

# **“Vantaggi e svantaggi nell’utilizzo di additivi in prodotti ittici”**

***Dr. Mila Nocentini***

*Istituto Zooprofilattico Sperimentale Lazio e Toscana,  
Sezione di Firenze*

Roma, 10 Ottobre 2017



Istituto Zooprofilattico Sperimentale  
delle Regioni Lazio e Toscana

# *Index of Presentation*

- ✓ *Implementation of fish product Shelf-life using different methods (MAP technique and or treatment with additives )*
- ✓ *Legislations*
- ✓ *Electronic absorption spectra in the UV-Vis regions of horse hearth myoglobin (hhMb)*
- ✓ *Comparison of absorption spectra of yellowfin tuna fish fillets untreated and differently treated with preservatives*
- ✓ *Study of the electronic absorption spectra of tuna meat drip and of hhMB treated*
- ✓ *Conclusions*



## *Implenetation of fish products shelf life*

**Colour is a prime sensory parameter that determines consumer acceptance of a meat.**

The bright red colour of tuna muscle is an economical important factor, due to the preference by consumers who consider red muscle colour as an indicator of freshness.

This assumption could be wrong because a technology (M.A.P) should be used that imparts a fresh colour.

This technology uses the strong affinity of CO against both ferric atoms of the blood pigment hemoglobin (Hb) and of the muscle pigment myoglobin (Mb).



# Implementation of fish products shelf life

Many strategies have been developed to inhibit lipid oxidation, not only to minimized rancidity, but also to improve color stability.

Additives, useful for food preservation, are commonly used.

We investigate a mixture of:

Ascorbic acid (E300)

Sodium ascorbate (E301)

Citric acid (E331)



## Implementation of fish products shelf life

It has been demonstrate\* that the lipid oxidation of skipjack tuna ordinary muscle, is closely related to metmyoglobin formation, and the addition of antioxidant, is effective at inhibiting lipid oxydation as well as myoglobin oxydation in post mortem meat.

The metmyoglobin content of muscle gradually increased, and was accompanied with darkening in the fish meat colour\*

\*Sohn, J.-H., & and Ohshima, T. (2010). Control of lipid oxidation and meat color deterioration in skipjack tuna muscle during ice storage. *Fish. Sci.*, 76, 703–710



# Legislation

## European Legislation

**Carbon monoxide is not included in the list  
of authorised additives**

**EC Regulation No 1333/2008**

of 16 December 2008

on food additives

**and further modifications**



## Legislation

**Other additives are permitted in unprocessed fish and fisheries products:**

- Ascorbic acid and ascorbate;**
- Citric acid and citrate;**
- Phosphoric acid, phosphates**
  - di-, tri- and polyphosphates;**



## Legislation

**Other additives are NOT permitted in unprocessed fish and fisheries products:**

- Nitrite and Nitrate;**
- Vegetable extract containing nitrite and nitrate;**
- H<sub>2</sub>O<sub>2</sub>;**
- Carbon monoxide;**







**EUROPEAN COMMISSION**  
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Crisis management in food, animals and plants  
Alerts, traceability and committees

Brussels,  
sante.ddg2.g.5(2016))6590657

Dear Sirs,

**Subject :**     **illegal treatment of tuna with vegetable extracts**

We would like to draw your attention to the use of cultivated vegetable extracts containing high concentrations of nitrates and nitrites to enhance the colour of tuna and its appearance. The fish industry, in its commitment to the fight against food fraud, reported these practices to the Commission.

Nitrites are not authorised for use in tuna. Furthermore, the addition of nitrites via the vegetable extract does not comply with the specifications for nitrites laid down in Commission Regulation (EU) No 231/2012. The use of nitrites not only deceives costumers regarding the tuna's colour but also masks the amounts of histamines present in the product. The products look fresh and of high quality but in reality may contain high amounts of histamines which can cause strong allergenic reactions. Financial gain is clearly the motive, given that it is estimated that 5 million tuna portions per week are treated this way (approximately 500 tons), involving a potential gain of approx. 200 million € per year.



# *Monossido di carbonio*

Nota del Ministero della Salute n. 10811 del 30/03/2012

Raccomandava agli organismi di controllo competenti di segnalare la non conformità del prodotto solo laddove il valore di monossido di carbonio riscontrato fosse stata superiore a **200 ng/g ( $\mu\text{g/Kg}$ )** (in linea con le indicazioni della DG SANCO – RASFF). Tuttavia il prodotto era considerato sospetto di trattamento in caso di riscontro di monossido tra 50 e 200 ng/g.

Il Ministero della Salute con la Nota n. 0020934 del 09/02/2014 ha poi comunicato che non essendoci metodi analitici validati a livello comunitario in grado di determinare quantitativamente il monossido di carbonio e/o distinguere il monossido naturalmente presente da quello utilizzato nel trattamento illecito.

Pertanto suddetta Circolare ha richiesto agli organi di controllo di non effettuare più campionamenti di prodotti ittici per la determinazione di monossido.



## *Esempi di trattamento a livello industriale*

Un momento del processo di iniezione della sogliola con una soluzione di polifosfati. Come si può vedere le sogliole vengono disposte intere con la parte cieca rivolta verso il basso. Sono numerose le specie ittiche che vengono additivate sia intere che in filetti.



**Eurofishmarket (2010) vol 3 pp 27-48. G. Arcangeli, A. Gallina**



# *Esempi di trattamento a livello industriale*

Filetti di pesce in ammollo in acqua ed additivi



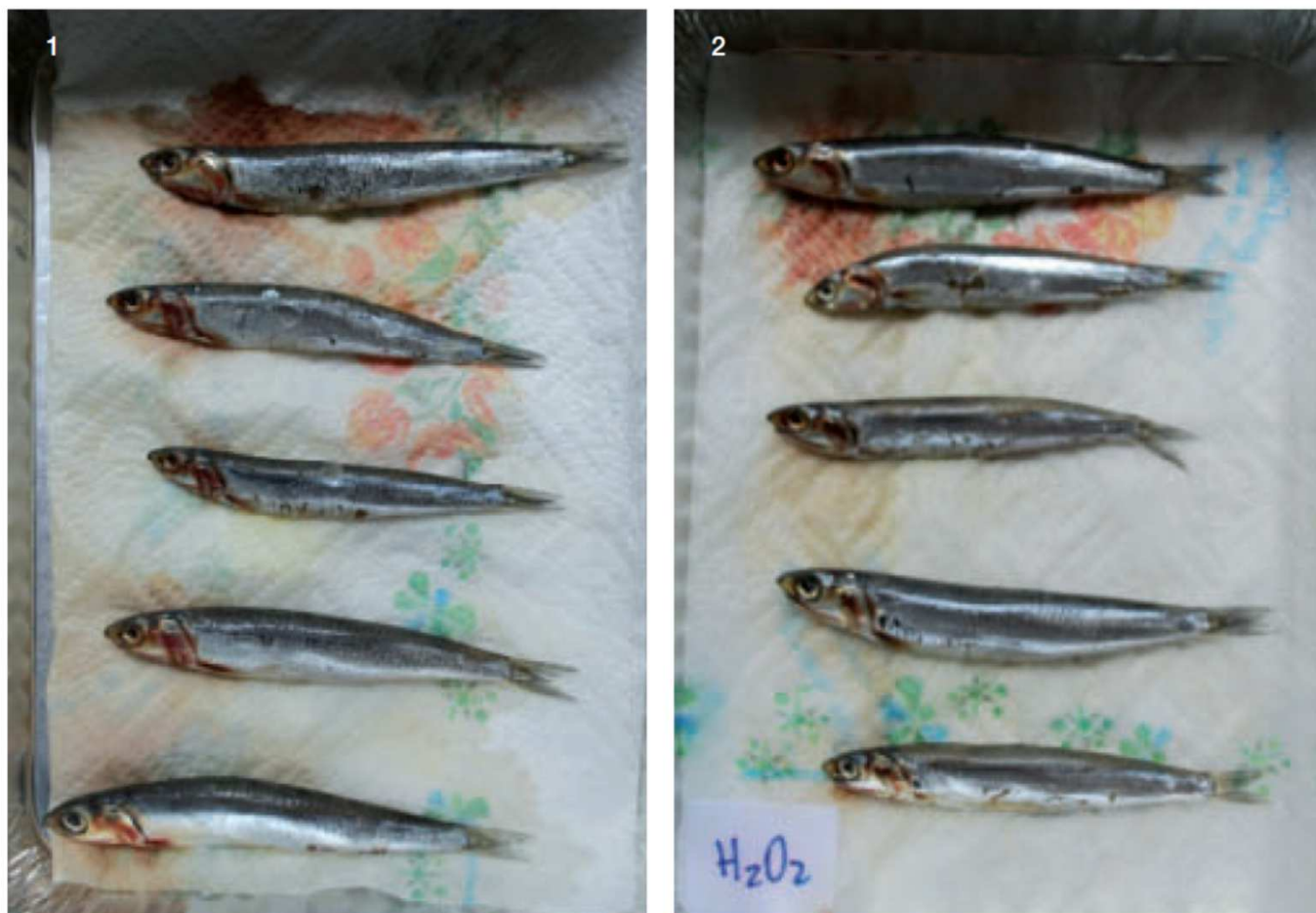
**Eurofishmarket (2010) vol 3 pp 27-48. G. Arcangeli, A. Gallina**





## *Esempi di trattamento a livello industriale*

A sinistra alici non trattate con acqua ossigenata, a confronto, a destra con alici sottoposte a trattamento con acqua ossigenata



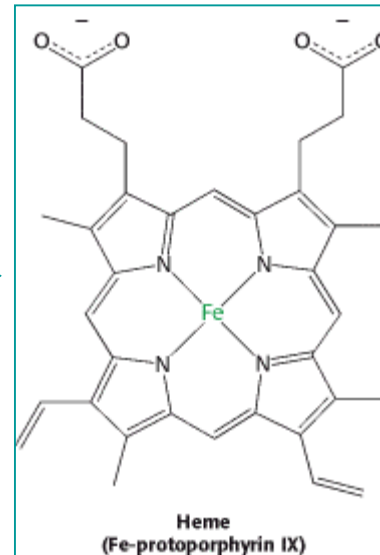
**Eurofishmarket (2010) vol 3 pp 27-48. G. Arcangeli, A. Gallina**



# Myoglobin structure

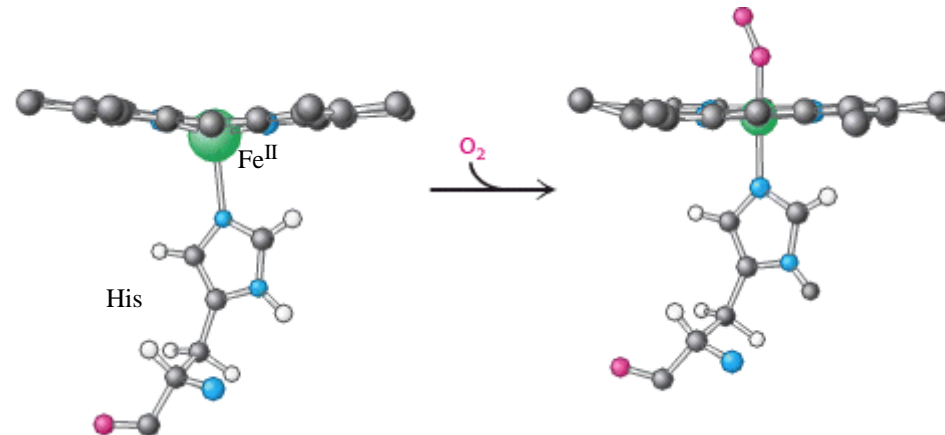
## Fe-protoporphyrin IX structure

(“Biochemistry fifth ed.”  
<http://www.ncbi.nlm.nih.gov/books>).



## Coordination of Iron Heme group with Oxygen

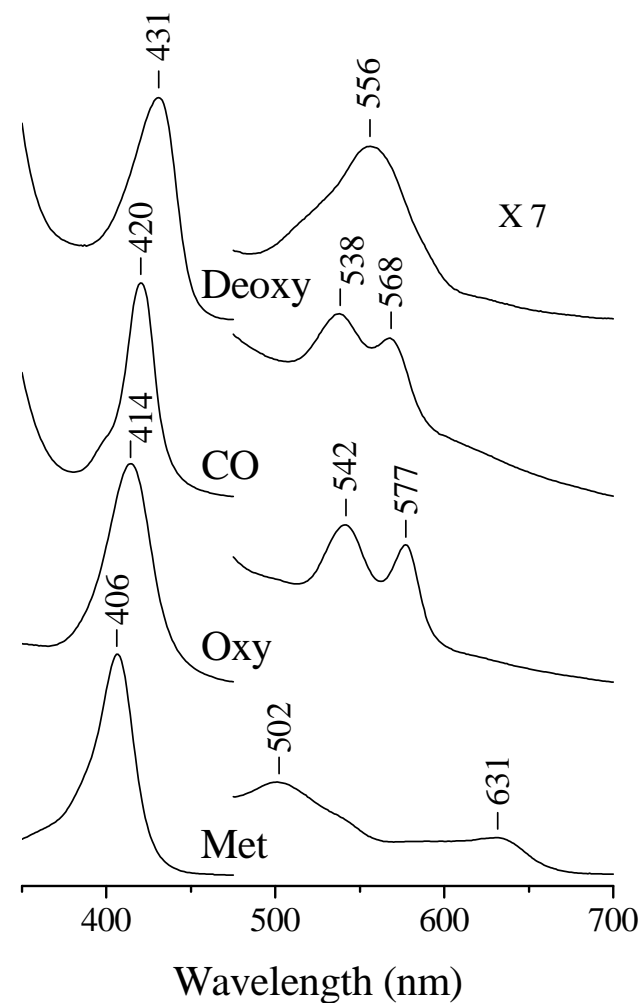
(“Biochemistry fifth ed.”  
<http://www.ncbi.nlm.nih.gov/books>).



# Absorption Spectra of Myoglobin

The chromophore and active site of Mb is the heme group which gives rise to two  $\pi \rightarrow \pi^*$  electronic transitions at about 400 nm (Soret or B band) which is very intense (with an extinction coefficient of about  $10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ) and at 500–600 nm (Q bands).

Deoxy	$\text{Fe}^{\text{II}}$
CO	$\text{Fe}^{\text{II}}\text{-CO}$
Oxy	$\text{Fe}^{\text{II}}\text{-O}_2$
Met	$\text{Fe}^{\text{III}}$



Smulevich, G., Droghetti, E., Focardi, C.,  
Coletta, M., Ciaccio, C. & Nocentini, M. *Food*  
*Chemistry*, 101, 1071-1077 (2007).

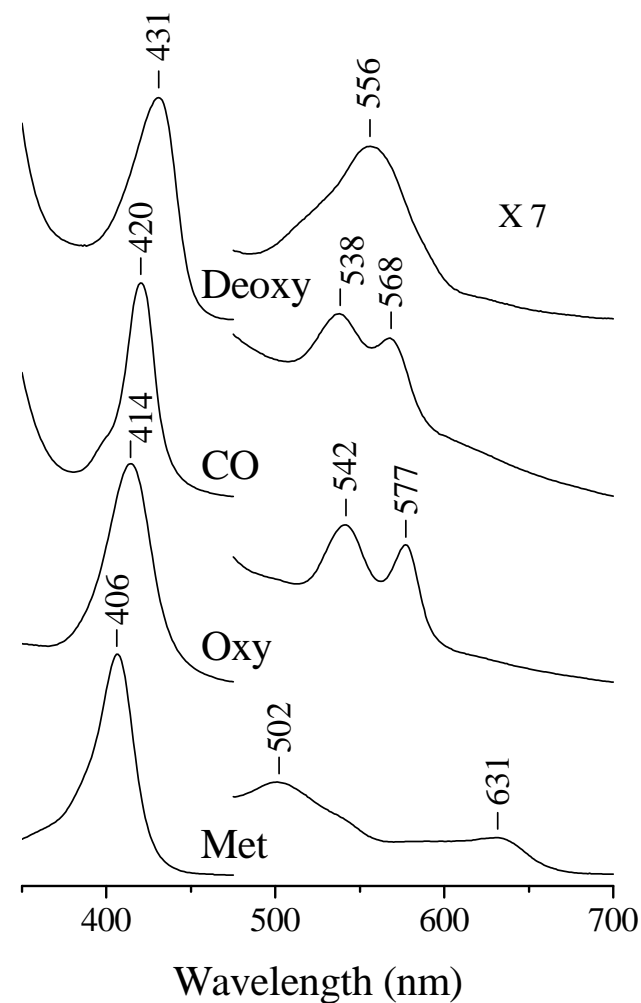
Absorption spectra of the met-, oxy-, CO-, and  
deoxy- myoglobin purified from tuna fish



# Absorption Spectra of Myoglobin

The chromophore and active site of Mb is the heme group which gives rise to two  $\pi \rightarrow \pi^*$  electronic transitions at about 400 nm (Soret or B band) which is very intense (with an extinction coefficient of about  $10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ) and at 500–600 nm (Q bands).

Deoxy	$\text{Fe}^{\text{II}}$
CO	$\text{Fe}^{\text{II}}\text{-CO}$
Oxy	$\text{Fe}^{\text{II}}\text{-O}_2$
Met	$\text{Fe}^{\text{III}}$



Smulevich, G., Droghetti, E., Focardi, C.,  
Coletta, M., Ciaccio, C. & Nocentini, M. *Food*  
*Chemistry*, 101, 1071-1077 (2007).

Absorption spectra of the met-, oxy-, CO-, and  
deoxy- myoglobin purified from tuna fish



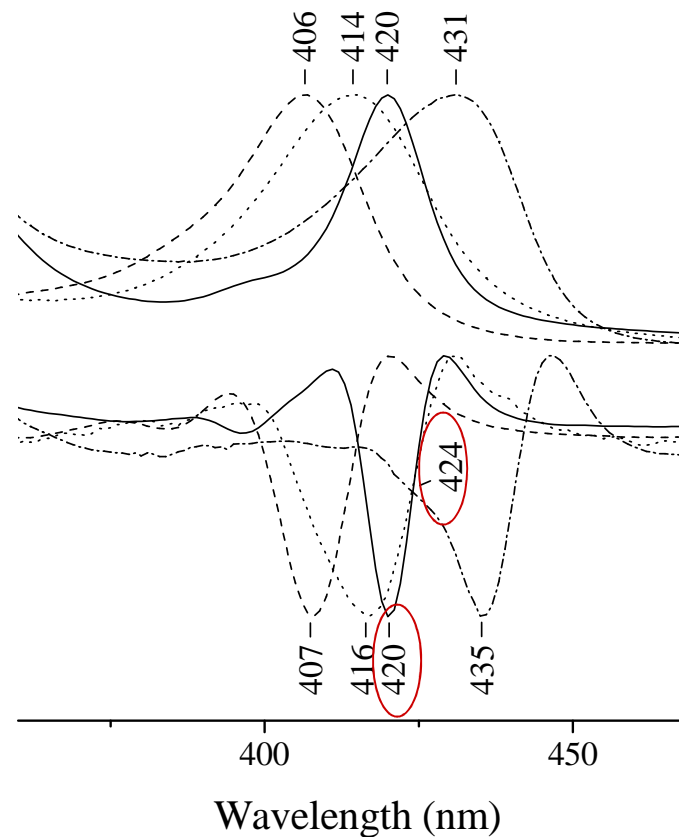


# Absorption spectra of myoglobin

Smulevich, G., Droghetti,  
E., Focardi, C., Coletta,  
M., Ciaccio, C. &  
Nocentini, M. *Food*  
*Chemistry*, 101, 1071-  
1077 (2007).

Deoxy	Fe <sup>II</sup>	431 nm
CO	Fe <sup>II</sup> - CO	420 nm
Oxy	Fe <sup>II</sup> - O <sub>2</sub>	414 nm
Met	Fe <sup>III</sup>	406 nm

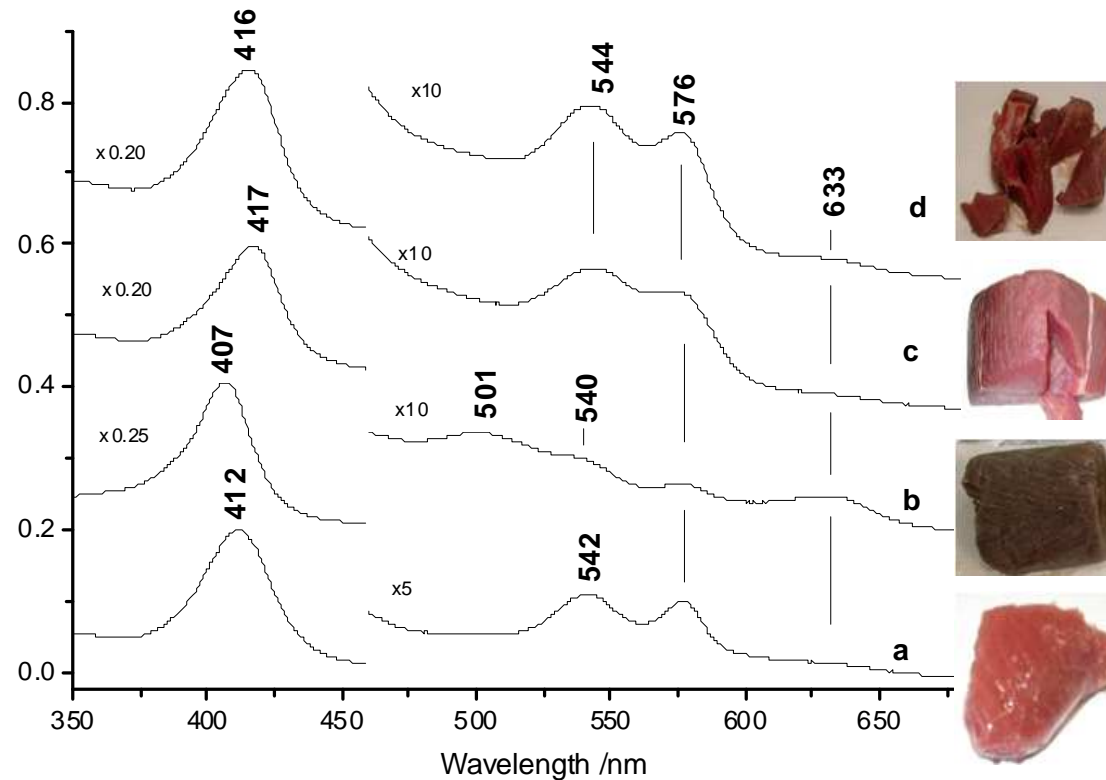
## Second derivative spectra



Absorption and second derivative spectra in the Soret region of the Met- (- - -), Oxy- ( ... ), CO- (—), and Deoxy ( - .- . ) myoglobin purified from tuna fish.



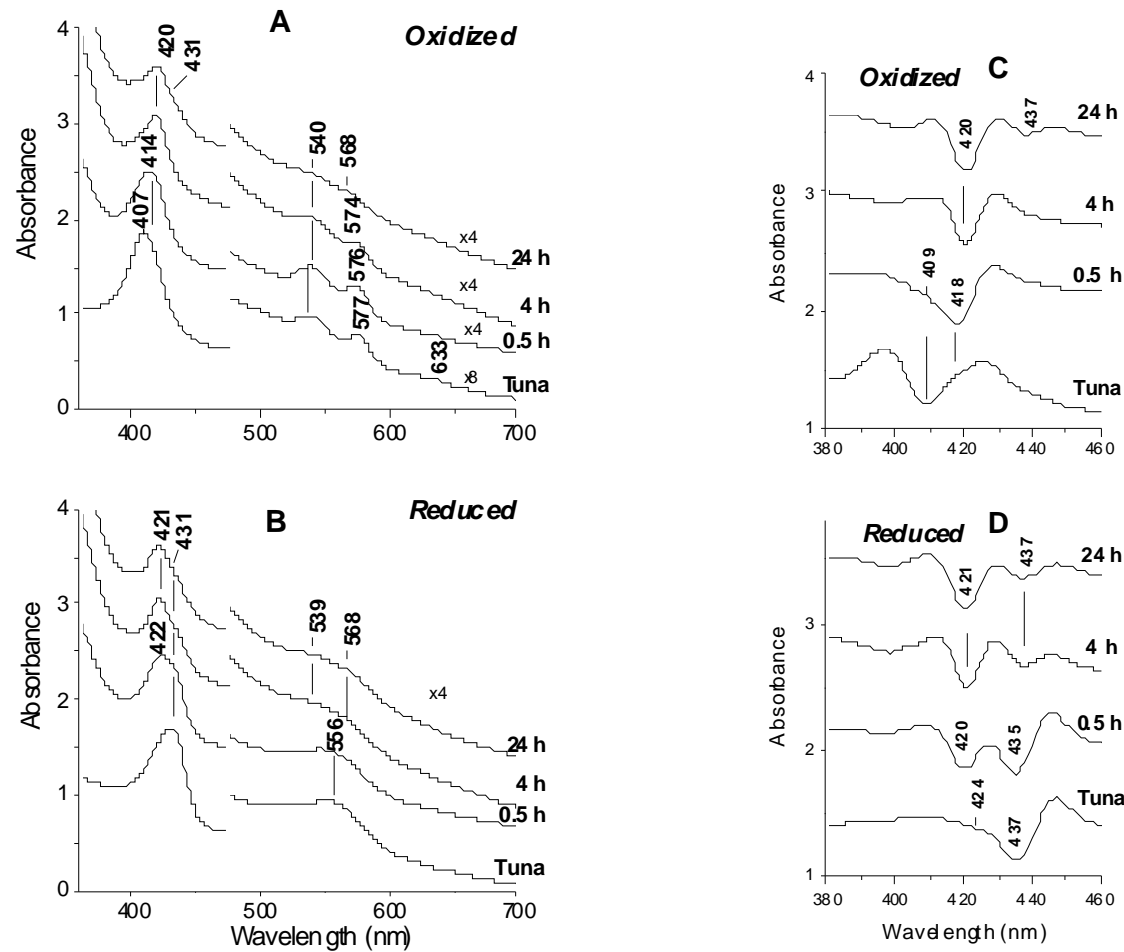
# Absorption spectra of tuna fish meat drip untreated and treated



**a)** untreated fresh sample; **b)** untreated aged sample; **c)** sample from the official control system treated with CO, and **d)** sample from the official control system declared to be treated with the mixture of additives



# Absorption spectra of tuna meat drip

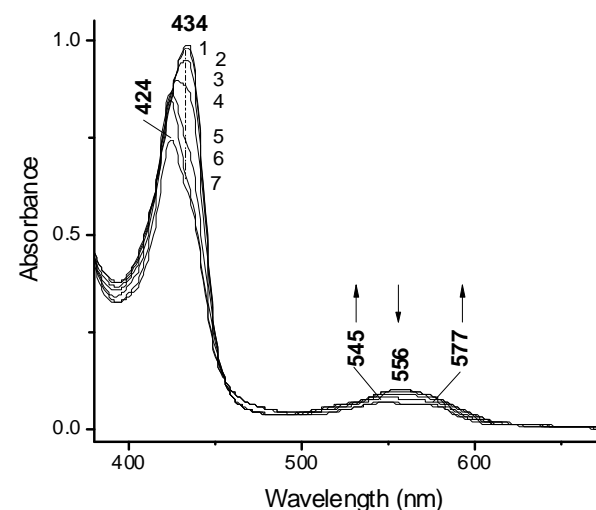
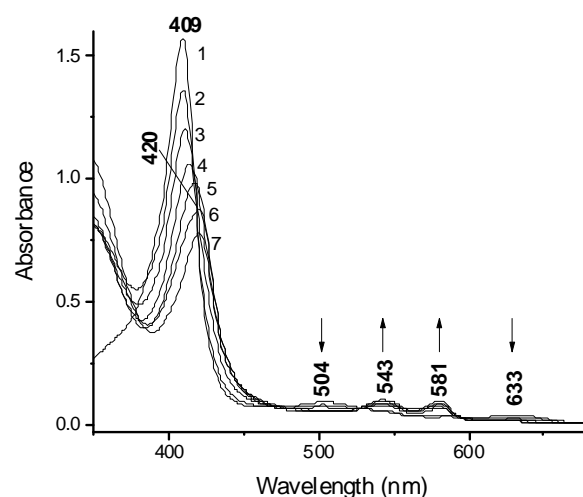


second derivative spectra

Panel A, C: treated with the mixture of preservatives; Panel B, D: same samples of Panel A, after addition of dithionite.



## Ferric hhMb spectral changes after addition of the mixture

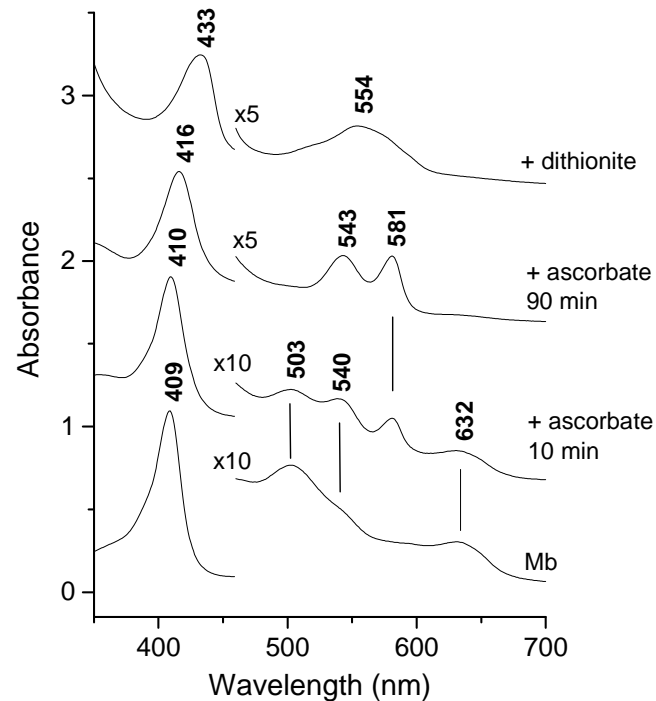


Spectral changes of the electronic absorption spectra of ferric hhMb (Left) and after reduction (right) following the addition of the mixture of additives in a time period.

1: no addition; 2: after 1 min; 3: after 10 min.; 4: after 30 min.; 5: after 70 min.; 6: after 4 hrs; 7: after 24 hrs



## Spectral changes for ferric hhMb after addition of Na ascorbate



Spectral changes in ferric hhMb after addition of Na ascorbic (5%).

a: ferric Mb; b: after 10 min; c: after 90 min; d: as c with dithionite



## Conclusions

- Ascorbic acid and sodium ascorbate give interferences with the spectrophotometric method due to the appearance with time of a broad band near 420 nm similar to that of the CO myoglobin complex.
- Tuna fish samples treated with the permitted preservatives show similar bright red color to that obtained by using carbon monoxide.
- The risk for the consumer is the same if it is used carbon monoxide or ascorbic acid and sodium ascorbate. In this latter case a very stable compound characterized by bright red color is formed, which might mask aging of the products.



## Conclusions

- A good parameter for the evaluation of aged of unprocessed fish should be the oxymyoglobin-metmyoglobin ratio.



## Conclusioni

Il Ministero della Salute con nota n° 0035856 del 08/09/2017 “Tonno – trattamenti illegali e sindrome sgombroide” dirama la segnalazione da parte dell’Unione Europea di un ulteriore aumento dei casi di istaminosi per consumo di tonno fresco riconducibili a pratiche fraudolente di uso di tonno congelato in deroga (destinato all’industria conserviera e quindi da destinarsi esclusivamente alla sterilizzazione) per la commercializzazione come fresco previo trattamento con additivi non consentiti. Vengono dunque indirizzati i controlli, oltre agli accertamenti analitici, sulla rintracciabilità allo scopo di risalire al tipo di materia prima o alla nave riconosciuta che ha effettuato la pesca, la nota fornisce anche un elenco di tali navi.

**FISHMARKET**

