NANOENCAPSULATED CLOVE ESSENTIAL OIL USED IN STUNNING DURING SLAUGHTERING OPERATION IMPROVES THE QUALITY AND SHELF-LIFE OF FARMED GILTHEAD SEABREAM (*Sparus aurata* L.), AND DECREASES FISH WASTES IN THE URBAN CONSUMPTION CHAIN

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Introduction

Waste in the food supply chain is characterized by a high ratio of product specific waste. Therefore, the generation of this waste could be avoidable if the quality of the finished product is enhanced and its shelf life increased (Jayathilakan et al., 2012). Pre-slaughter procedures should be carried out without causing avoidable excitement, pain, fear or stress conditions, so to assure not only acceptable standards of fish welfare, but also high quality fish. In aquaculture, sedative and anesthetic agents are very useful for reducing the stress caused by handling, sorting, transportation or artificial reproduction. Clove essential oil (CEO) is an effective, local and natural anaesthetic. Many hatcheries and research studies use CEO to immobilize fish for handling, sorting, tagging and to suppress sensory systems during invasive procedures (Javahery et al., 2012). In this study we evaluate the use of crushed ice and liquid ice including clove essential oil (CEO) encapsulated in β-Cyclodextrins (β-CDs) embedded in the ice crystals, during stunning at the slaughtering operation, to improve the quality and shelf life of gilthead seabream by reducing stress, in experimental (terrestrial tanks) and in farm/open sea conditions, analyzing its effect on fish stress (characterized by glucose, lactate, and cortisol levels in plasma), and shelf-life (characterized by sensory and microbial quality of the fish) in ice (including other antimicrobial essential oils in the ice crystals).

Results and Discussion

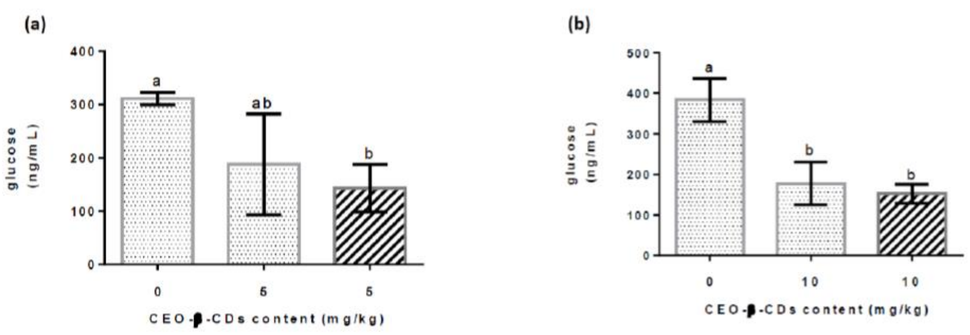

Figure 1. Glucose levels in plasma of gilthead seabream 2 h after slaughtering operation in open sea/farm conditions. Fish were stunned and slaughtered using different procedures: (a) Crushed ice (CI) with ice crystals of 15 mm size (black column) and 20 mm size (gray columns), mixed with seawater (SW) (ratio 1:1), at 1.0-2.0 ºC and including CEO-β-CDs at 0 and 5 mg/kg ice; (b) CI with 15 (black column) and 20 mm (gray columns) of ice crystals size) mixed with SW (ratio 1:1), at 1.5 ºC and with CEO-β-CDs at 0 and 10 mg/kg ice. Each value represents the mean ± S.E.M. of n fish per group in each sampling time. The letters indicate statistically significant differences among the groups according to an ANOVA and Bonferroni tests (P≤0.05), compared to control group, CI of 20 mm ice crystal size mixed with seawater (ratio 1:1) without CEO-β-CDs (letter a).

When fish are subjected to hypoxia and hypothermia during pre-slaughter and slaughter increase in stress level occurs resulting in increased muscle activity through vigorous movements, causing an increase in the anaerobic energy metabolism based on fermentation of glycogen or glucose (De Castro et al., 2017). This is the reason why glucose and lactate levels showed significant differences between the treatments studied in this work. Glucose levels decreased when CEO-β-CDs (15, 30 and 60 mg/kg) was added to CI and to LI, respectively, if comparing to treatments control (using stunning with CI-seawater at 1:1 ratio or LI at the same ratio, and without addition of CEO-β-CDs). Blood lactate has the same behaviour. According to other works, blood glucose and lactate levels appears to be sensitive and reliable indicator of stress in fish (Silbergeld, 1974; Well and Pankhurst, 1999). CEO-βCDs improve the stress conditions of gilthead seabream at slaughtering time by considering the decrease in the glucose levels in comparison to those levels of fish slaughtered without stunning with CO-βCDs (Figure 1). This decrease is higher when the concentration of the CEO used is low (10 to 15 mg/kg).

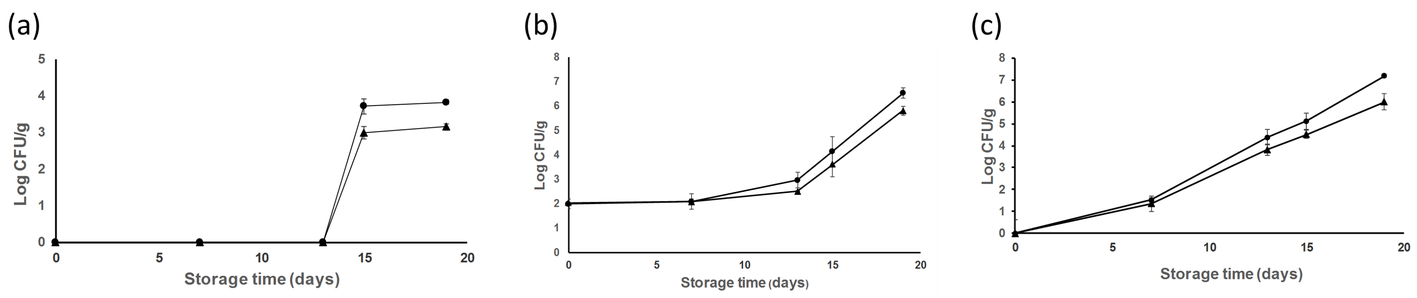

Figure 2 . Changes in the counts (Log CFU/g) of a) Pseudomonas, b) mesophilic aerobic bacteria, c) psychrotrophic bacteria of sea bream along storage at 2 ºC with different package icing conditions: control ice (●) and ice including EOs-β-CD (▲).

Pseudomonas is a bacteria responsible for fish deterioration. In fact, it has been used as spoilage indicator of fresh fish (Stamatis and Arkoudelos, 2007). In our study, after 12 days, Pseudomonas (Figure 1a) began to proliferate and the level grows during storage of chilled fish. Lactic acid bacteria showed similar evolution to Enterobacteriaceae and Pseudomonas. Mesophilic bacteria (Figure 1b) in the fishery products are a useful tool for evaluation of quality and shelf-life, and post-processing contamination (Huss, 1994). Samples from fish stunned and slaughtered with CEO-βCDs, and processed in ultraclean conditions, had lower counts throughout storage, and these groups did not reach 7 Log CFU/g at the end of storage time (day 19). These results demonstrated that EO had strong inhibitory effect on microbial growth in packaged sea bream during chilled storage, mainly in Pseudomonas, mesophilic aerobic bacteria and Enterobacteriaceae, so, extending the shelf life of fish.

Conclusion

- All the stress-related parameters analyzed indicated that CEO-βCDs incorporated into the ice crystals of CI mixed with seawater can be considered as a useful anesthetic on farmed gilthead seabream for stunning at slaughtering operation.
- Pseudomonas counts were reduced by more than 0.5 Log CFU/g after 19 storage days.
- Mesophilic aerobic bacteria counts reached 6.52 log CFU/g for control package icing and 5.8 Log CFU/g for package icing including EOs-β-CD after 19 days of storage.
- Psycrotrophic counts of samples packaged in ice with EOs-β-CD were reduced by 1 Log CFU/g after 19 days of storage.

Acknowledgement

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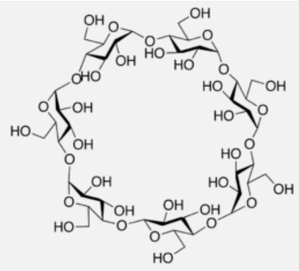
Material and Methods

1. Pre-slaughter procedure

Clove essential oil



β-Cyclodextrins

Crushed Ice

Stress parameters

Glucose
pH
pCO2
pO2
Lactate
HCO3
TCO2
SO2
Base Excess

Cortisol

Plasma analyses

Blood samples were extracted from the caudal vein immediately after death (Lerfall Jet al., 2015) .

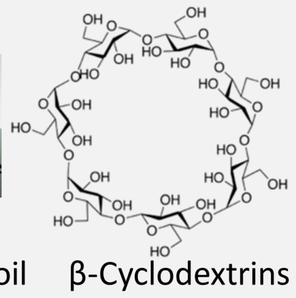
- Real time (PCR) (Chaves et al., 2008)

Gene expression samples

Total RNA was extracted from **head kidney** and **muscle** from each fish.

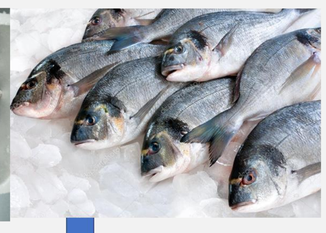
2. Microbiological analysis

Oregano essential oil



β-Cyclodextrins

Crushed Ice



Polystyrene boxes covered with 2 kinds of crushed ice:
- **Control ice (without antimicrobials)**
- **Ice with antimicrobial (EOs-β-CD, EOs forming an iclusion complex with β-CD)**

Stored in a cold room at 2ºC

Microbiological analysis: 0, 7, 13, 15 and 19 days

Las ciudades conectan naturalmente
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