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To cite this article: Ehsan Feizollahi & M. S. Roopesh (2021): Mechanisms of deoxynivalenol (DON) degradation during different treatments: a review, Critical Reviews in Food Science and Nutrition, DOI: [10.1080/10408398.2021.1895056](https://doi.org/10.1080/10408398.2021.1895056)

To link to this article: <https://doi.org/10.1080/10408398.2021.1895056>



Published online: 17 Mar 2021.



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REVIEW



## Mechanisms of deoxynivalenol (DON) degradation during different treatments: a review

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### ABSTRACT

Deoxynivalenol (DON) is one of the main trichothecenes, that causes health-related issues in humans and animals and imposes considerable financial loss to the food industry each year. Numerous treatments have been reported in the literature on the degradation of DON in food products. These treatments include thermal, chemical, biological/enzymatic, irradiation, light, ultrasound, ozone, and atmospheric cold plasma treatments. Each of these methods has different degradation efficacy and degrades DON by a distinct mechanism, which leads to various degradation byproducts with different toxicity. This manuscript focuses to review the degradation of DON by the aforementioned treatments, the chemical structure and toxicity of the byproducts, and the degradation pathway of DON. Based on the type of treatment, DON can be degraded to norDONs A-F, DON lactones, and ozonolysis products or transformed into de-epoxy deoxynivalenol, DON-3-glucoside, 3-acetyl-DON, 7-acetyl-DON, 15-acetyl-DON, 3-keto-DON, or 3-epi-DON. DON is a major problem for the grain industry and the studies focusing on DON degradation mechanisms could be helpful to select the best method and overcome the DON contamination in grains.

### KEYWORDS

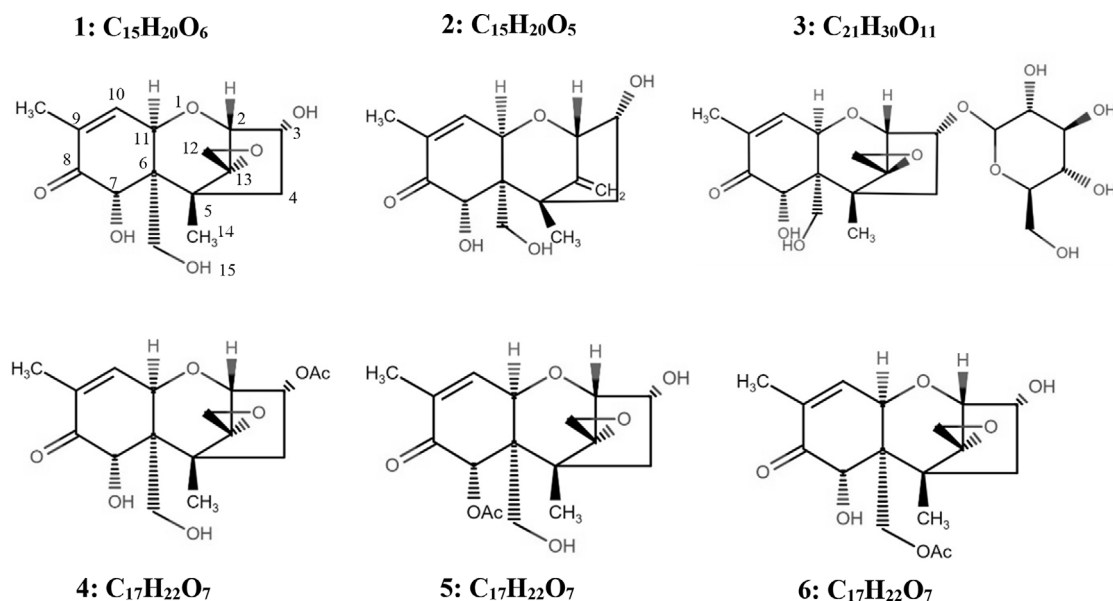
Deoxynivalenol; mycotoxin; ozone; irradiation; thermal treatment; atmospheric cold plasma; enzymatic treatment; chemical treatment

### Introduction

Cereal crops are infected by spoilage fungi in the field or during storage, and under optimum conditions, some of the fungi species can produce secondary metabolites named mycotoxins. Mycotoxins are toxic in low concentrations to humans and animals. Consumption of mycotoxin contaminated food can cause illnesses such as hepatic, gastrointestinal, and carcinogenic diseases (Fung and Clark 2004). Trichothecenes are a group of sesquiterpene mycotoxins and DON is one of the prevalently found trichothecenes on various cereal crops (Mishra et al. 2014). Trichothecenes have a 12,13-epoxytrichothec-9-ene ring as their basic structure. Type B trichothecenes share a keto substitution at C-8 such as deoxynivalenol (DON) (Bretz et al. 2006). DON is a common contaminant of cereal grains with 57, 40, 68, 59, 49, and 27% contamination rate in wheat, maize, oat, barley, rye, and rice, respectively (Joint FAO/WHO Expert Committee on Food Additives 2001). A recent review regarding the occurrence of DON in food commodities verified that in most of the assessed samples, DON concentration exceeded the permissible levels (Mishra et al. 2020).

DON produced by *Fusarium* spp. is a type B trichothecene. DON is the most predominant and economically important trichothecene but its toxicity is less than other trichothecenes, i.e., nivalenol and T-2 toxin (Mayer et al. 2017). Studies on animal specimens showed that DON can bind to the ribosome and inhibit protein synthesis. It can cause anorexia, and emesis, or exert an immunosuppressive

or immunostimulatory effect. It is cytotoxic to fibroblasts and lymphocytes related to protein synthesis inhibition at the ribosomal level with an effective concentration of 0.1–2 µg/ml of DON. Following the ingestion of DON, animals have lower feed consumption and lower weight gain. In higher dietary concentrations of DON, vomiting also has been reported in animals. However, the severity of the effects can differ based on the animal species, age and sex of the animal, concentration of DON, and the source of contamination. A detailed description of the toxic effects of DON can be found in the studies by Rotter, Prelusky, and Pestka (1996), Mayer et al. (2017), Sobrova et al. (2010) and Pestka and Smolinski (2005). DON can be converted to its acetylated derivatives, its conjugated form 3-O-glucoside (DON-3-Glc), or its de-epoxy metabolite (DOM-1) (Figure 1). The acetyl derivatives of DON (3-acetyl-DON (3-ADON), 7-acetyl-DON (7-ADON), and 15-acetyl-DON (15-ADON)) can exist together with DON but at much lower levels (Schmeitzl et al. 2015; Yan et al. 2020). The 3-ADON and 15-ADON are fungal metabolites that could be found on grains (Vidal et al. 2015). *Fusarium graminearum* responsible for the fusarium head blight (FHB) in North America has three chemotypes (i.e., 3-ADON, 15-ADON, and NIV). 3-ADON and 15-ADON chemotypes also produce DON. The in-vitro studies revealed that 3-ADON chemotype is more aggressive than the 15-ADON population based on the growth rate and production of the higher levels of DON (Puri and Zhong 2010). In a survey in Germany in



**Figure 1.** Chemical structure of deoxynivalenol (DON) (1), de-epoxy deoxynivalenol (DOM-1) (2), DON-3-glucoside (3), 3-acetyl-DON (4), 7-acetyl-DON (5), 15-acetyl-DON (6). Adapted from Wu et al. (2017).

1998 on 237 samples of cereal-based foods, 71, 4, and 4% of the samples had DON, 3-ADON, and 15-ADON with the average contents of 103, 17, and 24  $\mu\text{g/kg}$ , respectively (Schollenberger et al. 1999). In assessing 87 wheat samples in China, 95.4% of the samples contained 3-ADON at levels of 1.9–497.6  $\mu\text{g/kg}$ , and 87.4% were positive for 15-ADON at levels of 1.8–888.6  $\mu\text{g/kg}$  (Wu and Wang 2015). In a recent study of 579 wheat samples and 606 maize samples reported in China in 2017 indicated that all the wheat samples and 99.8% of maize samples tested positive for DON contamination with less frequent and lower concentrations of 3-ADON and 15-ADON (Yan et al. 2020). Also, another study in Lithuania in 2018 on wheat grains suggested high incidence of co-contamination of DON with 3-ADON and 15-ADON (Janaviciene et al. 2018). DON-3-Glc is formed during the germination of cereal grain by the UDP-glucosyltransferases enzyme. This enzyme conjugates DON with glucose to form DON-3-Glc as a biological detoxification mechanism in cereal plants showing FHB resistance (Jin et al. 2018). It also occurs in naturally contaminated grains as a phase II metabolite of the *Fusarium* mycotoxin DON (Berthiller et al. 2011). DON-3-Glc is the masked form of DON, as it cannot be detected by many routine analytical methods. In the human body, it can be converted back to DON by the action of colonic microbiota (Jin et al. 2018). A study on two-row barley indicated a significant contribution (26%) of DON-3-Glc from the total trichothecene content and hence its co-occurrence with DON should be considered (Tucker et al. 2019).

These modified forms of DON could be presumably transformed to DON, hence FAO/WHO Joint Expert Committee on Food Additives and Contaminants (JECFA) added 3-ADON and 15-ADON, as contributing factors to the maximum tolerable daily intake of DON (World Health Organization et al. 2011). The 3-ADON and 7-ADON are believed to be the precursors of DON during its production by *Fusarium* spp (Crippin et al. 2019). Toxicokinetic studies

in animals regarding the metabolism of DON showed that DON could be metabolized to DOM-1 (de-epoxy-DON) in the body (Yoshizawa, Takeda, and Ohi 1983), possibly due to the act of gut microbiota (Swanson et al. 1988). However, in a research by Turner et al. (2011) on UK adults, DOM-1 was not the major route of detoxification. DON can be further metabolized to the glucuronide-conjugated form of DON (as a major metabolite) by UDP-glucuronyltransferase to a less toxic metabolite (DON-glucuronides) by the addition of glucuronic acid (Schwartz-Zimmermann et al. 2017).

To reduce the concentration of DON on food/feed, pre-harvest, and post-harvest treatments have been used, such as pest control, varietal selection, sorting, dehulling, the addition of toxin binders, etc. Decontamination techniques such as the use of chemical agents, thermal treatment, ozonation, cold plasma, irradiation, and enzymatic/biological treatments have been evaluated in the previous years to understand their decontamination efficacy (Colović et al. 2019; Hojnik et al. 2017). The degradation mechanism/s of DON by each of these treatments are different. Knowing the degradation mechanisms of DON during treatments would allow the researchers to determine the relevant factors that impact the degradation rate and hence optimize these factors to achieve the highest decontamination efficacy. The knowledge about the DON degradation mechanism/s may help the industry to select the mycotoxin decontamination treatment based on their product, availability of the facilities, production method, final cost, etc. Only a few studies have addressed the degradation mechanisms of DON. These studies are scattered in the literature. No previous review discussed the effects of different decontamination treatments on DON degradation mechanisms. This review aimed to compile and discuss the studies that have presented the possible mechanism/s of DON degradation by thermal treatment, chemical treatment, biological/enzymatic and irradiation treatments,

**Table 1.** Thermal degradation of DON.

Thermal degradation method	Produce	Condition	DON content change (%)	Further information	Reference
Microwave heating	Corn	75, 100, and 175 °C	~ - (10, 20, and 40)	-	(Young 1986b)
Baking	Wheat bran and flour bread	230 °C, 20 min	- (7.6–9.9)	High stability of DON to high temperatures	(Israel-Roming and Avram 2010)
Baking	Wheat bread	240 °C, 14 min	-13	DON was converted to norDONs A-F and/or DON lactones	(Kostelanska et al. 2011)
Baking	Wheat bread	160 °C, 30 min	Crumb: 0 Crust: -10	-	(Kostelanska et al. 2011)
Heating	Model food	150–200 °C, 5–20 min	- (20–90)	Model food composition impacted the degradation rate of DON- Degradation products: norDON A (3), norDON B (4), norDON C (5), and DON lactone (6), and the new compounds 9-hydroxymethyl DON lactone (7), norDON D (8), norDON E (9), and norDON F	(Bretz et al. 2006)
Baking	Wheat bread	200 °C, 40 min	-81	Degradation of DON to DOM-1	(Vidal et al. 2015)
Baking	Wheat bread	190–240 °C, 30–50 min	-47.9	-	(Valle-Algarra et al. 2009)
Baking	cracker, biscuit, bread	Cracker: baked at 250 °C for 5 min and dried at 100 °C for 30 min Biscuits: baked at 180 °C for 8 min and dried at 100 °C for 10 min Bread: baked at 200 °C for 15 min	Cracker: -6 Biscuit: -5 Bread: -2	DON was degraded to iso-DON as the main derivative, and norDON B and norDON C as the minor degradation products	(Stadler et al. 2019)
Boiling	Noodle	5 min boiling	-52	-	(Zhang and Wang 2015)
Frying	Turnover pie	169, 243 °C, 1–15 min	- (>66, 38)	At higher temperature, DON reduction rate was lower!	(Samar et al. 2007)

light treatment, ultrasound treatment, ozone, and atmospheric cold plasma treatment.

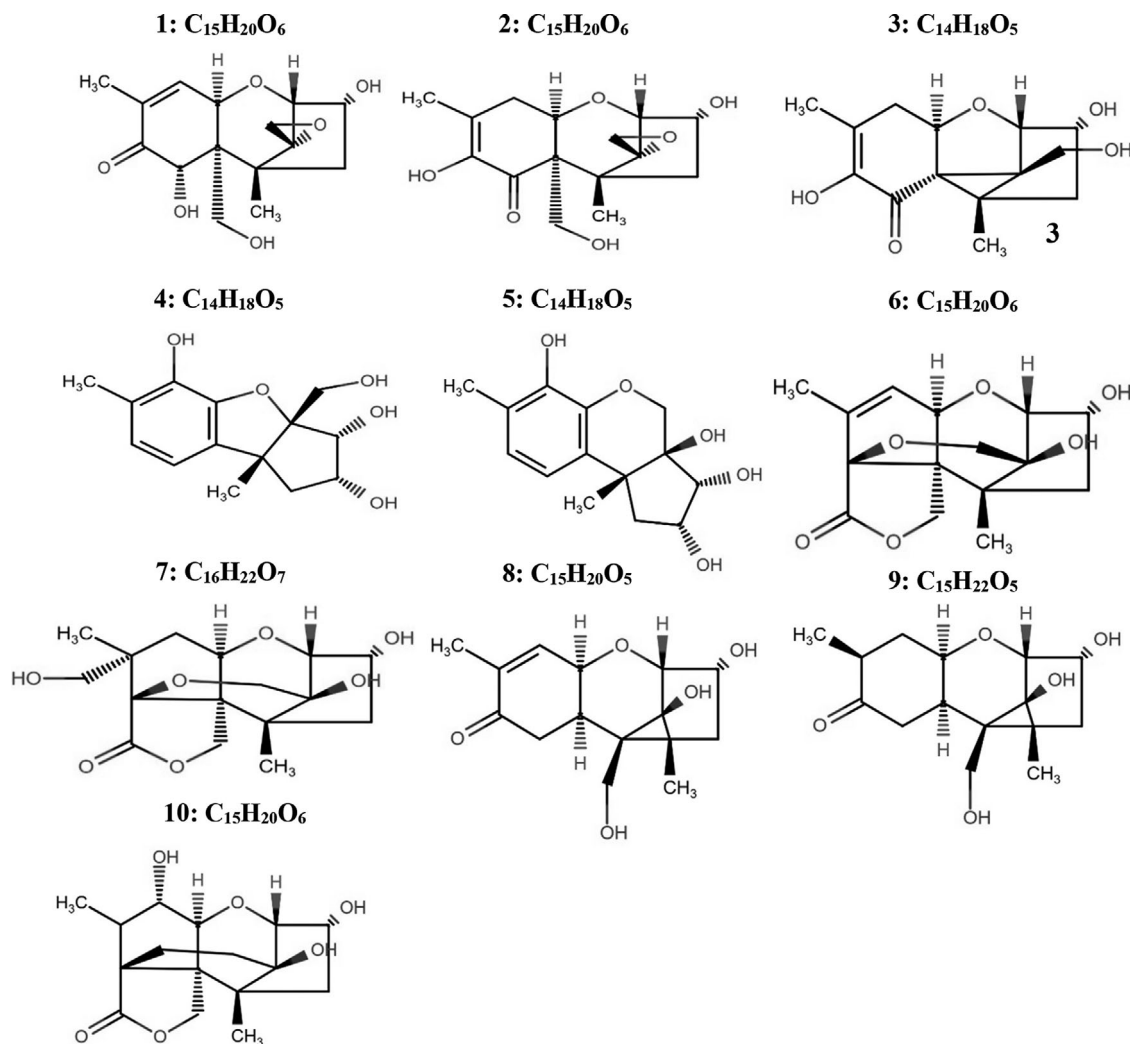
### Thermal degradation of DON

This section discusses the effect of various thermal treatments on DON (Table 1) and the mechanisms of degradation. Thermal treatment includes baking, frying, extrusion, steaming, and boiling, along with some relatively new approaches i.e., microwave heating. High temperatures used in thermal treatment can lead to the degradation of DON. Microwave heating of contaminated corn at 75, and 175 °C, reduced DON concentration by ~ 10, and 40%, respectively (Young 1986b). However, DON can be highly stable at high temperatures (Israel-Roming and Avram 2010). For instance, treating wheat bran and flour at 230 °C for 20 min resulted in a 7.6–9.9% reduction in DON concentration, which shows the high stability of DON to high temperatures (Israel-Roming and Avram 2010). Treatment time and temperature play important roles in the thermal degradation of DON (Kostelanska et al. 2011; Vidal et al. 2015). There are a number of studies on the degradation of DON during the baking process of bread (Samar et al. 2001; Young et al. 1984; Kostelanska et al. 2011; Kushiro 2008). The reported degradation rates in these studies have a wide range, depending on the temperature and time of baking, food

matrix, moisture content, pH, bread size, fermentation time, yeast strain, natural or spiked contamination, the initial concentration of the toxin, presence of the precursors of DON on the grain, etc. (Valle-Algarra et al. 2009; Samar et al. 2001; Bergamini et al. 2010). According to the FAO/WHO Expert Committee on Food Additives, trichothecenes are stable at 120 °C. At 180 °C, they are moderately stable and within 30–40 min at 210 °C, they degrade (Joint FAO/WHO Expert Committee on Food Additives 2001). Feizollahi, Arshad, et al. (2020) assessed the effect of sequential treatment consist of 30 min atmospheric cold plasma and 25 min heating at 80 °C on DON degradation and reported that the heat treatment had no effect on DON degradation.

Based on the temperature and time of thermal treatment, and the presence of the other precursors of DON, different DON thermal degradants can be formed i.e., norDONs A-F and/or DON lactones, NorDON-3-Glc A, B, C, D, and DOM-1 with reduced toxicity.

In the contaminated wheat and bread treated at 160 °C for 30 min, norDON A, B, and C were only found. The DON was not changed in the crumb of the bread where the temperature reached 85 °C, during the treatment. The degradation products were found only in the crust where the temperature was high there, and the DON concentration was reduced by 10% (Kostelanska et al. 2011). Bretz et al. (2006) heated R-D-glucose as sugar model, methyl-R-D-glucopyranoside as a starch model, and the amino acid



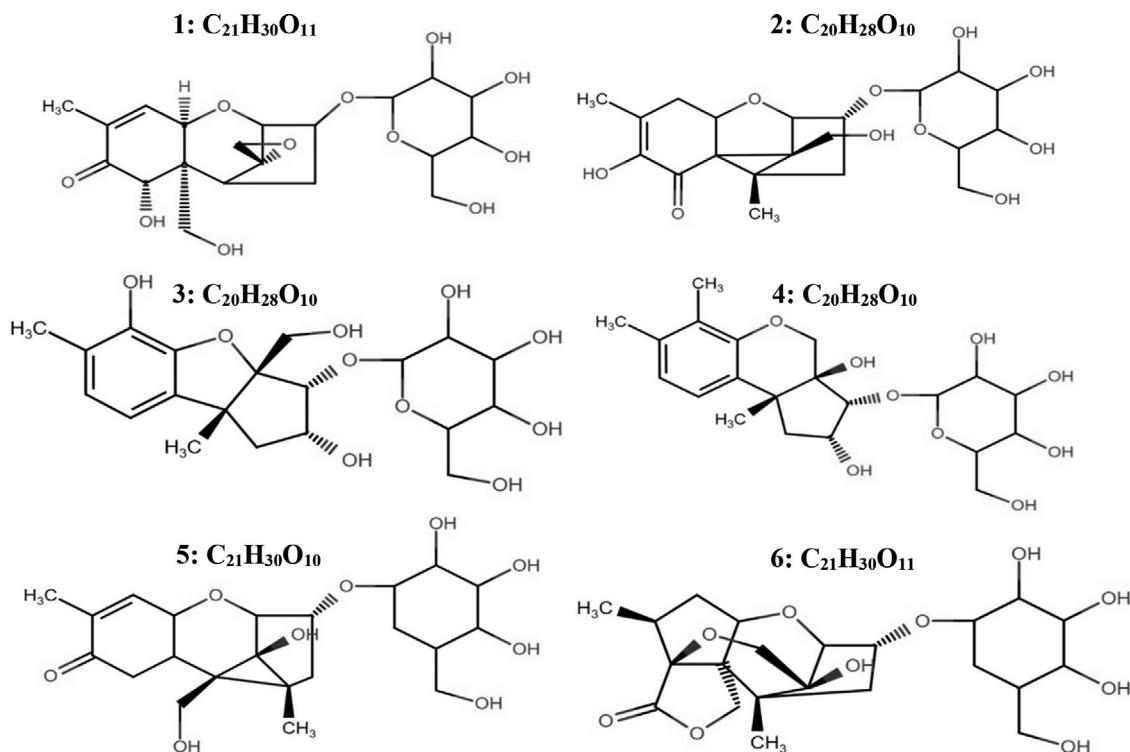
**Figure 2.** Structures of degradation products of DON (1) after thermal treatment. IsoDON (2), norDON A (3), norDON B (4), norDON C (5), and DON lactone (6), and the new compounds 9-hydroxymethyl DON lactone (7), norDON D (8), norDON E (9), and norDON F (10). Adapted from Kostelanska et al. (2011).

derivatives N-R-acetyl-L-lysine methyl ester and BOC-L-cysteine methyl ester as protein models consisted of 100 µg of DON in 1 mg of model food (simulating typical food constituents) at 150–200 °C for 5–20 min. Heating of the mixture resulted in DON degradants norDON A (3), norDON B (4), norDON C (5), and DON lactone (6), and the new compounds 9-hydroxymethyl DON lactone (7), norDON D (8), norDON E (9), and norDON F (10) as shown in Figure 2. However, norDON A (3), norDON B (4), norDON C (5), and DON lactone (6) were produced due to the alkaline decomposition by the amino group of the model sample as DON is unstable in alkaline condition at 75 °C (Bretz et al. 2006; Young, Blackwell, and ApSimon 1986). Bretz et al. (2006) also analyzed DON, norDON A, B, and C in thermally processed cereal products. The concentration of DON and its degradants were as follows: DON > norDON A > norDON B, and C. During the baking of crackers, biscuits, and bread, the reported DON degradation products were isoDON (1.3–3.9%), norDON B (0.2–0.9%) and norDON C (0.3–1.2%) (Stadler et al. 2019).

DON-3-Glc is the masked form of DON and it is important to be studied as DON can be released from DON-3-Glc by the action of the hydrolytic enzymes which hydrolyze

β-O-glucosidic bonds and cause the toxic effects. The effect of baking on DON and its conjugated form DON-3-Glc was studied by Kostelanska et al. (2011). Baking at 240 °C for 14 min reduced the concentration of DON and DON-3-Glc by 13, and 10%, respectively. They studied the degradation products of DON and DON-3-Glc in model samples (samples that were simulating the bread-baking process by naturally and artificially contaminated wheat) at 160 °C for 30 min. Based on LC-MS analysis, norDONs A–F and/or DON lactones (Figure 2) were determined to be the main degradation products of DON. Similarly, thermal degradation of nivalenol which is closely related to DON leads to the formation of norNIV A, norNIV B, norNIV C, and NIV lactone (Bretz et al. 2005). The degradation products of DON-3-Glc in analytical standard and contaminated wheat treated with heating at 160 °C for 30 min were determined based on the most intense peaks in UPLC-Orbitrap MS chromatograms. NorDON-3-Glc A, B, C, D, and DON-3-Glc-lactone were identified as the degradants of DON-3-Glc (Figure 3). Thermal treatment of DON-3-Glc also resulted in DON degradation products namely norDON A, B, and C which was due to the cleavage of the glycosidic bond





**Figure 3.** Degradation products of DON-3-Glc (1) after thermal treatment. NorDON-3-Glc A (2), norDON-3-Glc B (3), norDON-3-Glc C (4), norDON-3-Glc D (5), and DON-3-Glc lactone (6). Adapted from Kostelanska et al. (2011).

between DON and glucose during heat treatment (Kostelanska et al. 2011).

Vidal et al. (2015) studied the fate of DON, DON-3-Glc, 3-ADON, and DOM-1 during the baking of bread at different temperatures and times. The concentration of DON was reduced at different temperatures by increasing the baking time. On the other hand, in baked bread (140 °C, 35 min), the concentration of DON-3-Glc was increased from 9.7 µg/kg to 40.9 µg/kg. It was suggested that this could be due to the enzymatic releasing of DON-3-Glc from its insoluble forms bond to matrix or the generation of DON-3-Glc at the expense of DON. However, baking at 200 °C for 15 min reduced the concentration of DON-3-Glc to below the detection limit (Vidal et al. 2015). The concentration of 3-ADON was decreased by increasing the temperature and time of baking. 3-ADON could be transformed to DON, norDON A, norDON B, and norDON C when heat treated. On the other hand, the concentration of DOM-1 increased by increasing the baking time at different temperatures. The increase in DOM-1 concentration was 285% at 200 °C for 20 min baking, but at prolonged times, its concentration was below the detection limit. It was probable that DON was degraded to DOM-1 during heating (Vidal et al. 2015).

The baking stage of the bread has been reported to increase 3-ADON and 15-ADON content due to (i) the possible ADON release at high temperatures from the yeast cell wall, that was adsorbed during dough making; (ii) reconversion of DON to ADONs throughout the baking process (Wu and Wang 2015). Wu and Wang (2015) documented no ADONs in the samples that were spiked only with DON, due to the absence of free acetyl.

DON content was reported to be decreased in spaghetti and noodles by >40% by boiling (Zhang and Wang 2015; Vidal et al. 2016; Sugita-Konishi et al. 2006; Cano-Sancho et al. 2013). The main reason was that DON is soluble in water and it was leaching to the boiling water from the food (Vidal et al. 2016; Cano-Sancho et al. 2013; Zhang and Wang 2015). Due to the higher polarity of DON-3-Glc, its reduction rate is even better than that of DON during boiling (Zhang and Wang 2015). Zhang and Wang (2015) noted a reduction of 52, and 81% in DON and DON-3-Glc concentration in noodles, respectively. In contrast to the studies showing the greater degradation rate of DON at higher temperatures, frying of the turnover pie at 169 °C, 205 °C, and 243 °C reduced DON concentration by >66, 43, and 38%, respectively (Samar et al. 2007). A higher reduction of DON was observed in samples which were artificially inoculated (260 µg/kg) compared to naturally contaminated flour (1200 µg/kg) in the same study.

Research regarding the effect of extrusion cooking on DON degradation is inconsistent. Some studies showed the stability of DON during the extrusion cooking (Accerbi, Rinaldi, and Ng 1999; Wolf-Hall, Hanna, and Bullerman 1999; Scudamore et al. 2008); however, some other studies reported a significant DON reduction (Cazzaniga et al. 2001; Srecec et al. 2013). The variation in temperature, moisture content, compression level, and additives could be the possible reasons for the differences in the reduction of DON (Wu et al. 2011). Screw compression and higher moisture content during extrusion cooking led to a greater degradation rate of DON (Wu et al. 2017). Cetin and Bullerman

(2006) confirmed the reduced toxicity of DON after extrusion using mammalian cell cultures test.

A study on the cytotoxicity of DON and its modified forms showed that the toxicity of 15-ADON and DON was more potent than 3-ADON (Alizadeh et al. 2016; Eriksen, Pettersson, and Lundh 2004) and 15-ADON was slightly more toxic than DON. Based on the tissue and organ, and the evaluated cytotoxicity parameter, the toxicity of 3-ADON has been reported to be 2–100 times lower than both DON and 15-ADON in different studies (Eriksen, Pettersson, and Lundh 2004; Schmeitzl et al. 2015; Pinton et al. 2012); however, the acetylated forms of DON can be deacetylated to DON to a variable extent (Schmeitzl et al. 2015).

In contrast, DON-3-Glc and DOM-1 did not show any toxicity effect (Alizadeh et al. 2016). The low toxicity of DOM-1 was determined on porcine peripheral blood mononuclear cells, and 5-bromo-20-deoxyuridine (BrdU) incorporation assay (Dänicke et al. 2010b; Eriksen, Pettersson, and Lundh 2004). The concentration preventing 50% of the DNA synthesis of DOM-1 was 54 times more (less toxic) than DON (Eriksen, Pettersson, and Lundh 2004). The effect of de-epoxidation on reducing the toxicity of T-2 toxin, type A trichothecene, was demonstrated by Swanson et al. (1988) in which the toxicity was reduced by 400 times in the rat skin irritation bioassay. Valle-Algarra et al. (2009) documented 65.6, and 47.9% average reduction in 3-ADON, and DON levels, respectively, after baking at 190–240 °C for 30–50 min, respectively. The cytotoxicity assays revealed that 1.1 µmol/L as the approximate median effective concentration of DON. The norDON A, B, and C did not show any substantial effect up to 100 µmol. As norDON A, B, and C do not have the epoxy ring in their structure, the results of the cytotoxicity assay can prove the fact that the toxicity of trichothecene mainly depends on the epoxy group (Eriksen, Pettersson, and Lundh 2004; Rotter, Prelusky, and Pestka 1996; Dänicke et al. 2010). The effect of de-epoxidation on lowering the toxicity of the mycotoxin was reported by Swanson, Rood, et al. (1987), as well.

For quantification and the qualitative determination of thermal degradation products (degradants), ultra-performance liquid chromatography coupled to orbitrap mass spectrometer (Kostelanska et al. 2011), gas chromatography-mass spectrometry, and high-performance liquid chromatography-electrospray ionization tandem mass spectrometry along with nuclear magnetic resonance spectroscopy (Bretz et al. 2006) have been used. Overall, the degradation rate by thermal treatment is affected by the type of thermal treatment, temperature and time of the treatment, form of the treated DON (masked forms/conjugated forms, de-epoxide form, acetylated), nature of the substrate, type of contamination (natural or artificial), and the presence of the conjugated forms of DON. Aside from the aforementioned parameters, the variations in DON degradation rates in different studies could be due to the procedure that DON degradation rate was calculated. For instance, the different moisture content of the dough, or mixing of the dough with the ingredients which are non-contaminated such as water, fats, sugar, were

not accounted in the studies. Moreover, lack of consideration of recovery rates of DON could affect the variations seen in different studies (Stadler et al. 2019).

## Chemical degradation

This section discusses the effect of various chemical treatments (e.g., alkaline, chlorine, ammonium carbonate ((NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>), sodium bisulfite (Na<sub>2</sub>SO<sub>3</sub>), sodium metabisulfite (Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), L-ascorbic acid, L-cysteine) on DON degradation and its mechanisms. One of the popular chemical methods for DON reduction is using alkaline treatment. The effect of alkaline treatment on DON reduction has been evaluated in many reports (Wu et al. 2011; Young, Blackwell, and ApSimon 1986; Young 1986a; Nowicki et al. 1988). DON is unstable at pH = 10 at temperatures higher than 120 °C (Wolf and Bullerman 1998). The study by Mishra et al. (2014) revealed that at pH = 1, the peak of standard DON was reduced by 66% and an additional peak was formed. Since the pH of the stomach is 1–2, hence the results indicated that DON may be degraded in the stomach and form new degradation products. The study by Lauren and Smith (2001) determined the stability of DON at pH = 1–10, that were created in buffer solutions at 20–22 °C. Even at a pH of 10, there was <20% degradation rate of DON after 26 days of treatment. Increasing pH to 12 by Na<sub>2</sub>CO<sub>3</sub> as a nontoxic, water-soluble compound resulted in a 46% degradation rate and the addition of heating at 80 °C resulted in complete degradation of DON. Based on their results, using Na<sub>2</sub>SO<sub>3</sub> instead of citrate to create pH = 4 led to 80% DON degradation instead of 6% degradation by citrate and they observed a complete degradation by the addition of heating at 80 °C to Na<sub>2</sub>SO<sub>3</sub> solution. The results substantiated the importance of Na<sub>2</sub>CO<sub>3</sub> and Na<sub>2</sub>SO<sub>3</sub> on DON degradation. Likewise, in another study, heating of DON on barley at 80 °C resulted in a 20.1% degradation rate in 1-day treatment. However, 1 M of 20% v/w Na<sub>2</sub>CO<sub>3</sub> along with heating at 80 °C degraded 92.4% of DON on barley after 1 day (Abramson, House, and Martin Nyachoti 2005).

Treating DON with sulfur reagents such as Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> (Paulick et al. 2018; Dänicke et al. 2009) or Na<sub>2</sub>SO<sub>3</sub> solution results in the degradation of DON (Accerbi, Rinaldi, and Ng 1999; Young et al. 1987). Treatment of DON for 6 days with s Na<sub>2</sub>SO<sub>3</sub> at concentrations of >1% caused a complete elimination of DON in contaminated corn (500 µg/g) (Young 1986b). Treating DON with Na<sub>2</sub>SO<sub>3</sub> results in DON-sulfonate with no toxic effect in in-vivo and in-vitro studies (Young et al. 1987; Dänicke et al. 2010; Dänicke et al. 2008; Paulick et al. 2018). When an ethanolic solution of DON sulfonate was administered orally to pigs, no toxic effects of DON were observed (Young et al. 1987). Likewise, DON-contaminated maize treated with sodium sulfite had no toxic effect on pigs (Bahrenthien et al. 2020). Several factors influence the DON degradation rate by sodium sulfite. DON concentration decreases with increasing Na<sub>2</sub>SO<sub>3</sub> concentration, moisture content of the sample (14–30%), and the preservation period in a bi-exponential fashion when

Table 2. Biological/enzymatic removal of DON.

Biological/enzymatic degradation method	Condition	DON content change (%)	Further information	Reference
Fermentation	5–11 h at 25 °C	–21.6	Enzymatic release of DON from the precursors	(Neira et al. 1997)
Fermentation	1 h at 29–30 °C	0	–	(Valle-Algarra et al. 2009)
Fermentation	30, 60 min at 30 °C	0	Significant impact of temperature and time of fermentation on DON reduction- Importance of the amount of dough yeast in DON reduction	(Samar et al. 2001)
Fermentation	20, 40 min at 50 °C	–29, –41	Significant impact of the initial amount of DON on its reduction rate	(Samar et al. 2001)
Fermentation	45, 65, 85 min at 30, 40 °C	+46, +31	–	(Bergamini et al. 2010)
Fermentation	45 min at 30 °C	0	–	(Kostelanska et al. 2011)
Fermentation	80 min at 30 °C	0	–	(Wu and Wang 2015)
Malting	3-day steeping, 3-day germination at 14.5 °C	+210	Release of DON from its conjugated forms	(Lancova et al. 2008)
Biotransformation ( <i>Aspergillus tubingensis</i> )	14-day cultivation at 30 °C	–94.4	Biotransformation of DON to a compound which was 18.1 D (H <sub>2</sub> O) larger than that of DON	(He et al. 2008)
Biotransformation ( <i>Agrobacterium-Rhizobium</i> )	Incubation for 3 days at 30 °C	–100	Oxidative biotransformation to 3-keto-DON	(Shima et al. 1997)
Biotransformation (Microorganisms of the large intestines of chickens)	Anaerobic incubation for 96 h at 37 °C	–100	De-epoxidation of DON to DOM-1	(He, Young, and Forsberg 1992)
Biotransformation (Bovine rumen microorganisms)	Anaerobic incubation for 36 h at 38 °C	–39	De-epoxidation of DON to DOM-1	(Swanson, Nicoletti, et al. 1987)
Biotransformation (Bacterial strain BBSH 797 genus <i>Eubacterium</i> )	Anaerobic incubation for 3–5 days at 37 °C	–100	Transform DON into DOM-1 by epoxidase enzyme	(Fuchs et al. 2002; Schatzmayr et al. 2006)
Biotransformation (Strain WSN05-2, genus <i>Nocardioides</i> )	Incubation for 7 days at 28 °C	–90	Transform of DON into 3-epi-DON	(Ikunaga et al. 2011)
Biotransformation ( <i>Devosia insulae</i> A16)	Aerobic conditions for 48 h at 35 °C	–88	Oxidation of the OH group at C-3 of DON gave rise to 3-keto-DON	(Wang, Wang, Ji, et al. 2019)
Biotransformation ( <i>Bacillus</i> sp. LS100)	Anaerobic incubation for 3 days at 37 °C	–100	Transform DON to DOM-1	(Li et al. 2011)
Biotransformation (Microbial culture C133 from one catfish <i>Ameiurus nebulosus</i> )	Incubation for 72 and 96 h at 15 °C	–43.8, –100	Transform DON to DOM-1	(Guan et al. 2009)
Peroxidase enzyme	Incubation for 45 min at 15 °C	–40	The presence of co-factor such as H <sub>2</sub> O <sub>2</sub> was fundamental to enzymatic catalysis	(Feltrin et al. 2017)
Lipase purified from <i>Aspergillus niger</i>	Incubation at 40 °C for 5 min	–70	Transformation of DON to other products	(Yang et al. 2017)
Biotransformation (Enriched culture contained mainly enterobacterial genus <i>Serratia</i> )	Incubation for 144 h at 22–24 °C	–100	Optimum conditions for microbial de-epoxidation of DON: 25–30 °C, and pH = 7	(Islam et al. 2012)
Biotransformation ( <i>Eggerthella</i> sp. DII-9)	Incubation for 72 h at 37 °C	100 µg of DON/10 <sup>9</sup> –10 <sup>10</sup> cells	Transforming DON to DOM-1	(Gao et al. 2018)
Biotransformation (Bacterial consortium C20)	Incubation for 1–10 days at 20–40 °C, pH = 4–9	Max ~ –85	Optimal temperature and pH for DON degradation were 30 °C and pH 8.0	(Wang, Wang, Dai, et al. 2020)
Biotransformation ( <i>Pelagibacterium halotolerans</i> ANSP101)	72 h incubation under aerobic conditions at 28 °C	–80.2	Optimal temperature and pH for DON degradation were 40 °C and pH 8.0 by the cell lysate	(Zhang et al. 2020)
Biotransformation (Bacterial consortium IFSN-C1)	Incubation for 7 days at 37 °C	–86.5	3-keto-DON as the major, and 3-epi-DON as the minor	(Wang et al. 2020)

(continued)



Table 2. Continued.

Biological/enzymatic degradation method	Condition	DON content change (%)	Further information	Reference
Biotransformation ( <i>Bacillus subtilis</i> ASAG 216)	Incubation for 8 h at 37 °C	−81.8	degradation product of DON Broad temperature (35–50 °C) and pH (6.5–9.0) tolerance of <i>B. subtilis</i> ASAG 216	(Jia et al. 2021)
Adsorption (Fermentative bacteria strains)	Incubation at an optimal temperature of growth (30 or 37 °C) for 24 h	−(15–22)	<i>Enterococcus</i> had the highest adsorption rate	(Niderkorn et al. 2007)
Adsorption ( <i>Lactobacillus</i> sp., <i>S. cerevisiae</i> )	<i>Lactobacillus</i> sp.: 37 °C, 24 h <i>S. cerevisiae</i> : 30 °C, 24 h	<i>Lactobacillus</i> sp.: −(19–39) <i>S. cerevisiae</i> : −(22–43)	a significantly weaker DON binding activity compared to AF, ZEN or FUM by the analyzed strains of microorganisms	(Chlebicz and Śliżewska 2020)
Adsorption (Lactic acid bacteria strains)	Incubation for 4 h at 37 °C	−(16.4–71.2)	Smaller inactivation of DON by viable cells compared to heat-inactivated lactic acid bacteria cells	(Franco et al. 2011)
Adsorption (Lactic acid bacteria strains)	Incubation for at 37 °C for 4 h and 72 h	Max −34.3	DON metabolites (3-A-DON, 15-ADON, DOM-1, DON-3-Glc) were not detected in the incubated samples	(Zou et al. 2012)
Adsorption ( <i>Lactobacillus rhamnosus</i> RC007)	Incubation for 30, 60, 120 and 180 min at 37 °C	0	13.3 degradation of DON via biotransformation	(García et al. 2018)
Adsorption (Beer fermentation residue containing <i>Saccharomyces cerevisiae</i> )	Incubation for 60 min at 25 °C	pH = 3: −11.6 pH = 6.5: −17.6	Adsorption via $\beta$ -D-glucans from the yeast cell wall	(Campagnollo et al. 2015)
Adsorption ( <i>Aspergillus oryzae</i> , <i>Rhizopus oryzae</i> )	Keeping the unviable inoculum for 240 h at 30 °C	<i>Aspergillus oryzae</i> : −89 <i>Rhizopus oryzae</i> : −67	Inactivation of fungi in autoclave at 120 °C	(Garda-Bufferon, Kupski, and Badiale-Furlong 2011)
Adsorption (Strains of <i>Lactobacillus</i> and <i>Propionibacterium</i> )	Incubation for 1 h at 37 °C	<i>Lactobacillus</i> : −52 <i>Propionibacterium</i> : −54.5	No difference between viable and heat-killed bacteria in removing DON	(El-Nezami et al. 2002)
Adsorption (Different yeast strains, e.g., <i>Saccharomycopsis capsularis</i> , <i>Saccharomyces cerevisiae</i> , <i>Geotrichum fermentans</i> , <i>Rhodotorula glutinis</i> , <i>Kluyveromyces marxianus</i> )	Incubation for 10 days at 28 °C	Max −84.6	DON removal was affected by yeast strain, and type of substrate	(Bakutis, Baliukonienė, and Paškevičius 2005)

Na<sub>2</sub>SO<sub>3</sub> addition was  $\geq 3$  g/kg. There is a positive correlation between the Na<sub>2</sub>SO<sub>3</sub> concentration with pH value (Paulick et al. 2015). Based on the pH value, three different DON sulfonates (DONS 1, 2, and 3) could be formed. DON sulfonate 1 has no epoxide group whereas DON sulfonate 2 has hemiketal in its structure. At alkaline pH, rapid formation of DON sulfonate 1, and DON sulfonate 2 occurs. Slow formation of DON sulfonate 3 occurs at the acidic pH (Schwartz et al. 2013). Also, DON degradation rate by Na<sub>2</sub>SO<sub>3</sub> could be influenced by sample structure such as surface characteristics and particle sizes. It was clarified that the addition of 10 g Na<sub>2</sub>SO<sub>3</sub> per 1 kg maize at 30% moisture for 8 days resulted in a complete DON degradation (Paulick et al. 2015). Sodium metabisulfite can react with DON at the 9,10-double bond or at the keto group, which yields 10-DONS (major extent), and 8-DONS (minor extent), respectively. At alkaline conditions, DONS could be converted back to DON which depends on pH, time, and temperature (Dänicke et al. 2012). However, at alkaline pH, DON can be rearranged slowly to isoDON, and then rapidly degraded into nor-DON-A, nor-DON-B, nor-DON-C, and DON lactone (Young, Blackwell, and ApSimon 1986). A detailed

description of DON inactivation by sodium metabisulfite could be found in a review paper by Dänicke et al. (2012).

Young, Blackwell, and ApSimon (1986) reported norDON A, norDON B, and norDON C as the 3 isomeric degradation products of DON in alkaline treatment at 75 °C. DON was rearranged relatively slowly into isoDON and then degraded rapidly into norDON A, norDON B, and norDON C. In alkaline conditions, DON disappeared from the solution within 2 h and formed norDON A, norDON B, and norDON C. Maximum concentrations of norDON A, norDON B, and norDON C were at 65, 190, and 190 min of treatment, respectively. Also, the concentration of norDON A reached 0% within 5 h (Young 1986a), this could be due to the conversion of norDON A to norDON B and norDON C in alkaline conditions as has been explained by Young, Blackwell, and ApSimon (1986). Young (1986a) traced minor components in the alkaline solution of DON and speculated it to be isomeric epoxy lactone (DON lactone) as UV is not absorbed by this compound, using the UV detector in HPLC.

Treating corn with 5–100% chlorinated air resulted in the complete destruction of DON depending upon

concentration and time (Young 1986b; Young et al. 1984). However, chlorine levels of 0.1–0.5% (typical condition for milling) had little effect on DON degradation in biscuit and cake flour (Young et al. 1984).  $(\text{NH}_4)_2\text{CO}_3$  is another chemical, that exhibits a degradation effect on DON. It is used as a leavening agent and a pH controller agent and recognized as generally recognized as safe (GRAS) by the Food and Drug Administration of USA (FDA) (U.S. Food and Drug Administration 2020). It is believed that during the baking process, ammonia would be released and that could increase the degradation rate of DON (Young et al. 1984).

The effect of food additives on DON degradation in instant noodles was assessed by Moazami et al. (2014). Increasing the concentration of L-ascorbic acid,  $\text{Na}_2\text{SO}_3$ , or L-cysteine enhanced the degradation rate of DON. The authors noted that the observed reductions in DON levels could be due to the conversion of DON to other forms (Moazami et al. 2014). The effect of oxidants (potassium bromate and L-ascorbic acid), reductants ( $\text{Na}_2\text{SO}_3$  and L-cysteine), and ammonium phosphate on DON content reduction in bread was assessed by Boyacıoğlu, Heltiarachchy, and D'appolonia (1993).  $\text{Na}_2\text{SO}_3$  and L-cysteine were more efficient in reducing DON concentration (Boyacıoğlu, Heltiarachchy, and D'appolonia 1993).

It is hard to draw a conclusion to select the best chemical with the highest degradation rate on DON due to the different concentration and treatment times of the chemicals, different initial concentration of the DON, and type of exposure to the chemical, used in different studies; however, chlorine seems to be more effective than the other chemicals to degrade DON. During the chemical treatment of DON different degradation products such as norDON A, norDON B, and norDON C can be formed. The toxicity of the degraded products, quality changes of the treated substrates, costs, safety concerns, and the negative impact on grain quality and governmental regulations for using chemicals should take into account to opt-in the desired chemical for degrading DON. Sodium hydroxide is used for alkaline degradation of DON (Shalapy et al. 2020; Young, Blackwell, and ApSimon 1986),  $(\text{NH}_4)_2\text{CO}_3$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_2\text{SO}_3$ ,  $\text{Na}_2\text{S}_2\text{O}_5$ , L-ascorbic acid, L-cysteine, and ammonium phosphate are food additives, that are considered as GRAS when used in accordance with good manufacturing or feeding practice according to FDA (U.S. Food and Drug Administration 2020). Using alkaline or compounds such as  $\text{Na}_2\text{CO}_3$  that results in high pH need a neutralization step by chemicals such as gas-phase HCl that should be considered for the environmental issues and safety aspects. Chlorinated compounds are mainly used in the disinfection of food contact surfaces in food processing facilities to reduce the normal microflora of the fresh fruits and vegetables and poultry carcasses. These compounds result in the formation of potentially harmful chlorinated disinfection by-products. However, more research is needed to determine the toxicity of chlorinated disinfection by-products formed in water and food (World Health Organization 2009). In most of the DON degradation treatments involving chemicals, there is an additional cost of drying the

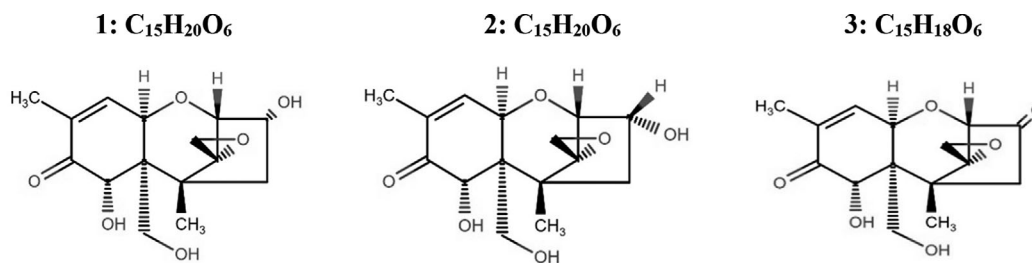
decontaminated soaked/washed grains. This could limit the usefulness of the grains intended for storage or use in dry products.

### Biological/enzymatic treatment

This section discusses the effect of various enzymatic treatments (e.g., fermentation, malting, enzymes, and bacterial enzymes) on DON degradation and its mechanisms (Table 2). Two of the main enzymatic methods for degrading DON, that have been studied widely are the fermentation and malting process. The principal type of fermentation in the dough is the action of yeast enzymes on fermentable sugars that leads to the production of  $\text{CO}_2$ , ethanol, and some aromatic compounds (Collado-Fernández 2003). The enzymes that are involved in the dough fermentation are  $\alpha$ -amylase, maltase, invertase, and zymase (Collado-Fernández 2003). The effect of fermentation during the baking process on DON degradation is contradictory. It was reported that the DON level increases within the fermentation during dough making. The release of DON from its precursors, i.e., 3-ADON, 7-ADON, 15-ADON, DON-3-Glc, or bound forms during fermentation (Young et al. 1984; Bergamini et al. 2010) was assumed to be the reason. However, in the high concentration of DON, the same phenomenon was not observed in which the release of DON from its bound form was negligible compared to the high concentration of free DON in the matrix. On the other hand, some studies determined a decrease in DON concentration after fermentation (Neira et al. 1997; Samar et al. 2001), and the DON reduction % increased with an increase in fermentation temperature and time, and the concentration of yeast (Samar et al. 2001).

The addition of bakery improver enzymes to wheat dough increased DON-3-Glc concentration by 145%, but DON content was not affected significantly. As the content of DON was not influenced, hence the  $\beta$ -glycosidic bond between DON and glucose unit in DON-3-Glc had remained undamaged, and probably  $\alpha$ -glycosidic bonds between DON-3-Glc and cell polysaccharides were cleaved (Kostelanska et al. 2011). In another study, fermentation decreased 20–40% of 3-ADON and 28–60% of 15-ADON content based on the spiking levels. Further reduction was observed during the proofing stage. The conversion of ADONs to DON by the active yeasts was mentioned by the authors during these steps (Wu and Wang 2015).

The malting process is an essential step in the brewing industry. The three main steps in the malting process are steeping, germination, and kilning/roasting. In the germination step, the enzymes (glucanases, proteases, amylases) are synthesized (Fox 2018). In the malting process of barley, mainly in the germination step, the concentration of DON and DON-3-Glc in artificially contaminated barley was increased by 2.1 and 8.6 times, respectively. In naturally contaminated barley, DON was the only toxin in barley; however, the malt contained DON, DON-3-Glc, and ADONs (Lancova et al. 2008). The release from their conjugated forms was the probable reason for an increase in the



**Figure 4.** DON (1), 3-epi-DON (2), 3-keto-DON (3).

concentration levels of DON, DON-3-Glc, and ADONs (Lancova et al. 2008).

The degradation of DON and its conjugated forms by enzymes has been studied in multiple studies. Feltrin et al. (2017) evaluated the effect of peroxidase (PO) enzyme on DON degradation and concluded the importance of the presence of H<sub>2</sub>O<sub>2</sub> as the co-factor for enzymatic catalysis and DON degradation (Feltrin et al. 2017). In an in-vitro model system, the degradation of DON-3-Glc via different enzymes was studied. DON-3-Glc is considered important due to the possibility of its hydrolysis to DON. It was determined that DON-3-Glc is resistant to acidic conditions as low as pH = 0.7 and hence it will not be hydrolyzed into DON in mammals' stomach. Also, in the same study, the highest degradation rate was observed with *Lactobacillus plantarum* after incubation for 8 h which degraded 62% of DON-3-Glc. The authors suggested that the position of the glucose in DON-3-Glc has a considerable effect on the cleavage of the molecule by enzymes (Berthiller et al. 2011). Also, the in vitro transcription/translation system (Poppenberger et al. 2003) and yeast growth and DNA microarray analysis (Suzuki and Iwahashi 2015) demonstrated that DON-3-Glc is significantly less toxic than DON. There is a possibility that DON-3-Glc has a marginal effect on the intercellular components due to its impact on inducing the DNA stress response genes and repressing the cellular metabolism genes like SER3 (Suzuki and Iwahashi 2015). It should be noted that DON-3-Glc can be cleaved to DON by bacteria in the gastrointestinal tract and hence metabolized similarly as DON. Study on pigs suggested that the bioavailability of DON-3-Glc is two times lower than DON and it is probable that DON-3-Glc is only absorbed in the form of DON in pigs but the experiments on poultry did not indicate that (Chain et al. 2017). A comprehensive human intervention study revealed that 58% of administered DON-3-Glc and 64% of DON were recovered in urine within 24 h in which the lower recovery rate of DON-3-Glc could be due to the lower bioavailability and lower absorption of DON-3-Glc by human intestinal cells and could be taken into consideration in risk assessment studies. Following the administration of DON-3-Glc, the concentration of DOM-1 in urine was increased (Vidal et al. 2018). It is possible that DON-3-Glc was converted to DON by the bacteria of the intestine and then biotransformed to DOM-1.

The utilization of biological methods for in situ detoxification of DON has been assessed in different studies. A bacterium belonging to the *Agrobacterium-Rhizobium* group

was capable to metabolize DON via oxidative biotransformation to 3-keto-DON (Figure 4). 3-keto-DON was identified as the main metabolite with one-tenth immunosuppressive toxicity compared to DON. Lower toxicity of 3-keto-DON indicated that the 3-OH group is the reason for the immunosuppressive toxicity of DON (Shima et al. 1997). The *Devosia* strain, *Devosia insulae* A16, can oxidize the hydroxyl group at C-3 of DON and result in 3-keto-DON. Increase in the concentration of DON reduced the degradation rate, and the optimum temperature and pH for degradation were 35 °C and pH = 7 (Wang, Wang, Ji, et al. 2019). During the microbial biotransformation, 3-ADON and 15-ADON could be deacetylated to form DON as an intermediate product before the oxidation of the hydroxyl group at C-3 of DON, then partly converted to 3-keto-DON as the final product (Wang, Wang, Ji, et al. 2019). It was described by He et al. (2017) that a novel aldo/keto reductase superfamily member, AKR18A1, of *Sphingomonas* sp. S3-4 oxidizes DON into 3-keto-DON (3-oxo-DON).

DON can also be converted to DOM-1 via microbial biotransformation that can reduce the 12,13-epoxide group to a carbon-carbon double bond (He, Young, and Forsberg 1992; Swanson, Nicoletti, et al. 1987; Li et al. 2011; Guan et al. 2009; Pierron et al. 2016; Li et al. 2020). It was determined that the epoxidase enzyme secreted by bacteria can transform DON into DOM-1 (Schatzmayr et al. 2006) with a no toxic to a very low toxic effect of DOM-1 compared to DON depending on the evaluated cytotoxicity parameter (Li et al. 2011; Eriksen, Pettersson, and Lundh 2004; Springler et al. 2017). The optimum temperature and pH for the transformation of DON to DOM-1 by catfish microbial culture, and enriched culture contained mainly enterobacterial genus *Serratia* was 15 °C, pH = 7.2–8.9 (Guan et al. 2009), and 25–30 °C, pH = 7 (Islam et al. 2012), respectively. It was determined that the transformation rate of DON to DOM-1 under aerobic conditions is higher compared to anaerobic conditions in enriched culture (Islam et al. 2012). It is noteworthy to mention that the biological degradation of DON is due to the encoding of enzymes capable of de-epoxidation of DON via bacteria.

DON can also be transformed into 3-epi-DON (epimer of DON) via particular strains of bacteria. The presence of a 12,13-epoxide group in the structure of 3-epi-DON may represent the same toxicity as DON (Ikunaga et al. 2011). However, in other studies in-vitro, and in-vivo experiments demonstrated the substantially lower toxicity of 3-epi-DON compared to DON (He et al. 2015; Pierron et al. 2016; Bracarense et al. 2020).

The other biological procedure for detoxification of DON is adsorption in the presence of specific substances such as glucomannan in the cell wall of the microorganism (Yao and Long 2020). The capacity of an adsorbent to bind to a mycotoxin depends on the chemical nature of the mycotoxin and the surface properties of the adsorbent. A strong bond between the adsorbent and mycotoxin can hinder the adsorption of the mycotoxin by the digestive tract. Bakutis, Baliukonienė, and Paškevičius (2005) assessed the adsorption efficiency of different yeasts. *Rhodotorula glutinis* was able to detoxify 84.6% of DON. Also, *Lactobacillus* sp. and *S. cerevisiae* have the ability to reduce DON concentration. The adsorption of mycotoxin by bacteria occurs through the peptidoglycans, polysaccharides, and the teichoic acid of the cell wall, primarily through hydrophobic interactions (Chlebicz and Ślizewska 2020). In a study by Franco et al. (2011) lactic acid bacteria cells were inactivated at 100 °C for 30 min and 121 °C for 15 min to determine whether the DON reduction is due to biotransformation or by adsorption. It was determined that the inactivated bacteria were able to significantly reduce DON levels, which suggests that the inactivation of DON was by absorption to the cell wall of lactic acid bacteria. Also, in another study, heat-treated and acid-treated lactic acid bacteria cells had more ability to remove DON compared to untreated cells. Moreover, none of the metabolites of DON were detected after incubation of bacteria with DON (Zou et al. 2012). The results proved the physical removal of DON by adsorption rather than biotransformation. The other studies that evaluated the adsorption efficiency of microorganisms are included in Table 2.

Overall, the biological degradation of DON could be achieved through absorption or enzymatic degradation. The enzymatic degradation of DON by bacteria could be accomplished by two pathways: I) the oxidation of DON into 3-keto-DON by the oxidation of the C3 hydroxyl group, along with isomerization to 3-epi DON under aerobic conditions (Wang, Wang, Dai, et al. 2020; Zhang et al. 2020), II) the reductive de-epoxidation into DOM-1 under anaerobic conditions generally by rumen or intestinal bacteria (Ikunaga et al. 2011; McCormick 2013; Ji, Fan, and Zhao 2016).

The degradation rate and the final degradation products of DON depends on the type of bacteria and hence the type of secreted bacterial enzyme, pH, temperature, initial concentration of DON, and the presence of other chemicals such as acetyl in the environment, that is necessary for ADON formation. However, further studies are needed to determine the degradation mechanisms and the genes that are responsible for the encoding of enzymes capable of degrading DON. This may help in developing DON resistant plant varieties to overcome DON contamination issue. Moreover, as the majority of the studies are focused on animal species and due to the difference in the microbiology of the gastrointestinal tract of humans and animals, more studies are needed to assess the degradation of DON in the human body and the toxicity of the degradation products for humans.

## Irradiation

Irradiation includes gamma rays, beta rays generated by electron accelerators, and X-rays (Harder, Arthur, and Arthur 2016). A number of food products are approved for

irradiation by the FDA and the European Commission legislation including poultry, beef and pork, fresh fruits and vegetables, spices, etc. (U.S. Food and Drug Administration 2020; European Commission 1999). Wheat, whole wheat flour, whole and ground spices, onions, dehydrated seasoning preparations, and potatoes are also approved by Health Canada for irradiation (Canadian Food Inspection Agency 2020). Gamma ( $\gamma$ ) irradiation (with Co-60 or Cesium-137 radioisotope) is one of the preferred sources of radiation in the food industry due to its high penetration and inactivation effect on microorganisms (Calado et al. 2018). DON in solution (dissolved in water) was identified to be less resistant to  $\gamma$ -radiation compared to the dry state on grain (O'Neill, Damoglou, and Patterson 1993). In solution, DON concentration reduced to below 20% by treating with 10 kGy gamma. However, in a dried DON on maize, DON was resistant to degradation at 10 kGy and 80–90% of its concentration remained at 50 kGy  $\gamma$ -irradiation. In the presence of water, free radicals are generated via radiolysis of water under  $\gamma$ -rays, which will then react with the mycotoxin (O'Neill, Damoglou, and Patterson 1993). Also, a higher degradation rate of DON in ultrapure water was observed compared to an organic solvent (acetonitrile/water (16:84 v/v)) which suggests a more severe reaction in water than in an organic solvent (Li, Guan, and Bian 2019a). Baby hamster kidney toxicity bioassay indicated the similar toxicity of the  $\gamma$ -treated extract on maize compared to the untreated sample. However, in the water solution, the toxicity was decreased, which was more evident in higher irradiation doses.

The food matrix is an important factor in the degradation of DON with  $\gamma$ -irradiation (O'Neill, Damoglou, and Patterson 1993). DON concentration in wheat was reduced by 41% at 2 kGy irradiation (Aziz, Attia, and Farag 1997), and by 2.4% at 5 kGy irradiation in Soybeans (Hooshmand and Klopfenstein 1995). Increasing the moisture content of soybeans from 9% to 17% did not significantly affect the degradation rate of DON (Hooshmand and Klopfenstein 1995). Increasing the doses of  $^{60}\text{Co}$   $\gamma$ -irradiation can improve the degradation rate of DON (Li, Guan, and Bian 2019a) and high initial DON concentrations require higher  $\gamma$ -irradiation doses (Li et al. 2013). Oral gavage technique of mice observing the feed consumption, body weight changes, ratios of the organ to body weight, serum biochemistry, and histopathological changes determined significantly lower toxicity of the irradiated DON compared to the untreated samples (Li, Guan, and Bian 2019a). The capability of  $\gamma$ -irradiation on degrading mycotoxin is dependent on the mycotoxin type and its concentrations, the matrix, water content, and radiation dose and time (Calado et al. 2018). The high dose of irradiation needed for the complete destruction of DON on grains makes  $\gamma$ -irradiation unsuitable for practical use.

Electron beam irradiation (EBI), produced by machines called electron accelerators, is the other irradiation technique that has been studied for its DON degradation capability. Like the  $\gamma$ -irradiation, the presence of water has a considerable effect on DON degradation via EBI. EBI doses of



51.4–55.8 kGy reduced DON content by 47.5, 75.5, and 74.4% in wet distillers grain, distillers solubles, and stillage, respectively. However, in unprocessed wheat (dry sample), the degradation rate was only 17.6% at 54.4 kGy, indicating the importance of the presence of water in improving the degradation rate by EBI (Stepanik et al. 2007). In a study by Kottapalli, Wolf-Hall, and Schwarz (2006), the 0–10 kGy EBI had no significant effect on DON degradation in raw barley. In malt samples prepared from irradiated barley, 54–100% reduction rates were observed at 4–10 kGy EBI. The prevention of DON synthesis by *Fusarium* spp. was the possible reason for higher reduction rates in malt compared to raw barley (Kottapalli, Wolf-Hall, and Schwarz 2006). The type of the substrate, irradiation dose, and water percentage are the crucial factors in the degradation of DON by EBI. Higher irradiation doses increase the degradation rate of DON; however, international committee experts consider food as safe and wholesome for consumption at an irradiation dose of up to 10 kGy (Morehouse and Komolprasert 2020). Based on the need for a high dose of ionizing radiation for DON degradation in dry foods, which can jeopardize the quality of the treated product, and since the current legislation of irradiation authorized by FDA and European Commission does not include the liquid foods (need less irradiation dose for DON degradation), this technology has limitations for the complete removal of DON. However, it could be applied in low doses along with other technologies in hurdle treatments to mitigate DON concentration in food products.

## Light treatment

Previous studies reported degradation of DON after treatment using light with a specific wavelength (e.g., ultraviolet-C (UV-C), 254 nm). UV-C treatment (intensity = 0.1 mW/cm<sup>2</sup>) of dried DON for 60 min reduced DON concentration from 30 ppm to 0.2 ppm. An increase in the irradiation dose, and dissolving DON in an aqueous solution can significantly improve DON degradation rate by UV light. Porcine neutrophils chemiluminescence experiment suggested the lower immunosuppressive effect of the UV treated DON (Murata, Mitsumatsu, and Shimada 2008). DON reduction efficacy of UV treatment is dependent on the food matrix or substrate used. UV treatment (1.5 mW/cm<sup>2</sup> at 254 nm UV-C) of DON on moist corn silage for 30 and 60 min reduced DON concentration by 22 and 21%, respectively (Murata et al. 2011). Using a UV-C dose of 15,000 mJ/cm<sup>2</sup>, a 97.3% reduction of DON (0.1 mg on filter paper) was achieved (Popović et al. 2018). The mycotoxin reduction on grain kernels was lower than on a model filter paper due to the difference in surface characteristics. Also, the DON content on maize and wheat was decreased by 30 and 14%, respectively (Popović et al. 2018). UV sensitivity of a molecule is dependent on (i) relative absorbance of UV light by the mycotoxin which relies on the extent of double bond conjugation and hence the power of light. UV light energy can be absorbed by the double bond before reaching breakable bonds. (ii) the number of low energy breakable

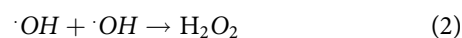
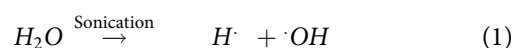
bonds (C–O and N–H) (7 in the case of DON) (Popović et al. 2018).

Pulsed light treatment, which can have greater effectiveness than UV treatment, is one of the decontamination techniques used to destroy bacteria, viruses, fungi, and spores. The light emitted by the pulsed light system consists of UV light to near-infrared light from 180 to 1100 nm. The lethal effect of pulsed light is believed to be related to the UV section of the spectrum (Moreau et al. 2013). UV light can have a photochemical effect i.e., altering the structure of pyrimidine bases and DNA in microorganisms, or a photothermal effect i.e., conversion of UV photons into energy resulting in a short, sharp, local temperature rise at the surface of the substrate (Moreau et al. 2013). The same photothermal effect can occur for mycotoxin's degradation by UV light. A pulsed light device, generating a light flux of 1 J/cm<sup>2</sup> during one 300 μs flash was used to degrade DON in water solution. One, and eight pulsed light flashes reduced DON by 14.3, and 72.5%, respectively. The higher number of flashes did not further enhance the degradation rate. The compact structure of DON compared to other mycotoxins was mentioned as the possible reason for the less effectiveness of UV on this toxin (Moreau et al. 2013). The degradation rate of DON by light treatment is dependent on the amount of light energy that is being delivered to the toxin which is influenced by the substrate matrix, the number of double bonds, and low energy breakable bonds, the wavelength of the light, and the exposure time.

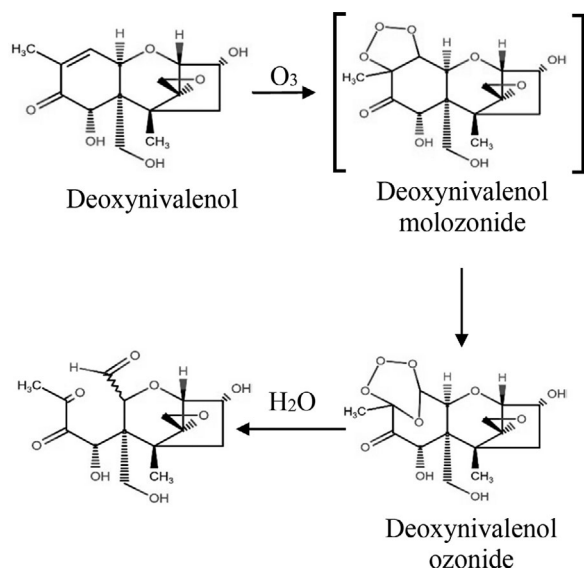
## Ultrasound

Ultrasound waves are sound waves that have a frequency of 20 kHz to 10 MHz (Gallo, Ferrara, and Naviglio 2018). Ultrasound can create cavitation bubbles in liquid media. When these bubbles collapse, high temperatures and pressures above 5000 °C and 1000 atm are created (Liu et al. 2019). In the bubble-liquid zone and the liquid mass zone, various reactive species can be formed. Hydroxyl radical (OH) is the most frequent reactive species in the medium that can attack any organic compound or recombine with another OH to produce H<sub>2</sub>O<sub>2</sub>. In the interface area that the temperature is very high, solutes can undergo thermal degradation or react with OH radicals which induce molecular sonolysis (Gallo, Ferrara, and Naviglio 2018). Previous studies reported the potential of ultrasound treatment on the degradation of DON. Ultrasound treatment at a power intensity of 4.4 W/cm<sup>3</sup> and a duty cycle of 66.7% for 50 min led to a reduction of 43.2% in DON concentration (Liu et al. 2019). Liu et al. (2019) reported a negative correlation between the initial concentration of DON with degradation efficacy via ultrasound. In mycotoxins with higher concentrations, ultrasound has a lower power to break the chemical bonds.

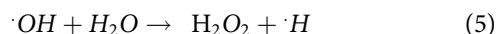
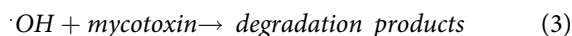
The reactions that can occur during ultrasound treatment of a mycotoxin are as follows (Liu et al. 2019):







**Figure 5.** Proposed mechanism by Young, Zhu, and Zhou (2006) for the addition of ozone to trichothecenes.



Increasing the power intensity and duty cycle of ultrasound to an optimum can improve DON degradation rate. Increasing the power intensity rise the number of cavitation bubbles, and hence hydroxyl radicals (Golash and Gogate 2012) besides, violent collapse of the cavitation bubbles can occur (Liu et al. 2019) and thereby improve the degradation rate of mycotoxins. However, increase the power intensity to more than optimum can results in an inadequate collapse of the cavitation bubbles and decelerate the increase in degradation rate (Liu et al. 2019). The duty cycle could affect the time taken for the growth of the cavitation bubbles and hence affect the extreme conditions for the degradation of a compound (Liu et al. 2019). Further research is needed to identify the degradation products of DON after ultrasound treatment to establish this green technology as a process for the reduction of DON from food and feed.

## Ozone

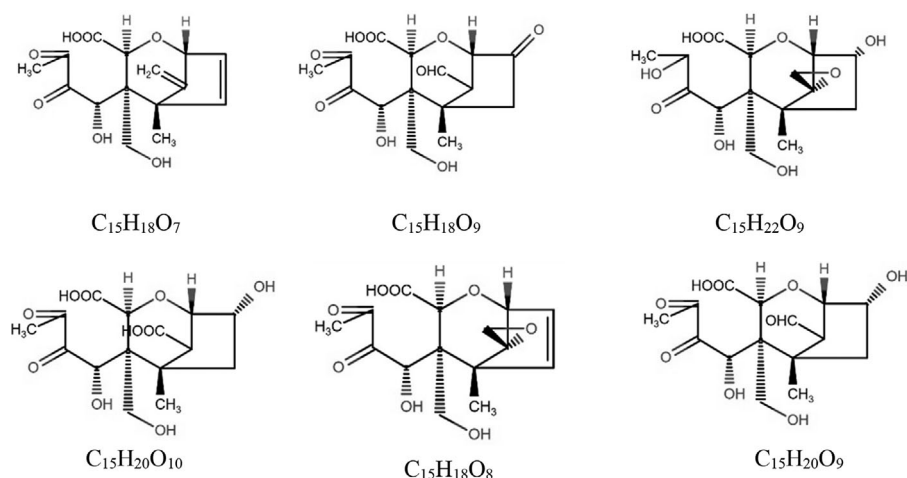
Ozone, as a potent oxidizing agent, has been assessed for its capability for DON detoxification. Ozone is unstable and its half-life is 20–50 min, and so can rapidly convert to oxygen and leaves no residue (Wang, Shao, et al. 2016). Ozone is recognized as GRAS by FDA to be used for the disinfection of bottled water with the maximum residual level of 0.4 mg of ozone per liter of bottled water (U.S. Food and Drug Administration 2020). In the food industry, it can be used for sanitizing food-contact surfaces, fresh fruit and vegetables for reducing pesticide levels on them, packaging and container material, or as an alternative to chlorine (Government of Manitoba 2021). Ozone treatment (75 mg/L  $O_3$ ) of naturally contaminated wheat samples for 30, and 90 min reduced DON concentration by 26.4%, and 53.5%,

respectively (Wang, Shao, et al. 2016). Increasing the ozone concentration, treatment time, and moisture content of the substrate improves the degradation rate of DON (Wang, Luo, et al. 2016; Li, Guan, and Bian 2015; Alexandre et al. 2017; Krstović et al. 2021). At higher moisture contents, ozone has higher oxidation power and penetration rate due to its solubility in water (Wang, Luo, et al. 2016; Santos Alexandre et al. 2018), which causes a higher degradation rate of DON. It was suggested that in the wet condition, ozone would move slower within the grain layers, allowing a greater exposure time (Tiwari et al. 2010).

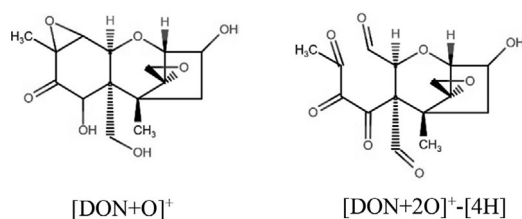
DON degradation rate in flour could be more than that in the wheat kernel because of higher exposure of DON to ozone in flour. However, flour coagulation due to maldistribution of moisture could limit the contact between ozone and flour, and hence affect the degradation rate of DON (Wang, Luo, et al. 2016). Wang, Luo, et al. (2016) reported no significant differences in the degradation rate of DON in wheat flour or wheat kernel at different concentrations of DON via ozone. Li, Guan, and Bian (2015) detected a lower degradation rate of DON solution at a higher concentration, 5  $\mu\text{g}/\text{ml}$  compared to 1  $\mu\text{g}/\text{ml}$  concentration. In lower concentrations of DON, more reactive species would have the chance to react with DON to lead to a higher degradation rate. When either the moisture content of the substrate or ozone is increased (wet sample and wet ozone, compared to dry sample and dry ozone), the reduction rate of DON increases (Young 1986b; Alexandre et al. 2017). In the presence of water, ozone can be broken into atomic oxygen and hydroxyl ions leading to the oxidative degradation of DON (Li, Guan, and Bian 2015). Ozone treatment for 30 min decreased DON content from 1065 to 206  $\mu\text{g}/\text{kg}$  (Savi et al. 2014). During the ozonation process, ozone reacts with double bonds and forms ozonides, which then reorganized to molozonide, an unstable compound, and finally generates a variety of aldehydes and ketones or organic acids (Figure 5) (Santos Alexandre et al. 2018).

Two primary oxidation pathways via ozone in water have been proposed: i) direct oxidation ii) indirect oxidation by free radicals that have been formed due to decomposition in water. These pathways are influenced by ozone concentration, UV light, pH, and the presence of radical scavengers (Young, Zhu, and Zhou 2006). One of the free radicals that is formed during ozone decomposition is hydroxyl radical which has more oxidation potential than ozone molecule. The reaction between the free radicals and organic compounds is believed to be faster but less selective compared to ozone (Young, Zhu, and Zhou 2006). The relative amount of ozone that is needed for a 50% reduction (R50) in trichothecenes is dependent on the oxidation state of  $C_8$ . R50 of trichothecenes is as follows: trichothecenes with group 8-methylene (no oxygen) < 8 hydroxy (free or esterified) < 8-keto. Trichothecenes have an olefinic double bond in the  $C_9$ - $C_{10}$  position. The olefinic position is one of the most reactive sites for the ozone to organic molecule reaction (Young, Zhu, and Zhou 2006).

Young, Zhu, and Zhou (2006) proposed a degradation pathway for trichothecenes such as DON via ozone (Figure



**Figure 6.** Structures of the six major degradation products of DON after ozone treatment. Adapted from Li, Guan, and Bian (2019b).



**Figure 7.** Structures of the two degradation products of DON after ozone treatment. Adapted from Sun et al. (2020).

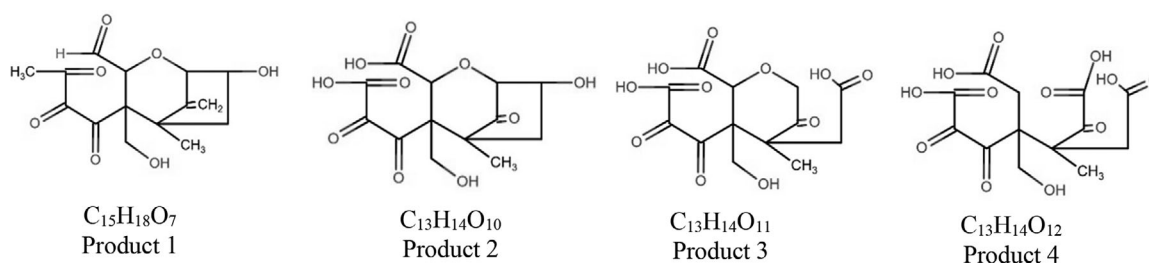
5). There is a possibility of further degradation in the presence of ozone to simple molecules such as acids, aldehydes, ketones,  $\text{CO}_2$ . Also, in the same study, the effect of pH on the degradation of DON via aqueous ozone was assessed. At pH = 4–6 all the mycotoxin was degraded, at pH = 7–8 the degradation rate was dependent on the oxidation state of the  $\text{C}_8$ , and at pH = 9, no degradation was observed (Young, Zhu, and Zhou 2006). This suggests the importance of the acidic condition in improving the degradation rate of DON by ozone. Cytotoxicity assays of DON on mice (Wang et al. 2017), and human hepatic carcinoma and Henrietta Lacks (Hela) cells (Ren et al. 2019) determined that the cytotoxicity could be highly reduced after ozonation. The toxicity of DON is believed to be due to the 12–13 epoxy ring, the unsaturated bond at  $\text{C}_{9-10}$ , and the presence of hydroxyl radical at position 3 (Li, Guan, and Bian 2019b). Increasing ozone concentration from 1 to 8 mg/L, improved the degradation rate of DON in ultrapure water from 21.9 to 95.7% after 15 s treatment. In higher concentrations of ozone, more free radicals such as hydroxyl radicals are generated that can react with DON in the solution (Li, Guan, and Bian 2019b). Six major ozonation byproducts of DON have been identified (Figure 6) by Li, Guan, and Bian (2019b). The change in the key toxicity functional groups of DON due to de-epoxy reaction, or change of hydroxyl group in  $\text{C}_3$ , and the loss of double bond in  $\text{C}_{9-10}$ , probably had significantly reduced the toxicity of DON after ozonation. Sun et al. (2020) assessed the degradation byproducts of DON in aqueous ozone and they determined two compounds as the ozonation byproducts of DON (Figure 7).

According to the degradation pathway and the ozonolysis products of DON that have been determined by Ren et al.

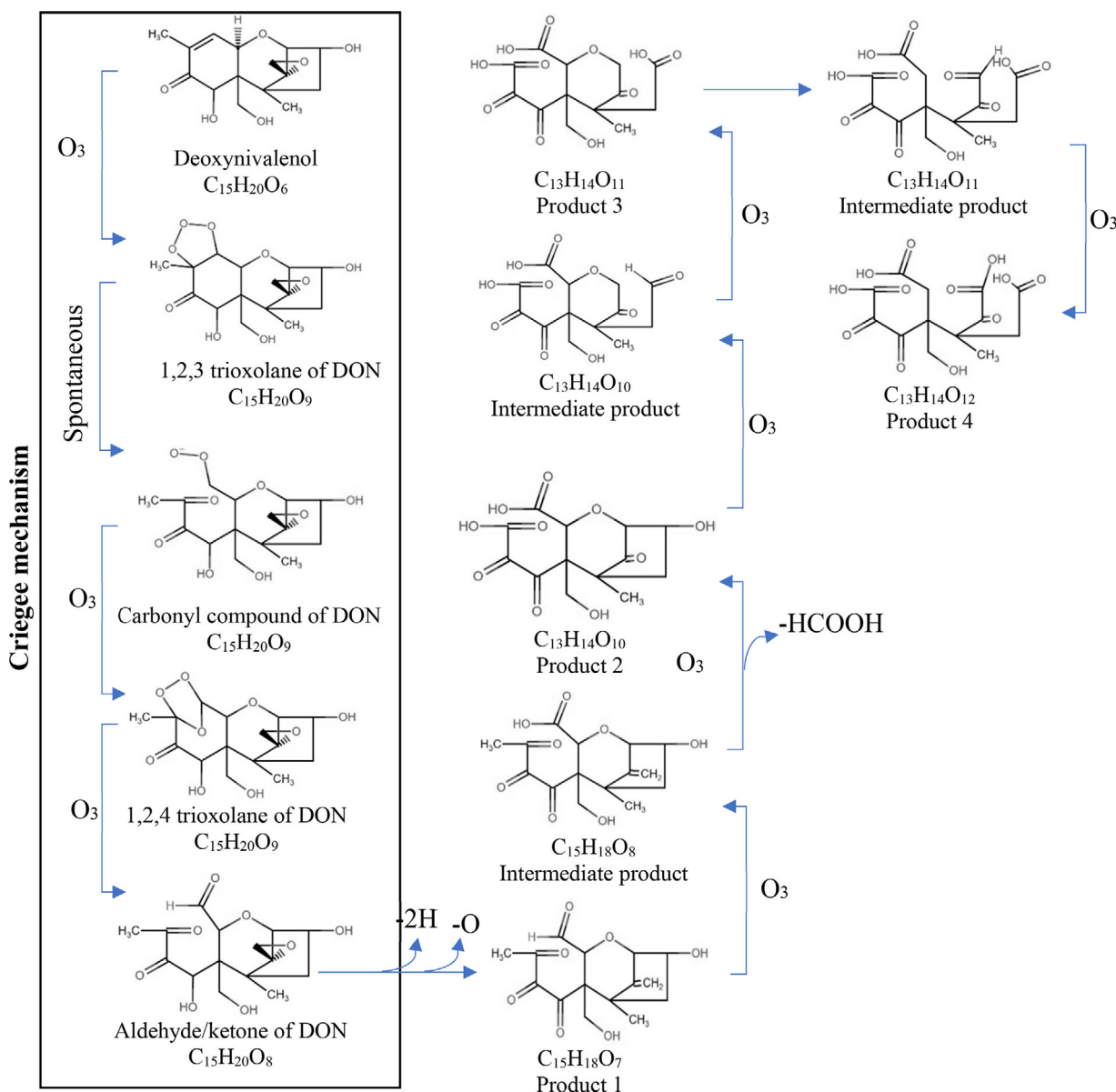
(2020) (Figure 8), ozonation cleaves the double bond at  $\text{C}_{9-10}$  and transforms it to an aldehyde, ketone or carboxylic acid. The hydroxyl group at  $\text{C}_7$  is oxidized to a carbonyl group, and the epoxy group is oxidized to a double bond. Based on the degradation pathway of DON by ozone defined by Ren et al. (2020) (Figure 9), the Criegee reaction is involved in the ozonolysis of DON. The degradation pathway of DON was deduced based on the accurate molecular masses and structures of ozonolysis products of DON. The differences in the degradation rates of DON in different studies could be due to various ozone concentrations and its flow rate, ozone state (aqueous or gaseous), treatment time, initial DON concentration, water content, and the surface characteristics of the substrate (Ren et al. 2019; Ren et al. 2020).

### Atmospheric cold plasma

Atmospheric cold plasma (ACP) is a novel non-thermal technology that has the potential for decontamination of microorganisms and mycotoxin. This system produces reactive species, ions, radicals, and UV light which are influenced by the process factors such as voltage, type of gas, relative humidity, treatment time, etc (Feizollahi, Misra, and Roopesh 2021). Previous studies reported the effect of ACP on the degradation of DON, however, no study assessed the degradation products of DON during ACP treatment. Feizollahi, Iqdam, et al. (2020) performed ACP treatment (34 kV, 2 mm gap) for 6 min, which reduced the DON levels on spiked barley by 48.9%. The chemical reaction of reactive species produced during ACP with mycotoxin, decomposition of the mycotoxin after collision with electrons and ions, and UV light are considered as the factors that contribute to the cleavage of DON or other mycotoxins by ACP treatment (Feizollahi, Iqdam, et al. 2020; ten Bosch et al. 2017). This could explain the higher degradation rate of DON via ACP compared to ozone treatment as numerous factors contribute to the degradation of the toxin in ACP. Among the reactive species produced during ACP treatment, reactive oxygen, and reactive nitrogen species (RONS) comprise the major part (Misra et al. 2019). Ozone which is one



**Figure 8.** Structures of the degradation products of DON after ozone treatment. Adapted from Ren et al. (2020).



**Figure 9.** Degradation pathway of DON in acetonitrile via ozone treatment. Adapted from Ren et al. (2020).

of the ROS in ACP probably plays a major role in the degradation of DON via ACP (Feizollahi, Iqdam, et al. 2020).

The presence of water could contribute to the formation of RONS in ACP and improve the degradation rate of DON (Feizollahi, Iqdiam, et al. 2020; Feizollahi, Arshad, et al. 2020). DON in solution (acetonitrile/water (20/80, v/v)) reached undetectable levels within 5 min of ACP treatment;

however in the dry state the degradation rate was 25.1%. In a solution, more ROS would be generated and the uptake of ozone which is soluble in water would increase, improving the degradation rate of DON (Feizollahi, Arshad, et al. 2020). Fourier-transform infrared spectroscopy results indicated the formation of carbonyl group or epoxy group in the degraded product after treating DON with ACP

(Feizollahi, Arshad, et al. 2020). The effect of dielectric barrier discharge plasma (ten Bosch et al. 2017), gliding arc plasma (Kříž et al. 2015), low-pressure microwave plasma (Kříž et al. 2015), and microwave-induced argon plasma (Park et al. 2007) on DON degradation have been assessed previously. UV radiation and etching by plasma were mentioned as the probable reasons for the degradation of DON by plasma (Park et al. 2007). It was postulated that the presence of aromatic rings in the structure of a toxin may contribute to its lower plasma-induced degradation compared to another toxin with a different structure (ten Bosch et al. 2017). Cytotoxicity assay has proved the complete decrease of DON cytotoxicity after 5 s treatment by microwave-induced argon plasma system (Park et al. 2007).

Plasma activated water (PAW) is another method of degrading DON and other mycotoxins. There are multiple studies on the application of PAW on bacteria inactivation. These studies have mentioned one reason for the inactivation of bacteria which could be relevant in the degradation of mycotoxin as well. Synergistic action of a high positive oxidation-reduction potential (ORP) and low pH (pH~ 2–3) (Oehmigen et al. 2010; Tian et al. 2015) are the main reasons for the inactivation of bacteria.  $\text{H}_2\text{O}_2$ ,  $\text{OH}^\bullet$ ,  $\text{O}$ ,  $\text{O}_3$ , and reactive nitrogen species (RNS) in PAW can increase the ORP. RNS also contributes to low pH (Feizollahi, Misra, and Roopesh 2021). A previous study reported that soaking the contaminated barley in PAW for 5, and 20 min, reduced the DON concentration by 22.5, and 25.8%, respectively (Chen et al. 2019). The degradation rate of DON was correlated to ORP and pH. In PAW, short-lived reactive species such as hydroxyl radical ( $\text{OH}^\bullet$ ), superoxide ( $\text{O}_2^-$ ), and singlet oxygen ( $\text{O}$  or  $\text{O}^-$ ) may not play any role in the degradation of DON; the major contribution for inactivation will be accomplished by long live reactive species such as  $\text{O}_3$ ,  $\text{H}_2\text{O}_2$ ,  $\text{NO}_3^-$  (Chen et al. 2019). It is important to state that during ACP treatments, the temperature of the samples does not rise to more than 60 °C, and hence no thermal degradation of the mycotoxin is expected (ten Bosch et al. 2017; Feizollahi, Iqdiem, et al. 2020).

The concentration and type of the reactive species during ACP could vary according to the process factors such as relative humidity of the air, voltage and current of the system, treatment time, type of the carrier gas, type of electrodes and dielectric materials, the gap between the electrodes, direct or remote exposure of toxin to the ACP, and the water content of the sample (Feizollahi, Misra, and Roopesh 2021). This makes the identification of degradation products and degradation pathways of mycotoxins via ACP challenging. However, advanced analytical techniques such as LC-MS and NMR (nuclear magnetic resonance) can facilitate the determination of degradation products of a mycotoxin after ACP treatment.

## Challenges and future research

The methods that have been elaborated in this article can be optimized and reduce DON amount below the required regulatory levels issued by authorized organizations. However, there is a crucial need to understand the

degradation mechanisms to be able to optimize each method for achieving an efficient degradation rate. A few studies have assessed the DON degradation mechanism and the by-products of DON for the mentioned methods including thermal and ozone treatments, but there is a need to understand the DON degradation mechanisms with regard to the other methods such as chemical, biological, irradiation, ultrasound, light, and atmospheric cold plasma treatments. The degradation mechanisms suggested for thermal and ozone treatments need to be validated by separate studies and clear the discrepancies in the mechanisms suggested for ozone treatment in different studies. To elucidate the degradation mechanisms of DON, LC-MS, HPLC-prep, and NMR should be performed by separating and purifying the degraded by-products. Determining the structure of the degraded by-products would be beneficial for assessing their cytotoxicity. To be able to separate and purify the degradation products by HPLC-prep followed by NMR, optimization of the experimental protocol should be performed, which is not economical as it requires a high concentration of DON. In the case of ACP treatment, inherently plasma is complex and there may be contributions from several factors on DON degradation, including the presence of different reactive species, UV light, ions, and energetic electrons. The concentration and type of reactive species and ions in ACP are heavily dependent on the treatment parameters such as voltage and frequency, treatment time, type of gas medium, relative humidity of the surroundings, and the surface characteristics of the substrate. These can change the degradation pathway of DON during ACP treatment. DON is a major problem for the grain industry and due to its harmful effects on human health, studies focusing on DON degradation mechanisms could be helpful to select the best decontamination method to provide safe products for consumers, as it is one of the sustainable development goals of Food and Agriculture Organization.

## Concluding remarks

Several treatment methods are used to reduce the concentration of DON on agricultural/food products. Among them, thermal, chemical, biological/enzymatic, irradiation, light, ultrasound, ozone, and atmospheric cold plasma treatments are the main processes, that were evaluated in different studies and discussed in this review paper. There is a distinct mechanism for each of the aforementioned methods in degrading the structure of DON. The DON concentration can be changed during these processes by the contribution of one or several of the following factors such as bond breakage in different segments of the DON structure, oxidation, isomerization, biotransformation, de-epoxidation, adsorption, etc. The changes in DON structure are caused by (i) energy supplied during thermal, irradiation, light, ultrasound, and ACP treatments; (ii) chemicals and enzymes during chemical and biological treatments; (iii) free radicals, reactive species, ions, and electrons produced during the ultrasound, ozone, and ACP treatments. To achieve a high degradation efficacy of DON by these methods, unfavorable



treatment conditions (e.g., long treatment times, or intense treatment) may be necessary, which could jeopardize the quality of the product. Moreover, the intense treatment applications are evaluated by regulatory authorities. For industrial applications of the degradation methods, the legislation of processes such as ultrasound and cold plasma should be established, considering the safety of the product. Also, without the appropriate in-vivo studies and necessary information about the degradation pathway and degradation products, reduction of DON during a process should not always be considered synonymous to its detoxification (Vanhoutte, Audenaert, and De Gelder 2016). There is a considerable lack of studies, focusing on advanced methods such as NMR to validate the proposed structures of the degraded DON products. Future research may focus on filling this research gap on identifying and confirming the structures of degradation by-products of DON after treating it with different processes.

### Author contributions

Ehsan Feizollahi: Writing original draft, review and editing. M. S. Roopesh: Review and editing, supervision.

### Disclosure statement

There are none to declare.

### Funding

We acknowledge the funding support from Alberta Agriculture and Forestry, Alberta Canola Producers Commission, and Natural Sciences and Engineering Research Council (grant nos. ACPC103MD2018; 2018F180R; RGPIN-2017-05051, CRDPJ532306).

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