



Review

The use of ozone gas for the control of insects and micro-organisms in stored products



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ABSTRACT

In this review paper, we analyze the potential of using ozone as an alternative pest control technology for use in stored products, and we also illustrate data on the efficacy of ozone against micro-organisms and its effect on qualitative characteristics and properties of the treated commodities. Ozone application is currently attracting attention, particularly due to the fact that a) there are no residues on the product and b) there is no need for aeration to remove the gas. Novel industrial applications and improvements in ozone technology together with new regulatory actions worldwide have emerged in recent years, making its use in the food industry easier and applicable in a wide range of cases. This review paper presents available literature on ozone in relation to its use against pests and other organisms, but also to its different application techniques and for large scale viability.

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1. Introduction

Control of insect pests in stored products is becoming increasingly difficult, partially due to the development of pesticide resistance in some species, but also due to the cryptic behavior of some major stored product species (Subramanyam and Hagstrum, 1996). Furthermore, the number of pesticides available for treatment of stored products is decreasing due to environmental and safety concerns and the consumers' demand for residue-free food (Phillips and Throne, 2010). With limited control options and the increased potential for resistance to insecticides (Zettler and Cuperusi, 1990; Benhalima et al., 2004; Lorini et al., 2007; Opit et al., 2012), additional methods are needed for management of insects in stored products. One potential candidate is ozone, which has proven effective against a wide range of storage insect pests and applicable in various stored commodities (Erdman, 1980; Strait, 1998; Kells et al., 2001; Leesch, 2003; Zhanggui et al., 2003; Isikber and Oztekin, 2009).

Ozone (O₃) is highly reactive and a strong oxidizing agent, which is classified as "GRAS" (Generally Recognized As Safe) by the United States Environmental Protection Agency (USEPA). Throughout the

world, ozone has been used to purify drinking water, kill bacteria, sanitize food, deodorize, and decrease aflatoxin contamination (Prudente and King, 2002; Sopher et al., 2002; Inan et al., 2007; Tiwari et al., 2010; White et al., 2010). Ozone is able to penetrate large masses of grain, is highly unstable and decomposes rapidly to oxygen without leaving residues (Kells et al., 2001; Mason et al., 1997; Pimentel et al., 2009; McDonough et al., 2011b). These attributes make ozone an attractive candidate for controlling insects and fungi in stored products (Mason et al., 1997; Kells et al., 2001). Ozone in its gaseous form kills insects in various commodities and this activity has been thoroughly evaluated by many researchers under laboratory and field conditions, with various application techniques (Erdman, 1980; Mason et al., 1997; Kells et al., 2001; Athanassiou et al., 2008; Pimentel et al., 2009; McDonough et al., 2010, 2011a,b). This paper reviews the efficacy of ozone for the storage and preservation of stored commodities, the effect of ozonation on product quality and the current status of ozone application in stored product protection strategies.

2. Characteristics of gaseous ozone

Ozone, an allotropic form of oxygen, is a triatomic molecule (O₃) that in its pure, concentrated, gaseous form has a pale blue color and a pungent characteristic odor (Weavers and Wickramanayake, 2001; Kim et al., 1999). In nature, small amounts of ozone (0.05 mg/L) are formed in the stratosphere, at 15–35 km altitude, by the reaction of solar ultraviolet radiation (<240 nm) with molecular

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oxygen (Horváth et al., 1985; Kim et al., 2003). The troposphere (<15 km altitude) contains approximately 10% of atmospheric ozone, and only a slight proportion of it is present on the surface of Earth (Wojtowicz, 1996). Ozone has a molecular weight of 48, a boiling point of $-111.9\text{ }^{\circ}\text{C}$, and a melting point of $-192.7\text{ }^{\circ}\text{C}$ at atmospheric pressure (The Merck Index, 2013). In addition, gaseous ozone has a higher density (2.14 g/L) than air (1.28 g/L) (Wojtowicz, 1996). The oxidation potential of ozone (2.07 V) is higher than that of hypochlorous acid (1.49 V) or chlorine (1.36 V) (Brady and Humiston, 1978). Ozone is partly soluble in water with a solubility ratio of 0.31–1.13 depending on water temperature (Horváth et al., 1985). Solubility of gaseous ozone in water increases at high pressure and decreases in the presence of ions, and at high pH (Kim et al., 2003). Generally, ozone in the stratosphere is considered as a “filter” for the harmful solar radiation, but at the same time, ozone is a serious pollution agent in the troposphere (Fiore et al., 2002).

Gaseous ozone is more stable than aqueous ozone. Half-life of gaseous ozone is 12 h at atmospheric pressure, and its decomposition depends on reactivity with surfaces, temperature, concentration, and pressure (Koike et al., 1998; Weavers and Wickramanayake, 2001). Conversely, the half-life of ozone in aqueous phase varies from 2 to 65 min in distilled water at $-20\text{ }^{\circ}\text{C}$ (Wynn et al., 1973; Wickramanayake, 1984; Graham, 1997). Stability of aqueous ozone depends on the presence of ozone demanding material in water, as well as ozone concentration, temperature, pH, UV light, and presence of metal ions and radical scavengers (Horváth et al., 1985; Weavers and Wickramanayake, 2001; Kim et al., 2003). Decomposition of aqueous ozone occurs in a stepwise mode, producing free radical species such as hydroperoxyl (HO_2^{\cdot}), hydroxyl (OH), and superoxide ($\text{O}_2^{\cdot-}$) (Hoigné and Bader, 1975; Grimes et al., 1983). These free radicals have a strong oxidizing power and a half-life of microseconds, and are responsible for the high reactivity of ozone (Khadre et al., 2001).

3. Toxicity and susceptibility of insects to gaseous ozone

Several studies have established that ozone treatments can kill stored product insects, including the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), the rice weevil, *Sitophilus oryzae* (L.), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the confused flour beetle *Tribolium confusum* Jacquelin du Val, the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), the Indianmeal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), and the Mediterranean flour moth *Ephesthia kuehniella* (Zeller) (Strait, 1998; Kells et al., 2001; Leesch, 2003; Zhanggui et al., 2003; Athanassiou et al., 2008; Isikber and Oztekin, 2009). The comparative efficacy of ozone against stored product insects under laboratory conditions is given Table 1. Based on the mortality results of various stored product insects presented in Table 1 it is obvious that ozone concentration and exposure time play an important role in ozone efficacy against stored product insects. The successful application of ozone technology requires a sufficient concentration of ozone for an appropriate amount of time, which varies according to the conditions prevailing. In laboratory bioassays, Athanassiou et al. (2008) reported that at 115 ppm, mortality of *R. dominica* and *S. oryzae* adults was close to 60% after 2 h of exposure, while 2 h later, all adults were dead. In the same study, mortality of *T. confusum* adults was extremely low after 2 h of exposure, but reached 100% at the 6 h interval. McDonough et al. (2011b) developed a model for insect mortality calculations based on CT (concentration \times exposure time) values, which are the products of the ozone concentrations (C) multiplied by the exposure time (T) needed to obtain a desired mortality level. In that study, the authors

Table 1

Summary of efficacy of empty space ozone treatment against stored product insects in laboratory studies.

Insect species	Concentration and exposure time	Stage	Mortality rate (%)	References
<i>Oryzaephilus surinamensis</i> ^a	5 ppm 3 days ^a	Adult	100% ^{a,b}	Mason et al. (1997)
<i>Tribolium confusum</i> ^b	5 days ^b	Adult	92.2% ^c	Kells et al. (2001)
<i>Tribolium castaneum</i> ^c	50 ppm	Adult	100% ^d	
<i>Sitophilus zeamais</i> ^d	3 days		94.5% ^e	Isikber and Oztekin (2009)
<i>Plodia interpunctella</i> ^e		Larva	100% ^b	
<i>Tribolium confusum</i> ^b	6482 ppm	Larva	87% ^f	Isikber and Oztekin (2009)
<i>Ephesthia kuehniella</i> ^f	(13.88 mg/L) 2 h	Pupa	100% ^b	
		Egg	17% ^f	McDonough et al. (2011b)
		Egg	3% ^b	
		Adult	64% ^f	McDonough et al. (2011b)
		Adult	100% ^b	
			4% ^f	McDonough et al. (2011b)
<i>Plodia interpunctella</i> ^e	1800 ppm	Larva	66.9% ^e	
<i>Tribolium castaneum</i> ^c	1 h	Larva	92.1% ^c	McDonough et al. (2011b)
		Pupa	92.9% ^e	
		Egg	79.2% ^c	Leesch (2003)
		Egg	66.9% ^e	
		Adult	88.3% ^c	Leesch (2003)
		Adult	100% ^e	
			84.3% ^c	Leesch (2003)
<i>Plodia interpunctella</i> ^e	170 ppm 4 h	Larva	10%	
	300 ppm 4 h	Pupa	98%	Hansen et al. (2013)
<i>Plodia interpunctella</i> ^e	33–37 ppm 6 days	Larva	100%	
		Pupa	100%	Hansen et al. (2013)
		Egg	100%	
		Adult	10%	Hansen et al. (2013)
<i>Tribolium confusum</i> ^b	35–42 ppm 6 days	Larva	100%	
		Pupa	100%	Hansen et al. (2013)
		Egg	100%	
		Adult	100%	Hansen et al. (2013)
		Adult	100%	
<i>Sitophilus oryzae</i> ^g	125 ppm 6 h	Adult	100% ^g	Athanassiou et al. (2008)
<i>Rhyzopertha dominica</i> ^h		Adult	100% ^h	
<i>Tribolium confusum</i> ^b		Adult	100% ^b	Athanassiou et al. (2008)

^a *Oryzaephilus surinamensis*.

^b *Tribolium confusum*.

^c *Tribolium castaneum*.

^d *Sitophilus zeamais*.

^e *Plodia interpunctella*.

^f *Ephesthia kuehniella*.

^g *Sitophilus oryzae*.

^h *Rhyzopertha dominica*.

calculated the CT values for ozone treatments required to achieve 100% mortality, and found that *T. castaneum* requires longer exposure or higher ozone concentrations than *P. interpunctella* (a mean exposure of 256,500 ppm-min and 183,000 ppm-min, respectively). Sousa et al. (2008) also conducted laboratory experiments with *T. castaneum* adults, treating these insects in plastic chambers with 150 ppm ozone to achieved 95% mortality with a treatment time between 23.35 h and 31.98 h (CT $\frac{1}{4}$ 210,150 ppm-min and 287,820 ppm-min, respectively). Kells et al. (2001) treated caged *T. castaneum* adults buried in corn with 50 ppm ozone. After a 3-d treatment, the authors obtained 92% mortality for a CT value of 216,000 ppm-min, which also agrees with the results reported by McDonough et al. (2011a,b) and Sousa et al. (2008). Similar results have been also reported by Athanassiou et al. (2008), where it was found that *T. confusum* adults were less susceptible to ozone than adults of *S. oryzae* or *R. dominica*. These studies confirm that *Tribolium* spp., at the adult stage, can be classified among the least susceptible stored product species to ozone. Nevertheless, even in this case, mortality occurs in a relatively short interval (usually at 24 h or earlier), which is by far shorter than the exposures that are needed for phosphine, and comparable with those required for methyl bromide, or other newer fumigants, such as sulfuryl fluoride

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