Effect of Ozone Treatment on Deoxynivalenol and Wheat Quality

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Abstract

Deoxynivalenol (DON) is a secondary metabolite produced by *Fusarium* fungi, which is found in a wide range of agricultural products, especially in wheat, barley, oat and corn. In this study, the distribution of DON in the wheat kernel and the effect of exposure time to ozone on DON detoxification were investigated. A high concentration of toxin was found in the outer part of the kernel, and DON was injected from the outside to the inside. The degradation rates of DON were 26.40%, 39.16%, and 53.48% after the samples were exposed to 75 mg/L ozone for 30, 60, and 90 min, respectively. The effect of ozonation on wheat flour quality and nutrition was also evaluated. No significant differences ($P > 0.05$) were found in protein content, fatty acid value, amino acid content, starch content, carbonyl and carboxyl content, and swelling power of ozone-treated samples. Moreover, the ozone-treated samples exhibited higher tenacity and whiteness, as well as lower extensibility and yellowness. This finding indicated that ozone treatment can simultaneously reduce DON levels and improve flour quality.

Introduction

Deoxynivalenol (DON), a secondary metabolite produced by *Fusarium* fungi, is a common contaminant in wheat, barley, oat, and corn[1,2]. *Fusarium* head blight, a fungal disease caused by several *Fusarium* species, occurs during wheat plant growth under humidity conditions suitable for fungal growth and temperate weather[3]. This disease causes two forms of agricultural damage: reduction in harvest because of grain shriveling and threat to food safety because of DON accumulation in grains by several *Fusarium* species[4]. Human and animal exposure to DON via ingestion of contaminated food can induce acute and chronic effects, such as immunosuppression, neurotoxicity, embryotoxicity, and teratogenicity[5–7]. The International Agency for Research on Cancer categorized DON as a Group 3 carcinogen[8]. Thus, safe, economical, and cost-effective technologies must be developed to reduce fungal growth and remove DON in wheat grains while mitigating post-harvest losses. DON detoxification involves physical, chemical, and biological methods[9–12]. However, each technique exhibits

certain limitations, such as nutrition loss, sensory attribute reduction, inconvenient operation, and high equipment cost, thereby hindering their practical applications.

Ozone has been widely used in the food industry as an effective antimicrobial agent for DON detoxification because of its potential oxidizing capacity[13]. The use of ozone in gaseous or aqueous phase as an antimicrobial agent has been approved by the US FDA according to the Federal Register provisions[14] and good manufacturing practices for processing and storage of food, bottled water, apple juice, and cider[15]. The advantages of ozone over traditional fumigants are as follows: (a) ozone rapidly decomposes (half-life of 20–50 min) to molecular oxygen, (b) leaves no residue, (c) can be generated on site, and (d) requires no storage and subsequent disposal of chemical containers[16]. Ozone can also kill pests in food and inactivate microbes, including bacteria, fungi, and viruses[17–19]. As such, ozone has been widely used to control the growth of various fungi in laboratory-scale trials in food, such as red pepper, wheat, dried fig, and peanut[20–24]. Since 1960, numerous studies have suggested that ozone treatment effectively degrades AFB_1, the most toxic compound among various fungi; moreover, the degradation products of AFB_1 after reacting with ozone has been widely studied[25–28]. However, data on DON degradation after ozone treatment are limited, and few studies have examined the effect of ozone treatment on the processing performance of wheat flour[29,30]. The limited studies on this field could be due to legislation that prohibits chemical treatment and uncertainty over the identity of reaction products.

Ozone treatment, a chemical detoxification method, can effectively control fungi in grains and may affect the quality of wheat in several aspects. Ozone can induce oxidation and/or modification of the physicochemical constituents of grains through lipid and starch oxidation, protein modification, grain discoloration, or changes in flour functionality. Sandhu et al.[31] found that ozone treatment can oxidize lipids, enhance brightness, reduce yellow hue, and increase peak and setback viscosities of flour. Violleau et al.[32] reported that ozonation can efficiently modify the technological properties of wheat grain.

Wheat and wheat products are consumed as food by approximately half of the world's population[15]; as such, the property of wheat after ozone treatment must be elucidated for human dietary purposes. A commercially acceptable method for detoxifying DON must be developed for application in the food industry. The present study aimed to (1) investigate the distribution of DON in the wheat kernel and the effects of duration of ozone exposure on DON detoxification to propose a practical method for ozonation detoxification, and (2) assess wheat flour quality and nutrition changes caused by ozonation.

Materials and Methods

Materials

Wheat samples. Wheat samples naturally contaminated with DON (*Wanmai 50*) were obtained from Suzhou City, Anhui Province, People's Republic of China (E116°09′–118°10′, N 33°18′ –34°38′) at the end of June 2013. The DON level reached 1.69 mg/kg.

Methods

Ozone treatment of wheat grains. Grains (20 kg) were subjected to ozone treatment using the procedure of Luo et al.[28] with minor modification. Wheat samples were placed in an airtight stainless steel reactor (70 cm in diameter and 140 cm in height). Ozone was produced using YG100 ozone generator (Beijing Shanmeishuimei Industry Co. Ltd., Beijing, China). Ozone gas (75 mg/L) was introduced into the ozone detector (Ideal-2000, Ideal Co. Ltd., Zibo, China) and then into the bottom of the reactor via a glass tube. Another ozone detector was connected to the outlet of the reactor to maintain the concentration of ozone. A

disposal apparatus (model DT250, Qingdao Guolin Industry Co. Ltd., Qingdao, China) was used to rapidly convert residual ozone into oxygen after the reaction with the samples. The ozone detector was used to detect ozone concentration in gas flow from the disposal apparatus. Wheat grains were exposed to ozone for 0, 30, 60, or 90 min at 25°C under 75% RH. During the treatment, grains were agitated in the reactor by using a central endless screw. After the ozone treatment, the grains were stored in clean polyethylene bags, which were either sealed and stored at 4°C for further analysis or used in milling.

Milling. Water (2% to 5% of dry weight) was added to the treated wheat grains to reach 15% to 16% (w/w) moisture content, depending on the initial water content. Each wheat sample was milled on a Buhler laboratory experimental mill (MLU-202) in accordance with the International Approved Methods 26–21.02[33] to obtain three samples of breaking flours (1B, 2B, and 3B), three samples of middling flours (1M, 2M, and 3M), and two samples of outer-layer fractions (bran and shorts). The milling room was maintained at 23°C–25°C under 40%–50% relative humidity. The milling recovery was 93% to 95%. The homogenous blend of 1B, 1M, 2B, 2M, 3B, and 3M fractions in a plastic bag (70 cm × 100 cm) was shaken 20 times to obtain a patent flour sample.

DON analysis. Wheat grain samples were analyzed using immunoaffinity columns for the cleaning step and LC/UV for detection according to the Chinese National Standard [34] with minor modifications. Briefly, 25 g of the sample was placed into a 250 mL Erlenmeyer flask and added with 100 mL of acetonitrile–water (84/16, V/V). The solution was mixed for 30 min under 200 r/min, filtered, and cleaned using a solid-phase extraction cleanup column (Mycosep227#, Romer Labs Inc., USA). Eluate (2 mL) was collected and evaporated using a heating block device at 50°C in a gentle nitrogen stream. Dry extract was then redissolved in 1 mL of acetonitrile–water (6:94, V/V) and filtered through a 0.22 μm filter membrane prior to high-performance liquid chromatography (HPLC) analysis.

The extract (20 μL) was injected into the LC/UV system at 218 nm, and the mobile phase was delivered at a constant flow rate of 0.8 mL/min. The total run time was 20 min, and the retention time of DON was 12.3 min. DON level was determined by comparing the measurement of the peak area at the DON retention time and that of the standard solutions used for the calibration curve (0.2, 0.5, 1, 3, 5, 10, 15, and 20 μg/mL; correlation $r = 0.997$).

Protein and lipid analyses and alveographic measurements. Protein content of wheat flour was determined according to the AACC International Approved Methods 46–12.01 by using nitrogen with a protein conversion of (N) × 5.7[33]. Alveographic measurements were performed based on Standard NF ISO 5530–4[35] with a Chopin NG alveograph (Chopin Technologies, Paris, France). The alveograph parameters were automatically recorded using the Chopin Alveolink-NG software; these parameters included the maximum overpressure or tenacity (P) needed to blow the dough bubble; abscissa at rupture (L), which measures dough extensibility; index of swelling (G) (measured as the square root of the volume of air necessary to inflate the dough bubble until it ruptures); deformation energy of dough (W), which represents the energy necessary to inflate the dough bubble to the point of rupture; deformation curve (P/L), and elasticity index (Ie). The alveograph W value could be considered as the indicator of gluten strength, whereas the alveograph P/L value represents the balance of elasticity and extensibility[36].

Fatty acid value was evaluated based on the method described by Rose et al.[37] to determine the effect of ozone exposure on fat components of wheat flour. The final results were expressed in terms of milligrams of KOH required to neutralize free fatty acid in 100 g of wheat flour (dry basis).

Color measurement of wheat flour. Color of the wheat flour samples was evaluated using Ultra Scan Pro 1166 (Hunterlab, USA) and expressed as L*, a*, and b*. L* denotes a

measurement of brightness (0–100), a* represents the red–green coordinates (− is green and + is red), and b* measures the blue–yellow coordinates (− is blue and + indicates yellowness) of the product. All these tests were conducted in triplicate.

Analysis of amino acids by HPLC. Amino acids were analyzed using HPLC according to the method developed by Yuetong et al.[38] with minor modifications. A sample (300 mg) was weighed into a screw-capped test tube and added with 6 M hydrochloric acid (8 mL) to determine amino acid content. The tubes were capped and hydrolyzed for 24 h at 110°C. After hydrolysis, the mixture was transferred to a 25 mL volumetric flask with ultrapure water and then decanted through filter paper (Whatman No. 1). The filtrate was evaporated to dryness under vacuum, and the hydrolysates were reconstituted in 1 mL of 0.02 M hydrochloric acid.

Analyses were performed on an Agilent 1100 HPLC (Palo Alto, CA, USA) equipped with a UV detector. Chromatographic separation was performed using an ACE HPLC column (C18-HL; HiChrom, Reading, UK) with a particle size of 5 μm (250 mm × 4.6 mm). Detection wavelength was 338 nm, and the column temperature was maintained at 40°C. The mobile phases used were as follows: (A) 0.10 mol/L sodium acetate buffer (pH 7.2 adjusted with acetic acid solution, 5% v/v) containing 0.0225% (v/v) triethylamine and 0.5% (v/v) tetrahydrofuran; and (B) 40:40:20 (v/v) mixture of acetonitrile, methanol, and 0.36 mol/L sodium acetate (pH 7.2 adjusted with acetic acid solution, 5% v/v). Amino acids were eluted under the following conditions: 8% B for 27.5 min and 60% B for 4 min (1.0 mL/min flow rate); 100% B for 1.5 min (1.5 mL/min flow rate); and 100% B for 2 min and 8% B for 1.5 min (1.0 mL/min flow rate). The target compounds were identified based on the retention times and UV-Vis spectral characteristics of the corresponding derivatized standards. Results were calculated and reported as gram of amino acid per 100 g of the sample.

Starch analysis. Starch content of flour was estimated using Megazyme kits (Megazyme International Ireland Ltd., Ireland) according to the approved AACC method 76–13[33]. Starch was isolated from wheat flour samples by using the procedures reported by Rupollo et al.[39] and Vanier et al.[40]. Starch was collected and dried in an oven at 40°C for 12 h. The starch powder was passed through a 100-mesh sieve and stored at ambient temperature.

Carboxyl content of the isolated starch was determined according to the procedure of Chattopadhyay et al.[41]. Carbonyl content of the isolated starch obtained from wheat grain was determined based on the titrimetric method reported by Smith[42]. Swelling power was determined on wheat flour samples by using the method described by McCormick et al.[43]. Swelling power was calculated as sediment weight divided by dried sample weight.

Statistical analysis

Data were evaluated by analysis of variance in SPSS 20.0 (SPSS Inc., Chicago, IL, USA). F-test was considered significant at $P \leq 0.05$. Means were separated using Fisher's protected least significant differences at $P = 0.05$.

Results and Discussion

DON analysis after ozone treatment

The distribution of DON in wheat kernel is shown in Table 1. The wheat grain sample, *Wanmai 50*, contained 1.69 mg/kg DON. Most toxins (DON) in the grain was distributed in the 1M flour, followed by bran, as shown in the "Fraction recovery" values presented in Table 1 [fraction recovery = fraction weight (%) × DON concentration (mg/kg)]. For example, the DON value was 0.44 mg/kg in 1M flour and 0.33 mg/kg in bran. The DON concentration gradually increased from 1B flour to 3B flour and then from 1M flour to 3M flour (Table 1). The retention of DON in the patent flour for human consumption was 53.25%. In the milled fractions of

Table 1. Milling properties of wheat and distribution of DON.

	Fraction weight (%)	DON concentration (mg/kg)[A]	Fraction recovery (mg/kg) [B]
Wheat kernel		1.69[a]	
1B flour	8.5	0.75[b]	0.06
2B flour	5.7	0.81[c]	0.05
3B flour	1.7	0.85[c]	0.01
1M flour	51.6	0.86[c]	0.44
2M flour	7.0	1.13[d]	0.08
3M flour	1.2	2.78[e]	0.03
Shorts	8.4	2.94[f]	0.25
Bran	15.9	2.05[f]	0.33
Total	100		1.26
Patent flour	75.7	0.90[cd]	0.68
Recovery after milling (%)[C]			74.56
Retention in patent flour (%)		53.25	

Means followed by the same small letters within columns are not significantly different ($P > 0.05$).

[A] Mean values ($n = 3$) are shown.

[B] Calculated by fraction weight (%) × DON concentration (mg/kg).

[C] Calculated by total fraction recovery (mg/kg)/concentration at wheat kernel (mg/kg).

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wheat, 1B and 1M fractions were located closest to the center of the endosperm, whereas 3B and 3M fractions were closest to the outer part of the kernel. In this study, a high toxin concentration was found in the outer part of the kernel, and DON level was higher inside than that outside the kernel. Most DON remained in the outer layer, where the majority of toxins accumulated. Despite different approaches used in various studies, researchers reported similar trends in mycotoxin distribution in different milled wheat fractions. Young et al. [44] indicated that the highest fungal infection is found on or near the kernel surface. Lancova et al.[45] demonstrated that the DON level of flour is approximately half the concentration of the whole wheat, and DON levels in bran can be two or more times higher than those in the wheat kernels; this finding indicated the presence of high toxin concentrations in the outer part of the kernel. During dry milling, toxins may concentrate on the bran and germ layers[46–48]. Interestingly, the total amount of DON in all fractions decreased by 25.44% compared with that in the whole wheat; this finding is consistent with the result reported by Zhang et al.[49] on the fate of DON during wheat milling. This reduction could not be attributed to experimental error. Moreover, Jackson et al.[50] reported that extrusion of maize-based foods, especially those with added glucose, leads to substantial decreases in AFB_1; in addition, 23%–38% AFB_1 in extruded grits with and without added glucose is bound to the component(s) of maize grits. We hypothesize that wheat grains are subjected to extrusion and shear forces of the grinder roll during milling, and the temperature increases with prolonged milling time. Thus, the mycotoxin structure may be modified by the interaction with wheat components.

Several researchers reported that milling effectively reduces mycotoxin concentrations in wheat flours by 30% to 50% compared with that in cleaned grains[51]. Thus, removal of the wheat bran layer can decrease toxin levels[52–54]. Although milling cannot be solely used as an effective treatment to reduce DON from wheat grains, this technique can effectively decrease DON levels in the patent flour. However, the extent at which *Fusarium* toxins in cereal grains are removed during processing may vary based on the milling method employed [49].
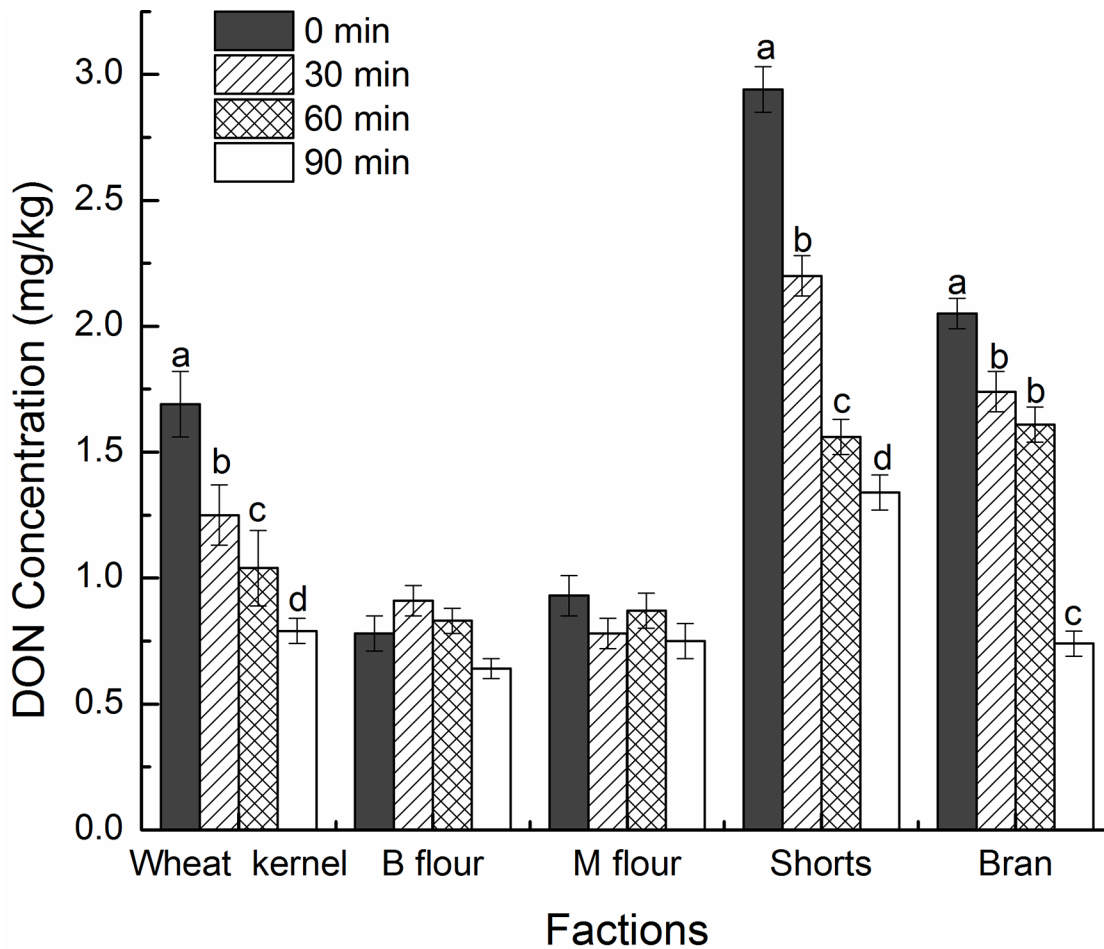
Fig 1. Distribution of DON in wheat kernel and milling fractions after ozone treatment. Values with different letters are significantly different (P < 0.05).

After 30, 60, or 90 min of exposure to 75 mg/L O_3, the DON degradation rates in wheat kernels were 26.40%, 39.16%, and 53.48%, respectively (data not shown). The same effect was observed in bran and shorts, whereas degradation rate was not significant different among all flour fractions (Fig 1). Ozone gas significantly affected the external part of wheat grain compared with than in the endosperm when eliminating DON. These results are in agreement with the trends reported by Savi et al.[55]; in this study, ozone treatment significantly decreases DON level in the pericarp compared with that in the endosperm. In summary, DON levels decreased after milling because of exposure to ozone treatment.

According to Criegee[56], DON degradation occurs when ozone molecules undergo a 1–3 dipolar reaction with a double bond. This phenomenon leads to the formation of ozonides (1, 2, 4-trioxolanes) from alkenes and ozone with aldehyde or ketone oxides as decisive intermediates, all of which have finite lifetimes[57]. Previous studies demonstrated that ozone is an effective agent for detoxification of aflatoxins, as evidenced by the less toxicity of ozone-treated products than the untreated samples and the absence of mutagenic activities or deleterious effects. Reports also show that detoxification efficiency increases with increasing treatment time and ozone concentration[20,58]. However, few studies have focused on DON. The present study shows that ozonation can effectively detoxify DON in grains. DON is regulated in several countries because of its harmful effects on human and animal health. In China, the National

Food Safety Standard of Maximum Levels of Mycotoxin in Foods (GB 2761–2012), which was issued in 2011, sets the maximum limit of 1 mg/kg for both wheat and flour. In the present study, both wheat kernel and flour samples treated with ozone can be used, thereby reducing the potential risk of illness compared with the untreated samples from the perspective of safety for consumption. In addition, ozone used before milling can enhance bran friability or the ability to separate bran from the starchy endosperm; ozone also significantly reduces (by 10%–20%) the required energy at the breaking stage without affecting grain hardness and flour yield[59].

Protein and lipid analyses and alveographic measurements of wheat grains after ozone treatment

For quality assessment, parameters evaluated in this study included protein and fatty acid oxidation. Despite the limited lipid content (2.2%) of wheat grains, lipid oxidation may occur when ozone oxidizes the unsaturated lipids of the grain. The value of fatty acid was used as an indicator for evaluating the degree of oxidation by ozone. Apparently, the observed fatty acid value was not significantly different between treated and untreated groups ($P > 0.05$), indicating that lipid was not affected by ozone under the experimental conditions. We conclude that the degree of oxidation using this method was acceptable. This result is consistent with the findings of Naito[60], who reported that the oxidation of lipids in cereal grains, cereal grain powder, peas, and beans was rarely observed after 0.05 to 5 ppm ozone treatment for 1 h. Similarly, the effect on lipid oxidation was not apparent in peanuts, pistachio, and Brazil nuts after O_3 application[58,61,62].

Wang et al.[63] found that the protein content of ozone-treated corn is lower than that of untreated corn; this finding indicated that protein could be destroyed by ozonation, thereby influencing the nutritional value of corn. Table 2 shows that no significant change ($P > 0.05$) occurred in protein content after the ozone treatment. These results are in accordance with the study by Mendez et al.[64], who reported that wheat treated with ozone showed no significant difference in protein content compared with that of untreated wheat. The difference between results of studies on wheat and corn may be attributed to the matrix effect. Corn was ground and thus porous, whereas the ozone cannot readily penetrate the whole wheat kernels.

In the present study, all flours from ozone treated wheat exhibited W and P/L values higher than those in the control (W = 312.10E^{-4}J, P/L = 2.50). This finding indicated that ozone treatment conferred flour with higher tenacity and lower extensibility (based on alveographic data) than native wheat. Gliadin is responsible for the viscous properties of dough, whereas glutenin contributes to dough strength. Disulfide bonds play a major role in determining dough properties. Exposure to oxidants can increase dough strength by oxidation of sulfhydryl groups to disulfide bonds[65]. We infer that disulfide bond formation is the main reaction underlying increased dough strength caused by ozone. Violleau et al.[32] also found that ozone treatment confers flour with higher force and tenacity and lower extensibility than those of the control. Ozone reacts with grain constituents (protein, lipid, and starch), depending on the

Table 2. Changes in the physicochemical properties of ozone-treated wheat flour.

Exposure time (min)	Fatty acid value [A]	Protein [A] (%)	W [B] (10E^{-4} J)	P/L [B]
0	61.1 ± 1.3	14.5 ± 0.3	312[ab]	2.50[ab]
30	60.9 ± 0.4	15.6 ± 0.5	316[b]	2.69[b]
60	61.1 ± 0.9	16.6 ± 0.8	322[c]	3.26[c]
90	59.0 ± 0.8	14.8 ± 0.0	338[d]	3.48[d]

[A] Values are presented as means ± standard deviations ($n = 3$) and are expressed on a dry weight basis.
[B] Mean value of five experiments.
Values with different letters are significantly different ($P < 0.05$).

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Table 3. Color changes of ozone-treated wheat flour.

Exposure time (min)	L*	a*	b*
0	93.64 ± 0.12^a	0.42 ± 0.02^a	6.70 ± 0.02^a
30	94.25 ± 0.02^b	0.39 ± 0.00^b	6.38 ± 0.05^{bc}
60	94.23 ± 0.11^b	0.36 ± 0.01^c	6.27 ± 0.10^c
90	94.18 ± 0.04^b	0.38 ± 0.02^{bc}	6.40 ± 0.09^b

Values are presented as means ± standard deviations ($n = 3$). Values with different letters are significantly different ($P < 0.05$).

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concentration and duration of ozone exposure. Other variables, such as temperature, moisture, and grain characteristics, as well as the presence of other organic matter, such as insects and microorganisms on the grain surface, may also affect the diffusion of ozone into the grain.

Color measurement of wheat flour after ozone treatment

The color of wheat flour is an important trait that strongly influences consumer acceptance of the flour itself and its end-products [66]. The color parameters (L*, a*, and b*) of the control and ozone-treated wheat flour samples are listed in Table 3. After ozone treatment, the L* value of wheat flour increased, whereas the a* and b* values significantly decreased; hence, ozone-treated wheat flour exhibited enhanced whiteness and reduced yellowness ($P < 0.05$). These results indicated that if ozone treatment was needed, such treatment would not decrease the acceptance of end users but can potentially improve the quality of flour. With regard to the color of wheat flour, ozone in gas or aqueous form can react with double bonds in carotenoid pigments, including β-carotene, xanthophyll, and flavones[16]. Previous studies also reported color changes in wheat flour. Chittrakorn[67] suggested that ozone could be used as a bleaching agent because flour treated with ozone gas is significantly less yellow and whiter than the control flour. Similar results were reported by Li et al.[66], who studied the effect of ozone treatment on the quality of wheat flour and shelf-life of fresh noodles. The current study demonstrated that the whiteness of flour and the noodle sheet increased after the ozone treatment.

Amino acid analysis

The nutritional quality of wheat is mainly related to the degree of balance of amino acids and essential amino acids. However, oxidation of amino acids by ozone can change the nutritional and metabolic value of grains, although few studies investigated this effect. Richard[68]_ENREF_1 reported that in aqueous solutions, amino acids that are readily oxidized by ozone include those containing the SH group, followed by tryptophan, tyrosine, and histidine. In the present study, the contents of essential amino acids (EAA) and total amino acids (TAA) slightly decreased; however, the ratio of EAA/TAA remained stable. In addition, no significant difference ($P > 0.05$) in tryptophan, tyrosine, or histidine was observed between the treated and untreated groups (Table 4). These results demonstrated that wheat treated under the experimental conditions were stable in terms of amino acid content. Similarly, Mendez et al.[64] indicated that treatment with 50 ppm ozone for 30 d did not change the amino acid content of hard and soft wheat, soybean, and maize. These data suggest that ozone did not adequately penetrate into the wheat kernel a less porous grain; thus, ozone did not reduce the nutritional value of the flour.

Starch analysis

Carbonyl and carboxyl contents, which are essential components of starch, and the total starch content are listed in Table 5. The total contents of starch, carbonyl, and carboxyl of wheat

Table 4. Effect of ozone treatment on the amino acid content of wheat flour.

Amino acid (g/100 g)	Exposure time (min)			
	0	30	60	90
Aspartic acid	0.85 ± 0.13	0.66 ± 0.10	0.88 ± 0.15	0.91 ± 0.18
Glutamic acid	5.78 ± 1.10	5.70 ± 0.90	5.56 ± 1.30	5.60 ± 1.20
Serine	0.60 ± 0.04	0.53 ± 0.06	0.58 ± 0.05	0.55 ± 0.07
Histidine	0.40 ± 0.02	0.39 ± 0.03	0.38 ± 0.04	0.39 ± 0.03
Glycine	0.63 ± 0.05	0.59 ± 0.06	0.59 ± 0.05	0.59 ± 0.04
Threonine*	0.43 ± 0.02	0.40 ± 0.04	0.40 ± 0.04	0.41 ± 0.03
Arginine	0.84 ± 0.04	0.95 ± 0.06	0.87 ± 0.04	0.88 ± 0.03
Alanine	0.55 ± 0.03	0.52 ± 0.03	0.50 ± 0.05	0.49 ± 0.06
Tyrosine	0.34 ± 0.03	0.39 ± 0.02	0.36 ± 0.05	0.36 ± 0.04
Cysteine	0.09 ± 0.01	0.11 ± 0.03	0.11 ± 0.02	0.11 ± 0.03
Valine*	0.82 ± 0.05	0.78 ± 0.04	0.77 ± 0.03	0.77 ± 0.03
Methionine*	0.16 ± 0.03	0.21 ± 0.04	0.17 ± 0.03	0.19 ± 0.02
Phenylalanine*	0.82 ± 0.03	0.79 ± 0.05	0.77 ± 0.04	0.78 ± 0.03
Isoleucine*	0.70 ± 0.04	0.66 ± 0.05	0.64 ± 0.03	0.64 ± 0.04
Leucine*	1.16 ± 0.08	1.10 ± 0.07	1.09 ± 0.05	1.10 ± 0.06
Lysine*	0.35 ± 0.03	0.33 ± 0.02	0.33 ± 0.03	0.34 ± 0.02
Proline	2.01 ± 0.06	2.04 ± 0.05	1.85 ± 0.09	1.92 ± 0.09
Tryptophan*	0.13 ± 0.01	0.11 ± 0.03	0.16 ± 0.05	0.14 ± 0.04
Essential amino acids	4.57 ± 0.29	4.38 ± 0.34	4.34 ± 0.30	4.36 ± 0.27
Total amino acids	16.66± 1.80	16.26 ± 1.68	16.01 ± 2.14	16.17 ± 2.04
EAA/TAA**	0.27 ± 0.16	0.27 ± 0.20	0.27±0.14	0.27 ± 0.13

*Essential amino acid.

**Calculated by [Essential amino acids/Total amino acids].

Values are presented as means ± standard deviations (n = 3). All treatments were not statistically significant when compared with the control group (P > 0.05).

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starch did not significantly change with increasing ozone treatment duration (P > 0.05). Meanwhile, swelling power was not significantly different (P > 0.05) between the control and ozone-treated samples.

Sandhu et al.[31] reported that oxidizing agents can oxidize hydroxyl groups at C2, C3, and C6 positions on glucose molecules to the carbonyl and carboxyl groups. The introduction of carboxyl and carbonyl groups could increase swelling power and alter the pasting properties of starch[69]. The increase in the swelling power of ozone-oxidized starch is related to the introduction of hydrophilic carboxyl groups[70]. The negative charges of carboxyl groups repel

Table 5. Effect of ozone treatment on the starch composition of wheat flour.

Exposure time (min)	Starch (%)	Carbonyl content (%)	Carboxyl content (%)	Swelling power
0	60.6 ± 1.1	0.000	0.000	8.22 ± 0.11
30	60.0 ± 2.5	0.059 ± 0.012	0.014 ± 0.005	8.16 ± 0.08
60	57.0 ± 2.3	0.035 ± 0.014	0.010 ± 0.001	8.06 ± 0.06
90	65.0 ± 2.3	0.074 ± 0.036	0.011 ± 0.004	8.04 ± 0.13

Values are presented as means ± standard deviations (n = 3). All treatments were not statistically significant when compared with the control group (P > 0.05).

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each other, thereby enhancing the swelling of starch granules during heating in water[71]; this finding suggested that the carbonyl and carboxyl contents of waxy rice starch gradually increases with increasing ozone treatment duration. Sandhu et al.[31] also demonstrated an increase in carboxylic groups and swelling power in isolated wheat starch (50 g) upon exposure to ozone gas for 30 min. However, ozone treatment used in the present study did not cause any alteration in wheat starch, which could be due to the greater effect of ozone treatment on the external part of the wheat grain. Similarly, Savi et al.[55] stated that the carboxyl and carbonyl contents of wheat starch are only affected by ozone treatment after 180 min. Other variables, such as botanical origin of starch, type of oxidizing agent, and reaction conditions, may result in differences in starch carboxyl and carbonyl contents, as discussed by Sangseethong et al. [72].

Conclusion

This study evaluated DON distribution in wheat kernel, effect of ozone treatment on DON degradation in wheat kernels, and changes in protein, lipid, dough quality, amino acids, color, and starch of wheat flour. A high toxin concentration was found in the outer part of the kernel, and the DON levels were higher inside than that outside the kernel. At a processing time of 90 min and ozone concentration of 75 mg/L, the DON degradation rate reached 53.48%. Ozone induced a more significant effect on the external part than that on the endosperm of wheat grains. No significant changes were observed in terms of protein content, fatty acid value, amino acid content, starch content, carbonyl and carboxyl contents, and swelling power of samples after ozone treatment. The tenacity and whiteness of the starch were higher, and its extensibility and yellowness were lower than those in the control samples.

Overall, this study reveals that ozone exhibits a remarkable potential in reducing fungal growth and removing various mycotoxins in wheat grains but minimally affects the quality of wheat naturally contaminated with DON. This study also provides information for potential users of ozone-treated grain. Future investigations are needed to determine the economic benefits of implementing ozone technology as a part of an integrated management program to remove various mycotoxins in wheat grains and study the degradation products of DON and their structures. Moreover, the mechanism for detoxifying DON, particularly in the wheat sample, can be elucidated. From the perspective of safety for consumption, the toxicity of ozone-treated wheat will be investigated through sub-chronic toxicity experiments.

Author Contributions

Conceived and designed the experiments: LW HLS ZXC XHL RW YFL YNL YPL. Performed the experiments: HLS XHL YPL. Analyzed the data: HLS. Contributed reagents/materials/analysis tools: ZXC RW YFL YNL. Wrote the paper: LW HLS.

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