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Chemical and microbiological quality of grass carp (*Ctenopharyngodon idella*) slaughtered by different methods

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Abstract

The effect of two slaughter methods (immersion in ice-water slurry and electrical stunning followed by ice slurry asphyxiation) on chemical and microbiological parameters of grass carp (*Ctenopharyngodon idella*) stored in ice for 20 days was evaluated. No differences in total volatile basic nitrogen (TVB-N), pH, carbohydrate or protein content of mucus were observed between the slaughter methods. Ice-slaughtered fish had lower bacteria counts at the beginning of storage, but higher counts than fish slaughtered by electricity at the end of storage ($p < 0.05$). However, no significant differences in the shelf life were observed between the slaughter methods evaluated (limit of acceptability – counts $> 3 \times 10^6$ CFU g^{-1} – attained after 13–16 days). Results indicated that the chemical parameters evaluated have a limited applicability to assess the shelf life of grass carp stored in ice, since pH limit (6.8) was exceeded after 4 days, while TVB-N limit (30 mg%) was not attained after 20 days of storage.

Keywords: Fish; *Ctenopharyngodon idella*; Slaughter; Shelf life; Freshness; Bacterial count

1. Introduction

Fresh fish is a highly perishable product (Ashie, Smith, & Simpson, 1996; Gram & Huss, 1996). The large number of bacteria found on mucus, gills, and gut of fish (González, López-Dias, García-López, Prieto, & Otero, 1999) makes microbial contamination the most important concern in fish processing, especially during refrigerated storage (Gram & Huss, 1996). In addition to microbiological assays, a variety of chemical and physical methods have been used to assess fish shelf life. These include pH (González-Rodriguez, Sanz, Santos, Otero, & Garcia-Lopez, 2001), total volatile basic nitrogen (TVB-N; Lakshmanan, Antony, & Gopakumar, 1996), trimethylamine (TMA; Gill, 1990), *K* value (Alasalvar, Taylor, Öksüz, Shahidi, & Alexis, 2002), and sensory (Özogul, Taylor, Quantick, & Özogul, 2000) analysis. Recently, Montagner et al. (2003) showed that carbohydrate and protein content of the fish surface mucus could be an alternative method to assess spoilage, since these parameters were highly correlated with microbiological count in refrigerated white croaker (*Micropogonias furnieri*).

Given the perishable nature of fish, researchers have been constantly searching for improved methods to preserve or extend their shelf life (Chang, Chang, Shiau, & Pan, 1998). Electric fields and currents have been used to disinfect drinking water and reduce the numbers of

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bacteria and yeast in foods (Castro, Barbosa-Canovas, & Swanson, 1993; Marquez, Mittal, & Griffiths, 1997; Patermarakis & Fountoukidis, 1990). These processes were used mainly in liquid foods (Jin & Zhang, 1999) and induce cytoplasm and cell membrane damage of microorganisms (Calderon-Miranda, Barbosa-Canovas, & Swanson, 1999; Detreux et al., 2000). Some studies indicated that post mortem electrical stimulation helps to reduce total viable counts and increase shelf life of red meat, poultry and fish under chilling conditions (Bawcom, Thompson, Miller, & Ramsey, 1995; Hata, 2000; Slavick, Griffis, Li, & Engler, 1991). However, in a recent study, post mortem treatment of white croaker with direct electric current did not reduce microbial count of flesh, but reduced total count of mesophilic bacteria in the water of treatment (Montagner et al., 2005).

Grass carp (*Ctenopharyngodon idella*) is one of the main freshwater fish species farmed in Southern Brazil. The increase in the intensive production of grass carp has raised concerns over the quality of the cultured products (Rangel, 1995). Therefore, investigations on the freshness quality during handling, distribution and storage in ice are of considerable interest (Scherer et al., 2004).

At present, most farmed grass carp in Southern Brazil are slaughtered either by immersion in ice-water slurry or asphyxia in ice. These methods do not induce immediate loss of brain function and therefore are not considered humane (Robb & Kestin, 2002; van de Vis et al., 2003). One of the reasons cited by producers for using these methods is that rapid chilling promotes flesh quality by reducing both autolytic degradation and muscular activity immediately before death (Robb & Kestin, 2002). Electricity has been used in the stunning or slaughtering of fish to reduce the stress and pain during fish slaughter (Robb & Kestin, 2002).

Despite previous evidence that electric fields and currents may reduce microbiological counts in some foods, there are no reports concerning the effects of the electrical stunning on microbiological parameters of fish flesh. Besides, there are no reports on the effects of electrical stunning on chemical and microbiological parameters of grass carp. Thus, the present study was aimed at comparing the effects of a conventional slaughter method (immersion in ice-water slurry) with those of a humane slaughtering (electrical stunning followed by ice slurry asphyxiation) on chemical and microbiological parameters of grass carp (*C. idella*) stored in ice.

2. Materials and methods

2.1. Fish and handling

Grass carps (*C. idella*; average weight and length: 822 ± 147 g and 40.8 ± 2.8 cm, respectively) were raised at Department of Zootecnia of Federal University of Santa Maria, in an earth pond (800 m^2) at a density of approximately 1 fish/3.2 m^2. During the growth period (July 2002–April 2003), chemical and physical quality of water was evaluated each other day, using test kits (Alfa Tecnoquímica, Florianópolis, SC, Brazil). Water alkalinity, pH, nitrite, ammonia, and transparency values remained almost constant (57.6 ± 2.6 mg $CaCO_3$/l, 7.5 ± 0.1, 0.06 ± 0.01 ppm, 0.49 ± 0.02 ppm, and 41.7 ± 7.6 cm, respectively) and within the range required for this species (Chen, Chen, & Ni, 1993). Dissolved oxygen content and temperature ranged between 4.3–9.2 mg/l and 15–29 °C, respectively. Fish were fed twice a day (in the morning and late in the afternoon) at a ratio of 5% of total biomass. The diet consisted of green fodder (*Penissetum americanum* and *Penissetum purpureum*) and a commercial feed (PURINA®, Paulínia, SP, Brazil) yielding 36% protein in the first month, 32% protein for the next 3 months, and then 28% protein up to the harvest. After catching, fish were fasted for 24 h before killing. Fish were randomly selected for one of the slaughter methods: immersion in ice-water slurry or electrical stunning followed by ice slurry asphyxiation. For ice-water slaughtering, fish were dipped in the ice-water slurry (2.4 kg ice : 3.6 l water : 1 kg fish) during 20 min. For electrical slaughtering, fish were dipped in fresh water (7.5 l/kg of fish) containing 2 g/l NaCl and direct current was applied (3 A/200 V for 1 min followed by 3.5 A/220 V for 2 min, DC). Immediately after slaughtering fish from both groups were transferred to polystyrene boxes covered with ice flakes and stored at 1.0 ± 0.5 °C. Boxes were provided with holes for drainage. Chemical, microbiological, and sensory analyses were performed on day 0 (2 h after death) and 1, 2, 4, 7, 10, 13, 16, and 20 days after slaughtering.

2.2. Chemical analysis

Mucus was carefully scraped from dorsal body surface (total area of 50 cm^2) using a cotton-tipped swab. After scraping, the cotton was immersed in 5 ml of distilled water, and the sample was used to determine carbohydrates (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and protein (Lowry, Rosebrough, Farr, & Randall, 1951). Total volatile basic-N (TVB-N) was determined in flesh by the method of Kjeldahl described by Furuichi, Taniguchi, and Murabayashi (1997), except that the protein fraction was separated by homogenizing the fish muscle with 5% trichloroacetic acid at a ratio of 1:2 (w/v) and samples were alkalified by adding MgO (2 g) during distillation. For pH analysis, muscle was homogenized 1:1 in distilled water (w/v) and the measurement was made with a model DMPH-2 Digimed pH-meter at room temperature (Pastoriza & Sampedro, 1994). Moisture, ash, and crude protein ($N \times 6.25$) were

assayed as described by AOAC (1995). Lipid content was determined as described by Folch, Lee, and Sloane-Stanley (1957).

2.3. Microbiological analysis

Enumeration of microorganisms in samples consisted of mesophilic and psychrotrophic plate count assessed as described by Swanson, Busta, Peterson, and Johnson (1992). For all analysis flesh samples (12.5 g of muscle with skin, without scale) were aseptically obtained by cutting slices from the dorsal, ventral and tail area, followed by blending in 0.1% (w/v) peptone for 2 min in a Stomacher. Appropriate serial dilutions were then plated onto plate count agar (Difco, Detroit, MI) by the pour-plate method. Mesophilic and psychrotrophic plate counts were determined by counting the colony forming units (CFU) after plates had been incubated at 35–37 °C for 48 h or 7–10 °C for 10 days, respectively.

2.4. Sensory analysis

On each day of analysis one fish from each slaughter method was assessed by an expert panel ($n = 7$–14). Panelists were asked to state whether the fish were acceptable or unacceptable for consumption. This judgment was used to determine fish shelf life.

2.5. Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA): 2 slaughter methods × 9 days of analysis. If there was a significant main effect or interaction ($p < 0.05$), the calculated mean values were post hoc compared using Duncan's test with significance defined at $p \leqslant 0.05$. The relationships between chemical and microbiological analysis were evaluated by a simple linear correlation. Data were analyzed using the Statistica® 6.0 data analysis software system (Statsoft Inc., 2001).

3. Results and discussion

The proximate analysis of grass carp is shown in Table 1. Fish muscle had a low lipid, intermediate protein, and high moisture content. Values found were similar to previous reports for this species (Bakir, Melton, & Wilson, 1993; Shahid, Sheri, & Raza, 1996).

Muscle pH values of grass carp slaughtered by immersion in ice-water slurry significantly increased on the 4th day of storage, remaining almost constant from this day onwards (data not shown). Fish slaughtered by electricity had a significant increase of pH from the 2nd to the 10th day of storage, reaching a plateau from this day onwards (data not shown). The rise in pH value is probably related to the accumulation of basic substances, such as ammonia and trimethylamine produced by microorganism development in fish (Huss, 1988). No significant differences were observed in pH values between the slaughter methods (data not shown). After 4 days of storage pH of both groups was above 6.8, which is pointed as a legal limit for human consumption of fish in Brazil (Brasil, 1974). However, sensory analysis revealed that the limit for the acceptability of the grass carp stored in ice was around 13–16 days regardless of the slaughter method used (data not shown). Other researchers have also observed that pH value is unsuitable to assess the freshness of some species (Ababouch et al., 1996; Kyrana & Lougovois, 2002).

Table 1
Proximate analysis (%) of grass carp

Composition	Mean ± SEM[a]
Moisture	77.34 ± 0.53
Protein	19.31 ± 0.60
Lipid	1.80 ± 0.11
Ash	1.22 ± 0.10
Carbohydrate[b]	0.33 ± 0.02

[a] $n = 4$.
[b] Calculated by difference [Carbohydrate=100 − (moisture + protein + lipid + ash)].

TVB-N values of carps slaughtered by immersion in ice-water slurry or electricity are shown in Fig. 1. Initial values of TVB-N were low and had no significant increase during the storage in either group. Until the end of the storage TVB-N did not reach the limit of 30 mg/100 g indicated for human consumption in various countries including Brazil (Brasil, 1974; Fig. 1). No significant difference between the slaughter methods was observed. Nevertheless, ANOVA revealed significant differences in TVB-N values with days after slaughter, while post hoc analysis revealed that TVB-N values of fish slaughtered by electricity were significantly lower from the 7th to the 16th days of storage when compared to the day of slaughter (day 0). TVB-N exhibits a great difference between species and seems to be a better index of spoilage for marine fish than for freshwater fish, since

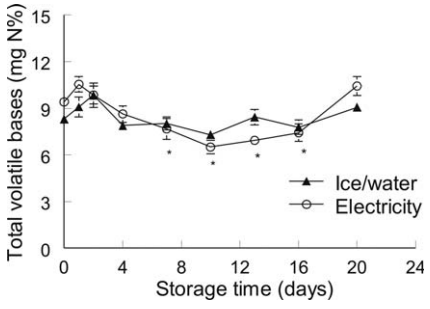

Fig. 1. Effect of the slaughter method on TVB-N levels of grass carp during storage in ice. Results are mean ± SE ($n = 4$). *, Significantly different from day 0 ($p < 0.05$).

freshwater fish have a minor content of trimethylamine oxide. Thus, TVB-N values of pearlspot (*Etroplus suratensis*) and tilapia (*Oreochromis* ssp.) stored in ice did not exceed 30 mg N/100 g after spoilage (Lakshmanan et al., 1996; Tome, Iglesias, Kodaira, & Gonzalez, 2000), while it was considered a good index to assess the spoilage of marine fish, like salted anchovies (Hernández-Herrero, Roig-Sagués, López-Sabater, Rodríguez-Jerez, & Mora-Ventura, 1999), hake (*Merluccius merluccius*, Ruiz-Capillas & Moral, 2001), and Mahi–Mahi (*Coryphaena hippurus*) (Antoine et al., 2002) stored in ice.

The fish body is externally covered by a layer of mucus mainly composed of water and glycoproteins. Montagner et al. (2003) showed that carbohydrate and protein content of the external mucus of white croaker (*M. furnieri*) linearly increased during spoilage. In order to determine if grass carp spoilage induces changes in the external mucus, surface carbohydrate and protein content were evaluated (Fig. 2(a) and (b)). A significant increase in surface carbohydrate content was observed after 4 and 7 days of storage for fish slaughtered by immersion in ice-water slurry and electricity, respectively (Fig. 2(a)). However, after 13 days of storage the carbohydrate content decreased ($p < 0.05$) in both groups. The protein content significantly increased from the 1st and 2nd days of storage onwards, for fish slaughtered by immersion in ice-water slurry and electricity, respectively (Fig. 2(b)). No significant difference in carbohydrate or protein content was observed between the slaughter methods. Changes in carbohydrate and protein content on the fish surface could be related to the microbial development. In fact, besides proteins present in the cell membrane of bacteria, the external capsule or glycocalyx of some bacteria is composed of secreted polysaccharides (Costerton, 1999).

The initial microbiological quality of fish used in this study was good, as indicated by the low initial bacterial counts (Fig. 3(a) and (b)). Wempe and Davidson (1992) reported that mesophilic counts gradually increased from 4.0 to 8.0–9.0 log CFU g^{-1} after 14 days in grass carp stored at 4 and 7 °C. These authors observed that the psychrotrophic bacteria *Pseudomonas* and *Acinetobacter* were the dominant spoilage organisms. In the present study, total count of mesophilic and psychrotrophic bacteria of grass carp significantly increased after the 2nd day of storage for both groups (Fig. 3(a) and (b)). Ice-slaughtered fish had lower bacteria counts in the initial days of storage, but due to a faster growth, in the last day of storage, bacterial counts of these fish were higher than those of fish slaughtered by electricity (significant treatment × days interaction, $p < 0.05$). Fish slaughtered by electricity had significantly higher mesophilic counts than ice-slaughtered fish in the initial period of storage (0, 4, and 7 days), but significantly lower counts in the 20th day of storage. Psychrotrophic bacteria counts of fish slaughtered by electricity showed a

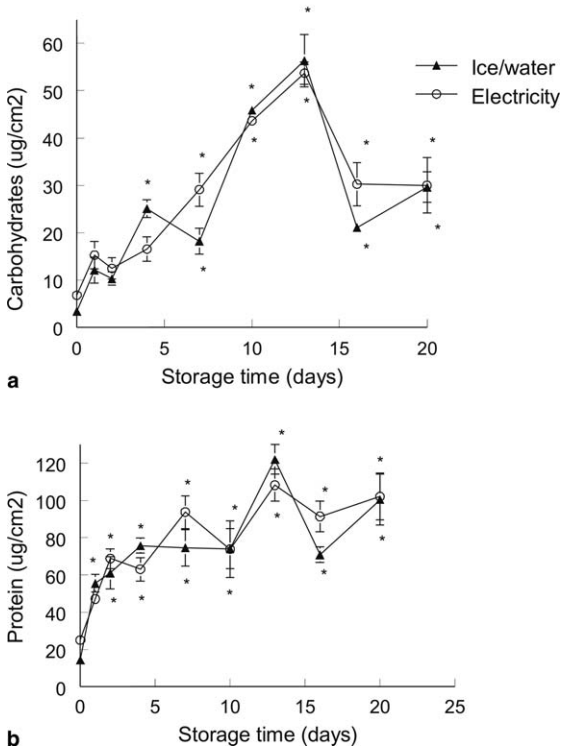

Fig. 2. Effect of the slaughter method on carbohydrate (a) and protein (b) concentration on the surface of grass carp during storage in ice. Results are mean ± SE ($n = 4$). *, Significantly different from day 0 ($p < 0.05$).

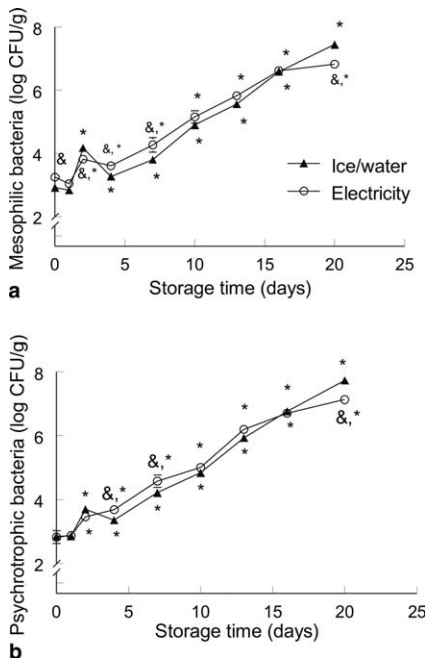

Fig. 3. Effect of the slaughter method on mesophilic (a) and psychrotrophic (b) bacteria count of grass carp during storage in ice. Results are mean ± SE ($n = 4$). *, Significantly different from day 0 ($p < 0.05$). &, Significantly different from ice/water in the same day ($p < 0.05$).

Table 2
Linear correlations (r) among the studied indexes of fish freshness

	TVB-N	CH	Protein	pH	Psychrotrophic count	Mesophilic count
Days of storage	−0.33*	0.64*	0.73*	0.76*	0.97*	0.94*
TVB-N		−0.49*	−0.23	−0.46*	−0.25*	−0.21
CH			0.71*	0.58*	0.56*	0.53*
Protein				0.63*	0.67*	0.62*
pH					0.68*	0.65*
Psychrotrophic count						0.98*

CH, carbohydrates; TVB-N, total volatile basic nitrogen.
* Significant at $p < 0.05$.

similar behavior, being higher than counts of ice-slaughtered fish in the initial period of storage (4 and 7 days), but lower in the 20th day of storage.

For fresh fish, the microbiological limit for human consumption proposed by ICMSF (1986) is 10^7 CFU g^{-1} in aerobic plate count analysis, while other authors recommend 3×10^6 CFU g^{-1} (Chang et al., 1998). Total count of mesophilic and psychrotrophic bacteria exceeded 3×10^6 CFU g^{-1} between the 13th and the 16th day, regardless of the slaughter method (Fig. 3(a) and (b)). Thus, according to the microbiological analysis the shelf life of grass carp stored in ice was around 13–16 days. This result is in agreement with the sensory evaluation of grass carp. The slaughter methods evaluated had no significant influence on the shelf life of grass carp. Hence, in contrast to the reduction of food and water bacterial counts induced by electric fields and currents (Castro et al., 1993; Marquez et al., 1997; Patermarakis & Fountoukidis, 1990), electrical stunning of fish did not affect bacterial counts. Previous studies that compared different slaughter methods found little or no effect on the microbiological quality of fish. Gilthead seabream (*Sparus aurata*) slaughtered in air had a slight delay in the initial bacterial growth when compared to fish slaughtered in ice slurry (Tejada & Huidobro, 2002). However, no significant difference in bacterial count was observed between rainbow trout slaughtered in ice slurry and by percussive stunning (Özogul & Özogul, 2004).

Table 2 shows the correlation coefficients between microbiological and chemical assessments. pH value had a positive correlation with storage time, mesophilic count, psychrotrophic count, carbohydrates, and protein, but a negative and poor correlation with TVB-N ($p < 0.05$, Table 2). Ruiz-Capillas and Moral (2001) found a moderate correlation ($r = 0.80$) between pH and TVB-N in hake stored in ice. TVB-N values showed a negative and poor correlation with storage time, carbohydrates and psychrotrophic count (Table 2). Baixas-Nogueras, Bover-Cid, Veciana-Nogues, and Vidal-Carou (2003) reported that TVB-N had no correlation with the storage time in Mediterranean hake stored in ice. Mesophilic and psychrotrophic bacteria had a highly positive correlation with storage time and moderate positive correlations with carbohydrate and protein content of mucus (Table 2). This finding is in agreement with Montagner et al. (2003) who reported a significant increase in carbohydrate and protein content of surface mucus after 3 days of storage and positive correlations between carbohydrates and total count of mesophilic bacteria in muscle and on the surface of fish.

The chemical parameters evaluated (pH, TVB-N, carbohydrates, and protein content of mucus) had a limited applicability to assess the shelf life of grass carp, since they were poorly correlated with microbiological counts. According to the sensory and microbiological analysis, grass carp stored in ice has a shelf life around 13–16 days. No significant differences were observed in the shelf life of grass carp between the slaughter methods evaluated (immersion in ice plus water and electricity). Hence, electrical stunning may be applied prior to ice-water slaughtering of grass carp. This practice would enable a humane slaughtering with no loss in microbiological or sensory quality.

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