Chapter

Innovation in Food Products Using Ozone Technology: Impact on Quality Assurance

Carlos Martín Enríquez-Castro, Manuel Pérez-Nafarrate and Jesús Enrique Gerardo Rodríguez

Abstract

Ozone application is a non-thermal technology used in food preservation, which is a powerful oxidant agent used in water and air treatment specially in disinfection processes for agriculture and food industry. The objective of this revision work is to publicize ozone applications in the growing, harvest, and postharvest handling of fruit and vegetables (F & V) across México. Ozonated water by foliar spraying and irrigation were proved to be effective in the control of pathogens, bacteria, and bugs. The use of Ozone was effective to heighten quality parameters of F & V, such as color, flavor, and soluble solids in mango, sugarcane, citric fruits, and nopal, increasing shelf life of fresh products up to 15 days after harvesting. Several protocols mentioned to fulfill the requirements of the producer were developed by TRIO3. The methodology proposed and the designed equipment by the company suggest a wider approach of this green technology in agriculture.

Keywords: ozone, climacteric fruits, ozonated water, foliar spraying, microbial growth control

1. Introduction

Ozone (O_3), also called trioxygen, is a gaseous substance whose molecule is formed by three oxygen atoms linked with an angular geometry. The ozone is formed by applying on the oxygen molecule enough energy to divide it forming various molecular structures [1]. This homonuclear molecule is the oxidant agent most powerful used for water and air treatment specially in disinfection processes for agriculture and food industry. Ozone is friendly with the environment and classified as Generally Recognized as Safe (GRAS) according to the Food and Drug Administration (FDA) [2].

Ozone is a universal disinfectant that reacts with the contaminant agents. It suppresses bad color and undesirable odor at the same time, destroying molds, bacteria, virus and algae [2, 3]. The deodorant action of ozone is due to the oxidation of chemical compounds such as ketones, hydrocarbons, acids, sulfides and nitrogenated derivatives [4]. Ozone oxidizes the cell wall, breaking its membrane and attacking to the DNA and RNA constituents. For this reason, the microorganisms are unable to develop immunity to the action of ozone as they do against other

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chemical compounds [1, 4, 5]. The use of ozone as sanitizer is effective without compromising health of consumers [6]. Traditional sanitizers are cheaper than ozone, however, sometimes they are not appropriate when a new outbreak emerges or when the presence of new food pathogens is detected [7, 8].

Ozone as a non-thermal technology is used in food preservation, which helps to improve the organoleptic characteristics of food following good manufacture practices [9]. Ozone can be pumped into a postharvest cold room. In water applications, ozone is drawn into a low negative pressure water stream using a Venturi injection system (Mazzei Company, LLT). The excess of not mixed ozone must be captured and destroyed to avoid the corrosion and personal injury [2]. A useful method of destroying the remanent ozone is combining UV light and catalytic agents such as granulated activated carbon [10]. The aim of this review is to describe several memories in the application of ozone in agriculture. Adhered to this, every project mentioned was adjusted to the state regulations achieving the expectations of the producer.

2. Biological factors involved in the deterioration of fruits and vegetables (F & V)

Some of the variables affecting the postharvest conditions of raw materials are related with their metabolic processes. Respiration as a process in living cells moderates the release of energy through the breakdown of carbon chains and the formation of new compounds, necessary for the maintenance and generation of synthetic reactions after harvest [11]. A low respiration rate in fresh materials increase postharvest life of perishable fruits. Ethylene concentration acts as a maturation hormone due to its biological activity in ripening climacteric fruits. Therefore, ethylene concentration can be monitored using precise instrumental methods [12]. Fundo et al. (2018) monitored the quality parameters of Cantaloupe melon juice using 30 min O_3 and 60 min O_3 treatments. They observed a diminution in color, vitamin C, total carotenoids, and antioxidant activity; but an increase in total phenolics registered in ozonated juices.

One of the most relevant stages of growth and development in F & V is probably the ripening or maturity. During ripeness, the enzymatic changes promote softening, hydrolytic conversion of storage material-starch to free sugars, and pigmentation changes, especially in climacteric fruits [13]. Flavor changes are considered an important topic in the metabolic pathway of F & V depending on sugars, acids and volatile compounds changes during ripening. As the fruit ripens, there is an increase in the synthesis of their volatile compounds [14].

2.1 Physical damage and pathological deterioration

In order to assure a good maturity index of raw materials, some factors such as water loss, changes in size, and changes in shape or surface must be considered, particularly when a marketable size is reached. Therefore, raw materials that presents physical damage must be separated to prevent postharvest losses. Measurement of texture, either manually or instrumentally can help to detect the maturity index of the fruit. Paciulli et al. [15] measured texture to frozen vegetables such as asparagus stems, zucchini and green beans. They observed a softening effect on the vegetable structure probably due to the blanching treatment affecting the inner cell wall. Depolymerization and/or solubilization reactions of the tissue occurred as a consequence of thermal treatment. In addition, it is advisable to avoid the improper handling or biodeterioration by microorganisms, insects, rodents or birds [16].

3. Applications of ozone

The harvesting of fresh F & V, its management during the freezing, packaging and processing involves the use of water. The presence of water on the agricultural products can increase the probability of contamination of plant pathogens and microorganisms of major concern in food safety. Inappropriate application of procedures related with prevention of contamination and disinfection of water can have severe consequences. Special attention on the microorganisms present in the water recirculation system must be taken because of a rapid reproduction and adaptability in adverse circumstances [16]. Ozone used for vegetable washing and disinfection either gas or dissolved in water has been effective in reducing several microorganisms such as *Escherichia coli*, Penicillium Italicum, and *P. digitatum* [17, 18]. In this way, damage and deterioration of the fruit can be prevented, and postharvest life increased [19]. Ozone is used to eliminate pests and insects without harming the food quality and the environment. Applied at proper doses, ozone diminishes the proliferation of bugs, and is an excellent choice to replace chemical products.

Feston et al. [20] used ozone and observed a reduction of bed bug (*Cimex lectularius* L.) in different life stages. They concluded that LC_{50} and LC_{90} values were higher in nymph and adults than the observed in eggs. The damage caused by insects it is not only restricted to what the vector physically damages and ingests, but also what is defecated over the plant. All these factors promote fungal development like Fusarium spp. and Aspergillus spp., and in most of the cases, they are responsible of promoting the development of mycotoxins [21].

3.1 Ozonated water by foliar spraying

Ozone applied in a foliar way allows the addition of nutrients in the plant and the elimination of pests and diseases. The effectiveness of this chemical agent in reducing microbial load of foods includes the presence of fungi, molds, spores, viruses, bacteria, eggs, and nymphs of insects as shown in **Figure 1**. Ozone has no unfavorable effects on the organoleptic characteristics of foods including textural and nutritional quality. Besides, ozone acts directly in the metabolism of the microorganism suppressing its development [19, 22]. During foliar spraying, plant diseases can be reduced due to the effect of nutrients and ozone added. Another benefit observed of using foliar spraying is that the dose of fertilizers applied in cultivars and the operating costs can be decreased. According to Ali [23] an appropriate foliar spraying improved the quality parameters of papaya using 2.5 ppm and 3.5 ppm ozone. Weight loss, firmness, ripening and soluble solids concentration were maintained and preserved for a longer storage period.

3.2 Ozonated water in the irrigation system

There are different ways to carry out an irrigation system: by gravity, sprinkling, dripping, micro sprinkling or sub-irrigation, and capillary diffusion as shown in **Figure 2**. The ozone irrigation system in cultivars allows the incorporation of nutrients and oxygen, the strengthening of roots, thickening of the tree trunk, and the increase of soluble solids and sugars. Ozonated water is effective to eliminate virus and bacteria and control in nopal-vegetable such as Fusarium spp. and Aspergillus spp. [24]. The nematodes present in their root are destroyed due to the sanitizing effect of ozonated water. The mature fruit (tuna or prickly pear fruit) can be sprayed with ozonated water to guarantee the innocuity and integrity of fruit [25].

Figure 1.
Ozonated water by foliar spraying in mango cultivars.

Figure 2.
Ozonated water in the irrigation system.

Ozonated water irrigation provides oxygen to the root and the stream that flows in its path reaching the root of the plant. The inner membrane of viruses, bacteria, fungi, algae, spores, and other microorganisms are destroyed using ozone dissolved

Commodity	Ozone treatment	Effects on quality	Reference
Broccoli	0.04 μL L^{-1}, 7 d, 10 °C	Delay of metabolic process & oxidative reactions	[2]
Cucumber	0.04 μL L^{-1}, 17 d, 3 °C	Enhancement of appearance. Higher values of Firmness (N) than the observed in control simples.	[2]
Mushroom	0.04 μL L^{-1}, 14 d, 4 °C	Blotch in cap surface was diminished.	[2]
Apple and pears	1.5 μL L^{-1}, 100 d, 20 °C	Total soluble solids and firmness were sustained.	[6]
Orange juice	Ozone variable dose	Control of decay, abatement of ethylene, removing of pesticides and residues.	[7]
Fresh cut lettuce	1 mg L^{-1}, 10 d, 4 °C	Storage life increased, decline of respiration rate & enzymatic browning.	[8]
Cantaloupe melon juice	7 ± 2.4 gL^{-1} for 30 & 60 min	Increase of polyphenols and vitamins.	[5]
Mango	10 μL L^{-1}, 3 d, 25 °C	Delay of ripening, Improvement of quality characteristics.	[7]
Papaya	2.5–3.5 ppm	Weight loss reduction, higher firmness values.	[8]
Soil and water deposits	Ozone variable dose	Bactericidal action on responsible for food delay (*P.aeruginosa*)	[6]

Table 1.
Uses of ozone in several fresh produces and its benefits.

in water, and promotes strength and productivity in the plant preventing diseases. This technique is widely applied in fruit trees, vineyards and nopal-vegetable among others [26]. **Table 1** shows uses of ozone in several materials and its benefits.

Several studies have been conducted to identify the vector disease in the plant as follows: leaf yellow curl virus in tomato [27], whitefly (*Bemisia tabici*) affects cotton [28], tropical fruits [29], and potato [30]. Ozonated water irrigation used in a regular way effectively eliminates plant disorders leaving no smell or trace [19]. Several researchers emphasize the effectiveness of ozone [30–34]. Contigiani et al. [35] applied ozonated water effectively in strawberries to reduce the risk of pathogen proliferation. They found significative differences ($P > 0.05$) in the fungal control (*B. cinedea*) using ozonated water for 5 min (2.73 mgL-1). Additionally, quality attributes were preserved. Ozone seems to be a very promising technology to reduce post-harvest losses.

4. Innovation project: processing of mangoes (*Mangifera indica*) using ozone technology

4.1 Introduction

Mango (Ataulfo variety) cultivated in the tropical areas of south of Mexico has a supreme quality but achieves a fast state of maturity and decomposes easily due to a high microbiological load. Local growers apply higher doses of fertilizers and pesticides several times per year to avoid the infestation of mango tree, specifically whitefly and their larvae. Effective post-harvest techniques to avoid major economic losses must be employed.

4.2 Methodology

The research team of Pérez-Nafarrate [36] designed processes using ozone gas and ozone water for disinfection and packaging of mango in an industrial plant in Guerrero, Mexico. The accomplishment of concise food safety regulations was according to studies realized by Tran et al. [37]. In order to preserve the physico-chemical characteristics of mango fruit, the next premises were followed:

Ozone gas used in a regular basis (one dose every third day) should eliminate gradually the presence of pests.

Gas monitoring was performed using an ozone analyzer (Model IN-2000, L2-LC, in USA Incorporated).

Ozone concentrations (1.5, 2.5, 3.5, 5 ppm) applied (96 h, 30 ± 3 °C) in the storage container should reduce the ethylene content generated as part of the fruit ripening process.

4.3 Results and discussion

The most effective ozone dose (3.5 ppm) preserved the organoleptic characteristics of the fruit and delayed the ripening for 15 days. According to producers, optimal shelf life of mango is 10 days [37]. Soluble solids, color, flavor and empiric firmness were improved (data not published) and a sensory evaluation of the fruit was developed by the operators. More and Rao [38, 39] used a combination of UV-C irradiation (210–280 nm), guar gum (2.5%), and ozone water (400 mg-h-1) to preserve organoleptic characteristics of mangoes at 32 ± 3 °C after 19 days.

Compliance with good manufacturing practices diminish the fruit deterioration, and the economic profit increased. Harvesting of climacteric fruits, including mangoes, papaya, and banana; requires storage temperature and relative humidity similar to that observed in subtropical environments. According to Pérez-Nafarrate [36], key factors to conduct an ozonated water experiment consist on reliability and accuracy of the measurement devices, type and growth stage of the microorganism, ozone concentration and time of contact with the product.

5. Innovation project: use of ozone in the harvesting and processing of nopal

5.1 Introduction

The nopal vegetable (*Opuntia ficus-indica*) grows in semi-arid zones around the world, it has a high dietary fiber content, and is usually consumed Mexico [40, 41]. Physical and morphological characteristics of nopal allow a quick adaptation to low precipitation and excessive heat (**Figure 3**). The nopal consist of two main parts: the edible part (84–90% water), and prickly pear (45–67% of reducing sugars) also called “tuna” [42]. Relative humidity is a crucial variable in nopal for development of pests and diseases. The most commonly microorganisms present in nopal are: cactus weevil *Cactophagus spinulae* (*Gyllenhal*), [43]; boll weevil (*Cylindrocopturus biradiatus Champs*), [40]; white worm (*Lanifera cyclades Druce*), zebra worn (*Olycella nephelepsa Dyar*), [44]; and cochineal (*Dactylopius coccus costa*), [45]. The most important include the bacterial stain (*Bacterium sp*.), rotting of the epidermis, anthracnose, prickly pear gold [46], the thickening of cladodes and the presence of bold among others [47]. The objective was to give added value to nopal-vegetable (raw and processed) using ozonated foliar spraying and ozonated water irrigation.

Figure 3.
Manual harvesting of nopal in México.

5.2 Methodology

Due to the nature of nopal, good manufacture practices in soil and water are required to control outbreaks of diseases. The experimental plantation was located in Matehuala, San Luis Potosi; México. The producers gave light watering to nopal seedlings every 8 or 15 days during the dry months as shown in **Figure 4**, and the irrigation program was monitored periodically to establish the ozone generator requirements. Salinity, hardness, pH and sedimented solids were registered for 15 days before installing the irrigation system. The ozone solubility control included the measurement of pressure and temperature, concentration of carbonates, nitrates and metals [6]. Once the nopal "pencas" (thick and fleshy central nerve that has leaves) were cut, they were placed in recyclable plastic boxes preserving the thorns to avoid the oxidation reactions [48].

5.3 Results and discussion

Ozonated water favored the organoleptic integrity of the nopal, reducing oxidation reactions and retaining green color in sliced raw nopal. Other goals achieved were the reduction in microbial load and the increase in shelf life (data not shown). The final product was packaged and sealed in plastic bags with 500 g and 1000 g. An investment proposal to install a rural cooperative company with capacity of processing 500 kg per day was presented to the cooperative.

Technical specifications and cost of a nopal sider, an immersion washing machine, a centrifugal dryer, and a vibrator dryer with cold were included in the investment project for $1,500,000.00 pesos.

Figure 4.
Sustainable management of the nopal irrigation system.

Good manufacturing practices are essential to accomplish nopal processing with ozone. Water quality and soil requirements monitoring were key factors to improve the postharvest life of nopal opuntia. An innovative option for rural population centers was presented to detonate the local economy. Almost 50% of inhabitants in Mexico are distributed in rural areas, therefore ozone technology should be affordable to most of the population.

6. Innovation project: use of ozone to counteract *Huanglongbing* in citrus trees

6.1 Introduction

The *Huanglongbing* (HLB) disease or "yellow dragon", affects the worldwide production of citrus (lemon, tangerine, orange). *Diaphorina citri* (Kuwayama), the insect vector of HLB, is the main responsible as shown in **Figure 5** [49, 50]. HLB is mainly distributed in Asia (Malaysia, Thailand and Vietnam) and Africa. Farmers invest up to 50 insecticide doses per year to control the vector and avoid the spread of the disease [51]. Symptoms of this plant disease include damage in tree and fruit, sparce yellow foliage, and stunting among others as shown in **Figure 6** [52], shortening of the productive life of the plant, and detriment of final quality product [53].

Figure 5.
HLB in citrus tree. Source: www.gob.mx/agricultura.

Figure 6.
HLB symptoms in Mexican lemon tree. Source: www.gob.mx/agricultura.

Since 2010, HLB has been spread to the American continent especially those areas with frequent rainfall and template temperatures. HLB in Mexico is a serious threat to the 526 thousand hectares of citrus distributed in twenty-three Federal Entities. This in turn represents a production risk of 6.7 million tons per year with a value of more than 400 million dollars. Around 67,000 producers engaged to citric industry generated approximately 70,000 direct jobs and 250,000 indirect jobs, all of them endangered by HLB [54]. Specific research in Mexico to counteract this pandemic is urgently recommended.

6.2 Methodology

In 2013, a collaboration protocol was signed between TRIO3 Food Technologies, the National Institute of Forestry, Agricultural and Livestock Research (INIFAP, for its acronym in spanish) and Los Limones Orchard, located in Tecoman; Colima (18°46′38.04′′ N, 103°48′56.74′′ W). Pérez-Nafarrate [55] evaluated the effect of ozone to eradicate the HLB disease in the productive region previously mentioned. Two main objectives were proposed: first, the early detection of infected trees in the growing season; and second to evaluate the damage spread by the vector in those trees with an advanced stage of the disease, and register its total loss [56]. Fifteen hectares planted with real lemon were micro-sprinkler irrigated with ozonated water, and nutrients were added to roots of trees every third day during six months. The sprinkler irrigation process was evaluated at the end of the experiment.

6.3 Results and discussion

Ozone applied in Los Limones Orchard considerably reduced the population of larvae and adult vectors increasing the citrus production (data not shown). After ozonated water process concluded in lemon trees, other benefits were observed by farmers and technical staff including the improvement of the foliage coloration, and strengthening of trunk and root trees, which are very promising. Showler [57] used an organic mixture composed of corn meal, humic acid, molasses and fish oil to suppress greasy spot in infected trees. An increase in soluble solids content and a weight loss reduction in treated trees, as a result of the healing treatment during three years, provided similar results as ozone did. According to Perez-Nafarrate [55], the insecticide and plaguicide action of ozonated water proved to be faster than traditional methods.

HLB, like many other plant diseases, be diminished effectively with ozonated water. Gas concentration used, soil quality and the effectiveness of the irrigation system were the critical parameters to observe in the experiment. This clean and affordable technology is efficient to control HLB, but more research is still needed to eradicate this bacterium from Mexico and its surroundings.

7. Innovation project: use of ozone to control diseases of sugarcane

7.1 Introduction

Sugarcane (*Saccharum spp hybrids*) is a tall perennial grass traditionally grown in tropical zones and used for obtaining sugar concentrates, molasses and other derivatives [58, 59]. Good manufacturing practices and biological control of diseases allow the correct development of this plant. It is common to find a considerable number of microorganisms in sugarcane such as bacteria, fungi and viruses, all these entities cause several diseases in the cultivar [60] as shown below:

Most common organisms found in sugarcane include: red rot (*Colletotrichum falcatum*) [61]; wilt (*Fusarium sacchari*) [62]; grassy shoot (*Exitanius indicus*) [63]; leaf scald (*Xanthomonas albilineans*) [64]; smut (*Sporisorium scitamineum*) [65, 66]; brown rust (*Puccinia melanocephala*) and orange rust (*Puccinia kuchnii*) [67].

The diseases of major economic importance in sugarcane are described as follows:

Mosaic disease. Is produced by Sugarcane mosaic virus and attacks young plants making areas appear on leaves pale green and yellowish within a normal green. The reeds become stunted causing production declines [68].

Eyespot. This name is derived from the powdery black mass of spores associated with this disease. The affected plants show a reddish elliptical lesion surrounded by a yellowish structure that varies in size. The spores of the fungus Ustilago scitaminea are transmitted by wind, rain, irrigation water, seeds or animals [69].

Rust. Produced by the fungus Puccinia melanocephala manifested by many elongated spots on the leaves, show no growth and the stem become thin. The yellowish spots look at the whole sheet [70].

Leaf scald. The causal organism Xanthomonas albineans is a bacterial disease first observed in Cuba in 1979. X. albineans causes sudden death of entire stems and seedlings affecting significantly sugarcane yields [64]. Leaf scald is also called in Latin America "gomosis", a plant disease characterized by an abundant production of gum. Gomosis caused by Xanthomona axonopodis involves widespread dwarfism in plant [70].

Ratoon stunting disease. RSD caused by Leifsonia xyli spp. was observed in Cuba for the first time in 1953. Several stunted stems very thin are present within the plant and short internodes. Damaged stems increase as the number of cuts in the field is higher [71].

The ozonated water irrigation provides a greater contribution of oxygen to the root. Ozonated water is free of viruses, bacteria, fungi, algae, spores and any other microorganism. Growth is achieved faster than usual with more liveliness and strength as well as more productivity. This irrigation method is beneficial for plants, and is currently used in fruit trees, vineyards and crops in general. Ozonated water achieves the prevention of plant diseases such as leaf scald and rust reducing the microbiological load. Chemical products such as pesticides and fungicides are reduced improving the shelf life of fresh products [60].

7.2 Methodology

On July 4 2008 was signed a collaborative project between the National Institute of Agrarian Innovation (INIA, for its acronym in spanish) and the National Institute of Research of Sugarcane in Havana-Pinar del Río. Red ferritic soil was used to grow up a 9-month agamic seed from 5 varieties identified as follows: C323–68, C86–56, C86–456, C88–380, and C90–530. The experimental design (divided plots 5 x 6 x 2) included 180 buds from each variety with two replicates, 5 varieties and 6 treatments. Finally, sugarcane certified quality seeds (P. Vista Florida cv. 115–2014) previously treated with ozonated water were introduced among the producers as shown in **Figure** 7.

The exposure time in ozonated water was 10, 20, and 30 min respectively. The treatments included a hot water-chemical treatment, a biological one, and a sample control [52]. The measurements were carried out one and six months after sowing. The main objective was to evaluate the effect of ozonated water on sugarcane seed growing. The viability and growth of agamic seeds of sugarcane used was also evaluated. The ozone equipment used is shown in **Figure 8**.

Figure 7.
Certified sugarcane seed P. Vista Florida. Cv. 115–2014.

Figure 8.
Equipment used for the ozonation of water.

7.2.1 Treatment with ozonated water

An irrigation and sprinkler system with ozonated water to promote the growth of sugarcane is shown in **Figures 9** and **10**. The fungicide was reduced progressively to control and eliminate fungus and pests as the amount of ozonated water increased. Previously, 90 buds were submerged in ozonated water during 24 h. Thirty buds per variety were immersed in ozonated water (1 ppm) for 10, 20, and 30 min respectively.

7.2.2 Witness without treatment

Thirty buds per variety were soaked on water for 24 h without applying any treatment.

Figure 9.
Ozonated water irrigation system as applied on sugarcane cultivation.

Figure 10.
Ozonized water by foliar spraying.

7.2.3 Hydrothermal + chemical treatment

Thirty buds per variety were submerged 24 h in water, then immersed in hot water at 51 °C for one hour. Immediately they were treated with propiconazole (Tilt EC 250) at 0.4% (w/w) for 15 min. This time was considered enough to eliminate leaf scald and red rot diseases.

7.2.4 Biological treatment

This treatment started on July 4 2008 with the immersion in water of 30 sugarcane buds per variety for 24 h, and then submerged for 30 min in a water solution of Nemacid (EDOCA, Amealco, Querétaro) at 2% (w/w). This organic insecticide is used in the control and inhibition of nemathodes. Quivican experimental station was used to plant the sprouted seeds treated with Nemacid. After 30 days, the sprouting count was carried out and analyzed in the laboratory. All the experiments were made by duplicate.

7.3 Results and discussion

All the treatments showed significant differences ($p < 0.0001$). Best results obtained on seeds immersed in ozonated water for different time intervals are shown in **Figure 11**. The sprouted seeds treated with 10 min-ozonated water immersion reached the highest germination level: 10 seeds in total. The sample control seeds and seeds treated with 30 min-ozonated water reached an acceptable germination rate: 9 and 8 respectively. Nemacid and hot water samples presented the lowest germination rate: 7 in total. Using ozonated water did not affect the viability of the seed. The experimental ozonated water immersion system proposed to producers of sugarcane reduced drastically eyespot and leaf scald. This treatment was beneficial for agamic seed viability showing a different response in every treatment, especially that with 10-min immersion time.

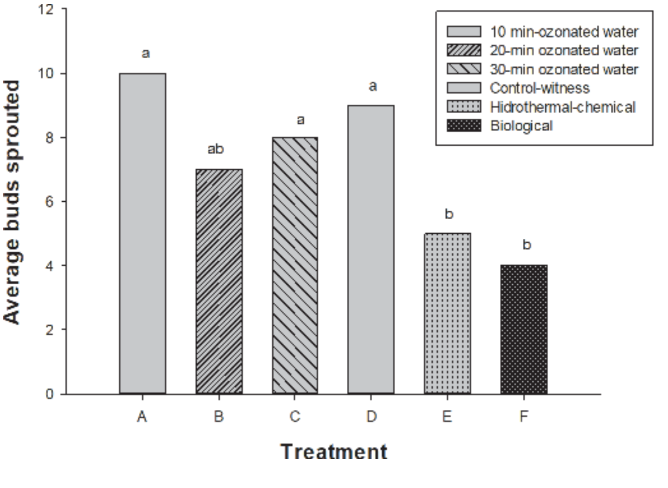

Figure 11.
Influence of ozonated water in sprouting of sugarcane agamic seed.

8. Conclusion

Ozone has demonstrated be useful for management of postharvest of climacteric fruits such as mangoes and papayas. This ecotechnology is cost effective and is very promising to use in developing countries of Latin América. Using ozone watering in Nopal opuntia crops, proved to be very effective to control diseases and increase plant health. An adequate ozone irrigation system prevents growth of bacteria and bugs in citrus trees, and other fruits, and is an alternative to reduce HLB infestation. Sugarcane and other important crops growing in tropical areas increase yield using ozonated water, and an early diagnose of diseases reduce losses and increase the economic benefit in productive areas. TRIO3 acknowledges all the producers and scientific investigators involved in the protocols developed during these last 10 years.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Carlos Martín Enríquez-Castro[1*], Manuel Pérez-Nafarrate[2] and Jesús Enrique Gerardo Rodríguez[3]

1 Superior Tecnological Institute Zacatecas Norte, Río Grande, Zacatecas, México

2 TRIO3 Food Technologies, Guadalajara, Jalisco, México

3 Department of Biochemistry and Molecular Biology and Robert M. Kerr Food and Agricultural Products Center, Oklahoma State University, Stillwater, OK, United States

*Address all correspondence to: carlos_enriquez_castro@hotmail.com

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