Optimisation of slaughtering method in gilthead sea bream (Sparus aurata). Industrial application in fish farms

Urbieta F.J., Ginés R.

Optimisation of slaughtering method in gilthead sea bream (*Sparus aurata*). Industrial application in fish farms

F.J. Urbieta* and R. Ginés**

*Centro Oceanografico de Vigo, Instituto Español de Oceanografia (IEO)
Apartado 1552, Cabo Estay-Canido, 36280 Vigo (Pontevedra), Spain
**Departamento de Producción Animal, Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria, Trasmontaña s/n, 35416 Arucas (Las Palmas), Spain

SUMMARY – It is well established that the slaughtering method affects the quality of cultured fish. Therefore the handling and post-harvesting procedures should be modified to develop products of the highest quality. Actually there are different methods: asphyxiation, percussive stunning, CO_2 narcotization, electrocution and ice with water, but in some cases their application in terms of operation and cost are limited. In this paper "liquid ice" is proposed, compared with "conventional ice", as an effective method to improve freshness for long time. Body and ice temperature, flesh texture (puncture and compression), freshness (K-value and degradation products of ATP) and external colour (L*, C*$_{ab}$ and H°$_{ab}$) was evaluated in 216 fish slaughtered in the ADSA fish farm (Canary Island, Spain). The analysis of texture was performed using an INSTRON 4465 Texturometer. High performance liquid chromatography (HPLC) was applied for identification and quantitative determination of nucleotides and K-value. External colour parameters were performed with a Minolta Chromameter CR-200. Economical and technical aspects of this technology are also described. Results showed higher values of puncture ($P<0.01$) and compression ($P<0.05$) with liquid ice at 2 and 7 days after slaughtering. K-value increased faster in conventional ice at 2 and 15 days *postmortem* ($P<0.01$). The body temperature decreased faster in liquid ice than conventional ice ($P<0.05$). Statistical differences in external colour were not found. In conclusion, the fish slaughtered with liquid ice showed better texture and freshness characteristics. The method is effective and faster than conventional ice and adaptable to the fish factory necessities.

Key words: Liquid ice, slaughtering, freshness, texture, K-value, colour.

RESUME – *"Optimisation des méthodes d'abattage pour la daurade royale (*Sparus aurata*). Application industrielle dans les exploitations aquacoles". Il a été bien établi que la méthode d'abattage affecte la qualité des poissons élevés. La manipulation et les procédures post-capture devraient être modifiées pour développer des produits de la plus haute qualité. Actuellement il y a différentes méthodes : asphyxie, étourdissement par percussion, CO_2, narcose, électrocution et glace liquide, mais dans certains cas leur application est limitée en termes d'opération et de coût. Cet article propose la méthode de la "glace liquide", comparée à la "glace conventionnelle", comme méthode efficace pour améliorer la fraîcheur pendant un temps long. La température corporelle et celle de la glace, la texture de la chair (poinçonnement et compression), la fraîcheur (valeur K et produits de dégradation de l'ATP) et couleur externe (L*, C*$_{ab}$ et H°$_{ab}$) ont été évalués chez 216 poissons abattus dans la ferme piscicole ADSA (Iles Canaries, Espagne). L'analyse de la texture a été effectuée en utilisant un Texturomètre INSTRON 4465. La chromatographie liquide à haute performance (HPLC) a été appliquée pour l'identification et la détermination quantitative des nucléotides et la valeur K. Les paramètres de couleur externe ont été examinés avec un Chromatomètre Minolta CR-200. Les aspects techniques et économiques de cette technologie sont également décrits. Les résultats ont montré une valeur supérieure du poinçonnement ($P<0,01$) et de la compression ($P<0,05$) avec la glace liquide à 2 et 7 jours après l'abattage. La valeur K a augmenté plus vite pour la glace conventionnelle à 2 et 15 jours post-mortem ($P<0,01$). La température corporelle a baissé plus vite dans la glace liquide que dans la glace conventionnelle ($P<0,05$). On n'a pas trouvé de différences statistiques dans la couleur externe. En conclusion, les poissons abattus avec la glace liquide ont montré une meilleure texture et de meilleures caractéristiques de fraîcheur. La méthode est efficace et plus rapide que la glace conventionnelle et adaptable aux besoins des usines de poissons.*

Mots-clés : *Glace liquide, abattage, fraîcheur, texture, valeur K, couleur.*

Introduction

The total production of gilthead sea bream (*Sparus aurata*) in Spanish coasts was 6330 tonnes in 1998. This is an increase of 34.5% since 1996. Thus, it is obvious that the quality assurance in the next years will be one of most important factors in the marketing of aquaculture products. In this

context, the slaughtering method should be considered as a traumatic procedure and may cause considerable physiological reactions in fish like, increased muscle activity, mobilization of energy stores in muscle and liver and changes in acid-base balance (Izquierdo-Pulido *et al.*, 1992; Lowe *et al.*, 1993; Sigholt *et al.*, 1997; Erikson *et al.*, 1997).

The influence of *antemortem* procedures on texture, has been described by different authors (Izquierdo-Pulido *et al.*, 1992; Orban *et al.*, 1996; Ambroggi *et al.*, 1996; Sigholt *et al.*, 1997). This is one of the most common attributes to evaluate the meat quality. A multiple regression analysis shows that the method of slaughtering explains 9% of texture changes (Færgemand *et al.*, 1995). The K-value or "freshness" index gives a relative freshness rating based primarily on the autolytic changes which take place during *postmortem* storage of the muscle. The effect of stress imposed by handling protocols in *Acipenser transmontanus* (Izquierdo-Pulido *et al.*, 1992), *Pagrus auratus* (Lowe *et al.*, 1993) and *Salmo salar* (Erikson *et al.*, 1997) resulted in higher K-values. In several studies where freshness conditions are evaluated, the fish were killed by percussive stunning or "iki jime" showing the highest quality index. However, the application of this method in fish farms is reduced. The external colour is also very important in the fish appearance. Some authors propose the application of instrumental methods like chromameter, as a rapid and accurate technique (Eifert *et al.*, 1992).

The objective of this study was to reduce post-harvest metabolism or physical activity during slaughter of gilthead sea bream. Meat texture, K-value and external colour were used to evaluate effects of handling stress on meat quality. Another objective was to show the feasibility of liquid ice in fish farms.

Materials and methods

Slaughter method

All fish were cultured and slaughtered in ADSA fish farm (Canary Islands, Spain). The sea breams were harvested and placed in slaughter tanks (350-400 kg in 600 l) with conventional ice "flake ice" (2/3 of total volume) and liquid ice (half tank). Salt water was also introduced at 1/4 of total volume in both groups. The temperature range was –0.5 to –1.0°C (conventional ice) and –2.3 to –2.8°C (liquid ice). A total of 216 gilthead sea bream (*Sparus aurata*) were used, with a mean weight of 451.17±4.52 g (commercial size). Both groups were packed (12 fish/box) in styrofoam boxes with ice and stored at 0°C. The liquid ice generator was a BRONTEC model B-105, with a total production capacity of 5 Tm/day and nominal cost of 12 KW. The dimensions are 100x80x190 cm (lengthxwidthxheight) and 500 kg (total weight) (Fig. 1).
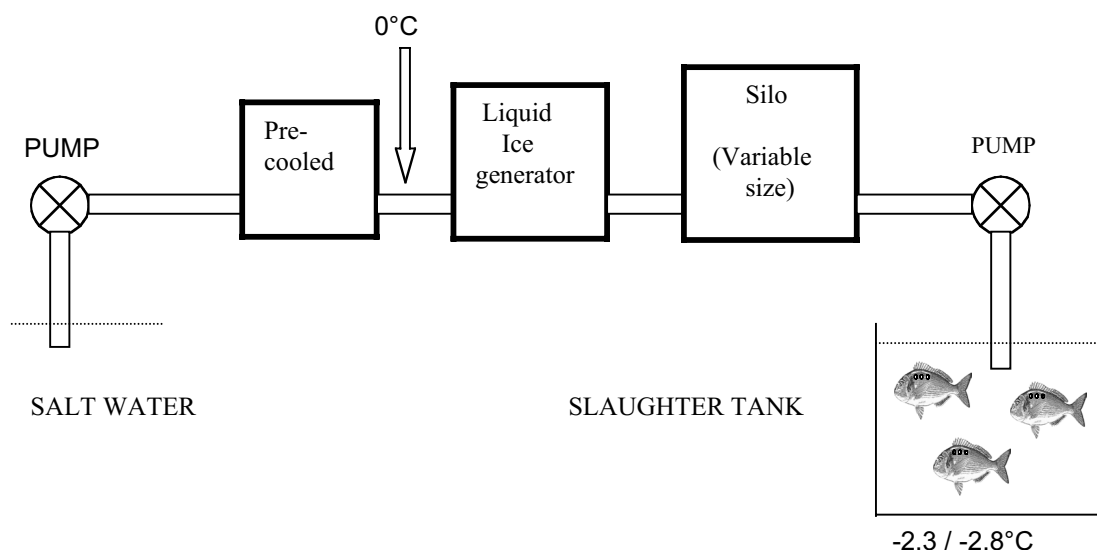

Fig. 1.   Liquid ice installation in fish farm.

Temperature recording

Body and ice temperature during harvest procedures (60 min) was measured randomly (10 fish) every 5 min with CRISON digital thermometer. The body temperature measurements was performed in fish anus.

Texture

Measurements of the force (Newtons) of puncture and compression was done with an INSTRON model 4465 Texturometer in raw fillets of anterior dorsal muscle. Puncture test was performed on circular pieces (diameter, 5.3 cm; height, 1.2 cm) with a cylinder piston (diameter, 0.8 cm) and a constant crosshead speed of 80 mm/min (Borderias *et al.*, 1983; Orban *et al.*, 1996). Compression test was defined as the force exerted to deform a cylinder fillet (diameter, 2.6 cm; height, 1.2 cm) at 30% of its height. The crosshead spead was 50 mm/min (Borderias *et al.*, 1983; Chamberlain *et al.*, 1993; Orban *et al.*, 1996).

Colour

External colour parameters were measured above the lateral line, behind the head, using a Minolta Chromameter model CR-200. After flashing, L*, a* and b* reflected light values were recorded. From a* and b* values the $H°_{ab} = tan^{-1} b*/a*$ and $C*_{ab} = (a*^2 + b*^2)^{1/2}$ were calculated according to Wyszecki and Stiles (1982). These three colour attributes: L* (lightness), $H°_{ab}$ (hue angle) and $C*_{ab}$ (chroma) are represented by a set of cylindrical coordinates (3D-space).

Determination of muscle nucleotides and K-value

K-value and ATP degradation products (nucleotides) were also measured on the anterior dorsal muscle of 20 fish for different slaughtering methods. For preparing the muscle samples, 0.25 g was dissected and homogenized in 1.5 ml of 0.6 M perchloric acid at 0°C. The homogenate was centrifuged at 1000 x g for 5 min and the supernatant neutralized to pH 6.5-7 with 0.5 M KOH. The extract obtained was passed through a 0.45 m filter and 1 ml was stored at –20°C until HPLC analysis. Concentrations (g/g wet muscle) of ATP and its degradation products were estimated by method of Ryder (1985). A Supelcosil LC-18-T (250X4.6 mm) column was used with an isocratic mobile phase of 0.04 M KH_2PO_4 and 0.06 M K_2HPO_2 with a flow rate of 1 ml/min. The K-value was calculated by substituting these concentrations in the equation proposed by Saito *et al.* (1959).

$$K\text{-value (\%)} = \frac{HxR + Hx}{ATP + ADP + AMP + IMP + HxR + Hx} \times 100$$

where:

ATP: adenosine 5´-triphosphate　　　　IMP: inosine 5´-monophosphate
ADP: adenosine 5´-diphosphate　　　　HxR: inosine
AMP: adenosine 5´-monophosphate　　　Hx: hypoxanthine

Statistical analysis

Analysis of variance (ANOVA) was performed with the factorial model, $Y_{ij} = \ + F_i + \ X_{ij} + \ _{ij}$, where is the overall mean, F_i is the fixed effect of slaughtering method, X_{ij} is the random effect of interaction between the variable *i* and the fish weigh *j* and $_{ij}$ is the residual error. Means were compared using Sheffé test.

Results and discussion

The body temperatures in fish slaughtered by liquid ice were significantly lower (P<0.05) than conventional ice during harvesting and transport to the packing area (1 h). The temperature declined from 20.5°C, when fish were placed in the slaughter tank, to 0.27°C in liquid ice and 2.02°C in conventional ice in 60 min. But never got the freezing point that usually is between –0.8 and –1.5°C for most marine species and depending of body salts concentration (Sikorski, 1990). Thus, the liquid ice keeps lower temperatures for long periods so the fish dies rapidly and minimize muscle activity. This method also gets the optimum packing temperatures (1-2°C) before than conventional ice (Fig. 2).
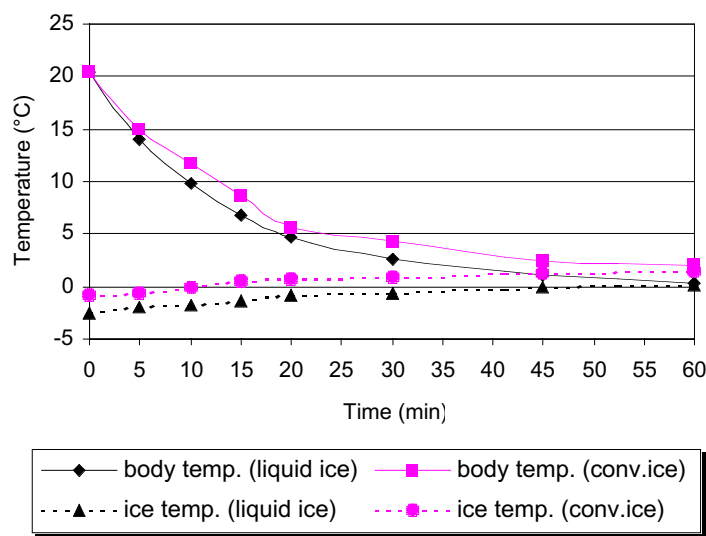

Fig. 2.   Body and ice temperature for different slaughtering methods.

The differences between treatments were significant for puncture (P<0.01) at 2 and 7 days. This parameter was applied in order to obtain the integrity of collagen and muscle myomeres. The compression test had also significant differences (P<0.05) at 2 days and (P<0.01) at 7 days, and evaluated the muscle firmness, cohesiveness and elasticity. All storage conditions (time, temperature, boxes and ice) were the same for the different methods. The results show higher firmness and integrity in fish slaughtered by liquid ice (Table 1).

Structural links between muscle cells and connective tissues provide the integrity that enables the flesh to withstand effects of post-harvest handling, processing and storage. *Postmortem* changes occur in the muscle cells in the elements of the cytoskeleton, in the interactions between proteins in the cells and in the myotendinous junction (Bremmer, 1992). Softening in fish flesh is due to changes in fine connections that anchor the three-dimensional structure of the tissue. Such changes are due to enzymes working at high activity in the myotendinous junctions (Bremmer and Hallett, 1989).

Some authors like Færgemand *et al.* (1995) and Ambroggi *et al.* (1996) proved that immediate killing with percussive stunning show higher texture attributes than electrocution and asphyxiation in rainbow trout (*Oncorhynchus mykiss*). This results should bee associated with the handling stress and muscle activity in the slaughter tank.

Relationship between body weight and texture were no significant, oppositely to Hatae *et al.* (1990) that suggests by comparison between species that the size of fibres and the characteristics of collagen partly explain the texture: fish with rather small fibres are more firm or tough than fish with large fibres. If such a concept is applied to the age/body weight effect, it could be proposed that the texture of large fish would be softer than that of small fish. The increasing amount of lipid and collagen more susceptible to gelification also participates in softening of the flesh of large fish

(Fauconneau *et al.*, 1995). The short weight range presented in this work could be the explanation of no significant correlation.

There was no effect of different methods on external colour parameters (L*, C*$_{ab}$ and H°$_{ab}$) (Table 1). The same results were found by Sigholt *et al.* (1997) in stressed and unstressed Atlantic salmon (*Salmo salar*) before slaughtered.

Table 1. Instrumental texture analysis (Newtons) and colour
parameters for different slaughtering methods[†]

		Liquid ice	Conventional ice
2 days	Puncture	6.83±0.19	5.85±0.20
	Compression	18.48±0.64	16.17±0.92
	L*	52.21±0.49	50.60±0.81
	C*$_{ab}$	1.64±0.12	1.58±0.16
	H°$_{ab}$	203.90±7.77	175.00±9.60
7 days	Puncture	5.65±0.20	4.83±0.16
	Compression	14.19±0.56	11.87±0.69
	L*	51.66±0.64	51.44±0.73
	C*$_{ab}$	2.17±0.18	1.87±0.18
	H°$_{ab}$	215.60±7.60	199.6±9.54

[†]Values are mean ± SE

Significant differences: (P<0.05); (P<0.01).

There was a clear effect of handling stress in K-value and nucleotides concentrations at 2 days (P<0.01) and 15 days (P<0.001) *postmortem* (Table 2). Comparison of K-value for different slaughtering methods, proves the effect of stress in fish freshness. The values were respectively 0% for liquid ice and 4.03% for conventional ice at 2 days, and 7.65% for liquid ice and 22.42% for conventional ice at 15 days.

The levels of more degraded products, inosine (HxR) and hypoxanthine (Hx) were higher in fish slaughtered by conventional ice. The average Hx concentrations in the muscle from conventional ice fish increased from 0 g/g to 641.7 g/g during 1he 15 days of storage, while in liquid ice remained constant

The low concentrations of ATP, ADP and AMP at 2 days, show their rapid degradation by autolytic enzymes. Ryder (1985) and Erikson *et al.* (1997) also found negligible concentrations of these nucleotides at 2 days that implies accumulation of IMP. Thus, Karube *et al.* (1984) proposed the K$_i$ index as fish quality indicator: K$_i$ = HxR + Hx / IMP + HxR + Hx.

K-value differences between species has been reported by Uchiyama *et al.* (1978), Sakaguchi and Koike (1992), Lowe *et al.* (1993) and Erikson *et al.* (1997). At 2 days *postmortem*, the values were nearly 20% for salmonids, 10% for yellow tail (*Seriola quinqueradiata*) and sardine (*Sardinops melanostictus*), 4-5% for snapper (*Pagrus auratus*) and 4% for red sea bream (*Pagrus major*). This results and that obtained in the present work, show less degradation for nucleotides in sparids than other marine fish. It seems reasonable to propose that this species keep freshness for long time.

Conclusions

(i) Body temperature of fish decrease faster during slaughtering procedures with liquid ice.
(ii) The fish slaughtered by liquid ice dies faster and present less stress than conventional ice.
(iii) The liquid ice fillets are firmer and have better texture attributes than conventional ice.

(iv) Fish freshness also improvement with liquid ice.

(v) The use of salt water and its feasibility are their best advantages to other traditional methods like conventional ice.

Table 2. ATP degradation products in g/g wet weight and K-value in (%) for different slaughtering methods[†]

		Liquid ice	Conventional ice
2 days	ATP	29.6±5.6	38.7±7.1
	ADP	30.9±7.3	46.1±8.9
	AMP	38.4±8.2	50.4±8.1
	IMP	1307.3±314.7	1809.8±283.0
	HxR	-	60.8±24.9
	Hx	-	-
	K-value[††]	0	4.03
15 days	ATP	17.6±2.8	68.7±12.4
	ADP	17.1±4.0	59.2±10.7
	AMP	18.8±3.7	84.9±15.0
	IMP	539.2±193.3	2457.2±426.7
	HxR	31.3±12.2	79.2±17.4
	Hx	-	641.7±112.9
	K-value[††]	7.65	22.42

[†]Values are mean ± SD

[††]K-value = HxR + Hx / ATP + ADP + AMP + IMP + HxR + Hx X 100

Significant differences: (P<0.01);   (P<0.001).

References

Ambroggi, F., Sebastio, P., Pirazzoli, P. And Baldrati, G. (1996). Influenza del sistema di sacrificazione su trote iridee (*Oncorhynchus mykiss*) di allevamento, II – Rigor mortis e consistenza del tessuto muscolare. *Industria Conserve*, 71: 157-161.

Borderias, A.J., Lamua, M. and Tejada, M. (1983). Texture analysis of fish fillets and minced fish by both sensory and instrumental methods. *Journal of Food Technology*, 18: 85-95.

Bremner, H.A. (1992). Fish flesh structure and the role of collagen – its *post-mortem* aspects and implications for fish processing. In: H.H. Huss *et al* (Eds). *Quality Assurance in the Fish Industry*. Elsevier Science Publishers B.V., pp. 39-62.

Bremner, H.A. and Hallet, I.C. (1989). Fish microstructure. In: L.W. Sze and F.C. Woo (Eds). *Trends in Food Science*. Institute of Food Science and Technology, Singapore, pp. 93-98.

Chamberlain, A.I., Kow, F. and Balasubramaniam, E. (1993). Instrumental method for measuring texture of fish. *Food Australia,* 45 (9): 439-443.

Eifert, J.D., Hackney, C.R., Libey, G.S. and Flick, G.J. (1992). Aquacultured hybrid striped bass fillet quality resulting from post-harvest cooling or CO_2 treatments. *Journal of Food Science*, 57 (5): 1099-1102.

Erikson, U., Beyer, A.R. and Sigholt, T. (1997). Muscle high-energy phosphates and stress affect K-values during ice storage of atlantic salmon (*Salmo salar*). *Journal of Food Science*, 62 (1): 43-47.

Færgemand, J., Ronsholdt, B., Alsted, N. and Borresen, T. (1995). Fillet texture of rainbow trout as affected by feeding strategy, slaughtering procedure and storage *post-mortem. Wat. Sci. Tech.*, 31 (10): 225-231.

Fauconneau, B., Alami-Durante, H., Laroche, M, Marcel, J. and Vallot, D. (1995). Growth and meat quality relations in carp. *Aquaculture*, 129: 265-297.

Hatae, K., Yoshimatsu, F. and Matsumoto, J.J. (1990). Role of muscle fibers in contributing firmness of cooked fish. *Journal of Food Science*, 55 (3): 693-696.

Izquierdo-Pulido, M.L., Hatae, K. and Haard, N.F. (1992). Nucleotide catabolism and changes in texture indices during ice storage of cultured sturgeon (*Acipenser transmontanus*). *Journal of Biochemistry*, 16: 173-192.

Karube, I., Matsuoka, H., Suzuki, S., Watanabe, E. and Toyama, K. (1984). Determination of fish freshness with an enzyme sensor system. *J. Agric. Food Chem*., 32: 314-319.

Lowe, T.E., Ryder, J.M., Carragher, J.F. and Wells, R.M.G. (1993). Flesh quality in snapper (*Pagrus auratus*), affected by capture stress. *Journal of Food Science*, 58 (4): 770-774.

Orban, E., Sinesio, F., Paoletti, F., Nicoli, S., Casini, Y. and Caproni, R. (1996). Caratteristiche nutrizionali de organolettiche di orate (*Sparus aurata*) da acquacoltura: un esempio di come le differenti tecniche di allevamento possono influenzare la qualitá del pesce. *La Rivista di Scienza dell´Alimentazione*, 25 (1): 27-36.

Ryder, J.M. (1985). Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. *J. Agric. Food Chem.,* 33: 678-680.

Saito, T., Arai, K. and Matsuyoshi, M. (1959). A new method for estimating the freshness of fish. Bull. *Japan Soc. Sci. Fish*, 24: 749-751.

Sakaguchi, M. and Koike, A. (1992). Freshness assessment of fish fillets using the Torrymeter and K-value. In: H.H. Huss *et al* (Eds). *Quality Assurance in the Fish Industry*. Elsevier Science Publishers B.V., pp. 333-338.

Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordvedt, T.S., and Seland, A. (1997). Handling stress and storage temperature affect meat quality of farmed-raised atlantic salmon (*Salmo salar*). *Journal of Food Science*, 62 (4), 898-905.

Sikorski, Z.E. (1990). *Seafood: Resources, Nutritional Composition and Preservation*. CRC Press, Inc., Boca Raton, Florida.

Uchiyama, H., Ehira, S., Uchiyama, T. and Masuzawa, M. (1978). Partial Freezing as a Means of Keeping Freshness of Cultured Rainbow Trout. *Bull. Tokai Reg. Fish Lab*., 95: 1-11.

Wyszecki, G. and Stiles, W.S. (1982). *Color science: Concepts and Methods, Quantitative Data and Formulae*. Wiley-Interscience Publication, New York.