Muscle proteomics as a tool to better understand texture defects in fish

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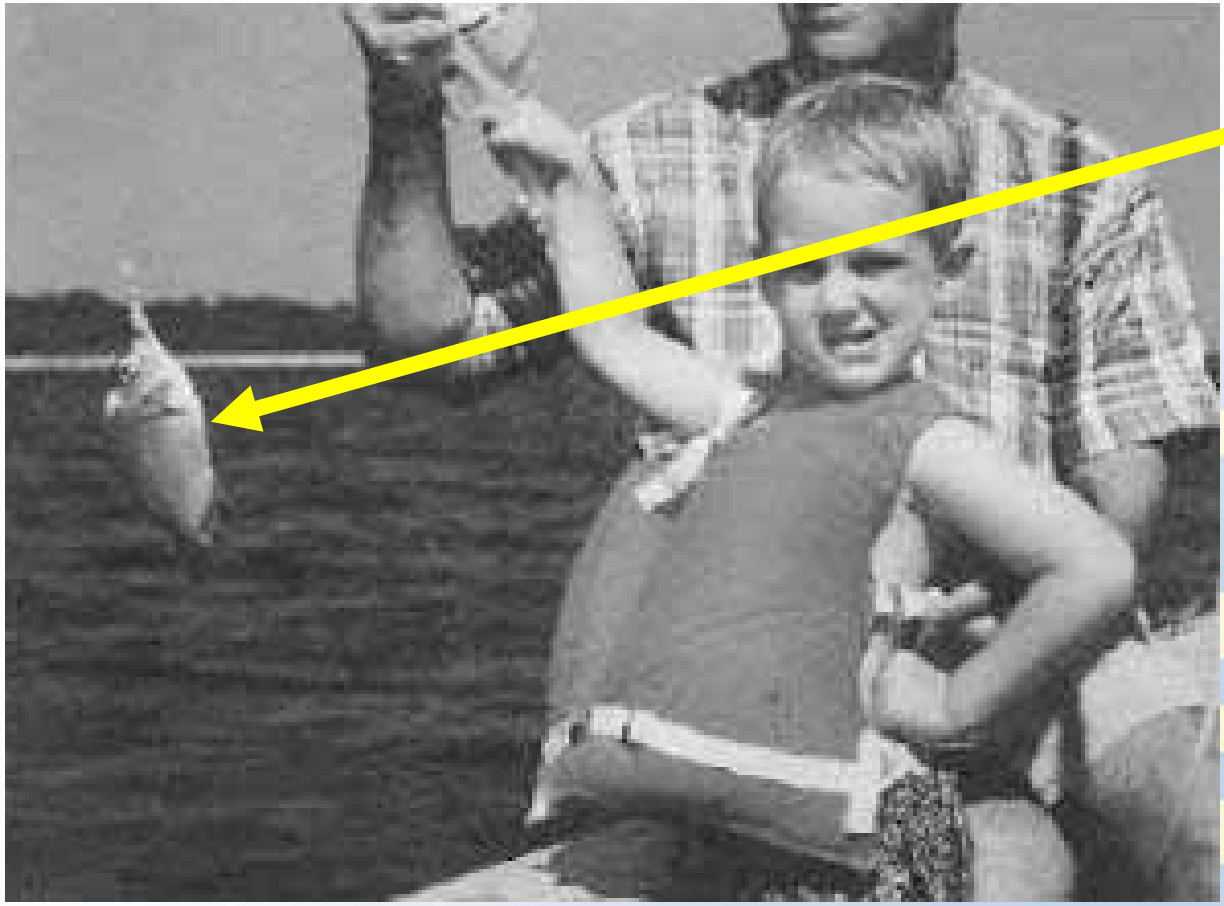
This is not the full presentation as presented at the meeting. It has been edited to preserve confidentiality of new research results, prior to their publication in a scientific journal.

Fish as food

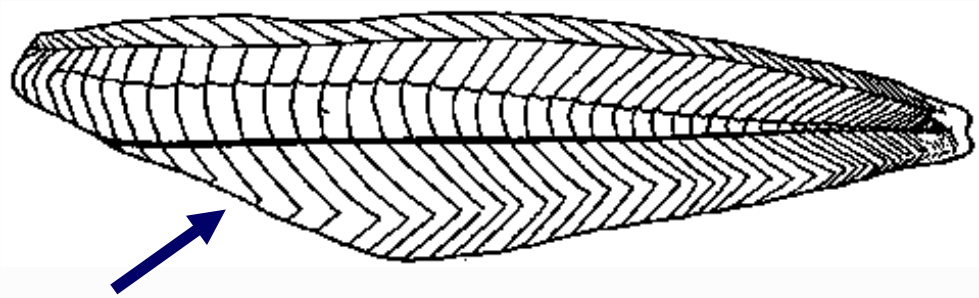


Fresh !

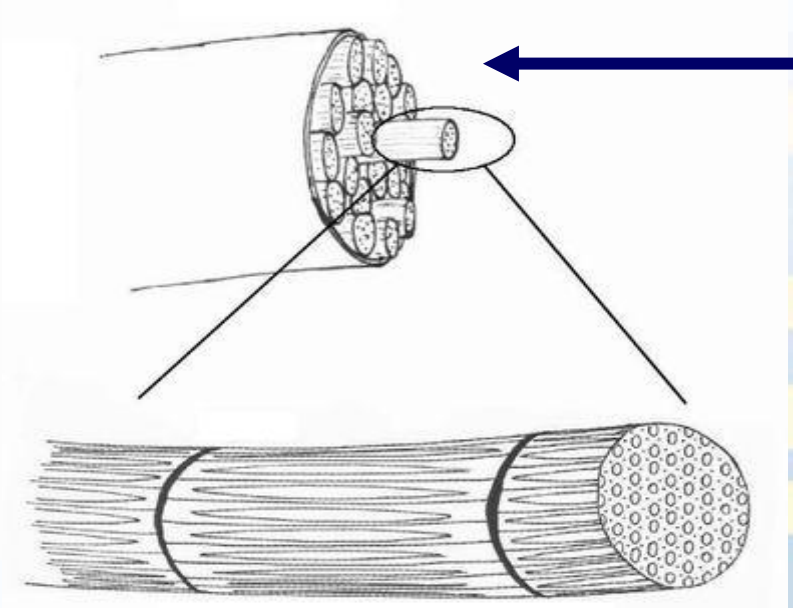
=

firm

elastic



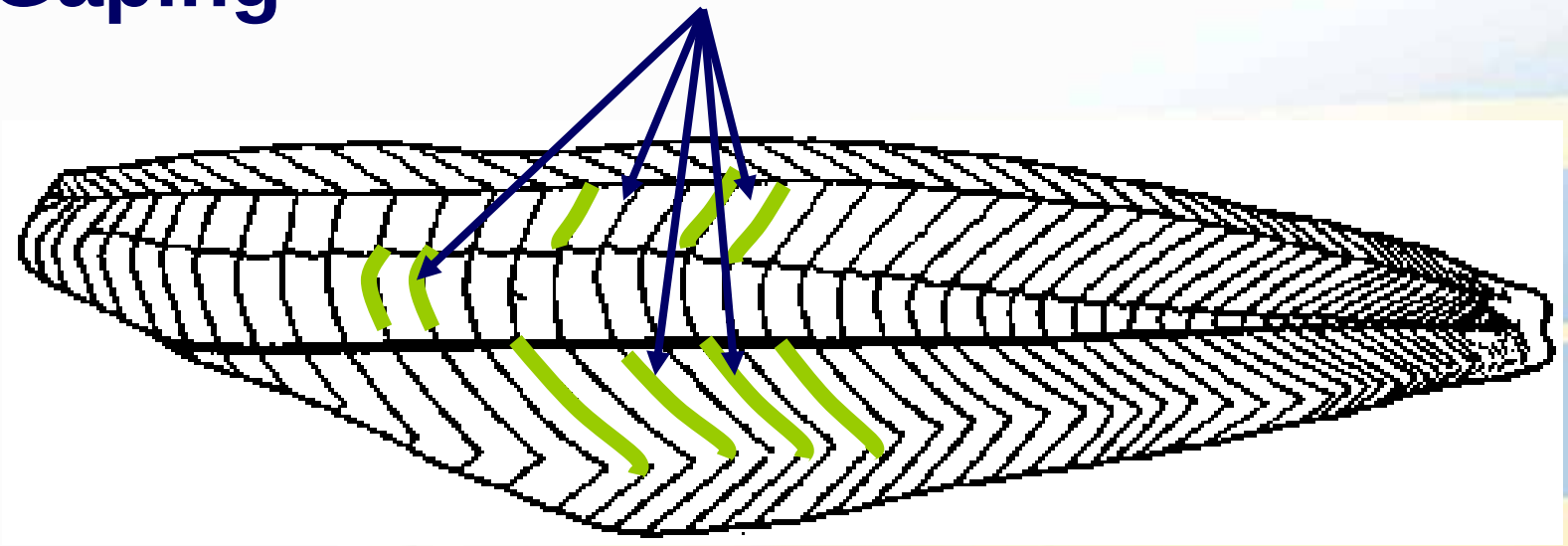
One block = myotome: muscle fibres



one fibre = one cell

Texture defects

Gaping



Connective tissue softening

Texture defects

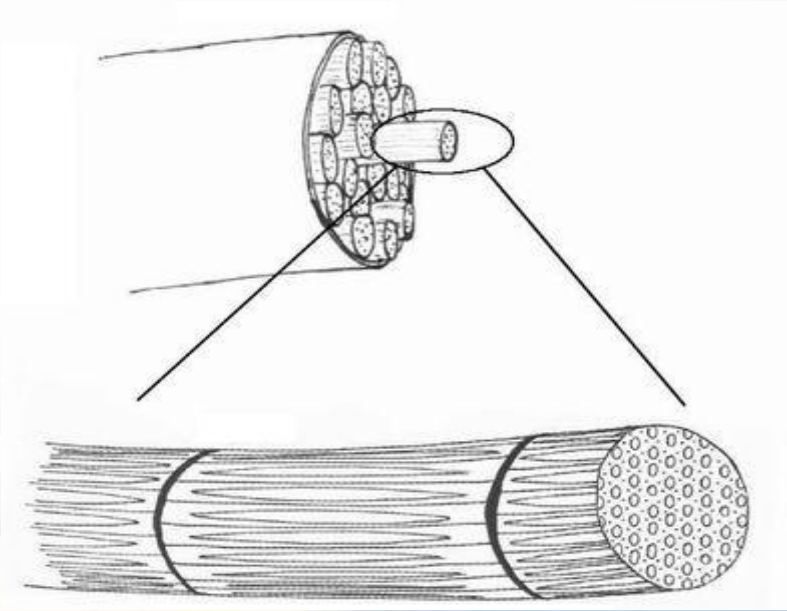
Tough and stringy

Lipid oxidation (e.g. frozen storage)

Protein oxidation - crosslinking

Texture defects

Too soft



Soft texture

Proteolytic enzymes are the main effectors of tissue softening

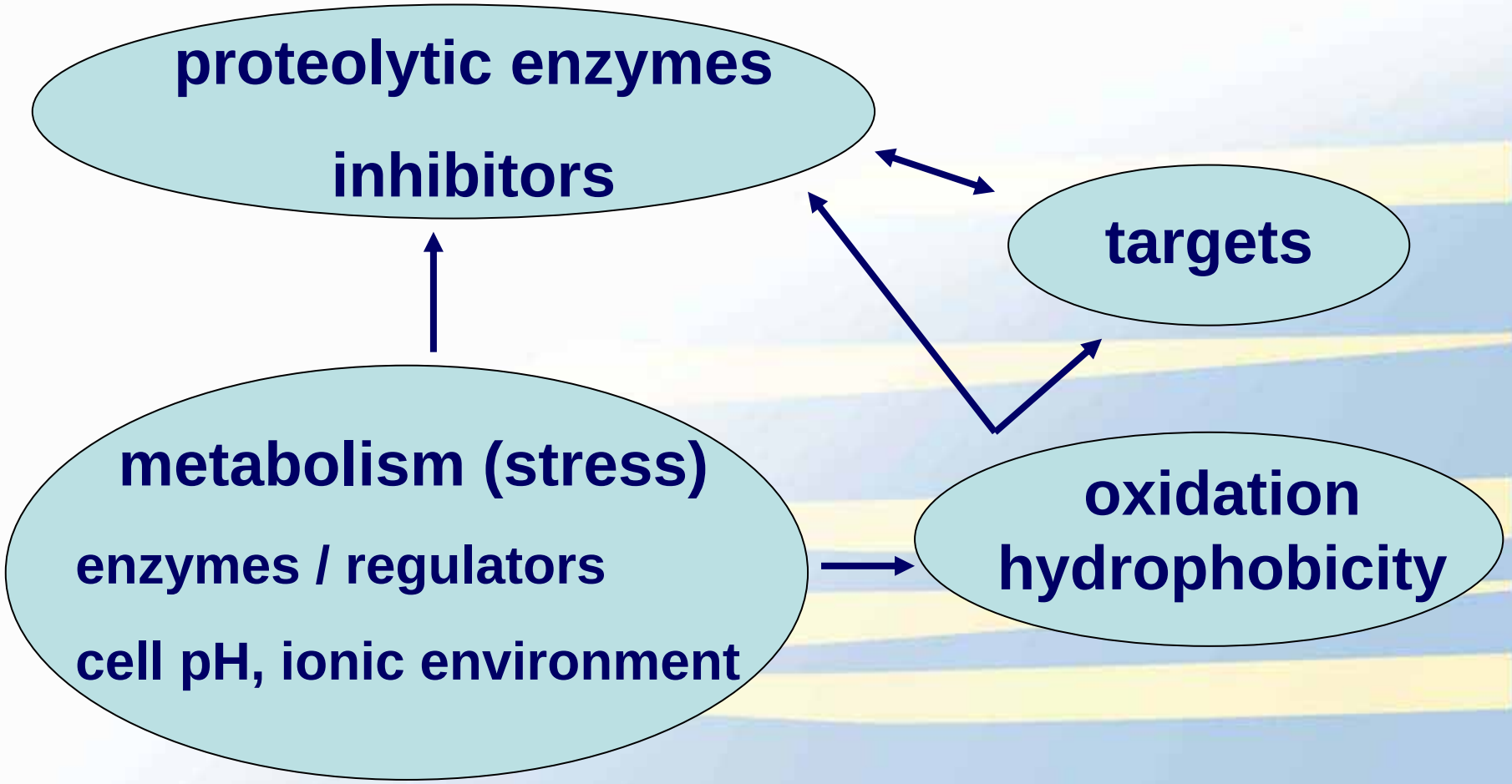
Godiksen et al., WEFTA 2005

Two enzymatic systems:

- **Calpains (fibre-fibre attachment)**
- **Lysosomal cathepsins**

Taylor, 1st SEAFOODplus conference

How are proteins involved?



Proteomics

Proteomics: study of proteome (all the proteins in a cell)

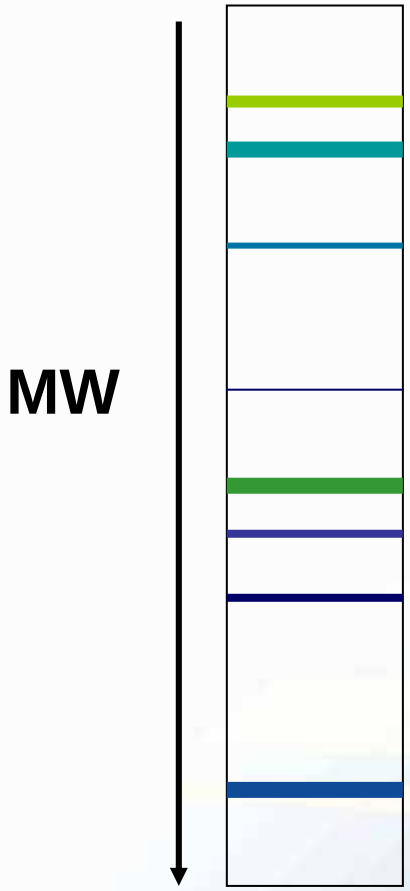
Muscle : ~ 10000 to 100000 protein types

High-resolution separative techniques

Quantification: image analysis

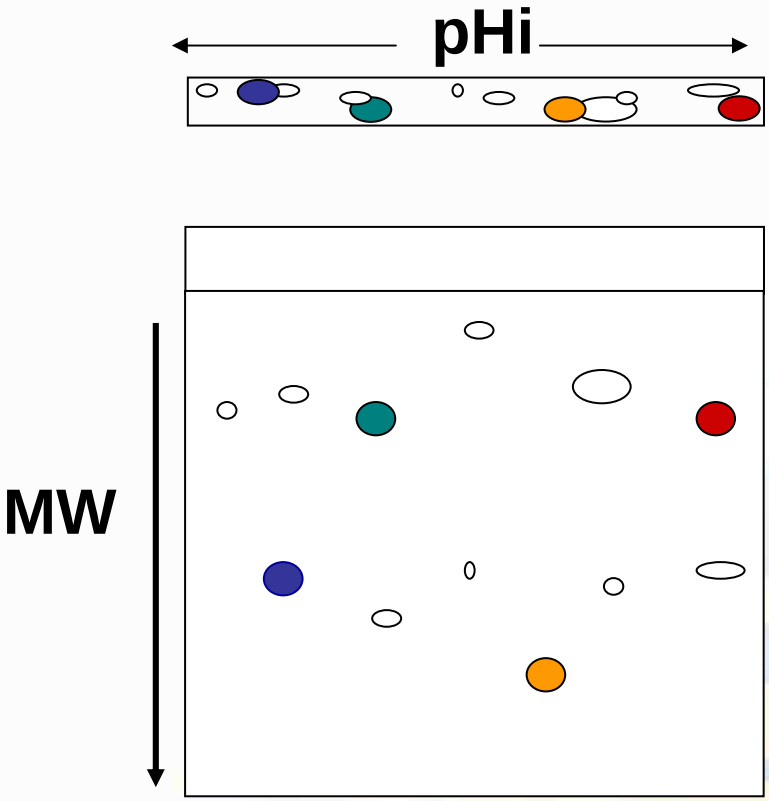
Protein identification: mass spectrometry

Electrophoresis: 1D

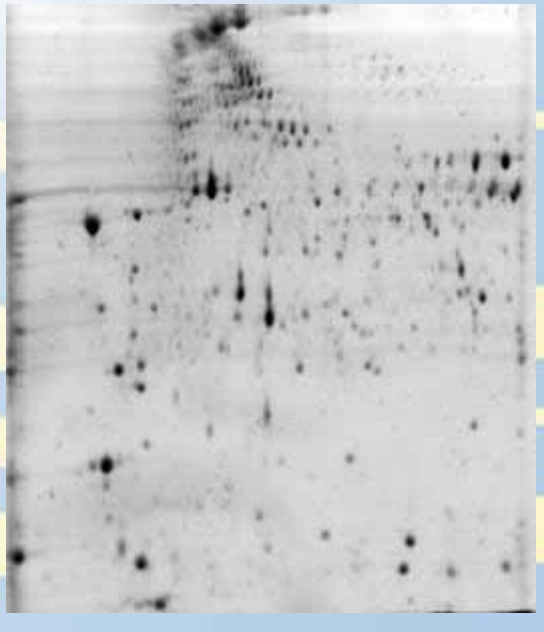


20-50 bands per lane

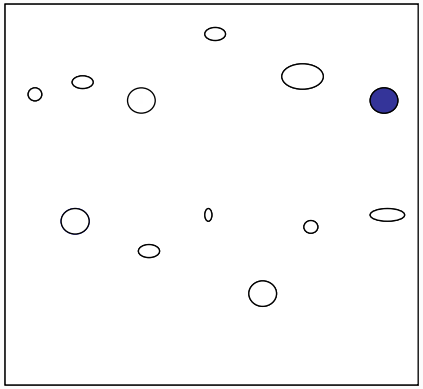
Electrophoresis: 2D

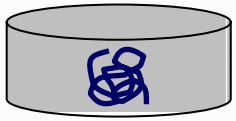


200-800 spots per gel



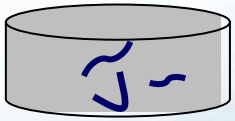
Mass spectrometry





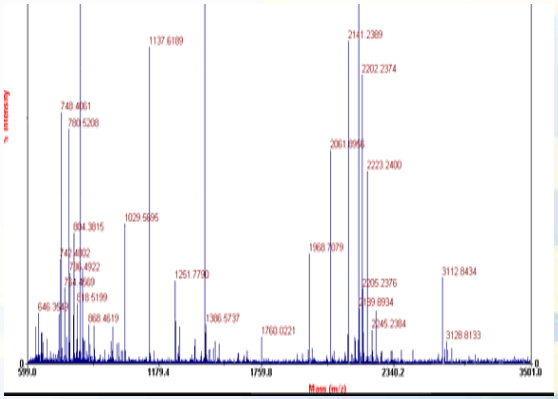
protein

trypsin



peptide residues

mass measurement (MALDI-TOF)



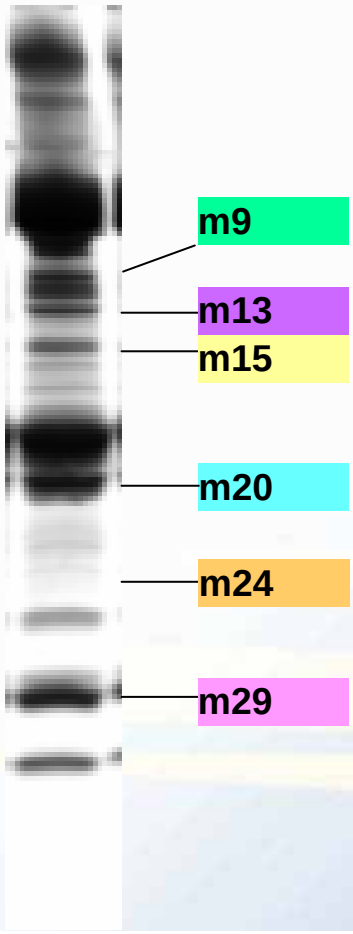
LIPIDTEXT: texture and cathepsin activity

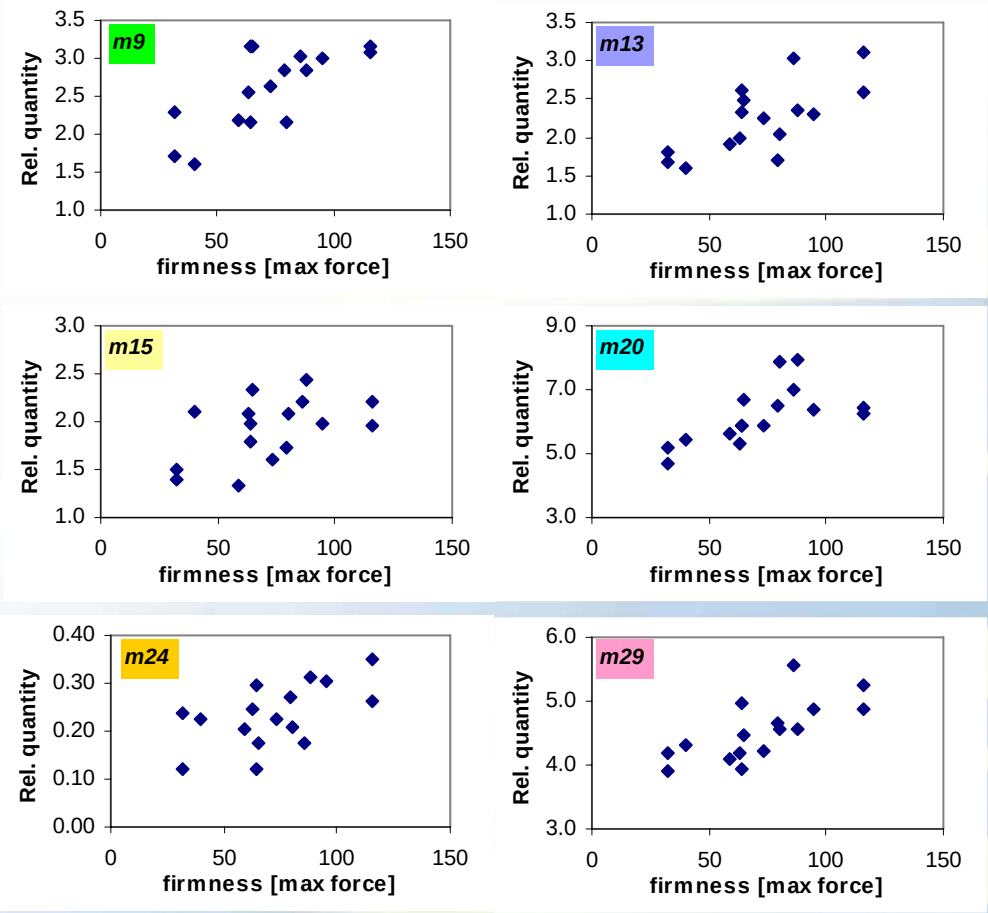
Rainbow trout – different farming conditions

- Mechanical / sensory texture measurement
- Cathepsin assays
- Proteolytic profiling (1D)
- *in vitro* incubation of proteins with cathepsins

Proteolytic profiling

Myofibrillar fraction





Ex: MyHC 70 kDa fragment

One fragment (spot) at ~ 70 kDa less soluble (45 minutes *post mortem*) after intense muscle activity

suggested in Morzel et al., JAFC, 2006

Generally softer texture

MyHC 70 kDa fragment: band

MSTDAEMQAY GK**AAIYLR**KS EKERMEAQAT PFDSKNACYV TDKVELYLKG LVTARADGKC TVTVTNPDGS
KEEGKEFEEA DIYEMNPPKY DK**IEDMAMMT YLNEASVLYN LK**ERYAAWMI YTYSGLFCAT VNPYKWLPVY
DEEVVNAYRG KKRMEAPPHI FSVSDNAFQF MMIDKENQSI LITGESGAGK TVNTKCVIQY FATIAVSGSK
KEVDPSK**MQG SLEDQIIAAN PLLESYGNAK** TVRNDNSSRF GKFIR**IHFQA GK**LAK**ADIET YLLEK**SRVAF
QLPDERGYHI FYQLMTGHKP ELVEMTLTT NPYDFPMISQ GHIAVPSIND KEELDATDDA ITILGFTNDE KMSIYKLTGA
VTHHGNLKFK QK**QREEQAEP DGTEVADKIG YLLGLNSAEL LKCLCYPR**VK VGNEYVTKGQ TVAQVYNAVM
ALAK**SIYERM FLWMVIR**INE MLDTKNPRQF YIGVLDIAGF EIFDYNSMEQ LCINFTNEK**L QQFFNHTMFV LEQEEYKK**EG
IVWEFIDFGM DLAACIELIE KPLGIFSILE EECMFPKASD TTFKNKFYDQ HLGKTKAFEK PKPAKGKPEA HFSLVHYAGT
VDYNITGWLD KNKDPLNESV ILMYGKASVK LLATLYPAAP PEDKAKKGGK KK**GGSMQTVS SQFR**ENLHKL
MTNLRSTHPH FVRCLIPNES KTPGLMENFL VIHQLRCNGV LEDLRICRKG FPSRIIYADF KQRYKVLNAS VIPEGQFMDN
KKASEKLLGS IDVNHEDYKF GHTKVFFKAG LLGVLEEMRD EKLAALVGMV QALSRGFLMR REFSKMMERR
ESIFSIQYNI RSFMNVKTWP WMKLYFKIKP LLQSAETEKE LANMKENYEK MKTDLAKALA TKKHLEEKLV
ALVQERADLA LQVASEGQSL NDAEERCEGL IKSKIQLEAK LKEMTERLED EEEMNAELTA KKRKLEDECS
ELKKDIDDLE LTLAKVEKEK HATENKVKNL TEEMASLDES VAKLTKEKKA LQEAHQQTLD DLQAEEDKVN
TLTKARTKLE QQVDDLEGSL EQEKKLRMDL ERAKRKLEGD LKLAQESIMD LENDKQQADE KIKKKEFETS
QLLSKVEDEQ SLGAQLQKKI KELQARIEEL EEEIEAERAA RAKVERQRAD LSRELEEISE RLEEAGGATS AQIDMNKKRE
AEFQKLRRDL EESTLQHEAT AAALRKK**QAD SVAELGEQID NLQR**VKQKLE KEKSEYKMEI DDLSSNMEAV
AKAKGNLEKM CRTLEDQLSE LKTKNDENVR QVNDISGQRA RLLTENGEFG RQLEEKEALV SQLTRGKQAF
TQQVEELKRQ IEEEVKAKNA LAHGVQSARH DCDLLREQFE EEQEAKAELQ RGMSKANSEV AQWRTKYETD
AIQRTEELEE AKKKLAQRLQ DAEETIEATN SKCSSLEKTK QRLQGEVEDL MIDVERANAM AANLDKKQRN
FDKVLAEWKQ KYEEGQAELE GAQKEARSMS TELFKLKNSY EEALDHLETL KRENKNLQQE ISDLTEQIGE
TGKSIHELEK AKKTVETEKS EIQTALEEAE GTLEHEESKI LRVQLELNQI KGEVDRKIAE KDEEMEQIKR NSQRVVDSMQ
STLDSEVRSR NDALRVKKKM EGDLNEMEIQ LSHSNRQASE AQKQLRNVQG QLKDAQLHLD DAVRVAEDMK
EQAAMVERRN GLMVAEIEEL R**VALEQTER**G RKVAETELVD ASERVGLLHS QNTSLLNTKK KLETDLVQVQ
GEVDDIIQEA RNAEEKAKKA ITDAAMMAEE LKKEQDTSSH LERMKKNLEV TVKDLQHRLD EAENLAMKGG
KKQLQKLEWR VRELETEVEA EQRRGVDAVK GVRKYERRVK ELTYQTEEDK KNVGRLQDLV DKLQMKVKAY
KRHAEEAEEA ANQHMSKFRK VQHELEEAEE RADIAETQVN KLRAKTRDSG KGKEVAE

MyHC 70 kDa fragment: band / spot

MSTDAEMQAY GKAAIYLRKS EKERMEAQAT PFDSKNACYV TDKVELYLKG LVTARADGKC TVTVTNPDGS
KEEGKEFEEA DIYEMNPPKY DKIEDMAMMT YLNEASVLYN LKERYAAWMI YTYSGLFCAT VNPYKWLPVY
DEEVVNAYRG KKRMEAPPHI FSVSDNAFQF MMIDKENQSI LITGESGAGK TVNTKCVIQY FATIAVSGSK
KEVDPSKMQG SLEDQIIAAN PLLESYGNAK TVRNDNSSRF GKFIRIHFQA GKLAKADIET YLLEKSRVAF QLPDERGYHI
FYQLMTGHKP ELVEMTLLTT NPYDFPMISQ GHIAVPSIND KEELDATDDA ITILGFTNDE KMSIYKLTGA VTHHGNLKFK
QKQREEQAEP DGTEVADKIG YLLGLNSAEL LKCLCYPRVK VGNEYVTKGQ TVAQVYNAVM ALAKSIYERM
FLWMVIRINE MLDTKNPRQF YIGVLDIAGF EIFDYNSMEQ LCINFTNEKL QQFFNHTMFV LEQEEYKKEG IVWEFIDFGM
DLAACIELIE KPLGIFSILE EECMFPKASD TTFKNKFYDQ HLGKTKAFEK PKPAKGKPEA HFSLVHYAGT VDYNITGWLD
KNKDPLNESV ILMYGKASVK LLATLYPAAP PEDKAKKGGK KKGGSMQTVS SQFRLNLHKL MTNLRSTHPH
FVRCLIPNES KTPGLMENFL VIHQLRCNGV LEDLRICRKG FPSRIIYADF KQRYKVLNAS VIPEGQFMDN KKASEKLLGS
IDVNHEDYKF GHTKVFFKAG LLGVLEEMRD EKLAALVGMV QALSRGFLMR REFSKMMERR ESIFSIQYNI
RSFMNVKTWP WMKLYFKIKP LLQSAETEKE LANMKENYEK MKTDLAKALA TKKHLEEKLV ALVQERADLA
LQVASEGQSL NDAEERCEGL IKSKIQLEAK LKEMTERLED EEEMNAELTA KKRKLEDECS ELKKDIDDLE
LTLAKVEKEK HATENKVKNL TEEMASLDES VAKLTKEKKA LQEAHQQTLD DLQAEEDKVN TLTKARTKLE
QQVDDLEGSL EQEKKLRMDL ERAKRKLEGD LKLAQESIMD LENDKQQADE KIKKKEFETS QLLSKVEDEQ
SLGAQLQKKI KELQARIEEL EEEIEAERAA RAKVERQRAD LSRELEEISE RLEEAGGATS AQIDMNKKRE
AEFQKLRRDL EESTLQHEAT AAALRKKQAD SVAELGEQID NLQRVKQKLE KEKSEYKMEI DDLSSNMEAV
AKAKGNLEKM CRTLEDQLSE LKTKNDENVR QVNDISGQRA RLLTENGEFG RQLEEKEALV SQLTRGKQAF
TQQVEELKRQ IEEEVKAKNA LAHGVQSARH DCDLLREQFE EEQEAKAELQ RGMSKANSEV AQWRTKYETD
AIQRTEELEE AKKKLAQRLQ DAEETIEATN SKCSSLEKTK QRLQGEVEDL MIDVERANAM AANLDKKQRN
FDKVLAEWKQ KYEEGQAELE GAQKEARSMS TELFKLKNSY EEALDHLETL KRENKNLQQE ISDLTEQIGE
TGKSIHELEK AKKTVETEKS EIQTALEEAE GTLEHEESKI LRVQLELNQI KGEVDRKIAE KDEEMEQIKR NSQRVVDSMQ
STLDSEVRSR NDALRVKKKM EGDLNEMEIQ LSHSNRQASE AQKQLRNVQG QLKDAQLHLD DAVRVAEDMK
EQAAMVERRN GLMVAEIEEL RVALEQTERG RKVAETELVD ASERVGLLHS QNTSLLNTKK KLETDLVQVQ
GEVDDIIQEA RNAEEKAKKA ITDAAMMAEE LKKEQDTSSH LERMKKNLEV TVKDLQHRLD EAENLAMKGG
KKQLQKLEWR VRELETEVEA EQRRGVDAVK GVRKYERRVK ELTYQTEEDK KNVGRLQDLV DKLQMKVKAY
KRHAEEAEEA ANQHMSKFRK VQHELEEAEE RADIAETQVN KLRAKTRDSG KGKEVAE

Conclusions

MyHc fragment

Spot – band (more than 1 protein?)

C terminal – N terminal fragments: implications?

Profiling

Higher proteolysis in softer fish

Perspectives in LIPIDTEXT

Targets of *in vitro* cathepsin proteolysis

Correlation texture / proteins separated by 2D

Link protein oxidation / lipid oxidation / texture

Many thanks to:

LIPIDTEXT partners

Helene Godiksen / Flemming Jessen (DIFRES)

Proteomic Platform (INRA Theix, France)

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