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HYGIENE INDICATORS OF PORK MEAT TREATED WITH ELECTROACTIVATED WATER

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ABSTRACT

Electroactivated water (EAW) has been growing in popularity in recent years in many countries. The use of EAW during meat production improves some hygiene indicators, and is a cheap, safe, non-toxic and effective option for improving the quality and extending the shelf life of meat.

In the present study, the influence of different types of EAW on some hygienic indicators of pork meat was studied. The following indicators were studied: total microbial count (TMC), amount of *Escherichia coli* and coagulase-positive staphylococci, as well as the presence of *Salmonella* spp. and *Listeria monocytogenes*.

All tested EAW solutions can be used to improve the hygiene in pork meat production, with the most effective in long term being the treatment with acidic water (AW), and in short term – the treatment with highly alkaline water (HALW).

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Key words: electroactivated water, hygiene indicators, pork meat, storage.

Introduction

Electroactivated water (EAW) is a new trend in water treatment that use nanotechnology principles. It was initially studied by Shimizu and Hurusawa (1992) in Japan, who described its bactericidal properties useful in the food industry. Hricova *et al.* (2008) noted the apparent increasing popularity of EAW as a cleaning agent used in the food industry in many countries.

Hsu (2005) provides a general description of an EAW generating device. It consists of a cylinder in which water is placed and two electrodes under a constant electric current. Positive and negative ions pass through a semipermeable membrane separating the two electrodes. A different solution is formed around each electrode. The anode produces an anolyte with a pH of 2.3-2.7 and a high (over 1000 mV) oxidation-reduction potential (ORP) containing free chlorine ions. At the other electrode, the cathode produces a catholyte with a pH of 10.0 - 11.5 and a very low ORP (-800 to -900 mV) containing dissolved hydrogen. According to Marriott and Gravani (2006), the presence of chlorine can disrupt protein synthesis, oxidative decarboxylation of amino acids to aldehydes and nitrites, and cause metabolic imbalance by destroying key enzymes in the microorganisms.

In their research, Suvorov *et al.* (2017) proved the positive influence of EAW on the freshness and hygiene of poultry and beef. Cloete (2015) investigates the use of catholyte and anolyte in different concentrations as a disinfectant in a poultry slaughterhouse during scalding and water cooling, as well as by spraying chicken carcasses before and after evisceration. Anolyte solution diluted 1:10 gave the best results in all forms of application. The use of catholyte did not give similar results. On the contrary, the author believes that catholyte can disperse bacteria during scalding without killing them, thereby leading to a higher level of contamination.

Fabrizio & Cutter (2004) studied the bactericidal effect of fresh anolyte water on artificially contaminated fresh pig carcasses with feces containing *Listeria monocytogenes*, *Salmonella Typhimurium* and *Campylobacter coli*. The study showed that a 15-second treatment (spraying) with anolyte could eliminate some undesirable bacteria, e.g. *Campylobacter* spp., however, for other pathogens such as *L. monocytogenes*, *S. Typhimurium*, *E. coli*, a longer exposure – more than 10 min – was required.

The effect of different concentrations of neutral EAW on pure cultures of *E. coli* O157:H7, *Salmonella Enteritidis* and *Yersinia enterocolitica*, and pork skins and meat contaminated with the same pathogens were studied by Han *et al.* in 2018. The results showed a good bactericidal effect as early as the second minute of exposure, both for pure cultures and for pork products. The organic matter in pork meat had a negative effect, which led to a weakening of the bactericidal effect of EAW, compared to the studied pork skins. The antibacterial effect is closely dependent on the EAW concentration, with a full bactericidal effect on pure cultures of the studied pathogens being achieved at an EAW concentration above 25% for 5 min.

The bactericidal effect of acidic and alkaline EAW, when sprayed on the surface of beef, goat and pork meat inoculated with *E. coli* K12, was investigated by Arya *et al.* (2018). The meat samples were subjected to different treatment times (from 2 to 12 minutes) and the reduction in the number of microorganisms was determined. Beef, goat and pork meat samples treated with acidic EAW showed the highest log reductions of approximately 1,16 (4 minutes), 1,22 (12 minutes) and 1,30 log CFU/mL (10 minutes), respectively. Treatment with alkaline EAW showed up to 1,61, 0,96 and 1.,52 log CFU/mL reduction, respectively.

Mansur *et al.* (2015) investigated the effect of slightly acidic anolyte, fumaric acid and their combination on the reduction of TMC, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *S. Typhimurium* in fresh pork. The authors also investigated the shelf life of the pork meat during storage at 4-10°C. The results showed that immersion of fresh pork meat in a mixture of slightly acidic anolyte and 0.5% fumaric acid at 40°C for 3 minutes gave the best effect compared to the other treatments. The same combination significantly slowed down the growth of TMC in the pork meat during storage at 4°C. Similar results were obtained in the study of shelf life – extension by 6 days when stored at 4°C and by 4-5 days when stored at 10°C, compared to the control sample of untreated pork meat.

Rahman *et al.* (2013) studied fresh pork meat inoculated with *E. coli* O157:H7 and *L. monocytogenes*. The authors investigated the effect of immersion in different solutions (distilled water, aqueous ozone solution, 3% lactic acid, 3% calcium lactate, sodium hypochlorite solution, low-concentration EAW, highly acidic EAW, and low-concentration EAW + calcium lactate) for 5 min at room temperature. The highest reduction (3.0–3.2 log CFU/g) was achieved with low-concentration EAW + calcium lactate, which was significantly different from the other treatments. The authors concluded that this combination also extended the shelf life of pork by up to 6 days at 4°C storage.

Accumulated scientific data in recent years around the world, as well as the lack of similar research in our country, led us in the present study to investigate the effectiveness of different types of EAW in their use to improve the hygiene of meat production in pig slaughter processing.

Materials and Methods

The subject of the study were pig carcasses of different weights obtained in a slaughterhouse. Sampling of the carcasses for microbiological analysis was carried out in accordance with ISO 17604:2015 in the following order: after the start of the slaughter of the animals in the respective company, the first 5 slaughtered and processed animal carcasses were passed; samples were taken from the 6th, 10th and 14th carcasses, before the wet toilet. Individual pieces of meat with fat were taken from the surface of each carcass with an approximate average thickness of about 2 cm, each piece having approximate dimensions of 30 cm/60 cm. The pieces of meat were put separately into plastic sterile sample bags and transported to the laboratory in a cooler box. Each piece was cut with a sterilized knife into 18 smaller pieces with a surface area of 100 cm^2 (10 cm/10 cm), each of which was hung on a sterilized hook.

The pieces were grouped by three from each carcass, and each group was showered with equal volume (1300 ml) of: tap water (TW), slightly acidic water (SAW), acidic water (AW), strongly acidic water (HAW) and strongly alkaline water (HALW). One group was also left as a control (C) – without washing. The washed hanging pieces were left to drain. After 30 minutes of exposure to the applied solutions, swab samples were made from each piece. The swabs were made with a dry sterile cotton swab from the surface of each piece, after which the swab was placed in a sterile tube with Amies transport medium.

Samples were taken immediately after treatment with EAW solutions (0 day), at 24 hours and on the 5th day of refrigerated storage (0-4℃).

An Ashbach-5 water ionizer was used to produce the electroactivated water solutions.

To determine the pH and ORP parameters of the EAW solutions, a pH-ORP meter – Consort C1010 was used. The parameters of the EAW solutions used in the experiment are shown in Table 1.

Table 1: Parameters of the EAW solutions used in the experiment.

EAW	ORP [mV]	pH
TW	+25,00	6,65
SAW	+87,33	5,55
AW	+206,33	3,51
HAW	+211,33	3,22
HALW	-217,00	10,47

Each sample from the different groups was tested for the following indicators: Total microbial count (TMC), according to the requirements of BDS EN ISO 4833-1:2013; Enumeration of *E. coli*, according to BDS ISO 16649-2:2014; Enumeration of coagulase-positive staphylococci according to BDS EN ISO 6888-1,2:2022. Presence of *Salmonella* spp., according to BDS EN ISO 6579-1:2020 and presence of *L. monocytogenes* according to BDS EN ISO 11290-1:2017.

The results were processed mathematically, and the mean and standard deviation were determined. To test the statistical dependence and reliability of the results, analysis of variance (ANOVA) single factor analysis for repeated measures was applied. The significance of the results was defined at a significance level of $P < 0,05$. The Microsoft ®Office Professional Plus Excel 2013 program (15.0.4569.1506) was used for the calculations.

Results and Discussion

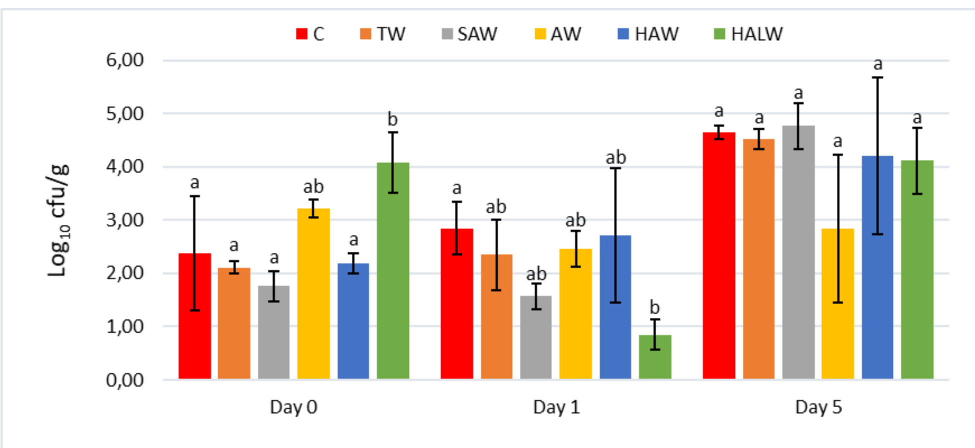
The obtained mean results for the indicator TMC are expressed as log_{10} CFU/g and are presented in Table 2. The obtained values for TMC for each individual day of the experiment differ significantly from each other. All TMC values are within the norm, according to European requirements (Regulation EC No. 2073/2005). Similar results for TMC were also obtained by Mansur *et al.* (2015).

Table 2: Total microbial count per treatment (mean and standard deviation presented as log_{10})

Sample	Day 0^{a}	Day 1^{b}	Day 5^{c}
	log_{10} (CFU/g)	log_{10} (CFU/g)	log_{10} (CFU/g)
C	$2.38^{a,x}$±1.07	$2.85^{a,x}$±0.50	$4.65^{b,x}$±0.13
TW	$2.11^{a,x}$±0.11	$2.35^{a,xy}$±0.66	$4.52^{b,x}$±0.19
SAW	$1.76^{a,x}$±0.28	$1.57^{a,xy}$±0.24	$4.77^{b,x}$±0.43
AW	$3.22^{a,xy}$±0.16	$2.46^{a,xy}$±0.34	$2.84^{a,x}$±1.38
HAW	$2.19^{a,x}$±0.19	$2.72^{a,xy}$±1.26	$4.21^{a,x}$±1.47
HALW	$4.09^{a,y}$±0.57	$0.85^{b,y}$±0.29	$4.12^{a,x}$±0.62

Designations with different letters (^{a,b} – in rows; ^{x,y} – in columns) indicates a significant difference at P<0.05.

The effect of treating pork meat with different types of EAW on TMC is presented in Figure 1. Treatment with SAW has the best effect immediately after washing, compared to the control sample. A similar result is also given by HAW. Although on the day of treatment HALW shows significantly worse results compared to the control sample, after 24 hours a significantly significant decrease in the number of microorganisms is observed, which, however, is not maintained and on the 5th day TMC again reaches high values. The effect of the other solutions is similar on the 24th hour after washing and storage. Washing with SAW continues to have the best results, but as with treatment with HALW, an increase in the number of microorganisms is again observed on the 5th day of refrigerated storage.



Designations with different letters (^{a,b} – for each individual day) indicates a significant difference at P<0.05.

Figure 1: Total microbial count per treatment

Figure 2 visualizes the trends in the change of TMC in dynamics during storage for the individual treatments. For most of the used aqueous solutions, a classic curve of development of microorganisms on the surface of pork meat is observed, as proven by other authors (Fabrizio & Cutter 2004; Mansur *et al.* 2015). An exception to this classic model is the curve of the AW. Although at the beginning of the experiment (Day 0) TMC is above the level of inoculation of the control sample, on the first day after treatment, and by the end of the experiment, a significantly lower microbial content is observed compared to the control sample. The reduction of TMC on the fifth day in this sample reaches 2,84 log, compared to 4,65 log in the control sample and 4.52 log in the sample treated with tap water, which is about 40% and 37% less, respectively. A similar decrease in the amount of TMC was observed on the fifth day of treatment with AW, compared to the first day, although to a lesser extent – 12%. All this indicates an effective reduction in TMC after treatment with AW solutions, both in short term (24 hours) and with longer storage. Treatment with HALW is also effective, where after 24 hours a significant and reliable decrease in the number of microorganisms is observed, and on the 5th day, although the TMC is increased it remains below the level of the control sample and the sample washed with tap water.

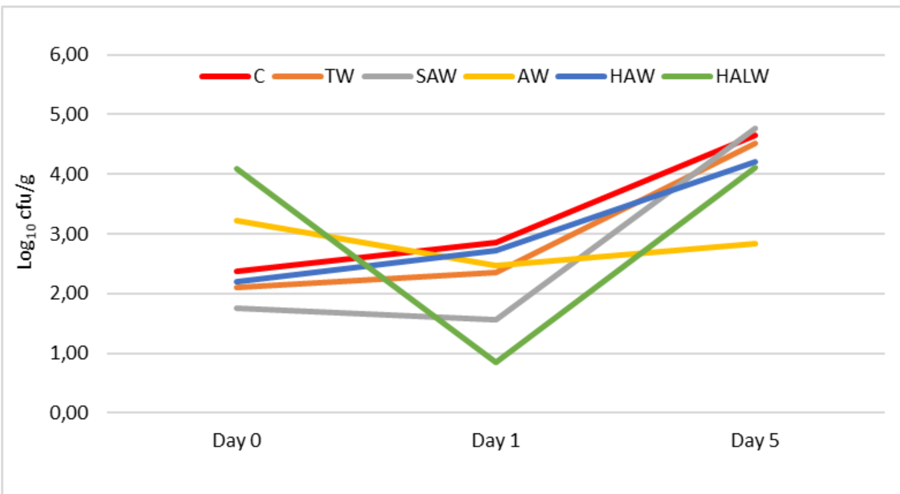


Figure 2: Changes of total microbial count during cold storage.

Throughout the entire period of the experiment, the TMC values in the HAW and TW treatments remained below the levels of the control sample, and at the end of the experiment (day 5) washing with HAW gave better results than washing with TW.

Other authors (Rahman *et al.* 2013; Mansur *et al.* 2015) obtained better and longer-lasting results when treating pork meat with EAW, which is probably due to the fact that they treated the meat by soaking it for a different period of time, rather than by washing, as in our experiments.

Table 3 shows the results of the conducted studies for the enumeration of *E. coli*. The presence of *E. coli* in the studied samples is sporadic and in insignificant quantities. In none of the studied samples was *Salmonella* spp. and *L. monocytogenes* detected. Similar results are observed in the studies for coagulase-positive staphylococci.

Table 3: *Escherichia coli* count of the samples.

№ Sample	Day 0 CFU/g	Day 1 CFU/g	Day 5 CFU/g
C-1	<1	<1	<1
C-2	0.7×10^1	<1	<1
C-3	<1	<1	<1
TW-1	<1	<1	1.4×10^1
TW-2	<1	<1	<1
TW-3	<1	<1	<1
SAW-1	<1	<1	<1
SAW-2	<1	<1	<1
SAW-3	0.6×10^1	<1	<1
AW-1	<1	<1	<1
AW-2	<1	<1	<1
AW-3	<1	<1	<1
HAW-1	<1	<1	<1
HAW-2	<1	<1	0.7×10^1
HAW-3	<1	<1	<1
HALW-1	0.8×10^1	<1	<1
HALW-2	1.1×10^2	<1	<1
HALW-3	<1	<1	<1

The obtained results indicate better hygiene in meat production and compliance with good manufacturing practices. Most likely, this is the reason why a number of other authors conducted their studies after preliminary controlled contamination. Results of Ding *et al.* (2010), Ye *et al.* (2017) and Arya *et al.* (2018) showed a significant decrease in log of *E. coli* when treated with acidic and neutral EAW. Other authors (Han *et al.* 2018) studied the effect of EAW on *Salmonella* spp. and proved a full bactericidal effect on pure cultures of the tested pathogens. In their studies on *L. monocytogenes* (Park *et al.* 2004) confirmed a full sterilization effect of EAW in a pH range between 2,6 and 7,0 and a residual chlorine level greater than 2 mg/l.

Conclusion and Recommendation

Treatment with AW, HALW, SAW and HAW can be used to improve the hygiene in pork meat production.

All tested EAW solutions have a beneficial effect on the hygiene in pork meat production in the short term, with the most effective treatment being HALW.

The use of AW, HALW and HAW for washing the surface of pork meat has a beneficial effect in the long term, with the most effective treatment being AW.

Hygiene in slaughterhouse meat production of pork carcasses is in accordance with the requirements in the country and European standards.

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