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Field effectiveness of gaseous ozone storage in alleviating damage to aged soybean seeds

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Abstract. Ozonation is a promising method for maintaining seed quality. However, the ability of ozone to preserve the quality of aged seeds, which have already deteriorated, is still open to question. This study evaluated the effectiveness of ozone in alleviating ageing soybean seed damage and also the economic feasibility of ozone technology under field conditions. Samples under ozonation storage (they were stored in open containers and woven sacks) were compared with those stored under conventional storage conditions (they were stored in woven bags without ozonation). Ozonated samples were exposed to 150 g h^{-1} of ozone gas for three hours a day for a six month storage period. All samples (with and without ozonation) were stored at 18±5°C and 50±5% relative humidity. Observations were conducted every month with regard to the seeds germination percentage, vigour index, moisture content, protein and free fatty acids. The results showed that ozonation has the potential to increase the viability of aged soybean seeds for up to 5 months. However, the decrease in the protein and free fatty acids levels in the ozonated seeds occurred more rapidly than in the control. Moreover, no significant difference was found between the ozonated seeds in open containers and sacks during storage, except for their moisture content. Ozone application on aged soybean seeds was found to be financially feasible with an ratio of revenue to cost value of 1.73, an return on investment of 74.63% and a payback period of 1.34 years.

Keywords: aged seed, soybean, ozone, quality, economic feasibility

1. INTRODUCTION

Soybean (*Glycine max* L.) is an essential protein and oilseed crop that is used for food, feed, and various industrial applications (Bantacut, 2017). Seed is one of the factors that have a positive effect on increasing soybean production (Rinaldi *et al.*, 2023). High-quality seed is the basis for the profitable production and expansion of soybean crops (Shelar *et al.*, 2008). The selection of quality seeds depends on the age of the seeds in question (Timotiwu *et al.*, 2022). High yields are obtained from soybean seeds with a high physiological potential, but this must be maintained at the postharvest stage (Jaques *et al.*, 2022). However, soybean seeds are susceptible to damage during storage due to their relatively high concentration of oil (Vijayakumar and Vijayakumar, 2015; Koskosidis *et al.*, 2022). Seeds deteriorate gradually and continuously during long-term storage thereby engendering quality loss, delayed germination, reduced vigour, and deprived viability, leading to genetic and financial losses (Zhang *et al.*, 2021; Adetunji *et al.*, 2021). An earlier study explained that during the seed ageing process, chromosomal damage (such as DNA strand breaks,

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SNA methylation, and aberrant gene expression) has the potential to accumulate and exceed a certain threshold, which results in seeds losing their ability to germinate (Zhang *et al*., 2021).

Even under optimal storage conditions, the ageing process has been proven unavoidable (Li *et al.*, 2017). As a result, cumulative losses from the postharvest process increase yearly (Jaques *et al*., 2022). Most conventional seed storage handling involves chemical treatment, however, this can degrade the physiological quality of the seeds due to the phytotoxic effects of some of the active constituents of chemical solutions (Silva *et al.*, 2019). Also, chemical approaches for enhancing seed germination include drawbacks such as being harmful to the environment and taking a significant amount of time and labour (Pandiselvam *et al*., 2019). These drawbacks have led to various research projects concerning the optimal physical and biological approaches to seed storage. However, information concerning the effectiveness of these non-chemical treatments in field applications is still lacking and requires further in-depth studies.

The relative humidity of the storage environment, the temperature and gas composition are the three essential elements influencing seed storability through direct and indirect impacts and also the interactions with biotic and abiotic factors (Dadlani *et al*., 2023). As a result, the use of the appropriate technology that can manage the temperature and relative humidity and maintain the moisture content of the storage facility in a state of hygroscopic balance is highly recommended for storing soybean seeds (Jaques *et al*., 2022). Ozone may be a viable alternative for protecting seed quality because it is both safer and more environmentally friendly than the standard methods used (Obadi *et al*., 2018). Ozone may be employed as either a gas or an aqueous solution (Pandiselvam *et al*., 2019). Several studies have demonstrated that ozone can enhance the quality of fresh seeds (Rodrigues *et al*., 2015; Normov *et al*., 2019) and increase both the seed germination and growth rate (Avdeeva *et al*., 2018; Mosneaga *et al*., 2020). Ozone possesses antifungal and disinfectant characteristics (Szumigaj-Tarnowska *et al*., 2020); thus, it is also potent agent for producing aseptic seeds (Çetinkaya *et al*., 2022), controlling insect pests (Shingala and Dabhi, 2022), and diminishing pesticide residues on stored seeds (Isikber and Athanassiou, 2015).

However, ozone's capability of improving the quality of aged seeds that have already been damaged remains questionable. Farmers typically employ long-term seed storage for planting in the following season. Seed producers must maintain their trade stocks, and the seed industry preserves the active genetic stock and germplasm for long-term use (Dadlani *et al*., 2023), thereby necessitating efforts to combat seed ageing. However, at present there is data scarcity concerning the effects of ozone treatment on aged seeds; therefore, this study aims to assess the efficacy of gaseous ozone on aged soybean seeds in field conditions with regard to their quality and economic feasibility. The findings of this study are intended to provide new insights into the impact of ozone and the ageing mechanism on seeds, as well as serving as a reference for seed industry management in terms of profitable seed storage supply and conservation.

2. MATERIALS AND METHODS

Fresh soybean seeds (var. Grobogan) were obtained from Grobogan, Central Java, Indonesia. Natural ageing was carried out by storing the soybean seeds in conventional farmers' warehouses with uncontrolled temperature and humidity for two months. After ageing, the seeds were evaluated for their initial condition before being treated with ozone. Meanwhile, the ozone treatment was performed in a seed producer warehouse that was provided with ozone producing equipment. Ozone was produced by a dielectric barrier discharge plasma reactor configured as a compact ozone generator (DIPO Technology, Semarang, Indonesia) equipped with a control device to set the exposure time. The ozone outlet valve was connected via a perforated stainless steel pipe attached to a metal rack cabinet in the storage room.

The study was conducted using a completely randomized factorial design. The experimental factors included the storage conditions and time; each test was repeated four times. The samples were divided into three storage conditions: 1) ozonated samples in open containers, 2) ozonated samples in woven sacks, and 3) control samples (without ozone) in woven sacks. Each treatment consisted of about 15 kg of aged soybean seeds. All of the samples were stored at 18±5°C and 50±5% relative humidity. In addition, the samples were periodically exposed to 150 g h^{-1} of ozone gas for three hours a day during storage for ozone treatment. In the meantime, the control samples were stored in conventional room storage which was free from ozone.

The observation parameters included the seeds germination percentage (*SGP*), vigour index (*VI*), moisture content as well as protein and free fatty acids (FFA). The *SGP* and *VI* were observed using the International Rules for Seed Testing (ISTA, 2015) method with slight modifications. About 25 seeds were rolled in parchment paper, moistened, and placed in an upright position in the germinator, alternating between 12 h of light and 12 h of darkness for five days at a constant temperature of 25°C. Moisture, protein, and FFA content were measured using the American Oil Chemist Society's recommended procedures (AOAC, 2010). The gravimetric method was used to determine the water content, while the Kjeldahl method was used to determine the protein content. Observations were made monthly for six months of storage (0, 1, 2, 3, 4, 5, and 6 months).

The *SGP* was calculated at the end of germination (on the fifth day) with the following formula:

$$SGP(\%) = \frac{Number\,of\,germination\,seeds}{Total\,seed\,amount} 100\% \,. \tag{1}$$

The *VI* was determined from the number of normal sprouts in the first count with the following formula:

$$VI(\%) = \frac{Number\, of\, normal\, germination\, seeds\, in\, first\, count}{Total\, seed\, amount} 100\% \,. \quad (2)$$

The financial feasibility of ozone technology on aged soybean seeds was determined based on the ratio of revenue to cost (*R/C*), return on investment (*ROI*), and the payback period value. The financial feasibility variable was calculated using the model used by Cheng and Rosentrater (2017), as follows:

$$R/C = \frac{Total\, net\, income}{Total\, cost} \,, \quad (3)$$

$$ROI(\%) = \frac{Net\, profit}{Total\, capital\, investment} 100\% \,, \quad (4)$$

$$Payback\, period = \frac{100}{ROI} \,. \quad (5)$$

Statistical analyses were performed using SPSS 20.00 software (SPSS Inc., Chicago, IL, USA). A further analysis used Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ to determine the treatment differences. A polynomial regression was used to determine the trend of the changes in the quality parameters of aged soybean seeds due to exposure to ozone during storage. A polynomial regression analysis was used because linear regression was found to be unsuitable for representing trends. The regression model was selected based on the coefficient of determination (R^2).

3. RESULTS AND DISCUSSION

The results showed that the conditions and duration of storage had a significant effect on the seeds germination percentage and vigour index of the soybean seeds ($p \leq 0.01$) (Table 1). The storage conditions and duration interacted to a significant extent with the physiological parameters of the soybean seed ($p \leq 0.01$). However, no significant difference was found between the open and closed ozonated seeds for all of the seed physiological parameters examined. The *SGP* and *VI* values tended to increase gradually during storage. Previous studies also showed that the *SGP* value increased during dry storage at room temperature (Finch-Savage and Bassel, 2016) and ozonation conditions (Avdeeva *et al*., 2018; Dong *et al*., 2022).

Table 1. Analysis of variance (ANOVA) of aged soybean seed

Quality parameter	Experiment factor	df*	F value	p value
Seeds germination percentage	Storage condition	2	1 142 456	0.00
	Period	6	131 250	0.00
	Condition x Period	12	87 272	0.00
Vigour index	Storage system	2	838 981	0.00
	Period	6	107 742	0.00
	Storage x Period	12	67 206	0.00
Moisture	Storage system	2	728 812	0.00
	Period	6	1 004 176	0.00
	Storage x Period	12	52 193	0.00
Protein	Storage system	2	1 232 783	0.00
	Period	6	659 261	0.00
	Storage x Period	12	42 412	0.00
Free fatty acid	Storage system	2	573 112	0.00
	Period	6	347 745	0.00
	Storage x Period	12	25 829	0.00

*df – degree of freedom.

A polynomial regression with degrees of three confirmed the physiological changes which were indicated by the experimental data produced by the aged soybean seeds (Table 2). A high R^2 value indicates that the regression model correctly describes the trend of changes in the aged soybean seeds during storage. The *SGP* value of the ozonated seeds increased significantly up to the fifth month of storage, whereas that of the control samples decreased significantly with the storage period (Fig. 1). The *SGP* value did not vary significantly between the ozonated seeds in the open and closed containers ($p \geq 0.05$).

Ozone treatment can increase the seeds germination percentage by stimulating their internal energy and activating the antioxidant potential of seeds (Normov *et al*., 2019). It was also assumed that ozone activates the internal energy of seeds to form essential compounds for cellular biosynthesis, such as adenosine triphosphate (ATP) and its derivatives

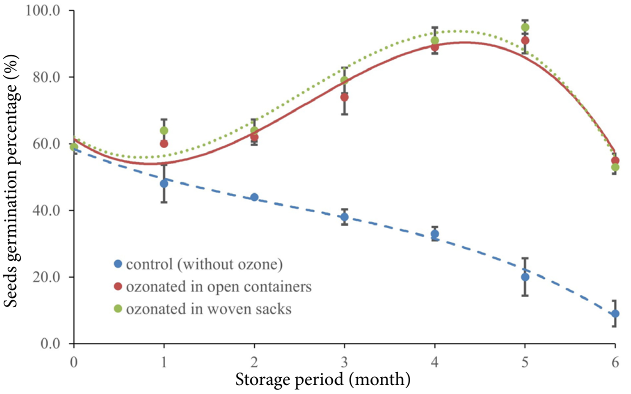

Fig. 1. Seeds germination percentage of aged soybean seeds stored under different conditions for a six month period.

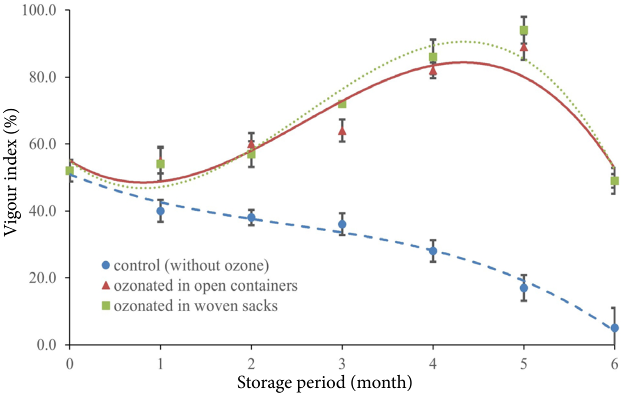

Fig. 2. Vigour index of aged soybean seeds stored under different conditions for a six month period.

Table 2. Adjusted regression models of aged seeds stored in different conditions

Variable	Storage	Equation	R^2
Seeds germination percentage	Ozonated, open container	$61.43 - 18.91x + 13.37x^2 - 1.72x^3$	0.929
	Ozonated, woven sack	$62.05 - 17.23x + 13.37x^2 - 1.78x^3$	0.904
	Control (without ozone)	$58.38 - 10.79x + 2.24x^2 - 0.31x^3$	0.994
Vigour index	Ozonated, open container	$55.05 - 17.24x + 12.64x^2 - 1.64x^3$	0.840
	Ozonated, woven sack	$54.95 - 21.29x + 15.48x^2 - 2x^3$	0.910
	Control (without ozone)	$50.91 - 10.74x + 2.82x^2 - 0.39x^3$	0.987
Moisture	Ozonated, open container	$11.43 - 1.27x + 0.12x^2$	0.976
	Ozonated, woven sack	$11.43 - 0.53x + 0.03x^2$	0.969
	Control (without ozone)	$11.75 - 0.53x - 0.001x^2$	0.966
Protein	Ozonated, open container	$38.60 - 3.66x + 0.16x^2$	0.985
	Ozonated, woven sack	$38.16 - 3.35x + 0.09x^2$	0.972
	Control (without ozone)	$39.36 + 0.533x - 0.29x^2$	0.997
Free fatty acid	Ozonated, open container	$0.25 - 0.026x + 0.002x^2$	0.972
	Ozonated, woven sack	$0.25 - 0.032x + 0.002x^2$	0.993
	Control (without ozone)	$0.25 - 0.004x - 0.0005x^2$	0.942

(Normov *et al.*, 2019), thereby increasing the rate of germination. Ozone triggers antioxidant activity to produce reactive oxygen species (ROS) through various defence mechanisms, anti-ageing, and healing abilities (Bhatia *et al.*, 2021). However, the *SGP* value of the ozonated seeds decreased significantly after six months ($p \leq 0.05$). A similar occurrence was also reported in corn seeds, it was found that further ozone exposure reduced the germination percentage (Normov *et al.*, 2019). This phenomenon indicates that the antioxidant system is no longer effective in controlling ROS levels, which results in a decrease in the germination percentage. Shingala and Dabhi (2022) also reported that ozone gas treatment increased the percentage of wheat germination to a peak and then it decreased with further treatment.

As was the case with the *SGP* value, the increase in the *VI* of the ozonated seeds was related to the antioxidant protection system released due to ozone exposure (Fig. 2). Both enzymatic and non-enzymatic antioxidants are essential in eliminating free radicals (Al-Aloosy *et al.*, 2019), the overall result is that the antioxidants released by the ozonated seeds trigger better vigour strength. However, the vigour index of the ozonated seeds also decreased significantly after the sixth month ($p \leq 0.05$). This occurrence indicates that exposure to ozone in the long term may harm the physiology of aged seeds by weakening their antioxidant activity and increasing the accumulation of ROS (Xin *et al.*, 2014), thereby resulting in a degradation of the *VI*. Therefore, an efficient antioxidant system is a prerequisite for inhibiting seed damage (Kaur *et al.*, 2021). This finding was consistent with the results of a study by Dong *et al.* (2022) that prolonged exposure to ozone may reduce vigour index and cause severe cell damage in seeds due to different levels of oxidative stress.

The moisture content, humidity, ozone concentration, pH, treatment time, form of application (gas/water form), and flow rate are some of the variables that affect ozone therapy (Sivaranjani *et al.*, 2021). The adjusted polynomial regression confirmed the physiochemical changes in moisture, protein, and FFA in the aged soybean seeds (Table 2). The storage conditions and storage duration had a significant effect on the moisture content, protein, and FFA of the soybean seeds stored for six months ($p \leq 0.01$) (Table 2). The results showed a significant interaction between the storage conditions and storage time on the soybean seed quality parameters ($p \leq 0.01$). However, no significant difference was found between the open and closed ozonated seeds with the exception of the moisture content for all of the seed quality parameters examined.

Moisture content is a crucial element in the effectiveness of ozone treatment (White *et al.*, 2013). This study reveals that the moisture content of soybean seeds in all treatments decreased significantly ($p \leq 0.05$) with the length of the storage time, and ozonated seeds in open containers were found to have the lowest moisture content (Fig. 3). The decrease in moisture

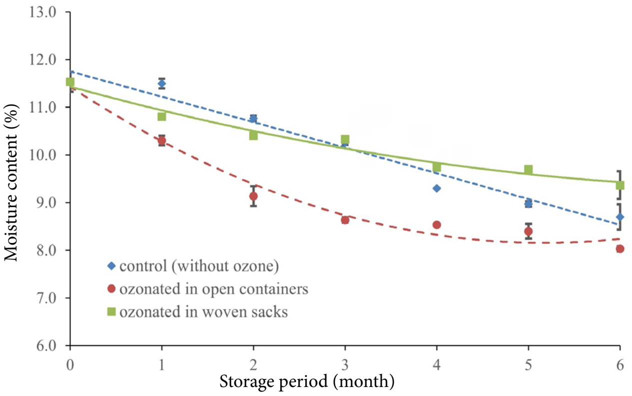

Fig. 3. Moisture content of aged soybean seeds stored under different conditions for a six month period.

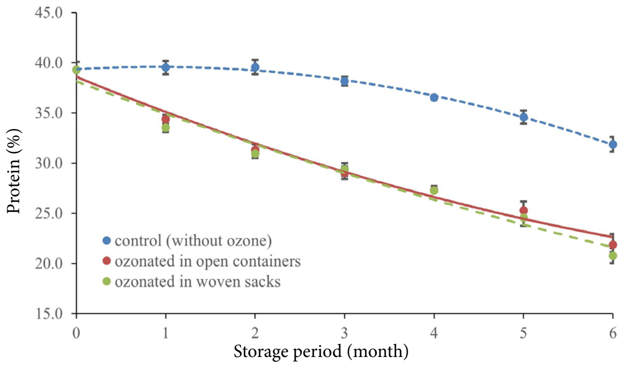

Fig. 4. Protein content of aged soybean seeds stored under different conditions for a six month period.

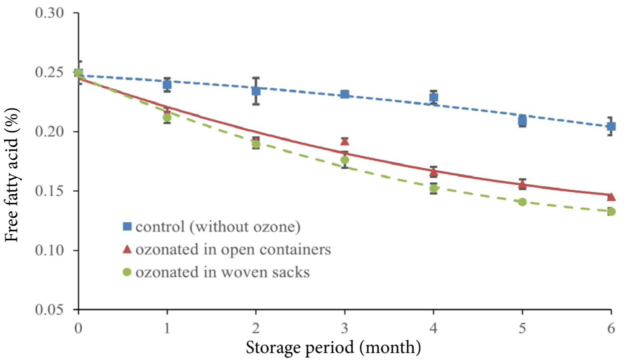

Fig. 5. FFA content of aged soybean seeds stored under different conditions for a six month period.

content during storage was caused by the movement of water from the seeds to the surrounding atmosphere in order to reach a hygroscopic equilibrium (Lamarca and Barbedo, 2012). This study revealed that low moisture content has a positive effect on ozone efficiency. This finding aligns with previous studies, which stated that ozone efficacy and grain moisture content have an inverse relationship (Pandiselvam *et al*., 2015; Sivaranjani *et al*., 2021). A higher moisture content reduces ozone efficiency in stored products (Pandiselvam *et al*., 2015).

The protein content of the soybean seeds also decreased significantly with more extended storage ($p \leq 0.05$), and the decrease in the protein levels for the ozonated seeds was sharper than that of the control sample (Fig. 4). This finding was consistent with the decreased protein levels due to ozone exposure in groundnuts (Sahab *et al*., 2013) and chickpea seeds (Nickhil *et al*., 2012). Ozone can reduce protein and amino acid levels (Baqasi *et al*., 2018). Ozone reactions with proteins can change the nature of polypeptide protein backbones, break peptide bonds, crosslink proteins, and modify amino acid side chains, which result in denaturation and to changes in protein solubility (Uzun *et al*., 2012), resulting in severe protein degradation in aged seeds. It was suspected that ozone stimulates the involvement of seed storage proteins in metabolic pathways and also enzyme synthesis to support germination and seed vigour, leading to a decrease in total protein content. Ehrhardt-Brocardo *et al*. (2022) assumed that there was a relationship between the mobilization of seed storage protein and the physiological quality of common beans.

Likewise, the FFA content decreased in all samples ($p \leq 0.05$) (Fig. 5). The reduction in FFA is thought to be related to the degradation of lipase activity and the inactivation of hydrolytic enzymes during low-temperature storage (Mohammadi *et al*., 2021) or to degradation through oxidative reactions during storage (Toci *et al*., 2013). Ozone can modify the lipase structure of the active site and inactivate some lipase enzymes in seeds (Obadi *et al*., 2018). Thus, it was found to be plausible that the FFA reduction in the ozonated seeds was higher than that of the control samples.

Table 3. Financial feasibility analysis of ozone application in aged soybean seeds over a five month storage period

Component	Number of unit	Unit price (USD)	Amount (USD)
Fixed cost			448.40
Depreciation of building (10 m^2)	1	84.03	84.03
Depreciation of equipment and ozone generator	1	364.37	364.37
Variable cost			12 746.30
Aged soybean seeds (kg)	25 000	0.50	12 605.04
Electricity (month)	5	16.81	84.03
Labour (person day^{-1})	5	13.45	67.23
Total cost (capital investment)			13 204.71
Revenue	24 500	0.94	23 058.82
Profit			9 854.12
R/C ratio			1.75
Return on investment (%)			74.63
Payback period (years)			1.34

Noted: 1 USD = IDR 14 875.

An analysis of the financial feasibility of ozone application on aged soybean seeds was calculated for a five month period based on its technical feasibility (Table 3). The investment allocated for the building (10 m^2) was approximately USD 2016.81, and the complete ozone generator with installation was approximately USD 4369.75. Each component has a different service life, subject to depreciation costs. The useful life of the building was assumed to be ten years, while the ozone generator and installation were assumed to have a useful life of five years. An operational cost of approximately USD 12756.30 was used to purchase aged soybean seeds, electricity services and labour. Based on baseline data, the initial price of the aged soybean seeds with an *SGP* of 52-60% was USD 0.50 kg^{-1}. At the end of the storage period after ozone treatment, the price of the aged soybean seeds becomes IDR 0.94 kg^{-1} as the *SGP* increases to 95%.

The estimated loss during ozonation storage was around 2%, thereby reducing the volume of seeds stored from 25000 to 24500 kg at the end of the storage period. However, the application of ozone for the storage of aged soybean seeds was found to be financially feasible, with an *R/C* value of 1.73 and an *ROI* of 74.63%. Also, the payback period was 1.34 years, this indicates that the capital investment obtained for the ozonated aged soybean seed storage will be returned in approximately one year. The application of ozone in seed storage is recommended for producing high-quality seeds (Baskakov *et al.*, 2020) and it has a high degree of potential for increasing environmentally friendly plant production (Sharaf-Eldin *et al.*, 2022).

4. CONCLUSIONS

Ozone effectively extends the shelf life of aged soybean seeds by up to five months. Ozone exposure triggers the antioxidant defence system and healing mechanism in aged soybean seeds, this is characterized by increased viability and a reduced FFA content. The intense protein decrease in ozonated aged soybean seeds is thought to be due to the activity of antioxidant proteins in fighting free radicals, maintaining ROS homeostasis, and restoring oxidative damage. The increase in the viability of ozonated aged soybean seeds contributes to a rise in the selling price of the seeds, this means that the use of ozone technology is financially feasible with regard to its implementation by seed producers.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

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