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Elimination of *Escherichia coli* from Oysters using Sodium Bicarbonate

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Abstract

Sodium bicarbonate is a disinfectant that can be used in food processing because it is safe for humans and is cost effective. In this study, sodium bicarbonate was used to eliminate *E. coli* from oysters (*Crassostrea belcheri*). The experiments were divided into 3 trials; in vitro disinfection of *E. coli* using sodium bicarbonate and the toxicity of sodium bicarbonate in oysters were determined in the first and second trials, and the effectiveness of sodium bicarbonate in eliminating *E. coli* in naturally contaminated oysters was performed in the final trial. From the in vitro study, it was found that sodium bicarbonate at 10 and 100 mM could inhibit *E. coli*; however, the high dose of 100 mM is not suitable to use on oysters, since the 50 % lethal concentration of sodium bicarbonate for oysters was 63 mM. An application of sodium bicarbonate to eliminate *E. coli* in naturally contaminated oysters at 10 mM for 1 h could decrease the *E. coli* to 96 MPN/100 g, lower than the standard level (230 MPN/100 g) after depuration. These results suggest that sodium bicarbonate could be potentially used to eliminate *E. coli* contamination in oysters.

Keywords: Sodium bicarbonate, elimination, oyster, *E. coli*

Introduction

Oysters are filter feeders that can concentrate pathogens, approximately from > 1 to 1000 fold higher than the levels of microorganisms than over laying waters [1]. Raw, or only lightly cooked, consumption of oyster may pose a risk to human health. Microbial contamination is chronic and pervasive in harvesting areas. Presently, measures for controlling public health problems associated with the consumption of sewage contaminated shellfish rely on the use of fecal contamination indicators, such as *Escherichia coli,* to determine the sanitary quality of shellfish harvesting areas [2,3]. Some *E. coli* strains that are pathogenic for humans and cause diarrheal illness may be categorized into specific groups based on virulence properties, mechanisms of pathogenicity and clinical syndromes [4,5]. Purification procedures (depuration or relaying) are required before shellfish can be marketed as live mollusks. Purification of oysters may be accomplished by relaying or depuration where the oysters are held in tanks of seawater that have been sterilized by physical or chemical means, mainly by Ultraviolet (UV) irradiation, ozone and chlorination [6]. However, several studies have shown that high doses of ozone produce seawater residues and are toxic for fish and bivalves [7,8]. Although UV irradiation is effective, it requires filtration of water to 1 μm and an extended exposure time to achieve maximum effectiveness. Moreover, UV irradiation causes higher costs of maintenance than other methods [9-13]. In the case of chlorine, reports suggested it to be a high efficiency disinfectant. However, the reaction between chlorine and organic nitrogen in water may produce residues which are toxic for marine organisms[13-16].

Sodium bicarbonate has a long history of use as an antimicrobial agent against bacteria and fungi [17-21] in foodstuffs, feed and industrial processes such as tooth pastes and mouth rinses [22-25]. This is because of its safety, low cost, and lack of toxicity. Consequently, the purpose of this study was to determine the efficiency of sodium bicarbonate for the elimination of *E. coli* in oysters.

Materials and methods

Bacteria

E. coli was obtained from laboratory of biotechnology, Walailak University and cultured in nutrient broth at 37 °C for 24 h before use.

Disinfection of *E. coli* using sodium bicarbonate in vitro

The completely randomized experimental design was applied with 4 treatments in triplicate. The treatments were devised following the concentration of sodium bicarbonate at 0, 1, 10 and 100 mM. Aqueous solutions of sodium bicarbonate were prepared in sterile distilled water with 0.85 % NaCl at the concentration of 1, 10 and 100 mM. *E. coli* was seeded into each aqueous solution of sodium bicarbonate at a final concentration of 10^3 CFU/ml. After incubation for 0, 1, 3, 6, 12 and 24 h., the number of *E. coli* were counted using the spread plate method on eosin-methylene blue agar (EMB) in triplicate.

The toxicity of sodium bicarbonate in oysters

In this experiment, live oysters (*Crassostrea belcheri*, n = 60) were harvested from oyster farms located in Suratthani, Thailand. The oysters were evenly distributed in tanks with 200 liters of sterilized seawater and acclimatized for 24 h. After 24 of bioaccumulation, the oysters were randomly divided into 4 groups in triplicates. Five oysters in each group were incubated in 5 liters of sterilized seawater with constant aeration. One group served as a negative control and received no sodium bicarbonate treatment. The other groups were exposed to 10, 50 and 100 mM of sodium bicarbonate. The mortality of oysters was observed for 24 h, following which the median lethal concentration (LC_{50}) of sodium bicarbonate was calculated by probit analysis method [26]. A safe concentration level was calculated by multiplying the LC_{50} value by factor of 0.1 [27].

Elimination of *E. coli* in contaminated oysters using sodium bicarbonate

Naturally contaminated oysters (n = 70) were harvested from oyster farms located in Suratthani, Thailand. Sea water was taken from the Shrimp Quarantine Center, with a temperature of 30 °C, salinity of 30 ppt, and a pH of 8. Initially, seven oysters were randomly checked for the amount of *E. coli*. The remaining oysters (n = 63) were divided into 3 groups. One group (n = 21) served as a negative control and received no sodium bicarbonate treatment. The other groups were exposed to 5 and 10 mM of sodium bicarbonate. The oysters were randomly sampled after 1, 3 and 6 h of disinfection and *E coli* number were determined using *E. coli* medium (EC) (Difco Co., Ltd.) and cultured up to 24 h at 44.5 °C according to the most probable number (MPN) technique and expressed as the MPN per 100 g of oyster tissue.

Statistical analysis

Data was analyzed using SPSS v13.0 (Statistical Package for the Social Sciences, Chicago, IL, USA). Significant differences ($p < 0.05$) between mean values were determined using Duncan's multiple range tests.

Results and discussion

Disinfection of *E. coli* using sodium bicarbonate in *vitro*

The results of the in vitro study showed that sodium bicarbonate in a certain concentration effectively reduced the number of *E. coli*. At 10 and 100 mM concentrations, numbers of *E. coli* continuously decreased within 12 h (**Figure 1**). A concentration of 100 mM reduces the initial number of

E. coli by 67 %. In contrast, growth of *E. coli* was still observed in the control group and in the group of 1 mM.

The toxicity of sodium bicarbonate in oysters

When the toxicity of sodium bicarbonate in oysters was investigated, it was found that the mortality of oysters was not observed in the control group and 10 mM of sodium bicarbonate. The LC_{50} concentration was obtained at 63 mM (**Figure 2**). So, the safe concentration level of sodium bicarbonate for oysters is 6.3 mM.

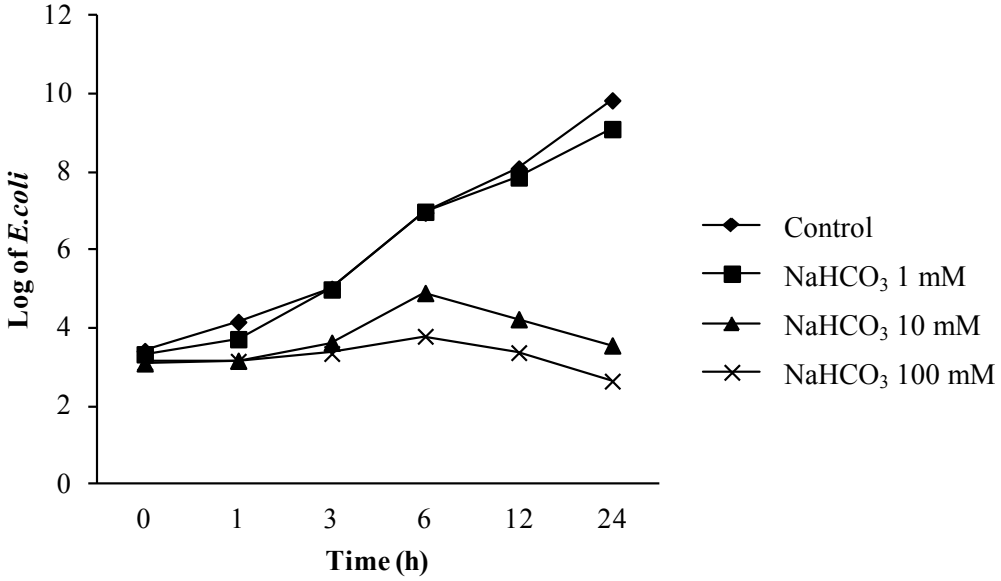

Figure 1 Disinfection of *E. coli* by using sodium bicarbonate in vitro.

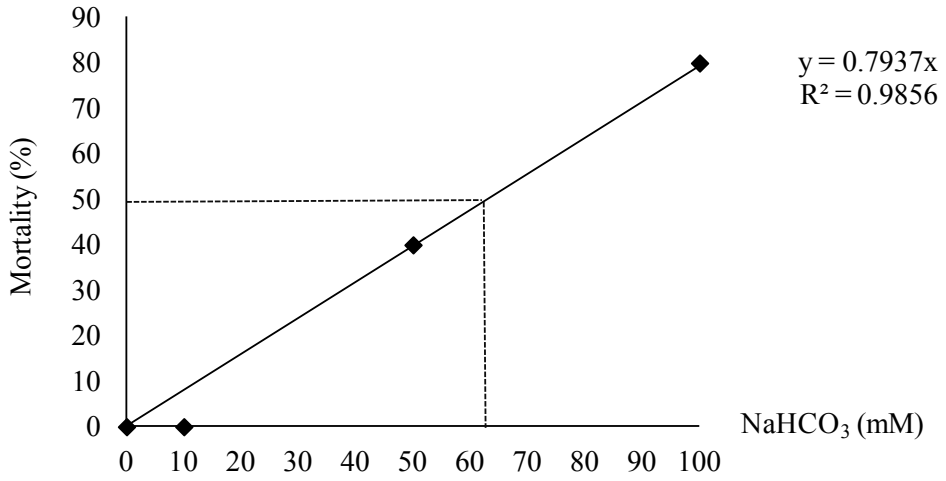

Figure 2 Median lethal concentration of sodium bicarbonate for oysters exposed at 24 h.

Elimination of *E. coli* in contaminated oysters using sodium bicarbonate

Oysters collected from the wild were found to be naturally contaminated with *E. coli* at an average amount of 2,276 *E. coli* MPN/100 g. The result from **Table 1** showed that there were significant ($p < 0.05$) differences of *E. coli* numbers among treatments. *E. coli* in naturally contaminated oysters depurated with 10 mM of sodium bicarbonate were significantly lower than the control and decreased below detection limits (96 *E. coli* MPN/100 g) after being depurated for 1 h.

Table 1 *E. coli* counts in naturally contaminated oysters after depuration with sodium bicarbonate.

	E. coli* counts (MPN/100 g)			
	0 h	**1 h**	**3 h**	**6 h**
Control	2276 ± 3148^{a}	403 ± 290^{a}	360 ± 294^{a}	110 ± 75^{a}
Sodium bicarbonate 5 mM	2276 ± 3148^{a}	361 ± 299^{a}	106 ± 112^{b}	93 ± 36^{b}
Sodium bicarbonate 10 mM	2276 ± 3148^{a}	96 ± 95^{b}	33 ± 11^{b}	34 ± 19^{b}

*The different superscripts in the same column indicate significant differences between treatments by DMRT ($p < 0.05$).

The problem of infection rising from the consumption of bivalve is widely recognized [28-29]. Live shellfish sold to the consumer must conform to a microbiological end product standard of less than 230 *E. coli* MPN/100 g [29]. A variety of methods have been developed to reduce the bacterial loading in water supplies in aquaculture, such as ozonization, UV irradiation and chlorination [30-32]. However, those methods are still too complicated for the farmer, so simpler alternative methods, such as use of sodium bicarbonate, which has antibacterial properties, is safe, has a low cost, and has a lack of toxicity to humans and marine animals, should be considered.

The antibacterial properties of sodium bicarbonate have been reported [33]; the *E. coli* number reduced to 4 log from the initial number when disinfected with 120 mM within 48 h by the changes in the physiochemical environment of the microorganisms. Moreover, the *E. coli* K12 number decreases after being treated with EDTA or carbonate, because lipopolysaccharides (LPS) were destroyed [34,35]. The high concentrations of sodium bicarbonate ranged from 182 to greater than 728 mM/L and was found to be bacteriocidal for oral gram negative bacteria, *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus* and *Eikenella corrodens* [22]. However, in this study, low concentrations of sodium bicarbonate 10 and 100 mM could inhibit *E. coli* after incubatation for 24 h. Even though the result from in *vitro* show that 100 mM was more effective than 10 mM, this level is higher than the 50 % lethal dose (63 mM). Thus, the concentration of sodium bicarbonate at 5 and 10 mM are suitable designed for oyster depuration. The result showed that the *E. coli* number was decreased to below the detection limit (96 *E. coli* MPN/100 g) after depuration with 10 mM sodium bicarbonate for 1 h. This dose was considered to be safe for humans because it is lower than the dose (1 % of sodium bicarbonate or 119 mM) that is used for washing adipose beef carcass [36]. Moreover, this method is more practical for the farmer due to the shorter time for depuration when compared to the other methods of chlorine, ozone, iodophores and UV irradiation, that usually take 2 days [37,38].

Conclusions

The results from this study showed that sodium bicarbonate could be potentially used to eliminate contamination of *E. coli* in oysters. The technique is simple and cost effective. The recommended dose is 10 mM sodium bicarbonate for 1 h.

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