EFFECT OF CARBON MONOXIDE ON WELFARE AND QUALITY IN ATLANTIC SALMON (*Salmo salar* L.)

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Abstract – Carbon monoxide (CO) has been used as a food preservative in the food industry. CO can be used to sedate, anaesthetize or kill animals without aversive reactions. The early application of CO in the slaughtering process seems to be beneficial by giving a more red stable colour, reducing microbial growth and lipid oxidation; this would be preferable for salmon which is highly vulnerable to lipid oxidation due to the high level of unsaturated fatty acids. Atlantic salmons (*Salmo salar* L.) were used to evaluate CO effects on shelf life and welfare. Fish were exposed to CO for 8 (CO8) and 20 minutes (CO20) and compared to stressed (SF) or control (C) groups. All fish were hauled from the tank and killed by percussion. Plasma Adrenaline and Noradrenaline contents were measured as stress indicators. Fish exposed to CO8 showed no stress signals, whereas Adrenaline content was significantly higher in CO20. The C and SF fish showed a lower catecholamine level than CO20. This reflects a challenge when conducting field studies with regard to stress. CO treatment affected positively colour and favoured a rapid onset of *rigor mortis* and a small but significant increase in drip loss.

Key words - Fish quality, Shelf life, Slaughter

I. INTRODUCTION

Carbon monoxide (CO) makes the colour of meat more stable and products might look fresh even though bacterial levels are high and the product is spoiled. For this reason CO application as packaging technology is banned in Norway and Europe. However, since 2002, in the USA, CO is permitted as a MAP gas during distribution [1]. It is well known that CO binds hemeproteins such as hemoglobin (Hb), myoglobin (Mb) and neuroglobin, the latter found in brain and neural tissue [2]. CO displaces oxygen, and it has been assumed that the animal dies due to oxygen shortage without sensing the deficiency. CO also affects cell respiration through inhibition of many enzymes (*e.g*. cytochromes) with porphyrin groups similar to Hb and Mb. Oxidative phosphorylation is suppressed and thus aerobic bacterial respiration and survival [3]. CO is also shown to enhance the colour and quality of fish [4, 5], even when live fish is exposed to CO [6]. CO may act as an antioxidant in muscle foods, then lipid oxidation and browning effect are reduced and the shelf life of the product is prolonged [7]. Preliminary experiments on CO exposure of pigs, chickens and salmon have proved promising from an ethical point of view, since the animals are anesthetized and die with limited stress responses. The effect of CO on the heme protein neuroglobulin in brain may explain why CO is sensed as unharmful towards animals [8].

The aim of the present study was to analyze how the exposition of Atlantic salmon to CO before slaughter can affect welfare parameters and shelf life in comparison with salmons that were only percussively stunned.

II. MATERIALS AND METHODS

A total of 60 Atlantic salmons (*Salmo salar* L.) with a mean weight of 1.11 ± 0.13 kg divided in 4 experimental tanks containing 900 L seawater, were used for this trial. The temperature of the seawater was constant at 7.3 ± 0.5 °C. Fish in tank 1 were used as control (C) and killed by percussion; fish in tank 2 were flushed with 100% food grade CO (Yara Praxair, Oslo, Norway) using a ceramic diffuser (wedge lock base unit, Point Four Systems Inc., Richmond, Canada) for 20 minutes at 2-3 bar (CO20); fish in tank 3 were also flushed with 100% food grade CO for approximately 8 minutes (CO8), a time at which they show reactions that could indicate stress. Fish in tank 4 were stressed by severe stirring (SF).

All groups were hauled out of the tanks and percussively stunned. For personnel safety the air

CO concentration was monitored and measured during the experiment by use of portable gas detectors (GasBadge Pro, Oakdale, PA, USA). The experiment was approved according to "The Regulations in Animal Experimentation" in Norway and conducted by certified personnel. Blood samples, immediately after percussive stunning, were collected from the caudal vein of 5 fish from each group, placed on ice and brought to the laboratory for plasma adrenaline (AD) and noradrenaline (NAD) determination. Plasma AD and NAD were analyzed using BI-CAT® - ELISA kit (DLD - Diagnostika, GMBH, Hamburg, Germany), according to the manufacturer's instructions.

Salmons from tank 1 and 2 were then individually tagged, weighed and stored in polystyrene boxes with ice. Immediately after slaughter *rigor mortis* was measured by tail drop and the Rigor Index (L_r) was calculated according to Bito *et al.* [9] as follows:

$$L_r(\%) = [(L_0 - L_t)/L_0] \times 100$$

where L_0 (cm) corresponds to the tail drop at the first measurement, whereas L_t (cm) to the rigor level at the actual time throughout the experiment. pH was measured at the cranial part of the epaxial region using a Mettler Toledo SevenGo pro™ pH meter (Mettler-Toledo Ltd, Leicester, UK) equipped with a Inlab puncture electrode (Mettler-Toldedo, Ltd). After *rigor mortis* resolution (64 h *post-mortem*, time_0_-_T0) all fish were gutted, filleted and weighed. Right fillets were *vacuum* packed and stored at -20 °C, whereas the left ones were stored for 14 days (T14) in PEHD trays with absorbent pads on the bottom, in a cold room at 2.5 °C.

From T0 until T14, every second day, L*a*b* colour (MiniScan™ XE Plus HunterLab [10]) and pH measurements were performed.

Drip loss (%) was determined by weighing the fillets at T0, T7 and T14, and calculated by the formula:

$$\text{Drip loss index (t)} = ((D_0 - D_{7,14})/D_0) \times 100$$

where D_0 is the fillet weight immediately after filleting, while $D_{7,14}$ correspond to the fillet weight after 7 or 14 days of storage. Data were analyzed using the General Linear Model procedures of the statistical analysis software SAS 9.1 for Windows [11]. A one-way ANOVA tested the stunning method as fixed effect.

III. RESULTS AND DISCUSSION

Compared to C and SF fish, those treated with CO20 showed significantly higher levels of catecholamines ($P<0.001$; Table 1). The fish stressed by stirring (SF) showed NAD values similar to those observed in C fish, but a significantly lower AD value ($P<0.001$). The fish exposed to CO8 presented AD and NAD values close to those found in C group. The high values found for both AD and NAD might depend on metabolic changes when CO starts inhibiting oxygen metabolism. Considering the behavioral pattern of the fish observed during the CO diffusion in the water, no aversive reactions such as those seen when treated with carbon dioxide [12, 13] were evidenced in our study. However, fish showed erratic swimming suggesting the presence of death cramps. A recent review [8] leads to support the hypothesis that the CO affinity to the oxygen binding protein neuroglobulin may induce immediate sedation and unconsciousness in fish, having then an important role in stress management in fish. From the results emerged by the present study it seems, however, that CO treatment was most stressful to fish as it increased catecholamine's release. On the other hand, the SF fish seemed unaffected by the stirring procedure, highlighting an ability to cope with this stressful *stimuli*. This certainly represents a challenge when conducting field studies with regard to stress, as technical difficulties in taking valid blood samples from undisturbed fish are still not overcome.

Table 1. Mean Adrenalin (AD) and Noradrenalin (NAD) values (ng/ml plasma) in blood samples collected from 5 Atlantic salmons per treatment: control (C), stressed fish (SF), CO8 and CO20.

	Treatment				*P*-value	RSD[(1)]
	C	SF	CO8	CO20		
AD	3.09^{b}	1.36^{a}	2.98^{b}	4.8^{c}	<0.0001	0.5
NAD	5.4^{a}	5.0^{a}	6.4^{ab}	8.1^{b}	<0.0001	0.9

(1) Residual Standard Deviation.

Salmons euthanized with CO (CO20) had earlier onset of *rigor mortis* and muscle *post mortem* pH reduction than the C fish (Figure 1). CO20 Fish reached full *rigor* approximately 10 hours *post*

mortem, whereas C fish after 24 hours *post mortem* (Figure 1A). Muscle pH decline was similar between the two groups (Figure 1B) during the first 24 hours; afterwards significantly lower ($P<0.05$) pH values were reported for the CO20 group at 30 and 64-hour measurements. The affinity of heme proteins for CO is at least 240 times higher than that for O_2 [14], this implies a dramatic reduction in O_2 transport and as a result the metabolism change from aerobic to anaerobic. This explains the rapid decrease of pH that resulted in an early onset of *rigor mortis* [15] with a slight but significant ($P< 0.05$) higher drip loss in CO20 compared to C group (Table 2).

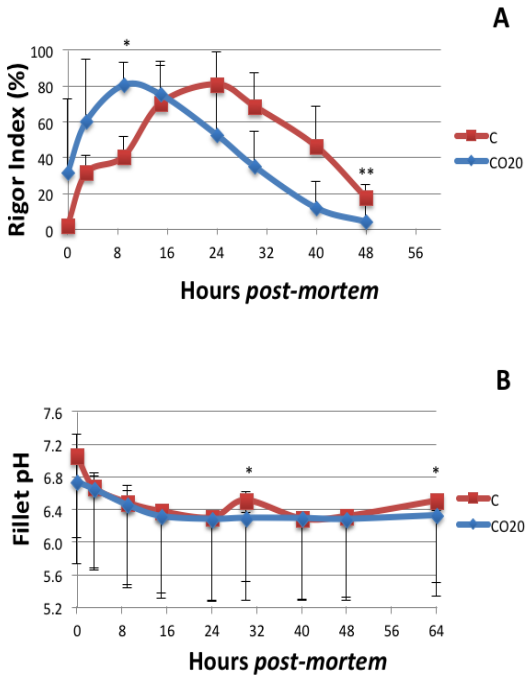

Figure 1. Rigor Index (A) and pH values (B) in Atlantic salmon fillets from control (C) and exposed to CO for 20 minutes (CO20) groups. The values are means (n = 6/group) ± SD

Table 2. Cumulative drip loss (DL, %) during cold storage of Atlantic salmon fillets from control (C) and exposed to CO for 20 minutes (CO20) groups.

	Treatment		*P*-value	RSD[(1)]
	C	CO20		
DL 0-7 days	2.3	2.9	NS	0.7
DL 7-14 days	1.4	1.4	NS	0.5
DL 0-14 days	3.7	4.3	<0.05	0.8

[(1)] Residual Standard Deviation

Fillets of CO20 fish showed a higher lightness (L*) and yellowness (b*) values than C fillets at T0, whereas at T14 no significant difference was detected between the two groups (Table 3) indicating that CO is able to yield a "natural" fresh looking fillets [7] restricted however to the fresh sample (T0).

After 14 days of chilled storage the b* value increased more in C than in CO20 fillets, perhaps attributed to both lipid and heme proteins oxidation; the latter, once oxidized to metHb/metMb, can produce a brown-yellowish appearance to red muscle, explaining the increase in b* value [16]. No significant difference for a* values at any considered time was found. The slightly higher a* value in CO20 at T0 could be attributed to CO binding to Mb, or Hb displacing oxygen, producing COMb or COHb that has a stable cherry red colour, and the degradation to metMb or metHb takes longer time [17] preventing discoloration. Indeed after 14 days of storage the redness for CO20 group was almost unchanged, highlighting the positive effect of CO. Salmon fillets contained astaxanthin that gives the characteristic red to orange colour and may have minimized the colour differences among the experimental groups.

Table 3.Colour parameters (lightness [L*], redness [a*], yellowness [b*]) measured every second day, from day (T) 0 to T-14 of storage in cold room (+2.5 °C), of Atlantic Salmon fillet subjected to two different killing methods, percussion (C) and CO euthanasia (CO).

Time (days)		Treatment		*P*-value	RSD[(1)]
		C	CO20		
0	L*	50.7	52.8	<0.01	1.6
	a*	19.9	21.1	NS	1.7
	b*	18.2	19.3	<0.05	1.3
14	L*	49.8	50.7	NS	1.4
	a*	21.2	21.2	NS	1.7
	b*	19.8	19.2	NS	1.2

[(1)] Residual Standard Deviation

IV. CONCLUSION

Salmons used in this trial were exposed to stirring that was supposed to generate high level of stress; however the cathecolamine levels found were lower than expected. At T0 the CO yielded

"natural" fresh looking fillets. During the 14 days of chilled storage CO treatment affected positively fillets colour, favoured a rapid onset of *rigor mortis* but also increased drip loss. Certainly further studies are needed to better understand how and to what extent the use of CO in the slaughtering process can affect welfare and quality aspects in Atlantic salmon.

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