Effects of different slaughtering methods on rigor mortis development and flesh quality of tench (Tinca tinca).

Published version:

DOI:10.1111/jai.12426

EFFECTS OF DIFFERENT SLAUGHTERING METHODS ON RIGOR MORTIS DEVELOPMENT AND FLESH QUALITY OF TENCH (*TINCA TINCA*).

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Summary

The effects of different slaughtering methods on *rigor mortis* and flesh quality parameters were investigated. Eighty tench were divided into 4 groups and each group was slaughtered following different methods: percussive stunning (PS), live chilling (LC), asphyxia (CO_2) and electrical stunning (ES). *Rigor mortis* (RM) progression was recorded (30 h) as well as pH, colour, cooking losses, texture, drip losses and free water after 24 h of storage at +2°C. PS group showed a delayed onset of RM if compared to the other groups and a later beginning of rigor resolution. The highest values of rigor index were shown by PS and CO_2 groups and were reached at 24 and 20 h *post mortem*, respectively. ES fish exhibit a significantly faster rigor onset if compared to PS group. pH at 24 hours was not affected by stunning methodology as well as colour and texture characteristics, even if a trend was observed (PS>ES>CO_2>LC) for this latter parameter. This research evidenced how stunning procedures before slaughtering have a significant effect on *rigor mortis* development while no differences were found concerning quality parameters.

Introduction

According to EU Council Directive 93/119/EC (1993), animals should be slaughtered in an appropriate manner in order to reduce pain, agitation, suffering, injury or contusions. Fish are part of the aim of this legislation and, although opinions differ in the academic world, because of the lack of tradition in considering fish as sentient animals, there are many references showing that fish possess anatomical features that allow them to feel pain and experience fear (Chandroo et al., 2004; Ashley, 2007). The concept of pain and welfare in fish is relatively new in aquaculture and, even if producers and consumers are become increasingly aware about it, is regarded as not sufficiently deemed and investigated (Poli, 2009).

Many authors reported how pre- and post-slaughter practices and quality parameters are intrinsically linked and that meat quality is reduced in badly treated fish; therefore, commercial and economics aspects arise beside ethical considerations (van de Vis et al., 2003; Acerete et al., 2009; Matos et al., 2010).

The Panel on Animal Health and Welfare was asked by the EU Commission to deliver an opinion on welfare aspects of the main systems of stunning and killing of farmed fish (EFSA, 2009) and, among the critical steps that can dramatically compromise fish welfare, slaughtering methods are of great importance (Poli, 2009). It was also specifically requested to set up recommendations in a species specific approach and it was stressed that stunning / slaughterer methods should be performed quickly in order to avoid stress and pain (EFSA, 2009).

The main stunning and slaughtering methods used in aquaculture are: electrical stunning, CO_2 stunning, asphyxia in air, ice slurry asphyxia (chilling), gill cut, percussion (Marx et al., 1997; Robb et al., 2000; Poli et al., 2005; Rahmanifarah et al., 2011).

Several biochemical and physical parameters have been used to evaluate the quality features of fish products (Parisi et al., 2002; Kiessling et al., 2004; Scherer et al., 2006; Wilkinson et al., 2008; Duran et al., 2008). One of the first changes that occurs postmortem is the onset of rigor mortis (RM). This happens when the muscles' ATP content decrease below a critical level and the bond between actin and myosin become irreversible. Stress or inappropriate handling before slaughter leads to a great consumption of the glycogen energy reserve at the expense of post mortem ATP regeneration. Similarly, ultimate muscle pH is due to the generation of H^+ ions associated with the lactic acid production as well as to the collapse of ATP reserves in muscle (Poli et al., 2005; Acerete et al., 2009). These two parameters are then very useful tools that reflect the pre-mortem status of reserve and stress before death (Bagni et al., 2007). Other parameters as meat color, texture, water holding capacity, drip losses or cooking losses are also good flesh quality indicators of pre-slaughter and slaughter practices (Matos et al., 2010).

The effects of an inadequate management during pre-slaughter procedures on quality of final products have been investigated for several fresh water or marine species (Poli et al., 2005; Håstein et al., 2005; Ashley, 2007; Wilkinson et al., 2008) while, to the best of our knowledge, there is any published data on the effect of slaughter methods on these parameters on tench.

Tench, *Tinca tinca* (L.), is a cyprinid pond fish species widely distributed around the world. Furthermore, in some specific area of Italy tench is considered as a high value niche product (18€/kg), has obtained the Protected Designation of Origin (PDO.) European recognition and is considered as an interesting new species for aquaculture development (Gasco et al., 2010).

Even if no official data exist on the killing methodologies, at present most tench farmed in Italy are killed by asphyxia in air, suffering a prolonged period before death. This methodology does not comply with the requirements of animal welfare for aquaculture and moreover could dramatically affect the final quality of the product.

Furthermore, the Panel on Animal Health and Welfare indicated as, for cyprinids, "*standard operating procedures to improve the control of the slaughter processes to prevent impaired welfare should be introduced, and validated, robust and practically feasible welfare indicators should be developed*".

In order to comply with these requests, the present study was aimed at comparing the effects of different slaughtering methods on rigor mortis and flesh quality parameters of tench.

Materials and methods

Animals and sampling

A total of 80 tench (*Tinca tinca*) (80.51±7.92g) were caught by gillnets, kept in a 4000 l aerated concrete tank (21±1°C) for a week and then fasted for 24h before killing. Fish were allocated to one of the four methods of slaughter: 1) live chilling (LC) by immersion of fish in chilled water (water : ice ratio = 1:1; T = 2-4°C) for 50 minutes; 2) asphyxia by CO_2 (CO_2) placing tench into a bath of water (10 l) where CO_2 was bubbled through the water to maintain CO_2 at saturation level; 3) percussive stunning (PS), by restraining manually and individually each of the 20 fish and stunning them by a blow on the head using a wood club; and 4) electrical stunning (ES) by placing simultaneously the 20 fish in a dry electro-stunner constructed following the indications of the local health service (51V, 30 sec).

Immediately after slaughtering fish were individually weighted, and carefully gutted. They were transferred into polystyrene boxes covered with ice flakes and stored in a refrigerated chamber at +2°C.

Rigor Mortis (RM) index

To record RM progression, the measurement was based on the tail curvature (Bito et al., 1983).

After evisceration (0) and for the successive hours (1 till 30), each fish was placed on a flat surface and an image was recorded with a digital camera (Nokia D3100). The image analyses were performed using the Image-Pro Plus. 5.1 software (Media Cybernetics Inc., Bethesda, USA). Measurement calibration was made to the nearest 0.01 mm. The rigor index (I_R) was then calculated following the formula $I_R = 100*(L_0-Lt)/L_0$ where L is the vertical distance between the base of the caudal fin and the table surface, measured immediately after death (L_0) and during the storage (Lt, with t = 1 to 30). For each group, the same 5 fish were used for *rigor mortis* measurements and between each measure fish were kept at +2°C.

Physical parameters

Physical parameters were determined 24h after death. Muscle pH was measured by a Crison MicropH 2001 (Crison Instruments, Barcelona, Spain) equipped with a combined electrode and an automatic temperature compensator.

Fillet colour was assessed using a Minolta CR-331C Minolta Colorimeter (∅ 25 mm measuring area, 45° circumferential illumination/0° viewing angle geometry) with the D65 illuminant and 2° standard observer. The results were expressed in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB colour space model (CIE, 1976). Chroma and Hue were calculated according to Boccard et al. (1981).

Cooking losses were assessed following Ramírez et al. (2004). Fillets hardness measurements were performed using a Zwick Roell® texturometer (software: Text Expert II) equipped with a 200 N load cell. One cycle compression test was done by using a 10 mm diameter cylindrical probe, at a constant speed of 30 mm/min until a 50% of total deformation. The textural attribute was measured in two locations of the epaxial muscle of each left fillet.

Drip losses (DL) were determined as fluid lost by the fillets stored in a polystyrene bag for 24 h in a refrigerated chamber, measured as difference between the weight of fillet before and after storage (previously dried with a paper towel), and expressed as percentage on the weight of fillets before the storage.

Water holding capacity (WHC) was determined according to Grau and Hamm (1953) method and expressed as free water (FW).

Statistical analysis

Parameters studied were analyzed by one way ANOVA using the slaughter method as predictor (R, 2005). In particular, for *rigor mortis* index, data were processed in the model for each time of sampling, separately. When ANOVA was significant, differences amongst groups were evaluated with Duncan's test. Values were reported as means of group ± standard deviation of the mean. Significance was accepted for $P<0.05$.

Results

Rigor Mortis index

The different slaughtering methods had a significant ($P\leq0.05$) impact on the RM onset (Figure 1). At the beginning, no differences between groups were recorded on rigor status. First differences emerged between ES (48.18%) and others groups (28, 17.84 and 14.57% for LC, PS and CO_2, respectively) since the second hour post mortem, ES showing a faster rigor onset if compared to LC, PS and CO_2 fish. After 4 hours, statistical differences emerged only between ES (68.28%) and PS (39.53%) groups. At 7 hours after death, I_R expressed by CO_2 fish (79.88%) was significantly different from ES (67.64%) and PS (65.6%) groups, while at 8 hours (CO_2 = 81.04%) this difference was only with regards to ES (64.08%) group.

As general trend, the PS group showed a delayed onset of RM if compared to the other groups and a later beginning (28h *post mortem*) of rigor resolution.

The highest values of I_R obtained were shown by PS (96.84%) and CO_2 (96.80%) groups and were reached at 24 and 20 h *post mortem*, respectively. From 8 till 25 hours, no significant differences emerged between CO_2 and PS groups.

ES fish exhibit a significantly faster rigor onset if compared to PS group. Value of I_R of these 2 groups was always statistically different except at hour 7. The maximum I_R value was reached at 25 h for ES group (83.96%), at 27 h for LC group (89.10%), at 22 h for CO_2 group (96.46%), and at 24 h for PS group (96.84%).

Physical parameters

Physical parameters at 24 h are reported in Table 1. No significant differences were highlighted between treatments.

Discussion

RM

Similarly to several authors, this research evidenced that slaughtering procedures have a significant effect on RM development (Ashley, 2007; Poli., 2009).

Acerete et al. (2009) compared 3 slaughter procedures and found that the RM onset was similar for all methods and resolution was similar between CO_2 and ice asphyxia groups that were different only at 24 h post death.

In our research, different results are showed. During the first 12 hours *post mortem*, LC showed the same trend than CO_2 group with not significant differences in I_R values. Afterwards the trend remained similar, but from h 17 I_R data were significantly lower for LC group (81.77 *vs* 91.35). The same trend can be noticed comparing LC and PS (90.71) fish. Scherer et al. (2005) showed that grass carp killed by electricity entered into RM earlier than ice-water slaughtered fish, as was found in this trial. Similarly to Marx et al. (1997) and Morzel et al. (2003) which reported that fish stunned by PS entered in rigor later than fish killed by electricity, in our study PS tench showed a more lower rigor onset rate than ES group as well as a higher intensity of RM. Roth at al. (2002) did nevertheless not observe these differences on *Salmo salar*. In this trial it has been noticed that in 3 case over 20 one percussive blow on head was not sufficient to kill tench so, in order to be sure that fish was died, two strokes were applied. Nevertheless, no external or internal damages were caused.

Abe and Okuma (1991) reported that on carps killed by electricity a 100% I_R was observed 20h after death whereas in group killed on ice-water slurry, the maximum muscle contraction was recorded 24 to 48 h post mortem (I_R = 92 to 97%). Similar values of I_R both for ES (83.96% at 24h) and LC (89.10% at 27h) groups were not reached for tench in this trial, indicating a lower capacity of ATP regeneration in these groups that could be due to high glycogen depletion.

In African catfish, Lambooij et al. (2006) reported an immediate induction of unconsciousness and insensibility using ES in water (300V). Nevertheless, as the death of fish was not obtained and since fish recovered from this procedure, a combination with decapitation and bleeding was suggested.

Conversely, Ashley (2007) reported as, if applied correctly, ES methods appear to achieved humane slaughter in several species and how the UK trout industry appears to be moving towards as preferred slaughter method (Lines et al., 2003). In this research, no tench recovered from ES treatment, indicating that death was immediately obtained. Nevertheless fish slaughtered by ES method evidenced the characteristics of a general epileptic insult, and 67% of tench showed blood spots in the flesh. Moreover, all fish stunned by ES procedure, showed two or three burn marks on the skin, originated from the contact point between skin and electrodes. Same kinds of observations were done by Erikson et al. (2012) observing cod skin after dry-electrical stunning. Then this procedure was efficient from a killing point of view but showed negative effects on appearance of the fish and fillet quality.

Hypothermia induces immobilization before unconsciousness and could be a suitable method for killing edible portion-sized sea bass for which individual stunning methods is not feasible (Parisi et al., 2002). Since immobilization do not mean lost of consciousness, some researchers found hypothermia highly stressful for fish (Robb et al., 2000; Lambooij et al., 2006; Van de Vis et al., 2003; Bagni et al., 2007).

On this trial, after some minutes LC tench showed a slowing down swimming behaviour and after 15 minutes fish shown convulsive and rapid movements completely ended only after 45 minutes, when no opercula movements were observed. Fish were removed from water after 50 minutes and no one recovered. This behaviour indicated that fish were subjected to a prolonged period of stress and suffering (at least 45 minutes) before death. In no case 50 minutes may be defined as “rapid” and then compatible with the notion of welfare. Analogously, Lambooij et al. (2006) concluded that hypothermia could not be considered as an acceptable stunning method because it prolongs the period of consciousness and does not reduce the ability to feel pain. Moreover, The Farmed Animal Welfare Council (FAWC, 1996) reported that cooling of live trout on ice should be prohibited.

Scherer et al. (2006) found no differences in shelf life of grass carp slaughtered by immersion in ice and water or by electricity and Rahamanifarah et al. (2011), even if reporting a slower rigor onset and resolution in carp stunned by hypothermia compared to CO_2, concluded that, in agreement with the FAWC (1996), this method should be deemed unacceptable from an animal welfare point of view because of prolonged induction time and abnormal behaviour.

Acerete et al. (2009) compared CO_2 and ice chilling methods on sea bass and evidenced in both cases struggling movements with a longer time to death for water-iced killed fish. Authors suggested that this could indicate a higher impact on muscle quality and concluded that CO_2 showed slightly better results regarding the response to stress indicators. Considering *rigor mortis* index as stress indicator, the same conclusions seem to emerge from this study as in full rigor stage, the I_R values showed by CO_2 group were higher than LC tench, even if significant differences emerged only at some hours. Nevertheless, struggling movements, erratic swimming and tentative to escape were recorded for CO_2 tench in this trial suggesting, as already reported by several authors (Håstein et al., 2005; Ashley, 2007; Poli, 2009), that slaughtering should not be performed with CO_2 for a question of welfare. Moreover, in this trial, RM resolution started before in CO_2 if compared to LC group.

Physical parameters

It has been reported that any kind of pre-mortem stress causes high glycogen depletion, leading to an increase of lactic acid in fish muscles and that pH is affected by pre-slaughter activity (Kiessling et al., 2004).

Rahamanifarah et al. (2011) reported no significant differences in pH value at 24h *post mortem* between CO_2 and hypothermia groups of carps while statistic differences were recorded at 72 hours of storage

In this trial, pH at 24 hours was not affected by stunning methodology even if PS group reached the lowest (but not significantly different) pH (6.02). Wedekind et al. (2003) reported pH values 24 hours after death ranging from 6.40 to 6.52 for different genetic strains of tench all stunned by a blow on head. These pH values were considered as indicators of high lactate level in tissues. Our results that were even lower (from 6.02 to 6.09) seemed to indicate a good nutritive status of tench before harvest, good condition of storage and no stressing handling before stunning. The lowest value of pH reached by tench slaughtered by PS would indicate that this method enable a minor depletion of stored reserves (glycogen).

Acerete et al. (2009) reported no differences of pH value at 24h *post mortem* between CO_2 and chilled water asphyxiate groups, while differences emerged comparing these treatments with air asphyxia group used as reference.

Duran et al. (2008) found the lowest pH value after 24 hours for carp and trout slaughtered by asphyxiation or percussion. Moreover, for carp the pH of the percussion group at 24 hours post death was lower than for asphyxiated fish. This was justified by a faster and early depletion of glycogen in the asphyxiate group because of the higher muscle activity pre mortem.

No differences emerged between groups considering colour parameters. Nevertheless, observing the table 1, it can be noticed that s.d. values resulted very high showing a dramatically variability between fillets on the same treatment that could have masked the effects of the slaughtering procedures.

Rahamanifarah et al. (2011) found that fillet colour was affected by pre-mortem stress, with carps of hyperthermia group having lower Hue value than CO_2 group. Moreover, they stated that biochemical effects of hypercapnea on carp blood and flesh could have caused paler fillets in CO_2 group even if further investigations were recommended.

Contrary to these Authors, in this research the lower Hue value is reported by CO_2 group and the highest by LC tench.

Robb et al. (2000) reported that in fish hyperactivity at slaughter (stress) causes the flesh to be significantly lighter, less red and more yellow. Fillets of tench stunned by LC resulted more yellow (b*) (no statistical differences) than other groups. Our results for L* values are slightly higher that what reported by Wedekind et al. (2003), while in a general way our tench resulted more red (a*) and yellow (b*) than the strains tested by these authors.

Cooking losses ranged between 15.39 (CO_2) and 17.55% (PS). Similar values were reported by Wedekind et al. (2003). In our trial, the cooking losses were not significantly different among treatments even if ES and PS groups reported the higher values of this important technological parameter.

Duran et al. (2008) comparing asphyxia and PS methods in carps found that fillet texture measures were significantly different between slaughter methods, in agreement with Roth et al. (2002) results on salmon. Differences were attributed to pre-mortem muscle activity: the more the activity, the more the muscle damage.

Low ultimate pH is reported to influence texture (Ginés et al., 2002) but no differences were found in this trial for muscle pH of tench at 24 hours post mortem suggesting that these methods have no major effect on muscle structure in terms of texture analysis. These results agreed with Huidobro et al. (2001) and with Matos et al. (2010) but did not agree with Urbieta and Ginés (2000) on sea bream or with studies on other species (Kiessling et al., 2004; Bahuaud et al., 2010).

As regards the texture, even though the values were not significantly different among the groups, the trend observed (PS>ES>CO_2>LC) was in agreement with the findings obtained in rainbow trout by Færgemand et al. (1995) and Ambroggi et al. (1996), that found higher texture attributes in fish slaughtered with PS in comparison with ES and asphyxiation, probably as a consequence of lesser handling stress and muscle activity in fish of the first group.

Very few information is available on the value of free water (FW) and drip losses (DL) on tench. Wedekind et al. (2003) found values different from those of this trial, probably due to different weight of fish analysed (200-300 g) and to the methodologies utilised for the analysis, not specified in that paper.

Both DL and FW are parameters largely affecting the commercial value and quality perception by the consumer, since they have an important role for the fish texture. Their behaviour is closely related to pH value of flesh (Fennema, 1990). Even though no significant differences were observed for those parameters, the results highlight a clear behaviour. Except for ES group, an inverse relationship could be deduced between the two parameters, since the PS group showed lower DL and higher FW (corresponding to a lower WHC) and vice versa, as the behaviour of LC also highlighted. ES group was characterized by reduced loss of water from muscle, when measured in terms both of DL and FW, probably as a consequence of the higher value registered for muscle pH, even though not significantly different from those of the other groups.

On turbot killed by percussion, electricity or bleeding, Morzel et al. (2003) found higher values of WHC in fish killed by percussion than in those slaughtered by electricity, whereas on sea bream Tejada and Huidibro (2002) did not find a clear influence of killing method on that parameter and on cooking drip.

In conclusion, our data show that different stunning procedures affect rigor onset and resolution in tench without significantly affecting quality parameters tested at 24 h post mortem.

Because of the long time needed to die, immersion in CO_2 saturated water as well as live chilling do not meet the criteria for humane slaughter. Concerning ES method, tench were immediately killed by the procedure. However, the exposure to electricity seemed to be either too long or too high and burns appeared on skin and blood spots on fillet. Percussive stunning method seems to be the more appropriate methodology as it induced a slower onset of RM, good I_R values and a later RM resolution. This procedure is considered as humane but is highly dependent on the adequate training and working condition of slaughterers that perform the operation. It is usually indicated for large fish as it is considered as too expensive and time consuming for small-sized species. Nevertheless, considering the high value of tench in specific area, this method could be recommended. A combination of various methods together could also be suggested.

This represent the first study on tench and, considering the increasing awareness of consumers about ethic and welfare aspects of aquaculture productions and the high impact of these conditions on quality parameters, further investigations are needed in order to define protocols for humane and less stressful slaughter practice for this species.

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Figure 1. Rigor index (I_R) evolution during 30 h of tench treated with different slaughter methods: live chilling (LC), CO_2, percussive stunning (PS) and electrical stunning (ES). Values reported for each sampling point (hours) are the means of the measurements recorded on five fish for each stunning procedure.

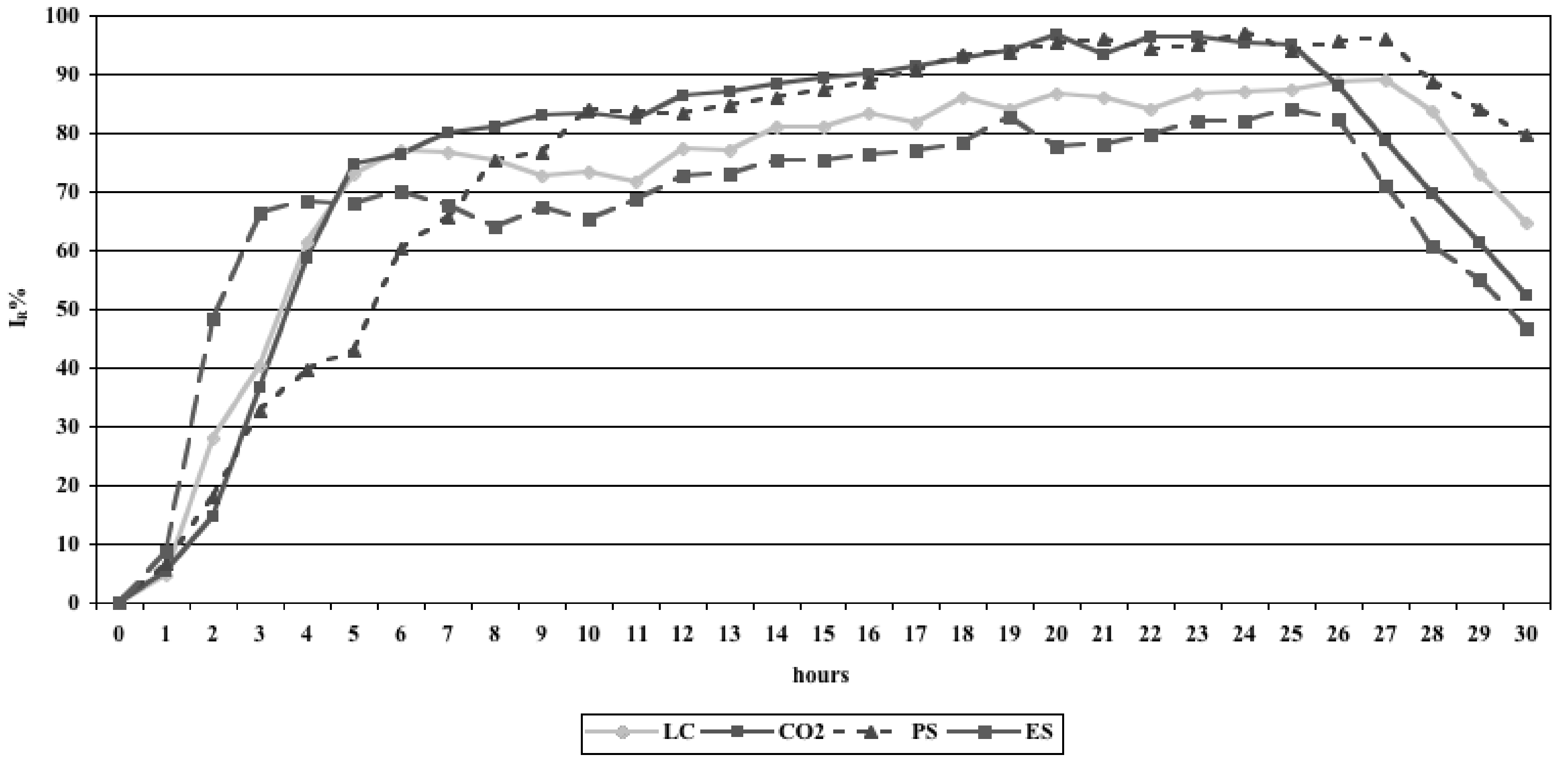

Table 1. Physical traits at 24 hours post mortem of fillets of tench treated with different slaughter methods: live chilling (LC), CO_2, percussive stunning (PS) and electrical stunning (ES) (mean ± s.d., n=5).

	LC			CO_2			PS			ES		
pH	6.07	±	0.14	6.08	±	0.13	6.02	±	0.11	6.09	±	0.10
Colour												
L	49.80	±	2.09	48.25	±	2.88	51.37	±	3.24	49.97	±	4.49
a*	0.39	±	1.36	0.46	±	1.37	-0.46	±	1.00	0.00	±	2.14
b*	0.16	±	1.07	-0.47	±	1.31	-0.59	±	1.02	-0.57	±	1.61
Hue	32.18	±	36.79	8.82	±	57.70	18.45	±	40.73	16.78	±	52.05
Chroma	1.52	±	0.75	1.78	±	0.71	1.27	±	0.92	2.26	±	1.34
Cooking loss (%)	15.83	±	2.26	15.39	±	1.16	17.55	±	2.69	17.52	±	2.47
Texture (N)	8.59	±	0.88	8.63	±	3.12	10.46	±	2.14	9.18	±	1.38
FW (cm^2)	10.96	±	2.98	11.68	±	0.52	12.44	±	1.85	10.53	±	2.01
Drip loss (%)	5.23	±	3.00	4.63	±	1.09	3.09	±	1.23	3.02	±	0.80