Evaluation of Chlorine Dioxide Gas Residues on Selected Food Produce

Valentina Trinetta, Nirupama Vaidya, Richard Linton, and Mark Morgan

Abstract: In recent years, the consumption of fresh fruits and vegetables has greatly increased, and so has its association with contamination of several foodborne pathogens (*Listeria*, *Salmonella*, and *Escherichia coli*). Hence, there is a need to investigate effective sanitizer systems for produce decontamination. Chlorine dioxide (ClO_2), a strong oxidizing gas with broad spectrum and sanitizing properties, has previously been studied for use on selected fruits and vegetables. ClO_2 gas treatments show great potential for surface pathogen reduction; however its use from a residue safety standpoint has yet to be assessed. Thus, the objective of this study was to evaluate residues of ClO_2, chlorite, chlorate, and chloride on selected fresh produce surfaces after treatment with ClO_2 gas. A rinse procedure was used and water samples were analyzed by N, N-diethyl-p-phenylenediamine and ion chromatography method (300.0). Seven different foods—tomatoes, oranges, apples, strawberries, lettuce, alfalfa sprouts, and cantaloupe—were analyzed after ClO_2 treatment for surface residues. Very low residues were detectable for all the food products except lettuce and alfalfa sprouts, where the measured concentrations were significantly higher. Chlorine dioxide technology leaves minimal to no detectable chemical residues in several food products, thus result in no significant risks to consumers.

Keywords: chlorine dioxide, disinfection byproducts, food safety

Practical Application: Potential for chlorine dioxide gas treatments as an effective pathogen inactivation technology to produce with minimal risk for consumers.

Introduction

Fresh fruits and vegetables are an important group of foods whose consumption has considerably increased during the past several years. However, since certain pathogenic microorganisms such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* have been associated with a number of foodborne illness outbreaks linked with fresh produce (CDC 2005), there is great interest in developing effective sanitizer systems for their decontamination. Irrigation or wash water, fertilizers from animal waste and municipal solids, infected operators and facilities with poor sanitation are believed to be some of the possible sources of contamination (FDA 1998).

Among the possible sanitizer options is chlorine dioxide (ClO_2), a strong oxidizing and sanitizing agent having a broad and high biocidal activity (Simpson 2005). Its use as an antimicrobial agent in water for washing fruits and vegetables has already been approved by FDA (FDA 1998). Regulation 21 CFR 173.300 allows the use of chlorine dioxide to disinfect fruits and vegetables and requires a potable rinse step in order to assure that there are no residues of concern in or on the products surfaces for consumers' consumption. Treatment of produces with gaseous ClO_2 without a following rinse step present chemical residues of concern.

MS 20100621 Submitted 6/4/2010, Accepted 9/27/2010. *Authors are with Dept. of Food Science, 745 Agriculture Mall Drive, Purdue Univ., West Lafayette, IN 47907-2009, U.S.A. Direct inquiries to author Trinetta (E-mail: vtrinett@purdue.edu).*

ClO_2 gas can freely participate in oxidation reactions and rapidly break down to chlorate (ClO_3^-) and chlorite (ClO_2^-) ions, which can further convert to chloride (Cl^-) (Gomez-Lopez and others 2009). Although it is well known that a major benefit of ClO_2 for disinfecting drinking water is the lack of organo-chlorine compounds, the residues from the direct treatment of fruits and vegetables have not been well established. The presence of chlorine, chloramines, and similar organic compounds from the disinfection may cause the formation of chlorinated byproducts. To ensure food product safety, there is a need to evaluate residues of these ion species for each treated product.

Similar to the aqueous form, ClO_2 gas also shows effective disinfecting ability for equipment and materials as well as raw products. Moreover, the gaseous form has greater penetration ability and leaves behind less residual (Knapp and Battisti 2001). Aqueous ClO_2 solutions are less desirable for fresh produce, because moisture is left on food surfaces after treatment, thereby promoting growth of molds.

In recent years, ClO_2 gas has been successfully used to reduce different foodborne pathogenic microorganisms. For example, a 5 log reduction/strawberry for *E. coli*, *L. monocytogenes*, and *S. enterica* was obtained by treating the fruit with 5 mg/L ClO_2 gas for 10 min (Mahmomud and others 2008). A similar log reduction was observed when apples were treated with 7.2 mg/L of ClO_2 for 10 min (Du and others 2003). Han and others (2001) also demonstrated the effectiveness of ClO_2 gas in reducing the growth of *L. monocytogenes* on uninjured and injured green pepper surfaces and found the treatment to be superior in comparison to aqueous ClO_2 and water washing. Thus, considering all these advantages, it can be concluded that ClO_2 gas

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shows potential as a good alternative sanitizer for fresh fruits and vegetables.

The Natl. Public Drinking Water Regulations (NPDWR): disinfectants and disinfection byproducts (Federal Register 1998) established the maximum residual disinfectant level for chlorine dioxide at 0.8 mg/L and a maximum contaminant level (MCL) for chlorite ions at 1.0 mg/L (in public drinking water). These regulatory limits were derived from the oral reference dose for chlorite, but no limits have been established for chlorate or chloride residuals (EPA 2003). The NPRWR specifies standard methods for analysis of ClO_2 and its byproducts in drinking water. The accepted methods include ion chromatography (IC) (EPA Method 300.1) for chlorite ion analysis, an amperometric method (Standard Method 4500-ClO2 E) for chlorite analysis, and colorimetric or amperometric methods (Standard Method 4500-ClO2 D or E) (APHA 1995) for chlorine dioxide residues. Previous research has examined the concentration of residual chlorite ion in vegetables and eggs treated with sodium chlorite by UV-IC (Suzuki and others 1997). This method used a C_{18} column to clean the sample after extracting sodium chlorite with water. The detection limit of sodium chlorite in vegetables and eggs was 1 mg/kg with recoveries of 90% to 100%.

The objective of this study was to investigate chlorine dioxide and byproduct residuals after ClO_2 gas treatments on fresh produce surfaces both by N, N-diethyl-p-phenylenediamine (DPD) method (for chlorine dioxide) and ion chromatography (for chloride, chlorate, and chlorite). Matrix and treatment conditions selected were based on previous microbial effectiveness studies (Du and others 2002; Mahmomud and others 2007; Mahmomud and Linton 2008; Bhagat 2009; Bhagat and others 2010).

Material and Methods

Fresh produce samples

The samples selected for this study were lettuce (*Lactuca sativa* iceberg), cantaloupe (*Cucumis melo* ssp. *melo* var. *cantaloupensis*), strawberries (*Fragaria x ananassa*), hydroponic tomatoes (*Solanum lycopersicum L. esculentum*), red delicious apples (*Malus domestica*), alfalfa sprouts (*Medicago sativa*), and navel oranges (*Citrus sinensis)*. The samples were purchased at a local market 1 d prior to testing and were stored at 4 °C (lettuce, strawberries, tomatoes, and alfalfa sprouts) or at room temperature, 25 ± 3 °C, (cantaloupes, apples, and oranges). The sample sizes for each replicate were: a lettuce leave, approximately 100 g strawberries, 1 tomato, 113 g alfalfa sprouts, 1 whole cantaloupe, 1 whole apple, and 1 whole orange.

Production of ClO_2 gas and treatment conditions

The ClO_2 gas was generated based on a method described by Simpson (2005). Briefly, 2% chlorine gas (Matheson Tri-Gas, Ill., U.S.A.) was passed through 3 sodium chlorite cartridges in series (Clordisys Solutions, Inc., N.J., U.S.A.) producing approximately 100 mg/L of ClO_2 gas in nitrogen at 20 L/min flow rate. The level of gas in a treatment chamber (internal dia 91.44 cm, length approximately 122 cm) was monitored and controlled using a ClO_2 sensor (Model-AF26, optek-Danulat, Wis., U.S.A.) and a programmable logic controller (DL-06, Automation Direct, Ga., U.S.A.), respectively. The gas was circulated within the treatment chamber using a fan (internal dia 20.16 cm, air velocity 0.04 to 0.10 m/s). Relative humidity (90% to 95% RH) during the treatment was controlled via a humidifier connected to a single-loop, feedback controller (Taylor Micro-scan 500) with a humidity sensor (Model - C1210032, Vaisala, Helsinki, Finland). Treatment conditions were based on maximum expected ClO_2 gas concentrations and exposure times used in previous studies to obtain 3 to 5 log reductions in surface pathogens (Du and others 2002; Mahmomud and others 2007; Mahmomud and Linton 2008; Bhagat 2009; Bhagat and others 2010) as shown in Table 1. The residues remaining on the surface of the products were monitored over time, immediately after treatment (day 0), after 24 h (day 1) of storage (room temperature for tomato, orange, apple, and cantaloupe; 4 °C for strawberries, lettuce, and alfalfa sprouts), and after 14 d (day 14). Untreated, unwashed samples of each product were used as the control for comparison purpose.

Rinse procedure

After ClO_2 treatment, the food surfaces were immediately rinsed with water to remove any remaining ClO_2 and byproducts. Only one rinse step was performed, since our previous research showed that all the detectable surface residues were removed in a single washing step.

Samples were rinsed with distilled water in a glass beaker and shaken for 10 min at 150 rpm. Four hundred milliliters of water was used to rinse tomatoes, oranges, apples, strawberries, lettuce, and alfalfa sprouts, and 2000 mL for cantaloupes. The rinse water was then collected: 10 mL were analyzed for chlorine dioxide content using DPD method, while the rest was analyzed for chloride, chlorate, and chlorite using IC at Underwriters Laboratories, South Bend, Ind., U.S.A., as described below.

Residual analysis

DPD method. The DPD colorimetric method was used to determine chlorine dioxide concentration in the rinse water solutions (Gates 1998). Briefly, 4 drops (approximately 2 mL) of glycine reagent were added to the sample in order to form chloramineoacetic acid and prevent free chlorine formation that interferes with the determination of chlorine dioxide content (Tinoco and others 1996). Then, a DPD Free Chlorine Powder Pillow was added and the sample was mixed well for 20 s. Chlorine dioxide reacts with DPD to form a pink color compound, the intensity of which is directly proportional to the ClO_2 present in the sample, by measuring absorbance at 530 nm. The limit of detection for this method is 0.04 mg/L. All experiments were conducted in triplicates and chlorine dioxide concentrations were measured in mg/L of rinse water and then converted to mg/kg of fruit.

Ion chromatography. IC was used to analyze the inorganic byproducts of chlorine dioxide by EPA Method 300 (Pfaff and Brockhoff 1990). The technique is able to determine multiple anions in a single analysis. Briefly, the method involves eluding an IC-pack column (Dionex, Sunnyvale, Calif., U.S.A.) with a solution of 2 mM Na_2CO_3/0.75 mM $NaHCO_3$ at a flow rate of 2 mL/min. Standard solutions were prepared concurrently at 4 concentration levels (0.1, 0.5, 1.0, and 5 mg/L) for each analyte and blank. The calibration curve was constructed for each chemical species by plotting UV detector response (peak area) against standard concentration. Sample filtration with 0.20 μm membrane-filters was required to prevent damage to the instrument columns and flow system. The concentration was calculated by comparing sample response to the standard curve, and then multiplying by the appropriate dilution factor (Pfaff and Brockhoff 1990). The detection limit for this method is 0.01 mg/L for chlorite, 0.01 mg/L for chlorate, and 2 mg/L for chloride. Measurements were conducted in triplicate, reported as mg/L of rinse water, and subsequently converted to mg/kg of fruit product.

Statistical analysis

All treatments were performed in triplicates, for each sampling day. However, for the controls, only one replicate for each day was measured with results pooled and averaged, since no significant difference was observed over time. Mean values were reported with a 95% confidence interval, and ANOVA was performed and Tukey's test, implemented in Minitab 15v (State College, Pa., U.S.A.), was used to differentiate between sample means (significant when $P < 0.05$).

Results and Discussion

Three of the products treated with ClO_2 gas (hydroponic tomatoes, red delicious apples, and navel oranges) had surface residues for all measured byproducts near the lower detection limits of the analytical methods. Of these 3 products, only apples had significant residues above the controls (chlorate) persisting more than 1 d. Strawberries, lettuce, alfalfa sprouts, and cantaloupes had surface residues that were significantly ($P < 0.05$) above their respective control samples (Table 2). The DPD method used to detect chlorine dioxide residues may have experienced some interference due to water-soluble components in the produce wash waters. For example, rinse water was pink from juice or pigments coming from strawberries during the rinse. Also, all of the untreated control samples for each product showed low detectable levels of ClO_2 using the DPD method. Since these samples were not exposed to chlorine dioxide, the low levels were assumed to be either interferences with the DPD color change reaction (also pink in color) or the absorbance measurement.

For tomatoes, no significant difference ($P > 0.05$) in chlorine dioxide residues was observed between the treated samples and controls on all 3 d. Chloride, chlorate, and chlorite concentrations were not significantly different ($P > 0.05$) as compared to the controls across days except for chlorite concentration on day 0, where the value obtained was significantly different from the control (0.06 ± 0.01 and $<0.01 \pm 0.01$, respectively). A decrease in chlorite concentration to levels below the detectable limits was also observed over time, which is in agreement with Pfaff and Brockhoff (1990).

For navel oranges, all measured residues were not significantly different from the control ($P > 0.05$) and were also near the detection limit of the methods. The concentration of chlorite on oranges decreased to be below detectable limits after 1 d. For treated red delicious apples, only the chlorate results were significantly different ($P < 0.05$) from the control. The residual chlorate levels remained on the treated apple samples for the entire 2 wk of storage.

Treated strawberries showed a significant difference ($P < 0.05$) in all the byproduct residue concentrations, as compared to the control samples on day 0. Moreover, a decrease in chloride, chlorate, and chlorite concentrations was observed over time as previously noticed for tomatoes, oranges, and apples. However, the decrease was not to an extent that the concentrations went below the detectable levels.

Higher levels of residues were measured for both lettuce and alfalfa sprouts. For almost all sampling days, chloride, chlorate, and chlorite residues were significantly different from the control ($P < 0.05$). On day 0, all the recorded values for treated lettuce were widely higher than the control. For example, the chlorite value, within day 0, for treated samples was 871.30 ± 216.19 while the control was 0.11 mg/kg. A decrease in residual concentrations was observed after 24 h, although the values were still statistically different than the control. Even after 2 wk of storage at 4 °C, the residual values were very high on both the lettuce and sprouts.

Table 1–ClO_2 treatment conditions used for the different produce surfaces and the corresponding microbial reduction achieved.

Produce surface	ClO_2 treatment conditions	Microbial reduction			References
		E. coli	*Listeria*	*Salmonella*	
Log CFU/cm^2					
Tomatoes	0.5 mg/L, 10 min, 90% to 95% RH	–	2.5	2.0	
	0.3 mg/L, 10 min, 90% to 95% RH	–	3.5	2.5	Bhagat and others (2010)
	0.5 mg/L, 10 min, 90% to 95% RH	–	4.5	5.0	
Log CFU/cm^2					
Oranges	0.1 mg/L, 10 min, 90% to 95% RH	–	–	2.2	
	0.3 mg/L, 10 min, 90% to 95% RH	–	–	n.d	Bhagat (2009)
	0.5 mg/L, 10 min, 90% to 95% RH	–	–	n.d	
Log CFU/spotted site					
Apples	3.0 mg/L, 10 min, 90% to 95% RH	–	3.3	–	
	4.0 mg/L, 10 min, 90% to 95% RH	–	5.5	–	Du and others (2002)
	4.0 mg/L, 30 min, 90% to 95% RH	–	n.d	–	
Log CFU/strawberry					
Strawberries	0.5 mg/L, 10 min, 90% to 95% RH	2.4	2.3	2.7	
	3.0 mg/L, 10 min, 90% to 95% RH	4.5	4.6	4.0	Mahmomud and others (2007)
	5.0 mg/L, 10 min, 90% to 95% RH	4.6	4.7	4.3	
Log CFU/cm^2					
Lettuce	0.5 mg/L, 10 min, 90% to 95% RH	1.6	–	1.9	
	3.0 mg/L, 10 min, 90% to 95% RH	3.3	–	2.5	Mahmomud and Linton (2008)
	5.0 mg/L, 10 min, 90% to 95% RH	3.9	–	2.8	
Log CFU/g					
Alfalfa sprouts	1.0 mg/L, 20 min, 90% to 95% RH	–	–	2.2	
	3.0 mg/L, 20 min, 90% to 95% RH	–	–	2.5	Bhagat (2009)
	5.0 mg/L, 20 min, 90% to 95% RH	–	–	2.7	
Log CFU/cm^2					
Cantaloupe	0.5 mg/L, 10 min, 90% to 95% RH	2.7	3.3	3.2	
	3.0 mg/L, 10 min, 90% to 95% RH	3.4	3.8	> 5	Mahmomud and others (2008)
	5.0 mg/L, 10 min, 90% to 95% RH	4.6	4.3	> 5	

The conditions in bold font were selected for the further residual analysis study.
n.d: not detectable.
-: Data not available, as the microorganisms were not outbreak-associated with the food matrix.

The residues on lettuce and sprouts exceeded the chlorite drinking water MCL on days 0, 1 and days 0, 14, respectively. The high residues for these products suggest that chlorine dioxide treatment at the selected conditions may cause concern with residues of chlorite and chlorate. The cause for the variation in residues over time for lettuce and sprouts is not well understood. Further research is needed to examine this phenomenon. However, this is not the only reason that chlorine dioxide may not be suitable for these products (under the treatment conditions studies). The visual quality of these products was also poor, making them unacceptable for consumers. The lettuce and sprouts showed significant discoloration, browning, and bleaching, due to the gas treatment. This quality change is likely due to chlorophyll oxidation reactions as reported by Singh and others (2002).

For cantaloupes, a significant difference between the treated and untreated samples was observed for chlorine dioxide and chlorate residuals for all the sampling days, while no significant difference was evident for the byproducts chloride and chlorite ($P > 0.05$).

This study was not the first one where chlorine dioxide byproducts were analyzed after ClO_2 treatment of food products. Analysis of chlorite, chlorate, and chlorine dioxide was also performed on potato skins treated to prevent microbial spoilage (Tsai and others 2001) and in seafood samples (Kim and others 1999). Low or undetectable amounts of chlorite and chlorate were recovered chromatographically. All measurements of byproduct residues were performed using the EPA Method 300 "Determination of inorganic anions in water by ion chromatography." However, the sample recovery protocols were different in each study. In our research, food surfaces were rinsed after treatment with chlorine dioxide gas; conversely potatoes were manually peeled and subsequently blended in water (Tsai and others 2001), while seafood samples were homogenized in buffer and after centrifugation the supernatant was analyzed (Kim and others 1999). In potatoes, no chlorite was detected by IC in treated samples, indicating that ion contents in skin extracts were below detectable limits. Authors concluded that the use of chlorine dioxide was effective to prevent potato spoilage and posed no significant risk of chemical residuals (Tsai and others 2001). Similar results were obtained by Kim and others (1999). No chlorite residues were detected in sea scallops, mahi-mahi or shrimp treated with ClO_2 (concentrations from 3.9 to 34.0 ppm), and low levels of chlorate were reported. The authors concluded that these levels were not expected to be of health concern to consumers.

The results obtained in the present study provide evidence that the method for removing residues from the product is food-dependent. Thus modification and adaptation of extraction methods should be considered in relation to the food matrix analyzed.

Table 2–Residual byproducts analysis by DPD and ion chromatography methodology.

	Free chlorine dioxide (mg/kg fruit)[a]	Chloride (mg/kg fruit)[b]	Chlorate (mg/kg fruit)[c]	Chlorite (mg/kg fruit)[d]
Hypodronic tomatoes				
Control	0.08 ± 0.03	<2.87 ± 0.06	<0.01 ± 1.8E^{-4}	<0.01 ± 1.8E^{-4}
Day 0	0.09 ± 0.03	<2.98 ± 0.07	0.05 ± 0.03	0.06 ± 0.01
Day 1	0.06 ± 0.01	<2.91 ± 0.10	0.01 ± 1.8E^{-4}	<0.01 ± 1.8E^{-4}
Day 14	0.07 ± 0.02	<3.05 ± 0.13	0.03 ± 1.2E^{-4}	<0.01 ± 1.2E^{-4}
Navel oranges				
Control	0.01 ± 0.03	<3.93 ± 0.23	<0.03 ± 0.01	<0.02 ± 2.1E^{-4}
Day 0	0.08 ± 0.02	<2.94 ± 0.07	0.12 ± 0.15	0.11 ± 0.07
Day 1	0.08 ± 0.03	<2.88 ± 0.02	<0.01 ± 1.7E^{-4}	<0.01 ± 1.7E^{-4}
Day 14	0.09 ± 0.05	<2.88 ± 0.15	0.02 ± 0.02	<0.01 ± 1.8E
Red delicious apple				
Control	0.15 ± 0.04	<5.78 ± 0.30	<0.03 ± 2.4E^{-4}	<0.03 ± 2.4E^{-4}
Day 0	0.28 ± 0.08	<5.74 ± 0.21	**0.16 ± 0.02**	0.05 ± 0.01
Day 1	0.21 ± 0.07	<5.90 ± 0.13	**0.18 ± 0.03**	<0.03 ± 3.9E^{-4}
Day 14	0.15 ± 0.03	<5.82 ± 0.09	**0.17 ± 0.03**	<0.03 ± 2.3E^{-4}
Strawberries				
Control	0.71 ± 0.04	7.78 ± 0.30	0.31 ± 0.32	0.04 ± 0.01
Day 0	**0.37 ± 0.06**	**25.74 ± 3.35**	**40.93 ± 6.11**	**5.71 ± 8.25**
Day 1	0.15 ± 0.02	13.90 ± 5.28	1.39 ± 0.60	0.07 ± 0.05
Day 14	0.35 ± 0.12	9.94 ± 1.77	**0.69 ± 0.10**	0.05 ± 0.02
Lettuce				
Control	0.62 ± 0.29	16.90 ± 5.66	<0.08 ± 0.01	0.11 ± 0.04
Day 0	11.15 ± 3.31	435.4 ± 334.01	**231.17 ± 35.95**	**871.3 ± 216.19**
Day 1	0.61 ± 0.31	**195.27 ± 5.28**	**13.04 ± 1.58**	**16.46 ± 6.14**
Day 14	0.52 ± 0.19	1892.27 ± 991.05	**356.04 ± 189.53**	0.07 ± 0.01
Alfalfa sprouts				
Control	3.96 ± 1.64	<7.08 ± 0.01	0.15 ± 0.21	0.37 ± 0.60
Day 0	7.88 ± 1.22	**5309.77 ± 936.55**	**18053.10 ± 2210.6**	**1259.58 ± 735.97**
Day 1	5.13 ± 0.96	**507.37 ± 20.44**	**165.19 ± 7.39**	<0.03 ± 1.8E^{-4}
Day 14	5.74 ± 0.14	**7669.62 ± 3172.7**	**6135.69 ± 2720.91**	43.77 ± 54.23
Cantaloupes				
Control	0.09 ± 0.04	3.17 ± 1.66	0.03 ± 0.03	0.04 ± 0.03
Day 0	**0.34 ± 0.04**	5.81 ± 0.98	**21.75 ± 3.12**	0.06 ± 0.01
Day 1	0.11 ± 0.04	4.64 ± 1.11	**1.81 ± 0.38**	<0.01 ± 1.8E^{-4}
Day 14	0.16 ± 0.02	3.81 ± 2.16	**0.86 ± 0.03**	0.36 ± 0.44

The numbers in bold font were significantly different as compared to the control for the same food matrix ($P < 0.05$).
[a]DPD detection limit 0.04 mg/L.
[b]IC detection limit 2 mg/L.
[c]IC detection limit 0.01 mg/L.
[d]IC detection limit 0.01 mg/L.

Conclusion

Overall, the results obtained in this study show that the use of ClO_2, as an antimicrobial agent to treat fresh produce, may leave detectable residual levels after treatment on products including lettuce and sprouts. However, treated products such as tomatoes, oranges, apples, strawberries, and cantaloupe were all found to have very low residuals when compared to the EPA acceptable levels for drinking water. Based on these residues, it is assumed that products with these low levels will not likely pose harm if consumed. However, since EPA does not officially have a tolerance established for residues on these products, either a petition for a tolerance or an exemption from tolerance will need to be made with EPA prior to using chlorine dioxide gas on fresh produce. In addition, EPA will require data on any organo-chlorine byproducts that may have formed during the treatment (not measured in this study). If the assumption is valid that no byproducts remain inside or on the products after rinsing, residue levels suggest that chlorine dioxide gas has great potential as a sanitizer technology for fruits and vegetables products, without any significant risks of chemical residual for consumers' consume.

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