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Antibacterial effects of combined slightly acidic electrolyzed water and UV light in chilled fish keeping quality

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ABSTRACT

The preservation of fish is critical for ensuring food safety, extending shelf life, and maintaining its quality. Therefore, the study was planned to investigate the impact of the application of slightly acidic electrolyzed water (SAEW) and/or ultraviolet light (UV) rays for 30 minutes maximum time of exposure, as innovative non-thermal for improving the bacteriological and keeping quality of mackerel fish samples during refrigeration storage. The obtained results indicated a significant inhibition in the bacterial counts with retardation in the protein and fat degradation through a noticeable reduction in the total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) levels in the treated fish samples as compared to the control samples during refrigeration. Furthermore, the sensory profile of the treated samples proved the promising beneficial effect as an accredited application in fish preservation and production, where the treated samples kept their organoleptic acceptability up to the 10th day of refrigeration storage (4±1^{O}C). In contrast, the control group showed spoilage signs after the 4th day of storage. Although all of the treated samples with SAEW or UV showed variable promising enhancement effects, the combined SAEW and UV treatment had higher sensory quality, besides its antibacterial effects. Accordingly, it is highly recommended that combined SAEW/UV treatment be applied to enhance the fish-keeping quality and ensure safe fish products.

1. INTRODUCTION

Fish are vital nutritious component offering a rich source of easily digestible protein, essential minerals and vitamins, and beneficial fatty acids, rich in omega-3 fatty acids, especially fatty varieties like salmon, mackerel, and sardines. Thus, the fish requires proper handling and storage to prevent contamination that affects its shelf life and its compatibility with human consumption (Awuchi et al., 2022).

The integration of slightly acidic electrolyzed water (SAEW) and ultraviolet (UV) light represents an innovative approach to enhancing the shelf life of fish products. This combination can maximize the antimicrobial properties of SAEW and the disinfecting capabilities of UV light to significantly improve food safety and quality (Sheng et al., 2020).

The SAEW is rich in hypochlorous acid, which effectively targets and eliminates a wide range of pathogens, including bacteria and viruses that can compromise fish safety. Further, UV light disrupts microbial DNA and inactivates harmful microorganisms. When SAEW is used in conjunction with UV light can provide a robust barrier against spoilage and contamination (Naka et al., 2020).

The synergistic effect of SAEW and UV light not only extends the shelf life of fish but also maintains its sensory qualities. The previous study applied by Zhong et al. (2024) showed that this combination can minimize oxidative damage by reducing lipid oxidation and preserving color and texture prolonging its freshness. Moreover, this method aligns with modern food safety standards, offering a chemical-free alternative to traditional preservation techniques.

Referring to the recorded data by Lan et al. (2021), UV light can reduce bacterial loads on fish surfaces, enhancing the overall effectiveness of the preservation method when combined with SAEW.

Therefore, the current study investigated the antibacterial and keeping quality effects of SAEW and/or UV application on the treated mackerel fish shelf life during chilling conditions.

2. MATERIAL AND METHODS

The research was performed after approval of Research Ethical Committee, Faculty of Veterinary Medicine, Benha University (BUFVTM 13-10-24).

2.1. Preparation of SAEW (Athayde et al., 2018)

SAEW was obtained from the Food Hygiene Dept., Animal Health Research Institute. SAEW was prepared in a diaphragmless electrolyzer and has a pH close to neutral (5.0–6.5), a relatively low *ORP* (800–900 mV), and a relatively low *ACC* (10–30 mg L^{-1}).

2.1. Collection and preparation of fish samples

Twenty frozen mackerel fish samples, with a mean weight of about 177.8 ± 5.0 g, were purchased from a local retail market in Benha City, Kalyobiya governorate, Egypt. The collected fish samples were eviscerated separately in

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hygienic conditions and then were grouped into 4 groups (5 samples\ group). First group (G1) was dipped in distilled water for 30 minutes as control, 2nd group (G2) was soaked in SAEW for 30 min. in the refrigerator (Tolba et al., 2020), 3rd group (G3) was exposed to UV (~260 nm) for 20 min about 45cm distance (Akgün and Ünlütürk, 2017); while, the 4th group (G4) was exposed to UV light, for 10 min, after soaking in SAEW for 30 min.

Control and treated groups were kept on chilling shelves at 4±1^{O}C. The sensory, bacteriological, and chemical examinations were performed at day zero (within 30 min after treatment), and then periodically every 48h of cold storage until organoleptic deterioration. The trial was repeated in triplicates.

2.3. Sensory evaluation

The color, odor, texture, and overall score were carried out following Mörlein (2019) in scores (1 to 5), where ≤1- represented the worst while 5- represented the excellent mark.

2.4. Bacteriological profile

2.4.1. Preparation of samples (ISO 6887-2, 2017): tenfold serial dilution was prepared on sterile peptone water (0.1%); from which the following parameters were examined.

2.4.2. Aerobic plate count "APC" according to ISO 4833-1 (2013): in APC agar and incubated at 30±1^{O}C for 72h.

2.4.3. Psychrotrophic bacterial count according to ISO 17410 (2019): on APC agar and incubated at 4±1^{O}C for 10 days.

2.4.3. Coliform count according to ISO 4832 (2006): in VRBA agar and incubated at 37±1^{O}C for 24h.

2.4.4. Staphylococcus aureus count according to ISO 6888-1 (2021): on Baird Parker agar supplemented with egg yolk tellurite and incubated at 35±2^{O}C for 24h.

2.5. Chemical profile

Total Volatile Nitrogen (TVN) and thiobarbituric acid (TBA) were measured according to the procedure of EOS (2006): ES N. 63-9/2006 and 63-10/2006, respectively.

2.6. Statistical analyses

The statistical analyses were performed by application of the Analysis of Variance (ANOVA) test on SPSS software v.20 according to Feldman et al. (2003).

Reduction (%) = $\left(\frac{(R1-R2)}{R1}\right) x\, 100$, where R1 and R2 indicate the microbial count of control and treated samples, respectively

3. RESULTS

The sensory scores of the treated mackerel samples showed a significant enhancement in the sensory quality that appeared as an elongation in the physical acceptability in relation to the control group; which started spoilage characteristics after the 4th day of storage. G2, while samples treated with dipping in SAEW, showed higher acceptability scores in comparison with that treated with UV light (G3) for the same time of exposure; whereas, G4, which was treated with a combination of SAEW followed by UV radiation, showed the highest acceptability time, where it still acceptable up to 10 days of refrigerated storage (Fig. 1).

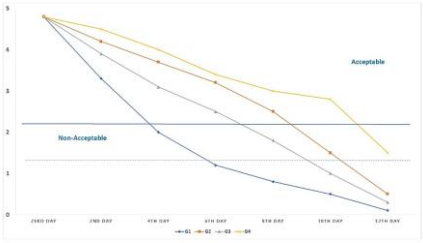

Fig. (1). Sensory profile of the examined mackerel fish groups during cold storage (4±1^{O}C): if the final quality score is 2, the sample's quality is marginally acceptable. If this score is less than 2, the sample is unacceptable. If this score is less than 1, the sample is apparently spoiled.

Regarding the bacteriological profile of the treated fish samples, Tables 1 to 4 revealed a significant bacteriostatic effect of the applied treatments appeared as significant retardation in the bacterial mean counts (log_{10} CFU/g) in relation to the control group; whereas, the combination treatment group (G4) showed higher keeping quality regarding the bacteriological quality of the treated samples than G3 and G2, respectively. Table 1 showed that the treated fish samples with SAEW (G2), UV light (G3), and SAEW-UV combination (G4) were still within acceptable limits of APC up to the 6th day for G3, and 8th day of storage for G2 and G4, respectively; but the control group exceeded the permissible limit of APC (1x10^{6} CFU/g) since the 4th day of storage; that indicating the positive effect of the applied treatments on the bacteriological counts and the product's shelf life consequently.

Table 1 The aerobic plate count (APC) (log_{10} CFU/g) of the examined groups during storage at 4±1ºC (Mean± SE)

Day	G1	G2	G3	G4
Zero-day	4.5 ± 0.1^{a}	4.3± 0.1^{a}	4.3± 0.1^{a}	4.1 ± 0.1^{a}
2nd day	5.6 ± 0.7^{a}	4.5 ± 0.2^{c}	4.6 ± 0.2^{b}	4.3 ± 0.1^{d}
4th day	6.2 ± 0.5^{a}	4.8 ± 0.2^{c}	5.0 ± 0.4^{b}	4.5 ± 0.3^{d}
6th day	xx	5.3 ± 0.4^{a}	5.5 ± 0.3^{b}	5.0 ± 0.3^{c}
8th day	xx	6.0 ± 0.3^{a}	6.2 ± 0.5^{b}	5.6 ± 0.4^{c}
10th day	xx	xx	xx	5.9 ± 0.^{5}
12th day	xx	xx	xx	xx

Values with different superscripts within the same row differed significantly at $P< 0.05$.

Psychrotrophic bacterial count, also, showed significant retardation in the bacterial growth as a consequence of the applied treatments (Table 2). The reduction effect began from zero days of the experiment, followed by slowly raising in comparison with the control untreated group, in which spoilage signs appeared since the 4th day of storage.

Referring to the recorded results of studying the antibacterial effect of the applied treatments on the coliform bacteria, as a fecal contamination indicator, coliform, Table 3 indicated significant inhibition of coliform growth rate; where the treated G4 was still within the acceptable limits up to the 10th day of storage; whereas, control group exceeded the permissible limit at the 4th day of storage. On the other hand, G2 and G3 still within limit up to the 6th day of chilled storage, respectively.

Table 2 The psychrotrophic count (log_{10} CFU/g) of the examined groups during storage at 4±1ºC (Mean± SE)

Day	G1	G2	G3	G4
Zero-day	2.5± 0.1^{a}	2.2 ± 0.1^{a}	2.3 ± 0.1^{a}	2.0 ± 0.1^{a}
2nd day	2.8 ± 0.3^{a}	2.4 ± 0.1^{c}	2.5 ± 0.2^{b}	2.2 ± 0.1^{d}
4th day	3.5 ± 0.2^{a}	2.7 ± 0.3^{c}	2.7 ± 0.3^{b}	2.5 ± 0.1^{d}
6th day	xx	3.2 ± 0.3^{a}	3.4 ± 0.4^{b}	2.9 ± 0.2^{c}
8th day	xx	3.6 ± 0.2^{a}	3.8 ± 0.3^{b}	3.4 ± 0.3^{c}
10th day	xx	xx	xx	3.9 ± 0.3
12th day	xx	xx	xx	xx

Values with different superscripts within the same row differed significantly at $P< 0.05$.

Table 3 The coliform count (log_{10} CFU/g) of the examined groups during storage at 4±1°C (Mean± SE)

Day	G1	G2	G3	G4
Zero-day	1.2 ± 0.1^{a}	1.1 ± 0.1^{a}	1.2 ± 0.1^{a}	1.0 ± 0.1^{a}
2nd day	1.5 ± 0.1^{a}	1.2 ± 0.1^{c}	1.4 ± 0.1^{b}	1.1 ± 0.2^{d}
4th day	2.3 ± 0.1^{a}	1.4 ± 0.2^{c}	1.7 ± 0.3^{b}	1.3 ± 0.2^{d}
6th day	xx	1.7 ± 0.2^{a}	2.0 ± 0.3^{b}	1.5 ± 0.3^{c}
8th day	xx	2.1 ± 0.3^{a}	2.3 ± 0.2^{b}	1.8 ± 0.2^{c}
10th day	xx	xx	xx	2.0 ± 0.3
12th day	xx	xx	xx	xx

Values with different superscripts within the same row differed significantly at $P< 0.05$.

Regarding the environmental superficial contamination indicator, *S. aureus*, Table 4 revealed a superior inhibitory effect of UV light on *S. aureus* than SAEW; whereas, SAEW-UV light combination revealed a significant synergistic effect on *S. aureus* count with mean values of 3.0, 2.8 and 2.9 for G2, G3 at the 8th day of storage, and G4 at the 10th day of chilled storage, respectively.

Table 4 The *S. aureus* count (log_{10} CFU/g) of the examined groups during storage at 4±1°C (Mean± SE)

Day	G1	G2	G3	G4
Zero-day	2.0 ± 0.1^{a}	1.8 ± 0.1^{a}	1.7 ± 0.1^{a}	1.6 ± 0.1^{a}
2nd day	2.6 ± 0.1^{a}	2.0 ± 0.1^{c}	1.8 ± 0.2^{b}	1.8 ± 0.1^{d}
4th day	3.3 ± 0.1^{a}	2.3 ± 0.2^{c}	2.1 ± 0.2^{b}	2.0 ± 0.1^{d}
6th day	xx	2.7 ± 0.3^{a}	2.4 ± 0.1^{b}	2.2 ± 0.2^{c}
8th day	xx	3.0 ± 0.3^{a}	2.8 ± 0.1^{b}	2.5 ± 0.1^{c}
10th day	xx	xx	xx	2.9 ± 0.2
12th day	xx	xx	xx	xx

Values with different superscripts within the same row differed significantly at $P< 0.05$.

Regarding the chemical indicators of keeping quality, Tables 5 & 6 showed that the treatment with SAEW and/or UV light had a significant favorable effect on the keeping quality of the treated fish samples appeared as staying of TVBN and TBA values within the permissible limits up to 6th day of chilled storage for G2 and G3; whereas still acceptable up to 8th day of storage for SAEW-UV light combination group (G4). On the other hand, control samples exceeded the permissible limits on the 4th day of storage.

Table 5 The TVB-N (mg/100 g) of control and treated groups during storage at 4±1°C (Mean± SE)

Day	G1	G2	G3	G4
Zero-day	20.1 ± 0.5^{a}	20.1 ± 0.5^{a}	20.1 ± 0.5^{a}	20.1 ± 0.5^{a}
2nd day	26.5 ± 0.9^{a}	22.8 ± 0.6^{c}	23.4 ± 0.8^{b}	22.1 ± 0.6^{d}
4th day	32.5 ± 0.6^{a}	25.4 ± 0.5^{c}	26.5 ± 0.5^{b}	24.3 ± 0.5^{d}
6th day	xx	28.1 ± 0.7^{a}	29.2 ± 0.6^{b}	26.8 ± 0.3^{c}
8th day	xx	32.2 ± 0.9^{a}	33.5 ± 0.4^{b}	28.2 ± 0.8^{c}
10th day	xx	xx	xx	30.4 ± 0.9
12th day	xx	xx	xx	xx

Values with different superscripts within the same row differed significantly at $P< 0.05$.

Table 6 The TBA (mg MDA/kg) of control and treated groups during storage at 4±1oC (Mean± SE)

Day	G1	G2	G3	G4
Zero-day	3.1 ± 0.5^{a}	3.1 ± 0.5^{a}	3.1 ± 0.5^{a}	3.1 ± 0.5^{a}
2nd day	4.0 ± 0.9^{a}	3.3 ± 0.6^{c}	3.7 ± 0.5^{b}	3.2 ± 0.7^{d}
4th day	4.7 ± 0.8^{a}	3.7 ± 0.5^{c}	4.1 ± 0.7^{b}	3.5 ± 0.4^{d}
6th day	xx	4.1 ± 0.7^{a}	4.5 ± 0.8^{b}	3.8 ± 0.6^{c}
8th day	xx	4.6 ± 0.9^{a}	4.8 ± 0.5^{b}	4.1 ± 0.8^{c}
10th day	xx	xx	xx	4.5 ± 0.7
12th day	xx	xx	xx	xx

Values with different superscripts within the same row differed significantly at $P< 0.05$.

4. DISCUSSION

The innovative technologies that have gained attention in recent years are Slightly Acidic Electrolyzed Water (SAEW) and Ultraviolet (UV) light treatments and both methods aim to reduce microbial contamination while preserving the sensory attributes of fish. Thus, the present study was planned out to investigate the impact of using SAEW and/or UV light on the safety and quality of mackerel fish during refrigeration.

In the present study, the sensory scores of the treated mackerel samples showed a significant enhancement in the sensory quality appeared as a longer physical acceptability while the SAEW-UV combination treatment recorded the highest acceptability scores up to twelve days of chilled storage which may be attributed to the significant antibacterial effects of SAEW and UV light that sharing in extension of the keeping quality and shelf life of treated fish samples; which was confirmed through recording the antibacterial effect of mackerel fish treatment with SAEW and\or UV light.

SAEW is produced by electrolyzing a diluted saltwater solution, resulting in water that contains a mixture of hypochlorous acid (HOCl) and sodium hydroxide (NaOH). This solution has been shown to possess antimicrobial properties effective against a range of pathogens commonly found in seafood; which was previously recorded by Speranza et al. (2021) who indicated that SAEW can significantly reduce microbial loads on fish surfaces, making it a promising alternative to traditional chemical sanitizers, that may be attributed to its ability to disrupt microbial cell membranes, leading to cell lysis and death. On the other hand, SAEW has been reported to cause minimal changes to the sensory attributes of fish products.

Additionally, UV light treatment is another non-thermal technology that has been extensively studied for its effectiveness in food preservation through its powerful antimicrobial efficacy by damaging the DNA of microorganisms, rendering them incapable of reproduction (Tchonkouang et al., 2023). Studies have shown that UV-C can effectively reduce pathogens such as *E. coli* and *L. monocytogenes* on fish fillets (Ahmed and Amin, 2019).

Tables (1-4) demonstrated the bacteriological quality of the control and treated fish samples; and the acceptability of tested fish samples were evaluated following the Egyptian Standard No. 3494 (2020) that noted the maximum permissible limit for APC, coliform and *S. aureus* counts must not not exceed $1x10^{6}$, $1x10^{2}$ and $1x10^{3}$ CFU/g, respectively. Depending on the relation to the bacteriological quality of the treated samples, the present recorded inhibitory effect of SAEW combined with UV treatment indicated the powerful synergism between each treatment which came in line with the recorded results by Safwa et al. (2023) who reported that the combination of these two methods has been shown to effectively reduce bacterial pathogens while preserving sensory attributes. Studies suggest that using SAEW in conjunction with UV light can maximize antimicrobial effects while minimizing oxidative damage.

Studying the effects of SAEW and UV light on the chemical indicators of keeping quality, TVBN and TBA, revealed that the treated groups showed significantly better chemical criteria and retardation of protein and fat degradation in relation to the control group, which exceeded the permissible limits within four days of refrigeration. The obtained results may be attributed to the previously noticed significant inhibitory effect of SAEW and UV on the bacterial population of mackerel fish samples that has a direct correlation with the acceleration of protein and fat degradation and raising TVB-N and TBA consequently; which came in line with the recorded attribution mentioned by Speranza et al. (2021).

Concerning the obtained results, combined SAEW-UV treatment revealed synergistic effects as to improve the sensory, bacteriological, and keeping quality of the treated fish samples. The synergistic application of SAEW and UV light presents a novel approach to enhancing both safety and sensory quality (Akther et al., 2023). The combination of these two methods has been shown to effectively reduce bacterial pathogens while minimizing oxidative damage. On another systematic review identified various combinations of UV-C treatments with other technologies, including SAEW, that resulted in improved safety outcomes with little

to no adverse effects on the sensory qualities of fish and meat products (Monteiro et al., 2023).

It is worth noting that UV-C light is a well-known effective technique for reducing microbial loads, but its application must be carefully controlled to prevent the adverse effect of high doses or prolonged exposure that can lead to oxidative degradation of lipids and proteins in seafood, negative impacting color, texture, and flavor. Therefore, combining UV-C with other preservation methods may help mitigate these adverse effects while enhancing safety (Baligad et al., 2023).

5. CONCLUSIONS

The application of SAEW and UV light represents a promising frontier in seafood preservation. Both technologies offer significant advantages in enhancing fish safety without compromising their sensory qualities. Generally, the combined treatment of SAEW and UV light revealed better impacts on the sensory, bacteriological and chemical quality of the treated fish samples as compared to untreated ones. Therefore, it is recommended to accredit this combination as a fish treatment before cold storage for safer and longer shelf-life fish production.

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