Effects of ozone treatment on physicochemical properties of Korean wheat flour

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Abstract The objective of this study was to investigate the effects of ozone treatment on the physicochemical properties of Korean wheat flour. Wheat flour samples were treated with ozone gas at 120 ppm for 15, 30, 45, and 60 min. Color b value, pH, and mold of flour decreased as exposure time to ozone increased. The water absorption index, peak viscosity, and final viscosity of flour increased by ozone treatment. Photomicrographs of flour suspensions under polarized light showed granules tended to lose birefringence owing to ozone during swelling. The result of SDS-PAGE showed that the intensity of protein bands at low molecular weights slightly increased in ozone-treated flours compared to the intensity in the control flour. The results of this study showed ozone gas affected the starch and protein of wheat flour, suggesting a need for further investigation on structural changes in starch and protein by ozone.

Keywords: wheat, ozone, flour, starch, protein

Introduction

Wheat (*Triticum aestivum* L.) is one of the three primary grains (1), and its worldwide production was 735 million tons in 2015 (2). Wheat is generally processed into wheat flour, which is composed of 65–73% starch and 8–16% protein for making bread, cookies, and noodles (3,4). Wheat starch is the primary component of wheat endosperm and is known to influence the structure by providing a rigid network in baked products during gelatinization (5). The content of protein in wheat flour is different, depending on the types of baked products such as snacks, noodles, and breads, and it can be used as an indicator of wheat quality, especially in yeast-leavened products (6). Thus, starch and protein in flour have a major impact on wheat quality and its final products.

Growing wheat requires specific weather conditions to maximize its production and to produce high-quality flour (7,8). Unsuitable climate conditions generate low-quality wheat, thereby resulting in substandard wheat products. For this reason, countries that do not have appropriate weather for growing wheat require additional efforts to improve the quality of wheat flour.

The application of ozone on wheat has been attempted as one of the methods to increase the quality of wheat. Sandhu *et al.* (9) reported that ozone can increase disulfide bond formation by oxidizing sulfhydryl groups in the protein of wheat flour. By increasing disulfide bonds, dough stability and the specific volume of bread are

increased. Ozone also easily breaks down into oxygen in the air (10); therefore, it does not leave any undesirable toxic residue after treatment (11). In 1997, the U.S. Food and Drug Administration announced that ozone was listed as “Generally Recognized as Safe” in the food industry as a disinfectant (12). As a strong oxidant, ozone can be used for decreasing microorganisms and disinfecting the surface of fruits, vegetables, meat, poultry, and dry foods in a gas or aqueous phase (13). As a role of a bleaching agent, ozone can diminish yellowness, which is a negative factor for wheat flour (14). The application of ozone gas for cereal treatment is reported for wheat, barley, and corn (15). Some research has been done on products made by ozone-treated wheat flour, such as breads and noodles (9,14). Changes in starch polymers and the formation of S–S bonds in dough were observed after ozone treatment (9). According to Li *et al.* (14), ozone could prohibit the growth of microorganisms and oxidize yellow pigments, resulting in an improvement in the whiteness of noodle sheets. Although previous studies reported the effects of ozone on dough, limited information is available regarding the effect of ozone gas on starch properties and the electrophoretic profiles of wheat flour. Therefore, the objective of this study was to investigate the physicochemical characteristics of Korean wheat flour treated with ozone gas for various periods of time. Starch properties were determined by viscosity and photomicrographs of flour suspensions under polarized light. Protein fractions were evaluated by the intensity of the bands by sodium dodecyl sulfate–

polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and Methods

Materials The wheat flour used in this study was obtained from CJ Cheiljedang Food Ingredient R&D Center (Suwon, Korea). Wheat (*Triticum aestivum* L. cv. Keumkang) was grown in various regions of Korea, harvested in 2013, and processed as wheat flour at CJ Cheiljedang pilot plant (Yongsan, Korea). This variety is the major wheat variety in Korea and accounts for approximately 70% of the total production. The moisture, ash, and fat contents of the wheat flour were 15.7% (dry basis, d.b.), 0.63% (d.b.), and 0.79% (d.b.), respectively.

Ozone treatment Ozone gas was produced by an ozone generator (HOZ-3G & HOX 3G; HNT Inc., Incheon, Korea) with maximum 3 g/h capacity (Fig. 1). Wheat flour (100 g) was placed in a polyvinyl chloride container (14.5 cm×18 cm) and ozone gas was passed through the container from bottom to top, as illustrated in Fig. 1. Wheat flour was exposed to ozone gas (120 ppm) at a flow rate of 5 L/min for 15, 30, 45, and 60 min. Then, flour samples were exposed to air at a flow rate of 5 L/min for 60 min to remove the ozone in the sample container.

Color, whiteness, pH, and particle size distribution of wheat flour

The color values (*L*, *a*, and *b*) of wheat flour were determined using a portable spectrophotometer (CM-700d; Konica Minolta Inc., Tokyo, Japan). The whiteness of flour samples was measured by a whiteness tester (C-300-3; Kett Electric Laboratory, Tokyo, Japan). The pH of the flour samples was determined according to AACC method 02-52 (16). The particle size distribution of flour samples was determined using a particle size analyzer (Particle Size Analyzer 1190; CILAS Co., Orleans, France).

Microbiological analysis of wheat flour

Aerobic plate count (APC)

and yeast and mold (YM) analyses were performed using an APC Petrifilm (3M Petrifilm™ AC; 3M Co., Maplewood, MN, USA) and YM Petrifilm (3M Petrifilm™ YM; 3M Co.). A mixture of wheat flour (1 g) and sterile 0.85% (w/v) NaCl (10 mL) was homogenized with a stomacher (HG400; Mayo International Ltd., Milano, Italy). After serial dilutions, diluents were plated on the Petrifilms. APC was incubated at 35°C for 24 h and YM was incubated at 25°C for 48 h. After incubation, each colony was counted and the number of CFU was calculated to log CFU/g.

Protein content, wet gluten, damaged starch, water absorption index, and water solubility index of wheat flour

The protein content of the wheat flour was determined by the AACC method 46-12 (16). The wet gluten content of the flour samples was measured in accordance with the AACC method 38-12A (16) using a Glutomatic apparatus (Glutomatic 2200; Perten Instruments Co., Stockholm, Sweden). The damaged starch of the flour samples was determined using the SDmatic instrument (SDmatic; Chopin Technologies Inc., Paris, France) in accordance with the AACC method 76-33 (17). The water absorption index (WAI) and water solubility index (WSI) of flour were determined, as described in the paper by Anderson *et al.* (18). The WAI and WSI were calculated using Eq. 1 and 2, respectively.

$$\text{WAI (g/g)} = \text{weight of residues} / \text{weight of flour} \quad (1)$$

$$\text{WSI (\%)} = (\text{weight of dried supernatant} / \text{weight of flour}) \times 100 \quad (2)$$

Pasting properties of wheat flour

The pasting properties of wheat flour were determined by a Rapid Visco Analyzer (Super 4; Newport Scientific Inc., Sydney, Australia) in accordance with the AACC method 76-21 (16). The flour samples (3 g based on 14% moisture content) were added to 25 mL of distilled water in an aluminum sample container. The flour suspension was uniformly mixed using a plastic paddle at 50°C for 1 min and raised up to 95°C for 3.7 min, held at 95°C for 2.5 min, cooled to 50°C for 3.8 min, and held at 50°C for 2 min. Peak, trough, breakdown, final, and setback viscosities

Fig. 1. Schematic of ozone-gas treatment system

were recorded via a software (Thermocline Window Software; Newport Scientific Inc.).

Photomicrographs of wheat flour suspension under polarized light

The effects of ozone gas on the properties of flour were observed using flour suspension under polarized light. A wheat flour suspension, 0.5% (w/v), was mixed for 30 s using a vortex mixer to homogenize. Several drops were placed on a slide glass and covered with a cover slip. The photomicrograph of the 0.5% wheat flour suspension was observed by a light microscope (CX 40; Olympus Co., Tokyo, Japan) with a polarized light filter. Each of the flour suspension samples was observed under normal and polarized light.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of wheat flour

For the preparation of protein samples, each flour sample (1 mg) was mixed with 1 mL of sample buffer (50 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 0.02% (w/v) bromophenol blue, and 1% (v/v) β -mercaptoethanol) and heated at 95°C for 4 min. The protein samples for non-reduced SDS-PAGE were prepared without β -mercaptoethanol in the sample buffer. The concentration of each protein sample was determined with a bicinchoninic acid protein assay kit (Thermo Fisher Scientific Co., Waltham, MA, USA). Protein samples (6 μ g) were loaded onto a 12% acrylamide separating gel well and electrophoresis was performed at 110 V. After gel electrophoresis, the gel was treated with a fixing solution (40% (v/v) ethanol and 10% (v/v) acetic acid) for 1 h and a rehydration solution (5% (v/v) ethanol and 5% (v/v) acetic acid) for 30 min three times. Then, the gel was stained with colloidal coomassie brilliant blue.

Statistical analysis The analysis of variance (ANOVA) was done to determine the difference among five samples using Statistical Analysis System software 9.2 (SAS, version 9.00; SAS Institute Inc., Cary, NC, USA). When a difference was found among the samples, a Student Newman-Keuls (SNK) multiple comparison was done to separate the means at $p < 0.05$.

Results and Discussion

Color, whiteness, pH, particle size distribution, and microbiological analysis of wheat flour

The color (L , a , and b), whiteness, pH, and particle size distribution of flour samples exposed to ozone gas for different amounts of time are presented in Table 1. The flour samples treated with ozone gas (0, 15, 30, 45, and 60 min) differed significantly in a , b , and whiteness values ($p < 0.001$), respectively. The a and b values of flour samples were gradually decreased with different exposure times to ozone gas. The b value is the degree of yellowness, which is related to the carotenoid content of wheat flour (19). The results of this study are comparable with the results of Li *et al.* (14), which reported a decrease in the b value from 15.67 to 13.62 and 13.39 with the exposure to ozone gas for 30 and 60 min, respectively. The decreased b value in this study might be due to the oxidation of lutein, which is a primary carotenoid with conjugated double bonds in wheat flour (10). As yellowness decreased, the whiteness of flour samples increased with the exposure time to ozone gas.

Significant differences were also observed in pH values and mean particle sizes in all samples ($p < 0.001$) (Table 1). The pH values of wheat flours treated with ozone gas decreased according to exposure time. These results are in agreement with Chitrakorn *et al.* (20), which showed a decrease in the pH from 6.07 to 5.96 and 5.66 with the exposure to ozone gas for 10 and 36 min, respectively. According to Langlais *et al.* (21), a decreasing pH value might be owing to the oxidation of carbohydrates, amino acids, and unsaturated fatty acids during ozone treatment, which results in acid-forming effects. Ozone treatment might decrease the pH value of the wheat flour as it changed the flour components such as hydroxyl groups and amino acids (22). In addition, the mean particle size of wheat flour increased after ozone treatment. Changes in the physical connections in flour might be the reason for the larger particle size, so that the ozone-treated flours were less dispersed than the control flour (22). Therefore, we inferred that the increase in particle size by ozone gas in this study led to the changes in physical connections between particles due to the oxidation of wheat flour by ozone gas.

Table 1. Color¹⁾ (L , a , and b), whiteness¹⁾, pH¹⁾, particle size¹⁾, and microbiological properties (aerobic plate count²⁾, yeast, and mold²⁾) of wheat flours exposed to ozone gas with time

Exposure time (min)	Color			Whiteness***	pH***	Particle size (μ m)***	Microbiological properties		
	L	a *** ⁴⁾	b ***				Aerobic plate count (log CFU/g)*	Yeast (log CFU/g)	Mold (log CFU/g)***
0	95.0 $\pm$ 0.3 ^{a3)}	0.28 $\pm$ 0.02 ^a	7.83 $\pm$ 0.02 ^a	78.2 $\pm$ 0.1 ^d	5.74 $\pm$ 0.07 ^a	30.2 $\pm$ 1.1 ^c	2.84 $\pm$ 0.04 ^a	3.69 $\pm$ 0.07 ^a	2.19 $\pm$ 0.21 ^a
15	93.9 $\pm$ 0.8 ^a	0.12 $\pm$ 0.04 ^b	6.62 $\pm$ 0.16 ^b	80.6 $\pm$ 0.3 ^c	5.30 $\pm$ 0.13 ^b	39.8 $\pm$ 0.7 ^b	2.40 $\pm$ 0.10 ^b	3.54 $\pm$ 0.28 ^a	0.00 $\pm$ 0.00 ^b
30	94.3 $\pm$ 0.3 ^a	0.06 $\pm$ 0.03 ^{bc}	6.09 $\pm$ 0.33 ^c	81.8 $\pm$ 0.4 ^b	5.05 $\pm$ 0.08 ^c	41.2 $\pm$ 1.2 ^b	2.50 $\pm$ 0.28 ^b	3.19 $\pm$ 0.21 ^a	0.00 $\pm$ 0.00 ^b
45	94.5 $\pm$ 0.3 ^a	0.03 $\pm$ 0.02 ^{cd}	5.83 $\pm$ 0.12 ^c	82.2 $\pm$ 0.2 ^b	4.85 $\pm$ 0.13 ^{cd}	41.0 $\pm$ 1.0 ^b	2.34 $\pm$ 0.00 ^b	3.69 $\pm$ 0.07 ^a	0.00 $\pm$ 0.00 ^b
60	94.7 $\pm$ 0.3 ^a	0.02 $\pm$ 0.04 ^d	5.75 $\pm$ 0.10 ^c	83.1 $\pm$ 0.2 ^a	4.66 $\pm$ 0.13 ^d	44.9 $\pm$ 0.6 ^a	2.46 $\pm$ 0.10 ^b	3.49 $\pm$ 0.21 ^a	0.00 $\pm$ 0.00 ^b

¹⁾Mean values by three replications

²⁾Mean values by two replications

³⁾Same superscripts within a column mean not significantly different at $p < 0.05$.

⁴⁾*, *** mean significantly different across the exposure time at $p < 0.05$ and $p < 0.001$, respectively.

Table 2. Protein¹⁾, wet gluten¹⁾, damaged starch¹⁾, water absorption index¹⁾, and water solubility index¹⁾ of wheat flours treated by ozone gas

Exposure time (min)	Protein (%) ^{*)2)}	Wet gluten (%) ^{*)2)}	Damaged starch (%) ^{*)2)}	Water absorption Index (g/g) ^{***}	Water solubility Index (%) ^{***}
0	11.9±0.1 ^{b3)}	31.2±0.5 ^{ab}	6.18±0.02 ^b	1.62±0.01 ^d	5.91±0.08 ^b
15	12.1±0.1 ^a	32.7±0.4 ^a	6.41±0.06 ^a	1.72±0.01 ^c	6.95±0.28 ^a
30	12.1±0.2 ^a	31.5±1.8 ^{ab}	6.41±0.12 ^a	1.78±0.01 ^b	7.20±0.42 ^a
45	12.1±0.1 ^a	30.4±1.5 ^{ab}	6.36±0.00 ^a	1.84±0.01 ^a	7.27±0.05 ^a
60	12.0±0.1 ^a	29.3±0.8 ^b	6.36±0.08 ^a	1.86±0.02 ^a	7.24±0.24 ^a

¹⁾Mean values by three replications²⁾Values are expressed on a dry weight basis.³⁾Same superscripts within a column mean not significantly different at $p<0.05$.⁴⁾*, *** mean significantly different across the exposure time at $p<0.05$ and $p<0.001$, respectively.**Table 3.** Pasting properties¹⁾ of wheat flours exposed to ozone gas with various treatment time

Exposure time (min)	Pasting properties				
	Peak viscosity (RVU) ^{***3)}	Trough (RVU) ^{***}	Breakdown (RVU) ^{***}	Final viscosity (RVU) ^{***}	Setback (RVU) ^{***}
0	113.2±1.7 ^{e2)}	73.1±1.7 ^d	40.1±1.0 ^c	152.1±1.9 ^e	79.0±2.3 ^c
15	139.6±4.5 ^d	91.1±3.0 ^c	48.4±1.6 ^b	183.8±4.6 ^d	92.7±2.5 ^b
30	155.5±3.3 ^c	104.3±1.9 ^b	51.2±3.4 ^b	193.0±3.2 ^c	88.7±5.0 ^b
45	167.5±2.2 ^b	114.8±2.2 ^a	52.8±0.3 ^b	207.1±4.8 ^b	92.3±3.7 ^b
60	175.9±3.9 ^a	114.2±4.8 ^a	61.6±2.5 ^a	214.2±3.4 ^a	100.0±3.1 ^a

¹⁾Mean values by three replications²⁾Same superscripts within a column mean not significantly different at $p<0.05$.³⁾***means significantly different across the exposure time at $p<0.001$.

The APC and mold of wheat flours treated with ozone gas were significantly different ($p<0.05$, $p<0.001$) according to exposure time. Mold is the key microbial organism to determine the quality of wheat flour even though wheat flour is generally regarded as a micro-biologically safe product due to low water activity (23). In this study, the APC of wheat flours decreased from 2.84 to 2.46 log CFU/g and the mold of wheat flours decreased from 2.19 to 0.00 log CFU/g as the ozone treatment time was prolonged. Similar decreasing patterns were observed by Li *et al.* (14) for wheat flour samples. The decreased APC and mold due to ozone gas in this study could be explained by the oxidation of the cell membrane and cell components such as proteins, sulfhydryl groups, polyunsaturated fatty acids, and amino acids of enzymes (24). It could be also explained by the degradation of unsaturated lipids in the cell envelope, resulting in cell death (12).

Protein content, wet gluten, damaged starch, water absorption index, and water solubility index of wheat flour

The protein and gluten contents of flour samples exposed to ozone for different amounts of time are shown in Table 2. Protein content was affected by ozone treatment as the protein content of wheat and rice increased by ozone treatments (25,26). The wet gluten content of wheat flour with 60 min of ozone treatment was the lowest among the samples. Mei *et al.* (27) reported that the wet gluten content of ozone-treated wheat flours was higher than that of the control wheat flour, and the wet gluten content of wheat flour exposed to ozone gas for 120 min decreased compared to samples exposed for

60 and 90 min. Appropriate ozone treatment seemed to aggregate insoluble gluten and form a gluten structure, whereas excessive ozone treatment damaged gluten structure, resulting in decreasing wet gluten content (27). Therefore, a decrease of wet gluten content in wheat flour treated with ozone for 60 min in this study might be caused by ozone treatment for an excessive amount of time at a high ozone concentration compared to the ozone concentration of Mei *et al.* (27).

The damaged starch, WAI, and WSI values of flours exposed to ozone for different amounts of time are presented in Table 2. The flour samples exposed to ozone had higher levels of damaged starch than the control flour. The wheat flours exposed to ozone gas (0, 15, 30, 45, and 60 min) differed significantly in terms of WAI values ($p<0.001$). The results of damaged starch and WAI in this study are in agreement with Mei *et al.* (27), who reported that an increase in damaged starch results in the increase of the WAI in wheat flour. The WSI value was higher in ozone-treated wheat flour (WSI: 6.95–7.24%) than in the control (WSI: 5.91%). However, the WSI values were not significantly different between ozone-treated wheat flours ($p>0.05$). Ozone treatment can damage wheat granules, resulting in an increased rate of water absorption (27).

Pasting properties of wheat flour

The pasting properties of wheat flour are related to swelling and gelatinization characteristics (4). The peak viscosity, trough, breakdown, final viscosity, and setback of wheat flour as affected by ozone treatment are presented in Table 3. The peak viscosity (from 113.2±1.7 to 175.9±3.9) and final viscosity

Fig. 2. Photomicrographs of wheat flour suspensions exposed to ozone gas with various treatment times under normal light (top) and polarized light (bottom) with 40 $\times$ magnification

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of protein extracted from wheat flours exposed to ozone gas with time. (A) the reduced pattern; (B) the non-reduced pattern. M means molecular weight standard. 0, 15, 30, 45, and 60 mean 0, 15, 30, 45, and 60 min, respectively.

(from 152.1 ± 1.9 to 214.2 ± 3.4) significantly increased according to the treatment time. The peak viscosity ranged from 113.2 to 175.9, which is lower than that demonstrated by Li *et al.* (172–224) (14). Trough, breakdown value, and setback value all increased as the exposure time to ozone increased. Hydroxyl groups were oxidized and carboxyl groups were formed in starch polymers during ozone oxidation (9). The carboxyl groups may absorb more water into granules during gelatinization (28). The swelling of granules increased as the negative charges of carboxyl groups repelled each other during gelatinization (9). For these reasons, peak viscosity and final viscosity increased by ozone treatment. The setback value is related to the retrogradation of starch, especially amylose (28).

Photomicrographs of wheat flour suspension Photomicrographs of ozone-treated wheat flour suspensions under normal and polarized light are shown in Fig. 2. Wheat starch granules are known to have bimodal size distribution (29). Park *et al.* (3) reported two types of granules—large granules called type A (generally $>10 \mu\text{m}$) and small granules called type B (generally $<10 \mu\text{m}$). Even though granules larger than $30 \mu\text{m}$ could exist, starch granules of up to $30 \mu\text{m}$ are frequently found. Photomicrographs of wheat flour suspensions (Fig. 2) confirmed the bimodal distribution of starch granules by showing

both the large granules, type A, and the small granules, type B. Under polarized light conditions, crystalline regions of starch granules represent birefringence known as a Maltese cross. Loss of birefringence occurs owing to loss of crystallinity in starch during irreversible swelling (30). Ozone gas used in this study might weaken the starch granule structure and increase the starch damage, resulting in an increase of water absorption and the swelling of starch granules. A distinctive Maltese cross, which was shown in the suspension of the control flour, was not exposed to ozone gas (Fig. 2). The results of this study confirmed that a less clear Maltese cross emerges in suspensions of wheat flour exposed to ozone than in suspensions of the control flour (30). Catal and Ibanoglu (30) reported that photomicrographs of corn and potato starch without ozone treatment were sharper than ozone-treated ones under polarized light. Similar to the way amorphous regions of starch granules were formed by oxidation as explained by Catal and Ibanoglu (30), the ozone gas used in this study would be involved in the oxidization of starch granules, resulting in a weakening starch structure and swelling starch granules.

SDS-PAGE patterns of protein fractions The protein in wheat flour after ozone treatment was separated by SDS-PAGE under reducing (Fig. 3A) and non-reducing (Fig. 3B) conditions. The protein patterns

separated by SDS-PAGE under reducing conditions demonstrated that the intensity of the bands was increased in the ozone-treated wheat flour proteins compared to the control flour within the range of 30–40 and 50–70 kDa. On the contrary, there was no difference in the intensities of the bands on the range of 70–100 kDa under reducing conditions. The results from this study are in contrast to the work of Li *et al.* (14) that reported that the intensities of bands from the ozone-treated samples increased in high molecular weight ranges (above 97.4 kDa), and decreased in low molecular weight ranges (31–43 kDa) of the reduced separated gel (31). The variation of wheat cultivars between this study and those in Li *et al.* (14) might change the result of protein patterns in SDS-PAGE. Differences in the types of glutenin subunits are related to wheat cultivars (31). Also, the intensity of protein bands at low molecular weights (28–35 kDa) of ozone-treated wheat flours under non-reducing conditions of SDS-PAGE (Fig. 3B) slightly increased, compared to the control wheat flour. This implies an increase in low molecular weight proteins by ozone gas. The results of protein patterns separated by SDS-PAGE support the idea that the treatment of ozone gas could contribute to an increase in the protein contents of low molecular weights in wheat flour. However, distinct changes in protein fractions by ozone gas were not found by SDS-PAGE analysis. Only a limited explanation of the effects of ozone gas on protein fractions in wheat flour could be made. Therefore, further investigation on changes in protein fractions owing to ozone gas is needed to explain the effects of ozone gas on the properties of wheat flour.

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