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ABSTRACTS

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Prospective Plasma Contribution to Fourth Agricultural Revolution

Masaharu Shiratani^{1,2}

1, Center of Plasma Nano-Interface Engineering, Kyushu University, Japan

2, Institute of Advanced Study

The third agricultural revolution or the Green Revolution is the period when agrichemicals had been intensively employed and genetically modified crops began to be used, leading to greater output of the products. In spite of the success, the Green Revolution has left our lands over-cultivated and over-used, making them unfit for use in the future and negatively impacting the ecological balance. Industrial nitrogen fixation, for instance, has increased exponentially since the 1940s, and human activity has doubled the amount of global nitrogen fixation [1]. In agricultural systems, fertilizers are used extensively to increase plant production, but unused nitrogen, usually in the form of nitrate, can leach out of the soil, enter streams and rivers, and ultimately make its way into our drinking water [2]. Such large changes in the availability of nitrogen can lead to severe alterations of the nitrogen cycle in both aquatic and terrestrial ecosystems. Hence, we need the fourth agricultural revolution, which supports the use of technology in order to realize sustainable farming. Plasma technology can contribute to solve such issues through the novel and effective ways of agricultural productivity enhancement in an ecofriendly manner [3-6].

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Everything You Need to Know to Make Sushi and Finding Fascinating

Japanese Foods Products

All-Japan Sushi Association, Director,
Masayoshi Kazato

sakaezushi8126@gmail.com

Today, with the world's population increasing and people becoming more health-conscious, there is a growing demand for seafood in countries around the globe. Raw seafood dishes, such as sashimi and sushi, from Japan's food culture are quickly gaining popularity in Europe, the US, and China, among other places. Japan's seafood quality is highly praised globally due to the careful handling of the catch and advanced cold-chain technology that ensures the fresh supply of safe and trustworthy raw seafood. As of 2013, more than 55,000 Japanese restaurants were open worldwide, and this number continues to grow year by year. However, in many countries where the food culture of eating raw fish has not taken root, sushi is often adapted to suit the local market. Unfortunately, accidents related to raw fish occur frequently, and restaurants that prioritize food safety still feel uneasy about this situation. The basic principle of sushi preparation is hygiene. To alleviate such concerns and ensure safe consumption of raw seafood, methods for making sushi rice to prevent bacteria growth caused by raw fish, techniques for preparing and processing fish (including marinading, salt-treating, vinegar-treating, blanching, searing, and washing), and fish preservation methods (such as refrigeration, freezing, thawing, and maintaining freshness) will be introduced.

Keywords: Sushi, Sashimi, Nigiri, gygiene, food safety

To boost rice productivity under a changing climate

Hiroyuki Shimono^{1,2},

1, Faculty of Agriculture, Iwate University

2, Agri-Innovation Center, Iwate University

Global food demand has been rising to support future population growth under changing climates. My research interest is to increase crop productivity (per unit land area) in response to environmental changes, genotype by environmental interaction, using methods of field phenotyping, mathematical simulation model, QTL and genome-wide association studies (GWAS) for mainly rice (*Oryza sativa* L.), a staple food for the more than half of the world's population. I want to share three topics of my current works, (1) Innovative technology: Early-winter direct-sowing cultivation of paddy rice, (2) Breeding new rice cultivars adapted to future elevated atmospheric CO₂, (3) Big data analysis for high yielding rice breeding.

Sustainable food production and supply is important issue in the direction of SDGs. I will introduce activities of Agri-Innovation Center, Iwate University, which was reinitiated from 2022.

Keywords: Atmospheric CO₂, Big data, Direct-seeding, Dormancy, Productivity, Rice, SDGs

Thermal Gelation Mechanisms of Fish Meat Paste - How Does Myosin Molecule Participate?

Shugo Watabe

School of Marine Bioscience, Kitasato University, 1-15-1 Kitasato, Minami, Sagami-hara, Kanagawa 252-0373, Japan

*Correspondence: swatabe@kitasato-u.ac.jp

We analyzed changes in the rheological properties of white croaker (*Pennahia argentata*) meat paste made from surimi added with 0.5 M NaCl during the following two-step heating procedure: first preheating at 0-70 °C with 5 or 10 °C intervals and subsequent secondary main heating at 85 °C. Changes in the breaking strength were hardly observed for both preheating and main-heating gels in the preheating temperature range of 0–30 °C. In the range of 35-45 °C, however, a marked increase was observed for the two gels with the increase of preheating temperature where the protein solubility in the SDS-urea solution and myosin heavy chain monomer content therein were rapidly decreased in a preheating temperature-dependent manner. Changes in viscoelastic properties such as storage modulus (G'), loss modulus (G'') and tangent delta (G'/G'') during preheating in the range of 35-45 °C and subsequent main heating were also markedly different from those during preheating in other temperature ranges and subsequent main heating. Interestingly, white croaker meat paste also showed prominent changes of viscoelastic properties at 35-45 °C in the temperature sweep analysis. We previously reported that the myosin molecule of white croaker is unfolded mainly at 34.8 and 44.1 °C due to the disruption of alpha helix and/or coiled-coil structure¹. The rod part of the myosin molecule has a unique coiled-coil structure with heptad repeats of amino acid residues where hydrophobic residues are buried inside the coiled-coil structure of two alpha-helices. Thus, it is likely that the unfolding of the myosin molecule at 35-45 °C, especially in the rod part, is important to rearrange myosin molecules in order to provide elastic thermal gels.

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Keywords: fish, myosin, surimi, thermal gelation, thermal unfolding

The Current State and Prospect of Recirculating Aquaculture Systems in China

○Ying Liu^{1*}

1, College of Biosystems Engineering and Food Science, Zhejiang University, 866 Yuhangtang Rd, Hangzhou 310058, P.R. China

*Corresponding author: liuyingzju@zju.edu.cn

Recirculating Aquaculture System (RAS) in China has developed rapidly in the past decade, and has also made great contributions to the provision of high-quality animal protein. This report covers the current status of RAS in China, developing opportunities of RAS, main production modes of RAS in China, technical level analyses of Chinese RAS, and perspective. It is hoped that this report will explore ways to upgrade the China's facility aquaculture engineering equipment to promote sustainable development of aquaculture, and provide a responsible development approach to world food security and economic growth.

Keywords: Recirculating Aquaculture System, Aquaculture engineering, Current state, Prospect

Studies on Measuring of Fruit and Vegetable Losses Throughout the Supply Chain and Awareness-Raising Campaign “Save Your Food” in Türkiye

Ruřan Özdemir Çifçi

Faculty of Agriculture, Ege University, 35100 İzmir, Türkiye
Ministry of Agriculture and Forestry Konak District Directorate, Umurbey Mah. İşçiler Cad.
No:139 Konak, İzmir, Türkiye
ruhsanozdemir@gmail.com, ruhsan.ozdemircifci@tarimorman.gov.tr

A study conducted between 2015-2017 on Measuring of Fruit and Vegetable Losses Throughout the Supply Chain in İzmir / Türkiye. The aim of the study was to determine the causes and extent of pre-harvest, harvest and post-harvest losses in agricultural production and to propose solutions that could be effective in reducing losses. Other purposes of the research are listed below.

- Identify the causes of food losses in the fruit and vegetable supply chain in the World and Türkiye,
- Determine the extent of food losses in the fruit and vegetable supply chain in the World and Türkiye.

After the studies for measuring food losses, the national “Save Your Food Campaign” is constructed. “Save Your Food Campaign” aims to create awareness and improve understanding of the causes and impacts of food losses and waste and to drive behavior change in individuals and communities in Türkiye. With this campaign, the Ministry of Agriculture and Forestry and FAO aims to combat food loss and waste both at the national and international levels, raise awareness of the issue, and make Türkiye a role model for efforts in this regard in the international arena.

Keywords: Food losses, food waste, fruit and vegetables, supply chain, save your food, Türkiye

Plant polyphenols as natural compounds to modulate muscle food quality attributes

Youling L. Xiong

University of Kentucky, USA
ylxiong@uky.edu

Polyphenol-rich herbs and spices are common food ingredients added in fish and meat processing to modify product flavor and appearance. When extracted plant phenolic compounds are mixed into minced muscle or proteins, their roles are beyond flavor and scavenging free radicals; they are found to modify product texture through the interaction with myosin or actomyosin. The ability of polyphenols to modulate gelation and emulsification of muscle protein, especially under oxidative conditions, explains their dose-dependent role in affecting the textural attributes of processed fish, poultry, and red meat products. In-depth studies have shown that phenolic compounds modify muscle proteins in a structure-dependent manner. For example, the variation in hydroxyl group distribution in flavonoid B-ring between mono-, di-, and tri-phenol compounds is an important factor affecting phenolic efficacy. The texture-modifying activity of plant phenolic compounds in muscle foods underscores their multi-functionality nature in fish and meat products.

Effective Utilization of Oyster Processing Byproduct

○Min-Jie Cao, Ling Weng, Yan Wang, Meng-Ya Ji, Qian Zhang

College of Ocean Food and Biological Engineering, Jimei University, Xiamen, 361021, China)

China is the major oyster production country in the world, with a production of 5.82 million tons in 2021. Shell is the major byproduct during oyster processing which occupies nearly 70% of the whole weight. Discarded oyster shell caused serious environmental problems and thus effective utilization of oyster shell is essential.

On the other hand, because of excessive application of chemical fertilizers and acid rain, soil acidification in China is quite serious, especially in southern east provinces. Acidified soil will cause low production and low quality of the agricultural products and impede sustainable development. Calcinated oyster shell mainly contains CaO, CaCO₃ as well as other metal ions and can be utilized as soil conditioner to neutralize acidified soil. We have applied this soil conditioner to more than 60 crops in 20 different counties in Fujian province. The results showed that application of oyster shell soil conditioner could significantly neutralize soil acidification, increase the content of soil organic matter and exchangeable calcium, and effectively improve the yield and quality of the crops while no side effect was observed.

Overview of Vietnam's aquatic sector during the period 2015-2022 and vision to 2045

Hanh Tran Thi My* and Duy Nguyen Xuan

Faculty of Food Technology, Nha Trang University, Khanh Hoa Province, Vietnam

*Corresponding author: myhanhtt@ntu.edu.vn

Vietnam is a country in Southeast Asia with a land area of 329,560 km² and a coastline of 3,260 km, an exclusive economic zone of 1 million km². In 2022, total fishery output was 9 million tonnes including 3.86 million tons from exploitation and 5.19 million tons from aquaculture. The export value of the fisheries sector in 2022 was \$11 billion. The workforce is more than four million people. Fisheries are defined as the country's leading economic sector, comprising about 4-5% of GDP, 9-10% of the country's total exports, ranking fifth in export value. In 2015 Vietnam's fisheries output increased 38% from 6.56 (2015) to 9.05 million tonnes (2022). Of these, aquaculture accounted for 57% and exploitation was 43%. Fisheries exports during 1998-2022 increased 13 times from \$817 million (1998) to \$11 billion (2022). The fisheries development objectives of the nation to 2030 and vision of 2045 are defined as follows: a 3.4-4% annual growth rate, total export output of 9.8 million tons, export value \$14-16 billion and employment of more than 3.5 million workers.

Keywords: Aquatic sector, export, Vietnam, vision

Rethinking on food system for sustainable world

○Noriyuki Tanaka^{1,2}

1, Food Society of Modern International Lifestyle Education(FSMILE)

2, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

**Corresponding author: norit22999@hotmail.com*

This presentation provides you a part of research outcome from coordinated study on bio-production (food production), ecosystems and humanity under the Integrated Research systems of Sustainability Science (IR3S), in which the presenter deeply involved. In my presentation,

Our environmental impact through food production, process, transportation, and consumption, is no longer trivial on earth. I try to convince you that food system science and education may play an important role for social and economical transformation of our society toward sustainability of the earth.

Keywords: Sustainability, Environment, Food System Science , Education

Food literacy behavior change toward post-pandemic society

○Kun Qian¹

1, Institute for Asian and Oceanian Studies, Kyushu University, Motooka 744, Nishi-ku, Fukuoka 819-0395, Japan

*Corresponding author: qk@kyudai.jp

The 2019 coronavirus (COVID-19) pandemic caused various changes in people's behaviors, particularly those related to eating and diet. Changes in eating behaviors caused by the pandemic have been investigated in diverse cultural and social contexts. In this presentation, the author will introduce several studies mainly conducted by his research team, focusing on the general food literacy behavior, including food waste, food consumption, cooking ability and preference. These studies were based on a series of longitudinal questionnaire surveys started from April, 2020. More than 30,000 samples were collected in these online surveys, from nationwide respondents in Japan. Based on the statistical analyses on and investigations into the dataset, we proposed a structural model of eating literacy behavior change during the pandemic. The model defined in our research provides informative suggestions for household food management during the COVID-19 pandemic, and similar situations in the future.

Keywords: SARS-CoV-2, cooking behavior, food waste, food consumption, stress, self-efficacy, communal eating

Functionality of natural pigments in food products

○Hayato Maeda *^{1,2}

1, Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan

2, United Graduate School of Agricultural Sciences-Iwate University, 3-18 Ueda, Morioka city, Iwate 020-8550, Japan

*Corresponding author: hayatosp@hirosaki-u.ac.jp

Foods contain various natural pigment components such as anthocyanins and carotenoids. These pigment components show various physiological functions such as anti-oxidation, anti-cancer, and anti-obesity activity. In this presentation, I introduce the functionality about new apple cultivars rich in anthocyanins and paprika rich in carotenoids.

Kurenai no Yume, literally “crimson dream”, is a red flesh apple cultivar developed by Hirosaki University. This apple is sweet, mildly tart, rich in anthocyanin, and delicious fresh or cooked. Its natural red color remains distinct even after cooking, making it excellent for the creation of richly colored apple products. It showed high anti-oxidant activity. Further, this apple extracts showed inhibitory effect of α -glucosidase and ameliorated blood glucose up-regulation in animal experiment¹⁾.

Paprika (*Capsicum annuum*) contain various carotenoids such as capsanthin, capsorubin, cryptocapsin cucurbitaxanthin A, β -cryptoxanthin, capsanthin epoxide, zeaxanthin, and β -carotene. Especially, capsanthin and capsorubin are characteristic carotenoid in paprika. They show strong anti-oxidative and anti-cancer promoting effects. Furthermore, these carotenoids show preventive effects of obesity related diseases^{2,3)}. Paprika carotenoids promoted adiponectin secretion by promoting differentiation of small size adipocyte cells. Moreover, Paprika carotenoids ameliorated chronic inflammation in obesity condition adipocyte cells.

Keywords: anthocyanin, carotenoid, apple, paprika, anti-obesity, diabetes, adipocyte

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Biological functions of phenethyl isothiocyanate for glucose and lipid metabolisms

Yoshiaki Ito^{1,2}, Moe Nagami¹, Maiko Chiba¹ and Takashi Nagasawa¹

¹*Biological Chemistry and Food Science, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan*

²*Agri-Innovation Center, Iwate University, Morioka, Iwate, Japan*

Keywords: phenethyl isothiocyanate, adipocyte, skeletal muscle, insulin resistance, Akt

Phenethyl isothiocyanate (PEITC), a natural dietary isothiocyanate, has been shown to have beneficial effects in improving cellular defense activities against oxidative stress through activation of nuclear factor erythroid-2 related factor 2 (Nrf2) pathway. However, little evidence exists if the antioxidative activity has beneficial effects on glucose metabolism. Here, we examined the preventive potential of PEITC for impaired insulin induced glucose uptake by oxidative stress in 3T3-L1 adipocytes. Treatment with PEITC increased the expression of antioxidative enzymes regulated by Nrf2 such as γ -glutamylcysteine-synthetase, heme oxygenase 1, NAD(P)H:quinone oxidoreductase 1 and glutathione S-transferase, and reduced oxidative stress induced by H₂O₂. Furthermore, PEITC restored impaired insulin-stimulated glucose uptake, translocation of glucose transporter 4 (Glut4) and insulin signaling by H₂O₂. These results indicate that PEITC protected insulin-regulated glucose metabolism impaired by oxidative stress through the antioxidative activity in 3T3-L1 adipocytes. Next, we tested whether PEITC directly promotes glucose utilization in mouse skeletal muscle cells, C2C12 myotubes. PEITC induced glucose uptake, Glut4 translocation to the plasma membrane, and activation of Akt and ERK in C2C12 cells. Inhibition of Akt suppressed PEITC induced Glut4 translocation and glucose uptake, whereas ERK inhibition did not. These results indicate that PEITC promotes glucose utilization through Akt pathway in C2C12 myotubes. Taken together, PEITC may serve as a dietary constituent with beneficial effects on the carbohydrate metabolism.

Anthocyanins food innovation: from laboratory bench to healthy dining table

De-Xing HOU^{1,2}

1, Department of Food Science and Biotechnology, Faculty of Agriculture,

2, The United Graduate School of Agricultural Sciences, Kagoshima University, Japan

*Corresponding author: hou@agri.kagoshima-u.ac.jp

Abstract

Anthocyanins are naturally occurring polyphenolic compounds that give the intense color to many fruits and vegetables such as berries, red grapes, red rice, purple sweetpotato and red cabbages. There are of great nutritional interest because their intakes have been estimated to be up to 9-fold higher than that of other dietary flavonoids. Thus, we have been working on chemical properties, bioactive functions and molecular mechanisms of anthocyanin-rich foods for almost two decades. In this talk, I will summarize some key results from our laboratory studies and our practices to innovate anthocyanin-rich foods for healthy dining table. First, we found that a clear chemical-bioactivity relationship is presented in kinds of anthocyanins. Thus, we clarified the quantity and quality of anthocyanins from a variety of fruits and vegetables, and provide chemical-bioactivity information to plant breeding scientists to breed fruit or vegetable that contains higher functional anthocyanins. Second, our animal experimental data demonstrated that anthocyanin-rich foods could ameliorate high fat diet-induced obesity, inflammation and dysbiosis of gut microbiome. Molecular analysis further revealed that active anthocyanins could upregulate Nrf2-mediated antioxidant signaling pathways and downregulated NFκB-mediated inflammatory pathways. These data provide the evidence and underlying mechanisms for the health-promoting properties of anthocyanin-rich foods. Third, we also observed that anthocyanins are easily oxidized and become pro-oxidants. The factors including light, pH and metals affect anthocyanin color and functions. Thus, we have notified these factors to keep anthocyanin color and functions in food processing. Our final goal is to make health-promoting properties of anthocyanin-rich foods into our dining table, thus, we are innovating the healthy menu from anthocyanin-rich foods to dining table, defined “KUROZEN”, basing on the scientific knowledges on chemical properties and functions of anthocyanins-rich foods, and nutrition-balance with other nutrients.

Keywords: Anthocyanin; Functions, Mechanisms; Processing, Healthy dining table

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Polysaccharides from brown algae: relationship between structure and function

○Yuya Kumagai*¹, Hiroki Kojima¹, Junpei BaBa¹, Hideki Kishimura¹

¹, Faculty of Fisheries Science, Hokkaido University, Minatocho 3-1-1, Hakodate, Hokkaido 041-8611, Japan

*Corresponding author: yuyakumagai@fish.hokudai.ac.jp

Kombu is an important ingredient used in the dashi soup of Washoku. Although the umami component is attracting attention as a feature of Kombu, it also contains fucoidan as a functional polysaccharide. Fucoidan is a sulfated polysaccharide with L-fucose as the main chain, and the structure differs sugar compositions, glycosidic bonds, and sulfate group content depending on the seaweed species and collection date. Fucoidan has been reported to have functions such as anti-inflammatory and antioxidant activity. Although the relationship between fucoidan structure and function has been pointed out, the detailed relationship remains unclear. In this study, we investigated the structural characteristics of Japanese Kombu (*Saccharina japonica*) depending on the collection date. Fucoidan was obtained from Kombu powder by hot water extraction. The supernatant was added calcium chloride to remove alginic acid, and the supernatant was lyophilized to obtain crude fucoidan after dialysis. Crude fucoidan was separated by anion-exchange column chromatography. Fucoidan structure was confirmed by FT-IR. The amount of crude fucoidan in Kombu from March to June was stable. Crude fucoidan was separated into three peaks by chromatography (P1, P2, and P3). Among them, the latest peak (P3) had a large number of sulfate groups compared to P2 and P1. We now plan to clarify the relationship between sulfate groups and functionality.

Keywords: Kombu, fucoidan, FT-IR, Polysaccharides

Make Your Idea a Cuisine: How Innovation is Made on Foods

○Weng Ling

College of Ocean Food and Biological Engineering, Jimei University, 43 Yindou Road,
Xiamen 361021, China

Email: weng1201@jmu.edu.cn

Foods are the most essential to our livelihood and shall not be defined to what satisfy taste buds, and quell hunger. Making foods is a nouveau life style and a fountain inspiring talents. How shall we make ordinary ingredients ascend into a masterpiece appealing to the mass, as well as being innovative to outcompete others in major events? This lecture aims to elaborate the winning design on their original idea, concept and points of innovation based on recent innovative food design competitions for university students held by Chinese Institute of Food Science and Technology (CIFST). Opening discussions on the topic of designing innovative foods are welcomed.

Keywords: Foods, Innovation, Food design competition, Chinese Institute of Food Science and Technology

Oxidation and reduction mechanisms of fish myoglobins

Yoshihiro Ochiai

Faculty of Agriculture, Tohoku University, Aramaki, Sendai, Miyagi 980-8572, Japan
E-mail: yochiai@tohoku.ac.jp

Myoglobin (Mb) is an oxygen-binding heme protein involved in intracellular oxygen storage and oxygen transport from the plasma membrane to the mitochondria. Mb forms various derivatives depending on its redox state. Especially, desirable bright red meat color is due to oxygenated form (oxyMb), while accumulation of oxidized form (metMb) results in browning or discoloration of meat. Due to the high contents of Mb in tuna meat as well as beef, undesirable discoloration proceeds very fast during postmortem storage. In addition, mitochondria involved in chemical energy production in live muscle could affect the redox state of Mb. In this presentation, molecular dynamics simulation of Mb oxidation mechanism will be discussed. The role of mitochondria in the reduction of metMb will also be referred to.

The simulation revealed the interaction between oxyMb and oxygen molecules. The number of water molecules in the heme pocket decreased under lower partial oxygen pressure, while higher oxygen pressure tended to reduce metMb formation. On the other hand, the mitochondrial fraction from tuna dark muscle reduced metMb. The involved enzyme is supposed to be cytochrome *b5* reductase located in the outer membrane of mitochondria. No significant change in the reduction activity was observed in the acidic pH range (5.7-6.8). NADH favored metMb reduction, while exhaustion of NADH resulted in metMb formation, suggesting that maintenance of NADH level is important for keeping the fresh meat color during chilled storage.

Keywords: myoglobin, oxidation, reduction, discoloration

Application of plant extracts as natural additives for the preservation of fishery products

○A. K. M. Azad Shah*

Department of Fisheries Technology, Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

*E-mail: azad@bsmrau.edu.bd

Fish and fishery products have become increasingly popular due to their high nutritional value owing to the presence of high-quality protein, n-3 polyunsaturated fatty acids, vitamins, and minerals. However, fish are recognized as being highly perishable, having a relatively short shelf life and being highly susceptible to enzymatic, microbiological, and chemical deterioration. To prevent these chemical deteriorations and delay the microbial growth in fish, various preservation methods are used. Among them, lowering the temperature of fish and using of synthetic preservatives are renowned; however, they do not completely inhibit the chemical deterioration and microbial growth in fish. Moreover, excessive use of synthetic preservatives has proven to be carcinogenic and potential health risks for humans. Therefore, there is an increasing interest in natural additives with potent antioxidant and antimicrobial properties to replace the synthetic preservatives in seafood preservation. Plant extracts with rich phenolic compounds from several plant sources, such as seaweed, guava leaves, water lily, and stevia leaves have demonstrated notable *in-vitro* antioxidant and antibacterial activity. Due to the presence of phenolic compounds, plant extracts retard bacterial growth as well as inhibit the formation of ammonia and other primary and secondary lipid oxidation products, which extends the shelf life, retains the quality, texture, and flavor of the fish fillets. Therefore, these plant extracts can be used as natural additives in the seafood industry for the preservation of fish and fishery products.

Keywords: Plant extract, Synthetic preservatives, Phenolic compounds, Antioxidant and antibacterial activity, Quality, Shelf life.

The study on marine natural product a new isoindolone FGFC1 for fibrinolysis

○Wenhui Wu¹, Chunli Gao¹, Haixing Zhang¹, Bin Bao^{*1, 2, 3}

1, Collage of Food Science and Technology, Shanghai Ocean University, Huchenghuan Road 999, Pudong New Area, Shanghai 201306, China

2, Marine Biomedical Science and Technology Innovation Platform of Lin-gang Special Area, Haiji Sixth Road 218-4, Shanghai 201306, China

3, Putuo Branch of International Combined Research Canter for Marine Biological Sciences, Zhongke Road 2, Putuo District, Zhoushan City 316104, Zhejiang Province, China

*Corresponding author: bbao@shou.edu.cn

Fungi fibrinolytic compound 1 (FGFC1) is a rare pyran-isoindolone derivative with fibrinolytic activity. The aim of this study was to further determine the effect of FGFC1 on fibrin clots lysis in vitro. We constructed a fibrinolytic system containing single-chain urokinase-type plasminogen activator (scu-PA) and plasminogen to measure the fibrinolytic activity of FGFC1 using the chromogenic substrate method. After FITC-fibrin was incubated with increasing concentrations of FGFC1, the changes in the fluorescence intensity and D-dimer in the lysate were measured using a fluorescence microplate reader. The fibrin clot structure induced by FGFC1 was observed and analysed using a scanning electron microscope and laser confocal microscope. We found that the chromogenic reaction rate of the mixture system increased from $(15.9 \pm 1.51) \times 10^{-3} \text{ min}^{-1}$ in the control group to $(29.7 \pm 1.25) \times 10^{-3} \text{ min}^{-1}$ for 12.8 μM FGFC1 ($p < 0.01$). FGFC1 also significantly increased the fluorescence intensity and D-dimer concentration in FITC fibrin lysate. Image analysis showed that FGFC1 significantly reduced the fiber density and increased the fiber diameter and the distance between protofibrils. The inhibition of fibrinolytic activity of FGFC1 by 6-aminohexanoic acid (EACA) and tranexamic acid (TXA) together with the docking results revealed that the lysine-binding sites (LBSs) play a crucial role in the process of FGFC1 binding to plasminogen. The action mechanism of FGFC1 binding to plasminogen was inferred, and FGFC1 was able to induce plasminogen to exhibit an open conformation by binding through the LBSs. The molecular docking results showed that docking of ligands (EACA, FGFC1) with receptors (KR₁–KR₅) mainly occurred through hydrophilic and hydrophobic interactions. In addition, the binding affinity values of EACA to KR₁–KR₅ were -5.2 , -4.3 , -3.7 , -4.5 , and -4.3 kcal/mol, respectively, and those of FGFC1 to KR₁–KR₅ were -7.4 , -9.0 , -6.3 , -8.3 , and -6.7 kcal/mol, respectively. The findings

demonstrate that both EACA and FGFC1 bound to KR₁–KR₅ with moderately high affinity. These results show that FGFC1 can effectively promote fibrin lysis in vitro and may represent a novel candidate agent for thrombolytic therapy.

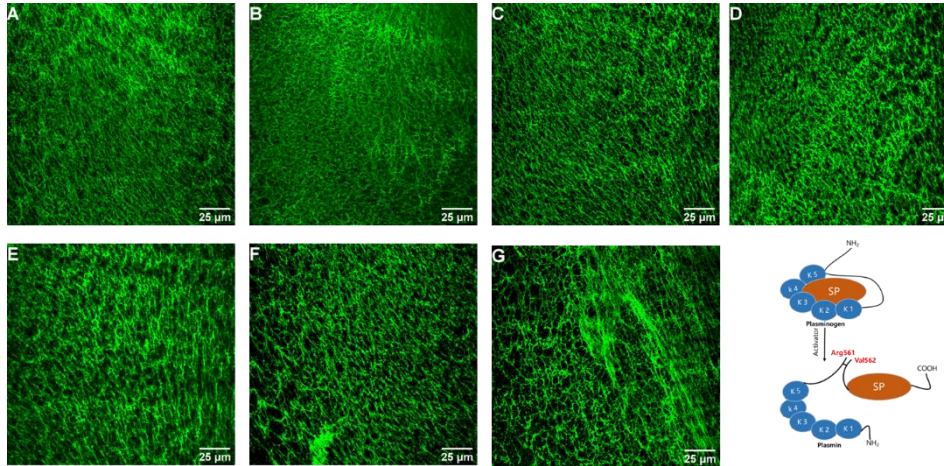


Fig. Confocal laser microscopy images of fibrin networks based on fibrinolysis by FGFC1

Keywords: Fibrinolytic compound, Marine natural product, Protofibrils, Molecular docking,

K -value, a freshness index of fish. Its application for modern demand

*Kunihiko Konno^{1,2}

1, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

2, Hokkaido University, Hakodate, Hokkaido 041-0843, Japan

*Corresponding author: konnokabuki2022@outlook.jp

One of the most important factors that determines the quality of fish is its freshness. Sensory methods are eventually used to judge the freshness. They are convenient but not quantitative. Among scientific freshness indices, the most popularly used one is K-value, which was proposed in 1960s and still valuable to show the freshness of fish. The value is based on ATP metabolism in fish muscle after its death.

ATP in fish muscle is sequentially degraded into much simpler compounds after its death. The degradation of IMP into HxR is the slowest among the steps, and the relative content of HxR and Hx derived from IMP is defined as K-value. As the IMP degradation is enzyme catalyzed reaction, the process is determined by the storage temperature and period, namely storage history. The index has been accepted by people. The reasons are;

1. A complete consumption of ATP leads "Rigor-mortis", traditional freshness index.
2. IMP gives synergistic effect with Soy sauce on the taste of fish meat (Sashimi).
3. K-value increase and freshness decrease judged by traditional methods fit very well.

Analysis technology was remarkably improved from ion exchange chromatography to HPLC. Although the separation mechanism of HPLC differs from original one, the sample preparation method designed for original method was used until now. The hardest part in the original method was pH adjustment to pH 7. As HPLC analysis does not require the sample to be pH 7, pH was adjusted to 3, where ATP was kept stable. Adjusting pH to 3 is simply achieved by adding a fixed volume of KOH solution. The simplified method makes it possible to analyze a large number of samples in short time even in night time by equipping autosampler.

K-value can be applicable not only to fresh fish but also to frozen stored and cooked seafood products. In the latter cases, the value gives the freshness of the raw material (fish) before the treatments.

Established K-value measurement protocol can guarantee the freshness of any seafoods distributed in the world scientifically.

Keywords: ATP, IMP, freshness, frozen fish, HPLC, Rigor-mortis,

Molecular interaction and properties characterization of anthocyanin-based film and its application in freshness monitoring and preservation

Yaqin Hu^{1*}

1, College of Food Science and Technology, Hainan Tropical Ocean University, Sanya, 572022

**Corresponding author: 1004346262@qq.com*

The increasing demand of food quality and safety has given rise to the intelligent and active packaging. The natural colorants with eco-friendly properties are integrated into packaging films. Among which, anthocyanins have been widely applied as the functional components due to their wide sources and multiple functions.

It is believed that various molecular interactions contained in the film matrix play the crucial role in the film properties. Hence, the kinds of molecular interactions between the bio-polymers and anthocyanins were studied based on our previous studies. Results demonstrated that molecular interactions could not only affect film physical properties by the formation of structural network, but also affect film functional properties (including pH-responsive, active and barrier characteristics) by controlling of anthocyanin release. Furthermore, the anthocyanin-based films with desirable functions were proved to show potent abilities in real-time freshness monitoring and efficient food preservation (such as better sensory profiles, lower TVB-N, TVC and k values, etc).

Our series of studies provided the basic insight into the molecular interactions, properties and applications of anthocyanin-based films. Nevertheless, further studies focusing on anthocyanin stability enhancement and preservation mechanism investigation of anthocyanin-based film are critically needed.

Keywords: Anthocyanin, Food packaging, Molecular interactions, Film properties, Real-time freshness monitoring, Food preservation

Changes in the K-value in several fishes and the benefits of applying K-value in the distribution of fisheries products

○Yuko Murata¹

1, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Fukuura, Kanazawa-ku 2-12-4, Yokohama, Kanagawa 236-8648, Japan

*Corresponding author: murata_yuko56@fra.go.jp

K-value is used as an index to determine the freshness of fish. It is especially suitable for determining the freshness of raw fish to be eaten such as sashimi and sushi. Our research so far determined that K-value is not only an indicator of freshness but may also be useful in quality control and determining the handling history of fish from catch to distribution. In this study, we present cases of the application of K-value in the distribution of fisheries products. In the case of red sea bream, *Pagrus major*, and rainbow trout, *Oncorhynchus mykiss*, it was found that the handling history of fish may be determined after the catch and during storage. It was also found that the time required to develop the best taste can be estimated in the case of yellowtail, *Seriola quinqueradiata*, and silver salmon, *Oncorhynchus kisutch*. The study finally presents the application of K-value from catch to consumption.

This research was supported by the research program on development of innovative technology grants from the Project of the Bio-oriented Technology Research Advancement Institution (BRAIN), and research and development projects for application in the new policy of Agriculture, Forestry and Fisheries from the Ministry of Agriculture, Forestry, and Fisheries, Japan. Also funded by Nippon Kaiji Kentei Kyokai.

Keywords: K-value, freshness, quality control, handling history, distribution, catch, storage

Validation of a simplified method for determination of K-value by interlaboratory study.

Takeya Yoshioka

Hokkaido Industrial Technology Center, Kikyo 379, Hakodate, Hokkaido 041-0801, Japan

**Corresponding author: yoshioka@techakodate.or.jp*

[Background] K-value is determined in many research institutes and testing laboratories as a fish freshness indicator. However, it was difficult to compare the K-value data from different laboratories because each laboratory used different determination procedure, reagents and apparatus. In addition, the conventional K-value measurement method was complicated and required skilled persons to perform accurate measurement. Therefore, in order to standardize the K-value determination method, simplification and validation were examined.

[Methods] To simplify the measurement method, the well-known conventional procedure was modified. An interlaboratory study to validate a simplified new method was designed based on the IUPAC protocol. As the test samples, frozen fish meat powder prepared from white muscle was packed in plastic bottles and distributed to each laboratory. The fish species selected considering white and red fish, natural and farmed fish, etc. According to a provided method, eleven laboratories quantified ATP-related compounds in the 10 test samples (5 pairs of blind duplicates) and calculated K-values.

[Results] The neutralization process of the perchloric acid extract was simplified by a two-step pH adjustment using alkaline solution and neutral phosphate buffer. In the interlaboratory study, specified statistical indices were calculated from K-value data reported from each interlaboratory. The discernible range of K-value ($4s_R$) calculated from the reproducibility standard deviation (s_R) was approximately 1% for high freshness samples (K-value 6-10%), approximately 2% for medium freshness samples (K-value 25-40%), and approximately 5% for low freshness samples (K-value 84%). These results were approved by a project committee composed of academic experts and others. Based on this method, a draft standard was submitted to the Ministry of Agriculture, Forestry and Fisheries (Japan), and "Test Method for Freshness (K-value) of Fish - High Performance Liquid Chromatography" was established as a Japanese Agricultural Standard in March 2022.

Keywords: K-value, standardization, interlaboratory study

Effect of Freshness before Freezing on the Quality of Thawed Meat in Japanese Pilchard Landed at Ports in Eastern Hokkaido

○Keisuke Moriya^{*1}, Akiko Miyazaki², Hiroyuki Kodama³, Koji Ebitani³

1, Food Processing Research Center, Hokkaido Research Organization, 589-4 Midori-machi, Ebetsu, Hokkaido, 069-0836, Japan

2, Abashiri Fisheries Research Institute, Hokkaido Research Organization, 7-8-5, Minato-cho, Mombetsu, Hokkaido 094-0011, Japan

3, Kushiro Fisheries Research Institute, Hokkaido Research Organization, 4-25, Nakahama-cho, Kushiro, Hokkaido 085-0027, Japan

*Corresponding author: moriya-keisuke@hro.or.jp

Fish with good freshness and quality for sashimi (sliced raw fish) are highly valued because Japanese consumers prefer sashimi. It is well known that freshness of Japanese pilchard (*Sardinops melanostictus*) that is migratory red-flesh fish deteriorates rapidly. The port in eastern Hokkaido is close to the fishing ground of Japanese pilchard, which is advantageous for maintaining the freshness of Japanese pilchard. The purpose of this study was to clarify the freshness of Japanese pilchard just after landing on eastern Hokkaido and the effect of ice storage period prior to freezing on the quality of thawed meat. Japanese pilchard which was landed at ports in eastern Hokkaido stored in ice for different periods (0, 5, 24 h) and then, these fishes were frozen in a liquid freezer. Each frozen pilchard was kept at -20 °C or -40 °C until analysis for 0 or 2 months and then, thawed for 15 h at 2 °C. Japanese pilchard just after landing was considered highly fresh due to K value of ≤ 4 % and some of which remained adenosine triphosphate (ATP). The K value of frozen meat of 5 h of ice storage after landing was about ≤ 5 %. The maximum strength as a physical property of the thawed meat decreased with ice storage period before freezing. The b^*/a^* value of thawed dark meat after keeping at -20 °C for 2 months with 0 h of ice storage, or after keeping -40 °C for 2 months with 0-24 h of ice storage were not changed during frozen storage. The pilchards, that were rapid-frozen just after landing or that were rapid-frozen within 5 h after landing and then stored at -40 °C, were suitable for consumption as an ingredient in frozen product for sashimi as they demonstrated good physical properties and insignificant color deterioration post-thawing.

Keywords: Freezing, Freshness, K value, Sashimi, Quality

Introduction to Standardization in ISO

Yoshiaki Yamano*

Standard Consulting Unit, Japan Standard Association, Mita MT Bldg., 3-13-12 Mita,
Minato-ku, Tokyo, 108-0073, Japan

**Corresponding author: yamano@jsa.or.jp*

This presentation is a general explanation of the standardization in ISO (International Organization for Standardization). International standards play an important role in world trade, import and export. They bring many kinds of benefits to our activities, such as food safety, environmental and quality management, etc. We also explain the acceptance criteria with a new work item proposal for the standardization in ISO.

Keywords: International standardization, ISO, Roles in world trade, Acceptance criteria,
New work item proposal

Optimisation of Virgin Coconut Oil Extraction of Natural Pigment from Microalgae by Response Surface Methodology (RSM)

○Eko Susanto*¹, Febriansyah Hanik Dwi saputri¹, Putut Har Riyadi¹, Lukita Purnamayati¹

1, Department of Fish Products Technology, Faculty of Fisheries and Marine Sciences, Universitas Diponegoro, Jl. Prof. Jacub Rais, Tembalang Semarang 50275

*Corresponding author: eko.susanto@live.undip.ac.id

The present study reports on the extraction of natural pigment from three different species of microalgae (*Arthrospira platensis*, *Nannochloropsis oculata*, and *Thalassiosira* sp). Response surface methodology (RSM) was used to investigate the effect of process variables on the extraction using virgin coconut oil (VCO). Two independent variables, temperature (50–60 °C) and extraction time (60–120 minutes), were investigated. According to the results, the optimal extraction condition with VCO were microalgae specific. For *Arthrospira platensis* and *Thalassiosira* sp, the ideal conditions were achieved at a temperature of 60 °C and an extraction period of 90 minutes; whereas, for *Nannochloropsis oculata*, the optimal conditions were achieved at a temperature of 59.83 °C for 88.09 minutes. The experimental results under ideal conditions were in good consistency with the predicted values, which showed that the extraction of pigments from microalgae using VCO will enhance the functionality of VCO itself and will be adaptable into products for functional foods.

Keywords: Virgin coconut oils, Microalgae, Carotenoids, Chlorophylls, RSM

Enhancement of anti-inflammatory activity of fish myofibrillar protein by Maillard-type glycation and its molecular mechanism

○Wenzhao Li¹, Ga-Hyun Joe¹, Yutaka Shimizu¹, Hiroki Saeki¹

Laboratory of Marine Food Science and Technology, Faculty of Fisheries Sciences, Hokkaido University, Minato 3, Hakodate, Hokkaido 041-8611, Japan

*lee19930206@eis.hokudai.ac.jp

[Objective] Myofibrillar protein (Mf) from spawned-out chum salmon gained strong anti-inflammatory activity *in vitro* and *in vivo* by the glycation with alginate oligosaccharide (AO) through the Maillard reaction. Considering that alginate oligosaccharide is a copolymer linked by two types of uronic acid possessing one carboxyl group in each molecule, the role of carboxyl group of the attached sugars in enhancing the anti-inflammatory activity of Mf was investigated in this study.

[Methods] The lyophilized Mf was glycated with monosaccharides and their oxidized derivatives (uronic acid) at 60 °C and 35% relative humidity through the Maillard reaction. The glycated Mfs were digested by pepsin-trypsin and evaluated the anti-inflammatory activity by measuring the secretions of inflammatory mediators in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. In addition, the effect on gene expression related to LPS-stimulated signaling pathways was also examined to discuss the molecular mechanism of the enhanced anti-inflammatory activity.

[Results] The anti-inflammatory activity of Mf was not affected by glucose and galactose, whereas carboxyl-containing glucuronic acid and galacturonic acid conferred strong anti-inflammatory activity upon addition to Mf, as the same as alginate oligosaccharide. These results indicate that the presence of carboxyl group in reducing sugar is an important factor enhancing the anti-inflammatory activity of Mf in the Maillard type glycation. In the gene expression analysis, the uronic acid-glycation suppressed LPS-stimulated inflammation by inhibiting the ability of CD14 recognizing LPS, thereby enhancing the suppressive effect of Mf in TLR4-MyD88-dependent inflammatory signaling pathway. In conclusion, the uronic acid-glycation, which modulates cell signaling and enhances anti-inflammatory function, would be a useful method for developing food-functional materials from the Mf.

Keywords: Fish myofibrillar protein, Anti-inflammatory activity, Maillard reaction, Uronic acid, Carboxyl group, Macrophage

Fresh-keeping effect of 3D printed surimi improved by Ca²⁺-nano starch-lutein and its freshness indicator mechanism based on printed anthocyanin

Gaoshang Li^{1,2}, Yaqin Hu² *

¹Institute of Food Engineering, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, Zhejiang, China, 310058

²College of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, PR China

*1004346262@qq.com

[Objective]

Protein-based surimi is suitable for 3D printing to produce customized foods. However, the insufficient bonding ability and weakened function (such as short shelf) become the weak points of 3D printed functional surimi foods. To facilitate its printing effects and fresh-keeping, a surimi added with a combination of Ca²⁺ and nano starch-lutein (NS-L) was used. Additionally, though the freshness of some materials changed during storage period, it could not be perceived due to the color of material itself. Hence, it is meaningful to research the printable freshness indicator materials.

[Methods]

The surimi was added to NS-L and Ca²⁺, printing properties (texture, gel strength, rheological properties) and freshness indexes of the materials were determined. Additionally, printable color-changing material--anthocyanin-hydroxypropyl methyl cellulose-xanthan gum-carrageenan (AHXK) was prepared. Its printing effects and discoloration mechanism (response to pH and ammonia) were determined. Furthermore, the real-time monitoring of freshness on printed surimi was explored.

[Results]

1. The NS-L and Ca²⁺ increased gel strength, hardness, τ , viscosity of surimi by 1.2-3 times, forming denser structure and strengthening supporting and bonding ability to enhance printing effects.
2. They also inhibited bacterial growth and spoilage bacteria of printed functional surimi, as well as amino acids decomposition of surimi was decreased by 12%, which limited the ATP decomposition (decreased K) and production of ammonia (decreased TVB-N).
3. The AHX with 5% K had suitable material properties (gel strength, texture, rheology) for printing.
4. The HXK with 0.75% A was sensitive to pH and ammonia, exhibiting significant color-changing on freshness monitoring of printed surimi during refrigeration.

[Conclusion]

Printed AHXK-functional surimi could achieve the aim of printing effect enhancement, fresh-keeping and freshness monitoring simultaneously, which could promote the application of 3D printing technology on food process.

Keywords: Printability; Functional surimi; Preservation; Color-changing; Freshness monitoring

Preservation and bacteriostasis functions of citral nanoemulsion on golden pomfret during 4 degrees C storage

Zhiheng Hu^{1,2}, Yaqin Hu^{*2}

1, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058

2, College of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022

*Corresponding author: 1004346262@qq.com

Golden pomfret is an economical marine fish specie which is easily prone to spoilage during process and storage. The spoilage in aquatic products is normally attributed to the oxidation, action of endogenous enzymes and the pollution of spoilage microorganisms. Hence, effective methods of preservation and bacteriostasis are vital for the fresh keeping of golden pomfret.

In our previous study, a citral nanoemulsion has been prepared, while its inhibition effects to *Shewanella putrefaciens*, one of the SSOs in golden pomfret, has also been clarified. In this study, the practical fresh keeping functions of citral nanoemulsion on golden pomfret were investigated. During storage, the citral nanoemulsion could significantly reduce the TVC value in fish. The SSOs of golden pomfret were mainly *Shewanella* sp. and *Pseudomonas* sp., among which *Shewanella putrefaciens* occupied the dominant status. The citral nanoemulsion could affect the distribution of microbial composition, which was mainly reflected by the stronger bacteriostasis on *Shewanella* sp.. Meanwhile, the nanoemulsion treatment effectively delayed the increase of freshness deterioration indexes such as TVB-N, TBARS and K value, following with higher texture parameters and sensory scores.

Overall, our study verified the ideal preservation and bacteriostasis functions of citral nanoemulsion on golden pomfret, the citral nanoemulsion could be applied as a potential fresh keeping agent in practical aquatic products.

Keywords: Golden pomfret, Citral nanoemulsion, Preservation, Bacteriostasis

Sensory Evaluation and Lipid Analysis of Marlin *Kajikia audax* for Sashimi

Kanako Hashimoto*¹, Tokifusa Kawashima², Yuko Murata¹, Takuya Seko¹, Shintaro Imamura¹, Kenji Ishihara¹

¹Fisheries Technology Institute, Japan Fisheries Research and Education Agency, 2-12-4 Fukuura, Kanazawa, Yokoyama, Kanagawa 236-8648, Japan

²Chiba Prefectural Fisheries Research Center, 2492 Hiraiso, Chikuramachi, Minamiboso, Chiba 295-0024, Japan

*Corresponding author: hashimoto_kanako05@fra.go.jp

Identifying the sensory properties of fish consumers is important for providing their preferred seafood. The sensory preference for fish is affected by odor, flavor, texture, and appearance. Moreover, several compounds are involved with sensory preference. In this study, we focused on fats that enhance the intensity of umami and examined the lipid content preference in the marlin *Kajikia audax* sashimi. First, we compared the lipid contents and fatty acid compositions of marlin by different individual size and body parts. Furthermore, the preference for total, taste, texture, odor, and lipid content in marlin sashimi taken from different parts of the body with different amounts of lipid was investigated by the paired-preference method. We also evaluated the relationship between the lipid content preference and total, taste, texture, and odor preferences by the chi-square test. The lipid contents in the bigger-sized marlin were higher than that in the small-sized. In bigger-sized marlin dorsal parts, the lipid contents in the anterior parts were the higher than that in the posterior parts. No differences were observed in the fatty acid composition of different size and body parts. The lipid content preference for the anterior dorsal part (high lipid part) was higher than that for the posterior dorsal part (low lipid part). The lipid content preference was associated with total and taste preferences. Therefore, the lipid content was found to be associated with the preference for marlin sashimi.

Keywords: sensory preference, sashimi, lipid content

Improvement Added Value of Fishery Byproduct as Halal Gelatin

○Mala Nurilmala*^{1,3}, Agoes M Jacobeb¹, Noviyan Darmawan^{2,3}, Tun Tedja Irawadi^{2,3},
Yoshihiro Ochiai⁴

1, Aquatic Product Technology Department, Faculty of Fisheries and Marine Sciences, IPB University, 16680, Bogor, Indonesia

2, Department of Chemistry, IPB University, 16680, Bogor, Indonesia

3, Halal Science Center, IPB University, 16129, Bogor, Indonesia

4, Graduate School of Agricultural Science, Tohoku University, Aramaki, Aoba, Sendai, Miyagi 981-8572, Japan

*Corresponding author: mnurilmala@apps.ipb.ac.id

Fishery industries leave waste such as heads, bones, skin, scales, swim bladder, and viscera. The amount of these byproducts is in the range of 30-50%. However, the utilization of them is still limited, then need efforts to increase its valorization. Recently, we have been developing gelatin from these byproducts to increase its added value and meet the requirement for 'halal' food as well as non-food such as cosmetics and pharmacy, since the common gelatin come from source of skin and bone of porcine. It has been known gelatin has been widely used as a multifunctional ingredient for many industries. Here, we use skin, scale, and swim bladder to produce halal gelatin by acidic method. The characteristic resulted gelatin were in accordance with those standard of Indonesian gelatin and GMIA (Gelatin Manufacturer Institute of America)

Keywords: Fishery byproducts, Gelatin, Halal, Valorization

Contribution of structural rearrangement and molecular interactions on quality improvement of frozen fish fillets

○Qingqing Jiang^{1*}, Shiyu Huang¹, Xichang Wang¹, Yaqin Hu², Emiko Okazaki³

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China

2, College of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, China

3, Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan

*Corresponding author: qqjiang@shou.edu.cn

The present study evaluated the effects and underlying mechanisms of light salting on quality properties of fish fillets during repeated freezing-thawing. Light salting was found to improve water-holding capacity and decelerated texture softening in fillets. Instead of tissue distortion and heterogeneous aggregates in control groups, light salting promoted myofibril disassembly and formation of an ordered protein network with the solubilized myofibrillar proteins. The myofibrils presented an overall amorphous appearance with the loss of M-lines, removing the restraints to myofibril swelling and solubilization from A-bands in salted groups during repeated freezing-thawing. The structural rearrangement caused by light salting facilitated the enlargement of water-holding space, transformation of tissue water, and tissue recoverability, improving water-holding capacity and texture properties of fillets during freezing-thawing. The finding provided novel insight into the improvement of quality properties of fish fillets by light salting when subjected to drastic temperature fluctuations.

Keywords: Structural rearrangement; Molecular interactions; Water transformation; Light salting; Freeze-thaw stability; Fish fillets

Involvement of Endogenous Proteases in Abalone Muscle Softening

○Chen Yulei^{1,2}, Zhang Minghui¹, Li Wanyu¹, Sun Lechang^{1,2}, Cao Minjie*^{1,2}

1, College of Ocean Food and Biological Engineering, Jimei University, 43 Yindou Road, Xiamen 361021, China

2, National & Local Joint Engineering Research Center of Deep Processing Technology for Aquatic Products, 43 Yindou Road, Xiamen 361021, China

*Corresponding author: mjcao@jmu.edu.cn

The extracellular matrix (ECM) is greatly degraded by endogenous proteases in aquatic animals after death, thus leading to muscle softening. We revealed collagenases to participate in postmortem tissue self-degradation of *Haliotis discus hannai* by degrading type I collagen. Hence, recombinant matrix metalloproteinase 1 and 14 (rMMP1 and rMMP14) with high purity and enzymatic activities were expressed using a prokaryotic expression system. rMMP1 and rMMP14 effectively degraded type I collagen into small fragments and peptides. Tissue inhibitor of metalloproteinase (TIMP), an endogenous inhibitor of MMP, was also expressed using HEK 293F cells. Recombinant TIMP (rTIMP) showed great inhibitory activity toward rMMP1, but not rMMP14. Hence, it can significantly inhibit rMMP1's degradation activity toward collagen. Inhibition kinetics analyses revealed rTIMP to be a competitive inhibitor of rMMP1. Biolayer interferometry revealed rTIMP can effectively bind with rMMP1, with an equilibrium dissociation constant value of 263 nM. Furthermore, we cloned the full-length cDNA sequence of prolyl endopeptidase (PEP) from abalone. Recombinant PEP (rPEP) was expressed and characterized in detail. We for the first time determined the 1.5 Å crystal structure of rPEP. Using collagen peptides as substrates, HPLC-ESI-MS analysis confirmed that rPEP specifically cleaved at the carboxyl side of proline residues, suggesting its role in the degradation of collagen peptides. These results elucidate the possible mechanism of abalone muscle softening in the aspect of endogenous proteases.

Keywords: Abalone, Muscle softening, Matrix metalloproteinase 1, Prolyl endopeptidase, Collagen degradation

Microstructure and physicochemical properties: Effects of different pretreatment combined with dual-frequency ultrasound on quality of large yellow croaker (*Pseudosciaena crocea*) during cold storage

Weiying LAN^{1,2,3} ○ Peiling YAN¹ Xinyu ZHAO¹ Jing XIE^{1,2,3*}

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China

2, Shanghai Aquatic Products Processing and Storage Engineering Technology Research Center, Shanghai 201306, China

3, National Experimental Teaching Demonstration Center for Food Science and Engineering (Shanghai Ocean University), Shanghai 201306, China

*Corresponding author: jxie@shou.edu.cn

Abstract: The quality changes of cold-stored (4 °C) large yellow croaker (*Pseudosciaena crocea*) were studied by using stable chlorine dioxide combined with dual-frequency ultrasound assisted-slightly acidic electrolyzed water (CUSS). The results of microbial indicators demonstrated that CUSS treatment inhibited microbial growth. Moreover, CUSS treatment had lower values of pH, total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA), and higher immobilized water content. CUSS treatment also inhibited the degradation of inosine monophosphate and the accumulation of hypoxanthine riboside and hypoxanthine. Through the results of texture profile analysis (TPA), protein degradation, and scanning electron microscopy (SEM), CUSS treatment could effectively protect the protein structure and maintain good texture characteristics of *Pseudosciaena crocea*. According to microbial indexes, K-value and sensory analysis, compared with dual-frequency ultrasound treatment, CUSS treatment could prolong the shelf-life of *Pseudosciaena crocea* for another 6 days. Therefore, CUSS treatment is a potential method to preserve *Pseudosciaena crocea* and improve its protein characteristics.

Key words: Stable chlorine dioxide, dual-frequency ultrasound, slightly acidic electrolytic water, *Pseudosciaena crocea*, quality

Condition-dependent Adenosine Monophosphate Decomposition Pathways by Endogenous Enzyme in Striated Adductor Muscle from Japanese scallop (*Patinopecten yessoensis*)

Huamao Wei¹, Wenge Yang¹, Tao Huang¹, Chunhong Yuan²

(1. College of Food and Pharmaceutical Sciences, Ningbo University, 315211; 2. Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan)

[Objective] The post-mortem ATP and related compounds have been extensively studied for quality evaluation of fish or shellfish for over 60 years. "K-value" is based on the ATP breakdown and the subsequent formation of inosine (HxR) and hypoxanthine (Hx). It is well-known that IMP is the main nucleotide present in most fish species such as mackerel, whereas AMP remains the major nucleotide in crustaceans and mollusks. It is controversial whether IMP in scallops generates. Therefore, the aim of this study was to investigate the AMP decomposition pathway in scallop adductor muscle and its influencing factors, such as EDTA or EGTA addition, heating, ions metal concentration change, et al.

[Methods] Crude enzyme solution was made from scallop striated muscle after chopping and homogenization at 4°C, and divided into three groups: control group, with EDTA or EGTA addition. Then, AMP, IMP and AdR were added into the crude enzyme solution as the substrate to detect the decomposition rate at 25°C. In addition, the crude enzyme solution was dialyzed to remove metal ions, and the deionized crude enzyme solution was divided into two groups: the control group (C) and the addition of 0.1% chloramphenicol (CP). Subsequently, AMP was added and changes in its ATP-related compounds was measured by HPLC at 25°C.

[Results] The results showed that IMP accumulated due to AMP decomposition via endogenous enzymes in scallops stored at both 4°C and 20°C. The AMP decomposition rate was highest in the supernatant of homogenized scallop adductor muscle, followed by the suspended solution and precipitate, while IMP could not be decomposed in scallop. AdR decomposed rapidly since high activity of AdR deaminase. The IMP generation rate increased dramatically in scallop crude enzyme solution containing 5 mM EDTA. Moreover, small amount of IMP also generated rapidly in scallop crude enzyme solution containing 5 mM EGTA within 2 h. Moreover, K⁺ was confirmed to promote the decomposition of AMP to IMP, Mg²⁺ promoted the decomposition of AMP to AdR, and Ca²⁺ slightly inhibit the decomposition of AMP. Meanwhile, the protein content and protein composition were also measure during dialysis and incubation. The results demonstrated that, the protein in the scallop enzyme solution of control group gradually denatured due to microbial activity during incubation, while CP group did not. Adenosine deaminase and adenosine kinase were detected in denatured protein fraction (40 kDa) by LC-MS/MS.

[Conclusion]. The decomposition path of AMP in scallops could be changed when concentration of metal ions change. In addition, the production of IMP in scallop was confirmed to be produced by endogenous enzymes via decomposition of AMP, while the generation of HxR and Hx is caused by endogenous enzymes and microbial activities.

Key words: AMP; endogenous enzyme; EDTA; IMP

Shape Analysis and Vitality Estimation of pacific oyster (*Crassostrea gigas*) Using Three-Dimensional Morphometric Techniques

○Xin Lu³, Shiliang Dong¹, Ryosuke Bizen², Sota Hayashi², Koichi Takaki^{3,4},

Kouichi Konno³, Kouichiro Kobayashi³, Katsuyuki Takahashi³, Chunhong Yuan^{2,4*}

1 United Graduate School of Agricultural Sciences, Iwate University,

2 Faculty of Agriculture, Iwate University

3 Faculty of Science and Engineering, Iwate University

4 Agri-Innovation Center, Iwate University

*Corresponding author: chyuan@iwate-u.ac.jp

Pacific oysters (*Crassostrea gigas*) are widely cultivated as an important marine fishery resource. Despite their production steadily rising over the years, their short survival and preservation period make the selection and maintenance of highly vital oysters a prime concern. While previous studies have explored the correlation between the shape of an oyster and its quality, this research proposes a novel, non-invasive, and immediate method for grading the freshness of Pacific oysters intended for brand sales. By exclusively applying 3D morphometric techniques to analyze oyster's external shapes, this method can replace the inconvenient but highly accurate chemical freshness assessment with a more streamlined approach..

In this study, the authors accurately describe the unique oyster shapes, including aspects like mean flattening ratio, the difference in this ratio at the front and back, the center axis curvature, and its orientation angle. Along with the shell height to length ratio, these five features form the external shape features to obtain detailed biochemical data about oyster vitality. They then use principal component analysis (PCA), a multi-variable technique, to simplify this five-dimensional feature space to one, and create a formula for classifying oysters as round or flat. Using this formula, they first separate the oysters into these two categories. Next, they arrange the oysters based on the A.E.C. value, a freshness and vitality indicator, separating the round (higher A.E.C.) and flat (lower A.E.C.) oysters again. Finally, they compare these classification results to calculate their accuracy.

The study used 90 oysters for the experiment, including 40 from Toyama-ken, purified in deep-sea water, and 50 from Yamada-machi, Iwate-ken. The proposed method accurately classified 70.0% of the round and flat Toyama oysters (20 each) and 76.0% of the round and flat Yamada Town oysters (25 each). As the two different species were collected at different attachment and growth periods in two major macaque production areas in different marine areas, the effectiveness of the classification method proposed in this study was demonstrated. The experimental results also prove that there are disparities in vitality maintenance between round and flat oysters defined by the proposed method. Applying this study in practical settings could enhance oyster branding and quality during distribution, such as at wholesale markets or during transportation.

Keywords: Pacific oyster, *Crassostrea gigas*, 3D morphometric techniques, vitality, shape features, flattening ratio, curvature, center axis, A.E.C. value, principal component analysis

Enzyme-assisted extraction and characterization of collagen from Basa fish (*Pangasius bocourti*) skin

○Quyen T.H. Tran*, Tang V. Nguyen, Thinh V. Phan

Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu, Nha Trang, Khanh Hoa 57000, Vietnam

*Corresponding author: quyentth@ntu.edu.vn

It's generally accepted that type I collagen is a fibrillar structure collagen, which plays an important role as the essential structural composition and mechanical scaffold of several tissues. The objective of this study is to extract type I collagen from Basa fish skin using acetic acid as the extraction solvent with the assistance of pepsin. The small pieces of Basa fish skin were immersed in 0.2 M sodium hydroxide solution under stirring in 24 h to eliminate lipids. After that, the skin was washed to neutral pH with distilled water. In the next step, the skin pieces were dipped and stirred in 0.003 M citric acid in 30 min to remove minerals. The skin pieces were immersed in 1% hydrogen peroxide solution for 3 h for colorant and odorant elimination. The collagen sample was obtained with 0.5 M acetic acid solution in addition to pepsin in 24 h. The extract filtration was performed through filter paper. The protein precipitation was done by adding dropwise sodium chloride solution in 24 h to the extract so that the final sodium chloride concentration was 2.5 M. The precipitate was collected after centrifugation at 4°C and 3000 rpm in 20 min. The extraction process was followed by dialysis in phosphate buffer with pH 7.8. Finally, the sample was lyophilized and stored in sealed containers until use at -20°C. The obtained collagen was characterized by quantitative and qualitative methods. The denaturation temperature of collagen samples was determined based on the change in viscosity of collagen solution as increasing temperature. The purity of collagen proteins was examined by ultraviolet-visible spectroscopy (UV-Vis). The molecular weight (Mw) of collagen subunits was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The amino acids composition was analysed by high performance liquid chromatography (HPLC). The surface morphology of collagen was observed by scanning electron microscope (SEM) images. The chemical groups were determined by fourier transform infrared spectroscopy (FTIR). The results showed that the collagen extraction yield was 520.5 mg/g of fresh fish skin on the basis of lyophilized dry weight. The denaturation temperature (T_d) was 34.8°C by measuring viscosity. UV-Vis spectrum with one peak at the wavelength of 230 nm confirmed the purity of the

collagen. Based on SDS-PAGE, Mw of collagen α_1 , α_2 , and β subunits were approximately 130, 118, and above 200 kDa, respectively. By HPLC, 17 proteinogenic amino acids were found in the collagen sample, in which the hydroxyproline content was 68.3 mg/g. SEM images confirmed the fibril structure of collagen. FTIR spectrum indicated characteristic bands according to the presence of amide A, B, I, II, and III bonds in collagen chemical structure. It is concluded that the preparation of collagen from Basa fish skin (*Pangasius bocourti*) was done. Therefore, the purified collagen obtained from this study can be further used in various fields of application.

Keywords: Basa fish skin, type I collagen, enzymatic extraction, proteinogenic amino acids, hydroxyproline

Factors influencing the nutritional composition, quality and safety of dried fishery products

OMd. Golam Rasul*¹, Chunhong Yuan², Kefeng Yu³, Koichi Takaki⁴, A.K.M. Azad Shah¹

1, Department of Fisheries Technology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur -1706, Bangladesh

2, Department of Food Production and Environmental Management, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Japan

3, Sanriku Fisheries Research Center, Iwate University, Heta 3-75-1, Kamaishi, Japan

4, Faculty of Science and Engineering, Iwate University, Ueda 4-3-5, Morioka, Japan

*Corresponding author: rasul@bsmrau.edu.bd

Dried fishery products are popular processed food items throughout the world. However, there is a frequent complain about the quality and safety of dried products. To reduce quality loss of dried fishery products, excellent quality raw materials with vacuum drying, oven drying, solar tunnel drying with different pre-treatments of chlorinated wash, bleeding, gutting, blanching, ultrasound, and treatment with various plant extracts have a broad potential application for the improvement of quality and safety of the dried products. Moreover, various chemical preservatives have also been found to be effective in increasing better organoleptic scores of the products although they have potential health hazards. Drying temperature and relative humidity should below 60°C and 10-43%, respectively to prevent loss of nutrients, quality decrease and consumer acceptability. Multilayer plastics, polyethylene films of high gauge, poly vinyl alcohol film containing 2% green tea extract along with vacuum packaging and oxygen scavenger packaging ensure the best quality protective effectiveness to prevent the dried fishery products from absorbing moisture and oxidizing of lipid. Recommended storage conditions for dried products are <60% relative humidity and temperature from 0-10°C; low oxygen levels of 0.5%. Low dose gamma irradiation (<5 kGy) reduces microbial loads, extends the shelf life and improves the taste and texture of dried fish. Ultraviolet light treatment, 10 mins cold oxygen plasma treatment, 3 mins atmospheric plasma treatment, corona discharge plasma (0-3 mins) and high hydrostatic pressure effectively inactivate the microbial contaminants from dried fishery products with better sensory properties. These findings will be helpful to develop an effective quality control for producing safe fishery products for domestic consumption and earning foreign currency.

Keywords: Dried fishery products, Factors, Nutritional composition, Quality, Safety

Effects of dry storage on mitochondrial structure and metabolic pathway of *Mizuhopecten yessoensis*

○Zhongzhuan Yin^{1,2}, Man Li¹, Yan Cai¹, Lin Qi, Chunhong Yuan³, Yuanyong Tian^{1*}

1, College of Food Science and Engineering, Dalian Ocean University, Dalian, 116023, Liaoning, China

2, Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7, Konan, Minato-ku, Tokyo 108-8477, Japan

3, Faculty of Agriculture, Iwate University, Morioka, 020-88550, Japan

*Corresponding author: tianyuanrong@foxmail.com

Postharvest dry storage was simulated by storing scallops at 4 °C for different periods. The quantification of 40 energy metabolites related to the pathways of glycolysis, tricarboxylic acid cycle (TCA) and oxidative phosphorylation in the scallop adductor muscle was performed by LC–MS/MS. The results showed that during the early stage, the TCA cycle was maintained at a low level. Meanwhile, the aspartate pathway and opine pathway were opened, and alanine and opines were the early metabolites during this stage. During the middle and late stages of dry storage, muscle pH decreased. Phosphoenolpyruvate carboxy kinase (PEPCK) was activated, leading to the competitive inhibition of pyruvate synthesis by PEPCK. Phosphoenolpyruvate enters the TCA reverse cycle via the phosphoenolpyruvate → malate pathway to produce succinate. Changes in metabolic pathways led to a change in the NADH level and mitochondrial membrane potential. The change of energy pathway is earlier than mitochondrial morphology change. The mitochondria just began to swell, the outer membrane was broken, and the matrix was cloudy after 12 h of dry storage. After 36h, mitochondria were basically completely broken. In summary, during the dry storage of scallops, the mitochondrial morphology was gradually destroyed, and the energy metabolism pathway was changed to maintain the ATP levels in the scallop adductor muscle.

Keywords: Dry storage; *Mizuhopecten yessoensis*; Energy metabolism, Mitochondrial morphology

Effects of stable chlorine dioxide combined with slightly acidic electrolyzed water on quality of large yellow croaker (*Pseudosciaen crocea*) during cold storage

Weiying LAN^{1,2,3}, ○Qi ZHOU¹, Xinyu ZHAO¹, Jing XIE^{*1,2,3}

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China

2, Shanghai Aquatic Products Processing and Storage Engineering Technology Research Center, Shanghai 201306, China

3, National Experimental Teaching Demonstration Center for Food Science and Engineering (Shanghai Ocean University), Shanghai 201306, China

*Corresponding author: jxie@shou.edu.cn

The quality changes of cold-stored large yellow croaker (*Pseudosciaena crocea*) were studied by using stable chlorine dioxide (ClO₂) combined with slightly acidic electrolyzed water (SAEW). The results of microbial indicators demonstrated that ClO₂ and SAEW treatment inhibited microbial growth. Moreover, ClO₂+ SAEW treatment had lower values of pH, total volatile basic nitrogen (TVB-N) and malondialdehyde (MDA) content, and higher water-holding capacity (WHC). Through the results of free amino acids (FAAs) and trimethylamine (TMA), ClO₂+ SAEW treatment could delay the degradation of umami amino acids and the accumulation of bitter amino acids of large yellow croaker. According to microbial indexes, sensory analysis and K-value, compared with sterile distilled water treatment, ClO₂+ SAEW treatment prolonged the shelf-life of large yellow croaker for another 4 days. Therefore, ClO₂+ SAEW treatment is a potential method to maintain the quality and flavor of large yellow croaker during cold storage.

Keywords: Stable chlorine dioxide, Slightly acidic electrolytic water, Large yellow croaker, Quality, Free amino acids

Insight into the antibacterial activity and mechanism of allicin and antioxidant of bamboo leaves against *Shewanella putrefaciens*

Weiying LAN^{1,2,3}, OZhe SHAO¹, Jintao DU¹, Jing XIE^{1,2,3,*}

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China

2, Shanghai Aquatic Products Processing and Storage Engineering Technology Research Center, Shanghai 201306, China

3, National Experimental Teaching Demonstration Center for Food Science and Engineering (Shanghai Ocean University), Shanghai 201306, China

*Corresponding author: jxie@shou.edu.cn

The antibacterial mechanism of allicin and antioxidant of bamboo leaves against *Shewanella putrefaciens* (*S. putrefaciens*) was studied. The inactivation effect of allicin combined with AOB against *S. putrefaciens* was evaluated by the growth curve. The changes in Na⁺ K⁺-ATPase activity, confocal laser scanning micrographs (CLSM), nucleic acid and protein release were measured to evaluate the inactivation mechanism. Scanning electron microscopy (SEM) was used to observe the morphology of bacterial cells. Crystal violet biofilm was reflected the ability to inhibit the formation of biofilm. The results showed that *S. putrefaciens* was effectively inactivated after single and combined treatments of allicin and AOB, and the inactivation effect of combined treatment was the best. The cell wall and cell membrane of bacteria were destroyed by allicin and AOB, resulting in the release of nucleic acid, protein. The results of Na⁺ K⁺-ATPase activity showed that allicin and AOB could change cell membrane permeability and destroy cell structural proteins. The results of SEM further proved the destructive effects of allicin and AOB on the bacterial morphology. Therefore, the combination of allicin and AOB can be used as a simple and effective method for the preservation of aquatic products.

Keywords: Allicin, AOB, antibacterial mechanism, *S.putrefaciens*

Effects of seaweed extracts on the quality and shelf life of Nile tilapia (*Oreochromis niloticus*) fillets during frozen storage

○Faria Afrin^{1,2}, Md. Monirul Islam², Md. Golam Rasul², Chunhong Yuan^{3,4}, A. K. M. Azad Shah^{*2}

1, United Graduate School of Agricultural Sciences, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

2, Department of Fisheries Technology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh

3, Department of Food Production and Environmental Management, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

4, Agri-Innovation Center, Iwate University, Morioka, Iwate 020-8550, Japan

*Corresponding author: azad@bsmrau.edu.bd

This study investigated the effects of seaweed (*Padina tetrastromatica*, *Sargassum muticum*, and *Spatoglossum asperum*) extracts on the quality and shelf life of Nile tilapia (*Oreochromis niloticus*) fillets during frozen storage for a period of 20 weeks. Fish fillets were dipped in seaweed ethanolic extracts solution (2%, w/v) separately for 10 min at 4°C. The control and seaweed extracts-treated fillets were stored at $-18\pm 1^\circ\text{C}$, and chemical, microbiological and sensory attributes were evaluated periodically. Results showed that *P. tetrastromatica* extract-treated fillets significantly ($P < 0.05$) delayed the increment of pH, total volatile basic-nitrogen, peroxide value, and thiobarbituric acid reactive substances in Nile tilapia fillets than other seaweed extracts-treated and control fillets. The aerobic plate count of fish fillets was within acceptable range during the storage period. Moreover, *P. tetrastromatica* extract-treated fillets displayed comparatively better sensory attributes than other seaweed extracts-treated fillets as well as control fillets. Results of this study indicated that ethanolic extract of *P. tetrastromatica* retains the quality and extends the shelf life of frozen Nile tilapia fillets for 8 weeks longer than control fillets. Thus, it can be concluded that *P. tetrastromatica* extracts can be used as a natural preservative for Nile tilapia fillets.

Keywords: Nile tilapia, Seaweed extracts, Shelf life, Sensory evaluation, Frozen storage

Screening the tyrosinase inhibitory activity of red algae extracts –applying in inhibition blackening of shrimp

Han The Nguyen, Quyen Le Vu, Huong Thi Thu Dang*

Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu, Nha Trang, Vietnam

*Corresponding author: dangthithuhuong@ntu.edu.vn

Tyrosinase (polyphenol oxidase -PPO) causes browning in fruits, vegetables, and blackening in seafood, especially shrimp. The activity of the tyrosinase can be inhibited by bioactive substances which can be found in red algae. In the present study, PPO inhibitory ability of the extract from 5 species of red algae (*Gelidiella acerosa*, *Gracilaria Salicornia*, *Acanthophora spicifera*, *Hypnea pannosa*, *Kappaphycus alvarezii*), collected in Nha Trang Vietnam were investigated. Screening was done under the same conditions for 5 species (methanol 80% for 60 minutes, at 60°C with material/solvent (1:20). The highest inhibitory ability of PPO was found in *Gelidiella acerosa* extract. The best extract was then fractioned through different polar solvents (n-hexane, ethyl acetate, butanol, distilled water) to determine the segment with the highest tyrosinase inhibitory ability. The optimum extraction conditions of *Gelidiella acerosa* were found in distilled water during 60 minutes at 60°C with material/solvent (1:40), fractioned through ethyl acetate. *Gelidiella acerosa* extract increased shelf-life of shrimp compared to the control sample.

Keywords: Red algae, extract, tyrosinase inhibitory ability, shrimp

Poster:

Extracting biomaterials from coconut shells and plant disease control applications

Duy Nguyen Xuan ^{1*}, Khoi Nguyen Le Ngoc² and Hanh Tran Thi My¹

¹Faculty of Food Technology, Nha Trang University, Khanh Hoa Province, Vietnam

²Faculty of Chemical Engineering, Vietnam National University Ho Chi Minh – University of Science, Ho Chi Minh city, Vietnam

*Corresponding authors: myhanhtt@ntu.edu.vn; duynx@ntu.edu.vn

The coconut is a popular tropical crop in Vietnam. The coconut can produce a wide variety of products. Coconut shells are considered a by-product of the processing of coconut products. The coconut shell contains many biologically active compounds. At present, however, they are not used properly and effectively. The study was carried out to obtain valuable substances from coconut shells and to find applications in the control of plant diseases. The pyrolytic method at the appropriate temperature is used to extract biologically active substances from the coconut shells. Results of the study indicate that extracts obtained from coconut shells have high antibacterial and antifungal activity. The typical class of substances found in extracts of the organic acid and phenolic groups. GC-MS analysis found that 17 groups of substances are phenolic derivatives. These are the principal substances that play an important role in the biology of the preparation. The results of early testing of preparations in the prevention and treatment of diseases on plants are quite good. These results open up the potential for using biological preparations obtained from coconut shells in controlling disease on plants.

Keywords: Biological preparations, coconut shells, clean agriculture, disease control

The water-soluble protein from walleye pollock attenuates obesity induced by high-fat, high-sucrose diet.

○Shu Shimada¹, Hiroki Saeki¹, and Ga-hyun Joe^{1*}

1, Laboratory of Marine Food Science and Technology, Faculty of Fisheries Sciences, Hokkaido University, Minato 3, Hakodate, Hokkaido 041-8611, Japan

*Corresponding author: Gahyun@fish.hokudai.ac.jp

[Objective] Obesity resulting from irregular eating habits induce chronic inflammation and various non-communicable diseases (NCDs). The waste effluent generated during surimi manufacturing process contains a large amount of water-soluble protein (WSP) from fish meat, but it is underutilized and mostly discarded. In this study, we examined the impact of WSP on metabolic syndrome associated with obesity, to develop advanced methods for utilizing WSP.

[Method] Waste effluent from surimi production was used to extract WSP. Frozen walleye pollack muscle was suspended in 50 mM NaCl and pressed with a screw-press to collect the effluent. WSP was then precipitated by shifting the pH from 6.8 to 4.0 to 7.0, and finally, collected by centrifugation. Male Wistar rats (aged 5 weeks) were fed with one of three types of diets for 65 days: (1) Control group (AIN-93G, 7% soybean oil, 10% sucrose and 20% casein), (2) HFHS group (7% soybean, 27% lard, 40% sucrose and 20% casein), and (3) HFHS + WSP group (7% soybean, 27% lard, 40% sucrose, 10% casein and 10% WSP). After the feeding experiment, plasma and tissues were collected and weighed. Also, lipid metabolism markers and adipokines (adiponectin and leptin) in the plasma and liver lipid content were measured.

[Results] There was no significant difference in total intake and total calories among the three groups during the experimental period. The HFHS group showed a significant increase in body weight compared to the control group, indicating the induction of obesity. On the other hand, WSP-ingested rats tended to lose weight 30 days after ingestion, and at the end of the experiment, the body weight was about 5% lower than that of the HFHS group. In addition, liver weight, adipose tissue weight, and plasma triglycerides tended to decrease in the WSP-fed group, while there was no difference between the groups in liver cholesterol, triglycerides, and free fatty acid concentrations. Interestingly, WSP intake improved the ratios of leptin and adiponectin, suggesting that the ingestion of WSP is involved in their secretion and improves obesity.

Keywords: Chronic inflammation, Obesity, water-soluble protein, metabolism.

Reduction of fish roe allergy risk in triploid female rainbow trout (*Oncorhynchus mykiss*).

○Saki Watanabe¹, Yutaka Shimizu¹, Shintaro Ishida¹, Yukiho Sasaoka², Takashi Todo¹,
Naoshi Hiramatsu¹, Takafumi Fujimoto¹, Gahyun Joe¹, Hiroki Saeki*¹

1, Laboratory of Marine Food Science and Technology, Faculty of Fisheries Sciences,
Hokkaido University, Minato 3, Hakodate, Hokkaido 041-8611, Japan

2, Hokkaido Research Organization, Fisheries Research Department Central Fisheries
Research Institute, Hamanaka 238, Yoichi, Hokkaido 046-8555, Japan

*Corresponding author: saeki@fish.hokudai.ac.jp

【Background and Purpose】

Fish roe is one of the most allergenic seafood, with high incidence of food allergy in Japan. Salmon roe is the primary causative agent of fish roe allergy and its labeling is recommended by the Japanese Food Labeling Act. The major allergen of fish roe is β' -component (β' -c), one of the yolk proteins. Vitellogenin (Vg), a precursor of the yolk proteins, also exhibits allergenicity similar to that of β' -c. Vg is synthesized in the liver of sexually mature female fish and is transferred to oocytes via the bloodstream. As a result, if such blood remains in the muscle, fish-roe-allergic patients are at risk of developing an allergic reaction to fish meat. Therefore, the purpose of this study is to identify types of salmonid fish that pose a lower risk of fish roe allergy in their meat. This study focused on triploid female rainbow trout (*Oncorhynchus mykiss*), which are commonly available on the market as sterile fish, and investigated their potential to cause fish roe allergy.

【Methods】

Twenty-two triploid female rainbow trout (TFRT 1-22) samples were sampled from two trout farms in Hokkaido and analyzed in this study. Muscle, gonad, liver and kidney of each fish were investigated as follows: TRFT 21 and 22 were excluded in (3).

(1)Vg concentration was measured by Sandwich ELISA using anti-rainbow trout β' -c rabbit IgG (anti-RTB), where the Vg concentration exceeds 0.25 μ g/g indicates a potential risk of causing fish roe allergy based on Japanese Food Labeling Act. (2)Cleavage state of Vg was analyzed using Immunoblotting (IB) with anti-RTB. (3)Development stage of oocytes was assessed by HE staining of gonad. (4)Polyploidy was measured by flow cytometry using liver as a sample.

【Results】

(1)In muscle, none of samples indicated a potential risk of causing fish roe allergy, but four out of twenty-two samples (TFRT 10, 13, 21 and 22) showed a potential risk in organs other than muscle. (2)Vg or its fragment were detected in 3 samples (TFRT 13, 21 and 22). (3)Gonads of 3 samples (TFRT 9, 10 and 13) showed developed oocytes at oil drop stage or yolk globule stage, which could also be observed visually. (4)Two samples (TFRT 13 and 21) were diploid, while all other samples were triploid.

【Conclusion】

Fish meat of triploid female rainbow trout can be eaten with a low risk of causing fish roe allergy, even though some samples may contain Vg due to triploidization failure or other reasons. However, the risk can be further minimized by visually inspecting the gonad development and eliminating samples with developed gonads.

Keywords: fish roe allergy, β' -component, vitellogenin, triploid, rainbow trout

The enhancement effects of roselle anthocyanin-based film on shrimp texture by water distribution controlling and protein structure maintenance

Jiayin Huang^{1,2}, Yaqin Hu^{2*}

1, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310058

2, College of Food Science and Technology, Hainan Tropical Ocean University, Sanya, 572022

*Corresponding author: 1004346262@qq.com

The perishable properties of food products and emerging eco-friendly requirements have driven the development of biodegradable active packaging. Several studies have confirmed the excellent preservation behaviors of active films by determination of primary biochemical parameters, such as pH, TVB-N and TVC. However, investigating some other inner quality traits are of vital importance to understand its preservative mechanism.

In this study, the enhancement effects of polyvinyl alcohol/hydroxypropyl methylcellulose/roselle anthocyanin-based (PHR) film on textural profiles of *Penaeus vannamei* were studied. Results demonstrated that shrimp packaged by PHR film exhibited better texture with higher hardness, springiness, chewiness and resilience values. The PHR films exerted positive influences on water migration by reducing drip loss and promoting conversion of water state. Meanwhile, the PHR film delayed protein denaturation through increasing myofibrillar protein and sulfhydryl group contents, decreasing trichloroacetic acid-soluble peptide contents and maintaining protein secondary structure. Furthermore, the correlation analysis revealed the close relationship among texture, water and protein profiles, indicating the better texture characteristics were resulted from the positive influences of PHR film exerted on the water state and protein structure of shrimp.

Overall, our study proved that the PHR film was a novel potential active packaging for shrimp texture modification.

Keywords: Active film, Textural profiles, Water migration, Protein conformation, Preservation mechanism

Involvement of MMP-9 in collagen degradation of sea bass (*Lateolabrax japonicus*): cloning, expression and characterization

○Ru-Qing Yang¹, Yu-Lei Chen^{1,2}, Le-Chang Sun^{1,2}, Wei Ou^{1,2}, Hai-Yan Liu^{1,2},
Ling-Jing Zhang^{1,2}, Min-Jie Cao^{1,2*}

1, College of Ocean Food and Biological Engineering, Jimei University, Xiamen, 361021, China

2, Collaborative Innovation Center of Marine Food Deep Processing, Dalian Polytechnic University, Dalian, 116034, China

*Corresponding author: mjcao@jmu.edu.cn

Disintegration of intramuscular connective tissue is responsible for post-mortem tenderization of fish muscle during chilled storage. Matrix metalloproteinase-9 (MMP-9) was reported to be involved in this process, while the mechanism has not been identified. In the present study, purified type I and V collagens from the connective tissue of fish muscle were firstly prepared. These two kinds of collagens comprise three polypeptide chains (α), forming a typical triple helical domain as determined by circular dichroism. The complete coding region of MMP-9 containing an ORF of 2070 bp encoding 689 amino acid residues was then cloned. The recombinant MMP-9 catalytic domain (rcMMP-9) was expressed in *E. coli* and exhibited high hydrolyzing activity toward gelatin. rcMMP-9 degradation to type V collagen was more effective than that to type I collagen at 4 °C. The enzymatic activity of rcMMP-9 was highly pH-dependent, and its enzymatic activity under neutral and basic conditions were higher than that under acidic conditions. Metal ion Ca²⁺ was necessary for the maintenance of rcMMP-9 activity, whereas Zn²⁺ inhibited its activity. Our present study indicated that MMP-9 is responsible for the disintegration of intramuscular connective tissues by cleaving type V collagen during post-mortem tenderization of fish muscle.

Keywords: *Lateolabrax japonicus*, collagen, matrix metalloproteinase-9, enzymatic activity, softening

Expression of bioactive polyphenol oxidase of *Litopenaeus vannamei* and its characterization

○Qian Hong¹, Yu-Lei Chen¹, Duanquan Lin¹, Ling-Jing Zhang¹, Kai-Yuan Cao², Yi-Ming Liu¹, Ling Weng¹, Le-Chang Sun¹, Min-Jie Cao^{1*}

1, College of Ocean Food and Biological Engineering, Jimei University, Xiamen, China, 361021

2, Department of Biological Science, National University of Singapore, Singapore, 117558

*Corresponding author: mjcao@jmu.edu.cn

Polyphenol oxidase (PPO) is an enzyme that plays a critical role in decrement of shrimp quality. Purification of native PPO is challenging because of its low content and unstability. To obtain active PPO and elucidate its enzymatic properties, PPO from *Litopenaeus vannamei* (Lv-PPO) was cloned, expressed by constructing an *Escherichia coli* prokaryotic expression system and then purified by affinity column chromatography. The Lv-PPO gene was 2076 bp in length and encoded 691 amino acid residues with deduced molecular weight of 78.8 kDa. The recombinant Lv-PPO (rLv-PPO) with a molecular weight of ~85.0 kDa was partially dissolved while mainly in inclusion body, and its sequence was verified by LC-MS/MS. rLv-PPO was biologically active with an optimal temperature of 40°C and an optimal pH of 6.0. Its denaturation temperature was $54.2 \pm 0.7^\circ\text{C}$ as measured by circular dichroism. Metal ions such as Cu^{2+} and Zn^{2+} altered the activity of rLv-PPO by influencing its secondary and tertiary structures. rLv-PPO showed catalytic activity towards L-Dopa and catechol. Its enzymatic characteristics were studied by kinetic analysis. A specific polyclonal antibody against rLv-PPO was prepared. Western blot analysis revealed that PPO levels were highest in hemolymph, followed by telson, carapace, and eyestalk. A trivial amount of PPO was identified in muscle while it could not be detected in hepatopancreas and midgut. Our present study expressed a biological active rLv-PPO for the first time. A specific polyclonal antibody against rLv-PPO was prepared for the prospect of assisting future studies on the enzymatic properties of PPO, and exploring its action mechanism in shrimp melanosis.

Keywords : polyphenol oxidase, *Litopenaeus vannamei*, melanosis, protein expression, enzymatic property, polyclonal antibody

Properties of Pacific white shrimp (*Litopenaeus vannamei*) collagen and its degradation by endogenous proteinases during cold storage

○Xu-Chen Xiao^{1#}, Duanquan Lin^{1#}, Kai-Yuan Cao², Le-Chang Sun¹, Yu-Lei Chen¹, Ling Weng¹, Ling-Jing Zhang¹, Min-Jie Cao^{1*}

1, College of Ocean Food and Biological Engineering, Jimei University, Xiamen, China, 361021

2, Department of Biological Science, National University of Singapore, Singapore, 117558

[#]These two authors contributed equally to this manuscript.

**Corresponding author: mjcao@jmu.edu.cn*

Many factors are responsible for the diminished quality of shrimp during cold storage, while the role of collagen has rarely been studied. This study therefore investigated the relationship between collagen degradation and changes of textural properties of Pacific white shrimp, and its hydrolysis by endogenous proteinases. The textural properties of shrimp decreased gradually along with disruption of shrimp muscle tissues, and the chewiness property of shrimp muscle showed a linear relationship with collagen contents in muscle during 6-day-storage at 4°C. Pepsin-solubilized collagen in shrimp muscle consisted of one $\alpha 1$ chain and two $\alpha 2$ chains, revealing a typical tripeptide sequence (i.e., Gly-X-Y) in their molecules. In addition, collagen could be hydrolyzed by crude endogenous proteinases extracted from shrimp hepatopancreas, and serine proteinase plays a critical role in the process. These findings strongly suggested that the quality reduction of shrimp during cold storage is closely associated with collagen degradation.

Keywords: collagen degradation; endogenous proteinase; shrimp preservation; cold storage; quality degradation.

Flash Boil-Shucking of Yesso Scallop (*Mizuhopecten yessoensis*) and Its Effect on Biochemical, Sensorial and Ultrastructural Properties of Adductor Muscle During Refrigeration

○Yabin Niu¹, Shiliang Dong¹, Nobuyoshi Wada², Huamao Wei³, Tetsuro Yamashita², Koichi Takaki^{4,5}, Chunhong Yuan^{2,5*}

1 United Graduate School of Agricultural Sciences, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

2 Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

3 College of Food and Pharmaceutical Sciences, Ningbo University, Ningbo, Zhejiang 315211, China

4 Faculty of Science and Engineering, Iwate University, Ueda 4-3-5, Morioka, Iwate 020-8551, Japan

5 Agri-Innovation Center, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan.

*Corresponding author:chyuan@iwate-u.ac.jp

Scallop adductor muscle has great popularity for raw consumption in Japan. However, the traditional method of shucking live scallops with a knife requires skill and is time-consuming. Sushi restaurants have adopted an alternative technique known as instantaneous boiling, which offers a convenient shucking method for live scallops. However, it is not clear about the effects of this treatment on the quality of its main edible part, adductor muscle. The objective of this study was to examine the effects of instantaneous boiling on the quality of raw-edible scallop adductor muscle, with a specific focus on flavor-enhancing components and ultrastructure. It was observed that the thermal treatment employed during instantaneous boiling contributed to the formation of flavor-enhancing nucleotides primarily in the external layer of the adductor muscle. Adenosine monophosphate content increased with the boiling time, which significantly increased to $1.47 \pm 0.42 \mu\text{mol/g}$ in the 30 s-boiled group compared with $0.38 \pm 0.13 \mu\text{mol/g}$ in the control group ($p < 0.05$). The rapid adenosine monophosphate accumulation after boiling, leads to a more rapid accumulation of inosine and hypoxanthine during subsequent refrigeration, which could bring bitterness to aquatic products. Despite the possibility of a decrease in total free amino acids, the proportion of free amino acids relevant to the flavor of scallop adductor muscle remained largely unaffected by instantaneous boiling. Instantaneous boiling has been found to potentially induce a hardening effect on the external layer of scallop adductor muscle. At the ultrastructural level, the muscle fibers demonstrate a tendency to aggregate transversely and contract longitudinally, with the extent of these changes depending on the duration of heating. Based on sensory evaluation, the 30 s-boiled samples were most suitable for immediate consumption, otherwise boiling for 8 seconds has been suggested as an alternative.

Keywords: Shellfish, Shucking, Ultrastructure, Flavor components, Sensory evaluation

Exploring Wakame Recipe Information and Cross-Region Food culture Analysis for seaweed marketing

○Natsumi Shimoyama¹, Kazuma Sugawara², Chunhong Yuan³

1, Fishery Innovation Program, Division of Regional Innovation and Management, Graduate School of Arts and Sciences, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

2, Marine Chemical Resource Development, Faculty of Fisheries Sciences, Hokkaido University, Hakodate 041-8611, Hokkaido, Japan

3, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

*Corresponding author: chyuan@iwate-u.ac.jp

Iwate Prefecture is one of the leading producers of wakame seaweed in Japan, but the production volume has been declining in recent years due to the low unit price. In order to improve the market value of high quality wakame, there is possibility to develop a marketing strategy for export which is rarely done at present. However, wakame is mainly consumed in Japan and South Korea, and many countries do not have a food culture. In this study, we analyzed the existing seaweed recipes from the global recipe service, and investigated how seaweed is eaten and consumer preference correlated to its food culture of each country for expanding overseas markets of seaweed.

The recipe dataset (n=494,963) provided to Kaggle in 2017 by Yummly, a global online cooking recipe service was used. The data were categorized according to, (1) regions, (2) search words, (3) frequency of use of ingredients and seasonings, and (4) types of dishes included in the dish names for seaweed recipes. Using R, we classified words such as ingredient names and regions included in each variable, and judged how seaweed was cooked from the frequency.

As a result of extracting data containing “wakame seaweed” in the variable “ingredient” from the dataset, 73 cases (0.015%) were obtained. From this, it turned out that seaweed dishes are rarely posted on English-speaking recipe sites. On the other hand, there were 281 cases on “seaweed”, which include another species. (1) We analyzed the area and other search words from the variable "search tag". As a result, “Japanese” was the most common area with 35 search tags, followed by “Chinese” with 3, “Korean” with 2, “Cajun”, “Irish” and “French” with 1. (2) The most frequently searched words were “vegetarian” in 28 cases, “dinner” in 26 cases, “soup” in 25 cases, “vegan” in 23 cases, and "salad" in 21 cases. It can see the keywords that are easy for users to search for seaweed recipes. This result shows that there are many tags indicating that it is a Japanese-style dish. Since there are many words such as vegetarian in the tag, it is thought that wakame recipes is targeting vegetarians. (3) As a result of examining the frequency of use of each ingredient and seasoning from the variable “ingredients”, the food ingredients were “water (30 cases)”, “onion” (26 cases), “garlic” (16 cases), “carrot” (13 cases), “green onion” (13 cases). Seasonings: “soy sauce” & “shoyu” (42 cases), “salt” (28 cases), “pepper” (25 cases). It is thought that the frequency increased because water is often used as a soup. And Few combinations with meat or fish. (4) As a result of analyzing the type of recipe from the variable "name", there were 25 "soup" recipes, 15 of which used miso. There were 22 cases for “salad” and 7 cases for “noodle”.

Based on these results, recipes using wakame seaweed are limited on English recipe sites, and it is thought that the development of recipes that match the taste of each country has not progressed. There are many search tags with vegetables and recipes for vegetarians, and it is considered that the current wakame is for people who like vegetables. To next step, we would like to conduct the same analysis on recipe information in formats other than English and clarify the differences in recipe information for each country.

Keywords: Wakame seaweed, Recipe, Data analysis, Food culture

Efect of " uzumaki process " on post mortem quality changes in cultured red sea bream at 4 °C

○Koma Nakahata¹, Keisuke Katsura², Xin Lu³, Chunhong Yuan^{*1}.

1. Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan
2. Japan Uzumaki Association, 726 Hachijima-cho, Nishi-ku, Kumamoto, 860-0057
3. Faculty of Science and Engineering, Iwate University, Ueda 4-3-5, Morioka, Iwate 020-8551, Japan

*Corresponding author:chyuan@iwate-u.ac.jp

In Japan, freshness preservation by Spinal cord destruction is widely practiced when wild or farmed live fish are landed. This slaughtering technique involves the destruction of both the brain and spinal cord, but it requires a high level of skill to perform.

To address this challenge, the Uzumaki process was developed. This method involves using a tube to suction the spinal cord out of the brain and is considered more reliable than traditional spinal cord destruction. In our study, we analyzed the postmortem changes in ATP, pH, rigor index, and impedance in red sea bream, a popular Japanese marine product.

Ten live cultured red sea bream were transported to lab and stored at 4°C for 72 hours. Four of the fish underwent spinal cord destruction while the remaining six were processed using the Uzumaki method. One half of each group was used to measure ATP and pH levels, while the other half was used for rigor index and impedance measurements and SEM image observation. HPLC (Asahipak GS-320HQ, 25°C, 0.6 mL/min) was used to measure ATP-related compounds, and a Fish Analyzer (Yamato seikou, DFA100) was used to measure impedance values.

The results revealed that the onset of rigor mortis was delayed by approximately 10 hours in the Uzumaki process group compared to the spinal cord destruction group. Additionally, the decrease in ATP content was slower in the Uzumaki process group, indicating a slower progression of postmortem rigor. While the pH decreased to 6.3 and 6.4 in both groups, there was no obvious difference observed. SEM images showed muscle contraction and cell membrane degradation, which could be associated with impedance fluctuations. Based on these findings, the Uzumaki process was deemed more effective in preserving the freshness of the fish compared to spinal cord destruction. However, it is necessary to further investigate the relationship between freshness retention and spinal cord removal rate in future studies.

Keywords: red sea bream, freshness, Spinal cord destruction, bioimpedance, ATP related compounds

Investigation of evaluation index of Wakame by Measuring Physical Properties and Analyzing Functional Components.

○Tomoya Suzuki¹, Kazuma Sugawara¹, Lu Xin²,
Gouki Mikawa¹, Chunhong Yuan¹

1. Faculty of Agriculture, Iwate University

2. Faculty of Science and Engineering, Iwate University

Objective: The market has introduced a system to certify wakame of the first grade landed in Iwate Prefecture. However, the grading of wakame is based on the findings of field staff based on their many years of experience. There are very few examples of wakame grading based on quantitative methods, so recently there has been a need to introduce an evaluation method that is more objective and quicker through mechanization and numerical methods. In order to develop a grading system for wakame seaweed using deep learning by correlating image data with the results of biochemical analysis, we conducted physical property measurements and biochemical analysis on wakame seaweed from several production areas and examined effective indices for grading evaluation.

Methods: Salted and frozen wakame from Iwate and Miyagi prefectures were used for the analysis. 1 x 3 cm pieces of wakame were cut and used for tensile strength measurements (rheometer, speed 300 mm/min). SPAD and L*a*b* values were measured to determine color-tone. Lyophilized powdered wakame seaweed was used for component analysis. The Folin-Ciocalteu method was used to determine total polyphenol concentration. Fucoxanthin was extracted by the same method as polyphenols, and its content was calculated by measuring the absorbance value at 449 nm.

Results: Correlation analysis of the measured indices showed a significant correlation between SPAD and b* values, SPAD is a simple measure of chlorophyll content and Lab is a value indicating the optical properties of the object. Since both indicators are related to color, it was suggested that they could be correlated with image data. Significant regional differences were observed in the correlation between optical properties and fucoxanthin concentration. In addition, there are some differences in correlations due to different trends in component content and physical properties depending on the region of production, so it is necessary to form an evaluation system that takes regional characteristics of wakame into account.

Keywords: Wakame, Seaweed, Food technology, Iwate prefecture

Effect of short-term rearing on changes in ATP related compounds and glycogen content during storage at -1°C

Sota Hayashi¹

1, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

*Corresponding author: g0123041@iwate-u.ac.jp

Short-term rearing is carried out with the aim of reducing fishing stress at the time of landing, thereby reducing the loss of vitality of the product by the fishery product. Among bivalves, there is a report that confirms the effect of rearing scallops, but there is little knowledge about oysters. In this study, we examined the effect of short-term rearing on oysters (*Crassostrea gigas*). Rearing oysters and non-reared oysters were stored in a refrigerator at -1°C for 14 days, and it were experimented on storage days 0, 1, 3, 7, and 14. ATP-related compounds, arginine phosphate, and glycogen were extracted, and changes in vitality over time during storage were examined. The ATP content was clearly higher in the 3 conditions with rearing oyster than without rearing on the 0th to 1st days of storage. The A.E.C value was higher for the oysters that were reared on the 0th day of storage. Compared to the arginine phosphate content in scallops, the arginine phosphate content was much lower, and we were unable to find a relationship with changes in ATP content over time. Glycogen was measured in this experiment as an indirect indicator of vitality, given that ATP is produced by glycogen. No relationship was found with ATP-related compounds or arginine phosphate, and no relationship with vitality could be found. It was suggested that short-term rearing might reduce the decrease in vitality of oysters on the 0th day of storage from the results of ATP-related compounds.

Keywords: oyster, short-term rearing, ATP-related, glycogen, A.E.C. value, vitality, syorage

Detection of ATP related compounds for judging Anisakis death killed by liquid nitrogen freezing

○Yuya Ota¹

¹Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

Anisakis is a dangerous parasite that poses a major problem when eating marine products raw in Japan, and is counted individually in food poisoning statistics. Furthermore, with the addition of Japanese food to the list of intangible cultural heritage, there is a possibility that the incidence of anisakiasis will increase as opportunities to eat raw fish increase worldwide. For this reason, guidelines have been established in various countries. For example, in the US FDA, Anisakis can be killed by freezing at -20°C or below for 7 days, freezing to -35°C and storing it at that temperature for 15 hours, or - It indicates that 24 hours of storage at 20°C or below is necessary but not suitable for large fish (e.g. fleshy fish over 6 inches). If the guidelines are followed, long-term frozen storage is required, and depending on the fish species, the quality will drop all at once.

Therefore, if Anisakis can be killed instantaneously by freezing liquid nitrogen, denaturation due to freezing will be less and it will be possible to eat something closer to the final taste and texture. However, there are few studies on the death of Anisakis. In previous research, the emphasis was placed on visual inspection to determine death, and there is also a method that uses malachite green to stain dead or injured individuals, but the result is that the injured part There is no certainty because it is not dyed. Therefore, this time, we compared Anisakis from ATP-related compounds under various conditions and verified whether there was a difference in vitality.

Anisakis was harvested from donko and squid. Freezing was frozen at -70°C for 3, 6, and 15 minutes using a liquid nitrogen freezer. As a control, ATP-related compounds were also extracted from unfrozen Anisakis.

As a result, only ATP and ADP were detected in Anisakis without freezing. In addition to ATP and ADP, AMP was also detected in 3 and 15 min freezing. However, after freezing for 6 minutes, the percentage of ATP decreased, but AMP was not detected.

From these results, it was found that there was a difference in vitality between those that were frozen and those that were not. However, the fact that the ratio of the 6 min individuals to the unfrozen specimens was closer to that of the other frozen specimens may be due to the difference in the vitality of Anisakis before freezing.

Therefore, it was suggested that the difference in the vitality of Anisakis could be observed by analyzing the components of ATP-related compounds. In the future, by establishing a method for extracting ATP-related compounds from Anisakis, it is expected that ATP-related compounds will be used as indicators of vitality in Anisakis and as a method for determining life and death.

Keywords: Anisakis, liquid nitrogen freezing,

Effect of ultrasound in conjunction with bromelain on the tenderness of *Litopenaeus Vannamei*

Xinyi Zhou¹, Lanxiang Su¹, Huamao Wei¹, Wenge Yang¹, Tao Huang¹, Chunhong Yuan²

(1. College of Food and Pharmaceutical Sciences, Ningbo University, 315211; 2. Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan)

[Objective] *Penaeus vannamei* is one of the most valuable aquaculture commodities globally due to its high nutritional and economic value. In China, the aquaculture output of *Penaeus vannamei* has shown an upward trend in the past three years, reaching 1.98 million tons in 2021. In recent years, shrimp can be processed into dried, frozen, boiled, and fried shrimp products. Among them, dried shrimps are more and more popular with consumers, because it is easy to carry, eat and store, as well as have unique flavor. However, the meat is accompanied by physical and chemical changes during the heating process, such as protein aggregation, severe oxidation, cross-linking, or increased disulfide (S-S) content, which increase the firmness of the meat and decrease the susceptibility of proteins during gastrointestinal digestion. Previous studies have found that sonication or enzymatic treatment can improve digestibility by increasing tenderness. To our knowledge, numerous publications are following closely in the poultry meat industry, but few have focused on the effect of ultrasound combined with enzyme treatments on aquatic products. So, this study aimed to investigate the combined effect of ultrasound with bromelain on the tenderization of dried shrimp and analyze the possible mechanism of tenderization.

[Methods] The samples were immersed in a bromelain solution (concentration 10 to 30 U/g with 2% salt, sample-to-solution ratio 1:1 (w/v)). At the same time, the packed meat was sonicated for 5 minutes (power 50-250 W, temperature 30-70 °C). During sonication, the temperature point was maintained by adjusting the temperature of the sonicator. After ultrasonic treatment, the meat was spread evenly on a wire mesh and dried using a hot air dryer. Drying is carried out at 100°C until the moisture content reaches 50%. After drying, the meat was subjected to textural analysis (TPA), shear force, and microstructural analysis. Changes in biochemical properties of raw shrimp without drying were determined by measuring SDS-PAGE, MFI, and active sulfhydryl content.

[Results] A single-factor experiment was used to determine the optimal processing conditions for the tenderization of dried shrimp meat. The result showed that the hardness and shear force of samples were decreased sharply and that the dried shrimp become redder in color. The optimal treatment conditions to achieve dried shrimp meat with minimum firmness and shear force were obtained: power of 100 W, temperature of 50°C, and bromelain concentration of 20 U/g. Under these conditions, the predicted value of the MFI was highest among the examined groups. The bromelain has the ability to destroy myofibril protein and connective tissue into smaller peptides and amino acids. Whereas ultrasound treatment not only facilitates the penetration of the enzyme, but ultrasound itself has the effect of disrupting cell membranes and basic structures. The combination of ultrasound and enzymes causes protein degradation in shrimp meat. In addition, the tissue cells exhibited severe damage, with fractures and large spacing within loose muscle fibers. From among the treatments explored throughout this study, the combined ultrasound and bromelain yielded the best tenderness results, especially when an ultrasound radiation of 100 W was applied at 50 °C and 20 U/g concentration of bromelain. Therefore, it can be concluded that the combined treatment with ultrasonic radiation and bromelain could be a promising technique for the production of dried shrimp meat and may have a high potential for application in the meat industry.

Keywords: *Penaeus vannamei*, tenderness, bromelain

Effect of protease treatment on the quality of Pacific oysters during refrigeration

Lanxiang Su¹, Xinyi Zhou¹, Huamao Wei¹, Wenge Yang¹, Tao Huang¹, Chunhong Yuan²

(1. College of Food and Pharmaceutical Sciences, Ningbo University, 315211; 2. Faculty of Agriculture, Iwate

University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan)

[Objective] Pacific oysters (*Crassostrea gigas*) are one of the main economic fish species in the East China Sea region and their popularity is mainly due to their flavor and freshness. Currently, Pacific oysters are usually distributed as shucked meat and their high moisture content tends to cause the freshness of the product to deteriorate rapidly. The content of ATP-related compounds in oysters varied with freshness, with AMP and IMP contributing to flavor. Consumers demand for fresher, more flavorful oyster products is increasing daily. Therefore, the enhancement of oyster flavor during the preservation process has become a major research priority. Many studies have shown that neutral proteases are beneficial in increasing peak flavor nucleotide (IMP) levels in the short term. However, the introduction of enzymatic hydrolysis techniques has accelerated the autolysis process in oysters, ultimately leading to a reduction in shelf life. Therefore, it is essential to investigate the shelf life of enzyme-treated oysters.

[Methods] Fresh Pacific oysters were bought from the aquatic market, shucked and immersed in diluted water containing 0.5% neutral protease (w/v 1:2) for 1.5 h. The control group was immersed directly in diluted water. After washing with sterile saline, the oysters were packed in sealed bags containing 3.5% sterilized saline and stored at 4 °C for 7 days. Biochemical parameters (glycogen, pH, ATP-associated compounds, TVB-N and total viable count) were measured on days 0, 1, 3, 5 and 7 for the four parts of the mantle, adductor muscle, gills and body trunk, respectively, as well as for the whole oyster.

[Results] AMP and IMP content in mantle, adductor muscle, gill and body trunk increased after protease treatment. During refrigeration, all four fractions of the enzymatic hydrolysis group exhibited higher levels of IMP. The pH of each group decreased with increasing refrigeration time, with the enzymatic hydrolysis (EH) group showing a more pronounced downward trend, probably due to the accelerated autolysis process by neutral proteases. Glycogen content decreased in the mantle and adductor muscle but did not change significantly in the gill and body trunk. In terms of TVB-N value, those of the control group and EH group increased from 5.85 mg/100 g and 5.37 mg/100 g to 10.19 mg/100 g and 12.74 mg/100 g, respectively. On day 5, the total viable count reached 10 mg/100 g in the control and enzymatic hydrolysis groups. The overall results indicated a minimum shelf life of 3 days for the muscle of Pacific oysters (*Crassostrea gigas*) treated under experimental conditions, with increasing levels of AMP and IMP.

Keyword: *Crassostrea gigas*, neutral proteases, AMP, IMP