Evaluation of ozonated water's efficacy in sanitizing surfaces in a processed fishery products industry

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Abstract

In response to the modern consumer demands for safe and high-quality food, industries are adopting innovative decontamination methods. Ozone emerges as a promising one, being economical, safe, and chemical-free. It effectively inactivates bacteria, molds, yeasts, parasites, and viruses with short contact times and at low concentrations.

This study aims to explore the effectiveness of ozonated water as an eco-friendly, chemical-free, and safe alternative to conventional sanitizers in the processed fishery products industry. More specifically, it compares ozonated water efficacy with the company's standard method of sanitizing boxes used for soaking cod (spraying the boxes with hydrogen peroxide before placing them in the dishwasher) and its efficacy on boxes stored for 21 days after the standard sanitation. A total of 175 boxes were tested for microbial contamination. The standard sanitization method significantly reduced the microbial load on the boxes after their use compared to the ozone one, resulting in a reduction of 98.29% compared to 48.29% for total mesophilic count (TMC) and 97.96% compared to 37.32% for total psychrophilic count (TPC), respectively. On day 21, the ozonated water achieved a TMC reduction of 80.46% and 98.87% for the TPC on stored boxes. The percentage reduction given by ozonated water on stored boxes was higher than on dirty ones; the presence of organic matter reduces the sanitizing efficacy of ozone. Based on the obtained evidence, ozonated water would appear to be a good sanitation method for surfaces in the processed fishery products industry after adequate cleaning, contributing to the industry's push toward sustainable practices.

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Introduction

Modern consumers require safe, high-quality (less-processed, ready-to-eat, and ready-to-cook) food, leading to continuous research of innovative decontamination methods characterized by low impact on the environment (Brodowska *et al.*, 2018).

In the food industry, surfaces can be significant sources of microbial contamination of the product: even if they appear clean, surfaces can still be contaminated by large numbers of viable microorganisms (Chawla *et al.*, 2008) and must be cleaned properly. After cleaning and removal of food residues, dirt, grease, and other objectionable matter, disinfection is needed to reduce the number of viable microorganisms on surfaces to a level that does not compromise food safety and/or suitability (FAO and WHO, 2011).

In the food industry, disinfection is traditionally achieved by heat in the form of hot water, steam, or liquid chemicals such as chlorine (Moore *et al.*, 2000). The use of liquid chemicals can lead to food contamination by chemical by-products that are harmful to human health and the development of bacterial disinfectant resistance.

Ozone treatment emerges as an effective method for decontamination that enhances the safety and quality of food products (Brodowska *et al.*, 2018). Ozone is a strong antimicrobial agent with high reactivity and penetrability, and decomposes into oxygen. In both gaseous and aqueous forms, ozone can inactivate bacteria, molds, yeasts, parasites, and viruses with short contact times and low concentrations (Kim *et al.*, 1999).

Ozone proves its efficacy across different areas of food production (Cullen and Norton 2012): from ozonated water applied directly to food processing equipment, floors, drains, walls, and tanks; through mobile or centralized systems for the application in personal protective equipment and hand sanitation (Breidablik *et al*., 2020; Epelle *et al*., 2022).

This study aims to provide original data concerning the efficacy of ozonated water as a safe and effective method for sanitizing surfaces (boxes used for the soaking process of cod) compared to the company standard. For this purpose, a total of 175 boxes were sampled in groups of five under various conditions: after being used for soaking cod, after the treatment with ozonated water, and after the standard sanitization procedure. Samples were taken during a 21-day storage period (at 7, 14, and 21 days) and after treatment with ozonated water on day 21.

Materials and Methods

This study compares the company's standard sanitation procedure with the use of ozonated water.

The company's standard sanitation procedure consists of spraying the boxes with hydrogen peroxide (5% for 15 minutes) before rinsing and placing them in the dishwasher (cycle of 5 minutes at 55°C, with alkaline detergent (Cristal, Rhütten s.r.l, Montalto, Italy). After that, the boxes are either used immediately or stored (from 1 to 21 days) and reused after a further cycle in the dishwasher.

Ozonated water was applied for 60 seconds through a portable ozone system (Dino Purification Co.Ltd, Guangzhou, China) that delivers an applied ozone dose of 2.0 ppm, with an ozone water volume of ≤10 LPM through a hand-held spray wand.

Sample collection

Sample collection was structured by five collection moments covering a period of 3 months from April to June 2024, involving groups of five boxes. Each one of them presented an internal surface area of 1784 cm^2 that was sampled using the sponge technique (Nasco Whirl-pak®, Speci-sponge®) in accordance with the UNI EN ISO 18593:2018 (ISO, 2018).

The boxes were initially tested at the following moments: after being used for soaking cod (n. 5); after the treatment with ozonated water at a known concentration of 2 ppm (Co.Ltd, Guangzhou, Guangdong, China)) (n. 5), and after the standard sanitization procedure (n. 5). A total of 20 boxes that had been sanitized using the conventional technique were stored in the washing machine room for 21 days at a constant, controlled temperature of 10°C. The environmental contamination was evaluated during the storage period, and the boxes were tested at 7, 14, and 21 days of storage (n. 5 per time interval). At the end of the storage period, on day 21, 5 boxes were treated with ozonated water and tested before being put back in use, to assess how effectively ozonated water works on surfaces that are contaminated but clear of organic matter.

The scientific design is illustrated in Figure 1.

Microbiological analysis

The 175 collected samples were tested for the following microbiological parameters: total mesophilic count (TMC) and total psychrophilic count (TPC), according to the ISO 4833-1:2013 and ISO 17410:2019 standards, respectively (ISO, 2013 and 2019).

Sponges used for the sampling were aseptically introduced into stomacher bags (BagMixer®, Interscience, Puycapel, Cantal, France), adding 90 mL of maximum recovery diluent (Oxoid Ltd., Basingstoke, Hampshire, UK).

Samples were subsequently stomached for 90 seconds at room temperature to produce 10-fold dilutions. Plate count agar (PCA) (Oxoid Standard Plate Count Agar, Oxoid Ltd., Basingstoke, Hampshire, UK) was used to plate all incubated specimens, and the obtained quantitative data were expressed in log CFU/g.

Statistical analysis

Statistical analyses were performed using IBM® SPSS 20.0 statistics software version (SPSS Inc., Chicago, IL, USA). The descriptive statistical analysis was performed to assess the normal distribution of the data using the Shapiro-Wilk test.

The two-tailed *t*-test was used as a non-parametric method in evaluating the effectiveness of the treatment with the standard procedure compared with the independent variable treatment with ozone at a known concentration. The two dependent variables taken into consideration were the TMC and the TPC, respectively. The values obtained were considered statistically significant with $p<0.05$.

Results and Discussion

The obtained results on the boxes after the use for soaking cod were a TMC average value of 2.92±0.70 log CFU/cm^2 and a TPC average value of 3.43±0.83 log CFU/cm^2. The TMC and TPC values detected on dirty boxes, after the standard sanitation procedure and after the treatment with ozonated water, are represented in Figure 2. After washing according to standard procedure, a reduction of 2.87±0.81 log CFU/cm^2 (98.29%) was observed for TMC and 3.36±0.73 log CFU/cm^2 (97.96%) for TPC. After the exclusive use of ozonated water, the reduction was 1.41±0.47 log CFU/cm^2 (48.29%) for the TMC and 1.28±0.87 log CFU/cm^2 (37.32%) for the TPC. Washing the boxes according to the standard procedure showed a statistically significant difference ($p<0.01$) compared to the treatment with ozonated water.

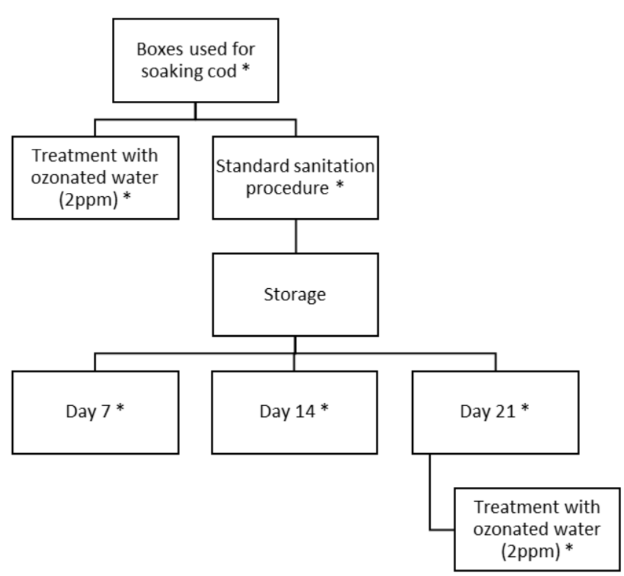

Figure 1. Sampling flowchart. *Sampling of 5 boxes.

As represented in Figure 3, the microbial load increased during the storage period and reached 0.37±0.45 log CFU/cm^2 on day 7 and 0.72±0.27 log CFU/cm^2 on day 14 for the TMC and 1.37±0.87 log CFU/cm^2 on day 7 and 1.44±0.57 log CFU/cm^2 on day 14 for the TPC.

After 21 days of storage, the boxes showed a contamination level of 1.74±0.64 log CFU/cm^2 for TMC and 1.77±0.45 log CFU/cm^2 for TPC. After the treatment with ozonated water, the TMC average value of 0.43±0.76 log CFU/cm^2 and a TPC average value of 0.28±0.58 log CFU/cm^2 were obtained.

The treatment with ozonated water resulted in reductions of microbial loads of 1.4±0.95 log CFU/cm^2 (80.46%) for the TMC and 1.75±0.81 log CFU/cm^2 (98.87%) for the TPC, showing statistically significant differences ($p<0.01$) compared to the absence of the treatment.

Table 1 summarizes this study's results expressed as the means of the obtained values and the standard deviations.

There are few studies available regarding the application of ozonated water on surfaces in the food industry, evaluating its direct application on dirty ones.

Crapo *et al.* (2008) evaluated ozonated water efficacy on polyethylene cutting boards contaminated by spreading fish slime containing high bacterial loads over them. Bacterial contamination of the tested surfaces resulted in 10^4 to 10^6 bacteria/cm^2 of surface. They observed a reduction of aerobic plate count (PCA plates were incubated for 48-72h at 25°C) by <1 log ($p>0.05$) when ozonated water at 1.5 ppm was sprayed on the contaminated cutting boards. With the same method on stainless steel, they observed a reduction of 2-3 logs, concluding that the nature of the processing surfaces apparently influenced the effectiveness of ozone treatment.

Our study shows a higher logarithmic reduction in TMC, 1.41±0.47 log CFU/cm^2, after the exclusive use of ozonated water on dirty boxes. The material of the tested boxes (high-density polyethylene) is the same as the cutting board. It is important to consider, however, that the initial microbial load determined in their study by the fish slime (10^4 to 10^6 bacteria/cm^2 of the surface) is higher than ours (10^2 to 10^3 CFU/cm^2).

Hampson (2000) evaluated ozonated water efficacy on plastic shipping containers used in the wine industry by spraying at 2 ppm through a 10 gpm hand-held spray wand. Test areas were not

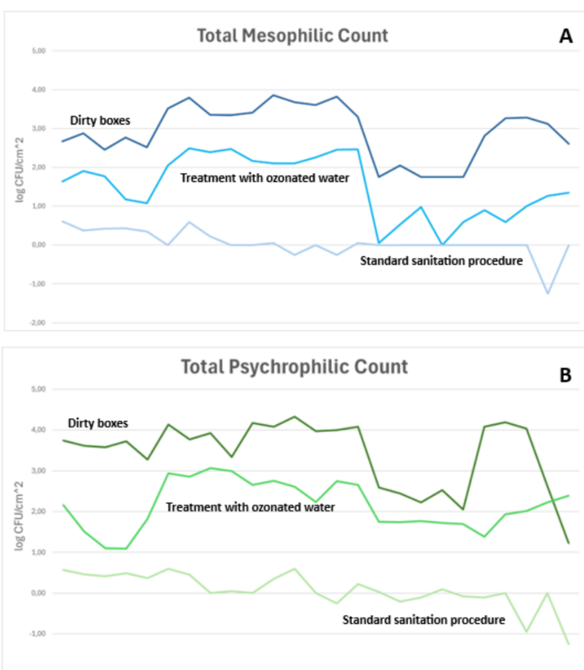

Figure 2. Representation of total mesophilic count (**A**) and total psychrophilic count (**B**) values revealed on dirty boxes, after the treatment with ozonated water and after the standard sanitation procedure.

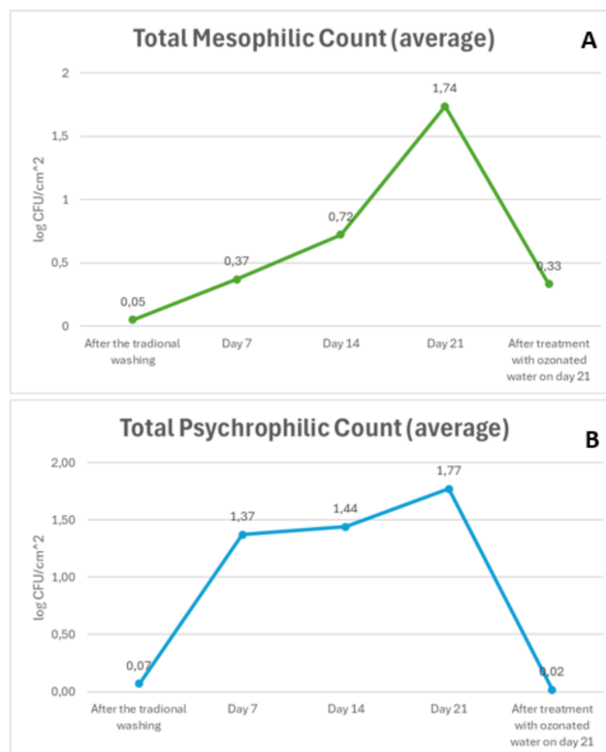

Figure 3. Representation of total mesophilic count (**A**) and total psychrophilic count (**B**) average values during the 21-day storage time and after the treatment with ozonated water on day 21.

Table 1. Means (log CFU/cm^2) and standard deviations of the obtained values at the different sampling times.

		Dirty boxes	Treatment with ozonated water	Standard sanitation procedure	Storage Day 7	Storage Day 14	Storage Day 21	Treatment with ozonated water on day 21
TMC	Mean	2.92	1.51	0.05	0.37	0.72	1.74	0.33
	SD	0.70	0.79	0.35	0.45	0.27	0.64	0.87
TPC	Mean	3.43	2.15	0.07	1.37	1.44	1.77	0.02
	SD	0.83	0.59	0.44	0.87	0.57	0.45	0.83

TMC, total mesophilic count; TPC, total psychrophilic count; SD, standard deviation.

cleaned prior to sanitation, so only the effect of the ozone spray wash was measured. They observed a percentage reduction of aerobic plate count from 96.9 to 97.2, much higher than our reduction on TMC after the exclusive use of ozonated water on dirty boxes (48.29%) and slightly higher than that reelevated on 21 days stored boxes (80.46%).

Crapo *et al.* (2008) in their study concluded that fish processing residuals on surfaces significantly reduce ozonated water sanitizing effectiveness. Our results show that the presence of fish organic matter reduces the sanitizing efficacy of ozone since the percentage reduction given by ozonated water on stored boxes was higher than on dirty ones. In agreement with this, Moore *et al*. (2000) state that, in relation to food surfaces, ozone should be used after adequate cleaning.

To use ozonated water directly on boxes after their use, they should be subjected to pre-cleaning procedures with water and detergents, or ozonated water should be implemented in a clean-in-place system and used in standard washing procedures.

Therefore, the ozonated water treatment, which almost resets the microbial load, is an effective substitute for a second passage in the dishwasher.

Compared to the normal sanitization procedure, the application of ozonated water for washing boxes has the advantage of requiring less time and water, does not require the use of chemicals harmful to operators, is economical, and can be carried out by a single operator.

Ozonated water can be used in cold suspensions between 15 and 30°C, which is convenient regarding energy savings (Dobeic, 2017; Pandiselvam *et al*., 2019). In this study, the portable ozonated water generation system was directly connected to the company water system using cold water instead of hot water normally used for rinsing surfaces or in the dishwasher.

The use of ozonated water as a sanitizer potentially contributes to decreasing the carbon footprint of food production facilities, reducing the carbon emissions in the atmosphere (also called in industrial systems as a calculation of the "Sustainability Report", ISO 14001/2015) (ISO, 2015).

Since ozone is created on-site, the use of this technology allows for the reduction of the need to store disinfectants and their cost. While ozone generators involve an initial investment (equipment, personnel training) for small-scale businesses, over time, the long-term benefits, such as reduced chemical costs, fewer water resources, and less energy consumption, justify their use (Pandiselvam *et al*. 2019).

The use of ozonated water in food processing has significant potential to positively impact consumer perceptions of food safety. Since ozone naturally decomposes into oxygen and leaves no chemical residues, it meets growing consumer demand for cleaner, safer, and more natural food products.

Conclusions

This study highlights the potential of ozonated water as an effective and sustainable sanitization method for surfaces in the processed fishery products industry, contributing to safer and cleaner production environments.

While the company's standard sanitization procedure showed superior microbial reduction compared to ozonated water on boxes immediately after use, due to the presence of organic matter, ozone proved to be a viable alternative for stored boxes.

Ozonated water not only provides substantial energy and water savings but also eliminates the need for harmful chemicals, making it a more eco-friendly and economical option.

Indeed, it is possible to affirm that innovation in the food industry requires companies to be supported by research groups that can provide solutions to internal problems. Ozone is increasingly replacing traditional sanitization techniques due to its chemical-free, economical, and safe attributes. By reducing cross-contamination and microbial loads, advanced hygiene practices involving ozone treatment could extend the shelf life of food products, contributing to the production of more sustainable products.

Further research into optimizing ozone concentration and contact time for sanitization in the food industry is essential, especially when organic residues are present. It is important to properly remove any organic matter before using ozonated water for sanitization on surfaces. Studies should focus on determining the optimal balance between concentration and exposure time to ensure maximum microbial inactivation without compromising material compatibility or food quality.

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