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

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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\textsubscript{3}) in food processing. Due to its high potential as an oxidant, O\textsubscript{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\textsubscript{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.

Keywords
Food safety
Chemical contaminants O\textsubscript{3}
Gaseous ozonation
Ozonized water

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).
Mycotoxins are other food chemical contaminants 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; Chelé 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 environmentally 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, O$_3$ 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)
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 trichothecces (Rocha et al., 2014; Wu et al., 2014).

In cereal grains, Fusarium toxins, such as trichothecces, zearalenone and fumonisins, are the most commonly detected, particularly in the pre-harvest phase (De Ruyck et al., 2015). Trichothecces, 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).

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

![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- ochratoxin A, 7- patulin, 8- zearalenone. Reference: Adapted from PubChem (2015).](image-url)
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 pointed 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 \(AFB_1\) 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

<table>
<thead>
<tr>
<th>Food</th>
<th>Mycotoxin</th>
<th>Conditions</th>
<th>Reduction levels (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>AFT</td>
<td>6.0 mg of (O_3)/L for 30 min</td>
<td>65.8%</td>
<td>Chen et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>AFB(_1)</td>
<td>50 mg of (O_3)/L for 60 h</td>
<td>65.9%</td>
<td>Diao et al. (2013)</td>
</tr>
<tr>
<td>Brazil nut</td>
<td>AFT</td>
<td>21 mg of (O_3)/L for 96 h</td>
<td>30%</td>
<td>Alencar et al. (2012)</td>
</tr>
<tr>
<td>(in-shell)</td>
<td>AFB(_1)</td>
<td>14 mg of (O_3)/L for 30 days</td>
<td>100%</td>
<td>Giordano et al. (2010)</td>
</tr>
<tr>
<td>Corn flour</td>
<td>AFB(_1)</td>
<td>13.6 mg of (O_3)/L for 180 min</td>
<td>95.2%</td>
<td>Luo et al. (2014b)</td>
</tr>
<tr>
<td>Dried figs</td>
<td>AFB(_1)</td>
<td>Ozonized water (1.7 mg of (O_3)/L) for 180 min</td>
<td>88.6%</td>
<td>Zorhengen et al. (2008)</td>
</tr>
<tr>
<td>Flaked red</td>
<td>AFB(_1)</td>
<td>66 mg of (O_3)/L for 60 min</td>
<td>91%</td>
<td>Inan et al. (2007)</td>
</tr>
<tr>
<td>pepper</td>
<td>AFT</td>
<td>9 mg of (O_3)/L for 420 min</td>
<td>23%</td>
<td>Albas and Ocaldemir (2006a)</td>
</tr>
<tr>
<td>Pistachio</td>
<td>AFB(_1)</td>
<td>0.604% of (O_3) in air for 20 min</td>
<td>96.6%</td>
<td>El-Desouky et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>AFT</td>
<td>60 (\mu)mol/mL for 180 min</td>
<td>84.5%</td>
<td>Savi et al. (2014b)</td>
</tr>
<tr>
<td>Wheat grains</td>
<td>AFB(_1)</td>
<td>Ozonized water (1.7 mg of (O_3)/L) for 180 min</td>
<td>75.2%</td>
<td>Savi et al. (2014b)</td>
</tr>
<tr>
<td></td>
<td>AFB(_1)</td>
<td>0.604% of (O_3) in air for 20 min</td>
<td>96.6%</td>
<td>El-Desouky et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Citrinin</td>
<td>80 (\mu)mol/mL for 180 min</td>
<td>75.2%</td>
<td>Savi et al. (2014b)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Patulin</td>
<td>$1.2%$ of (O_3) (w/w) bubbled into juice</td>
<td>100%(^1)</td>
<td>Cataldo (2008)</td>
</tr>
</tbody>
</table>

\(^1\) Lower than the Limit of Detection (LOD) for the method used. AF: Aflatoxins.
AFT: Total aflatoxins. \(O_3\): Ozone.
the deleterious effects of AFB₁ 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 O₃/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 AFB₁ 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 O₃ 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 O₃ 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 O₃. 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 O₃ 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 O₃ 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 O₃, which is highly insoluble in water, to be dissolved easily, generating higher amounts of hydroxyl radicals, which are very
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 carrots 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 O₃ 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 O₃ 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 O₃ 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 O₃/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 O₃ 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, O₃ 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 O₃ for 45 min.

Sandhu et al. (2012) described that the use of O₃ 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 O₃/h for 60 min, and suggested that ozone can be used as a bleaching agent for wheat flour.

An undesirable effect of O₃ 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 Chitrtrakorn 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, O₃ 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 O₃. Kamotani et al. (2010) used gaseous O₃ (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 O₃ 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 O₃ usage (USDA, 2011). Other
regulatory agencies in different countries do not make restrictions on the use of O₃ 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, O₃ 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 O₃ 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.

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