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Ozonated Washing Water for Quality Carrot Preservation

Carrots are washed before packaging to remove tare and organic debris and to make them appealing to the consumer. However, this increases the risk that carrots which are strongly infested with microorganisms might infect previously uncontaminated tap roots, potentially causing large product losses. For this reason the washing water must be effectively disinfected, e.g. by adding ozone. The bactericidal and fungicidal effects of ozone are well established and no hazardous residues remain. On the other hand, ozone is a strong oxidant and might damage the produce. The inactivation effect of ozone on Pectobacterium carotovora, which causes bacterial soft rot, was evaluated, as well as whether practice-relevant ozone concentrations might damage the products.

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Keywords

Carrots, ozonized water, quality of product

Literature

Literature references can be called up under LT 05611 via internet http://www.landwirtschaftsverlag.com/landtech/local/literatur.htm.

According to the marketing standard for fruit and vegetables minimum requirements demand, among others, that the produce must be "clean" and "practically free of any visible foreign matter" [1]. Hence, many produce need to be carefully washed to fulfil these simple demands. In addition, washing helps to reduce the product temperature and to decrease their microbial load. Many different approaches have been tested during recent years. Besides the application of ultra sound, irradiation (UV-C, γ), high pressure, electrolyzed water, heat treatment and chlorine, the use of ozone has been proposed [2, 3]. Many investigations have established the bactericidal and fungicidal properties of gaseous and water-dissolved ozone [4, 5]. Furthermore, ozone can be relatively easily implemented into existing washing processes and does not generate hazardous residues which have to be processed and depolluted with financial efforts.

On the other hand, ozone is a strong oxidant that might affect the produce metabolism [6] or even damage it, thus reducing the quality and the shelf life of the product [7]. Hence, it is essential to verify that the proposed ozone treatment does not impair treated produce.

Especially the bacterial soft rot, caused by the bacterium *Pectobacterium carotovora*, represents a high risk for root vegetables that eventually results in a total loss of the entire harvest. An infestation of produce with P. carotovora spp. results in a disintegration of the tissue structure [8] because this putrefactive agent segregates enzymes that degrade the pectins of the middle lamellae in the cell walls of adjacent cells.

The presented investigation aimed to point out the inactivation effect of ozonated water on P. carotovora, both in pure culture and, as a practical example, on wash carrots. Furthermore, it should be established that concentrations with practical relevance of water dissolved ozone do not damage the ozone treated carrots. For this purpose, the effects of ozonated water on the respirational and transpirational properties and on the vitamin C content of the carrots were studied.

Materials and Methods

Ozonated water was generated using the „Bewazon 1" ozone generator (BWT Water Technology Ltd. Schriesheim, Germany). Ozone measurement was performed using the LASAR 2plus photometer with the complementary chlorine/ozone cuvette test (Bruno Lange, Germany).

10 µl of suspensions of *Pectobacterium carotovora* (DSMZ 30168) were mixed with 10 ml of ozonated water (4 ppm) or of distilled water, respectively. The final strain concentrations in the mixtures were $1{,}3 \cdot 10^5$ cfu/ml. After exposure times of 10, 20 and 30 sec. the mixtures were shaken for 1 minute using a whirler to destroy the residual ozone.

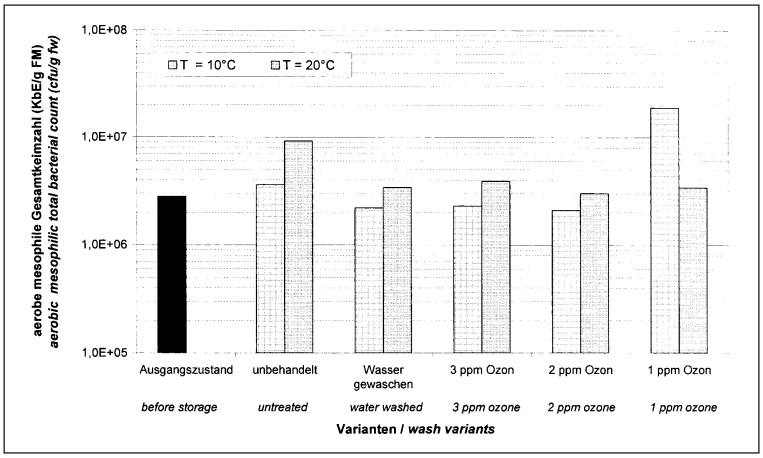

Fig. 1: Microbial counts of carrots after storage (10 d) at 10 °C and 20 °C

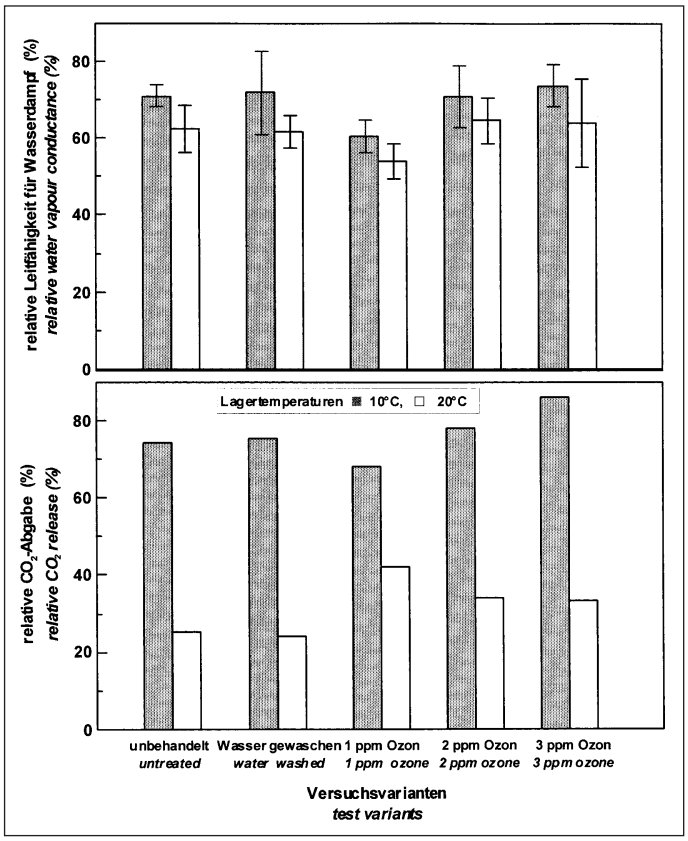

Fig. 2: Relative vapour conductance and relative CO_2 release rates (%, related to initial values measured before storage) of carrots after ten day storage at 10°C and 20°C, respectively

Then, aliquots of the different mixtures were plated (Mac-Concey-Agar plates, Merck, Germany) and incubated at 25°C for 2 d. The experiment was performed twice.

Fresh carrots were purchased from a local market. The investigations were accomplished within two days due to the extensive sample size. Untreated (control 1), water washed (control 2), and ozonated water washed ($c(O_3)$ = 1, 2 and 3 ppm) carrots were stored in water vapour saturated atmosphere at 10°C or 20°C for 10 days. During this time the samples were analyzed repeatedly. Ozone treatment was performed in a continuous system. The carrots (3 carrots per method) were washed for 30 seconds in ozone solution or running water (control).

The initial level of microbial population on the carrots was determined before and after the ozone treatment while their final levels were analysed after 10 days of storage at 10°C or 20°C. The carrots were mashed under sterile conditions. Then 10 g of the resulting slurry was added to 90 ml of Ringer solution, aliquot diluted, plated, (PCA plates, Merck, Germany), incubated at 25°C for 2 d and subsequently counted.

Respirational CO_2 release of intact carrots was measured in a closed system fitted with infrared sensors ($FYA600_{CO2}$, Ahlborn Mess- und Regeltechnik, Germany). From the increase in CO_2 concentration over time within a closed Perspex cylinder and the tuber fresh mass, respiration activity was calculated as mg_{CO2} kg^{-1} h^{-1}. Transpirational water losses (mmol min^{-1} kg^{-1}) of each carrot were repeatedly determined by consecutive weighing of the carrots under controlled environmental conditions. From the transpiration rates and the respective data of air and tuber temperatures and humidity of the surrounding air, surface conductance was calculated [9].

For determination of the Vitamin C content each carrot was cut longitudinal into two halves. The Vitamin C content of one half was determined after washing with 2.0 L tap water for 0.5 min, the second half was washed for 0.5 min in 2.0 L ozonated water ($c(O_3)$: 1.0, 1.5, 2.0, 2.5 and 3.0 ppm). A juice extractor was used to obtain the fruit juice. The samples were filtered und diluted with distilled water (1:2) before their Vitamin C content was measured using the „Reflectoquant“ test set (Merck, Germany).

Results

Treating the P. carotovora suspensions with ozonated water (4 ppm) for 10, 20, and 30 sec., completely inactivated the bacteria and no colonies growth was found on the agar plates. This clearly indicates that P. carotovora is sensitive to ozone. Even a 10-second treatment with an ozone concentration from 4 ppm reduces the bacterial content from $1.3 \cdot 10^5$ cfu/ml to less than 10^2 cfu/ml, the limit of the detection method.

The aerobic mesophilic bacterial count was initially $2.8 \cdot 10^6$ cfu/g. After 10 days of storage the number of microorganisms increased marginally. Growth was slightly more pronounced at 20°C than at 10°C because of the better growing conditions at the higher temperature (*Fig. 1*). The highest growth was observed for the untreated control. On the other hand, washing with ozonated water has no unspecific bactericidal or inhibitory effect in comparison to tap water washing. The natural microbial flora seems not to be affected by treatments with ozonated water up to 3 ppm. This aspect is positive because in the drinking water purification ozone treatment resulted in accelerated growth of surviving microorganisms due to the bioavailability of the killed microorganisms [10].

During storage, water vapour conductance and respirational activity as indicated by the CO_2 release rates declined in all carrots. This response was irrespective of the type and the ozone concentration of the treatment (*Fig. 2*). Additionally, neither of the treatments did affect the Vitamin C content of the stored carrots (data not shown). This points out that short term washing with ozonated water did not significantly affect the metabolic activity of the carrots. In contrast, it has been reported that continuous fumigating of carrots with high ozone concentrations resulted in noticeable damages that largely reduce produce quality [7]. One effect of ozone damage is a largely increased respiration activity [6]. Destruction of the dermal tissue directly exposed to ozone should have significantly increased the water vapour conductance of the treated carrots. However, the presented results prove that the short term disinfection of carrots by dipping in ozonated water with concentrations of up to 3 ppm does not affect the physiological efficiency and hence the inner quality of the produce.

Conclusions

The short term addition of 4 ppm ozone to vegetable washing water results in a reduction of P. carotovora by 3 log units in a pure bacterial suspension. On carrots, artificially infected by P. carotovora, the success of ozone treatment somewhat declined to a reduction of the microbial density by 1 to 2 log units (unpublished data).

Washing carrots with ozonated water (up to 3 ppm) for 30 sec. did not affect their Vitamin C content, respiration and water vapour conductance. Furthermore, their natural microbial flora remained stable. Hence, the presented results indicate that ozonated water is an effective though gentle means to disinfect vegetable washing water.