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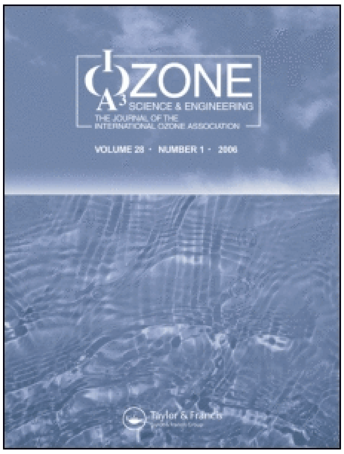

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The use of ozone for postharvest sanitation and decay control of fruits, vegetables and their products during handling, processing and storage has been investigated for commercial applications. Due to their significant contribution to world trade and human nutrition, citrus fruits are thought to be important commodities. Decay can be observed in these products because of microbial activity and ethylene accumulation throughout storage. Residues of pesticides and other compounds in citrus fruit and food-borne illness outbreaks caused by consumption of contaminated citrus juice are important health issues. In this study, the possible uses of ozone in citrus industry for all these problems, and efficacy, benefits and/or detrimental effects of these applications are discussed.

Keywords Ozone, Citrus, Decay Control, Ethylene Abatement, Illness Outbreak, Pesticide Residues, Mycotoxins

INTRODUCTION

After being used for years to disinfect water for drinking purposes, ozone was declared as a GRAS (generally recognized as safe) substance and has recently been approved for use as a disinfectant or sanitizer in food processing. It has been tested for many applications in steps of various food processing industries such as fruit and vegetable (Olmez and Akbas, 2009; Karaca and Velioglu, 2007), cereal (Wu et al., 2006; Ibanoglu, 2002), dairy (Hwang et al, 2006; Serra et al., 2003), red meat (Novak and Yuan, 2003; Castillo et al., 2003), poultry (Al-Haddad et al., 2005; Diaz et al., 2001), sea food (Pastoriza et al., 2008; Gelman et al., 2005), etc. Moreover, a number of commercial processors, mainly in developed countries, have started to employ various ozone applications in industrial scale. Due to its quick decomposition to oxygen with no safety concerns about residues, it could be an acceptable technology to use with commodities marketed under "organic" classification (Gabler et al., 2010).

With their tremendous production amount (totally 90–110 million tons), citrus fruits rank first among fruits in the world. They are grown commercially in more than 50 countries and this provides jobs to many people in harvesting, handling, transportation, storage, and marketing operations. The contribution of the citrus industry to the world economy was estimated at more than 10 billion US $ annually (Ladaniya, 2007).

Researchers have considerable interest in methods to control postharvest diseases in citrus fruit. It is very important to maintain the quality and extend the shelf life of the product. Practices in use generally ensure a limited self life and producers widespread use pesticides. Pesticides both increase the yield and improve the quality of the product. However, public concerns increase due to the potential health hazards of pesticide residues. As a result of these concerns, organic production of many foods, including citrus fruit, has recently been very popular. However, shortness of shelf lives of these products constitutes a practical problem for the producers (Anon, 2009). Outbreaks associated with consumption of fruit juices, including citrus juices, are also thought as serious health problems. Many illnesses have occurred after consumption of pathogen contaminated juice.

Surprisingly, a recently approved agent, ozone, has shown potential solutions for all these critical issues related with citrus fruit. In this work, the studies on citrus fruits about decay control, ethylene abating, juice treatment and residue removing by ozone were reviewed. Discussions on efficiency and potential for practical applications of ozone were also included.

DECAY CONTROL IN CITRUS FRUIT BY OZONE

Fruit decay due to pathogens is one of the main causes of citrus losses. Postharvest blue and green molds caused by *Penicillium italicum* Wehmer and

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P. digitatum (Pers.:Fr.) Sacc., respectively, are among the most economically important postharvest diseases of citrus worldwide (Valencia-Chamorro et al., 2008). Therefore, ozone studies on citrus fruits have mainly focused on these fungi.

Exposure to low levels of ozone gas did not reduce final incidence of postharvest blue or green molds on artificially wound-inoculated and cold stored oranges and lemons. However, infections developed more slowly on fruits stored in an ozonated atmosphere than the ones stored in an ambient atmosphere. Ozone gas also inhibited the normal aerial growth of the mycelia and greatly prevented sporulation of *P. italicum* and *P. digitatum* from lesions among infected fruit once lesions developed. But, aerial mycelia growth and sporulation resumed afterward in ambient atmosphere (Palou et al., 2001). It could be concluded that ozone could significantly suppress the sporulation of fungi as long as the produce was stored in ozone atmosphere. However, it was not able to kill fungal spores on fruit surface. For this aim, higher ozone concentrations could be tested, but in this case detrimental effects of ozone on produce (Smilanick, 2009) should be taken into consideration.

Smilanick et al. (2002) reported that green mold and sour rot on citrus fruit, caused by *P. digitatum* and *Geotrichum citri-aurantii*, respectively, were not reduced by 20 min immersion in 10 ppm ozone. The failure of ozone in aqueous applications might be related with the low solubility of ozone in water. In addition, efficiency of ozone in microbial inactivation depends on the amount of ozone demanding substances in the treatment medium (Karaca and Velioglu, 2009).

Especially, the presence of dirt, soil, fruit residues, and any organic material in wash water can protect the microorganisms to be inactivated by ozone. Nonetheless, Renzo et al. (2005) mentioned a synergistic effect between ozone wash and gaseous ozone exposure in inhibiting mycelia growth preventing sporulation on citrus inoculated with *P. italicum* and *P. digitatum*. After washing with 0.6 ppm ozonated water, they stored inoculated oranges for 8 weeks in an intermittent ozone exposure at 0.25 ppm. They reported that gaseous ozone did not reduce mold incidence in control (not washed) samples, apparently the fungal structures in wounds remain protected from the oxidant effect. Ozone could decrease the load of pathogenic spores in the storage room and inhibit the surface growth of mold on packages, walls and floors, with a subsequent reduction in the amount of inoculum available for re-infections of stored products. Moreover, ozone exposure reduced ageing and weight loss of oranges more than those stored in a non-ozonated environment.

Metzger et al. (2007) claimed that low level atmospheric ozone-enrichment could reduce the level of waste in citrus stored in a semi-commercial environment. After a 35-day storage in an ozone-enriched atmosphere (0.18–0.20 ppm), they observed that the percentage of the rotten fruits was significantly reduced by 7%. Their subsequent experiments focusing on fungicide-treated versus wax and pesticide free oranges revealed that ozone-treated fruits exhibited significantly less (27%) spoilage than equivalent controls. As far as we can understand from the conducted studies; ozone, especially in gaseous phase, is able to prevent microbial activity even at low doses owing to its high oxidizing potential. However, factors related with produce and treatment conditions have a tremendous influence on the action of ozone.

Antimicrobial efficiency of a gaseous agent obviously depends on its penetration ability into packages of food products. After working on ozone gas penetration into various kinds of packages, Palou et al. (2003) indicated that sporulation inhibition of *P. italicum* and *P. digitatum* was satisfactory only on oranges packed naked in ‘highly vented’ returnable plastic containers. Ozone could not control sporulation on oranges bagged in polyethylene bags or packed in fiberboard cartons due to its low penetration through these commonly used commercial packages. It is most likely that the nature of packaging and air circulation in the room determines the effectiveness of gaseous ozone. Due to the high reactivity of ozone, its penetration into packages is critical for effective treatments to be achieved. Recently, many studies about this phenomenon such as the effects of ozone on properties of packaging materials (Ozen et al., 2002) and ozone penetration through materials (Shanbhag and Sirkar, 1998) have been conducted to make the relation between ozone and packaging material more clear.

ABATEMENT OF ETHYLENE BY OZONE

Potential storage life of citrus fruits with fairly good appearance and eating quality can be obtained if fruits are stored under the most optimum conditions after harvest. Ethylene, also known as stress hormone, has a special role in fruit humidity, ripening and senescence, and therefore has its own importance in postharvest management of citrus. Citrus fruits have a very low rate of ethylene evolution, in the amount of $<0.1\ \mu L/kg/h$. Even this rate can slowly build up ethylene concentration in closed chambers (Ladaniya, 2007).

Postharvest actions that reduce the accumulation of ethylene around citrus and other non-climacteric produce during marketing can result in an increase in postharvest life. Ozone has shown a potential to meet this criterion and given encouraging results. Skog and Chu (2001) claimed that ozone could reduce the level of ethylene in the air in a cold storage room, so that ethylene-sensitive and ethylene-producing commodities could be shipped or stored together for longer time. Indeed, ozone was found to be effective in removing ethylene from export containers (Palou et al., 2001). A theoretical mechanism by which ozone destroys ethylene was offered by Anglada et al. (1999). After studying the energetics of possible reactions, they suggested that ozone combines with ethylene to form

1,2,3-trioxolane, an energy-rich ozonide. This unstable compound then breaks down through several possible mechanisms to form various radicals, formaldehyde, carbon dioxide, carbon monoxide, hydrogen and water. Since removal of ethylene may have beneficial effects in extending produce life by delaying ripening or senescence, detailed studies must be carried out about ethylene destruction by ozone and physiological response of the produce to this action.

Many factors such as freezing, drying or high carbon dioxide concentrations can increase ethylene evolution from agricultural products. Controversial results are found in the literature about ozone from this point of view. For instance, ozone treatment did not affect ethylene productions in tomato and peach fruits (Tzortzakis et al., 2007; Palou et al., 2005). However, ethylene production significantly increased in leaves of 3-year-old trees of Clementina mandarin after ozone exposure in open top chambers. Authors claimed that it could be a result of triggering protective mechanisms against oxidative stress in citrus (Iglesias et al., 2006).

Actually, ozone is known to impose oxidative stress and cause many physiological changes, including ethylene synthesis in crops (Forney, 2003). During ozone exposure, plants attempt to maintain a constant redox potential in their cells. In many cases, this results in an increase in the concentrations of antioxidants enzymes and compounds that play a major role in the defense system of the plants against oxidative stress. Ascorbic acid levels in spinach (Luwe et al., 1993), anthocyanins in blackberries (Barth et al., 1995) and concentrations of phytoalexins in grapes (Sarig et al., 1996) were reported to increase after ozone exposure. More research is needed to identify and define the physiological responses of each commodity to ozone.

OZONE TREATMENTS OF CITRUS JUICES

Outbreaks of illness associated with consumption of fruit juices, including citrus juices, have been a growing public health problem. In a ten-year-period, just in the United States, 21 juice-associated outbreaks occurred; 10 implicated apple juice or cider, 8 were linked to orange juice, and 3 involved other types of fruit juice. These outbreaks caused 1,366 recorded illnesses, with a median of 21 cases per outbreak. Among the 13 outbreaks of known etiology, 5 were caused by *Salmonella*, 5 by *Escherichia coli* O157:H7, 2 by *Cryptosporidium*, and one by Shiga toxin-producing *E. coli* and *Cryptosporidium* (Vojdani et al., 2008).

To ensure food safety related with juice consumption, efficient methods that can inactivate these pathogens are strongly needed. To achieve this goal, ozonation of fruit juices was tested (Steenstrup and Floros, 2004; Dock, 1999). Moreover, a number of commercial fruit juice processors in Europe and the US have started to employ ozone for pasteurization of juices. Ozonation of liquid phases is most frequently accomplished by injecting ozone gas through a sparger into the liquid. Typically, studies on ozone absorption in the aqueous system are carried out in stirred-tank reactors or bubble columns (Tiwari et al., 2008a).

Williams et al. (2004) reported that in orange juice samples *E. coli* O157:H7 and *Salmonella* populations were undetectable after 75 and 15 min of ozonation process at 50 °C, respectively. Ozonation at 4 °C reduced *E. coli* O157:H7 and *Salmonella* by 4.8 log CFU/mL and 4.2 log CFU/mL in orange juice, respectively. As a result, ozone was suggested as an effective alternative to heat pasteurization of juices. Same authors also studied the effect of ozone in combination with dimethyl dicarbonate and hydrogen peroxide for orange juice preservation. They reported that a 5 log reduction of *E. coli* O157:H7 could be achieved using ozone in combination with mentioned antimicrobials (Williams et al., 2005). It is obvious that inactivation rates vary depending on many factors related with target microorganisms, treatment conditions and fruit juice as the treatment medium. For instance; pulp content of orange juice was reported to be an important factor on the effectiveness of ozone in *E. coli* inactivation (Patil et al., 2009a). Fast inactivation rates for *E. coli* reduction were achieved in model orange juice and in juice with low pulp content whereas inactivation needed more time in unfiltered juice. This indicated that juice organic matter interferes with antibacterial activity of ozone.

Effects of a process on product quality and consumer acceptability as well as on important constituents of the food are critical issues in evaluating for industrial application. From time to time, ozone treatments have been reported to have a significant influence on important constituents of juices. Studies on the effects of ozone on juice properties reported by various researchers are summarized in Table 1. Ascorbic acid, the most known nutritional constituent of orange juice, was reported to be significantly affected by ozonation process. Its content was found to decrease from 41.59 to 12.70 mg/L in freshly squeezed orange juice after 10 min of treatment time. Additionally, ozonated samples were observed to be lighter in color. L^* value increased while a^* and b^* values decreased significantly at the end of ozonation process. Ozone concentration, gas flow rate and treatment time were all found to be critical factors influencing color degradation in orange juice (Tiwari et al., 2008b). These factors should be considered prior to industrial adoption of ozone technology in fruit juice applications. Parameters that govern microbial inactivation in each product and for specific applications should be optimized to meet food quality and safety requirements (Patil et al., 2009b).

TABLE 1. Effects of Ozone on Properties of Fruit Juices

Fruit juice	Ozone treatment	Results	Ref.
Blackberry	Various ozone concentrations (1.6–7.8%, w/w) up to 10 min treatment time	Lightness, yellowness, redness and anthocyanin content values changed significantly by ozone treatments	Tiwari et al., 2009a
Strawberry	Various ozone concentrations (1.6–7.8%, w/w) up to 10 min treatment time	Significant reductions in anthocyanin (98.2%) and ascorbic acid (85.8%) levels	Tiwari et al., 2009b
Tomato	Various ozone concentrations (1.6–7.8%, w/w) up to 10 min treatment time	Titratable acidity, pH, Brix, cloud value and non enzymatic browning were not affected but color values and ascorbic acid content changed significantly	Tiwari et al., 2009c
Apple	Ozone gas was applied at 0.5 L/min at 860 ppm for 28 min	Treated samples had greater sedimentation. Lower sucrose content and a decrease in soluble solids	Choi and Nielsen, 2005
Orange/Apple	Ozone (0.9 g/h) was pumped for up to 240 min	Orange juice was darker and apple cider was lighter in color after treatment	Williams et al., 2004
Apple	Different gaseous ozone concentrations above 1000 ppm were applied	Turbidity and sedimentation were not affected	Dock, 1999,

REMOVAL OF PESTICIDES AND OTHER RESIDUES BY OZONE

The yield of agricultural products can severely be reduced as a result of plant diseases and pest infestations. To prevent yield reduction, pesticides are commonly used worldwide. This case has increased public concern about possible health risks from residues on agricultural products. To ensure the preservation of human health, maximum residue limits (MRLs) have been established for many pesticides.

Pesticides are also commonly used in citrus fruits production to ensure pre- and postharvest protection. It is known that an adequate deposit of fungicide left on the fruit skin gives satisfactory inhibition of mold sporulation. Numerous postharvest treatments, including dipping and treating with a water emulsion wax containing fungicide are extensively used for preventing deterioration and moisture loss during storage, shipment and marketing (Kader, 2002).

Many countries regularly monitor domestic and imported food products for pesticide residues. If residues are detected over the tolerance levels for a sample, then all the fruits which have been stored in the same container have to be recalled and discarded to protect consumer health (Ito et al., 2003). It is obvious that, in this case, economically important loss of product is unavoidable.

Raising consciousness against hazardous effects of chemicals make many people pay higher prices for organically produced foods. Additionally, some international buyers are requesting "chemical-free" products from citrus packers. There is no universal standard for chemical-free products, but this is usually defined as citrus packed without the use of artificial pesticides. To achieve this aim, chemical-free fruit packers need to take the same precautions as the packers of organic products (Hall, 2006).

Organic products are expected and needed to be free of any chemicals in accordance with ecological growing conditions. However, existence of pesticide residues in these products has been reported from time to time. For instance, 3 of 23 (13%) organic citrus products sold in Swiss markets were found to contain residues of pesticides (Ortelli et al., 2005). Moreover, organophosphorus and organochlorine pesticides were found in citrus essential oils from biologically grown fruits (Di Bella et al., 2006). Although citrus essential oils are not alimentary products as such, this case constitutes a health risk, since they can be an ingredient of perfumes, drugs and antiseptic agents. They are also used as aromatizers for liqueurs, tea, sweets and candied fruits in confectionery industry.

In order to ensure food safety, the amount of pesticide residues in food should be as low as possible. Products with much lower pesticide residues are demanded by some buyers in international trade (Hall, 2006). Therefore, practices designed to maintain pest control using minimum pesticide levels or with less toxic alternatives are required. Also, methods that can effectively and safely reduce or remove pesticide residues are needed to be developed.

Ozone, with its high oxidation potential, seems to be a good alternative that can substitute for many toxic pesticides. By this way, current concerns about proliferation of resistant fungal pathogens (Bus et al., 1991) due to widespread use of pesticides will be decreased. Moreover, ozonation is apparently a key technology to solve the pesticide residue problem. It is considered as one of the most promising variations of chemical oxidation and has a long history of investigation for aqueous pesticide degradation (Ikehata and Gamal El-Din, 2005).

Washing or dipping treatments with ozonated water were shown to be effective in reducing pesticide residues on products. Most of the studies dealt with pesticide residues on apples (Choi et al., 2008; Hwang et al., 2002). Ong et al. (1996) reported that dipping into ozonated water (0.25 ppm) resulted in reducing levels of azinophos-methyl, captan and formetanate hydrochloric acid on the surface of apples in the ratio of 75%, 72% and 46%, respectively. Hwang et al. (2001) observed that mancozeb residues decreased 56–97%, ethylene-thiourea completely removed after ozonated water treatment. Even low level of ozone (1.4-2.0 ppm) in water was shown to be effective in reducing diazonin, parathion, methyl-parathion and cypermethrin insecticides on Pak Choi (*Brassica rapa*) vegetable (Wu et al., 2007a; Wu et al., 2007b).

Compared to aqueous ozonation studies, very little work has been carried out to examine the effectiveness of gaseous ozone in pesticide degradation. After storing waxed Navel oranges for 35 days, Metzger et al. (2007) observed lower levels of imazalil, malathion and chlorpyrifos on samples stored in ozone atmosphere (0.18-0.20 ppm). This finding is important because it shows that ozone could be useful in lowering the pesticide residues on citrus fruit. Degradation of pesticide residues on agricultural products by ozone could have a potential for practical application. This approach may be feasible if the degradation products of the parent pesticides are known and proven to be harmless.

Apart from pesticides, some other contaminants can also be found and cause severe problems in citrus fruits and their products. Besides causing fruit decay and subsequent quantitative loss, mold infections can also constitute potential health risks due to the production of toxic secondary metabolites known as mycotoxins. Aflatoxins, ochratoxin A, patulin, *Alternaria* toxins, cyclopiazonic acid, trichothecenes are some important kinds of mycotoxins that can be encountered in many processed and non-processed foods and feeds (Anon., 2003). Because of their toxic properties, mycotoxin levels in food and feed are restricted by legislation in many countries.

Alternaria mycotoxins such as alternariol and alternariol monomethyl ether are the main toxins found in citrus fruits. They have been detected in mandarins (Logrieco et al., 1990) and tangerines (Magnani et al., 2007). Aflatoxins and cyclopiazonic acid productions were also reported (Bamba and Sumbali, 2005; Mahmoud and Omar, 1994). Moreover, *Fusarium poae*, a producer of trichothecenes and fusarin C toxins, has been isolated from decayed citrus fruits (Filtenborg et al., 1996).

Mycotoxin diffusion may vary depending on the structure of the contaminated product. In oranges the diffusion is slow leaving the major part of the fruit uncontaminated (Filtenborg et al., 1996). Magnani et al. (2007) analyzed different tissues of tangerines and found that flavedo was contaminated with high amounts of *Alternaria* toxins (up to 17.4 ppb) while albedo tissue was free of these mycotoxins. It indicates that these toxic compounds are not accumulated inside fruits, suggesting that flavedo tissues might act as a barrier for penetration. This superficial contamination of mycotoxins can be removed by a strong oxidizer like ozone that can easily affect the surface of the fruits and vegetables.

Indeed, ozone was used to remove various kinds of mycotoxins. Studies on the effects of ozone on different mycotoxins in various products are summarized in Table 2. As can be seen from the table, generally the degradation rates of mycotoxins with ozone are quite high. Apparently, ozone is strong enough to destroy many of these toxic compounds. In addition, the results of embryo assays and feeding experiments with various kinds of animals showed that many deleterious effects of toxins were prevented by ozone treatments (Dollear et al., 1968; Maeba et al., 1988; Chatterjee and Mukherjee, 1993; McKenzie et al., 1997). Hence, the application of ozone on mycotoxin-contaminated food crops seems to be a promising treatment. After conducting necessary toxicological experiments, ozone treatment can be suggested as an industrial practice to reduce or eliminate mycotoxins from contaminated agricultural crops including citrus fruit.

CONCLUSIONS

Ozone, a recently approved agent, has been tested for many applications of various food processing and encouraging results have been revealed. There are also many opportunities for use of ozone in citrus industry such as controlling decays, abating ethylene, treating citrus juice as well as removing pesticide and other residues. Due to its low penetration power, ozone will probably fail in inactivating microorganisms that located in the deeps of the product. However, microbial contaminations usually start from the product surface. Additionally, mycotoxins and pesticide residues are also surface-related problems in general. Therefore, it is reasonable to examine the superficially effective agent, ozone, for solving all these problems. As ozone decomposes into simple oxygen with no safety concerns about consumption of residual by-products, it shows a great promise to be used in treating organically grown commodities.

Ozone applications seem to allow citrus producers to provide safe products to their consumers. Ozone can be applied in the gas as well as aqueous phase, providing additional processing benefits. However, if it is

TABLE 2. Ozone Treatment Studies for Mycotoxins Degradation in Various Products

Food/Matrix	Mycotoxin	Ozone treatment	Results	Ref.
Model system	patulin	Aqueous ozone at 0.17 ppm	Up to 98% of initial toxin concentration was oxidized in 1 min	Karaca and Velioglu, 2009
Dried fig	aflatoxin B_1	13.8 ppm gaseous or 1.7 ppm aqueous ozone	About 90% degradation was achieved with both applications. Gaseous ozone was more effective	Zorlugenc et al., 2008
Apple juice	patulin	Gaseous ozone was bubbled into sample	2.4×10^{-3} mM patulin disappeared almost completely by bubbling a little amount of ozone	Cataldo, 2008
Red pepper	aflatoxin B_1	Gaseous ozone at various concentrations	Reductions were 80 and 93% after exposure to 33 and 66 ppm ozone for 60 min, respectively	Inan et al., 2007
Model system	Trichothecenes	Aqueous ozone at various concentrations	25 ppm ozone resulted in degradations up to materials that were not detected. At low ozone doses (0.25 ppm) intermediate products were formed	Young et al., 2006
Pistachio	aflatoxins	Gaseous ozone at 5–9 ppm for 140 or 420 min	Ozonation (9 ppm) for 420 min resulted in 5–24% reduction in total aflatoxin levels	Akbas and Ozdemir, 2006
Peanut	aflatoxins	Gaseous ozone (4.2% per weight) for 5–15 min	Significant degradations in both kernels and flour	Proctor et al., 2004
Corn	aflatoxin	Gaseous ozone (10 to 12 wt%) for 96 h	Treatment reduced aflatoxin levels by 92%	Prudente and King, 2002

improperly used, ozone can cause some deleterious effects on produce, such as losses in sensory quality and nutritional value. Therefore, optimum conditions such as ozone concentration and treatment time should be separately defined for each application to achieve effective and successful results.

An application can be thought as "suitable" if it provides cost benefits besides helping to produce high quality products with increased shelf life. Since ozonation is still in development stages for commercial use in food products, the cost of ozone generators is unfortunately still quite high. Therefore, a large amount of capital investment is required before putting ozone into use. However, once the ozone system is installed, the producer will be able to find cost savings due to significantly reduced maintenance, operation and labor costs. This additional income would pay out the capital investment for the system based on ozone in a relatively short time. Detailed feasibility studies should be conducted for each product and application for comparing economically ozone with the other methods.

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