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Effect of ozonation on microbiological and chemical traits of wheat grain

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ABSTRACT

Ozone, as a strong oxidative agent, is used to eradicate microbial, but this treatment affects also the quality of grain. The objective of this study was to evaluate the effect of ozonation of winter wheat grain harvested in different cropping and tillage systems on the number and composition of fungi colonizing grain surface and on the contents of total-phosphorus (P), phytate-P and phenolic acids. Wheat was sown in a two-factor experiment established with the method of randomized sub-blocks. The first order factor included cropping systems: (a) crop rotation and (b) monoculture, whereas the second order factor included tillage systems: (1) conventional (CT); (2) reduced (RT); and (3) herbicide. Ozonation significantly reduced the count of fungi on the surface of grain, especially on the grain harvested from wheat monoculture. In addition, it increased the content of phytate-P but decreased the content of total-P. A higher total-P content was determined in the grain harvested from monoculture than from crop rotation, whereas phytate-P content in the grain from crop rotation was higher than from monoculture. The ozonated grain harvested from CT plots was characterized by a higher content of phytate-P and a lower content of total-P, compared to the non-ozonated grain. Ozonation also increased the content of phenolic acids in the grain, especially in that harvested from the RT system.

Keywords: *Triticum aestivum*; disinfection; fungicidal efficiency; polyphenol; microbial organism

Ozonation is applied in many domains of life. As a strong oxidative agent, ozone is used to eradicate bacteria, viruses and fungi (Jouany 2007, Dubois et al. 2008). Water mixed with ozone serves for disinfection of fruit and vegetables as well as installations and tanks of various types (Kahforoushan et al. 2005). Ozonation of food products ensures their microbiological purity and extends their shelf-life (Kim et al. 1999). Ozonation of air in closed rooms destroys storage pests (Kells et al. 2001). This treatment affects also the quality of cereal products. In a study by Kholodova (2011), a mixture of ozone and air increased the elasticity of gluten proteins in wheat flour and dough elasticity and decreased its dilution during fermentation. Dough obtained from ozonated flour was characterized by longer freshness, which results from good binding of water with a protein-starch complex. However, a research by Ibanoglu (2001) did not confirm the positive effect of ozonation on contents of protein and ash and on the colour of flour. Also milling and baking indices of grain turned out to be similar in ozonated and non-ozonated grain. According to Mendez et al. (2003), these differences may result from the method of ozonation, grain quality and thickness of ozonated grain layer. Mariotti et al. (2006) points to poorer water absorption by starch and degradation of amylase in the ozonated

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grain. In addition, ozonation was found effective in destroying fungi and fungal spores present on the surface of grain (Nowakowicz-Dębek et al. 2013). These fungi produce toxins that pose risk to both men and animals as well as they induce an inhibiting effect on plants (Jouany 2007).

The objective of this study was to evaluate the effect of ozonation of winter wheat grain harvested from different cropping and tillage systems on the number and composition of fungi present on the surface of grain and on contents of total-phosphorus (P), phytate-P and phenolic acids in the grain.

MATERIAL AND METHOD

Grain of winter wheat cv. Ozon used in the study originated from a field experiment conducted in the years 2013–2015 at the Experimental Station in Uhrusk (51°18'10''N, 23°36'44''E) belonging to the University of Life Sciences in Lublin, Poland. The analysed wheat was sown in a two-factorial experiment established with the method of randomized sub-blocks. The first order factor were systems of crop succession: (a) crop rotation (pea-winter wheat-spring wheat) and (b) monoculture (winter wheat-winter wheat-winter wheat). The second order factor were tillage systems: (1) conventional (CT); (2) reduced (RT); and (3) herbicide (HT). Field cultivation under winter wheat in the CT system included shallow ploughing and sow ploughing; in the RT system included a cultivator and a tillage system, whereas in the HT system included the use of glyphosate herbicide (4 L/ha) and a cultivator before wheat sowing. Wheat was sown in the first week of October. The experiment was established on Rendzic Phaeozem soil (IUSS Working Group WRB 2006) with the composition of sandy clay with 24.4% of the silt fraction and 13.0% of dust fraction.

Samples of ozonated and non-ozonated grain were determined for: (1) the number and species composition of fungi, and (2) contents of total-P, phytate-P and phenolic acids. In order to determine the count and species composition of fungi, six primary samples were collected from each plot. The samples were poured together and then divided into 2 collective samples with the mass of 250 g each. Afterwards, each collective sample was randomly divided into 2 groups: (1) grain intended for ozonation, and (2) non-ozonated grain (control sample). Thus prepared samples of grain (20 ± 0.1 g) were placed in sterile and hermetically-sealed containers. Grain was ozonated for 1 h with an ozone generator with the capacity of 2.8–3.2 mg/h. The samples of ozonated and non-ozonated grain were transferred to glass containers with a sterile diluting fluid, and serial dilutions were prepared. The serial dilutions were plated in 0.1 mL solution on a solid Sabouard medium and incubated at a temperature of 25°C ± 0.2 for 7 days according to PN-R-64791 (1994) standard. In order to compute the total count of fungi, the grown colonies were counted using an electronic colony reader. For species identification, the grown colonies were transferred to microcultures and determined according to the Watanabe's key (Watanabe 2002). Determinations were conducted in 4 replications and the results were presented as geometric means, by expressing the number of fungi in the form of colony-forming units in 1 g of the analysed material (CFU/g).

Determination of total-P consisted in grinding and incineration of 1-g samples of the grain at 620ºC. The resultant ash was dissolved in 5 mL of 6 mol/L HCl and diluted to the volume of 50 mL with distilled water. The analysis was conducted based on PN-EN ISO 6878 (2006) standard. Determination of phytate-P consisted in grinding 0.2-g sample of grain and shaking with 20 mL of 2.5% HCl on a shaker at the speed of 150 rpm. The resultant extract was centrifuged for 10 min with the speed of 3000 rpm. The supernatant was 10-fold diluted with water. Then, 1 mL of a diluted solution of the extract and 0.35 mL of Wade reagent (0.3 g $FeCl_3$ 6 H_2O + 3 g sulfosalicylic acid in 1 L) were collected for determination. Absorbance was determined with the spectrophotometric method (λ = 500 nm) (Latta and Eskin 1980, Dragičević et al. 2011). The content of phenolic acids was determined with the Arnova method (Farmakopea 2010). The sample (2 g) was shaken with 20 mL of water for 20 min. Next, 1 mL of the analysed extract, 1 mL of 0.5 mol/L HCl, 1 mL of Arnova reagent, and 1 mL of 1 mol/L NaOH were added to 5 mL of distilled water and the whole sample was filled up with distilled water to the volume of 10 mL. Absorbance of the solutions was measured against standard at a wavelength of 490 nm. The content of phenolic acids (expressed per caffeic acid) was calculated from the following formula:

$$X = 1.7544\ A/m$$

Where: A – absorbance; m – mass of the weighed portion (g).

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Table 1. Number of fungi (colony forming unit/g) on wheat grain in crop rotation and monoculture (n = 48)

Cropping system (CS)	Grain (G)		Mean
	ozonated	non-ozonated	
Crop rotation (CR)	195.7	403.8	299.8
Standard deviation	89.7	257.5	
Monoculture (M)	213.0	844.5	528.7
Standard deviation	139.9	47.4	
Mean	204.3	624.2	–
$HSD_{0.05}$ for CS = 195.4; G = 195.4; CS × G = 311.0			

HSD – honest significant difference

Table 2. Number of fungi (colony forming unit/g) on wheat grain in tillage systems (n = 72)

Tillage systems (TS)	Grain (G)		Mean
	ozonated	non-ozonated	
Conventional tillage (CT)	311.3	601.9	456.6
Standard deviation	154.1	457.5	
Reduced tillage (RT)	217.1	674.1	445.6
Standard deviation	73.6	186.4	
Herbicide tillage (HT)	159.9	654.3	407.1
Standard deviation	57.5	496.3	
Mean	229.4	643.4	–
$HSD_{0.05}$ for TS = ns; G = 201.2; TS × G = ns			

HSD – honest significant difference

The obtained results were developed statistically with the method of the analysis of variance (ANOVA), whereas the significance of differences between mean values was evaluated with the Tukey's *HSD* test, $P < 0.05$.

Table 3. Species composition of fungi on wheat grain in crop rotation and monoculture (%)

Specification	Cropping system			
	CR	M	CR	M
	ozonated grain		non-ozonated grain	
Proteus sp.	5.2	0.6	4.3	2.2
Saccharomyces sp.	9.9	20.5	10.2	15.5
Paecilomyces sp.	2.4	2.1	1.9	4.0
Cladosporium sp.	–	3.5	3.5	6.1
Cladosporium macrocarpum	–	2.4	2.1	2.3
Muccor sp.	8.3	7.7	7.6	5.7
Rhizopus sp.	–	–	0.8	0.3
Aspergillus niger	–	–	4.4	–
Aspergillus sp.	5.6	16.3	5.4	11.2
Aspergillus flavus	–	1.3	–	4.1
Alternaria sp.	–	–	–	0.2
Ulocladium sp.	21.4	13.6	8.8	15.3
Rhodotorula sp.	8.4	6.3	4.5	3.9
Fusarium sp.	12.3	13.4	12.3	12.5
Phoma sp.	7.9	–	4.2	–
Penicillium sp.	10.7	0.6	10.0	4.4
Basipetospora sp.	–	–	–	0.9
Unidentified	7.9	11.7	20.0	11.4
Number of identified genera and species	10	12	14	15

CR – crop rotation; M – monoculture

RESULTS AND DISCUSSION

Regardless of the plant succession system, the treatment of ozonation caused over a three-fold decrease in fungi concentration on the surface of wheat grain, compared to the non-ozonated grain (Table 1). Higher effectiveness of ozonation was determined in the grain from the monoculture than from crop rotation. It certainly results from a higher number of fungi on the surface of grain harvested from monoculture than from crop rotation. Also other investigations (Struik and Bonciarelli 1997, Woźniak 2001) demonstrated significantly greater infestation of wheat by fungi in the monoculture than in crop rotation, which consequently reduced grain yield. In our study, the effect of tillage systems (CT, RT, HT) on the fungi number on grain surface was insignificant (Table 2). In turn, the number of fungi was decreased by ozonation, compared to the non-ozonated grain. A high fungicidal efficiency of ozonation was also confirmed in a research by Jouany (2007) who demonstrated that this treatment significantly reduced the count of fungi on grain surface. In our experiment, the ozonated grain from crop rotation and from monoculture was colonized by, 10 and 12 genera (and species) of fungi, whereas the non-ozonated grain by 14 and 15 genera, respec-

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Table 4. Species composition of fungi on wheat grain in tillage systems (%)

Specification	Tillage system					
	CT	RT	HT	CT	RT	HT
	ozonated grain			non-ozonated grain		
Proteus sp.	7.8	–	1.0	4.8	–	4.9
Saccharomyces sp.	20.9	18.5	6.3	13.7	17.7	7.3
Paecilomyces sp.	3.6	3.2	–	2.9	5.9	–
Cladosporium sp.	2.1	3.2	–	5.2	5.0	4.3
Cladosporium macrocarpum	–	3.6	–	–	5.4	1.3
Muccor sp.	8.4	7.3	8.4	6.3	7.0	6.7
Rhizopus sp.	–	–	–	–	–	1.7
Aspergillus niger	–	–	–	1.9	1.0	3.8
Aspergillus sp.	8.4	7.2	17.3	11.3	6.2	7.6
Aspergillus flavus	–	–	1.9	–	–	6.2
Alternaria sp.	–	–	–	–	–	0.3
Ulocladium sp.	5.7	10.3	36.6	7.6	8.7	19.9
Rhodotorula sp.	6.3	15.7	–	3.2	9.5	–
Fusarium sp.	15.5	21.1	1.9	14.5	15.6	7.2
Phoma sp.	3.6	–	8.4	2.8	1.0	2.5
Penicillium sp.	7.8	–	9.3	9.2	3.7	8.7
Basipetospora sp.	–	–	–	–	–	1.4
Unidentified	10.4	10.2	9.2	16.9	13.7	16.5
Number of identified genera and species	11	9	9	12	12	15

CT – conventional tillage; RT – reduced tillage; HT – herbicide tillage

tively (Table 3). Among them, the most numerous were: *Ulocladium* sp., *Fusarium* sp., *Penicillium* sp., *Saccharomyces* sp., and *Aspergillus* sp. The species composition of fungi was also influenced by tillage systems (Table 4). In the CT system, the predominant species found on ozonated and non-ozonated grain included: *Saccharomyces* sp., *Fusarium* sp., *Muccor* sp. and *Aspergillus* sp., in the RT system: *Fusarium* sp., *Saccharomyces* sp., *Rhodotorula* sp. and *Ulocladium* sp., and in the HT system: *Ulocladium* sp., *Aspergillus* sp., *Penicillium* sp. and *Muccor* sp. In a study by Jouany (2007), the surface of grain was colonized mainly by fungi of the genus *Aspergillus*, *Penicillium*, *Fusarium*, *Helminthosporium* and *Alternaria*, however ozonation significantly reduced their counts. The overall effect of ozonation, cropping system and soil tillage on microbial contamination is presented in Table 5. Analysis of variance components (*F*-value) showed that microbial contamination depended on the interaction of the cropping system (CS) with the tillage system (TS).

Table 5. Effect of ozonation (O), cropping system (CS) and tillage (TS) on microbial contamination

Specification	SS	MS	*F*	*P*
Ozonation	10575641	057564	13.381	0.001
Cropping system	314543	314543	3.980	0.059
Tillage	16197	8098	0.092	0.911
O × CS	268916	268916	3.402	0.081
O × TS	70580	35290	0.399	0.675
CS × TS	1974566	1974566	24.881	0.428
O × CS × TS	560533	50957	0.642	0.775
Error	1904424	79351	–	–
Totally	5215529	–	–	–

Table 6. Chemical composition of wheat grain in cropping systems (n = 48)

Cropping system (CS)	Grain (G)		Mean
	ozonated	non-ozonated	
Total-P (g/kg DM)			
Crop rotation (CR)	1.97	2.11	2.04
Standard deviation	0.32	0.07	
Monoculture (M)	2.08	2.12	2.10
Standard deviation	0.10	0.25	
Mean	2.02	2.12	–
$HSD_{0.05}$ for CS = 0.04; G = 0.04; CS × G = ns			
Phytate-P (g/kg DM)			
Crop rotation (CR)	0.81	0.80	0.81
Standard deviation	0.06	0.09	
Monoculture (M)	0.79	0.75	0.77
Standard deviation	0.04	0.04	
Mean	0.80	0.78	–
$HSD_{0.05}$ for CS = 0.01; G = 0.01; CS × G = ns			
Phenolic acids (g/kg DM)			
Crop rotation (CR)	0.17	0.18	0.18
Standard deviation	0.04	0.02	
Monoculture (M)	0.17	0.16	0.17
Standard deviation	0.02	0.02	
Mean	0.17	0.17	–
$HSD_{0.05}$ for CS = ns; G = ns; CS × G = ns			

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Table 7. Chemical composition of wheat grain in tillage systems (n = 72)

Tillage systems (TS)	Grain (G)		Mean
	ozonated	non-ozonated	
Total-P (g/kg DM)			
Conventional tillage (CT)	2.03	2.24	2.13
Standard deviation	0.38	0.15	
Reduced tillage (RT)	2.15	2.19	2.17
Standard deviation	0.11	0.16	
Herbicide tillage (HT)	2.13	2.16	2.15
Standard deviation	0.08	0.27	
Mean	2.10	2.20	–
$HSD_{0.05}$ for TS = ns; G = 0.06; TS × G = 0.14			
Phytate-P (g/kg DM)			
Conventional tillage (CT)	0.80	0.72	0.76
Standard deviation	0.04	0.06	
Reduced tillage (RT)	0.81	0.75	0.78
Standard deviation	0.04	0.08	
Herbicide tillage (HT)	0.78	0.80	0.79
Standard deviation	0.05	0.09	
Mean	0.80	0.75	–
$HSD_{0.05}$ for TS = ns; G = 0.04; TS × G = 0.07			
Phenolic acids (g/kg DM)			
Conventional tillage (CT)	0.17	0.17	0.17
Standard deviation	0.03	0.03	
Reduced tillage (RT)	0.20	0.15	0.18
Standard deviation	0.04	0.02	
Herbicide tillage (HT)	0.17	0.16	0.16
Standard deviation	0.01	0.02	
Mean	0.18	0.16	–
$HSD_{0.05}$ for TS = ns; G = 0.01; TS × G = 0.04			

Ozonation was observed to increase the content of phytate-P and to reduce the content of total-P in grain (Table 6). The higher content of total-P was also found in the grain harvested from monoculture than from crop rotation, whereas that of phytate-P in the grain from crop ration compared to the grain from monoculture. In turn, in a study by Woźniak and Makarski (2013), the content of total-P in wheat grain was significantly higher in crop rotation than in monoculture. In our experiment, phytate-P constituted 39.6% and 36.8% of the total-P in ozonated and non-ozonated grain, respectively. As reported by Sandberg (2002), phytate-P forms complexes with iron and zinc, which may induce deficiencies of these elements in human diet. Phytates are the main form in which phosphorus is stored in plant tissues (Kumar et al. 2010). They are synthesized during grain maturation when their content constitutes from 60–90% of the total-P (Loewus 2002). The analysis of the grain harvested from different tillage systems demonstrated that in the CT system ozonation significantly increased the content of phytate-P and decreased the content of total-P, compared to the non-ozonated grain (Table 7). The effect of ozonation, cropping system and soil tillage on total-P, phytate-P and phenolic acids in grain are presented in Table 8. Analysis of variance components showed that total-P and phytate-P depended on the interaction of CS × TS. In turn, Woźniak et al. (2014) showed a higher content of phytate-P in the grain from herbicide than from ploughing and reduced tillage systems. Ozonation also increased the content of phenolic acids in the grain, especially that from RT system. Kholodova (2011) reports that ozonation of flour had a beneficial effect on dough properties and bread quality, however a study by Ibanoglu (2001) showed no differences between the ozonated and non-ozonated grain.

In summary, it should be concluded that ozonation effectively reduced the number of fungi on wheat grain surface, especially in the case of grain harvested from wheat monoculture. In addition, it increased phytate-P content and decreased total-P content in the grain. A higher content of total-P was also found in the grain harvested from monoculture than from crop rotation, whereas that of phytate-P in the grain from crop rotation compared to the grain from monoculture. In the grain from

Table 8. Effect of ozonation (O), cropping system (CS) and tillage (TS) on chemical composition

Specification	Total-P		Phytate-P		Phenolic acids	
	F	*P*	*F*	*P*	*F*	*P*
Ozonation	1.104	0.304	0.848	0.368	0.021	0.886
Cropping system	0.582	0.454	2.223	0.150	0.605	0.445
Tillage	0.084	0.919	0.728	0.490	0.554	0.580
O × CS	0.325	0.575	0.618	0.441	1.292	0.268
O × TS	0.700	0.504	2.593	0.091	2.298	0.117
CS × TS	9.210	0.678	16.231	0.679	3.332	0.435
O × CS × TS	7.923	0.608	0.932	0.478	0.978	0.438

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CT system, ozonation increased phytate-P content and decreased total-P content, compared to the non-ozonated grain. Ozonation also increased the content of phenolic acids in the grain, especially in that from the RT system.

REFERENCES

Dragičević V.D., Sredojević S.D., Perić V.A., Nišavić A.R., Srebrić M.B. (2011): Validation study of a rapid colorimetric method for the determination of phytic acid and inorganic phosphorus from seeds. Acta Periodica Technologica, 42: 11–21.

Dubois M., Canadas D., Despres-Pernot A.G., Coste C., Pfohl-Leszkowicz A. (2008): Oxygreen process applied on nongerminated and germinated wheat: Role of hydroxamic acids. Journal of Agricultural Food Chemistry, 56: 1116–1121.

Farmakopea Polska (2010): The Office for Registration of Medicinal Products, Medical Devices and Biocidal Products. Warszawa. (In Polish)

Ibanoglu S. (2001): Influence of tempering with ozonated water on the selected properties of wheat flour. Journal of Food Engineering, 48: 345–350.

IUSS Working Group WRB (2006): World Reference Base for Soil Resources 2006. World Soil Resources Reports No. 103. Rome, FAO, 132.

Jouany J.P. (2007): Methods of preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. Animal Feed Science and Technology, 137: 342–362.

Kahforoushan D., Chitsaz M., Adeli S. (2005): Use of ozone in food industrial of Iran. Iranian Journal of Chemical Engineering, 4: 54–60.

Kells S.A., Mason L.J., Maier D.E., Woloshuk C.P. (2001): Efficacy and fumigation characteristics of ozone in stored maize. Journal of Stored Products Research, 37: 371–382.

Kholodova E.A. (2011): The improvement of wheat flour ozonation technology for the bakery goods production. [Ph.D. thesis]. Kyiv, National University of Food Technology of the Ministry of Education, Youth and Sports of Ukraine, 20. (In Ukrainian)

Kim J.G., Yousef A.E., Dave S. (1999): Application of ozone for enhancing the microbiological safety and quality of foods: A review. Journal of Food Protection, 62: 1071–1087.

Kumar V., Sinha A.K., Makkar H.P.S., Becker K. (2010): Dietary roles of phytate and phytase in human nutrition: A review. Food Chemistry, 120: 945–959.

Latta M., Eskin M. (1980): A simple and rapid colorimetric method for phytate determination. Journal of Agricultural and Food Chemistry, 28: 1313–1315.

Loewus F.A. (2002): Biosynthesis of phytate in food grains and seeds. In: Reddy N.R., Sathe S.K. (eds.): Food Phytates. Boca Raton, CRC Press, 53–61.

Mariotti M., Pagani M.A., Lucisano M. (2006): Starch damage: An interesting index for the determination of wheat flour quality. Tecnica Molitoria, 57: 397–403.

Mendez F., Maier D.E., Mason L.J., Woloshuk C.P. (2003): Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. Journal of Stored Products Research, 39: 33–44.

Nowakowicz-Dębek B., Bojarczyk M., Krukowski H., Misztal-Majewska B., Wlazło Ł., Trawińska B. (2013): Ozone disinfection of feed wheat. Annales Universitatis Mariae Curie-Skłodowska, Sectio EE: Zootechica, 31: 43–48.

Sandberg A.S. (2002): Bioavailability of minerals in legumes. British Journal of Nutrition, 88: S281–285.

Struik P.C., Bonciarelli F. (1997): Resource use at the cropping system level. Developments in Crop Science, 25: 179–189.

Watanabe T. (2002): Pictorial Atlas of Soil and Seed Fungi. Boca Raton, CRS Press LLC, 486.

Woźniak A. (2001): Studies on the yielding, weed infestation and state of health of spring triticale, spring wheat and spring barley in crop rotations and short-term monoculture on soil rendzina central eastern Lublin region. Lublin, Monograph 247, Agricultural University of Lublin, 127. (In Polish)

Woźniak A., Makarski B. (2013): Content of minerals, total protein and wet gluten in grain of spring wheat depending on cropping systems. Journal of Elementology, 18: 297–305.

Woźniak A., Soroka M., Stępniowska A., Makarski B. (2014): Chemical composition of spring barley (*Hordeum vulgare* L.) grain cultivated in various tillage systems. Journal of Elementology, 19: 597–606.

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