CHAPTER

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The Effect of Carbon Monoxide on Slaughter and Processing of Fish

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CHAPTER POINTS

- CO is an efficient sedative and anesthetic agent in fish.
- CO affects the brain directly by binding to heme proteins in *Saccus vasculosis* and neuroglobin.
- CO can be detected together with gases like nitrogen, oxygen, and carbon dioxide using gas chromatography
- Visible and near infrared spectroscopy can be used to study color and protein, fat and water content online.
- CO stabilizes the color of red fish muscle.
- CO increases product stability by inhibition of microbial growth and lipid oxidation.

INTRODUCTION

Fish are regarded as a highly perishable food, since they are very susceptible to microbial and chemical decay. The type and rate of decay varies with fish species and is significantly influenced by the immediate handling before and after slaughter, and by processing and packaging systems. Optimizing these processes will lower the two main challenges, bacterial growth and lipid oxidation, the latter being particularly challenging in fatty fish species.

In the aquaculture industry, there is a growing awareness of maintaining animal welfare all the way through the slaughter process. The ethics involved in fish husbandry require that the process proceed with a minimum amount of strain. Normally, the fish should be anesthetized before being slaughtered and bled. This has proven to be a challenge for the industry. The use of chemicals like clove oil had been suggested as a non-toxic anesthetic (Iversen *et al.*, 2003). However, the use of chemicals is troublesome as traces may remain in the flesh at consumption and will cause concern to some consumer groups. Further, the use of chemicals is likely to be banned in some countries while allowed in others. This creates a challenge when fish are sold on a global market. Consequently, in most countries, the aquaculture industry relies on non-chemical anesthetic methods. In Norway, only percussion and electrical stunning are allowed by the authorities, while other countries also use liquid ice and carbon dioxide (CO_2). Many of these methods have potential welfare issues that may limit their use in the future. For example, electrical stunning may cause muscular contraction promoting rapid onset of *rigor mortis*. However, when properly applied on sedated fish, the method is very useful (Roth *et al.*, 2003). Immersion in liquid ice is considered stressful by many authors and is a questionable approach (Robb 2001; Kestin *et al.*, 1991). The use of CO_2 is basically to asphyxiate the fish, and the exposure generally elicits a flight response, causing the fish to swim erratically, trying to escape; this method is therefore regarded as unacceptable (EFSA, 2009).

Carbon monoxide has been used in animal euthanasia for a long time (Smith, 2001) but is not widely used in fish. However, recent data in Atlantic salmon (Bjørlykke *et al.*, 2011, 2012), tilapia (Mantilla *et al.*, 2008), pollack

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(*Pollachius pollachius*), herring (*Clupea harengus*), and mackerel (*Scomber scombrus*) (Slinde *et al.*, unpublished data) suggest that CO is an excellent fish sedative agent that does not appear to cause any visible stress response.

The mode of action of CO as sedative is not fully understood. However, it is well known that CO will bind to the heme group of hemoglobin and myoglobin displacing oxygen and producing carboxy-myoglobin (COMb) and carboxy-hemoglobin (COHb) that are incapable of oxygen transport. Both COMb and COHb are stable compounds, and it has been assumed that the animal will die due to oxygen shortage without sensing the deficiency. Recent data also suggest that CO binds to the oxygen-storage proteins in *Saccus vasculosus*, and neuroglobin (Ngb) (Figure 51.1) of the brain. It is believed that *Saccus vasculosis* is an oxygen depot with functions during hypoxia and stress (Burmester and Hankeln 2009), while Ngb is an oxygen transporter mainly located in neurons of the central and peripheral nervous systems (Figure 51.1A, B) and in some endocrine tissues (Reuss *et al.*, 2002). Blocking these with CO may induce immediate sedation and unconciousness in fish.

HOW FISH COMPOSITION IS ALTERED

Processing Methods

The dominant commercial method for applying CO to fish during processing is by pretreatment with filtered smoke (Kowalski 2006). Fish rich in red muscle containing heme proteins, like tuna (various *Thunnus*) and mahi mahi (*Coryphaena hippurus*), are suitable for this technology. Filtered smoke is generated from natural sawdust by removal of some taste and odor components, carcinogen compounds, and gases. Usually filtered smoke contains 15–40% CO, and the fish are treated in chambers for 2–48 h, depending on the size and thickness of fillets. Thereafter, the treated fillets are vacuum packaged, frozen, and transported to the markets.

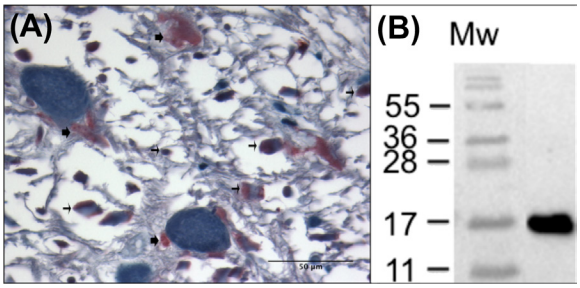

FIGURE 51.1 (A) Immunostaining using anti salmon neuroglobin in 1:70 dilution. Neuroglobin is found in perikardion (thick marker), and axon (thin marker) in thalamus of brain of Atlantic salmon. The arrows mark some of the positive staining. (B) Western blot analysis using anti-salmon neuroglobin to detect recombinant salmon neuroglobin (Bjørlykke *et al.*, Unpublished data) at the expected MW of 17,000.

In addition to filtered smoke, fish may be pretreated, packaged or stored in high concentrations of CO, close to 100%. Packaging of fresh meat with low levels of CO, up to 0.4%, combined with high levels CO_2 and no oxygen is well established, in particular in the USA (Cornforth and Hunt, 2008). Based on the beneficial experiences obtained with low CO packaging of meat over the last two to three decades, there is a potential for implementing this technology in the fish processing industry, yielding better color, longer shelf-life and inhibition of lipid oxidation. The application of CO already to the live fish is in this connection regarded as beneficial.

Flesh Color

In normal tissue, most color is caused by myoglobin. But some hemoglobin will also be present, particularly if fish have not been bled. Fresh tissue contains only oxymyoglobin/oxyhemoglobin (OMb/OHb) which has a bright-red color. Shortly after death, oxygen is lost producing deoxyglobins (DMb/DHb) that have a dark-red color. With further decay, iron is oxidized to its ferric state, producing the meth-form of the globins (MMb/MHb) which has a brown color. This color change is an unattractive feature to consumers, and producers therefore aim to maintain the bright-red color as long as possible.

When CO is added it binds directly to oxy- or deoxyglobins displacing oxygen, producing COMb/COHb that has a cherry red color. They are stable compounds and the degradation to MMb/MHb takes a long time (Chow *et al.*, 1998) and will thus prevent discoloration. The attribute has been used by some producers to maintain color in products like tuna for a long period of time. The process (gas or filtered wood smoke) may also be used to stabilize globins of white flesh fish, improving the color appearance over time (Mantilla *et al.*, 2008; Kowalski, 2006). Table 51.1 shows redness, a* values, of salmon, herring, and mackerel treated with CO compared to control groups. In herring and mackerel the COMb in the red muscle show a persistent cherry-red color due to binding of CO. CO-treated fish did not develop the typical rancid smell even after 6 days of storage as was the case for the controls. Storage of Atlantic salmon in 100% CO and consequent binding to heme demonstrated that

TABLE 51.1 Color (a*, i.e., change in redness) of cold-room-stored (4°C) Atlantic salmon (1.30 kg), herring (0.16 kg), and mackerel (0.56 kg) after being anesthetized with CO

	Salmon		Herring		Mackerel	
Day	Control	CO	Control	CO	Control	CO
1	22.3 ± 0.7	23.0 ± 2.0	2.7 ± 1.1	10.0 ± 2.0	9.5 ± 1.0	8.9 ± 2.0
6	19.4 ± 1.0	21.9 ± 2.0	3.7 ± 0.3	10.3 ± 2.0	5.1 ± 0.9	10.0 ± 0.7

The L* and b* values were very similar for treated and untreated fish.

this pigment contributes slightly to the color in addition to the dominant astaxanthin pigment (Bjørlykke *et al.*, 2011; Ottestad *et al.*, 2011).

Lipid Oxidation

After slaughter, oxidative processes will start in meat. Oxymyoglobin is a relatively unstable compound and has the potential to contribute significantly to oxidation through several pathways. For example, OMb (Fe^{2+}) is easily oxidized to MMb (Fe^{3+}) producing superoxide anion that can dismutate to hydrogen peroxide and thus initiate lipid peroxidation. Next peroxides (lipid peroxides and hydrogen peroxide) are strong oxidizers that can oxidize OMb $Fe^{2+} > Fe^{3+}$ (Tajima and Shikama, 1987) and thus propagate peroxidation. Finally, many peroxidation products like aldehydes, e.g., 4-hydroxy-2-noenal, may themselves attack sites on myoglobin facilitating its oxidation (Faustman *et al.*, 2010). COMb does not contain oxygen and will thus not facilitate the production of superoxide radicals. Furthermore, introducing a ligand like CO to the 6th coordination orbital increases the stability of the Fe^{2+} in the heme moiety. This will increase the shelf-life, and reduce lipid oxidation and browning of the product (Cornforth and Hunt, 2008; Hsieh *et al.*, 1998). This is especially important in fatty fish where the high level of unsaturated fatty acids makes them more susceptible to lipid oxidation.

Microbial Growth

CO is known to have an inhibitory effect on microbial growth at levels above 5% (Gee and Brown, 1980). Fish treated with filtered smoke benefits from this by having extended microbiological shelf-lives (Kowalski, 2006). In a study of aerobic bacteria in stored yellowfin tuna (*Thunnus albacares*), filtered smoke efficiently reduced bacteria caused by high levels of CO, carbon dioxide, and smoke components (Kristinsson *et al.*, 2007). The storage of tuna under 100% CO reduced bacterial growth, but to a smaller extent than filtered smoke. The mechanism of CO induced inhibition on bacterial growth is still relatively unclear, however. CO will affect cell respiration through inhibition of many enzymes (e.g., cytochromes) with heme groups similar to Hb and Mb. With cytochromes, CO inhibits oxidative phosporylation and thus aerobic bacteria respiration and survival (Prescott *et al.*, 1996). During prolonged storage of marine fish with red muscles, bacteria may penetrate the flesh and convert free histidine to histamine. Although histamine is toxic at very low concentrations, it does not cause appreciable visual or organoleptic changes. This increases the risk of intoxication by the consumer. Treating (directly or indirectly) fish with CO can reduce aerobic bacterial growth and histamine formation and increase shelf-life (Garner and Kristinsson, 2004).

ANALYTICAL TECHNIQUES

Visible (VIS)/Near Infrared Spectroscopy (NIRs) Spectra of CO in Fish and SRI Dissolved Gas Analyzer–Gas Chromatography (DGA-GC) System

Ottestad *et al.* (2011) used spectroscopic measurements on mackerel muscle to study how spectral changes correspond to color variations under three different storage conditions; air, vacuum and CO (Figure 51.2). The spectral color properties were dominated by myoglobin (and hemoglobin) at different oxidation states and bound to different ligands. The formation of COMb was positively correlated to the a* value on the L*a*b* scale (lightness, redness and yellowness). This implies the presence of different myoglobin species in fish, as reported by other authors (Mantilla *et al.*, 2008; Smulevich *et al.*, 2007). It will be interesting for future studies to use spectroscopy as a non-destructive way for online measurements of water, lipid, and protein (Folkestad *et al.*, 2008) together with visible color.

Detecting the soluble gasses like CO in the water (Figure 51.3) has been challenging both for scientific trials and as a dosage-control system in the industry. Using gas–liquid chromatography may offer long-awaited solutions to these challenges. For example, using a dissolved gas analyzer–gas chromatography (DGA-GC) it is now possible to quantify the amount of CO in the water, in addition to oxygen and waste gaseous products.

PRACTICAL CONSIDERATIONS

Human Toxicity

It is important to be aware of the possible toxic effect of CO on humans. It is a colorless, odorless, tasteless, and non-irritant gas. Inhalation of CO decreases the amount of O_2 delivered to the tissues. The affinity of hemoglobin for CO is over 200-times higher than its affinity for O_2. However, a low concentration of CO is not considered a hazard. The uptake of CO to hemoglobin is reversible and the half-life of COHb is 4–6 h. The rate of absorption and excretion of CO from the body is relatively slow. When working with CO a security alarm should be worn at all times. The administrative Norwegian working norm of CO is 25 ppm.

Legal Issues

Presently, CO is not permitted for foods in the EU and Norway. The regulations in the use of CO in treatment and processing of muscle foods however do differ between countries and regions. United States Food and Drug Administration stated 'tasteless smoke' or filtered smoke as GRAS (Generally Recognized as Safe) in 2000

FIGURE 51.2 Absorption spectra from mackerel fillet stored in vacuum, air, and CO. The Soret maximum in Visible Spectra for mackerel packed in air was 421 nm; in CO 423 nm; and in vacuum 431 nm. This shows the presence of the various myoglobin species depending on storage conditions. Fillet stored in vacuum (black solid line), air (dotted line), and CO (hyphened line). *Courtesy of Jens Petter Wold, Nofima.*

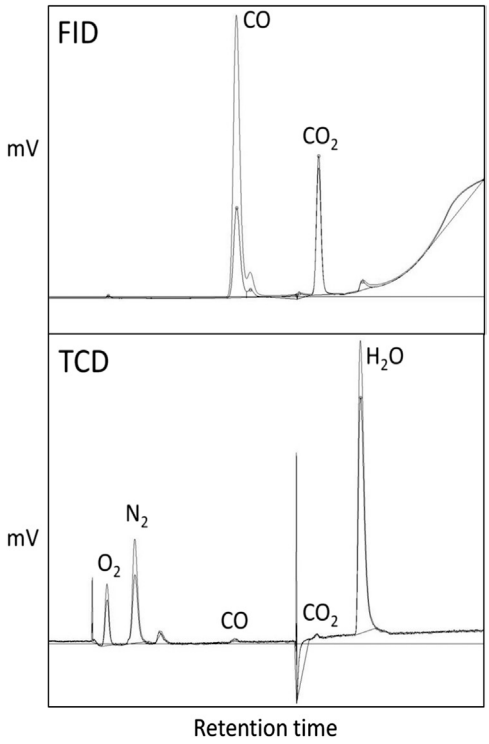

FIGURE 51.3 Detection of soluble gasses in water using an SRI DGA-GC system equipped with a Thermal Conductivity Detector (TCD) and a Flame Ionisation Detector (FID). Two different standards (i.e., water samples) with different amounts of CO were used. For each detector the individual traces are overlaid, with the upper trace representing the sample having twice the CO content compared to the lower trace sample. This detection method shows excellent reproducibility and allows detailed analysis of the water gas atmosphere. A detailed explanation of the system can be found at the SRI homepage (http://www.srigc.com).

(USFDA, 2000). Later, packaging of meat with up to 0.4% CO has been permitted in the USA to master-bags and case-ready meat. Until 2004, low CO concentrations were widley used for packaging of meat in Norway, but at that time CO packaging was prohibited due to trade agreements with the EU. The positive effects of CO might cause a change in legislation in the EU in the future. However, there are no regulations in the EU on the use of CO as a sedative or anesthetic component for fish.

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