



# Nutraceutical properties of mullet *bottarga*

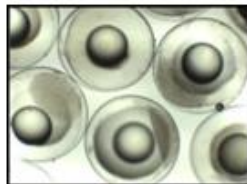
Dott.ssa **Antonella Rosa**

Department of Biomedical Sciences  
University of Cagliari, Italy



Workshop on grey mullet aquaculture: state of the art and perspectives.

14<sup>th</sup> May 2018, Palace Hotel, Bari (Italy)





# Mullet roes

A wide spectrum of fish roe products is consumed throughout the world (raw or as salted, smoked, or boiled products)



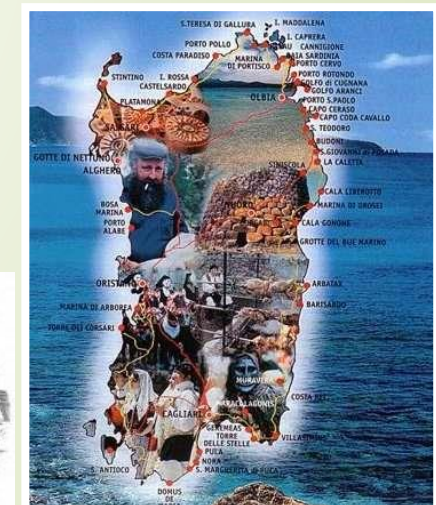
**Mullet (*Mugil cephalus*) roe** is regarded as a delicacy

The **salted** and **dried** mullet ovary product is produced in several countries in the world (known with the name of "**bottarga**" in Italy, "karasumi" in Japan, and "avgotaracho" in Greece)



Among the Mediterranean countries, **Sardinia** (Italy) has a **long tradition** in making a high quality **bottarga**

Popular in the international market

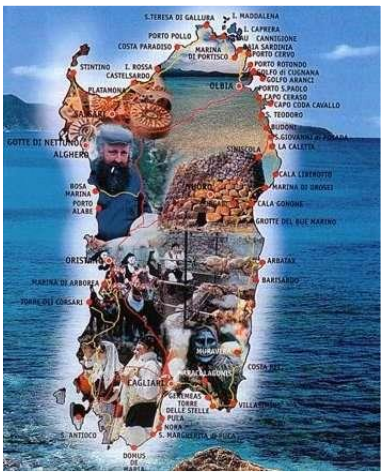






# Salted and dried mullet ovary product (bottarga)

Bottarga is a **Traditional Sardinian Food**  
(art.8 Decreto Legislativo n. 173/98, art. 2 Decreto Ministeriale n. 350/99)



“Mediterranean caviar”  
“Gold of Sardinia”

Final product of several treatments on ovaries of mullets



To obtain a good bottarga is an art that requires a great deal of skill. Traditional and industrial procedures



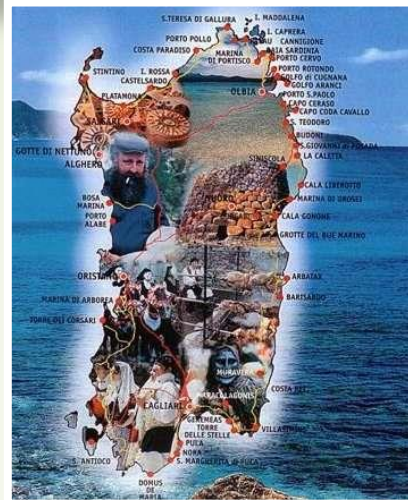
Bottarga has an **amber colour** and its unique chewy texture is due to its peculiar lipid composition (wax esters)







# Salted and dried mullet ovary product (bottarga)



In the Mediterranean countries, **mullet bottarga** is commonly eaten grated with spaghetti, mozzarella, and vegetables or cut into thin slices with extra virgin olive oil



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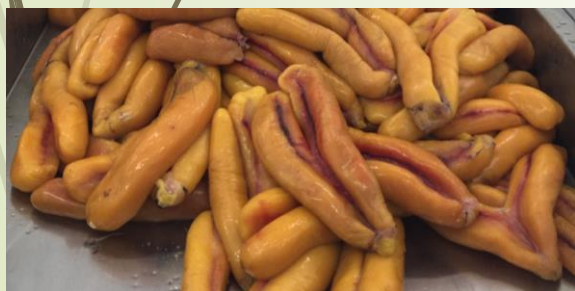
# Preparation of the salted and dried mullet ovary product (bottarga)



Mulletts are caught at the end of summer and the beginning of autumn (September-October)



Whole mature ovaries are removed from the fish (eviscerated roes)



Ovaries are salted with natural sea salt



Eviscerated roes are washed with water (to remove blood/blood vessels)





# Preparation of mullet bottarga

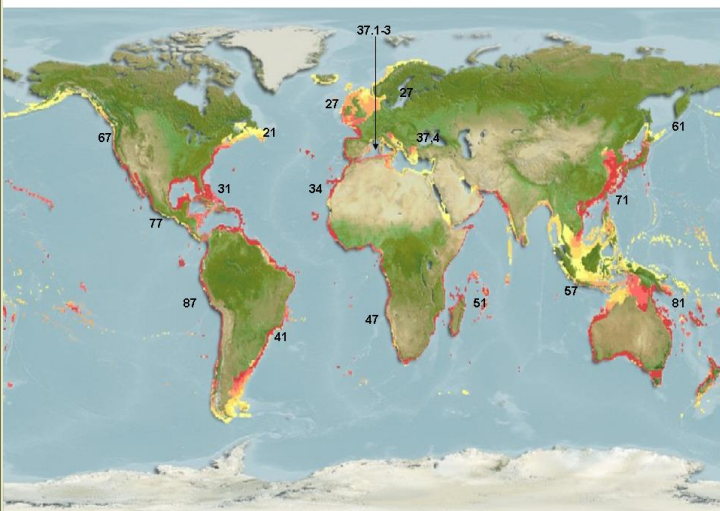
Roes are **pressed** and **dried** in rooms with controlled humidity and temperature



The final product can be sold as whole ovaries under vacuum packaging, or grated in jars

Bottarga production with local fish is not sufficient to meet the demand

AREE DI PESCA FAO



The raw material is acquired from distributors located in several areas of the globe and is processed (**salted** and **dried**) by Sardinian companies (according to tradition)





# Chemical composition of mullet raw roe

- Proteins 23% (fresh weight)
- Lipids 14-20%
- Moisture 50-61%
- Ash 2%

Food Chemistry 115 (2009) 891–896

Contents lists available at ScienceDirect

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journal homepage: [www.elsevier.com/locate/foodchem](http://www.elsevier.com/locate/foodchem)

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Eur. J. Lipid Sci. Technol. 2009, 111, 505–512

Research Paper

NMR study of the lipid profile of mullet raw roe and bottarga

Paola Scano<sup>1</sup>, Antonella Rosa<sup>2</sup>, Emanuela Locci<sup>1</sup>, M. Assunta Dessi<sup>2</sup> and Adolfo Lai<sup>1</sup>

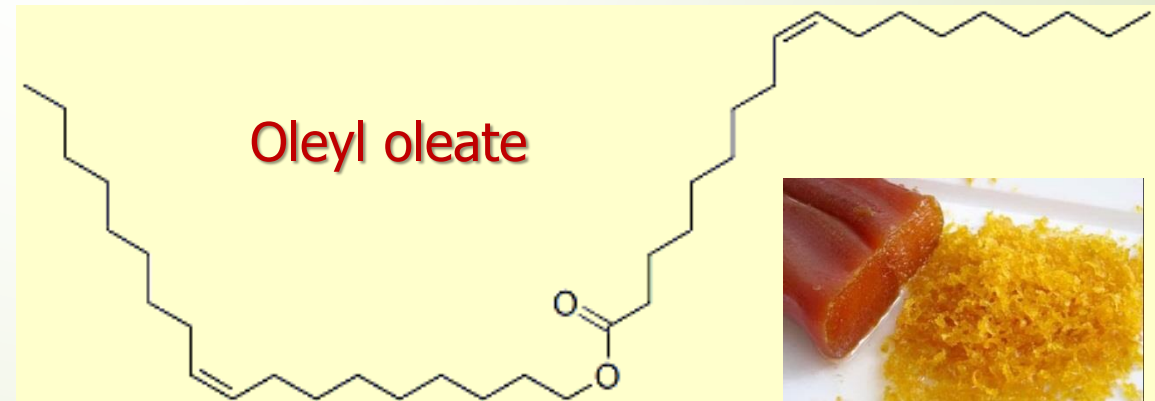
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Total lipids 150 - 200 mg/g of fresh weight  
Cholesterol 4.8 mg/g of fresh weight (represented ca. 3-4% of total lipids)

Table 2. Lipid class composition of raw roes from different fishing areas and of the corresponding processed products.

	Percentage of total lipids [mol-%] <sup>†</sup>			
	Fishing area 1		Fishing area 2	
	Roe	Bottarga	Roe	Bottarga
<u>Wax esters</u>	71.7	60.5	75.9	65.3
Triacylglycerols	6.0	4.5	11.9	7.8
Phospholipids	13.8	9.9	9.7	7.0
Free fatty acids	4.8	19.0	0.0	12.8
Free fatty alcohols	n.d.	3.6	n.d.	6.2
Cholesterol	3.7	2.4	2.9	2.2



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# Chemical composition of mullet raw roes



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**Table 1**  
Fatty alcohols composition (%) of roe samples by GC.

Fatty alcohol	Roe 1	Roe 2
14:0	14.4 ± 0.9	7.3 ± 0.2
15:0	1.1 ± 0.1	3.4 ± 0.1
16:0	49.2 ± 2.7	55.9 ± 0.9
18:0	5.8 ± 0.5	7.0 ± 0.3
16:1 n – 7	11.6 ± 0.58	7.5 ± 0.1
18:1 n – 7	4.4 ± 0.3	2.2 ± 0.1
18:1 n – 9	2.1 ± 0.1	3.8 ± 0.2
SFa	70.5 ± 3.8	73.5 ± 10.2
MUFa	18.1 ± 1.0	13.3 ± 0.6

SFa, saturated fatty alcohols; MUFa, monounsaturated fatty alcohols.  
Mean and standard deviation of six samples.



**Table 2**  
Fatty acids composition (%) of roe samples by GC.

Fatty acid	Roe 1	Roe 2
12:0	0.03 ± 0.01	0.02 ± 0.00
14:0	1.81 ± 0.03	1.61 ± 0.02
15:0	0.15 ± 0.01	0.55 ± 0.01
16:0	10.30 ± 0.29	11.96 ± 0.24
18:0	3.52 ± 0.28	3.21 ± 0.28
16:1 n – 7	14.70 ± 0.18	20.59 ± 0.12
18:1 n – 7	7.45 ± 0.07	6.72 ± 0.12
18:1 n – 9	7.71 ± 0.06	17.94 ± 0.12
16:2	1.74 ± 0.05	1.38 ± 0.04
16:3	1.06 ± 0.09	1.68 ± 0.02
16:4	0.81 ± 0.15	0.32 ± 0.20
18:2 n – 6	1.33 ± 0.10	0.94 ± 0.06
18:3 n – 3	0.47 ± 0.02	0.43 ± 0.03
18:3 n – 6	0.42 ± 0.04	0.34 ± 0.02
18:4 n – 3	2.29 ± 0.06	0.25 ± 0.02
20:3 n – 3	0.04 ± 0.01	0.06 ± 0.01
20:3 n – 6	0.22 ± 0.03	0.40 ± 0.03
20:4 n – 6	0.76 ± 0.04	3.37 ± 0.11
20:5 n – 3	12.75 ± 0.15	4.33 ± 0.02
22:4 n – 6	0.26 ± 0.05	0.59 ± 0.05
22:5 n – 3	8.06 ± 0.05	4.31 ± 0.10
22:6 n – 3	12.93 ± 0.18	8.29 ± 0.14
SFA	15.80 ± 0.48	17.35 ± 0.49
MUFA	29.87 ± 0.20	45.25 ± 0.14
PUFA	43.11 ± 0.33	26.68 ± 0.33

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.  
Mean and standard deviation of six samples.

**Mullet roe lipids** represent an important natural health source of long chain omega-3 (or n-3) polyunsaturated fatty acids (n-3 PUFA), in particular eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), 5–10 mg/g and 10–15 mg/g of fresh weight, respectively (17–21% of total fatty acids)





# Chemical composition of mullet raw roes

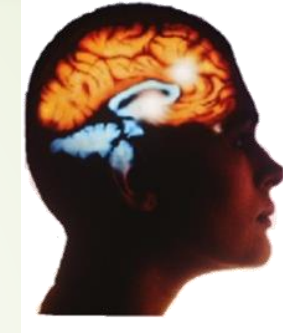


Long chain omega-3 (or n-3) polyunsaturated fatty acids (n-3 PUFA), **eicosapentaenoic acid** (EPA, 20:5 n-3) and **docosahexaenoic acid** (DHA, 22:6 n-3), have an important role in human health:

- cardiovascular disease prevention
- tumor growth and metastasis decrease
- anti-inflammatory activity
- prevention of age-related cognitive decline

U. Gogus and C. Smith, *Int. J. Food Sci. Technol.*, 2010, 45, 417–436

G. Calviello et al., *Biomed. Res. Int.*, 2013, 2013, 743171

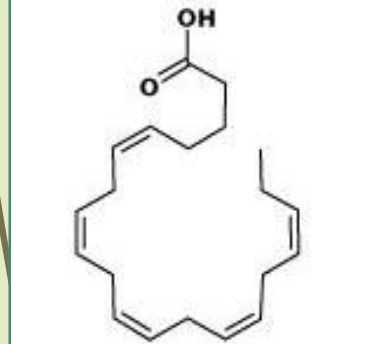


Humans have a very limited ability to synthesize these fatty acids from the essential precursor  **$\alpha$ -linolenic acid (18:3 n-3)**

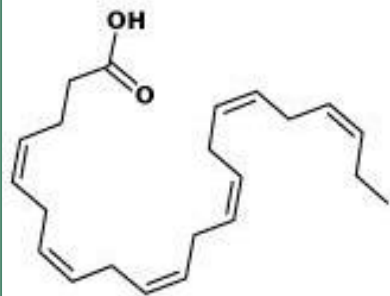
Dietary intake of these functional constituents



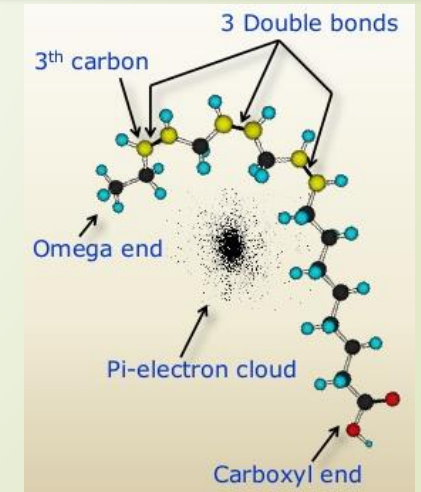
a key aspect of human nutrition



Eicosapentaenoic acid  
EPA (20:5n-3)



Docosahexaenoic acid  
DHA (22:6n-3)





# Chemical composition of mullet bottarga

- Proteins** 35-57%  
(fresh weight)
- Lipids** 25-30%
- Moisture** 17-31%
- NaCl** 5-8%
- Ash** 5-9%

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Chemistry and Physics of Lipids 151 (2008) 69–76

CPL  
CHEMISTRY AND PHYSICS OF LIPIDS  
www.elsevier.com/locate/chemphyslip

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Research Paper

**NMR study of the lipid profile of mullet raw roe and bottarga**

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The lipid class composition of whole or grated **bottarga** reflects that of raw roes

## Whole bottarga

- Wax 60-65%
- Phosphatidylcholine 7-10%
- Triacylglycerols 4-8%
- **Free fatty acids 13-19%**
- Free fatty alcohols 4-6%
- Free cholesterol 2-3%
- Cholesteryl esters 0.3%



## Grated bottarga

- Wax 51-66%
- Phosphatidylcholine 6-7%
- Triacylglycerols 7-10%
- **Free fatty acids 16-29%**
- Free fatty alcohols 2%
- Free cholesterol 2-4%
- Cholesteryl esters 1.2%

Lipids **230-325** mg/g  
Cholesterol 7-9 mg/g of edible portion (3% of total lipids)

Lipids **264-344** mg/g  
Cholesterol 9-11 mg/g of edible portion (2–4% of total lipids)

Higher content of FFA due to hydrolysis processes induced by manufacturing procedures

Bottarga samples show higher values of lipid components per edible portion with respect to fresh roes, due to the lower moisture level

AROMI ADDITIVI SEMILAVORATI

**INGREDIENTI ALIMENTARI**

STUDIO ANALITICO DELLA COMPONENTE LIPIDICA DELLA BOTTARGA DI MUGGINE







# Chemical composition of mullet bottarga

**Table 2**  
Fatty alcohols composition (%) of bottarga products by GC

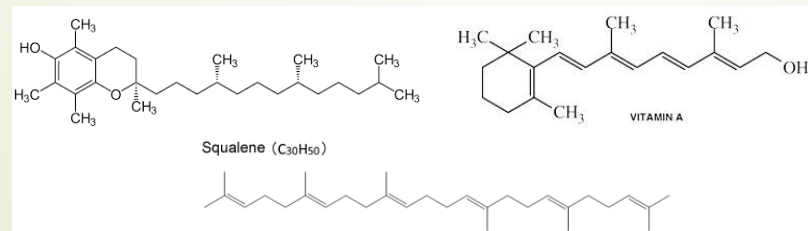
Fatty alcohol	Product 1	Product 2
14:0	10.9 ± 0.1	7.3 ± 0.1
15:0	3.1 ± 0.0	3.3 ± 0.2
16:0	52.8 ± 0.4	51.7 ± 0.6
18:0	6.8 ± 0.1	7.9 ± 0.1
16:1 n-7	10.4 ± 0.1	9.4 ± 0.3
18:1 n-7	3.3 ± 0.1	3.6 ± 0.1
18:1 n-9	3.9 ± 0.1	4.4 ± 0.1
SFA	73.6 ± 0.5	70.2 ± 0.5
MUFA	17.7 ± 0.2	17.3 ± 0.4

SFA, saturated fatty alcohols; MUFA, monounsaturated fatty alcohols. Mean and standard deviation over six samples.



Bottarga is also a source of antioxidant compounds (carotenoids,  $\alpha$ -tocopherol, vitamin A and squalene)

Mullet bottarga represents an important natural source of long chain n-3 PUFA, EPA (20:5 n-3) - 10–20 mg/g, and DHA (22:6 n-3) - 18–30 mg/g of each edible portion



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STUDIO ANALITICO DELLA COMPONENTE LIPIDICA DELLA BOTTARGA DI MUGGINE

elevated (n-3)/(n-6) ratio, with important health benefits

**Table 3**  
Fatty acids composition (%) of bottarga products by GC

Fatty acid	Product 1	Product 2
12:0	0.03 ± 0.01	0.04 ± 0.01
14:0	2.07 ± 0.11	1.75 ± 0.06
15:0	0.44 ± 0.08	0.52 ± 0.02
16:0	10.90 ± 0.19	12.69 ± 0.28
18:0	3.18 ± 0.12	3.07 ± 0.07
20:0	0.15 ± 0.07	0.14 ± 0.08
16:1 n-7	17.92 ± 0.43	14.71 ± 0.35
18:1 n-7	7.22 ± 0.20	6.23 ± 0.11
18:1 n-9	10.61 ± 0.13	13.70 ± 0.18
20:1 n-9	0.16 ± 0.02	0.37 ± 0.04
16:2	0.70 ± 0.03	0.56 ± 0.02
16:3	1.46 ± 0.14	0.63 ± 0.18
16:4	1.98 ± 0.45	Trace
18:2 n-6	1.27 ± 0.04	1.58 ± 0.14
18:3 n-3	0.57 ± 0.02	0.97 ± 0.05
18:3 n-6	0.40 ± 0.01	0.38 ± 0.08
18:4 n-3	1.42 ± 0.03	1.16 ± 0.05
20:3 n-3	Trace	0.18 ± 0.03
20:3 n-6	0.25 ± 0.05	0.15 ± 0.00
20:3 n-9	0.11 ± 0.05	0.29 ± 0.15
20:4 n-6	1.69 ± 0.08	2.21 ± 0.10
20:5 n-3	9.40 ± 0.17	6.73 ± 0.31
22:4 n-6	0.49 ± 0.06	0.48 ± 0.14
22:5 n-3	5.95 ± 0.12	3.96 ± 0.14
22:6 n-3	11.62 ± 0.20	15.28 ± 1.03
SFA	16.77 ± 0.27	18.22 ± 0.43
MUFA	35.90 ± 0.32	35.01 ± 0.27
PUFA	37.27 ± 0.36	34.47 ± 1.48

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Mean and standard deviation over six samples.

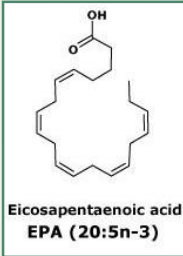
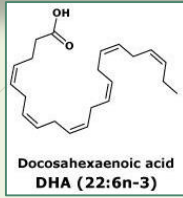


# Oxidative stability

n-3 PUFA are subject to rapid and/or extensive **oxidation** (processing/storage):

- potential alteration in nutritional composition and quality of food
- generation of toxic compounds

Bottarga samples during storage (under domestic or local market/supermarket conditions) are subject to **non-enzymatic browning**, a process with controversial effects on food and human health



- Effect of manufacturing procedures (salting and drying) on mullet roe lipids



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Antonella Rosa<sup>a,\*</sup>, Paola Scano<sup>b</sup>, M. Paola Melis<sup>a</sup>, Monica Deiana<sup>a</sup>, Angela Atzeri<sup>a</sup>, M. Assunta Dessì<sup>a</sup>

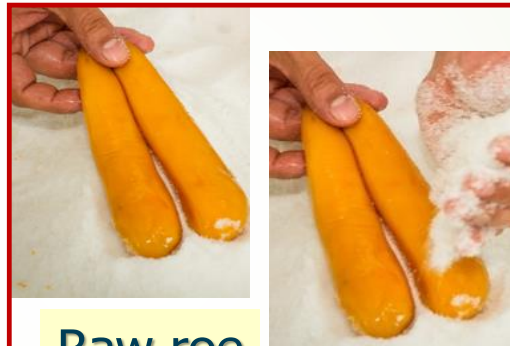
<sup>a</sup> Dipartimento di Biologia Sperimentale di Sezione Patologia Sperimentale, Università degli Studi di Cagliari, Cittadella Universitaria, SS 554, Km 4.5, 09042 Monserrato (CA), Italy

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## Effect of Storage Conditions on Lipid Components and Color of *Mugil cephalus* Processed Roes

Antonella Rosa, Paola Scano, Angela Atzeri, Monica Deiana, Simone Mereu, and M. Assunta Dessì

Journal of Food Science A Publication of the Institute of Food Technologists



Raw roe



Salted and dried roe

Research Article

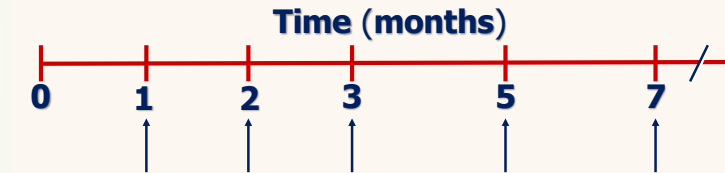
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(wileyonlinelibrary.com) DOI 10.1002/mrc.3819

**Modifications of the <sup>1</sup>H NMR metabolite profile of processed mullet (*Mugil cephalus*) roes under different storage conditions**

Paola Scano,<sup>a,\*</sup> Antonella Rosa,<sup>b</sup> Emanuela Locci,<sup>a</sup> Giorgia Manzo<sup>a</sup> and M. Assunta Dessì<sup>b</sup>

## Bottarga

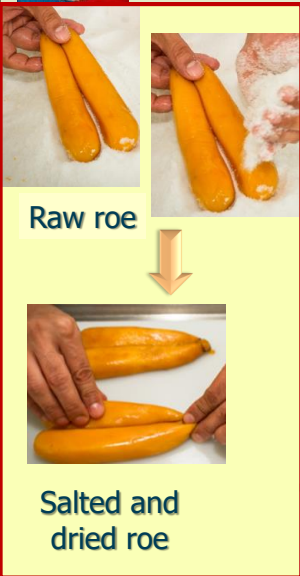


-20 °C  
2-3 °C  
Room temperature/absence of light  
Room temperature/light

- Colour/oxidation of a grated bottarga sample immediately after preparation and during 7 months of storage in different conditions



# Oxidative stability of mullet roes/bottarga



Raw and cured roes exhibited the same level of lipid compounds (cholesterol, fatty alcohols and fatty acids)

The procedure to obtain bottarga did not affect the level of n-3 fatty acids. Bottarga samples showed similar oxidation than raw materials

Major biochemical change observed: increase of free fatty acids in bottarga samples, originated from lipid hydrolysis (salting procedure)

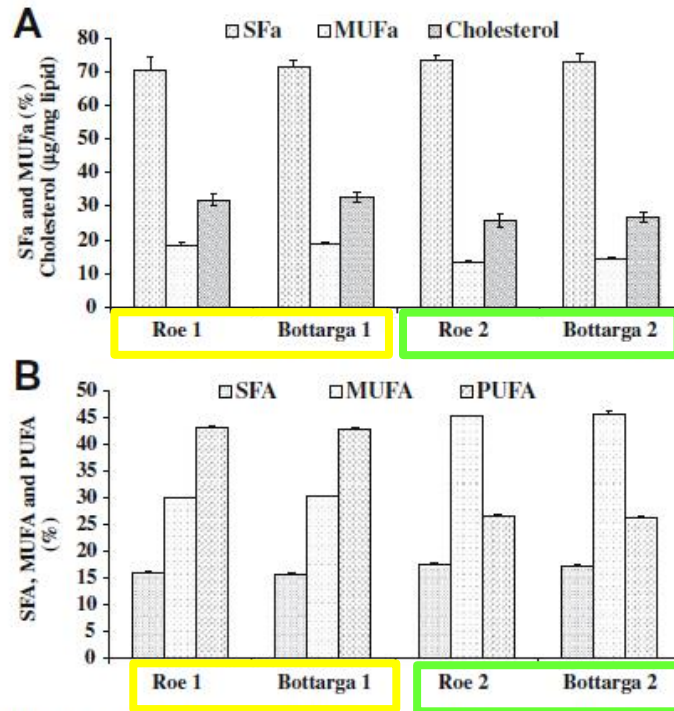


fig. 1. Values of the total saturated (SFA) and monounsaturated (MUFA) fatty alcohols, expressed as percentage of total fatty alcohols (%), cholesterol (µg/mg lipid) (A), the total saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids (% total fatty acids) (B), measured in mullet raw roes and cured samples; (n = 6).

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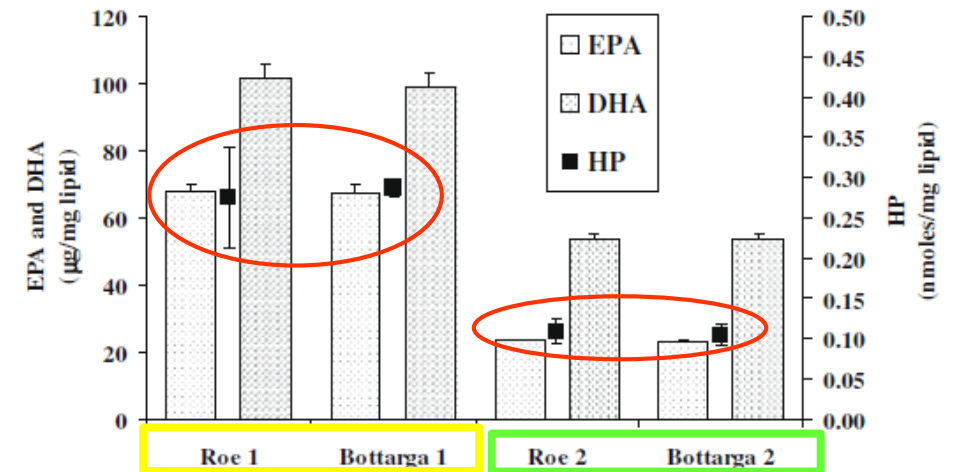
journal homepage: www.elsevier.com/locate/foodchem

Oxidative stability of lipid components of mullet (*Mugil cephalus*) roe and its product "bottarga"

Antonella Rosa<sup>a,\*</sup>, Paola Scano<sup>b</sup>, M. Paola Melis<sup>a</sup>, Monica Deiana<sup>a</sup>, Angela Atzeri<sup>a</sup>, M. Assunta Dessì<sup>a</sup>

<sup>a</sup> Dipartimento di Biologia Sperimentale di Sezione Patologia Sperimentale, Università degli Studi di Cagliari, Cittadella Universitaria, SS 554, Km 4.5, 09042 Monserrato (CA), Italy

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Mullet roe n-3 PUFA show a high oxidative stability during processing

A significant amount of n-3 PUFA in mullet roe are **WE components**. WE enriched in n-3 fatty acids have a low degree of susceptibility to oxidation

# Oxidative stability of mullet roes/bottarga

## Effect of Storage Conditions on Lipid Components and Color of *Mugil cephalus* Processed Roes

Antonella Rosa, Paola Scano, Angela Atzeri, Monica Deiana, Simone Mereu, and M. Assunta Dessì

Journal of Food Science

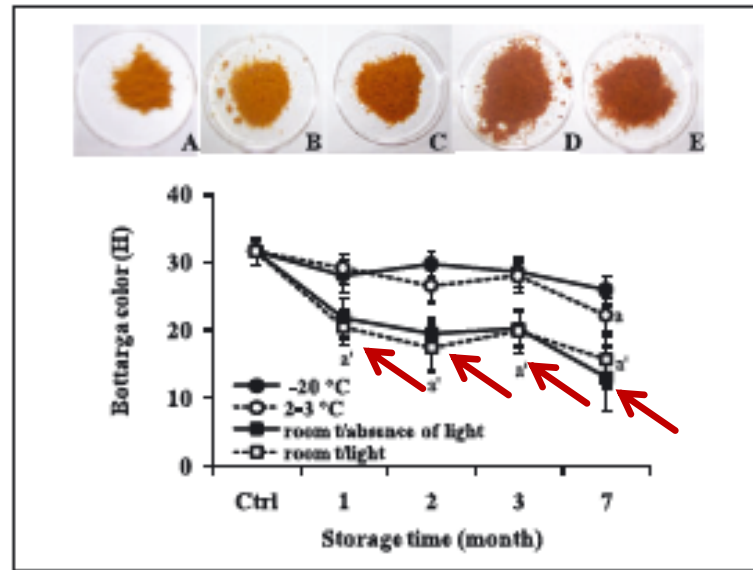


Figure 1—Color, expressed as H values, measured in the bottarga control sample (Ctrl) and samples stored for 1, 2, 3, and 7 mo at  $-20\text{ }^{\circ}\text{C}$ , 2 to  $3\text{ }^{\circ}\text{C}$ , room temperature in the absence and in the presence of light.  $a = P < 0.001$ ;  $a' = P < 0.01$  for both refrigerated temperatures versus Ctrl ( $n = 12$ ). Digital images of the bottarga control sample (A) and samples stored for 7 mo at  $-20\text{ }^{\circ}\text{C}$  (B), 2 to  $3\text{ }^{\circ}\text{C}$  (C), room temperature in the absence (D) and in the presence of light (E) are also reported on the upper side of the figure.

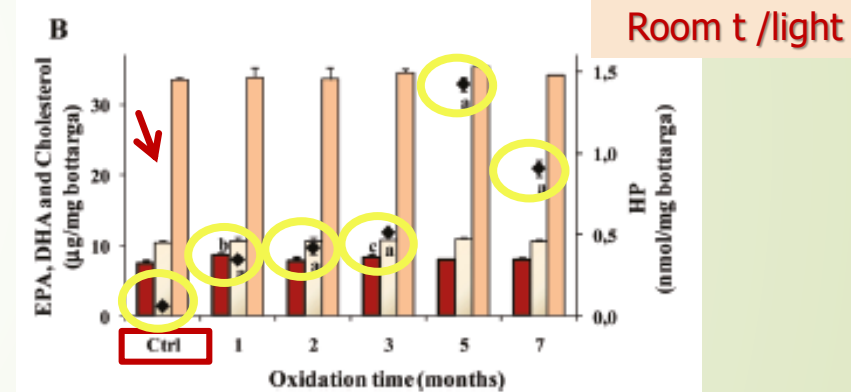
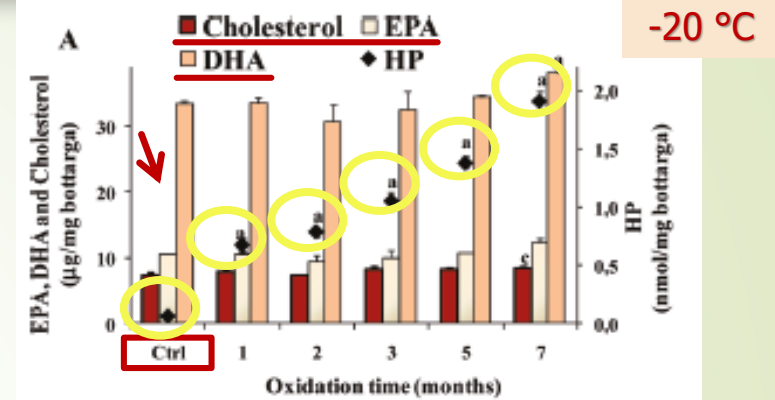


Figure 3. Values of 20:5  $n-3$  (EPA), 22:6  $n-3$  (DHA), cholesterol (expressed as  $\mu\text{g}/\text{mg}$  of edible portion), and conjugated diene fatty acid hydroperoxides (HP) (nmol/mg of edible portion) measured in the bottarga control sample (Ctrl) and after 1, 2, 3, 5, and 7 months of storage at (A)  $-20\text{ }^{\circ}\text{C}$  and (B) room temperature under light exposure.  $a, p < 0.001$ ;  $b, p < 0.01$ ; and  $c, p < 0.05$  versus Ctrl ( $n = 4$ ).

Oxidative stability of lipid components of mullet (*Mugil cephalus*) roe and its product "bottarga"

Antonella Rosa<sup>a,\*</sup>, Paola Scano<sup>b</sup>, M. Paola Melis<sup>a</sup>, Monica Deiana<sup>a</sup>, Angela Atzeri<sup>a</sup>, M. Assunta Dessì<sup>a</sup>

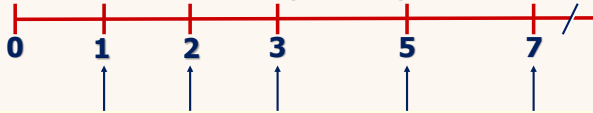
<sup>a</sup>Dipartimento di Biologia Sperimentale e Scienze Patologiche Sperimentale, Università degli Studi di Cagliari, Cittadella Universitaria, SS 554, Km 4.5, 09042 Monserrato (CA), Italy

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### Bottarga



Time (months)



$-20\text{ }^{\circ}\text{C}$

2-3  $^{\circ}\text{C}$

Room temperature/absence of light

Room temperature/light

7 months of storage - Bottarga samples placed at room temperature in the absence and in the presence of light showed a **marked browning process** over time

Different storage conditions did not significantly affect n-3 fatty acids and cholesterol levels (vs Ctrl). Significant **hydroperoxide** increase in all the storage conditions

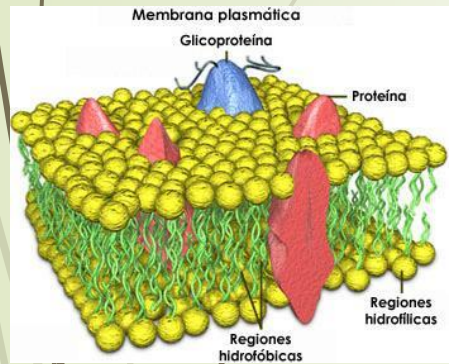
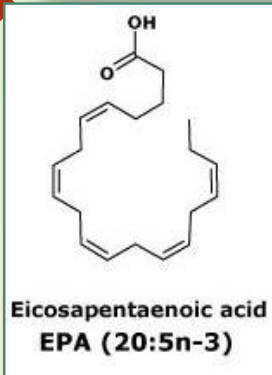
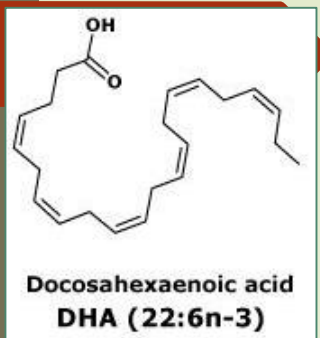
Mullet bottarga is a **stable natural source** of n-3 PUFA – the correct preparation and proper storage (at low temperatures) is essential to preserve bottarga from browning/oxidation



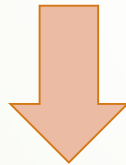
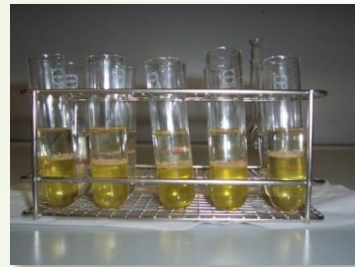
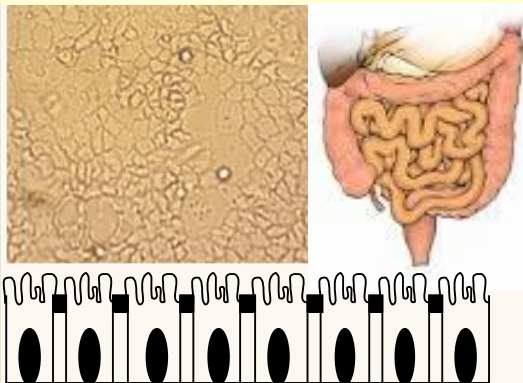


# Nutraceutical properties of mullet bottarga

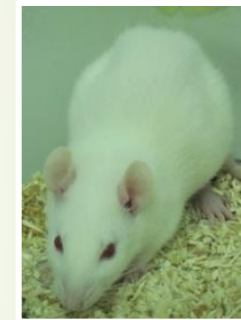
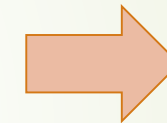
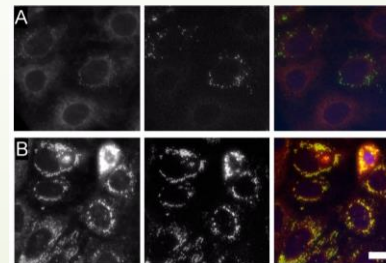
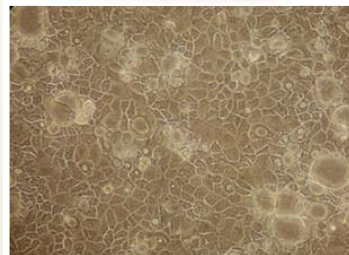
The beneficial health effect of **n-3 PUFA** in humans is strictly correlated to their ability to modify the cell membrane structure, tissue profile and to affect cellular functions



## Intestinal epithelial cells



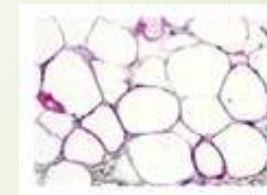
## Cancer cell lines



Brain



Liver



Adipose tissue



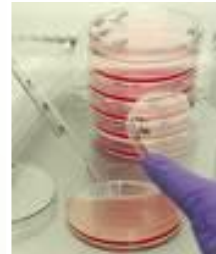
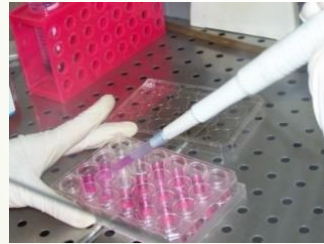
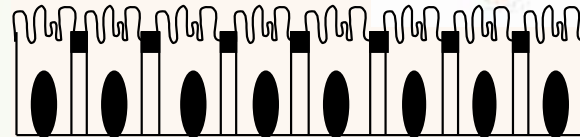
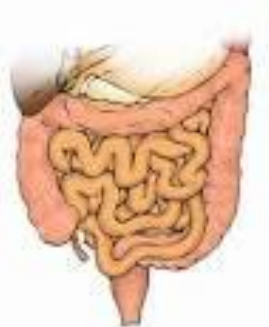
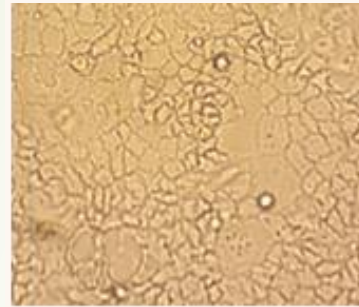
Kidney



Plasma



# Activity in normal cells



## Effect of Aqueous and Lipophilic Mullet (*Mugil cephalus*) Bottarga Extracts on the Growth and Lipid Profile of Intestinal Caco-2 Cells

Antonella Rosa,\* Angela Atzeri, Monica Deiana, M. Paola Melis, Debora Loru, Alessandra Incani, Barbara Cabboi, and M. Assunta Dessi

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MATTIOLI 1885

## PROGRESS IN NUTRITION

Giornale Italiano del Metabolismo e della Nutrizione

LAVORO ORIGINALE

A. ROSA, A. ATZERI,  
M. DEIANA, M.P. MELIS,  
A. INCANI, D. LORU,  
B. CABBOI, M.A. DESSI

Bottarga di muggine come fonte di acidi grassi n-3: stabilità ossidativa e modulazione del profilo lipidico in cellule epiteliali intestinali Caco-2

Differentiated Caco-2 cells are a model of **enterocytes**, extensively used in studies of intestinal absorption and toxicity

Evaluation of **cell viability** and **lipid composition** and **peroxidation** in intestinal cell monolayers after 6-48 h of incubation with bottarga extracts

Lipid/hydrophilic extracts were obtained from bottarga samples with a different browning/oxidation (7 months at -20 °C and room t under light exposure)

Uptake and effect of bottarga extracts in intestinal cells (**monolayers of differentiated Caco-2 cells**)



Caco-2 monolayer



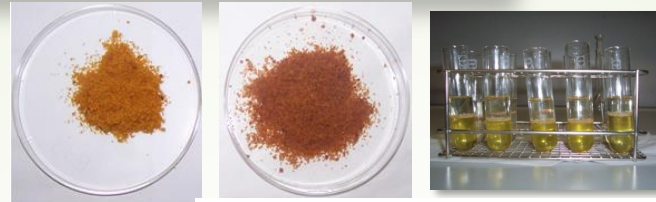


# Activity in normal cells

## Effect of Aqueous and Lipophilic Mullet (*Mugil cephalus*) Bottarga Extracts on the Growth and Lipid Profile of Intestinal Caco-2 Cells

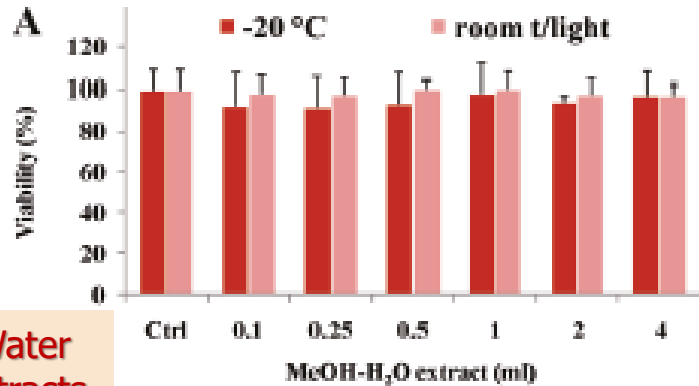
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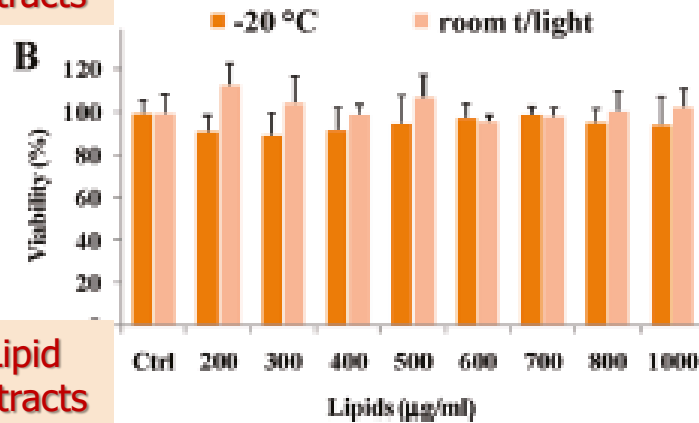


Epithelial cells incubated with bottarga oil showed significant **changes in fatty acid composition** but not in cholesterol levels, with an accumulation of EPA, DHA, and 22:5 n-3

- **bioavailability** of bottarga n-3 PUFA in intestinal cells
- the correct preparation/ proper storage is necessary to reduce oxidation products and preserve the nutritional properties



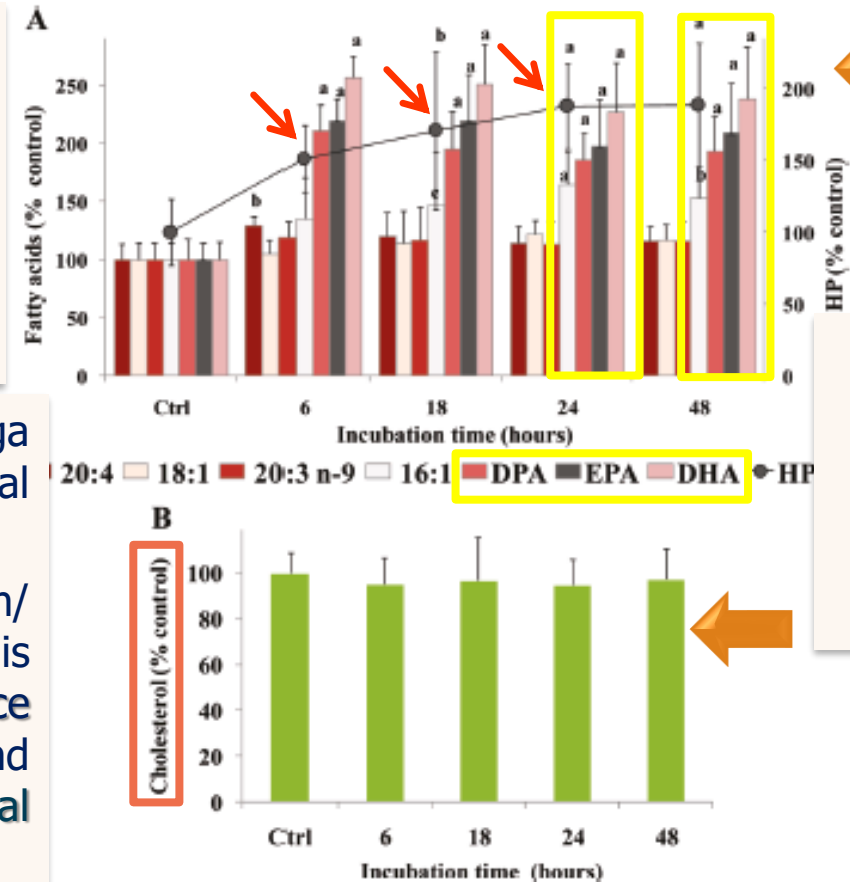
Water extracts



Lipid extracts

Figure 4. Viability, expressed as a percentage of the control, induced by 24 h of incubation with different amounts of the (A) aqueous (MeOH-H<sub>2</sub>O) and (B) lipophilic extracts in the human differentiated Caco-2 cell culture (alamarBlue assay) (n = 9).

No tested bottarga extracts showed a **toxic effect** on intestinal cells (differentiated Caco-2 cells)



Cell hydroperoxides were higher in treated cells, in relation to the **oxidation of bottarga oil**

Figure 5. Values (expressed as a percentage of the control) of (A) fatty acids 20:4, 18:1, 20:3 n-9, 16:1, 22:5 n-3 (DPA), 20:5 n-3 (EPA), 22:6 n-3 (DHA), conjugated diene fatty acid hydroperoxides (HP) and (B) cholesterol measured in differentiated Caco-2 control cells (Ctrl) and after 6, 18, 24, and 48 h of incubation in the presence of the lipid fraction (100 µg/mL) obtained from the bottarga sample stored at -20 °C. a, p < 0.001; b, p < 0.01; c, p < 0.05 versus Ctrl (n = 9).



# Activity in cancer cells

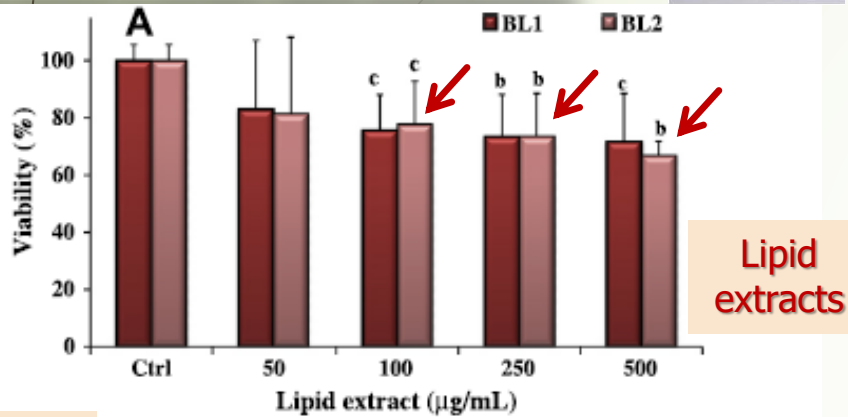
Potential anti-tumor effects of *Mugil cephalus* processed roe extracts on colon cancer cells

Antonella Rosa<sup>a,\*</sup>, Paola Scano<sup>b</sup>, Angela Atzeri<sup>a</sup>, Monica Deiana<sup>a</sup>, Angela Maria Falchi<sup>a</sup>  
<sup>a</sup>Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, SS 554, Km 4.5, 09042 Monserrato, Cagliari, Italy  
<sup>b</sup>Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria, SS 554, Km 4.5, 09042 Monserrato, Cagliari, Italy

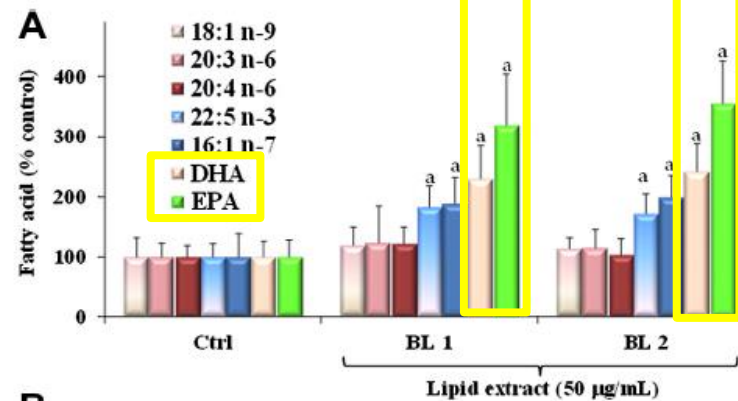
**Lipid/hydrophilic extracts**  
 (7 months of storage at -20 °C and room t under light exposure)



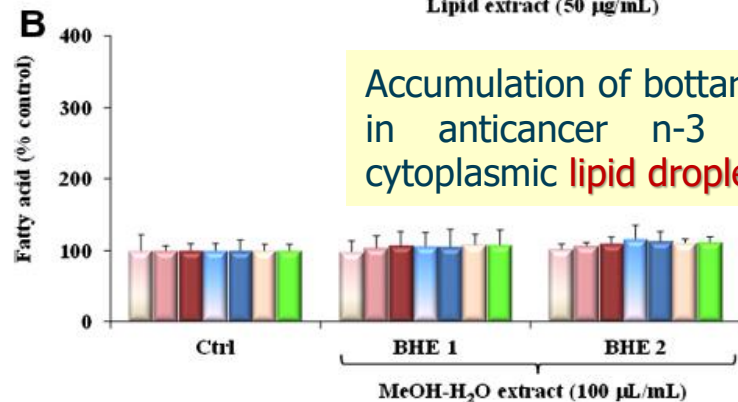
**Colon cancer cells**  
 (undifferentiated Caco-2 cells)



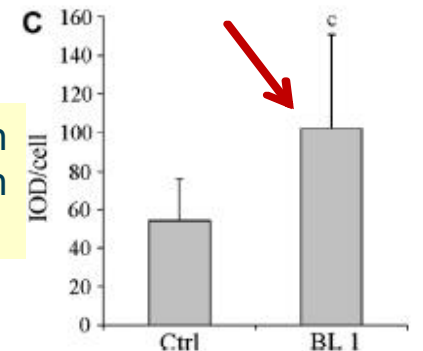
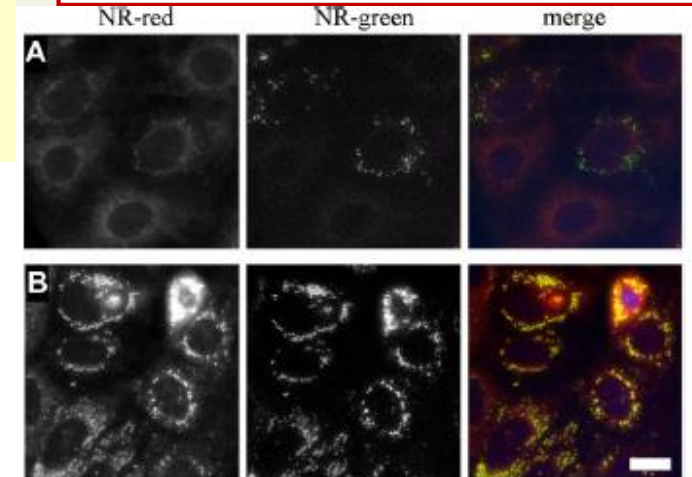
**Lipid extracts**



Accumulation of bottarga lipids, rich in anticancer n-3 PUFA, in cytoplasmic lipid droplets



MeOH-H<sub>2</sub>O extract (100 µL/mL)

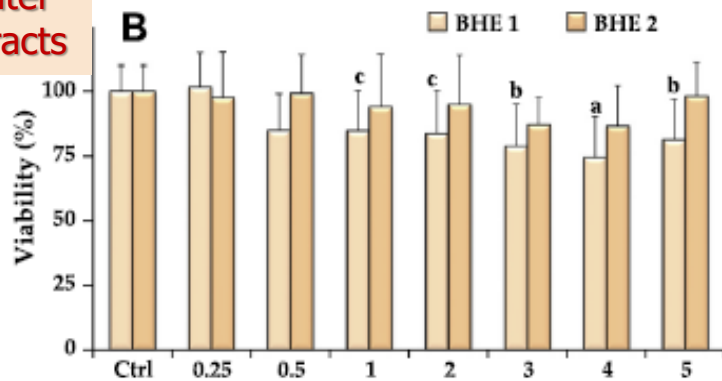


Bottarga lipids have **nutraceutical properties** and potential benefits in colon cancer prevention and treatment



Fig. 6. Values, expressed as % of the control, of the fatty acids 20:4 n-6, 18:1, 20:3 n-6, 16:1 n-7, 22:5 n-3, 20:5 n-3 (EPA), 22:6 n-3 (DHA), measured in cancer Caco-2 control cells (Ctrl) and after 24 h of incubation in the presence of the bottarga lipid (BL 1 and BL 2, 50 µg/mL) (A) and hydrophilic (BHE 1 and BHE 2, 100 µL/mL) (B) extracts.  $\alpha = P < 0.001$  versus Ctrl; (n = 9).

**Water extracts**



Bottarga lipid extracts showed (24 h) a significant toxic effect in cancer cells. Bottarga oil induced marked changes in fatty acid composition (significant accumulation of EPA and DHA)



# Activity in cancer cells

*Mugil cephalus* roe oil obtained by supercritical fluid extraction affects the lipid profile and viability in cancer HeLa and B16F10 cells

A. Rosa,<sup>a\*</sup> A. Piras,<sup>b</sup> M. Nieddu,<sup>a</sup> D. Putzu,<sup>a</sup> F. Cesare Marincola<sup>b</sup> and A. M. Falchi<sup>a</sup>



Supercritical extraction with CO<sub>2</sub>

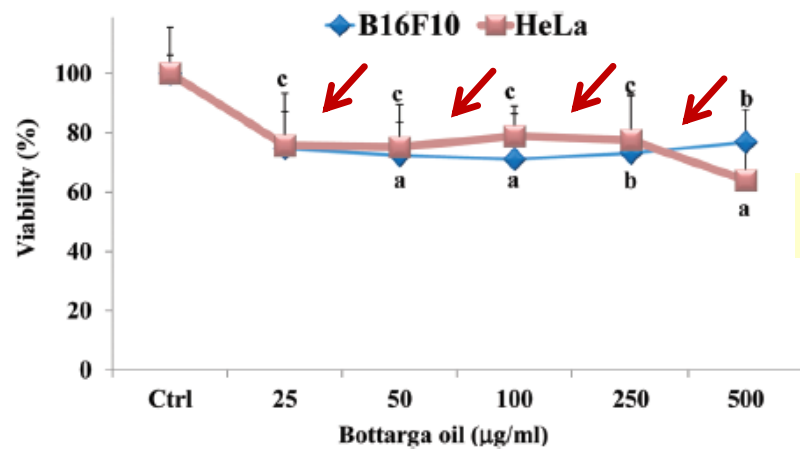
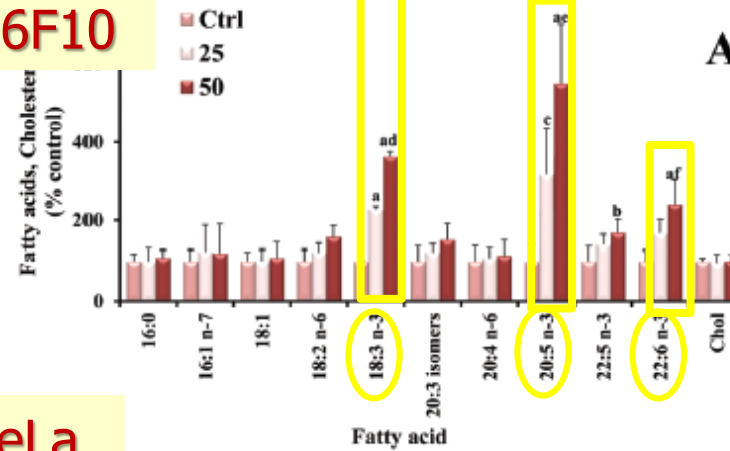


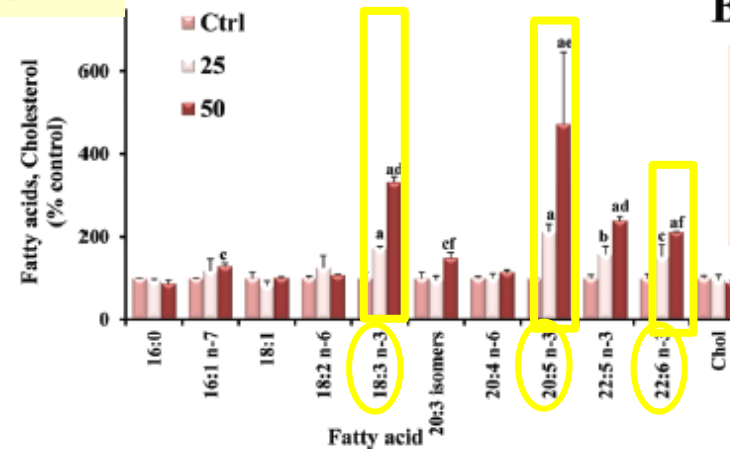
Fig. 4 Viability, expressed as the % of the control, induced by incubation for 24 h with different concentrations of bottarga oil (25–500 µg mL<sup>-1</sup>) in cancer HeLa and melanoma B16F10 cells (MTT assay). Three independent experiments were performed and data were presented as means ± SD, a = p < 0.001, b = p < 0.01, c = p < 0.05 versus Ctrl.

The treatment of murine melanoma cells (B16F10 cells) and human cervical carcinoma cells (HeLa cells), for 24 h with bottarga oil **reduced the viability** and affected the fatty acid profile, with a significant **n-3 PUFA increase** in treated cells

## B16F10



## HeLa



The bottarga oil is absorbed by cancer cells and influences the cell growth and lipid profile, confirming the potential role of this Mediterranean food in **cancer prevention**

Bottarga oil uptake

Influencing the synthesis of intracellular membranes

accumulation of cytoplasmic lipid droplets in cancer cells

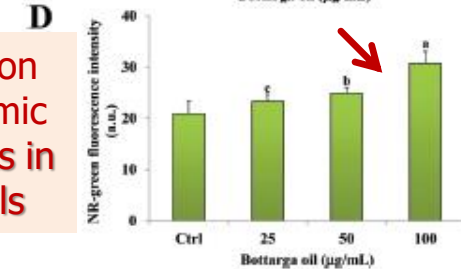
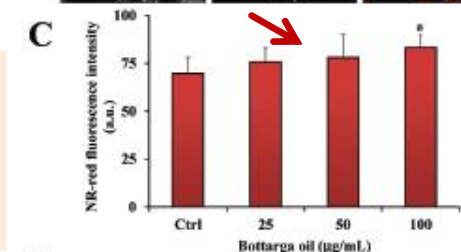
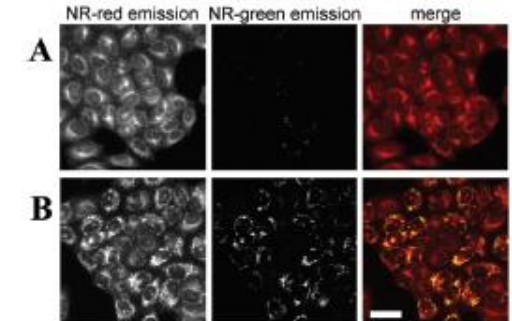


Fig. 8 Representative images of red (cytoplasmic membranes), green (lipid droplets, LD) and merged emissions of untreated-control (A) and oil-treated (100 µg mL<sup>-1</sup>) (B) HeLa cells loaded with Nile red. NR-fluorescent quantification of cytoplasmic membranes (C) and lipid droplets (D) of HeLa cells exposed, for 24 h, to different amounts (25, 50 and 100 µg mL<sup>-1</sup>) of bottarga oil. Results are expressed as means ± standard deviation (SD) of three independent experiments involving duplicate analyses for each sample. a = p < 0.001, b = p < 0.01, c = p < 0.05 versus Ctrl.

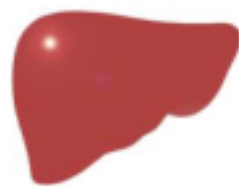
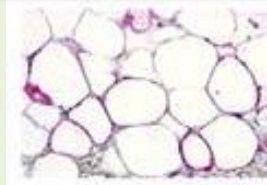
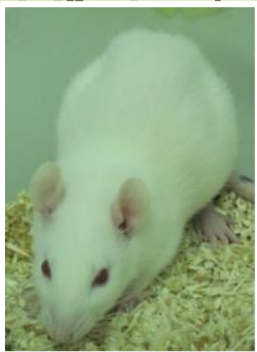
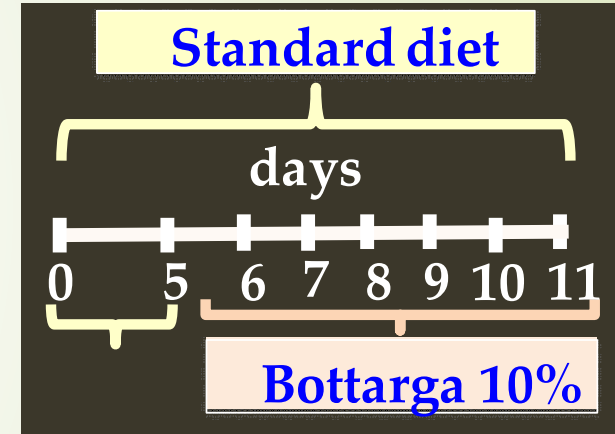


# Animal model



Effect of a **diet** enriched with mullet bottarga (**10%**) on the lipid profile of **plasma, liver, kidney, brain, and adipose tissue** of healthy rats

- short term *in vivo* **bioavailability** of **n-3 PUFA** when given in the form of wax esters
- extent of **DHA** and **EPA** absorption into tissues



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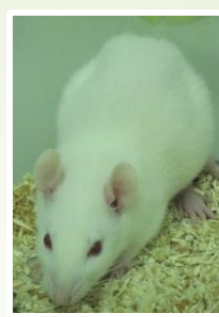
A diet enriched with *Mugil cephalus* processed roes modulates the tissue lipid profile in healthy rats: a biochemical and chemometric assessment

A. Rosa,<sup>a\*</sup> A. Atzeri,<sup>a</sup> D. Putzu<sup>a</sup> and P. Scano<sup>b</sup>





# Animal model



**Table 1** Levels of total lipids (TL) (expressed as mg per g diet), total cholesterol (TC) (mg g<sup>-1</sup>),  $\alpha$ -tocopherol ( $\alpha$ -Toc) ( $\mu$ g g<sup>-1</sup>), and conjugated diene fatty acid hydroperoxides (HP) ( $\mu$ moles per g) measured in control diet (Ctrl diet) and diet supplemented with 10% bottarga (Diet Bott 10%). Data are the means and standard deviations (sd) over 4 samples

Treatment	TL (mg g <sup>-1</sup> )	TC (mg g <sup>-1</sup> )	$\alpha$ -Toc ( $\mu$ g g <sup>-1</sup> )	HP ( $\mu$ moles per g)
Ctrl diet	40.36 $\pm$ 1.25	0.13 $\pm$ 0.01	38.10 $\pm$ 2.65	2.50 $\pm$ 0.78
Diet Bott 10%	32.34 $\pm$ 1.35	0.83 $\pm$ 0.06	38.12 $\pm$ 1.99	1.70 $\pm$ 0.08

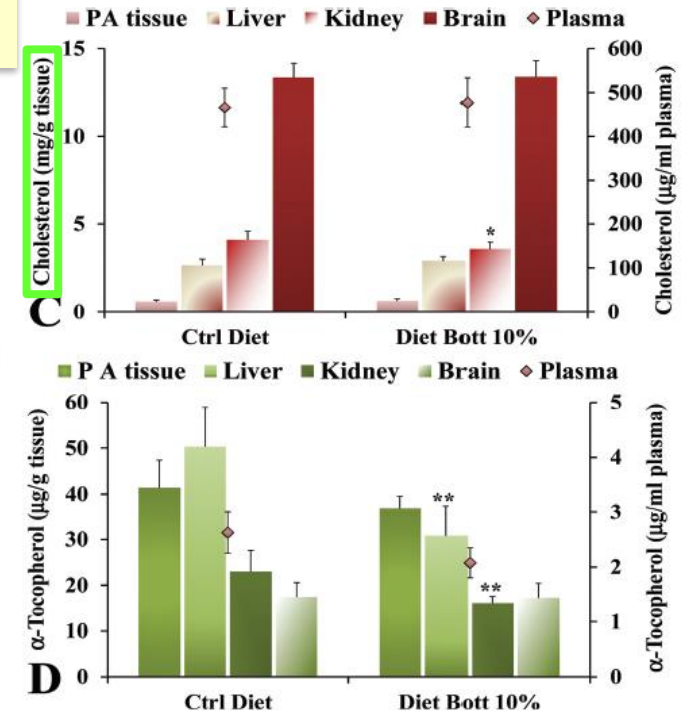
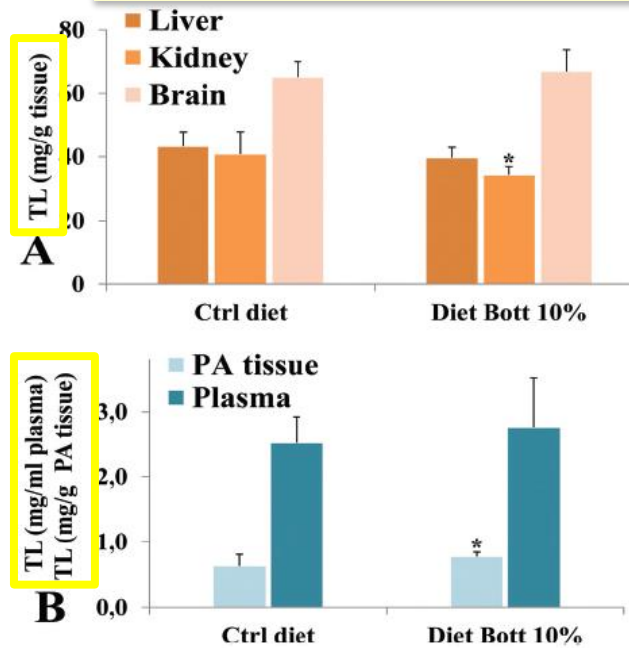
Rats fed a 10% bottarga enriched-diet (5 days) showed **body weights** similar to those of animals fed a control diet

No marked differences in the total lipid amount in tissues of the two groups

Bottarga diet did not result in an increase of total cholesterol levels in all tissues of the experimental rat group

**Table 2** Values (expressed as mg per g diet) of the main unsaturated fatty acids measured in control diet (Ctrl diet) and diet supplemented with 10% bottarga (Diet Bott 10%). Data are the means and standard deviations (sd) over 4 samples

Fatty acid	Ctrl diet (mg g <sup>-1</sup> )	Diet Bott 10% (mg g <sup>-1</sup> )
16:1 n-7	0.15 $\pm$ 0.02	2.43 $\pm$ 0.17
16:2	—	0.11 $\pm$ 0.01
16:3	—	0.08 $\pm$ 0.00
16:4	—	0.04 $\pm$ 0.01
18:1 n-7 + 18:1 n-9	7.30 $\pm$ 0.39	8.77 $\pm$ 0.23
18:2 n-6	15.22 $\pm$ 0.92	14.09 $\pm$ 0.13
18:3 n-3	1.76 $\pm$ 0.08	1.91 $\pm$ 0.07
18:3 n-6	0.01 $\pm$ 0.00	0.09 $\pm$ 0.01
18:4 n-3	0.03 $\pm$ 0.00	0.20 $\pm$ 0.01
20:3 n-3 + 20:3 n-6	0.04 $\pm$ 0.01	0.15 $\pm$ 0.03
20:4 n-6	0.05 $\pm$ 0.00	0.24 $\pm$ 0.02
20:5 n-3	0.25 $\pm$ 0.12	1.85 $\pm$ 0.10
22:5 n-3	—	1.10 $\pm$ 0.21
22:6 n-3	0.59 $\pm$ 0.03	2.15 $\pm$ 0.11



**Fig. 2** Values of the total lipids (TL) measured in liver, kidney, brain (A), plasma, and perirenal adipose (PA) tissue (B) and values of total cholesterol (C) and  $\alpha$ -tocopherol (D) measured in tissues of rats alimented with control diet (Ctrl diet) and diet supplemented with 10% bottarga (Diet Bott 10%). \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ .



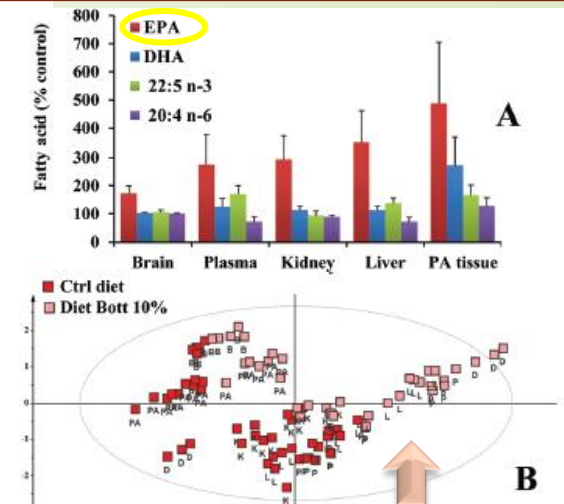
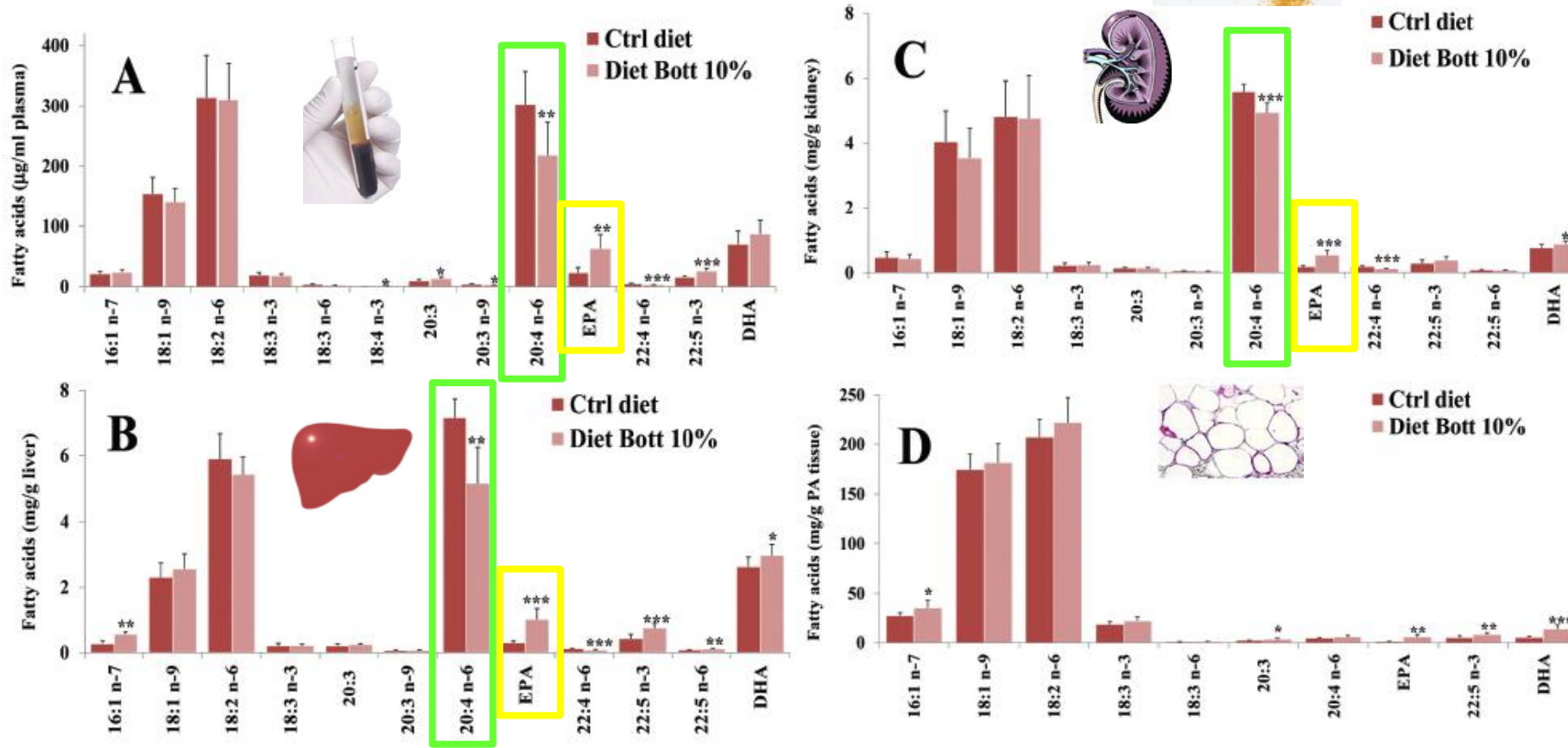
# Animal model



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A diet enriched with *Mugil cephalus* processed roes modulates the tissue lipid profile in healthy rats: a biochemical and chemometric assessment

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Diet enrichment with grated mullet bottarga induced marked changes into the lipid profile of rat tissues and their enrichment with **n-3 PUFA**

Fig. 4 Values of EPA, DHA, 22:5 n-3, and 20:4 n-6 (expressed as % Ctrl group value) measured in the brain, plasma, kidney, liver, and perirenal adipose (PA) tissue of rats alimented with diet supplemented with 10% bottarga (Diet Bott 10%) (A). PLS-DA of HPLC fatty acid data, normalized to 100, for Ctrl diet vs. Diet Bott 10% groups (5 components, R2Y = 0.75, Q2Y = 0.68). Score plot along the first 2 components. B = brain, L = liver, K = kidney, PA = perirenal adipose tissue, P = plasma, D = diet (B), and loading scatter plot (C).

Fig. 3 Values of unsaturated fatty acids measured in the plasma (expressed as  $\mu\text{g ml}^{-1}$ ) (A), liver (B), kidney (C), and perirenal adipose (PA) tissue (D) (expressed as mg per g of tissue) of rats alimented with control diet (Ctrl diet) and diet supplemented with 10% bottarga (Diet Bott 10%) (A). \*\*\* =  $P < 0.001$ ; \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ .

Bottarga supplementation induced a marked increase of **n-3 PUFA**, particularly **EPA**, in all rat tissues, together with a 20:4 n-6 decrease in plasma, liver, and kidney

Mullet bottarga may be considered as a natural bioavailable source of **n-3 PUFA** and qualifies as a traditional food product with **functional properties**



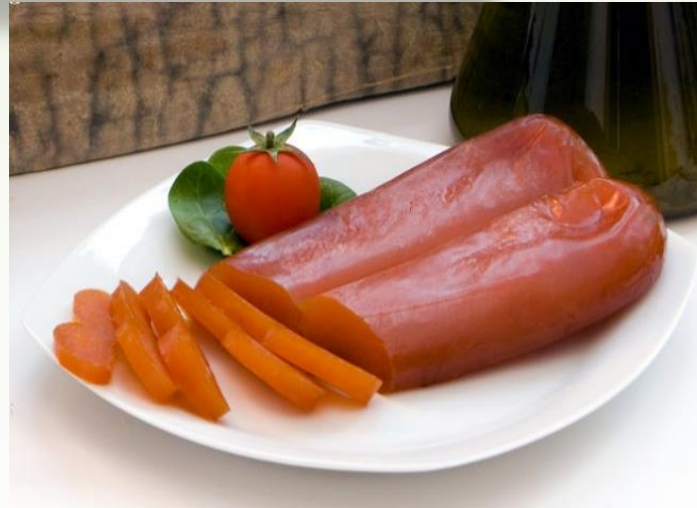




# Conclusion

Mullet bottarga has important **nutritional properties** as a source of:

- Essential amino acids
- Antioxidants
- **n-3 Fatty acids** with beneficial health effects



Low storage temperatures are optimal for preserving bottarga samples from **lipid oxidation** and **browning**



A correct preparation and proper storage is necessary to preserve the **nutritional properties** of this food delicacy

Mullet bottarga qualifies as a traditional food product with **nutritional** and **nutraceutical properties** and **potential health benefits**

THANK YOU  
FOR YOUR  
ATTENTION

