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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.

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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, tenfold 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

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