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Letter to the editor

# Comments on Botta et al. (2018). Potentially active spoilage bacteria community during the storage of vacuum packaged beefsteaks treated with aqueous ozone and electrolysed water. International Journal of Food Microbiology, 266, 337–345

*Dear Editor,*

We write to present our point of view on the information provided in the above scientific paper. We are concerned that, if some details are left without comment, the information given in the article could be considered correct, which would be detrimental to the body of knowledge on the biocidal efficacy of ozonated water and electrolysed water, and sanitation approaches applied in food products in general.

As stated in the paper's abstract, the microbial contamination that occurs during the slaughtering process and the handling of the meat results in a shortening of the shelf life of meat. Botta et al. looked at the possibility to extend the shelf life of beefsteaks by spraying the products with either ozonated water (6.00 ± 0.25 ppm of ozone) or water containing active chlorine (100 ppm). However, after a careful reading of this study, significant doubts have arisen in regards to the methodology used, as the reported trends are in contrast with all evidences available in the scientific literature.

The results presented by the study are mostly focussed on the microbial dynamics and GC–MS analysis of volatile compounds; however, it is noteworthy the first part of the study investigated the effects of biocides on microbial load over time. The experimental set-up comprised an untreated control, a treatment with only water, and two sanitising treatments, namely ozonated water (AO) and electrolysed water (EW).

To synthesise the ozonated water and the "electrolysed water", the authors relied on equipment provided by Industrie De Nora S.p.A. (IDN): a C32-AG ozone generator and an "Eva System 100". As briefly detailed in the Materials and Methods section of the article, the first machine has a nominal productivity of 32 g $O_3$/h, when using pure oxygen as the input gas, while the second machine produced a solution containing approx. 4000 ppm of active chlorine (AC), with a pH of 9.

Coincidentally, the Eva System was developed by IDN in the frame of a collaboration with researchers of the University of Ferrara (Italy) and, at that time, one of the commenting authors was contributing to the research in the same lab group, thus significantly understands and appreciates the technology. In brief, the Eva System comprises an undivided electrochemical reactor that allows synthesising a hypochlorite solution, whose final concentration depends on the initial chloride content in the fed solution (a relatively diluted brine), the current and the electrolysis time (Rossi et al., 2012). At that time, the use of KCl (which has strangely been considered also in the study by Botta et al.) was justified by the subsequent use of the AC-containing solution to fight against some vegetable pathologies in the field (unfortunately, some plants do not tolerate sodium, while potassium is generally used as a fertiliser).

By electrolysing a solution containing 10 g/L of KCl, it was typical to obtain a solution with 2500 ppm of AC and a pH close to 9.7. Botta et al. reported the production of a solution with 4000 ppm of AC with a 1% residual KCl (this implies that the starting KCl concentration is at least 14 g/L) and a pH of 9; however, based on our experience, we would expect a final pH closer to 10, or even higher. Therefore, to obtain a solution with 100 ppm of AC, Botta et al. must have diluted their "neat solution" 40 times, thus obtaining a pH variation of about 1.5 units (in the absence of any buffering effects). The pH of their final solution (containing 100 ppm of AC) can thus be estimated to be around 8.5, which means that about 95% of the active chlorine is present in the rather ineffective hypochlorite form.

As mentioned above, the Eva System produces a hypochlorite solution using an undivided reactor, and, to our understanding, this is not an "electrolysed water". To the best of our knowledge, the latter terminology is currently used to refer to an "electrochemically activated solution" produced in a divided electrochemical reactor, i.e. a system equipped with a diaphragm or ion-exchange membrane to prevent that species synthesised at the anode can react with those produced at the cathode, thus leading to the hypochlorite solution. Depending on how hydraulic flows are managed, the EW (also called "anolyte", because it is obtained from the anodic compartment of the divided reactor) may present an acidic to neutral pH (Ferro, 2015). In any case, in the EW, the active chlorine is mainly present as hypochlorous acid, which has been reported to be a biocide more effective than the hypochlorite form, with a difference of about two orders of magnitude (Fukuzaki, 2006).

In the scientific literature, there is a huge number of contributions discussing the effectiveness of one or the other type of EW. In addition, a number of studies were specifically addressed to meat and the possible increase of shelf life (Bosilevac et al., 2005; Ding et al., 2010; Jadeja and Hung, 2014; Tango et al., 2014; by the way, why none of these works was mentioned in the article by Botta et al.?). Investigations have been carried out both in vitro and in real applications, demonstrating that, in the latter case, both the AC content and the contact time must be correctly optimised.

Regarding ozone and ozonated water, the lack of activity reported in the article is even more surprising, as ozone has been used as a food preservative for the cold storage of meats since 1909 (Rice et al., 2002). In 2001, the US Food and Drug Administration (FDA) formally approved the use of ozone as an antimicrobial agent for the treatment, storage and processing of foods in gas and aqueous phases (US FDA, 2001). In the same year, the Food Safety and Inspection Service of the US Department of Agriculture (USDA/FSIS) approved the use of ozone in contact with meats and poultry, from raw product up to fresh cooked and products just prior to packaging (USDA FSIS, 2001). First evidences in the scientific literature date back to 1969, with an article discussing the preserving effect of ozone on fish (Haraguchi et al., 1969). Ten years later, Yang and Chen (1979) discussed the effect of ozone treatment (at either 2.48 or 3.88 ppm) on the poultry meat's microflora, while Hampson (2000) investigated the use of

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ozone for winery and environmental sanitation. Interestingly, good results were obtained in the latter case by spraying ozonated water containing approx. 2 ppm of ozone. In a more recent report, Food Science Australia stated that ozonated water allows achieving a reduction in microbial load comprised between 1 and 2 Log, on meat treated for a time comprised between 15 and 60 s. Unfortunately, the report does not mention the ozone concentration, yet the "possible discolouration of lean at high concentrations" has been pointed out (Food Science Australia, 2006).

Interestingly, Botta et al. reported no significant difference in the microbial reduction when beefsteaks were either treated with water containing 6 ppm of ozone, water containing 100 ppm of AC (even if as KOCl), or pure water. It is also noteworthy that these treatments provided insignificant modifications to the microbial load also when compared to the untreated controls. As shown in Table 1, at day 0 the different treatments provided no significant difference in the microbial load, and even less reasonable results were obtained in the following days, as the control (untreated sample) was found to have a lower microbial load (though not significant) when compared to the treated samples (see the counts of total bacteria, total coliforms, and yeasts, in particular).

It appears that the spraying system that Botta et al. used for their investigation had design limitations, or perhaps the active/biocidal components were consumed within the system, before making contact with the beef samples. Moreover, the spraying time (90 s) could have been too short or the amount of sprayed solution was not enough to cover the beef samples (unfortunately, this volume is not reported in the article), or the test environment was not sufficiently sterile.

It is obviously difficult to imagine what the reason for the failure was but, certainly, there were some inaccuracies as to how the experiments were executed.

In summary, it is not really relevant (in our opinion) whether the subsequent investigation on spoilage bacteria and possible correlation with aroma (volatile) changes is sound or not; we believe that the initial results, i.e. the lack of any significant changes in microbial load after different sanitation treatments, are erroneous and, as a minimum, should have been explained. The authors should have developed a careful investigation to clarify the reasons of the failure of their sanitation approach, especially because their results are in contrast with all previous reports.

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