Carbon monoxide residues in vacuum-packed yellowfin tuna loins (*Thunnus Albacares*)

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Abstract

The use of carbon monoxide (CO) in fresh fish has generated considerable debate. Carbon monoxide is used to treat fresh fish in order to retain its *fresh* red appearance for a longer period. It reacts with the oxy-myoglobin to form a fairly stable cherry red carboxy-myoglobin complex that may mask spoilage, because the CO-complex can be stable beyond the microbiological shelf life of the meat. The presence of CO in tuna fish (*Thunnus Albacares*) has been investigated by means of optical spectroscopy. Formation of the CO adduct can be easily detected by the combined analysis of electronic absorption spectra in their normal and second derivative modes, monitoring the intense Soret band at 420 nm. Samples were judged as CO treated when their levels were higher than 200 ng/g. Only two positive samples out of 29 analyzed were detected. The high level of uncertainty (0.30) of the method requires the use of more specific and sensitive methods for confirmatory analysis.

Introduction

The seafood industry is continuously attempting to develop new technologies aimed at extending the shelf life of fish products, without changing their nutritional and sensory characteristics. The freshness of tuna meat is generally judged by its bright red colour. The desirable colour is due to the level of oxygenated myoglobin, which is present in the red muscle fibres. In order to retain its *fresh* red colour for a longer period, carbon monoxide (CO) gas has been used to treat meat in the modified atmosphere packaging (MAP) system. The carbon monoxide reacts with the oxy-myoglobin to form a very stable carboxy-myoglobin complex (CO-Mb) as the partition coefficient for CO is about 50-fold higher than that of oxygen (Antonini and Brunori, 1971). There are no direct health implications from eating CO-treated tuna. However, CO may mask spoilage, because the stable cherry red colour can last beyond the microbiological shelf life of the meat. In this respect, it has to be considered that tuna fish, like other Scombroid fish, is commonly associated with cases and outbreaks of histamine intoxication. CO-treatment of fish and meat is allowed at industrial level in the United States and in the Netherlands (Schubring, 2008), but is not permitted in the European Community. In fact, CO is not included in the list of allowed food additives (EU Directive 95/2/EC; European Commission, 1995). Recently, frozen yellowfin tuna loins (*Thunnus Albacares*) with the characteristic cherry red colour typical of the treatment with CO, have been introduced into the EU market. In accordance to the EU Regulation No 178/2002 (European Commission, 2002) a rapid alert system has been notified on these products. Different methods are available for the analysis of CO in food, useful for forensic purposes, including spectrophotometric methods (Bylkas and Andersson, 1997; Cruz *et al.,* 1993; Sano and Hashimoto, 1958; Watts *et al.,* 1978). Aim of this work was the research of the presence and concentration of CO in tuna fish loins (*Thunnus Albacares*) with a spectrophotometric method.

Materials and Methods

From October to December 2013, twenty-nine tuna loins of *Thunnus Albacares* coming from eight batches and individually vacuum packaged were examined. The samples were sampled randomly from the distribution establishment and immediately transported under conditions of constant temperature (0-4°C) to the laboratory of the Food Inspection Section of the Department of Veterinary Medicine and Animal Productions in Naples, where the label data (lot, capture date, freezing and thawing time, vacuum and capture time) were verified and recorded. Three parts of about 10 g from the dorsal, ventral and lateral parts of the each loin were singly analyzed for the qualitative and quantitative determination of carbon monoxide using UV-VIS spectrophotometry (Stonek *et al.,* 2004; Smulevich *et al.,* 2007). The relative expanded uncertainty from the validation data was found to be equal to 0.30. The results obtained from the average of three determinations are expressed in ng/g of carbon monoxide.

Results

The CO content in 27 out of 29 examined loins was found to be less than 200 ng/g, a value that is considered the internationally accepted limit for a physiological CO content in muscle tissues (Ministry of Health and Welfare of Japan, 1995). Results indicated only two positive samples out of 29 analyzed when the average value of the three parts of each loin were considered. In F2 loin, sampled on 23/10/13, an average value of 777 ng/g of CO was found (Table 1). In F4 loin, sampled on 29/10/13, the CO concentration was higher than the *natural* limit within the margin of uncertainty provided by the method used. On the whole out of the eighty-seven analyzed samples (Fa dorsal; Fb lateral and Fc ventral parts of each loin) only twelve samples (equally distributed between the dorsal and ventral parts) showed values higher than 200 ng/g (Table 2).

Discussion

Since 2003, EU Commission has notified to all member countries through the Rapid Alert System for Food and Feed (RASFF) the presence of carbon monoxide in tuna (*Thunnus Albacares*) from South-East Asian countries (FAO Area 71 and 57) and from some Member States. According to many authors, very low concentrations of carbon monoxide (0.3-0.5%) are necessary in the atmosphere for the formation of the CO hemoglobin in food. When this gas increases (0.1-0.5%) the reduction of deoxy-myoglobin is favored and the browning of meat is tempered. Carbon monoxide is not included in the positive list of food additives of EU Regulation N.o 1333/2008 (European Commission, 2008). In the treated product, the

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colour may mask the deterioration associated with potential risk of *scombroid syndrome*. Therefore CO treatment is not allowed within the EU with the exception of the Netherlands where the treatment (cold smoking method clear smoke®) is accepted, restricting the marketing of the processed products to the national territory. Today, in different parts of the world (USA, Japan, Southeast Asia), the seafood industry requires the approval of the treatment with carbon monoxide based on the absence of toxicity of the compound. For the Food and Drug Administration (USA) the tuna is wrongly labelled if it is treated with carbon monoxide but the presence of the additive in the label is not declared. Obviously, the target is to determine the minimal CO dose to obtain the desired colour.

Conclusions

Results from the present research showed only two positive samples out of 29 analyzed (3/3 rates). In some aliquots of the negative samples concentrations near limit of 200 ng/g were observed and this results show the high variability in CO distribution. The significant margin of uncertainty (0.30) of the spectrophotometric method requires the adoption of more specific and sensitive methods. The Japanese government has developed a chromatographic method (GC/FID nickel catalyst) to regulate the import/export of fishery products (Hsieh *et al.,* 1998; Ishiwata *et al.,* 1996). At the National and European level, a confirmatory method is strongly required for a trade harmonization of these products, in most cases imported.

Table 1. Carbon monoxide content and uncertainty in tuna loins analyzed in first step (October-November 2013).

Sample	Sample weight (g)	Cco (ng/g)	U (ng/g)	Result
F1 (dorsal)	10.09	188.20	56.50	Negative
F1 (lateral)	10.01	24.20	7.30	Negative
F1 (ventral)	10.01	252.60	75.80	Negative
Mean		155.00	46.50	
F2 (dorsal)	10.00	746.10	223.80	Positive
F2 (lateral)	10.10	787.60	236.30	Positive
F2 (ventral)	9.80	797.40	239.20	Positive
Mean		777.00	233.10	
F3 (dorsal)	10.50	266.00	79.80	Negative
F3 (lateral)	10.10	163.30	49.00	Negative
F3 (ventral)	10.10	25.80	7.70	Negative
Mean		151.70	45.50	
F4 (dorsal)	10.10	136.20	40.90	Negative
F4 (lateral)	10.08	3.60	1.10	Negative
F4 (ventral)	10.05	199.40	59.80	Negative
Mean	-	113.10	33.90	

Cco, carbon monoxide content; U, uncertainty. The results obtained from the average of three determinations are expressed in ng/g of carbon monoxide.

Table 2. Carbon monoxide content and uncertainty in tuna loins analyzed in second step (November-December 2013).

Sample	Sample weight (g)	Cco (ng/g)	U (ng/g)	Result
F1 (dorsal)	10.02	92.90	27.90	Negative
F1 (lateral)	10.01	16.50	4.90	Negative
F1 (ventral)	10.01	133.01	39.90	Negative
Mean		80.8	24.20	
F2 (dorsal)	10.00	289.3	86.8	Positive
F2 (lateral)	10.00	142.4	42.7	Negative
F2 (ventral)	10.05	254.8	76.4	Negative
Mean		228.8	68.6	
F3 (dorsal)	10.08	115.6	34.7	Negative
F3 (lateral)	10.00	208.7	62.6	Negative
F3 (ventral)	10.04	71.3	21.7	Negative
Mean		131.9	39.6	
F4 (dorsal)	10.18	243.2	73.0	Negative
F4 (lateral)	10.08	305.6	91.7	Positive
F4 (ventral)	10.05	302.5	90.8	Positive
Mean		283.8	85.1	

Cco, carbon monoxide content; U, uncertainty. The results obtained from the average of three determinations are expressed in ng/g of carbon monoxide.

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