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Efficacy of Sanitizers on the Shelf Life of Rohu Steaks Stored at two Refrigeration Temperatures

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Steaks of rohu (*Labeo rohita*) were washed and dipped in 0, 30 and 50ppm concentrations of either Chlorine (Cl) or Chlorine dioxide (ClO_2) for 20min, packed in trays and stored at one of the two refrigeration temperatures; 4-6°C and 1-2°C. Changes in moisture of steaks were not significant. The pH of steaks stored at both temperatures increased significantly from 6.55 to 7.37 at 4-6°C and 6.5 to 7.32 at 1-2°C during storage across all treatments and especially in the last phase. Mesophilic bacteria in steaks treated with either sanitizer recorded low or nil counts initially, but quickly rebounded after 4-6 days storage at both temperatures and increased with no significant differences between treatments. Psychrophilic bacteria followed a similar pattern of increase during storage, but 50ppm ClO_2 treated steaks had significantly lower counts than others when stored at 1-2°C. Total volatile base nitrogen (TVBN) levels increased significantly at 1-2°C, but not at 4-6°C. At 1-2°C, TVBN increased markedly between 8-14 days, but more slowly in ClO_2 treated steaks. Organoleptically, the 4-6°C stored steaks were inedible by the 10th day, but were still acceptable when stored at 1-2°C. Sensory signs of spoilage, mainly appearance of off-odours were found to be delayed in ClO_2 treated steaks at both temperatures. The lower temperature of storage for steaks appeared to be the primary cause of extension in shelf life, but ClO_2 treatment did appear to improve the sensory properties of steaks.

Keywords: Chlorine, chlorine dioxide, fish steaks, *Labeo rohita*, refrigerated storage, sanitizers, spoilage

Chlorine in various forms is widely used in food industry for disinfection and sanitization of process surfaces, equipment and raw materials. Chlorine in the form of sodium hypochlorite solution has long been used in fish processing industry. Liquid chlorine and hypochlorites are generally used in the 50 to 200 ppm concentration range with a contact time of 1 to 2 min to sanitize produce surfaces and processing equipment, but studies indicate those chlorine concentrations traditionally used with produce (<200 ppm) are not particularly effective at reducing microbial populations on foods like lettuce (USFDA/CFSAN, 2001). The Codex Alimentarius Commission observed that the high concentrations of chlorine used in treatment wash water for foods such as frog legs may not necessarily be found in the end product (FAO/UN, 1979). However, concerns have also arisen about its safety, due to corrosive effects of chlorine and the reported formation of chloramines and trihalomethanes during its use. Chlorine dioxide (ClO_2) is one of the relatively newer alternatives used for disinfection and sanitization of foods and water which has been found to be more effective and leaves lesser harmful residues compared to chlorine (Imaeda *et al*, 1994; Elphick, 1998; Arora *et al*, 2001). Chlorine dioxide is the principal species that is

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formed at neutral pH range when sodium chlorite is acidified, which is the usual way ClO_2 is generated for use. Further, chlorine dioxide through reaction with microbial organisms / organic matter is reduced to chlorite and the typical degradation pathways for chlorite would then follow with oxidation to form a small amount of chlorate and reduction to form the major residue component: chloride (FSANZ, 2003).

It is also to be noted that chlorine disinfectants with the exception of chlorine dioxide are readily inactivated by the presence of organic soil (FAO/UN, 1979) and thus are less likely to be effective in such circumstances. White shrimp (*Penaeus setificus*) and pond raised crawfish (*Procambarus clarkii*) meat samples treated with ClO_2 had significantly lower aerobic and psychrophilic counts than chlorine treated ones with no residues, while significant total organic halogen residues were present in chlorine treated samples (Andrews et al., 2002). The FDA permitted in 1998, the use of chlorine dioxide as an antimicrobial agent in water used in poultry processing in an amount not to exceed 3 parts per million (ppm) residual chlorine dioxide and at the same level for washing of fruits and vegetables (FDA 1998). In August 1999, U.S. Food & Drug Administration (FDA) also approved the use of a commercial 5% stabilized chlorine dioxide preparation as an antimicrobial agent in water and ice that are used to rinse, wash, thaw, transport, or store seafood (International dioxicide Inc., 2006). The New Zealand food safety authority permits the use of both Chlorine and ClO_2 at a maximum available chlorine concentration of 1ppm in all foods ClO_2 provided the final food contains no more than the maximum permitted level specified (NZFSA, 2008). ClO_2 has been used in poultry processing for washing of carcasses and in seafoods for washing of round fish (Kim *et al,* 1999), for reduction of post-harvest pathogen inoculum during handling of fruits (Roberts & Reymond 1994), improving colour and shelf-life of white mushrooms (Bartley et al, 1991), but reports of its use in dressed fish portions are limited (Huang *et al,* 1996).

Dressed fish portions such as fillets and chunks are more amenable to cook than round fish and have been preserved by storing on ice and more recently under modified atmosphere packaging (MAP). Farmed tilapia fillets in frozen condition are a major import item into USA (Picchietti, 1996). Storing dressed fish portions on ice necessitates re-icing to replace the melted ice, and also leaches soluble compounds more easily from the exposed fish flesh. Strict temperature control is necessary in MAP to prevent the occurrence of *Clostridium botulinum* (Garcia–Mendez 1988). Due to the use of minimal heat processing, high water activity, absence of preservatives, and the use of many different often exotic ingredients, these products have a high risk potential (Martens, 1995). While the efficacy of chlorine and ClO_2 on process ice, fish and shellfish have been tested before (Iyer & Choudhuri, 1966, Huang et al, 1996, Kim *et al,* 1999) reports on comparison of their efficacy in extending the shelf life of refrigerated fish steaks are again limited. We report in this paper, the effectiveness of different concentrations of chlorine and ClO_2 sanitization on steaks of a fresh water fish *Labeo rohita* and its subsequent shelf life when stored at two refrigeration temperatures.

Materials and Methods

Rohu (*Labeo rohita)* of 600 to 800g size in good, iced and post rigor condition were purchased from the fish market. Fish were brought to the laboratory within 1h in iced condition. The processing floor as well as all processing surfaces such as boards, knives, trays, bowls etc., were sanitized with 100ppm ClO_2 using a commercial chlorine dioxide preparation made following the manufacturer's instructions (Vetcare, Bangalore, India) and a contact time of 20 min. Fish were weighed and dressed immediately by descaling, definning and evisceration. The

dressed fishes were thoroughly washed once with tube well water and then cut into 1-1.2 cm thick steaks of around 40-50g weight using fresh pre-sanitized boards and knives. Processing of fish into cut steaks was completed within 30 min.

Fish steaks were dipped in 5x their weight of ice-water mixture (0.5:1) containing effective chlorine concentrations of either chlorine or chlorine dioxide (ClO_2) at 0, 30 and 50 ppm for 20 min. Actual chlorine concentrations were verified using a chlorine test kit (Microquant chlorine test kit, E. Merck (India) Ltd) immediately after preparation of the chlorine or ClO_2 solutions. The steaks were then drained for 5min and packed in 750 ml polystyrene display trays (16.7x14x5 cm) with lids. Tray bottoms were lined with paper napkins and overlaid with perforated 100guage polypropylene (PP) sheets. Trays, lids and PP sheets were sanitized by dipping in 100ppm ClO_2 solution for 20 min, while napkins were sanitized by exposure to UV light for 2h. About 150g steaks were packed in each tray with lids. In separate set of experiments, the packed steaks were stored at either at 4-6 or 1-2°C. Samples were drawn on 0, 4, 6, 7/8, 10 & 14d of storage.

Aerobic plate count of samples was determined essentially as per FDA (2001) by pour plating on Plate count agar (Himedia Laboratories Ltd., Mumbai). One set of plates were incubated at 35°C for 24-48 h and another set at 10-12°C for 48-72 h for mesophilic and psychrophilic counts respectively. In addition the following parameters were also analyzed: Moisture as per AOAC (1984), Total volatile base nitrogen by Conway microdiffusion of 10% TCA extract (Conway, 1950). The pH of a 10g sample dispersed in 100ml of distilled water was recorded using a digital pH meter. Organoleptic evaluation was performed after cooking bite sized pieces of fish in 3% brine for 5min and graded by a trained panel on a 10-point hedonic scale. All chemicals and reagents used were of Analytical grade. Results were analyzed by two-way ANOA using Microsoft Excel XP software.

Results and Discussion

Moisture in fish steaks ranged between 69-77% (Table-1) during storage. Although moisture content varied during the course of storage at both storage temperatures, and decreased slightly towards end of the storage across the treatments, the changes, either between the treatments or during storage were not significant ($p<0.05$).

Table 1. Moisture content (%) of fish steaks treated with hypochlorite and chlorine dioxide and stored at 4-6 or 1-2°C

Storage period, d	Stored at, °C	Treatments				
		Control	30Hc	50Hc	30ClO_2	50ClO_2
0	4-6	75.52 ± 0.99	75.85± 1.45	75.88 ± 2.35	76.38 ± 1.09	76.68 ± 1.30
4		76.23 ± 2.08	74.17 ± 1.62	74.75 ± 0.75	71.98 ± 3.27	75.23 ± 1.43
7		74.81 ± 1.73	74.91 ± 1.28	76.88 ± 0.94	73.59 ± 2.85	77.23 ± 2.09
10		77.09 ± 1.84	74.48 ± 1.25	74.73 ± 0.86	74.99 ± 1.19	76.85 ± 2.31
14		ND	ND	ND	ND	ND
0	1-2	74.19 ± 0.99	74.12 ± 1.24	72.96 ± 0.84	74.61 ± 1.78	74.24 ± 1.53
4		74.40 ± 0.98	73.02 ± 0.42	72.30 ± 1.41	72.34 ± 1.64	73.26 ± 0.98
6		73.34 ± 2.04	73.23 ± 0.88	74.37 ± 0.89	72.25 ± 1.54	74.12 ± 1.52
8		72.39 ± 2.50	72.68 ± 1.60	75.46 ± 0.86	73.04 ± 1.20	73.47 ± 3.70
10		72.45 ± 1.74	72.92 ± 1.63	73.70 ± 0.96	72.78 ± 0.92	68.90 ± 5.54
14		71.48 ± 1.88	72.38 ± 1.11	74.53 ± 1.69	72.60 ± 1.69	72.15 ± 1.41

Hc- Hypochlorite,
ND – No data – study discontinued due to spoilage

The pH of the samples stored at 4-6°C initially ranged between 6.54 and 6.58 but increased after 10 days to between 7.12-7.54 (Table-2). Corresponding increase in 1-2°C stored steaks was from 6.42-6.55 initially to 7.20-7.52 after 14d. The increase in pH at both storage temperatures were similar, occurring mostly in the last phase of storage, i.e., between 7-10 days at 4-6°C and between 10-14 days at 1-2°C. Thus, increase in the mean pH of steaks during the earlier storage period (0-4d or 0-8d at 4-6 and 1-2°C respectively) were not significant, but the increase in pH from 7/8 to 10/14d were significant ($p<0.05$). The rate of pH increase was lower at the colder storage temperature. The colder storage temperature (1-2°C) did not appear to bring out significant differences ($p<0.05$) if any, between the sanitizer treatments, but at 4-6°C, the ClO_2 treated steaks had significantly ($p<0.05$) higher pH than control ones.

The initial microbial load was comparatively higher in the 4-6°C stored batch of fishes than 1-2°C stored batch of fishes (Table-3), notably in gills and viscera. The difference was mainly due to the market samples procured on different days. Mesophilic counts in 4-6°C stored steaks were not recordable (<25cfu/plate) in the sanitizer treated sets at 0d storage (Table-4), but reappeared after 4d, with marginally lower counts in ClO_2 treated steaks. Counts gradually increased to 9.27 log cfu/g in control and around 9.04 log cfu/g in treated steaks after 10d storage with no significant ($p<0.05$) differences discernable between the treatments. In steaks stored at 1-2°C, the average initial mesophilic counts in Cl/ClO_2 treated steaks were slightly lower than control (2.25 vs 2.39 log cfu/g) and were not recordable on 4d. Counts gradually increased to 9.93 log cfu/g in control and 7.85 log cfu/g (average) in treated sets on 14d. Chlorine dioxide treated sets recorded slightly but not significantly ($p<0.05$), lower counts than the control & hypochlorite treated steaks.

Initially the psychrophilic counts could not be recorded at both the storage temperatures as in case of mesophiles (Table-5). Later changes in the psychrophilic flora during storage were similar to that observed in mesophiles, increasing significantly ($p<0.05$) as the storage progressed when steaks were stored at either temperature. There were no significant differences ($p<0.05$) in the psychrophilic counts between the treated and

Table 2. The pH of steaks treated hypochlorite and chlorine dioxide and stored at 4-6 or 1-2°C

Storage period, d	Stored at, °C	Treatments				
		Control	30Hc	50Hc	$30ClO_2$	$50ClO_2$
0	4-6	6.54 ± 0.06	6.55 ± 0.09	6.59 ± 0.03	6.58 ± 0.02	6.56 ± 002
4		6.62 ± 0.04	6.72 ± 0.04	6.58 ± 0.04	6.70 ± 0.03	6.86 ± 0.02
7		6.73 ± 0.15	6.88 ± 0.03	6.83 ± 0.12	6.91 ± 0.01	6.93 ± 0.03
10		7.12 ± 0.11	7.27 ± 0.04	7.44 ± 0.03	7.54 ± 0.03	7.49 ± 0.01
14		ND	ND	ND	ND	ND
0	1-2	6.42 ± 0.02	6.53 ± 0.03	6.55 ± 0.02	6.52 ± 0.03	6.51 ± 0.02
4		6.55 ± 0.02	6.54 ± 0.02	6.56 ± 0.03	6.67 ± 0.03	6.61 ± 0.07
6		6.53 ± 0.02	6.62 ± 0.03	6.56 ± 0.01	6.55 ± 0.02	6.57 ± 0.03
8		6.55 ± 0.02	6.74 ± 0.02	6.54 ± 0.04	6.64 ± 0.02	6.63 ± 0.03
10		6.67 ± 0.02	6.78 ± 0.01	6.91 ± 0.02	6.79 ± 0.12	6.63 ± 0.02
14		7.52 ± 0.02	7.20 ± 0.02	7.33 ± 0.02	7.22 ± 0.01	7.31 ± 0.18

Hc- Hypochlorite,
ND – No data – study discontinued due to spoilage

Table 3. Initial microbial load (log cfu /g) of fish stored at 4-6 and 1-2°C

Stored at, °C	Skin (log cfu/ cm²)	Gills	Viscera
4-6	4.57	7.92	7.2
1-2	4.55	5.99	6.62

control steaks in steaks stored at either temperature. However, the rate of bacterial growth of both meso and psychrophiles in steaks stored at either temperature, were initially higher in Cl/ClO_2 treated steaks, but after 6-8d, generally decreased to rates lower than control.

The total volatile base nitrogen (TVBN) levels in the 4-6°C stored steaks increased slightly after 10d storage though decreasing briefly on the 4d (Table-6). The increase in TVBN occurred after only 4d in control and 30HC treated steaks compared to ClO_2 and 50HC treated steaks where the increase was delayed till 7d storage. Overall increase in TVBN was not significant ($p<0.05$) between storage periods or treatments. In steaks stored at 1-2°C TVBN levels increased sharply and significantly ($p<0.05$) after 10-14d of storage, mainly in control steaks. The 50ClO_2 steaks had significantly lower TVBN levels than control and HC30 treated steaks.

At 4-6°C the organoleptic scores decreased rapidly from an initial score of 8.70 to 6.33 by 7d and all steaks were inedible by 10d of storage (Table 7). ClO_2 appeared to delay the onset of off-odour, which became evident by the 7d in control and HC treated steaks, but did not occur in ClO_2 treated steaks till 10d (Table 8). These changes were however not reflected in the organoloeptic scores. Steaks stored at 1-2°C were acceptable at 10d, but with significantly decreased scores after 8d and inedible on 14d. Signs of spoilage were evident in control and HC treated steaks by 8d, but delayed till 10d in ClO_2 treated steaks, and evident by 14d. Physical and sensory observations on the stored steaks showed concomitant changes in colour, texture, odour and appearance of microcolonies on the steaks (Table 8) with organoleptic scores. Greater turbidity in dilution water of microbiological analysis was noticed as the spoilage progressed.

Table 4. Mesophilic count (log cfu/g) of steaks treated with hypochlorite and chlorine dioxide and stored at 4-6 or 1-2°C

Storage period, d	Stored at °C	Treatments				
		Control	30Hc	50Hc	30ClO_2	50ClO_2
0	4-6	6.81	nd	nd	nd	nd
4		6.39	6.95	7.81	6.04	5.78
7		7.69	8.17	7.23	8.00	6.72
10		9.27	8.94	9.23	9.04	8.94
14		ND	ND	ND	ND	ND
0	1-2	2.39	2.30	2.57	1.95	2.07
4		2.95	ND	ND	ND	ND
6		3.63	4.39	4.30	5.17	4.34
8		5.55	6.32	5.27	6.56	6.54
10		6.30	7.14	7.04	7.07	5.39
14		9.93	7.87	7.96	8.95	6.63

Hc- Hypochlorite, nd- Not detected (<25cfu/plate)

ND – No data study discontinued due to spoilage.

Table 5. Psychrophilic count (log cfu /g) of steaks treated with hypochlorite and Chlorine dioxide and stored at 4-6 or 1-2°C

Storage period, d	Stored at, °C	Treatments				
		Control	30Hc	50Hc	$30ClO_2$	$50ClO_2$
0	4-6	5.00	nd	nd	nd	nd
4		6.88	6.83	7.81	6.66	7.11
7		7.97	8.81	8.81	7.00	8.04
10		9.63	9.57	9.39	9.04	9.44
14		ND	ND	ND	ND	ND
0	1-2	2.57	ND	ND	ND	ND
4		3.34	4.00	4.00	3.83	3.55
6		4.23	5.30	5.43	6.07	5.32
8		5.55	7.46	7.17	8.14	7.34
10		7.17	8.17	7.77	7.70	6.89
14		8.84	8.90	8.97	9.17	9.11

Hc- Hypochlorite , nd- Not detected (<25cfu/plate),
ND no data- study discontinued due to spoilage.

Table 6. TVBN (mg N/100g) of steaks treated with hypochlorite and chlorine dioxide and stored at 4-6 or 1-2°C

Storage period, d	Stored at, °C	Treatments				
		Control	30Hc	50Hc	$30ClO_2$	$50ClO_2$
0	4-6	75.17 ± 1.76	61.77 ± 1.57	61.37 ± 1.52	62.97 ± 0.55	28.23 ± 0.74
4	4-6	43.20 ± 1.01	26.03 ± 1.27	49.47 ± 0.72	48.93 ± 0.86	44.30 ± 0.66
7	4-6	77.13 ± 0.32	63.27 ± 1.62	32.20 ± 1.57	31.43 ± 0.67	32.97 ± 0.15
10	4-6	94.73 ± 4.61	81.37 ± 1.17	56.37 ± 0.40	62.07 ± 0.80	41.23 ± 1.00
14	4-6	ND	ND	ND	ND	ND
0	1-2	44.17 ± 0.57	30.87 ± 0.31	34.07 ± 0.75	32.60 ± 0.56	27.37 ± 2.05
4	1-2	45.10 ± 0.60	42.90 ± 0.89	40.33 ± 2.46	41.30 ± 0.89	27.83 ± 0.25
6	1-2	46.87 ± 1.01	45.77 ± 0.97	31.83 ± 1.04	41.00 ± 0.80	32.30 ± 0.62
8	1-2	46.47 ± 0.45	54.53 ± 0.64	30.40 ± 1.50	49.10 ± 1.28	33.13 ± 1.00
10	1-2	54.43 ± 0.49	58.10 ± 0.46	47.97± 0.45	50.83 ± 1.04	36.97 ± 0.87
14	1-2	94.30 ± 1.15	61.60 ± 0.96	57.57 ± 1.02	52.50 ± 1.28	35.13 ± 0.42

Hc- Hypochlorite,
ND no data - study discontinued due to spoilage

Treatment of fish steaks with hypochlorite and chlorine dioxide was aimed at reduction of initial microbial load on steaks and also comparing their efficacy on shelf life. Processing operations can greatly increase bacterial loads on fish and shellfish (Chytiri *et al.*, 2004; Thampuran and Gopakumar, 1988), and thus require steps in processing where they can be reduced. The pH recorded a significant increase ($p<0.05$) during storage at both storage temperatures as spoilage products such as amines of alkaline nature accumulated in the steaks. A number of biochemical, genetic and physiological processes are impaired by chlorine exposure under differing conditions and

Table 7. Organoloeptic Scores of steaks treated with hypochlorite and chlorine dioxide and stored at 4-6 or 1-2°C

Storage period, d	Stored at, °C	Control	30Hc	50Hc	30ClO_2	50ClO_2
4-6	0	9.17±0.29	8.67±0.58	8.67±0.58	8.67±0.58	8.33±0.58
	4	7.67±0.76	8.08±0.52	8.00±0.52	8.00±0.50	7.67±0.76
	7	6.17±0.29	6.00±0.50	6.67±0.50	6.67±0.29	6.17±0.15
	10	IE	IE	IE	IE	IE
	14	ND	ND	ND	ND	ND
1-2	0	9.0±0.50	9.0±0.40	9.0±0.47	9.0±0.58	9.0±0.61
	4	8.25±0.29	8.25±0.29	8.25±0.50	8.50±0.50	8.50±0.58
	6	8.00±0.29	8.25±0.29	7.75±0.50	8.00±0.50	7.50±0.76
	8	7.75±0.29	8.25±0.14	8.05±0.14	7.75±0.58	8.00±0.0
	10	6.00±0.40	6.50±0.0	6.50±0.50	6.75±0.50	6.50±0.82
	14	IE	IE	IE	IE	IE

Hc- Hypochlorite, IE- Inedible,
ND No data - study discontinued due to spoilage

alternative water disinfection agents such as ozone, chlorine dioxide, and ultraviolet irradiation, will also probably lead to the same result. As a result, a significant portion of the total population of indicator bacteria in water might not be enumerated (using currently employed selective procedures) and inaccurate water quality determinations could result (McFeters & Camper 1983). The absence of recordable counts in chlorine / ClO_2 treated steaks in our study could be due to this reason and the lack of any repair period or special media for recovery of injured bacteria as adapted by other workers to recover ClO_2 injured bacteria (Han et al, 2002, Thampuran *et al*, 2006). The intervening period of storage in this study could have provided the necessary repair period for resuscitation of the bacteria, accounting for their sudden reappearance during the next sampling. Further, incomplete sterilization of bacteria in food systems by sanitizers can also result due to formation of biofilms where reaction-diffusion limited penetration may result in only low levels of antimicrobial exposure to deeper regions of the biofilm. Sheltered cells are then able to enter an adapted resistant state if the local time scale for adaptation is faster than that for disinfection (Szomolay *et al*, 2005). Karthikeyan *et al* (1999) observed initial decrease in total plate counts and total coliforms (MPN) up to 4d in cultured *Penaeus indicus* that had been disinfected with Chlorine and stored in ice, as was observed in mesophiles of steaks stored at 1-2°C in this study. The cultured shrimps treated with 100ppm sodium hypochlorite for 20 min and stored in flake ice initially had TPC and Total coliform counts of 5.06, 2.36 cfu/g, but decreased gradually through 4d storage to 3.95 and 1.38 cfu/g and increased with further storage (*Ibid*). The decrease in microbial counts post treatment during storage was attributed by these workers to cold shock and destruction of mesophilic bacteria during iced storage, which could have also happened to the mesophiles of chlorine and ClO_2 treated steaks stored at 1-2°C in this study, A treatment of 12.0 mg l^{-1}/10 min, 7.2 mg.l^{-1}/ 20 min or 4.8 mg l^{-1}/30min of ClO_2 was found to completely inactivate the 8 log cfu.site^{-1} of *Escherichia coli* O157:H7 bacteria initially inoculated on the skin of apples (Du et al, 2003). Sanitization of fish steaks with chlorine and chlorine dioxide was able to reduce both mesophilic and psychrophilic counts immediately after treatment, but the counts quickly rebounded during the storage period. Significantly lower ($p<0.001$) counts of

Table 8. Physical and sensory observations of raw Rohu steaks treated with hypochlorite and chlorine dioxide and stored at 4-6 or 1-2°C

Storage period, d	Stored at, °C	Control	Hypochlorite	Chlorine Dioxide		
			30Hc	50Hc	$30ClO_2$	$50ClO_2$
0	4-6		Firm, bright pink flesh, characteristic odour			
4		Firm, bright pink, no odour	Firm, bright pink, no off odour	Firm, slight bleached, characteristic odour	Firm, bright pink, characteristic odour	Firm, bright pink, characteristic odour
7		Soft, bleached & slimy, distinct off-odour	Soft, bleached & slimy, slight to distinct off-odour	Soft, bleached & slimy, slight to distinct off-odour	Fairly firm, slimy, faint off odour	Fairly firm, slimy, faint off odour
10		Soft, Slimy, Micro-colonies, Strong ammo-niacal & sulfu-rous odour	Soft, Slimy, micro-colonies, Strong ammo-niacal & sulfu-rous odour	Soft, slimy, micro-colonies, distinct off odour	Soft, slimy, micro-colonies, distinct off odour	Soft, Slimy, micro-colonies, distinct off odour
14		ND	ND	ND	ND	ND
0	1-2		Firm, bright pink flesh, characteristic odour			
4	1-2	Firm, bright pink, no off odour	Firm, bright pink, no off odour	Firm, bright pink, characteristic odour	Firm, bright pink, characteristic odour	Firm, bright pink, characteristic odour
6	1-2		Firm, no drip or slime or colour change or off odour			
8	1-2	Firm, no drip or slime or colour change or off odour	Firm, no drip, slime or colour change or odour	Firm, no drip or slime or colour change or odour	Firm, no drip or slime or colour change or odour	Firm, no drip or slime or colour change or odour
10	1-2	Soft, dull & slimy flesh, Slight drip & off odour, micro-colonies and turbidity	Soft, dull & slimy flesh, Slight drip & off odour, micro-colonies and turbidity	Soft, dull flesh, slight off odour & slime, micro-colonies and turbidity	Soft, Dull flesh, micro-colonies, no turbidity, drip or slime	Firm, No colour change, off-odour or slime
14	1-2	Soft, while & slimy flesh, Strong off-odour, micro-colonies, high turbidity and drip	Soft, while & slimy flesh, Strong off-odour, micro-colonies, high turbidity and drip	Soft, while & slimy flesh, Strong off-odour, micro-colonies, high turbidity and drip	Soft, Slimy with strong off dour and micro-colonies, turbidity and drip	Slight softening, strong off-odour, micro-colonies and drip

Hc- Hypochlorite,
ND – no data study discontinued due to spoilage

aerobic and psychroptropic bacteria have been reported in shrimp and crawfish treated with 10-40ppm ClO_2 compared to aqueous chlorine (Andrews *et al.*, 2002). Even application of antimicrobial ice containing 100ppm ClO_2 had similar effects on microflora of mackerel skin, reducing pathogenic microflora like *E. coli* 0157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* (Shin *et al.* 2004).

Psychrophilic counts were not recorded on first day in this study, and in the later storage periods similar behaviour on mesophiles at both storage temperatures and in control as well as treated steaks was noted. Fillets of salmon and red grouper fillets treated with 20-200 ppm ClO_2 showed markedly reduced bacterial counts after treatment and even after 3-7 d of storage in ice, lower bacterial numbers prevailed in treated fillets (Huang *et al.*, 1996). This study used only 30/50ppm of chlorine (hypochlorite)/ClO_2 in comparison. Initial washing of the dressed fillets before chlorine/ClO_2 treatment as followed in this study further helps to reduce the initial bacterial loads. Karthikeyan *et al* (1999) have demonstrated the importance of washing and chlorine disinfection in reducing the bacterial counts of cultured shrimp prior to icing. Spray washing of beef with ClO_2 water appeared to confer no advantages over plain water spray (Cutter & Dorsa, 1995), but dips in ClO_2 water appeared to have better effect, as seen in this study. Though the chlorine and ClO_2 levels used in this study ranged from 30-50 ppm in the dip water, no traces of chlorine could be detected when the treated steaks were tested for chlorine using a chlorine test kit. Interest in the use of ClO_2 is spurred by the fact that it has ~2.5times the oxidizing capacity of chlorine on a equal molecular basis, but reacts more selectively on microbes. This led the Environmental Protection Agency in 1983 to suggest its use as an effective means of controlling trihalomethanes in drinking water that arose when water was treated with chlorine (Simpson *et al*, 2002).

Increase in TVBN values in fish is a reliable index of spoilage, with good correlationn to TVBN levels, aroma scores and aerobic plate counts (Antoine et al., 2002). Although TVBN levels increased more slowly in sanitizer treated Rohu steaks especially in case of ClO_2 treatment, the differences between the treatments were not significant ($p>0.05$). TVBN of whole Rohu stored in ice remained steady up to 7 days and showed slight decrease on further storage, indicating it to be not very reliable as a spoilage index for Rohu (Joseph *et al.*,1988). Although the shelf life of steaks at 4-6°C in this experiment was short at less than 7d in all treatments, sensory signs of spoilage (Table 8) were delayed by ClO_2 treatment and even in case of control, shelf life at 4d was greater than the 2d reported for Saithe, Redfish and Salmon fillets at 6-9°C by Ranau & Meyer (2000). In comparison, steaks stored at 1-2°C in this study had better storage characteristics, greater shelf life (10d), lower bacterial counts and other parameters than those stored at 4-6°C. ClO_2 treated steaks were acceptable till 10 days compared to control and hypochlorite treated steaks stored at 1-2°C, where onset of off odour was seen by 7d itself. 'Salmide Super' (a stabilized form of redox-buffered sodium chlorite) containing ice when used to store haddock fillets nearly doubled their shelf life (18d) compared to fillets stored in ice alone (Zhang *et al*, 2003). Unlike chlorine, ClO_2 does not lead to undesirable reaction products or taints due to which it is also used in drinking water treatment plants (Kindt, 2002). Salmon, red grouper, Calico scallops and brown shrimps, treated with 20, 40, 100 and 200ppm ClO_2 had lower bacterial counts than non treated controls, with significant differences in the 100 and 200ppm treated fish. But slight bleaching of skin colour was also noticed in red grouper and salmon at these concentrations (Kim *et al*, 1999). ClO_2 has already been approved by USFDA and USDA as a disinfectant in poultry chiller water (Tsai *et al.*, 1997).

Thus, spoilage signs were delayed in ClO_2 treated Rohu steaks stored at 4-6 or 1-2°C, but all the steaks were unacceptable by 10d when stored at 4-6°C. However, storage of steaks at a temperature 1-2°C extended their shelf life upto 14d in comparison with storage at 4-6°C for 10d.

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References

Andrews, L.S., Key, A.M., Martin, R.L., Grodner, R., and Park, D.L. (2002) Chlorine dioxide wash of shrimp and crawfish: an alternative to aqueous chlorine *Food Microbiol.* **19**, pp 261-267

Antoine, F.R. Wei,C.I., Otwell, W.S., Sims, C.A., Littell, R.C., Hogle, A.D. and Marshall, M.R. (2002) TVB-N Correlation with Odor Evaluation and Aerobic Plate Count in Mahi-Mahi (*Coryphaena hippurus*) *J. Food Sci.* **67**, pp 3210-3214

AOAC (1984) Official methods of Analysis. Association of the Official Analytical Chemists. Washington DC

Arora, H., LeChevalier, M. and Battigelli,D. (2001) Effectiveness of chlorine dioxide in meeting the enhanced surface water treatment and disinfection by-products rules *J.Water Supply* **50**, pp 209-228

Bartley, C.E., Beelman, R.B. and Winnett, J.R. (1991) Factors affecting colour of cultivated mushrooms (*Agaricus bisporus*) prior to harvest and during postharvest storage. In *'Science and Cultivation of edible fungi'* pp 689-694 (Maher, M.J. Ed) Dublin, Republic of Ireland.

Chytiri, S., Chouliara, I., Savvaidis, I.N. and Kontominas, M.G. (2004) Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout *Food Microbiol.* **21**, pp 157-165

Conway, E.J. (1950) *Microdiffusion analysis and volumetric error.* 3rd edition, Crosby Lockwood and sons Ltd., London, England pp 87-107

Cutter, C.N. and Dorsa, W.J. (1995) Effect of chlorine dioxide spray washes for reducing fecal contamination on beef. *J. Food Prot.* **58**, pp 1294-1296.

Du, J., Han, Y and Linton, R.H. (2003) Efficacy of chlorine dioxide gas in reducing *Escherichia coli* O157:H7 on apple surfaces. *Food Microbiology* **20**, pp 583-591

Elphick, A. 1998. The Growing use of Chlorine dioxide. *Processing* 1998 March, p24

FAO/UN (1979). *Report of the fifteenth session of the codex committee on food hygiene* Washington. D.C., USA. 18-22 September 1978, 105p

FDA (1998) U.S. F.D.A. § 173.300 Chlorine Dioxide 21 CFR part 173. *Federal Register* Vol. 63, No. 138 / Monday, July 20, 1998 / Rules and Regulations p38747

FDA (2001) U.S. Food and Drug Administration. *Bacteriological Analytical manual.* http://www.cfsan.fds.gov

FSANZ (Food Standards Australia and New Zeland) 2003 . *Final Assesment Report. Application A476. Acidified Sodium Chlorite as a Processing aid.* 12/03. 8 October 2003

Garcia Mendez, G.W. (1988) Quantitative Evaluation of *Clostridium botulinum* Nonproteolytic types B,E and F growth risk in Fresh Salmon stored under Modified Atmospheres *Diss. Abst.Int. Pt.B. Sci & Engg* **48**, p 95

Han,Y. , Linton R. H., Nielsen, S. S. and Nelson, P. E. (2002) A comparison of methods for recovery of chlorine dioxide-injured *Escherichia coli* O157:H7 and *Listeria monocytogenes Food Microbiology* **19**, pp 201-210

Huang, T.S., Kim, J.M, Du,W.X., Marshall, M.R. & Woi.C.I. (1996) Determination of the effectiveness of using chlorine dioxide in controlling bacterial loads and quality of various seafood products over a 7 day period In *1996 IFT Annual meeting: Book of Abstracts.* Institute of Food Technologists. p133. ISSN 1082-1236

Imaeda, K., Yamamoto, D., Watanabe, T., Yoshimura, Y., Uchiyama, K., Kato, K., and Ohsawa, K. 1994 Formation of trihalomethanes during production of bean-curd. *Jap. J. Toxicol. Environ. Health.* **40**, pp 527-533

International dioxicide Inc. (2006) Fresh seafood enhancement with chlorine dioxide. http:// www.idiclo2.com/ indexapp.seafood.html

Iyer, T.S.G. & Choudhuri, D.R. 1966 Influence of ice on the bacteriological quality of the processed fishery products. *Fish. Tech.* **3**, pp 113-117

Karthikeyan, M., Abraham, T.J., Shanmugam, S.A., Jasmine, G.I., Jeyachandran, P. (1999) Effect of washing and chlorine disinfection on the quality and shelf-life of iced cultured shrimp. *J. Food Sci. Technol.* **36**, pp 173-176

Kim, J.M., Huang. T.S., Marshall, M.R., Wei. C.I. (1999) Chlorine dioxide treatment of seafood to reduce bacteria loads *J. Food Sci.*, **64**, pp 1089-1093

Kindt, C. (2002) Taint-free disinfectant finds new opportunities. *Eur. Food Drink Rev.* 2002(4), pp 15-16

Martens, T. (1995) Safety aspects of "sous vide" products and prevention of microbial risks. *Microbiologia* **11**(1), pp 33-42

McFeters, G.A. and Camper, A.K. (1983) Enumeration of indicator bacteria exposed to chlorine. *Adv. Appl. Microbiol.* **29**: 177-193

NZFSA (2008) New Zealand Food safety authority Appendix 5: Australia New Zealand Food Standards Code – Standard 1.3.3 (Excerpt only) http:// www.nzfsa.govt.nz/ index.htm).

Picchietti, M. (1996) Tilapia Marketing Maturity. *Aquaculture Magazine* **22**, pp 19-26

Ranau, R. and Meyer, C. (2000) Quality changes in raw fish fillets during refrigerated storage. *Inform. fuer die Fisch. aus der Fischereiforschung* **47**, pp 102-105

Reyes, J.E., Aguirre, C., Vargas, C., Petzold, G., Valencia, C., Luarte, F. (2003) Efficacy of washing by spraying with chlorine dioxide, ozone or Kilol (R) for reduction of bacterial contamination of pieces of beef. Alimentaria A **344,** pp 17-21

Roberts, R.G., Reymond, S.T. (1994) Chlorine dioxide for reduction of postharvest pathogen inoculum during handling of tree fruits. *Appl. Environ. Microbiol.* **60**(8), pp 2864-2868

Shin, J.H., Chang, S., Kang, D.H. (2004) Application of antimicrobial ice for reduction of foodborne pathogens (Escherichia coli O157:H7, Salmonella Typhimurium, Listeria monocytogenes) on the surface of fish. *J. Appl. Microbiol.* **97**, pp 916-922

Simpson, G.D., Miller, R.F., Laxton, G.D. and Clements, W.R. (2002) A Focus On Chlorine Dioxide: The "Ideal" Biocide. Paper No. 472, Presented at CORROSION/93 (March 8 to March 12, 1993, New Orleans, LA), Sponsored by NACE http://www.clo2.com/index.html

Szomolay, B., Klapper, I., Dockery, J. and Stewart, P.S. (2005) Adaptive responses to antimicrobial agents in biofilms ***Environ. Microbiol.*** **7**, p 1186

Thampuran, N. and Gopakumar, K. (1988) Impact of handling practices on microbial quality of shrimp *Metapenaeus dobsoni.* In FAO Fisheries report No.401 Suppl. Indo Pacific Fisheries Commission. 7th session of IPFC working party on fish technology and marketing. Bangkok, Thailand. 18-22 April 1988. pp 47-50.

Thampuran, N., Sreeganga, K. and Surendran, P.K. (2006) Effect of Chlorine on the survival of *Vibrio cholerae* on shrimp. *Fish. Technol.* **43**(2), pp 180-185

Tsai, L.S., Wilson, R. and Randall, V. (1997) Mutagenecity of poultry chilled water with either chorine di oxide or chlorine *J. Agric. Food Chem.* **45**, pp 2267-2272

USFDA/CFSAN (2001) (U. S. Food and Drug Administration and Center for Food Safety and Applied Nutrition) *Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/ Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce . Chapter V. Methods to Reduce/Eliminate pathogens from Fresh and Fresh-Cut Produce.* http:// www.cfsan.fda.gov./~comm/ift3 Accessed 03.03.2008

Zhang, Y., Lu, H., and Levin R.E. (2003) Enhanced storage-life of fresh haddock fillets with stabilized sodium chlorite in ice *Food Microbiology* **20**, pp 87-90