Article

Efficacy of disinfection methods and effects on nutraceutical properties in coriander and strawberry

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Abstract

Cultivation conditions and postharvest handling of coriander and strawberries promote their microbial contamination. There are several procedures to disinfect these products, however, it is necessary to establish advantages and disadvantages of their application. The objectives of this work were to compare the effectiveness of some disinfection treatments to reduce the bacterial load in coriander and strawberry and evaluate the antioxidant properties of these foods, before and after disinfection. The disinfection treatments compared were ozone generated in an appliance at a flow of 6.25×10^{-5} mol min^{-1} and ozone obtained in the laboratory by an electrolytic reaction at a flow of 7.75×10^{-7} mol min^{-1}. Likewise, commercial disinfectants based on chlorine dioxide, colloidal silver and two products obtained from citrus extracts were used, one applied after washing with detergent (standardized extract of citrus seeds and glycerin) and the other used directly (citrus seed extract based on lactic and ascorbic acids). After the disinfection treatments, the colony forming unit count was carried out and the characteristic pigments of the disinfected products, the total phenolic content and the antioxidant capacity were determined by the FRAP test. The results showed that the standardized extract of citrus seeds and glycerin proved to be more effective ($p< 0.05$) to disinfect both strawberries and coriander, as colony forming units decreased by 97 and 99.9%, respectively; however, it reduced the concentration of anthocyanins in strawberries and the antioxidant capacity in coriander.

Keywords: citrus extracts, commercial disinfectants, food safety.

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Introduction

Strawberries and coriander are crops that grow a few centimeters from the ground and can become contaminated with microorganisms throughout their vegetative cycle or be inoculated during pre-harvest and post-harvest management (Wang *et al*., 2004; Gil *et al*., 2010). Gastrointestinal diseases are one of the first causes of medical consultation and death in Mexico and in the world, therefore, it is urgent to ensure the quality and safety of fruits and vegetables, eliminating as much as possible the pathogenic microorganisms that can affect the health of the consumer.

On the other hand, commercial disinfectants are generally oxidizing agents that could affect the nutraceutical properties of fruits and vegetables, which are related to the content of phenolic compounds, carotenoids, vitamin C and essential oils (Rastkari *et al*., 2015). In recent years it has been shown that fruits and vegetables contain antioxidant substances such as vitamins C and E, β-carotene, lycopene, lutein, flavonoids and anthocyanins (Murcia *et al*., 2001). These bioactive compounds reduce the risk factors associated with cardiovascular diseases, cancer, diabetes and obesity (OMS, 2002). Mexico is located within the ten main fruit and vegetable producing countries, among which are strawberry and coriander (Demirsoy and Serçe, 2016; Hojilla-Evangelista and Evangelista, 2017).

Coriander is used in different dishes for its unique aroma, high content of chlorophyll and vitamin A (Laribi *et al*., 2015). On the other hand, the strawberry is consumed by its attributes of flavor, color, high content of anthocyanins and vitamin C (Da Silva Pinto *et al*., 2008). To date, various investigations have been carried out to study the efficacy of some disinfectants in reducing microbial load in fresh fruits and vegetables, previously inoculated with microorganisms (Karaca and Velioglu, 2007). However, the advantages and disadvantages of the most common commercial disinfectants to reduce it, nor the effect of these products on their antioxidant properties, have not been studied.

The objectives of this work were to compare the efficacy of some disinfection treatments to reduce the bacterial load in coriander and strawberry, as well as to evaluate their effect on the antioxidant properties of these products after the disinfection treatment.

Materials and methods

Chemical reagents

Folin-Ciocalteu reagents, gallic acid (GA), anhydrous sodium carbonate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), glacial acetic acid, sodium acetate trihydrate, hydrochloric acid and ferric chloride hexahydrate were purchased from Sigma Aldrich. Bacteriological agar and nutrient broth were purchased from Dibico and Bioxon, respectively.

Vegetal material

Strawberries and coriander were purchased at a local market in Mexico City. In the case of strawberries, peduncle and calyx were removed. The products were washed with drinking water to remove the sludge and placed on paper towels for drying. The disinfection procedure, in the case of commercial products, was done following the instructions specified on the label. Before applying the disinfection treatments, the products were cut into squares (strawberries) and strips (coriander) of 1 cm using a sanitized stainless-steel knife. This procedure was done in triplicate.

Commercial disinfectants and ozone

Commercial products applied to disinfect strawberries and coriander, with the exception of ozone generated by electrolysis in the laboratory (OEL), were purchased at a mall

Disinfection and colony forming units (CFU) treatments

Strawberries (70 g) and coriander (5 g) previously cut were subjected to the disinfection treatments described in Table 1. Commercial disinfectants were applied according to the manufacturer's instructions. The total aerobic microorganism count was made according to the method of Luksiene and Paskeviciute (2011) with modifications, the disinfected samples were treated with 200 mL of a 0.9% sterile physiological saline solution and allowed to stand for 10 min. After that time, an aliquot (600 µL) of the saline solution, in which the strawberries were submerged, was transferred to a Petri dish.

Table 1. Disinfection treatments applied to strawberries and coriander.[1]

Treatment	Content	Application form
OED	Ozone flow to 6.25×10^{-5} mol min^{-1} [2]	Ozone bubble in water for three minutes
OEL	Ozone flow 7.75×10^{-7} mol min^{-1} [3]	Ozone bubble in water for three minutes
DC	Chlorine dioxide at 10%	It was immersed in an aqueous solution with 5 drops L^{-1} of 10% DC (20 min) and then drained
PC	Colloidal silver at 0.35%	Immersed in an aqueous solution with 8 drops of 0.35% PC L^{-1} (10 min), drain
EC	Citrus seed extract based on lactic and ascorbic acids	Sprayed with disinfectant and wait 10 minutes
ECD	Standardized extract of citrus seeds and glycerin	Pre-wash with water and liquid detergent, spray with the disinfectant and wait for 10 min

[1]= strawberries and coriander were rinsed before disinfection treatments; [2]= generated with an equipo Biozon 2000 home appliance; [3]= Obtained in the laboratory by electrolytic reaction.

Also, after making serial dilutions, an aliquot (100 µL) of the saline solution that was in contact with the cilantro was measured and inoculated in another Petri dish. The incubation temperature on the plates was 37 °C for 48 h and the results were reported in CFU mL^{-1}. According to the official Mexican standard nom-092-ssa1-1994, dilutions were made to obtain a count in the range of 25-250 colonies. Each disinfection treatment was done in triplicate.

Evaluation of antioxidant properties

Preparation of extracts

Samples of strawberry and coriander previously disinfected were extracted according to the method of (Hernández-Rodríguez *et al*., 2016). They were first mixed with 80% methanol in a 1/10 (w/v) ratio, adjusting the pH to 3 with 10% HCl. The extraction was carried out by vortex agitation (5 min at 3 000 rpm), sonication (15 min) and incubator agitation (30 min at 30 °C). Finally, the mixture was centrifuged (1 277 g, 15 min), the supernatants were recovered and titrated to 10 mL with 80% methanol. The extracts were stored protected from light and refrigerated for later analysis. Each sample was processed in triplicate.

Total phenol content

The total phenolic content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965) adapted to microplates. In each well of a microplate 25 µL of the sample to be analyzed, 125 µL of distilled water, 20 µL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and 30 µL of 20% Na_2CO_3 were placed. The mixture was stirred and allowed to react for 30 min in the absence of light; target absorbance was measured at 765 nm in a Synergy 2 microplate multidetector, equipped with Gen5 data analysis software (Biotek Instruments Inc., Winoosky, VT, USA).

The results were expressed as equivalent milligrams of gallic acid per gram of fresh-based sample (mg EAG g_{bf}^{-1}); the gallic acid calibration curve was prepared in a concentration range of 0.001-0.01 mg mL^{-1}.

Antioxidant capacity

The antioxidant capacity of strawberry and coriander extracts was determined by the FRAP assay described by Benzie and Strain (1996), adapted to microplates. First, the following solutions were prepared: pH 3.6 buffer (4.624 g of $C_2H_3NaO_2 \cdot 3H_2O$ and 18.2 mL $C_2H_4O_2$), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The FRAP solution was prepared at the time of use, mixing the buffer solution pH 3.6, 10 mM TPTZ and 20 mM $FeCl_3 \cdot 3H_2O$ in 10:1:1 (v/v) proportions, respectively.

In a 96-well microplate, 20 µL of extract, 60 µL of distilled water and 200 µL of FRAP solution were placed, 260 µL of FRAP were placed blank. After 10 min the absorbance at 600 nm was measured. The results were expressed as equivalent micromoles of Trolox per gram of fresh-based sample (µmol ET g_{bf}^{-1}). The range of the Trolox calibration curve was 3.84 - 46.1 µM.

Total anthocyanin content

The quantification of anthocyanins in strawberries was carried out by the pH difference method described by Lee *et al*. (2015), adapted to microplates. Two samples were prepared separately: 1 mL of strawberry extract was mixed with 1 mL of buffer pH= 1, on the other hand, the same amount of extract was mixed with 1 mL of buffer pH= 4.5. Both mixtures were stirred for 3 min in a vortex at 1 000 rpm. Subsequently, 100 µL of each of the mixtures were placed in a 96-well microplate and the absorbances at 513 and 700 nm were measured. The concentration of total anthocyanins was expressed as milligrams of cyanidine 3-glucoside per gram of fresh-based sample (mg Cyd-3-glu g^{-1}_{bf}), according to the following equation:

$$\text{Total anthocyanins}\left(\text{mg } g^{-1}_{bf}\right)=\frac{(A*PM*FD*10^3)}{(\varepsilon*0.38)}$$

Where: $A = (A_{513\ nm}-A_{700\ nm})_{pH1.0}-(A_{513\ nm}-A_{700\ nm})_{pH\ 4.5}$; PM = 449.2 g mol^{-1} is the molecular weight of cyanidine 3-glycoside; FD= is the dilution factor of the samples; ε= 26 900 mol^{-1} cm^{-1} (molar extinction coefficient of cyanidine 3-glucoside) and 0.38 is the path length or correction factor by microplate use.

Total chlorophyll content

The chlorophyll content was determined according to Lichtenthaler (1987). First, the coriander was mixed with 90% methanol in a 1:20 (w/v) ratio. Then, the extraction was carried out by vortexing (10 min at 3 000 rpm), sonication (15 min) and centrifugation (10 min, 1 300 g). Subsequently, the supernatants were recovered and titrated to 10 mL with the solvent used for extraction. Finally, absorbances at 665 and 652 nm were measured in the microplate reader. The determination was made in triplicate. The total chlorophyll concentration was expressed in milligrams per 100 g of fresh sample (mg 100 g^{-1}_{bf}) and was calculated according to the following equation.

$$C_{total}=\frac{(0.28A_{665}+27.64A_{652})\times V}{m}\times 100$$

Where: A= is the absorbance at 665 and 652 nm; V= is the volume in liters of 90% methanol and m= is the mass in grams of the sample on a fresh basis.

Statistical analysis

The experiments were conducted according to a completely randomized design with the treatments and repetitions described previously. An analysis of variance and comparison of treatment means (Tukey, $p< 0.05$) was applied using the SAS statistical package (version 9.4).

Results and discussion

The most frequent way to disinfect food is to immerse it in a solution that contains a disinfectant (Nascimento *et al*., 2003; Rastkari *et al*., 2015). Recently, disinfectants are also applied by spraying (Chang and Schneider, 2012). The products most used to reduce the microbial load of fruits and vegetables are formulated from chlorinated compounds and organic acids, although ozone generated *in situ* is also used (Martinelli *et al*., 2017; Chen and Hung, 2018; Gómez-Aldapa *et al*., 2018).

The results of the different disinfection treatments are shown in Table 2. As can be seen, the ECD treatment was found to be more effective ($p< 0.05$) in both strawberries and coriander as the CFU decreased 97 and 99.9%, respectively. The percentage of effectiveness of each treatment with respect to the control was calculated with the following formula.

$$\%\text{ of effectiveness} = 100 - \frac{\text{surviving cells CFU mL}^{-1}}{\text{initial viable account}}$$

Table 2. Colony forming units in strawberry and coriander after applying different disinfection treatments.

Treatment	Strawberry (CFU mL^{-1})	Effectiveness regarding control (%)	Coriander (CFU mL^{-1})	Effectiveness regarding control (%)
Control	1351.25 a		63082.5 a	
OED	170 cde	87.42	6940 de	89
OEL	203.89 cd	84.91	7333.33 d	88.38
DC	135.83 de	89.95	3493.33 ef	94.46
PC	203.89 bc	84.91	2630 f	95.83
EC	262.08 b	80.6	13173.33 c	79.11
ECD	42.78 f	96.83	50 f	99.92

OED= ozone generated in an appliance; OEL= ozone generated electrolysis in the laboratory, commercial disinfectants based on; DC= chlorine dioxide at 10%; PC= colloidal silver at 0.082%; EC= citric extract with lactic and ascorbic acid; ECD= standardized extract of citrus seeds and glycerin. Means with equal letters within the same column are not statistically different (Tukey, $p< 0.05$). The data is the average of three repetitions.

Citrus seeds and ascorbic and lactic acids have antimicrobial effects (Tajkarimi and Ibrahim, 2011; Damian-Reyna *et al*., 2017; Jung *et al*., 2017). According to the information provided by the manufacturer, the ECD product contains a standardized extract of citrus seed and glycerin; in general, among the components found in citrus seeds are (2E)-hydroxycinnamic, gallic, syrinic and rosmarinic acids among others (Moulehi *et al*., 2012), whose non-ionized form predominates at acidic pH, crosses the cell membrane until reaching the cytoplasm.

Because the intracellular pH is close to neutrality, the acid dissociates within the microbial cell inhibiting enzymatic reactions and transport systems (Foegeding, 1991). The commercial ECD product is applied by spraying and its effectiveness can be explained by the surfactant action of glycerin (López *et al*., 1998), which allows a longer contact time between active agents and microorganisms. Another aspect that could influence the decrease of microbial load when applying the ECD, with respect to the rest of the treatments used, is the recommendation of a previous washing with liquid detergent.

ECD and EC treatments have a similar formulation (citrus seed extract). However, EC also contains ascorbic and lactic acids. Previous studies have reported the synergistic effect exerted by ascorbic and lactic acids, at a concentration of 0.2%, in inhibiting the growth of *Escherichia coli* O157: H7 in carrot juice (Tajkarimi and Ibrahim, 2011). In this case, EC was 18.57%, on average, less efficient than ECD to inhibit CFU. This could be explained considering the low concentration of organic acids in the disinfectant and why a prewash with detergent is not applied.

In 1998, the Food and Drug Administration (FDA), federal agency of the Department of Health of the Government of the United States of America, allowed the use of chlorine dioxide to disinfect fruits and vegetables. Its advantages over sodium hypochlorite, widely used, are as follows: 1) it is 2.5 times more oxidizing; 2) does not react with phenolic compounds and, therefore, does not produce chlorophenols with an unpleasant taste and smell; and 3) when it reacts with organic matter it produces chlorites and chlorates, instead of toxic compounds such as trihalomethanes (Gómez-López *et al*., 2009).

The treatment containing colloidal silver (PC) was statistically ($p< 0.05$) equal to ECD in the case of coriander. The silver ion (Ag^+) could generate superoxide radicals capable of oxidizing the lipids present in the cell membranes of microorganisms (Hwang *et al*., 2008). Likewise, silver ions can cause damage to cells by different mechanisms: 1) formation of DNA and RNA bonds causing loss of biological function; 2) reaction of Ag^+ ions with sulfur-containing peptides, inside and on the cell membrane, affecting their viability; 3) destabilization of the cell membrane of proteins and inhibition of various intracellular enzymes; and 4) a high concentration of Ag^+ ions affects the cytoplasm and nucleic acids, while at low concentrations the Ag^+ ions tend to prevent the permeability of protons and phosphates in the membrane (Sintubin *et al*., 2011).

The disinfection of fruits and vegetables with colloidal silver is easy to apply and low toxicity (Zhao and Stevens, 1998), for this reason the consumer abuses the use of such disinfectant, which can result in an accumulation of silver ions in the surface of fruits and vegetables. In addition, the water used in the disinfection process becomes a polluting waste. For a long time, ozone has been used to disinfect water, although in recent decades some research has been directed towards the possibility of applying ozone in different fields of the food industry.

According to the FDA, ozone is a substance generally recognized as safe, generally recognized as safe (GRAS) because it does not generate toxic waste when used to control the growth of microorganisms during storage or processing of food. On an industrial level, ozone has been applied, both in gaseous form and in ozonated water (by washing or immersion), to disinfect fruits and vegetables (Öztekin *et al*., 2006; Ibrahim *et al*., 2012; Bermúdez-Aguirre and Barbosa-Canovas, 2013; Chitravathi *et al*., 2015).

Regarding ozone disinfection treatments, the flow generated by the OEL ($7.75x10^{-7}$mol min^{-1}) was 80 times lower than the OED ($6.25x10^{-5}$ mol min^{-1}), although no significant differences were observed in the CFU mL^{-1}. Therefore, the ozone flow generated by the electrolytic reaction was sufficient to decrease the bacterial charge. On the other hand, when comparing OEL, OED, DC and PC treatments in the case of strawberry, no significant differences were observed in CFU mL^{-1}. Similar results were reported by Singh (2002) when comparing the effectiveness of ozone and chlorine dioxide dissolved in water to decrease the microbial load on baby lettuce and carrots; however, ozone disinfection is more advisable because residual ozone decomposes spontaneously within 15 minutes of being generated (Jin-Gab *et al*., 1999).

In addition, the water used during this treatment can be reused after applying a filtration, to remove suspended solids. This is recommended in places where there is a shortage of water.

Content of polyphenols, anthocyanins and total chlorophylls

The content of phenols for strawberries and coriander without disinfection treatment was 0.92 ± 0.21 and 0.84 ±0.04 mg EAG $g^{-1}{}_{bf}$, respectively. After applying disinfection treatments (OED, OEL, DC, PC, EC, ECD), no significant differences ($p< 0.05$) were observed for these values (Table 3), which is consistent with that reported by Beltrán *et al*. (2005); López-Gálvez *et al*. (2010) in lettuce and by Restuccia *et al*. (2014) in artichoke. Regarding the total anthocyanin content in strawberries, most of the treatments were statistically equal, except ECD, in which a significant decrease (46%) was observed with respect to the control.

This could be explained by the enzymatic degradation of anthocyanins or their transformation to secondary phenolic compounds (Chitravathi *et al*., 2015). The total chlorophyll quantified in the coriander after applying disinfection treatments was found in the range of 38.27 ±2.64 to 51.11 ±4.04 mg 100 g^{-1} and no significant differences ($p< 0.05$) were observed with respect to the control (50.10 ±5.98 mg 100 g^{-1}). This is consistent with that reported by Wang *et al*. (2004) in coriander disinfected with ozone, electrolyzed acid water, chlorine and a combination of ozone with electrolyzed acid water and in chard when using chlorine, hydrogen peroxide and ozone as disinfectants (Karaca, 2016).

Table 3. Total phenols (FT) and total antioxidant capacity (FRAP), anthocyanins and total chlorophyll in strawberry and coriander after applying different disinfection treatments.

Treatment	Fresas			Cilantro		
	Phenols mg EAG $g^{-1}{}_{bf}$	FRAP µmol Trolox $g^{-1}{}_{bf}$	Anthocyanins mg Cyd-3-glu $g^{-1}{}_{bf}$	Phenols mg EAG $g^{-1}{}_{bf}$	FRAP µmol Trolox $g^{-1}{}_{bf}$	Total chlorophyll mg 100 $g^{-1}{}_{bf}$
Control	0.92 a	5.17 a	0.11 a	0.84 a	4.2 a	50.1 ab
OED	0.81 a	4.15 ab	0.085 ab	0.8 a	4.74 ab	51.11 ab
OEL	0.81 a	4.5 ab	0.095 ab	0.75 a	3.51 a	41.44 ab
DC	0.71 a	4.02 ab	0.083 ab	0.8 a	3.67 a	50.79 a
PC	0.74 a	4.14 ab	0.078 ab	0.84 a	4.07 ab	47.2 ab
EC	0.63 a	3.35 b	0.1 ab	0.87 a	3.86 ab	38.27 b
ECD	0.75 a	4.05 ab	0.059 b	0.69 a	2.96 b	50.79 ab

OED= ozone generated in an appliance; OEL= ozone generated electrolysis in the laboratory, commercial disinfectants based on; DC= chlorine dioxide at 10%; PC= colloidal silver at 0.082%; EC= citric extract with lactic and ascorbic acid; ECD= standardized extract of citrus seeds and glycerin. Means with equal letters within the same column are not statistically different (Tukey, $p< 0.05$). The data is the average of three repetitions.

The OED, OEL, DC and PC treatments showed no significant differences ($p< 0.05$) with respect to the control. This is contrary to that described by Liu *et al*. (2016), who found that to extend the shelf life of apples, the application of aqueous ozone decreases the antioxidant capacity during the first days. In general, the disinfection treatments studied here have an acceptable efficiency to reduce the bacterial load in strawberry and coriander and can be used according to their availability in certain places. However, citrus seed extracts are very accessible and do not generate toxic waste. On the other hand, EC and ECD treatments affect the nutraceutical properties of strawberries and coriander, respectively.

Conclusions

The treatment with the standardized extract of citrus seeds and glycerin (ECD) proved to be more effective for disinfecting both strawberries and coriander, as colony forming units decreased 96.83 and 99.92%, respectively; however, the concentration of anthocyanins in strawberries was reduced 46% and the antioxidant capacity in the coriander by 30%, with respect to the samples without disinfecting.

The treatment with colloidal silver (PC) also proved to be useful to reduce CFU mL^{-1} in 95.83% coriander.

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