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EFFICIENCY OF SLIGHTLY OXIDIZED ELECTROLYZED WATER (EO) FOR IMPROVEMENT OF FERMENTED SAUSAGE

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ABSTRACT

Electrolyzed water (EW) is a brand-new technology that arisen as a novel technology with probable application in foods industry, primarily in microbiological aspects, with various application modes as washing, spraying or dipping the food using solution containing such any different types of such water. The antibacterial activity of slightly oxidized electrolyzed water (EO) was tested using fermented sausages and exhibited a considerable decline in coliform, and increase in lactic acid bacteria (LAB) counts compared to control sample, while psychotroph, mold & yeast counts showed an increase slightly lower in treated samples in comparison to control one. Total coliform count recorded (mean $\log_{10}$cfug/±SD) 1.60±0.02, and 1.55±0.06 at zero time for both control and EO treated samples, respectively reaching to 1.97±0.4 at the end of storage for control sample, while the treated EO samples decreased to <1. In addition (LAB) showed means of 6.38±0.07 and 8.15±0.03 at zero time and the 18th day of storage for control, meanwhile the EO treated samples results were 6.43±0.028 and 8.96±0.0 at zero time and the 25th day of storage, respectively. Psychotropic counts were 1.08±0.07 and 1.05±0.06 for control and treated EO at zero time and reached 3.86±0.02 at the 18th for the control and 2.79±0.02 at the 25th day for the treated EO. Mold and yeast count revealed a mean of 2.74±0.13 for control, moreover, EO treated samples exhibited 2.12±0.04 at zero time, reaching 5.88±0.03 at the 18th for the control and 4.36±0.23 at the 25th day for the treated EO, also *E. coli* and *S. aureus* recorded < 1 log cfu/gm in all examined samples. Neither *Salmonella enterica* nor *L. monocytogenes* were isolated from either control or treated sample. Chemical analysis (TVB-N, TBA and pH) also was determined to relay the freshness of the sample groups; spoilage of the control samples was noticed at the 22th day, while the treated EO acceptance was prolonged to the end of the work. Sensory examination revealed that EO can maintain the sensory attributes of the fermented sausages and increase its shelf life, at the 22nd day of storage, control samples were completely spoiled organoleptically while the EO treated ones was sound (tissues still hard, adhesive and cohesive) and it remained healthy until the end of the experiment (day 25th) recording (5.37±0.15, 5.67±0.21, 5.97±0.15 and 5.67±0.30) for odor, appearance, texture and overall acceptability, respectively. The objective of this study was to examine the efficacy of slightly oxidizing electrolyzed water (EO) for reducing the microbial load and extension of shelf life of fermented sausage.

KEYWORDS: Slightly oxidized electrolyzed water (EO), fermented sausage, quality, Sensory criteria, microbial counts, TVB-N, TBA, Ph.

INTRODUCTION

Raw meat is an ideal medium for growth of many microorganisms due to its high moisture content (70–80%), also its content of proteins, amino acids, fatty acids, peptides, vitamins and minerals, so meat should be preserved in a manner to prevent its contamination with food-borne microorganisms as it is considered a highly perishable product **(Askild *et al.*, 2017**). Fermented sausage is one of the most famous treasured traditional foods, nowadays, a massive number of different recipes and manufacturing processes are used in its production **(Pereira and Vicente, 2013).** Fermented sausage consumption has been allied with harmful effect caused by the pathogenic microorganisms which can be imported by way of raw materials contamination or via cross-contamination from personnel, equipment's, throughout processing or at retail points **(Askild *et al.*, 2017**).

Achieving the aim of food safety and quality is an important point of concern for food manufacturers, retailers, researchers, regularity authorities, and policymakers in developed as well as developing countries **(Kang, 2019**). Food-borne illness outbreaks

incidences are still predominant in the food service sector, including food stores, institutions, and fast-food restaurants, where food commodities receive multiple treatments to ensure their safety for consumption **(Mun, 2020).** With increasing demands for processed food, the food chain is becoming complicated in terms of transportation, handling, storage, and processing, rendering the maintenance of a safe food chain supply a challenging task **(King *et al.*, 2017).** A lot of techniques have been designed to control incidences of foodborne diseases to provide a safe food supply **(Davidson *et al.*, 2017**). Electrolyzed water (EW) is a new trend that appeared in the last years with potential application in foods, mainly in microbiological aspects, with variable application modes, either dipping the food in solution, or in the form of spray or washing like in fresh vegetables and other products **(Athayde *et al.*, 2018).** Electrolyzed water including EO is a promising strategy for preservation of raw meat, ready-to-eat meat, chicken, fish, and many other food products without affecting their sensory characteristics. In this concern, EW can be applied to different types of food and against different pathogens **(Khalid *et al.*, 2020 & 2023).**

Electrolyzed water is a green chemical technology which has attained demand as a disinfection technique **(Leães *et al.*, 2020),** also it has been concerned as a sanitizer due to its antimicrobial activity against a broad spectrum of microorganisms within a brief time. The formulations of EW are occurred in an electrolysis chamber, containing a solution of hydrogen chloride (HCl^-) or dilute salt (NaCl), which all-inclusive a separate two chambers as one containing cathode and the other chamber containing the anode pole. According to the production conditions, the electrolyte solution and the used apparatus, EW can be classified as neutral, acidic, or alkaline **(Pangloli *et al.*, 2013)**. Plentiful studies noted the potential antimicrobial activity of electrolyzed water against a lot of microorganisms **(Zhao *et al.*, 2021).** Lately, EW has applied in medicine, dentistry, agriculture, and the food industry **(Yan *et al.*, 2021; Rahman *et al.*, 2016).** Electrolyzed water has been used as antimicrobial agent for poultry carcasses production and as a detergent and sanitizing agent for cutting tools and processing equipment's in different food establishments **(Moghassem *et al.*, 2020)**. Comparing EW with other sanitizers revealed an overall reduction in pathogens population, **Al-Holy and Rasco (2015)** concluded that *E. coli* O157:H7 and *Salmonella typhymurium* was decreased by 1.5–1.6 $\log_{10}$ cfu/g in alkaline EW treated beef after soaking for 10 min. Electrolyzed water considered cheap and effective product than other ordinary cleaning and sanitizing agents **(Afari and Hung, 2018 and Hsu *et al.*, 2019).** Preference of EO is as a result of its safety, it is not corrosive for tissues, more ever, no hazardous chemicals added during the production, has less adverse effect on the environment and it becomes ordinary water again, also, very little side effects, almost low cost than other sanitizers, easy to process, and microorganisms do not achieve resistance. **(Al-Holy and Rasco 2015; Xuan and Ling 2019).** Moreover, EW eradicates pathogenic microorganisms **(Li *et al.*, 2020)** and protects the environment from the unfavorable impacts of hazardous chemical disinfectants **(Han *et al.*, 2017).** Electrolyzed oxidized water (EO) is considered more efficient in pathogens reduction of contaminated food in comparison to other hazardous chemical sanitizers, and it has attained powerful aspects in the food, agriculture, and pharmaceutical industries, as it is formed in an environmentally friendly way from sodium chloride and distilled water **(Afari and Hung, 2018)**. Electrolyzed water recovered to its initial form without assuming any risk to the environment or consumers after usage, and the performing cost included the original cost of purchasing a generator, water, chemical salts, and electricity charge, and it is onsite production is beneficial, therefore, it can be produced and used without storage or time consuming as a result of transportation **(Hricova *et al.*, 2008).** Electrolyzed water can be practiced in a varied field of food products and by that is a convenient alternative for synergistic microbial control in the food industry to assure safety and quality of food without altering its sensory properties **(Rebezov *et al.*, 2022).** Slightly oxidized water (EO) is made in a single or double cell unit(s) with a pH of between 5.5 - 6.5, an oxidation-reduction potential (ORP) from 800 to 900 mV, and an available chlorine concentration (ACC) between 10 and 80 ppm **(Guentzel *et al.*, 2008; Bansal *et al.*, 2018 and Rivera-Garcia *et al.*, 2019).** Slightly oxidized water (EO) has been produced by electrolysis of 2-6% HCL or NaCl of 0.1-0.2% **(Athayde *et al.*, 2018).**

MATERIAL AND METHODS

Fermented sausages production

Two batches of fermented sausage were produced using 5 kg of lean beef and fat (2:1), 1st one, as a control; contained seasoning formula per 1 kg beef meat consisted of 1.9% sodium chloride and 120 ppm sodium nitrate (Sigma chemical co., St. Louis, Mo), then adding of spices including 5% cumin, 0.42% paprika, 0.42% black pepper and 0.25% dextrose for curing (Difco laboratories inc., Detroit, MI). Following the mixing, the batter was inoculated with starter culture, *Lactobacillus acidophilus*, *Sacromyces cerevisiae* and *Bifidobacterium longum* to achieve a cell concentration into the batter of 10^7 cfu/g, for each starter. The 2nd batch, in which the lean beef was immersed in EO for 5 mints before mincing, with addition of the aforementioned ingredients as in batch one except sodium nitrate. The batter was stuffed into natural large diameter beef casing, hand tied with cotton strings at 15 cm intervals. Each sausage link was clearly labeled to differentiate between both of control, EO. Sausages were hung vertically in an environmentally controlled incubator for fermentation at 22^{o}C for 5 days, the sausages were then dried at 18^{o}C for 7 days then stored at 4±1^{o}C (**Asmaa *et al.*, 2013).** Finally, the sausage was sampled for sensory, bacteriological and chemical analysis at days 0, 3, 6, 9, 12, 15, 18, 22, 25 during storage.

Preparation of oxidized electrolyzed water

Sufficient amount of potable drinking water was prepared with addition and dissolving of 2 g sodium chloride (NaCl) / liter of potable water 9-10-volt amber current (VA) was passed through water using an electrolysis cell with two poles of anode (+) and cathode, NaCl was dissociated into Na^+ and Cl^-. Meanwhile, at the anode side, water was oxidized to give O2 gas according the following equations: -

- **$2\ H2O\ (l) \rightarrow 4\ H^+$ (ions) + O2 (gas) + 4 e^-,**
- **$2\ NaCl \rightarrow Cl2$ (gas) + 2 Na^+**

The final results are the formation of acidic solution (pH 5 – 6.5) containing Hypochlorus acid (**HOCl**), Hypochlorite ions (**OCl^-**), Hydrochloric acid (**HCl**) and chlorine gas (**Cl2**) **(Athayde *et al.*, 2018; Khalid *et al.*, 2023).**

Samples preparation

The aforementioned prepared two batches was divided according to the timeline of analysis and packed separately in polyethylene bags and stored at 4±1°C and examined at zero-time, 3rd, 6th, 9th, 12th, 15th, 18th, 22th and 25th days of storage. The experiment was repeated in triplicate.

Sensory analysis

It was carried out based on odor, appearance, texture and overall acceptability by (10) specialized panelists, the panelists were asked to score independently using 10-point hedonic scale according to **Chen *et al.*, (2016)**. All samples were evaluated in triplicate and the evaluation was performed according to the following Evaluation Sheet Key.

Sensory evaluation sheet key									
Very poor (Dislike)	**poor**		**Border line**	**Good**		**Excellent**			
1	2	3	4	5	6	7	8	9	10

Bacteriological examination

Preparation of food sample homogenate according to APHA (2001)

Ten grams of fermented sausage sample of both batches were homogenized for 1 min. with 90 ml of sterile peptone water (0.1% w/v). One ml from homogenate was transferred to a separate sterile test tube containing 9 ml of sterile peptone water, then tenth fold serial dilution were prepared up to 10^6**.**

Lactic acid bacteria count according to APHA (2001)

0.1 ml of tenfold serial dilution was streaked on MRS (Man -Rogosa-Sharpe) agar media. The inoculated plates were incubated anaerobically at 37°C for 72 hrs. The number of colonies were counted and recoded as $\log_{10}$cfu/g sample.

Total coliform count according to FDA (2002)

One ml of each serial dilution of samples homogenate was poured in sterile petri dishes, then 15 ml of violet red bile agar (VRBA) was added to each plate; after solidification, 10 ml of VRBA over layer was added and let to solidify. Inoculated plates were incubated at 35°C for 24 hours. The number of colonies were counted and recorded as $\log_{10}$cfu/g sample.

Mold and yeast count according to ISO 21527/1 (2008)

From each previously prepared serial dilution, 1 ml. was transferred to DG18 dechlorane rose Bengal agar plates (DRPC), distributed by sterile glass spreader, plates were incubated at 25°C±1°C for 5 to 7 days, counts were recorded as $\log_{10}$cfu/g sample.

Psychotropic count according to APHA (2001)

One ml of serial dilutions of each sample streaked on the surface of Standard Plate Count (APC) agar medium (Oxoid, CM0463). The plates were incubated at 7±1°C for 10 days. The number of colonies were counted and recorded as $\log_{10}$cfu/g sample.

Enumeration of *S. aureus*: According to FDA (2001)

One ml of each serial dilution was streaked on 3 plates of Baired Parker agar media (0.4, 0.3 and 0.3 ml) and distributed on the surface of the plates using sterile bended glass spreader, let to dry, then incubated at 35°C for 24- 48 hrs. The number of colonies was counted and recorded.

Enumeration of β-glucuronidase-positive *E. coli* according to ISO (16649- 2:2001) (TBX method)

This method used for enumeration and isolation of B-glucuronidase – positive *E. coli* forming typical blue green colonies after incubation at 44°C for 18h to 24h on Tryptone Bile Glucuronide selective agar medium (TBX) for all kinds of food and feed of animal origin

Isolation and identification of *Salmonella enterica* according to (ISO, 6579-1/2017)

The previously prepared sample homogenate of both batches was incubated for 18±2h at 37 ±1°C, then, 0.1 ml of pre- enrichment broth culture was inoculated in 10 ml Rappaports Vassiliadis broth with soya (RVs broth), incubated at 41.5±1°C for 24±3hr as well as, one ml of pre-enrichment broth was inoculated to Muller-Kauffmann Tetrathionate/novobiocin broth (10ml MKTTn), incubated at 37±1°C for 24±3 hrs. Loopful from both RVs and MKTTn was streaked over the surface of both Xylose Lysine Deoxycholate agar (XLD agar) and Brilliant Green (BG) agar, incubated at 37±1°C for24±3 hrs. Suspected colonies were inoculated in nutrient agar slant for further biochemical and serological identification.

Isolation of *L. monocytogenes* according to FDA 2017

Twenty -five gm of each sample batches were weighted and mixed with 255ml of buffer listeria enrichment broth (BLEB), and incubated at 30 ^{o}C for 24-48hr, then loopful from each sample homogenate was streaked on Oxford and Aloa agar media, and incubated at 35 ^{o}C for 24-48 hr. Transfer five or more *Listeria* colonies to Trypticase Soya Agar (TSA) with yeast extract streaking for purity at 30 ^{o}C for 24-48hr, carry out the biochemical and serological identification for *L. monocytogenes.*

Chemical analysis

Total volatile basic nitrogen (TVB-N) according to ES (63-9/ 2006)

Accurately 10 g of each sample batches as added to two gm magnesium oxide + 300 ml distilled water were added. The distillation step generally takes 20 min. about 100 ml of distillate was received in flask containing 25 ml boric acid 2% and two drops of indicator. Flask was boiled tell 100 ml distillate was obtained. Sample was titrated with 0.1 M H_2SO_4 (R1). Steps were repeated using distilled water instead of sample as blank (R2). TVBN expressed as mg/100 gm = (R1- R2) X 14.

Thiobarbituric acid (TBA) according to (ES 63-10/2006)

Accurately 10 g sample was homogenized with 97.5 ml distilled water for two min., then washed in distillation flask with 47.5 ml water. 2.5 ml of 4 N HCl was added to adjust pH to 1.5, few drops of antifoam emulsion or 3 to 5 glass beads were added to prevent bumping. Contents well swirled and distilled rapidly until 50 ml distillate is collected. The distillation step generally takes 15 to 20 min. Five ml distillate were pipetted into a screw cap tubes then 5ml of 0.02 M. TBA reagent was added. A reagent blank was prepared (i.e., 5ml of water and 0.02 M TBA), during this, vortex, and heated for 35 min in a boiling water bath, then cooled under running tap water for 10 min, and then the absorbance. The test samples were measured at 538 using a glass cuvette. TBA value mg/kg of sample = Absorbance x 7.8

Reagents and Chemical

HCL 4N [One part conc. HCl: two-part D.W (1:1)].
TBA reagent (0.2883gm/100ml glacial acetic acid 90%).

Measurement of pH according to ES (63-11/2006)

For pH determination, 50 g sample was blended with 200 ml of distilled water for 2 min. the supernatant was filtered, 50 ml portion of the filtrate was diluted with 50 ml of distilled water. After mixing for 10 min, the pH was measured at room temperature using a digital pH meter (Suntex TS-1, Taiwan) equipped with a probe-type combined electrode (Ingold) through direct immersion of electrode into the mixture.

Statistical analysis

Statistical analysis was done in triplicate and results were recorded as mean values and standard deviation (Mean $\log_{10}$cgu/g ± SD) using independent sample T-test of Statistical Packaging for the Social Science (SPSS) Ver. 20. A p-value less than 0.05 ($p \leq 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Sensory criteria for accepting or rejecting Control and Treated fermented sausage during the preservation period

As always known that LAB could retard or even inhibit the growth of food spoilage as well as food poisoning microorganisms, but it resulted in acidic flavor with increasing the storage time which may makes the product undesirable or repulsive for consumers and this also may accompanied with product chemical changes represented by elevation of TVB-N and TBA levels. Therefore, the measure of rejection and acceptance of fermented sausage in the current research depends on several factors including the sensory characteristics and the extent of consumer acceptance during storage periods which was determined according to the experience of the specialist panelists in examining foods with sufficient experience in identifying any changes in the product's sensory characteristics at the due time and also the examination of food for assessing their safety and quality for consumption through matching the bacteriological and chemical criteria with the Egyptian Standards **(ES-4177/2005)** which its safety parameters are excerpted from international regulations regarding food safety standards.

There are no significance differences ($P>0.05$) of all sensory parameters of **Table (1)** including odor, appearance, texture and overall acceptability between control and EO treated samples at zero, 3rd, 6th and the 9th day of storage. At the 12th day of storage, the difference as clear ($p<0.05$) as the changes of odor, color and overall acceptability appears to be started in control samples (6.77±0.25, 6.87±0.09 and 6.90±0.36) which appear to be loose in texture and slightly variable in color and odor as compared with EO treated one (7.73±0.31, 8.10±0.10 and 7.94±0.19) respectively, but still accepted. By time and specifically, at the 15th day, the difference ($P<0.05$) was almost clear between all sensory parameters of control and EO treated samples which indicates that control samples are on the verge of corruption. At the 18th day the deterioration of control samples was rapid and the difference was very clear between the two groups. At the 22nd day of storage, control samples were completely spoiled organoleptically while the EO treated ones was sound (tissues still hard, adhesive and cohesive) and it remained healthy until the end of the experiment (day 25th) recording (5.37±0.15, 5.67±0.21, 5.97±0.15 and 5.67±0.30) for odor, appearance, texture and overall acceptability, respectively.

Table 1: Sensory evaluation of control and EO treated samples during refrigerator storage.

Storage Days	Control				EO			
	Odor	appearance	Texture	Overall acceptability	Odor	appearance	Texture	Overall acceptability
Zero	9.17±0.21	9.07±0.038	9.10±0.03	9.11±0.05	9.23±0.21	9.17±0. 15	9.47±0.15	8.29±0.16
3rd	9.17±0.15	9.10±0.10	9.17±0.15	9.15±0.04	9.23±0.21	9.17±0.21	9.47±0.15	9.29±0.16
6th	8.37±0.15	8.90±0.10	8.80±0.10	8.69±0.28	9.03±0.06	9.13±0.5	9.33±0.21	9.16±0.15
9th	7.60±0.26	7.97±0.21	7.77±0.40	7.90±0.15	8.63±0.15	8.57±0.5	8.77±0.25	8.66±0.10
12th	6.77^{A}±0.25	6.93±0.15	6.90^{B}±0.36	6.87^{C}±0.09	7.73^{a}±0.31	8.00±0.10	8.10^{b}±0.10	7.94^{c}±0.19
15th	5.87^{D}±0.15	5.90^{E}±0.10	6.00^{F}±0.10	5.92^{G}±0.07	7.43^{d}±0.25	7.57^{e}±0.21	7.63^{f}±0.38	7.54^{g}±0.10
18th	4.37^{H}±0.25	4.20^{I}±0.20	4.20^{J}±0.17	4.26^{K}±0.10	7.00^{h}±0.10	7.03^{i}±0.21	7.00^{j}±0.10	7.01^{k}±0.02
22nd	SPOILED				6.83±0.21	6.47±0.25	6.57±0.21	6.62±0.19
25th	SPOILED				5.37±0.15	5.67±0.21	5.97±0.15	5.67±0.30

- **There are significance differences (P<0.05) between means having the same superscripted small and capital letter in the same row for each parameter and its counterpart (A,a; B,b; C,c; etc).**
- **Sensory parameters were evaluated according to the aforementioned table.**

In this regard, **the obtained results in the current study listed in Table (1) were in line with Rahman *et al.*, 2012** who comparing treated samples to untreated controls and showed that EO treatments extended the shelf life of chicken meat with marginal changes of sensory quality. Although EO treatments showed similar antimicrobial effects and it found to be more beneficial in practical application for its semi neutral pH and low chlorine content. In the same context, several investigators (**Kim *et al.*, 2006; Alam *et al.*, 2020; Khalid *et al.*, 2023)** found that EW can be effectively used to reduce bacterial spoilage and extend shelf life of fish during distribution and marketing that improving its sensory parameters and chemical properties. This agreed with the results in the present study. On contrary, the obtained results were higher than **Sheng *et al.*, (2018)** whose claimed that sensory properties including odor, appearance, texture and overall acceptability were 4.52±0.12, 4.43±0.13, 4.31±0.13, and 4.42±0.025 respectively, at the end of 16 storage day for slightly acidic EO beef treated samples.

Microbiological status of examined dried sausage

Statistical analytical results listed in **Table (2)** revealed that there was a significance differences between control and EO treated samples concerning LAB from the beginning of the experiment (Zero time) till the end of the storage period. The number of LAB were significantly increased (P<0.05) from the first day of storage in EO treated as compared with control, and the population reached approximately 8.91±0.01 log cfu-g^{+1} and 8.96±0.01 at the end of 22^{th} and 25^{th} day for EO treated samples. Total coliforms (TC) also showed significance difference (P<0.05) from day zero of storage (1.6±0.02 & 1.55±0.06) till the 12^{th} day (1.86±0.03 &1.01±0.6) for control and treated samples, respectively. Control samples spoiled at the 22^{nd} day and they recording mean TC of 1.97±0.4 log cfu/g at the 18^{th} day while TC were not detected (<1 log cfu/g) in EO treated samples from the 15^{th} day of storage till the end of the storage time (25^{th} day). Egyptian standard (No. 4177/2005) mentioned that TC should not exceed 2 log cfu/g. Moreover, mold & yeast as well as Psychotropic counts were increased significantly (P<0.05) around 1log cfu/g. reduction or more in EO treated samples as compared to control ones all over the storage period. In addition, *Staph. aureus* and *E. coli* counts recorded (<1 log_{10} cfu/g) all over the experimental time either in control or EO treated samples. Otherwise, both organisms were not detected in examined samples.

The number of LAB incriminated in EO treated samples in the present study were compliant with **Seon *et al.*, 2015** (9 log cfu/$g^{1)}$, and higher than that obtained by **Gurbuz *et al.*, 2009** (7.88 log cfu/g) and **Lebert *et al.*, 2007** (6.5 and 7.9 log cfu/g^{-1}) in handmade fermented sausages obtained from two small food factories in France. **Macedo *et al.*, 2008** noticed that >8 log cfu/g^{-1} in LAB count in probiotic cultures prepared sausage after 150 days of storage under refrigeration. In this regard, **Rahman *et al.*, 2010** found that EW sprays can decrease the anaerobic bacteria by 3.5 logs cfu/100 cm^2 for 7 days of storage at 4 ◦C.

Also, as expected, Total coliforms of control sample increased progressively than EO treated .The decrease in coliform count in EO treated fermented sausage confirmed the competitive superiority of lactic acid bacteria and the acids originated from water electrolysis of EO over the endogenous microbiota as well as those coliform bacteria do not grow well at low pH, in this context, **Wang *et al.*, (2018)** discovered that practically 1log cfu/cm^2 microbial reduction was attained by using innovative spraying electrolyzed water technology. This agreed with the results in the present study. In this respect**, Chevalier *et al.*, (2006**) stated that coliform count declined very quickly and totally inhibited within 7 days, also, TC in the present study, were completely inhibited (< 1 log10 cfu/g) from the beginning of 15^{th} day of storage.

Gurbuz *et al.*, (2009) cleared that coliform count was not detected at the end of storage of fermented sausage. This substantiates the data in the present work. Furthermore, **Macedo *et al.*, (2014)** recorded a mean of <1 log cfu/g at the end of storage period (14^{th} day) in

both groups of different starter cultures of Italian fermented sausage. **Cheng *et al.*, (2016**) explained that the bacterial surface was altered from smooth, consecutive, and bright into rough, shrunken, and even lysed after EW treatment. Moreover, it was mentioned in **Egyptian Standard (ES 4177/2005)** that coliform count should not exceed 10^2 cfu/g in fermented sausage.

Mold and yeast count in Table (2), Sarah *et al.*, (2024) clarified that contamination caused by fungi considered as a significant microbiological problem in the food industry, particularly leading to early spoilage of various food products, including dry-fermented meat industry. The emergence of undesired fungi on product surfaces results in substantial economic losses. Once microorganisms infiltrate the food, contamination ensues, and their presence led to adverse impact the product's appearance, odor, flavor, and texture. This, in turn, not complies with the consumer requirements and loss of its confidence, and subsequently leads to consumer rejection and negatively affects the company products. Given the detrimental effects of spoilage fungi in the food industry, practices such as thorough cleaning and sanitization become crucial to prevent contamination and subsequent premature deterioration.

Table (2): Statistical analysis of microbial count (log_{10} cfu/g) of control and treated fermented sausage throughout storage period

Microbial counts		Storage period/days								
		Zero	3rd	6th	9th	12th	15th	18th	22nd	25th
Lactic acid bacteria (LAB)										
	Control	6.39±0.07	6.51±0.03	6.6±0.02	7.08±0.07	7.78±0.04	7.91±0.01	8.15±0.03	SPOILED	
	Treated	6.43±0.02	6.67a±0.03	6.7a±0.05	7.39a±7.07	7.95a±0.02	8.02a±0.05	8.84a±0.01	8.91±0.01	8.96±0.01
Total Coliforms (TC)										
	Control	1.60a±0.02	1.61a±0.08	1.71a±0.04	1.78a±0.04	1.86a±0.03	1.88±0.04	1.97±0.4	SPOILED	
	Treated	1.55b±0.06	1.27b±0.09	1.16b±0.08	1.09b±0.10	1.01b±0.6	<1	<1	<1	<1
Mold and Yeast										
	Control	2.74a±0.13	3.53a±0.47	3.95a±0.05	4.81a±0.02	4.87a±0.02	5.88a±0.03	5.88a±0.03	SPOILED	
	Treated	2.12b±0.04	2.27b±0.47	2.65b±0.16	3.00b±0.08	3.45b±0.02	3.70b±0.09	3.89b±0.03	3.98±0.02	4.36±0.23
Psychographic bacteria										
	Control	1.08a±0.07	1.21a±0.12	1.84a±0.07	2.68a±0.05	2.88a±0.02	3.36a±0.04	3.86a±0.02	SPOILED	
	Treated	1.05b±0.06	1.09b±0.05	1.18b±0.04	1.95b±0.04	2.02b±0.11	2.17b±0.12	2.70b±0.01	2.75±0.02	2.79±0.02
Staph. Aureus										
	Control	< 1 log_{10} cfu/g							SPOILED	
	Treated	<1 log_{10} cfu/g								
E. coli										
	Control	<1 log_{10} cfu/g							SPOILED	
	Treated	<1 log_{10} cfu/g								

There are significant differences between the control and treated samples with different superscripted small letters for each day separately

These measures play a pivotal role in ensuring the quality and safety of food, while also extending the shelf life of products. This substantiates the findings in the present research. In this regard, **Davies *et al.*, (2021) and Visconti *et al.*, (2021)** stated that proliferation of fungi in food is associated with adverse effects on the sensory attributes of products, such as appearance, texture, and flavor properties. These consequences not only prompt consumer rejection but also contribute to economic losses for producers.

Regarding psychotrophic mean count. **Huda *et al.*, (2022)** proved that rinsing with slightly acidic EW remains the psychotrophic count around $1log_{10}$ till 7^{th} day, and increased gradually recording 2.18±0.03 at the 9^{th} of storage which was nearly similar to the obtained results in the present study. In this respect, **Huang *et al.*, (2008)** stated that AcEW found to have strong bactericide activity and could be able to limitation of the growth and multiplication of food spoilage microorganisms over the surface of food products. Moreover, the obtained results coincide with **Khalid *et al.* (2018**) who mentioned that AcEW and neutral electrolyzed water (NEW) have been reported to have a strong bactericidal effect on various types of foodborne pathogens and food spoilage microorganisms for most of food products and food contact equipment and surfaces and subsequently increase the food products shelf life. Also, it was estimated by **Brychcy *et al.* (2015)** that there was a reduction in psychotropic count by 3 log cfu/g $^{-1}$ when acidic electrolyzed water was used in spraying form, which was higher than those obtained through the current study. **Cichoski *et al.* (2019)** noticed one log reduction of psychotrophic count by 0. 76 when combination of SAEW and ultrasound US (25 kHz) was used in chicken breast. Furthermore **Khalid *et al.* (2020)**

recorded 4.8 log10 cfu/g) in EO shrimps at the 11th day of storage

Various studies have addressed the importance of Electrolyzed oxidized (EO) water as a new friendly environmental green technology in eliminating bacterial contamination specially *S. aureus* and *E. coli* in shrimps (**Lin *et al.*, 2013; Ratana-Arporn and Jommark 2014);** fish as whole (**Al-Holy and Rasco, 2015**), beef (**Al-Holy** and **Rasco, 2015; Mansur *et al.*, 2015b**), pork (**Rahman *et al.*., 2016)** and poultry carcasses (**Rahman *et al.*, 2012** and **Al-Holy and Rasco, 2015**). Most studies have found that Gram-positive bacteria were more resistant to EW exposure than Gram-negative bacteria (**Kim *et al.*, 2000b; Park *et al.*, 2004; Guentzel *et al.*, 2008; Khalid *et al.*, 2023)**. The pH of the sausages decreases due to lactic acid bacteria that produce lactic acid from metabolizing sugar and create an extra margin for safety. The pH drop causes the proteins to give up water, resulting in a drying effect that creates an environment unfavorable to spoilage organisms. Drying continues after the fermentation stage and more moisture is removed from the sausage. In this regard, **Adam and Stanley, 2009**; **New Zealand food Safety Authority Guidelines, 2009** & **FSIS Guideline, 2023** concluded that fermented sausages should attain a pH of 5.3 or lower within the proper time frame in order to control the growth of pathogenic microorganisms including pathogenic *E. coli* and *S. aureus*.

Isolation of some pathogenic microorganisms

In the present work, neither *Salmonella enterica* nor *L. monocytogenes* were isolated from either control or EO treated samples. Absence any of *Salmonella enterica* and *L. monocytogenes* in control and treated samples in the current study indicated that meat and other ingredients used is fermented sausage production is produced and handled under good hygienic measures, as well as the Good Manufacture Practices (GMP) that were followed during manufacturing including good design of fermentation, sat curing and drying processes. This complies with the scientific data published by **FSIS (2023)**.

Physico-chemical properties of examined fermented sausage

Fig. (1) Illustrated the TVB-N (mg/100g) of both control and treated group which showed that significance difference was clear (P<0.05) between both groups from the 12th day of storage (14.1 for control & 11.9 for EO treated samples), in control samples, TVB-N begins to be elevated hanging at the 15th day (16.1 mg/100g) reaching the critical limit (18.7 mg/100g) at the 18th day. While, they were completely deteriorated (Rejected) at the 22th day of storage. On contrary, the EO treated samples remained sound (19.8 mg/100g) till the 25th day of storage.

Total volatile basic nitrogen (TVB-N) content (mg/100 g) is used as an indicator for tissue protein breakdown caused by proteolytic enzymes due to microbial activity during the storage of meat products **(Ruan *et al.*, 2019; Wang *et al.*, 2020.** Overtime, storage of fermented sausage leads to an increase in TVB-N which goes parallel to other spoilage biomarkers, and the increase in enzymatic activities particularly of protease enzyme produced by certain microorganisms **(Huang *et al.*, 2014).** This agreed with the obtained results as the control samples deteriorated at the 22nd day of storage which attributed to the microbial enzymatic activity. The obtained data in the present research inconsistent with **Rahman *et al.* (2012)** who found that SAEW treated group had a lower TVB-N and TBA as compared with control group due to the presence of OH^- and HOCL that has antioxidant effect, and can maintain the oxidation stability of meat. Meanwhile, results in the present study were little higher (9.5 for control and 9.0mg/100g for EO treated) than those obtained by **Sheng *et al.*, 2018** (8.40±0.41, and 8.19±0.63 mg/100g) initially for both control and treated SAEW group samples, then rapidly increased at the 6th day of storage to 16.94±1.29 in control samples which considered much more than the results of current research and 9.25±0.43 in SAEW treated samples which nearly similar to the obtained results in the present study.

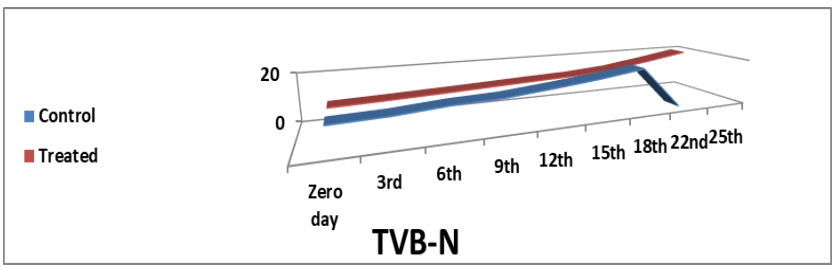

Fig. 1: Mean total volatile basic nitrogen (mg/100g) of control and EO treated samples during refrigeration storage.

NB: results represent the mean of triplicates of each group

Fig (2) showed the mean TBA (mg/kg malonaldehyde). The significance variation was not clear (P>0.05) between both groups which indicated that TBA cannot be taken alone as a measurement of meat and its products spoilage but it is necessary to be combined with other analysis for accurate judgment of samples fitness to

human consumption including sensorial and bacteriological examination. It is noteworthy that the control samples were spoiled organoleptically at the 22nd day of storage, while the EO treated samples were valid until the 25th day of storage.

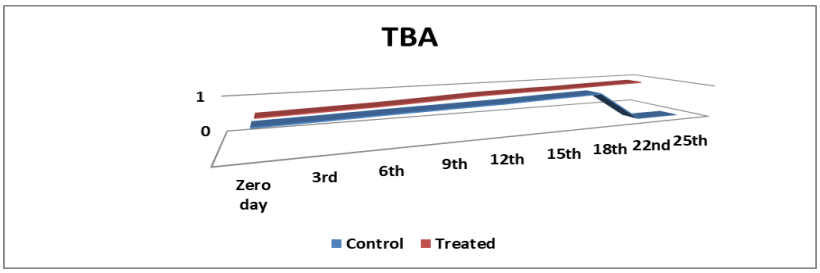

Fig. 2: Mean Thiobarbituric acid content (TBA mg/kg) of control and EO treated samples during refrigeration storage.

TBA values are applied as a lipid oxidation index for many fatty foods, the acceptable limit of TBA (0.9 mg/kg) as set by **ES (4177/2005**). Lipid oxidation (rancidity) is mediated by the act of, lipases and due to unsaturated fatty acids and molecular oxygen reaction causing fat deterioration **(Mariutti and Bragagnolo 2017).** A gradual but not significant increase (P<0.05) of TBA content was observed from the beginning of refrigeration of all examined samples, and their spoilage was observed at 22th day for control group, which recorded (0.89 mg/kg) at the 18th day. This agreed with **Chen *et al.* (2016)** whose reported that SAEW does not have immediate antioxidant activity and found that TBA content of SAEW treated sample was not better than those of control sample. In this respect, **Sheng *et al.*, (2018)** and **Khalid *et al.*, (2023)** concluded that there were no significant differences (p > 0.05) between the untreated and SAEW treated fish group in the content of thiobarbituric acid, suggesting that SAEW does not possess antioxidant activity. On contrary, **Sheng *et al.*, (2018)** concluded that there was significant difference between both samples throughout the experiment except at zero – day, the increase in TBA content was observed during the whole storage period from the initial 0.17 and 0.18 to 0.73±0.03 and 0.53±0.02 mg/kg at end of storage for control and SAEW treated samples, respectively, which is not compliant with the results of the current study.

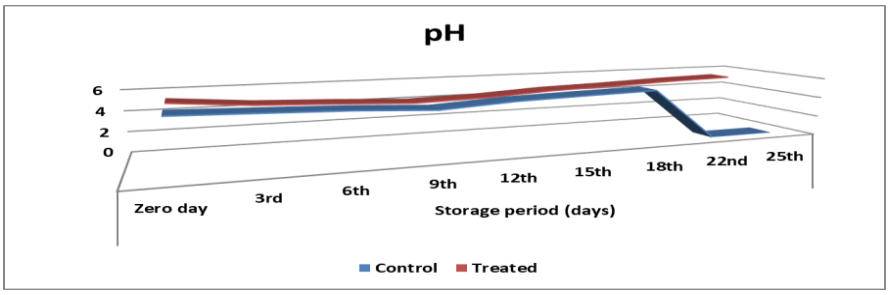

Fig. 3: Mean pH of Control and EO treated samples during refrigeration storage.

Fig (3) cleared that mean of pH of control samples vise EO treated recorded 5.29±0.03 & 5.26±0.02; 5.1±0.2 & 4.69±0.07; 4.9±0.1 & 4.44±0.03; 4.54±0.03& 4.12±0.03; 4.96±0.04 & 4.31±0.04; 5.15±0.04 & 4.7±0.04; 5.3±0.2 & 4.88±0.1 at zero, 3rd, 6th, 9th, 12th, 15th and 18th storage days, respectively. The significance difference between control and EO treated samples was obvious from the 3rd day of storage and continued till the 18th day at which the control samples were corrupted while EO treated ones remained fit till the 25th day of storage. Fig. (3) also showed that both control and treated samples witnessed a gradual decrease in pH from third day until the 9th day of storage, where pH began to increase from the 12th day, in both groups (control & EO treated) but gradually and less progress increase in EO treated samples compared to control ones, the control samples reached a maximum limit of PH (5.3) on the 18th day recommended by scientific references that pH should be 5.3 or less for fermented products, while EO treated samples did not exceeded this pH (5.3) until the 25th day of storage. In this respect, **Garrido *et al.* (2004)** identify dry fermented sausage as that product made of chopped or ground meat of maximum pH of 5.3. The rapid growth of LAB bacteria in fermented meat products may explain the cause of reduction in pH as mentioned by **Mitrovic *et al.* (2019),** who claimed that the mean pH of the sausage declined at the end (after 18 days) to 5.32 ± 0.03, which was agreed with the current study, although the control samples were significantly higher, in which their final pH recorded 4.76 to 4.85, which was higher than the current study. In this respect**, Sean *et al.* (2015)** concluded a sharp decline was observed in pH value from 6 to 4.76 in fermented sausages, it was suggested it was due to LAB had becoming the dominant

microorganism. The current results were doesn't match with those obtained by **Gurbuz *et al.* (2009)** who stated that pH value was 5.11 at the end of storage and explained that because of the production of lactic acid by increasing the LAB population.

CONCLUSION

Electrolyzed water proved strong antimicrobial properties and considered an environment-friendly sanitizer, used in various industries, EW can be applied in a wide range of food products and that why it is convenient prime for microbial control in the food industry to assure food safety and quality without alternating the sensory parameters of the food. Overall, the microbial, chemical and sensory properties correlated highly with the freshness of the meat. In order to prolong the shelf- life and to improve the microbiological quality of fermented sausage, lower initial microbial load of raw meat and the other ingredients, maintenance of appropriate chill temperature during storage. This study showed that EO could be used as an antibacterial and to expand the shelf life of fermented sausage without influencing the sensory quality. Also, it delayed chemical deterioration of fermented sausage. Furthermore, many studies are needed to focus on other benefits of electrolyzed water in food industry to gain a safe and good quality food.

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