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Sanitization system in horticultural sector

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Abstract: The food industry has recognized the importance of environmental sanitation, and fruit control, a renowned leader in controlled atmosphere, has invested in sanitation through the use of ionizers to eliminate microorganisms in agri-food environments. This report presents results of tests conducted on various products, both in experimental and real scenarios in fruit and vegetable distribution platforms, to evaluate the effectiveness of ionization on vegetable products. The report covers three different situations: the first two focused on the effects of ionization on radish and table grape cells, while the last test verified the impact of ionization on a distribution platform that processes and markets various types of fruits and vegetables, such as pepper, tropical fruits, blueberry, apples, pears, table grape, chicory, and more.

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The authors declare no competing interests.

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1. Introduction

The preservation of fruit and vegetables has always gone hand in hand with the need to ensure the healthiness of the stored products. In fact, it is necessary that the products are stored in the best conditions, from the organoleptic but also from the health point of view (Gnanasekharan *et al.*, 1992; Karabulut *et al.*, 2004; Del-Valle *et al.*, 2005). Lately this aspect has become increasingly important, especially for greater protection for consumers using physical means such as irradiation (γradiation), ultraviolet-C light, sub-atmospheric pressure, high hydrostatic pressure and ionized gases (gas plasma) (Romanazzi *et al.*, 2001; Moreau *et al.*, 2008; Tappi *et al.*, 2014; Papoutsis *et al.*, 2019).

Ionization consists in the generation of one or more ions due to the removal or addition of electrons from a neutral molecular entity which can be caused by collisions between particles or by absorption of radiation (Lin and Lin, 2017; Baggio *et al.*, 2020; Tanaka, 2022) The atoms or molecules that have a number of electrons lower than the atomic number remain positively charged and are called “cations”; those that have a number of electrons greater than the atomic number, remain negatively charged and are called “anions” (Forney *et al.*, 2001; Fan *et al.*, 2002; Lin and Lin, 2017; Tanaka, 2022).

Many tests and researches have been done in collaboration with research institutes and universities on many fruit and vegetables (i.e. mango, melon, lettuce, apple, kiwifruit) taking into consideration multiple parameters (acidity, hardness, color, etc.) (Perni *et al*., 2008; Tamaki and Uyama, 2008; Bernardinelli *et al*., 2012; Tappi *et al*., 2014; Ramazzina *et al*., 2015; Tappi *et al*., 2016, Woo *et al*., 2017).
This report is a demonstration of some of the tests done in last years, especially at industrial level, and they take in consideration the results relating to the phytosanitary aspects, the subject of this symposium. The tests were conducted with the equipment of our production called “IONNY”. IONNY is produced in different versions and sizes depending on the volume of the cells in which it is installed. There are also cabinet versions intended for research institutes.

The tests carried out and reported are essentially aimed at verifying the elimination of micro-organisms during the conservation and processing of fruit and vegetables, with the aim of verifying whether ionization can actually be considered a valid system for preventing rotting and sanitizing such products. The machines used were sized according to the volumes of the cells and the environments in which they were installed.

2. Materials and Methods

Plants material and experimental setup

Three separate tests were conducted:

First test done in cold room containing radish (*Cicorium intybus*) using a Ionny 400;

Second test done in cold room containing table grape (*Vitis vinifera,* cv. Italia) with Ionny 400;

Third test done in a cold warehouse (CE.DI.OR, Zelo Buon Persico, Lodi, Italy). Inside this cold warehouse three different areas were tested (cold rooms, processing, and shipping rooms) using ionizers mod DUCT as compared to control (without ionizer).

Radish test

The test was carried out at GEOFUR, Gallese Verona, Italy, in a storage warehouse where radishes were stored in a refrigerated cell with a volume of 400 m^3. The ionization process was performed using an IONNY 400 and the storage room was maintained at a temperature of -1°C and a relative humidity of 95%. The radishes were stored in bins measuring 110 cm x 11 cm x 56 cm. The IONNY 400 ionizer was capable of covering a volume of up to 400 m^3, making it suitable for the test.

Table grape test

To ionize table grapes cv Italia that were stored in a refrigerated cell measuring 400 m^3, an IONNY 400 (mod. Industrial, NOACOOP - Noicattaro BA - Italy) was used. The ionizer effectively covered the entire volume of the cell. The grapes were harvested from the same orchard and stored in wooden boxes weighing 15 kg each. The storage warehouse had a volume of 300 m^3 and was maintained at a temperature of 4°C and a relative humidity of 95%.

Warehouse platform test

The experiment was carried out in a storage warehouse located in Zelo Buon Persico LO, Italy, called CE.DI.OR. The warehouse stored and processed various types of fruits and vegetables under real conditions. To ensure the ionizator machines had the appropriate capacity for each area, one or more units (model DUCT) were installed in each room without any construction work needed. The machines were fixed in the upper part of the rooms, near the evaporator positioned in the suction area. The products were stored in different types of packaging, such as bins, wooden boxes, and cardboard boxes of varying sizes. The storage temperature ranged between 0-10°C. Specifically, during the experiment, the temperature was between 1-4°C in the cold storage and 10-15°C in the processing and shipping areas of the logistic structure CE.DI.OR. The humidity level was between 85-90% in the cold rooms and 75-89% in the processing and shipping areas. Additionally, during the experiment, the amount of ozone generated (tested only for this experiment with a manual analyzer) did not exceed 0.06 ppm inside the room, even after 8 hours of operation.

Petri dishes containing Tryptic Soy Contact Agar + LT + ICR were positioned in the storage room and other areas at ground level, specifically in the corner of the cold room store (Fig. 1): cold room (which was totally full) for test 1 and 2; cold room, processing area, and shipping room for test 3. These Petri dishes were placed at a height of approximately 70 cm. The air sampling procedure and incubation conditions of the plates were carried out as described below.

Microbial analysis during storage

For each of the tests described above, Petri dishes (plates) (90 mm diameter) containing Tryptic soy contact agar + LT + ICR (Merck, Darmstadt, Germany)

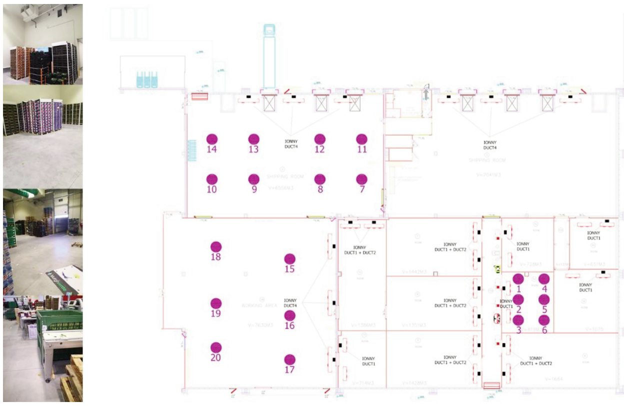

Fig. 1 - Cedior logistic platform, position of Petri dishes (violet) and model of IONNY used.

were positioned by hand (with the use of gloves in order to ensure absolute non-contamination by the operator) in the area of interest according to the test performed in each case investigated. Air samples were taken before the use and after the operation of the ionizers used in each case. During air sampling, plates remained open for 1 min and then closed again. Incubation of the plates took place at 18-22°C for 7 days and the colonies developed in each Petri dish were counted, after dividing them into fungi (black-grey), bacteria (yellow) and yeasts (white). For the determination of total yeast and mold counts, plates were incubated at 20-25°C for 5-7 days (for all the test done). Results were calculated as colony forming units (CFU) per m^3 using the following equation proposed by Omelyansky (1940), $N(CFU\ m^{-3})=5a \times 10^4 (bt)^{-1}$, where: a is the number of colonies counted per Petri dish, b the Petri dish area (in cm^2), and t the exposure time (in min) and further expressed as CFU $m^{-3} \times 10^3$ (Viani *et al.*, 2020).

Ion cluster generator and its principle

When a voltage is applied to electrode plate with the air between them, if the applied voltage exceed a certain level, the air layer is ionized to flow the current between electrode plates, creating, so-called, an arc discharge. If electrode plates are separated by 1 cm, when at 30.000 volts is applied, a discharge occurs (in the air, if the intensity of electric field is more than 30 kv/cm, a discharge occurs). At this time, ions are created, they are bonded with surrounding water molecules to produce, so-called, ions. There is no other fluid used except air. Ventilation is due to evaporator ventilation (ducted model) or internal fan (in case of industrial model).

3. Results and Discussion

The effects of IONNY operation in a cold room for radish storage and in a general purpose fruit and vegetable distribution platform (industrial test) are presented in figure 2. Not using IONNY in a cold room containing radishes, bacterial counts ranged between 7.07 and 20.43 CFU $m^{-3} \times 10^3$, while fungi populations reached up to 2.36 CFU $m^{-3} \times 10^3$. The use of IONNY resulted in a dramatic decrease of both bacteria and fungi, with numbers found at levels of 0.79 CFU $m^{-3} \times 10^3$ for both. Overall, the reduction of air microorganisms (fungi and bacteria) inside the cold rooms containing radishes with the use of IONNY is clearly indicated in figure 2. This microbial reduction might be attributed to the formation of hydroxyl radical ($OH^{\cdot}$) during air ionization. This radical can compromise the function of the microbial membranes due to oxidation of the unsaturated fatty acids in the membranes (Zhang *et al.*, 2019). In addition, the different structure of the bacterial and fungal cell wall could also influence the susceptibility to ionization, as it has been reported that chitin the main component of

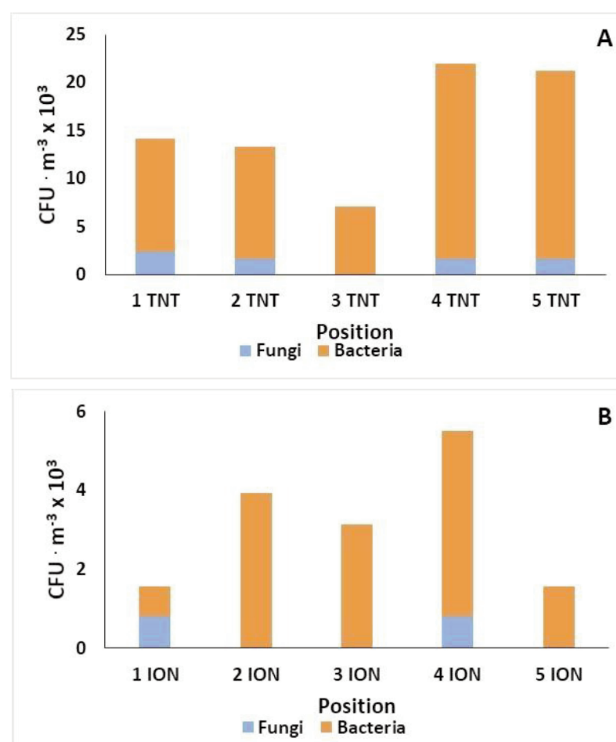

Fig. 2 - Microorganism inside a cold room during storage of radish without (A) and with (B) IONNY use after 48h from switching on of ionizator. TNT: not treated commodity; ION: IONNY use; 1-5: positions inside the cold room where the Petri dishes were placed.

fungal cell wall is a more rigid material compared to peptidoglycan (Liang *et al*., 2012).

A diversity among bacteria, fungi and yeasts populations was observed in different areas of a cold storage in a distribution platform (industrial test) when the IONNY was not used (for 48 h) (Fig. 3A). However, the use of IONNY for 48 h resulted in significant decrease of the tested microorganisms. In this case, the total effectiveness of IONNY was equal to approx. 70% and the effect of this reduction was found similar to all the investigated microorganisms (fungi, bacteria and yeasts). These findings are not in accordance with a previous study were cold atmospheric plasma treatment (created by dielectric barrier discharge-DBD) on ginseng seed surface presented great bactericidal and fungicidal activity, with fungi found to be more susceptible than bacteria (Lee *et al*., 2021). This difference might be attributed to the time/duration of application, the type of ionizer used and the microorganisms exposed; among other factors (Arnold *et al*., 2004; Tappi *et al*., 2014; Zhang *et al*., 2019).

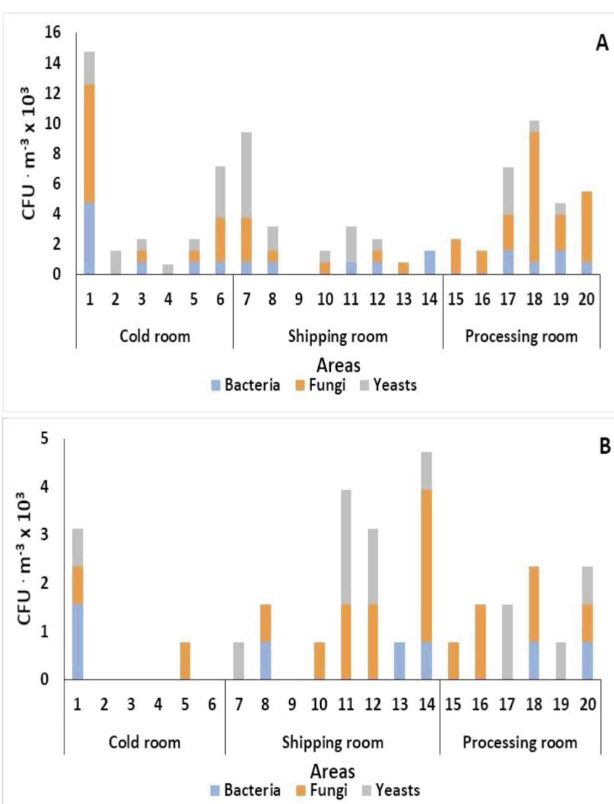

Fig. 3 - Bacteria, fungus and yeasts inside a cold room without (A) and with (B) IONNY use for a period of 48 h inside different areas of a commercial distribution warehouse platform. 1-20: positions inside the warehouse where the Petri dishes were placed.

The effectiveness of IONNY on the different areas examined (cold room, processing room and shipping room) was more or less similar. Moreover, fungi were absent and/or in low populations after the treatment with IONNY (except in some cases) (Fig. 3B). These findings suggest that the use of IONNY can lower the risk of fresh fruit and vegetables contamination in an industrial level. A previous study showed a reduction of airborne and surface *Salmonella* Enteritidis artificially (generated aerosol) ranging from 72 up to 98% when exposed to negative air ions (Seo *et al*., 2001). The differences between the microorganisms examined inside the three investigated areas in the distribution warehouse especially prior the use of IONNY might be attributed mainly to the different actions that take place there. For instance, more movement and increased air exchange might be observed more frequently in the processing and shipping room compared to the cold room. Also, the different temperatures of the areas might also affect the air's microbial load i.e. cold room temperature: 1-4°C, whereas processing and shipping room temperature: 10-15°C.

The use of IONNY during cold storage of table grapes for 21 days, showed a decrease in molds populations inside the room after 10 and 21 days, compared to non-treated room (Fig. 4A). This phenomenon was more evident on the 21st day of storage. When high voltage atmospheric cold plasma was applied against *Aspergillus flavus* spores, inactivation of spore forms was observed as well as degradation of fungal culture and its mycotoxin (deoxynivalenol) (Ott *et al*., 2021). Moreover, in the present work yeasts population was found to decrease in a room used for cold storage of table grapes when IONNY was applied, 10 and 21 day after application (Fig. 4B). The numbers of yeasts were decreased during the 21 days of storage for both treated and un-treated with IONNY samples, with greater decrease been seen on the 21st day with the IONNY use (1.02 and 1.57 CFU m^{-3} x 10^3, respectively).

Kikuchi *et al*. (2020) showed that dielectric barrier dischargers presented great antifungal activity against an airborne fungi (*Penicillium italicum*) (up to 2.5 log decrease), whilst spores adhered to the ionizers were not affected. The antimicrobial effects of ionization on molds and yeasts might be attributed to the ionization of hydroxyl groups, atomic oxygen and nitrogen and the subsequent production of reactive oxygen and nitrogen species (ROS and RNS) i.e. ozone (O_3), nitrogen dioxide (NO_2), nitrate (NO_3), dini-

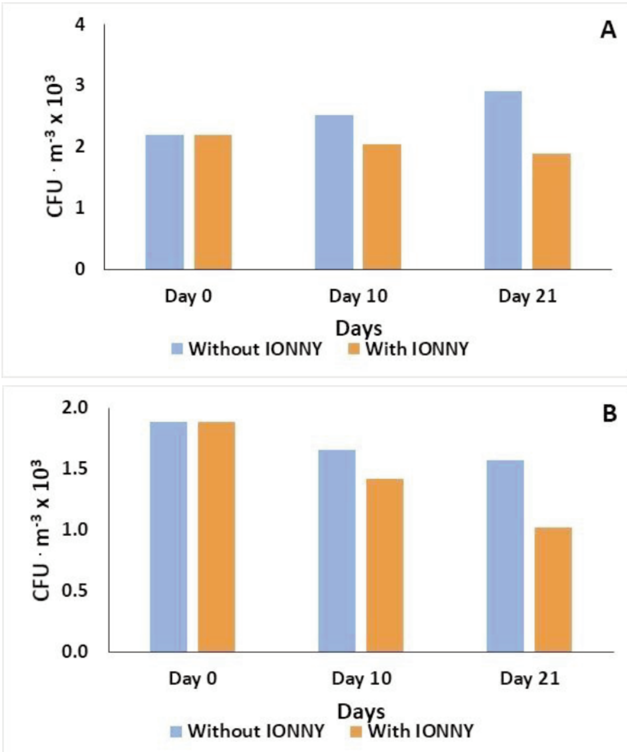

Fig. 4 - Populations of molds (A) and yeasts (B) inside a table grapes room without and with IONNY use for a period of 21 days.

trogen tetroxide (N_2O_4), and dinitrogen pentoxide(N_2O_5) (Ott *et al*., 2021). These oxidizing forms have been previously mentioned to interfere and decrease *Cordyceps pruinosa* spore viability, by damaging the fungal cell wall (deformation and deterioration), increasing permeability and cell components leakage (Kim *et al*., 2016). In table 1, different IONNY sanitization system models are available for different uses are shown.

Table 1 - Different IONNY sanitization system models are available for different uses

Model	Area coverage	Fluid used
IONNY 150 HOME AND OFFICE	up to 150 m^3	air
IONNY 300 HOME AND OFFICE	up to 300 m^3	air
IONNY 400	up to 400 m^3	air
IONNY EVO 1000	up to 800 m^3	air
IONNY EVO 2000	up to 1600 m^3	air
IONNY DUCTED 1	up to 1200 m^3	air
IONNY DUCTED 2	up to 2400 m^3	air
IONNY DUCTED 4	up to 4800 m^3	air
IONNY DUCTED 6	up to 7200 m^3	air
IONNY DUCTED 8	up to 9600 m^3	air

4. Conclusions

Ionization is a valid alternative sanitizing, physical method for the reduction of microorganisms (bacteria, fungi and yeasts), which can replace and eliminate the use chemical and other sanitizing systems in the food sector. This method does not cause harm to human health; neither does damage metallic surfaces. These benefits among others suggest that ionization can be used in a continuously manner without endangering the environment. In fact, if the ion source is properly sized relatively to the application volume, recommendations and limits issued by the USA Agency for Safety and Health at Work (OSHA) 0.06 ppm (50 ppb) for a continuous 8 h/5 days exposure (MAC 8 hrs) 0.3 ppm (300 ppb) for a 15 min exposure (MAC15min), can be completely respected ensuring human health and protection.

Ionny reduces ethylene, molds, fungi, virus, yeast and air born bacteria, extending extends the fruit shelf life, preserving value of the produce and lowering costs with economic benefits for the producer(Buglia *et al*., 2013; Fadanelli *et al*., 2017).

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