Fish Muscle postmortem change and salting performance - Impact of muscle microstructure on salt transfers

Arno Germond, Christelle Blavignac, Camille Rivard, Laurence Dubreil, Pierre-Sylvain Mirade, Thierry Astruc

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Impact of muscle microstructure on salt transfers.

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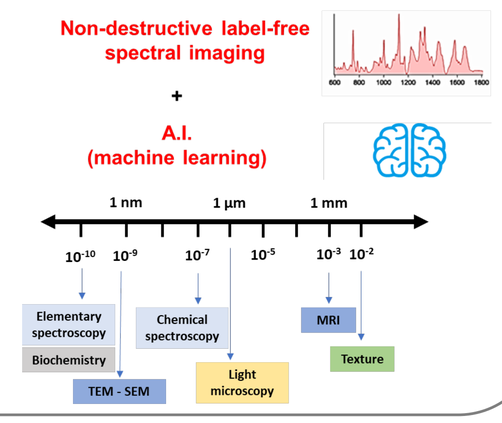
Objectives

This study aimed to characterize fish muscle change with *postmortem* time, and predict the impact of ageing on salt transfers.

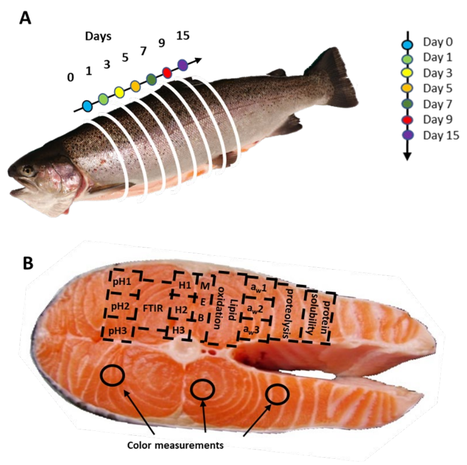


Highlights

- Combinatory approaches of **physicochemical measurements and machine learning** helped characterize the impact of aging and salting in trout muscle.
- Biomarkers accurately **predicted the *postmortem* time of fish** and salt concentrations.
- **Label-free fast characterization** open perspectives to optimize the salting process, improve food quality, and reduce the environmental impacts of salting in the processing industry.

Methods

6 rainbow trouts (3 Kg), from 0 to 15-day *postmortem*
One filet was salted and its contralateral serves as control



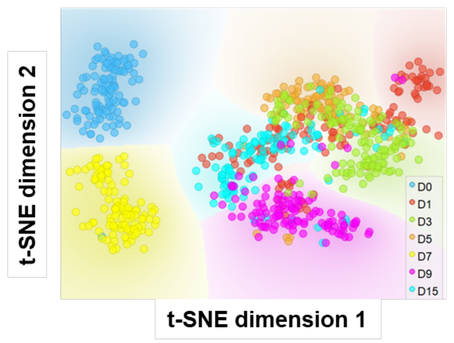
Physico-Chemical Analyses

- pH
- Colour (L, a*, b*)
- Microscopy
- HPLC for Na^+ & Cl^- quantification
- Water activity (a_w)
- Lipid oxidation (T bars)
- Proteolysis
- Solubility

Imaging

- Synchrotron X-ray fluorescence
- MRI of H^+ et Na^{23}
- SEM+ EDS
- TEM
- FTIR
- SHG

Machine learning coupled with FTIR revealed biomarkers of *postmortem* time



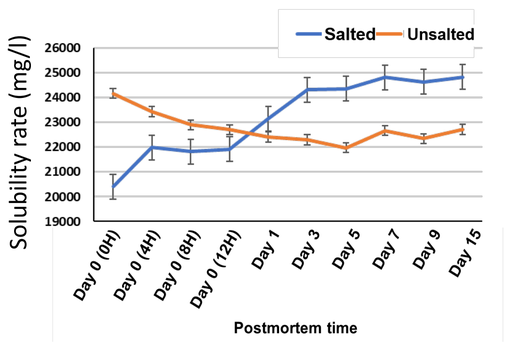
True values \ Predicted values	D0	D1	D3	D5	D7	D9	D15	Σ
D0	100.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	111
D1	0.0 %	93.1 %	0.0 %	0.7 %	0.0 %	0.0 %	22.1 %	122
D3	0.0 %	0.0 %	82.9 %	27.6 %	0.0 %	0.0 %	0.0 %	129
D5	0.0 %	0.0 %	8.1 %	70.9 %	0.0 %	3.6 %	0.8 %	109
D7	0.0 %	0.0 %	0.0 %	0.0 %	100.0 %	0.0 %	0.0 %	114
D9	0.0 %	3.0 %	0.0 %	0.0 %	0.0 %	91.1 %	6.6 %	113
D15	0.0 %	4.0 %	9.0 %	0.7 %	0.0 %	5.4 %	70.5 %	107
Σ	111	101	111	134	114	112	122	805

Wavenumber	Biomarker for	Molecular bond	Biological compounds
1002 cm^{-1}	D0	Phe, Tyr	aromatic compound, protein
1024 cm^{-1}	D0	CO	polysaccharides, glycogen
1172-1176 cm^{-1}	D0	CC of DNA, PO_2 stretch	DNA, PO_2, nucleic acids, carbohydrates
1204-1206 cm^{-1}	D7	amide III	amide III, collagen
1247 cm^{-1}	D0	PO_2^-	nucleic acids (RNA)
1363 cm^{-1}	D0	CH_2, CC	polysaccharides
1469 cm^{-1}	D0	CH_2 bending of lipids	saturated lipids
1490 cm^{-1}	D7	CC, CH	amide I, fatty acids
1553 cm^{-1}	D0	amide II	predominantly α-helix of amide II
1635 cm^{-1}	D0	ß-sheet structures of amide I	ß-sheets, proportion of secondary protein structures (shoulder peak of amide I)
1670 cm^{-1}	D7	ν(CC) trans, antiparallel ß-sheet structures of amide I	antiparallel ß-sheets (shoulder peak of amide I), fatty acids
1712 cm^{-1}	D0	CO	glycogen, lipid oxidation, unsaturated fatty acids (PUFA)
1760 cm^{-1}	D0	CO, COO-R	glycogen

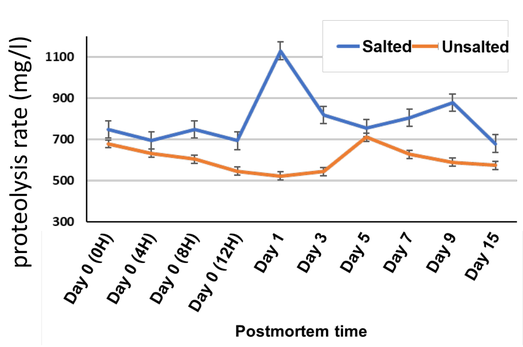
Major physicochemical and structural changes

- **In the absence of salt, biochemical analyses revealed little changes over the *postmortem* time. Surprisingly, the proteolysis index and solubility of proteins varied very little during the *postmortem* time. A fast pH drop was observed soon after stunning and bleeding and it stabilized close to 6,5.**

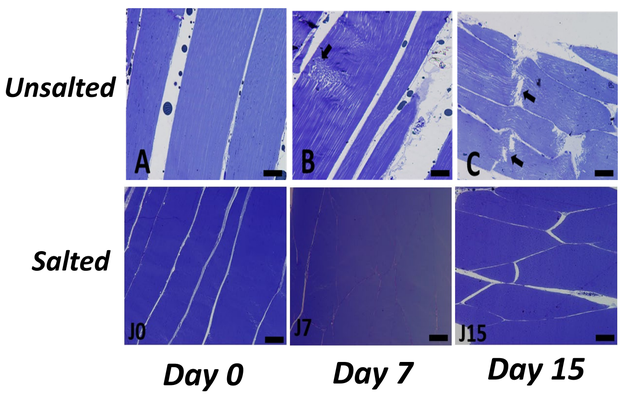
Protein solubility during *postmortem* time



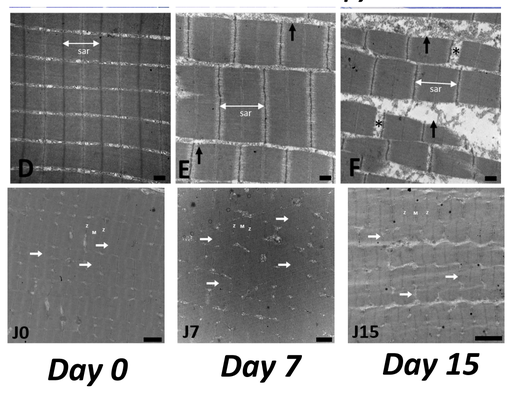
Proteolysis during *postmortem* time

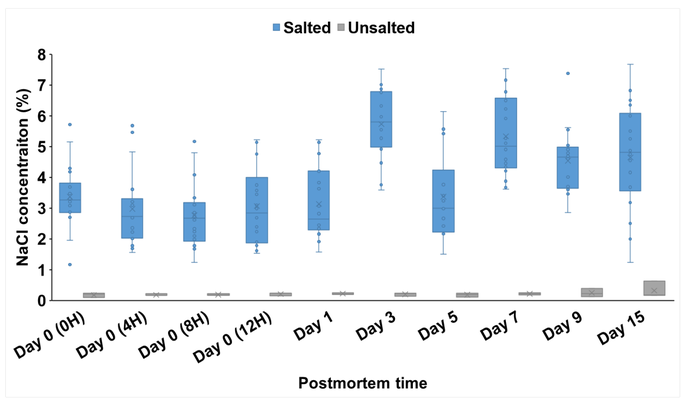


Microstructural changes
Light microscopy



Ultrastructural changes
Electron microscopy



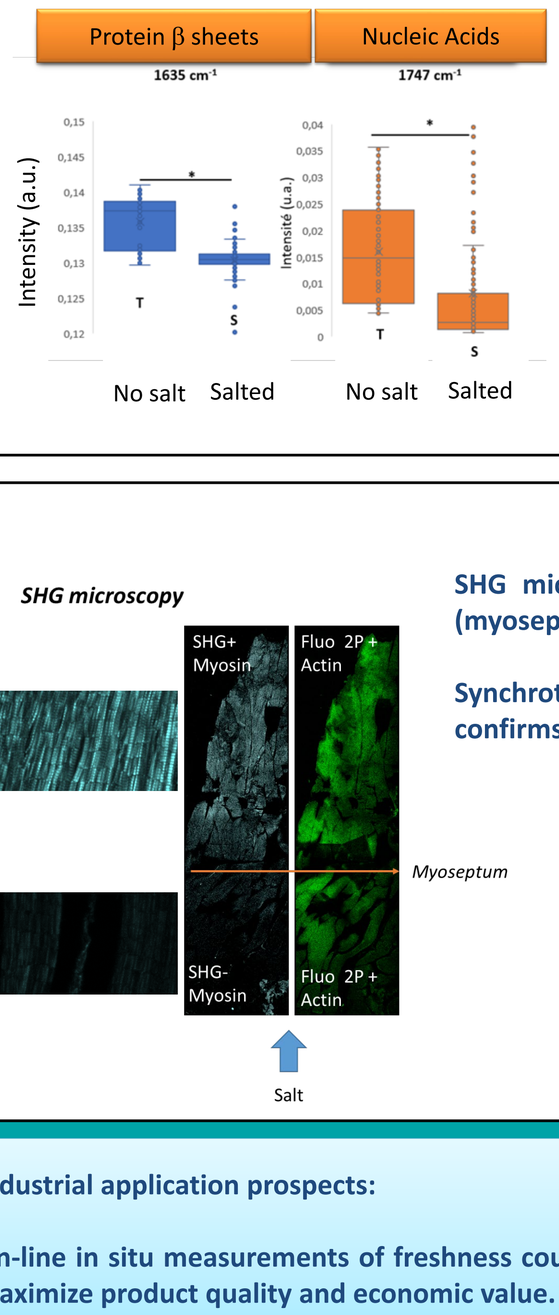


Final salt concentrations in trout muscles increased with advanced *postmortem* times

Salt content increases with *postmortem* time, from 2,5% (salting before 3 days *postmortem*) up to 6% when the filet is salted from 3 days *postmortem*.

European standard imposes a minimum of 3% final salt concentration

Biomarkers toward the fast characterization of filets



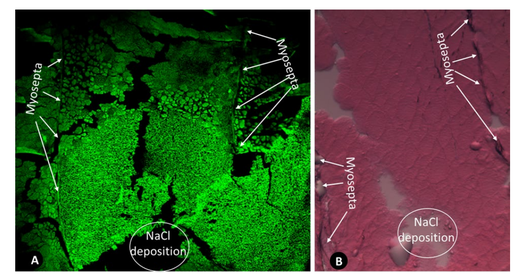
By increasing the ionic strength, salting decreases absorbance at 1635 cm^{-1} and 1747 cm^{-1} assigned to β-sheet structure and nucleic acids respectively

SHG microscopy

SHG microscopy suggests that connective tissue (myoseptum) slows down the diffusion of salt.

Synchrotron X-Ray Fluorescence of Na and Cl confirms the barrier effect of myoseptum

X-ray fluorescence of Na and Cl



Industrial application prospects:

On-line in situ measurements of freshness could be used to adjust salt concentrations to maximize product quality and economic value.