

Comparison of methods for anaesthetizing Senegal sole (*Solea senegalensis*) before slaughter: Stress responses and final product quality

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Abstract

The aim of this work was to evaluate several anaesthetization methods such as clove oil, hypothermia and asphyxia in Senegal sole (*Solea senegalensis*), a highly resistant species to hypoxic conditions. The overall intent of the study was to determine a method that minimizes *pre mortem* stress and maximizes final product quality in sole for market sale. Several *pre* and *post mortem* indicators were considered for this study, namely rigor mortis, muscle pH, ATP/IMP ratios, eye refraction index and sensorial attributes. In addition, the stress experienced by the fish *pre mortem* was assessed by measuring cortisol, glucose and osmolality levels in blood plasma just after death (*post mortem*). Based on these physiological indicators, the results revealed that of the three methods considered, 1 ml·L⁻¹ clove oil was the best method for stunning sole because it not only ensures a good final quality product, but also is acceptable for a direct human consumption.

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1. Introduction

The aquaculture industry, as well as the overall animal production sector, is concerned with ethical and welfare issues related to fish husbandry. The Scientific

Panel on Animal Health and Welfare of the European Food Safety Authority has issued an opinion related to the animal welfare during stunning and killing procedures in farms, recommending further investigations on the mechanism and effect of different stunning and killing methods (EFSA, 2006). Besides the ethical considerations, *i.e.*, avoiding any possible fish suffering from pain or distress (Huntingford et al., 2006), there are also economical and commercial reasons for using human procedures for slaughter, as poorly treated fish results in lower quality meat. In addition, managers in the distribution and supermarket sectors are increasingly

Abbreviations: AS, Asphyxia; CO, clove oil; HY, Hypoxia; RI, Refraction index; RM, rigor mortis.

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requiring quality assurances and inclusion of welfare procedures for fish farms.

Senegal sole (*Solea senegalensis*) is being gradually incorporated into commercial aquaculture production in Europe, mainly due to its high acceptance by consumers and also its potential as a substitute species for gilthead sea bream (*Sparus aurata*) or turbot (*Scophthalmus maximus*) in land-based facilities in areas around the Mediterranean Sea. The volume of farmed sole in the markets is still low and a standard method for slaughtering this species for commercial sale is not yet available. A specific method for slaughtering sole should take into account the high resistance of this species to hypoxic conditions, otherwise slaughtering could result in a considerable distress to the fish when kept out of the water, which would not be an acceptable practice from both ethical and commercial perspectives. Therefore, an efficient stunning method should be established to shorten and desensitize fish before the actual slaughter. A considerable amount of research on the effect of different stunning methods on the welfare and quality of fish has been done in the recent years because of the interests of the aquaculture sector, with most of them proposing anaesthetizing methods (Savenije et al., 2002). Among them, the methods based on the use of chemical anaesthetics are not suitable because of the potential risk for human consumption of the residues that are retained in the fish muscle. Marx et al. (1997) found that stunning fish using carbon dioxide (CO₂) causing death by asphyxia was a very rapid method for trout and carp species (3.2 and 9.3 min, respectively), but very slow in hypoxia-resistant species such as eel (*Anguilla anguilla*) (109.7 min), and probably would be for sole species, which show signs of increased mucus production during CO₂ narcosis that consequently affects muscle quality.

Clove oil is a relatively new product in the aquaculture sector as a potential method for fish euthanasia. Its active ingredient is eugenol (4-allyl-2-methoxyphenol) and it derives from the stem, flowers and leaves of clove (*Eugenia caryophyllata* and *E. aromatica*) (Lee and Shibamoto, 2001; Martini et al., 1996). Although it has been recently used as an anaesthetic in many fish species with good results, its physiological effects are still poorly understood. Fishes in which clove oil has been used successfully for this purpose include: rabbitfish (*Siganus lineatus*) (Soto and Burhanuddin, 1995), rainbow trout (*Oncorhynchus mykiss*) (Anderson et al., 1997; Keene et al., 1998), the coral reef fish, *Pomacentrus amboinensis* (Munday and Wilson, 1997), channel catfish (*Ictalurus punctatus*) (Waterstrat, 1999), rockpool fishes (Griffiths, 2000), gilthead sea bream (Tort et al., 2002), sockeye salmon (*Oncorhynchus. nerka*) (Woody et al., 2002),

Atlantic salmon (*Salmo salar*) (Iversen et al., 2003), silver perch (*Bidyanus bidyanus*) (Kildea et al., 2003) and steelhead (anadromous rainbow trout) (Pirhonen and Schreck, 2003; Woolsley et al., 2004).

Another way for anaesthetizing fish in aquaculture is hypothermia by which low temperature affects activity, metabolic rate and oxygen consumption, and finally the fish is immobilised (Solomon and Hawkins, 1981; Hovda and Linley, 2000). Hypothermia is more or less efficient for stunning fish depending on the thermal gradient between the cooling bath and the culture water. For this reason, cooling is easier when species live in relatively warm water, as Mediterranean fishes do, as well as being an extensive method described mainly for fish transport (Acerete et al., 2004).

Thus, the aim of this work was to evaluate the effectiveness of stunning sole with clove oil, asphyxia and hypothermia to determine the best method that minimizes *pre mortem* stress and maximizes final product quality. Several indicators were chosen to assess the efficacy of the stunning methods mentioned previously for use with sole. First, rigor mortis (RM) was examined, as it is widely used as an indicator of *pre mortem* stress both in duration and intensity (Lowe et al., 1993; Nakayama et al., 1992). Second, muscle pH was measured, as changes in pH help to assess the expected shelf-life of the product. Several studies revealed that *pre mortem* stress caused lower muscle pH immediately after death in white sturgeon (*Acipenser transmontanus*) (Izquierdo-Pulido et al., 1992), carp (Nakayama et al., 1992) and salmonids (Azam et al., 1989; Fletcher et al., 1997; Sigholt et al., 1997). In addition, the ATP/IMP ratio was also measured as it is considered as a good indicator of the energetic consequences of the deterioration process, and finally, eye refraction index (IR) was measured as it changes depending on the time after death and freshness conditions.

Other than biochemical and physical properties of the fish muscle, sensorial methods are very relevant in quality studies because they assess fish quality in the same way that consumer would perceive it (Nielsen et al., 1992). When specific sensorial methods for a particular species are not available, which is the case for sole, more generic methods can be used (skin, eyes, gills and odour freshness), as described by FAO assessment (FAO, 1997), and are very convenient to compare among different species and investigators. The stunning procedure induces a severe acute stress experience in fish that, as a result, develop a response in which hormonal, biochemical, osmoregulatory and energetic alterations quickly take place (Barton, 2002; Tort et al., 1998). Those changes can be assessed to evaluate the level of stress in a specific situation such as a particular stunning method.

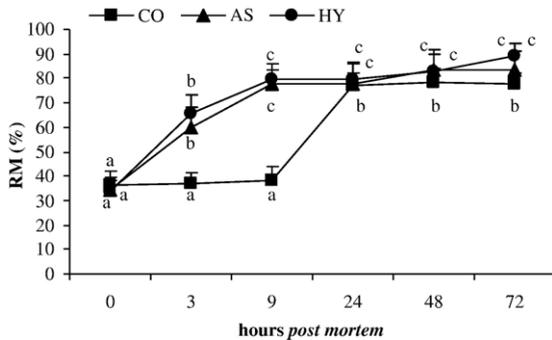


Fig. 1. Dynamics of rigor mortis (RM) in Senegal sole treated with different stunning methods: clove oil (CO), asphyxia (AS) and hypothermia (HY). Data are expressed as percent in tail bending classified in one of the steps of rigor states at each sampling time ($t=0, 3, 9, 24, 48$ and 72 h *post mortem*; $N=8$). Data are expressed as mean (<0.05).

Plasma cortisol has been long considered as a reliable indicator of acute stress in fish (Wedemeyer et al., 1990) along with other blood variables, such as metabolites involved in metabolism of glucose, lactate and osmolality (Barton et al., 2005).

2. Materials and methods

2.1. Fish and sampling

A group of 126 Senegal sole (mean \pm SD body weight of 155.3 ± 43.5 g and standard length of 22.9 ± 1.8 cm) were obtained from raceways of a commercial fish hatchery in the Ebre Delta area near Tarragona, Spain. These raceways were squared pools of 200×3 m and 1 m deep. Before experiments, some fish were grouped to one corner of the raceway with grids but without changing density or water characteristics and were subjected to food deprivation for 24 h. After that time, three different stunning methods were performed. One method was hypothermia (HY) by direct immersion of fish into chilled water (from ambient 16 °C to $2-4$ °C), the second method tested was clove oil anaesthetization (CO) by immersing fish in a bath containing a previous water dilution of $1 \text{ mL} \cdot \text{L}^{-1}$ clove oil prepared after vigorous shaking. Clove oil was obtained from pure (100%) preparation of stem, flowers and leaves of clove (*E. caryophyllata* and *E. aromatica*) distributed by Reform Products Solnatural (Cabrera de Mar, Barcelona, Spain). To compare with the standard method used for stunning sole before slaughter, a third group was subjected to asphyxia (AS) during transport on ice in styrofoam boxes to simulate current operating conditions. Tests were carried out at “time of immobilisation” on eight fish per method after 0, 3, 9, 24, 48, and 72 h ($N=126$) *post mortem* (i.e., after a complete immobilisation), and kept on ice during all period of ex-

periment. At each sampling time, several indicators were evaluated: rigor mortis (RM), muscle pH, eye refraction index (RI) and sensorial analysis. Muscle samples were extracted and kept at -80 °C for further determinations of muscle ATP/IMP ratios. For assessment of physiological stress, blood was taken from caudal vessel of eight fish for each method by puncture with a syringe at the first sampling time *post mortem* ($t=0$ h). In parallel, blood was also taken from control group ($N=8$) obtained directly from the raceways in order to have resting (unstressed) values of physiological indicators. Blood was placed immediately in heparinised tubes and centrifuged shortly thereafter for 5 min at 4000 rpm at 4 °C. Plasma was separated and frozen at -20 °C until analysis.

2.2. Analytical methods

For whole fish RM, a tail-bending measurement method was followed (Bito et al., 1983) and results were expressed as Rigor Index (%) = $100 \times (\text{current height} - \text{height before entering rigor}) \times (\text{height before entering rigor})^{-1}$. At each sampling time, fish were individually classified in one of the three stages of rigor in the following way: a percentage of Rigor Index below 33% is considered pre-rigor state; a value between 33 and 77% is classed as onset of rigor; full rigor is shown by a percentage above 77%.

For muscle pH, a penetration electrode TESTO 320 (TESTO GmbH & Co., Germany) inserted into the cephalic dorsal muscle was used. Eye refraction index (RI, expressed as °Bx), was directly measured by taking a few drops of aqueous ocular liquid and measuring its refractometry (ATAGO N-1 refractometer, Japan) as previously described by Eskin (1990). The ATP/IMP ratio in muscle was determined using the methodology described by Khan and Khan (1971) as the ratio of the absorbance at 258 nm to that at 250 nm of perchloric acid muscle extracts. Sensorial analysis of several fish freshness attributes (skin, eyes, gills and odour) was

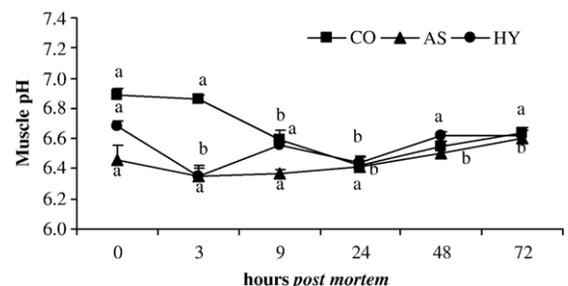


Fig. 2. Dynamics of muscle pH in Senegal sole treated with different stunning methods: clove oil (CO), asphyxia (AS) and hypothermia (HY). Data are expressed as mean (<0.05).

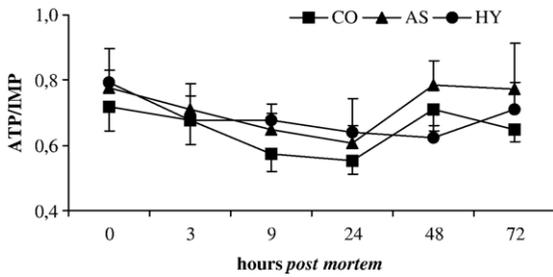


Fig. 3. Dynamics of ATP/IMP ratio of Senegal sole treated with different stunning methods: clove oil (CO), asphyxia (AS) and hypothermia (HY). Data are expressed as mean (<0.05).

assessed by 4 trained evaluators, according to the freshness classification of the scoring from 0 (least fresh) to 3 (most fresh) (FAO, 1997).

Plasma cortisol levels were measured by radioimmunoassay following the procedure described previously by Rotllant et al. (2001). The antibody used was purchased from Biolink S.L. (BioLink, Barcelona, Spain). Plasma glucose and lactate were determined by enzymatic colourimetric methods in ELISA plates using commercial kits (BioMérieux 61270 and 61192, France). Plasma osmolality was assessed by direct reading using a freezing-point depression osmometer (Osmomat, Berlin, Germany).

The statistical analysis on the effectiveness of HY, AS and CO as stunning methods for sole was performed by one-way analysis of variance (ANOVA) with a Duncan *post-hoc* analysis. In all statistics $P < 0.05$ was accepted as the level of significance.

3. Results

3.1. Rigor mortis

The evaluation of final percentages found in RM analysis, classifying individuals in one of the three steps of rigor dynamics is shown in Fig. 1. Rigor onset and resolution was markedly affected by the stunning

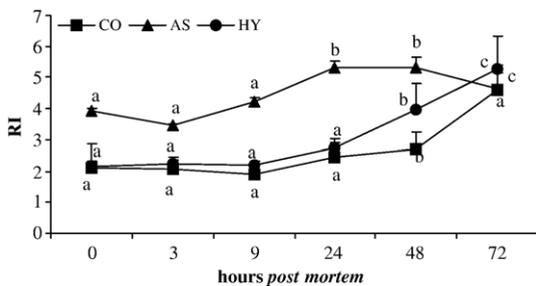


Fig. 4. Dynamics of refraction index (RI) ratio in Senegal sole treated with different stunning methods: clove oil (CO), asphyxia (AS) and hypothermia (HY). Data are expressed as mean (<0.05).

Table 1

Dynamics of sensorial analysis in Senegal sole treated with different stunning methods: clove oil (CO), asphyxia (AS) and hypothermia (HY)

| | Skin | | | Eyes | | | Gills | | | Odour | | |
|-------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | CO | AS | HY |
| 0 | 3.00±0.00 | 1.86±0.26 | 2.43±0.18 | 3.00±0.00 | 2.29±0.18 | 2.86±0.14 | 3.00±0.00 | 2.43±0.30 | 3.00±0.00 | 2.00±0.00 | 1.86±0.34 | 2.00±0.00 |
| 3.00 | 3.00±0.00 | 1.71±0.18 | 1.86±0.34 | 3.00±0.00 | 1.29±0.18 | 2.14±0.14 | 3.00±0.00 | 1.43±0.20 | 2.57±0.20 | 2.00±0.00 | 1.29±0.18 | 2.00±0.00 |
| 9.00 | 2.71±0.18 | 1.29±0.18 | 2.00±0.00 | 2.14±0.14 | 1.00±0.00 | 1.43±0.20 | 2.43±0.20 | 0.43±0.20 | 1.57±0.20 | 2.00±0.00 | 1.43±0.20 | 1.86±0.14 |
| 24.00 | 1.71±0.18 | 1.00±0.22 | 1.43±0.20 | 1.43±0.20 | 0.86±0.14 | 1.29±0.18 | 1.29±0.18 | 0.86±0.34 | 1.00±0.22 | 2.00±0.00 | 1.29±0.18 | 1.57±0.20 |
| 48.00 | 1.43±0.20 | 0.29±0.18 | 0.29±0.18 | 1.00±0.22 | 0.29±0.18 | 0.57±0.20 | 0.43±0.20 | 0.14±0.14 | 0.43±0.20 | 2.00±0.00 | 1.29±0.18 | 1.57±0.20 |
| 72.00 | 0.14±0.14 | 0.00±0.00 | 0.00±0.00 | 0.43±0.20 | 0.71±0.18 | 0.29±0.18 | 0.29±0.18 | 0.14±0.14 | 0.00±0.00 | 1.71±0.18 | 0.71±0.18 | 1.29±0.18 |

Data are expressed mean±SE values, where freshness attributes (skin, eyes, gills and odour) were assessed by trained evaluators, according to the freshness classification of the scoring from 0 (least fresh) to 3 (most fresh) (FAO, 1997).

method used in sole. In the group of fish stunned with CO the onset was clearly delayed compared with fish stunned by HY and slaughtered by AS, not including individuals in full rigor until 24 h after death. In addition, the resolution was also faster, and the intensity of contraction was lower. In both HY and AS groups, the RM results revealed a similar pattern independent to the treatment. Indeed, just after 3 h after death, an onset level was achieved and in 9 h all individuals were classified in full RM level.

3.2. Muscle pH

A slower onset of rigor mortis (3 h *post mortem*) in sole stunned by AS and HY than those by the CO method (9 h *post mortem*) induced higher pH decreases, reaching significantly lower minimum values than the latter (pH 6.33 vs. pH 6.87, $P < 0.05$) (Fig. 2). The AS group showed pH values smoothly constant through all sampling time, whereas dynamics from fish subjected to HY significantly changed pH values depending on each sampling time.

3.3. ATP/IMP ratio

Fish anaesthetized by the three different methods showed similar muscle ATP/IMP ratios throughout the

72 h *post mortem* monitoring period (Fig. 3). Muscle ATP/IMP ratios followed a similar trend independently to the treatment. However, it could be considered that fish stunned by AS showed higher ATP/IMP ratio at 48 and 72 h *post mortem* (Fig. 3), although not significantly.

3.4. Refraction index

Sole anaesthetized by the AS method showed significantly higher RI values with respect to the other two groups, while sole of the CO group showed the lowest RI value throughout the whole *post mortem* period, although were only significantly different at 48 and 72 h *post mortem* (Fig. 4). Similar dynamics were found among fish subjected to HY; that is, RI values increased through time reaching the highest RI at 72 h.

3.5. Sensorial analysis

All features studied to assess fish freshness (skin, eyes, gills and odour) showed a decreasing change from 0 to 72 h *post mortem*. Table 1 shows sensorial analysis dynamics for the three fish treatments through the whole experiment. Fish anaesthetized by AS showed a more rapid loss of freshness in all features studied. In the skin, a significantly brighter and more iridescent pigmentation together with an absence of decolouration

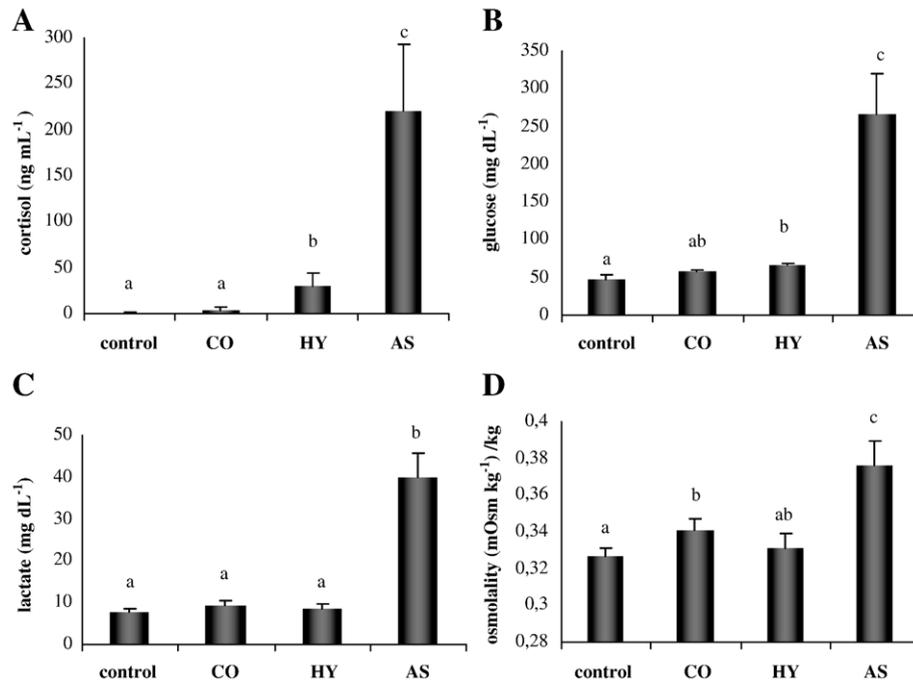


Fig. 5. Mean (< 0.05).

was observed in sole from group CO relative to those from AS and HY groups from 9 to 72 h *post mortem*. Fish stunned by HY showed intermediate scores, and those by AS the lowest. Skin pigmentation turned to pale as early as 9 h *post mortem* in the AS group, but not until 48 and 72 h *post mortem* in fish of HY and CO groups, respectively. As early as 3 h *post mortem*, the AS group showed plain eyes, while this did not occur until approximately 48 h *post mortem* in the other groups of fish. Freshness was more rapidly lost in the gills than in the skin or eyes of the sole. Gill scores decreased from 0 to 9 h *post mortem* significantly faster in AS fish than in the other groups. Scores reached the minimum quality value from 24 to 72 h *post mortem* (decolouring to yellowish) and no differences were evident among fish groups. Sole from group CO demonstrated significantly higher odour scores compared with those of group AS from 3 to 72 h *post mortem*. Sole of group HY showed intermediate scores, while those of group AS showed the lowest ones or a slightly acidic odour.

3.6. Stress analysis

Concentrations of the stress indicator variables measured were compared with values from resting (unstressed) sole. Plasma cortisol concentration of fish in group CO was similar to the resting value, whereas in fish in groups HY and AS, 35- and 263-fold higher concentrations, respectively, than the resting value of plasma cortisol were measured (Fig. 5A). Fish of group CO had plasma glucose concentrations similar to unstressed fish, while those of group HY and group AS were 1.4- and 5.6-fold higher, respectively (Fig. 5B). Lactate concentrations in plasma were 5.2-fold higher than the resting level in fish of group AS ($P < 0.05$), while the other groups had similar concentrations (Fig. 5C). Finally, sole in the AS group showed higher plasma osmolality than in the unstressed group, but no other significant differences were found in the other groups (Fig. 5D).

4. Discussion

One of the critical steps for quality maintenance in the aquaculture industry is the stunning/slaughtering procedure. Pre-slaughter chilling combined with reduction of stress also delays the onset of RM, creating a time window long enough to allow pre-rigor filleting and processing to take place, thus, avoiding the problem that during the rigor process the fish becomes stiff and mechanical handling is liable to cause muscle rupture

(Skjervold et al., 2001). RM is the most important *post mortem* physical process, which is characterized by a decline in adenine triphosphate (ATP) content of the muscle below a critical level and a decrease of muscle pH. Muscle ATP content at the time of death is affected by stress (Berg et al., 1997; Korhonen et al., 1990; Lowe et al., 1993; Mochizuki and Sato, 1994; Sigholt et al., 1997; Thomas et al., 1999). In addition, improper handling before slaughtering may lead to more rapid flesh deterioration (Lowe et al., 1993; Sigholt et al., 1997), which was recently observed in sea bass (*Dicentrarchus labrax*) and gilthead sea bream (Bagni et al., 2007). Both capture and sacrifice methods have an influence on the *post mortem* muscle degradation, causing changes in *rigor mortis* onset and resolution, a drop of muscle pH and loss of water retention capacity, protein denaturation, lipid oxidation and increase of microbiological flora (Tornberg et al., 2000). All of these effects have been found for different fish species including: snapper (*Pagrus auratus*) (Lowe et al., 1993), rainbow trout (Choubert and Baccaunaud, 2005), Atlantic halibut (*Hippoglossus hippoglossus*) (Kiessling et al., 2006), eel (Özogul et al., 2004), gilthead sea bream (Huidobro et al., 2000), white sturgeon (Izquierdo-Pulido et al., 1992) and Atlantic salmon (Kiessling et al., 2006; Olsen et al., 2006), there are few works on flatfish. Recently, some research have been published in turbot (Aubourg et al. 2005; Rodríguez et al. 2006), but less data is available describing *post mortem* muscle quality in sole. Our results suggest that in order to have a good muscle quality in sole, the slaughtering/stunning method is critical. Fish stunned by CO had a better RM index as well as better condition of the freshness features examined compared with AS- and HY-treated fish. The onset of RM together with the depletion of ATP obtained from our results confirmed findings from other fish species (Nakayama et al., 1992; Watabe et al., 1991). Thus, lower muscle pH immediately after death was coincident with a more rapid ATP decline during storage in stressed fish (Izquierdo-Pulido et al., 1992).

Our results are coincident with those observed in Atlantic salmon in which the Rigor Index (%) increased from initial slaughter to 24 h of storage time, and then gradually decreased from 24 to 36 h of storage (Sigholt et al., 1997). Therefore, the moderately high ATP/IMP ratios observed over 72 h *post mortem* in AS fish could be due to low IMP rather than to high ATP levels. The post-rigor status increased pH in fish muscle, whereas no effects were observed beyond 24 h *post mortem* irrespective of the anaesthetization method used. In the fish group stunned with CO the onset of rigor is clearly

delayed compared with fish stunned by HY and AS, not including individuals in full rigor until 24 h after death. Additionally, after 72 h *post mortem* following HY most of the individuals were in full rigor state, which could, in part, be due to the cold. Sensorial analysis revealed that fish freshness was lost more rapidly in the AS group than in fish from HY and CO groups, respectively. Results from this study clearly indicated that sole sacrificed by CO would suffer less stress and show improved fish quality compared with the other stunning methods tested.

There is a close relation between the *pre mortem* handling of fish and the stress suffered before and during slaughtering in quality of the final product (Azam et al., 1989; Erikson et al., 1997; Jerret et al., 1996; Morzel et al., 2002). Fish experiencing severe acute stress prior to death develop a stress response in which hormonal, biochemical, osmoregulatory, immune and energetic alterations take place (Barton, 2002; Tort et al., 1996, 1998), and consequently have a poor quality fillet (Taylor et al., 2002). Severe handling often results in an increase of plasma lactate, acidification and energy depletion and usually has strong effects on fish physiology and behaviour (Ross and Ross, 1999). Physiological and endocrine results reported here reveal high levels of cortisol, glucose, lactate and osmolality in plasma of the AS group, which could corroborate a previous ATP decline found. Highly altered levels of physiological stress indicators, particularly cortisol, in the AS fish may be related to the length of time required for AS to induce complete anaesthetization compared with more rapid stunning effect of the HY and CO methods, especially the latter. The higher lactate concentrations observed in fish of group AS could also be reflected in the low pH values recorded at 0 h *post mortem*. These results are in concordance with sensorial analyses where sole in the AS group showed a more rapid decline in flesh quality. Cold treatment did cause significantly increased plasma cortisol levels but at much lower degree. Chilling itself is known to be stressful to fish. Rainbow trout similarly subjected to a rapid temperature drop from 8–9 °C to 1–3 °C showed significantly elevated plasma cortisol levels that took a long time to recover indicating that cold shock alone causes appreciable stress (Barton and Peter, 1982). Interestingly, the fish group treated with CO did not show significant changes in the cortisol response. Similar results were found in steelhead 4 h after being anaesthetized with CO (Pirhonen and Schreck, 2003), in channel catfish after 30 min of exposure to CO (Small, 2003) and more recently in fathead minnows (*Pimephales promelas*) (Palic et al., 2006). Certain anaesthetics reduce

or block activation of the hypothalamo–pituitary–interrenal (HPI) axis associated with handling stressors (Iwama et al., 1997; Ross and Ross, 1999) resulting in a suppression of the HPI axis and cortisol release, the principle hormone to help fish overcome stress (Tort et al., 1998). Plasma cortisol results suggest that CO may act as a suppressor or depressor of the cortisol response in sole with better efficiency than cold treatment or asphyxia, in part by acting much more quickly to induce anaesthesia, thereby arresting the normal release of cortisol.

In addition, aquaculture production of sole is being perceived as having a potential due to a high and increasing appreciation by consumers. Sole is highly resistant to hypoxic conditions, which requires an efficient stunning method to ensure a quality product. Slaughtering is one of the critical steps for welfare in aquaculture and consequently in muscle quality for human consumers (Olsen et al., 2006). One way to minimize the possible suffering during slaughter is to use methods that render the fish insensitive to pain before actual death. Various anaesthetizing methods meet with this demand and do not negatively affect the final quality of fish. However, anaesthetics based on the use of chemicals are not desirable considering the potential risk for human consumption (Larsen et al., 1992). Many studies concerning anaesthetic consequences in different fish species have been published and include the use of tricaine methane sulphonate (*i.e.*, MS-222), quinaldine and etomidate (Summerfelt and Smith, 1990), CO₂ (Wagner et al., 2002), benzocaine (Iversen et al., 2003) and 2-phenoxyethanol (Tort et al., 2002). As a fish anaesthetic, CO has good qualifications in terms of efficacy, cost, availability, ease of use and limited side effects on fish, humans and the environment (Marking and Meyer, 1985). Clove oil is considered a non-mutagenic and safe substance (Nagababu and Lakshmaiah, 1992) and its use does not require a withdrawal period as some others do (Anderson et al., 1997).

5. Conclusions

The overall results of this study describe the efficacy and benefits of CO on muscle quality and sensorial analysis and the effect on physiological stress indicators in the Senegal sole. The data shows that stunning fish using CO at a dosage of 1 mL·L⁻¹, can be an efficient method that ensures a good quality product for market. These results for CO may also be applicable to other hypoxic-resistant species used in aquaculture production as a stunning method to ensure a high level of fish quality suitable for human consumption.

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