



TAFT 2012

4TH TRANS-ATLANTIC FISHERIES TECHNOLOGY CONFERENCE

Program Book
October 30 - November 2, 2012
Clearwater Beach, Florida, USA

WELCOMEOn behalf of the host organization, the Seafood Science and Technology Society of the Americas (**SST**), I cordially welcome you to the 4th Trans-Atlantic Fisheries Technology Conference (**TAFT**) featuring talent and cooperation from our sister organizations, the Atlantic Fisheries Technology Conference (**AFT**) and the Western European Fisheries Technology Association (**WEFTA**), and guests. We want you to enjoy Florida, enjoy the conference and continue to enjoy seafood!

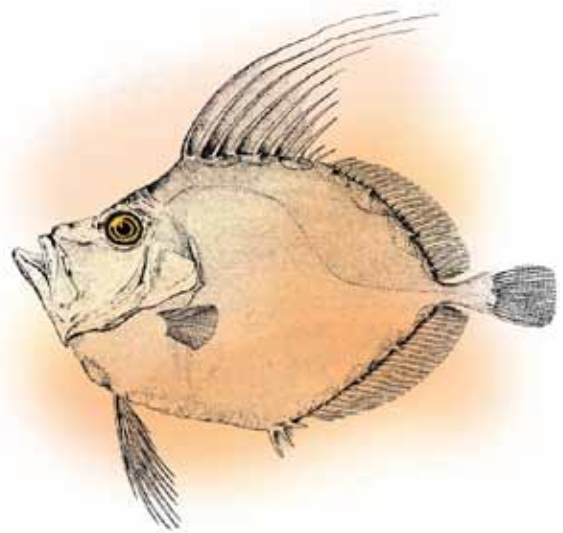
Steve Otwell
*Seafood Science and Technology
Society of the Americas*

THE HISTORY OF ZEUS

This picture of the American John Dory, *Zenopsis conchifera* has accompanied all communications of the Tropical and Subtropical Seafood Science and Technology (SST) Society of the Americas since the first annual conference held March 1976 in Corpus Christi, Texas. The reasons for the selection of this fish are somewhat of a mystery bordering on humor, but the beast has silently endured for over thirty-four years. He was pictured on the first publication of this professional organization when it was known as the Tropical and Subtropical Fisheries Technological Society. He remains a true resident of our region swimming along the continental shelf and slopes to about 600 meters from southern Brazil to Nova Scotia. He is not harvested commercially in this region, but his foreign cousins from the fish family Zeidae are regarded as choice food in Europe and an established fishery is reported in Australia. Today he also swims in animation across the SST website – <http://sst.ifas.ufl.edu>. He has survived attempts to replace him with more meaningful seafood or scientific images. Some have even called him ugly and degrading, but he has endured transitions and critics.

For his dedicated service, he remains SST's officially recognized mascot with the honored name, 'Zeus'. This name is based on his original genus title, Zeus. He deserves special recognition and status because he exemplifies dedication, resilience to criticism, and indifference to vogues, while he swims in the regional waters that bind the Seafood Science and Technology Society of the Americas.

Background Information: *Bigelow and Schroeder's 2002, 3rd Edition, Fishes of the Gulf of Maine. Edited by R. Collette and G. Klein – MacPhee. Smithsonian Institution Press, Washington, DC. 748 pp. Picture by H.C. Todd (1953).*



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Fisheries Technology
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Zina Williams, Aquatic Food Products Program, University of Florida, Gainesville, USA

CONFERENCE AGENDA

Monday, October 29

3 pm Pre-Registration Desk open to assist participants that may arrive in advance

Tuesday, October 30

9:00 am Registration Desk Opens
Special Group Meetings (space available)

11:30 **Welcome and Opening Ceremony Luncheon for all registrants (Rusty's)**
"Past, Present and Future TAFT"
Torgjer Børresen, Steve Otwell and David Green – TAFT 2012 Coordinating Chairmen

Concurrent Poster Session for Technical Sessions 1 and 2 is open 1:00-6:30 pm (Ball Room) with Social and Refreshments from 5:00-6:30 pm. Dinner on own.

TECHNICAL SESSION 1 Functional Seafood and Ingredients (Ball Rooms)

Chair Person – Torgjer Børresen, Danish Institute for Fisheries Research, Technical University of Denmark

1:00 pm	Functional Foods and Ingredients from Marine Resources Hordur G. Kristinsson, Matis, Iceland.....	1
1:20	Development of Cardioprotective Fish Products by Using Isoelectric Solubilization/Precipitation and ω -3 Rich Oils Reza Tahergorabi et al, West Virginia University, Morgantown, WV, USA.....	2
1:40	Antioxidative, DPP-IV and ACE Inhibiting Peptides from Fish Protein Hydrolyzed with Intestinal Proteases Susan S. Falkenberg et al, Technical University of Denmark, Lyngby	3
2:00	Antioxidant Property and Average Size of Nanocapsules from Fish Protein Hydrolysates for Application in Edible Coatings Carlos Prentice et al, Federal University of Rio Grande, Brazil	4
2:20	Effect of Oxidation during Enzymatic Hydrolysis on Antioxidant Protection of Cod Protein Hydrolysates Halldorsdottir SM et al, Matis, Iceland	5
2:40	Break	

Concurrent Poster Session for Technical Sessions 1 and 2 is open 1:00-6:30 pm. with Social and Refreshments 5:00-6:30 pm. Dinner on own.

TECHNICAL SESSION 2 Seafood Utilization

Chair Person – David Green, Food, Bioprocessing & Nutrition Sciences, North Carolina State University

3:00 pm	Novel Insights into Pigmentation and Salt Solubility of pH-Shift Produced Protein Isolates Ingrid Undeland (2011 WEFTA Distinguished Awardee) et al, Chalmers University of Technology, Gothenburg, Sweden	6
3:20	Restructured Seafood Products with Glucomannan as Principal Ingredient: Effect of Salt and Oil Addition Beatriz Herranz et al, Instituto du Ciencia y Tecnologia de los Alimentos y Nutrición, Madrid, Spain	7
3:40	Optimal Enzymatic Hydrolysis of Discarded Crustaceans in North Atlantic Fisheries Luis T. Antelo et al, IIM-CSIC. C, Vigo, Spain.....	8
4:00	Quantification of Low Molecular Weight Nitrogen Containing Compounds in Red Salmon and Alaska Pollock Whole Fish, Fish Meal and Byproduct Peter J. Bechtel et al, USDA/ARS New Orleans and University of Alaska, USA	9
4:20	Effect of Addition of Antioxidants to Herring By-products on the Production of Oil Ana Karina Carvajal et al, SINTEF Fisheries and Aquaculture, Trondheim, Norway	10
4:40	Purification of Alaska Cod (<i>Gadus macrocephalus</i>) Liver Oil Using Short-Path Distillation Alexandra C. M. Oliveira et al, University of Alaska, USA	11
5-6:30	Poster Session Social and Refreshments. Dinner on own.	
6-7:30	SST, AFT and WEFTA Executive Committee Meetings	

Wednesday, October 31

7-8:00 am Continental Breakfast

Concurrent Poster Session for Technical Sessions 3-6 is open 8:00 am-6:30 pm with Social and Refreshments 5-6:30 pm. Dinner on own.

TECHNICAL SESSION 3 Muscle Chemistry

Chair Person – Paul Sarnoski, Food Science & Human Nutrition, University of Florida

8:00 am	Kinetic Modeling of Kramer Shear Resistance and Water Holding Capacity of Frozen Stored Hake (<i>Merluccius merluccius</i>) Muscle Mercedes Careche et al, ICTAN-CSIC, Madrid, Spain	12
8:20	Quality of Frozen Fillet of Alaska Pollock as Assessed by Myosin Denaturation Study Kunihiko Konno and Yoshiko Konno, Hokkaido University, Hokkaido, Japan	13
8:40	Effect of Mechanical Impact, Salt Concentration and Storage Temperature on Rainbow Trout (<i>Oncorhynchus mykiss</i>) Erythrocyte Lysis; an Approach to Decrease Hb Leakage into Fish Muscle Linnea Eriksson et al, Chalmers University of Technology, Gothenburg, Sweden	14

9:00	A Study of the Microstructure Sizes of the Pre-Rigor Fillets of Atlantic Salmon during Superchilling Process and Superchilled Storage Lilian Daniel Kaale et al, Norwegian University of Science and Technology, Trondheim, Norway.....	15
9:20	New Insights Regarding Lipid Oxidation in Fish Muscle and Its Inhibition Mark P. Richards et al, University of Wisconsin, Madison, Wisconsin, USA.....	16
9:40	Emulsifying and Antioxidant Properties of a Shrimp Hydrolysate (<i>Pandalus borealis</i>) Conjugated with Xylose and Dextran Nicolas Decourcelle et al, Université de Brest, Quimper, France	17
10:00	Break	

TECHNICAL SESSION 4 Seafood Processing 1

Chair Person – Stina Frosch, Division of Industrial Food Research, Technical University of Denmark

10:20	Lipid Degradation of Cod Liver during Frozen Storage as Influenced by Temperature, Packing Methods and Season Magnea G. Karlsdottir, et al, Matis and University of Iceland, Reykjavik, Iceland	18
10:40	Linking Pre-harvest with Post-harvest Data for Process Optimization in the Salmon Value Chain Gine Ø. Johansson et al, Technical University of Denmark, Lyngby.....	19
11:00	Low Field Nuclear Magnetic Resonance Spectroscopic Analysis of Hake (<i>Merluccius merluccius</i> L.) upon Freezing: A Possibility for Authentication of Fresh vs Thawed Muscle Mercedes Careche et al, Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), Madrid, Spain.....	20
11:20	Temperature Control during Containerized Sea Transport of Fresh Fish Sæmundur Eliasson et al, Matis, Iceland.....	21
11:40	Quality Consequences of Fish Bleeding and Methods in Commercial Fisheries Heidi Nilsen et al, Nofima, Tromsø, Norway	22

12:00 p.m. Informal Lunch and Special Award

◆ WEFTA Outstanding Scientist Award – Western European Fisheries Technology Association
Presented by Dr. Torger Børresen, Technical University of Denmark

TECHNICAL SESSION 5 Seafood Processing 2

Chair Person – Denise Skonberg, Food Science & Human Nutrition, University of Maine, Orono

1:20 p.m.	Automatic Detection of Nematodes in Cod Fillets (<i>Gadus morhua</i> L.) by Interactance Hyperspectral Imaging Agnar H. Sivertsen and Karstenh Heia, Nofima, Tromsø, Norway	23
1:40	Non-Destructive Freshness Assessment of Atlantic Salmon (<i>Salmo salar</i> L.) Fillets by Visible/Near-Infrared Spectroscopy Tukashi Kimiya et al, National Research Institute of Fisheries Science, Yokohama, Japan and Nofima, Tromsø, Norway	24
2:00	Near Infrared Spectroscopy (NIR) an Ultimate Method for Quality Control Christel Solberg and Chris Andre Johnsen, University of Nordland, Norway.....	25

2:20	Automated Sorting of Pelagic Fish based on 3D Machine Vision Ekrem Misimi et al, SINTEF, Trondheim, Norway.....	26
2:40	Separating and Sorting Shrimp for Market Grades, Quality and Uniformity with Machine Vision Robert Lane et al, Virginia Tech, Hampton, VA, USA	27
3:00	Break	

TECHNICAL SESSION 6 Seafood Processing 3

Chair Person – Mercedes Careche, Institute of Food Science, Technology & Nutrition, Madrid, Spain

3:20	Removal of Allergens from Fish Infected with <i>Anisakas simplex</i> Larvae in the Washing Process of Surimi Production Margarita Tejada (2011 WEFTA Distinguished Awardee) et al, ICTAN-CSIC. C, Madrid, Spain.....	28
3:40	Acoustic Measurements for Grading of Ice-Glaze Content of Single Frozen Prawn Stina Frosch et al, Technical University of Denmark, Lyngby	29
4:00	Temperature Fluctuations and Quality Deterioration of Chilled Cod Fillets Packaged in Palletized Wholesale Boxes Under Dynamic Temperature Conditions – Simulation of Air and Sea Freight Björn Margeirsson et al, Matis, Iceland	30
4:20	Spice-Cured Sprats Ripening, Flavor Development and Quality Properties Loreida Timberg et al, Competence Center of Food and Fermentation Technologies and Tallinn University of Technology, Tallinn, Estonia	31
4:40	Processing Pasteurized Crabmeat: Critical Factors for Best Quality Thomas E. Rippen, University of Maryland-Eastern Shore, Princess Anne, MD, USA.....	32
5-6:30	Concurrent Poster Session Social and Refreshments. Dinner on own.	

Thursday, November 1

7- 8:00 am Continental Breakfast

Concurrent Poster Session for Featured Session Topics 1-3 is open 8:00 am-6:30 pm. Gala Dinner and Awards at 6:30 pm.

FEATURED SESSION 1 Seafood Product Integrity: Moisture Control

Chair Person – Tyre Lanier, Food, Bioprocessing & Nutrition Sciences, North Carolina State University

8:00 am	Chem-Free Products and Clean Labels Steve Otwell, University of Florida, Gainesville, Florida, USA	33
8:20	Commercial Perspectives Lisa Weddig, National Fisheries Institute, Better Seafood Board, McLean, Virginia, USA	34
8:45	Regulatory Perspectives - United States Patti Ross, Food and Drug Administration, College Park, Maryland, USA	35
9:15	Seafood Marinating – A Tool for Adding Value, Extending Shelf-Life and Providing Added Safety to Seafood Products Juan L. Silva et al, Mississippi State University, MS, USA	36

9:35	Seafood Integrity Elucidated by Novel Analytical Authentication Tools Saskia van Ruth, Samuel Heenan et al, RIKILT Wageningen UR, Netherlands	37
	Updating Latest EU Rules on Food Additives and Additional Considerations Jaime Forn, Budenheim Altesa, Valencia, Spain.....	38
10:20	Break and Displays	

FEATURED SESSION 2 Seafood Product Integrity: Sodium Levels in Seafood

Chair Person – Heidi Nilson, Nofima, Tromsø, Norway

10:40 am	Using the Right Treatments to Reduce Sodium in Seafood Yan Huang, Innophos Inc, Cranbury, New Jersey.....	39
11:00	Investigating Methods to Reduce and Control Sodium Levels in Shrimp Molly Sims et al, University of Florida	40
11:20	Improving Sensory and Nutritional Quality of Lightly Salted Fish Fillets by the Aid of Sodium Bicarbonate Magnus Åsli and Turid Møkøre, Nofima, Norway	41
11:40	Use of Sodium Nitrate in Cold-Smoke Processing of Farmed Atlantic Salmon: Evaluation of Fillet Quality and Food Safety during Refrigerated Storage Jorgen Lerfall and Marianne Østerlie, Sør-Trøndelag University College, Trondheim, Norway.....	42
12:00 pm	Informal Lunch	

FEATURED SESSION 3 Seafood Product Integrity: Seafood Safety

Chair Person – Tom Rippen, Seafood Technology, University of Maryland, Princess Ann

1:20 p.m.	<i>Vibrio parahaemolyticus</i> Prevalence in New Zealand Shellfish Cristina D. Cruz et al, New Zealand Institute for Plant & Food Research Limited, Auckland.....	43
1:40	Assessing a Heat Shock Method of Control for <i>Vibro vulnificus</i> and <i>Vibro parahaemolyticus</i> in Raw Oysters Abdallah Al-Dakheelallah et al, North Carolina State University, Raleigh, North Carolina, USA.....	44
2:00	Lessons Learned from Sushi Salmonellosis Outbreaks Douglas L. Marshall, Eurofins Scientific Inc., Fort Collins, Colorado, USA.....	45
2:20	Extraction of Enteric Virus Indicators from Seawater Using Activated Carbon Jiemin Cormier et al, Louisiana State University, Baton Rouge, Louisiana, USA	46
2:40	Impact of Red-Colored Halophile Bacteria on Salted and Dried Cod Ann Helen Hellevik et al, Møreforskning Marin, Ålesund, Norway	47
3:00	Break	

Chair Person – Steve Otwell, Food Science & Human Nutrition, University of Florida

3:20 p.m.	Validation for Pre-Cooking as a Control for Potential Histamine Production in Tuna Loins Used for Subsequent Canning Farzana Vogl et al, Bumble Bee Foods and Clover Leaf Foods, Vancouver, Canada	48
3:40	End Point Internal Product Temperature (EPIPT) Control of Histamine in Tuna Pre-Cooking Step Fred Nolte et al, Bumble Bee Foods and Clover Leaf Foods, Vancouver, Canada	49
4:00	Effectiveness of Various Sanitizers Against Natural Bacterial Flora, <i>Listeria monocytogenes</i> and <i>Salmonella enterica</i> on the Outer Skin Surface of Tuna Fish Aubrey F. Mendonca et al, Iowa State University Ames, Iowa, USA.....	50
4:20	An Integrated Approach to the Assessment of Risks and Benefits Associated with the Consumption of Seafood in Different World Regions Leonor Nunes et. al, Portuguese Institute of the Sea and the Atmosphere, Portugal.....	51
4:40	Seafoodhealthfacts.com: Developing Informational Materials for Healthcare Professionals Michael T. Morrissey et al, Oregon State University, Portland, Oregon, USA.....	52
6:30 p.m.	Gala Dinner and Award Ceremonies ♦ McFee Award - Atlantic Fisheries Technology Conference (AFT) Presented by Pamela Tom, University of California-San Diego ♦ Lifetime Achievement Award by Seafood Science and Technology Society (SST) Presented by Dr. Steve Otwell, University of Florida	

Friday, November 2

7– 8:00 am Continental Breakfast

Poster Session for Featured Session 4 is open 8:00 am-Noon.

FEATURED SESSION 4 Seafood Product Integrity: Species Identification and Product Origin

Chair Person – Lisa Weddig, National Fisheries Institute, McLean, Virginia, USA

8:00 am	State of Technology for Proper Species Identification in USA LeeAnn Applewhite, Applied Food Technologies, Alachua, Florida	53
8:30	Development of a Rapid Seafood Species Identification Technique Using Chip-based Capillary Electrophoresis and Species-Specific Protein Patterns Calvin C. Walker et al, National Marine Fisheries Service, Pascagoula, Mississippi	54
9:00	Tailor-Made Authentication Tools for Storytelling Begona Perez-Vilarel et al, AZTI, Spain.....	55
9:30	Breaking Seafood Identity: Geographic Origin Perspective Miguel Angel Pardo et al, AZTI, Spain	56
10:00	Break	

Chair Person – Barbara Blakistone, National Fisheries Institute, McLean, Virginia, USA

10:20 am	How to Define Traceability Petter Olsen and Melania Borit, Nofima and University of Tromsø, Norway	57
10:40	LABELFISH: The Atlantic Network on Genetic Control of Fish and Seafood Labeling and Traceability Carmen G. Sotelo et al, Instituto de Investigaciones Marinas CSIC, Vigo, Spain.....	58
11:00	Costs, Benefits and Human Challenges when Implementing Traceability in the White Fish Processing and Packing Industry: A Case Study Kathryn A-M Donnelly, Nofima, Norway.....	59
11:20	Louisiana Wild Certified Seafood: Controlling Traceability and Species Identification from Harvest to Sale via Geographic Authenticity Branding and Marketing Program Jon W. Bell et al, Louisiana State University and LAWF Department.....	60
11:40	Traceability in the Fish Sector from Research to Commercial Business Erling P. Larsen, Technical University of Denmark, Charlottenlund.....	61
NOON	Targeted Time to Adjourn the TAFT 2012 Conference (Announce Next Conference Sites)	

CONFERENCE POSTERS

Tuesday, October 30

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Concurrent Poster Session for Technical Sessions 1-2 is open 1:00-6:30 pm. with Social and Refreshments 5:00-6:30 pm. Dinner on own.

1	Inhibition of α -amylase and α -glucosidase by Icelandic Seaweed Hamaguchi, P.H. et al, Matis, Iceland.....	63
2	HPP – Automatic, Electric and Environmentally Friendly Small Fish Meal Factory Magnus V. Gislason et al, University of Iceland, Reykjavik	64
3	Environmental Impacts of a Fishmeal Processing Factory: Advantages of Discards Valorization against Disposal Carla Lopez et al, IIM-CSIC, Vigo, Spain.....	65
4	A One Year Survey of Pacific Cod (<i>Gadus macrocephalus</i>) Livers as a Source of Fish Oil Alexandra C. M. Oliveira et al, University of Alaska, USA	66
5	Quality Changes of Alaska Fish Meals during Storage at 4°C and 40°C: One Year Study Alexandra C. M. Oliveira et al, University of Alaska, USA	67
6	Characterization of Effluents from the Marinated Herring Industry Part I: From Boat to Barrel Ingrid Undeland et al, Chalmers University of Technology, Gothenburg, Sweden	68
7	Characterization of Effluents from the Marinated Herring Industry Part II: From Barrel to Glass Jar Nina Gringer et al, Technical University of Denmark, Lyngby.....	69

Wednesday, October 31

POSTERS for Technical Sessions 3, 4, 5 and 6

Concurrent Poster Session for Technical Sessions 3-6 is open 8:00 am-6:30 pm with Social and Refreshments 5:00-6:30 pm. Dinner on own.

8	Automatic Box-Freezer – An Energy Efficient Freezing process with Improved Product Quality of Pelagic Fish Hagalin Á. Gudmundsson et al, Matis, Reykjavik.....	70
9	Innovative On-Board Technologies and Solutions Toward the Sustainability of Marine Resources: The FAROS Initiative Luis T. Antelo et al, IIM-CSIC. C, Vigo, Spain	71
10	Pre-rigor Produced Fillets of Atlantic Cod Show No Cold Shortening Tone Friss Aune et al, Nofima, Tromsø, Norway	72

11	Effect of Heme Oxidation States on Spectral Variations of Atlantic Salmon (<i>Salmo salar</i> L.) during Cold Storage Izumi Sone et al, Nofima, Tromsø, Norway	73
12	Vibrational Spectroscopic Analysis of Hake (<i>Merluccius merluccius</i> L.) Lipids during Frozen Storage Mercedes Careche et al, IEM-CSIC, Madrid, Spain	74
13	Myofibrils Are Not a Suitable Model Material to Study Muscle Protein Denaturation in Frozen Shrimp Thitima Jantakoson and Kunihiko Konno, Hokkaido University, Hokkaido, Japan.....	75
14	Modeling Time and Temperature History of Frozen Thawed Hake (<i>Merluccius merluccius</i> L.) Muscle by Low Field Nuclear magnetic Resonance spectroscopy Mercedes Careche et al, ICTAN-CSIC, C, Madrid , Spain	76
15	Effect of Bleeding on Quality Changes of Cod (<i>Gadus morhua</i>) Muscle during Chilled Storage Magnea G. Karlsdottir et al, Matis, Iceland.....	77
16	Effect of High Pressure Processing on Abalone Texture and Color Brianna H. Hughes et al, University of Maine, Orono, Maine, USA	78
17	Work Procedures in Icelandic Fish Markets and the Use of Requirement Analysis for Identifying Potential Improvement Areas Gígja Eyjólfsdóttir et al, University of Iceland, Reykjavik, Iceland	79
18	Economic Analysis on the Effects of New Regulation that Obligates Icelandic Freezer Trawlers to Bring Ashore Cod Heads Gísli Eyland et al, University of Iceland, Reykjavik, Iceland	80
19	Instrumental Quality Grading of Atlantic Salmon Fillets (<i>Salmo salar</i> L.): Detection of Surface and Embedded Blood and Melanin Spot by Hypersecral Imaging Karsten Heia anjd Agnar H. Siverlsen, Nofima, Tromsø, Norway.....	81
20	Automatic Detection and Quantification of Red Pigmented Halophilic Archaea's in Dried Salt-Cured Cod Using Hyperspectral Imaging Agnar H. Sivertsen et al, Nofima, Tromsø, Norway	82
21	Use of Microarray Technology for Studying the Molecular Basis of Fillet Firmness in Atlantic Salmon (<i>Salmo salar</i> L.) Thomas Larsson and Turid Mørkøre, Nofima, Norwegian University of Life Sciences	83
22	CFD Modeling of Combined Blast and Contact Cooling of White Fish Valur O. Bjarnason et al, Matis , Iceland	84
23	Monitoring Spatial Distribution of Quality Parameters in Salmon Using Computer Vision Bjørn S. Dissing et al, Technical University of Denmark, Lyngby	85
24	Novel Processing of Pre-Rigor Farmed Atlantic Cod (<i>Gadus morhua</i>) Fillets by Combining Direct Fileting and Superchilling Bjørn Tore Rotabakk et al, Nofima, Stavanger, Norway	86
25	Effect of Different Diet Supplementation on Several Muscle Collagen Parameters in Farmed Atlantic Salmon (<i>Salmo salar</i> L.) Moreno H.M., Javier Borderias et al, ICTAN (CSIC) Madrid, Spain	87

Thursday, November 1

POSTERS for Seafood Product Integrity Topics 1, 2 and 3

Concurrent Poster Session for Featured Session Topics 1-3 is open 8:00 am-6:30 pm. Gala Dinner and Awards at 6:30 pm.

26	Method to Determine Added Polyphosphates in Seafood John Reuther, Eurofins Central Analytical Labs, New Orleans, Louisiana, USA	88
27	Bacterial Growth and Histamine Production in Tuna Salad Preparations Susan McCarthy et al, U. S. Food and Drug Administration, Dauphin Island, Alabama, USA	89
28	Studies on High Salinity Relaying and Rapid Cooling of Oysters Michael Jahncke et al, Virginia Tech, Hampton, Virginia, USA	90
29	Developing Cooking Controls for Potential <i>Vibrio</i> Pathogens in Oysters Chris Hanna et al, University of Florida, Gainesville, Florida, USA	91
30	Framing the Message About Seafood: Outcomes of a Conference About Communicating Seafood Safety Doris Hicks et al, University of Delaware, Lewes, Delaware, USA.....	92
31	Seafood Health Facts: Making Smart Choices, Balancing the Benefits and Risks of Seafood Ken Gall, et al, Cornell University, and others across USA.....	93
32	Overview of the Risks and Benefits of the Consumption of Different Classes of Seafood Products Leonor Nunes et al, Portuguese Institute of the Sea and the Atmosphere, Portugal.....	94
33	Validation of Microwave Cooking Instructions for Not-Ready-to-Eat (NRTE) Seafood David P. Green et al, North Carolina State University, Raleigh, North Carolina, USA	95
34	Food Safety and Screening of Contaminants in Icelandic Seafood: Emphasis on PFC's Hrönn Jorundsdóttir and Helga Gunnlaugsdóttir, Matis, Iceland.....	96
35	Assessment of Contaminant Levels on Discarded Species as a Key Step on the Definition of Optimal Valorisation Strategies Luis T. Antelo et al, IIM-CSIC.C, Vigo, Spain.....	97
36	The Effect of K-lactate Salt and Liquid Smoke on Bacterial Growth in a Model System Simulating a Cold Smoke Process Trond Løval et al, Nofima, Norway	98
37	Effect from Digested Cod Liver Oil of Different Quality on Oxidation, Energy Metabolism and Proteome in Yeast (<i>Saccharomyces cerevisiae</i>) Ingrid Undeland et al, Chalmers University of Technology, Gothenberg, Sweden	99
38	Louisiana Direct Seafood: Using a Successful Direct Marketing Program to Deliver Best Practices and Technology Transfer to Seafood Harvesters Julie Falgout et al, Louisiana State University, New Orleans, USA.....	100
39	Communicating Seafood Sustainability from the Gulf Coast: A Two-Pronged Approach Rene LeBreton, LA Department of Wildlife and Fisheries, New Orleans, USA	101

40	Adding Value through Sustainable Fisheries Michaela Aschan et al, University of Tromsø, Norway	102
41	Microbiologically Food Security and Sustainable Development of Exportable Shrimp Product through Value-Addition and Its Contribution to the National Economy Uddin M. Kapel, Seamark (BD) Limited, Chittagong, Bangladesh	103
42	Ultrasound Reflection as a Tool for Improving Sustainability of the Crab Fisheries Ulrik Cold and Bo M. Jørgensen, DTU National Food Institute, Lyngby, Denmark	104

Friday, November 2

POSTERS for Seafood Product Integrity Topic 4

Concurrent Poster Session for Seafood Product Integrity Topic 4 is open 8:00 am-Noon.

43	Rapid Identification of Species of Gadiformes of Commercial Interest by Means of High Resolution Melting Analysis Taboada Iglesias, Leticia et al, Instituto de Investigaciones Marinas, Consejo Superior de Investgaciones Cientificas, and AZTI, Vigo Spain	105
44	Dipstick Test for DNA-based Cod Fish Products Authentication Taboada Iglesias, Leticia et al, Instituto de Investigaciones Marinas, Consejo Superior de Investgaciones Cientificas, and AZTI, Vigo Spain	106
45	DNA Probes for <i>Thunnus</i> species Identification in Canned Products Miguel Angel Pardo et al, AZTI, Spain	107
46	Use of Otolith Microchemistry to Identify Nursery Origin and Track Migration Pathways of Gulf of Mexico Fishes David L. Jones and Ernst B. Peebles, University of South Florida, St. Petersburg, FL, USA.....	108
47	Consumer Behavior Analysis for Understanding the Dissonance between Attitude and Sensory Perception of Wild and Farmed Fish Garcia-Quiroga M. and Gartzia I., AZTI, Spain	109
48	Brown Trout – Suitability of an ‘Old’ Species for Organic and Conventional Aquaculture Monkika Manthey-Karl, Max Rubner Institute, Hamburg, Germany	110
49	Is Organically Farmed Seafood a Better Quality? Horst Karl and Manthey-Karl, Max Rubner Institute, Hamburg, Germany	111
50	The Use of Microalgae as a Protein Source in a Finfish Diet Paul J. Sarnoski et al, University of Florida, Gainesville, Florida, USA	112
51	Effects of Dietary Mineral Supplements on Quality of Fresh and Salt Cured Fillets from Farmed Atlantic Cod, <i>Gadus morhua</i> Hilde Herland et al, Nofima, Tomsø, Norway	113
52	Automated Quality Assurance in Industrial Fish Feed Pellet Production Michael E. Nielsen et al, Technical University of Denmark, Brande	114
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ORAL PRESENTATION ABSTRACTS

Technical Session 1: Functional Seafood and Ingredients

Tuesday, October 30

1-2:40 pm

Functional Foods and Ingredients from Marine Resources

Dr. Hordur G. Kristinsson

Research Director, Matis, Vinlandsleid 12, IS-113, Reykjavik, Iceland

Vast amounts of marine based raw materials are still in large part underutilized. Major opportunities exist with these raw material sources as they are rich in various natural and highly functional compounds, which with proper extraction, isolation and processing techniques can find use in various foods, specialty feeds, nutraceuticals, cosmeceuticals and even medical products. The market for natural products is growing very rapidly, particularly products which possess bioactive properties which can have positive effects on health and performance. The past few years have seen significant advances in the isolation and production of novel ingredients from underutilized raw materials. This includes the production of fatty acids, enzymes, cartilage compounds such as chondroitin sulfate,

glucosamine, functional fish proteins, bioactive fish peptides and various seaweed based compounds, to name a few. Some of these ingredients have very unique functions compared to their non-marine counterparts, and display very high activity. The industry is realizing that very significant value addition can be achieved with underutilized raw materials. However for this industry to become successful and compete in the marketplace, continued and significant support of research and development is needed, as well as patience. Particular attention to marketing strategies is also important for these ingredients to stand out in the marketplace.

Presentation 1

Development of Cardioprotective Fish Product by Using Isoelectric Solubilization/Precipitation and ω -3 Rich Oils

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Fish resources are limited; therefore it is desirable to devise a processing strategy allowing recovery of functional and nutritious muscle proteins from low-value fish processing by-products for subsequent inclusion in value-added food products. Furthermore, Western diet is characterized as low in ω -3 fatty acids (FAs) and high in ω -6 FAs. Due to the cardioprotective effect of ω -3 FAs there is an increasing demand to enhance diet with these oils. The objectives of this study were to characterize effects of ω -3 FAs addition on (1) FA profile and

oxidation, (2) total volatile basic nitrogen (TVBN) and (3) texture. Whole gutted rainbow trout was used as a model for fish processing by-products. Fish were homogenized and solubilized at pH=11.5. Bones, skin, and fat were removed by centrifugation. Solubilized proteins were precipitated at pH=5.50 and de-watered by centrifugation. Salt substitute, ω -3 PUFA-rich oils (flaxseed, algae, fish, krill, or blend [flaxseed: algae: fish at 8:1:1]) were added.

Presentation 2

Antioxidative, DPP-IV and ACE Inhibiting Peptides from Fish Protein Hydrolysed with Intestinal Proteases

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Proteins from fish tissue could be a promising source of peptides with a nutritional and pharmaceutical value, e.g. as treatment of type 2 diabetes with dipeptidyl peptidase IV (DPP-IV) inhibiting peptides, and could be used in health and functional foods and thereby increasing the value of secondary marine products.

The approach in this study is to hydrolyse skin and belly flap tissue from Salmon with the use of mammalian digestive proteases from pancreas and intestinal mucosa and test hydrolysates for antioxidative capacity, intestinal DPP-IV and angiotensin converting enzyme (ACE) inhibiting properties.

10kDa dialysis bags containing 10ml water were added to homogenized fish tissues, which were subsequently hydrolysed for 24 hours at 37°C and pH 8 with intestinal mucosa extract and/or pancreatin solution from pig. Dialysis bags were then removed and content were analyzed for free amino groups, antioxidative capacity by ABTS (2,2-

azinobis(3-ethylbenzothiazoline-6-sulfonicacid)), DPP-IV and ACE inhibiting activity.

Degree of hydrolysis (DH) of hydrolysates was approximately 13% and 10% for belly flap and skin respectively. No clear difference was observed in DH between pancreatin and pancreatin + mucosa hydrolysates. No DH was obtained for tissues hydrolysed with only intestinal mucosa extract.

Preliminary results showed antioxidant activity and intestinal DPP-IV and ACE inhibiting activity in 10 kDa fraction from both belly flap and skin hydrolysates but with a higher antioxidative capacity in belly flap hydrolysates. No difference between hydrolysates with pancreatin and pancreatin+mucosa was observed.

Hydrolysates will be further fractionated by gelfiltration. Fractions will be analyzed for the three bioactivities and also presented.

Presentation 3

Antioxidant Property and Average Size of Nanocapsules from Fish Protein Hydrolysates for Application In Edible Coatings

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Enzymatic hydrolysis of food proteins is an efficient way to recover potent bioactive peptides. Fish protein hydrolysates have been shown to have potential for nutritional or pharmaceutical applications. Some proteins of fish may have biological activity, for example, antioxidant, which can be associated with the bioactive peptide sequences in specific protein released after the enzymatic hydrolysis. Numerous peptides derived from hydrolysed food proteins have been shown to have antioxidant activity. The nanoencapsulation is used to protect a sensible substance in a capsule or wall physically. This barrier can allow a controlled release of the substance and prevent contact with other components in a mixture.

The objectives were to prepare nanocapsules from fish protein hydrolysate using proteins of muscle and residue from Whitemouth croaker (*Micropogonias furnieri*) and to evaluate the antioxidant activity, the average size and distribution of particles nanoencapsulated. Hydrolysates were prepared using the enzyme Flavourzyme in a buffer

solution of pH 7 for 60 min of reaction at 50°C. After inactivation of enzymes, the hydrolysates were lyophilized and encapsulated by liposomes method using phosphatidylcholine as wall material, prepared by purification of soy lecithin with ethyl acetate and acetone. The muscle and residue hydrolysed presented an absorbance of 0.146 and 0.079, respectively, and then the hydrolysate of muscle protein presented more reducing power as compared with hydrolysate of residue protein. Nanocapsules of croaker muscle hydrolyzed had an average size of 266.8 nm and 0.298 polydispersity and the nanocapsules of residue showed an average size of 263.9 nm and 0.197 polydispersity. The polydispersity values show that the particle size slightly deviated from the average size demonstrating that the nanocapsules formed a uniform suspension. This nanocapsules will be applied on coatings produced from fish protein isolate to evaluate their antioxidant action during the protection in foods.

Presentation 4

Effect of Oxidation During Enzymatic Hydrolysis on Antioxidant Properties of Cod Protein Hydrolysates

Haldorsdottir SM, Kristinsson HG, Sveinsdottir H, Thorkelsson, G and Gudmundsdottir A.

Matis, Vinlandsleid 12, IS-113 Reykjavik, Iceland

Fish protein hydrolysates (FPH) possess various bioactivities making them a desirable ingredient in health foods. During the hydrolysis process heating and shifts in pH can cause negative changes in the properties of the resulting FPH due to lipid oxidation. Thus it is critical to understand the oxidative processes during hydrolysis to aid in the commercial development of bioactive FPH products. The objective of this study was to investigate oxidative processes during enzymatic hydrolysis of cod protein isolates and a washed cod model system. A washed cod model system was prepared. The cod protein isolate was produced according to the alkali-aided pH-shift method. Different levels and combinations of hemoglobin, iron and fish oil were added to both systems. Protease P "Amano" 6 was used to hydrolyze the two systems (2% protein) at pH 8 and 36°C to achieve 20% degrees of hydrolysis (%DH). Lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) were analyzed at 0, 5, 10, 15 and 20 %DH. Sensory properties were investigated

before and after the hydrolysis process. The effect of oxidation on antioxidant properties of the FPH products was investigated by ORAC, radical scavenging, reducing power, metal chelation and cellular antioxidant activity assays. Results showed different levels of oxidation in the FPH samples, demonstrating that all variation factors in the study had an impact (system type, level and combination of pro-oxidants and %DH). While results obtained by chemical based assays showed a negligible impact of oxidation products on antioxidant properties the results from the cellular assay indicated a negative impact of oxidation products on cells. This study provides important information for producers of FPH, particularly those aiming at producing bioactive FPH. Also, it shows that FPHs may have similar antioxidant properties in chemical based assays but different effects in cell based assays.

Presentation 5

Technical Session 2: Seafood Utilization

Tuesday, October 30
3-5:00 pm

Novel Insights into Pigmentation and Salt Solubility of pH-Shift Produced Protein Isolates

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The pH-shift method, also referred to as isoelectric solubilization/precipitation, gives a unique opportunity to isolate functional muscle proteins from bony complex raw materials such as whole fish and fishery by-products. During the last 10 years, the Food Science group in Chalmers has applied the pH-shift method on a long series of raw materials, including whole herring, whole vendace, whole shell-on mussels, cod frames and cod heads. Average protein yields have ranged from 50-75%, and gels produced have been decent to good. In addition, dioxins and toxins have been efficiently removed from contaminated raw materials. We have

thus considered this processing method a very promising strategy to produce food ingredients from low value raw materials that currently go to feed or biogas production. Based on both lab scale and pilot larger scale trials, we have however identified two challenges that may prevent an optimal use of the protein isolates. One of them is a drastically reduced protein salt solubility of the isolates compared to the non-processed muscle, the other is the difficulty to remove enough pigments to make the color of the isolates appealing. Research has recently been conducted to overcome both of these difficulties.

Presentation 6

Restructured Seafood Products with Glucomannan as Principal Ingredient: Effect of Salt and Oil Addition

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Previous works have described the ability of konjac glucomannan (KGM) to form a gel net in which non-functional mince muscle could be incorporated as a filler in order to elaborate restructured seafood products. This was done by making mixtures of aqueous KGM solutions (AGD) at 3 and 6% (w/v) and minced fish muscle with poor gelling properties at ratios of 50:50 and 25:75. A good prototype gel, with flavour and texture similar to that of fish (hake) muscle was obtained when AGD at 6% (w/v) was mixed with minced fish muscle at a ratio of 25:75. The final glucomannan concentration was 1.25% and the pH rose to 11.0 with 0.6N KOH to deacetylate the glucomannan and form a certain texture and a thermostable character. Afterwards, the samples were neutralised. In order to improve sensory and functional properties 5% of fish oil (Lot *A*) and 0.8 % of salt (Lot *S*) were added and then evaluated and

compared with the control (Lot *C*) during 0, 1 and 3 months of frozen storage at -18°C. Water binding capacity (WBC), cooking loss (%), puncture and viscoelasticity from creep-recovery tests were performed. Before freezing, *S* had the lowest gel strength (GS) and *A* the highest. Similar elasticity and high WBC >85% were shown for *C* and *S*. As the frozen storage time increased, there was a slight decrease in WBC and a noticeable increase in breaking force (BF) and GS in all of them. As a result, *C* and *A* lots experienced an important loss of elasticity after 3 months. However, *S* maintained better elasticity and network connectivity, indicated by the low relaxation exponent (*n*). Therefore, the addition of salt would seem to be beneficial in the elaboration of this kind of fish restructured product as it protects its structure maintaining a good degree of cohesion.

Presentation 7

Optimal Enzymatic Hydrolysis of Discarded Crustaceans in North Atlantic Fisheries

Carla Lopes¹, Diana Rivas¹, Tatiana Ordóñez¹, Amaya Franco-Uría², Ricardo Pérez-Martín¹, Antonio A. Alonso¹, Luis T. Antelo¹

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It is a well-recognized fact that worldwide fishing activity has been responsible for producing high quantities of discards (overboard dumped dead, unwanted fish), which generate important negative economic and environmental impacts. This problem is nowadays perceived by society as a serious problem that threatens sustainability of marine resources. This issue must be addressed as a high priority in actual and future fisheries policies, aiming both a drastic reduction of discards as well as to make the best possible use of the captured resources/biomass avoiding its waste. As a consequence, the creation of new high-added value marine products has been the aim of intense research during the last decades.

During the work developed in the LIFE+ Project FAROS, it was stated that Northern Spanish and Portuguese coastal bottom otter trawl fleets discard up to 2,250 t/year of crustaceans (mainly *Polydora* spp. and *Munida* spp.). It is well known that the exoskeleton of crustaceans is a significant source of chitin, a marine polysaccharide with biological

and physicochemical properties of interest in the nutraceutical and medical sectors.

In this work, the enzymatic hydrolysis of muscle of *Munida* spp. has been studied in order to define optimal experimental conditions which improve the hydrolysis degree as a key step on the production of chitin. Such conditions were obtained through a complete factorial design with three experimental control variables (temperature, pH and enzyme-substrate ratio).

In addition, experimental data were used to define a robust and reliable mathematical kinetic model based on the Michaelis-Menten rate expression. The objective is to give an accurate description of the enzymatic hydrolysis dynamics and their influence on final product quality and process productivity. Moreover, this model is used as the core of the proposed control problem for systematically computing optimal operation policies, aiming the maximization of the hydrolysis degree.

Presentation 8

Quantification of Low Molecular Weight Nitrogen Containing Compounds in Red Salmon and Alaska Pollock Whole Fish, Fish Meal and Byproduct

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There is interest by fish processors in the identification and eventual extraction of higher valued low molecular weight nitrogen compounds from fish byproducts such as stickwater, hydrolysates and other byproducts. Hydrophilic interaction chromatography (HILIC) can be used for the separation and quantification of amino acids, small nitrogenous acids and bases, as well as other nitrogen containing metabolites. The objective of this study was to use a non-destructive, non-derivatizing, HILIC—HPLC method for analyses of abundant low molecular weight nitrogen containing compounds found in red salmon (*Oncorhynchus nerka*) whole fish, stickwater, and fishmeal; pollock (*Theragra chalcogramma*) whole fish, stickwater, fishmeal, heads and head hydrolysates. Three separate samples of all material were made. Samples were extracted and then centrifugally filtered through 3000 MW membranes. The identification of abundant low molecular weight compounds was accomplished using HILIC—HPLC.

Abundant low molecular weight nitrogen containing compounds were quantified and comparisons made. Hypoxanthine was found in

higher concentrations (7.1 g/kg dry wt) in salmon stickwater samples than pollock stickwater samples (4.4 g/kg dry wt). Creatine and creatinine were both concentrated in salmon stickwater fractions. Taurine concentrations were elevated in salmon (29 g/kg dry wt) and pollock (16 g/kg dry wt) stickwater samples. This study suggests that creatine, creatinine, taurine, and hypoxanthine are found in elevated concentrations in stickwater and show preferential partitioning to the stickwater fraction. Pollock heads and hydrolysates made from heads had similar concentration of taurine, and creatine.

The HILIC method developed allowed for the quick, separation, identification, and quantification of low molecular weight compounds in fish byproduct fractions. The method had a high percent recovery (98 ± 3%). The quantified results indicated there is a great deal of partitioning of these low molecular weight nitrogen containing components to the stickwater fraction during fishmeal processing. Fractions of stickwater may eventually be used as ingredients.

Presentation 9

Effect of Addition of Antioxidants to Herring By-Products on the Production Of Oil

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Annually, the herring industry in Norway generates almost 300 000 tons of by-products. Most of it is used for production of animal and fish feed. However, the by-products can be a valuable source for production of fish oil and fish protein hydrolysates for human consumption.

In the industry, the goal is to produce a crude oil with best possible quality and stability. The quality of the raw material together with the processing conditions will affect the oxidative status and stability of the crude oil. Low content of free fatty acids (FFA), low oxidative status (low peroxide and anisidin value) and high stability (resistance towards oxidation) are desired properties for oils intended for use as supplements or functional food ingredients. A way to protect the raw material during processing and produce oil with low oxidative status and high stability can be by adding antioxidants to the raw material prior to oil production.

The objective of the work was to study the effect of addition of antioxidants to herring by-products prior to oil production on the quality and stability of the produced oil. Several antioxidants were tested in lab scale using a screening test developed to study the inhibition effect of the antioxidants on the oxidation of the by-products. The most potential antioxidants (butylhydroxytoluene (BHT) and propyl gallate (PG)) together with citric acid were chosen for further testing in a mobile production plant. Thermal treatment and enzymatic hydrolysis were used as production methods.

Addition of antioxidants to the raw material prior to oil extraction showed to influence both the oxidative quality and stability of the produced oils.

Presentation 10

Purification of Alaska Cod (*Gadus macrocephalus*) Liver Oil Using Short-Path Distillation

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In Alaska large quantities of fishery byproducts are used for production of fishmeal and fish oil. However, most fish oil produced is crude or unrefined. Unrefined fish oils are typically used as an ingredient for animal feeds. If the fish oil is to be used as a food ingredient, further processing steps are necessary to purify the product to meet specifications for human use.

The aim of this research was to use short-path distillation (SPD) to purify oil rendered from cod livers having the lowest (lipids<30% w/w; March) and highest (lipids>50% w/w; December) lipid content within a one-year harvest season. Plate-frozen liver blocks (3 x 7.5 Kg) were obtained from Alaska Leader Fisheries (Kodiak) in Dec. 2011 and Mar. 2012. Livers were comminuted and oils rendered multiple times until a quantity of 1.5 L of oil was obtained. Rendering was conducted under nitrogen atmosphere and continuous stirring (65°C; 30 min), and oils separated from solids by centrifugation (7,500 rpm; 30 min). Oils were

distilled using a bench-top 2" wiped-film SPD (Pope Scientific, Saukville, WI). The distillation process was repeated nine times for each oil type.

Purification of all cod liver oils with SPD resulted in significant ($P<0.05$) decreases in water content (Karl-Fisher method), free fatty acid values (AOCS # Ca 5a-40), peroxide values (AOCS # Cd 8b-90), and *p*-Anisidine values (AOCS # Cd 18-90). Crude and SPD-purified cod liver oils had similar ($P>0.05$) fatty acid profiles; accordingly, SPD did not affect the contents of nutritionally important fatty acids such as EPA and DHA. All SPD-purified cod liver oils met edible fish oil specifications.

Crude fish oils can be easily purified to nutraceutical-grade using SPD. Main advantages of SPD, as compared to the traditional four-step fish oil refinement protocol, are that the process is quick and doesn't require use of chemicals.

Presentation 11

Technical Session 3: Muscle Chemistry

Wednesday, October 31

8-10:00 am

Kinetic Modeling of Kramer Shear Resistance and Water Holding Capacity of Frozen Stored Hake (*Merluccius merluccius*, L.) Muscle

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The analytical methods developed to authenticate seafoods usually address the verification of the species, geographic origin and production method, but there is a lack of methods to authenticate its processing and to estimate the shelf life of frozen fish products. Yet, this is necessary not only to comply with the current legislation about labelling but also as a demand from the fish sector.

The Arrhenius equation relates the specific reaction rate (k) with the absolute temperature (T) so that if a given physical change or chemical reaction follow an Arrhenius type behavior, the measurement of this process can provide an estimation of the storage time if the temperature is known and *viceversa*. It has been successfully used to correlate the temperature dependence of the rate of the reactions of many biological materials including food systems. Given that WHC and Kramer shear resistance are both important parameters to evaluate the quality of frozen stored lean species and that

they are easy to perform in a quality control laboratory, the aim of the present work was to establish whether the temperature dependence of these two parameters followed an Arrhenius type pattern during frozen storage of hake (*Merluccius merluccius* L.) muscle and if so, to determine whether they could be used to estimate quality changes in terms of time-temperature history.

For each of the three storage temperatures examined (-10 -20 and -30 °C), Kramer shear resistance was adjusted to zero-order kinetics, whereas WHC was fitted to a logarithmic function. The time and temperature dependence of both parameters was modeled in one-step by using non-linear regression. A good agreement was found between predicted and observed values for both of them, which allowed good physical interpretation of parameter estimates and indicated their potential usefulness for quality management.

Presentation 12

Quality of Frozen Fillet of Alaska Pollock as Accessed by Myosin Denaturation Study

Kunihiko Konno and Yoshiko Konno

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Fish and its meat-based products are distributed in frozen form world-widely. We have proposed that myosin denaturation is the sensitive index to evaluate the quality of fish meat (Bluefin tuna and salmon). To study myosin denaturation, various indices such as salt solubility, monomeric myosin content (myosin aggregation), chymotryptic digestion (internal structural change of myosin molecule) as well as ATPase activity were adopted. In the presentation, myosin denaturation in three frozen fillets of Alaska Pollock (two imported (A and B) and one domestic (C)) was studied using fresh meat (D) as control.

Chopped meat (1 g) was once washed with 0.05 M NaCl, pH 7.0 (40 ml) and homogenized in the same buffer to convert into meat homogenate. Protein contents in 1 g of sample (D) was 112 mg, while ones from frozen fillets are 116 mg (C), 84 mg (A), and 62 mg (B). Ca^{2+} -ATPase activity for (D) was 0.075 $\mu\text{mol}/\text{min}/\text{mg}$, while three frozen samples gave decreased activities of 0.047 (C), 0.027 (A), and 0.021 (B). Remarkably decreased salt-solubility

and monomeric myosin content was found with (A and B) but they were kept high for (C). Chymotryptic digestion revealed severe damage at subfragment-1 region for (A) and (B). Microscopic observation of the homogenate revealed myofibril-like structure with striation on the surface.

Myosin denaturation in commercially available frozen Surimi of Alaska Pollock, Pacific whiting, southern blue whiting, and Arabesque greenling were also studied. Loss of salt solubility without Mg-ATP was commonly observed, while practically all of myosin was salt-soluble in the presence of Mg-ATP. A slight decrease of the monomeric myosin content was commonly observed. However, morphological structure of the homogenate was completely different from the muscle homogenate showing large aggregates without clear striation on the surface.

Presentation 13

Effect of Mechanical Impact, Salt Concentration and Storage Temperature on Rainbow Trout (*Oncorhynchus mykiss*) Erythrocyte Lysis; An Approach to Decrease Hb Leakage into Fish Muscle

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Fish-hemoglobins (Hbs) have a strong pro-oxidative capacity. In vivo, Hbs are located in the erythrocyte. During post mortem handling of fish Hbs can however contaminate the fish muscle when erythrocytes burst, thereby triggering oxidation. The erythrocytes can become disrupted for example by mechanical treatment, after washing with hypo- or hypertonic solutions or after being subjected to temperature changes. With the purpose to optimize post mortem handling of fish from an oxidation perspective, the aim of this study was to determine the effect of mechanical treatment, hypotonicity and temperature on rainbow trout erythrocyte lysis over time. The effect on metHb formation was also investigated. The treatments were chosen to simulate handling that may occur during realistic fish processing conditions. For methodological reasons, the effect of different anticoagulants on erythrocyte lysis was also tested.

The effects of mechanical impact were tested by dropping the tube with whole blood or washed erythrocytes using two different test conditions; a drop of 2 meters

twice and a drop of 2 meters 5 times plus a control. The concentrations of NaCl tested ranged from 0 % to 3% and the temperature ranges tested were: 0°C (on ice), 4-6°C and 10-12°C. Mechanical impact and temperature were evaluated at 0.9% NaCl. The anticoagulants evaluated were heparin, EDTA and citrate. The hemolysis was studied during time periods of up to two weeks.

Initial results from the study show benefits of using a 0.9 % salt solution and minimizing mechanical impact to limit erythrocyte burst. Temperatures of 0°C and 4-6°C limited hemolysis compared to 10-12°C. Of the anticoagulants EDTA induced erythrocyte lysis in comparison to heparin and citrate. Small adjustments during early post mortem handling of fish may thus limit the contact between Hb and muscle components susceptible to oxidation. Results from a factorial design approach will also be presented.

Presentation 14

A Study of the Microstructure Sizes of the Pre-Rigor Fillets of Atlantic Salmon during Superchilling Process and Superchilled Storage

Lilian Daniel Kaale¹, Trygve Magne Eikevik¹, Turid Rustad², Tom Ståle Nordtvedt³, Tora Bardal⁴ and Elin Kjorsvik⁴

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The pre-rigor salmon fillets were superchilled in an impingement freezer using two different treatments, F (-20°C, 4.2 min) and G (-30°C, 2.1 min) and stored at $-1.7 \pm 0.3^\circ\text{C}$ for 29 days. The objectives of this work were to compare between the ice crystal sizes of pre-rigor salmon fillets superchilled at fast and slow rates and to assess the change of these microstructure sizes during superchilled storage. The influence of these treatments on the microstructure of pre-rigor salmon fillets was studied. The equivalent diameter of the intracellular ice crystals formed at the surface of the salmon fillet were 60 ± 5 and $23 \pm 1 \mu\text{m}$ for the samples subjected to treatments F and G, respectively.

The size of the ice crystals increased significantly after one day of superchilled storage. This is due to two important factors during superchilled storage: Firstly, the large difference between the superchilling process temperature (-20 and -30°C) and the

superchilled storage temperature ($-1.7 \pm 0.3^\circ\text{C}$). This large temperature difference will cause the growth of the ice crystals, particularly the small ones that were formed at the surface of the salmon fillets. Secondly, the thermal gradient effect that was created during the superchilling process. These gradients were observed to result in slight melting of the small ice crystals formed at the surface layer and the subsequent water diffusion to larger ice crystals. Results showed that after one day of storage, when temperature equalisation was achieved within the samples, the growth of the intracellular ice crystals was not significant ($P < 0.05$) at any storage times

It was concluded that, formation of the ice crystals within the food muscles regardless the superchilling rates is most important factor for reducing damage of food muscles and hence maintains their quality.

Presentation 15

New Insights Regarding Lipid Oxidation in Fish Muscle and Its Inhibition

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The primary mechanism of lipid oxidation in fish muscle is difficult to determine due to the multitude of potential reactants that are endogenously present including lipoxygenases, myeloperoxidases, low molecular weight metals, myoglobin (Mb), and hemoglobin (Hb) from residual blood. ApoShp, a bacterial protein, can specifically inactivate Hb by acquiring hemin (ferriprotoporphyrin IX) that is released from metHb. Hemin was transferred from carp metHb to apoShp at pH 6.0 and 4°C whereas no hemin was transferred from carp metMb at the same conditions. ApoShp strongly inhibited lipid oxidation in macerated, trout muscle during iced storage which suggests that endogenous Hb was the primary catalyst of lipid oxidation.

Hb from bighead carp was a better catalyst of lipid oxidation in washed muscle compared to Mb at pH 6.0, yet Mb did incur some lipid oxidation. Carp metMb released hemin at pH 5.5 and 4°C but not at pH 6.0 using apoH64Y reagent. These findings suggest that the mechanism of fish Mb-

mediated lipid oxidation may switch from a peroxidase to hemin-mediated as pH is decreased from 6.0 to 5.5.

Hemin loss is fastest in perch Hb (4 glycines at site E14), intermediate in trout IV Hb (2 glycines and 2 alanines at E14) and slowest in trout I Hb (4 alanines at E14). Wild-type bovine Mb which contains E14(Ala) was used as a model globin to examine the effect of glycine substitution at E14 on hemin loss. Hemin loss from the E14(Gly) mutant was 45-fold faster compared to E14(Ala). E14(Gly) provides a large gap for protons to enter the heme pocket which disrupts the linkage between the porphyrin and the globin via protonation of the proximal histidine.

Future antioxidant strategies should consider the role of released hemin as a major pathway that leads to quality deterioration during storage.

Presentation 16

Emulsifying and Antioxidant Properties of a Shrimp Hydrolysate (*Pandalus borealis*) Conjugated With Xylose or Dextran through Maillard Reaction by Dry-Heating Under Mild Conditions

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Emulsifying and antioxidant properties of a shrimp hydrolysate (HN) prepared through Maillard Reaction (MR) by dry-heating (50°C for 48h ; relative humidity = 75 %) with dextran (D) or xylose (X) and at sugar/HN ratios of 0.6 (HX0.6, HD0.6) or 1.2 (HX1.2) were investigated. They were concurrently compared to the emulsifying and antioxidant properties of native sodium caseinate (CN) and its glycoconjugates obtained under same MR conditions at sugar/CN ratio of 0.6 (CD0.6, CX0.6). Important modifications of HN after Maillard Reaction were found with xylose. Size exclusion chromatography (SEC-HPLC) showed the occurrence of new fractions with higher molecular weights which were ~2.7-fold higher and ~3.6-fold higher for HX0.6 and HX1.2, respectively, compared to HN. Therefore, antioxidant properties obtained through reducing power and ORAC value were increased by MR while xylose/HN ratio increased. As a result of a 4h ageing period,

emulsions (oil/water 50/50 w/w at pH = 7) containing HN or its glycoconjugates at 0.5 % (w/w) showed an oil phase volume fraction higher ($\alpha_f \sim 0.8$) compared to those obtained with CN and its glycoconjugates ($\alpha_f \sim 0.5$) at the same concentration. Yet, in comparison to HN and HX1.2, emulsions in the presence of HX0.6 had the highest consistency index values (K), the highest apparent viscosities (η), and the lowest flow index (n). MR with dextran had not significantly modified the functionality of HN or CN, except the solubility of CN. Furthermore, sugar-conjugate CX0.6 showed important insolubility. Therefore, using MR between xylose and HN under the present conditions could be a useful approach to modulate both antioxidant properties and the role of HN on rheological properties of emulsions, depending on the sugar/HN ratio.

Presentation 17

Technical Session 4: Seafood Processing 1

Wednesday, October 31

10:20 am - Noon

Lipid Degradation of Cod Liver During Frozen Storage as Influenced by Temperature, Packing Methods and Season

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Lipid deterioration is one of the most common and detrimental changes in food products during storage, resulting in quality loss and reduced shelf life. The presence of highly unsaturated lipid in marine raw material makes them highly susceptible to oxidation and to enzymatic hydrolysis leading to unpleasant flavour and reduction of nutritional value. The deterioration of marine lipids can be influenced by internal factors like enzymes, transition metals and microbial growth and external factor like temperature, light and oxygen accessibility. Frozen storage has been widely employed to retain fish products properties before consumption or employed in other technological processes. During the frozen storage of marine raw material, lipid hydrolysis and oxidation have been shown to occur and become an important factor of fish acceptance as influencing e.g. rancidity development. This study was concerned with cod liver stability during frozen storage.

Lipid damage of cod liver during frozen storage was studied, where the effects of storage temperature, packing methods and seasonal

variation were evaluated. For this, hydrolytic and oxidative lipid damages were analysed. Lipid damage within different part of the liver was also considered. Increasing lipid hydrolysis and oxidation were observed for most of the samples throughout the frozen storage. Vacuum packing and lower frozen storage temperature had significantly stronger preservative effect. Some higher lipid hydrolysis could be observed in cod liver captured in June than in its counterpart from the September trial. Concerning the peroxide formation, generally more increase between storage times were observed for cod liver captured in June when compared to their corresponding September liver. Significant difference in lipid oxidation was observed in different layer of the liver while lipid hydrolysis showed minimum variation between the surface and the middle part of the cod liver. Based on the present results, packing method and storage temperature have significant effect on lipid hydrolysis and oxidation in frozen cod liver.

Presentation 18

Linking Pre-Harvest Data with Post-Harvest Data for Process Optimization in the Salmon Value Chain

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To ensure a sustainable and optimal utilization of the salmon resource, the salmon value chain (SVC) has to be optimized. This calls for all players in the SVC to jointly develop strategies for optimizing quality and yield together with a trim down of waste and energy consumption. By tradition the SVC is regarded as linear, however our hypothesis is that to optimize the SVC a coupling of data from different links in a circular way is essential to understand their impact on each other and to reveal their impact on the overall SVC.

Based on interviews, field trips and literature review the SVC will be mapped in this study. The work includes a survey of the information capture and information exchange along the SVC, an analysis and validation of existing SVC including advanced data analysis to clarify the environmental, economic and quality impact from the individual links and their effect on the overall SVC. The

analysis will also point out which information needs to be improved and thus which information will be beneficial to collect to optimize the SVC in the future. A data-driven modelling approach will be used to develop decision support tools for the SVC partners. Access to the database from an industrial processing industry consisting of huge amounts of historical data relating to purchasing, production and economy will be the basis for this. The scientific challenges include how to couple data from the different links e.g. raw material history, production parameters, processing data, economic data and distribution, and understand mutual impacts.

The developed underlying assessment tools will make it possible to carry out decisions based on predictive, mathematical models both for improvements in the production and for quality grading throughout the complete SVC in the future.

Presentation 19

Low Field Nuclear Magnetic Resonance (LF NMR) Spectroscopic Analysis of Hake (*Merluccius merluccius*, L.) Upon Freezing. A Possibility for Authentication of Fresh vs Thawed Muscle

Isabel Sánchez-Alonso¹, Pilar Moreno² and Mercedes Careche²

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Current EU regulation states that fishery products to be consumed raw or preserved by light treatments must be frozen at a core temperature of -20 °C or lower for at least 24 h in order to inactivate parasites (CD 91/493/EU of 22 July 1991). It is also obligatory to inform customers about the species, origin, method of production and whether the fish is fresh or thawed (CR EC No. 2065/2001 of 22 October 2001). In this context analytical methods need to be developed to authenticate seafood processing conditions.

Low field nuclear magnetic resonance (LF NMR) is non-destructive and non-invasive spectroscopic technique which requires minimal preparation and gives information of water distribution and mobility which are relevant parameters affected by freezing and frozen storage. T_2 transversal relaxation times measured by LF NMR have been shown to detect in muscle the presence of several populations of water related to frozen storage conditions.

The aim was to describe to what extent LF NMR data from hake (*Merluccius merluccius* L.) fillets

subjected to different freezing methods (air blast freezer, liquid N₂, and *walk in* freezers), *post mortem* time before freezing (3 and 14 days), and storage conditions (-20 °C and -10 °C for 1, 8 and 18 weeks) were different from those of unfrozen muscle. The second objective was to explore if these differences could be used to discriminate between fresh and frozen/thawed hake.

LF NMR distribution function obtained by the CONTIN algorithm showed that unfrozen samples displayed a major band centered 40-80 ms whereas when samples had been previously frozen, an additional band appeared with varying amplitude and relaxation time depending on the conditions. A discriminant analysis of all data rendered that 95% of the cases were correctly classified as fresh or frozen/thawed. The results show the potential of LF NMR for authentication of fresh vs frozen/thaw in a wide variety of processing conditions.

Presentation 20

Temperature Control During Containerized Sea Transport of Fresh Fish

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Insufficient temperature control in cold chains has negative effects on fresh fish quality and storage life. Until recent years fresh fish fillets and other high-value, short storage life products have generally been transported by air freight but large volume exporters now take advantage of better temperature control and the lower cost option of refrigerated sea transport. The focus of this study was to analyse temperature control in sea transport of palletised, chilled fish products in reefers. The temperature distribution inside reefers was mapped in an outside, stationary environment and influences of variable pallet stowage patterns, reefer types and seasons were analysed. A field test of real sea transport was also conducted.

The results showed that a room can be found for improvement in sea transport cold chains. The field test demonstrated the importance of correct operating procedures during loading of reefers and their handling from the processor to the end location. Furthermore, it showed that the temperature control during sea freight may be improved by selecting the reefer types most suitable for fresh fish transport and selecting different set point temperatures during summer and winter. The mappings of temperature distribution inside the reefers showed spatiotemporal variability and imply that a more uniform distribution can be achieved by means of forced air circulation and modification of pallet setups.

Presentation 21

Quality Consequences of Fish Bleeding and Methods in Commercial Fisheries

Leif Akse, Sjúrdur Joensen, Karsten Heia and Heidi Nilsen

Nofima, P.O.Box 6122, NO-9291 Tromsø, Norway

In commercial fisheries large quantities of fish must be handled and processed within a short time period after catch. With an increasing focus and request for quality there is need for methods and handling practices that support and maintain good quality of the fish. It is well known that bad or insufficient bleeding of the fish is detrimental to quality. Remains of blood in fish muscle and in blood vessels will affect both the quality of the raw material as well as the quality of further processing and fish products.

In this work we documented how both the method of bleeding and time before bleeding starts impact on how well the fish are bled. The experiment verifies this for different bleeding methods and time intervals from capture to bleeding that are realistic in commercial cod fisheries. The quality of the raw material was assessed by sensory

evaluation of blood discoloration in muscle and blood vessels as well as instrumental measurements of blood in fillets by hyperspectral imaging.

When cod was bled alive, immediately after catch, two-stage bleeding methods, where the fish is first bled and afterwards gutted, provided better bleeding, as compared to direct gutting methods. Bleeding became poorer depending on the time from catch to start of bleeding. Time before bleeding was evaluated at intervals of 0, 30, 60, and 180 minutes from catch to start of bleeding. There was significant ($p < 0.01$) difference between all the tested time intervals. The main difference was found between fish bled immediately after death and that bled at later time stages after death, documenting the quality effect of immediate bleeding of fish after catch.

Presentation 22

Technical Session 5: Seafood Processing 2

Wednesday, October 31

1:20-3:00 pm

Automatic Detection of Nematodes in Cod Fillets (*Gadus morhua* L.) by Interactance Hyperspectral Imaging

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Nematodes, often referred to as cod worms, constitute an aesthetic as well as a food safety problem. Today all cod fillets are skinned and manually inspected from both sides on a candling table, and remnants such as nematodes are located and removed by hand. Due to the high cost of manual inspection, it is of great interest to have this operation automated. The goal for such a system is to sort out fillets with remnants that need manual processing, and hence reduce the amount of manual labor required. The manual detection efficiency of nematodes in cod fillets under industrial conditions is reported to be between 60 and 70 %.

Detection of objects embedded in tissue, using visible light, is difficult due to light scattering. The optical properties of the surrounding tissue will influence the spectral characteristics of the light interacting with the object, and the spectral signature observed from the object will be directly affected. A novel method for calibrating the spectral

signature of small objects embedded in translucent material, by the estimated local background spectrum, has been developed. The method was evaluated under industrial conditions in a fillet processing plant, using a new hyperspectral imaging system for automatic detection of nematodes in cod fillets. The system can operate at a conveyor belt speed of 400 mm/s, which meets the industrial required speed of one fillet per second. For one or more false alarms in 60 % of the fillets sampled after the trimming station, a Gaussian maximum likelihood classifier detects 70.8 % and 60.3 % of the dark and pale nematodes, respectively. This is better than what is previously reported using a higher resolution instrument on a slow moving conveyor belt under lab conditions, and comparable or better to what is reported for manual inspection under industrial conditions.

Presentation 23

Non-Destructive Freshness Assessment of Atlantic Salmon (*Salmo salar* L.) Fillets by Visible/Near-Infrared Spectroscopy

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It is a common conception among consumers that fresh fish is caught/harvested and then stored chilled for a brief period of time prior to use. Frozen fish usually has a lower market price than fresh fish. A system capable of measuring the quality of every fillet in production would be of great value for preventing unfair competition by false labeling of catching/harvesting date and frozen-thawed, and for documenting the raw material quality. Visible/near-infrared spectroscopy has shown promising results for assessing the frozen-thawed status and the freshness as storage days in ice for a range of different fish species. Previous works for non-pigmented fish, such as cod and mackerel, have shown that most of the spectral changes in the visible region observed during storage, can be explained by changes in the heme proteins. However, it can be problematic to directly apply the previous findings to salmon. This is because Atlantic salmon muscle contains high levels of the carotenoids with high absorption in the wavelength region below 600 nm overlapping most of the absorption bands of heme proteins.

In this work we have evaluated visible/near-infrared spectroscopy for freshness prediction and frozen-thawed classification of farmed Atlantic salmon fillets. Both a handheld interactance probe for rapid measurements on single fillets and an imaging spectrometer for online analysis at industrial speed of one fillet per second have been explored. Freshness as storage days in ice can be determined with an accuracy of 2.3 days, whereas frozen-thawed fillets can be completely separated from fresh fillets. The region between 605-735 nm, which excludes interference by carotenoids and water, is well suited to both freshness prediction and frozen-thawed classification of salmon fillets. The results indicate that the spectral changes are explained mainly by oxidation of heme proteins during freeze-thaw cycle and chilled storage in ice for salmon fillets.

Presentation 24

Near Infrared Spectroscopy (NIR) an Ultimate Method for Quality Control

Christel Solberg and Chris Andre Johnsen

Faculty of Biosciences and Aquaculture, University of Nordland, 8009, Bodø, Norway.

Farming of Atlantic salmon is a new very successful industry in Norway. Approximately 800.000 tons of Atlantic salmon is produced yearly and this is expected to increase. NIR-analysis is non-destructive and very rapid with a measuring time from seconds to less than a minute. One can also get the results for several constituents simultaneously as fat, water and protein. NIR technology has therefore been adapted in several different areas from feed development, breeding program to the slaughter quality.

NIR spectroscopy of the feed has been adopted by the feed producing factories and is in regular use for at line quality control. The feed producers are constantly improving the feed composition making it necessary to follow up the results of the new feed on the growth and the biological composition of the fish. Analysis of the proximate composition of

minced fish samples has therefore been extremely useful because it make it possible to analyse large number of fishes during the whole production period and make it possible to merge biological and chemical data for multivariate data analysis. Analysis directly on the fillet or on the live fish also allows a truly non-destructive measurement but to the price of lower accuracy.

Analysis of different types of samples (live, fillet or minced) make it necessary to develop separate calibration. Different fish species will also demand separate calibrations. Variation in the sample temperature can be avoided by storing the samples on ice before measurement with the exception for live fish where calibration must be performed with samples covering the temperature variation.

Presentation 25

Automated Sorting of Pelagic Fish based on 3D Machine Vision

Ekrem Misimi, John Reidar Mathiassen, John Andre Fossum, Morten Bondø, Bendik Toldnes, Stein Ove Østvik, Harry Westavik

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Pelagic industry needs to sort fish individually according to pre-determined weight classes. Weight is an important parameter by which the price of whole pelagic fish (*Clupea harengus* and *Scomber Scombrus*) is determined. Current mechanical weight graders are capable of a high throughput but have a relatively low accuracy. Consequently, there is a need for a more accurate weight estimation of whole pelagic fish, for sorting pelagic fish according to species, and for separating bits and pieces from whole fish, in order to make the processing system more effective. To address these issues, a 3-dimensional (3D) machine vision system was developed for weight estimation of whole pelagic fish and for grading according to species. A machine vision based concept was chosen due to its versatility i.e. ability to estimate weight of fish, control quality, and determine species by a single image generated from camera. The machine vision subsystem uses a 3D laser triangulation system above a conveyor belt

moving at a speed of 1000 mm/s. Weight prediction models for both herring and mackerel were developed for several feature sets, and a linear regression model consisting of several 2-dimensional (2D) and 3D features enabled accurate weight estimation.

Physical grading of pelagic fish in pre-determined weight classes was done using dropping flaps which were pneumatically actuated by a control system, integrating the 3D machine vision, conveyer belt and actuating subsystem. Both 3D machine vision and actuation of dropping flaps is done from a main program designed in LabVIEW. The entire architecture of the system was designed in such a way that it enables an easy implementation in a commercially automated industrial system for sorting of pelagic fish.

Presentation 26

Separating and Sorting Shrimp for Market Grades, Quality and Uniformity with Machine Vision

Robert M. Lane¹, Dah-Jye Lee², and Dong Zhang³

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Machine Vision can be defined as the ability of machines to see, comprehend, or figure out something based upon a mathematical application in a repeatable manner with accuracy and precision

This discussion will consider a machine vision systems' flexible capabilities, given specific shrimp parameters, to detect, measure, analyze, sort, grade transport and evaluate based on size, color, defect and completeness.

Refinement of the technology incorporates and address industry needs. These include food quality parameters, addressing some USDC defined grade characteristics, and values as defined by users in the market in order to move, hold, account for and grade shrimp. Design considerations in the equipment incorporate using local business

replacement for materials and parts which move and transport shrimp while grading. This is to keep down time for maintenance and repair to a minimum.

The proposed system is an example of a machine vision system designed to incorporate the aforementioned technology and capabilities while providing the capability to change grade sizes based on changing markets.

Research described in this presentation takes into consideration the current economic, business, market, climate, technology and regulatory changes.

Presentation 27

Technical Session 6: Seafood Processing 3

Wednesday, October 31

3:20-5:00 pm

Removal of Allergens from Fish Infected with *Anisakis simplex* Larvae in the Washing Process of Surimi Production

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Most of the commercial species of marine fish and cephalopods are infected with *Anisakis* L3 larvae, degrading the product and causing big economic losses to the fishing industry as food safety and quality issues related to parasites in seafood are growing worldwide. Human diseases due to eating fish parasitized with anisakid larvae represent one of the most important health problems related to fish parasites nowadays. The ingestion of infected fish may cause infection to the consumer by the live larvae when consuming raw or undercooked fish that do not kill the larvae and also allergy due to sensitization by ingesting the live larvae or the allergens excreted/secreted by the larvae into the fish muscle.

Freezing and heating fish muscle at certain time-temperature conditions kill the larvae avoiding human infection. However part of the known *Anisakis simplex* s.s. allergens are extremely resistant to heat and freezing treatments, therefore allergic consumers often have to exclude fish from their diet. Moreover, the visual detection of larvae in fish muscle causes rejection of the fish at purchasing.

The object of the work was to know if allergenic proteins are removed in the washing process to

obtain surimi from fish infected with *Anisakis simplex* [*A. simplex* s.s. (97.15%) and *A. simplex* s.s. and *A. pegreffii* hybrids (2.85%)] L3 larvae.

Artificially infected minced hake muscle (50 L3 larvae/100g mince) was washed in a pilot plant (3 cycles washing-decanting) with water or different solutions (1:4; w:v). *Anisakis* and *Anisakis simplex* antigens were quantified by immunodetection (Dot blot) in the three solutions recovered in each washing step (S_n) and the final washed muscle.

The results indicate that both types of allergens were removed in the 3 washing steps, even when the total protein in the recovered washing solutions decreased. This approach opens a line to utilize parasitized fish in a safer way.

Work financed by the Spanish Project AGL2009-12485-C03-01/02/03 (ANIDET). Fabiola Olivares carried out her work at the ICTAN on a grant provided by Science and Technology Program of the Government of Peru (FINCyT) and managed by LASPAU.

Presentation 28

Acoustic Measurements for Grading of Ice-Glaze Content of Single Frozen Prawns

Stina Frosch, Maria H. Ekgreen and Bo M. Jørgensen

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Ice-glazing is an important step in the production of frozen cooked and peeled prawns. The ice-glaze content is commonly in the range of 8-12% of the gross weight, but coatings of ice-glaze up to 25-45% have occurred. By law, the net weight of frozen seafood may not include the weight of ice-glazing, thus standardized procedures for determination of ice-glazing are required.

In this study, we investigated whether acoustic measurements combined with advanced data analysis can be used as a fast, semi-automated procedure for determination of the ice-glaze content of single-frozen prawns. Our hypothesis is that since ice and prawn meat have different acoustic characteristics, it is reasonable to assume that the acoustic signals obtained during transport of glazed prawns contain information about the ice-glaze content.

The data set consisted of three groups of samples (High, Medium and Low ice-glaze content). The sample size was either 50 g gross weight or single

prawns. The acoustic measurements were conducted on a special designed unit. The prawn samples are placed in the top of the unit, and when a valve is opened, the prawns fall down to the bottom of the tube and four microphones placed close to the bottom of the unit simultaneously record the sound. The acoustic signals are then pre-processed before principal component analysis (PCA) is conducted. The score values from the PCA with 50 g sample size clearly showed a discriminative performance according to the ice-glaze content which was not the case when measurements was conducted on single prawns.

The results suggest that acoustic measurements has the potential as a fast and semi-automated determination of ice-glaze content of single frozen prawns although to even out the within batch variation in ice-glaze content a certain amount of prawns should be measured simultaneously.

Presentation 29

Temperature Fluctuations and Quality Deterioration of Chilled Cod Fillets Packaged in Palletised Wholesale Boxes under Dynamic Temperature Conditions – Simulation of Air and Sea Freight

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Temperature variations in cod fillets packaged in four levels of expanded polystyrene (EPS) boxes and corrugated plastic (CP) boxes stored on pallets under thermal load were studied numerically and experimentally. In the experiment fillet temperature at multiple positions on the pallets along with environmental temperature and humidity were monitored during dynamic temperature storage. Differences in product temperature of up to 10.5 °C were recorded on the thermally abused pallets stored for 6.4 hours at mean ambient temperature of 18.5 °C. 3-D, time dependent heat transfer models were developed using the ANSYS FLUENT Computational Fluid Dynamics (CFD) software. A satisfactory agreement was yielded between experimental and numerical results. The model of EPS boxes was further developed in order to simulate temperature evolution inside a fully loaded, 12-level pallet under the same dynamic temperature conditions as a four-level pallet studied before. The mean temperature after 9-hour thermal load was 1.0

°C lower in the 12-level pallet but the maximum temperature evolution was similar in both pallets.

Parallel to this study, a storage life study was performed to evaluate the effect of the fish temperature variation on quality deterioration of the cod fillets as influenced by the box type used and their position on pallets. Storage life of thermally abused fillets was compared to that of fillets stored at steady temperature simulating well-controlled, containerised sea transport. Compared to storage life of fillets stored at steady temperature conditions, the dynamic temperature storage resulted in a storage life reduction of 1.5–3 days. The results from the current study suggest that the storage life difference between the most and the least sensitive boxes on a full size pallet in a real air transport chain can exceed 1–1.5 days, depending on the level of ambient thermal load.

Presentation 30

Spice-Cured Sprats Ripening, Flavor Development and Quality Properties

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The aim of this work was to describe the ripening process of spice-cured sprats and to identify the key quality attributes and their relationships to raw material pretreatment and package materials, and to evaluate the competitiveness and development opportunities.

Baltic sprat (*Sprattus sprattus balticus*) is the most caught fish species among the Estonian seacoast fishermen, and therefore it is important to understand catching season effects on sprat sensory and nutritional quality. Variation in fish water and lipid content during catching season was perceived by the sensory panel.

Spice-cured sprat products from fresh and frozen-thawed fish in glass and plastic package were prepared. Sensory attributes of the spice-cured sprats correlated well with free amino acids content and storage and loss modules.

It was found that the predominant species in spice-cured sprats were *Brochothrix thermosphacata*, *Lactobacillus sakei/curvatus* and in case fresh fish was used as raw material also *Aerococcus viridans*. Plastic

packaging compared to glass packaging promoted the growth of the genus *Lactobacillus* and/or *Aerococcus* and increased the rate of spoilage, which can be explained by permeability of plastic to oxygen.

Estonian spice-cured sprat products competitiveness was evaluated in local market (Estonia) and in new market (Thailand). The main drivers of consumer acceptance for the spice-cured sprat products were different, flavor and appearance in local market and appearance and odor in new market.

It can be concluded that spice-cured sprats made from frozen-thawed fish and/or packed into plastic package ripen faster and their sensory properties are different in hardness, sourness and rancidness. Those findings can be utilized by the sprat processing industry in product development, quality and stability control. To obtain a high and stable quality spice-cured sprat product fresh fish and air-tight packaging materials would be recommended.

Presentation 31

Processing Pasteurized Crabmeat: Critical Factors for Best Quality

Thomas E. Rippen

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A large international industry depends on successfully pasteurizing the meat picked from a variety of swimming crab species. Fifty years after the U.S. seafood industry first began pasteurizing Chesapeake Bay blue crab meat, these products remain among the most difficult to produce with consistent quality and shelf-life. At \$½ million per shipping container, the stakes are high for U.S. importers. Common quality defects include bluing and other appearance problems, bland or off-odors and flavors, mealiness and other textural defects, struvite, excess free liquid and premature spoilage. The basic controls for most of these defects have been known and implemented for many years so why do problems persist? The answers often lie in secondary causes little understood and under-appreciated by the industry. For example, bluing can

be triggered by pasteurizing at temperatures above 190°F (88°C). However, it also occurs when lower processing temperatures are used, occasionally even in the presence of appropriate additives. The role and sources of secondary factors, including trace metals, crabmeat properties and quality, post-process microbial growth, SAPP type and application methods will be discussed. Excessive free liquid in pasteurized products is commonly attributed to undercooking crabs. However, other factors are more often responsible requiring, for example, proper design and operation of cooked crab coolers, and prevention of condensate formation in picking rooms. Each major quality problem will be similarly addressed in this presentation with recommendations for its control.

Presentation 32

FEATURED SESSION: Moisture Control

Thursday, November 1
8-10:20 am

Chem-Free Products and Clean Labels

Steve Otwell

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Moisture control for seafood products is a critical factor both in terms of product yield and consumer satisfaction. Commerce must account for changes that can occur from the moment of harvest through processing, distribution, storage and final preparation. Beyond the initial practices with basic salt (sodium chloride), commercial breading operations introduced the use of moisture control agents during the mid-1960's to prevent moisture migration from the whole fish or shrimp muscle into the surrounding batters and coatings during frozen storage. This control was necessary to assure compliance with established product standards for specific percent breading. The moisture retention agents of choice were borrowed from the poultry and red meat industry that had been successfully using various blends of sodium tripolyphosphates (STP) and sodium chloride. The success progressively moved into applications for non-breaded items to maintain yields and net weights. This practice eventually advanced into questionable methods to primarily boost product weight. As efforts favored the addition of weight, buyers and regulatory authorities began to question abuse and potential economic fraud. Although research continued to demonstrate consumer preferences for products processed with reasonable amounts of moisture retention to provide appeal and prevent dehydration, some commercial practices had grown

dependent on larger moisture margins. Regulation was not consistent or effective, and lacked for reasonable measures that also accounted for the necessary conversion of animals to food and related consumer benefits. Frustrated buyers opted for controls that would not tolerate any use of moisture retaining chemicals. New buyer specifications began to feature the so-called phosphate-free or chem-free seafood. This trend merged with the current demands for clean labels suggesting no prior treatments or exposure to any food additives or processing aids that would require ingredient statements. In response, continued dependence on the moisture margin introduced use of less expensive non-phosphate blends involving bicarbonate salts, rice flours, buffers and a variety of ingredients that offered some moisture control while averting detection. The prevailing situation is further complicated by the increasing reliance on new sources for both seafood and retention agents, more dependence on farm-raised species, and needs for alternative product forms to suit the persistent demand for affordable seafood selections. Market implications mindful of the shifting supply and product costs suggest at least three categories linked with the moisture margin, i.e., buyers willing to pay, buyers beware and buyer indifference.

Presentation 33

Commercial Perspectives on Moisture Control Ingredients

Lisa M. Weddig

Director, Regulatory and Technical Affairs, National Fisheries Institute, McLain, Virginia, USA

In 2007, NFI implemented the Seafood Economic Integrity Initiative and formation of the Better Seafood Board to ensure consumer confidence that the seafood they purchase is honest. While the initial focus of the initiative was the commitment to properly label products for net weight, species identity and country of origin, NFI members have also pledged to adhere to all other pertinent FDA labeling laws.

The U.S. consumers is now more aware of and demanding a clear understanding of what is added to the foods that they eat. Ensuring that seafood

products are labeled properly will maintain consumer confidence. In addition, proper disclosure of the addition of moisture control ingredients allows for a competitive market place by creating a level playing field.

Many challenges exist for ensuring proper labeling of seafood products that have added moisture control ingredients – these will be outlined in the presentation.

Presentation 34

U.S. Regulatory Perspective on Moisture Control

Patti Ross

US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Food Safety, Division of Seafood Safety, 5100 Paint Branch Pkwy, College Park, MD

Moisture control has long been a concern for the seafood industry, and efforts to limit the loss of moisture during the processing and storage of fish and fishery products have led to the use of a variety of control methods. Moisture retention agents, or humectants, are intended to promote the retention of moisture in a food. One of the more commonly used moisture retention agents is sodium tripolyphosphate, or STP, but more recently the list of agents has grown. These agents may be used as single ingredients or in combination with each other.

FDA requires all ingredients be listed on the product label, except those ingredients exempted by Section 101.100 of the Code of Federal Regulations (CFR). Exemptions may include incidental additives, such as processing aids. These are defined

in 21 CFR 101.100(a)(3)(ii). FDA has been asked whether moisture retention agents may be classified as incidental additives or processing aids in order to exempt them from labeling requirements. FDA's regulations for additives and labeling are found at <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfr/cfrsearch.cfm>.

Recently, there has also been a great deal of interest on the use of "clean labels" and how incidental additives and processing aids are reflected on a "clean label." This presentation will give an overview of FDA's perspective on moisture retention agents with regard to ingredients, incidental additives, processing aids, labeling and "clean labels."

Presentation 35

Seafood Marinating – A Tool for Adding Value, Extending Shelf-Life and Providing Added Safety to Seafood Products

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Marination provides a long list of benefits to muscle foods: added value, enhanced flavor and yield, moisture retention, added shelf-life, enhanced safety, uniform texture and flavor (quality), enhanced nutritional profile, and other advantages. Red meat and poultry products have been marinated for decades with enhanced yield and quality. Marination can be as simple as soaking product in a solution allowing the solution to diffuse into the seafood or as complex as highly automated multi-needle injection equipment. Many researchers and technologists have shown that marination and/or acidification enhances shelf-life and sensory acceptance of seafood products; and that marinating fish filets increased their acceptance, shelf-life, and safety. Injection may be superior because it minimizes protein extraction which coagulates on the surface of the filets. A high pH, agglomerated phosphate with various phosphate chain lengths (including monophosphates) works well. The monophosphates are very important in a lower pH solution (or acidified) due to their powerful buffering properties. Agglomerated phosphate

blends function better than STP due to superior solubility and chemical make-up.. Potassium lactate and acetate can be used to extend refrigerated shelf-life and have been shown to enhance consumer preference. Marinated products are not as prone to be 'overcooked' (dry texture) by consumers who are often overzealous in trying to assure food safety by overcooking. Marination could enhance and equilibrate the dielectric properties of seafood products, making them easier to be microwavable. Marination also serves as a hurdle(s) in seafood products. Numerous studies have shown bacteriostatic and even bactericidal properties of different marinades, including some spices like rosemary and mustard, or their derivatives, against pathogens like *Listeria monocytogenes*, *Salmonella*, and others. Many times this is synergistic with enhanced antioxidant and/or functional properties of the marinade components. Inclusion of the right marinade solution could also lead to a better nutritional profile for seafood products.

Presentation 36

Seafood Integrity Elucidated by Novel Analytical Authentication Tools

Saskia van Ruth, A. Durá de Miguel, A. Koot, A. Tres, E. Capuano, S. Heenan, A. Lommen, E. Kok, M. Alewijn, and G. van der Veer

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People's growing awareness of health, environment and animal welfare has led to an increased public interest in the quality of foods and food production systems. Counterfeiting and adulteration is a complex global issue that causes severe economic damage and exposes consumers to unacceptable health and safety risks. Due to ongoing globalization of markets and advancing technologies, adulteration and counterfeit production is growing at an alarming rate. Any product with some sort of added value is susceptible to fraud. Fair competition between producers and sustained consumer confidence favour genuine production. Although administrative controls are pivotal, innovative approaches to understand, detect and deter counterfeiting, mislabelling and adulteration of seafood substantiate the confirmation of the uncompromised integrity of seafood considerably. Seafood authentication generally concentrates on compositional aspects (species, and addition of water and water retaining agents), processing (defrosted vs. fresh), geographical origin, production systems (wild/farmed/organic) and typicality (for specialty products).

Traditional analytical strategies for guaranteeing quality and uncovering adulteration have relied on the determination of the amount of a marker

compound or compounds in a material and a subsequent comparison of the value(s) obtained with those established for equivalent material. With complex quality characteristics, such as production systems or geographical origin, it is unlikely to find a single marker that allows discrimination between the genuine product and others. Even for a particular product, this approach is challenging. Selective fingerprinting, which involves analysis of a range of compounds which are considered potential discriminators, is the more promising approach. Techniques cover a wide range and include GC(-MS), LC(-MS), ICP-MS, SELDI-TOFMS, IR-MS, PTR-MS, NIRS, NMR, DSC, DNA-based techniques, bio-assays and visual image analysis. As these techniques result in a high number of variables the application of multivariate statistical methods greatly facilitate the evaluation of the data. In the presentation an overview of analytical approaches based on fingerprint type of methodology will be presented. Examples will be shown with regard to the species determination, detection of water retaining agents in seafood, provenancing, and discrimination between wild/farmed/organic production of salmon with these fingerprint tools.

Presentation 37

Updating Latest EU Rules on Food Additives and Additional Considerations

Jaime Forn

Budenheim Altesa S.L.U., Valencia, Spain

Everyone benefits from food's globalization thanks to additives. Additives play a key role in the complex food supply chain, for example: the final consumer not usually live near the production areas, therefore the use of additives help to keep the food safe, wholesome and appealing from farm to fork.

Previous 1995 first Food additives regulations needed an update and the European union went through it, issuing in 2008 incomplete rules which were implemented at the end of 2011.

Latest regulations (2008 and 2011) have included some articles on labeling, in order to secure even more food safety. Even though, there are products which contain none declared additives not easily found by analytical method. These chemicals are added to food because they increase food industry profits by reducing production costs, extending shelf life and increasing sales. In some cases the additive supplier is the responsible as he doesn't declare all ingredients included in the formulation while in other cases is the processor the responsible for hiding information when labeling all ingredients incorporated in the final product.

Belonging to the well-know Oetker's Group and following its strict policy on food quality, safety and compliance, Budenheim food additives are safe elements of our diet. Of course, our clear labeling adds to consumers' ability to make informed choices about the foodstuffs they buy.

In conclusion, food additives help assure a constant food supply of safe, healthy, nutritious, varied and appealing products at an affordable price, something we have come to expect. But make sure you approach a reliable partner to source such ingredients.

Budenheim Altesa, located in Valencia (Spain) is the subsidiary fully dedicated to the development, manufacturing and commercialization of functional ingredients dedicated to seafood processing. Their address is: Calle de les Rotes, 13 Pol.Ind. 7 46540 El Puig (Valencia) Spain. Cell Phone: +34 639 682 170 Email: jaime.forn@budenheim.com

Presentation 38

FEATURED SESSION: Sodium Levels in Seafood

Thursday, November 1
10:40 am - Noon

Using the Right Phosphate(s) to Reduce Sodium in Seafood

Yan Huang

Innophos Inc., 259 Prospect Plains Road, Cranbury, NJ, 08512

Seafood is very popular in the world due to its being high in protein, low in calories and rich in polyunsaturated fatty acids. It is also considered a value source of minerals and vitamins. However, the loss of nutrients and water in seafood right after capture and during processing can be so severe that phosphates are usually added during processing in industry for better quality.

Phosphates are natural components in almost all foods and are also used as functional food additives in food processing. In the seafood industry, among the functional goals for the use of phosphates in seafoods are improving water holding capacity, retention of natural moisture and flavor, improving texture and tenderness, and inhibiting oxidation of lipids and flavors by cyroprotection and Fenton-related oxidation. In the seafood applications, the most commonly used phosphates contain from 23-35% sodium. Thus phosphates overall contribute a significant part of sodium in finished seafoods such as cocktail shrimp, scallops and fish fillets.

The Center for Disease Control (CDC) recent report "Where's the Sodium" estimated that about 90% of Americans eat more sodium than is recommended for a healthy diet. Too much sodium increases a person's risk for high blood pressure the leading preventable risk factor for heart disease and stroke.

Innophos has special low sodium phosphate blends for the seafood industry both from the seafood substitute and the leavening system.

Curavis® So Lo has sodium range from 2-15% for different seafood varieties. Balanced ratio of sodium and potassium has no effect on sensory and texture of the products, depending on the application a sodium reduction of 10-50% can be achieved.

Catfish fillets were tumbled to approximately 10% over green weight prior to storage at -15 C° for 21 days. Fillets were evaluated for cook yield, pH, cooking loss, freeze and thaw loss, tenderness and sodium and phosphorus as P2O5 analysis. Low sodium phosphate treated fillet has similar pick up cook yield, freeze and thaw loss, tenderness and sensory acceptance as to sodium tripolyphosphate (STPP). Sodium content in finished with low sodium phosphate was 220mg/100g fillet after cooking compared to 340mg/100g with STPP treated fillet, a 35% reduction compared to that of catfish fillet with STPP.

Medium size South American Shrimp was soaked with sodium tripolyphosphate or low sodium phosphate blend for 2 hours containing 3% phosphate and 0.5% salt. Shrimp was then evaluated for cook yield, pH, appearance and sodium analysis. There is no significant difference between STPP and low sodium phosphate treated shrimp after cooking in cook yield, pH and appearance and sensory acceptance. The sodium content, however, in low sodium treated shrimp was 280mg/100g compared to 470mg/100g in STPP soaked shrimp. It was a 40% sodium reduction in cooked shrimp.

Presentation 39

Investigating Methods to Reduce and Control Sodium Levels in Shrimp

Molly Sims, Laura Garrido and Steve Otwell

Aquatic Food Products Lab, Food Science and Human Nutrition Department, University of Florida, Gainesville, FL, USA

As the majority of Americans consume well over the recommended 2,400 mg sodium per day, the seafood industry is facing the possibility that they will have to reduce sodium levels in seafood products. Shrimp in particular often has increased levels of sodium due to the increasing use of processing aids to maintain favorable moisture and flavor. The challenge is to select moisture retention treatments that result in lower sodium levels while maintaining consumer acceptance. The basic approach used to determine preference were untrained consumer panels using cooked shrimp with prior exposure to variety of different processing aids. *Litopenaeus vannamei* farmed in Ecuador were treated to a variety of sodium and moisture levels utilizing many different processing aids a *add details. All processing aids were compounds or blends currently available for commercial applications. Low sodium treatments utilized potassium functioning as a sodium replacement. Consumer panelists (n = 100) were presented five different shrimp and asked to rate overall likeability of the following characteristics on a 9-point hedonic

scale: texture, saltiness, flavor, and color. Consumers were additionally asked to rate the saltiness, firmness, moistness, and purchase intent on a 5-point Just About Right scale. The results were collected on Compusense™ and analyzed using ANOVA and Tukey's test for significant differences. It was found that shrimp in the sodium level 500-700 mg/100g and moisture 80-82% were best liked. In one panel in which four low sodium shrimp ranging from 253-347 mg/100g sodium were compared to the standard STPP treated shrimp containing 631 mg/100g sodium. In this trial it was found that there was no significant difference between the shrimp that had 347 mg/100g sodium and 263 mg/100g sodium in comparison with full sodium shrimp. The shrimp with 253 mg/100g sodium and 259 mg/100g sodium were given less preferential ratings. The results indicate that although it will be a challenge for the shrimp industry to decrease sodium levels, it is possible to still create a product that consumers will enjoy and purchase.

Presentation 40

Improving Sensory and Nutritional Quality of Lightly Salted Fish Fillets by the Aid of Sodium Bicarbonate

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The integrity and quality of fish fillets are largely dependent on the water content, which may be >80%. The many steps of processing; slaughter, filleting, packing, freezing and thawing can lead to a high liquid loss, and thereby affect the palatability and consumer acceptance of the meat.

Sodium chloride (NaCl) is widely used in processing of meat products as it helps retaining liquid, and improves texture and flavor. However, excessive dietary sodium consumption is in human related to cardiovascular diseases, and therefore the food industry is encouraged to reduce the amount in processed products. Sodium bicarbonate (NaHCO₃) is mostly known as a leavening agent in flour based products such as bread and cakes, but has also been used to tenderize meat and vegetables. The present study was conducted to investigate the nutritional and technological fillet quality of lean and fatty fish species (cod and salmon, respectively) after injecting brines containing NaHCO₃.

Cod and salmon fillets were injected with five different brine concentrations; with or without

additional NaHCO₃. The cod raw materials were either unfrozen or frozen/thawed prior to injection, and analyzed after 24 hours. One group was also injected and freeze stored prior to analysis. The salmon fillets were unfrozen and analyzed 24 hours after injection.

The liquid losses were generally lower in the groups injected with brines added NaHCO₃. Volatile components like 1-penten-3-ol and hexanal, known to correspond with sensory rancidness and off-flavors were significantly lower in the NaHCO₃ treated fillets. Assessment of baked fillets by a trained sensory panel (cod and salmon) and consumer evaluation (cod) revealed improved flavor, odor and texture attributes of fillets injected with brines containing NaHCO₃. The NaCl content of fillets injected with intermediate brine concentrations ranged between 0.4 and 1.8%, with the benefit of qualifying as low salt products (<2% NaCl).

Presentation 41

Use of Sodium Nitrite in Cold-Smoke Processing of Farmed Atlantic Salmon – Evaluations of Fillet Quality and Food Safety during Refrigerated Storage

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The use of sodium nitrite in cold-smoke processing of farmed Atlantic salmon (*Salmo salar* L.) is known to improve the colour of the smoked fillet and protect the product against microbiological growth, which is important for the salmon industry due to the possible presence of *Listeria monocytogenes*. However, the use of sodium nitrite in food and especially fish preservation is disputed due to negative health consequences of sodium nitrite itself and the possibility of N-nitrosoamines formation in the products. Sodium nitrite is prohibited in fish preservation in EU.

This study determined positive and negative consequences of nitrite treated dry salted cold-smoked salmon fillets during 44 days refrigerated storage (4 °C). The use of sodium nitrite induced only minor changes in chemical composition which included contents of fat, dry matter, sodium chloride, astaxanthin, peroxides (peroxide value) and nine different N-nitrosoamines. However, nitrite

treated fillets had higher contents of residual nitrite and showed an increased isomerisation of astaxanthin in the fillet surface as compared to corresponding fillets treated with vacuum salt. Improved colour (formation of nitrosomyoglobin) observed after smoking (day 1) of nitrite treated fillets fade during storage which resulted in a more similar colorimetric characteristic between the protocols at day 24 and 44. Moreover, nitrite treated fillets showed improved microbiological stability. It is concluded that the improved colour fade during storage and benefits of using sodium nitrite in production of cold-smoked Atlantic salmon are therefore limited to increased microbiological stability. A higher content of residual nitrite in the smoked fillet was presumably the unhealthiest consequence of nitrite treatment of Atlantic salmon. The contents of N-nitrosoamines were low and did not increase during storage.

Presentation 42

FEATURED SESSION: Seafood Safety

Thursday, November 1
1:20-5:00 pm

Vibrio parahaemolyticus Prevalence in New Zealand Shellfish

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The food-borne pathogen *Vibrio parahaemolyticus* (*Vp*) is present in New Zealand (NZ) seawaters, but there are no reported cases of food poisoning from NZ-grown seafood. Concern has been expressed that risk may increase with global warming. Our study determined the current numbers of *Vp* in NZ oysters and Greenshell™ mussels and the prevalence of pathogenic strains of *Vp*. Only a few strains of *Vp* are pathogenic and little is known about their presence in NZ. Pacific oyster (233), Greenshell™ mussel (54) and dredge oyster (19) samples were obtained from commercial shellfish-growing areas between December 2009 and June 2012. *Vp* levels were determined using the FDA Most Probable Number method and the presence of pathogenic genes *tdh* and *trh* was assessed by conventional PCR. In 2012 samples were also analysed by Real Time PCR (RT-PCR).

Pathogenic *Vp* levels were low, only detected in 10/215 North Island oyster samples (three *tdh* and seven *trh*) using the FDA methodology and a further

two *tdh* and four *trh* *Vp* positive samples using RT-PCR. Pathogenic *Vp* reached a maximum level of 42/g, well below the FDA recommendation (< 10⁴/g). From the South Island, non-pathogenic *Vp* were detected in just 1/37 oyster and 2/16 mussel samples, all at 0.36/g. Non-pathogenic *Vp* was detected in 81% of Pacific oysters and 34% of mussel samples harvested from the North Island, reaching peak numbers of 2.4 x 10⁴/g and 95/g, respectively. Numbers increased with increasing seawater temperatures, peaking in late summer when most shellfish-growing farmers do not harvest. Samples only exceeded 1,000/g when seawater temperatures exceeded 19°C so seawater temperature could be used as a warning of potential hazard. There was little evidence of change in total *Vp* numbers compared with those from 1981 to 1984 and there was no evidence of human health risk from pathogenic *Vp*.

Presentation 43

Assessing a Heat Shock Method of Control For *Vibrio vulnificus* and *Vibrio parahaemolyticus* in Raw Oysters

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Pathogenic *Vibrio* species are the most common cause of foodborne disease associated with the consumption of raw or undercooked seafood, especially molluscan shellfish. Failure to adequately control these pathogens prompted the U.S. Food and Drug Administration in 2009 to propose that Gulf Coast oysters harvested in warm months and intended for the raw (half-shell) be subjected to a post-harvest process (PHP). The purpose of this study was to evaluate a commercial heat-shock method to determine its efficacy in reducing pathogenic *Vibrio* levels to meet FDA requirements as a recognized PHP method. Naturally contaminated Gulf Coast oysters having levels of *V. vulnificus* and *V. parahaemolyticus* exceeding 1×10^4 MPN/g were subjected to a mild heat process under pilot-scale laboratory conditions and commercial-scale conditions. Treatments consisted of $60 \pm 0.5^\circ\text{C}$ for 2, 4, 6, 8, and 10 min followed by rapid chilling. Control and treated oysters were quantitatively analyzed for surviving *V.*

vulnificus and *V. parahaemolyticus* populations using the Most Probable Number (MPN) method. F-values were calculated and the F-value/Dref ratio was used to evaluate and compare process efficacy. Using the most conservative F-value obtained for each treatment trial, a \log_{10} inactivation of 0.37 was projected for the 6 min treatment; 6.5 for the 8 min treatment; and 7.7 for the 10 min treatment. Microbiological testing revealed near completed inactivation (up to $4.5 \log_{10}$ MPN/g) of both *V. vulnificus* and *V. parahaemolyticus* after the 10 min treatment. These F-value calculations suggest that 8 to 10 min commercial treatment times are adequate to provide the targeted $3.52 \log_{10}$ reduction in pathogenic *Vibrio* species in keeping with the NSSP definition of a PHP method. These results indicate the commercial heat-shock treatment has potential to be used by industry as a PHP method for the control of pathogenic *Vibrio* spp. in raw oysters.

Presentation 44

Lessons Learned from Sushi Salmonellosis Outbreak

Douglas L. Marshall

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Reported incidence rates of *Salmonella* in raw seafoods can be high (>50%). Incidence rates in ready-to-eat (cooked, smoked, raw) seafoods are generally low (<5%). This year a U.S.-wide outbreak of salmonellosis was linked to consumption of raw Yellowfin tuna nakauchi scrape, an ingredient popularly used in spicy tuna sushi rolls. The outbreak affected nearly 400 individuals in 27 U.S. States. The nakauchi scrape was manufactured in SW India and imported as vacuum-packaged frozen blocks. FDA investigators revealed that the manufacturing plant had several HACCP, GMP, and SSOP violations that likely lead to widespread contamination of the ingredient. Noted deficiencies included the absence of CCPs for pathogen control during cutting, scraping, and vacuum packaging,

insufficient monitoring of sanitary conditions and practices, and insufficient monitoring of water used on food and food contact surfaces, hand washing, toilets, and for manufacturing of ice. Bird feces, insects, and filth were found in ice manufacturing equipment and product residue was found on utensils and on ceiling after cleaning. FSMA-related preventive control programs and strategies to control *Salmonella* in RTE seafoods will be discussed. Example strategies include time/temperature control, prevention of recontamination after kill steps, and proper monitoring and verification of control programs.

Presentation 45

Extraction of Enteric Virus Indicator from Seawater Using Activated Carbon

Jiemin Cormier*¹, Miguel Gutierrez¹, Lawrence Goodridge² and Marlene Janes¹

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Colorado State University², Fort Collins, Colorado, USA

Enteric virus-contaminated shellfish represents a significant health threat to shellfish consumers as well as an economic threat to the seafood industry. Male specific bacteriophage MS2 has been identified as a suitable indicator for water-borne enteric virus outbreaks, and its presence indicates a recent contamination. The use of activated carbon has been tested for extraction of MS2 bacteriophage from seawater and has the potential to be developed into a rapid concentration and detection method for enteric water-borne viruses.

The effects of pH (4, 5, 6, 7, 8, and 9), salinity (0, 10, 20, 30, and 40 ppt) and contact temperature (4 °C, 20 °C and 37 °C) on the absorbance efficiency of activated carbon were investigated in artificial seawater. 10^8 - 10^9 PFU of MS2 bacteriophage and 1 g of activated carbon were inoculated into 500 ml of artificial seawater. After 3 h of constant stirring, activated carbon was separated from the seawater, incubated with 1 ml of trypsin-

EDTA solution for 2 h at room temperature to release MS2 bacteriophage from the activated carbon, and RNA was extracted. qRT-PCR was conducted to determine the PFU of MS2 bacteriophage released from the activated carbon.

Results indicated that warmer temperature provides significantly better efficiency ($P < 0.01$) for activated carbon and pH has no significant effect on the absorbance efficiency. Activated carbon has significant higher absorbance efficiency in seawater of salinities 10 ppt, 20 ppt and 40 ppt at 37 °C ($P < 0.01$). In 20 ppt seawater at 37°C, 1.0 g of activated carbon was able to absorb up to 2.5×10^6 PFU of MS2 from $\sim 10^9$ PFU MS2 inoculated in the 500 ml seawater. Since shellfish harvesting area is usually around 20 ppt seawater, activated carbon can efficiently concentrate enteric viruses and assist the monitoring of enteric virus outbreak.

Presentation 46

Impact of Red-Colored Halophile Bacteria on Salted and Dried Cod

Ann Helen Hellevik, Trygg Barnung, Kristine Kvangarnes and Ola Ween

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In 2010 a research program was started aiming at studying the effect of red-colored halophile bacteria on salted and dried fish. Red-colored halophile (salt-loving) bacteria attack the fish flesh by breaking down the fish protein resulting in unpleasant odor. The main source of infection is the use of common sea salt. Most of these bacteria have a red dye (pigment) that makes them visible when they occur in large numbers (1-10 million per cm²). They are called "Rødmidd" by the Norwegian salt-cure and dried cod industry. Until now, the knowledge has been based on experiments conducted during the 1960-70's. These results have formed the basis for the Norwegian regulation and demands for using salt in the production of salt-cured and dried cod.

The present study tells a quite different story compared to the experiments conducted during the 1960- and 1970-ies. The results have therefore

contributed to change the Norwegian regulatory system for producing salted and dried fish. One important aim of the project was to study the effect of using salt twice before destruction, aiming at increasing the profit of the industry as well as environmental impact. The results show that the amount of halophile bacteria in salt is reduced when fish is produced with second-time used salt. Further, second-time used salt result in a product with higher yield implying a potential for higher profit. A new and faster method for detection and quantification of the bacteria in fish flesh is established using DNA technology (PCR). Detecting halophile bacteria in new, as well as second-time used salt the method was, however, difficult due the fact that few bacteria being present in the salt. Therefore, further work is required to develop a suitable method.

Presentation 47

Validation for Pre-Cooking as a Control for Potential Histamine Production in Tuna Loins for Subsequent Canning

Farzana Vogl¹, R. Salazar², F. Nolte³, G. Kontoh⁴, and G. Ybanez⁵

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⁵Bumble Bee Foods, LLC, San Diego CA, 92123.

A series of commercial trials involving 30 test variables, 765 fish and a total of 3842 individual histamine tests were designed to demonstrate that pre-cooking tuna is an effective control for potential histamine formation. Long line caught Albacore tuna (*Thunnus alalunga*) sized from 10-20 kg, caught in the South Pacific Ocean were chilled on board and delivered within 3-13 days post harvest from customary fishing vessels. These raw fish were intentionally spoiled to exceed 50ppm histamine to simulate harvest vessel time-temperature abuse beyond worst case conditions, and then frozen to represent standard commercial processing conditions. The fish were thawed and cooked in industrial atmospheric steam pre-cookers using

commercial pre-cooking schedules. Results demonstrate that achieving an end point internal product temperature of 60C controls histamine formation and previous studies have shown that 60C provides a 5D reduction in *Morganella morganii*, a heat resistant and prolific histamine forming bacteria. No further histamine formation was observed for up to 18 hours. This is more than adequate time to convert pre-cooked fish into frozen tuna loins or canned tuna. These critical limits need to be incorporated as part of a HACCP plan built on a sound foundation of proper GMP's, SSOP's and Pre-requisite Programs.

Presentation 48

Use of End Point Internal Product Temperature to Control Histamine Formation in Tuna at the Pre-Cooking Step

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There has been renewed attention to the control of histamine in canned tuna, corresponding to the publication of the 4th edition of the FDA Fish and Fishery Products Hazards and Controls Guide in April, 2011. Control of histamine levels is seen as the primary means of mitigating the risk of scombroid fish poisoning. Tuna for the canned trade are mostly processed from a fresh catch that is frozen at sea and later thawed, butchered, partially cooked (or "pre-cooked") and cooled. Cooking and cooling facilitates separate the skin, bones, and dark meat from desirable white or light meat. Histamine formation takes place primarily at temperatures above 25°C and for this reason regulators have limited the exposure time of previously frozen tuna to a maximum of 12 h if temperatures rise above 21°C at any point during these steps. Tuna processors have proposed that the pre-cook step stops spoilage and histamine formation for at least an additional 12 h by destroying or retarding the growth of histamine-producing bacteria (HFB) and have validated pre-cooking as a critical control point (CCP). A recent thermal death time (TDT) study on five high HFB has established *Morganella morganii* as the most heat resistant of the commonly reported histamine forming bacteria, and established conservative *D* and *z*-values for *M. morganii* as a reference organism. A 5- log reduction

of *M. morganii* has been proposed as a conservative critical limit for the pre-cook CCP, and validation studies have shown histamine formation is inhibited for at least 12 h after pre-cooking, even when the process has delivered less than a 5-log reduction. Despite this, it is not practical to base the pre-cook CCP on a cook schedule. Pre-cook times vary for a number of complex reasons, including individual variation in fish size, the need to limit the number of size categories used in processing, variable fish proportions, lipid content, initial temperature (I.T.) variations inevitable in processing, as well as variations in pre-cooker temperature distribution. These issues have long been recognized and tuna processors have always relied on end point internal product temperature (EPIPT) to validate adequate pre-cooking. Confirming a 5-log reduction of *M. morganella* for a pre-cook process can be accomplished by applying *D*- and *z*-values to the cold spot temperature history of a fish in that cook, and using the General Method for determining the lethality of a process. This report uses a worst case cold spot temperature history to establish that a minimum EPIPT of 60°C/140°F will reliably assure a 5-log reduction of *M. morganii* at the cold spot, provided key conditions are met.

Presentation 49

Effectiveness of Various Sanitizers Against the Natural Bacterial Flora, *Listeria monocytogenes* and *Salmonella enterica* on the Outer Skin Surface of Tuna Fish

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Tuna fish are often caught and processed for preparation of sushi products without any previous microbial reduction step applied to their skin. To evaluate the efficacy of various sanitizers for reducing aerobic plate count (APC), *Enterobacteriaceae* (ENT), *Listeria monocytogenes* (LM) and *Salmonella enterica* (SAL) on tuna fish skins, the skins were aseptically cut into squares (20 or 40 cm²). Skins (20 cm²) were inoculated with mixtures of 5-strains of LM or 5-serotypes of SAL and held (4 °C for 20 h) before exposure for 2.0 minutes to sterile distilled water (DW) or sanitizers [sodium hypochlorite (CHL; 100 ppm) or PROSAN (0.5, 1.0 and 2.0 oz/gallon)]. Skins were treated by brushing solutions onto their surfaces or by immersion and then rinsed (4 seconds) in DW. Skins were analyzed for viable bacterial counts by: i) surface swabbing (40-cm² skins) and ii) pummeling in buffered peptone water (BPW; 20-cm² skins). Aliquots (0.1 ml) of serially diluted BPW (10-fold

obtained from both types of sampling were plated on appropriate agar media. Inoculated agar plates were incubated at 30 °C (APC), or 37 °C (ENT, LM and SAL) and colonies were counted at 48 h. Initial bacterial counts were 6.92 and 4.42 (log₁₀ CFU/cm²) for APC and ENT, respectively. For pathogens, initial counts (log₁₀ CFU/skin) were 5.90 (LM) and 6.09 (SAL). Log reductions from CHL were 1.02 (APC), 0.97 (ENT), 1.06 (LM) and 0.80 (SAL). For all bacterial groups log reductions were greater with increasing PROSAN concentrations. Following PROSAN (1.0 oz/gal) treatment, log reductions for APC, ENT, LM, and SAL were 1.48, 1.34, 1.62 and 1.77, respectively. Log reductions from PROSAN (2.0 oz/gal) were 1.95 (APC), 1.65 (ENT), 2.22 (LM) and 2.39 (SAL). The use of PROSAN (1.0 or 2.0 oz/gal) has good potential for reducing bacterial populations on the skin surface of tuna fish.

Presentation 50

An Integrated Approach to The Assessment of Risks and Benefits Associated with the Consumption of Seafood in Different World Regions

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Main risks and benefits associated to seafood consumption in different world regions were assessed. This involved a thorough research of the available scientific literature in order to identify risks and benefits for eight different countries associated to the SECUREFISH project: Portugal, Netherlands, and United Kingdom (Europe), Ghana, Kenya, Namibia (Africa), Malaysia (Asia), and Argentina (South America). The risks and benefits considered significant were combined with consumption frequencies and/or scenarios. Accordingly, the intake of nutrients and contaminants through seafood consumption in the selected regions as well as the associated probability of exceeding the recommended daily intake (RDI) or the provisional tolerable weekly intake (PTWI) were estimated. Two estimators were used: plug-in (PI) and tail estimation (TE).

Seafood consumption levels varied between countries, being highest in Portugal and low in Argentina and African countries. The main consumed seafood products were also different between countries. The most common identified risk was methylmercury (MeHg) contamination and

the main benefit derived from the intake of omega-3 polyunsaturated fatty acids (n-3 PUFA), mainly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Unfortunately, there was a positive correlation between higher n-3 PUFA intakes and higher MeHg intakes. The associated probabilities of exceeding the respective thresholds were also positively correlated. Accordingly, Portugal displayed the highest probability of exceeding the RDI of EPA+DHA, 66.05 % (PI estimator), but also the highest probability of surpassing the MeHg threshold, 6.71 % (TE estimator). For the other countries, risks and benefits were much smaller. Namely, the probability $P(X_i > PTWI)$ was 0.22 % (TE) for the Netherlands and further lower for the remaining studied countries. Moreover, it was confirmed that TE was most accurate for small probabilities and PI yielded best estimates for larger probabilities.

Acknowledgments: This work was supported by the project "SECUREFISH", Ref. KBBE.2011.2.5-02 from FP 7 EU PROJECT THEME (Grant agreement No 289282).

Presentation 51

Seafoodhealthfacts.com: Developing Informational Materials for Healthcare Professionals

Michael T. Morrissey¹, Ken Gall², Doris Hicks³, Lori Pivarnik⁴, Steve Otwell⁵, Pamela D. Tom⁶, and Heather Mann⁷

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Communication about the benefits and risks of seafood consumption is critically important in this rapidly changing world where information is at one's fingertips or the nearest iPhone. While the tide has shifted in this often acrimonious debate and the positive benefits of seafood consumption have been shown to outweigh the risks, there is still a need to improve messaging of this information to health professionals and consumers. A collaborative effort among several universities and a non-profit organization was undertaken to address this issue. An initial survey involving over 600 health professionals (doctors, nurses, dieticians, etc.) was conducted and showed that few understood the facts regarding the benefits and risks of seafood consumption and how easy it is for groups to misinterpret official advisories, such as the joint 2004 EPA and FDA advisory for women who might become pregnant, pregnant women, nursing mothers and young children. The survey also showed that health professionals wanted the information for themselves and for their clients/patients in three different formats: printed materials, Internet access and/or brochures. The information needed to be clear, concise and science-based.

After several discussions, it was decided that the best method to reach a large audience was through a

science-based, simple to use website. As there are numerous websites about seafood consumption the question became: "How could we distinguish ourselves and from all the others?" Since the project team experts have been involved in seafood research, extension and HACCP training for more than two decades and are competent seafood safety and outreach professionals, the team decided that our overall seafood knowledge would be our strength. As the website was developed, sections on seafood choices, often missing when discussing seafood benefits and risks, were included. Thus, with a general outline of seafood topics that needed to be addressed, diverse assignments were made for the team to complete.

Following several iterations, major changes and strong debates plus subsequently a few key focus groups, a website was developed targeting the major points in the debate of seafood consumption. To further refine the web site design a workshop was held, where several stakeholder groups convened to discuss several of the contentious seafood issues about benefits and risks. This presentation will describe the creative process in developing material about benefits and risks in seafood consumption and the continued need for outreach for this topic.

Presentation 52

FEATURED SESSION: Species Identification and Product Origin

**Friday, November 2
8:00 am - Noon**

State of Technology for Proper Species Identification in the USA

LeeAnn Applewhite

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www.appliedfoodtechnologies.com

While mislabeled seafood in commerce and species identification testing have been hot topics for years it has really heated up in the US over the last 15 months. The 2010 report by the U.S. Government Accountability Office (GAO) on seafood fraud in the US exposed the current inadequacies in the detection and prevention of seafood fraud by federal agencies and caught the eye of the largest international organization focused on ocean conservation – Oceana. Oceana launched a new campaign to fight seafood fraud in May 2011. Since, they have performed studies and testing across the country drawing the attention of federal and state regulators, national media groups, as well as consumers. FDA has stepped up to the plate and validated a DNA Barcoding Method for Seafood Species Identification; published a Guidance for this method; set up sequencers in nine field labs; and trained scientists to perform DNA barcoding. In addition, FDA has embarked on a national seafood surveillance and testing program, targeting 100 seafood distribution centers and warehouses across the country. FDA is currently testing random commercial seafood samples at their regional labs.

The results will be published when the testing is complete.

FDA's Guidance is thorough in outlining the protocols and procedures for the DNA barcoding method. It is quite clear on the importance of using reference libraries containing voucher specimens with authoritative taxonomic identification in DNA barcoding protocols and states "FDA will only make regulatory decisions based on identifications using adequately authenticated standards." To support the Guidance, FDA has publicized their database of sequences of validated seafood references. So, do we now have all the tools needed to combat seafood mislabeling in the US? Absolutely not!

In this presentation, as well as others in this session, additional methods for DNA-based seafood speciation will be discussed as well as stream-lined and automated DNA extraction protocols. These new and innovative approaches not only complement FDA's DNA Barcoding Method but also strengthen the barcoding method and broaden capabilities for monitoring seafood mislabeling in commerce.

Presentation 53

Development of a Rapid Seafood Species Identification Technique Using Chip-Based Capillary Electrophoresis and Species-Specific Protein Patterns

Calvin C. Walker^{1*}, Cheryl L. Lassitter¹, Shannara Collins¹, Courtney Ford¹, Kevin R. Rademacher², Alonzo N. Hamilton, Jr.², Mark A. Grace²

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Seafood species substitution creates economic hardship on industry and food safety issues for consumers. Fraudulently labeled seafood decreases the value of legally harvested seafood, places honest fishermen at a competitive disadvantage, increases consumer costs for less desirable species, and lowers consumer confidence in seafood. Seafood substitution can pose serious health risks from several toxins and from allergies to substituted species. The enormous volume of seafood product commerce is an overriding issue in any species substitution detection program. DNA-based techniques are most commonly used for detection of seafood species substitution. While DNA analysis provides results with a high degree of confidence, downsides include high capital output, staff and analysis cost and low sample throughput. One

approach to improving seafood species identification throughput is the use of a tiered approach utilizing a rapid screening assay for primary analysis and a confirmatory method for verifying screening positives. A tiered approach to testing seafood will help ensure that seafood marketed in the USA is accurately labeled and safe. The presentation will describe seafood labeling concerns, propose a tiered approach to seafood testing to better ensure accurate product labeling, and present a new seafood speciation technique utilizing species-specific protein profiles to rapidly classify seafood samples by species. An overview of the performance and application of the proposed technique for high throughput screening of muscle tissue to determine seafood species will be presented.

Presentation 54

Tailor-Made Authentication Tools for Storytelling

Begoña Pérez-Villareal, Kepa Escuredo and Miguel Ángel Pardo

AZTI-Tecnalia. Parque Tecnológico de Bizkaia. Astondo bidea, 609 - 48160 Derio, Bizkaia, Spain

Storytelling is a food trend that responds to the demand for transparent, attractive and nearby information by associating messages and stories with food products. The message brings unmistakable added value to products, brands and manufacturers. This trend referred to the seafood sector, connects and brings the consumer closer to the seafood they eat, and at the same time claims for exquisite and very exigent identity and authenticity requirements applied to the actual seafood products. However, the expectations of consumers, seafood industry, and public administrations frequently cannot be fulfilled due to lack of specific methodologies for seafood authentication purposes.

In the current market, the consumer is the main motor of innovation. The trends are related to the natural evolution resulting from the consumers' motives, tastes, needs and preferences, and which therefore affect their behavior when they consume something. Detecting and analyzing these trends means visualizing and anticipating what consumers will demand in the future, detecting business opportunities early, new niches in the market, and anticipating the competition. At the same time, it is very important to align scientific efforts with the real

requirements of the market and its stakeholders. Following these demands, very explicit in some cases and hidden in others, has guided us in the design and development of new molecular authentication tools for making seafood storytelling trustworthy and detect mislabeling.

During this presentation some practical cases will be addressed including the detection and authentication of fish species mixtures in (i) ready to eat presentations such as canned tuna and (ii) surimi based products. The development of these authentication methodologies was the consequence of market requirements, pushed by consumers, processors and by the public administration. These tailor-made authentication tools are based on the use of DNA fluorescent probes in a Real Time PCR detection system. The same molecular tools have been developed to identify the origin of a number of very valuable and sustainable seafood products from specific areas: (iii) white tuna and (iv) anchovy from the Cantabrian Sea, and (v) young eels, products considered as 'delicatessen' and very appreciated by some consumers.

Presentation 55

Breaking Seafood Identity: Geographical Origin Perspective

Miguel Ángel Pardo, Kepa Escuredo and Elisa Jimenez

AZTI-Tecnalia. Parque Tecnológico de Bizkaia. Astondo bidea, 609 - 48160 Derio, Bizkaia, Spain

Nowadays, there is a major concern regarding the identity of high value seafood products. Moreover there is a growing interest in protecting both local food industry as well as the final consumer from the introduction of questionable quality food in the local market. Commercialization of seafood products under sustainable local or geographical denominations is indeed one of the most promising EU strategies to foster internal food industry against the introduction of less quality food products in this globalized market. Competent authorities are making significant efforts concerning the development of legislation that clearly marks the quality requirement to be accomplished in the case of protected denominations and catalog non-compliance established standards as fraud. In this sense, the application of genomics based techniques is capable of meeting this objective.

We have developed a series of methodologies based on high resolution genotyping. These analyses led to SNP marker discovery and further analysis in order to provide the level of discrimination that is required to distinguish different geographical origins. The analytical systems developed allow population assignation in terms of breed or geographical origin by means of SNP genotyping and subsequent comparison of the results from previously characterized reference populations. We successfully applied this technology to the major challenges of breed and geographical origin confirmation in a series of valuable and sustainable seafood products. Once validated and standardized, these techniques could be employed by regulatory authorities and industrial control laboratories.

Presentation 56

How to Define Traceability

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While food and in particular seafood product traceability has become increasingly important in recent years, there is no consensus on what the term "traceability" means. This presentation gives an overview of relevant traceability definitions as pertaining to food products, outlining similarities, differences, and the consequences of choosing one definition over another. To ascertain which definitions are most commonly used, we reviewed 101 scientific articles relating to food traceability. The review process gave rise to the following observations:

The term traceability, as pertaining to food production and products, is not well defined; several conflicting definitions exist, and definitions are interpreted in widely varying ways

In scientific articles, the most frequently used definitions are the EU General Food Law definition and the old ISO 8402 definition

Both these definitions and the other relevant definitions have clear faults, ambiguities or omissions, and they do not match the functionality and content of traceability systems as described in many articles

Finally, by combining the best parts of the existing definitions, this presentation offers a new possible definition of traceability as pertaining to food and seafood products.

Presentation 57

LABELFISH: The Atlantic Network on Genetic Control of Fish and Seafood Labeling and Traceability

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Traceability of fish and seafood is mandatory since 2005 within the EU. Full implementation requires an adequate management of information and also the availability of techniques which allow the verification of the information transmitted. One of the relevant aspects of the verification of traceability and labelling legislation is the capability of identify and authenticate biological species. In recent years a substantial amount of research has been invested in the development of genetic methods for the identification of commercially fish. However, there is still a need to standardize these methodologies among the user laboratories and also to update databases and genetic profiles of commercially relevant species in order to provide a standardized response regardless of the country, or laboratory, where the analyses are performed.

LABELFISH is a project funded by the Atlantic Area Programme and includes participants of six countries in Europe, mainly from the Atlantic area, which are characterized by an intense economic and social relationship with marine resources.

The main objective of **LABEL FISH** is to set up a network of laboratories and national control

bodies with experience and interest in the development of a common strategy and in the use of harmonized analytical techniques for the control of genetic traceability and labelling of seafood products which are sold in the European market and in particular in the respective countries involved in the project. This general objective will have as specific objectives: I) the development of a new database which will gather different existing genetic data (mostly DNA genetic markers) and ii) the selection of tested and validated analytical tools for the identification of the selected fish species with commercial importance in the Atlantic area regions. Expected outcomes of the present proposal are the protection of the both European consumers and SMEs involved in fisheries and aquaculture in the Atlantic regions. The former by helping to ensure their rights to correct product information and the latter by implementing reliable traceability and authentication genetic tools which will ultimately protect their own produce and protect their market niche.

Presentation 58

Costs, Benefits and Human Challenges when Implementing Traceability in the White Fish Processing and Packing Industry: A Case Study

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Food consumers are becoming much more aware of the attributes of the food they eat. The ability to access this information is affected by the information flow within a food processing company. Methods and technologies for such information collection have been the focus of research and development over the last 10 years. Some of the effects of this implementation are specific while other effects are more difficult to describe or observe such as human factors but are equally important. These factors play an important role in the profitability of a company and the ability to implement the appropriate measures for their market.

For many companies the ability to trace every aspect of production from reception of raw material to end product is beneficial and necessary. Protection from law suits and other legal claims has often been highlighted as a major driving force for traceability systems. In this case it was reported that this was not a factor. In the case of any food safety

incident the system would be essential but there was no lowering of insurance costs reported in this case.

This study showed that an initial 'low tech' system implementation was not expensive in terms of equipment. This exemplifies the fact that traceability systems do not necessarily have to be synonymous with cost. It has been discussed that electronic and therefore 'high tech' systems are necessary for satisfactory information exchange throughout a supply chain. 'Low tech' may be sufficient internally within small to medium size companies and where appropriate.

One the main findings was that the full advantages of the traceability system were not seen immediately in fact it was observed that even after 10 years the company are only now beginning to reap the full rewards, this was also associated with advances in the traceability system. This may be attributable to external factors such as changing demands in the market.

Presentation 59

Louisiana Wild Certified Seafood: Controlling Traceability and Species Identification from Harvest to Sale via a Geographical Authenticity Branding and Marketing Program

Jon W. Bell¹, Rene LeBreton², and Jason Froeba³

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The U.S. seafood market relies on products produced from both wild harvest and aquaculture sources from around the globe. The awareness of both intentional and unintentional mis-stating of seafood species of products in the U.S. market is growing, resulting in an increased interest among industry personnel, consumers, and regulators to know and verify the seafood species of products in packages and on labels. As a result of these two market realities, a growing response is the development and expansion of analytical procedures to test and verify for species identification at or near the end of the distribution chain and sale of these products.

Instead of relying on routine end-product testing, another approach to provide assurance of proper labeling and species identification is to develop an

authenticity program that focuses on the links in the seafood production process to control species information as well as additional key market information. The Louisiana Wild Certified Seafood Program (LWCS) authenticates that branded seafood products are harvested from Gulf of Mexico or Louisiana waters by licensed commercial fishermen and are processed in Louisiana in compliance with federal food safety regulations. The program provides product and document traceability through product identification and trip tickets and invoices by program participants, and links the authenticity of species as well as Louisiana origin from harvest to packaging. Testing for species identification can then be used as a verification activity.

Presentation 60

Traceability in the Fish Sector – From Research to Commercial Business

Erling P. Larsen¹

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The last twenty years, the focus on traceability has grown from total obscurity to full attention of the European Parliament legislation activities. Due to the latest legislation the fish sector has been introduced to more strict legislation than the rest of the food sector. To meet these demands it was decided by the Danish Government four years ago, to create a consortium with representatives from research, applied research, private innovative companies, the sectorial branch organizations and the authorities to meet the future challenges.

The result of the work with traceability are solutions that have the backing of all the links in the value chain from the primary producer (the fisherman) to the retailer both nationally and internationally. The Danish fish and shellfish export is the fifth biggest in the world.

The development of a central database structure, the specification demands, the individual needs from companies and the applications to the existing

systems both at the authorities and private levels have been solved. A study of the perception of traceability has been done in the processing industry and interviews with all the major retailers in Europe have been conducted. To collect and use the traceability information from the fishermen, it has been necessary to develop a new IT platform that can communicate with the official data collecting systems.

At present nearly 90 % of all fish landed in Denmark is registered in the central database, and the number of processing industries, wholesalers and retailers that are using the database are increasing steeply. In the near future another major step will be taken, due to an extensive investment program financed by the European Community, which will use the traceability data, from showing interactive information to the consumers to processing planning in the processing industries.

Presentation 61

POSTER ABSTRACTS

Technical Sessions 1 and 2

Tuesday, October 30
1-6:30 pm

Inhibition of α -Amylase and α -Glucosidase By Icelandic Seaweed

Hamaguchi, P.H., Sveinsdottir, H., Jonsdottir, R., Vrac, A.J., Kristinsson, H.G.

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Hydrolysis of dietary carbohydrates is the major source of blood glucose and is carried out by enzymes such as pancreatic α -amylase and intestinal α -glucosidase. The inhibition of these enzymes can be important to control type II diabetes by retarding the rapid utilization of dietary carbohydrates and suppress postprandial hyperglycemia. The current therapeutic drug for diabetes type II come at a high cost and with gastrointestinal disturbances as side effects. There is a need to look for a natural source that could potentially be used as an effective therapy for postprandial hyperglycemia with minimum side effects. In this study, the potential of the seaweed *Fucus vesiculosus* to control type II diabetes through α -amylase and α -glucosidase inhibition was investigated. Seaweed was collected monthly to characterize seasonal variations in composition and activity. Samples were analyzed for proximate composition and minerals. Total polyphenols were determined with the Folin-Ciocalteau method. Functional seaweed extracts were prepared and their bioactive properties studied with various *in-vitro*

antioxidative assays and α -amylase/ α -glucosidase inhibition assays. Seaweeds were found to vary significantly in proximate composition depending on the season. Seaweed samples had high amounts of polyphenols, varying from 71–97 g phloroglucinol equivalent per 100 g extract, depending on season. Seaweed extracts demonstrated high anti-hyperglycemia activities evidenced by their strong ability to inhibit pancreatic α -amylase and intestinal α -glucosidases, with some variation in activity with season. Seaweed extracts were also found to have very high radical scavenging activity according to ORAC, DPPH and cellular antioxidant tests. This study showed that seaweed harvested in different seasons can vary significantly in composition and activity. It also suggests strong *in-vitro* antioxidant and antihyperglycemia activities of seaweed extracts. Seaweed extracts may find use as an alternative natural choice to control type II diabetes.

Poster 1

HPP – Automatic, Electric and Environmentally Friendly Small Fish Meal Factory

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The aim of the project is to develop an automatic fish meal factory (HPP). The factory is automatic, environmentally friendly and runs on electricity. The manufacturing process and equipment for fish meal has been redesigned in various ways. An energy model of a pipe heat exchanger is under development, in order to maximize energy efficiency. The knowledge on the process management and the properties of the raw material based on fish meal processing will serve as a basis for the companies to develop new equipment for the full processing of marine products.

Experiments with HPP consist of two main parts: 1) testing new equipment and manufacturing process and 2) examination of mass- and energy flow through the process. Focus is on byproducts from

processing fish for human consumption e.g. viscera from whitefish and bones. Also experiments have been conducted on shell from shrimp and pelagic fish which has been used for fish meal processing for decades with its well-known properties.

Results have shown that HPP can produce fish meal and fish oil from previously little used byproducts of many species, quality of the fish meal and oil depends on freshness on the raw material. For a small factory that can be stationed close to a fish processing plant, the freshness of raw material should not be a problem. Measurement of low water content in fish oil and low fat content in the meal, states that the new equipment and process are giving results as hoped.

Poster 2

Environmental Impacts of a Fishmeal Processing Factory: Advantages of Discards Valorization Against Disposal

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Reuse and valorization of fish discards is a key process for marine resources conservation. Generally, fishmeal processing factories collect the byproducts and discards generated by fishing port activities. This symbiosis reports an economical benefit to both parts, since the port avoid the costs of their disposal as a residue. However, it is important to evaluate the advantages of valorization processes not only in terms of economic income but also in terms of environmental impacts. Thus, if the valorization process of a residue provokes higher impacts than its corresponding waste management process, then its advantages are maybe not sufficient for guarantying a sustainable reuse, and the net benefit obtained must be analyzed with caution.

Methodologies such as Ecological Footprint (EF) or Life Cycle Assessment (LCA) are being extensively applied to evaluate environmental impacts of different types of processes, including those of waste management. These tools help to identify the stages susceptible to be modified in order to optimize process sustainability. In this case,

the environmental burdens of a fishmeal process were evaluated considering the raw materials and energy consumed and the residues generated. Preliminary results showed an important contribution of wastewater to EF. This was due to the large quantity of water handled in the process, which comes as part of the fish byproduct (70 %). This high water content results in low process efficiency and in a significant volume of liquid effluents. Water reutilization through the process plant would decrease environmental impact and improve global efficiency. A comparative assessment with EF and LCA between the fishmeal process and different waste management scenarios (incineration, landfilling or both) will provide quantitative results of the advantages of fish byproduct valorization. This comparison is a necessary step for the development and industrial implementation of these processes as the best alternative treatment for fish discards.

Poster 3

A One-Year Survey of Pacific Cod (*Gadus macrocephalus*) Livers as a Source of Fish Oil

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The 2010 catches of Pacific cod (PC) in Alaska averaged 200,000 t, generating about 95,700 t of byproducts coming from human-food processing lines. Livers can be segregated from viscera and used for production of cod liver oil; notwithstanding, catcher-processor vessels in Alaska discard a majority of PC byproducts at sea. The objective of this study was to record monthly variations in composition of PC livers and their respective liver oils.

Livers were plate-frozen monthly in 7.5 Kg blocks, in triplicate, onboard catcher processor vessels (Alaska Leader Fisheries, and Aleutian Spray Fisheries) harvesting cod in the Bering Sea from December of 2010 to November of 2011. Livers were comminuted and oil rendered under nitrogen atmosphere and continuous stirring (65°C; 30 min). The oil was separated from liver solids by centrifugation (7,500 rpm; 30 min; 20 °C). Protein, moisture, and ash contents were determined using AOAC methods. Lipid content was determined by the Folch method and fatty acids were quantified by gas chromatography.

Total lipids were remarkably different between seasons. From August to December lipid content was consistently above 50% w/w, while a drop in lipids was observed after January with lowest values occurring in March (35% w/w). From March to July (45% w/w) lipid content increased, and in May liver lipids had the highest increment change from 40 to 48% w/w. As expected, moisture content of livers was inversely proportional to lipid content, while ash and protein contents showed little variation through the year. Significant differences were also recorded in the quantities of saturated, monounsaturated and polyunsaturated fatty acids, and in the total content of highly unsaturated fatty acids such as DHA and EPA throughout the year.

Results from this study are readily applicable to seafood processors interested in recovering fish oil, rich in omega-3 fatty acids, from Pacific cod livers.

Poster 4

Quality Changes of Alaska Fishmeals During Storage at 4°C & 40°C: A One-Year Study

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The pollock and salmon fisheries account for more than 50% of the yearly fish harvest in Alaska. Processing of salmon and pollock generate over 1,00,000 t of byproducts annually. In 2009 Alaska produced 31,000 t of fishmeal, representing 1% of the global supply. Production of fishmeal from salmon byproducts is a seasonal, while fishmeal made from pollock byproducts is produced year-round. Understanding differences in shelf life of Alaska fishmeal products is important to seafood processors. The objective of this study was to determine shelf life of commercial fishmeals produced from pollock and salmon byproducts.

Fishmeal from pink salmon and pollock were obtained from processing plants in Kodiak (2 x 22.7 Kg) and Seward (2 x 4.5 Kg) in August 2007. Ethoxyquin, an antioxidant, was added to fishmeals at each processing plant immediately after production. Fishmeals were frozen immediately upon arrival at KSMSC pilot plant. Sampling commenced in October 2007 (Time 0). Samples

were subdivided and duplicate independent samples of each fishmeal type (salmon vs. pollock) and source (Kodiak vs. Seward) were stored at both 4°C and 40°C. Fishmeals were chemically characterized at time 0, 60, 180 and 360 days. Proximate composition and fatty acid profiles were determined using AOAC methods, and free fatty acid values (FFA) and TBA values using AOCS methods.

The effects of fishmeal type, production location, and storage temperature and time were statistically investigated using General Linear Models followed by Tukey's HSD Test ($P < 0.05$). All factors contributed to observed differences in composition and shelf life of fishmeal products. Protein content was significantly higher and moisture content was significantly lower after 360 days at 40°C for all but the salmon meals from Seward. The FFA values were significantly higher and EPA and DHA levels were significantly lower after 360 days storage for most fishmeal products studied.

Poster 5

Characterisation of Effluents from the Marinated Herring Industry Part I: From Boat to Barrel

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Traditional herring processing industries yield large volumes of effluents with high organic loads. The cost imparted for their discharge is therefore substantial, and the discharge *per se* can be considered a waste of molecules with potential good market values. Although systematic quantitative and qualitative investigations are lacking, it is known that herring proteins, lipids and smaller molecules leach out at different stages during the production chain where water is used. The project “Pelagic Industry Processing Effluents Innovative and Sustainable Solutions” (PIPE) is aiming at adding value to herring processing waters through an interdisciplinary research effort engaging both industry and academia. The specific goal of this study was to quantify and identify proteins and lipids in processing waters generated during the early steps of a herring production chain; from boat to barrel.

Process waters referred to as refrigerated sea water (RSW, 3% salt), storage water (3% salt), filleting water (tap water) and pre-salting brine (3 or 5%

salt) were collected during the spring and fall of 2012 at a local herring producer. All waters were analyzed for total protein, polypeptide profile and fatty acid pattern. For the storage water and salt brines, leaching time (0-4 days, and 0-1 day, respectively) was also taken into consideration.

Spring data revealed that RSW waters contained the least protein and fat; 0.05% and 0.003% w/w, respectively. Storage waters reached ~2% protein and 0.04% fat after 4 days, and filleting waters 0.2-0.4% protein and 0.01-0.2% fat, depending on the type of herring cut produced. Salt brines reached up to 1.3% proteins and 0.16% fatty acids after 1 day. Sarcoplasmic proteins <30 kDa dominated in the three first process waters while also myofibrillar proteins, including myosin, were present in salt brines. Omega-3 fatty acids contributed to 15-50% of the total fatty acids. Data from fall samples, expected to be considerably richer in fat, will also be presented.

Poster 6

Characterisation of Effluents from the Marinated Herring Industry: Part II: From Barrel to Glass Jar

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Volumes of effluents from marinated herring processing industries are substantial and are discarded as waste water in communal sewage. In addition, the organic loads of these effluents are high, which means that the cost imparted for their discharge is substantial. All effluents from the marinated fish processing industries are usually pooled and treated at the “end of the pipe” without any attempt to collect the organic matter or recycle the water. However, these effluents may contain molecules with good marked potential, which are currently discarded. Therefore, there is an urgent need for a detailed mapping of the composition of the effluents in order to indentify interesting compounds.

With this background the industry has expressed that any process that would add value to the effluents, and/or that would allow recycling of water

would be of high interest. This project “Pelagic Industry Processing Effluents Innovative and Sustainable Solutions” (PIPE) is aiming at adding value to herring processing waters though an interdisciplinary research effort engaging both industry and academia. The specific goal of this study is to quantify and identify proteins and lipid leaching out in the last steps of a herring production chain, from barrel to jars. As a starting point, the effluents from each of the processing steps, at a local marinated herring product producer, were collected and characterised. These included samples coming *after* the maturation step; the mother brine, the marinade/spice brine and the sugar brine. All samples were analysed for dry matter, ash, salt, lipid and protein content. The results from the characterisation will be presented.

Poster 7

Technical Sessions 3, 4, 5, and 6

Wednesday, October 31
8:00 am – 6:30 pm

Automatic Box Freezer – An Energy Efficient Freezing Process with Improved Product Quality of Pelagic Fish

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The automatic box freezer is an improved freezing method as compared to the regular plate freezing method where the separation between plates is kept floating, i.e. the floating plates allow the product to thermally expand. The reduced pressure on the fish keeps the product from being deformed, thereby limiting gapping, drip and outside appearance defects. In addition, the reduced freezing time compared to blast freezing is known to cause smaller-sized ice crystallization which results in improved quality with respect to drip. The aim of the study is to compare the automatic box freezer method to the traditional blast freezer with respect to product quality and energy consumption and to optimise the design of the automatic box freezing process.

In order to estimate the energy consumption, temperature loggers are inserted into fish samples

which are placed in different boxes distributed according to a specific pattern inside the freezer. The result is a map of the temperature distribution estimated with a three dimensional scattered interpolation based on the measured temperatures and the respective coordinates inside the freezer. These experiments confirm that a more even temperature distribution and a more efficient freezing method, which the automatic box freezer provides as compared to traditional blast freezing, yields higher quality product with respect to drip, gapping and appearance. The results also reveal that precooling pelagic fish in slurry ice for 30 minutes at $-2.5\text{ }^{\circ}\text{C}$ can reduce the freezing time in the automatic blast freezer by 10–25%, which further increases the freezing capacity and reduces energy consumption.

Poster 8

Innovative On-Board Technologies and Solutions Towards the Sustainability of Marine Resources: The FAROS Initiative

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In marine harvesting, fish waste due to the discard of non-targeted species represents a risk for the sustainability of fisheries, a loss of potentially valuable living resources and a threat for the ecological equilibrium. The LIFE+ project FAROS aims to develop and implement an efficient, integral management network for discards (named Management Geoportal Network – MGN), to reduce global impact of this established practice, while putting in value and optimising the use of the by-catch.

The development of on-ship automated systems for discards evaluation, species biomass estimation and real-time data transmitting is presented in this contribution. The Biomass Estimation Optical System (BEOS) integrates machine vision technologies and optical information processing and feature extraction by means of nonlinear modeling based on artificial neural networks. The proposed identifying strategy includes: a) *Species classification by body shape pattern matching*; b) *species classification by spatial colour modeling* and; c) *biomass calculation*

by weight estimation modeling. The achieved percentage of correct discarded species identification and mass estimation is up to 95%.

The aim of BEOS (in combination with the data transmitting device denoted as RED BOX) is to feed with real catch/discards data the developed MGN virtual environment to get a global map of fishing activity in the European Atlantic coast. In this work, it is presented how proposed solutions will be very useful: a) to assess the most suitable areas to host a particular fishing gear/fishing operation; b) to perform a spatial rating of the fishing areas based on the proportion target/discard and; c) to quantify the spatial/temporal distribution and abundance of discards, which allow to apply statistical methods to derive density maps. With this complete information, fishing fleets could plan in advance (in port) their future activity, minimizing discards, the associated fishing pressure or other negative environmental impacts over stocks while maximizing their profit.

Poster 9

Pre-Rigor Produced Fillets of Atlantic Cod Show No Cold Shortening

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In modern fish farming industry the slaughtering procedures normally delay the onset of *rigor mortis* so much that *pre-rigor* filleting is industrially feasible. *Pre-rigor* produced fillets have several advantages compared to *post-rigor* produced fillets. Examples are fresher fillets, extended sale period, reduced cost of transportation and improved properties such as reduced fillet gaping and firmer texture. Possible drawbacks are fillet contraction and increased weight loss. To increase the shelf-life of fresh fish products it is important to reduce the temperature to 0°C as soon as possible after slaughter. Rapid cooling to 0°C of *pre-rigor* excised meat from warm-blooded animals and from fish in temperate waters, result in very extensive muscle contraction (“cold shortening”) and adverse quality.

The aim of this work was to study if cold shortening occur in Atlantic cod (*Gadus morhua* L.),

a cold water species, and how relevant temperatures after slaughter affected contraction and other quality parameters in *pre-rigor* produced cod fillets. The fillets were stored at 0, 4 or 7°C for 48 hours before continued storage at 0°C for 8 days for all fillets. The results showed that cold shortening do not occur in *pre rigor* produced fillets of Atlantic cod. Fillet contraction was significantly stronger and weight loss was larger in fillets initially stored at 7°C compared to those stored at 0 and 4°C. The small differences in storage temperature during the first 48 hours *post mortem* clearly reduced the shelf-life of the fillets. After ten days of storage *post mortem* the level of Total Volatile Nitrogen was 19.5, 38.2 and 44.8 mg/100 g in the fillets initially stored at 0, 4 and 7°C, respectively.

Poster 10

Effects of Heme Oxidation States on Spectral Variations of Atlantic Salmon (*Salmo salar* L.) During Cold Storage.

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The objective of this study was to demonstrate how different oxidation states of hemoglobin affect spectral properties of fresh salmon fillets during storage. In addition, the origin of a shoulder peak appearing around at 600 nm was investigated to determine whether it originated from absorption of water.

Absorption spectra of salmon hemoglobin and its derivatives (methemoglobin and deoxyhemoglobin) were collected at a post mortem pH. A PCA model was constructed based on the spectra of hemoglobin in the three oxidation states and 24 randomly selected spectra of salmon fillets stored in air, under 60% CO₂/40% N₂ and vacuum on each storage day up to 14 days post mortem. In addition, hyperspectral interactance images of salmon mince with different water content were obtained during storage in air and under 100% N₂. Changes in the absorption features of the shoulder peak were

studied during mince storage by estimating the total peak area around at 600 nm by cubic spline interpolation.

The PCA score and loading plots illustrated the increased similarity between the spectra of air stored fillets and methemoglobin, resulting from changes in the spectral features at 636 nm. The results demonstrated that heme oxidation is the primary source of spectral variations occurring in air-stored salmon. Higher water content in the muscle resulted in a more distinctive shoulder peak and the total peak area changed distinctively under different storage conditions. The results showed that the shoulder peak is related to absorption due to water in the salmon muscle. The visibility of the water shoulder peak varies in the spectrum depending on the dominant oxidation state of heme in the muscle under different atmospheres.

Poster 11

Vibrational Spectroscopic Analysis of Hake (*Merluccius merluccius*, L.) Lipids during Frozen Storage

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There is a need for new procedures to monitor changes of fish muscle during processing or storage that ideally are non-destructive and fast. Fish lipids undergo a series of changes during frozen storage which in principle can be used as markers of muscle quality. Vibrational spectroscopy including mid infrared (IR) and Raman techniques is a useful analytical technique to characterize edible oils and to investigate the lipid deterioration process. The objective of this work was to characterize hake (*Merluccius merluccius* L.) lipids by vibrational spectroscopy (mid IR and FT-Raman) as well as to investigate possible lipid changes in this fraction during frozen storage. Kramer shear resistance was used as a marker of texture changes and lipid damage was also investigated by following the development of conjugated dienes and free fatty acids by spectrophotometric methods. Since the rate of deterioration keeps a close relationship with the storage temperature, $-10\text{ }^{\circ}\text{C}$ was chosen to

accelerate these changes. Additionally fish muscle was stored at $-80\text{ }^{\circ}\text{C}$ and analyzed periodically as an internal control.

Results show that the intensity of the free fatty acid carboxylic $\nu(\text{C}=\text{O})$ band measured by ATR-FTIR spectroscopy can be used for monitoring the development of lipid hydrolysis in hake lipids. Changes in the Raman $\nu(\text{C}=\text{C})$ stretching region (1658 cm^{-1} band), partially attributed to conjugated dienes development, were the only observed spectroscopic alterations related to lipid oxidation of hake lipids during frozen storage at $-10\text{ }^{\circ}\text{C}$. The high correlation of free fatty acids with instrumental texture and the disappearance of $\nu_{\text{as}}(\text{PO}_2^-)$ band are consistent with membrane lipid hydrolysis as being one of the factors directly related with toughening of lean fish flesh.

Poster 12

Myofibrils are Not a Suitable Model Material to Study Muscle Protein Denaturation in Frozen Shrimp

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Shrimp is an important commodity globally and its products are generally exported in frozen form. Although frozen storage is an excellent method that has been used to preserve meat for long periods with small damage of muscle, many reports indicate myosin still denatures during freezing and affects meat quality. Myofibrils (Mf) have been commonly used as a model material to study muscle protein denaturation in meat because the basic structure of filament myosin and actin is kept intact. It is interesting to know how muscle protein in Mf was denatured upon freezing and whether Mf are a suitable model material to study freeze denaturation. For this purpose, myosin and actin denaturation in Mf and meat (Japanese Tiger Shrimp (*Masupenaeus japonicus*)) were compared during the frozen storage at -20°C . Muscle homogenate was prepared by repeated homogenization of meat in 0.1 M NaCl 20 mM Tris-HCl buffer (pH 7.5) for denaturation

analysis. Myosin denaturation was assessed by measuring Ca-ATPase activity and salt solubility. Actin denaturation was assessed by chymotryptic digestion. With Mf, Ca-ATPase decreased to 70% in a day, whereas meat took 27 weeks to reach the same inactivation. Salt solubility decrease was slower than Ca-ATPase inactivation in Mf, whereas both proceeded similarly in meat. Surprisingly, actin in Mf denatured in a day almost completely, which was faster than myosin denaturation. On the other hand, actin in meat was slightly damaged during the storage. It was concluded that myosin and actin were kept stable in meat, and that Mf are not a suitable model material to study freeze denaturation of myosin and actin in shrimp meat.

Poster 13

Modeling Time and Temperature History of Frozen Thawed Hake (*Merluccius merluccius*, L.) Muscle by Low Field Nuclear Magnetic Resonance Spectroscopy (LF NMR)

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Freezing, frozen storage and thawing of fish muscle products induce changes in their water distribution and mobility evidenced by several populations of water detected by the T_2 transversal relaxation times, a parameter easily measured in a non-destructive, non-invasive manner by low field nuclear magnetic resonance (LF NMR). The aim of this work was to explore the potential of LF NMR to authenticate the time and temperature history of frozen thawed fish fillets.

The T_2 relaxation times of hake (*Merluccius merluccius* L.) fillets stored at -10, -20, and -30 °C for up to 120 weeks were measured using a LF NMR minispec mq 20. Several populations of water were observed including the so called “trapped” water (T_{21}) and “free” water (T_{22}). The relaxation times and the relative abundance of these water pools displayed higher changes the higher the storage time, whose change rate was higher the higher the storage temperature.

Principal Component Analysis of the NMR distribution function obtained by CONTIN

analysis, showed that 7 Principal Components (PC) explained 91% of the total variance. Interestingly, the PC1 was found to have an Arrhenius type behavior with a coefficient of determination of 0.91. The Arrhenius equation relates the specific reaction rate (k) with the absolute temperature (T) of many physical and biological systems and is therefore potentially useful to model the time and temperature of frozen thawed samples. The results of the model presented here on LF NMR data were comparable to the results of modeling the water holding capacity, a well-known marker for the sensory quality of fish muscle.

In conclusion, modeling of the results obtained by LF NMR and their close relationship with well-known quality parameters, suggests that this technique has a clear potential to be used for the authentication of frozen storage time and temperature in fishery products.

Poster 14

Effect of Bleeding on Quality Changes of Cod (*Gadus morhua*) Muscle during Chilled Storage

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Bleeding of fish is generally carried out to eliminate most of blood from the tissues. Bleeding is a good nutritional source for microbes and its presence in the flesh of fish contributes towards shorter shelf life of the catch. Without bleeding, the residual blood in fish tissues is one of the main factors inducing the development of undesirable odour and unpleasant flavour of the fish flesh during chilled storage. The aim of this study was to increase the value of seafood and seafood products by analysing the optimum bleeding conditions of wild cod which could prevent defects in cod products due to blood damages.

Quality loss of cod fillets during chilled storage where the effects of four different bleeding methods were evaluated: (i) Bled in seawater for 5-15 min., (ii) bled in seawater for 30-40 min., (iii) bled in

slurry ice for 5-15 min., and (iv) bled in slurry ice for 30-40 min. The temperature history of each group was studied using temperature loggers. The samples were analysed with sensory, microbiological and chemical methods for up to 14 days from catch. The results from microbial and chemical measurements were generally in good agreement with the results from sensory evaluation. Comparison of the groups showed that the use of slurry ice in the bleeding tank resulted in at least two day longer storage life than the groups bled in seawater. The bleeding time showed minimum effect when the cod was bled at lower temperature (slurry ice) but if it was bled at higher temperature (seawater), the storage life increased with longer bleeding time.

Poster 15

Effect of High Pressure Processing on Abalone Texture and Color

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Abalones are a high-value shellfish delicacy consumed in Asia, Australia, New Zealand, and the west coast of the U.S. Abalones typically require mechanical tenderization prior to consumption due to the toughness of the meat. High pressure processing (HPP) has been shown to tenderize and lighten the color of meat, such as beef and chicken, while HPP studies with abalones have been focused on shelf-life extension using 500 MPa or more, without specifically investigating the potential tenderizing effects of HPP. The effect of rigor status during processing on texture of HPP meat has not been conclusively determined. The objectives of this study were to evaluate the texture and color of abalone processed pre- or post-rigor using lower pressures than previously reported.

A total of 20 treatments (6 abalones/treatment), including pre- and post-rigor controls, were used at varying pressures (100, 200, 300 MPa) and processing times (1, 3, 5 min). Pre-rigor abalones were processed within 6 h of shucking, and post-

rigor abalones were processed at least 30 h after shucking. Sample plugs (20x20x5 mm) were cut from the adductor and foot of each abalone for texture and color analyses. Texture profile analysis demonstrated that post-rigor processed abalone meat, irrespective of muscle type, was significantly ($p < 0.05$) less firm and less chewy than pre-rigor processed meat, however there were no significant effects of pressure or processing time. Additionally, the foot was significantly ($p < 0.05$) firmer and chewier than the adductor regardless of processing parameters. HPP increased whiteness of the foot as pressure increased.

This study demonstrates the importance of rigor status during HPP on meat texture, and suggests that processing of abalone meat take place after resolution of rigor to maximize tenderness. Additionally, in markets desiring whiter abalone meat the increase in whiteness with HPP may be advantageous.

Poster 16

Work Procedures in Icelandic Fish Markets and the Use of Requirement Analysis for Identifying Potential Improvement Areas

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Sales records show that supply is the primary decider of prices paid at Icelandic fish auctions and that various quality factors have typically very little affects on the setting of prices. Despite recommendations to connect prices and quality better, from government and various industry stakeholders, limited progress has been achieved. With a new computer system that Reiknistofa fiskmarkaðanna hf. (the fish markets information center) began operating in 2012 it has become easier to store and display various information that has previously not been possible. The aim of this research project was to identify which information and services are most important for stakeholders linked to the Icelandic fish markets. To carry out this work, a requirement analysis was done for different groups of stakeholders, laws and regulations of this industry analyzed along with a

study of operations within two of the largest Icelandic fish markets. Data was collected through a survey, interviews with various stakeholders, reviews and readings of related laws and regulations. The data was then analyzed and evaluated to identify the requirements of different stakeholders. The results were used in a requirement analysis where potential improvement areas were identified.

The main findings of the project are that fish markets work hard to maintain good operations, but still there is a great potential for improvements. The results of this work identifies the areas where there is a need for improvements and furthermore provides recommendations on where to make improvements that can potentially increase value and/or reduce costs for fish producers, fish processors, retailers.

Poster 17

Economic Analysis on the Effects of a New Regulation that Obligates Icelandic Freezer Trawlers to Bring Ashore Cod Heads

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According to a new regulation (810/2011) Icelandic freezer trawlers with on-board filleting will, from September 1st 2012, be obligated to bring ashore part of the cod heads deriving from the on-board processing. Vessels with 600-800 m³ of effective storage space in the freezer hold will be obligated to land at least 30% of the cod heads deriving from their processing and vessels with more than 800 m³ of effective storage space will be obligated to land at least 40% of the cod heads deriving from their processing. Alternatively they will be able to land corresponding volumes of by-products produced from cod heads i.e. tongs, cheeks, faces etc.

The objective of this research project was to explore the applicability of this new regulation and to analyze how it will affect operating conditions of the freezer trawlers that the regulation applies to.

Economic model, incorporating all operation costs and returns, was developed for each of the trawlers affected by the regulation. Available solutions to meet the requirements of the regulation were explored and feed into the model; returning economic analysis on the return on investment.

The results of the project are that the regulation has very little effects on operation costs and revenues of the vessels in question. It also suggests that total volume of landed cod heads will only increase by 1.5% due to this regulation. The regulation applies to eleven vessels, of which seven were already meeting the requirements in the quota year 2010/11. The available technical solutions that were considered applicable were feed into the economic model and the results suggest that the required investments will in most cases return marginal profits.

Poster 18

Instrumental Quality Grading of Atlantic Salmon Fillets (*Salmo salar* L.): Detection of Surface and Embedded Blood and Melanin Spots by Hyperspectral Imaging

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Atlantic salmon is an important farmed fish species in Norway and the trend is that more of the salmon is exported as fillet instead of whole fish. A major problem in salmon farming is the presence of blood and melanin spots in the final product. The reasons for these spots are not fully understood, and as it is today they represents a quality problem. There exist technologies for detection of dark spots in the fillet surface, but not for embedded spots. In this study an instrumental technique based on hyperspectral imaging in interactance mode has been developed. The applied imaging geometry allows both surface and embedded spots to be identified. Furthermore, it's possible to separate between surface and/or embedded spots and to classify the spots as blood or melanin.

The analysis was performed in two steps. First the recorded spectra were transformed into chemical spectra using a modified Extended Multiplicative

Signal Correction (EMSC) model. Then these chemical spectra were sent through a constrained spectral unmixing algorithm with a set of selected absorbance spectra as end-members. At the end of the study an industrial test was carried out with more than 250 fillets. All fillets were manually inspected before entering the experiment and all visible dark spots were registered. After imaging the fillets were manually inspected once more (including slicing) to look for embedded spots and to register depth information. The outcome of the test was that 92% and 98% of the manually identified melanin and blood spots were detected. Furthermore, it was possible to separate blood and melanin spots based on their spectral features, and to classify the spots as embedded or not by combining the result of the hyperspectral imaging analysis with a color image of the fillet.

Poster 19

Automatic Detection and Quantification of Red Pigmented Halophilic Archaea's in Dried Salt-Cured Cod Using Hyperspectral Imaging

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Red discoloration is occasionally observed on the surface of dried salt-cured cod. This is related to a high concentration of halophilic archaea's. According to *Codex Alimentarius*, products with such discoloration are not accepted for commercial use, and should be discarded through manual grading by the producer or by inspection by the resellers. Manual grading of dried salt cured cod is both expensive and time consuming, and lately there has been an increased attention to optimizing this process. An automatic grading system should be able to grade products based on their most important quality features, and the amount of halophilic archaea's is an important quality feature for dried salt-cured cod. To our knowledge, there exist no previous research on this topic.

In this work we have evaluated diffuse reflectance hyperspectral imaging as a method for detection of halophilic archaea's on dried salt-cured cod. By extracting the pigment from halophilic archaea's obtained from dried salt-cured cod, the spectral fingerprint of the microorganism has been identified. Using this fingerprint in combination with a constrained spectral unmixing technique the concentration as well as the spatial distribution across the product can be estimated. The method has been evaluated on 16 dried salt cured cod. The results show that the method is able to detect all visible red discolored regions, corresponding to a level of minimum 10^6 halophilic archaea's per gram.

Poster 20

Use of Microarray Technology for Studying the Molecular Basis of Fillet Firmness in Atlantic Salmon (*Salmo salar* L.)

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Fillet firmness of farmed Atlantic salmon (*Salmo salar* L.) is an important quality trait for the suitability for processing and for consumer acceptance. Despite the importance of fillet firmness for commercial salmon aquaculture, there is only limited knowledge about molecular features associated with this trait. Microarray technology presents a powerful tool for revealing expression patterns and genes associated with phenotypic characteristics, and is well suited for work with poorly investigated traits.

Microarray was used to determine gene expression in skeletal muscle that covered the whole range of fillet firmness in farmed Norwegian salmon. The transcriptomic analyses revealed that firmness of salmon fillets is associated largely with intracellular metabolic processes of the skeletal muscle. Similar expression profiles were observed in several functional groups. Aerobic metabolism using lipids as fuel, and a rapid removal of damaged proteins, appeared to play major roles.

These findings were further investigated in salmon fed a standard diet or the same diet

supplemented with L-glutamate. Glutamate is a metabolically versatile non-essential amino acid that is important for maintenance and promotion of cell function. Salmon fed the glutamate diet had firmer fillets and a metabolic profile similar to that of salmon with firm fillets described above.

In order to further investigate the effect of metabolism on fillet firmness, salmon suffering from metabolic disturbances due to loss of pancreatic tissue caused by pancreas disease (PD), was studied. Pancreas is the primary organ damaged by PD, and salmon with loss of pancreatic tissue were runts with abnormally hard fillets, concurrent with metabolic changes in hearts similar to those associated with softer fillets in the other studies mentioned.

It is concluded that an active aerobic metabolism is important for obtaining preferred fillet firmness, and that these molecular processes can be stimulated by glutamate supplementation, resulting in improved fillet firmness.

Poster 21

CFD Modelling of Combined Blast and Contact Cooling for Whole Fish

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Fish quality is highly influenced by the cooling method which is applied during processing. Earlier research has shown that precooling whitefish fillets to a superchilled temperature ($T_{f,i} = -0.91^{\circ}\text{C}$) in a SuperChiller by Marel, Garðabær, Iceland (formerly referred to as CBC-cooler) results in prolonged storage life. A CFD model which can simulate the effects of the CBC-cooling might save time and cost when predicting the necessary chilling time for a product when its thermal properties are known.

CFD models were created in two and three dimensions in FLUENT to simulate the

superchilling process, and were compared with experimental results from two tests. The temperature inside the SuperChiller and the chilling period for the first test were -7.4°C and 6 minutes, respectively. The corresponding values for the latter test were -14.1°C and 14 minutes.

The thermal contact resistance between the Teflon-coated aluminium-droplets belt and fish was

determined to be $R = 0.028 \text{ m}^2\text{K/W}$ and the k- ϵ RNG turbulence model was selected to simulate the air flow in the computational domain. A good agreement between the simulated 2D-results and the experimental results from the latter test was obtained. Three meshes were compared and the most refined one at the fish surface yielded the best results. The 3D-model was applied to the latter test to investigate if the thickness variations along the fish had an effect on the temperature distribution. Good agreement was obtained between the measurements and the 3D-CFD model, which predicted 3D-effects to take place. A simulation of a 30-minute CBC-cooling and a storage period of one hour showed that the fish flesh did not reach the initial freezing point. Hence, it was concluded that a lower temperature or a longer chilling period are required for a whole fish weighing 2.5 kg.

Poster 22

Monitoring Spatial Distribution Of Quality Parameters in Salmon Using Computer Vision

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The salmon industry has very strict demands for determination of both in- and output quality. Early grading and sorting of fish according to different quality parameters e.g. fat distribution and the number of melanin spots are of utmost importance in order to keep production costs low and ensure the desired quality. Even though, several methods for fast and automated control of selected quality parameters before and during filleting can be found in the literature, the industrial applications are still very sparse.

In this project we combine knowledge from the literature and processing industry with experiments to develop fast and automated methods based on spectral vision technology for quality control in the salmon industry.

Vision technology is able to rapidly evaluate the entire surface of a fillet, quantify the quality of each part of the fillet and grade the fillet based on a pre-calibrated statistical model. Furthermore, spectral imaging will allow simultaneously incorporation of

information from both visual and non-visual electromagnetic reflection from the fish fillet and thereby make grading of the fillets based on a pre-calibrated statistical models possible.

We demonstrate a spatial fat prediction model for whole salmon fillets based on spectral images of biopsy punches from different locations within the same fillet. Each biopsy is subsampled in the depth dimension in order to further check the accuracy of the prediction in three dimensions. A chemical fat determination is carried out for each subsample in order to have reference values for all acquired images. We see a large variation of fat in both the spatial and depth direction, and based on these results and knowledge from the industry a method for quality control have been established. Moreover, we demonstrate how spatial detection of blood and melanin spots followed by locally removing of the downgraded part can be used for process optimization.

Poster 23

Novel Processing of Pre-Rigor Farmed Atlantic Cod (*Gadus morhua*) Fillets by Combining Direct Filleting and Superchilling

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The traditional slaughter and filleting process is in short stunning, bleeding, gutting and filleting followed by shipment with wet ice. Direct gutting and filleting without prior bleeding combined with superchilling is a novel way to process farmed fish.

To attain knowledge about how direct gutting and filleting followed by superchilling affects the flesh quality, a full factorial design was set up, giving 4 variants. After 7 days in EPS-boxes stored under chilled conditions (approx. 0.5 °C), the fillets were assessed for drip loss, shrinkage, color and texture.

The new filleting protocol was performed by direct heading, gutting and filleting without the pre step of bleeding in chilled sea water. Directly after skinning, the fillets were rinsed with chilled fresh water for 10 minutes to remove blood from the fillet.

The superchilling was performed in a Nitrogen freezer. The freezer was preset at -60 °C, and the exposure time was four minutes, giving an equilibrium temperature in the fillets of -0.7 ± 0.1 °C. The traditionally iced fillets obtained a core

temperature of 0°C after 24 hours which was maintained, while the superchilled fillets stayed subzero throughout the observation period.

Bleeding regime had no significant ($P>0.07$) effect on the color parameters, indicating that direct gutting and filleting did not give more residual blood in the fillets.

Superchilling reduced the drip loss ($P<0.001$) with 57 % compared to traditionally icing and gave a significantly ($P<0.001$) decreased contraction of the fillets compared to traditionally iced fillets.

Both superchilling and the direct filleting ($P<0.047$) gave a softer texture of the surface of the fillets than traditional processing and cooling.

The novel process gave a decreased temperature during storage, and reduced contraction and drip loss. The method did not affect the quantity of residual blood, while it resulted in a slightly softer surface texture.

Poster 24

Effect of Different Diet Supplementation on Several Muscle Collagen Parameters in Farmed Atlantic Salmon (*Salmo salar* L.)

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Norway is one of the most important producers of Atlantic salmon (*Salmo salar* L.) worldwide. Salmon production has been subjected to different processes to improve quality, including diet supplementation. Moreover, it is well known that farmed salmon usually present a softer texture when compared with the wild variety, and this fact has been correlated with collagen crosslinks and muscle tensile strength. Since collagen is the most abundant intramuscular connective tissue protein, changes in it have been linked with variations in muscle texture.

The effect of diet supplementation with Arginine (Arg) and Glutamate (Glu) has been studied in order to determine the differences and changes in connective tissue that affect muscle texture. For this study, samples undergoing two different processing procedures have been studied, one frozen immediately after death and filleting (pre-rigour, Lot A) (-20°C/1 month) and one frozen after 5 days kept on ice (post-rigour, Lot B).

The results indicated that though supplementation with Arg and Glu did not produce changes in connective tissue strength, changes were produced in the mechanical properties of the

muscle. The latter could be due to the fact that both amino acids are good fish attractants and could consequently induce an increase in feed rate, which in turn would give rise to muscle cell proliferation, thus improving muscle texture. Despite these findings, feeding supplementation did not induce changes in connective tissue yield, so, although muscle cell proliferation could take place to some extent, connective tissue cell proliferation seemed to be unaffected. Furthermore, many different analyses carried out on connective tissue such as, collagen solubility, glycation, differential scanning calorimetry and pyridinoline bonds, did not reveal any clear difference in connective tissue properties regarding feed supplementation. With respect to the different processing procedures, the lot frozen after rigour (Lot B) presented a higher amount of pyridinoline cross-links leading to a more aggregated collagen, but with no observed effect on texture.

In conclusion it can be stated that salmon diet supplementation with Arg and Glu, did not modify connective tissue characteristics in any of the processing procedures studied.

Poster 25

Featured Session Topics 1, 2, and 3

Thursday, November 1
8 am – 6:30 pm

Method to Determine Added Polyphosphates in Seafood

John Reuther

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Polyphosphates are added to seafoods to retain natural edible muscle moisture content that may be lost during processing, storage, and preparation. In addition to moisture retention and reduction of thaw drip loss, polyphosphates also contribute to maintaining certain texture attributes in many products. Some believe that polyphosphate use may contribute to economic fraud due to ability to hold added water. Detection of added polyphosphates in seafoods is complicated by the presence of naturally occurring phosphates and the dissolution of

phosphate moieties into numerous species (poly, tri, hexa, meta, ortho, tri, pyro, etc.). Also, commercial suppliers use proprietary mixtures of phosphates and other agents for specific applications. An HPLC method was developed to detect, differentiate, and quantify various polyphosphates species as well as citrates in frozen fish. This method allows detection of polyphosphates and other chemicals agents at levels expected to be found in normally treated and over treated fillets.

Poster 26

Bacterial Growth and Histamine Production in Tuna Salad Preparations

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Histamine (Hst) poisoning is associated with consumption of scombroid fish such as tuna. Contamination of commercial tuna salad with Hst-producing bacteria (HPB) from raw tuna, processing equipment, or added ingredients, combined with temperature abuse during processing/storage, can present a food safety hazard. The objectives of this study were to isolate HPB from onions and celery to determine their role in contamination of commercially-produced tuna salad and evaluate Hst production by HPB during storage of tuna salad.

Bacteria were isolated from onion and celery and characterized by real-time PCR, API 20E, 16S sequencing, and/or Hst production. Twenty-five g of tuna salad (3:1 tuna:mayonnaise) and tuna salad with added ingredients [sanitized onion or celery, or 20% DV (vinegar-based product, WTI, Inc.)] were inoculated with $2 \log_{10}$ CFU/g *Pantoea/Erwinia*, *Erwinina persicina*, *Erwinia* spp, or *Enterobacter pyrinus* isolated from celery (30°C, 3 d). Tuna salad preparations were also inoculated with a four-strain

cocktail of *Morganella morganii* isolated from scombroid fish (Mm; 18°C or 30°C, 3 d). Plate counts and MPN-PCR were performed on all samples; Hst was determined fluorometrically.

HPB from celery were 0.7-4.3 \log_{10} CFU/g higher in the presence of sanitized celery and onions vs. plain tuna salad; only *E. pyrinus* produced significant Hst levels (513-2046 ppm; 30°C, 3 d). Mm increased by 2-3 \log_{10} CFU/g in plain tuna salad and tuna salad with onion and by 4-5 \log_{10} CFU/g in tuna salad with celery held at 18°C for 3 d. Mm produced 1315-3083 ppm Hst and increased by 2.5-4 and 5-6 \log_{10} CFU/g in tuna samples after 1 and 3 days at 30°C, respectively. DV inhibited growth of all isolates by 1-5 \log_{10} CFU/g. Introduction of raw celery into tuna salad can add HPB that may cause Hst poisoning if the product is temperature abused. Addition of DV to tuna salad can inhibit growth of HPB and/or production of Hst.

Poster 27

Studies on High Salinity Relaying and Rapid Cooling of Oysters

Michael Jahncke¹, Salina Parveen², Helen Crocker³, Sara Elmahdi⁴, Chanelle White⁵ Stephanie Gray⁶, Bob Lane⁷, and Amanda Morris⁸

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Most seafood related illnesses in the United States (U.S.A). can be attributed to the consumption of raw or undercooked molluscan shellfish. Oysters are filter feeders that can concentrate pathogens. *Vibrio vulnificus* and *Vibrio parahaemolyticus* are two naturally occurring estuarine bacteria that can accumulate in oysters and cause illnesses in consumers. *Vibrio vulnificus* is the leading cause of death related to seafood consumption in the U.S.A. Gastroenteritis associated with *V. parahaemolyticus* is usually self-limiting with diarrhea and flu-like symptoms. Infections with *V. parahaemolyticus* rarely cause death, but closures and loss of business due to this bacterium can be devastating to the oyster industry. A variety of post-harvest-processing (PHP) methods have been studied and several of them have been validated and are currently being used by industry to

reduce *V. vulnificus* and *V. Parahaemolyticus* numbers to non-detectable levels. At Virginia Tech and at the University of Maryland Eastern Shore, we have been conducting studies to determine the effect of high salinity relaying in both on-shore closed system recirculating tanks and outside in the Chesapeake Bay to reduce *V. vulnificus* and *V. parahaemolyticus* numbers in oysters. In addition to the high salinity relay studies, we have also conducted studies on post-harvest cooling rates of oysters to limit *V. vulnificus* and *V. parahaemolyticus* growth after harvest. Our results to date, show that we were able to achieve 2-5 log₁₀ reductions in *V. vulnificus* and *V. parahaemolyticus* in high salinity relaying studies. We were also able to chill oysters from 35°C to less than 10°C within 10 hours of harvest.

Poster 28

Developing Cooking Controls for Potential *Vibrio* Pathogens in Oysters

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Additional controls are necessary to prevent continuing illnesses associated with *Vibrio spp.* in oysters, *Crassostrea virginica*. The primary bacterial culprits have been *Vibrio parahaemolyticus* and *V. vulnificus* with continuing concerns for associated *Vibrio spp.* (i.e. *V. cholerae* serogroup 01). Despite efforts to introduce post harvest processing (PHP) methods designed to reduce the presence of naturally occurring *Vibrio spp.*, cooking remains the most effective recommendation. Use of cooking is advanced by directives in the National Shellfish Sanitation Program (FDA) and the Model Ordinance guidelines maintained by the Interstate Shellfish Sanitation Conference (I.S.S.C), but these recommendations lack clarity for proper cooking methods or appropriate determinations for thermal lethality.

In response, a series of trials were conducted to determine the standard thermal parameters for lethality (D and z-values) of the aforementioned *Vibrio spp.* The results are based on routine survival in a phosphate buffered saline (PBS) media, followed by comparison with naturally occurring *Vibrio spp.* in raw oysters. These lab trials were further substantiated in cooking trials to mimic and measure actual commercial restaurant operations.

The obtained D and z-values in media for *V. vulnificus* CMCP6 were as follows: D48=2.24 min, D50=2.05 min, D55=0.50 min and z-value=10.19°C; *V. cholera* 01 N16961: D48=2.36

min, D50=1.96 min, D55=0.52 min, and a z-value=10.31°C; with *V. parahaemolyticus* TX2103 being the most heat stable with D48=3.02 min, D50=1.99 min, D55=0.72 min with a z-value=11.3°C. Trials with whole oysters with elevated levels of *V. vulnificus* and *V. parahaemolyticus* were conducted (at 48, 50 and 55°C) to determine a protective effects of the food system on the bacteria. It was determined that at lower temperatures a protective effect is noted, but progressively diminished at higher temperatures. Finally, an evaluation of the effectiveness of the Food and Drug Administration's (FDA) U.S. Food Code, which recommends cooking seafood products to an internal temperature of 145°F (62.8°C) for 15 seconds in order to reduce or eliminate human pathogenic threats, was conducted on chargrilled half-shell shucked oysters containing enhanced levels of *Vibrio spp.* Additional testing was conducted at 200°F (93.3°C) which was the observed temperature during standard cooking practices from commercial restaurant settings. Results utilizing the restaurant standard cooking practices as well as those recommended in the U.S. Food Code proved effective in reducing or eliminating the potential *Vibrio spp.* pathogens. These results support the integration of HACCP based concepts for more effective cooking controls in restaurant settings and operations.

Poster 29

Framing the Message about Seafood: Outcomes of a Conference about Communicating Seafood Safety

Doris Hicks¹, Ken Gall², Michael Morrissey³, Heather Mann⁴, Pam Tom⁵, Dr. Lori Pivarnik⁶ and Steve Otwell⁷

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Seafood is a nutrient-rich food that provides high quality protein, long-chain polyunsaturated fatty acids and several important minerals and vitamins. Research over the last two decades have shown that seafood consumption is closely linked to health benefits such as improved coronary health in adults and improved cognitive development in infants and children. While health benefits greatly outweigh the risks for the general population, there is concern that contaminants in some species of fish may pose risks for some select populations. However, the problem of reduced seafood consumption, due to price, availability, or undue caution may be the greatest risk for populations throughout the U.S. and other countries.

A consensus building conference was facilitated to conduct a one and a half-day workshop that brought together participants from the private sector, government, academia, and advocacy communities to discuss the challenges and opportunities of a risk-based approach to seafood

safety, and the coordinated roles of government and industry in such a system. The workshop focused on the issues and implications of messaging efforts currently being used by these groups (i.e. seafood guide cards and other advisories), with the goal of making concrete and actionable recommendations for implementation of a new science-based message.

The Framing the Message About Seafood conference represented the first time that a very diverse group of stakeholders have been convened to discuss the information that has been presented to the public on seafood health benefits and risks in a format designed to specifically explore and identify alternative approaches to reduce confusion and misinformation. It was remarkable that a consensus was reached on an alternative approach that could be readily translated to an existing Web-based resource that would be easy to use and could provide a model for others.

Poster 30

Seafood Health Facts: Making Smart Choices. Balancing the Benefits and Risks of Seafood Consumption

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The Seafood Health Facts Website is designed to be a comprehensive resource on seafood products for healthcare providers and practitioners and their patients. It is also intended to be a resource for consumers to obtain objective information on seafood products. The information on this site is organized by topic and includes resources for seafood nutrition and the benefits of seafood consumption, seafood safety and the risks associated with certain types of seafood, a comparison of the risks and benefits of seafood consumption, and the

seafood supply in the U.S. It is also organized to provide different types of resources appropriate for different groups of people. The educational materials and other resources for each of the seafood and health related topics are organized into three different sections based on their usefulness for: the general public; healthcare professionals; and scientific publications for all groups. This project was supported through USDA/CSREES Award No. 2007-51110-03815.

Poster 31

An Overview of the Risks and Benefits of the Consumption of Different Classes of Seafood Products

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Seafood is a nutrient-rich food source that is widely available, being its consumption recommended due to several nutritional benefits. In general, dietary recommendations advise weekly consumption of one to two portions of fat fish. In fact, fish and other seafood products are a good source of high quality protein, low in saturated fat, and rich in many micronutrients (like selenium and some vitamins). They are also a good source of long chain polyunsaturated omega-3, particularly eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids. But, seafood can be a source of contaminants. Particularly, some fish species, such as black scabbard fish, swordfish, tuna, or dogfish, present high toxic metal contents. Accordingly, this study aimed to compare and assess the risks and benefits associated to the consumption of different classes of seafood products: bony and cartilaginous wild fish, farmed fish, canned fish, cephalopods, bivalves, and crustaceans.

The overall comparison showed that toxic metal levels are higher in cartilaginous fish, which contain relatively low EPA+DHA contents. Nevertheless, some bony fish display high amounts of methylmercury due to their ecology and feeding

habits. Farmed fish typically present low contaminant concentrations due to the greater environmental and diet control they are subjected to. However, the omega-3/omega-6 ratio is more unfavourable when compared to the wild fish. Cephalopods are lean seafood and, as such, a poor source of EPA+DHA, but also contain low amounts of contaminants with exception of cadmium. Likewise, the bivalves exhibit substantial cadmium and, in addition, lead levels. Regarding crustaceans, a distinction must be done between the muscle with low contamination and the viscera (also consumed as a delicatessen) where toxic metals are particularly abundant. Finally, among canned fish, canned tuna warrant some caution, given their methylmercury contents. Accordingly, a balanced seafood diet is advised.

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Poster 32

Validation of Microwave Cooking Instructions for Not-Ready-To-Eat (NRTE) Seafood

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Control of potential hazards associated with foods involves application of preventative measures in the food chain from primary production through processing and consumption. Unlike ready-to-eat (RTE) food products which are safe for consumers to consume regardless of the form purchased, not-ready-to-eat (NRTE) food products must be properly cooked by consumers to assure their safety. Therefore cooking instructions provided by industry for NRTE foods must be accurate and effective, and consumers must understand the instructions and be able to follow them to assure food safety. The aim of this project was to assess and validate microwave cooking instructions used by industry for consumers of NRTE seafood products. A Microwave Work Station (MWS) was purchased from FISO Technologies, Inc. in Québec, Canada. The MWS includes a microwave oven equipped with a turntable, an eight-channel fiber optic rotating unit for temperature measurements, and the FISO Commander Workstation Edition software. The

Simple Steps™ packaging platform supplied by CryoVac/Sealed Air Corporation in Duncan, South Carolina was used to package a variety of NRTE seafood products at Fresher than Fresh, Inc. in Gastonia, North Carolina. Real-time temperature data was obtained during microwave cooking to assess internal product temperature and uniformity of heat distribution in the food package. End-product temperature data were used to determine validity of consumer cooking instructions. Factors affecting microwave heating of seafood products were examined and examples of proper consumer cooking instructions were developed. Results and recommendations for seafood industry members with an interest in distribution of NRTE seafood are presented and outcomes are summarized in a technical publication and consumer fact sheet.

Poster 33

Food Safety and Screening of Contaminants in Icelandic Seafood: Emphasis on PFC's

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Seafood has been identified as one of the major sources of persistent organic pollutants and inorganic trace elements for humans. Food safety is an issue of great concern worldwide, consequently the safety of seafood will be of prime importance in the future. Monitoring and surveillance of seafood products to evaluate their status regarding undesirable chemicals is therefore an important tool to ensure food safety. For this reason Matis conducts a surveillance program to gather information on various inorganic trace elements as well as persistent organic pollutants (POPs) and pesticides in Icelandic seafood products. The data collected provides the foundation for scientific evidence that Icelandic food products conform to regulations on food safety and can therefore help to ensure high level of consumer protection. The information can also be utilized to carry out risk-benefit assessments of fish consumption. Different fish species and fisheries products as well as fish meal and fish oil for the feed industry are included in the surveillance program.

The project fills in gaps of knowledge regarding the level of undesirable chemicals in Icelandic seafood products. It is considered to be a long-term project and every year a priority chemical group is

selected in order to fill in gap of knowledge regarding chemical contaminants in the Icelandic marine biosphere. In 2011, main emphasis was laid on poly- and perfluorinated compounds (PFCs), since the information on the amount and distribution of this chemical group in the Icelandic environment is scarce. Altogether 11 different PFCs were analysed, including PFOS and PFOA, in two fish species, cod (*Gadus morhua*) and lumpfish (*Cyclopterus lumpus*) flesh and roe as well as liver and sperm (cod only). PFCs were also analysed in capelin meal and oil (*Mallotus villosus*) and mackerel meal (*Scomber scombrus*). PFOS was the only congener detected in the samples, where PFOS was below limit of detection in the flesh samples of cod and lumpfish and was not detected in the oil samples. In the cod, the PFOS concentration was as follows: roes > liver \approx sperm, indicating an effective mother-offspring transfer. PFOS concentration was higher in capelin meal compared to mackerel. From the presently available data the highest concentration of PFOS was measured in capelin meal 13 ug/kg. No official maximum residue level is available for PFCs in food or feed.

Poster 34

Assessment of Contaminant Levels on Discarded Species as a Key Step on the Definition of Optimal Valorisation Strategies

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Increased by-catch utilisation interest may come from a greater demand for fish products: the development of new markets for previously discarded species, use of low-value by-catch specimens for aquaculture and animal feed or the creation of value-added products for food or pharmaceutical industries. However, the contribution to a sustainable management of this biomass through their optimal valorisation highly both depends on the quality of this new raw material as well as on the products to be obtained from them.

Many studies in related literature, including surveys of fish products in markets of different countries or monitoring reports of Public Administrations and the European Commission, reported the presence of significant levels of pollutants (especially of dioxins, polychlorinated biphenyls-PCBs, organochlorinated pesticides-OCPs and heavy metals) in commercial species of different fisheries. Hence, it is logical to assume the existence of contaminants in other non-commercial discarded species present in mixed fisheries, although their levels are not usually assessed.

In this work, developed on the framework of LIFE+ Project FAROS, the evaluation of pollutant content on most discarded species identified in Spanish fleets operating in Great Sole Bank and Coastal waters is presented. A complete spatio-temporal monitoring of heavy metals (Hg, Pb, Cd), PCBs, dioxins and pesticides together with a statistical analysis of obtained data were performed as a key step on the proper definition of valorisation strategies that could overcome drawbacks regarding the fact that some marine valorised by-products (mainly concentrates such as fish meal, oil, etc.) present pollutant levels of concern.

Based on obtained results, some species (like *Actinauge Richardi* or *Capros Aper*) that were at first considered of interest for valorisation purposes (due to the high amounts of discards obtained by year) could not be appropriate due to determined excessive metal concentrations (especially in the case of Cd), requiring decontamination actions.

Poster 35

The Effect of K-Lactate Salt and Liquid Smoke on Bacterial Growth in a Model System Simulating a Cold Smoke Process

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The effects of a commercial K-lactate salt formulation (KL) and a commercial liquid smoke flavor (LS) on the growth of selected fish spoilage bacteria (FSB), lactic acid bacteria (LAB), and *Listeria innocua* (as a non-pathogenic substitute for the human pathogen *L. monocytogenes*) were investigated in a model system simulating a cold smoke process for cold smoked salmon (CSS). Tryptic soy broth with yeast extract (TSBYE) supplemented with 3 or 6% KL (Purasal Opti.Form PPA Plus; Purac) with or without 0.07 or 0.14% LS (Arosmoke P-50; Red Arrow) were inoculated with two strains of *L. innocua*, three FSB (*Photobacterium phosphoreum*, *Pseudomonas putida*, and *Vibrio vulnificus*), and five LAB (*Carnobacterium maltaromaticum*, *Carnobacterium inhibens*, *Lactobacillus curvatus*, *Lactococcus lactis*, and *Enterococcus faecalis*), respectively, in mono-cultures. Bacterial growth at 20 °C was measured for up to one week by recording absorbance at 600 nm every

10 minutes using a microplate incubator and reader (Bioscreen C). The treatments had very variable effects on growth depending on species. Most detrimental was the effect of KL on the growth of *L. curvatus*. Interestingly, growth of this species appeared to be enhanced by the supplement of LS, and it was able to grow in the presence of 6% KL + 0.07% LS, but not when 6% KL was the sole preservative. However, the apparent lag time in this situation (106 h), was threefold longer compared to 0.07% LS alone. Another unexpected result was the inhibiting effect of KL on the growth of *V. vulnificus*. This halophilic species was not able to grow in any combination with 6% KL (all other species where), and was significantly inhibited by the lower KL concentration. In general, the isolated effect of LS was minor compared to the effect of KL, but in most cases, a combinatorial effect of the two preservatives was observed.

Poster 36

Effect from Digested Cod Liver Oil of Different Quality on Oxidation, Energy Metabolism, and Proteome in Yeast (*Saccharomyces cerevisiae*)

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It has lately been recognized that the conditions of the gastrointestinal (GI) tract appear to be pro-oxidative. Thus, PUFA-containing food items may oxidize not only during storage, but also during the GI-passage. We have recently shown that in vitro GI digestion of cod liver oil stimulated TBA-reactive substances (TBARS) formation in both the gastric and intestinal steps, while levels of lipid hydroperoxides remained nearly constant. Preformed oxidation products in the cod liver oil resulted in further elevated TBARS levels during the digestion. The aim of this study was to investigate how in vitro digested fresh and slightly rancid cod liver oils affected metabolic activity, intracellular oxidation and proteome in yeast (*Saccharomyces cerevisiae*) cells.

Cod liver oils with two initial levels of TBARS (3.8 $\mu\text{mol/kg}$, fresh and 21.8 $\mu\text{mol/kg}$, slightly rancid) were subjected to a static in vitro digestion model. Yeast in the stationary growth phase was then exposed to the digested oils at a concentration of 1.7 mg/ml. After 2 h incubation, the effect of both digests was studied at a cellular level by

measuring cell energy metabolic activity, intracellular oxidation and proteome. The latter was done by analyzing mitochondrial proteins with 2-D electrophoresis. Differentially expressed proteins were identified by mass spectrometry.

Results showed that TBARS values increased to 71 and 273 $\mu\text{mol/kg}$ lipid during digestions of the fresh and slightly rancid cod liver oils, respectively. Both digests increased intracellular oxidation and cell energy metabolic activity compared to untreated cells. No differences between digested rancid and fresh oils were measured. At the proteome level mostly down-regulation of proteins was observed and was more intensive for digested rancid oil compared to digested fresh oil. Among the down-regulated proteins were enzymes involved in organic acid metabolism, in regulation of intracellular acetyl-CoA pool and in regulation of the metabolic flux through the citric acid cycle. The enzymes might be inhibited by high cell energy charge resulting from the increased intracellular oxidation and energy metabolism.

Poster 37

Louisiana Direct Seafood: Using a Successful Direct Marketing Program to Deliver Best Practices and Technology Transfer to Seafood Harvesters

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Over the years, Sea Grant college programs in different states have developed numerous outreach materials to support seafood harvesters. Many of these materials have focused on recommendations for best practices, ranging from business operations to harvest and product handling technologies and techniques. Poor access to an interested audience has been a key barrier to the delivery and eventual adoption of these recommended practices by fishermen and clientele. Recent efforts, such as the USDA's Trade Adjustment Assistance (TAA) for Shrimp Fishermen program and other funded projects, have required attendance to technical assistance and outreach workshops to individual monetary assistance on a fishery-wide basis.

The Louisiana Sea Grant Program has helped develop the Louisiana Seafood Direct program in response to a small, grass roots demand. The program goal is to support individual, independent generational fishermen which comprise the majority of the state's fisheries, and who want to attain a

higher price for their seafood products and improve their financial viability. The program utilizes websites, electronic newsletters and other efforts to link interested customers with participating fishermen. The initial success of the program in Delcambre, Louisiana, to connect customers who are willing to pay higher prices for high quality fresh seafood resulted in significantly increased profitability to the participating fishermen. These higher prices have in return produced an audience of fishermen interested in learning practices that can help them transition to a product quality-focused approach from a quantity-based model. The result of the technology transfer that has taken place includes the adoption and implementation of new vessel technologies for seafood harvest and refrigeration, as well as recommended practices concerning improvements in business management, product quality and food safety, and electronic marketing and media.

Poster 38

Communicating Seafood Sustainability from The Gulf Coast: A Two-Pronged Approach

Rene LeBreton

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Pressure from the eNGO community on seafood buyers to demonstrate that the seafood they are sourcing is “sustainable”, has created an influx of sustainability models and programs. This has created an increased amount of pressure on government fishery management agencies to provide communications and assurances to the supply chain with limited budgets and staff. LDWF is working on two programs to help create ‘home-grown’ programs which will provide a transparent source of data on our fisheries and to provide a level of confidence to the buyers of our seafood.

1. One of the key projects the Gulf Coast states are embracing is a “GulfWatch” website. This would be similar to the NOAA FishWatch website, but would emphasize those species managed at the state level – species not currently covered by the federally managed species on FishWatch. This site will be a transparent resource of information for buyers and consumers to make educated decisions about our fisheries.

2. The second key project is to combine efforts with the Audubon Nature Institute to develop a program that is based on the concept of fishery improvement plans (FIPs). The Audubon Nature Institute is a conservation organization with a strong reputation on the Gulf Coast, and will lend third-party credibility to this program. Many seafood buyers don’t require an eco-label to be associated with the seafood they source (in fact many view the additional logo as competition to their own brand), what they do want is the confidence that they are sourcing seafood from a responsible fishery. This program will highlight the strengths of the major Gulf Coast fisheries and indicate areas that need improvement to conform to the FAO Code of Conduct for Responsible Fisheries. As this program develops regular input from stakeholders will be critical to ensure its success.

Poster 39

Adding Value through Sustainable Fisheries

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There are three main aspects of sustainability; the environmental, the social and the economic. When it comes to fisheries, stock sustainability is particularly important and the stock and ecosystem health has significant effects both on the environment and on the sector economy. In order to add value to a product, sustainability needs to be documented and communicated to the buyer and the end-consumer. Recent studies in British supermarkets have shown that this price premium exists, and commonly it is between 10 and 20%. Sustainability discourses in marine resource management have tended to emphasize biological sustainability, and value adding through product certification predominantly comes in the form of

green certification schemes. This implies that there is a scope for achieving value adding through social and economic sustainability dimensions. The potential for adding value through documentation along the three dimensions of sustainability in fisheries creates an incentive for the operators (organized groups of fishermen) to develop management plans. This poster summarizes various sustainability indicators for the fisheries sector; it explains what the indicators mean, and how they are interrelated, and illustrates how they may be used in management plans to achieve value added fish products.

Poster 40

Microbiologically food security and sustainable development of exportable shrimp product through value-addition and its contribution to the national economy

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Fisheries is the fastest growing enterprise within the agriculture sector and is gaining significant momentum in several parts of the world. Shrimp fisheries has emerged and exhausted on a valuable foreign exchange earner for developing countries and as an alternative to meet the protein requirement of the increasing world population. (An effort was made to improve the broodstock by following the guideline for healthy broodstock.) Effort was made to assess a comparative study on cost benefit analysis as well as microbiological view from a same batch of black tiger (*Penaeus monodon*) shrimp through 1 traditional head less shell on block frozen (Product A) and 4 value-added individual quick frozen (Product B-E) processing line. Weight loss or gain at different key points during processing was determined. Percentage of weight gain increased to 10.6 due to value addition in comparison to traditional head less shell on block frozen products. Moreover, the net profit of traditional block frozen product (Product A) and value-added ready-to-cook (Product B-C) and value added ready-to-eat (Product D-E) were 13.52%, 23.69%, 38.42%, 50.89% and 41.86% respectively. (The microbiological quality and safety aspects of raw

shrimp at the receiving stage and processed finished products were been carried out.) / (In the microbiological study) the average standard plate count (SPC), total coliform (TC) and faecal coliform (FC) of received raw shrimp were found 27.5×10^5 cfu/g , 287 cell/g (MPN) and 67 cells/g (MPN) respectively. After freezing the microbiological flora showed 51.6% decrease in traditional block frozen product A, whereas in the value-added products B, C, D and E showed 51.6%, 89.5%, 90.2%, 7.8% and 98.6% decrease respectively. *Salmonella* and *V. cholerae* were detected 1 occasion each during handling at landing center. In the present study a plea was also undertaken to prepare a Hazard Analysis Worksheet, HACCP Plan Form and Traceability Checklist for uplifting the value-added shrimp safety and quality. It is hoped that if the findings of these efforts were been implemented in the shrimp processing plant, it could be helpful to sustain our export trade and to ensure safety of health of the consumer from microbial food poisoning, food-borne infections by offering excellent value-added products.

Poster 41

Ultrasound Reflection as a Tool for Improving Sustainability of the Crab Fisheries

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Fishery of edible (or brown) crab (*Cancer pagurus*) is of increasing importance in the North Sea and North Atlantic Ocean and takes place most of the year. Male crabs are killed onboard the fishing vessel and only the claws are kept, taken ashore and sold, whereas females are sold alive e.g. to gourmet restaurants. When the crab approaches the time for change of shell, its' claws are full of meat and of a high commercial value. However, in a period after the new shell has been formed, the claw content is mostly water. As this is not realised until after the claws have been sold, the fisherman gets a lower, "average" price. A much more preferable situation would be if the "unwanted" crabs could be released alive (undamaged) and only the ones with high meat

content landed. This would improve the sustainability of the fisheries and at the same time increase the profit.

The poster demonstrates how ultrasound imaging technology – like the one used for medical applications – can assist in sorting crabs according to the claws' meat content. The images show a clear contrast between the claw shell and between water and tissue inside the claw. The method is fast, although not quite fast enough to enable measurement on each single individual. However, as the crabs in a pot probably do not vary much in meat content, representative samples may be used.

Poster 42

Featured Session Topic 4

Friday, November 2
8 am – Noon

Rapid Identification of Species of Gadiformes of Commercial Interest By Mean of High Resolution Melting Analysis

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There is a growing interest in authenticating food products in general, Commercialization of seafood products under *Gadus morhua* labeling, yet being other gadiformes species is becoming a major concern. Several species of gadiformes can be easily mistaken in the market.

Regulatory authorities are becoming aware of the necessity of confirmatory techniques to allow seafood products authentication. In this sense, molecular biology techniques, mainly DNA sequencing are the most commonly used for seafood species genetic screening.

In this study, we report an alternative method to traditional sequencing analysis using High Resolution Melting Analysis to perform genomic variant scanning. An assay integrating real-time quantitative PCR (Q-PCR) with high-resolution melting (HRM) curve analysis was developed and assessed for rapid identification of four species of gadiformes. This method is based on analysis of the PCR product melting curve that discriminates PCR

products according to their lengths and base sequences.

We describe a novel HRM-PCR (high-resolution melting) assay, using LightCycler480 instrument, capable of an accurate identification of *Molva molva*, *Theragra chalcogramma*, *Brosme brosme* and *Gadus morhua*.

We have evaluated High Resolution Melting (HRM) analysis as a method for one-step fish identification. Using common primers, designed to amplify a fragment located in the 3' end of the Cytochrome B gene, coupled with high resolution melting (HRM) analysis, we are able to distinguish four different species of gadiformes generating of four different HRM curve profiles.

HRM is a fast, reliable, accurate and cost-effective screening method for species of gadiformes, which can be applied to the identification of any species of interest.

Poster 43

Dipstick Test for DNA-Based Cod Fish Products Authentication

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In recent years, food authentication has become one of the major issues in quality control. While morphological identification is generally easy in fresh and unprocessed fishes, for many fish species there is the possibility of fraudulent or accidental species substitution when products are imported or sold without informative morphological characters.

This has triggered the need to develop innovative cost-effective methods for testing the authenticity of food products.

Gadus morhua represents one of the most important commercial species worldwide and it is sometimes replaced by cheaper species, which are introduced in the market labeled as *Gadus morhua*, of superior quality. Suitable methods are required to differentiate these species.

In this sense disposable dipstick-type DNA biosensors are particularly useful for DNA authentication in small facilities or field analysis due to their simplicity, low cost and portability. This method also allows a visual genotyping without the need of specialized instruments.

We have developed a series of dipsticks for the rapid DNA-based identification of four cod species which are commonly sold both fresh and processed.

Colored Gold nanoparticles are employed as reporters to enable visual detection. This detection is performed through capture of nanoparticles by hybridization to complementary oligonucleotide immobilized at the test zone of the dipstick.

A 140 bp fragment of the mitochondrial cytochrome *b* gene is amplified by means of PCR using a set of primers common to the four species. This is followed by primer extension reaction of the unpurified amplification product using several species-specific internal primers which were designed for detection of *Molva molva*, *Theragra chalcogramma*, *Brosme brosme* and *Gadus morhua*. These primers incorporate a tag able to hybridize with gold nanoparticles.

The developed lateral flow device enables *Gadus morhua* DNA-based authentication by simple visual detection, providing a mean to simplify nucleic acid detection.

DNA Probes for *Thunnus* Species Identification in Canned Products

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Thunnus genus constitutes eight tuna species: albacore (*Thunnus alalunga*), bigeye tuna (*T. obesus*), blackfin tuna (*T. atlanticus*), longtail tuna (*T. tonggol*), northern bluefin tuna (*T. thynnus*), Pacific bluefin tuna (*T. orientalis*), southern bluefin tuna (*T. maccoyii*), and yellowfin tuna (*T. albacares*). Albacore, bigeye and yellowfin are caught in much higher quantities and commercialized by canned industry under “white tuna” and “light tuna” denominations with a higher value than “tuna” denomination. Therefore, since each tuna species has a different quality and price, a fraudulent replacement of valuable species by less valuable ones may occur. As a matter of fact, for effective food and fishing management and the protection of consumer rights, molecular methods are needed for the species identification of tuna canned products.

Sequencing is the most robust molecular technique for tuna species identification. However, its robustness is limited since DNA is extremely degraded in canned products. Moreover, although most tuna is canned in brine or oil alone, nowadays canned tuna include also peppers, onions, spices and

sauces that affect negatively to the DNA quality by inhibiting the PCR reaction. For that reason, in many cases is completely impossible tuna identification by sequencing. Thus, the analysis of short DNA fragments with fluorescent probes has been revealed as the method of choice.

In this work we have developed the first ever method based on a battery of ten fluorescent DNA TaqMan™ probes for rapid and accurate identification of any of the eight *Thunnus* species in canned tuna. This innovative method has been successfully validated *in silico* (after analyzing more than 600 mitochondrial sequences), and methodologically with 122 authentic reference tissue samples and 30 different commercial tuna presentations. These results make the established method a relevant tool to deter illegal activities that undermine sustainable tuna fisheries, obstruct socioeconomic development, and impede consumer information and protection.

Poster 45

Use of Otolith Microchemistry to Identify Nursery Origin and Track Migration Pathways of Gulf of Mexico Fishes

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The living marine resources that occur along the Gulf Coast of Florida support multibillion-dollar commercial and recreational fisheries and associated cultural heritages. These fisheries are managed by state and federal scientists who increasingly rely on the novel use of new technologies in their search for independent guidance. The analysis of the chemical composition of fish otoliths (ear bones) is a well established tool used to answer a variety of questions in fisheries biology by: (1) defining the separation of fish stocks; (2) tracking ontogenetic migrations of fishes; and (3) determining the origin of adult populations. Trace elements dissolved in the ambient water mass are incorporated into microscopic, crystalline layers of the otoliths of fishes during growth. Thus, chemical analysis of these microscopic layers (referred to as “otolith microchemistry”) provides a detailed, well-preserved chronological record of an individual’s exposure to

changes in ambient water conditions that can arise as it migrates between water masses or large-scale environmental perturbations occur. The presence and relative proportions of these elements define a distinct, permanent microchemical signature (i.e., natural tag) that varies among fishes exposed to different water masses and environmental conditions. We are using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to characterize the trace elemental composition of otoliths from a variety of juvenile and adult fishes collected from estuaries along the west coast of Florida and offshore waters in the Gulf of Mexico. Ongoing studies focus on gag, red snapper, spanish mackerel, and red drum in order to track migration pathways and identify those habitats that contribute most to replenishment of adult spawning stocks.

Poster 46

Consumer Behavior Analysis for Understanding the Dissonance Between Attitude and Sensory Perception of Wild and Farmed Fish

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Senegalese sole (*Solea senegalensis*) and turbot (*Psetta maxima*) are high-value commercial fish species with increasing importance in aquaculture. The access to a wider variety of farmed fish species is an increasing market demand.

The study intends to point out the characteristics of modern consumers' behavior and perception towards wild versus farmed fish species.

A sensory assessment score sheet for cooked fish was designed and a blind test of acceptability for the sensory parameters was performed following the method UNE.ISO 4121: 2006 for sensory evaluation through quantitative categorical response scales. Qualitative "free listing" method has been applied in which each consumer free and spontaneously had to assign terms (descriptors) associated with wild fish or farmed fish. Altogether 96 consumers completed the test (males and females

from 18 to 75 years, who eat fish at least twice a week).

As a result of the acceptability blind test, consumers liked more the taste of farmed fish but they did not found significant ($p < 0.01$) differences in the rest of sensory attributes like texture, odor and overall acceptability. However, in the free association study using qualitative terms, it was noted that consumers considered farmed fish to have inferior organoleptic characteristics while the wild fish had stronger flavor and better texture.

Therefore there is a significant dissonance between the attitude and the sensory perception that consumers have to fish from aquaculture origin. The result in this study with Spanish consumers shows a significant difference ($p < 0,01$) for the sensory attribute that measures the flavor in favor of fish from aquaculture.

Poster 47

Brown Trout – Suitability of an “Old” Species for Organic and Conventional Aquaculture

Monika Manthey-Karl

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Background: German aquaculture concentrates mainly on rainbow trout and carp. To increase the product range farmers turn back to well known but non target species like Brown trout that is a less robust for farming.

Fish feed contain increasing amounts of plant material which can cause nutritional, physiological and ecological effects. The influence of different feed composition on the growth performance and the product quality of the fish at market size was investigated.

Materials and methods: Brown trout (*Salmo trutta fario* L.) were raised according to the organic guidelines of “Naturland”, a German founded association for organic agriculture. Three certified organic trout feeds of different suppliers were tested. A control group was fed a standard trout diet.

The fillets were analysed for proximate, fatty acid and free amino acids composition and selenium. Instrumental methods include colour, texture and water-binding capacity. Sensory assessment was accompanied by electronic nose measurements.

Results: All brown trout groups showed comparable and acceptable growth performances. The fillet composition of the organic groups was similar and rich in long chain n-3 fatty acids. High values of oleic acid mirrored the increased amount of plant material in the conventional feed. This led to lower taurine and selenium contents as well.

Conclusions: The fish quality of all groups was good. The tested modern organic feed stuffs yielded similar production values compared to standard diets. High taurine and omega-3 and omega-6 fatty acids highlight the healthy aspects of fish consumption.

Although the overall costs for organic fish production is higher, due to higher feed and production prizes and because of strict maximum production limits requested by organic certifiers it was shown that organic Brown trout production is economically feasible.

Poster 48

Is Organically Farmed Seafood a Better Quality?

Horst Karl and Monika Manthey-Karl

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The market share and variety of aquaculture fish and products thereof is increasing each year in Germany. Salmon from Norway, pangasius from Vietnam and rainbow trout and carp from German farms are the main species, but also sea bass and sea bream from Mediterranean countries or tilapia and prawns from Asia gain more importance. Most fish and crustaceans originate from conventional farms but there is an increasing demand for organically raised species.

The consumer expects organically produced food to be healthier and to have a better taste and less contaminants. However, comparative scientific investigations in the aquaculture field are rare.

During the last years we compared the quality of various conventionally and organically produced seafood available on the German market. Studies included chemical, physical, instrumental and sensory assessment.

Results will be presented for Atlantic salmon, rainbow trout, pangasius, tilapia and prawns. No differences were observed in the proximate

composition, contaminant levels and sensory assessment of organically and conventionally farmed salmon. Rainbow trout of both rearing conditions and products thereof were of high quality but again no significant differences were observed.

For pangasius fillets large differences in the proximate composition were found due to addition of water during processing of conventional products. Low protein content and $\text{pH} > 7.5$ are reliable indicators

for the use of water and water binding additives. Sensory assessment yield divergent results. Organically farmed tilapia fillets had a high quality whereas conventionally farmed Tilapia fillets varied considerably from excellent up to bad. The quality of prawns on the German market ranged widely but organic prawns achieved high scores.

In conclusion, organically and conventionally produced seafood were mostly of high quality but conventionally produced seafood did not always assure the clean label.

Poster 49

The Use of Microalgae as a Protein Source in a Finfish Diet

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Until recent times it has been thought that food supplies derived from the oceans were inexhaustible. However, we are now facing the realization that fishery stocks are in decline and in danger in many locations. The advent of aquaculture has been able to mitigate some of these declines presently, but as we look to the future, we need to increase our efforts to improve our aquaculture harvests while adopting sustainable practices if we are to meet the needs of a growing population. A possible sustainable source of feed for aquaculture is microalgae. Microalgae are currently being used in the rearing process for many aquatic organisms and cultivated for use as feed for aquacultured bivalves, such as oysters and clams.

However, relatively little work has been done to determine if algae could serve as an effective feed for finfish. In this study Nile tilapia (*Oreochromis niloticus*) were fed pelletized diets containing two types of microalgae over the course of 12 weeks. Based upon the feed conversion ratio (FCR) data, the diets containing algae were found to perform as well as a diet containing fish meal and better than a diet containing soybean meal as the major protein source.

Poster 50

Effects of Dietary Mineral Supplementation on Quality of Fresh and Salt Cured Fillets from Farmed Atlantic Cod, *Gadus morhua*

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In intensive fish farming, the feed provided is most often extruded and formulated with high quality protein meals and oils (lipids). Minerals are added in surplus to the formulated feed to ensure that mineral deficiency is avoided, as little is known about marine fish mineral requirement. Effects of dietary mineral supplementation on product quality of farmed fish have been studied to a limited degree. However, it has been shown that feeding farmed cod with diets containing supplementary copper may result in reduced fresh fillet quality due to black stripes in the muscle (blood vessel melanosis). The aims of this study were to investigate effects of dietary mineral supplementation on chemical and sensory quality parameters of fresh farmed cod fillets and on the quality of salt-cured farmed cod.

Farmed cod were fed three experimental diets with different levels of mineral supplements (no supplementation, supplementation without zinc and copper, full supplementation) for approximately 2

years. After slaughter, one-third of the experimental fish were subjected to chemical and physical analysis, another third were used for sensory analysis and the remaining fish were salt cured. Potassium, copper, and muscle protein were higher in muscle tissue of cod fed full supplementation than cod fed without supplementation. Instrumental color analysis showed that the fillets of cod fed full supplementation were slightly more green and yellow than fillets of cod fed without extra supplements. A sensory panel could, however, not detect any differences between heated fresh cod given feed with or without mineral supplements. However, the quality of salt ripened cod which had received a complete mineral supplement in the diet was reduced because of increased yellowness, probably caused by the increased level of copper in the muscle.

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Automated Quality Assurance in Industrial Fish Feed Pellets Production

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Intensive aquaculture production is expanding rapidly throughout the world and further development is highly dependent on secure supply of quality-warranted fish feed. Industrial quality parameters beside the nutritional of importance in extruded fish pellets are factors as; size, additive content, storage life and oxidative stability.

Quality assessment of non-nutritional parameters is at the present achieved by a variety of methods of which many are labor intensive and not necessarily accurate and robust. The aim of the presented study is to link vision technology and multivariate data analysis with measurements of chemical properties of non-nutritional quality parameters in industrial fish feed production. Laboratory experiments as well as experiments directly in the industrial process-line was conducted to prove the possible usage of vision technology.

Salmon fish pellets containing different astaxanthin concentrations were applied with controlled light and temperature conditions and monitored by vision analysis and sampled regularly for more than six months. The effects of light and temperature on weight, oxidative stability and astaxanthin content were chemically determined as reference for the image analysis. Additionally size and astaxanthin concentration were also investigated directly in the production line of extruded fish feed.

The experimental results proved vision technology and data analysis to be a fast, robust and reliable method to make assessment of non-nutritional quality parameters of extruded fish feed faster. Furthermore extruded fish feed containing astaxanthin was found to be interestingly stable even in a very harsh environment with constant light and high temperature.

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Profiling of Shrimp to Identify Source

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Interest has been growing among industry, consumers, and regulators to know, and verify, where seafood products are produced. Recent studies have shown that shrimp can reflect the mineral content of the waters from which they are harvested. The mineral profiles of wild caught Louisiana shrimp have been compared to the profiles of imported shrimp found in local grocery stores. These mineral profiles clearly allow us to

differentiate the sources of the Louisiana and imported shrimp. The differences in Louisiana shrimp are less clear but may offer some information on different harvest waters within Louisiana. We have also compared total cholesterol in the Louisiana shrimp and the levels are remarkably consistent. Limited profiling of fatty acid distribution may reflect location within the fishery.

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Post-Harvest Quality of Pond-Raised Shrimp (*Macrobrachium rosenbergii*) in the Mid-Atlantic States

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Many agricultural landowners in Southwest and Southside Virginia are converting their land, or a portion of their land, from traditional crops and animal production to shrimp production. Shrimp farming has significant economic potential especially in geographic regions that are close to major retail markets. Shrimp were obtained during the end of the growing season to determine product quality/safety and identify good manufacturing practices for the shrimp which can deteriorate quickly due to extrinsic and intrinsic spoilage.

Shrimp were harvested and: (1) immediately headed and stored at 0° and 4° C and (2) placed in ice slush for 4 hrs, headed, and stored at 0° and 4° C. The aerobic plate count (log₁₀) of shrimp headed immediately and stored at 0° increased from 4.9 to 5.6 by the 14th day of storage while the shrimp stored at 4° C increased to 7.7. Shrimp held on ice for 4 hrs, headed, and stored at 0° C increased from 3.9 to 3.8 by the 14th day of storage while the shrimp stored at 4° C increased to 5.3. The *Vibrio*

and *Aeromonads* counts were determined on the shrimp. The (log₁₀) of shrimp headed immediately and stored at 0° decreased from 3.8 to 3.5 by the 14th day of storage while the shrimp stored at 4° C increased to 5.7. Shrimp held on ice for 4 hrs, headed, and stored at 0° C decreased from 3.9 to 3.0 by the 14th day of storage while the shrimp stored at 4° C increased to 4.1. Less than 45 fecal coliforms/100 g of shrimp were isolated from every storage time and temperature. No *Salmonella* or *Listeria monocytogenes* was isolated from any sample. Bacteria isolates included: *Aeromonas hydrophila* (which was the predominate microorganism in the *Vibrio* medium and aerobic plate count), *Citrobacter braaaki*, *Edwardsiella tarda*, *Enterobacter cloacae*, *Plesiomonas shigelloides*, *Vibrio cholerae*. Some of the isolates are recognized as shrimp and human pathogens.

The effects of holding times and temperatures as determined by a sensory panel.

	<u>Appearance</u>		<u>Taste</u>		<u>Texture</u>	
	<u>Day 2</u>	<u>Day 11</u>	<u>Day 2</u>	<u>Day 11</u>	<u>Day 2</u>	<u>Day 11</u>
Immediately headed, stored 0°	6.8	7.3	6.4	6.4	6.3	6.7
Immediately headed, stored 4°	6.9	6.9	6.2	4.6	6.5	6.5
4 hrs hold, headed, stored 0°	6.8	7.0	4.5	6.3	5.8	6.4
4 hrs hold, headed, stored 4°	6.5	7.1	6.3	9.0	6.5	6.2

The rating used a scale of 1 to 9 with 50 panelists.

Compensatory Growth as a Means To Optimize Feed Utilization and Minimize Waste Production in Fish Aquaculture

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Aquaculture industry is searching for more efficient modes of production, including the reduction of operating costs, as well as environmental impacts. Feed management is a key point in the achievement of those objectives.

The concept of compensatory growth — i.e. accelerated growth following a period of starvation and concomitant slowed growth — has been tested in the brook trout *Salvelinus fontinalis*. Experimental groups have been subjected to a starvation period of 4 or 8 weeks to quantify the impact of fasting and then re-feeding on fish performance parameters: weight, growth, condition index, as well as key digestive and energy metabolism enzyme activities.

Starvation had significant negative impacts on individual mass and growth rate, regardless of the fasting period. The 8 weeks starvation period led to a decrease in hepatosomatic index, pyloric caeca

index and is trypsin, chymotrypsin and citrate synthase activities. Recovery of those physiological parameters was achieved following a re-feeding period of ca. 10 days for the 4 weeks starvation group and of ca. 20 days for the 8 weeks starvation group. Even if individual weight was not entirely recovered during the experimental re-feeding period (1 month only), re-feeding induced a marked stimulation of growth, expected to efficiently ensure recovery in body weight. This growth rate enhancement was much pronounced following the longest fasting period. Compensatory growth appeared to be a promising physiological/nutritional approach in fish production, as a potential strategy to optimize feed utilization by a reduction in required quantities, as well as a diminution of costs and amounts of uneaten feed and organic waste.

Poster 55

The Secret of the Extreme Longevity (Over 500 Years) of the Ocean Quahog

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Cellular aging is believed to be fostered by the oxidation of polyunsaturated fatty acids in biological membranes. The susceptibility to peroxidation of membrane lipids peroxidation index (PI) is negatively correlated with longevity in birds and mammals. Long-living marine molluscs are increasingly studied as longevity models, and the presence of different types of lipids in the membranes of these organisms raises questions on the existence of a PI-longevity relationship. We address this question by comparing the longest living metazoan species, the mud clam *Arctica islandica* (maximum reported longevity = 507 year) to four other sympatric bivalve molluscs greatly differing in longevity (28, 37, 92, and 106 year). We contrasted the acyl and alkenyl chain composition of phospholipids from the mitochondrial membranes of these species. The analysis was reproduced in

parallel for a mix of other cell membranes to investigate whether a different PI-longevity relationship would be found. The mitochondrial membrane PI was found to have an exponential decrease with increasing longevity among species and is significantly lower for *A. islandica*.

The PI of other cell membranes showed a linear decrease with increasing longevity among species and was also significantly lower for *A. islandica*. These results clearly demonstrate that the PI also decreases with increasing longevity in marine bivalves and that it decreases faster in the mitochondrial membrane than in other membranes in general. Furthermore, the particularly low PI values for *A. islandica* can partly explain this species' extreme longevity.

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Seafood Forensics: Development of a Handheld Sensor for the Real-Time Identification of Grouper

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Grouper are one of the most economically important seafood products in the state of Florida and their popularity as a high-end restaurant dish is increasing across the U.S. There is an increased incidence rate of the purposeful, fraudulent mislabeling of less costly and more readily available fish species as grouper in the U.S., particularly in Florida. Currently, the U.S. Food and Drug Administration recognize 56 species of fish that can use “grouper” as an acceptable market name for interstate commerce. This group of fish includes species from ten different genera, making accurate taxonomic identification difficult especially if distinguishing features such as skin, head, and tail have been removed. This is leading regulatory agencies, as well as members of the seafood wholesaler community, to employ genetic identification methods which tend to have much

higher species-level resolution than phenotypic methods. Standard genetic identification methods are highly technical and require expensive lab-based equipment to perform, which often leads to very long turnover times. We have developed a generic grouper assay that detects the majority of the grouper species listed on the 2011 FDA Seafood List, including all of the species found in Florida waters. This assay is based upon real-time nucleic acid sequence-based amplification (RT-NASBA) targeting 16S rRNA for the accurate detection of grouper. This assay can be performed in fewer than 90 min with little potential for cross-reactivity from non-target species. We have also developed a handheld, four-sample thermoregulated fluorometer to perform RT-NASBA in the field.

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