

## Evaluation of stabilised hydrogen peroxide as sanitiser in seafood processing industry

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### ABSTRACT

The efficiency and period of effectiveness of three different sanitisers viz., sodium hypochlorite, hydrogen peroxide and commercial stabilised hydrogen peroxide were studied at different locations in seafood processing factories. The results were analysed using the analysis of variance technique and means were separated using the Tukey 'B' test at 5% level of significance using SPSS16.0. All the treatments were found to be significantly different from each other ( $p < 0.05$ ). The commercial stabilised  $H_2O_2$  gave a distinctly better overall sanitising action at 5% level of significance. The study has shown that the commercially available stabilised hydrogen peroxide is the most effective sanitiser compared to sodium hypochlorite and plain hydrogen peroxide. Stabilised  $H_2O_2$  is a better disinfectant for achieving sanitation and hygiene in food processing industries, particularly in the protein and moisture rich seafood processing industry. It is ecofriendly and least harmful compared to chlorine and its derivatives.

Keywords: Hygiene, Processing, Sanitiser, Seafood, Stabilised  $H_2O_2$

### Introduction

Seafoods are one of the most perishable food items, highly susceptible to bacterial and enzymatic spoilage unless handled, processed, packed and stored scientifically. High standard of hygiene and sanitation are the pre-requisites for safety and quality in food production and they form the foundation on which HACCP and other food safety management systems work. Prevention of contamination from food contact surfaces and workers is controlled by adopting standard sanitations operating procedures (SOP). Sanitisation and cleaning of equipment used for processing is to be evaluated for controlling cross-contamination during the production process. Clean-up and disinfection need to be considered as regular procedures since they can remove most microorganisms that can contaminate equipments. All processing equipment surfaces are subject to adhesion of microorganisms, and thus being a possible source of diseases caused by contaminated food (Silva *et al.*, 2010).

Sanitisers are used to reduce the pathogenic and spoilage microorganisms in food processing facilities and equipments including personnel. The major types of sanitisers in use are heat, radiation and chemicals. Heat and radiation techniques are less practical for food processing as compared to sanitising with chemicals. A wide variety of chemical sanitisers such as iodine

compounds, quaternary ammonium compounds, peracetic acid, ozone *etc.* are now available. Presently seafood industry mainly uses sodium hypochlorite as sanitising agent. Gram negative and Gram positive bacteria are equally susceptible to chlorine compounds (Hayes, 1985). Chlorine levels of 20-25 ppm were shown to be effective in killing strains of both *Escherichia coli* and *Listeria monocytogenes* in fish processing industry (FAO and WHO, 2000). *Staphylococcus* required 10 ppm chlorine to reduce their population by 99% (Overdahl and Zottola, 1991). Sizable population of *Vibrio cholera* was reduced to less than  $1 \text{cfu ml}^{-1}$  after exposure to 100 ppm chlorine for 2 min (McCarthy and Miller, 1994)

While hydrogen peroxide and ozone are well known for their decomposition to harmless products, their effectiveness as a sanitiser especially in protein and moisture rich seafood activities is questionable. In this background, a study was organised with the main objective to evaluate the efficiency of different sanitisers. They were used at different locations in fish processing plants and performance was evaluated. The period of effectiveness of sanitisers were also studied.

### Materials and methods

The study was undertaken in three different seafood processing plants located at Kochi, Kerala, India. The

sanitisers were applied after primary cleaning with neutral detergent and brush as per standard operating procedures (SOP). The sanitisers used for efficiency testing were sodium hypochlorite, hydrogen peroxide and a commercially available stabilised hydrogen peroxide (containing 571 mg l<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> and colloidal silver 0.36 g l<sup>-1</sup> as declared by the manufacturer). The concentrations of sanitisers used in each surface and contact time are given in Table 1.

Stainless steel processing table top, conveyor belt, tray, floor, wall, worker's hand, foot dip and insulated vehicle were identified as experimental food contact surfaces. A unit area of 5x 5 cm<sup>2</sup> was selected and bacteria associated with it were aseptically transferred to 100 ml sterile buffer water using sterile swab. Total plate counts per unit area, was estimated following standard method (APHA, 2001) by plating on to Tryptone Glucose Beef Extract Agar (Himedia, Bombay) medium. Plates were incubated at 37 °C for 48 h. After incubation, the number of colonies developed on the plates were counted for total bacterial load and expressed as cfu cm<sup>-2</sup>. In order to see the duration of effect, the swab samples were taken after 2 h and 4 h and TPC cm<sup>-2</sup> were determined. A control sample was also taken from the same surface without any sanitiser application.

The experimental design was an 8x3x3 factorial arrangement of treatment in a completely randomised

design with three repetitions. The results were analysed using the analysis of variance (ANOVA) technique and means were separated using the Tukey 'B' test at 5% level of significance using SPSS16.0.

## Results and discussion

Sanitisers need to be used for cleaning and disinfection of food processing equipments and food contact surfaces to maintain a clean processing and storage environment for any food. There are some sanitisers that cannot be used on contact surfaces due to the effect they have on food. Some sanitisers alter the odour of fish and other food materials and may affect the taste. Some may leave residues that can cause food safety problems. Of the three chemicals used for the present study, none of them leave any objectionable odour, flavour or taste to the product at recommended levels of treatment. The sanitiser used in foot dip and hand dip should not cause any irritation or any discolouration to skin. The processing area should also be clean, disinfected and free from pungent odours, to have a healthy environment.

The total plate count cm<sup>-2</sup> (TPC cm<sup>-2</sup>) values obtained for control and after sanitiser treatment for various food contact surfaces during the present study are given in Table 2. In general, the results reveal that application of

Table 1. Concentrations (ppm) of sanitisers used in different surfaces related to sea food processing industry for 20 min contact time

Surface	Sodium hypochlorite	Hydrogen peroxide	Commercial stabilised Hydrogen peroxide
Processing table	100	1000	1000
Conveyor belt	50	1000	1000
Tray	50	1000	1000
Floor	200	1000	1000
Wall	100	1000	1000
Worker's hand	20	200	200
Foot dip	100	500	500
Vehicle	200	1000	1000

Table 2. Total plate count per cm<sup>2</sup> from different processing areas after the application of different sanitisers for 20 min contact time (mean ±SE)

Surface	Control	Sodium hypochlorite	Hydrogen peroxide	Commercial stabilised H <sub>2</sub> O <sub>2</sub>
Processing table	756±3.21	560±5.86	56±5.77	20±2.08
Conveyor belt	280±5.51	84±0.58	12±1.53	4±1.15
Tray	40±3.21	24±2.65	16±1.53	12±1.73
Floor	512±0.58	76±1.53	176±3.46	52±1.0
Wall	52±1.86	32±0.58	16±2.52	12±2.52
Worker's hand	4116±8.33	1568±6.81	32±0.58	4±0.58
Foot dip	2816±11.37	8±0.58	4±1.15	4±1.15
Vehicle	4340±18.48	3664±8.08	1636±11.37	596±3.46

sanitiser has significant effect on bacterial load, particularly, stabilised hydrogen peroxide has shown good effectiveness on reducing bacterial load compared to the other sanitisers used in the study. While stabilised H<sub>2</sub>O<sub>2</sub> was found to be three to four times more effective than sodium hypochlorite, plain H<sub>2</sub>O<sub>2</sub> was found slightly less effective than stabilised hydrogen peroxide. In all cases, bacterial levels were significantly ( $p < 0.05$ ) reduced compared to control, where no sanitiser was used. It is also seen that for processing table, worker's hand and insulated vehicle, the levels of chlorine is ineffective to achieve desired sanitation. H<sub>2</sub>O<sub>2</sub> and stabilised H<sub>2</sub>O<sub>2</sub> were found ineffective only for the heavily contaminated insulated vehicles. On looking at the values for the control sample, it is clear that those surfaces which were not sanitised to desired level before the application of sanitisers, were originally having high bacterial load. The prior cleaning of these surfaces was not satisfactory for the sanitiser to act effectively. It was found that all the sanitisers tested in the present study had significantly different sanitising property on the locations studied ( $p < 0.05$ ).

Table 3 summarises the sustainability of sanitary condition of the three different sanitisers after 2 h. Generally it is seen that, as the time increases, the rate of retention of sanitising action decreases. In the case of hydrogen peroxide, the sanitising action was found to decline prior to 2 h post-application.

Table 4 gives the retention of sanitising property after 4 h of application. Hydrogen peroxide as such is having very poor sanitising power after 4 h of application where as commercial stabilised H<sub>2</sub>O<sub>2</sub> showed retention of sanitising action even after 4 h. This is because commercial sanitiser being stabilised hydrogen peroxide is available for a longer period.

After 20 min of application, all the treatments differed significantly ( $p < 0.01$ ) among themselves in their effect on the surface of application (Table 5). When the swab samples were taken and analysed after 2 h of sanitiser application, the resultant count significantly differed ( $p < 0.01$ ) among different sanitisers tested. The same was the observation at 4 h of application. From the same table, it could also be inferred that sanitising power of stabilised hydrogen peroxide was the best among the sanitisers taken up for comparative study.

The analysis of data using ANOVA indicated significantly different sanitising effects between the four treatments. In the case of commercial stabilised H<sub>2</sub>O<sub>2</sub>, it gave a distinctly better overall sanitising action at 5% level of significance. The same effect was observed even when each treatment area was taken separately. When both treatment and time factors were taken together, also distinctly different sanitising action was shown by each sanitiser, and the most significant sanitising action and

Table 3. Total plate count per cm<sup>2</sup> from different processing areas, 2 h after the application of different sanitisers (mean  $\pm$  SE)

Surface	Control	Sodium hypochlorite	Hydrogen Peroxide	Commercial stabilised H <sub>2</sub> O <sub>2</sub>
Processing table	776 $\pm$ 11.32	28 $\pm$ 1.15	56 $\pm$ 3.46	8 $\pm$ 3.46
Conveyor belt	324 $\pm$ 2.30	36 $\pm$ 1.15	20 $\pm$ 1.15	20 $\pm$ 3.05
Tray	66 $\pm$ 3.05	56 $\pm$ 2.30	20 $\pm$ 1.15	4 $\pm$ 0.58
Floor	644 $\pm$ 1.15	32 $\pm$ 1.15	196 $\pm$ 1.15	8 $\pm$ 1.15
wall	76 $\pm$ 3.05	24 $\pm$ 1.52	24 $\pm$ 2.08	4 $\pm$ 0.58
Worker's hand	4216 $\pm$ 11.25	360 $\pm$ 12.50	144 $\pm$ 9.56	116 $\pm$ 4.91
Foot dip	3028 $\pm$ 16.77	56 $\pm$ 2.08	84 $\pm$ 0.58	28 $\pm$ 2.18
Vehicle	5340 $\pm$ 2.30	2360 $\pm$ 8.66	228 $\pm$ 4.04	104 $\pm$ 4.50

Table 4. Total plate count per cm<sup>2</sup> from different processing areas 4 h after the application of different sanitisers (mean  $\pm$  SE)

Surface	Control	Sodium hypochlorite	Hydrogen peroxide	Commercial stabilised H <sub>2</sub> O <sub>2</sub>
Processing table	752 $\pm$ 38.2	20 $\pm$ 1.92	160 $\pm$ 8.1	4 $\pm$ 0.03
Conveyor belt	360 $\pm$ 3.5	20 $\pm$ 1.45	28 $\pm$ 1.75	8 $\pm$ 1.04
Tray	56 $\pm$ 2.9	52 $\pm$ 5.45	16 $\pm$ 0.84	0
Floor	712 $\pm$ 20.2	36 $\pm$ 3.7	224 $\pm$ 3.65	0
wall	80 $\pm$ 7.1	32 $\pm$ 3.2	28 $\pm$ 1.94	0
Worker's hand	4004 $\pm$ 90.5	344 $\pm$ 7.6	184 $\pm$ 13.01	80 $\pm$ 2.75
Foot dip	2816 $\pm$ 109.1	72 $\pm$ 5.4	36 $\pm$ 2.13	8 $\pm$ 0.43
Vehicle	6320 $\pm$ 201.3	1252 $\pm$ 65.5	244 $\pm$ 12.33	40 $\pm$ 0.91

Table 5. Comparison of effects of different sanitisers at different time periods (in terms of total plate count per cm<sup>2</sup>; mean ±SE)

Period	Control	Sodium hypochlorite	Hydrogen peroxide	Commercial stabilised H <sub>2</sub> O <sub>2</sub>
20 min	1614.0±359.5 <sup>a</sup>	752.0±252.03 <sup>b</sup>	243.5±110.29 <sup>c</sup>	88.0±40.16 <sup>d</sup>
2 h	1808.8±406.6 <sup>a</sup>	369.0±158.46 <sup>b</sup>	96.4±16.24 <sup>c</sup>	36.4±9.04 <sup>d</sup>
4 h	1887.4±446.3 <sup>a</sup>	228±83.47 <sup>b</sup>	116.0±45.979 <sup>c</sup>	17.6±5.55 <sup>d</sup>

Values bearing different superscripts differ significantly (p<0.05)

retention of sanitising property was for commercial stabilised H<sub>2</sub>O<sub>2</sub> (p<0.05).

The effectiveness of sanitisers is also related to the cleanliness of the surface to be sanitised. If organic materials such as blood, slime and dirt are not removed by scrubbing with detergents and washing with potable water, the disinfectant applied rapidly combines with them and neutralises the disinfecting ability of any sanitiser solution. Simply soaking baskets, crates, knives and processing equipments in a sanitiser is ineffective. According to Maurer (1987) and Giese (1991), a concentration of chlorine compound, which gives 100–200 ppm of available chlorine is recommended for the disinfection after cleaning the food processing equipment and food contact surfaces with suitable neutral detergent. Concentrations ranging from 50 to 200 ppm available chlorine with a contact time of 20–30 min are normal in food plant sanitation for eight hour shift cleaning and disinfection (Hayes, 1985).

The advantages of commercially available sanitisers like stabilised hydrogen peroxide are: long term effectiveness, effective even in low concentrations, gentle to the skin, colourless, odourless and tasteless. It fulfils not only all the requirements for the disinfectants but also superior to conventional products in its ability to eliminate bacteria, viruses, mold, fungi, amoeba, spores as well as to remove biofilm. It can disinfect reliably all areas where reduction of pathogens and food safety are a prime necessity. In the case of chlorine, it is seen that there is no further reduction of bacterial load after 2 h. In case of stabilised H<sub>2</sub>O<sub>2</sub>, the reduction in bacterial load continues even after 2 h and a nano level combination of silver nitrate and a weak organic acid with hydrogen peroxide, the proportions of which are kept secret by the manufacturers.

The main difference between stabilised hydrogen peroxide and ordinary hydrogen peroxide lies in the presence of the nano quantity of silver nitrate and organic acid which serve as a 'stabiliser and activator' at the same time. This stabiliser prevents the decomposition of hydrogen peroxide. The nano quantity of silver nitrate also serves as a disinfectant to boost up the effect of H<sub>2</sub>O<sub>2</sub>. In contact with bacterial, cell and other biological material, the silver reacts and loses its stabilising function which leads to the activation of the hydrogen peroxide. The capacity of the silver to activate the hydrogen peroxide is

preserved until the silver nitrate is fully used by biological material. As long as some silver nitrate is left over, some H<sub>2</sub>O<sub>2</sub> will remain stable and will be available for long time disinfection effect

In case of contact with organic material, one of the oxygen atoms from the peroxide group will tend to release itself from the H<sub>2</sub>O<sub>2</sub> molecule to form water. The nascent oxygen atoms released from the H<sub>2</sub>O<sub>2</sub> in this way possess high kinetic energy, which efficiently oxidise the bacteria and other organic materials. In contrast to the normally activated hydrogen peroxide, the reaction is quick and all H<sub>2</sub>O<sub>2</sub> decompose quickly and the excess nascent oxygen generated acts as disinfectant and remaining part combine to form molecular oxygen without any disinfection property. Thus, in many respects, stabilised H<sub>2</sub>O<sub>2</sub> is a better disinfectant for achieving sanitation and hygiene in food processing industry, particularly in the protein and moisture rich fish processing industry. It is ecofriendly and least harmful compared to chlorine and its derivatives.

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