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Application of advanced oxidation processes (ozone/UV) to reduce *Escherichia coli* contamination in flour slurry from rice noodles production

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ABSTRACT

This paper aimed to demonstrate another potential use of AOPs for reclaiming flour slurry from rice noodle making process by destroying spoilage microorganisms and preventing further microbial growth using *Escherichia coli* as a model microorganism. A prototype AOPs system, consisting of UV sterilizer (45 W), ozone generator (2 L/min) and combined ozone/UV, was fabricated and tested on water and flour slurry (53 g/L) systems using the initial *E. coli* concentration at approximately, 7 log CFU/ml. The *E. coli* cell counts showed that UV disinfection was very effective in water, enabling the reduction of cell density from 7 to 0 log CFU/ml within 15 min at all mass circulation flow rates (0.4, 0.6, and 0.8 kg/s). For flour slurry, the blockage of UV radiation by flour suspension substantially compromised the effectiveness of UV sterilization and *E. coli* destruction. Although, the ozone treatment by itself showed slower *E. coli* inactivation kinetics on both transparent and turbid flour slurry, it produced powerful disinfecting effect when it installed prior to the UV sterilizer. The synergic combination of UV and ozone treatments in inactivating *E. coli* was demonstrated on the flour slurry experiment and able to eliminate *E. coli* viability within 8-10 min. Faster mass circulation rate facilitated higher *E. coli* elimination due to higher UV exposure of *E. coli* and better mixing/dissolution of ozone gas in flour slurry.

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INTRODUCTION

The principle of AOP technology encompasses the two key oxidation processes: 1) the generation of strong oxidants (e.g., hydroxyl radicals) and 2) the reaction of these oxidants with compatible substances (e.g., organic or inorganic contaminants). Hydroxyl radicals are highly reactive and non-selective oxidizing agents and can be used to degrade toxic organic and inorganic compounds to less harmful and much simpler molecules (Glaze *et al*., 1987). High concentration of hydroxyl radicals can be achieved from a variety of advanced oxidation processes (AOPs), including but not limited to ozone, UV, H_2O_2, Fenton, etc. (Ollis, 1993). The application of ozone with UV technology serves as a good combination for disinfection and/or oxidation of drinking water and food products with respect to micro pollutant and microbial destruction (Meunier *et al*., 2006).

The hydroxyl radicals in the ozone/UV process were generated by photocatalytic reaction (Beltran, 2004; Vilve *et al*., 2007). Its synergistic effects in sequential or combined disinfection processes were shown to be beneficial in improving disinfection effectiveness, reducing operating costs and decreasing disinfection byproduct formation (Cho *et al*., 2006). Despite these findings, little investigation has been done to study the effect of the combined disinfection processes involving ozone and UV on microbial inactivation. Much less has been investigated on how the turbidity of disinfecting samples affects the effectiveness of the ozone/UV process. Some authors have demonstrated the combination of ozonation and UV treatments leading to the improvement of water quality and enabling the reduction of the ozone dosage in the treatment process (Meunier *et al*., 2006). Ozone by itself is a strong oxidizing and disinfecting agents and can cause elevation of reactive oxygen species in living cells, leading to oxidative stress and causing cell deaths (Kim *et al*., 2003). The combination of ozonation and UV radiation to disinfect fecal coliform in municipal wastewater effluent was successfully implemented in a pilot scale (Venosa *et al*., 1984). However, the use of ozone and UV application for the disinfection of microorganism in a highly turbid medium are relatively scarce.

In this paper, the application of ozone and UV combination was performed on turbid rice flour slurry that is commonly used to prepare rice noodles. The goal was to prolong the fermentation of rice flour slurry for noodle production using the combine ozonation and UV radiation. The successful implement of this technology should have the least impact to the physicochemical properties of the flour slurry while the growth of spoilage microbes was ceased.

MATERIALS AND METHODS

E. coli inoculum preparation

Frozen stock of *E.coli* strain (DMST 4609 received from Department of Medical Science of Thailand) was thawed and recovered in 100 ml of Trypticase Soy Broth (TSB, BD™, Germany) for incubated 24 h at 37 °C to reach the final cell density at 7 log CFU/ml. The strain of *E. coli* was confirmed and enumerated in Chromocult® Coliform Agar (CCA) using the micro-inoculation technique as described elsewhere (Khueankhancharoen *et al*., 2010; Saeaung and Boonyaprapasorn 2010 and Supanivatin *et al*., 2010).

Spiked water and flour slurry preparation

For the flour slurry sample, 800 g of rice flour was mixed in distilled water to reach the final volume of 15 L. Both flour slurry and water samples were spiked with 200 ml of 10^7 CFU/ml *E. coli* culture. The 15.2 L solution was placed in the reservoir of the O_3/UV system in Figure 1 and the circulation mass flow rates were set at 0.4, 0.6 and 0.8 kg/s for different treatments of UV, ozone and ozone/UV. Sampling time intervals were 0, 2, 4, 6, 8, 10 and 15 min.

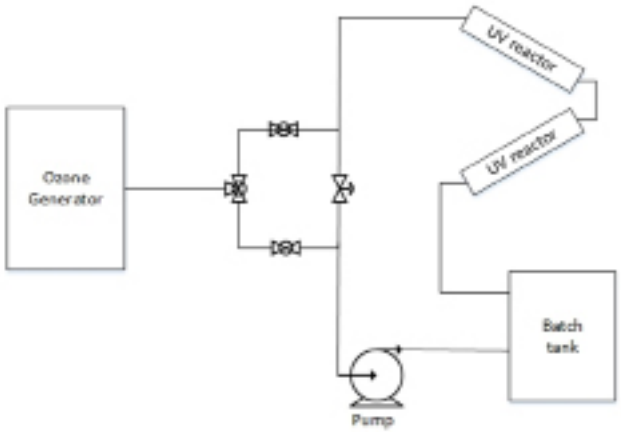

Figure 1 Schematic diagram of the Advanced Oxidation Processes (AOPs) prototype.

UV treatment

The ultraviolet sterilizer allowed the liquid sample to flow into annular space between quartz sleeve and inside chamber wall and ensure uniform exposure of suspended microorganisms to lethal ultraviolet rays. The UV germicidal lamps (9047A481 Hanovia lamp 45 W, YUP OEM, China) operated at the radiation peak of 254 nm.

Ozone treatment

Ozone gas was generated using a water-cooled ceramic ozone tube enabling the production of ozone gas from oxygen at 16-34 g/h. The ozone flow rate was set at 2 L/min and the ozone gas was dissolved in liquid using a Venturi mixer. The final dissolved ozone concentration was validated at 4-15 ppm.

O_3–UV treatment

The prototype ozone/UV reactor was a closed circulation system with the sample liquid transferred from the reservoir using a centrifugal sanitary pump through a Venturi gas-liquid mixer followed by two sets of UV sterilizers in series (Figure 1). The holdup volume in the piping system was estimated at 5 L. The mass flow of the pump can be adjusted using an inverter. This prototype equipment can be configured to operate using ozone and UV sterilizer independently or ozone/UV combination.

Enumeration of surviving *E. coli*

E. coli enumeration was achieved by taking 1 ml aliquot of sample and preparing serial dilution. Sample dilutions were then plated on Chromocult® Coliform Agar (CCA, Merck, Germany) and incubated at 37 °C for 24 h (Maria *et al*., 2008). The colony count was performed using the micro-inoculation technique.

Statistical analysis

Prior to the statistical analysis microbial counts were logarithmically transformed. The data were statistically analyzed by analysis of variance (ANOVA) using SPSS 16.0.

RESULTS AND DISCUSSION

UV treatment on water

When the UV light radiated inside the annular cavity of the UV sterilizers, its energy was partly reflected and partly absorbed by the artificially contaminated water. The series of UV sterilizers with a 45 W lamp each were able to bring down the total cell count in the water within 8 minutes from the initial cell loading at approximately 7 log CFU/ml (Figure 2a). The cell count of the control treatment without the UV treatment remained at 7 log CFU/ml throughout the course of experiment. The faster mass flow rate of water circulation resulted in faster viable cell reduction (Figure 2a). As the mass flow rate increased from 0.4 to 0.8 kg/s (equivalently 37.5 to 18.8 s of cumulative exposure time), the total sterilized time reduced from 8 to 4 min. The similar reduction (more than 5-log10 reduction) in *Listeria monocytogenes* in goat's milk was observed after the milk was passed 12 times through the 8 UV lamp system (a cumulative UV dose of 15.8 mJ/cm^2) corresponding to a cumulative exposure time of 18 s (Matak *et al*., 2005). The requirement of more UV lamps to achieve similar viable cell reduction perhaps was essential to compensate the higher turbidity and less transparency nature of goat's milk.

In a less opaque medium, like apple cider, the same 5-log10 elimination on *Cryptosporidium parvum* oocyst viability was reported using the equivalent UV dose at 14.32 mJ/cm^2. Since the apple cider has less turbidity than the goat's milk sample, the treatment time was much shorter, only 1.2-1.9 s (Hanes *et al*., 2002). Several authors have reported the use of UV sterilizers with great success in other systems, like other juices, milk or transparent agricultural media and lesser degree of microbial inactivation on more turbid solutions (Koutchma *et al*., 2006; Hakguder, 2009; Unluturk *et al*., 2010).

Ozone treatment of on water

In comparing to the UV experiment, the effectiveness of the ozone treatment produced more sluggish reduction of *E. coli* count and longer sterilization time (Figure 2a). In the lower water flow treatments (i.e., 0.4 kg/s circulation flow rate), the final cell count remained at approximately 2 log CFU/ml (Figure 2b). Both the 0.8 and 0.6 kg/s treatments achieved total sterility. The lower flow rates may compromise the dissolubility of ozone at the venture gas-liquid mixture due to less turbulent mixing and gas-liquid interface area. Hence, lesser ozone exposure to *E. coli* cells resulted in poorer *E. coli* destruction despite the fact that ozone has been shown to effectively reduce *E. coli* O157:H7 count in clear phosphate buffer (Byun *et al*., 1998). Hunt and Marinas (1997) suggested that the antibacterial activity of ozone highly related to its diffusion capability into the liquid phase. Higher ozone solubility and treatment exposure was able to improve the bactericidal effect of gaseous ozone at 2.1–7.6 µL/L (Singh *et al*., 2002). They reported that there was a minimal time of ozone exposure to notice the decrease of *E. coli* O157:H7 cells on lettuce and baby carrots and the 15 min threshold of ozone exposure

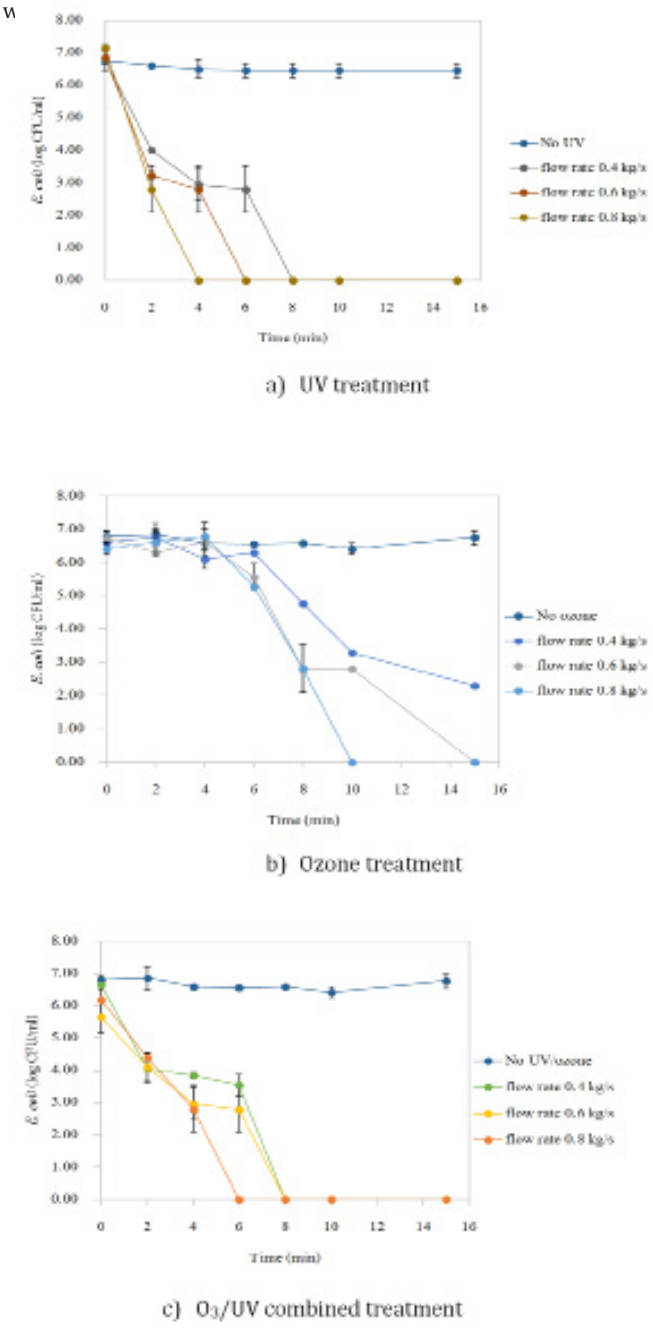

Figure 2 Effect of UV, ozone and Ozone/ UV treatments on the *E. coli* viable cell counts at various circulation flow rates (0.4, 0.6 and 0.8 kg/s) of spiked water.

1.11–2.64 log CFU/g, respectively. In our experiment, there was a minimum exposure time of 6 min to inflict a noticeable reduction of *E. coli* cells in the tested water (Figure 2b). The key benefit of ozone treatment is the general acceptance as a GRAS (i.e. Generally Recognized as Safe) disinfectant or sanitizer to use in food industry (Alexandre *et al*., 2011). Ozone disinfection technology has outstanding antimicrobial properties and is effective against broad-spectrum microorganisms, including bacteria, fungi, viruses and bacterial and fungal spores (Xu 1999; Kim *et al*., 2000). Dissolved ozone produces

high oxidation potential and powerful oxidizing effect. The antimicrobial mechanism lies on its ability to lyse bacterial cell wall causing oxidative rupture and irreversible damage to both the fatty acids in the cell membrane and internal macromolecules, like some essential protiens and DNA (Hoffman, 1971). Ozone is rather unstable and gradually breaks down into free oxygen, eliminating the potential residue of hazardous chemicals (McDonough *et al*., 2011). The property makes ozone technology one of the best antimicrobial agents to use in both gaseous and aqueous forms. (Kim *et al*., 1999)

Ozone/UV combined treatment on water

The combined ozone/UV process showed sharp reduction of *E. coli* counts initially and sluggish destruction response toward the rest of treatment course when comparing to the UV treatment (Figure 2a and c). However, these *E. coli* inactivation patterns were the improvement of the ozone treatment alone (Figure 2b). The delay of *E. coli* reduction presumably was as a result of the UV radiation blockage due to ozone bubbles. To maximize the production of hydroxyl radicals from the excitation of UV ray on dissolved ozone in the spiked water, the Venturi ozone-water mixer was strategically placed in front of the UV sterilizers. As a result, the turbulence of ozone micro-bubbles may play some role in hindering the emitting of UV inside the UV sterilizer cavity. However, the faster circulation flow rate (i.e., the 0.8 kg/s treatment) produced some improvement of *E. coli* reduction. Perhaps the better mixing and more contact surface of gas-liquid interface can facilitate gas transfer and ozone solubility in the liquid phase. Several authors have witnessed the synergistic enhancement using ozone/UV systems on a wide array of foodborne pathogen inactivation, like *B. subtilis* spores (Von 1986; Lazarova *et al*., 1999; Selma *et al*., 2008).

UV treatment on flour slurry

In Figure 3a, the effectiveness of the UV sterilizer on the flour slurry was largely compromised as opposed to the spiked water experiment (see Figure 2a). On flour slurry system, the turbidity of flour slurry limited the depth of UV penetration significantly despite the use of high circulation flow rate up to 0.8 kg/s in the 15 L reaction volume. There was no absolute *E. coli* reduction as seen in the water experiment. Acra *et al*. (1990) also reported that the transparency of the liquid medium and the properties of the UV light including the duration, intensity, and spectrum influenced the efficacy of UV sterilization technique. In successful implementation of UV sterilization, UV light should be absorbed directly and effectively into the microorganisms. Therefore, the blockage from flour suspension to prevent UV from reacting with the microorganism was able to decrease the disinfection efficacy. Clumping or aggregation of microorganisms, turbidity and medium color played a subtle role in affecting the disinfection efficiency of UV.

The approaching cell concentration toward the end of the UV treatment course suggested a transition to a new dynamic equilibrium between cell death rate from restricted UV radiation and *E. coli* growth rate in nutrient rich flour mixture. The increase of mass flow rate from 0.4 to 0.8 kg/s only shifted the asymptotic viable cell density from 4 to 3 log CFU/ml. Perhaps only was *E. coli* localized in very thin layer of contaminated flour slurry surrounding the UV lamp exposed to the 254 nm UV radiations causing biomolecule and nucleic acid destruction and leading to microbial DNA and RNA damage (Aguiar *et al*., 1996; Hoffman *et al*., 2004).

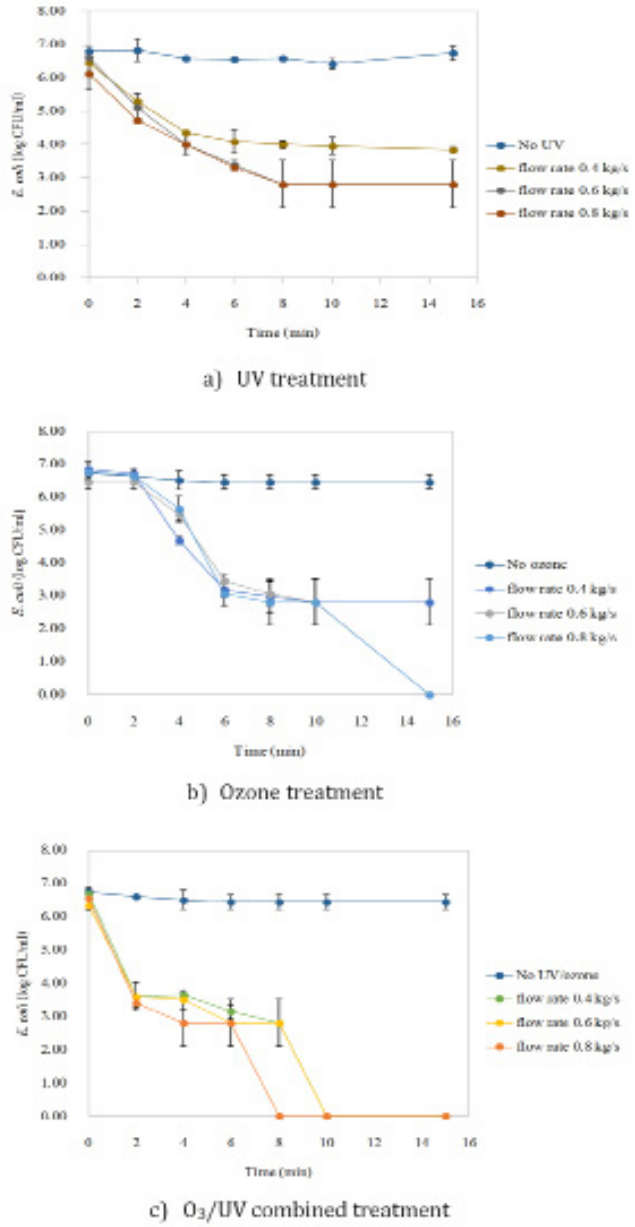

Figure 3 Effect of UV, ozone Ozone/ UV treatments on the *E. coli* viable cell counts at various circulation flow rates (0.4, 0.6 and 0.8 kg/s) of spiked flour slurry.

When DNA and RNA in microbial cells effectively absorb UV light, there is a formation of dimers as a result of covalent bonding between the same nucleic acids. Dimers disrupt the transcription process from DNA to RNA and interfere cell replication. This was not an abrupt destructive process; therefore, microorganism may be able to survive but cannot reproduce effectively. There may be a chance that the microbes can revive and repair damages caused by the UV light. This hypothesis should explain the poor performance of the UV treatment in this flour slurry experiment. Yaun *et al*. (2004) also suggested that the success of UV inactivation varied greatly depending on the optical properties of the liquid medium (e.g., turbidity, particulate matter, and flocculation of microorganisms).

Ozone treatment on flour slurry

As opposed to the UV treatment (Figure 3a), ozone treatment was able to bring down the *E. coli* counts to zero at the circulation flow rate higher than 0.8 kg/s (Figure 3b). Kim *et al*. (1999) reported the decrease of UV inactivation efficacy in a variety of translucent food products, including milk, gelatin, albumin, casein, and meat products. In the case of limited UV transmission media, ozone treatment provided distinct effects on the microorganism reduction. Guzel-Seydim *et al*. (2004) showed significant reduction of the total plate count (TPC) in wheat flour when the exposure time was longer than 30 min. Short ozone treatment between 1 min and 10 min, however, had nearly no impact on microbial reduction. Since the wheat flour had low water content, the microbial inactivation speed was slow and the disinfection ability of ozone gas was not able to achieve total sterility.

Similarly, the lag period at the initial phase of the ozone treatment was considered the accumulation of dissolved ozone concentration to reach an antimicrobial concentration. Singh *et al*. (2002) reported 15 min delay after applying 5.2 mg/L of gaseous ozone to inactivate *E. coli* O157:H7 on shredded lettuce and baby carrots for 1.6 and 2.5 log reduction, respectively. The faster flow rates promoted more ozone solubility in the flour slurry and maximized the exposure of viscous and thick medium to ozone gas.

Ozone/UV combined treatment on flour slurry

The combined ozone and UV light has considerable potential to reduce microbial contamination in flour slurry samples (Figure 3c). The use of ozone/UV sterilization was able to achieve similar *E. coli* reduction profiles as in the UV treatment in water as seen in Figure 2a. No viable *E. coli* count was observed after 10 min of ozone/UV exposure in all treatments. The fastest flow rate treatment returned zero *E. coli* count within 8 min. The use of ozone was able to eliminate the slow or no cell reduction of the UV treatment towards then end of the UV treatment course (Figure 3a). The fast UV inactivation compensated the initial inactivation delay due to low ozone dissolution below the critical threshold. The improvement of combined ozone/UV system was hypothetically contributed to the multiple oxidation effects from ozone, UV and generated OH radicals (Von, 1986).

The synergic effect of ozone/UV system was hypothesized to be derived from better production of OH radicals. Munter (2001) proposed the mechanism of hydroxyl radical generation from the combination of ozone/UV system beginning with the adsorption of UV radiation by ozone gas as in Equation 1. In an aqueous solution, hydrogen peroxide was instantly formed and further decomposed to hydrogen peroxide radicals (Equation 2).

$$O_3 + hv \rightarrow O_2 + O\ (^1D) \quad \text{.....(1)}$$

$$O\ (^1D) + H2O \rightarrow H_2O_2 \rightarrow 2HO^{\cdot} \quad \text{.....(2)}$$

By photolysis, hydrogen peroxide was broken down into two hydroxyl radicals (Equation 3).

$$H_2O_2 + hv \rightarrow 2HO^{\cdot} \quad \text{.....(3)}$$

Depending on the physical pH, HO_2^- in the aqueous H_2O_2 solution also absorbs UV light and form a hydroxyl radical (Equations 4, 5):

$$H_2O_2 \leftrightarrow HO_2^- + H^+ \quad \text{.....(4)}$$

$$HO_2^- + hv \rightarrow + HO^{\cdot} + O^{\cdot -} \quad \text{.....(5)}$$

High concentration of OH radical in Equation (5) from the ozone/UV system enabled faster and more powerful oxidative rupture to *E. coli* cell membranes as proposed earlier in the effect of ozone. Together with UV radiation and background of high ozone concentration, this proposed ozone/UV scheme was highly effective to reduce spoilage microorganism such as *E. coli* in a flour slurry system.

CONCLUSION

The combined ozone/UV treatments have been shown to be more appropriate treatments for cloudy and turbid medium, like flour slurry, than UV or ozone alone. The use of combined treatment was able to achieve zero *E. coli* count within 10 min. In a highly transparent system, like water, UV radiation alone was adequate and very effective. The introduction of ozone in the UV treatment should concern the bubble effect that may interfere with the UV transmission. The faster flow circulation rate enabled more UV exposure and enhanced mixing and ozone solubility in both water and flour slurry. However, the appropriate choice of treatments may not only determine by the microbiological quality alone but also depends on cost-effectiveness criteria and specific reuse requirement of each individual application as well.

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