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The Sanitizing Effect of Peracetic Acid on Microbial Contamination of Pistachio (*Pistacia vera* L.)

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A R T I C L E I N F O

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Microflora;
Peroxide value

A B S T R A C T

The economic importance along with the high nutritional value of pistachio has necessitated further research on this agricultural product. Its contamination with various microorganisms causes several problems for the production, consumption and export of pistachio every year. Pistachio as a fatty nut is very susceptible to fungal and bacterial contamination and thereby, its shelf life would be reduced. Peracetic acid is a strong oxidizer which can rapidly remove a wide range of microorganisms and is also environmentally safe. In this research, pistachio seeds were firstly treated with different concentrations (0, 1, 2 and 2.5%) of peracetic acid for 0.5, 1, 1.5, 2 and 5 min. Then the growth of fungi and bacteria were evaluated. The results indicated that 2% peracetic acid for 0.5 min led to 99% reduction in contaminating microflora . In the next step, the most common microflora contaminating pistachio seeds were detected and exposed to different concentrations of peracetic acid for 0.5, 1, 1.5, 2 and 5 min. The results showed that 1% peracetic acid for 0.5 min inhibited the growth of the dominant microflora more than 99%. Peracetic acid treatment in the tested range had no significant effect on lipid content but reduced peroxide value. Therefore, disinfection of pistachio product with a concentration of 1-2% peracetic acid in the washing basins of pistachio processing terminals may be an applicable approach to inhibit their microbial (bacteria and fungi) contamination up to 99% and improve their quality before introducing to the market. However, this idea requires further field research.

Introduction

Pistachio (*Pistacia vera* L.) is one of the most popular nuts in the world due to its high nutritional value and dehisced shell (Eslami et al., 2019; FAO, 2018), and Iran is known as an important country producing pistachio (Sharifkhah et al., 2020; Shamshiri et al., 2015) and ranked as second producer of pistachio around the world (Norozi et al., 2019). Microbial contamination adversely affect pistachio quality and lead to restriction of its export every year. For example, various species of *Aspergillus* fungus such as *A. flavus* and *A. parasiticus* contaminate pistachio and other fatty seeds, and producing aflatoxins as the most toxic fungal poison (Bennett and Klich, 2003; Shephard, 2003; Elsanhoty *et al.*, 2014; Rastegar *et al.*, 2017; Mahbobinejhad *et al.*, 2019). In some countries, there are restricting laws for *pistachio* export according to the amount of aflatoxin. In European Union, the limits for aflatoxin B1 and total aflatoxins are 8 and 10 ppb, respectively (European Food Safety Authority, 2020). Reports

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indicate that at least 25% of agricultural products are infected with fungal toxins annually (FAO, 2018). In recent years, it was indicated that pistachio seed can also be infected by pathogenic bacteria (Centers for Disease Control Prevention, 2009). Infection of pistachio seeds with some bacteria as *Escherichia coli*, *Bacillus cereus*, *Klebsiella*, *Salmonella* etc. causes many problems for human health (Umeh and Berkowitz, 2002; Akbas and Ozdemir, 2006; Centers for Disease Control Prevention, 2009).

Peracetic acid ($C_2H_4O_3$), also known as peroxyacetic acid or percidine, is an antimicrobial substance derived from a combination of hydrogen peroxide (H_2O_2) and acetic acid (CH_3COOH) (Zoellner *et al.*, 2018). This compound, not only disinfecting a wide range of microorganisms but also provides environmental benefits (Gehr *et al.*, 2003; Pietrysiak *et al.*, 2019; Suurnäkki *et al.*, 2020). One reason for priority of peracetic acid to other disinfectants, such as chlorine dioxide, is the lack of production of harmful byproducts. Peracetic acid in contact with organics, decomposes into oxygen (O_2) and acetic acid, and finally into H_2O and CO_2 (Zhao *et al.*, 2008).

Alvaro *et al.* (2009) studied and compared disinfecting effects of peracetic acid and sodium hypochlorite on some vegetables such as tomato, sweet pepper and cucumber. This research was conducted in three different parts of studying organoleptic properties of vegetables from the consumer overview; comparison of disinfectant capacity of peracetic acid and sodium hypochlorite; evaluating of phytotoxic effects of disinfectant compounds (Alvaro *et al.*, 2009). The results indicated that the composition of peracetic acid, in comparison with sodium hypochlorite, is more suitable for washing vegetables and increases the post-harvest shelf life. In addition, peracetic acid is more important due to lower toxicity for environment and also human health, and it does not affect sensory characteristics of products.

Spraying peracetic acid on fresh slices of strawberry showed that its concentration (1-140 mg l^{-1}) and the duration of spraying (11-138 s) had no effect on the quality characteristics of the fruit (Méndez-Galarraga *et al.*, 2019). On the other hand, this method reduced the contaminating microorganisms, molds, yeasts and bacteria.

Peracetic acid has attracted much attention in recent years and is widely used as disinfectants in some factories as cheese and dairy, food processing, and beverage (Joshi *et al.*, 2013; Thomas *et al.*, 2016; Zoellner *et al.*, 2018). Given the importance of pistachio quality in exports and its economic value, the use of peracetic acid as a safe or low-risk disinfectant can be valuable. In the current study, the ability of this compound to reduce the microbial contamination of pistachio nuts, and to improve the quality of the product was evaluated.

Materials and Methods

At first, peracetic acid was produced by the combination of acetic acid and hydrogen peroxide (Fig. 1).

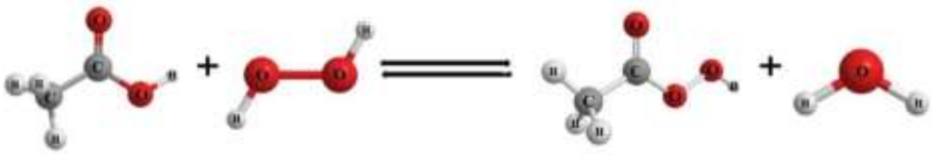

Fig. 1. The equation of peracetic acid production (Kitis, 2004).

The seeds of *P. vera* cv. Fandoghi were prepared from Pistachio Research Center (PRC), Rafsanjan, Iran. They were treated with different concentrations of peracetic acid (0, 1, 2 and 2.5%) for 0.5, 1, 1.5, 2 and 5 min. Then some parameters as the inhibitory effect of peracetic acid on common as well as predominant microflora (fungi and bacteria) of pistachio, lipid content and peroxide value of the treated seeds were evaluated as follow.

The inhibitory effect of peracetic acid on common microflora of pistachio

To evaluate the restricting effect of peracetic acid on common microflora, 100 pistachio seeds were added to 1 liter flasks containing 500 ml of sterile distilled water and 0.2% peptone (as the source of nitrogen). The flasks were shaken (150 rpm) for 2 h at room temperature (Moradi *et al.*, 2014). The resulting suspension was exposed to concentrations of 0, 1, 2 and 2.5% peracetic acid for 0.5, 1, 1.5, 2 and 5 min. Then 100 µl of diluted (10^{-3}) suspension were cultured in petri dishes containing NA (Nutrient Agar, HiMedia™, India, 28 g l^{-1}) and MA (Malt Extract Agar, HiMedia™, India, 35 g l^{-1}) for the growth of fungi and bacteria, respectively. It is noteworthy that the suspension untreated with peracetic acid was cultured as the control in NA and MA media. The petri dishes were incubated at 28° C in the dark. After 48 h, the number of grown colonies was counted. The inhibitory effect of peracetic acid was calculated based on the number of colonies grown in each treatment compared to the control, according to the following formula:

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

The inhibitory effect of peracetic acid on the dominant microflora of pistachio

For the separation and identification of predominant fungi and bacteria, their morphological characteristics as the quantity and manner of growth on the culture medium, the color of surface and back of the colony, appearance and colony topography as well as how producing vegetative reproductive organs by using microscope and stereomicroscope, were evaluated. After identification, the dominant isolates were prepared from the Department of Technology and Production Management, PRC, and used in the next steps of the research. In order to investigate the effect of peracetic acid on the isolated and predominant microflora contaminating pistachio, concentrated suspensions of the most common microflora prepared in sterile distilled water, separately. Then, they were added to the flasks containing different concentrations (0, 1, 2 and 2.5%) of peracetic acid and 0.2% peptone. After 0.5, 1, 1.5, 2 and 5 min, 10^{-3} dilutions of the resulting suspension were cultured on NA and MA media. Then, they were incubated at 28°C in the dark. After 48 h, the number of microflora-grown colonies and the percentage og inhibitory effect of peracetic acid were evaluated as described above.

Lipid content

Pistachio seeds treated with the concentrations 0, 1, 2 and 2.5 % peracetic acid for different times (0.5, 1, 1.5, 2 and 5 min), were washed and dried. For pistachio oil extraction, the kernels were crushed carefully by a mortar and mixed with *n*-hexane. After 24 h, the resulting *n*-hexane extract was filtered and the solvent was evaporated by using rotary apparatus (model RV8, Germany) (Kaviani *et al.*, 2015). The content of lipid was evaluated with the following formula:

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

Peroxide value

To measure the peroxide value, 5 g of the extracted oil (described above) 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 added and the mixture was placed in the dark for 1 min. Then, 25 ml of distilled water and a few drops of 1% starch adhesive indicator were added. The resulting solution was titrated with 0.1 N sodium thiosulfate until the solution blue color disappeared (Pasquariello *et al.*, 2015). Peroxide number was calculated by the following formula:

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

Statistical analysis

This research was conducted as a factorial experiment in a completely randomized design with 3 replications for each treatment. Statistical analysis of data was performed using SPSS (version 16). Duncan's test (P<0.05) was applied to compare the means.

Results

Common microflora of pistachio

The effect of time periods of treatment with different concentrations of peracetic acid on the growth of common microflora (fungi and bacteria) contaminating pistachio seeds was shown in Fig. 2. As the results show pistachio treatment with 1% peracetic acid for 0.5 and 1 min decreased the population of contaminating fungi by more than 85% (Fig. 2a). This inhibitory effect on fungal population reached to more than 99% at the concentrations of 2 and 2.5% peracetic acid. At time periods of 1.5, 2 and 5 min, seed treatments with 1% or higher concentrations (2 and 2.5%) of peracetic acid, inhibited the population of contaminating fungi by about 90% or more than 99%, respectively (Fig. 2a).

Treatment with 1% peracetic acid for 0.5 min decreased pistachio bacterial contamination by more than 60% (Fig. 2b). By increasing the concentration of peracetic acid to 2% during this time period, the growth of contaminating bacteria would be inhibited to about 97%. There was no significant difference between concentrations of 2 and 2.5% peracetic acid. They reduced bacterial contamination by more than 99% (Fig. 2b). Increasing the time period of treatment with 1% peracetic acid, reduced the population of infectious bacteria, significantly (Fig. 2b). At higher concentrations (2 and 2.5%) of peracetic acid, there was not any significant difference between the time periods of treatment.

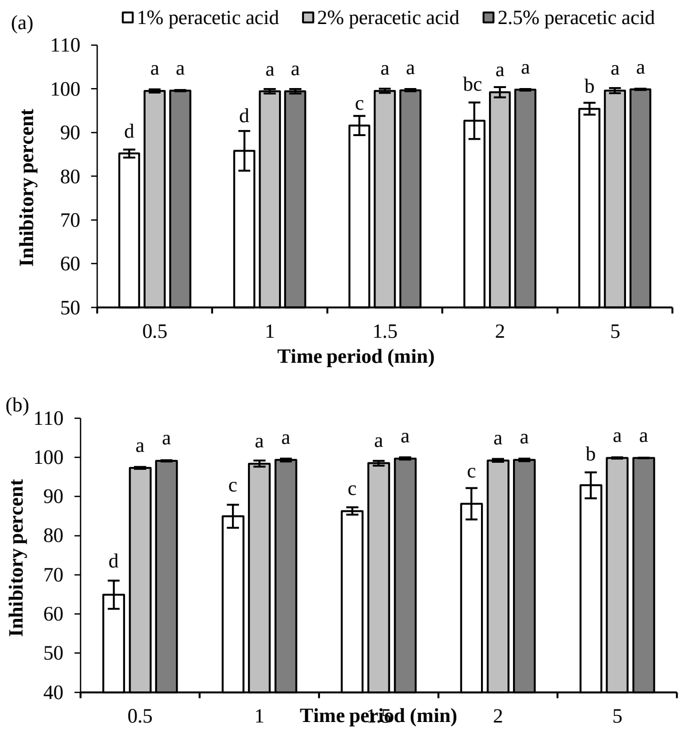

Fig. 2. The effect of different time periods and concentrations of 1, 2 and 2.5% peracetic acid treatment on the growth inhibitory percent of common fungi (a) and bacteria (b) contaminating pistachio seeds. The values were evaluated compared to the control (without peracetic acid) and the columns with common leters are not significantly different according to Duncan test (P<0.05).

Dominant microflora of pistachio

The inhibitory effects of different concentrations (1, 2 and 2.5%) of peracetic acid at time periods of 0.5, 1, 1.5, 2 and 5 min on the growth of some identified predominant fungus contaminating pistachio seeds, as *A. flavus*, *A. niger* and *Alternaria sp.* was indicated inFig. 3. As the results showed, treatment with the lowest concentration of peracetic acid (1%) for 0.5 min completely (100%) inhibited the growth of *A. flavus* (Fig. 3a). No significant difference was observed between different concentrations and time periods of treatment for this fungal species (Fig. 3a). Treatment of pistachio seeds with 1% peracetic acid for 0.5 min decreased the growth of *A. niger* by more than 99.9% (Fig. 3b). Increasing the concentration of peracetic acid to 2 and 2.5 in a period of 0.5 min caused a significant increase in the percentage of growth inhibition of *A. niger* (Fig. 3b). Growth inhibition of this fungus at the concentrations of 2 and 2.5% peracetic acid was significantly higher than the concentration of 1% in the treatment period of 1 min. There was not any significant difference between 2 and 2.5% (Fig. 3b). Such results were somewhat similar at periods of 1.5, 2 and 5 min. Sanitizing of pistachio seeds with 2 and 2.5% peracetic acid for 1.5, 2 and 5 min completely inhibited the growth of *A. niger* (Fig. 3b). As the results in Fig. 3c show, 1% peracetic acid for 0.5 min inhibited the growth of contaminating *Alternaria sp*. by 100% (Fig. 3c).

The effect of peracetic acid on the identified predominant bacteria as *B. pumilus*, *Actinobacter radioresistens* and *Pseudomonas fluorescens* contaminating pistachio seeds was shown in Fig. 4. As the results showed, the lowest treated concentration (1%) peracetic acid at time periods of 0.5 min caused completely inhibited growth of these contaminating bacterial species. There was not any significant difference between different concentrations of peracetic acid and different time periods of treatment (Fig. 4).

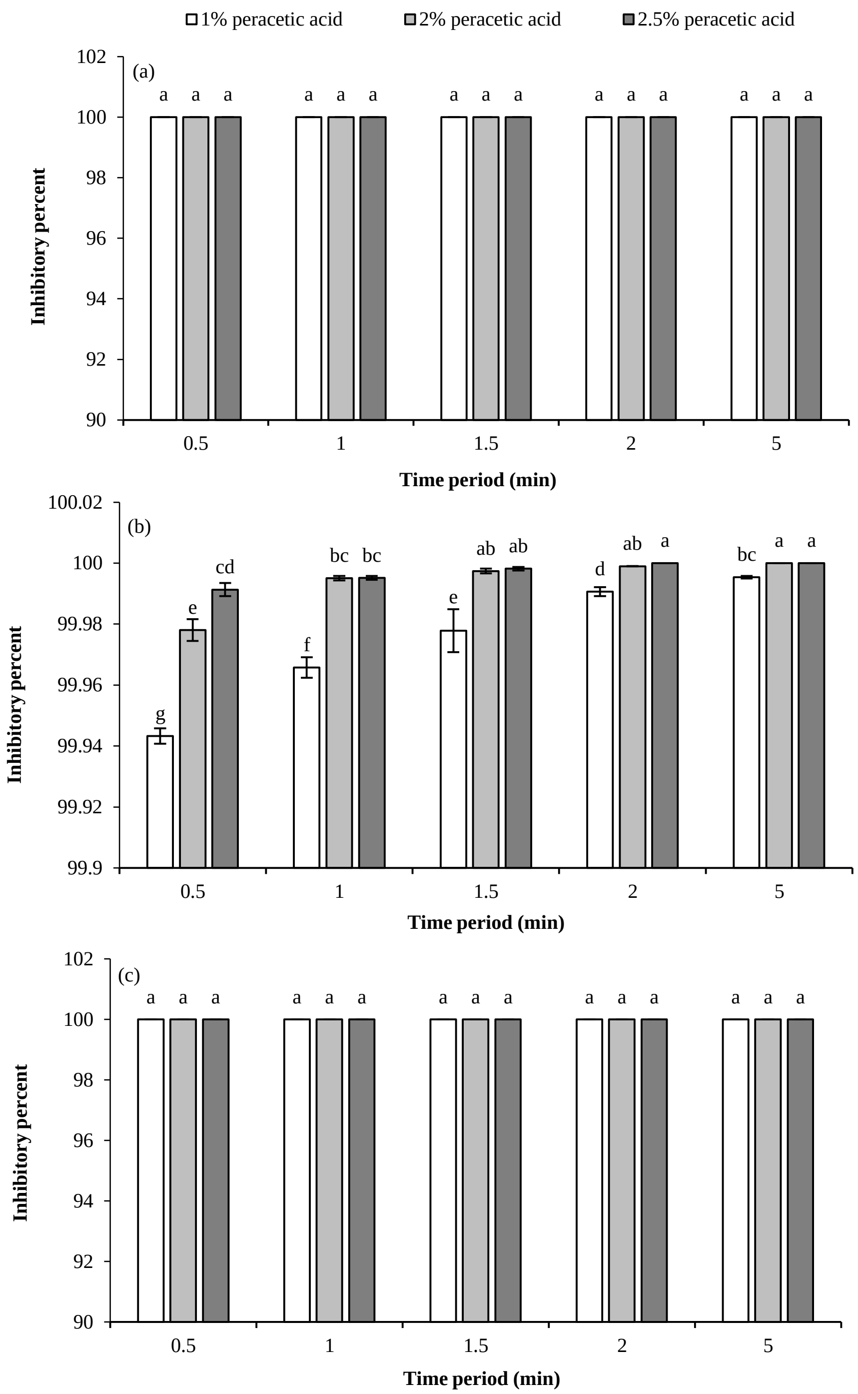

Fig. 3. The effect of different time periods (0.5, 1, 1.5, 2 and 5 min) and concentrations of 1, 2 and 2.5% peracetic acid treatment on the growth inhibitory percent of some identified predominant fungus as *Aspergillus flavus* (a), *A. niger* (b) and *Alternaria sp.* (c) contaminating pistachio seeds. The values were evaluated compared to the control (without peracetic acid) and the columns with common leters are not significantly different according to Duncan test (P<0.05).

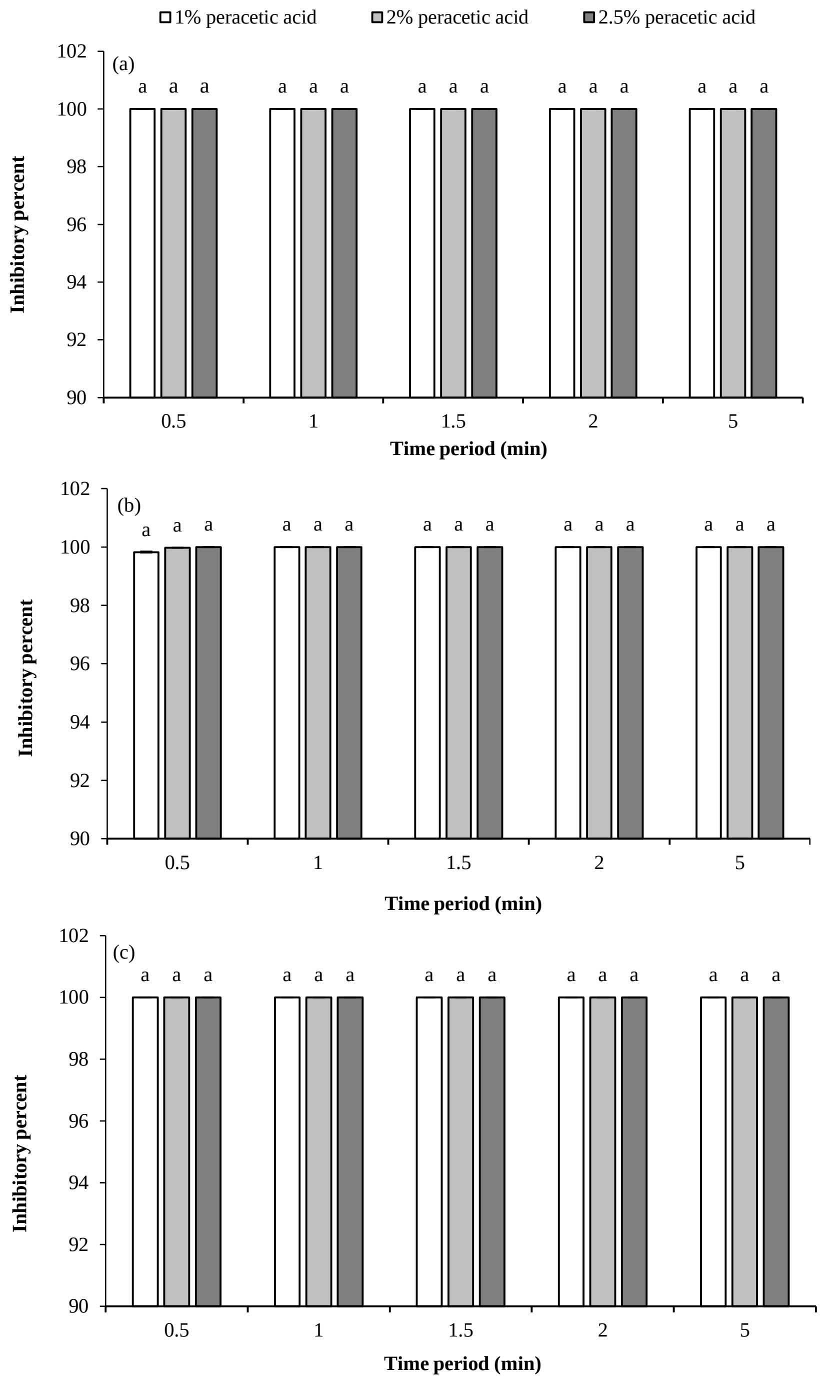

Fig. 4. The effect of different time periods (0.5, 1, 1.5, 2 and 5 min) and concentrations of 1, 2 and 2.5% peracetic acid treatment on the growth inhibitory percent of some identified predominant bacteria as *Bacillus pumilus* (a), *Acinetobacter radioresistens* (b) and *Alternaria sp.* (c) contaminating pistachio seeds. The values were evaluated compared to the control (without peracetic acid) and the columns with common leters are not significantly different according to Duncan test (P<0.05).

Lipid content

The effect of different concentrations (0, 1, 2 and 2.5%) of peracetic acid for time periods of 0.5, 1, 1.2, 2 and 5 min on the lipid content (%) of pistachio kernels was shown in Fig. 5. Lipid percent of pistachio kernels (about 35-40%) was not affected by peracetic acid sanitizing.

Peroxide value

The results related to the effect of peracetic acid (0, 1, 2 and 2.5%) treatment on the peroxide value of pistachio kernel was shown in Fig. 6. As can be seen, peroxide value decreased significantly by increasing the concentration of peracetic acid from 1 to 2% in every time periods. There was no significant difference in peroside value between 0 (control) and 1% and also between 2 and 2.5% peracetic acid.

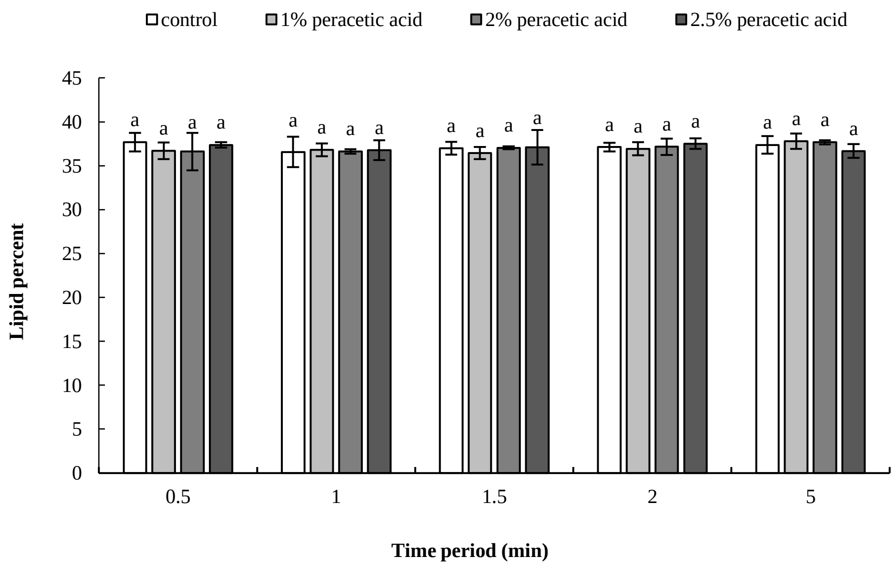

Fig. 5. The effect of different time periods (0.5, 1, 1.5, 2 and 5 min) and concentrations of 1, 2 and 2.5% peracetic acid treatment on the lipid content (%) of pistachio kernel. The columns with common leters are not significantly different according to Duncan test (P<0.05).

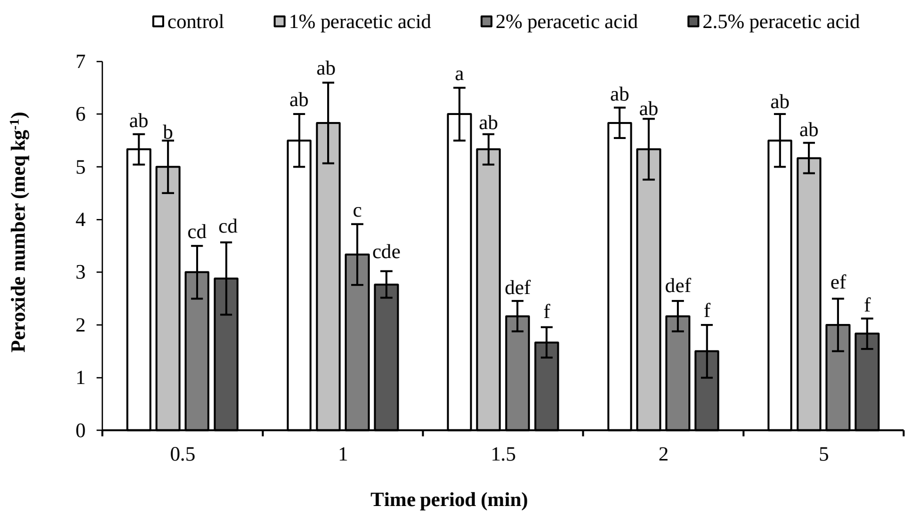

Fig. 6. The effect of different time periods (0.5, 1, 1.5, 2 and 5 min) and concentrations of 1, 2 and 2.5% peracetic acid treatment on the peroxide value (meq kg^{-1}) of pistachio kernel. The columns with common leters are not significantly different according to Duncan test (P<0.05).

Discussion

Extensive use of chemical compounds can have many adverse effects on the environment and human health. The results of current research showed that concentration of 2% peracetic acid could inhibit the growth of common microflora contaminating pistachio nuts up to 99%. However, when separate suspensions of predominant microflora were treated, 1% peracetic acid inhibited the growth of fungi and bacteria up to 99.9 and 100%, respectively.

In a study conducted by Ribeiro *et al.* (2020), the antiseptic effects of sodium hypochlorite and peracetic acid on Brazilian nuts were investigated. So that, they counted total bacteria and fungi as well as the *Aspergillus* section *flavi* to evaluate nuts microflora. Their results indicated that aflatoxin was not detected in any of treated samples, despite the presence of microbial contamination, moisture and high initial water activity. The count of total bacteria and fungi, and the measurement of aflatoxin showed that the optimal disinfection conditions were 250 mg l^{-1} and 8.5 min for sodium hypochlorite, and 140 mg l^{-1} and 15 min for peracetic acid. These sanitizers in the used concentrations did not have any action on aflatoxin, despite being efficient to control fungi. The treatments had also no unacceptable sensorial effect on the nuts.

Concerning decrease, the adverse effect of chemical pesticides, some researchers combined the biocide effects of chemical fungicides with the peracetic acid one (Alvaro *et al*., 2009). They used different concentrations of peracetic acid and commercial fungicides (SIGNUM and SWITCH) to inhibit the growth of mycelium and germination of *Botrytis cinerea* spores contaminating tomato. The concentrations of 16.77 and 14.47 µg ml^{-1} of SIGNUM and SWITCH, respectively and 1.5% peracetic acid completely (100%) inhibited fungal growth. When these chemical fungicides were combined to peracetic acid, their 100% effective concentration reduced to 0.5 µg ml^{-1}. Therefore, this approach minimized the consumption of fungicides by more than 95%.

The effect of peracetic acid on bacterial contamination was also investigated with previous researchers. Walter *et al.* (2009) studied the antiseptic effect of peracetic acid and sodium hypochlorite on coconut fruit contaminated with 5 bacteria strains of *Listeria monocytogenes*. In this regard, the fruits were soaked in 200 mg l^{-1} sodium hypochlorite and 80 mg l^{-1} peracetic acid solutions at room temperature for 2 min. The results indicated that disinfection of fruits with sodium hypochlorite and peracetic acid reduced bacterial population by 2.7 and 4.7 $\log_{10}$ CFU, respectively. The inhibitory effect of peracetic acid on bacteria may be achieved through delaying bacterial growth and destroying their cell wall (Wang *et al.*, 2020).

The current research also indicated that the treatment with peracetic acid was not affected the lipid content of pistachio nuts and on the other hand reduces its peroxide number. Decreasing the peroxide number can improve the shelf life of the product (Manzocco *et al.*, 2020). In this regard, we can refer to a research conducted by Park *et al.* (1991) which used a buffered solution of sodium hypochlorite, Bionox, to disinfect poultry meat. Their results showed that the peroxide value reduced by immersing whole poultry tight in sanitizing solution for 30 min. In another study, the disinfecting effect of chlorine dioxide on green walnut was evaluated and the peroxide value did not change during storage (Jiang *et al.*, 2015). Habibie *et al.* (2019) also proved positive effect of ascorbic acid incorporated with walnut green husk extract for preserving the postharvest quality of cold storage fresh walnut kernels.

In general, it is concluded that peracetic acid can eliminate a wide range of contaminating microorganisms more effectively than other sanitizers. Another factor that makes peracetic acid more important is the lack of production of harmful by-products. Our results indicated that the use of peracetic acid can be significantly (up to 99%) effective in reducing the microbial contamination of pistachios. Applying this sanitizing compound in pistachio processing terminals can be considered as a key factor to decrease pistachios contamination, which requires more field research.

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