Study on the antimicrobial activity of strongly acidic electrolysed oxidising water for large yellow croaker

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RESEARCH ARTICLE

Abstract

This study was to evaluate the bactericidal efficiency of strongly acidic electrolysed water (SAEW) and to consider SAEW used as a safe sanitiser for large yellow croaker (LYC). More specifically, bactericidal activities of four kinds of electrolysed water (SAEW1, SAEW2, SAEW3 and SAEW4) were compared by suspension quantitative bactericidal test. Morphological changes of *Escherichia coli*, amino acid composition and texture of LYC were evaluated during the SAEW1 treatments and the bacteriostatic mechanism of SAEW was observed. Suspension quantitative bactericidal test showed that bactericidal efficiency of SAEW for *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis* and *Candida* were 62, 48, 28 and 57%, respectively. Increasing the oxidation-reduction potential of SAEW from 640 to 1,250 mV modified the bactericidal efficiency for the bacteria above-mentioned to 100, 10, 97 and 100%, respectively. The damage process of *E. coli* O157:H7 under the SAEW1 treatment was observed after mixing the bacterium suspension with SAEW1 for 5 min. Finally, amino acid composition and texture analysis test showed that there were no significant differences (P>0.05) between the treated and control group. SAEW was proved to be a safe and effective sanitiser of the tested bacteria.

Keywords: food safety, food quality, antimicrobial activity, acidic electrolysed water

1. Introduction

Strongly acidic electrolysed oxidising water (SAEW) is also known as electrolysed oxidising water or high oxidation-reduction potential water. SAEW has a pH<2.8, a high oxidation-reduction potential (ORP >1000 mV) and a high available chlorine concentration (ACC) (Zhu *et al.*, 2008). SAEW was invented in the 1980s in Japan and has been used as an antimicrobial agent for many years (Guentzel *et al.*, 2008; Huang *et al.*, 2008). The primary component of SAEW is water. The antimicrobial mechanism is different from other sanitisers such as alcohol, peracetic acid and chlorhexidine acetate (Koseki *et al.*, 2004; Russell, 2003). The reports during the past decade indicate that it has the advantages of effective disinfection, easy operation, relatively inexpensive, and environmentally friendly compared to the traditional sanitisers (Ma and Li, 2008; Que, 1999). Therefore, SAEW was used extensively in food processing.

Larger yellow croaker (LYC), belonging to the family *Perciformes* and the genus *Sciaenidae*, is one of the principal fish cultivated throughout the world for its fleshy meat and daintiness of taste. With increasing demands for fresh and healthy food, easy and cost-effective food preservation becomes increasingly necessary in order to maintain the nutritional value, texture and flavour of food. A main challenge in this respect is to inhibit food-borne putrefactive bacteria during storage.

Using SAEW was proven to be a safe and effective method to prolong the shelf life of LYC. (Pei *et al.*, 2012; Shoji *et al.*, 2011). The objective of this study is to evaluate the effectiveness of SAEW on sanitizing bacteria at different pH, ORP and ACC and to reveal the relationship between the sanitizing rate and the chemical parameter of SAEW. Furthermore, antimicrobial mechanism, amino acid composition and texture analysis were studied to observe the impact of SAEW treatment on LYC.

2. Materials and methods

Materials and reagents

Fresh samples of large yellow croaker were obtained from Zhoushan Marine Fisheries Aquaculture Companies (Zhoushan, China P.R.), weighing 280±15 g. Sodium chloride, sodium thiosulfate, acetate buffer and phosphate buffer were used as analytically pure and purchased from Sinopharm Group Co. Ltd. (Shanghai, China P.R.). Tryptic soy broth medium were purchased from Qingdao Hope Biol-Technology Co., Ltd. (Qingdao, China P.R.). *Escherichia coli* O157:H7(pH value ranged from 4.5 to 8.0), *Bacillus subtilis* (pH value ranged from 3.0 to 8.0), *Staphylococcus aureus* (pH value ranged from 4.5 to 9.0) and *Candida* (pH value ranged from 4.2 to 6.0) were obtained from Faculty of Food and Pharmacy, Zhejiang Ocean University, Zhoushan, China P.R.

Main equipment and apparatus

An AR224CN precision balance from Ohuas Co., Ltd. (Shanghai, China P.R.); 539-WT refrigerator from Haier Co., Ltd. (Qingdao, China P.R.); MDF-U4186S ultra low temperature refrigerator from Sanyo Co., Ltd. (Tokyo, Japan); HD-108 ice crusher from Hongda Co., Ltd. (Guangzhou, China P.R.); TMS-PRO food texture analyser from Food Technology Corporation (Sterling, VA, USA); BX53 microscope from Olympus Co., Ltd. (Tokyo, Japan); L-8900 amino acid analyser from Hitachi Co., Ltd. (Tokyo, Japan); Q7 water electrolyser from Hitachi Co., Ltd. (Tokyo, Japan); and 510M-01 multi-parameter measuring meter from Thermo Fisher scientific Co., Ltd. (Waltham, MA, USA), were used.

Strongly acidic electrolysed water preparation

Diluted salt solution was added into the electrolytic cell of Q7 water electrolyser first. Then the water electrolyser was set to produce SAEW with a sequential pH around 3, 4, 5 and 6. SAEW was collected and stored in a brown reagent bottle at 4 °C while maintaining the pH. Finally, the pH and ORP were measured by multi-parameter measuring meter. ACC was measured according to the method of iodimetric analysis (Ruan *et al.*, 2010). The SAEW obtained with different pH value were named SAEW1, SAEW2, SAEW3 and SAEW4, corresponding to the sequential pH solutions, respectively.

Cultures and cell suspension

E. coli O157:H7, *B. subtilis* and *Candida* were cultured for investigating the bacteriostatic action of SAEW. Bacteria were individually enriched in 9 ml of tryptic soy broth. *B. subtilis* and *E. coli* O157:H7 were cultured at 37 °C for 24 h and *Candida* was 25 °C for 24 h. Enriched cultures were centrifuged at 5,000×*g* for 30 min at 4 °C. Pelleted cells were washed three times with 5 ml of buffered peptone water and re-suspended in buffered peptone water to produce a culture cocktail of approximately 10^6 cfu/ml.

Suspension quantitative bactericidal test

In order to evaluate the germicidal efficacy of SAEW, suspension quantitative bactericidal test was used. There were two groups: ‘treated group’ and ‘control group’. Each group contained 6 test tubes. 0.1 ml bacterial suspension was added into 4.9 ml acidic electrolysed oxidising water (SAEW1, treated group) or into 9.9 ml physiological saline (control group) for a selected time of 1, 2, 3, 4, 5 and 10 min. At the end of defined hold time, 0.5 ml suspension bactericidal liquid of each tube in the treated group was taken out and mixed with 4.5 ml neutralising agents (1 g/l sodium thiosulfate, 5 g/l Tween 80 and phosphate buffer) for 10 min. The neutralising agents prevent the residual action of SAEW on the bactericidal liquid suspension. Finally, 1 ml miscible liquid of every tube was transferred to tryptic soy broth medium at 37 °C for 24 h, respectively. Total colony counts were recorded to investigate the germicidal efficacy. The sanitizing effectiveness of SAEW was calculated as follows:

$$\text{Sanitizing effectiveness} = \frac{A\text{-}B}{A} \times 100\%$$

Where A = total colony count of treated group, and B = total colony count of control group.

Effect of the structure of *Escherichia coli* O157:H7 after the SAEW1 treatment

The suspension of *E. coli* O157:H7 (10^6 cfu/ml) was added into two tubes named A and B. 4 ml SAEW1 was then added into the tube A (treated group) and same volume of physiological saline was added into the tube B (control group). 5 min later, 200 µl miscible liquid each from the tube A and B was transferred to slides and stained using lycopene. Morphological changes of *E. coli* O157:H7 before and after the SAEW1 treatment were observed under the microscope.

Bacteriostatic mechanism of SAEW

0.1 mol/l HCL was diluted with distilled water to make DA1, DA2, DA3 and DA4; the ACC of these four reagents were made the same as of SAEW1, SAEW2, SAEW3 and SAEW4, respectively, by adjusting with sodium hypochlorite containing 5 g chlorine/l. Sodium hypochlorite containing 5 g chlorine/l was diluted with distilled water to make DH1, DH2, DH3 and DH4; the pH value of these four reagents were made the same as of SAEW1, SAEW2, SAEW3 and SAEW4, respectively, by adjusting with 0.1 mol/l HCL.

LYC fish samples were soaked in 300 ml of a reagent for 5, 10, 15, 20 and 25 min. The test was repeated for the four reagents to study the bactericidal efficacy of the reagents against the LYC surface pathogens. At the end of each time interval, each LYC sample (280±5 g) was transferred to a large beaker containing 2.8 l physiological saline and shaked for 10 min to prepare bacteria suspension. 1 ml of bacteria suspension with different dilution ratios (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) was transferred to nutrient agar and cultured at 37 °C for 24 h. Finally, the total colony count was calculated.

Amino acid composition test of large yellow croaker before and after SAEW treatment

Amino acid analyses were used for observing the impact of using the SAEW treatment for LYC, which were performed according to (Adeyeye, 2009). About 25 mg of fresh fish meat was weighted into glass ampoules. 7 ml of 6 M HCL was added. Nitrogen was used to expel the oxygen from the glass. Then, the glass ampoules were sealed with a Bunsen flame and put into an oven at 110 °C for 22 h. The content was filtered to remove the humins after the ampoule was allowed to cool. The filtrate was then evaporated to dryness at 40 °C under vacuum in a rotary evaporator. Each residue was dissolved with 5 ml acetate buffer (pH 2.0). Amino acids composition was analysed in the L-8900 amino acid analyser by separation in an ion-exchange column and post-column reaction with ninhydrin. Finally, it was calculated with a standard solution of amino acids. Tryptophan was not detected in this study.

Effect of the quality and structure of large yellow croaker after the SAEW treatment

Fish samples were assigned into two groups including the treated group and the control group, each group contains 3 fish samples. The fish sample quality and structure was assessed using the FTC texture analyser with 100 N load cell and computer software. A flat-ended cylindrical probe of stainless steel having 5 mm diameter was used for the compression study. Due to non-homogeneity in the fish flesh the sampling area was selected from the upper portion with respect to the lateral line of the fish body. The sample was placed under the probe that moved downwards to a distance of 35 mm at a constant speed of 40 mm/min.

Statistics

Analysis of variance (ANOVA) were performed to analyse the data obtained in the study by using the software of SPSS17.0 (SPSS Inc., Chicago, IL, USA), where $P<0.05$ presented the significant difference between the means of two independent tests in ANOVA.

3. Results

Parameters of strongly acidic electrolysed oxidising water

As Figure 1 shows, the water electrolyser was comprised of an anode and a cathode which are separated by a two-way ion exchange membrane. When adding a diluted salt solution into the electrolytic cell, negatively charged ions such as chloride hydroxide in the diluted salt solution move to the anode to give up electrons and become oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid and hydrochloric acid, while positively charged ions such as hydrogen and sodium move to the cathode to take up electrons and become hydrogen gas and sodium hydroxide. SAEW with a low pH value, a high ORP and a high ACC is produced from the anode side. Four different kinds acidic electrolysed oxidising water were obtained according to different electrolysed time and be named SAEW1, SAEW2, SAEW3 and SAEW4. The detail parameters are shown in Table 1. SAEW1 had the lowest pH value (2.62), the highest OPR (1,250 mV) and ACC (45.12 mg/l), which spent the longest electrolysed time.

Results of suspension quantitative bactericidal test

Suspension quantitative bactericidal test was used to indicate the bactericidal efficiency of different kinds of SAEW. *E. coli* (Gram-negative bacteria), *S. aureus*, *B. subtilis*

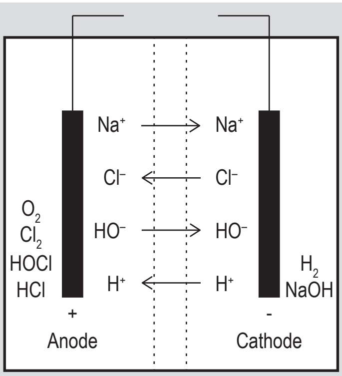

Figure 1. Schematic diagram of electrolysing process.

Table 1. Parameters of 4 strongly acidic electrolysed oxidising water (SAEW) treatments.

	SAEW1	SAEW2	SAEW3	SAEW4
pH	2.62	3.8	5.4	6.2
ORP[1] (mV)	1,250	1,120	812	640
ACC[2] (mg/l)	45.12	37.05	32.08	26.45

[1] ORP = oxidation-reduction potential water.
[2] ACC = available chlorine concentration.

(Gram-positive bacteria) and *Candida* (fungal) were selected as the test objects. As can be seen from Table 2, 2 min later after the SAEW1 treatment, the bactericidal rate for *S. aureus, E. coli* and *Candida* were 90, 85 and 82%, while it was only 23% for *B. subtilis*. This was probably owing to the spore present in the *B. subtilis*. That would make the *B. subtilis* more difficult to be sanitised. When the treatment time increased to 10 min, the bactericidal rate for *S. aureus, E. coli* and *Candida* all increased to 100 and 97% for *B. subtilis* as well. The same phenomenon could be seen in the other groups. The bactericidal rate decreased when the pH of SAEW increased from 2.62 to 6.2. The bactericidal rate for *S. aureus, E. coli*, *B. subtilis* and *Candida* were 62, 48, 28 and 57% when treated with SAEW4 for 10 min at pH of 6.2. Generally speaking, the optimum pH of bacteria is from 4 to 8 (Yang *et al.*, 2003). The pH values of SAEW3 and SAEW4 are within this range. Another reason for SAEW1 to have an increased antimicrobial activity over the other SAEW was the high ORP and ACC, which could cause the modification of metabolic fluxes and ATP production, probably due to the change in the electron flow in cells (Liu *et al.*, 2006). SAEW1 was selected as the antimicrobial agent observed in the subsequent test due to the best antimicrobial activity against a variety of microorganisms compared with other SAEW.

Results of morphological changes of *E. coli* O157:H7 before and after SAEW treatment

As is presented in Figure 2, detail morphological changes of *E. coli* O157:H7 before and after the SAEW1 treatment were observed. The cell shape was intact under the microscope before the SAEW treatment. Once the bacteria contacted with SAEW1, the cell shape began to shrink and fracture. Ten min later, all the bacteria were dead. One reason reported for the germicidal efficacy of strongly acidic electrolysed oxidising water is the high ORP (Lu *et al.*, 2010; Park *et al.*, 2009), which can change the membrane potential of the bacteria and facilitate the transfer through the cell membrane. Finally, it makes the cell membrane fractured (Ozer and Demirci, 2006; Panglolip *et al.*, 2009).

Antimicrobial mechanism of SAEW

In order to explore the antimicrobial mechanism, DA and DH (containing the same value of ACC or ORP compared with SAEW) were used. The bactericidal efficiency of the reagents on inactivating the bacteria on the surface of large yellow croaker was observed. As can be seen from Table 3, the bactericidal efficiency of the reagents in group 1 and 2 was better than reagents in group 3 and 4. The

Table 2. Inactivation of microbes using strongly acidic electrolysed oxidising water (SAEW).[1]

	Concentration (log cfu/ml)	1 min	2 min	3 min	4 min	5 min	10 min
SAEW1							
Staphylococcus aureus	5.83±1.05	5.01±0.35	4.83±0.46	4.31±0.15	3.83±0.22	-	-
Escherichia coli	5.72±1.75	5.02±1.45	4.89±1.26	4.80±1.13	4.72±0.45	3.72±0.84	-
Bacillus subtilis	5.88±0.50	5.88±0.83	5.76±0.67	5.64±1.08	5.54±0.29	5.35±0.57	4.35±1.38
Candida	5.74±0.35	5.14±0.34	5.00±1.26	4.64±0.79	4.74±1.48	-	-
SAEW2							
S. aureus	5.83±1.05	5.23±0.35	4.95±100	4.68±1.24	4.31±0.52	-	-
E. coli	5.72±1.75	5.19±0.42	4.90±1.96	4.80±1.37	4.56±2.37	4.41±2.56	-
B. subtilis	5.88±0.5	5.88±2.42	5.79±1.11	5.71±0.54	5.62±0.49	5.38±1.23	4.95±1.14
Candida	5.74±0.35	5.28±0.83	5.17±0.73	4.85±1.49	4.64±1.05	4.44±2.21	4.04±2.03
SAEW3							
S. aureus	5.83±1.05	5.58±2.38	5.47±1.08	5.38±1.47	5.28±2.33	5.23±1.51	5.06±2.45
E. coli	5.72±1.75	5.49±2.08	5.41±0.74	5.32±0.94	5.23±1.02	5.16±0.70	5.11±1.04
B. subtilis	5.88±0.50	5.88±2.70	5.85±2.48	5.82±0.39	5.74±0.69	5.69±1.77	5.59±0.77
Candida	5.74±0.35	5.55±1.53	5.46±2.34	5.37±0.68	5.27±1.55	5.22±0.40	5.08±0.37
SAEW4							
S. aureus	5.83±1.05	5.72±1.91	5.67±0.90	5.59±1.96	5.51±0.60	5.49±1.09	5.41±1.55
E. coli	5.72±1.75	5.65±0.84	5.59±1.22	5.55±0.62	5.51±2.14	5.49±1.82	5.43±0.21
B. subtilis	5.88±0.50	5.88±1.69	5.87±0.65	5.84±2.57	5.82±1.72	5.80±0.96	5.73±1.23
Candida	5.74±0.35	5.66±0.65	5.62±2.09	5.57±0.53	5.50±0.47	5.46±1.67	5.37±0.49

[1] See Table 1 for the parameters of the 4 SAEW treatments.

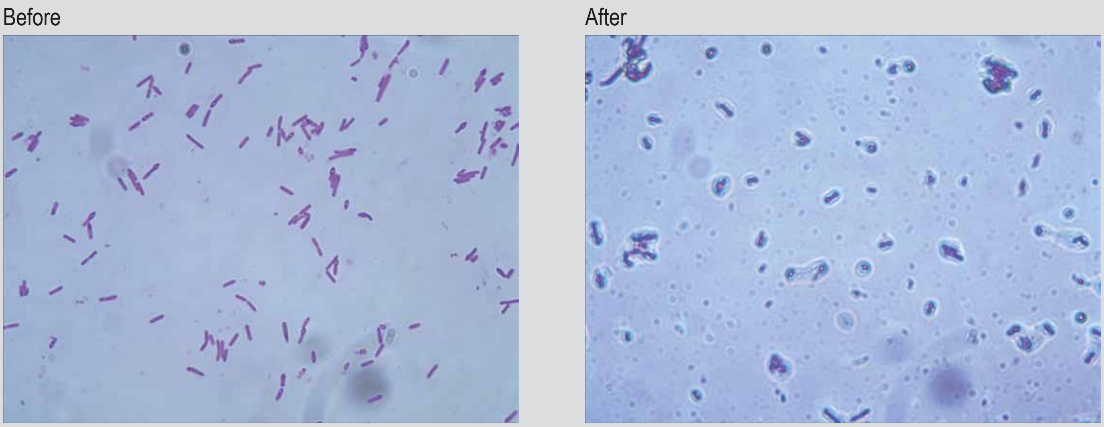

Figure 2. Morphological changes of *Escherichia coli* O157:H7 due to SAEW treatment.

Table 3. Total plate count on the surface of large yellow croaker after different kinds of bactericides treatment.[1]

	5 min	10 min	15 min	20 min	25 min
Group 1					
SAEW1	4.26±1.19	3.65±1.00	3.30±0.64	2.81±0.49	2.80±0.94
DH1	4.40±2.14	4.18±1.28	3.89±0.53	3.68±1.56	3.58±1.20
DA1	4.30±0.99	3.72±0.41	3.45±1.49	3.21±1.39	3.10±0.74
Group 2					
SAEW2	4.48±0.65	4.18±1.57	3.93±0.57	3.75±1.75	3.63±0.35
DH2	4.56±1.70	4.43±0.47	4.23±7.5	4.18±1.18	3.95±0.29
DA2	4.51±2.34	4.32±1.82	4.12±2.10	3.88±0.59	3.74±1.05
Group 3					
SAEW3	4.60±1.21	4.54±1.04	4.43±0.79	4.30±0.69	4.26±0.29
DH3	4.61±1.31	4.56±0.79	4.48±2.09	4.34±0.99	4.32±1.05
DA3	4.60±1.67	4.55±1.48	4.46±1.75	4.33±2.03	4.30±0.80
Group 4					
SAEW4	4.62±0.75	4.57±1.27	4.46±0.35	4.41±1.75	4.40±0.76
DH4	4.61±0.39	4.58±1.94	4.54±0.60	4.51±0.93	4.51±0.68
DA4	4.61±1.03	4.57±1.35	4.47±1.50	4.45±2.19	4.40±1.94

[1] SAEW = strongly acidic electrolysed water; DH = SAEW samples diluted with 0.1 mol/l HCL; DA = SAEW samples diluted with sodium hypochlorite containing 5 g/l chlorine.

total plate count (log_{10} cfu/g) of group 1 was 4.4 when treated with DH1 for 5 min, and it was 4.3 for DA1 and 4.26 for SAEW. When the treatment time increased to 25 min, the total plate count decreased to 4.26, 4.4 and 2.8, respectively. The same phenomenon also existed in group 2, but the decrease in total plate count was not obvious in group 3 and 4. SAEW showed better bactericidal efficiency than DA and DH in the same group. Therefore, ORP was largely responsible for inactivating bacteria, which was not present in DA and DH. Sanitisation mechanisms of available chlorine are: (1) disruption of protein synthesis; (2) oxidative decarboxylation of amino acids to nitrites and aldehydes; (3) reactions with nucleic acids, purines, and pyrimidines; and (4) unbalanced metabolism after the destruction of key enzymes. Thus, it was the comprehensive action of low pH, ORP and ACC that allowed the SAEW to inactivate the bacteria (Kim *et al.*, 2001; Xiong *et al.*, 2010; Zhang *et al.*, 2012).

Different of amino acid composition of large yellow croaker treated with SAEW1

LYC was rich in amino acid compared with other marine fishes (Xu *et al.*, 2004). As can be seen from Table 4, the average total amino acid (TAA) was 65.5 mg/g, the essential amino-acids such as Thr, Val, Met, Phe and Lys contained

Table 4. Amino acid composition of large yellow croaker.[1]

Amino acid	Control group	Treated group
Asp	6.80±0.74	5.90±0.22
Thr	3.20±1.08	3.20±0.35
Ser	2.90±0.19	2.50±0.28
Glu	10.90±0.97	9.50±1.43
Pro	1.30±0.77	1.30±0.12
Gly	2.50±1.00	2.50±0.51
Ala	4.10±0.25	4.00±0.70
Cys-Cys	1.00±1.21	1.10±0.24
Val	3.20±0.22	3.20±0.18
Met	2.00±0.40	1.80±0.11
Ile	3.20±0.17	3.00±0.70
Leu	5.60±1.32	5.20±0.72
Tyr	2.80±0.70	2.50±0.68
Phe	3.00±0.61	3.00±0.25
Lys	7.00±0.56	6.80±0.76
His	1.60±0.39	1.50±0.19
Arg	4.40±0.93	4.20±0.23
Total amino acids	65.50±2.30	61.20±1.70

[1] Values are averages of duplicate determinations of pooled samples of each trial, expressed as mg/g of fish carcasses, dry basis.

in LYC were 3.2, 3.2, 2.0, 3.0 and 7.0 mg/g. In particular, LYC contained abundant Glu. It is ordinarily used as a food additive in monosodium glutamate. The high content of Glu in LYC (10.9 mg/g) indicated the fish samples had a high freshness. LYC is consumed as a major protein source in food. It is very important that the amino acid contents should not be compromised during the sanitisation processing. The TAA in the treated group was 61.2 mg/g and the essential amino-acids in treating groups were not destroyed. The Thr, Val, Met, Phe and Lys content were 3.2, 3.2, 1.8, 3.0 and 6.0 mg/g, respectively, slightly lower than the control group. Using SAEW for inactivating bacteria was different from the traditional sanitisation methods like high temperature or high pressure, which will not break the protein structure. Results of amino compositions test indicated SAEW could be considered as an acceptable sanitiser in the food industry.

Texture analysis

The variation in textural parameters of the fish samples is presented in Table 5. There was a slight variation in fish hardness between the control group and the treated group, which was 22 N for the control group and 21 N for the treated group. The brittleness of the fish sample was 22 N for the control group and 18 N for the treated group. Other texture indexes were not significantly different (P>0.05) between the treated and control group.

4. Conclusions

Using SAEW for inactivating bacteria has already been reported. Li *et al.* (2012) studied the effect of SAEW, sanitisation of total bacteria numbers of pork samples and changes of physicochemical parameters of SAEW during treatments. Results showed that bactericidal efficiency

Table 5. Texture analysis of fish cakes.

	Hardness (N)	Brittleness (N)	Viscosity (mJ)	Cohesion (P)	Elasticity (mm)	Tackiness (N)	Chewiness (mJ)
Control group	22±1.67	22±1.54	0.18±0.04	0.35±0.12	1.65±1.50	7.60±2.5	12.56±1.13
Treated group	21±2.03	18±1.07	0.15±0.03	0.42±0.05	1.65±1.30	7.86±3.8	15.31±1.06

increased from 88.2 to 96.3%, as the ACC of SAEW increased from 29.62 to 88.87 mg/l (Li *et al.*, 2012). Xie *et al.* (2010) studied the efficiency of electrolysed water washing on the inhibition of bacteria for raw shrimp. Almost no bacterial colonies were observed after treated with SAEW for 20 min. However, the detail antimicrobial mechanism and the impact of LYC before and after using the SAEW had not been evaluated sufficiently. In this paper, the detail antimicrobial mechanism of SAEW had been discussed. Amino acid composition and texture analysis test showed that the amino acid concentration of LYC was comparable when using SAEW to inactive the bacteria of large yellow croaker. SAEW was proved to be an effective sanitiser of sea food.

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