



Letter to the editor

Replies to the Authors' responses to “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,

In light of the responses of Botta et al. to the criticisms received with our first correspondence, we feel compelled to make further comments.

It is understandable that the EVA System® may have been improved, yet it is difficult to judge the efficiency aspects based on the data made available in the paper or from the technical sheets found on the De Nora website.

We commented that “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” (this was realised in 2012, using distilled water, analytical-grade salt and the first prototype patented; chemical conversion yield: 26.3%).

With the improved EVA System®, Botta et al. state that a solution with 4000 ppm of AC and a pH of 9.0 can be obtained by electrolysing a brine of 15 g/L of KCl, with a chemical conversion yield of 28%. Without knowing the applied current and the electrolysis time (which are the deciding factors to judge the efficiency) and focusing only on the chemical conversion yield, the two systems do not seem very different. On the other hand, it is well established that chlorine synthesis takes place at the anode together with water oxidation, while water reduction is the only significant reaction at the cathode. On these bases, in an undivided reactor initially filled with a neutral saline solution, the higher the chlorine production (or the active chlorine in the solution), the higher is the pH shift of the solution towards alkaline values. Accordingly, it is simply not possible to avoid the pH shift without changing the chemistry of the involved reactions (e.g. by electrolysing a solution made acidic by adding some hydrochloric acid). We do not know how the characteristics of the EVA System® have been improved, but no significant benefits can be expected in terms of pH shift if only water and salt are used.

In regards to the acronym BEW, it has probably first been used by Al-Haq et al. (2005) to refer to the catholyte, i.e. the solution produced at the cathode side of a two-chamber system; this solution has an alkaline pH (typically comprised between 10 and 13) and a negative value of ORP, due to dissolved hydrogen. Because of the presence of the diaphragm, the catholyte is not expected to contain active chlorine, and thus its inclusion among the “electrolysed waters” is somewhat misleading (as commented by Al-Haq et al. “Scientists have given various names to the water collected from the anode...”, and it is exactly to this water that a correct use of the terminology should refer).

The use of the abbreviation ‘NEW’ or the term ‘Neutral Electrolyte Water’ to refer to solutions with pH between 7 and 8 is also misleading: the neutral pH is the pH of pure water, in which the activities of protons and hydroxyl anions are equal. The exact value of neutral pH depends on temperature and ionic strength. In a soil, the pH is considered as neutral when comprised between 6.6 and 7.3. It is therefore acceptable

to tolerate a difference of a few decimal units, but in presence of a displacement of a pH unit (i.e. a pH of 8) the solution can no longer be considered ‘neutral’.

We explained that “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. 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”. This clearly indicates that EW can have different pH values, from those strongly acidic to perfectly neutral ones, depending on the management of hydraulic flows, in contrast with the statement by Botta et al. that “ECAS, also called ‘oxidising waters’ or ‘anolytes’ ... should refer to AEW”.

Regarding the choice of the salt, the paper by Sharan et al. (2010) is not relevant, as it discusses the effect of different salts present in water stored in copper and glass vessels. On page 107 of their article, Sharan et al. stated “Control experiments using glass-stored water containing the same inorganic constituents showed little change in counts in all cases (Fig. 1a–e), confirming that the observed results were due to storage in copper, rather than being an effect of time of storage”.

It follows that the biostatic/biocidal activity mentioned by Botta et al. is not linked to salts, but to the effects of the salts themselves on the copper vessel (the biocide activity was therefore due to copper ions).

Also the comment on the different levels of corrosiveness of NaCl and KCl is misleading: the work of Enestam et al. (2013) had the objective of comparing the effects of the two salts “on commonly used superheater steels at typical superheater tube temperatures (400–650 °C)”. As expected from a purely chemical point of view, Enestam et al. concluded their abstract saying “for practical applications, the two chlorides are equally corrosive on the tested steels under the experimental conditions considered”.

After trying unsuccessfully to respond to our comments on the chemical part of their investigation, Botta et al. have then tried to respond to our observations on the efficacy of active chlorine and ozonated water. However, evidence to support their opinions is weak.

Kalchayanand et al. (2008) investigated the effects of various antimicrobial compounds for the possible inactivation of *E. coli* O157:H7 on bovine heads with removed skin. An acidic electrolysed water (pH 2.8, 60 ppm of chlorine) was applied either as a rinse for 26 s, or as a final treatment for 13 s after an initial spray with alkaline water (for 13 s). Log reductions of 0.76 and 0.50 were obtained in the two cases, respectively, which were commented as follows “EO was not as effective in reducing *E. coli* O157:H7 on the surfaces of cheek meat as the other compounds tested. This may be due to organic materials like proteins, fats, and oils that could shield the bacterial cells from bactericidal activity of chlorine and low pH of EO”. Kalchayanand et al. found EO “not as effective as the

<https://doi.org/10.1016/j.ijfoodmicro.2018.10.018>

other compounds tested”, which does not mean that EO was ineffective (which is instead the result obtained by Botta et al.)

Veasey and Muriana (2016) investigated the use of EW (with 50 ppm of AC) and found that EW was not effective in reducing the levels of generic *E. coli* inoculated on the surface of beef carcasses. They commented “This could be due to a greater degree of soluble protein running off the surface of freshly slaughtered beef carcasses” and “the data suggests that the high organic load immediately beneath the inoculated areas (or soluble protein dripping over the inoculated cells) buffers the surface-inoculated cells from the hypochlorous acid, rendering it ineffective even though there is residual activity sufficient to kill bacteria in the recovered rinse solutions that is not observed with tap water”. It is clear that the experimental approach followed by Botta et al., in which no freshly slaughtered beef surfaces were considered (steaks were obtained 24 h after slaughter), is quite different from that considered by Veasey and Muriana, and therefore it is still quite misleading to compare the results obtained in the two cases.

As for Table 1 in the article by Botta et al., it is worth commenting that the article does not clearly allow to clearly understand how many samples have been taken into consideration: section 2.1 reports “3 different batches of beefsteaks [...] each batch divided equally into 4 parts of 7 beefsteaks per part” (84 steaks in total?); section 2.2 informs “five surface portions of 1 cm², cut from each side of the beefsteaks”; how many steaks were considered at each sampling time (bringing to 10 surface portions per steak)?

Table 1 reports means and SDs for each sampling time and each treatment; even without knowing the real numerosity of each sample, it seems quite curious that all the surveys provided the same mean and exactly the same SD, on Day 0. If these numbers are not independent replicas, there is still a misleading presentation of data.

We commented “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”. In our opinion, it is not only unreasonable that the treated samples (theoretically sanitised) provided higher microbial loads (although statistically not significant) than the untreated samples, but above all that EW and AO proved to be absolutely ineffective, without this inducing the authors to wonder if what they were doing made sense.

To answer the question asked by Botta et al. in their response to our comments (“Would a significant effect of the treatments have been a

success? In our opinion, this is not an independent scientific approach”), the problem is not to have an independent scientific approach, but to understand what is being done. If sanitisers are called in this way, it means that they allow for sanitisation; the presence of high organic loads can certainly limit their effectiveness, to the point that no effects are found, but the direct consequence is that the treatment was useless, and therefore... what is the interest to perform the subsequent investigation on microbiota dynamics? Considering that “Meat spoilage microbiota is known to show a high lot-to-lot variation that decrease progressively along the storage time” and based on the lack of sanitisation effects discussed above, the conclusions of the article (“the here performed treatments with EW and AO were not able to reduce the initial microbial counts of the products. Moreover, they were incapable of modifying the microbiota composition, dynamics and the related volatime to any great extent during chilled VP storage”) seem to be a logical consequence that could have been foreseen a priori. In other words, Botta et al. could have carried out their study considering only untreated samples and obtaining the same results; in this hypothetical case, the title would have been different and all these criticisms would never have been raised.

References

- Al-Haq, M.I., Sugiyama, J., Isobe, S., 2005. Applications of electrolyzed water in agriculture and food industries. *Food Sci. Technol. Res.* 11, 135–150.
- Enestam, S., Bankiewicz, D., Tuiremo, J., Mäkelä, K., Hupa, M., 2013. Are NaCl and KCl equally corrosive on superheater materials of steam boilers? *Fuel* 104, 294–306.
- Kalchayanand, N., Arthur, T.M., Bosilevac, J.M., Brichta-Harhay, D.M., Guerini, M.N., Wheeler, T.L., Koohmaraie, M., 2008. Evaluation of various antimicrobial interventions for the reduction of *Escherichia coli* O157:H7 on bovine heads during processing. *J. Food Prot.* 71, 621–624.
- Sharan, R., Chhibber, S., Attri, S., Reed, R.H., 2010. Inactivation and sub-lethal injury of *Escherichia coli* in a copper water storage vessel: effect of inorganic and organic constituents. *Antonie Van Leeuwenhoek* 98, 103–115.
- Veasey, S., Muriana, P., 2016. Evaluation of electrolytically-generated hypochlorous acid (“electrolyzed water”) for sanitation of meat and meat-contact surfaces. *Foods* 5, 42. <https://doi.org/10.3390/foods5020042>. (1–15).

Sergio Ferro^{a,*}, Tony Amorico^a, Permal Deo^b

^a *Ecas4 Australia Pty Ltd, 8/1 London Road, Mile End South, SA 5031, Australia*

^b *Sansom Institute for Health Research, School of Pharmacy and Medical Science, University of South Australia, Adelaide, SA 5000, Australia*
E-mail addresses: sergio@ecas4.com.au, fre@unife.it (S. Ferro).

* Corresponding author.

¹ On leave from Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara 17, 44121 Ferrara, Italy.