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”

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Dear Editor,

herein our response to criticisms posed by the Authors to our scientific paper 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. All comments are referred to specific sections of the original text of the letter and reported in parenthesis.

Despite the first author is included within the inventors (**Lines 32-33**) of the European Patent EP 2207415 B1, we want (the company owning the patent and us) to clarify that the current electrolyzed water (EW) generator named EVA system® has been improved from the first prototype patented, especially it has being modified in several efficiency aspects. For instance, it is known that important parameters for efficacy of the produced EW, such as oxidation reduction potential (ORP), free chlorine (AC) and pH can be significantly changed by the electrical current value, flow rate, electrode material, electrolysis time and amperage of EW generator (Rahman et al., 2016). Therefore, we confirm that EVA System® provided by De Nora S.P.A. produces a mother solution of 4,000 ppm of AC at pH 9.00 and a KCl residual of 1 %, which was obtained electrolyzing a brine of 15 g/L. More information on the EVA System® and its technical sheet can be easily and freely found at the company website for a further confirmation of this set up and machine features.

Besides this, we strongly disagree about the Author's criticisms over the terminology (**Lines 50-60**). To best of our knowledge the term "electrolyzed water" includes basic electrolyzed water (BEW), acid electrolyzed water (AEW), neutral electrolyzed water (NEW) and slightly acid electrolyzed water (SAEW). Most of the EW machines can be divided into 2 types: machines (i) with 2-cell chambers separated by diaphragms that produce

separately from NaCl brine the AEW (HOCl, $^{-}$OCl, HCl, O_2, Cl_2) and BEW (NaOH, H_2), respectively from anode and cathode compartments; and machines (ii) without diaphragm that produce in a single chamber NEW and SAEW, respectively from NaCl or HCl (alone or in combination with NaCl) (Rahman et al., 2016). In this frame the "Electrochemically activated solutions" (ECAS), also called "oxidizing waters" or "anolytes" by the Authors (Ferro et al., 2018) should refer to the AEW, which (by the way) has shown limited efficacy on utensils, food products, and surfaces owing to various factors, the most important of which include the type of surface, presence of organic matter, and type of tape water used (Rahman et al., 2016). In any case, it is not our intention to debate here the pros and cons shown in scientific literature by the different type of EW.

As far as the final free chlorine of the "net solution" is concerned (**Lines 41-49**), the AC has been measured by iodometric titration (APHA, 1992) before and after the dilution with distilled water (Laureano et al., 2016; Yaseen et al., 2016). In relation to these measurements the "net solution" were freshly prepared for each experiment at 100 ppm of free chloride (pH ~ 7.0; thus a NEW). We are aware of the pH effect on free chlorine concentration, the decrease of chlorine due the interaction with organic matter and along the time (investigation on the chlorine decomposition in different storage condition is currently ongoing for this NEW), as well as the differential effectiveness existing between hypochlorous acid and hypochlorite (Athayde et al., 2017; Rahman et al., 2016). Anyway, we do not want go into the merits of pH variations and dilutions hypothesized by the authors, because are based on their personal experience and not supported by data. Moreover, we believe we have provided all information necessary to repeat the preparation of electrolyzed water in the Material and Methods section, which has already been peer reviewed, as all the article.

Regarding the choice of the salt (**Lines 37-40**), we simply preferred to avoid any unwanted biostatic/biocide activity by any residual NaCl during the subsequent storage (Sharan et al., 2010). Moreover the potential residual NaCl has been proven more corrosive than KCl on some type of steels (grid containing the beefsteaks during treatments) (Enestam et al., 2013).

As rightly stated by the Authors (**Lines 61-67**), in three of the studies cited (Ding et al., 2010; Jadeja and Hung, 2014; Tango et al., 2014) different types of EW or their combination have been effective in reducing bacterial

count of meat surfaces, contrariwise to our outcomes, even if only Jadeja and Hung (2014) have tested a neutral EW. On the other hand we cannot compare results from Bosilevac et al. (2005) to ours, since they sprayed beef hides with BEW and AEW at 52 and 60 °C respectively, and high temperature can significantly improve the sanitizing efficacy of EW (Cao et al., 2009; Fabrizio and Cutter, 2003). Anyway, in literature we can also find studies that highlight a limited or absent reduction of microbial loads on beef carcass sprayed with EW (from 60 to 250 ppm of free chlorine) (Kalchayanand et al., 2008; Veasey and Muriana, 2016).

As far as the ozone is concerned, it is surprising the Authors' attempt (**Lines 68-78**) to demonstrate the efficacy of aqueous ozone (AO) in the sanitization/decontamination of meat by citing Rice et al. (2002) (a magazine article dealing on the use of ozone in food industry as surface sanitizer and its legislation in USA) and another magazine article dealing the use of AO and ozone in winery environments (Hampson et al., 2000). It is also noteworthy that in the report proposed in **lines 79-84** (Hallam and Hiscock, 2006), which collects references on this topic and does not shown original data, some of the cited studies confirm the efficacy of AO in decontamination of meat/beef carcass (Bosilevac et al., 2005; Gorman et al., 1995) and others a limited or absent activity (Castillo et al., 2003; Reagan et al., 1996).

Regardless from the number of references demonstrating or not the efficacy of AO and EW, in the Table 1 of our article (Botta et al., 2018) at day 0 the value refer to the beefsteak before the treatments, contrariwise of what argue by the Authors (**Lines 85-89**) and in agreement to what is reported in our Material and Methods section: " *The samplings were performed before the treatments, for each treatment and each batch, on the first day and after 5, 9 and 15 days of storage at 4 °C*". Unlike most of the studied cited above, in our study we wanted to check the efficacy of the two treatments along the shelf life of the product on the market , which thus starts from the first day after the packaging of the meat (day 1). The authors focused their attention on some "not reasonable" differences between EW/AO treated and untreated meat, but neither significantly lower nor significantly higher values can be observed between them looking at the microbial and pH dynamics shown in Table 1.

Concerning the experimental set up, both treatments timing and concentrations (**Lines 94-98**) have been chosen to avoid the risk of discoloration, which is reported also by the same Authors (**Lines 82-84**) as an effective side effect, especially for AO. The reasons of our choices also reported in the Discussion part: "*However, the limiting factors for applying AO and EW to raw beef re- main undoubtedly their concentrations and exposure times, which were here chosen on the basis of the acceptability of the colour of the meat after treatments and considering the effectiveness of treatments per- formed by spraying AO and EW on different meats and seafood products*."

Finally, we firmly disagree with the biases expressed by the Authors in the **lines 99-106**: "*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*."

As all experimental methods applied in small-scale processing line, this approach can be improved and implemented, but however the experiments performed in Botta et al. (2018) were executed with the maximum scientific rigor. It is strange, from our point of view, that Authors consider a limited/non significant effects of the two experimental sanitizing treatments as an unacceptable "failure", which was "certainly" caused by inaccuracies that however are "difficult to imagine". The failure is the state or condition of not meeting a desirable or intended objective, in opposite of success. Would a significant effect of the treatments has been the success? In our opinion, this is not an independent scientific approach. The objective of our work, considering all its possible methodological limitations, was not to confirm at any cost the efficacy of these two specific treatments with EW and AO, but to define their action on meat microbiota dynamics by means of culture-dependent and -independent approaches along the shelf-life. The overall evaluation of EW or AO as meat sanitizer need undoubtedly further investigation, and the final judgment on the suitability of these treatments is absolutely beyond the purpose of this work. In light of this we cannot consider our outcomes detrimental for the body of knowledge of this subject.

Despite the Author consider our results not relevant (**Lines 101-104**) in relation to the initial "failure" of the treatments, we believed that the RNA-based analysis integrated with the volatilome has helped to unravel the complexity of the potentially active microbiota, in this way expanding the current knowledge on the spoilage dynamics of vacuum packaged beefsteaks (as reported in the conclusions). This because the spoilage dynamics of raw meat have mainly been studied with DNA-based HTS approaches so far, which cannot be associated to the volatilome only produced by active microbiota.

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