

Ozone-Aided Corn Steeping Process

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ABSTRACT

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The present research evaluated the feasibility of using ozone (O_3) to replace sulfur dioxide (SO_2) in corn steeping. Traditionally, steep water contains 0.1–0.2% sulfur dioxide to promote starch-protein separation and high starch yields, and to control microbial growth. However, residual SO_2 in starch products affects product quality and jeopardizes the “organic products” claims. Also, SO_2 discharged to the environment pollutes water and air. Ozone is a strong oxidant and disinfectant with a capability to control the growth of putrefactive microorganisms in steeping systems, and

to break down the endosperm protein matrix and, hence, improve starch release. This study demonstrates that an ozone-aided steeping (OAS) process had starch yields as high as conventional SO_2 steeping. OAS processes can be conducted at a lower temperature (20°C vs. 50°C) and for shorter times (36 hr vs. 48 hr) than the conventional SO_2 processes, suggesting significant energy savings and increased productivity. We have found that the timing of ozone application is of great importance to the performance of the OAS process.

In corn wet milling, sulfur dioxide (SO_2) is traditionally used in the steeping process designed to facilitate high starch yields. Sulfur dioxide has two major functions: 1) it controls the growth of putrefactive microorganisms, and 2) it helps break down disulfide bonds so that starch can be easily separated from its encompassing protein matrix (Blanchard 1992). Typically, 0.1–0.2% SO_2 is added to the steep water. Although most of the added SO_2 leaves the process at some point during wet milling, some remains in the final products (Committee on Food Chemicals Codex 1997). Sulfur dioxide in the waste water or emitted into the air is hazardous both to the environment and to human health. Sulfur dioxide is a primary component of acid rain, as its chemical reaction with water produces sulfurous acid. Human exposure to SO_2 can cause impairment of respiratory function, aggravation of existing respiratory disease (especially bronchitis), and a decrease in the ability of the lungs to clear foreign particles (Office of Air Quality Planning and Standards 1994). Manufacturers often must devote considerable resources to reducing residual SO_2 to approved levels in final products. In recent years, demand for organic starch products has been rising and residual SO_2 in the final products will certainly jeopardize manufacturers’ “organic” claim for their products. Therefore, manufacturers who wish to process organic corn must find an alternative steeping process.

An alternative to SO_2 is ozone, which is a disinfectant and an oxidant with the ability to suppress the growth of putrefactive organisms and break down protein matrix. The benefits of using an ozone-aided steeping (OAS) process are threefold: 1) no residual ozone will remain in the products, and thus manufacturers need not be concerned with residual O_3 or SO_2 levels in the final products (products can be labeled as “organic”); 2) ozone reverts to oxygen after use and thus does not pose a threat to the environment; and 3) production costs required for the OAS process may be lower than those associated with SO_2 processing. The objectives of this study were to investigate the feasibility of using ozone to replace SO_2 , and to determine the effects of some processing variables on the performance of the OAS process.

MATERIALS AND METHODS

Materials

Unless stated, Yellow Dent #2 corn harvested in 1997 at the University of Minnesota Rosemount Research and Outreach Center was used in the experiments. Yellow Dent #2 corn harvested in 2001, provided by Cargill, was used in some experiments as stated. The initial moisture contents of 1997 and 2001 samples were 12.91 and 12.77% (wb), respectively, determined by the oven method (Approved Method 44-19, AACC 2000). Samples were screened to remove foreign materials and hand-picked to remove broken kernels, then stored at approximately -18°C in sealed plastic containers. Before each test, the samples were removed from the freezer and left to equilibrate to room temperature. Any cracked, round, or odd-shaped kernels that were immature and undeveloped were discarded to reduce sample variations. Chemical-grade sodium meta-bisulfite ($Na_2S_2O_5$, Lot no. 010294, Fisher Scientific) and lactic acid (DL-Lactic acid, Lot No. N35625, J. T. Baker, Phillipsburg, NJ) were used.

Ozonation System and Ozone Treatment

The ozonation system consisted of an ozone generator and glass cylinders (Fig. 1). The ozone generator was designed and built in our laboratory using an ozone reactor, an electric power

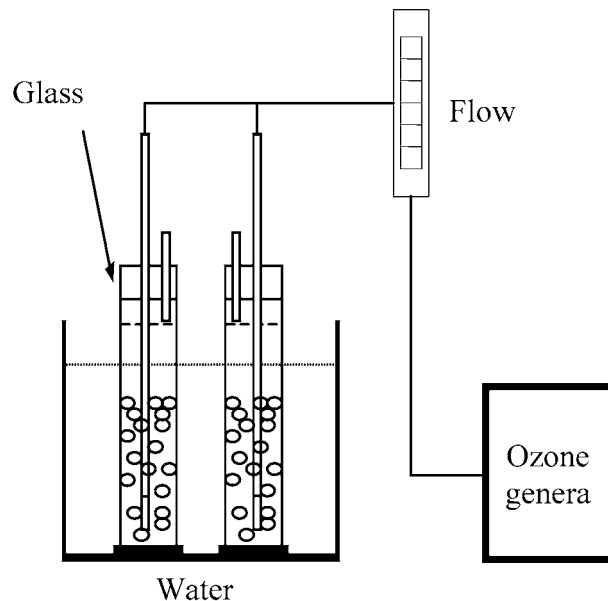


Fig. 1. Ozone-aided steeping system.

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supply, a variable autotransformer, an air pump, and a cooling fan. Ozone is generated by means of nonthermal plasma working at the voltage of 10,000V. The ozone generator could produce 10 g/m³ ozone as determined by the H1 ozone analyzer, (In USA, Inc., Needham, MA.) at an airflow rate of ≈ 7.5 standard cubic feet/hr (SCFH) (SI equivalent 2.832E-2 m³/hr). Ozone was introduced to the steep water in the glass cylinder through a glass tube and a small diffuser made of sandstone.

For each steeping experiment, three replicate samples (100 g each) were placed in the glass cylinders containing 180 mL of steep water. Steep water compositions used in the experiments were 1) deionized water (180 mL) (blank); 2) deionized water (179 mL) + lactic acid (1 mL) (LA); 3) deionized water (180 mL) + Na₂S₂O₅ (0.53 g) (SO₂); 4) deionized water (179 mL) + lactic acid (1 mL) + Na₂S₂O₅ (0.53 g) (LA + SO₂, control). Ozone treatment methods used in the experiments were 1) continuous O₃ treatment (ozone mixed into steep water for 12 or 24 hr continuously during corn steeping); 2) 1-hr O₃ treatment (ozone mixed into steep water for 1 hr [at 1st or 13th hr during corn steeping]); 3) multiple O₃ treatment (1-hr ozone treatment applied at 1st, 13th, and 24th hr) for a total of 3 hr during corn steeping.

The amount of ozone used and the time at which ozone was introduced into the steep water are indicated in Figs. 2 to 10. Lactic acid was added to the steep water to simulate an industrial steeping process (Eckhoff et al 1991). The presence of lactic acid in steep water is believed to make the cell walls easier to break (MacMasters 1962). The temperature of the steep water was maintained at 52°C (unless stated) with a temperature-controlled water bath. Steeping time varied from 24 to 48 hr.

Corn Processing System

The laboratory corn wet-milling procedure was similar to that described by Eckhoff et al (1996). Upon completion of steeping, the steep water was drained and the corn kernels were placed in a kitchen-type food blender (model 51BL32, Waring Commercial, New Hartford, CT). Some deionized water was used to rinse the steeping cylinders and to adjust the liquid volume in glass

cylinders to 200 mL. The corn and liquid were then poured into the blender and blended for 3 mins to produce a corn-water slurry. The slurry was drained through a #7 sieve placed on a bucket (B1) installed on a sieve shaker for 5 min. The sieve was rinsed with deionized water and the rinse water was added to B1. After sieving, endosperm chunks remaining on the sieve were removed with a forceps and put into B1. We now had coarse fiber and germ on the sieve and starchy material in B1. The starch contents of bucket B1 were slowly poured into a grinding mill. B1 was rinsed with deionized water and the rinse water was also poured into the grinding mill. The output from the grinding mill was collected in a bucket (B2) and allowed to settle for 30 min. The upper layer of thin liquid was decanted off into another bucket (B3). The remaining contents in B2 were poured onto a #200 sieve placed on bucket (B4), and the sieve was shaken for 5 min. The thin liquid from B3 was also poured onto the sieve. B2 and B3 and the sides of the sieve were rinsed with deionized water and the rinse water was poured onto the sieve. After sieving was complete, a spatula was used to drain and press the fine fiber on the sieve. The filtrate in B4 was allowed to settle for 35–40 min and then the thin liquid was decanted slowly into bucket (B5). The specific gravity of the filtrate in B5 was adjusted to 1.040–1.045 sp gr determined by a hydrometer (Fisher). Next, a starch table was used to separate starch and gluten. The starch table was a U-shaped gutter 8 ft long, 2 in. wide, and 1.5 in. high with a slope of 1.5 in. (SI equivalent 2.54 cm/in.). A stir-bar was placed in B4, and B4 was placed on a magnetic stirrer to produce mixtures with a homogeneous density. The flow rate of starch slurry from B4 onto the starch table was set at 50 mL/min using a console drive (model 7521-50, Cole-Parmer Instrument Co., Barrington, IL). The contents of B4 were poured onto the starch table, ensuring that the liquid covered the bottom surface of the table. The decanted water in B5 was also added to the table. Starch remained on the table and gluten slurry flowed into a bucket (B6). After the starch sat on the table overnight at room temperature ($\approx 20^\circ\text{C}$), it was removed and placed in small weighing cans that were put into a 135°C oven for 2 hr. The dry starch was weighed and starch yield was calculated. All starch and by-product yields (db) were calculated based on 100-g whole-corn samples unless indicated otherwise.

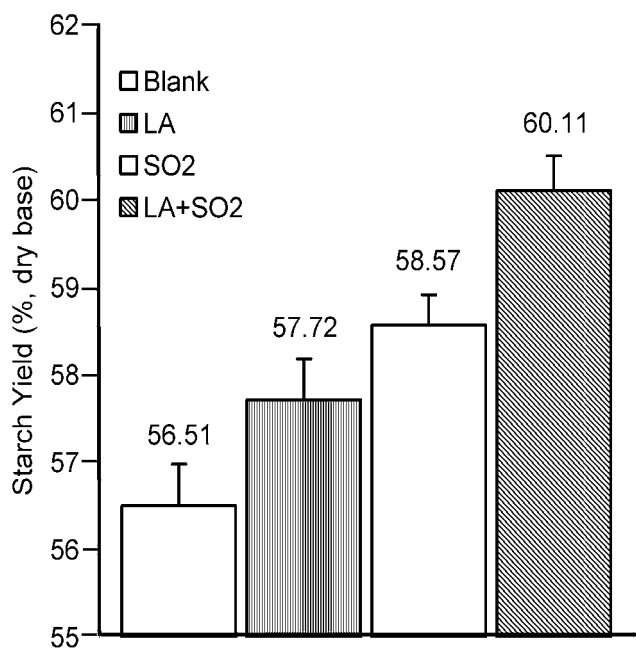


Fig. 2. Corn starch yields under different steeping conditions without ozone treatment at 52°C. Steeping conditions: blank, 180 mL of deionized water; LA, 179 mL of deionized water + 1 mL of lactic acid; SO₂, 180 mL of deionized water + 0.53 g of Na₂S₂O₅; LA+SO₂, 179 mL of deionized water + 1 mL of lactic acid + 0.53 g of Na₂S₂O₅. Error bars indicate standard deviations.

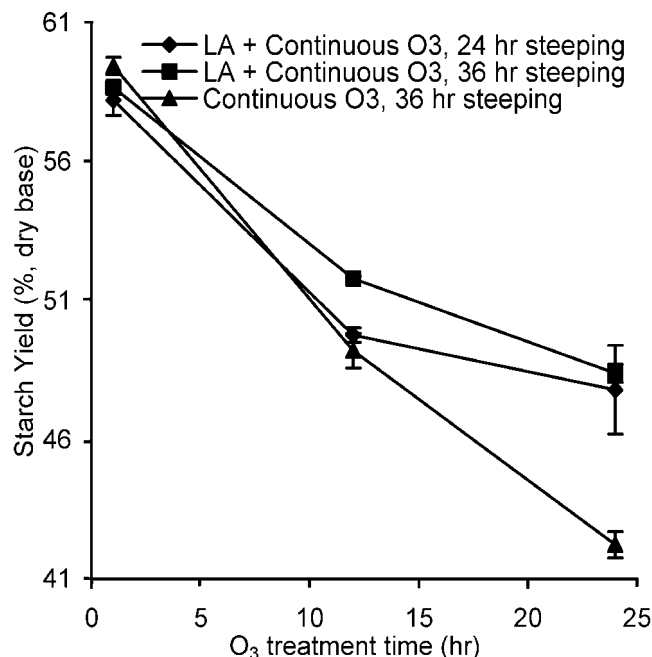


Fig. 3. Corn starch yields when corn was steeped for 24 and 36 hr at 52°C with different ozone treatment times. Error bars indicate standard deviations.

Experiments were designed to quantify how starch yield was affected by steep water composition and steeping conditions. The steep water contained lactic acid, SO₂, or ozone, or none, or combinations of these chemicals; the processing variables included steeping temperature and time. The experiments fall into two categories: nonozone-aided steeping (non-OAS) processes and OAS processes. The non-OAS experiments were intended to quantify the effects of lactic acid and SO₂, and to provide starch yield baseline information. The OAS experiments were designed to determine whether ozone is indeed effective and how ozone interacts with lactic acid and SO₂.

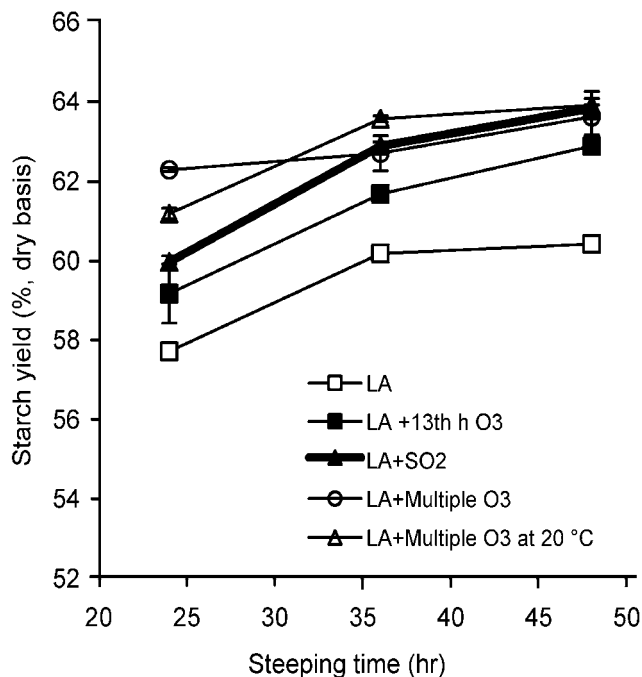


Fig. 4. Corn starch yields as a function of steeping time at 52°C (unless stated otherwise). LA+O₃, 1 hr O₃ at 13th hr; multiple O₃, 1 hr O₃ at 1st hr + 1 hr O₃ at 13th hr + 1 hr O₃ at 24th hr. Error bars indicate standard deviations.

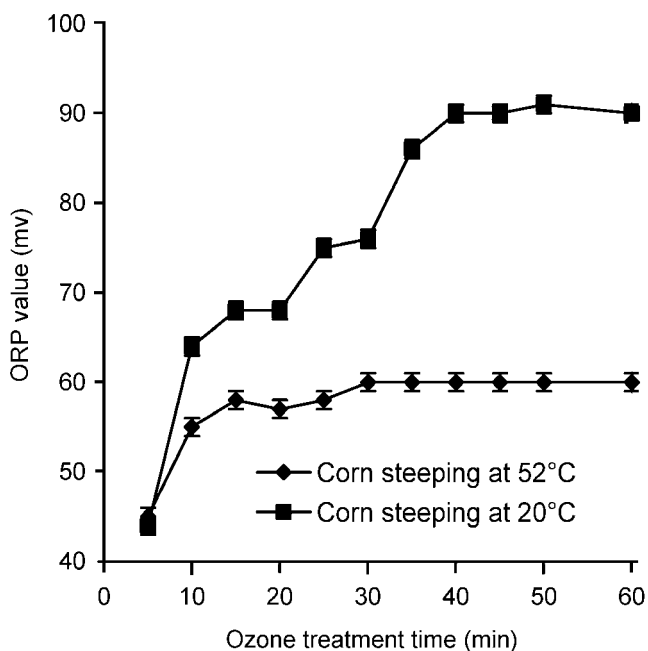


Fig. 5. Ozone concentration, oxidation reduction potential (ORP) value, for corn steeping at 52 and 20°C with continuous ozone treatment.

Non-OAS Processes

In the non-OAS experiments, the steep water contained (A) deionized water (blank), or (B) deionized water + lactic acid (LA), or (C) deionized water + SO₂, or (D) deionized water + LA + SO₂ (control). Steep water D represents the composition currently used by industry and will be referred to herein as the standard process or standard sample.

OAS Processes

Ozone processes included three different treatments: 1) injecting ozone continuously to the steep water from the beginning of steeping to a preset time (1, 12, or 24 hr) during the entire steeping process; 2) injecting ozone only during the 13th hr of the steeping process (1 hr of O₃ treatment); and 3) injecting ozone continuously to the steep water during the 1st, 13th, and 24th hr of the steeping process (a total of three 1-hr O₃ treatments).

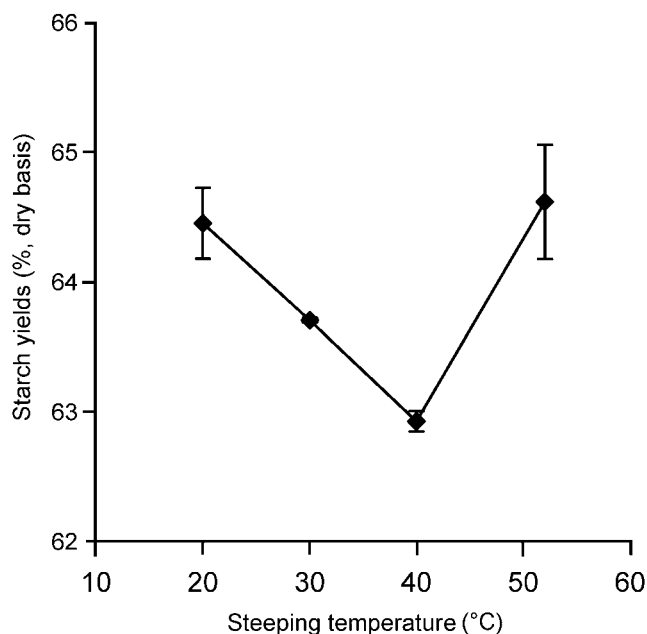


Fig. 6. Corn starch yields at different steeping temperatures for multiple ozone treatments and 48 hr of steeping.

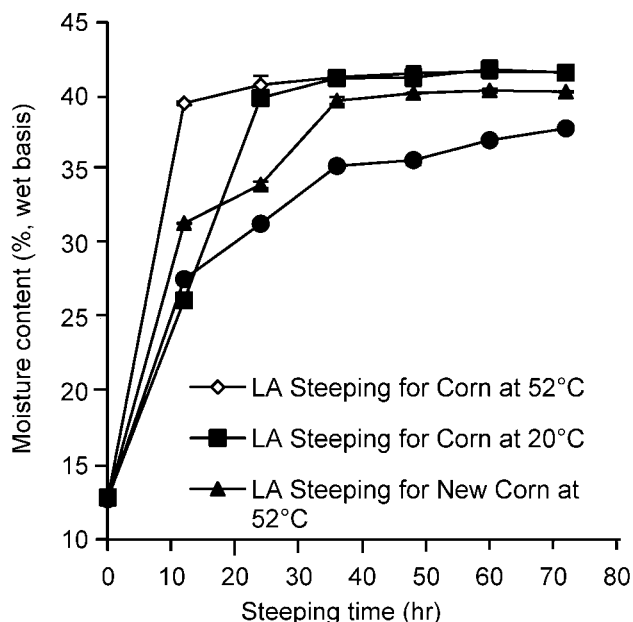


Fig. 7. Water absorption of corn steeping in lactic acid solution at 52 and 20°C. Error bars indicate the standard deviations.

RESULTS AND DISCUSSION

Non-OAS Processes

Figure 2 shows results from four experiments without ozone treatment. The samples were soaked at 52°C for 24 hr before milling. LA and SO₂ alone or in combination increased the starch yield compared with the blank as expected. Samples treated with SO₂ exhibited higher starch yields than those treated with LA. The use of LA and SO₂ in combination produced the highest starch yield. The increase in yield caused by combination of LA and SO₂ was slightly greater than the sum of increase by LA and SO₂ alone, suggesting some synergy between LA and SO₂.

OAS Processes

Figure 3 shows the starch yields obtained with continuous ozone treatments when corn was steeped in deionized water with or without lactic acid for different steeping times. The starch yields obtained with 1-hr ozone treatment are compatible with, or slightly higher than, those with non-OAS treatments except for LA and SO₂ combined, indicating that ozone did help starch release. Surprisingly, prolonged ozone treatments substantially decreased the starch yields, especially for treatments without lactic acid. It was observed that the prolonged ozone treatments resulted in extremely viscous milled slurry. The increased viscosity of the slurry is probably due to the reaction of ozone with the lignin of the corn fiber or due to hydrolysis of hemicellulose (Holen et al 1998). Viscous slurries made sieving very difficult, which in turn might have lowered the starch recovery efficiency, even though starch granules might have been more effectively released from the protein matrix with the help of ozone. Another hypothesis is that ozone inhibited the function of lactic acid. If this were the case, allowing lactic acid to take effect before ozone treatment would produce a higher yield than when ozone was applied in the beginning. The experiment with 1-hr O₃ treatment applied at the 13th hr during steeping did produce starch yields (Table I) higher than those obtained with the continuous O₃ treatments (Fig. 3), and highly compatible with those obtained with SO₂ or combination of LA and SO₂ treatments. This seems to support the above hypothesis.

A 12-hr continuous ozone treatment from the beginning of the steeping produced higher yield at 20°C (53.59%) (Table I) than at at 52°C (51.80%) (Fig. 3), suggesting that ozone is more effective at lower temperatures, which may be attributed to the fact that ozone has a higher solubility in water and a longer lifetime at lower temperatures.

TABLE I

Experiment with 1-hr O₃ Treatment Applied at 13 hr During Steeping

Steeping Temp. (°C)	Steeping Time (hr)	Starch Yield (% db)
52	24	59.21
52	36	61.68
20	36	53.59

TABLE II

Mean Fraction Yields for SO₂ Steeping and Ozone Treatment Methods

Processing Conditions	Starch	Protein	Steeping Solids	Coarse Fiber	Fine Fiber	Total Fiber	Total
LA+SO ₂ , 52°C, 24 hr	59.97 ± 0.16	8.37 ± 0.75	3.34 ± 0.09	1.87 ± 0.18	23.93 ± 0.49	25.81 ± 0.44	97.49 ± 0.75
LA+SO ₂ , 52°C, 36 hr	62.88 ± 0.24	8.59 ± 0.22	5.00 ± 0.12	2.16 ± 0.06	19.62 ± 0.59	21.77 ± 0.53	98.24 ± 0.33
LA+SO ₂ , 52°C, 48 hr	63.84 ± 0.21	7.92 ± 0.82	5.34 ± 0.007	1.91 ± 0.2	19.44 ± 0.47	21.34 ± 0.27	98.44 ± 1.06
13th hr O ₃ +LA, 52°C, 24 hr	59.17 ± 0.74	8.86 ± 0.69	4.09 ± 0.07	1.72 ± 0.21	23.76 ± 0.72	25.47 ± 0.51	97.59 ± 0.51
13th hr O ₃ +LA, 52°C, 36 hr	61.68 ± 0.15	8.46 ± 0.66	4.88 ± 0.06	1.88 ± 0.36	21.52 ± 0.64	23.40 ± 0.28	98.42 ± 0.67
13th hr O ₃ +LA, 52°C, 48 hr	62.89 ± 0.21	9.78 ± 0.53	4.85 ± 0.24	1.27 ± 0.23	19.90 ± 0.67	21.16 ± 0.44	98.68 ± 0.60
Multiple O ₃ , 52°C, 24 hr	62.29 ± 0.11	9.19 ± 0.77	4.2 ± 0.01	2.00 ± 0.22	20.57 ± 1.89	22.56 ± 1.66	98.24 ± 1.02
Multiple O ₃ , 52°C, 36 hr	62.70 ± 0.79	9.92 ± 0.77	4.53 ± 0.04	2.13 ± 0.12	19.33 ± 0.56	21.46 ± 0.68	98.61 ± 1.07
Multiple O ₃ , 52°C, 48 hr	63.62 ± 0.65	9.70 ± 0.41	4.85 ± 0.06	1.64 ± 0.16	18.23 ± 0.11	19.87 ± 0.05	98.04 ± 0.71
Multiple O ₃ , 20°C, 24 hr	61.19 ± 0.14	7.05 ± 0.32	0.87 ± 0.31	1.55 ± 0.009	27.57 ± 0.70	29.12 ± 0.69	98.23 ± 0.66
Multiple O ₃ , 20°C, 36 hr	63.57 ± 0.18	7.30 ± 0.01	1.56 ± 0.16	1.77 ± 0.09	23.81 ± 0.43	25.58 ± 0.51	98.01 ± 0.76
Multiple O ₃ , 20°C, 48 hr	63.91 ± 0.79	6.41 ± 0.26	1.50 ± 0.08	2.05 ± 0.07	24.41 ± 1.39	26.46 ± 1.32	98.28 ± 0.66

To find out whether additional O₃ treatment could improve starch yield, multiple O₃ treatments were applied at the 1st, 13th, and 24th hr during steeping. Figure 4 shows results from several different treatments, including multiple O₃ treatments, compared with those from the standard procedure (LA+ SO₂) at 52°C (unless stated). The starch yields for LA or LA+O₃ at the 13th hr were lower than those for the standard samples at all steeping times. The multiple O₃ treatment (LA+multiple O₃) substantially increased the yields in the beginning, and approached a level similar to the standard after 35 and 48 hr of steeping. When the multiple O₃ treatment was conducted at 20°C, the starch yield started with a lower level but increased gradually to levels higher than all other treatments conducted at 52°C after 38 or 48 hr of steeping. These results indicate that higher starch yields can be achieved at room temperature with increased ozone treatment times. The fact that steeping for 36 hr at 20°C with multiple O₃ treatments could achieve the same starch yield as steeping for 48 hr at 52°C with the standard process suggests that energy savings can be achieved and steeping time can be reduced for corn steeping at room temperature (20°C) with increased discontinuous ozone treatment times.

The increased effectiveness of ozone at low temperature may be attributed to the long half-life of ozone molecules at low temperature, as mentioned earlier. This is supported by the data shown in Fig. 5, which indicates that the measured average concentration

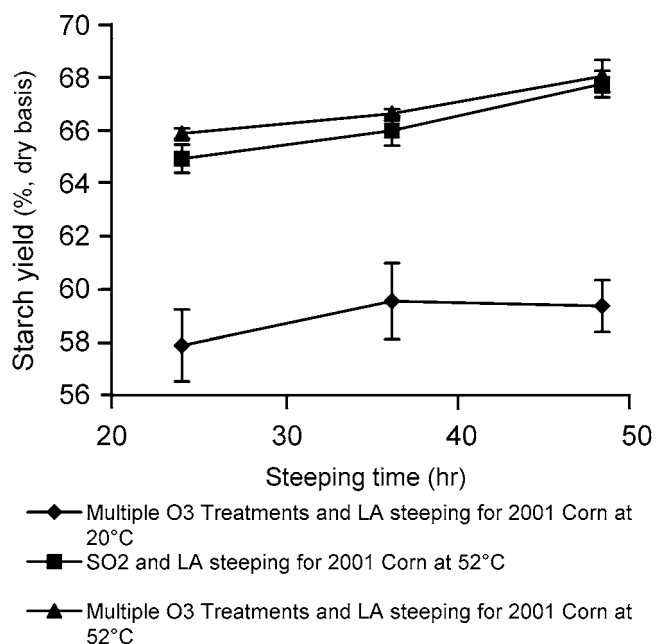


Fig. 8. Corn starch yields for 2001 corn as a function of steeping time at 20 and 52°C. Error bars indicate the standard deviations.

of O₃ at room temperature (20°C) is 40–50% greater than that at 52°C. However, at 30 and 40°C, the starch yields were lower than at 20 or 52°C (Fig. 6), which could be due to larger populations of bacteria that convert simple sugars into alcohol or carbon dioxide at these temperatures.

At temperatures of 20°C and 52°C, the growth of undesirable microorganisms is limited. Nevertheless, more study is needed to address this issue.

Water Uptake

The breakage of the protein disulfide bonds can not occur without the presence of sulfur dioxide, which must be carried into the matrix by water (Haros et al 2001). This could be another possible explanation for increased effectiveness of ozone treatment in a later time, when ozone could diffuse into corn kernels effectively after the kernels absorbed sufficient water.

Figure 7 shows the moisture absorption by corn kernel samples from 1997 and 2001 at 20 and 52°C during steeping in LA solutions. For 1997 corn, water absorption at 20 and 52°C was similar, except for a lag time for 20°C, which coincides with the low starch yield in the early stage at 20°C (Fig. 4). For 2001 corn, water absorption was much faster at 52°C than at 20°C, which coincides very well with the starch yields for 2001 corn at these two temperatures (Fig. 8). It appears that the effect of temperature on OAS process is twofold. Low temperature conditions favor ozone's effect but result in low water absorption by corn kernel during steeping. Methods to speed up water absorption rate at room temperatures should help OAS processing under ambient conditions.

Mean Fraction Yields

Mean fraction yields, another important parameter in corn steeping and milling, are reported in Table II. Total recovery was >98% for both sulfur dioxide steeping and ozone treatment methods. Corn steeped in room temperature with multiple ozone treatment had significantly lower protein yields and steep solids yields at $P < 0.05$. Multiple ozone treatment at 52°C steeping had a slightly higher standard deviation for total solids recovery. Germ was not separated from the fiber. Both fine fiber and coarse fiber contained germ.

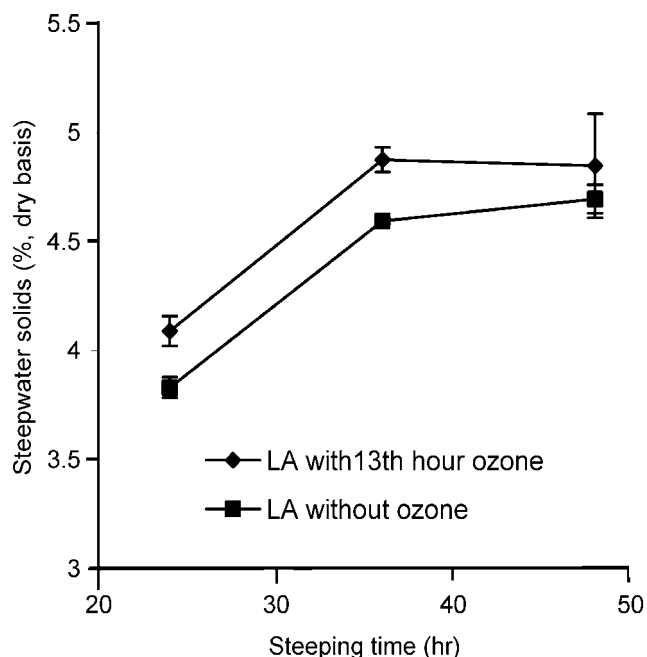


Fig. 9. Solids content of steep water for lactic acid steeping with and without treatment at 13th hr at 52°C. Error bars indicate standard deviations.

Soluble Solids

According to Boundy et al (1967), the sulfur dioxide weakens the protein matrix by breaking disulfide bonds and forming soluble S-sulfo-proteins, which prevent the reformation of disulfide bonds. Haros et al (1995) observed a significant increase in total solids in the steep water with SO₂ compared with plain water. Neuman et al (1984) indicated that the breakage of the disulfide bonds by SO₂ promotes solubilization of the endosperm proteins, which increases starch yields.

Figure 9 indicates that the solids content in the steep water with ozone treatment at the 13th hr was higher than that in steep water without ozone. Figure 10 shows that at 52°C, the solids content in steep water for corn steeped in SO₂+LA and for corn steeped in LA with multiple ozone treatments was almost the same. However, at room temperature, the solids content of steep water with multiple ozone treatments was significantly lower than that at 52°C. It seems that ozone helped to leach corn nutrients into steep water at 52°C, similar to the effects of SO₂. A low soluble solids content in steep water may be an advantage for OAS processing because less ozone is consumed by oxidation of soluble solids.

CONCLUSIONS

This study showed that ozone-aided steeping (OAS) is a valid alternative to conventional SO₂ steeping. The application of ozone at a later stage of steeping was more effective than at the beginning of steeping. The mechanisms responsible for this are unknown. However, in practice, an early small dose of ozone may be necessary to control microbial growth.

When ozone was applied at the beginning of steeping, prolonged ozone treatments reduced the starch yield. When ozone was applied multiple times during steeping, high starch yield could be obtained, even though steeping was conducted at room temperature for 36 hr. Steeping at room temperature represents a significant saving in energy and time compared with conventional steeping.

How ozone works in corn steeping is largely unknown. More studies on the mechanisms of ozone action in corn steeping and effects of processing conditions are needed before OAS can replace conventional SO₂ processes. Developing an integrated ozone approach (steeping, sanitation, water treatment, waste treatment, etc.) for the corn milling industry is highly desirable.

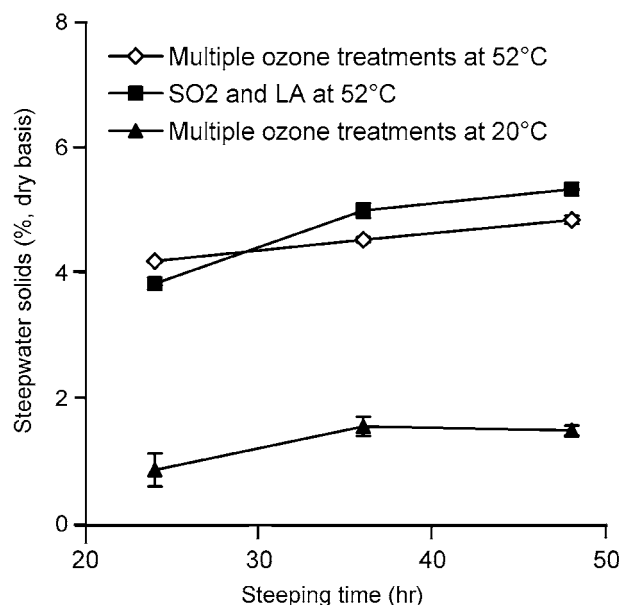


Fig. 10. Solids content of steep water with multiple ozone treatments and SO₂ treatment.

It was also observed that ozone treatment gave the corn kernels a golden color after steeping, similar to that obtained with SO₂ treatment. Generally, corn kernels have a darker color (from the growth of putrefactive microorganisms) without SO₂ treatment.

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