Optimization of bacteria-reducing of oyster by ozone combined with slightly acidic electrolytic water

Ying BU, Yueyue LV, Guizhi TAN, Wenhui ZHU*, Jianrong LI*

College of Food Science and Engineering, Bohai University, National & Local Joint Engineering Research Center of Storage, Processing and Safety Control Technology for Fresh Agricultural and Aquatic Products, Jinzhou 121103, China

Corresponding author’s e-mail: zhuwenhui@bhu.edu.cn; lijr6491@163.com

Abstract. In this paper, oysters were treated with ozone purification, and then the response surface methodology (RSM) was used to optimize the bacteria-reducing process with slightly acidic electrolytic water (SAEW) based on total viable counts (TVC). The factors affecting the sterilization effect were the available chlorine concentration (ACC) > soaking time > solid-liquid ratio. The optimization results showed that the best effect was obtained by soaking the oyster in SAEW with effective chlorine concentration of 30 mg/L and solid-liquid ratio of 1:3 for 13 min after purification 9 h. The total colony of oyster decreased by 0.84 lgCFU/g under this condition.

1. Introduction
Oysters, which belong to the phylum Mollusca, are one of the most important marine bivalves for global human consumption [1]. In 2020, China’s oyster production has reached more than 5,000,000 t [2]. Oysters are a valuable marine shellfish and an excellent source of quality nutrition [3-5]. However, it grows in a relatively fixed location, easily filtering seawater sediment and bacteria into the body [6]. Therefore, the first step in the treatment of live oysters is purification. Ozone technology, which is commonly used in shellfish purification, was used for preliminary purification of oyster [7]. After opening oyster shells, without the protection of tissues, they are more vulnerable to microbial contamination, which affects their edible quality and shelf life.

Electrolytic water refers to the use of electrochemical methods to dilute salt solution or dilute hydrochloric acid solution in the electrolysis device for electrolysis, which produces functional water with sterilization characteristics. Electrolytic water exists as acidic electrolytic water and alkaline electrolyzed water. Acidic electrolytic water has been authorized to sterilize. SAEW has the best bactericidal effect among strongly acidic, weakly acidic and SAEW. Currently, SAEW has been used in the processing and storage of aquatic products [8].

Therefore, the purpose of this paper is to study the purification and bacteriostatic effects of ozone combined with SAEW on oyster.

2. Materials and methods
The fresh oysters were cleaned with a brush, which oysters of equal size and quality were selected as the research object. According to Tao et al. [9] and the results of our previous study, first of all, the selected oysters were put into the prepared artificial seawater to spit sand for 15 h.
2.1 Oysters ozone purification
Put the air outlet pump head of the ozone generator into the temporary tank which contain artificial seawater, then opened the ozone generator and measured the concentration of ozone in seawater at regular until the concentration reached 0.25 mg/L. The experimental seawater without ozone gas and the seawater with ozone concentration up to 0.25 mg/L were taken to detect the total number of bacteria. The survival rate of oysters was calculated every 3 h and a few oysters were taken out to determine the total number of bacterial colonies. The concentration of ozone was determined by the neutral potassium iodide method.

2.2 Preparation of treatment solutions
SAEW was generated in a SAEW generator, and diluted hydrochloric acid (0.1%) and tap water were continuously pumped into the SAEW generator. According to the preparation time of different preparation needed for the slightly acidic electrolytic water. The pH and ORP (Oxidation-reduction potential) were determined using a pH/ORP meter equipped with pH and ORP probes. The free chlorine concentration was determined using iodine quantity method\[10\].

2.3 Response surface methodology
In order to optimize the bacteria-reducing process of slightly acidic electrolytic water, the Box-Behnken design (BBD) was employed. Such designs were used to evaluate the interaction between selected experimental factors and yield responses accordingly. On the basis of the previous single factor experiment, the BBD was constructed using three independent variables (A: ACC, B: soaking time, C: solid-liquid ratio).

2.4 Microbiological analyse
Oyster samples (25 g) were homogenized in 225 mL of sterile 0.85% NaCl solution for 1 min. Decimal dilutions were performed and 1 ml of each dilution was transferred to plate count agar (PCA, Qingdao Hope Bio-Technology Company Ltd., China). Plates were incubated at 30℃ for 48 ± 3 h. Total viable counts (TVC) were determined by counting the number of colony forming units \[11\].

2.5 Statistical analyses
The experimental data was analysed by one-way analysis of variance (ANOVA). Duncan procedure was used to reveal intergroup differences. Figures were obtained using OriginPro9 (Origin Lab Co., Northampton, MA, USA).

3. Results and discussion
3.1 Effect of ozone purification on the survival rate of oyster
In order to explore the purification effect, several oysters were taken out every 3 h to measure the TVC and calculate the survival rate at the same time. As can be seen from Fig 1 and Fig 2, there were significant differences in the changes of TVC and survival rate of oyster (P< 0.05). The purification effect of oyster was the best in the first 3 h, which the bactericidal value was 0.38 lgCFU/g. With the extension of purification time, the purification effect decreased. Both the control groups and the experimental groups showed a decreasing trend in the survival rate of oysters. The TVC in oysters decreased slowly from the 9 h, and the survival rate also began to decrease from the 3 h, but the survival rate in the first 9 h was always greater than 80%, and the decrease trend was not significant. Therefore, 9 h was selected as the best time for oyster purification in this experiment.
3.2 Response surface test design analysis

As shown in Table 1, the TVC of oysters was between 3.65 lgCFU/g and 4.04 lgCFU/g. The regression equation of the TVC (Y) on the ACC (A), soaking time (B) and solid-liquid ratio (C) was obtained by Design Expert, and the response surface equation was predicted as follows:

\[ Y = 3.68 - 0.10A - 0.064B - 0.052C + 0.028AB + 0.015AC + 0.030BC + 0.11A^2 + 0.099B^2 + 0.046C^2 \]

The analysis results of the regression model were shown in Table 2. The results of the variance analysis showed that the \( P < 0.001 \), which implied a satisfactory and significant model. The missing term was not significant because \( P = 0.7950 > 0.05 \), so the quadratic model was established. This model can be used to predict the TVC and optimize the sterilization process. The factors affecting the sterilization effect were the ACC > soaking time > solid-liquid ratio. Studies have shown that the model has a good fit when the regression coefficient \( R^2 \) is more than 0.8 when explaining the relationship between data changes and parameters. It can be seen that \( R^2 = 0.9875 \), indicating that the model was in a good agreement with the actual situation.

Among the three factors, the interaction of the ACC and soaking time, ACC and solid-liquid ratio, and the soaking time and solid-liquid ratio were significant. The steeper the response surface curve is, the more significant the interaction of the three factors is. As can be seen from Fig 3, the response surface slope of interaction terms AB and BC are relatively steep, indicating that the interaction between AB and BC has a significant impact on the experimental results. The slope of the response surface of the interaction term AC is relatively gentle, indicating that the interaction of AC has no significant influence on the experimental results.

The optimum conditions for sterilization by response surface optimization were the effective chlorine concentration was 33.78 mg/L, the soaking time was 13.11 min, and the solid-liquid ratio was 1:3.27, and the TVC was 3.64 lgCFU/g. To facilitate the actual operation, the sterilization conditions were set as the effective chlorine concentration was 30 mg/L, the soaking time was 13 min, and the ratio of solid to liquid was 1:3. Finally, the TVC of 3.66 lgCFU/g was optimized by Design-Expert 8.0 software (Table 3). It was close to the 3.64 lgCFU/g predicted by the model, which indicated that the model was highly reliable and the bacteria reduction conditions using the model were reliable.

| Run | ACC (mg/L) | Soaking time (min) | Solid-liquid ratio (w/v) | TVC (lgCFU/g) |
|-----|------------|-------------------|-------------------------|--------------|
| 1   | 20         | 5                 | 1:3                     | 4.04         |
| 2   | 40         | 5                 | 1:3                     | 3.89         |
| 3   | 20         | 15                | 1:3                     | 3.95         |
### Table 2: Analysis of variance (ANOVA) for the developed regression equation

| Source                        | Sum of squares | Degree of freedom | Mean square | F-value | P-value | Significance |
|-------------------------------|----------------|-------------------|-------------|---------|---------|--------------|
| Model                         | 0.26           | 9                 | 0.029       | 61.55   | <0.0001 | **           |
| A (ACC)                       | 0.082          | 1                 | 0.082       | 175.29  | <0.0001 |              |
| B (soaking time)              | 0.033          | 1                 | 0.033       | 69.49   | <0.0001 |              |
| C (solid-liquid ratio)        | 0.022          | 1                 | 0.022       | 47.13   | 0.0002  |              |
| AB                            | 3.025E-003     | 1                 | 3.025E-003  | 6.47    | 0.0385  |              |
| AC                            | 9.000E-004     | 1                 | 9.000E-004  | 1.92    | 0.2080  |              |
| BC                            | 3.600E-003     | 1                 | 3.600E-003  | 7.69    | 0.0275  |              |
| A^2                           | 0.054          | 1                 | 0.054       | 116.45  | <0.0001 |              |
| B^2                           | 0.041          | 1                 | 0.041       | 87.76   | <0.0001 |              |
| C^2                           | 9.007E-003     | 1                 | 9.007E-003  | 19.25   | 0.0032  |              |
| Residual                      | 3.275E-003     | 7                 | 4.679E-004  |         |         |              |
| Lack of fit                   | 6.750E-004     | 3                 | 2.250E-004  | 0.35    | 0.7950  | ns           |
| Pure error                    | 2.600E-003     | 4                 | 6.500E-004  |         |         |              |
| Corr Total                    | 0.26           | 16                |             |         |         |              |

R^2=0.9875 \quad R_j^2=0.9715

### Table 3: The predicted and experimental values under the optimum process conditions

| Condition                        | ACC (mg/L) | soaking time (min) | solid-liquid ratio (w/v) | TVC (lgCFU/g) |
|----------------------------------|------------|--------------------|--------------------------|---------------|
| Model expectation                | 33.78      | 13.11              | 1:3.27                   | 3.64          |
| actual value                     | 30         | 13                 | 1:3                      | 3.66          |

### 4 Conclusion

In this paper, live oysters were immersed in purified seawater with ozone concentration of 0.25 mg/L for 9 h, and the total colony of oyster could be reduced by 0.67 lgCFU/g. The optimization results showed that the best effect was obtained by soaking the oyster in SEAW with effective chlorine concentration of 30 mg/L and solid-liquid ratio of 1:3 for 13 min, the total colony of oyster decreased
by 0.84 lgCFU/g on this condition. This study provides theoretical reference for shellfish purification and bacteria-reducing in factories.

Acknowledgements
The National Key Research and Development Program (No.2019YFD0901702).

References
[1] LIU C S, JI W Z, JIANG H Z, et al. 2020. Comparison of biochemical composition and non-volatile taste active compounds in raw, high hydrostatic pressure-treated and steamed oysters *Crassostrea hongkongensis*. Food Chemistry, 344,128632.
[2] FAO. 2017. Fisheries and aquaculture information and statistics service. Available at: http://www.fao.org/fishery/statistics/en.
[3] HE Y, YANG R J, and WANG Z. 2003. Nutritional components and proteolysis of oyster meat. Journal of Fisheries of China, 27(2):163-168.
[4] WANG Q, LI W, HE Y, et al. 2014. Novel antioxidative peptides from the protein hydrolysate of oysters (*Crassostrea talienwhanensis*). Food Chemistry, 145, 991-996.
[5] MEI X, LUAN L, HONG P C, et al. 2020. Anti-fatigue property of oyster polypeptide fraction and its effect on gut microbiota in mice. Food & Function, 11(10):8659-8669.
[6] LIN Q, MA Q T, LI C X, et al. 2017. Effect of combined ozone-ultraviolet on the total colony count and the coliform group in *Crassostrea rivularis*. Journal of Tropical Biology, 8(4), 404-408,423.
[7] YANG H, LOU Y J. 2004. Current situation of shellfish purification and research progress of purification technology. China Fisheries, 5: 72-73.
[8] SUZUKI K, NAKAMURA T, DOI T, et al. 2005. The disinfectant effect of slightly acidic electrolyzed water prepared with hydrochloric acid as a raw material for lettuce. Journal of Antibacterial and Antifungal Agents, 33, 589-597.
[9] TAO J, YANG R J, ZHANG W B, et al. 2008. Purification technique of fresh oysters. Food Science and Technology, 7: 108-112.
[10] ISSA-ZACHARIA A, KAMITANI Y, MIWA N, et al. 2011. Application of slightly acidic electrolyzed water as a potential non-thermal food sanitizer for decontamination of fresh ready-to-eat vegetables and sprouts. Food Control, 22, 601-607.
[11] Chinese National Standard. 2016. GB/T 4789.2-2016 Food microbiological examination: Aerobic plate count. Beijing: The Chinese National Hygiene Ministry.
[12] PARMAR P, SHUKLA A, GOSWAMI D, et al. 2020. Optimization of cadmium and lead biosorption onto marine Vibrio alginolyticus PBR1 employing a Box-Behnken design. Chemical Engineering Journal Advances. 4, 100043.
[13] LI Y, CUI F, LIU Z Q, et al. 2007. Improvement of xylanase production by *Penicilliu moxalicum* ZH-30 using response surface methodology. Enzyme and Microbial Technology, 40(5): 1381-1388.