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Efficacy evaluation of a new water sanitizer for increasing the shelf life of Southern Australian King George Whiting and Tasmanian Atlantic Salmon fillets

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ABSTRACT

The bacterial species and specific spoilage organisms associated with the Southern Australian King George Whiting (KGW) and Tasmanian Atlantic Salmon (TAS), and the efficacy of a HOCl-containing water-based sanitization product (Electro-Chemically Activated Solution, by ECAS4) in extending the shelf life of KGW and TAS fillets were evaluated. Fillets were washed with an ECAS4 solution containing either 45 ppm or 150 ppm of free chlorine and bacterial species enumerated on selective and non-selective media, followed by identification of pure isolates by 16 S rRNA gene sequencing. The dominant spoilage microbiota in KGW and TAS fillets stored at 4 ± 1 °C were *Pseudomonas* spp. and *Shewanella* spp. At either concentration, ECAS4 significantly reduced total bacterial load and specific spoilage organisms on KGW and TAS fillets (approx. 1–2 log colony-forming units) during storage and significantly extended the shelf life of the fillets by 2 and 4 days, respectively. The significant increase in shelf life and quality of fillets was corroborated by raw and cooked sensory evaluation. ECAS4 sanitization could have a significant impact on the overall food industry, translating into health and economic benefits through reduction of food spoilage bacteria and potentially, foodborne pathogens without many of the disadvantages of currently approved biocides.

1. Introduction

Food spoilage represents a growing economic concern worldwide, with approximately one-third designated for human consumption being lost or wasted annually, particularly in medium- and high-income countries (FAO, 2011). Additionally, it has been estimated that approximately 30% of people living in the developed world are experiencing foodborne diseases (at different levels) each year (Bondi et al., 2014). A thorough understanding of the biology of food-spoilage organisms (particularly in seafood) is critical to the development of ways to prolong product shelf life as well as for quality management systems in the food industry. Concerns regarding the spread of pathogenic and spoilage bacteria in foods and food production environment, coupled with limitations associated with existing biocides (Pfuntner, 2011), continue to drive the development of novel sanitizing methods. One of these strategies involves the use of acidic electrolyzed water to reduce bacterial load on seafood (Mahmoud et al., 2004; Ozer and Demirci, 2006). In this context, the pH-neutral Electro-Chemically Activated Solution that can be obtained by using a special reactor with 4 chambers (ECAS4) represents a relatively new technology in the field of water sanitization and surface disinfection. In the USA, the Department of Agriculture Food Safety and Inspection Service (FSIS) has included "Electrolytically generated hypochlorous acid" among the allowed antimicrobial treatment products (FSIS Directive 7120.1). In Europe, ECAS4 is currently used in the healthcare industry to control *Legionella* in water supplies (Migliarina and Ferro, 2014), and has recently been introduced in Australia, where trials have focused mostly on the food industry.

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Unlike the various 2-chamber predecessors that have been used for many years with limited success, the ECAS4 technology relies upon a 4-chamber system for the generation of a genuinely neutral (pH 7.0 ± 0.1) anolyte, through the electrolysis of a dilute solution of sodium chloride (NaCl) in a recently patented electrochemical reactor (Ferro, 2015; Migliarina and Ferro, 2014). The saline solution is initially passed through two cathodic compartments (two chambers hydraulically connected in parallel) and then through two anodic compartments (chambers connected in series), thus allowing for the production of the pH-neutral anolyte. The obtained solution contains active chlorine, mainly in the form of hypochlorous acid (about 75% at pH 7, the remaining 25% being sodium hypochlorite); it has a high oxidation-reduction potential (≥850 mV), is non-hazardous, non-corrosive, and has been demonstrated to be effective in controlling a variety of microorganisms in the hospital environment (Robinson et al., 2012; Thorn et al., 2012). However, the efficacy of this technology is yet to be demonstrated against foodborne pathogens and spoilage bacteria, particularly those associated with seafood.

Given the reported activity of ECAS4 treatment against bacteria, we were interested in evaluating its efficacy in prolonging the shelf life of Southern Australian King George Whiting (KGW; *Sillaginodes punctatus*, family *Sillaginidae*) and Tasmanian Atlantic Salmon (TAS; *Salmo salar*, family *Salmonidae*). In particular, KGW is endemic to the southern coast of Australia, where it is often the sole target for fishermen who seek it for its excellent eating quality and high commercial value (McKay, 1992). Unfortunately, the shelf life of KGW fillets is less than 3 days, even when properly maintained at 0 ± 1 °C: during this time, the product remains safe and retains desirable sensory and physical characteristics, largely influenced by the growth of microbial populations and autolysis (Jeyasekaran et al., 2005). TAS are farmed in the marine waters off the coast of the southern Australia state of Tasmania with an annual production in excess of 48,000 tonnes per annum for the Australian market, and output valued at around AUD497 million (ABARES, 2014). TAS is favored for its visual appeal, high protein content, rich source of omega-3 essential fatty acids, versatility for use in a variety of recipes, as well as for its quality, being harvested from very clean waters.

Major spoilage microorganisms, implicated in decreased shelf life in other fish during aerobic refrigerated storage, consist typically of Gram-negative psychotropic bacteria [*Alteromonas, Flavobacterium* spp, *Pseudomonas* and *Shewanella*] (Gram and Huss, 1996; Parlapani et al., 2015). While acidic electrolyzed water has been used to reduce bacterial load on seafood (Mahmoud et al., 2004; Ozer and Demirci, 2006), to our knowledge, there have been no studies on the effects of a neutral anolyte (like that produced by ECAS4) in prolonging fish shelf life in general and no specific studies into the spoilage microbiota of KGW. Therefore, the objective of this study was to characterize the bacterial species associated with spoilage of the KGW and TAS, and evaluate the efficacy of this new electrochemically activated solution in prolonging shelf life.

2. Methods

2.1. Preparation of ECAS4 solution

ECAS4 solution was prepared at the ECAS4 Australia site in a patented electrochemical reactor (Quadrelli and Ferro, 2010) as described previously (Ferro, 2015; Migliarina and Ferro, 2014). The anolyte contained approximately 300 mg/l of free available chlorine (FAC) and was characterized by a measured oxidation-reduction potential (ORP) of ≥850 mV (Oakton pH/mV meter, Eutech Instruments, Vernon Hills, IL), a neutral pH (7.0 ± 0.1) and a residual chloride level (RCL) of less than 0.5% (Chlorine Ultra HH meter, Hanna Instruments, Woonsocket, RI). To perform the investigations, the fresh as-prepared solution was diluted with tap water in order to obtain ECAS4 solutions at 50% (v/v) and 15% (v/v), respectively.

2.2. Sampling and experimental design for ECAS4 treatment

Fresh whole KGW, approximately 24–30 cm in length and weighing between 90 and 120 g, and whole TAS were stored on ice on arrival at a seafood outlet for 2 days prior to commencement of the experiments, according to Industry protocols.

2.2.1. KGW

Three independent experiments were designed to attain the most effective concentration and duration of ECAS4 treatment required to increase the shelf life of the KGW fillets while retaining overall eating quality as well as desired sensory and physical characteristics. For each experiment, a total of 30 KGW fish were randomly assigned to three treatment groups (n = 10 fish per group), as follows: control (tap water), 15% ECAS4 solution and 50% ECAS4 solution. For each group, 5 fish were used for bacterial analysis and another 5 fish for sensory evaluation. In Experiment 1, the fish were eviscerated, filleted and then washed for 10 s using tap water, 15% ECAS4 solution, or 50% ECAS4 solution at 14 ± 1 °C. In Experiment 2, the fillets were washed in tap water, 15% or 50% ECAS4 solution for 5 min at 14 ± 1 °C. The final experiment (Experiment 3, two-step wash) was designed based on the results obtained from the earlier experiments: the fish were eviscerated and initially washed either in tap water, 15% or 50% ECAS4 solution for 10 sec at 14 ± 1 °C. Then, they were filleted and treated for a second time in the respective solution for 5 min at 14 ± 1 °C. After draining the fillets for 5 min, each sample was packed in a zipped bag and transported to the laboratory on ice for analysis. Specimens of tap water, 15% and 50% ECAS4 solutions prior to and after washing fillets were analyzed for pH, ORP and temperature and also for bacterial enumeration.

2.2.2. TAS

Having achieved the best ECAS4 washing conditions for KGW (the two-step wash protocol), we assessed the efficacy of the ECAS4 regime in reducing the microbial load and extending the shelf life of TAS. For this experiment, whole TAS were initially washed for 10 s either in tap water (control), 15% ECAS4 solution or 50% ECAS4 solution. Thereafter, each fish was filleted, cut into 25 g portions with the skin on or off, and then dipped again in the respective wash solution for 5 min. Samples (5 × 25 g fillets per treatment) were collected, separately bagged, transported to the laboratory on ice and stored at 4 ± 1 °C until needed for microbial analysis.

2.3. Microbiological analysis

KGW fillets were prepared on days 0, 3 and 6 post-treatment (days 0, 3, 7 and 10 post-treatment for TAS) for bacterial enumeration as described previously (Rodrıguez et al., 2004). Briefly, 25 g samples from each of 5 fillets in each treatment group were homogenized in 225 ml peptone water for 2 min using a Stomacher Lab-blender 400 (Seward, London, UK) for bacterial analysis.

For bacterial enumeration and preliminary identification, ten-fold serial dilutions of each sample were plated in duplicate on non-selective and selective media. Total aerobic viable counts (TPC), coliform and *Pseudomonas* counts were determined using plate count agar (PCA), *E. coli*/Coliform Petrifilm™ plates and *Pseudomonas* CN selective agar (PCN), respectively. Additionally, iron agar (IA) was used for the isolation of H_2S-producing bacteria

Table 1
Quality assessment scheme for raw King George Whiting and Tasmanian Atlantic Salmon fish fillets, as recommended by the FAO[a].

Quality parameter and attributes			Score
Appearance	Texture	Odor	
Translucent, glossy	Firm	Marine fresh	1
Natural color, opaque	Elastic	Neutral	2
Dull	Soft	Sour	3
Blood-stained	Rubbery	Spoiled	4
Discolor	Plastic	Putrid	5

[a] FAO/WHO, 2001. Codex Alimentarius, Fish and Fishery Products, Rome, Italy.

and *Photobacterium* spp. All plates were incubated at 24 ± 1 °C for 2–3 days, except for PCA plates, which were incubated at 4 ± 1 °C for 7 days (to enumerate psychotropic bacteria) and Petrifilm™ plates (incubated at 35 °C ± 1 °C for 24 h initially and incubated for another 24 h as required). Colony-forming units (CFU) were calculated for each type of agar. Colonies of each discernible type from each plate were sub-cultured onto the corresponding fresh agar plate and re-streaked to ensure purity. Pure cultures were harvested and stored in Brain Heart Infusion broth (BHI) with 20% glycerol in cryo-vials at −20 °C for identification.

2.4. Bacterial identification

An initial identification of each pure isolate was carried out using standard phenotypic bacterial identification schemes including: colony morphology, microscopic morphology, Gram

Table 2
List of bacterial isolates associated with King George Whiting (KGW) and Tasmanian Atlantic Salmon (TAS) fillets by 16 S rRNA gene sequencing.

	Order	Family	Genus	Species (source of isolation)		Accession[a]
				KGW	TAS	
1	Aeromonadales	Aeromonadaceae	*Aeromonas*	*salmonicida* **(PCA, IA)**	-	X74681.1
2	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	-	*maltophilia* **(PCA, IA)**	AJ409153.1
3	Actinomycetales	Micrococcaceae	*Arthrobacter*	*psychrolactophilus* **(PCA, IA)**	-	AF134183.1
			Leifsonia	*ginsengi* **(PCA, IA)**	-	DQ473536.1
4	Flavobacteriales	Flavobacteriaceae	*Chryseobacterium*	*scophthalmum* **(PCA, IA)**	-	EU057842.1
				yeoncheonense **(PCA)**	-	JX141782.1
			Epilithonimonas	*lactis*	-	EF204460.2
			Flavobacterium	-	*hercynium* **(PCA)**	JQ966057.1
5	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*gessardii* **(PCA, IA, PCN)**	*gessardii* **(PCA, IA, PCN)**	NR_024928.1
				fluorescens **(PCA, IA, PCN)**	*fluorescens* **(PCA, IA, PCN)**	AB204715.1
				fragi **(PCA, IA, PCN)**	*fragi* **(PCA, IA, PCN)**	AM933514.1
				jessenii **(PCA, IA, PCN)**	-	AM933510.1
				mandelii **(PCA, IA, PCN)**	-	AY039828.1
				synxantha **(PCA, IA, PCN)**	*synxantha* **(PCA, IA, PCN)**	D84025.1
				syringae **(PCA, IA, PCN)**	-	AJ576247.1
				veronii **(PCA, IA, PCN)**	-	AY972408.1
				-	*reactans* **(PCA, IA, PCN)**	AF255337.1
				-	*graminis* **(PCA, IA, PCN)**	NR_026395.1
		Moraxellaceae	*Psychrobacter*	*glacincola* **(PCA, IA, PCN)**	-	AJ312213.1
			Psychrobacter	-	*arcticus* **(PCA, IA, PCN)**	AY444823.1
6	Alteromonadales	Shewanellaceae	*Shewanella*	*baltica* **(PCA, IA, PCN)**	*baltica* **(PCA, IA, PCN)**	AB205580.1
				putrefaciens **(PCA, IA, PCN)**	*putrefaciens* **(PCA, IA, PCN)**	AB205575.1
7	Enterobacteriales	Enterobacteriaceae	*Pantoea*	-	*agglomerans* **(PCA, IA, PCN)**	AF157694.1

[a] Accession numbers were obtained using BLASTN 1.8.4-Paracel from GenBank database; PCA = Plate Count Agar; IA =Iron Agar; PCN = *Pseudomonas* CN selective agar.

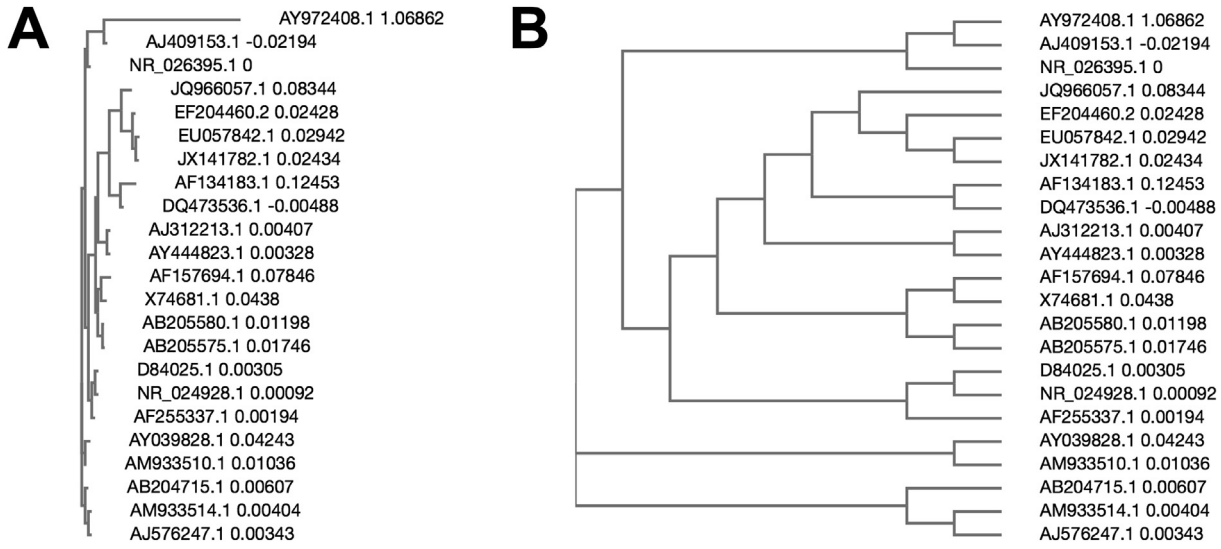

Fig. 1. Phylogenetic tree (Newick/PHYLIP format) of the dominant bacterial isolates associated with King George Whiting and Tasmanian Atlantic Salmon fillets based on 16S rRNA sequences. Phylogram (**A**) and Cladogram (**B**) were obtained using Simple Phylogeny neighbour-joining clustering method provided through the ClustalW2 package from the European Bioinformatics Institute (EMBL-EBI; http://www.ebi.ac.uk/).

stain, growth characteristics, motility, oxidase and Microbact 12A and 12B kits (Thermo Fisher Scientific, Australia) and other standard individual biochemical tests (Hogan et al., 1999). DNA was extracted from each pure culture using the MyTaq™ Extract-PCR Kit (Bioline Cat No: BIO-21126) and isolates were identified at species level by 16 S rRNA gene sequencing essentially as described previously (Khazandi et al., 2014; Milinovich et al., 2006). Briefly, a 1.5 kb region of the 16 S RNA was amplified using primer pairs 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492r (5′-TACGGYTACCTTGTTACGACTT-3′). The PCR reaction was performed using MyTaq™ Extract-PCR Mix in a 25 μl reaction volume containing 50 ng of template DNA, 12.5 μl of 2 × Master mix and 100 nM of each primer. The cycling parameters consisted of initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s (denaturation), 55 °C for 30 s (annealing) and 72 °C for 2 min (extension) with a final extension of 72 °C for 5 min. Specific PCR products were visualised by running 5 μl of each sample on a 1% TAE agarose gel electrophoresis and sequenced on an Applied Biosystems 3700 DNA analyzer. Analysis of sequences of dominant phylotypes associated with the KGW and TAS fillets was carried out by constructing a phylogenetic tree using Simple Phylogeny neighbour-joining clustering method (Goujon et al., 2010; Larkin et al., 2007) provided through the ClustalW2 package from the European Bioinformatics Institute (EMBL-EBI; http://www.ebi.ac.uk/).

2.5. Sensory index on raw fillets

The sensory evaluation of raw KGW and TAS fillets was carried out by three trained panelists using a Sensory Index (SI) technique and examining three characteristic qualities (appearance, texture and odor) for raw fillets, as established by Food and Agriculture

Table 3
The mean values (±SEM) of the pH, ORP and temperature for tap water, 15% and 50% ECAS4 before and after washing King George Whiting and Tasmanian Atlantic Salmon fillets.

Treatment	Parameters					
	Before washing			After washing		
	pH	ORP (mV)	Temperature (°C)	pH	ORP (mV)	Temperature (°C)
Control	7.3 ± 0.1	225 ± 5	14 ± 1	7.2 ± 0.1	NA	15 ± 1
15% ECAS4	7.0 ± 0.1	840 ± 10	14 ± 1	6.9 ± 0.1	855 ± 15	15 ± 1
50% ECAS4	7.0 ± 0.1	885 ± 5	14 ± 1	6.5 ± 0.1	915 ± 15	15 ± 1

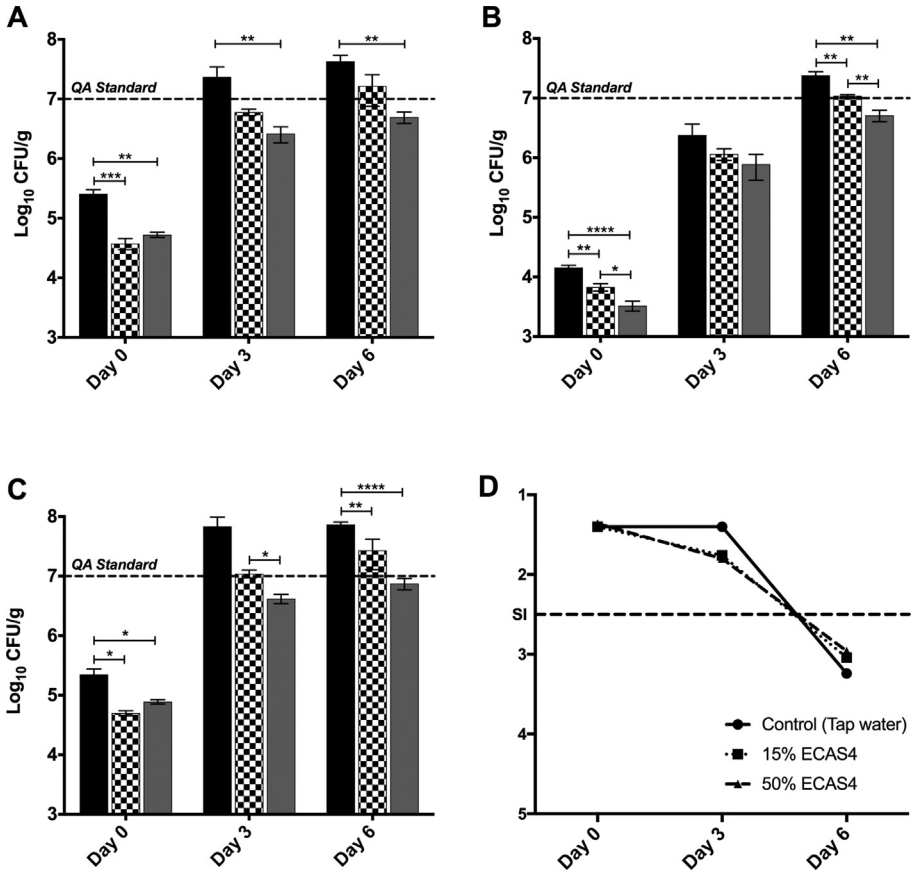

Fig. 2. Effect of a 10 s wash with tap water, 15% or 50% ECAS4 on microbial load and sensory attributes of King George Whiting fish fillets. At days 0, 3 and 6, 25 g of fillet from each treatment ($n = 5$) was assessed for microbial load, and total plate counts (**A**), total coliform counts (**B**), spoilage bacteria (**C**) as well as sensory attributes (**D**), were determined. The Quality Assurance (QA) standard was set at 10^7 colony-forming units; the sensory cut-off score was fixed at a Sensory Index (SI) of 2.5. Differences in microbial load between treatments were determined using unpaired *t*-test (two tailed). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Organization (FAO, Table 1) (FAO/WHO, 2001). A weighted Sensory Index (SI) technique based on the scoring system from 1 (best) to 5 (worst) was adopted.

On days 0, 3 and 6 post-treatment (for KGW) or days 0, 3, 7 and 10 post-treatment (for TAS), SI values were independently determined for KGW using a modification of the equation described by Kreyenschmidt et al. (2010), and for TAS (Miks-Krajnik et al., 2016) as follows: SI = (2 × C+2 × O + T)/5, where C is the appearance (color), O the odor and T the texture. The color and odor were weighed twice, as these attributes showed the first and most noticeable changes in sensory quality of the fillets. The evaluated demerit points were summed and averaged to give overall sensory scores between a minimum of 1 and a maximum of 5, where a higher score represented poorer quality. The cut-off SI score was fixed at score ≥2.5.

2.6. Sensory evaluation of cooked KGW and TAS fillets

For this analysis, KGW and TAS (skin-on and skin-off) fillets were subjected to the two-step wash protocol using 15% ECAS4 or 50% ECAS4 solution in a controlled double blind experiment after which fillets were separately bagged and stored in a container with ice packs at 0 ± 1 °C for 72 h. Thereafter, fillets were pan-fried and seven sensory panellists assessed the cooked fillets as per the sensory attributes, using freshly prepared fillets washed in tap water as control. A hedonic scoring scale was used to assess the attributes of cooked fillets as recommended (FAO, 1999). Panellists were asked to evaluate the overall acceptability with regards to odour intensity, flavour intensity, juiciness, tenderness, colour, off-odour, off-flavour and off-taste. A ten-point hedonic scoring scale with 10 = Excellent and 1 = very poor, was employed for odour, flavour, juiciness, tenderness and colour respectively. In addition, a ten-point hedonic scoring scale with 10 = not-detected and 1 = extremely-off was used for assessment of off-odour, off-flavour and off-taste.

2.7. Statistical analysis

All experiments were performed in replicates ($n = 5$ per group) and reported as means ± SEM. The effect of 15% and 50% ECAS4 solutions on reducing bacterial load on fillets was analyzed by the Student's *t*-test, two-tailed ($p < 0.05$) using GraphPad Prism version 6 software.

3. Results

3.1. Identification of bacterial populations on KGW and TAS

Examination of culture plates indicated that the majority of bacterial samples recovered from the three treatment groups (control tap water, 15% ECAS4 solution and 50% ECAS4 solution) showed a range of colony types on PCA, three colony types on IA, and three colony types on PCN, at day 0. Distinct bacterial morphological type colonies were re-streaked onto fresh agar

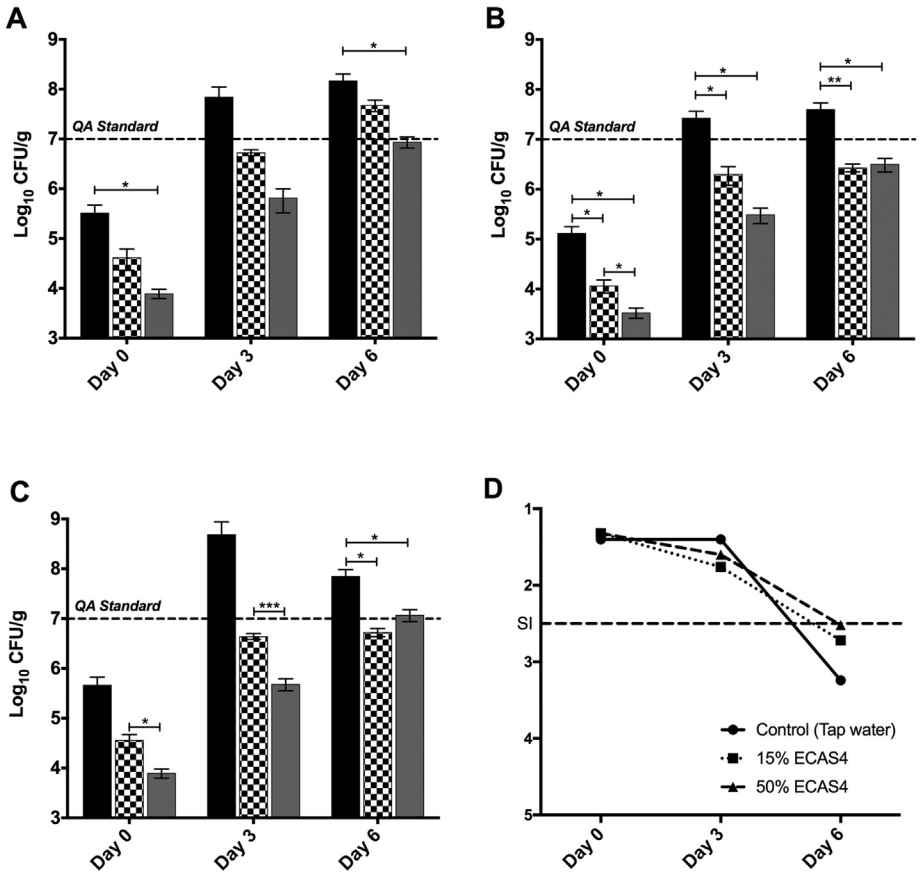

Fig. 3. Effect of a 5 min wash with tap water, 15% or 50% ECAS4 on microbial load and sensory attributes of King George Whiting fish fillets. At days 0, 3 and 6, 25 g of fillet from each treatment ($n = 5$) was assessed for microbial load, and total plate counts (**A**), total coliform counts (**B**), spoilage bacteria (**C**) as well as sensory attributes (**D**), were determined. The Quality Assurance (QA) standard was set at 10^7 colony-forming units; the sensory cut-off score was fixed at a Sensory Index (SI) of 2.5. Differences in microbial load between treatments were determined using unpaired *t*-test (two tailed). $^*P < 0.05$; $^{**}P < 0.01$.

plates to obtain pure cultures. Following examination of the growth and morphological characteristics, 55 representative pure colonies from PCA (10 yellow, 5 white and 5 creamy colonies), IA (10 black, 5 creamy colonies), and PCN (10 green, 5 creamy and 5 white colonies) were selected for further identification by 16 S rRNA gene sequencing. Results showed that 56.5% of the initial bacterial population belong to the family Pseudomonadaceae (12 different species); 11.5% of the isolates belong to the family Shewanellaceae; 4% each to Enterobacteriaceae, Flavobacteriaceae and Moraxellaceae, while the remainder were identified as *Stenotrophomonas* and Xanthomonadaceae (4% each, Table 2). In this study, the dominant Gram-negative isolates in KGW fillets were identified as *Pseudomonas* spp., *Shewanella* spp., *Aeromonas* spp., *Arthrobacter psychrolactophilus,* and the only Gram-positive bacteria identified belonged to a *Leifsonia* sp. However, the dominant Gram-negative isolates in TAS fillets were *Pseudomonas* spp. and *Shewanella* spp. Phylogenetic analysis of sequences of dominant phylotypes associated with the KGW and TAS fillets using Simple Phylogeny neighbour-joining clustering method (Fig. 1) suggests the isolates are closely related.

3.2. Efficacy of ECAS4 washing on bacterial load on fish fillets

Our analysis of the pH, ORP and temperature of tap water, 15% or 50% ECAS4 solution showed that the ECAS4 anolyte has a reliable chlorine content, a high ORP (≥850 mV), and a consistent neutral pH before and after fillet washing for 10 s, 5 min or for the two-step (10 s + 5 min) wash (Table 3). Analysis of bacterial counts showed that a 10-s dip of KGW fillets in either the 15% or the 50% ECAS4 solution had a significant effect in reducing the TPC, total coliform and spoilage bacterial counts at day 0; a similar trend was observed at day 3 and the difference was also significant at day 6 post-filleting (Fig. 2). In addition, a longer dip (5 min) in either the 15% or the 50% ECAS4 solution caused further reduction of the total bacterial load at day 0, and there was a consistent and significant reduction in the overall bacterial load in fillets washed with ECAS4 up to 6 days post-filleting (Fig. 3).

The TPC and total coliform counts for KGW fillets after a two-step wash in either 15% or 50% ECAS4 were significantly reduced by approximately 0.5 and 1 $\log_{10}$ CFU/g, respectively, at day 0 compared to fillets washed in tap water (Fig. 4). On day 3, the differences in bacterial populations were significantly higher (>1.8 $\log_{10}$ CFU/g) for fillets treated with 15% and 50% ECAS4 in comparison to the control (Fig. 4A–C). This significant reduction was maintained throughout the 6 days of storage. Furthermore, the two-step wash in either 15% or 50% ECAS4 solutions resulted in a better sensory index than the one-step wash (10 s or 5 min).

3.3. Sensory index of raw fillets

The sensory attributes of KGW fillets subjected to a 10 s wash with either 15% ECAS4 or 50% ECAS4 was not appreciably better compared to control (tap water) wash (Fig. 2D). However, a 5 min dip in either 15% ECAS4 or 50% ECAS4 water resulted in a

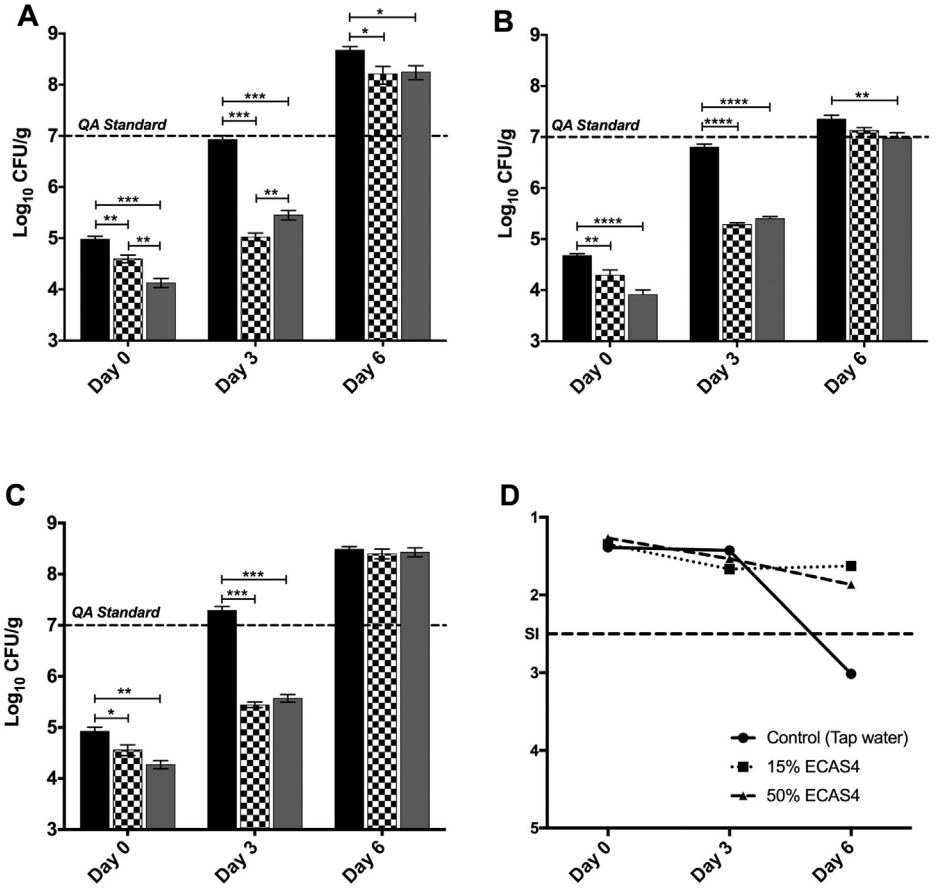

Fig. 4. Effect of a 2-step wash (10 s + 5 min) with tap water, 15% or 50% ECAS4 on microbial load and sensory attributes of King George Whiting fish fillets. At days 0, 3 and 6, 25 g of fillet from each treatment ($n = 5$) was assessed for microbial load, and total plate counts (**A**), total coliform counts (**B**), spoilage bacteria (**C**) as well as sensory attributes (**D**), were determined. The Quality Assurance (QA) standard was set at 10^7 colony-forming units; the sensory cut-off score was fixed at a Sensory Index (SI) of 2.5. Differences in microbial load between treatments were determined using unpaired *t*-test (two tailed). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

substantially improved sensory attribute, with the latter showing a better sensory index (Fig. 3D). For the two-step treatment, overall freshness characteristics, including appearance, texture and odor at 4 °C remained acceptable at day 6 for treated fillets ($p < 0.05$), while control fillets were discolored on day 6 and their odor was putrid, which led to their being classified as unfit (grade > 2.5 of hedonic scale) and therefore rejected (Fig. 4D). Additionally, regarding fillets treated with tap water, spoilage occurred by day 3, at which time the TPC reached $\geq$7 $\log_{10}$ CFU/g. In contrast, this level was only achieved on day 6 in the case of the fillets treated with either 15% or 50% ECAS4 solution. Furthermore, maximum bacterial densities (particularly spoilage levels) were generally lower for the fillets treated with either 15% or 50% ECAS4 solution at all time points examined (Supplementary Table 1).

For TAS, the microbial counts post-treatment with tap water, 15% and 50% ECAS4 for the skin-on and skin-off fillets are shown in Fig. 5 and Fig. 6. The initial bacterial population in TAS fillets was approx. 1 $\log_{10}$ CFU lower than that obtained for KGW fillets, and the corresponding initial spoilage (including H_2S-producing) bacterial populations was also approximately 1 $\log_{10}$ CFU lower than that obtained for KGW fillets. We found that 15% or 50% ECAS4 dosing already had a significant effect in reducing the TPC at day 0. The TPC, total coliform, and *Pseudomonas* spp. counts after treating fish fillets in 15% or 50% ECAS4 were significantly reduced by up to 0.95, 0.55 and 0.9 $\log_{10}$ CFU/g, respectively, at day 0 in comparison to untreated fillets, and a similar trend was observed on days 3 and 7. We found a consistent and substantial decrease in the overall bacterial load in fillets dipped in either 15% or 50% ECAS4 water up to 10 days post-filleting. As observed for the KGW experiment, bacterial densities (particularly spoilage levels) were generally lower for the fillets treated with either 15% or 50% ECAS4 solution over the duration of the experiments (Supplementary Tables 2 and 3).

The sensory qualities of raw skin-on and skin-off TAS fillets were evaluated by three trained panellists using the SI technique as described above, examining three characteristic qualities (appearance, texture and odour) for raw fillets. We observed that a 2-step wash with either 15% or 50% ECAS4 (10 s + 5 min dip) significantly increased the shelf life at days 7 and 10 relative to the control (tap water wash) group, and there was no significant difference between the 15% vs. 50% ECAS4-treated fillets at days 7 and 10 (Fig. 5D, Fig. 6D).

3.4. Quality assessment of cooked KGW and TAS fillets

The blinded sensory assessment on cooked KGW fillets by 7 panellists at 3 days post-treatment showed no detectable difference in organoleptic qualities with respect to the freshly prepared control fillets (Fig. 7A). Likewise, sensory assessment on cooked TAS fillets between tap-water-treated and ECAS4-treated fillets at 3 days post-treatment showed no detectable difference in organoleptic qualities (Fig. 7B–C). However, sensory assessment on cooked TAS was not performed beyond day 3 post-treatment, owing to a deterioration of the raw sensory attributes of the

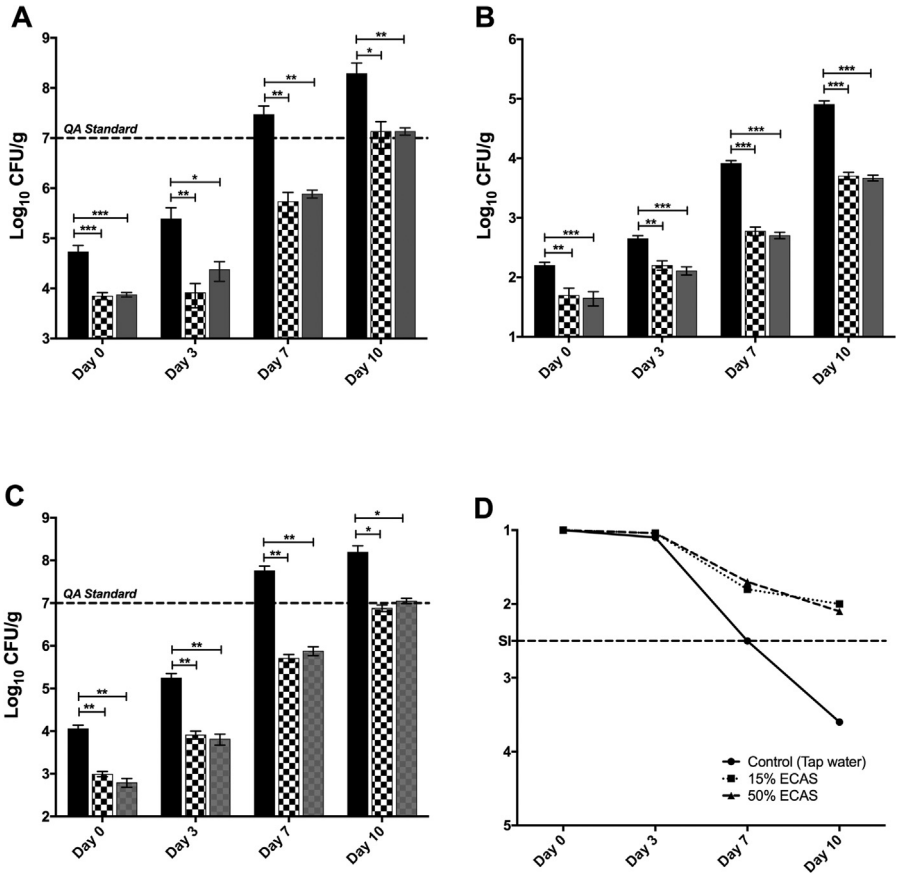

Fig. 5. Effect of a 2-step wash (10 s + 5 min) with tap water, 15% or 50% ECAS4 on microbial load and sensory attributes of skin-on Tasmanian Atlantic Salmon fillets. At days 0, 3, 7 and 10, a 25 g sample of fillet from each treatment ($n = 5$) was assessed for microbial load, and total plate counts (**A**), total coliform counts (**B**), spoilage bacteria (**C**) as well as sensory attributes (**D**). The Quality Assurance (QA) standard was set at 10^7 colony-forming units; the sensory cut-off score was fixed at a Sensory Index (SI) of 2.5. Differences in microbial load between treatments were determined using unpaired *t*-test (two tailed). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

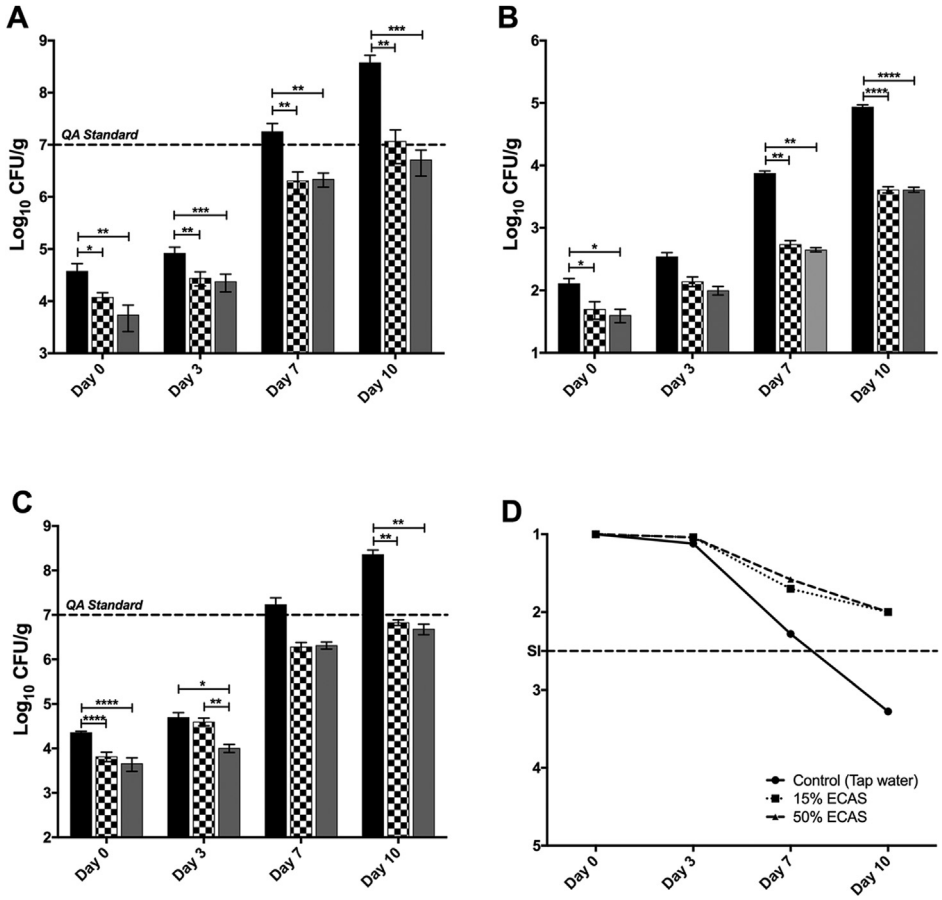

Fig. 6. Effect of a 2-step wash (10 s + 5 min) with tap water, 15% or 50% ECAS4 on microbial load and sensory attributes of skin-off Tasmanian Atlantic Salmon fillets. At days 0, 3, 7 and 10, a 25 g sample of fillet from each treatment ($n = 5$) was assessed for microbial load, and total plate counts (**A**), total coliform counts (**B**), spoilage bacteria (**C**) as well as sensory attributes (**D**). The Quality Assurance (QA) standard was set at 10^7 colony-forming units; the sensory cut-off score was fixed at a Sensory Index (SI) of 2.5. Differences in microbial load between treatments were determined using unpaired *t*-test (two tailed). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

fillets during storage, which was more pronounced in the tap water-treated group.

4. Discussion

In this study, we evaluated the efficacy of a relatively new water sanitization and surface disinfection technology (ECAS4) in reducing the bacterial load of Southern Australian KGW and TAS, thereby increasing the shelf life and maintaining the overall quality of fillets. The ECAS4 solution has proven to be highly effective against a variety of microorganisms found in planktonic and biofilm environments, including healthcare settings (Ferro, 2015; Migliarina and Ferro, 2014; Thorn et al., 2012).

With the present results, we are showing for the first time that six genera of Gram-negative psychrotrophic bacteria (belonging to the families Pseudomonadaceae, Flavobacteriaceae, Shewanellaceae, Aeromonadaceae and Moraxellaceae) and two genera of Gram-positive bacteria (belonging to the family Micrococcaceae) were found to be the most common cultivatable microbiota on fillets of the Southern Australian KGW. In particular, the predominant microbiota were identified as *Shewanella baltica, P. fluorescens*, *P. syringae*, *P. fragi*, *P. synxantha*, *S. baltica* and *C. scophthalmum*. All these species have previously been reported as spoilage organisms in various types of fish (Gennari et al., 1999; Parlapani et al., 2013; Wang et al., 2014). We also identified eight bacterial species in KGW fillets that have previously been reported in the marine environment, but not as fish spoilage organisms. These include: *A. psychrolactophilus* (Wang et al., 2009), *C. yeoncheonense* (Hoang et al., 2013), *E. lactis* (Hantsis-Zacharov and Halpern, 2007), *Leifsonia* sp., Antarctica (Ganzert et al., 2011), *P. gessardii* (Arnau et al., 2015), *P. mandelii* (Keller-Costa et al., 2014) and *P. synxantha.* We also found that *Pseudomonas* spp. and *Shewanella* spp., were the predominant cultivatable spoilage microbiota of KGW and TAS fillets kept in cold storage. This is in agreement with the spoilage microbiota reported for farmed sea bream and carp in other studies (Beaz-Hidalgo et al., 2015; Parlapani et al., 2013). However, our molecular approach (16 S rRNA gene sequencing) detected different bacteria at the species level, in some cases. During the early stages of cold storage, the dominant bacteria belonged to the *P. fluorescens* group, including *P. fluorescens*, *P. gessardii*, *P. mandelii*, *P. veronii and P. synxantha*, thus differing from earlier reports.

The shelf life of KGW fillets traditionally washed with tap water is less than 3 days when stored at 4 °C, similar to the shelf life of sea bream fillets stored at 5 °C (Parlapani et al., 2015), while the shelf life of TAS fillets washed with tap water is less than 7 days. However, our results indicate that the use of 15% and 50% ECAS4 solution along the production line of KGW fillets significantly extended the shelf life of the KGW fillets by 2 days. Similarly, TAS fillets treated with either 15% or 50% ECAS4 water had a significant extension of shelf life by 4 days. This extension is most likely due to the significant reduction on the initial bacterial populations as a result of the antibacterial activity exerted by the ECAS4 solution.

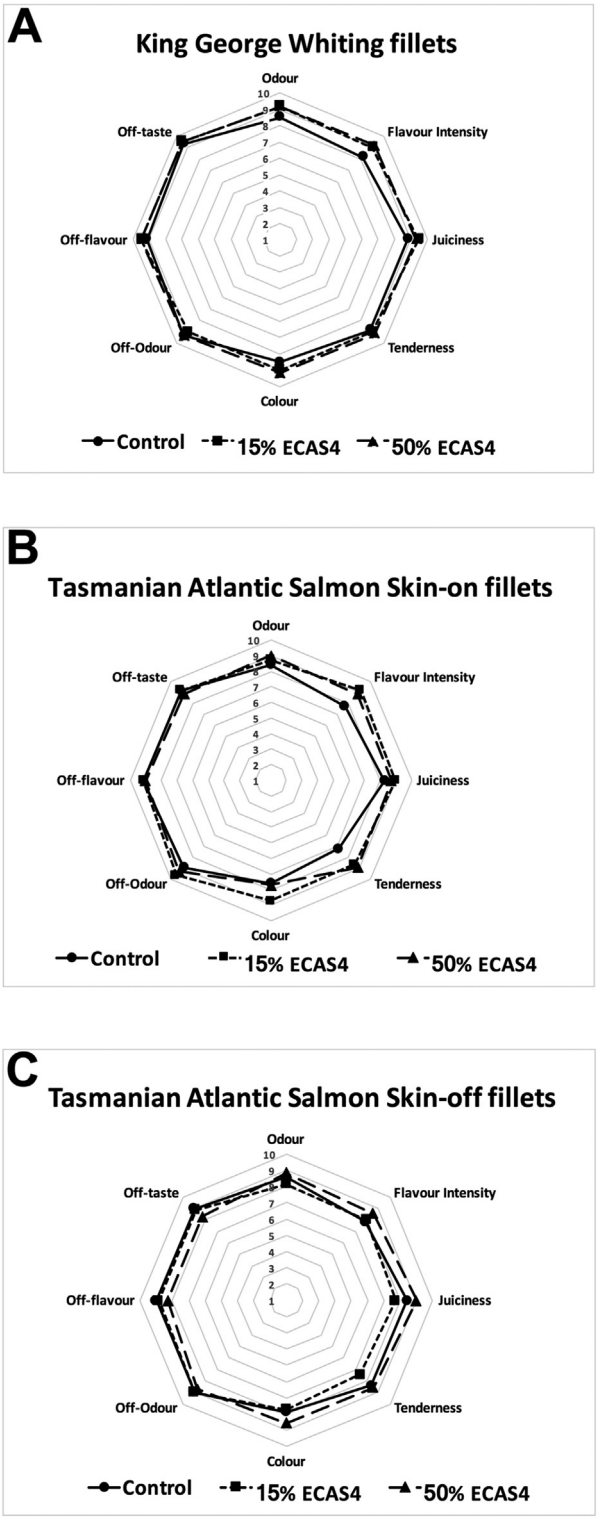

Fig. 7. Sensory attribute scores of cooked King George Whiting and Tasmanian Atlantic Salmon fillets treated with ECAS4 in two-step wash protocol. The results are presented mean score of 7 sensory panelists that assessed on the following sensory attributes: odour intensity, flavour intensity, juiciness, tenderness, colour, off-odour, off-flavour and off-taste.

This is corroborated by the consistent reduction in the number of specific spoilage organisms and an overall reduction in bacterial load throughout the 6 days of storage for KGW, and 10 days of storage for TAS. Similar results were obtained for Gram-negative bacteria when acidic electrolyzed oxidizing water was used in previous studies (Al-Holy and Rasco, 2015; Huang et al., 2006). Importantly, a detailed blinded sensory assessment on cooked KGW and TAS fillets treated with 15% and 50% at 3 days post-treatment showed no detectable difference in organoleptic qualities in comparison to freshly cut fillets washed with the control (tap water).

Our results also showed that 50% ECAS4 solution was more effective against the bacterial load compared to the 15% ECAS4 solution, likely due to the different free chlorine contents of the two ECAS4 solutions. Our results are in agreement with previous studies. For example, Rajkowski and Sommers found a 1 $\log_{10}$ reduction for *Salmonella* on catfish fillets washed for 3 min with electrolyzed water (FAC of 300 ppm, pH of 6.0–6.5) (Rajkowski and Sommers, 2012). In another study, dipping of carp fillets in an electrolyzed solution (FAC of about 40 ppm, pH of 5.5) for 15 min reduced total microbiota by 2 $\log_{10}$ CFU/cm^2 (Mahmoud et al., 2004). Inoculated salmon fillets treated with acidic electrolyzed water (FAC of 70–90 ppm, pH of 2.6) for different times, showed reductions of *L. monocytogenes* and *E. coli* of up to 1 $\log_{10}$ CFU/g (Ozer and Demirci, 2006). The differences between our study and those of others could be attributable to the different experimental conditions used: the FAC, treatment time and pH are probably the most influencing parameters (FAC and pH are related, in determining the active form of the biocide), the temperature and type of food product being examined may also play a role.

5. Conclusions

The use of 15% or 50% ECAS4 solution for the treatment of KGW and TAS fillets significantly reduced the initial microbiota, considerably prolonging shelf life without affecting the overall raw and cooked qualities of the fillets. Owing to the fact that it is non-hazardous and, most importantly, non-corrosive, the ECAS4 solution can be developed as a safe and affordable water sanitization system, which will significantly reduce bacterial load to below quality assurance standards; the technology may find widespread use in a variety of primary food industries, including those producing beef, pork, eggs and poultry, and minimally processed vegetables. We also suggest that implementation of this new technology at the end of the food production chain will be advantageous in minimizing disruption to existing food manufacturing processes, and will ensure that the foods reaching the consumer are of the highest quality. This could translate into health and economic benefit through reduction and/or elimination of food spoilage bacteria and foodborne pathogens.

Conflict of interest statement

Sergio Ferro, Simon Crabb and Tony Amorico are technical manager, national business manager and managing director, respectively, of ECAS4 Australia.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fm.2017.06.008.

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