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The effect of using peracetic acid in the processing terminal to reduce microbial contamination of pistachio

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ABSTRACT

Purpose: Pistachio is a crusial agricultural product in Iran, but its contamination with various micro-organisms can cause problems in production, consumption, and export. Peracetic acid is an antimicrobial substance that can eliminate a wide range of micro-organisms quickly. This study aimed to evaluate the effectiveness of peracetic acid in reducing microbial contamination of pistachios in processing terminals. **Research method:** Harvested pistachios were treated with concentrations of 0 (control), 1, and 2% peracetic acid at the washing basin of the terminal. Then microbial growth, bacterial and fungal population, lipid percentage, and peroxide number of pistachio kernels were evaluated at 0 and 6 months after treatment. **Findings:** The results showed that both 1 and 2% peracetic acid inhibited bacterial and fungal growth by about 100% and reduced microbial flora growth by more than 90%. As there was no significant difference between 1 and 2% peracetic acid, the final recommendation is to use 1% concentration. After 6 months, the contamination level increased by about 5%, possibly due to storage conditions and contamination in subsequent stages. Different concentrations of peracetic acid did not have a significant effect on lipid percentage or peroxide number of pistachio kernels. **Research limitations:** As the treatment time with peracetic acid in the washing basin is uncontrollable, this factor has been eliminated in this research. **Originality/Value:** The study confirms the importance of using peracetic acid in the terminals to reduce and control pistachio contamination without producing harmful by-products. Economic evaluation also showed that using 1% peracetic acid for disinfecting pistachios in processing terminals is cost-effective.

INTRODUCTION

Iran and the United States of America are the largest pistachio (*Pistacia vera* L.) producing countries in the world, holding nearly 90% of the global pistachio market (FAO, 2021). Microbial contamination is one of the most important factors that have affected the pistachio market in recent years. In 1997, the European Union returned all pistachios exported from Iran due to the aflatoxin level of 11-400 ppb (Bui-Klimke et al., 2014). Some countries have also established laws restricting the allowable levels of aflatoxin in foodstuffs. In 2003, the European Union defined a maximum limit of 4 ppb for aflatoxin, which was changed to 10 ppb in 2009 (Wu, 2007; Bui-Klimke et al., 2014, European Food Safety Authority, 2020). However, the product quality seems to be varying among countries. Iranian pistachios contain an average of 54 ppb aflatoxin, while most pistachios of the United States have levels below the European Union standard of 10 ppb (Bui-Klimke et al., 2014). Despite such standards, the global pistachio market has changed and Iran's pistachio exports, which higher contamination had compared to American pistachios, decreased to European and other countries with higher aflatoxin limits at a lower price (Wu, 2007; Bui-Klimke et al., 2014). In this regard, Abdolahi Ezzatabadi (2010) showed that removing aflatoxin from Iranian pistachios increased their price by up to 26% and would benefit producers.

Peracetic acid ($C_2H_4O_3$), also known as peroxyacetic acid or percidine, is an antimicrobial substance obtained from the combination of hydrogen peroxide and acetic acid (Moghassem Hamidi et al., 2021; Lin et al., 2023). This compound, in addition to its environmental advantages, is capable of affecting a wide range of micro-organisms (Gehr et al., 2003; Pietrysiak et al., 2019; Suurnäkki et al., 2020). Due to its antibacterial, antiviral, and antifungal properties, peracetic acid has gained significant attention in recent years (Kitis, 2004; Garg et al., 2018; Lin et al., 2023). Another reason for the increased importance of this disinfectant compared to others such as chlorine dioxide is the absence of harmful by-products (Huang et al., 2022; Danielewicz, 2023; Pant et al., 2023). Peracetic acid rapidly decomposes into oxygen and acetic acid when in contact with organic compounds, ultimately resulting in water and carbon dioxide (Zhao et al., 2008; Pant et al., 2023). This compound is widely used in cheese and dairy processing plants, food processing equipment, as a disinfectant for various beverages, as well as for nuts and grains (Joshi et al., 2013; Thomas et al., 2016; Zoellner et al., 2018; EnviroTech, 2021; Pant et al., 2023).

Considering the importance of pistachio health in exports and its economic value, the use of peracetic acid as a safe disinfectant can be significant. As review of literature shows there is no report so far regarding the effect of peracetic acid on pistachios under terminal conditions. Therefore, in this study, the antimicrobial effect of peracetic acid in pistachio processing terminals was evaluated in terms of reducing microbial contamination and improving product quality.

MATERIALS AND METHODS

P. vera cv. Fandoghi were harvested from a commercial garden in September 2021 and then transferred to Hejri processing terminal located in Rafsanjan city, Kerman province, Iran. After peeling, pistachio nuts were disinfected with 0 (control), 1, and 2% peracetic acid in washing basin of the processing terminal. In fact, different concentrations of peracetic acid (formulated by Barafza Keshavarz Pars Company) were prepared inside a 1000-liter tank. The terminal washing basin was filled with these concentrations and the other processes were carried out according to the usual procedure. As the treatment time with peracetic acid in the washing basin is not controllable, the time factor has been eliminated in this research.

A portion of the treated product was transferred to the laboratory, and 100 pistachios were selected in three repetitions and placed in one-liter flasks. Then, 500 ml of sterile distilled water containing a concentration of 2 in 1000 peptone (as a nitrogen source) was added, and the flasks were shaken for 2 h on a shaker (150 rpm) at room temperature (Moradi et al., 2014). Suspensions with dilutions of 10^{-3} were prepared, and 100 µl were cultured in petri dishes (8 cm diameter) containing Malt Extract Agar (MA) medium (HiMedia^{TM}, India, 35 g l^{-1}) for fungal growth, Nutrient Agar (NA) medium (HiMedia^{TM}, India, 28 g l^{-1}) for bacterial growth, and Potato Dextrose Agar (PDA) medium (HiMedia^{TM}, India, 39 g L^{-1}) with pH=5.6 for microbial population growth. It should be noted that suspensions prepared from untreated pistachios (zero concentration of peracetic acid) were cultured as control in MA, NA and PDA culture media. Then, the petri dishes obtained from the above steps were placed in the darkness at the temperature of 28 °C. After 48 h, the number of grown colonies was examined and counted. The percentage of inhibition by peracetic acid was calculated based on the number of grown colonies in each treatment compared to the control using the following formula (1):

$$\text{Inhibition (\%)} = \frac{\text{number of colonies under control condition} - \text{number of colonies under different peracetic acid treatments}}{\text{number of colonies under control condition}} \times 100 \quad (1)$$

For the extraction of pistachio oil, dried kernels were ground carefully and mixed with 120 ml *n*-hexane. After 24 h, the mixture was passed through a filter to separate the oil and hexane from the pistachio residue. Then, *n*-hexane was separated using a rotary device (model RV8, Germany) (Kaviani et al., 2015) and the percentage of obtained oil was evaluated by the following formula (2):

$$\text{Lipid content (\%)} = (\text{initial weight of sample} - \text{final weight of sample}) \times 100 \quad (2)$$

In order to evaluate the peroxide value, 5 g of extracted oil was mixed with 25 ml of the solvent consisted of acetic acid and chloroform in a ratio of 3:2. After 5 min, 1 ml of saturated potassium iodide solution was also added. The mixture was placed in a dark place for 1 min. Thereafter, 25 ml of distilled water and 0.5 ml of 1% starch indicator were added to it, and titration continues until the blue color of the solution disappears using a normal sodium thiosulfate solution. The peroxide value was calculated using the following equation (3):

$$\text{Peroxide value (meq kg}^{-1}) = \frac{1000 \times \text{normality of sodium thiosulfate} \times \text{used amount of sodium thiosulfate (ml)}}{\text{sample weight (g)}} \quad (3)$$

It should be noted that microbial contamination, lipid percentage, and peroxide value were also evaluated for disinfected pistachio nuts that were stored 6 months at room condition (the temperature of 20-25 °C and relative humidity of 30-50%).

The economic analysis of the cost of this pistachio disinfection method (using peracetic acid) at the processing terminal was conducted according to partial budgeting method (Soleiman et al., 2010). Partial budgeting is a basic method designed to evaluate economic consequences of partial changes in farm or orchard activities. This method is based on the logic that a small change in farm activity structure reduces some costs and benefits while simultaneously increasing other costs and benefits. The net economic effect of a change is equal to the sum of positive economic effects minus the sum of negative effects (Table 1). In other words, partial budgeting method is not designed to express the total costs and benefits of the entire farm, but rather aims to express the net increase or decrease in farm income.

In this study, using data extracted from the technical section of the project, costs were calculated and using the results of Abdolahi Ezzatabadi's study (2010), benefits were

evaluated in two scenarios of using and not using peracetic acid. Net benefits were calculated using Table 1. This research was conducted as a completely randomized design with 3 repetitions. Statistical analysis of data was performed using SPSS software, and Dunken's multiple range tests was used at a significance level of 5% to compare means.

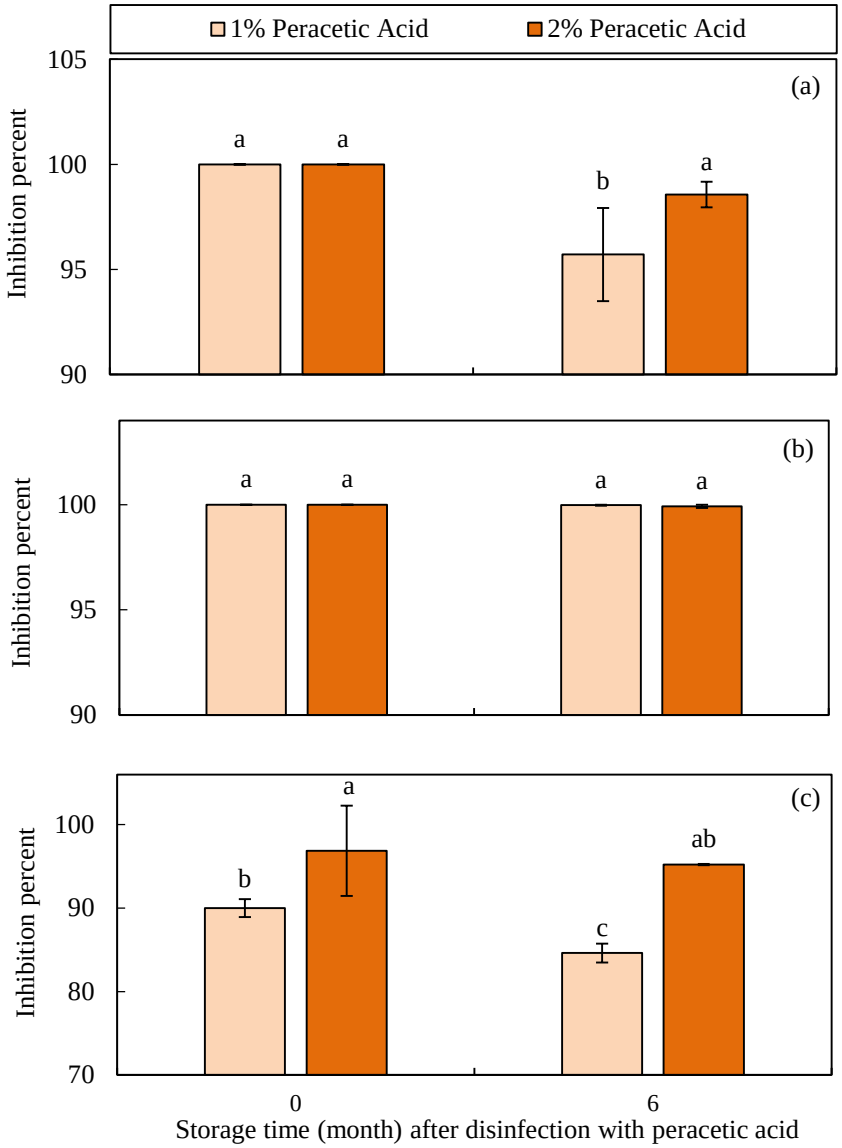

Fig. 1. The effects of 1 and 2% concentrations of peracetic acid and the duration (0 and 6 months) after treatment on the growth inhibition percent of bacteria (a), fungi (b), and microbial flora (c) contaminating pistachio nuts, respectively in NA, MA, and PDA culture media. The values were evaluated relative to the control (without peracetic acid), and columns with common letters do not have a significant difference according to the Duncan test at a 5% level.

Table 1. Partial Budgeting Map (Soleiman et al., 2010).

Costs	Benefits
A- Added costs: Costs incurred in alternative options that do not exist in the current situation.	C- Added revenues: Revenues in alternative options that do not exist in the current situation.
B- Reduced revenues: Revenues in the current situation that do not exist in the alternative option.	D- Reduced costs: Costs in the current situation that do not exist in the alternative option.
Total cost: A + B	Total benefits: C + D
Net profit: C + D - A - B	

Partial budgeting: Comparing the current situation (not using acid plastic) with the alternative option (using peracetic acid).

RESULTS AND DISCUSSION

The effect of 1 and 2% concentrations of peracetic acid and storage period (0 and 6 months) after treatment on the inhibition percentage of the growth of bacteria, fungi, and microbial flora of pistachio seeds compared to the control is shown in Figure 1. As observed, disinfecting pistachios with 1 and 2% peracetic acid causes approximately 100% reduction in bacterial growth compared to the control (without peracetic acid) at time zero (Fig. 1a). At this stage of the experiment, no significant difference was observed between the concentrations of 1 and 2% of peracetic acid. However, when bacterial growth was investigated at 6 months post peracetic acid treatment, a significant difference between 1 and 2% concentrations was observed, with an inhibition rate of approximately 96% in the 1% peracetic acid (Fig. 1a).

The concentrations of 1 and 2% peracetic acid limit fungal contaminants of pistachios by approximately 100%, and the results remained consistent even after six months (Fig. 1b). These Concentrations also caused a reduction in microbial flora contamination by approximately 90% and 96%, respectively, compared to the control (without peracetic acid) (Fig. 1c). Six months after peracetic acid treatment, the inhibition rate for microbial flora of pistachios treated with 1 and 2% peracetic acid reached approximately 85% and 95%, respectively (Fig. 1c). The results of this study demonstrated that the use of 1 and 2% peracetic acid in the washing basin of pistachio processing terminals can effectively inhibit the growth of bacteria and fungi by up to 100% and reduce the overall microbial flora by more than 90%. The increase in pistachio contamination after six months may be due to storage conditions and subsequent contamination.

In a study conducted by Hasani et al. (2020), two disinfection methods were evaluated for inactivating the important foodborne pathogens of *Salmonella* or *Listeria monocytogenes* on de-shelled pistachios. One method was based on the combination of peracetic acid-ethanol sanitizer (PAA-ethanol), and the other involved an Advanced Oxidation Process (AOP) utilizing UV-C, ozone, and hydrogen peroxide simultaneously. Disabling *Salmonella* in pistachios using 2.5 ‰ (part per thousand), PAA-ethanol was significantly more effective than the AOP method. *L. monocytogenes* had a higher sensitivity to hydrogen peroxide and AOP, so it can be eliminated from the samples. They showed that these two disinfection methods are used to reduce *Salmonella* and *L. monocytogenes* on pistachios, although their efficacy depends on the pathogen and product type (Hasani et al., 2020).

Pao et al. (2006) treated almonds contaminated with *Salmonella* with water, acetic acid, citric acid, acidified sodium hypochlorite, peroxyacetic acid, and a combination of citric, hydrochloric, and phosphoric acids. Their results showed that these acids caused a reduction in the bacterial population contaminating almonds. Furthermore, increasing the application number (1 to 3 times) and the concentration of acid treatments resulted in increased effectiveness and further reduction in the population of pathogenic bacteria (Pao et al., 2006).

Research on the effect of peracetic acid on bacterial growth has shown that this disinfectant compound delays bacterial growth and also causes destruction of bacterial cell walls (Wang et al., 2020; Liu et al., 2023).

Spraying peracetic acid on fresh strawberry cuts showed that its concentration (1-240 mg l^{-1}) and spraying duration (11-138s) did not have any effect on fruit quality characteristics (Méndez-Galarraga et al., 2019). On the other hand, this method led to a reduction in contaminating micro-organisms such as molds, yeasts, and bacteria.

The effect of using different concentrations of peracetic acid (0 as control, 1, and 2%) in the washing basin of the processing terminal and the duration after treatment (0 and 6 months) on the lipid content of pistachio kernels is shown in Figure 2. As observed, the amount of lipid present in pistachio kernels is approximately 30-35%, and treatment with different concentrations of peracetic acid does not have a significant effect on the lipid content at both time points (Fig. 2).

Figure 3 illustrates the effect of different concentrations of peracetic acid (0 as control, 1, and 2%) and the duration after treatment (0 and 6 months) on the peroxide value of pistachio kernels. As observed, different concentrations of peracetic acid do not have a significant effect on the peroxide value at a level of 5%.

Excluding the effect of peracetic acid disinfectant on lipid content and peroxide value in pistachio kernels can be important for preserving product quality and increasing shelf life (Tavakolipour, 2015; Akhavan-Mahdavi et al., 2023). In another study, the effect of chlorine dioxide disinfectant on green walnuts was investigated, but it did not cause any changes in the peroxide value during storage period (Jiang et al., 2015).

Ribeiro et al. (2020) investigated the disinfectant effect of different concentrations (20, 80, and 140 $mg.l^{-1}$) peracetic acid on Brazil nuts for 2, 8.5, and 15 min. Their results showed that the optimal sanitizing condition was 15 min treatment with 140 $mg.l^{-1}$ peracetic acid. It was demonstrated that the used concentrations of peracetic acid have no effect on aflatoxins, despite their effectiveness in controlling fungi. Peraceic acid treatment did not have any sensorial properties of the products (Ribeiro et al., 2020).

Since the use of peracetic acid almost eliminates the contamination of pistachio by fungi, bacteria, and microbial flora, the likelihood of incompatibility between Iranian exported pistachio samples and global health standards, especially those set by the European Union, has decreased and even reached zero. This issue leads to a decrease in the number of returned Iranian pistachio samples from global markets, resulting in an increase in the price of pistachio at the Iranian producer level. According to Abdolahi Ezzatabadi (2010), removing aflatoxin from Iranian pistachios increases their price at the produce level by 26%. Based on current prices (spring 2023), the average price per kilogram of pistachios (different varieties with various qualities) at the producer level is around five million Rials. If we multiply this number by 0.26, it will amount to 1,300,000 Rials. Considering that currently there are no other costs for disinfecting pistachios at terminals, 1,300,000 Rials per kilogram is considered as the only benefit of using this disinfectant.

The costs of using peracetic acid include the price of purchasing this material itself, and due to the automation of the machines in the terminals, there is no need for the labor. Since the washing basins are already present in the pistachio processing terminals, no additional costs were included in this regard. Considering that the final research recommendation for the use of peracetic acid is 1% concentration, the cost of using this type of acid was also taken into account in the economic evaluation. For the final application, one liter of 1% peracetic acid is required to disinfect one kilogram of pistachios, with a price of 77,600 Rials per liter. Therefore, the cost of using peracetic acid is equivalent to 77,600 Rials per kilogram. Since the use of peracetic acid does not eliminate any pre-existing benefits, the costs associated with

using this material are only additional costs resulting from its application. The summary of benefits and costs resulting from the use of peracetic acid is provided in Table 2. As shown, the net profit resulting from peracetic acid in pistachio processing terminals is positive and equivalent to 1,222,400 rials per kg of pistachios. In other words, its use is economically viable.

Table 2. Partial budgeting for the application of acid plastic for pistachio disinfection in processing terminals.

Costs	Benefits
A- Additional Costs: 77,600	C- Additional Revenues: 1,300,000
B- Reduced Revenues: 0	D- Reduced Costs: 0
Total Costs: 77,600	Total Benefits: 1,300,000
Net Profit: 1,222,400	

(unit: Rials per kilogram of pistachios).

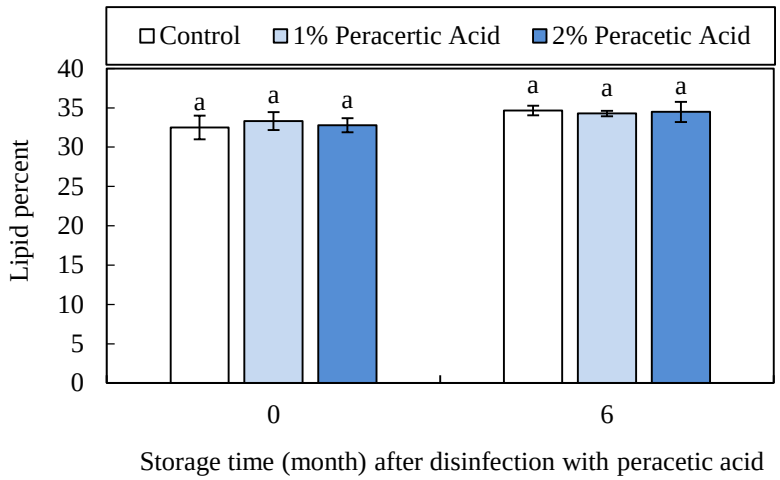

Fig. 2. The effect of using concentrations of 0 (control), 1, and 2% peracetic acid in the terminal washing basin and the duration after treatment (0 and 6 months) on lipid content of pistachio kernels. Columns with common letters do not show a significant difference according to the Duncan test at a 5% level.

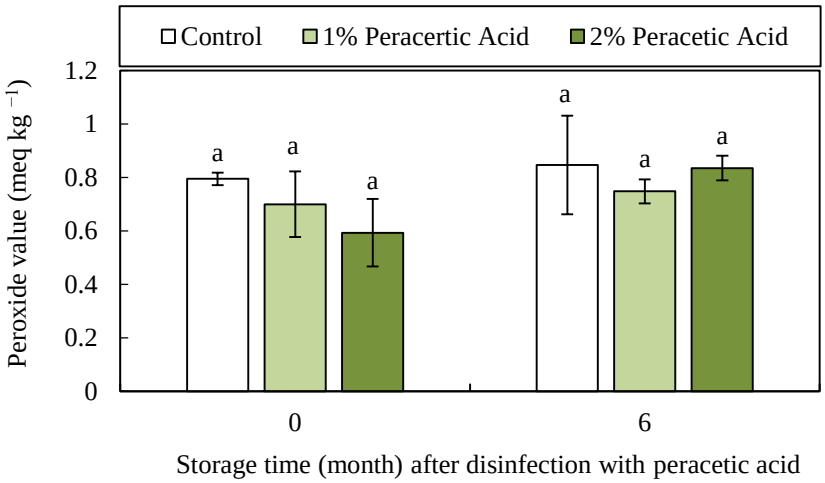

Fig. 3. The effect of using concentrations of 0 (control), 1, and 2% peracetic acid in the terminal washing basin and the duration after treatment (0 and 6 months) on the peroxide value (meq kg^{-1}) of pistachio kernels. Columns with common letters do not show a significant difference according to the Duncan test at a 5% level.

CONCLUSION

Microbial contaminations are among the most important factors that affect the quality of pistachios and restrict pistachio exports. Peracetic acid is a compound that, besides not producing harmful by-products and having environmental benefits, is capable of eliminating a wide range of micro-organisms in the shortest possible time, which gives it an advantage over other disinfectants. The results of this study showed that the use of 1 and 2% concentrations of peracetic acid in the washing basins of pistachio processing terminals can inhibit the growth of bacteria and fungi by up to 100% and reduce the general microbial flora by more than 90%. The significant reduction of microbial population (fungi and bacteria) contaminating pistachios, along with not producing harmful by-products, can confirm the importance of using peracetic acid disinfectant to reduce and control pistachio contamination. As there was not any significant difference between 1 and 2% peracetic acid in controlling microbial contamination of pistachios, the final recommendation is the treatment with 1% peracetic acid. In addition, economic evaluation also showed that the use of 1% peracetic acid in pistachio processing terminals for disinfection is cost-effective.

Conflict of interest

The authors have no conflict of interest to report.

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