Assessing the Efficacy of Percidine as an Eco-Friendly Disinfectant to Control Microbial Contaminations in Pistachio Nut

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Abstract

Background: One of the primary challenges in pistachio production, consumption, and export is contamination by various microorganisms at different stages. Pistachio kernels have a high lipid content, which makes them highly susceptible to fungal and bacterial spoilage, thus reducing their shelf life during storage. Percidine is a compound known for its strong oxidizing properties, enabling it to rapidly eliminate many microorganisms. It also holds environmental significance.

Materials and Methods: In this study, the microbial flora of contaminated pistachio was subjected to different concentrations (0, 1, 2, and 2.5%) of percidine for 0.5, 1, 1.5, 2, and 5 min.

Results: The results showed that a 1% concentration of percidine for 0.5 min can inhibit microbial growth by over 99%.

Conclusion: Reducing microbial contamination in pistachios is crucial for producing a safe product and boosting its economic viability.

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1. Introduction

Pistachio (*Pistacia vera* L.) due to its high nutritional value and dehisced shell, is one of the most popular nuts in the world. Iran is recognized as an important pistachio-producing country [1]. Microbial contamination is one of the most important factors affecting the quality of pistachios and limiting their exports. For example, various species of *Aspergillus* fungi such as *A. flavus* and *A. parasiticus* produce aflatoxin, the deadliest fungal toxin, which contaminates pistachios and other oil-rich nuts [2-5]. *Aspergillus* fungi have a strong tendency to grow on oil-rich nuts. The spores of these fungi are present in the air, soil, and agricultural environments [6] and can grow at temperatures between 10°C and 45°C, with the optimum temperature being between 25-28°C [1, 7]. Some countries have laws limiting the allowable levels of aflatoxin in food.

In addition to fungal contamination, pistachios can be a suitable source of pathogenic bacteria [8]. Contamination of pistachios with some bacteria such as *Escherichia coli*, *Bacillus cereus*, *Klebsiella*, *Salmonella*, etc. can cause various problems for humans [8-10]. Although low water activity in dried products can limit bacterial growth, it does not have much effect on their survival. Under favorable environmental conditions, the spores of bacteria can grow and cause food poisoning in consumers [11].

To ensure pistachio safety and prevent microbial contamination, several measures are implemented throughout production, including good agricultural practices during cultivation, proper handling and processing techniques, regular microbial testing, and strict hygiene standards.

There have been many methods tested to control microbial contamination, maintain quality, reduce waste, and increase the storage life of horticultural products. For example, sodium hypochlorite (NaOCl) is often used as a sanitizing agent in postharvest practices for fruits and vegetables [12]. NaOCl is cost-effective and easy to apply. The effectiveness of hypochlorite solutions is influenced by temperature changes, organic matter residues, and pH levels. Chlorine compounds react with organic substances, potentially leading to the formation of harmful by-products like chloramines and trihalomethanes, which are known carcinogens [13]. Consequently, there is a growing trend towards eliminating chlorine compounds from sanitizing processes [14]. Generally recognized as safe (GRAS) sanitizers such as organic acids (acetic acid, citric acid, lactic acid), hydrogen peroxide (H_2O_2), sodium carbonate (Na_2CO_3), and potassium carbonate (K_2CO_3) can serve as alternatives to chlorine compounds for controlling postharvest diseases in fresh horticultural products [15-17].

Percidine ($C_2H_4O_3$), also known as peroxyacetic acid or peracetic acid, is an antimicrobial substance obtained from the combination of hydrogen peroxide and acetic acid. This compound, in addition to having environmental advantages, can quickly affect a wide range of microorganisms [18-20]. Due to its antibacterial, antiviral, and antifungal properties, percidine has attracted a lot of attention in recent years and is widely used for disinfecting fruits and vegetables, cheese and dairy processing plants, various drinks, and food processing equipment [13, 21-24]. Another reason that makes percidine more important than other disinfectants such as chlorine dioxide is that it does not produce harmful byproducts. Percidine decomposes upon contact with organic compounds to oxygen and acetic acid, ultimately producing water and carbon dioxide [25].

Percidine is also more potent than hydrogen peroxide, is unaffected by catalase and peroxidase, the enzymes that break down hydrogen peroxide [26], and retains its activity in the presence of organic loads or food residue when kept at acidic pH [22]. It has been utilized by the food industry for many years because percidine activity is consistent across several critical factors such as temperature (from 0 to 40°C) and pH (from 1 to 8) and does not require the use in high concentrations.

In this study, the ability of percidine to reduce the population of contaminating microorganisms in pistachios and improve their quality is evaluated.

2. Materials and Methods

2.1. Product preparation

In this study, samples of *P. vera* cv. Fandoghi were collected from 4 different directions of trees in a commercial garden in the Rafsanjan region in October 2019. After peeling and drying, pistachios were used for various next stages of the experiment. The measured parameters included the percentage of inhibition of fungal (mold and yeast) and bacterial growth on pistachio.

2.2. Cultivation and identification of Microbial population contaminating pistachios

Three sets of one hundred dried pistachios were placed in one-liter flasks. Then, 500 ml of sterile distilled water containing a concentration of 2 g l^{-1} peptone (as a source of nitrogen) was added to each flask. The flasks were shaken for 2 hours at 150 rpm on a shaker at room temperature [27]. Suspensions with densities of 10^{-3} were prepared and 100 µl were cultured on petri dishes with a diameter of 8 cm containing Malt Extract Agar (MA, HiMedia™, India, 35 g l^{-1}) and Nutrient Agar (NA, HiMedia™, India, 28 g l^{-1}) media for fungal (mold and yeast) and bacterial growth, respectively. The petri dishes were incubated in the dark at 28 °C for 48 h. A stereomicroscope and microscope were used to differentiate fungi and bacteria based on their morphological characteristics. The dominant isolates were identified and collected from the collection of the Technology and Production Management Department at the Pistachio Research Center (PRC) and used in subsequent stages of the research.

2.3. Inhibitory percent of microbial growth by percidine

Initially, 15% percidine was prepared from Barafza Keshavarz Pars Company. To investigate the effect of percidine on microbial populations, a suspension of the most common microbial flora identified in sterile distilled water was prepared, and different concentrations of percidine (0, 1, 2, and 2.5%) were added separately to the suspensions. After 0.5, 1, 1.5, 2, and 5 min, suspensions were cultured on MA and NA media. These concentrations and treatment durations were selected with pre-treatment being carried out.

The cultured petri dishes were incubated in the dark at 28 °C for 48 h. The inhibitory percent of microbial growth was calculated based on the number of colonies grown on each treatment compared to the control (without percidine treatment), as following formula:

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

2.4. Statistical analysis of results:

This research was carried out using a completely randomized factorial design with a minimum of three replicates for each treatment. The treatments consisted of four different concentrations of percidine (0%, 1%, 2%, and 2.5%) and five different time points (0.5, 1, 1.5, 2, and 5 minutes). Statistical analysis was conducted using SPSS software, and mean values were compared using the Duncan multiple range test at a significance level of 5%.

3. Results

3.1. Inhibition effect of percidine on pistachio contaminating fungi (mold and yeast)

The inhibition percent of different concentrations of percidine (1, 2, and 2.5%) over various time intervals (0.5, 1, 1.5, 2, and 5 min) on the growth of toxigenic (strain 4) and non-toxigenic (strain 17) strains of *A. flavus* are shown in Fig. 1.

As the results show, treatment with the lowest concentration of percidine (1%) for 0.5 minutes completely inhibited the growth of this toxigenic strain (Fig. 1a). No significant difference was observed between the different concentrations and treatment times in this stage (Fig. 1a). Similar results were obtained for the non-toxigenic strain of *A. flavus* (Fig. 1b).

The effect of different concentrations and treatment times of percidine on the inhibition percent of the growth of *Pichia kudriavzevii* (strain yp3-5), *Saccharomyces* sp. (strain 224), and *Lachancea thermotolerance* (strain 8-47) yeast species is shown in Fig. 2. As the results show, in all cases, percidine treatment inhibited the growth of these species by more than 99%.

3.2. Inhibition effect of percidine on pistachio contaminating bacteria

The effect of percidine concentration and treatment time on the inhibition percent of the growth of *Bacillus subtilis* (strain B5VRV) and *Pseudomonas fluorescens* (strain 58-8) bacteria is shown in Fig. 3. As the results show, the lowest concentration of percidine (1%) for the shortest treatment time (0.5 minutes) completely inhibited the growth of these bacteria. Increasing the concentration and treatment time of percidine did not have a significant effect on the results.

4. Discussion

Microbial contamination is a significant factor that impacts the quality of pistachios and hinders their exportation. Fungal toxins, also known as mycotoxins, pose a major challenge to food safety. Reports from the Food and Agriculture Organization (FAO) indicate that approximately 25% of food products are contaminated by mycotoxins annually [1]. This contamination not only has adverse effects on consumer health but also results in substantial economic losses across various levels of the food supply chain [28]. In certain countries, including those in Europe, regulations have been implemented to limit the permissible levels of fungal toxins such as aflatoxin (10 ppb) and ochratoxin A (5 ppb) in food products [29-32]. A study conducted in 2010 revealed that the removal of aflatoxin alone from Iranian pistachios leads to a 26% increase in the price of pistachios at the producer level [33].

The extensive use of chemical disinfectants can have many adverse effects on human health and the environment. The findings of the current study suggest that treating pistachio contaminants with an eco-friendly disinfectant called percidine at a 1% concentration can inhibit fungal growth (mold and yeast) by up to 99.9% and completely prevent bacterial growth.

The study by Ribiero et al. [34] focused on the antimicrobial effect of percidine on nuts. Despite the presence of microbial contamination and high initial moisture, no aflatoxin was found in any of the treated samples. The results showed that optimal disinfection conditions involve using 250 mg l^{-1} of sodium hypochlorite for 5.8 minutes, and 140 mg l^{-1} of percidine for 15 minutes. The study confirmed that both sodium hypochlorite and percidine, at the concentrations used, do not impact aflatoxin levels, but instead help control fungal populations.

In a different study, Ayoub et al. [35] explored the impact of percidine fungicide, SWITCH, and SIGNUM on the fungus *Botrytis cinerea*, which is a major threat to tomato plants in Morocco. Various concentrations of percidine and commercial fungicides were tested to inhibit the growth of B. cinerea mycelium and spore germination. The results indicated that concentrations of 16.77 and 14.47 µg ml-1 of SWITCH and SIGNUM and 1.5% percidine completely halted the growth of the fungus. However, when these fungicides were combined with 5% percidine, their effectiveness was reduced to 5.0 µg ml-1, achieving a reduction in fungicide use of over 95%.

Wang et al. [36] conducted a study on the antimicrobial properties of percidine and its mechanism of action against *Morganella psychrotolerant* bacteria. They found that when the bacteria were exposed to 20 ppm of percidine for 5 minutes, their population decreased to an undetectable level. Additionally, percidine delayed the growth of the bacteria. The results indicated that percidine destroys the bacterial cell wall.

Hasani et al. [37] compared two methods for disinfecting de-shelled pistachios to eliminate pathogenic bacteria *Salmonella* and *Listeria*. One method involved using a disinfectant combination of peracetic acid and ethanol, while the other method utilized an Advanced Oxidation Process (AOP) using UV-C, ozone, and hydrogen peroxide. The study found that using a 2.5% concentration of peracetic acid and ethanol was more effective than the AOP method in inactivating *Salmonella* in pistachios. However, *Listeria* was more susceptible to hydrogen peroxide and the AOP method. The research concluded that both disinfection methods can reduce contamination of pistachios with Salmonella and Listeria, but their effectiveness depends on the specific pathogen and product.

The use of peracetic acid significantly reduces the risk of pistachio contamination by fungi, bacteria, and microbial flora. This makes it a crucial factor in reducing the microbial population of pistachios in processing terminals, especially in washing basins. As a result, the likelihood of Iranian exported pistachio samples not meeting global health standards, particularly those of the European Union, is greatly reduced, potentially even reaching zero. Consequently, this decrease in rejected Iranian pistachio samples from global markets may lead to an increase in the price of pistachios at the Iranian producer level.

5. Conclusion

Microbial contamination is among the most important factors that affect the quality of pistachios and limit their export. The results of this study showed that the use of percidine can significantly (up to over 99%) reduce the contamination of pistachios with fungi, yeast, and bacteria. Given the importance of pistachio health in exports and its economic value, the use of peracetic acid as an eco-friendly disinfectant can be important.

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Conflict of Interest

All authors declare that they have no conflict of interest.

References

1- FAO. 2018 [Available from: http://www.fao.org/faostat/en/#rankings/countries_by_commodity.

2- Bennett El-Sayed RA, Jebur AB, Kang W, El-Demerdash FM. An overview on the major mycotoxins in food products: characteristics, toxixity, and analysis. J Future Foods. **2022**;2:*91-102.*

3- Shephard Kluczkovski AM. Fungal and mycotoxin problems in the nut industry. Curr Opin Food Sci. **2019**;29:*56-63.*

4- Elsanhoty RM, Salam SA, Ramadan MF, Badr FH. Detoxification of aflatoxin M1 in yogurt using probiotics and lactic acid bacteria. Food Control. **2014**;43:*129-34.*

5- Rastegar H, Shoeibi S, Yazdanpanah H, Amirahmadi M, Khaneghah AM, Campagnollo FB, Sant'Ana AS. Removal of aflatoxin B1 by roasting with lemon juice and/or citric acid in contaminated pistachio nuts. Food Control. **2017**;71:*279-84.*

6- Campbell BC, Molyneux RJ, Schatzki TF. Current research on reducing pre- and post-harvest aflatoxin contamination of US almond, pistachio, and walnut. J Toxicol: Toxin Rev. **2003**;22:*225-66.*

7- Giorni P, Leggieri MC, Magan N, Battilani P. Comparison of temerature and moisture requirements for sporulation of Aspergillus flavus sclerotia on natural and artificial substrates. Fungal Biology. **2012**;116:*637-42.*

8- Centers for Disease Control and Prevention CDC. Salmonella in pistachio nuts 2009 [Available from: https://www.cdc.gov/salmonella/pistachios/update.html.

9- Al-Moghazy M, Boveri S, Pulvirenti A. Microbiological safety in pistachios and pistachio containing products. Food Control. **2014**;36:*88-93.*

10- Akbas Haendiges J, Davidson GR, Pettengill JB, Reed E, Ramachandran P, Blessington T, Miller JD, anderson N, Myoda S, Brown EW, Zheng J, Tikekar R, Haffmann M. Genomic evidence of environmental and resident Salmonella Senftenberg and Montevideo contamination in the pistachio supply-chain. PLOS ONE. **2021**;16:*1-22.*

11- Kinsella K, Prendergast D, McCann M, Blair I, McDowell D, Sheridan J. The survival of Salmonella enterica serovar Typhimurium DT104 and total viable counts on beef surfaces at different relative humidities and temperatures. J Appl Microbiol. **2009**;106:*171-80.*

12- Sheikhi A, Mirdehghan SH, Arab MM, Eftekhari M, Ahmadi H, Jamshidi S, Gheysarbigi S. Novel organic-based postharvest sanitizer formulation using Box Behnken design and mathematical modeling approach: A case study of fresh pistachio storage under modified atmosphere packaging. Postharvest Biol Technol. **2020**;160:*111047.*

13- Zoellner C, Aguayo-Acosta A, Siddiqui MW, Dávila-Aviña J. Peracetic acid in disinfection of fruits and vegetables. Postharvest disinfection of fruits and vegetables. **2018**;53-66.

14- Pablos C, Romero A, de Diego A, Vargas C, Bascón I, Pérez-Rodríguez F, Marugán J. Novel antimicrobial agents as an alternative to chlorine with potential applications in the fruit and vegetable processing industry. Int J Food Microbiol. **2018**;285:*92-7*.

15- Feliziani E, Lichter A, Smilanick JL, Ippolito A. Disinfecting agents for controlling fruit and vegetable diseases after harvest. Postharvest Biol Technol. **2016**;122:*53-69*.

16- Romanazzi G, Lichter A, Gabler FM, Smilanick JL. Recent advances in the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes. Postharvest Biol Technol. **2012**;63:*141-7*.

17- Wisniewski M, Droby S, Norelli J, Liu J, Schena L. Alternative management technologies for postharvest disease control: The journey from simplicity to complexity. Postharvest Biol Technol. **2016**;122:*3-10*.

18- Julio FR, Hilario TP, Mabel VM, Raymundo LC, Arturo LR, Neftali RVM. Disinfection of an advanced primary effluent using peracetic acid or ultraviolet radiation for its reuse in public services. J Water Health. **2015**;13:*118-24*.

19- Pietrysiak E, Kummer JM, Hanrahan I, Ganjyal GM. Efficacy of surfactant combined with peracetic acid in removing Listeria innocua from fresh apples. J Food Prot. **2019**;82:*1965-72*.

20- Suurnäkki S, Pulkkinen JT, Lindholm-Lehto PC, Tiirola M, Aalto SL. The effect of peracetic acid on microbial community, water quality, nitrification, and rainbow trout (Oncorhynchus mykiss) performance in recirculating aquaculture systems. Aquaculture. **2020**;516:*734534*.

21- Kitis Souza Santos D, Cesar Teixeira LA, Volschan Jr I. Fast disinfection of contaminated stormwater using peracetic acid. J Urban Environ Eng. **2021**;15:*42*.

22- Joshi K, Mahendran R, Alagusundaram K, Norton T, Tiwari B. Novel disinfectants for fresh produce. Trends in Food Science & Technology. **2013**;34:*54-61*.

23- Thomas P, Bruns A, Ganzer A, Jünemann T, Kordex L, Krone N, Metz H-N, Sandmann I, Schorling D, Schröder A. Peracetic acid and atmospheric plasma as alternatives for packaging disinfection in the dairy industry. Milchwissenschaft. **2016**;69:*2-6*.

24- Garg A, Namboodiri V, Smith B, Al-Anazi A, Murugesan B, Bowman T, editors. Disinfection of wastewater with peracetic acid (PAA) and UV combined treatment: a pilot study. Disinfection and Reuse Symposium. **2013**;2018:*76-89*.

25- Dagher D, Ungar K, Robison R, Dagher F. The wide spectrum high biocidal potency of bioxy formulation when dissolved in water at different concentrations. PLOS One. **2017**;12:*e0172224*.

26- Gad S. Cadmium GS. Encyclopedia of Toxicology: Academic Press, San Diego; 2014.

27- Moradi M, Fani SR, Masoumi H. Population density of Aspergillus species belong to section Flavi and Nigri on pistachio nut in Kerman Province. J Appl Res Plant Prot. **2014**;3:*79-91*.

28- Soares Mateus AR, Barros S, Pena A, Sanches Silva A. Mycotoxins in pistachios (Pistacia vera L.): Methods for determination, occurrence, decontamination. Toxins. **2021**;13:682.

29- Wu F. A tale of two commodities: how EU mycotoxin regulations have affected US tree nut industries. World Mycotoxin J. 2008;1:95-102.

30- Bui-Klimke TR, Guclu H, Kensler TW, Yuan J-M, Wu F. Aflatoxin regulations and global pistachio trade: insights from social network analysis. PLoS One. **2014**;9:*e92149*.

31- European Food Safety Authority EFSA. The outcome of a public consultation on the draft risk assessment of aflatoxins in food. 2020;17(3):1798E.

32- European Tabarani A, Zinedine A, Rocha JM, Sanaa M, Abdennebi EH. Comparative study of ochratoxin A exposure through the intake of cereal products in two climatic Moroccan regions. Toxins. 2023;15:452.

33- Abdolahi Ezat Abadi M. An investigation of the role of aflatoxin in the instability of pistachio market and measuring WTP for reducing contamination. Iran J Agric Econ Dev Res. **2010**;2:*159-69*.

34- Ribeiro MS, Freitas-Silva O, Castro IM, Teixeira A, Marques-da-Silva SH, Sales-Moraes AC, Abreu LF, Sousa CL. Efficacy of sodium hypochlorite and peracetic acid against Aspergillus nomius in Brazil nuts. Food Microbiol. **2020**;90:*103449*.

35- Ayoub F, Chebli B, Ayoub M, Hafidi A, Salghi R, Jodeh S. Antifungal effectiveness of fungicide and peroxyacetic acid mixture on the growth of Botrytis cinerea. Microb Pathog. **2017**;105:*74-80.*

36- Wang D, Yamaki S, Kawai Y, Yamazaki K. Sanitizing efficacy and antimicrobial mechanism of peracetic acid against histamine-producing bacterium, Morganella psychrotolerans. LWT. **2020**;126:*109263.*

37- Hasani M, Wu F, Hu K, Farber J, Warriner K. Inactivation of Salmonella and Listeria monocytogenes on dried fruit, pistachio nuts, cornflakes, and chocolate crumb using a peracetic acid-ethanol based sanitizer or Advanced Oxidation Process. Int J Food Microbiol. **2020**;333:*108789.*