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# The fate of deoxynivalenol through wheat processing to food products

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## Highlights

- Deoxynivalenol is not completely removed through wheat processing.
- Milling and ozonation are the food processes with the highest deoxynivalenol reduction.
- Deoxynivalenol can increase during breadmaking process, due to release from flour.
- There is a high increase of deoxynivalenol-3-glucoside during breadmaking process.
- More information about deoxynivalenol degradation products is necessary.

## **Abstract**

Deoxynivalenol (DON) is one of the most frequently occurring mycotoxin in wheat crops worldwide and it poses a risk to human and animal health due to its wide range of adverse effects. As its accumulation at field seems to be unavoidable, it is very important to investigate its stability during food processing. Recent outcomes of DON stability during milling, fermentation, and baking show some opportunities to reduce DON. In-depth knowledge of such processes is required. Moreover, DON-3-glucoside seems to increase during the breadmaking process, thus actions to prevent this to occur are required. Finally, recent studies have pointed out that ozonation may help reducing DON content in wheat, which provides a new alternative to the food industry.

## **Introduction**

*Fusarium* species produce a heterogeneous blend of mycotoxins known as trichothecenes. The most abundant trichothecene is deoxynivalenol (DON), also known as vomitoxin. It is mainly produced by *Fusarium graminearum* and *Fusarium culmorum* [1] and it is not classified as to its carcinogenicity to human by IARC (International Agency for Research on Cancer) [2]. However, DON can inhibit the synthesis of proteins when humans or animals ingest contaminated food, causing immune dysregulation, chronic autoimmune diseases and aberrant intercellular signalling [3]. Recent exposition studies showed the high exposure of human to DON, with high percentages of population exceeding the tolerable daily intake [4,5]. The high presence of DON in human urine is mostly attributed to consumption of contaminated wheat derived products [6].

Wheat is highly susceptible to DON accumulation in field, and a high percentage of analysed wheat samples, often above 85 %, contain DON [7-9]. Due to the high presence of DON in raw wheat, studying the stability of DON during food processing is critical. In addition, some studies showed that despite cereal processing, wheat based products also contain relatively high DON concentrations such as 246 ng/g [6] and 437 ng/g [10] in bread, 137.1 ng/g in pasta [11] and 14.6 ng/mL in beer [12]. In recent years research has been mainly focussed in DON stability during milling and breadmaking (Fig. 1). Moreover, wheat treatment with ozone has been proposed as a new alternative in the past few years [13].

The co-occurrence of conjugated DON forms has been documented in raw wheat, especially deoxynivalenol-3-glucoside (DON-3-glucoside) [14,15] which is a plant metabolite of DON [16]. Despite the high presence of DON-3-glucoside in wheat, few studies exist on its occurrence.

The present review summarizes the latest outcomes in DON and DON-3-glucoside stability during different food processing, in particular milling, fermentation and baking, as well as ozonation.

## **Wheat processing and DON**

### ***Milling***

Most of the wheat harvested in the world is subjected to milling. A large amount of studies have been made about how wheat milling can affect DON reduction [17-20]. DON is not eliminated during this step but it is only redistributed and concentrated in certain milling fractions. Milling of DON contaminated wheat results in less contaminated fractions intended for human consumption (flour or semolina); while concentration is observed in the animal feed fractions (bran, shorts and middlings). The significantly lower DON levels in finished flour may be attributed to the potential of the bran layer to behave as a physical barrier preventing the mycelia from penetrating further into the kernel structure [21]. The major DON concentration in

the external parts is of concern because although these parts are almost always used for animal feed, sometimes they are used for direct human consumption due to their benefits (improved large bowel function to slowed digestion and absorption of carbohydrate and fat and reduced risk for certain diseases). As Vidal et al. [22] showed wheat bran consumption can be an important source of DON intake. For instance, DON was found in 19 % of wheat bran samples, at concentration above the EU legal limit (750 ng/g).

Recent studies were performed using various kinds of wheat, natural and spiked contaminated, with different DON levels, from a low (0.001 mg/kg) to a high level (36.72 mg/kg) and different milling methods. This results in certain variability in the reported distribution factors. The levels of DON reduction in flour are extremely different, from a high reduction (> 80 %) [23\*\*] to a very low one (4 %) [24]. The reason for this variability may be the initial DON concentration, the higher the initial DON concentration the higher is the reduction in the final flour [23\*\*]. Another possible explanation may be the extent of mould penetration in the grain; although there is usually more DON in the external grain layers mould can penetrate inside the grain which would end in a higher DON concentration in the inner part and a lower DON reduction in the final flour than in other grains where less mould penetration occurred. Almost all the studies used laboratory mills (mainly the Bühler laboratory mill MLU-202) [10,23\*\*,24,25,26\*\*,27,28] and few of them used industrial equipment [29,30]. Anyway, few differences exist between the results of the different methods. In addition, wheat moisture is usually increased to 14-17 % to get a good split of the grain; although Zhang et al. [23\*\*] got the highest DON reductions (> 80 %) in the final flour with the highest moisture (16.5 %), Schwake-Anduschus et al. [26\*\*] using the same moisture (16.5 %) only got a 15 % of DON reduction in the flour. Relative distribution is the percentage of DON content in each milling fraction relative to the initial DON content in the initial grain, thus it is necessary to know the weight of each fraction to calculate it. Unlucky, few studies include this information and the absence of DON degradation cannot be confirmed. For instance, Zhang et al. [23\*\*] recovered the 97 % of the initial DON content in the different milling parts (shorts, bran and flour).

Regarding durum wheat, the milling process has the same impact in DON content, and different types of cereal did not cause different levels of reduction [21,29,31]. All in all, milling causes a reduction of the DON concentration in the final flour or semolina, while a DON increase in the external parts of wheat grains (shorts and bran) is detected. More studies using industrial conditions are needed.

### ***Fermentation***

Most studies in the fate of DON during fermentation deal with breadmaking. Although a huge amount of studies have been made in the past, they showed contradictory results and while some of them have suggested that DON concentrations are reduced during fermentation [32], others have shown that DON concentration significantly increases after fermentation [33,34]. Firstly, the presence of enzymes can have an important role. Suman et al. [35] detected an

increase of up to 14 % in DON using non-specific enzymes. Moreover, Vidal et al. [36], using flour improvers with non-specific enzymes, detected a 30 % increase in DON during fermentation. Studies in which either malt flour or other enzymes were added reported increases in DON levels [27,35,36], while in the absence of added enzymes, reductions in DON content often occur [32]. The importance of enzymes in detected DON was showed by Vidal et al. [37\*]. They tested different enzymes presence compared to fermented doughs without enzymes. Without the use of enzymes DON was reduced, 5 % when the fermentation was at 30 °C and 23 % when it was at 45 °C. On the other hand, the use of  $\alpha$ -amylase during the fermentation at 30 °C led to an increase of 10 % in the DON level. Moreover, xylanase, cellulase, protease and glucose-oxidase use resulted in an increase of DON of 15, 63, 75 and 78 %, respectively, in the fermented dough compared with the fermented dough without enzymes when the fermentation was at 45 °C. This could be associated with the release of DON from the wheat matrix through enzyme catalysis. Recently, Wu and Wang [38\*] and Wu and Wang [39] working at 38 °C for 1 hour without neither improvers nor enzymes, showed no significant change in DON. Temperature, apart from modifying enzyme activity, may directly affect to DON stability; Samar et al. [40] assayed fermentation temperatures from 30 to 50 °C, and found higher DON reduction as the temperature increased (from 0 to 56 %). The highest reduction in DON levels was observed at the highest temperature (50 °C) and the longest time tested (60 min). This result is consistent with the results by Vidal et al. [37\*] who observed only a 5 % of DON reduction when fermentation was at 30 °C, and 23 % when it was at 45 °C. Similarly, Generotti et al. [41\*\*] assayed different fermentation temperatures (from 26 to 46 °C), and they found reduced DON stability with higher fermentation temperatures. Thus, fermenting at high temperatures may be a feasible alternative to reduce DON content in bread, as long as bread quality is not affected.

On the other hand, Generotti et al. [41\*\*] pointed out that DON is more affected by initial yeast amount in the recipe than by fermentation time and temperature; a possible explanation for the observed DON reduction is DON adsorption on the yeast wall [42], however more studies to confirm this point are necessary. By contrast, sourdough use led to increases in DON content during fermentation [43]; it was suggested that sourdough bacteria could be able to either transform DON precursors into DON or to release bound DON, increasing the DON content at the end of the fermentation. For example, the increase of DON can also be favoured by the conversion of acetyldeoxynivalenol (3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol) forms to DON. Wu and Wang [38] showed that almost all 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol were converted to DON during kneading and fermentation.

In conclusion, DON can be either reduced or increased in dough fermentation. The use of high fermentation temperatures and avoiding enzyme use should produce DON reductions at the end of the fermentation, while use of enzymes (especially xylanase and  $\alpha$ -amylase) can cause important increases of DON due to enzymatic release.

## **Baking**

Mycotoxins are considered thermostable compounds; however, baking affects the stability of DON to a certain extent. Studies are contradictory: some studies have observed reductions in DON levels [32,33], while other studies have reported no changes or even increases in DON levels [36,44]. Firstly, the inconsistencies may exist because these studies have been conducted on different scales: some studies have been conducted in laboratories, while other studies have been conducted at the industrial level [20,32]. Moreover, Vidal et al. [45\*\*], using small items, demonstrated that DON levels may be widely reduced only in the external part of the loaves due to the reduced heat transmission. Thus, the size of the baked items may also provide an explanation for the inconsistent results reported for baking studies. Moreover, DON is rather more affected by the time of baking than the baking temperature [20,36]. Generotti et al. [41\*\*] even considered that baking temperature had negligible importance, for instance they found that the fermentation temperature is a more important factor to reduce DON than baking temperature. The reason is that temperature inside the bread loaves is always below 100 °C regardless the baking temperature [36]. The use of enzymes could modify the potential DON reducing effect of baking. Hence, xylanase and  $\alpha$ -amylase use caused an increase of DON after baking [37\*], however, the increase of DON for each enzyme depended on the fermentation temperature, as these enzymes have different optimum temperatures. The initial DON concentration affects the level of DON reduction, when the initial DON concentration is higher more reduction is got [33,45\*\*].

Kinetics for DON degradation during baking have been studied [45\*\*], it followed a first-order equation and the Arrhenius model showed a good fit.

### ***Ozonation***

The use of ozone to reduce DON concentration has been tested recently with important outcomes [13\*,46,47,48\*\*]. Although ozone has not been widely tested in DON in the past, previous studies have proven that ozone can detoxify mycotoxins, especially aflatoxins, effectively in food, such as corn [49] and wheat [50]. Ozone gas is a strong oxidizing reagent that can destroy the double bonds in organic compounds, and further produce simple products with low molecular weight [51,52]. In addition, ozone has favourable penetration, and can decompose to oxygen with no toxic residual [53]. Another positive point of ozone is that it does not cause alterations to the physical and biochemical characteristics of the whole wheat grains showing that the ozone treatment would not decrease the quality of wheat [46,47].

Some factors can affect the level of DON reduction during ozone treatment. The higher the contact time the higher the DON reduction [46–48\*\*], even Savi et al. [47] achieved a total DON reduction after 2 h of ozone treatment at 60 mg/L of ozone. However, they used spiked wheat samples and DON artificial contamination could be easier to reduce, because lower reductions were observed in naturally contaminated samples with longer treatments. Some of the studies [46–48\*\*] followed the evolution of DON during ozone treatment time with decreasing DON

concentration through the time. Moreover, DON is more reduced with higher wheat moisture [46,48\*\*]. Ozone can quickly be decomposed into atomic oxygen with strong oxidation and hydroxyl ions in water, which are effective in oxidative degradation of DON. Under higher water contents more reactive ions can be generated because ozone is soluble in water, so it has a stronger effect on the degradation of DON. Another important factor is ozone concentration; some studies showed highly variable results with higher ozone concentration [46,48\*\*]. For instance, Li et al. [46] only obtained a reduction of 3.5 % of DON with an ozone concentration of 20 mg/L, while they got a reduction of 26 % using an ozone concentration of 80 mg/L, and similar tendency has been observed in Wang et al. [48\*\*] who tested 25, 50, 75 and 100 mg/L of ozone. Few studies checked the impact of initial DON concentration in wheat. Wang et al. [48\*\*] assured that ozone effects had no correlation with initial DON concentration, but their tested DON range was very narrow. On the other hand, Li et al. [46] who worked in aqueous solutions (1 and 5 µg DON/mL) observed higher reduction in lower DON concentrations when the remaining factors (moisture, ozone concentration and time) were constant. It can be hypothesized that ozone generated reactive ions in solution was roughly the same under the same ozone concentration, therefore the lower the concentration of DON, the higher chance to react with reactive ions in solution, so the lower concentration of DON in the same concentration of ozone had higher rate of decline. Finally, when ozone treatment is made in whole wheat grain the reduction of DON in ozone treatment is mainly produced in the external layers of wheat grain and a small reduction is detected in the internal layers of wheat grain [46]. In the same way, Savi et al. [47] showed that the reduction is higher in whole wheat flour than in wheat kernels, so an ozone treatment after milling could be theoretically implemented as a critical control point.

For ozone degradation in wheat also a first order reaction was followed [48\*\*]. According the  $t_{1/2}$  values, ozone treatment is slower than baking for DON reduction. To sum up, long time, high ozone concentration, high percentage of moisture and low initial DON concentration increase the effectivity of ozone treatments. Up to now, ozone studies have been only made in raw material.

### **Increasing interest in DON-3-glucoside**

The availability of DON-3-glucoside standards has triggered the number of studies. Results show that DON-3-glucoside and DON in milling fractions is similar and the bran fractions were clearly higher contaminated with the DON-3-glucoside [26\*\*,28,54]. Recently, Vidal et al. [45\*\*] showed that DON-3-glucoside may be released under mild baking conditions of temperature and time (for instance, 140 °C for 35 min or 200 °C for less than 10 min) but reduced under harsher baking conditions (i.e., longer periods of time and higher temperatures). On the other hand, the enzyme use can also produce an increase of DON-3-glucoside concentration during the breadmaking process, in particular with the use of xylanase, α-amylase, cellulase and lipase



(> 100 % of increase of DON-3-glucoside) [37\*]. Although DON-3-glucoside is a DON conjugate, authors pointed out the DON-3-glucoside behaviour is not linked to DON [28,37\*] and Kostelanska et al. [28] suggested that a possible splitting of glycosidic bonds between DON-3-glucoside and cell polysaccharides may cause the release.

The increase of DON-3-glucoside during the breadmaking process agrees with the high ratio DON-3-glucoside/DON found in some analysed breads. While the ratio DON-3-glucoside/DON in raw cereals is from 10 to 30 % [14,15], some studies showed higher ratios in breads: De Boevre et al. [55\*] found the same concentration of DON-3-glucoside than DON, Vanheule et al. [7] found a ratio of 1.55 DON-3-glucoside/DON and De Boevre et al. [56] found a ratio of 0.91.

Finally, the increase of DON-3-glucoside is of concern because, although DON-3-glucoside is far less active as protein biosynthesis inhibitor than DON [57], DON-3-glucoside will likely be cleaved in the gastrointestinal tract due to chemical hydrolases or, more important, to microbial activity in the intestine as shown *in vivo* in swine and *in vitro* using human intestinal microbiota [58], thus its presence is important for food safety and the Joint European Commission FAO/WHO Expert Committee (JEFCA) considered DON-3-glucoside as an additional contributing factor of the total dietary exposure to DON [59].

## Conclusions

Although difficult to remove, some possibilities for DON reduction through wheat processing exist. In-depth knowledge of the food processes is, however, required to avoid those practices which could lead to increasing DON presence. DON-3-glucoside is of concern during breadmaking process because high increases of concentration are detected at the end of the baking process. There are still many factors to investigate which may affect DON stability. In recent years, ozonation has been proven to be a promising decontamination alternative, thus implementation of an additional step in food processing could be a choice. Food treatments can reduce DON concentration but attention should be paid to degradation products. There is little information on them and their associated toxicity. Degradation products have been only analysed in baking products and norDONs A-F and DON lactones have been described. All of them are less toxic than DON itself. The losses that cannot be ascribed to the formation of degradation products are most likely caused by pyrolysis or polymerization reactions [60]. On the other hand, two unknown compounds have been described after ozone treatment [61] which had a lower molecular weight ( $C_{11}O_4H_{22}$  and  $C_{13}O_2H_{25}$ ) than DON ( $C_{15}O_6H_{20}$ ), but their toxicology has not been studied.

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This paper is one of the few studies which described clearly the relative distribution of DON in the different milling parts (whole wheat, bran, shorts, flour and lost). Moreover, they also studied the relative distribution of DON-3-glucoside and the fate of DON and DON-3-glucoside during steamed bread.

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This study is very interesting because they described with a high level of detail the process of milling. It is very useful to understand deeply the milling process and how to build correctly a milling study. Moreover, they analysed DON in the milling fractions during the process. On the other hand, they also analysed the level of conjugated mycotoxins.

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It is an interesting study because they observed the stability of DON during breadmaking process and moreover they also checked the transformation of acetyl DON forms to DON during breadmaking process. They also detected degradation products of DON in the crust.

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This paper is important because they studied a high level of factors to see the importance of them in the stability of DON and DON-3-glucoside during the breadmaking process in an industrial process. The obtained results provided interesting information about the trends and directions useful to be followed exactly in this industrial perspective.

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Figure 1. Studies on DON stability for each type of food process since 2010.

