DEVELOPMENT AND APPLICATION OF NOVEL METHODS FOR FISH HARVESTING AND PROCESSING FOR QUALITY PRESERVARTION AND SHELFLIFE EXTENSION

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Abstract
The objective of the study was the design and application of alternative postharvest treatments of marine cultured fish for quality preservation and shelf life extension. Slurry ice was applied as an alternative cooling medium during harvesting and transportation of gilthead seabream and European sea bass, using different concentrations (0.5 or 100%) of slurry ice prepared from sea water, in conventional flake ice. The mild surface disinfection during gutting and filleting of fish by the incorporation of organic acids (i.e. citric acid, lactic acid, peracetic acid) at different concentrations and treatment durations (0-7500 ppm and 0-10 min, depending on the tested acid and application) in the washing water of gutted and filleted fish was also investigated. Whole, gutted and filleted fish were stored isothermally at 0 ± 0.2°C for shelf life evaluation. Quality evaluation was based on microbial growth (total viable count, *Pseudomonas* spp., *Brochothrix thermosphacta*, H_2S-producing bacteria, yeasts / molds and Enterobacteriaceae), colour, texture, lipid oxidation, proteolytic enzyme activity and sensory evaluation. The replacement of conventional flake ice with slurry ice resulted in improved quality and microbial stability during refrigerated storage, resulting in up to 6 days shelf life extension of fish, without affecting the sensory properties of the final products. Fish surface decontamination up to 2.0 log cfu / g was achieved by the addition of citric acid in the washing water, resulting in up to 4 days shelf life extension of gutted fish.
Keywords: *Slurry ice*, *Surface Disinfection, Fish Washing, European Sea Bass, Gilthead Seabream, Spoilage*
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1. Introduction

Fish is highly susceptible to spoilage, which can be caused by both intrinsic chemical reactions and microbial growth. An estimated 25% of primary agricultural and fishery products are lost every year, mostly because of chemical deterioration and microbial spoilage (Baird-Parker 2000). The deterioration process is accelerated by increased temperatures, physical damage and contamination. Therefore, the key to fish preservation is the immediate chilling upon catch or harvest to a temperature slightly above the freezing point and maintaining this temperature throughout the cold chain (Kauffeld *et al.* 2010). New minimal and non thermal food processing methods are sought by the industry in the pursuit of producing better quality fish products with extended shelf life with retention of nutritional and sensory properties (Tsironi & Taoukis 2019).

Slurry ice, is a biphasic system consisting of small spherical ice particles surrounded by seawater at subzero temperature (Cakli *et al.* 2006). Its reported advantages over traditional fresh-water ice (such as flake, tube, and block ice) include its lower temperature, faster chilling (due to a more rapid heat exchange), and lower rate of physical damage (due to its spherical microscopic particles) (Bellas & Tassou 2005; Kauffeld *et al.* 2010). The ability of adjusting the ice concentration up to 60% and the salt content in the range of 2-3% in the ice slurry ensures maximum preservation results without damage to delicate fish and avoids excessive salt uptake by the fish (Kauffeld *et al.* 2010). Ntzimani *et al.* (2021) evaluated the effect of slurry ice as an alternative cooling medium during harvesting and transportation on the quality and shelf life of whole European sea bass.

Sodium salts of the low molecular weight organic acids, as for example acetic, lactic and citric acid, have been applied with the aim to delay microbial growth, preserve sensory attributes and extend the shelf life of various food systems. Several studies have been conducted recently on the efficacy of washing and sanitizing

treatments in reducing microbial populations on perishable products. Limited work on the effect on fish has been published and no industrial scaling-up has been reported (Sallam 2007; Thi *et al.* 2015).

The objective of the SlurryFish project (slurryfish.chemeng.ntua.gr, 2018-2022) is to develop and optimize environmentally friendly and cost effective postharvest treatments of marine cultured fish for quality preservation and shelf life extension (Figure 1). The aim of the study was the design and application of slurry ice as an alternative cooling medium during harvesting and transportation and a mild surface disinfection during gutting and filleting of farmed gilthead seabream and European sea bass.

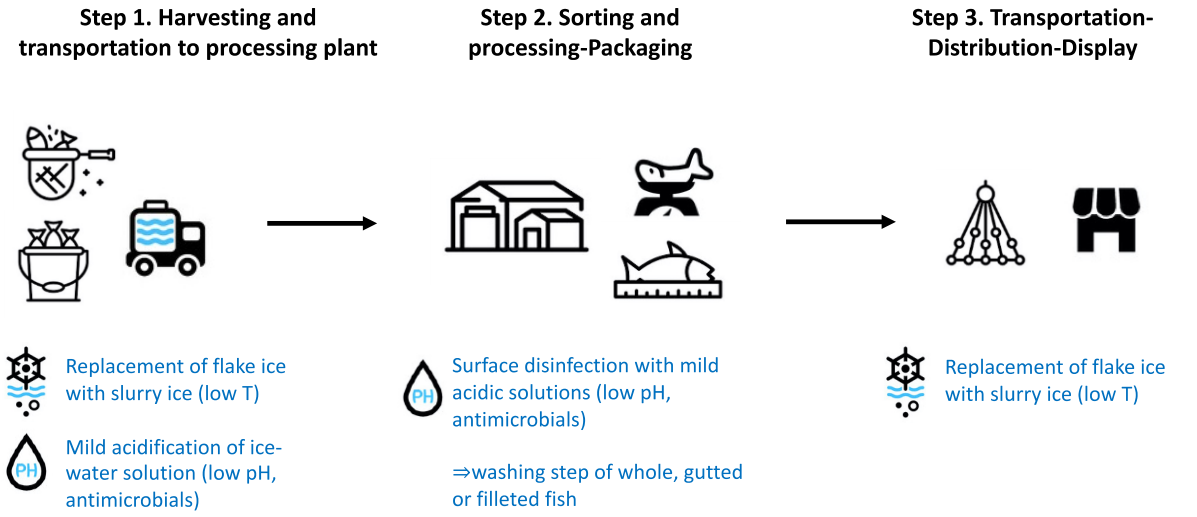


Figure 1. Technological improvements at postharvest fish processing stages developed and optimized within the SlurryFish project.

2. Material and Methods

Slurry ice was prepared from filtered seawater (salinity: 3.5%) using a semi-industrial scale slurry ice machine (ZIEGRA, Germany) in Philosofish S.A. farming facilities (Larymna, Fthiotida, Greece). The temperature of the slurry ice mixture was -3.2°C. Whole gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) was slaughtered and transported in different mixtures of slurry ice and conventional flake ice (C: slaughtered and transported in 100% flake ice-Control samples, SC: slaughtered in 100% slurry ice and transported in 100% flake ice, S50: slaughtered and transported in 50% slurry ice-50% flake ice, S100: slaughtered and transported in 100% slurry ice). Upon receipt at the laboratory (24 hours after harvesting), all fish samples (C, SC, S50, S100) were stored in high-precision low temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Japan) under controlled isothermal conditions at (0 ± 0.2°C). The temperature in the incubators was monitored using miniature data-loggers (COX TRACER, Belmont, NC).

The incorporation of mild organic acids (i.e. citric acid, lactic acid, peracetic acid) at different concentrations and treatment durations (0-7500 ppm and 0-10 min, depending on the tested acid and application) in the washing water of gutted and filleted fish was also investigated. The microbial load reduction of total viable count (TVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, H_2S-producing bacteria, yeasts / molds and Enterobacteriaceae was evaluated as a function of acid concentration and washing time of gutted fish and fillets.

Whole, gutted and filleted fish were stored isothermally at 0 ± 0.2°C for shelf life evaluation. Quality evaluation was based on microbial population changes (total viable count, *Pseudomonas* spp., *Brochothrix thermosphacta*, H_2S-producing bacteria, yeasts / molds and Enterobacteriaceae), colour, texture, lipid oxidation, proteolytic enzyme activity and sensory evaluation.

TVC was enumerated on plate count agar (PCA, Merck, Darmstadt, Germany) after incubation at 25°C for 72 h, whereas *Pseudomonas* spp. were enumerated on Cetrimide agar (CFC, Merck, Darmstadt, Germany) after incubation at 25°C for 48 h. For H_2S-producing bacteria and Enterobacteriaceae enumeration, the pour-plate method was used. H_2S-producing bacteria were enumerated on Iron Agar (Iron agar with L-cysteine) followed by incubation at 25°C for 48 h. For Enterobacteriaceae enumeration violet red bile glucose agar (VRBG, Merck, Darmstadt, Germany) was used, which was incubated at 37 °C for 18-24 h. Two replicates of at least three appropriate dilutions were enumerated.

The microbial growth was modelled using the Baranyi Growth Model (Baranyi and Roberts 1995). For curve fitting the program DMFit (IFR, Institute of Food Research, Reading, UK) was used (available at http://www.combase.cc/index.php/en/). Kinetic parameters such as the rate (k) and lag phase (λ) of microbial growth were estimated. To evaluate lipid oxidation, 2-thiobarbituric acid reactive substances (TBARs) assay was performed according to the method of Loovas (1992). Color of all fish samples was measured on the dorsal part of the body with the color meter Minolta CR-200 (Minolta Company, Chuo-Ku, Osaka, Japan).Texture parameters were defined using a texture analyzer with a load cell of 5 kg (TA-XT2i, Stable Micro Systems, Godalming, Surrey, United Kingdom). The sensory attributes of raw and cooked fish were evaluated by a sensory panel of eight trained evaluators using descriptive tests with practice evaluation methods of determining spoilage characteristics in fish (Botta, 1995). The proteolytic enzyme activity was assayed by the methods described by Barrett & Kirschke (1981). The protein content of enzyme extracts was quantified with the Bradford (1976) method using bovine serum albumin as a standard. Two replicates per sample were performed.

3. Results

The replacement of conventional flake ice with slurry ice as a slaughtering method led to improved quality stability during subsequent refrigerated storage and shelf life extension, in terms of microbial growth, flesh quality and sensory degradation of fish. The microbial growth (TVC, *Pseudomonas* spp. and H_2S-producing bacteria) in whole sea bass stored isothermally at 0°C is illustrated in Figure 2. The use of slurry ice at slaughter and flake ice in transportation was accompanied by low activities and late peaks of all enzymes that are expected to lead to delayed proteolytic degradation and extended freshness (Figure 3).

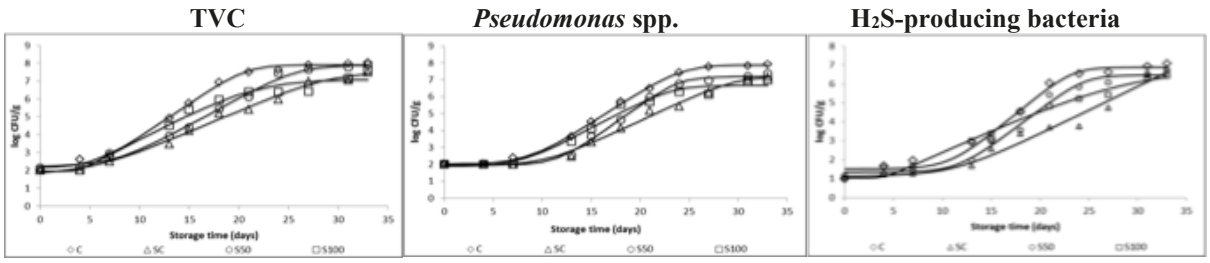


Figure 2. Effect of slurry ice during harvesting and transportation of whole European sea bass on microbial growth during subsequent isothermal storage at 0 °C (◇ C, △ SC, ○ S50, □ S100).

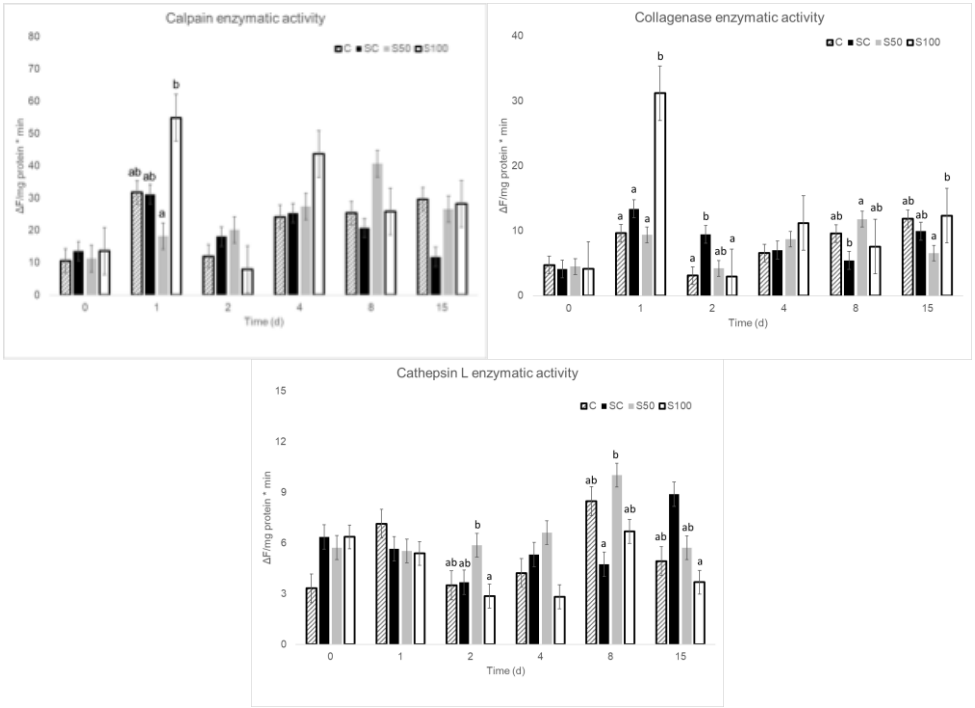
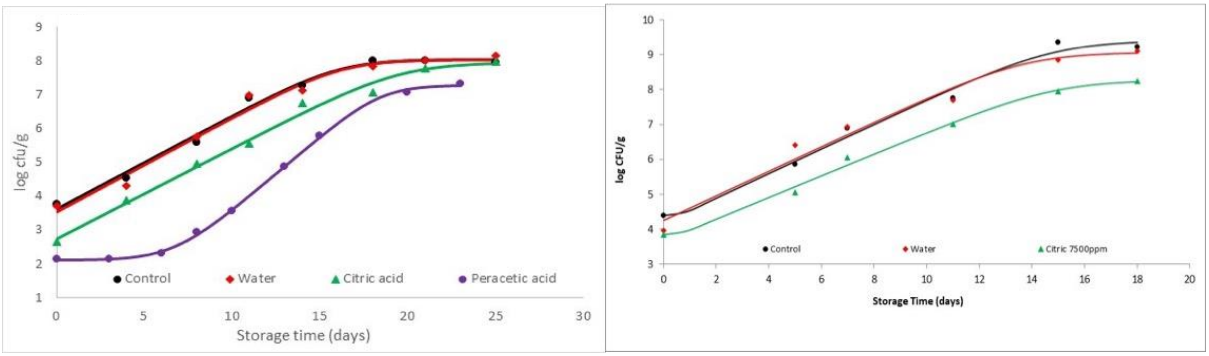


Figure 3. Enzymatic activity of calpain, collagenase and cathepsin L in all slaughter and storage methods. Superscripts indicate statistically significant differences (p<0.05) between treatments on each sampling day

Initial surface decontamination in the range of 1.0-2.0 log cfu / g by the addition of organic acids in the washing water, resulted in 3-4 days shelf life extension of fish stored at 0°C. Increased microbial load reduction was achieved for higher washing solution concentrations and longer treatment. Higher reduction of the initial microbial load was observed after treatment with citric acid for TVC, *Pseudomonas* spp. and H_2S-producing bacteria, with lactic acid for Enterobacteriaceae and with peracetic acid for *Pseudomonas* spp. and Enterobacteriaceae, compared to other bacteria tested. Microbial growth during subsequent refrigerated storage of untreated (Control) and treated fish is illustrated in Figure 4. Limit of sensory shelf life of gutted fish (score 5 by the sensory panel for overall impression) coincided with a level of 10^7 cfu / g of *Pseudomonas* spp. for gutted samples and of TVC for fillets, respectively, stored at 0 °C (Tsironi *et al.* 2019).



(a) (b)

Figure 4. (a) *Pseudomonas* spp. (log cfu/g) in gutted sea bass after surface washing with citric acid (200 ppm for 10 min) and peracetic acid (200 ppm for 4min), or water and Control during storage at 0°C. (b) TVCs (log cfu/g) in filleted sea bass after surface washing with citric acid (7500 ppm for 10 min), water and Control during storage at 0°C.

The results of the study indicated that the application of washing treatment with acids may result in significant deactivation of spoilage microorganisms (*Pseudomonas* spp, H_2S-producing bacteria) in gutted fish and fillets. Washing of fish using organic acids can reduce initial microbial load and significantly extend the shelf life of gutted fish and fillets. The replacement of conventional flake ice with slurry ice as a slaughtering method led to improved quality stability during subsequent refrigerated storage and shelf life extension, in terms of microbial growth, flesh quality and sensory degradation of fish. Shelf life extension of fish could open new distant markets currently inaccessible to fresh fish products and contribute to reduction of food waste. The systematic evaluation of the effect of harvesting, processing and transportation conditions on the quality and shelf life of fish may provide technological solutions for fish handling to improve quality and shelf life of fresh fish and reduce food losses during distribution and storage from harvesting up to the consumer level.

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