, 2005). The contents of minerals, proteins, lipids, and carbohydrates were expressed on a dry basis. Total titratable acidity was measured according to the method by the Adolf Lutz Institute (2008).

Grain color was evaluated via direct reflectance readings of the coordinates $L*$, $a*$, and $b*$ with a colorimeter model CR410 (Konica Minolta, Osaka, Japan). Color hue $h*$ (Eq. 1) and color saturation or chroma $C*$ (Eq. 2) were determined based on the values of $L*$, $a*$, and $b*$ (Cie, 2004).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad \text{(Eq.1)}$$

$$h^* = arctang\left(\frac{b^*}{a^*}\right) \quad \text{(Eq.2)}$$

The electrical conductivity of the grain soaking water was determined through the method used by Vieira et al. (1999). Samples of 50 beans were weighed and put into plastic containers filled with 75 mL of distilled water. The sample sets were stored in a BOD-type climate chamber at 25 ºC for 24 h. The electrical conductivity of the grains was measured with a conductivity meter model MCA-150 (Tecnopon, Piracicaba, São Paulo, Brazil), and the results were expressed in $\mu S\ cm^{-1}\ g^{-1}$.

The water absorption capacity (WAC) of the grains was established according to Garcia-Vela & Stanley (1989) and Plhak et al (1989) following the guidelines of SNPCMAPA (Directive No. 294/98 – Annex IV). Bean samples with 30 g were put into plastic cups, and 100 mL of distilled water was added to each set. Then, they were stored in a BOD-type climate chamber at 25 ºC for 16 h. The water absorption capacity of the grains was given by Eq. 3.

$$WAC\ (\%) = \frac{Mf - Mi}{Mi}\ x\ 100 \qquad \text{(Eq.3)}$$

Where M_i and M_f correspond to the initial and final grain masses (g), respectively.

Bean samples of 100 g were soaked in 100 mL of distilled water inside disposable plastic cups covered with aluminum foil to determine their hydration coefficient (HC) and hydration capacity (HCA). The grains remained immersed in distilled water for 24 h inside a BOD-type climate chamber at 25 ºC. Then, the superficial water was drained, and the grain mass was measured. The hydration coefficient was given by Eq. 4 (Saba et al., 2016), and the hydration capacity by Eq. 5 (Adebowale et al., 2005).

$$HC\ (\%) = \frac{Mb}{Mo}\ x\ 100 \qquad \text{(Eq.4)}$$

$$HCA\ (\text{ggrain}^{-1}) = \frac{M_b - M_0}{N} \qquad \text{(Eq.5)}$$

M_b is the mass of the hydrated beans (g) after 24h, M_o is the mass of beans (g) before hydration, and N is the number of grains.

The percentage of tough-skin beans was established according to Corrêa et al. (2010). Bean samples of 100 g were immersed in distilled water for 8 hours. After that, the grains that had not absorbed water were counted. The grains were visually evaluated for skin wrinkling, and those without this characteristic were classified as tough-skin beans. The result was expressed as a percentage of tough-skin beans (without water absorption capacity).

The cooking time of the beans was determined according to Wang & Daun (2005). A bean sample of 30 g was submerged in distilled water for 16 h inside a BOD-type climate chamber at 25 ºC. After that, whole beans with the skin still attached to the cotyledons were selected to establish the cooking time. The grains previously soaked in distilled water were put into a stainless-steel Mattson cooker (*STANHOF*, Lavras, Minas Gerais, Brazil). Then, 400 mL of distilled water was added to the beans, and the cooking time was defined as the time between the moment the concoction started to boil (90 ºC) and the fall of the thirteenth plunger of the apparatus.

2.2.2 Phenolic compounds and antioxidant capacity of beans during storage

Extracts of beans treated or not with ozone were used to determine the total phenolic compounds content and the antioxidant capacity of the grains, according to the methodology by Ranilla et al. (2007) modified by Alexandre et al. (2018). Ground-bean samples of 1.0 g were weighed in 50-milliliter beakers. Next, 20 mL of an extractant mixture consisting of water, methanol, and acetic acid (30:70 v/v) was added to the beakers. The mixture was stirred with an orbital shaker model GEHAKA AO-370 (Ramos, Rio de Janeiro, Brazil) for 20 min at 240 rpm. Then, it was centrifuged with a centrifuge model ALB-12-CC (Uberlândia, Minas Gerais, Brazil) at 2000 × g for 20 min at 25 ºC. The resulting supernatant was collected for further analysis.

The total phenolic compounds content was determined according to the guidelines of Singleton & Rossi (1965) with some modifications. First, the bean extracts were oxidized with the Folin-Ciocalteu reagent in alkaline conditions. Subsequently, an analytical curve was drawn based on different dilutions of a stock solution of gallic acid (200 mg L^{-1}), ranging from 0 to 150 mg L^{-1}. Aliquots of 0.6 mL of each gallic acid dilution (mg L^{-1}) and 3.0 mL of the Folin-Ciocalteu reagent diluted in water (1:10 v/v) were transferred to test tubes. These mixtures were homogenized for 3 min with a vortex mixer model CRALTECH (Cotia, São Paulo, Brazil). After that, 2.4 mL of saturated Na_2CO_3 solution (7.5% w/v) was added to the tubes. Then, they reposed for 1 h away from the light. The absorbance readings were performed at 760 nm with a spectrophotometer Rayleigh model UV-9200 **(**Ramos, Rio de Janeiro, Brazil) previously calibrated with distilled water. The blank corresponded to 0.6 mL of distilled water, which underwent the above mentioned procedure. The results of total phenolic compounds content were expressed as milligrams of gallic acid equivalent per 1 g of beans (mg GAE g^{-1}).

The antioxidant capacity was assessed through the assays DPPH (1.1-diphenyl-2-picrylhydrazyl radical) and ABTS (2.2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical). The DPPH method was performed according to Brand-Williams et al. (1995). A methanolic solution of DPPH (Sigma-Aldrich, St. Louis, USA) was concocted at 0.6 mmol L^{-}

^{1}. Next, an analytical curve was drawn with different methanolic dilutions from a stock solution of Trolox (1.0 mmol L^{-1}) ranging from 0 to 1.0 mmol L^{-1}. Aliquots of 0.1 mL of Trolox dilutions were transferred to test tubes in a dark ambient. Each tube was added of 2.9 mL of the methanolic solution of DPPH, and the sets were homogenized in a test-tube shaker model CRALTECH (Cotia, São Paulo, Brazil). After that, the tubes reposed in a dark ambient for 25 min. The absorbance readings were done at 515 nm with a spectrophotometer Rayleigh model UV-9200 **(**Ramos, Rio de Janeiro, Brazil) previously calibrated with methanol.

The ABTS method was performed according to Camargo et al. (2012) and Re et al. (1999). In a container, ABTS solution at 7 mmol L^{-1} (Sigma-Aldrich, St. Louis, USA) was mixed with potassium persulfate 2.45 mmol L^{-1} (1:1 v/v). The mixture was kept in the dark for 12 to 16 h to generate the chromophore cation. It was then diluted in ethanol 80% until reaching the absorbance of 0.700±0.005 at the wavelength of 734 nm (Rayleigh spectrophotometer model UV-9200). Aliquots of 0.5 mL of different sample dilutions and 3.5 mL of the ABTS solution were added to test tubes, and these sets were homogenized with a test-tube shaker model CRALTECH (Cotia, São Paulo, Brazil). The absorbance was read after 6 min of reaction at the wavelength of 734 nm.

The antioxidant activity of the samples was given according to the Trolox equivalent antioxidant capacity (TEAC value), and the results were expressed as µmol of Trolox per gram of beans (µmol of Trolox g^{-1}) in both methods. The TEAC value was calculated based on the analytical curve of Trolox, drawn from dilutions of the standard solution at concentrations ranging from 4.00 mmol L^{-1} to 100.00 mmol L^{-1}, employing the same conditions as the samples.

2.3. Statistical Analysis

Initially, the data were submitted to normality and homogeneity analyses with the Shapiro-Wilk and Levene tests, respectively. Subsequently, the data were subjected to analysis of variance (ANOVA) and regression analysis. For the qualitative factor (treatment), Tukey's test was used to establish a comparison between means, and Dunnett's test was employed to compare each treatment with the control group – both tests considered a 5% significance level. For the quantitative factor (storage time), the models were chosen based on the significance of the regression coefficients by applying Student's *t*-test. A 5% significance level was adopted for both the determination coefficient (r^2) and the behavior of the phenomenon under study.

3. RESULTS

3.1. Bean quality

3.1.1. Technological properties of the beans

Table 1 contains the average values of percentage composition and total titratable acidity of beans exposed to ozone gas, oxygen, or neither (untreated control). Ozone did not significantly affect (p>0.05) the percentage composition values of the beans. This variable varied in the grains as follows: minerals from 3.34 to 3.55 %, water content from 11.54 to 11.76%, proteins from 18.74 to 18.81%, lipids from 1.10 to 1.49%, and carbohydrates from 64.01 to 65.15%. Total titratable acidity was significantly affected (p<0.05) by ozone. Beans exposed to ozone or oxygen had higher total titratable acidity values (5.39 mL sol N% in each case) than the control treatment, which averaged 4.80 mL sol N%.

Table 2 presents the average values of brightness (L^*), color saturation or chroma (C^*), color hue (h^*), electrical conductivity, water absorption capacity, hydration coefficient, hydration capacity, percentage of tough-skin beans, and cooking time of beans stored for 180 days. There was no significant effect (p> 0.05) of the interaction between the storage time or treatments and the technological properties of the beans. Furthermore, the isolated factors (treatments) did not significantly affect (p> 0.05) the color variables (L^*, C^*, and h^*), water absorption capacity, hydration coefficient, hydration capacity, percentage of tough-skin beans, and cooking time. Nonetheless, a significant effect (p< 0.05) of ozone was observed on the electrical conductivity values. The electrical conductivity of ozone-treated beans was higher than that observed in beans exposed to oxygen and the control. The values ranged from 62.49 to 82.5 $\mu S\ cm^{-1}\ g^{-1}$.

Table 1 - Average values of percentage composition and total titratable acidity of beans.

Treatments	Minerals (% w/w)	Water content (% w/w)	Protein (% w/w)	Lipids (% w/w)	Carbohydrates (% w/w)	Total titratable acidity (mL sol N%)
Control	3.55 a	11.54 a	18.81 a	1.10 a	65.15 a	4.80 b
Ozone	3.42 a	11.69 a	18.74 a	1.37 a	64.36 a	5.39 a*
Oxygen	3.34 a	11.76 a	18.74 a	1.49 a	64.01 a	5.39 a*

* Means followed by an asterisk in the row differ from the control at a 5% probability level according to Dunnett's test; ±SD - standard deviation.

Source: Sitoe (2023)

Table 2 - Average values of luminosity (*L**), color saturation or chroma (*C**), color hue (*h**), electrical conductivity, water absorption capacity, hydration coefficient, hydration capacity, percentage of tough-skin beans, and cooking time over 180 days of storage.

Treatment	Storage time (days)				
	0 days	45 days	90 days	135 days	180 days
Luminosity (*L**)					
Control	45.18 aA	45.60 aA	45.33 aA	45.10 aA	45.56 aA
Ozone	45.28 aA	45.57 aA	45.51 aA	45.52 aA	45.62 aA
Oxygen	45.94 aA	45.36 aA	45.47 aA	45.45 aA	45.38 aA
Color saturation or chroma (*C**)					
Control	8.40 aA	8.50 aA	8.62 aA	8.30 aA	8.24 aA
Ozone	8.10 aA	8.55 aA	8.48 aA	8.66 aA	8.44 aA
Oxygen	8.94 aA	8.94 aA	8.68 aA	8.82 aA	8.85 aA
T Color hue (*h**)					
Control	30.05 aA	30.49 aA	30.34 aA	330.50 aA	30.51 aA
Ozone	30.24 aA	30.16 aA	30.25 aA	30.60 aA	30.42 aA
Oxygen	30.45 aA	30.40 aA	30.37 aA	30.38 aA	30.20 aA
Electrical conductivity (µS cm^{-1} g^{-1})					
Control	64.51 bA	64.69 bA	62.49 bA	65.82 bA	64.46 bA

Ozone	82.05 aA*	82.37 aA*	82.21 aA*	82.55 aA*	82.39 aA*
Oxygen	64.27 bA	64.44 bA	64.29 bA	64.83 bA	64.46 bA
Water absorption capacity (%)					
Control	94.42 aA	94.26 aA	94.45 aA	94.49 aA	94.36 aA
Ozone	94.25 aA	94.24 aA	94.34 aA	94.49 aA	94.35 aA
Oxygen	94.78 aA	94.40 aA	94.30 aA	94.48 aA	94.80 aA
Hydration coefficient (%)					
Control	2.03 aA	2.06 aA	2.02 aA	2.04 aA	2.04 aA
Ozone	2.04 aA	2.05 aA	2.04 aA	2.05 aA	2.06 aA
Oxygen	2.04 aA	2.04 aA	2.00 aA	2.01 aA	2.01 aA
Hydration Capacity ($g.grain^{-1}$)					
Control	0.34 aA	0.34 aA	0.35 aA	0.34 aA	0.34 aA
Ozone	0.34 aA	0.34 aA	0.34 aA	0.34 aA	0.35 aA
Oxygen	0.34 aA	0.34 aA	0.34 aA	0.35 aA	0.34 aA
Percentage of tough-skin beans (%)					
Control	1.16 aA	1.19 aA	1.16 aA	1.16 aA	1.16 aA
Ozone	1.22 aA	1.16 aA	1.16 aA	1.22 aA	1.16 aA
Oxygen	1.22 aA	1.19 aA	1.19 aA	1.16 aA	1.16 aA
Cooking time (min)					
Control	27.34 aA	27.52 aA	27.40 aA	27.27 aA	27.44 aA
Ozone	27.36 aA	27.44 aA	27.52 aA	27.67 aA	27.75 aA
Oxygen	27.36 aA	27.46 aA	27.52 aA	27.68 aA	27.76 aA

* Means followed by an asterisk in the row differ from the control at a 5% probability level according to Dunnett's test.

Source: Sitoe (2023)

3.1.2. Phenolic compounds and antioxidant capacity of beans during storage

Table 3 shows the average values of phenolic compounds content and antioxidant capacity in the different treatments applied to bean grains. The values of phenolic compounds observed in untreated common beans (control), exposed to oxygen and ozone were 1.46, 1.41 and 1.45 mg EAG g^{-1}, respectively. The antioxidant capacity measurement values observed by the ABTS method in untreated bean grains, treated with oxygen and ozone were 9.96, 9.78

and 9.83 μmol de Trolox g^{-1}, respectively. In the DPPH method, measured values of antioxidant capacity in bean grains of 6.47, 6.47 and 6.46 μmol de Trolox g^{-1}, respectively, were observed.

Table 4 presents the mathematical models and respective determination coefficients (r^2) describing the behavior of bioactive compounds of beans stored for 180 days. The interaction between the storage time and treatments had a significant effect ($p < 0.05$) on the values of phenolic compounds and antioxidant capacity. The determination coefficient (r^2) of the phenolic compound values of stored beans ranged from 0.98 to 0.99%. As for the antioxidant capacity measurement, the determination coefficient (r^2) ranged from 0.97 to 0.98% and 0.98 to 0.99% in the ABTS and DPPH methods, respectively. An isolated effect ($p < 0.05$) of the ozone treatment on the quantification of phenolic compounds was verified. However, ozone had no significant effect ($p > 0.05$) on the antioxidant capacity of the beans.

Table 3 - Average values of phenolic compounds content and antioxidant capacity of beans.

Treatments	Phenolic compounds (mg EAG g^{-1})	Antioxidant capacity (μmol de Trolox g^{-1})	
		ABTS	DPPH
Control	1.46 a	9.96 a	6.47 a
Ozone	1.41 b*	9.78 a	6.47 a
Oxygen	1.45 ab	9.83 a	6.46 a

*Means followed by the same letter in the row do not differ statistically ($p < 0.05$) according to Tukey's test. Means with an asterisk in the row differ from the control at a 5% probability level, according to *Dunnett's* test. Source:
Sitoe (2023)

Table 4 - Fitted equations of total phenolic compounds content and antioxidant capacity of beans stored for 180 days and their respective determination coefficients (r^2)

Bioactive properties of beans	Fitted Equation	r^2
Phenolic compounds (mg EAG g-1)		
Control	$\hat{y}_i$ = -0.0004t + 1.5072	0.99
Ozone	$\hat{y}_i$ = -0.0005t + 1.4592	0.98
Oxygen	$\hat{y}_i$= -0.0004t + 1.4939	0.99
Antioxidant capacity measurements (µmol de Trolox g-1)		
ABTS		
Control	$\hat{y}_i$ = -0.0089t + 10.5634	0.98
Ozone	$\hat{y}_i$ = -0.0074t + 10.4524	0.98
Oxygen	$\hat{y}_i$ = -0.0085t + 10.5998	0.97
DPPH		
Control	$\hat{y}_i$ = -0.0007t + 6.5375	0.99
Ozone	$\hat{y}_i$ = -0.0005t + 6.5126	0.98
Oxygen	$\hat{y}_i$ = -0.0005t + 6.5092	0.99

t – time (days). The estimated parameters were significant at a 5% significance level ($p < 0.05$) according to the *t*-test. Source:
Sitoe (2023)

4. DISCUSSION

The ozone concentration of 21.20 mg L^{-1} did not alter the mineral, water, protein, lipids, and carbohydrates contents of beans. The results of grain quality in the present study corroborate those of other authors. Nickhil et al. (2021) investigated the effect of ozone at concentrations from 1.63 to 3.30 mg L^{-1} on protein, amino acid, and fatty acid profiles, mineral content, and micro-structure of chickpeas and found that ozone did not significantly affect the contents of the total minerals and protein. A similar finding was reported by Trombete et al. (2016) who reported that the ozonation process of wheat did not affect the mineral content of the flour. Savi et al. (2014) proved that applying ozone gas at 60 mg L^{-1} for up to 120 min did not compromise wheat-grain quality attributes, such as lipid peroxidation and total protein profile.

In this work, the values obtained for minerals, proteins, lipids, and carbohydrates are within the typical ones in beans. Previous research indicated that the mineral content in common beans varies between 3 and 4% (Lovato et al., 2018; Nascimento-Silva et al., 2020), protein between 17.72 and 31.59% (Pires et al., 2005; Marquezi et al., 2016; Lovato et al., 2018), lipids from 1.15 to 1.93% (Barros & Prudencio, 2016; Lovato et al., 2018; Nascimento-Silva et al., 2020), and carbohydrates from 55.80 to 66.4% (Wani et al., 2017; Lovato et al., 2018).

This study verified a higher total titratable acidity in beans exposed to ozone or oxygen compared to the untreated grains (control). Similar results were verified by Porto et al. (2022) when applying ozonation to corn grains. The high total titratable acidity observed in ozone- and oxygen-treated bean samples may be related to the oxidative breakdown of triacylglycerol and the release of free fatty acids (Tiwari et al., 2010).

The lower concentration of phenolic compounds in the bean samples exposed to ozone or oxygen could be associated with the degradation of organic compounds. Besides, due to acidification, the ozone-treated grains showed higher electrical conductivity. The resulting values, however, are similar to those reported by other authors (Coelho et al., 2013; Sáez et al., 2017).

Bean-grain color is one of the decisive variables for acceptance by the market (Ribeiro et al., 2008). Ozone and storage time did not affect the luminosity (L^*), color saturation or chroma (C^*), and color hue (h^*) of the beans. The results were consistent with those of Bento et al. (2020). Sitoe et al. (2023) investigated the effects of low-pressure ozone injection on the quality variables of common beans and verified that the gas treatment did not affect grain color. Silva et al. (2022) demonstrated that ozone-treated popcorn kernels kept their quality throughout all storage period.

Water absorption capacity, hydration coefficient, hydration capacity, percentage of tough-skin beans, and cooking time are paramount for cooking beans. These factors were not significantly affected by the ozone treatment or storage time. The average values of these cooking properties were consistent with other works (Corrêa et al., 2010; Saba et al., 2016; Wani et al., 2017; Boros & Wawer, 2018). According to those authors, beans with higher water absorption capacity, hydration coefficient, and greater swelling capacity require less time for cooking. The literature points to a correlation between the percentage of water absorption and cooking time and states that higher absorption levels (remarkably over 80%) are linked to a faster cooking process.

Water absorption and hydration capacities determine the extent to which beans take in water when soaked. According to Saha et al. (2009) the hydration capacity of beans varies between 0.31 and 0.59 $g.grain^{-1}$. Beans with high water absorption and hydration capacity tend to have better cooking quality (less time required), thus reducing energy usage during preparation (Corrêa et al., 2010; Wani et al., 2017).

Tough skin is a characteristic that pertains to the grain integument and refers to the incapacity of beans to absorb water properly when soaked or cooked (Saba et al., 2016). Long cooking times may be associated with tough skin in beans, which hinders water absorption or cause the water adsorbed not to soften the bean integument during soaking and cooking (Guzmán-Maldonado et al., 2000). Thus, consumers and grain-processing industries prefer beans with high water absorption capacity, hydration coefficient, and hydration capacity but low skin toughness and short cooking time.

The total phenolic compounds content was affected by ozone. The reduction is probably associated with direct or indirect ozone-induced oxidative stress on the grain surface (Sachadyn-Król et al., 2016; Jia et al., 2020; Lang et al., 2020; Ziegler et al., 2020). The total phenolic compounds are primarily found in the grain integument (Vaher et al., 2010), which makes them prone to oxidation when subjected to ozonation. Phenolic compounds can undergo direct oxidation through interaction with the ozone molecules, or indirect oxidation due to the production of hydroxyl radicals (Amado-Piña et al., 2017; Diao et al., 2019). The reduction of the total phenolic compounds content in the food matrix by ozone can be induced by high concentrations, lengthy exposure times, the application method, and reactivity of the grain surface with the gas (Alexandre et al., 2018; Diao et al., 2019; Kaur et al., 2022).

High ozone levels cause the degradation of phenolic compounds into aliphatic compounds (mainly oxalic acid) (Amado-Piña et al., 2017). Sarooei et al. (2019) verified a reduction in the total phenolic compounds content in wheat grains as the ozone concentration and exposure time increased. This behavior is attributed to ozone auto degradation, generating various free radical species, such as hydroperoxyl (HO_2^-), hydroxyl (OH^-), and superoxides (O_2^-), which diminish the total polyphenol content (Pandiselvam et al., 2019).

The loss behavior of the total phenolic compounds throughout storage was studied in mung beans (*Vigna radiata* L.) (Ziegler et al., 2020). It may be related to the antioxidant action of these substances, which inhibits lipid peroxidation and the chelation of reactive metals. Rosa et al. (2013) observed that using the enzymes xylanase and feruloyl esterase type-*A* caused cell wall destruction and reduced the total phenolic compounds. On the other hand, the decline in the phenolic compounds content over storage may be due to interactions between free phenolics and other cellular components after lignification (Ziegler et al., 2020). In their studies, Htwe et al. (2010) stored rice grains for four months at three temperatures (20, 30, and 40 ºC) and observed that storage time was responsible for lowering the total phenolic compounds content, regardless of the temperature. Cheng et al. (2006) attributed this reduction to the enzymatic and oxidative processes during storage.

The assays employed in the present study for measuring the antioxidant capacity (ABTS and DPPH) produced discordant results. Even though both methods work by neutralizing radicals and transferring electrons, they follow distinct methodologies, which may explain the value differences. Notwithstanding, both methods revealed loss of antioxidant capacity in beans of all treatments (ozone, oxygen, and control) during storage for 180 days at 25 ºC and 60±5% relative humidity. Cheng et al. (2006) reported a loss of antioxidant capacity in wheat bran after storage for nine days at 25 °C (38% loss) and 60 °C (47% loss). In research conducted by Do et al. (2015) the loss of antioxidant capacity in barley was checked after four months of storage. The decrease in the antioxidant capacity of grains during storage may be a consequence of degradation due to the aging process of the grain, as observed by Zhou et al. (2014).

The results of this work and the information available in the literature make it evident that ozone gas applied at low-pressures is a promising alternative for insect-pest management and fungal decontamination without compromising the quality of the grains.

5. CONCLUSION

The ozone concentration of 21.20 mg L^{-1} did not alter the percentage composition, color, cooking properties, and antioxidant capacity of common beans. Exposing the grains to ozone gas increased the total titratable acidity and electrical conductivity values. However, the changes observed did not compromise the marketability of the grains. During the 180-day storage period, there was a reduction in both phenolic compounds content and antioxidant capacity in all treatments. Further research is necessary to understand the ozone-treatment effects on protein, amino acid, and fatty acid profiles.

REFERENCES

Abreu, A. O., Faroni, L. R. D. A., Assis Silva, M. V., Sousa, A. H., Alencar, E. R., & Silva, G. N. (2022). Ozone as an alternative fumigant for controlling *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in cowpea beans. *Journal of Stored Products Research*, *97*, 101969.

Adebowale, Y. A., Adeyemi, A., & Oshodi, A. A. (2005). Variability in the physicochemical, nutritional and antinutritional attributes of six Mucuna species. *Food chemistry*, *89*(1), 37-48.

Alexandre, A. P. S., Miano, A. C., Brandão, T. R. S., Miller, F. A., Fundo, J. F., Calori-Domingues, M. A., & Augusto, P. E. D. (2018). Ozonation of Adzuki beans (Vigna angularis): Effect on the hydration kinetics, phenolic compounds and antioxidant capacity. *Journal of Food Process Engineering*, *41*(8), e12893.

Amado-Piña, D., Roa-Morales, G., Barrera-Díaz, C., Balderas-Hernandez, P., Romero, R., del Campo, E. M., & Natividad, R. (2017). Synergic effect of ozonation and electrochemical methods on oxidation and toxicity reduction: phenol degradation. *Fuel*, *198*, 82-90.

AOAC. (2005). Official methods of analysis of the Association of Official Analytical Chemists International. 18.ed. *Washington*.

ASABE- American Society of Agricultural and Biological Engineers. (2013). Moisture measurement-unground grain and seeds. *ASABE Standards*. St. Joseph, Michigan. ASAE S352.2 APR1988 (R2017).

Barros, M., & Prudencio, S. H. (2016). Physical and chemical characteristics of common bean varieties. *Semina: Ciências Agrárias*, *37*(2), 751-761.

Bento, J. A. C., Lanna, A. C., Bassinello, P. Z., Oomah, B. D., Pimenta, M. E. B., Carvalho, R. N., & Moreira, A. S. (2020). Aging indicators for stored carioca beans. *Food research international*, *134*, 109249.

Bernardes, W. A., Silva, E. O., Crotti, A. E., & Baldin, E. L. (2018). Bioactivity of selected plant-derived essential oils against *Zabrotes subfasciatus* (Coleoptera: Bruchidae). *Journal of Stored Products Research*, *77*, 16-19.

Boros, L., & Wawer, A. (2018). Seeds quality characteristics of dry bean local populations (*Phaseolus vulgaris* L.) from National Center for Plant Genetic Resources in Radzików. *Legume Research-An International Journal*, *41*(5), 669-674.

Brand-Wiliams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology, 28*, 25-30.

Camargo, A. C., de Souza Vieira, T. M. F., Regitano-D'Arce, M. A. B., Calori-Domingues, M. A., & Canniatti-Brazaca, S. G. (2012). Gamma radiation effects on peanut skin antioxidants. *International Journal of Molecular Sciences*, *13*(3), 3073-3084.

Cheng, Z., Su, L., Moore, J., Zhou, K., Luther, M., Yin, J. J., & Yu, L. (2006). Effects of postharvest treatment and heat stress on availability of wheat antioxidants. *Journal of agricultural and food chemistry*, *54*(15), 5623-5629.

Cie. (2004). CIE technical report. Colorimetry (3rd ed.). 552. Colorimetry (pp. 24–). ISBN 3-901-906-33-9.

Coelho, S. R., Prudencio, S. H., Christ, D., Sampaio, S. C., & Schoeninger, V. (2013). Physico-chemical properties of common beans under natural and accelerated storage conditions. *Ciencia e investigación agraria: revista latinoamericana de ciencias de la agricultura*, *40*(3), 629-636.

Corrêa, M. M., de Carvalhoa, L. M. J., Nuttib, M. R., Luiz, J., de Carvalhob, V., Netob, A. R. H., & Ribeiroa, E. M. G. (2010). Water absorption, hard shell, and cooking time of common beans (*Phaseolus vulgaris* L.). *African Journal of Food Science and Technology1*, *1*, 13-20.

Diao, E., Wang, J., Li, X., Wang, X., Song, H., & Gao, D. (2019). Effects of ozone processing on patulin, phenolic compounds and organic acids in apple juice. *Journal of food science and technology*, *56*, 957-965.

Do, T. D. T., Cozzolino, D., Muhlhausler, B., Box, A., & Able, A. J. (2015). Antioxidant capacity and vitamin E in barley: Effect of genotype and storage. *Food Chemistry*, *187*, 65-74.

FDA. (2001). Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice. *Federal Register*, *66*, 6137– 6202.

Gad, H. A., Laban, G. F. A., Metwaly, K. H., Al-Anany, F. S., & Abdelgaleil, S. A. (2021). Efficacy of ozone for *Callosobruchus maculatus* and *Callosobruchus chinensis* control in cowpea seeds and its impact on seed quality. *Journal of Stored Products Research*, *92*, 101786.

García-Vela, L. A., & Stanley, D. W. (1989). Water-holding capacity in hard-to-cook beans (*Phaseolus vulgaris*): Effect of pH and ionic strength. *Journal of Food Science*, *54*(4), 1080-1081.

Guzmán-Maldonado, S. H., Acosta-Gallegos, J., & Paredes-López, O. (2000). Protein and mineral content of a novel collection of wild and weedy common bean (*Phaseolus vulgaris* L). *Journal of the Science of Food and Agriculture*, *80*(13), 1874-1881.

Hou, L., Johnson, J. A., & Wang, S. (2016). Radio frequency heating for postharvest control of pests in agricultural products: A review. *Postharvest Biology and Technology*, *113*, 106-118.

Htwe, N. N., Srilaong, V., Tanprasert, K., Tongchitpakdee, S., Kanlayanarat, S., & Uthairatanakij, A. (2010). Effects of storage time and temperature on radical scavenging activities and bioactive compounds in colored rice varieties. *Journal of Food, Agriculture & Environment*, *8*(3-4), 26-31.

INSTITUTO ADOLFO LUTZ. (2008) "Métodos Físico-Químicos para Análise de Alimentos" - 4ª Edição. 1ª Edição Digital. *São Paulo*. Capítulo 04, p. 813-814.

Jia, X., Li, J., Du, M., Zhao, Z., Song, J., Yang, W., & Li, X. (2020). Combination of low fluctuation of temperature with TiO2 photocatalytic/ozone for the quality maintenance of postharvest peach. *Foods*, *9*(2), 234.

Kaur, K., Pandiselvam, R., Kothakota, A., Ishwarya, S. P., Zalpouri, R., & Mahanti, N. K. (2022). Impact of ozone treatment on food polyphenols–A comprehensive review. *Food Control*, *142*, 109207.

Lang, G. H., Rockenbach, B. A., Ferreira, C. D., & de Oliveira, M. (2020). Delayed drying interval of red rice: Effects on cooking properties, in vitro starch digestibility and phenolics content. *Journal of stored products research*, *87*, 101613.

Los, F. G. B., Zielinski, A. A. F., Wojeicchowski, J. P., Nogueira, A., & Demiate, I. M. (2018). Beans (*Phaseolus vulgaris* L.): whole seeds with complex chemical composition. *Current Opinion in Food Science*, *19*, 63-71.

Lovato, F., Kowaleski, J., Silva, S. Z. D., & Heldt, L. F. S. (2017). Composição centesimal e conteúdo mineral de diferentes cultivares de feijão biorfortificado (*Phaseolus vulgaris* L.). *Brazilian Journal of Food Technology*, *21*, e2017068.

Marquezi, M., Gervin, V. M., Watanabe, L. B., Bassinello, P. Z., & Amante, E. R. (2016). Physical and chemical properties of starch and flour from different common bean (*Phaseolus vulgaris* L.) cultivars. *Brazilian Journal of Food Technology*, *19*, e2016005.

Nascimento-Silva, A., Ramos, M. L. G., Júnior, W. Q. R., Alencar, E. R., da Silva, P. C., Lima, C. A., & Silva, M. A. V. (2020). Water stress alters physical and chemical quality in grains of common bean, triticale and wheat. *Agricultural water management*, *231*, 106023.

Nesci, A., Barra, P., & Etcheverry, M. (2011). Integrated management of insect vectors of *Aspergillus flavus* in stored maize, using synthetic antioxidants and natural phytochemicals. *Journal of stored products research*, *47*(3), 231-237.

Nickhil, C., Mohapatra, D., Kar, A., Giri, S. K., Tripathi, M. K., & Sharma, Y. (2021). Gaseous ozone treatment of chickpea grains, part I: Effect on protein, amino acid, fatty acid, mineral content, and microstructure. *Food Chemistry*, *345*, 128850.

Nickhil, C., Mohapatra, D., Kar, A., Giri, S. K., Verma, U. S., & Muchahary, S. (2022). Gaseous ozone treatment of chickpea grains: Effect on functional groups, thermal behavior, pasting properties, morphological features, and phytochemicals. *Journal of Food Science*, *87*(12), 5191-5207.

Ogecha, J. O., Arinaitwe, W., Muthomi, J. W., Aritua, V., & Obanyi, J. N. (2019). Incidence and Severity of common bean (*Phaseolus vulgaris* L.) pests in agro-ecological zones and farming systems of Western Kenya. *East African Agricultural and Forestry Journal*, *83*(3), 191-205.

Oktavianawati, I., Arimurti, S., & Suharjono, S. (2020). The Impacts of Traditional Fermentation Method on the Chemical Characteristics of Arabica Coffee Beans from Bondowoso District, East Java. *forest*, *9*(2), 133-141.

Pandiselvam, R., Singh, A., Agriopoulou, S., Sachadyn-Król, M., Aslam, R., Lima, C. M. G., & Khaenegah, A. M. (2022). A comprehensive review of impacts of ozone treatment on textural properties in different food products. *Trends in Food Science & Technology*, *127*, 74-86.

Pandiselvam, R., Subhashini, S., Banuu Priya, E. P., Kothakota, A., Ramesh, S. V., & Shahir, S. (2019). Ozone based food preservation: A promising green technology for enhanced food safety. *Ozone: Science & Engineering*, *41*(1), 17-34.

Pires, C. V., Oliveira, M. G. A., Cruz, G. A. D. R., Mendes, F. Q., De Rezende, S. T., & Moreira, M. A. (2005). Composição físico-química de diferentes cultivares de feijão (*Phaseolus vulgaris* L.). *Alimentos e Nutrição, Araraquara*, *16*(2), 157-162.

Plhak, L. C., Caldwell, K. B., & Stanley, D. W. (1989). Comparison of methods used to characterize water imbibition in hard-to-cook beans. *Journal of Food Science*, *54*(2), 326-329.

Porto, Y. D., Trombete, F. M., da Silva, B. R., de Castro, I. M., Direito, G. M., Ascheri, J. L. R., & Silva, O. F. (2022). Ozonização de grits de milho (*Zea mays* L.) em altas concentrações promove alteração na cor e aroma do produto. *Research, Society and Development*, *11*(5), e46311528462-e46311528462.

Ranilla, L. G., Genovese, M. I., & Lajolo, F. M. (2007). Polyphenols and antioxidant capacity of seed coat and cotyledon from Brazilian and Peruvian bean cultivars (*Phaseolus vulgaris* L.). *Journal of agricultural and food chemistry*, *55*(1), 90-98.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237.

Ribeiro, D. F., Faroni, L. R. D. A., Pimentel, M. A. G., Prates, L. H. F., Heleno, F. F., & De Alencar, E. R. (2022). Ozone as a fungicidal and detoxifying agent to maize contaminated with fumonisins. *Ozone: Science & Engineering*, *44*(1), 38-49.

Ribeiro, N. D., Storck, L., & Poersch, N. L. (2008). Classificação de lotes comerciais de feijão por meio da claridade do tegumento dos grãos. *Ciência Rural*, *38*, 2042-2045.

Rosa, N. N., Dufour, C., Lullien-Pellerin, V., & Micard, V. (2013). Exposure or release of ferulic acid from wheat aleurone: impact on its antioxidant capacity. *Food chemistry*, *141*(3), 2355-2362.

Saba, I., Sofi, P. A., Zeerak, N. A., Bhat, M. A., & Mir, R. R. (2016). Characterisation of a core set of common bean (*Phaseolus vulgaris* L.) germplasm for seed quality traits. *SABRAO Journal of Breeding and Genetics*, *48*(3), 359-376.

Sachadyn-Król, M., Materska, M., Chilczuk, B., Karaś, M., Jakubczyk, A., Perucka, I., & Jackowska, I. (2016). Ozone-induced changes in the content of bioactive compounds and enzyme activity during storage of pepper fruits. *Food Chemistry*, *211*, 59-67.

Sáez, G. D., Hébert, E. M., Saavedra, L., & Zárate, G. (2017). Molecular identification and technological characterization of lactic acid bacteria isolated from fermented kidney beans flours (*Phaseolus vulgaris* L. and *P. coccineus*) in northwestern Argentina. *Food Research International*, *102*, 605-615.

Saha, S., Singh, G., Mahajan, V., & Gupta, H. S. (2009). Variability of nutritional and cooking quality in bean (*Phaseolus vulgaris* L) as a function of genotype. *Plant foods for human nutrition*, *64*(2), 174-180.

Sarooei, S. J., Abbasi, A., Shaghaghian, S., & Berizi, E. (2019). Effect of ozone as a disinfectant on microbial load and chemical quality of raw wheat germ. *Ozone: Science & Engineering*, *41*(6), 562-570.

Savi, G. D., Piacentini, K. C., Bittencourt, K. O., & Scussel, V. M. (2014). Ozone treatment efficiency on Fusarium graminearum and deoxynivalenol degradation and its effects on whole wheat grains (*Triticum aestivum* L.) quality and germination. *Journal of Stored Products Research*, *59*, 245-253.

Silva, M. V. D. A., Faroni, L. R. D. A., de Alencar, E. R., de Sousa, A. H., Cecon, P. R., Nogueira, J. V. F., & Mason Filho, V. (2022). Ozone injection at low pressure: decomposition kinetics, control of Sitophilus zeamais, and popcorn kernel quality. *Ozone: Science & Engineering*, *44*(1), 66-78.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Sitoe, E. D. P. E., Faroni, L. R. D. A., de Alencar, E. R., Silva, M. V. D. A., & Salvador, D. V. (2023). Low-pressure ozone injection system: relationship between reaction kinetics and physical properties of grains. *Journal of the Science of Food and Agriculture*, *103*(3), 1183-1193.

Sivaranjani, S., Prasath, V. A., Pandiselvam, R., Kothakota, A., & Khaneghah, A. M. (2021). Recent advances in applications of ozone in the cereal industry. *LWT*, *146*, 111412.

Sousa, A. D., Faroni, L. D. A., Guedes, R. N. C., Tótola, M. R., & Urruchi, W. I. (2008). Ozone as a management alternative against phosphine-resistant insect pests of stored products. *Journal of Stored Products Research*, *44*(4), 379-385.

Thompson, H. J. (2019). Dietary bean consumption and human health. *Nutrients*, *11*(12), 3074.

Tiwari, B. K., Brennan, C. S., Curran, T., Gallagher, E., Cullen, P. J., & O'Donnell, C. P. (2010). Application of ozone in grain processing. *Journal of cereal science*, *51*(3), 248-255.

Trombete, F., Minguita, A., Porto, Y., Freitas-Silva, O., Freitas-Sá, D., Freitas, S., & Fraga, M. (2016). Chemical, technological, and sensory properties of wheat grains (*Triticum aestivum* L) as affected by gaseous ozonation. *International Journal of Food Properties*, *19*(12), 2739-2749.

Vaher, M., Matso, K., Levandi, T., Helmja, K., & Kaljurand, M. (2010). Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chemistry*, *2*(1), 76-82.

Vieira, R. D., Paiva, J. A., Perecin, A., & Perecin, D. (1999). Electrical conductivity and field performance of soybean seeds. *Seed Technology*, *27*(1), 67-75.

Wang, N., & Daun, J. K. (2005). Determination of cooking times of pulses using an automated Mattson cooker apparatus. *Journal of the Science of Food and Agriculture*, *85*(10), 1631-1635.

Wani, I. A., Sogi, D. S., Wani, A. A., & Gill, B. S. (2017). Physical and cooking characteristics of some Indian kidney bean (*Phaseolus vulgaris* L.) cultivars. *Journal of the Saudi Society of Agricultural Sciences*, *16*(1), 7-15.

Zhou, Z., Chen, X., Zhang, M., & Blanchard, C. (2014). Phenolics, flavonoids, proanthocyanidin and antioxidant activity of brown rice with different pericarp colors following storage. *Journal of Stored Products Research*, *59*, 120-125.

Ziegler, V., de Almeida Veeck, I., Ugalde, M. L., Lang, G. H., Hoffmann, J. F., dos Santos, A. C. M., & Ferreira, C. D. (2020). Effects of storage period and temperature on the technological properties, starch digestibility, and phenolic compounds of mung beans (*Vigna radiata* L.). *Journal of Stored Products Research*, *89*, 101694.

GENERAL CONCLUSION

The physical properties of the grains affected the reaction kinetics of ozone gas during the low-pressure injection process. The half-life of the gas ranged from 17.86 to 52.91 and from 16.43 to 52.91 minutes, outside and inside the package in polished rice and beans, respectively. The low-pressure ozone concentration of 32.10 mg L^{-1} effectively controlled adult *S. zeamais* in popcorn kernels and corn, *C. maculatus* in cowpeas, and *Z. subfasciatus* in beans. The initial concentration of 61.37 mg L^{-1} applied in 10 injections created a modified atmosphere inside the package that efficiently controlled the egg and larval phases of *Z. subfasciatus*, reduced mass loss, and inactivated *A. flavus* in the grains. The ozonation in 10 injections did not change the quality of the beans stored for 180 days.