International Food Research Journal 23(6): 2545-2556 (December 2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Ozone against mycotoxins and pesticide residues in food: Current applications and perspectives

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Article history

Received: 1 December 2015
Received in revised form:
20 February 2016
Accepted: 27 February 2016

Keywords

Food safety
Chemical contaminants O_3
Gaseous ozonation
Ozonized water

Abstract

Food safety may be compromised by the presence of chemical contaminants, such as mycotoxins and pesticide residues. Mycotoxins are natural contaminants produced by certain species of filamentous fungi and can cause toxic effects on human health. Pesticide residues are any specified substance in food resulting from the use of a pesticide with toxicological significance. To protect consumers from these toxic substances, different food regulatory agencies have set maximum levels permitted in different raw materials and processed foods. However, recent research has demonstrated a high incidence of both mycotoxins and pesticide residues (not simultaneously) in foods marketed all around the world, sometimes with levels above the regulated limits. One way to reduce such contaminants is to use ozone (O_3) in food processing. Due to its high potential as an oxidant, O_3 or the radicals generated in the ozonation process react with mycotoxins and pesticide residues that lose their toxicity due to molecular degradation. In this review paper the recent research into using O_3 for gaseous ozonation and ozonized water to decontaminate food by eliminating and/or reducing mycotoxins and pesticide residues are discussed. Also the changes promoted in food quality attributes, the possible formation of degradation products of toxic relevance, as well as some perspectives for the future use of this technology in food processing are explored.

Introduction

Every day more and more consumers, worldwide, are becoming aware of food safety and the risks associated with its contamination by microorganisms and by toxic compounds (Kher *et al*., 2013). The presence of contaminants in food, such as pesticide residues or mycotoxins, raises concerns in terms of public health and food safety.

Pesticides are used in agriculture to improve productivity by protecting crops from disease and infestation. However, they must be applied in accordance with the Good Agricultural Practices (GAPs) and the levels present in foods must be below the Maximum Residue Levels (MRLs). MRLs of pesticide vary greatly worldwide, because countries have different requirements and different legal limits (EFSA, 2015; Handford *et al.*, 2015).

Nowadays, the high global usage of pesticides, approximately two million tons per year (De *et al.*, 2014), allied with the increased resistance of pests and pathogens, has posed a renewed concern on the use of pesticide in food (Liu *et al*., 2015). Despite the surveillance carried out by the competent authorities, recent research has shown significant incidence of pesticide residues at levels above the MRLs in various foods, such as: vegetables (Akoto *et al.*, 2015; Chourasiya *et al*., 2015), coffee (Oliveira *et al*., 2016), honey (Bargańska *et al*., 2013; López *et al*., 2014) apples (Lozowicka, 2015), guava, kaki and peach (Jardim *et al*., 2014) and cereal grains (Min *et al*., 2012; Lozowicka *et al*., 2014).

The ingestion of food contaminated with pesticide residues is associated with endocrine, reproductive and nervous disorders, as well as the risk of cancer (US EPA, 2014; Blaznik *et al*., 2015; Chiu *et al*., 2015). This contamination is of particular concern for infants and adolescents due to their lower detoxification capacity and high intake of food per kg body weight (Lombard, 2014).

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Mycotoxins are other food chemical contaminant with a rising concern in public health. Mycotoxins are harmful metabolites produced by a number of filamentous fungi, such as, *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. that may contaminate food in the field and/or when improperly stored (EFSA, 2013; Cheli *et al*., 2014). Their effects on human health depend on the mycotoxin and the level consumed. These effects may vary from mild intoxication to cancer and death (IARC, 2002; Marroquín-Cardona *et al*., 2014). GAPs include procedures to prevent infestation by fungi in stored products and avoid consequent contamination by mycotoxins; however, as they are natural contaminants, all humans and animals are exposed to such hazard. Recent studies, using urinary and breast milk biomarkers, carried out all over the world, showed a high human exposure to different mycotoxins, such as aflatoxins, ochratoxin A, zearalenone and deoxynivalenol (Srey *et al*., 2014; Iha *et al*., 2014; Rubert *et al*., 2014; Gerding *et al.*, 2015).

Recent research has demonstrated that ozonation can be used to decontaminate and remove mycotoxins and pesticide residues in food, especially in fresh fruits, vegetables and grains. The molecular ozone (O_3) or the hydroxyl radicals generated in the process, especially in ozonized water, react with these contaminants promoting their degradation and form lower molecular weight products, thus eliminating or reducing the biological activity of these contaminants in terms of toxicity (Ikehata *et al*., 2006; Diao *et al*., 2012; Luo *et al*., 2014a). The United States Food and Drug Administration (FDA) has recognized, since 2001, that O_3 is GRAS (Generally Recognized as Safe) for the treatment, storage and processing of food and water (FDA, 2001). The advantages of using O_3 to decontaminate food products over other oxidants is that it is environmental friendly to produce and its use does not leave any residues in the food, as the O_3 dissociates into oxygen. Consequently it is recognized as a “green technology” (O’Donnel *et al*., 2012; Greene *et al*., 2012).

Ozonation efficacy to degrade mycotoxins and pesticide residues depends on the O_3 concentration, exposure time, type of food, moisture content, mode of application (gas or water), among others factors. Moreover, ozonation may cause positive changes in the quality of the food, such as, increasing the volume of breads and cakes or increasing strength and clarity of flours (Caballero *et al*., 2007; Li *et al*., 2012). However, as it is not a universally beneficial process, O_3 can also cause negative alterations, such as oxidation of lipids, changes in sensory characteristics, color loss, degradation of phenolic compounds and some vitamins, among other adverse effects (Patil *et al*., 2010; Gabler *et al*., 2010). These negative effects must be studied in greater depth to define the limitations of this technology.

This paper reviews the most recent studies on the reduction of mycotoxins and pesticide residue levels promoted by ozonation, including changes in the quality of the food due to its treatment. The possible formation of degradation products of toxic relevance and the future prospects for scientific research and the industrial use of O_3 in the food science and technology field are also discussed.

Industrial production of O_3 and legislation for food processing

Ozone must be produced on-site for immediate use in ozonation processes due to its instability and rapid dissociation into O_2. When O_3 is used to decontaminate food, it is usually produced with ozonizers based on corona discharge. Ozonizers expose O_2 molecules to a high voltage electrical discharge which initiates the formation of free radical oxygen and thereby generates O_3 (WCBBC, 2006). The corona discharge method can obtain high concentrations of O_3 at a low cost; however, UV radiation can also be used for commercial production of O_3 but with a lower concentration and yield (Tapp and Rice, 2012).

Ozone can be applied in the gaseous form directly into the food, as occurs in cereal grains, or it can be bubbled into water to produce ozonized water, which is especially suitable for the raw materials that require an aqueous disinfection step (Coelho *et al*., 2015). In the gaseous form, the half-life of ozone is a few hours in the presence of food, however, in still air at 0% humidity it can have a half-life of up to 25 h (McClurkin *et al*., 2013). When bubbled in water, O3 dissolves partially, forming hydroxyl radicals (OH^-) that can oxidize the contaminants more efficiently than molecular O_3 (Takahashi *et al*., 2007). Ozonized water can be used for washing a variety of foods, such as fruits and vegetables, and it can even be used on cereal grains that require water as a conditioning step (tempering) prior to the milling process, like wheat grains (Ibanoglu, 2001).

There are no concentration limits for the application of O_3 in food; however, as it is a GRAS substance its concentration should be as low as reasonably achievable (ALARA) and also in accordance with the Good manufacturing practices of the food industry. The American conference of governmental industrial hygienists set a limit of 0.2 mg/m^3 of O_3 exposure for an 8-hour-day (FDA, 2014), while the World Health Organization (WHO) recommends 0.1 mg/m^3 for an 8-hour mean (WHO, 2006). As O_3 is a toxic gas, levels higher than these limits may cause undesirable physiological effects on the central nervous system, heart, and vision (PubChem, 2015).

Current concerns related to mycotoxins and the use of O_3 to reduce food contamination

The contamination of food with mycotoxins is a serious public health concern and it can lead to many health problems due to the diverse toxic effects promoted by these substances, such as, cytotoxicity, genotoxicity, immunotoxicity, carcinogenic or teratogenic effects (Stoev, 2015). The main mycotoxins related to food contamination are the aflatoxins, fumonisins, zearalenone, citrinin, patulin, ochratoxin A, deoxynivalenol and other trichothecenes (Rocha *et al*., 2014; Wu *et al*., 2014).

In cereal grains, *Fusarium* toxins, such as trichothecenes, zearalenone and fumonisins, are the most commonly detected, particularly in the pre-harvest phase (De Ruyck et al., 2015). Trichothecenes, like deoxynivalenol (DON), are known for their strong capacity to interfere with protein synthesis and induce immunosuppression (Antonissen *et al*., 2014). Fumonisins are associated to esophageal cancer and can also interfere in the biosynthesis of sphingolipids with consequent cell activity disorders. Zearalenone is a potent estrogenic metabolite and can cause infertility, abortion and other reproduction problems (Yazar and Omurtag, 2008).

Aspergillus spp. and *Penicillium* spp. fungi are of great importance during food storage. If adequate moisture and temperature conditions exist, *Aspergillus* spp. can produce mycotoxins, such as aflatoxin, especially in oilseeds and cereals (Gorayeb *et al*., 2009). Aflatoxins (AF) are one of the most important environmental toxins and the AFB_1 is the mycotoxin with the most toxicity in this group, with hepatocarcinogenic and immunosuppressive activities (Magnussen, 2013). Both *Aspergillus* spp. and *Penicillium* spp. may produce ochratoxins that mainly contaminate cereals, but they can also contaminate grapes and their derivatives, like wine. Ochratoxin A is the most relevant in this group and has been reported to be nephrotoxic and carcinogenic to humans (Sorrenti *et al*., 2013). These fungi may also produce patulin in apples, which is a genotoxic and cytotoxic substance (Glaser and Stopper, 2012). Certain species of *Penicillium, Aspergillus* and mainly *Monascus* may produce citrinin in rice, which has nephrotoxic, hepatotoxic and carcinogenic activity (Li *et al*., 2012b). Figure 1 illustrates the molecular structure of the main mycotoxins that occur in food.

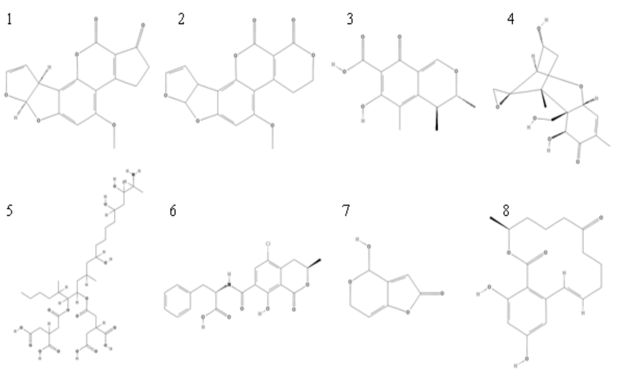


Figure 1. Molecular structures of the main mycotoxins found in food. 1- aflatoxin B1, 2- aflatoxin G1, 3- citrinin, 4- deoxynivalenol, 5- fumonisins B1, 6- ocratoxin A, 7- patulin, 8- zearalenone. Reference: Adapted from PubChem (2015).

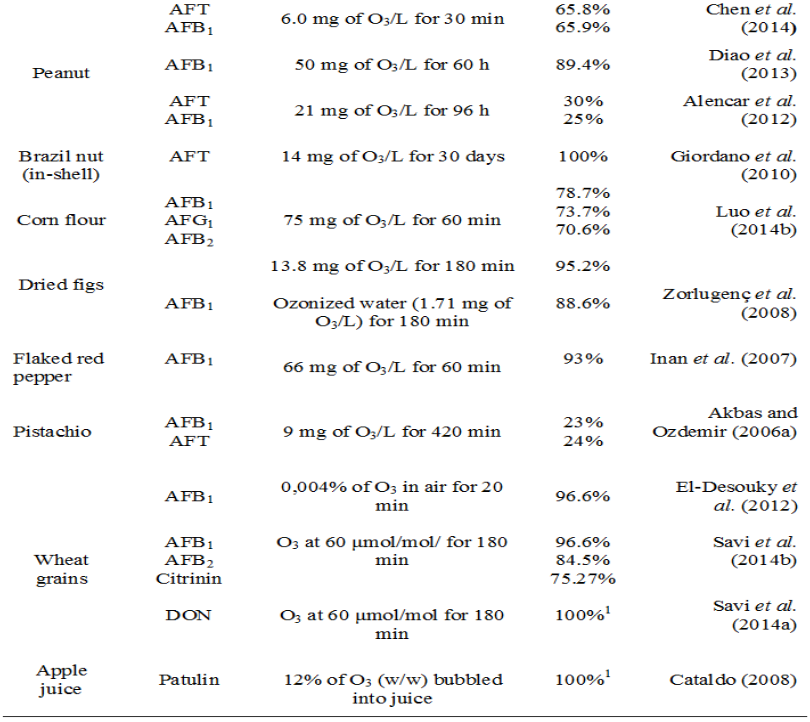
The production of mycotoxins depends on the environmental conditions during plant growth and subsequent food storage, consequently their presence is sometimes unavoidable in food (Stoev, 2015). Mycotoxins are stable to tradicional industrial processes applied to raw materials; thus if the raw food is contaminated, these mycotoxins will also be present in the processed foods, posing a health risk to consumers (EFSA, 2013; Tibola *et al*., 2015).

When O_3 is applied at low concentrations to the storage of fruits and cereals with a long exposure time it can control or inhibit growth, germination and sporulation of fungi, thus preventing the production of their toxins (Giordano *et al*., 2012; Feliziani *et al*., 2014; Hansen *et al*., 2013). However, these effects are very dependent on the fungal species, growth stage, O_3 concentration and exposure time (Freitas-Silva and Venâncio, 2010).

On the other hand, to promote the molecular degradation of mycotoxins, high concentrations levels of O_3 are needed. The exposure time, type of food, moisture content and temperature are also factors that directly affect the efficacy of this decontamination (Ikeura *et al*., 2011; Li *et al*., 2014). The degradation rates of mycotoxins by O_3 reported in the literature vary widely due to the different experimental conditions used in each study. Table 1 summarizes some recent experimental studies involving mycotoxin decontamination by ozonation in different kinds of foods, including the conditions applied and the degradation percentage obtained.

Besides experimental laboratory studies, McDonough *et al.* (2011) evaluated the use of ozonation on a commercial scale by applying O_3 at 4.7% in air to corn kernel using a continuous-flow system. The O_3 was delivered into a screw conveyor with retention time for the grains moving through the system equal to 1.8 min. Under these conditions

Table 1. Summary of some recent studies involving mycotoxin decontamination in food by O_3



Food	Mycotoxin	Conditions	Reduction levels (%)	Reference
Peanut	AFT AFB_1	6.0 mg of O_3/L for 30 min	65.8% 65.9%	Chen *et al.* (2014)
	AFB_1	50 mg of O_3/L for 60 h	89.4%	Diao *et al.* (2013)
	AFT AFB_1	21 mg of O_3/L for 96 h	30% 25%	Alencar *et al.* (2012)
Brazil nut (in-shell)	AFT	14 mg of O_3/L for 30 days	100%	Giordano *et al.* (2010)
Corn flour	AFB_1 AFG_1 AFB_2	75 mg of O_3/L for 60 min	78.7% 73.7% 70.6%	Luo *et al.* (2014b)
Dried figs	AFB_1	13.8 mg of O_3/L for 180 min	95.2%	Zorlugenç *et al.* (2008)
		Ozonized water (1.71 mg of O_3/L) for 180 min	88.6%	
Flaked red pepper	AFB_1	66 mg of O_3/L for 60 min	93%	Inan *et al.* (2007)
Pistachio	AFB_1 AFT	9 mg of O_3/L for 420 min	23% 24%	Akbas and Ozdemir (2006a)
Wheat grains	AFB_1	0,004% of O_3 in air for 20 min	96.6%	El-Desouky *et al.* (2012)
	AFB_1 AFB_2 Citrinin	O_3 at 60 μmol/mol/ for 180 min	96.6% 84.5% 75.27%	Savi *et al.* (2014b)
	DON	O_3 at 60 μmol/mol for 180 min	100%[1]	Savi *et al.* (2014a)
Apple juice	Patulin	12% of O_3 (w/w) bubbled into juice	100%[1]	Cataldo (2008)

[1] Lower than the Limit of Detection (LOD) for the method used. AF: Aflatoxins. AFT: Total aflatoxins. O_3: Ozone.

there was an approximate reduction of 30% in the aflatoxins.

Some studies have demonstrated the efficacy of O_3 to degrade mycotoxin standards in solution, which is also a method that helps explain how O_3 promotes the mycotoxin degradation. Young *et al.* (2006) studied the effects of ozonized water at 25 ppm in the degradation of DON, nivalenol (NIV) and other trichothecenes. These authors concluded that the degradation begins with attack of the C9-10 double bond by O_3 causing the mycotoxin to breakdown into organic acids, aldehydes, and ketones. This process effectively reduced the trichothecenes levels in solution. Dudziak (2012) studied zearalenone degradation by ozonized water at 1 mg/L for 20 min. This treatment reduced the concentration of the mycotoxin to undetectable levels. The authors concluded that the use of a high exposure time contributes to a more effective degradation. Freitas-Silva (2011) also demonstrated the potential of ozonized water at 20 mg/L to degrade a solution of cyclopiazonic acid. The author point out that the ozonized water can decontaminate not only mycotoxin standards with efficacy but also raw materials, laboratory equipment and reagents for disposal.

In order to study the practical use of O_3 treatment, besides the proven reduction of mycotoxins levels that can be verified by analytical techniques, it is essential to identify the molecular products formed by the degradation of mycotoxins, and to have knowledge of their toxicity. Luo *et al.* (2014a) identified six degradation products of aflatoxin B_1 by ozonation in aqueous solution at different O_3 concentrations, through the structure–activity relationship, authors confirm that the ozonation eliminated the toxicity of aflatoxin B_1. According to these authors, the unsaturated molecules are more easily attacked by O_3, while the saturated ones are more resistant to detoxification. Diao *et al.* (2012) reported that the oxidation of AFB_1 in acetonitrile, using gaseous ozone at 6.28 of O_3 mg/L, formed thirteen degradation products and it eliminated the molecular structure of AFB_1 responsible for its toxic effects. According to the authors, the toxicity of aflatoxin was significantly reduced because of the disappearance of the double bond on the terminal furan ring or the lactone moiety on the benzene ring.

Another way to verify if ozonation can eliminate or significantly reduce the toxic effects of food contaminated by mycotoxins was demonstrated using animal models. Diao *et al.* (2013) showed that peanuts contaminated with AFB1 and then ozonized at 50 mg of O_3/L for 60 h did not cause any symptoms of toxicity or changes in the appearance and behavior of female Wistar rats. These authors showed that the deleterious effects of AFB_1 were reduced by the ozonation to such an extent that the health of the animals was not affected. Gaou *et al.* (2005) investigated if the treatment of wheat grains with 5 g of O_3/kg grains would promote adverse effects on the health of Dark Agouti rats due to grain consumption. After 4 weeks, clinical, hematological, biochemical blood, urinary and histopathological determinations were not significantly affected; thus the consumption of the ozonized grains was considered safe.

Luo *et al.* (2014c) studied the toxicity of the degradation products from AFB_1 formed in artificially contaminated ozonized corn using the human hepatocellular carcinoma cell line (HepG2) as the model. The authors reported that the toxicity of ozone-treated corn had no significant difference with the corn free of mycotoxins. These different techniques indicate that the gas ozonation process and the use of ozonized water are effective to reduce food contaminated by mycotoxins and that they do not produce degradation products of any known relevant toxicity.

Applications of O_3 to reduce pesticide residues in food

Pesticide residues are any specified substance in food, agricultural commodities, or animal feed resulting from the use of a pesticide, including its conversion products, metabolites, reaction products and impurities considered to be of toxicological significance (FAO, 2001). The impact on human health due to the use of pesticides in agriculture is of increasing concern in the eyes of the public due to the evidence between human exposure to these residues and chronic diseases, such as cancers, diabetes, neurodegenerative, as well as birth and reproductive disorders (Mostafalou and Abdollahi, 2013; Parrón *et al*., 2014).

Food containing pesticide residues is a direct source of exposure and although industrial or domestic processing of food, such as peeling and cooking, can help reduce the contamination, these residues will partially remain in the final product, representing a health risk if ingested in high concentrations (Kaushik *et al*., 2009). The MRLs for pesticides in food samples are regulated throughout the world, and are basically concerned with the quality, efficacy and safety in the use of pesticides; however, there is not a global harmonized legislation (Malik *et al*., 2010). In general, developed nations have more stringent regulations than developing countries, which lack the resources and expertise to adequately implement and enforce regulation, posing a technical barrier to trade and to public health protection (Handford *et al*., 2015).

In recent years, powerful analytical methods, especially mass spectrometry, have played a vital role in the identification and quantification of these substances in a variety of matrices (Malik *et al*., 2010; Romero-González, 2015). These up-to-date tools have identified pesticide residues in human blood, urine, breast milk and hair by various authors, indicating a high level of exposure to humans worldwide (A El-Morsi and Rahman, 2012; Dewan *et al*., 2013; Yusa *et al*., 2015).

The number of studies investigating the potential use of ozonation to reduce pesticide residue levels in food has increased in recent years, especially studies concerning ozonized water to wash vegetables and gas ozonation to treat cereals. The degradation of these pesticide residues can be carried out via the molecular O_3 reaction pathway with the food, which gives rise to selective reactions, especially with unsaturated and aromatic hydrocarbons; or, by an indirect pathway, involving radicals of higher oxidation potential that can attack organic and inorganic molecules with non-selective reactions (Ikehata *et al*., 2006; Ormad *et al*., 2008).

For the treatment of drinking water, more drastic conditions of ozonation in terms of concentrations and exposure time may be applied compared with the treatment for food. Lafi and Al-Qodah (2006) studied ozonation associated with UV radiation for the treatment of drinking water and reported a 100% degradation of the insecticide deltamethrin and also an 80% reduction of halogenated and non-halogenated compounds. Ormad *et al.* (2008) investigated the reduction of 44 pesticides by chlorine or O_3. These authors concluded that ozonation was more efficient than chlorine as it eliminated about 70% of the pesticides studied, and with an advantage over chlorine as it did not form trihalomethanes after the treatment. When these authors incorporated a carbon activated absorption step together with ozonation, the levels of pesticides in water were reduced by 90%, demonstrating the high potential of O_3 when applied together with other technologies in the treatment of drinking water.

Ikeura *et al.* (2011; 2013) studied the application of ozone microbubbles (<50 µm in diameter) in water at low O_3 concentrations (1 to 2 ppm) to wash fruits and vegetables, concluding that the use of this technique is highly effective in quickly removing pesticide residues. According to the authors, the microbubbles allowed the O_3, which is highly insoluble in water, to be dissolved easily, generating higher amounts of hydroxyl radicals, which are very

Table 2 - Summary of recent studies involving pesticide residue degradation in food by O_3

Food	Pesticide residues	Experimental conditions	Reductions obtained	Reference
Wheat grains	Fenitrothion Deltamethrin	O_3 at 60 µmol/mol for 180 min	66.7% 89.8%	Savi *et al.* (2015)
White cabbages	Chlorfluazuron Chlorothalonil	Ozonized water exposed to 250 mg of O_3/h for 15 min	60% 55%	Chen *et al.* (2013)
Persimmon leaves	Fenitrothion Benomyl	Microbubbles in water at 2 ppm of O_3 for 15 min	56% 50%	Ikeura *et al.* (2013)
Citrus fruits	Chlorothalonil Tetradifon Chloropyrifos	10 ppm of O_3 for 5 min	100% 98.6% 94.2%	Kusvuran *et al.* (2012)
Lettuce	Fenitrothion	Microbubbles in water at 1 ppm of O_3 for 5 min	58%	Ikeura *et al.* (2011)
Table grapes	Fenhexamid Cyprodinil Pyrimethanil Pyraclostrobin	Fumigation for 1 h (10 mL of O_3/L)	68.5% 75.4% 83.7% 100.0%	Gabler *et al.* (2010)
Brassica rapa	Diazinon Parathion Methyl-parathion Cypermethrin	Ozonized water using 1.4 to 2.0 mg of O_3/L for 30 min	Reduction from 60 to 90%	Wu *et al.* (2007)

effective at decomposing organic molecules.

In contrast to its use for drinking water, ozonation of food is limited by the undesired changes that occur in the quality of these due to the high oxidizing capacity of O_3. Consequently, an optimization of the conditions for decontamination must be studied for each food and for each pesticide residue. Table 2 summarizes some recent studies involving pesticide residue degradation in food through ozonation.

Food alterations due to ozonation treatment

When O_3 is used during storage or food processing in order to reduce the levels of residues or contaminants, its high oxidation power may promote unwanted changes in the food quality. Fruits and vegetables are the most affected by the negative effects of ozonation due their high moisture content, enzymes and phenolic compounds. Patil *et al.* (2010) bubbled apple juice with 0.048 mg of O_3 for 10 min and observed a change of color and reduction of phenolic compounds. These authors suggested that the O_3 and the hydroxyl radicals (OH-) generated may have opened the aromatic rings of the phenolic compounds which lead to the oxidation of organic acids, aldehydes and ketones. They also suggested that the loss of color was a consequence of the breakdown of conjugated double bonds. Gabler *et al*. (2010) also reported color changes and other injuries when they used ozonized water at 5 mg of O_3/L to wash grapes for 1 h. However, such changes can also be promoted by other oxidant processes, for instance; loss of flavonoids in fresh-cut onion slices due to washing with sodium hypochlorite (Pérez-Gregorio *et al*., 2011); induced browning of lettuce due to gaseous ClO_2 (Mahmoud *et al*., 2008); color alterations of red bell peppers and strawberries due to sanitization with H_2O_2 solution (Alexandre *et al*., 2012).

Several other studies involving ozonation have reported a significant loss in vitamin C content. Beltrán *et al*. (2005) reported a loss of ascorbic acid in lettuce when ozonized water at 20 mg of O_3/L was used. Tiwari *et al*. (2009) reported the same reduction and also a loss of anthocyanin in strawberry juice bubbled with 7.8% of O_3 for 10 min. A loss of ascorbic acid as well as carotenoids was reported by Chauhan *et al.* (2011) when they used ozonized water to wash carrots for 10 min.

On the other hand, Karaca and Velioglu (2014) used 12 mg/L of ozonized water for 15 min to wash lettuce, spinach and parsley but did not observe any changes in the levels of chlorophyll, ascorbic acid, total phenolic contents or antioxidant activity. Aguayo *et al.* (2013) also did not reported changes in the quality of tomato slices when 0.4 mg/L of ozonized water was applied for 3 min. Tzortzakis *et al.* (2007) did not observed changes in the quality of tomatoes stored for 6 days at 1 µmol/mol of O_3 .

Positive changes may also occur, as reported by Ali *et al.* (2014) when stored papaya fruit was ozonized with O_3 at 2.5 ppm for 10 days. Higher total solid values, ascorbic acid, β-carotene, lycopene and antioxidant activity were obtained in relation to the control sample, meaning a lower decay in these compounds with time. Similar results were reported by Yeoh *et al.* (2014), when fresh-cut papaya was treated with O_3 at 9.2 mg/L for 20 min. The authors observed that the total phenolic content increased by 10.3%, which occurred due to the activation of certain enzymes that are stimulated by different abiotic stresses.

When applied to cereal grains, the effects of O_3 may be confirmed through the changes of certain quality parameters in the flour obtained from the ozonized grains. Violleau *et al.* (2012) applied 5 g of O_3/kg grains and reported greater force and less extensibility in dough due to the oxidation of gluten, which interfered in the technological properties of the flour. Li *et al*. (2012) used 5 g of O_3 for 60 min and reported greater dough development time and an increased stability in the flour, which also occurs when other oxidizing agent, such as potassium bromate or chlorine are used. In this case, O_3 has the advantage of promoting the same effects, but without leaving potassium bromate or chlorine residues in the food. Sandhu *et al.* (2011) reported similar changes when they exposed wheat flour (100 g) to 1500 mg of O_3 for 45 min.

Sandhu *et al.* (2012) described that the use of O_3 at 1500 mg/kg for 45 min on wheat flour resulted in depolymerization of high molecular weight amylopectin, with a consequent increase in low molecular weight polymers, which may be useful for flours with low viscosity, high clarity, and low temperature stability requirements.

As occurs in fruits rich in pigments, ozonation may also react with the conjugated double bonds in the carotenoid of wheat flour decreasing the yellowness (Sandhu *et al*., 2011). Li *et al*. (2012a) reported this effect when they used 5 g of O_3/h for 60 min, and suggested that ozone can be used as a bleaching agent for wheat flour.

An undesirable effect of O_3 reported in some studies, is the formation of an unpleasant smell in the flour after the ozonation process, due to the formation of volatile low molecular weight compounds, as described by Chittrakorn *et al*. (2014) and Li *et al.* (2012). However, according to these authors, the aeration of the flour using storage ventilation can easily eliminate this problem.

In animal products, O_3 can also be applied to control microorganisms without promoting unwanted changes in quality, as reported by Iacumin *et al.* (2012) when sausages were stored in at 1 ppm atmosphere of O_3. Kamotani *et al.* (2010) used gaseous O_3 (9.7% in oxygen for 40 min) for processing eggs as an alternative treatment to pasteurization and reported no significant changes in the characteristics of the product, and with the advantage of not having to use heat. On the other hand, Uzun *et al*. (2012) observed reduced solubility of whey protein isolates and egg yolk proteins as a consequence of both aqueous and gas ozonation. Ozone treatment also negatively affected the emulsion activity of whey protein isolates and reduced their stability.

Perspectives for the use of O_3 in food processing

Based on the recent evolution of studies involving ozonation of food, more research can be expected with the goal to integrate gas ozonation or ozonized water into the traditional or innovative food processing procedures in order to reduce residues and contaminants, especially pesticide residues and mycotoxins. In fact, some studies have already been done, demonstrating interesting results. Chauhan *et al.* (2011) studied the washing process of carrots using ozonized water followed by storage in a controlled atmosphere, which reduced the lignification and kept the quality of fresh-cut carrots for 30 days. Chen *et al*. (2013) evaluated the effects of ultrasound combined with ozonized water on the degradation of organophosphorus pesticides residues in lettuce. With the use of both technologies the average levels of reduction peaked 82%, without negatively affecting the quality of the vegetable. Puzyr *et al*. (2010) studied the efficacy of ozonation followed by adsorption on nanodiamonds hydrosol to decrease the aflatoxin B1 content. These authors suggested that the use of these technologies together is a new approach to mycotoxin decontamination, where the ozonation degrades the toxin and the nanodiamond adsorbs the residual levels with high efficacy. Dudziak (2012) evaluated the application of an integrated system of ozonation and nanofiltration using a cellulose acetate membrane to remove the mycotoxin zearalenone in water. This study showed that with the combined use of these technologies it was possible to eliminate 100% of the zearalenone.

Ozone has a significant potential to be applied as a substitute of the normal chemical agents used in vegetable sanitization, fumigation of grains and in food storage, especially as it does not leave residues due to the treatment. Special attention should be given to the processing of organic food. According to the United States Department of Agriculture, organic food can be treated with ozonation, and the food can be classified as "100% organic" or "organic", depending on the O_3 usage (USDA, 2011). Other regulatory agencies in different countries do not make restrictions on the use of O_3 as a sanitizing agent for organic food, which makes its use very promising in this sector.

Due to the consumer's interest in new food processing technologies and the excellent results promoted by ozonation in improving the quality of food, new types of ozonizers are expected to appear on the market not only for industry but also for domestic use. Based on such expectations, more scientific research should be conducted, evaluating the effects of ozonation on the removal of different residues and contaminants, the possible formation of toxic degradation products and, also, the processing cost studies in order to disseminate a more practical and commercial application of this technology.

Conclusion

Current consumer perceptions concerning food safety and the rising concern about the presence of residues and contaminants has opened new fields of study for emerging food processing technologies. Gaseous ozonation and ozonized water are interesting nonthermal methods and with high efficacy for the decontamination of pesticide residues and mycotoxins in different types of foods. According to recent research, O_3 can degrade and reduce both mycotoxins and pesticide residues in food. As verified by mass spectrometry, using structure–activity relationships and also according to the studies with animal models, the toxic effects of food contaminated by mycotoxins can be eliminated or significantly reduced using ozonation processes. Some negative effects of ozonation are the undesirable changes that may occur in food quality, such as, a loss of phenolic compounds and ascorbic acid, inactivation of some enzymes and changes in color, especially when applied to fresh vegetables and fruit products. However, when optimal conditions are determined for each food, these effects will be greatly reduced. More studies are needed to clarify in depth the effects of ozonation on a higher number of mycotoxins and pesticides as well as the influence of the process on a greater variety of foods. The most recent studies that have demonstrated the excellent effects of O_3 in improving the quality of food should result in greater interest of this technology by the food industry, and consequently a wider acceptance and popularization of ozonized products by consumers.

Acknowledgements

Authors are grateful for the financial support provided by CNPq (National Counsel of Technological and Scientific Development, Brazil), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil) and FAPERJ (Rio de Janeiro State Foundation to Support Research).

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