ABSTRACTS

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Contents

Combined effects of ultrasound and slightly acidic electrolyzed water on quality of sea bass (*Lateolabrax Japonicus*) fillets during refrigerated storage

Weiqing LAN[1,2,3] Ai LANG[1] Dapeng ZHOU[1] 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
* *jxie@shou.edu.cn*

[Objective]

The aim of present study was to investigate the combined effectiveness of ultrasound (US) and slightly acidic electrolyzed water (SAEW) on inactivating bacteria and maintaining the quality of sea bass (*Lateolabrax Japonicus*) fillets.

[Methods]

Fillets were divided into four groups: (1) samples were immersed in distilled water for 10 min (CK); (2) samples were immersed in the distilled water with ultrasound treatment (20 kHz, 600 W) for 10 min (US); (3) samples were immersed in SAEW (pH 6.35, oxidation reduction potential (ORP) of 861.6 mV and available chlorine concentration (ACC) of 30.0 mg/L) for 10 min (SAEW); (4) samples were immersed in SAEW with US treatment for 10 min (US+SAEW). Then, they were put in polyethylene bags and stored at 4 °C. Different indexes, such as microbial (total viable count (TVC), *Pseudomonas* bacteria counts and H_2S-producing bacteria counts), physicochemical (texture profile analysis (TPA), color difference, pH value, total volatile basic nitrogen (TVB-N), K value, Thiobarbituric acid (TBA), Intrinsic Fluorescence Intensity (IFI)), combined with sensory evaluation, were analyzed at 2-days interval for 14 days.

[Results]

The results confirmed that US+ SAEW treatment could retard the increase of TVC, *Pseudomonas* bacteria counts and H_2S-producing bacteria counts, which also inhibit the rise of TVB-N, TBA, pH and K value. In addition, compared with SAEW or US treatment alone, US+SAEW treatment had greatly effects on inhibiting protein degradation, maintaining better texture and sensory scores. When compared with CK group, the shelf-life of sea bass treated with US+SAEW was increased for another 4 days at least.

[Conclusion]

The combined treatment of US and SAEW may provide useful tools for sea bass on the microbial deactivation and quality improvement, which could enhance food safety and optimize the food processing in food industries.

Keywords: Ultrasound; slightly acidic electrolyzed water; *Lateolabrax Japonicus*; refrigerated storage

Allergenicity Change of Shrimp Tropomyosin During Production of *Terasi,* Indonesian Fermented Shrimp Paste

Amalia Ulfah*[1,2], Yutaka Shimizu[1], and 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, Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Central Java 50275, Indonesia
* *ulfah.amalia@live.undip.ac.id*

[Objective]

It is known that crustaceans, including shrimp and crab, are the most common causative foods that cause marine allergies. *Terasi* is a naturally fermented small shrimp paste and a popular food ingredient in Indonesia. In order to evaluate *terasi* as a low allergenic food, changes in behaviour of tropomyosin (TM: major allergen in shrimp) and its IgG/IgE-binding abilities were investigated during the manufacturing process.

[Methods]

Terasi was made from three kinds of small Japanese shrimp, *Akiami (Acetes japonicus), Okiami (Euphasia pacifica)* and *Isazami (Neomysis awatschensis)* by the conventional method consisting salt-curing and three-times repeating dry-fermentation steps. Protein degradation and the IgE-binding ability in TM during the manufacturing process were examined by SDS-PAGE and immunoblotting (IB) using anti-TM rabbit IgG and by competitive ELISA (C-ELISA) using crustacean-allergic patients' sera, respectively.

[Results]

Products of *Akiami and Isazami terasis* were matched the Indonesian quality standard based on moisture, water activity and protein contents. However, regardless of the quality differences, TM in the shrimps were degraded to small-digested peptides during *terasi* manufacturing process, and the specific IgG response to TM in IB disappeared markedly at the second fermentation step. The loss of the IgE-binding ability measured by C-ELISA was observed in all *terasi*, whereas a marked decrease in the allergenicity was found in *Akiami terasi* in which the effective protein degradation occurred.

[Conlusion]

In conclusion, fermentation process, the major proteolytic stage in the *terasi* manufacturing process is an important factor to reduce allergenicity caused by shrimp TM. Therefore, it is necessary to clarify an appropriate raw material and an optimum fermentation condition in order to ensure the reduction of allergenicity in *Terasi.*

Keywords: allergenicity, fermentation, low allergenic food, shrimp, *terasi*, tropomyosin.

Dynamically optical and highly stable pNIPAM @ Au NRs substrate for sensitive SERS detection of malachite green in fish

Bingxue Hu, Da-Wen Sun*, Hongbin Pu, Qingyi Wei

School of Food Science and Engineering, South China University of Technology, Guangzhou 510641, China.
**dawen.sun@ucd.ie*

[Objective]

Malachite green (MG) is an illegal fungicide in aquaculture that has been restricted or banned in many countries because of its serious health risks. However, because of its low cost and high efficacy, MG is still being abused in aquaculture. Variety of techniques have been proposed for MG detection. However, most of them are associated with a number of constraints, such as high cost, complicated pre-treatment process. Hence, it is urgently necessary to develop promising method for rapid and sensitive MG inspection. As a finger-print vibrational spectroscopy, surface enhanced Raman scattering (SERS) exhibits great potentials in varies fields. However, the instability or aggregation tendency of noble metal nanoparticles colloids are serious issues that hinder their widespread application. Herein, Au NRs were combined with temperature-sensitive material pNIPAM to tune their optical and plasmon nature through external stimulate, and to avoid their self-aggregation when in storage.

[Methods]

A universal SERS substrate pNIPAM @ Au NRs with tuneable plasmonic behaviour and long-term stability was synthesized using seed-mediated method, and layer-by-layer self-assembly method; then characterized using transmission electron microscopy (TEM) and field-emitting scanning electron microscope (FESEM), and ZetasizerNano ZS system. The tuneable thermo-responsive optical properties, photo-thermal properties, temperature-sensitive SERS, and SERRS properties were systematically analyzed using Vis-NIR spectroscopy, Vis and NIR laser lines, and Raman spectroscopy. The SERS performance evaluation of the pNIPAM @ Au NRs composites in food safety inspection was performed, especially for the detection of the banned fungicide MG in fish tissues.

[Results]

1. PNIPAM @ Au NRs with tuneable plasmon behaviour and high stability was synthesized.
2. Excitation wavelength-dependent SERS efficiency can be tailored by temperature.
3. The synergistic versus competitive mechanisms of SERS and SERRS were first proposed.
4. Three analytes (MG, SC, SS) exist in food safety issues were detected in trace level.
5. The LOD of MG in fish tissues was 0.73 ng/g with recoveries 80.7-115.6% obtained.

[Conclusion]

The pNIPAM @ Au NRs SERS substrates allowing trace level detection of analytes present in food safety issues. This work reveals a new path for Au NRs colloids preservation by separating them from each other on the polymers' network of pNIPAM, while providing high SERS efficiency by facilitating Au NRs proximity and aggregation to generate high density "hot-spots" when they be used. In addition, this work also provided considerable fundamental study to guide the application of pNIPAM @ Au NRs hybrids in practical food safety inspection scenario.

Keywords: Gold nanorods, thermo-responsive microgels, SERS and SERRS, malachite green

Effects of slightly acidic electrolyzed water combined with compound preservative on the protein characteristics of Pacific white shrimp (*Litopenaeus vannamei*) during refrigerated storage

Weiqing LAN [1,2], Bingjie ZHANG [1], Xi ZHANG[1], Jing XIE[1,2*]

1, Shanghai Ocean University College of Food Science and Technology
2, Shanghai Aquatic Products Processing and Storage Engineering Technology Research Center / Shanghai Ocean University National Experimental Teaching Demonstration Center for Food Science and Engineering, Shanghai 201306

[Objective]

The effects of slightly acidic electrolyzed water combined with compound preservative on the protein characteristics of Pacific white shrimp (*Litopenaeus vannamei*) during refrigerated storage were measured.

[Methods]

Samples were impregnated with slightly acidic electrolyzed water (SAEW), compound preservatives (rosemary extract + citric acid, RC), slightly acidic electrolyzed water and compound preservative (SAEW+RC) and sterile water (CK) treatment for 10 min respectively. Then drained them for 15 min, put them into PE bag and stored in refrigerator at (4±1) ℃. Different indexes, such as pH, TVB-N, total sulfhydryl content, Ca^{2+}-ATPase activity, chemical bond, MFI and IFI, which also combined with SDS-PAGE, were analyzed respectively.

[Results]

SAEW+RC treatment could delay the increase of pH, MFI, TVB-N value, inhibit the decrease of total sulfhydryl content and Ca^{2+}-ATPase activity. It was also indicated that the speed of protein decomposition, oxidation, and denaturation in SAEW+RC group was relatively slower than other groups. The chemical bond of protein and IFI were relatively stable.

[Conclusion]

Slightly acidic electrolyzed water combined with compound preservative treatment could inhibit the oxidation and decomposition of protein, delay the quality decrease of pacific white shrimp during refrigerated storage.

Keywords: slightly acidic electrolyzed water; rosemary extract; citric acid; Pacific white shrimp; protein characteristics.

Effect of ascorbic acid and citric acid on bioavailability of iron from *Tegillarca granosa* via an *in vitro* digestion/Caco-2 cell culture system

Bolun Sun, Beibei Tan, Nan Sun, Ping Huang, Jingxia Hong, Chao Li*, Wenge Yang*

College of Food and Pharmaceutical Sciences, Ningbo University, Ningbo 315211, PR China
** lichao@nbu.edu.cn *yangwenge@nbu.edu.cn*

[Objective]

Iron deficiency anaemia (IDA) has been brought to worldwide attention. Developing safe and effective iron supplements is of great significance for IDA treatment. *Tegillarca granosa* (*T. granosa*), a traditional aquaculture bivalve species in China, is considered as an excellent source of micronutrients, but the distribution and bioavailability of minerals are yet to be investigated.

[Methods]

The present research was conducted to determine the contents and solubility of minerals in *T. granosa* using ICP-MS. Meanwhile, two iron-binding proteins, hemoglobin and ferritin, were extracted from *T. granosa*, with their structures investigated using Fourier transform infrared (FT-IR) spectroscopy analysis and circular dichroism (CD) spectroscopy analysis. The iron accessibility and bioavailability of samples were analyzed using an *in vitro* simulated digestion/Caco-2 cell model. Moreover, the effects of ascorbic acid (AA) and citric acid (CA), two commonly applied dietary factors, on these parameters were evaluated.

[Results]

1. The *T. granosa* derived iron-binding proteins appeared to have more disordered secondary structures with the presence of AA/CA, likely due to the unfolding of protein and transformation of α-helix into random coil content.
2. The FT-IR results indicated a decrease in hydrogen bond intensity of proteins, which could be due to the binding of organic acids (AA/CA) to the proteins via hydrophilic interaction.
3. AA/CA had a beneficial effect on the iron absorption of iron-binding proteins, which might be related to the disordered structures induced by the two organic acids.
4. The results of the iron uptake in Caco-2 cells revealed that *T. granosa* exhibited excellent iron bioavailability compared with food matrices, suggesting that it could be a good source of dietary iron.

[Conclusion] *T. granosa* is rich in hemoglobin and ferritin that are recognized as multifunctional iron-binding proteins. The results of this study provided a basis for the development of *T. granosa* derived proteins-based iron supplements, promoting the diverse utilization of marine aquatic resources.

Keywords: iron bioavailability, *Tegillarca granosa*, Caco-2 cell model, simulated gastrointestinal digestion, protein secondary structure

Development of bio-functionally enhanced collagen by alginate oligosaccharide glycation

Boxue Yang*, Ga-Hyun Joe, Hiroki Saeki

Laboratory of Marine food Science and technology, Faculty of Fisheries Sciences, Hokkaido University, Minato 3, Hakodate, Hokkaido 041-8611, Japan
** you_h_g@eis.hokudai.ac.jp*

[Objective]
Chronic inflammation leads to disease development in non-communicable diseases (NCDs) like non-alcoholic fatty liver disease, and control chronic inflammation by diet has been performed as a countermeasure to NCDs. Continuous oxidative stress may also cause chronic inflammation and further leads to NCDs, whereas intake of edible antioxidants could reduce the oxidative stress in our body. Therefore, anti-oxidative and anti-inflammation foods derived increasing attention worldwide. Food protein-glycation using the Maillard reaction is an effective manner to improve some health functions of food proteins such as antioxidant capacity and anti-inflammatory activity. The objective of this study is to clarify the effectiveness of the glycation using alginate oligosaccharide (AO) on improving the antioxidant capacity and anti-inflammatory function of fish collagen.

[Methods]
Lyophilized collagen mixed with a half weight of reducing sugars was incubated at 60 °C and 35% of relative humidity up to 18 h for producing the Maillard-type glycated collagen; SDS-PAGE, available lysine content, and UV-absorbance at 294 and 420 nm were examined for monitoring production of the glycated collagens conjugated with AO (C-AO), glucose (C-Glu). As a control, the collagen-sorbitol mixture was prepared at the same incubating condition. After in vitro gastrointestinal digestion of the glycated collages by pepsin and trypsin, the digested samples (dC-AO, dC-Glu, and dC-Sor) were subjected to ABTS and DPPH assays. Subsequently, the cytoprotective effect on H_2O_2-induced cell oxidative damage and the suppressive effect on inflammatory cytokine (TNF-alpha) secretion were examined by pretreating Raw 246.7 cells with digested samples then stimulated with 0.8 mM-H_2O_2 and 20 ng/mL-LPS, respectively.

[Results]
(1) The glycation reaction rate of C-Glu group was higher than that of C-AO group.
(2) Radical scavenging activity of dC-AO and dC-Glu groups was enhanced with glycation progress. Furthermore, dC-AO obtained more enhancing antioxidant capacity without marked available lysine loss as dC-Glu
(3) The cytoprotective effect of collagen on the cell oxidative damage was enhanced to the same level by AO and glucose modification, despite the lower reaction rate of C-AO.
(4) Only dC-AO suppressed TNF-alpha secretion, indicating enhanced anti-inflammatory activity.

[Conclusion]
These results indicate that glycation using the Maillard reaction could be useful to prepare the improved antioxidative and anti-inflammatory collagen material, and AO is a suitable reducing sugar for the glycation of collagen in the consideration of reaction rate and function enhancement.

Keywords:
Maillard reaction, collagen, alginate oligosaccharide, antioxidant, anti-inflammation.

Preparation and Quality of Astaxanthin Self-microemulsion Functional Yogurt

Chaoyan Zhang*[1, 2], Na Li[1], Min Lei[1], Wentao Ma[1], Yingying Huang[1], Jinyu Yi[1]
1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
2, Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, Shanghai 201306, China
*chyzhang@shou.edu.cn

[Objective]
Haematococcus pluvialis is a new type of food resource, containing a lot of astaxanthin. Astaxanthin has many functions such as antioxidant, anti-inflammatory, and prevention of cardiovascular and cerebrovascular diseases. Therefore, astaxanthin can be added to food as a dietary functional factor which has important practical significance to enhance human health. The objective of this study is to explore the effect of astaxanthin contained *Haematococcus pluvialis* powder self microemulsion (HP(AST)-SMEDDS) on the quality and flavor of fermented yogurt.
[Methods]
In this study, HP(AST)-SMEDDS was directly added to sterilized pure milk, added starter, fermented at 43 ℃ for 6 hours, and then refrigerated for 12h to obtain HP(AST)-SMEDDS yoghurt samples. Yoghurt samples were detected with the changes of viable number of lactic acid bacteria, acidity, water holding capacity, odor and astaxanthin content as evaluation indexes.
[Results]
The results showed that the water holding capacity of yogurt increased with the increase of the addition amount, when the addition amount of HP(AST)-SMEDDS (mg/100g) was 0-0.8%, and the water holding capacity of yogurt decreased with the increase of the addition amount, when the addition amount of HP(AST)-SMEDDS was 0.8-2%. When the addition of HP (AST) - SMEDDS was 0.8%, there was no significant change in the number of live lactic ation acid bacteria in yogurt, and there was no significant discrepancy in the pH, odor, dehydration and condensation rate of yogurt. The retention rate of astaxanthin reached 78.9% after the yoghurt samples with 0.8% addition were stored in a dark environment at 4 ℃ for 14 days.#
[Conclusion]
There was no significant difference in the viability of ferment bacteria between yogurt samples with HP(AST)-SMEDDS and ordinary yogurt. In the detection of electronic nose, the group with the addition of HP(AST)-SMEDDS of 0.8% and 0.4% overlapped more with the blank yogurt, but the fishy smell of astaxanthin was not completely covered. This study provides a reliable reference method for the preparation and quality evaluation of astaxanthin functional yogurt.
Keywords: *Haematococcus pluvialis,* astaxanthin, yogurt, quality, storage

Anterior Cruciate Ligament Transection and Medial Meniscectomy induced Osteoarthritis in High Fat Diet-induced Obese Rats: Effect of *Lactobacillus plantarum* Fermented Lemon Peel Extract

Chi-Shung Tseng[1], Wei-En Chen[1], Zwe-Ling Kong[1*]
1, Department of Food Science, National Taiwan Ocean University, Keelung City 20224, Taiwan (R.O.C.)
* *kongzl@mail.ntou.edu.tw*

[Objective]

It's generally accepted that *Citrus limon* (L.) Brum. f. peels are common flavonoid source. The objective of this study is to compare effects of fermented lemon peel extracts (FLPs) and lemon peel crude extracts (CEs) on high-fat diet fed osteoarthritis rats.

[Methods]

To get FLPs, CEs from lemon peel extracted by autoclave, then fermented them with *Lactobacillus plantarum.* For *in vivo* study, obesity and osteoarthritis (OBOA) rats model was induced by high-fat diet and meniscus meniscectomy and anterior cruciate ligament transection. The OBOA rats were then given CEs and FLPs by oral gavage for six weeks.

[Results]

The results showed that FLPs reduced nitric oxide (NO) released levels in RAW264.7 cells, reduced IL-6, and increased Col2a1 expression levels in SW1353 cells, according to *in vitro* study. *In vivo* study, FLPs shows to reduce IL-1β, NO, PGE2 levels and inhibit MMP-13 activity according to plasma analysis. FLPs appear to reduce synovitis inflammation and glycoprotein loss, according to histopathological analysis. In this study, FLPs (75 mg/kg b.w.) show the strongest effect to decrease pro-inflammatory cytokines in both cell and animal models, increasing antioxidant enzyme activity and alleviating knee joint destruction.

[Conclusion]

In conclusion, FLPs show enhance its effectivity for OA treatment through the fermentation process by *Lactobacillus plantarum*.

Keywords: Osteoarthritis, Obesity, *Citrus limon* (L.) Brum., *Lactobacillus plantarum*, Fermentation

Current Status and Issues of *Shokuiku* in Japan

Chunhong Yuan*[1], Lan Xiao[2], Masahide Kaeriyama[3]
1, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, 020-8550, Japan
2, Institute for the Advancement of Higher Education, Hokkaido University, Kita 17 Nishi8 N104, Kita-ku, Sapporo, 060-0817, Japan
3, Arctic Research Center, Hokkaido University, N21 W11, Kita-ku, Sapporo, 001-0021, Japan
* chyuan@iwate-u.ac.jp

Japan established the Basic Act on *Shokuiku* in 2005, which defined *Shokuiku* as the "acquisition of knowledge about food and nutrition, as well as the ability to make appropriate decisions through practical experience with food, with the aim of developing people's ability to live on a healthy diet".

It is well-known that good foods supply essential nutrients necessary to support human life and health. Humans have acquired knowledge and experience about food and nutrition and created diverse food cultures. A theory of “Medicine and food have the same origin” has been believed by the people in China since ancient times, which means a balanced diet leads to a healthy body, and healthy food both prevents and cures sickness. In Western countries, there are also similar expressions such as "You are what you eat", or "One apple a day keeps a doctor away". As lifestyles have changed, people are concerned with food more than ever, such as food safety, local products, supply rate, lifestyle-related diseases, irregular and nutritionally unbalanced meals, and the diet of the elders. Japanese have the highest life expectancy of any major country. Life expectancy is 86.9 years for women and 81.5 years for men in 2020, and they could expect to live 75 of those years disability free and fully healthy, according to the World Health Organization report (2015). In 1985, the Ministry of Health, Labor and Welfare in Japan called for eating 30 different kinds of food in one day, which was a nationwide campaign. Now *Shokuiku* prevails in Japan, which is seen as a basis of the education on morality, intelligence and physique. The ‘Dietary guidelines for Japanese´ was launched in 2000. The ‘Japanese food guide spinning top’ was published in 2005 and revised in 2010. The guidelines were developed by the Ministry of Education, Science and Culture, the Ministry of Health and Welfare, and the Ministry of Agriculture, Forestry and Fisheries with the aim of promoting better dietary patterns.

Based on the situation surrounding food education such as the health of the people, changes in the environment surrounding food, and the digitization of society, the 4th Basic Plan for Promotion of Food Education focuses on the following three items (dietary education that supports physical and mental health throughout life; dietary education that supports sustainable food; dietary education corresponding to "new daily life" and digitalization) as basic policies and will be promoted by Japanese government at all levels comprehensively.

This presentation will introduce the background, contents, importance, status, and issues of *Shokuiku*. It is the most important subject to promote food and nutrition education in the world.

Keywords: *Shokuiku*, food and nutrition education, dietary guideline

Multifunctional food packaging nanofibers containing curcumin

Mengxia Duan[1], Jishuai Sun[1], Yequn Huang[1], Haixin Jiang[1], Yaqin Hu[3 *], Jie Pang[1,2,4 *], Chunhua Wu[1,2,4 *]

1.College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian350002, China;
2. Engineering Research Centre of Fujian-Taiwan Special Marine Food Processing and Nutrition, Ministry of Education, Fuzhou, Fujian, 350002, China;
3. College of Food Science and Technology, Hainan Tropical Ocean University, Sanya572022, China;
4. Key Laboratory of Marine Biotechnology of Fujian Province, Institute of Oceanology, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China

**yqhu@hntou.edu.cn (Y. Hu); *pang3721941@163.com (J. Pang); *chwu0283@163.com (C. Wu).*

[Objective]

Traditional food packaging has been unable to meet the market demand due to the frequent occurrence of food safety incidents. Multifunctional food packaging not only has the function of traditional food packaging but also has the function of extending food shelf life and monitoring food environment changes. Curcumin as a natural colorant has the above functions.

[Methods]

Curcumin (CUR, from Curcuma longa, purity ≥65%, HPLC) was supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Chitosan (CS) was dissolved in 60% (v/v) acetic acid under vigorous magnetic stirring for 4 h at 45 °C to obtain the CS solution (3%, w/v). Gelatin (GA) was dissolved in 80% (v/v) acetic acid under vigorous stirring for 4 h at 45 °C to obtain the GA solution (25% w/v). The obtained CS and GA solutions were mixed at a ratio of 3:7 to obtain the CS/GA mixture under vigorous stirring for 1 h. Subsequently, different amounts of CUR (0%, 0.1%, 0.2% and 0.3%, based on the dry weight of GA and CS) were added into the above obtained mixture, and recorded as GA/CS, GA/CS/CUR I, GA/CS/CUR II and GA/CS/CUR III, respectively. The nanofibers were then prepared using electrospinning technology. The electrospinning equipment used in this study was uniaxial blend electrospinning with a set voltage of 20 kV, a receiving distance of 10 cm, and a jet speed of 0.5 mL/h.

[Results]

1. Scanning electron microscopy results indicated that CUR could be well embedded in the GA/CS nanofiber-forming matrix and no obvious changes were observed in the morphological properties of the produced nanofibers upon increasing the concentration of CUR except for the slight increase of diameter.
2. The DPPH free radical scavenging rate increased with the increase of CUR content. Therefore, CUR can play a good antioxidant role in food packaging as an indicator.
3. The results of antibacterial activity showed that CUR could effectively inhibit the growth of *E. coli* and *S. aureus*. Therefore, the addition of CUR to food packaging can effectively inhibit the growth of microorganisms around food and the GA/CS/CUR II and GA/CS/CUR III nanofiber have similar antibacterial activity.
4. Food packaging nanofibers loaded with CUR are sensitive to ammonia, which allowed rapid monitoring of changes in ammonia concentration around food to convey information about food quality. And the GA/CS/CUR II nanofiber showed faster to ammonia than other nanofibers.

[Conclusion]

The nanofibers loaded with CUR have excellent antioxidant and antibacterial effects and can monitor the changes in food quality, so the CUR can be added to food packaging to increase its functionality.

Keywords: multifunctional food packaging, curcumin, nanofibers

In Vitro Study of the Fibrinolytic Activity via Single Chain Urokinase-Type Plasminogen Activator and Molecular Docking of FGFC1

Chunli Gao[1] , Quan Shen [1] , Pengjie Tang [1] , Yuling Cao [1] , Houwen Lin [2] , Bailin Li [1,3] , Peng Sun [4] , Bin Bao[1,3]and Wenhui Wu[1,3] *
1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China;
chunlgao@163.com (Chunli Gao)

[Objective]

Fungi fibrinolytic compound 1 (FGFC1) is a rare marine-derived compound that can enhance fibrinolysis both in vitro and in vivo. The objective of this study is to further evaluate the fibrinolytic activity characterization of FGFC1 mediated by plasminogen (Glu-/Lys-) and a single-chain urokinase-type plasminogen activator (pro-uPA).

[Methods]

The binding sites and mode of binding between FGFC1 and plasminogen were investigated by molecular docking. The fibrinolytic activity characterization of FGFC1 was mediated by plasminogen (Glu-/Lys-) and a single-chain urokinase-type plasminogen activator (pro-uPA) by constructing a fibrinolytic system composed of plasminogen (PLG) and pro-uPA in vitro and measuring the absorbance using a microplate reader.

[Results]

A 2.2-fold enhancement of fibrinolytic activity was achieved at 0.096 mM FGFC1, whereas the inhibition of fibrinolytic activity occurred when the FGFC1 concentration was above 0.24 mM. 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.

[Conlusion]

It is concluded that the mechanism of the interaction between FGFC1 and Glu-plasminogen may be related to its conversion to Lys-plasminogen and the existence of polymorphism. FGFC1 interacted with the LBSs in plasminogen via hydrogen bonds. Overall, this study provides a theoretical basis for the clinical pharmacology of FGFC1 and a reference for the development of noveplasminogen activators.

Keywords: FGFC1; pro-uPA; plasminogen; molecular docking; fibrinolytic properties

Optimization of spray drying process for fish oil microcapsule and study on its storage stability

Chunrong Chen, Hui Wang*, Yan Bai

Department of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, PR China
** hwan072@163.com*

[Objective]

Fish oil is easy to oxidize and deteriorate. Microencapsulation of fish oil can reduce the impact of environmental factors on fish oil oxidation, shield the fishy smell of fish oil, and make fish oil evenly added to food. The processing conditions of microencapsulation of fish oil by spray drying method are studied and the storage stabilities of microcapsules under high temperature and high humidity conditions are analyzed. This study provides a theoretical basis for the application of fish oil microcapsules in food

[Methods]

Spray drying process for fish oil microcapsule was studied using maltodextrin and arabia gum as wall materials with adding a certain amount of nano-montmorillonite. The effects of four factors including nano-montmorillonite content, solid content, core-wall ratio and inlet temperature on the embedding rate of fish oil were investigated. The optimum conditions for preparation of fish oil microcapsules were optimized through orthogonal experiment.

[Results]

1. The embedding rate of fish oil was 77.6% under the optimum conditions: the content of nano-montmorillonite 4%, solid content 24%, wall core ratio 1:2.5, inlet temperature 180 ℃.
2. The water content, angle of repose and surface oil content of fish oil microcapsules containing 4% nano montmorillonite were lower than those without nano montmorillonite.
3. The oxidation rate of fish oil gradually increased with the increase of humidity and temperature, and the oxidation rate of fish oil microcapsules significantly slowed down compared with non-embedded fish oil.
4. The oxidation rate of fish oil microcapsules containing 4% nano montmorillonite was lower than that without nano-montmorillonite under the same environmental conditions, .

[Conlusion]

Microencapsulation of fish oil can slow down the oxidation rate of fish oil compared with non-embedded fish oil.. The proper addition of nano-montmorillonite in the embedding wall materials used by natural polymer can reduce the oxidation rate of embedded fish oil and extend its shelf-life.

Keywords: fish oil microcapsule; nano-montmorillonite; spray drying; stability

Shelf Life Extension of Refrigerated Nile Tilapia (*Oreochromis niloticus*) Fillets using Seaweed Extracts

Faria Afrin*[1], Md. Jaki Shahrier[1], Md. Golam Rasul[1], Md. Rabiul Islam[2], A K M Azad Shah[1]

1, Department of Fisheries Technology, Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh
2, Department of Aquaculture, Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh
**faria3019@gmail.com*

[Objective]

Quality deterioration and shelf life reduction are the main concerns in Nile tilapia (*Oreochromis niloticus*) fillets during storage at chilled condition. This study was aimed to evaluate the effects of ethanolic extracts of *Padina tetrastomatica*, *Sargassum natans* and *Sargassum fluitans* on extending the shelf life of Nile tilapia fillets during refrigerated storage.

[Methods]

Ethanolic extracts were prepared from the seaweeds. Nile tilapia fillets were treated with 2% extracts of each seaweed and stored at 4±1°C in air tight polyethylene bags. The chemical (pH, thiobarbituric acid reactive substances (TBARS) and total volatile basic nitrogen (TVB-N)), microbiological (aerobic plate count (APC)) and sensory evaluation was performed at every 4 days intervals until apparent unacceptable for human consumption.

[Results]

Results of this study showed that the pH values were gradually increased and reached to 6.83, 7.14, 6.97 and 7.02 for control, *P. tetrastomatica*, *S. natans* and *S. fluitans* at 8th, 20th, 12th and 16th days of storage period. Similarly, TBARS values of all the treated and untreated samples were increased with the increasing of storage period, and the values ranged from 0.62 mg MDA/kg to 0.91 mg MDA/kg. The TBARS values observed in this study was within acceptable limit (1-2 mg MDA/kg). However, TVB-N values were exceeded the acceptable range (30 mg N/100g fish muscle) at 8th, 20th, 12th and 16th days for control, *P. tetrastomatica*, *S. natans* and *S. fluitans* extracts treated fillets, respectively. The APC values were gradually increased in all the samples and reached to 6.53 log CFU/g, 7.11 log CFU/g, 6.75 log CFU/g and 7.10 log CFU/g at 8th, 20th, 12th and 16th days of refrigerated storage for control, *P. tetrastomatica*, *S. natans* and *S. fluitans* extracts treated fillets, respectively. Overall, *P. tetrastomatica* extracts treated fillets showed significantly ($P < 0.05$) the lowest pH, TBARS, TVB-N and APC values than those of other seaweed extracts treated and untreated fillets. Moreover, *P. tetrastomatica* extracts treated fillets had acceptable sensory characteristics up to 16th days of storage period followed by *S. fluitans* (12 days), *S. natans* (8 days) and control (4 days) fillets.

[Conlusion]

Results of this study demonstrated that ethanolic extracts of *P. tetrastomatica* retains the quality and extends the shelf life for 12 days longer than untreated fillets in refrigerated condition and thus, the extracts can be used as natural additives for the preservation of Nile tilapia fillets.

Keywords: Nile tilapia, seaweed extracts, quality, shelf life, sensory evaluation

Microalgae as sustainable feedstocks for food, feed, and cosmetics

Fengzheng Gao*[1], Iago Teles (Cabanelas, ITD)[1], René H Wijffels[1,2], Maria J Barbosa[1]

1, Wageningen University, Bioprocess Engineering, AlgaePARC, P.O. Box 16, 6700 AA, Wageningen, Netherlands
2, Faculty Biosciences and Aquaculture, Nord University, N-8049 Bodø, Norway
* *fengzheng.gao@wur.nl*

Microalgae are small unicellular photosynthetic organisms that can grow fast using (sun)light, carbon dioxide, and nutrients to produce different compounds such as proteins, pigments, fatty acids, and carbohydrates. These microalgal compounds could be sustainable feedstocks for food, feed, and cosmetics. The use of microalgae as feedstocks has several advantages compared to conventional crops. For instance, microalgae can grow in seawater using non-arable land, easing up the crises of water and land availability. In addition, microalgae can fix carbon dioxide, reducing its atmospheric concentration. During the past decades, several microalgal species have been produced at industrial scale, such as *Spirulina*, *Chlorella*, *Haematococcus*, and *Dunaliella*, with a global production ≈ 25000 tons dry weight/year of which more than half is produced in China.

Today there are only market opportunities for high-value applications because the production costs are still too high to make microalgae as competitive feedstocks for commodities. There is presently a large production capacity for a limited number of microalgae strains in Europe. However, the number of concrete products and market applications is still limited. The Bio-Based Industries consortium (BBI-Horizon 2020) project MAGNIFICENT aims to develop and validate a sustainable and economically feasible new value chain based on cultivation and processing of microalgae, with the aim to transform microalgae biomass into valuable ingredients for food, aquafeed and cosmetics applications. The MAGNIFICENT consortium has 16 partners from 7 European countries including 10 Small and Medium sized Enterprises. 3 Large Enterprises, 1 University, and 2 Research and Technology Organisations, and comprises commercial partners in the entire value chain and the 3 target markets. The outcomes of this project will lead to new business opportunities for microalgal products, industrialize the biotechnology and provide the knowledge and experience required to enter the medium to low-value market within 5-10 years.

Keywords: microalgae, food, feed, cosmetics, MAGNIFICENT, new business opportunities

3D printing properties and printability definition of *Pennahia argentata* surimi and rice starch

Gaoshang Li, Zhiheng Hu, Jiayin Huang, Xunyu Luo, Chunhong Yuan, Koichi Takakid, Yaqin Hu*

College of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, PR China
** yqhu@hntou.edu.cn*

[Objective]

3D printing technology is a new technology of additive manufacturing. It is gradually used in food processing due to the personalized customization and forming without a mold. Most researches about 3D printing of surimi and starch focus on the effect of material properties on 3D printing, such as texture, gel strength and rheological properties. While the properties can not accurately characterize the printability of materials and there are no report about this. Thus, based on the properties of *Pennahia argentata* surimi and rice starch, a method for judging the 3D printability of materials was established.

[Methods]

The surimi with different moisture (75%, 76%, 77%, 78%, 79%, 80%), starch with different content (10%, 15%, 20%), surimi (80% moisture content) with different starch content (4%, 6%, 8%, 10%) were prepared. The different materials were printed to square frustum with different inclination angles (75°, 60°, 45°, 30°), characterizing the printing effect. Moreover, texture, gel strength and rheological properties of materials were determined. Based on the correlation analysis of the results of different properties, the measurement results of different indicators were normalized. Then sum of the product of normalized values and correlation indexes was used as an equation for judging printability of materials.

[Results]

1. *Pennahia argentata* surimi with 78 moisture content, rice starch with 20% content and surimi with 8% starch could be printed in the smallest angle shape, meaning that they had best printability.
2. Texture, gel strength and rheological properties of materials influenced the printing effect and they had correlation with each other.
3. The result of the printability equation and actual printing effect are consistent, indicating that it could better characterize the printability of materials.

[Conlusion]

The established 3D printability equation could characterize the printability of materials through specific values. It greatly simplifies the steps and difficulty of evaluating materials printability, facilitates the selection of 3D printing food materials, promoting the application of 3D printing in food processing.

Keywords: 3D print, texture properties, gel strength, rheological properties, printability equation

Potential adverse effects induced by foodborne Titanium dioxide nanoparticles

Hang Xiao, Ph.D., Professor
Department of Food Science
University of Massachusetts, Amherst, MA, U.S.A.
* *hangxiao@foodsci.umass.edu*

Abstract: Titanium dioxide (TiO_2), a commonly used food additive, contains an appreciable fraction of particles in nano-scale. There is increasing concern about the potential health risks associated with foodborne TiO_2 nanoparticles (NPs), especially within certain susceptible populations, such as the obese. Our recent studies aimed to determine the potential adverse effects of TiO_2 NPs in obese male and female individuals and the potential role of gut microbiota in mediating the adverse effects. Two types of TiO_2 (30 nm and E171-food grade TiO2, 0.1% wt%) were fed to two mice populations (high-fat diet-fed obese mice and low-fat diet-fed non-obese mice), and each mice population contained male and female individuals. Meanwhile, fecal samples of male mice from the above groups were collected for orally transplanting to mice fed a low-fat diet for 10 weeks. Transmission electron microscope imaging, serum biochemical parameters, histological analysis, immunohistochemistry, qRT-PCR, 16s rRNA gene amplicon sequencing and short-chain fatty acid (SCFA) analysis were utilized to characterize the nanoparticle distribution in tissues, inflammation status, the composition of the microbiota and the effects of altered gut microbiota on the inflammation status of mouse colon. The results showed that dietary intake of TiO_2 NPs significantly exacerbated the high-fat diet-induced abnormality in serum biochemical parameters related to liver function and lipid metabolism, these effects were more pronounced in the obese mice than in non-obese mice. Administration of TiO_2 NPs led to accumulation of TiO_2 NPs in the kidney and liver, and the NPs caused damages to specific cellular organelles in the renal and liver tissues, including mitochondrial swelling, disappearance of mitochondrial cristae, and enlargement of endoplasmic reticulum. Furthermore, the TiO_2 NPs profoundly modulated the mRNA levels of genes involved in oxidative damage in liver and kidney. The abundance of inflammation-related cytokines and myeloperoxidase in colonic mucosa were significantly altered by TiO_2 NPs to produce an inflammatory state. The results showed that dietary TiO_2 NPs led to a significant dysbiosis of gut microbiota with stronger alterations in the high-fat diet-fed obese mice than the low-fat diet-fed non-obese mice. TiO_2 NPs decreased the cecal levels of SCFAs such as butyrate. After 10 weeks of microbial transplant, microbiota from the obese male mice consuming a high-fat diet with TiO_2 NPs led to an increase of pro-inflammatory cytokines and loss of healthy colonic morphology in the colon of the low-fat diet-fed recipient mice, indicating a significant colonic inflammation. Overall, these findings provided a valuable new perspective on the potential adverse effects and underlying mechanisms of foodborne TiO_2 NPs among obese vs. non-obese populations.

Keywords: Foodborne Titanium dioxide, Nanoparticles, Obesity, Gut microbiota, Colonic Inflammation

The simulated interaction between myoglobin and ATP

Hideo Ozawa*[1], Yoshihiro Ochiai[2]

1, Kanagawa Institute of Technology, Atsugi, Kanagawa, Japan
2, Tohoku University, Sendai, Miyagi, Japan
* *ozawa@bio.kanagawa-it.ac.jp*

[Objective]

The deterioration of red-fleshed fish meat is represented by browning, where oxy or deoxy myoglobin (Mb) changes to metMb. The metMb contains Fe(III) in the heme, whereas the bioactive Mbs contain Fe(II). The browning of fresh meat is generally slow, partially because ATP prevents oxyMb from metMb formation. In the present study, molecular dynamics (MD) simulation of the interaction between oxyMb and ATP was performed to understand the effect of ATP on oxyMb structure and its dynamics.

[Methods]

The oxyMb structure of yellowtail *Seriola quinqueradiata* was obtained by homology modelling with the counterparts of blackfin tuna *Thunnus atlanticus* (PDB ID: 3QM5). MD simulation and its analysis were performed by Amber 18 suite. To the oxyMb, around ten thousand of water molecules were added and one ATP molecule was randomly added. Then, sodium and chloride ions were randomly added to 100 mM. After energy minimization and equilibration, MD simulation of 500 ns at 300 K was performed for 30 times. The data of the first 100 ns were discarded and those of the remaining 400 ns were used for the analysis. To test the difference on average or standard deviation, Welch's t test and F test were performed, respectively. To control the positive false discovery rate, q value was used.

[Results]

Since fish Mbs lack D helix, CE and EF loops were highly fluctuated as evaluated by the RMSF, irrespective of the presence of ATP. In some trajectories, ATP interacted with this region, although there were no difference in RMSF values for averages nor in the standard deviations, irrespective of the presence of ATP. Including these regions, RMSF values did not show significant difference ($q > 0.05$) between oxyMbs, irrespective of the presence of ATP. This would be partially because oxyMb dose not have any specific binding site for ATP. Rather, ATP would bind to multiple sites on the surface of oxyMb.

[Conclusion]

The present study showed that, by RMSF, it was difficult to understand the effect of ATP binding on oxyMb dynamics. Therefore, further analysis should be performed.

Keywords: molecular dynamics simulation, myoglobin, yellowtail

Physicochemical properties of fish gelatin-pectin -based edible films

Hui Wang*, Sheng Huang

State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China
** 5037565710@qq.com*

[Objective]

Most commercial preservative films are made by petroleum base materials which are non-renewable resources and due to the large environmental pollution. To make the better properties films, modification materials and compound materials are used. In order to meet the antioxidant requirements of food production, sales and storage, resveratrol (RSV) as active antioxidant ingredient was added into the FG-LMP films in this research.

[Methods]

The final total concentration of FG and LMP in this study were 5% (w/v). Different proportions groups of FG and LMP (3:1, 2:1, 1:1, 1:2, 1:3) with RSV were designated as $TPR_{3:1}$, $TPR_{2:1}$, $TPR_{1:1}$, $TPR_{1:2}$, $TPR_{1:3}$. The pure FG or LMP films loaded RSV were named FGR and LMPR, respectively. FG and LMP were heated to 50°C and 60°C respectively, with continuous stirring for their dispersions. RSV was dissolved in ethanol. FG were thoroughly mixed well with diluent RSV (0.05 wt. %, final concentration) solution and 15 wt. % glycerol (on total dry basis) at 50°C for 1 h. After cooled down to room temperature, the blends were magnetic stirring with LMP dispersions for 1 h. 15 mL solutions were pooled into a Petri dish (9 cm × 9 cm) and casting at 40°C for 10 h.

[Results]

1. The complex formed by the FG-LMP bring the better mechanical properties and low waster solubility to films.
2. UV resistance of films were enhanced owing to the addition of LMP.
3. The hydrophobic/hydrogen bond/electrostatic interaction between LMP and FG affected the physicochemical of films.
4. The antioxidant ability of the coating films was tested on DPPH tests and beef tallow, and the films containing RSV present significant delay in visible decay in preserving the beef tallow against oil deterioration.

[Conlusion]

The potential release behavior mechanism was proposed that the LMP compete the combination of FG with RSV, and shorted the release path of RSV in the film

.

Keywords: Fish gelatin; Low-methoxyl pectin; Resveratrol; Films; Controlled release

Study on Percutaneous Absorption of Collagen in Medical Dressing

Huishu Zhang[1], Tian Zhang[1], Wenhui Wu[2]*

1, Shanghai Ocean University, Shanghai 201306, China
2, college of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
* *whwu@shou.edu.cn*

[Objective]

There is a medical dressing, we need to test the percutaneous absorption of the medical dressing.

[Methods]

The collagen in dressing was marked by fluorescein isothiocyanate(FITC) and apply to the mice. If the collagen is percutaneous absorbed into the blood, the fluorescence intensity of the blood will rise. Subcutaneous injection and HE stain was used for further explanation.

[Results]

We found the fluorescence intensity of the blood did not change significantly and the skin tissue of mice was normal.

[Conlusion]

The collagen in this medical dressing is not absorbed through the skin, which proves this medical dressing is safe for use.

Keywords: medical dressing, percutaneous absorption, FITC

Effect of sorbitol, ethanol, and vitamin C on sensory quality, water content, peroxide value and microorganism of semi-dried tilapia fillet

Huong Thi Thu Dang
The Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu street, Nha Trang City, Vietnam
dangthithuhuong@ntu.edu.vn

The effects of sorbitol, ethanol, and vitamin C on sensory quality, water content, peroxide value and microorganism of semi-dried tilapia fillets were investigated. Fish fillets were immersed in a 10% brine solution (ratio of v/w: 3:1) for 15 minutes. After being drained, the fish were divided into 3 Groups: Group 1: (Control Group): Salted fish were sun dried for six hours; Group 2: salted fish were seasoned (sorbitol 8% combined with ethanol 39.5%-30ml/kg salted fish); Group 3: salted fish were seasoned (sorbitol 8% combined with vitamin C 0.4%). After being seasoned for 20 minutes, then drained for 20 minutes, fish fillets were dried in the sun for 6 hours. Fish were then dipped in a 0.5% chitosan solution for 20 seconds, drained, then vacuum packed (85%) in PA bags and then chilled stored at 2±2℃. Using sorbitol combined with ethanol reduced water content faster than the Control Group and vitamin C Group in the same drying time. This decrease was from 80% to 50%. The sensory evaluation score was also higher in Group 2 compared to others. On the other hand, Group 3 showed the lowest peroxide value. After 3 weeks of chilled storage, sensory quality of all groups was satisfactory according to TCVN 3215-79, microorganism was still guaranteed at a safe level of use according to 46/2007/QĐ-BYT.

Keywords: *ethanol, semi- dried, tilapia fillet, sorbitol, vitamin C*

Physicochemical and sensory properties of biscuits with added fish protein isolate

Huynh Nguyen Duy Bao*[1], Nguyen Ngoc Thuy Dung[1]

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

* *hndbao@ntu.edu.vn*

[Objective]

Biscuits are one of the most popular consumed baked items. However, traditional biscuits are frequently poor in protein and lack some of the essential amino acids. Meanwhile, fish protein isolate (FPI) is proven to contain all the essential amino acids. The addition of FPI to biscuits can improve their nutritional value but may change their physicochemical and sensory properties. The present study was, therefore, conducted to investigate the physicochemical and sensory properties of biscuits with added FPI.

[Methods]

The FPI was prepared from yellowfin tuna trimmings using the pH-shift method. The ingredient formulation of control biscuits based on dough weight included 53.9% wheat flour, 16.2% butter, 6.5% sugar, 1.6% edible oil, 0.6% salt, 1.6% cilantro, 1.3% baking powder, and 18.3% water. A certain amount of wheat flour was substituted with FPI for ingredient formulations of biscuits with FPI added. The mixture of ingredients was well kneaded and then incubated for 15 minutes at room temperature. Subsequently, the dough was sheeted to a thickness of 3 mm and cut into a square shape (30 × 30 mm^2). The shaped biscuit dough was baked in an electric oven at 175 ^oC for 25 minutes. The baked biscuits were cooled to room temperature and packed in polyethylene ziplock bags for evaluation of their physicochemical and sensory properties.

[Results]

The results showed that the moisture and water activity of biscuits with 1.6 – 5.4% FPI added significantly decreased compared with the control biscuits without FPI. The hardness of the biscuits with FPI added compared to the control biscuits, however, was not significantly different. The results of sensory evaluation also showed that the appearance, texture, color, flavor, taste, crunchiness, and overall acceptability of the biscuits with FPI added and the control biscuits did not differ significantly except for the color and flavor of the biscuits with 3.8 – 5.4% FPI added. The addition of 2.7% FPI to biscuits was found to have no negative impact on the biscuits' quality. Biscuits with 2.7% FPI added had a proximate composition of 1.58% moisture, 11.93% protein, 23.2% lipid, 2.43% ash, and 60.08% total carbohydrates.

[Conlusion]

The physicochemical and sensory properties of the biscuits with 2.7% FPI added were not significantly different from those of control biscuits without FPI, except for their moisture and water activity. These findings suggested that FPI prepared from tuna trimmings could be a good source of protein for making protein-rich cakes.

Keywords: fish protein isolate, biscuits, tuna, value-added products

Sensory and Metabolites Migration in the Boiling Process of Tilapia Skin

Jiahui Chen[1], Yinghua Nie[1], Jiamin Xu[1], Shudan Huang[1], Jie Sheng[1], Xichang Wang[1,2], Jian Zhong[1,2,*]

1,College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306,China
2, Collaborative Innovation Center of Seafood Deep Processing, Dalian Polytechnic University, Dalian 116034, Liaoning, China
**cjh16636184361@163.com*

[Objective]

In order to realize the high-value utilization of fish skin, metabolomics technology is used to explore the migration law of small molecular metabolites after boiling the fish skin.

[Methods]

In this experiment, physical and chemical properties, electronic tongue, electronic nose, GC-TOF-MS and LC-MS/MS were used to analyze the relationship between sensory and metabolites in fish skin and fish skin soup at different boiling times.

[Results]

The results show that during theboiling process, the soluble substances flow out after the fish skin absorbs water and swells. The number of different metabolites decreases significantly, and the types decrease. Among them, linoleic acid metabolism, taurine and hypotaurine metabolism, and purines mainly occur, which leads to an increase in the sweetness of the fish skin and a decrease in the bitterness of the fish skin soup.

[Conlusion]

At the same time, it is found that as the boiling time increases or the boiling temperature increases, the metabolites in the fish skin are destroyed. This experiment provides a theoretical basis for solving this problem.

Keywords: fish skin, fish skin soup, sensory, metabolites, GC-TOF-MS, LC-MS/MS

Effects of different sources of anthocyanins on developing intelligent and active food packaging

Jialin Liu, Yaqin Hu*

College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China
* *yqhu@hntou.edu.cn*

[Objective]

In this work, active and intelligent films were investigated based on CS/PVA/nano-ZnO incorporated with anthocyanins extracted from purple potato or roselle. The effects of anthocyanins content and composition on the physical and functional properties for packaging films were also investigated and compared, including micromorphology structure and mechanical, barrier, pH-sensitive, antibacterial properties. The films were also applied in the freshness of shrimp for their function examination.

[Methods]

FT-IR, XRD, SEM were employed to characterize films. Physical properties of films were tested through physical appearance, thickness and mechanical properties, moisture content and water vapor permeability, light transmittance and opacity. Functional properties of films were tested through pH-sensitive property and antibacterial activity.

[Results]

1. CS/PVA/Nano-ZnO films, incorporated with various concentrations of PPE or RE, were successfully developed by a solution casting method.
2. When incorporated with PPE or RE, mechanical resistance of film was significantly enhanced ($P < 0.05$), while the moisture contents and flexibility of film significantly reduced ($P < 0.05$).
3. CPZ-RE film exhibited darker color and lower light transmittance than CPZ-PPE film at the same incorporation level, which was due to different compositions and contents of anthocyanin.
4. CPZ-PPE films exhibited higher antimicrobial activity against *E. coli* and *S. aureus* than CPZ-RE films.

[Conlusion]

Our results suggested CPZ-PPE and CPZ-RE films have promising potential as active and smart packaging materials for applications in food industry.

Keywords: Chitosan/Polyvinyl alcohol/Nano-ZnO; anthocyanins; active and intelligent packaging

Effect of Transglutaminase Crosslinking on the Structural, Physicochemical, Functional, and Emulsion Stabilization Properties of Three Types of Gelatins

Jiamin Xu[1], Lili Yang[1], Yinghua Nie[1], Mengyang Yang[1], Wenjuan Wu[1], Zhengquan Wang[1], Xichang Wang[1], Jian Zhong[1,2*]

1, National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China

2, Collaborative Innovation Center of Seafood Deep Processing, Dalian Polytechnic University, Dalian 116034, Liaoning Province, China

*First author: jiamin921@163.com, * jzhong@shou.edu.cn*

[Objective]

The objective of this study is to study the effects of transaminase crosslinking on the molecular structure, functional properties and emulsion application of three types of gelatin (bovine bone gelatin, cold-water fish skin gelatin and pig skin gelatin).

[Methods]

we comprehensively explored TG modification of three types of gelatins (bovine bone gelatin, BBG; porcine skin gelatin, PSG; and cold-water fish skin gelatin, CFG) and analyze their structural, physicochemical, functional, and emulsion stabilization properties. First, we synthesized TG-modified BBG (BBG-TG), TG-modified PSG (PSG-TG) and TG-modified CFG (CFG-TG) and characterized the structural properties by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy, scanning electron microscopy (SEM), and atomic force microscopy (AFM). Second, we analyzed the physicochemical properties of TG-modified gelatin solutions such as solution turbidity, textural properties, molecular surface hydrophobicity, and molecular flexibility. Third, we determined the functional properties of TG-modified gelatins such as foaming properties and emulsifying properties. Finally, we observed the storage stability of fish oil-loaded TG-modified gelatin-stabilized emulsions for 28 days at room temperature.

[Results]

The results demonstrated that TG modification increased the molecular aggregation behaviors and change the physicochemical and functional properties of three types of gelatins. Further, TG-modified aquatic gelatin showed comparable foaming properties and higher emulsifying properties to mammalian gelatins, which suggested it could be potential mammalian gelatin replacer. Finally, TG-modified gelatins decreased the droplet sizes and increased creaming stability for fish oil-loaded emulsions.

[Conlusion]

All these results suggested TG modification was an efficient method to improve the emulsifying properties of gelatins. Further, these results also showed significant differences among these three types of gelatins with and without TG modification.

Keywords: bovine bone gelatin; cold-water fish skin gelatin; emulsion; porcine skin gelatin;

Slightly acidic electrolytic water pretreatment combined with compound preservatives on the quality and microflora changes of Pacific white shrimp (*Litopenaeus vannamei*) during refrigerated storage

Weiqing Lan[1,2], Jiaxin Zhao[1], Xi Zhang[1] , Jing Xie[1,2*]

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

[Objective]

The effects of slightly acidic electrolyzed water (SAEW) pre-treatment combined with rosemary extract (RE)-citric acid (CA) compound preservatives on the quality and bacterial community of Pacific white shrimp (*Litopenaeus vannamei*) during refrigerated storage were studied.

[Methods]

Samples were impregnated with sterile water (CK), RC, SAEW and SAEW+RC for 10 min respectively, then drained and refrigerated at (4 ± 1) ℃. Microbial (total viable count (TVC), *psychrophilic* bacteria count, *shewanella* bacteria count and *pseudomonas* bacteria count) and chemical (total volatile basic nitrogen (TVB-N), biogenic amines contents (putrescine and cadaverine)) indexes were measured respectively at 2-days interval. The quality and microflora changes were analyzed by high-throughput sequencing technique and heatmap.

[Results]

Slightly acidic electrolyzed water combined with compound preservatives can delay the increase of various indexes of *Litopenaeus Vannamei* during refrigerated storage. On day 10, the TVC of sample with SAEW+RC treatment was (6.10±0.14) log CFU/g, TVB-N value was (16.06±0.34) mg/100 g, putrescine content was (4.40 ± 0.06) mg/kg and cadaverine content was (6.73 ± 0.06) mg/kg. All indexes were significantly lower than those in the control group ($P<0.05$). Compared with the control group, the shelf-life of *Litopenaeus vannamei* during refrigerated storage was prolonged for another 4 days at least. *Vibrio*, *Shewanella* and *Psychrobacter* were the dominant spoilage bacteria of *Litopenaeus Vannamei* at the end of storage.

[Conclusion]

Slightly acidic electrolytic water combined with compound preservative had a good inhibitory effect on *Shewanella* and *Psychrobacter*, and had the obvious preservation effect on *Litopenaeus Vannamei*.

Keywords: *Litopenaeus Vannamei*; slightly acidic electrolyzed water; rosemary extract; citric acid; quality; microbial flora

Preparation of HPMC modified PVA film incorporating roselle anthocyanin for shrimp freshness monitoring and preservation

Jiayin Huang[1,2], Yaqin Hu[2*]
[1]Institute of Food Engineering, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, Zhejiang, China, 310058
[2]College of Food Science and Technology, Hainan Tropical Ocean University, Yucai Rd 1#, Sanya 210027, China
**1004346262@qq.com*

[Objective]

Intelligent packaging, which can provide consumers with real-time food information and preserve food freshness has been attracted much attention. The objective of our study is to prepare a biodegradable film with better functional characteristics for shrimp freshness monitoring and preservation.

[Methods]

Indicator films incorporating roselle anthocyanin extracts (RAE) were developed by casting, and Hydroxypropyl methylcellulose (HPMC) modified polyvinyl alcohol (PVA) was applied as film-forming substrates. The structural properties of PVA/HPMC/RAE (PHR) films were characterized by rhelogical behavior studies, FT-IR and SEM; physical properties were characterized by mechanical and hydrophobic performances, while indication properties were characterized by pH-responsive ability. Furthermore, the PHR film was applied on shrimp freshness monitoring and preservation by the determination of film color and chemical spoilage indexes.

[Results]

Structural characterization results suggested RAE, HPMC and PVA were well dispersed. The addition of HPMC would improve film functional characteristics, and the films with a ratio (HPMC-to-PVA) of 3:1 exhibited better physical properties and larger color variations in response to pH changes. The films were successfully applied for shrimp freshness real-time monitoring. The film color changed from rose-red to light green at 4 d, corresponding to the onset of spoilage, and then turned into yellow at 8 d, when the shrimp was severely spoilage. Furthermore, the PHR films were applied for shrimp preservation, the shrimp wrapped by PHR film exhibited lower TBA, TVB-N, TVC values and higher sensory scores, indicating the excellent preservation performances of PHR film.

[Conlusion]

The incorporation of HPMC could improve film functional characteristics by structural modification of new interaction formation, and the PVA/HPMC/RAE film could be applied as for real-time shrimp freshness monitoring and preservation.

Keywords: intelligent packaging, freshness monitoring, food preservation

Effect of hypotaurine on melanosis and quality changes of *Penaeus vannamei* during refrigeration

Jiaying Zhou[a b]
a College of Biosystems Engineering and Food Science, National-Local Joint Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang Key Laboratory for Agro-Food Processing, Fuli Institute of Food Science, Zhejiang University, Hangzhou 310058
b College of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, China

[Objective]

Due to the existence of melanosis, *Penaeus vannamei* will cause waste in processing and transportation, resulting in the loss of market value. Therefore, a safer, natural and efficient melanosis inhibitor is sought to prolong the shelf life of shrimp. At present, hypotaurine has been used in the preservation of fruits and vegetables, but there are few studies on the preservation effect of shrimps. Also, melanosis and quality changes of *Penaeus vannamei* during storage are not clear. Hence, the research aimed to determine the impact of hypotaurine treatments at different concentrations on melanosis prevention, muscle physicochemical properties and extending shelf-life during storage, hoping to provide some references for the application of hypotaurine in the preservation of aquatic products.

[Methods]

The used white shrimp, *Penaeus vannamei*, were captured near Zhoushan sea area and treated with different concentrations of hypotaurine (1 g / L, 10 g / L, 20 g / L). The effects of hypotaurine on melanosis, pH, TVB-N, TBA, total bacterial count and other indexes of *Penaeus vannamei* during storage were measured.

[Results]

1 The antioxidant activity of taurine was evaluated by DPPH radical scavenging ability method. It was found that different concentrations of taurine showed antioxidant activity positively correlated with concentration.

2 Higher concentration of hypotaurine has better effect on delaying shrimp melanosis, which may be because hypotaurine has better antioxidant activity, can slow down the oxidative denaturation of protein, and reduce the degree of color change.

3 Taurine treatment could delay the rise of TVB-N value, pH value and TBA value of shrimp.

4 The results of texture and tissue section showed that high concentration of taurine could reduce the decrease of hardness and elasticity and maintain the integrity of muscle.

5 The microbial results showed that compared with the control group, taurine could inhibit the growth rate of the total number of colonies in the later stage of storage, but there was little difference among the concentration groups.

[Conlusion]

Hypotaurine had good antioxidant activity and could significantly inhibit the melanosis of *Penaeus vannamei.* The inhibition efficiency of hypotaurine depended on the concentration. The lipid oxidation, TPC and muscle structure loss of shrimp treated with 20g / L hypotaurine during storage were low ($P < 0.05$). Therefore, hypotaurine can be used as a safe substitute for commercial melanosis inhibitors to control melanosis during storage and prolong the shelf life of shrimp.

Effect of Acetylated Distarch Adipate on the Physicochemical Characteristics and Structure of Shrimp (*Penaeus vannamei*) Myofibrillar Protein

Jie Mi [a, b], Wei Ni [a], Ping Huang [a], Jingxia Hong [a], Ru Jia [a, b], Shanggui Deng [c], Huamao Wei [a, b*], Wenge Yang [a, b*]

a . College of Food and Pharmaceutical Sciences, Ningbo University, 315211, China
b . Key Laboratory of Animal Protein Food Deep Processing Technology of Zhejiang Province, 315211, China
c . College of Food and Pharmacy, Zhejiang Ocean University, 316000, China
** weihuamao@nbu.edu.cn; *yangwenge@nbu.edu.cn*

[Objective]
This study was to investigate the effect of Acetylated Distarch Adipate (ADA) on the physiochemical properties and structure of myofibrillar protein (MP) and MP gel (MPG) by means of chemical bonds, protein secondary structure, microstructure, water state, water hold capacity (WHC) value and texture. Besides, the results of this work would contribute to the utilization of ADA for the functionality of surimi products improvement.

[Methods]
Shrimps (*Penaeus vannamei*) were purchased from Lulin market (Ningbo, Zhejiang, China). Acetylated distarch adipate was provided from Starpro Company (Hangzhou, China). MP were prepared from the shrimp muscle using 10 mM phosphate buffer (pH 7.0) containing 2 mM $MgCl_2$, 0.1 M NaCl and 1 mM EDTA. MPGs were obtained by adding different ratios (w/w) of ADA as gel enhancer and heating at 90 °C for 30 min. Chemical bonds, water state, gel strength and water holding capacity were employed to characterize the physicochemical properties of MP during gel formation. The spectrum of FTIR and electron microscopic observation were used to characterize the structure properties.

[Results]
1. The MPG with 1.0% ADA showed the highest gel strength and WHC value.
2. Excess starch will destroy the gel network structure after absorbed water and swelled.
3. The relaxation time (T_2) of MPG reduced with the increased of ADA addition.
4. Heating changed the secondary structure of MPG significantly while ADA did not.

[Conclusion]
On the one hand, the starch swelled by water absorption and was embedded in the protein gel network, which caused the gel strength improvement. On the other hand, the added starch competes with the protein for water indirectly led to the water content of protein decreased, which resulted in an increase in gel strength. However, excessive starch could also compress the protein matrix by absorbing water and swelling, thereby disrupted the gel network structure and caused a reduction in gel strength. Furthermore, ADA addition had little effect on the protein secondary structure content and the protein composition of shrimp MP and MPG. Therefore, based on our study, ADA could be used as an effective gel enhancer to improve the quality of shrimp surimi products.

Keywords: Myofibrillar protein; Shrimp; Gel strength; Microstructure; Water state

A comparison study of recovered protein from pearl oyster *Pinctada fucata martensii* isolated by various recovery methods

Jie-Ting Geng*, Rong-Hao Chen, Nonthacha Thanathornvarakul, Ya-Fang Shi, Kazufumi Osako

Department of Food Science and Technology, Tokyo University of Marine Science and Technology,Tokyo, Japan
**gengjieting@kaiyodai.ac.jp*

[Objective]

Japanese pearl oyster (*Pinctada fucata martensii*) is a mother oyster for Akoya pearl. Some surfactant was added to separate the pearl and after collecting pearl the meat and viscera is cut into pieces hardly to separate. Therefore, it is not being utilized and discarded as a by-product. However, A pearl oyster meat contains a valuable protein source because of the high protein level (about 10%) and multiple nutrients such as glycogen ceramide. Meanwhile, the fat accounts are very low. This study aims to explore a method of utilization of meat from Japanese pearl oyster. Firstly, various methods were used to evaluate the feasibility of protein recovery from Japanese pearl oyster.

[Methods]

Two types of Japanese pearl oyster meat (wih or without surfactant) were used for this study. The protein was recovered from Japanese pearl oyster using pH-shift processing (pH 2~13) and saltwater treatment (1~10% NaCl). The recovery proteins were compared by colour, proximate analysis, relative protein recovery yields, protein composition and amino acid composition.

[Results]

1. The sample without surfactant showed significant higher protein recovery yield.
2. The protein recovery yield and essential amino acid composition for using alkaline-aided treatment were highest, followed by the acid-aided and saltwater treatments, regardless with or without surfactant.
3. The recovered protein using alkaline-aided and saltwater treatments showed similar surface color, although both of the two types showed the lower L^*, a^* and b^* values than raw sample.
4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis indicated the recovered protein by alkaline-aided treatments showed a similar protein composition to raw sample, while the myosin heavy chain degraded significantly in the acid-aided and saltwater treatment.

[Conlusion]

The recovered protein using alkaline-aided treatment was superior to those using acid-aided and saltwater treatment regardless the sample with or without surfactant, suggested alkaline-aided is a suitable method for recovered protein from pearl oyster.

Keywords: Japanese pearl oyster, protein recovery, colour, amino acid composition

Effect of coating with apple polyphenols and chitosan on microbiological and quality properties of large yellow croaker (*Pseudosciaena crocea*)

Weiqing LAN[1,2,3] Jintao DU[1] Jiali LIU[1] Meng WANG[1] 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
* *jxie@shou.edu.cn*

[Objective]

The objective of this study was to evaluate the effects of apple polyphenols (AP) with chitosan (CS) coating on quality enhancement of large yellow croaker (*Pseudosciaena crocea*) during ice storage.

[Methods]

Fillets were randomly divided into four groups (16 fillets in each group): (1) samples were treated by deionized water (CK); (2) samples were treated by 1.0% (v/v) acetic acid (AA); (3) samples were treated by 1.0% AP with 1.0% chitosan (AP+1.0%CS); and (4) samples were treated by 1.0% AP with 2.0% chitosan (AP+2.0%CS). Various groups were dipped into the corresponding treatment solutions for 10 min in a refrigerator. The samples were removed from the solutions and drained well. After that, all samples were immediately packaged in polythene sterile bags, layers of fillets were covered with crushed ice and stored at 4 °C for 16 days.

[Results]

The results showed that the results presented in this study indicated that the combination of AP and CS coating was more effective in retarding lipid oxidation and moisture migration through fluorescence spectroscopy and LF-NMR analysis. Meanwhile, AP+1.0% CS and AP+2.0% CS groups showed a lower nucleotide breakdown and microbial growth than AA treated group or CK group. Moreover, AP+2.0% CS group could better maintain the color characteristics and physicochemical properties during ice storage. Compared with the CK group, the shelf life of AP+1.0%CS group was extended for 8 days, while AP+2.0%CS group reached more than 8 days. Hence, the antioxidant, antimicrobial and gas barrier effects of AP+2.0%CS coating may be a promising method of maintaining quality and extending the shelf-life of large yellow croaker.

[Conclusion]

The shelf-life of larger yellow croaker in control (CK) group was 8 days, while 1.0%AP +2.0%CS could delay the quality deterioration of iced large yellow croaker and extend the shelf life for another 8 days.

Keywords: Large yellow croaker; apple polyphenols; chitosan; low field nuclear magnetic resonance; fluorescence spectroscopy

Effect of Glycation on Physicochemical Properties and Volatile Flavor Characteristics of Silver Carp Mince

Junya Liu, Wenzheng Shi*

College of Food Science and Technology, Shanghai Ocean University, Shanghai, 201306, China
* *wzshi@shou.edu.cn*

[Objective]

It's generally accepted that Maillard reaction can enhance food aroma. However, the effect of protein glycation induced by Maillard reaction on the volatile flavour of fish substrate is less studied. The objective of this study is to explore the effect of glycation on physicochemical properties and volatile flavour characteristics of silver carp mince (SCM).

[Methods]

The changes in the degree of grafting, chemical composition, pH, colour, total amino acid composition, and volatile flavour compounds of SCM with or without glucose were studied at different heating times.

[Results]

The results showed that the addition of glucose could promote the glycation reaction rate of SCM. Lysine and cysteine were the main amino acids involved in glycation. Glycation enhanced the overall aroma of SCM by accelerating lipid oxidation and Strecker degradation.

[Conclusion]

It is concluded that glycation can enhance the volatile flavour of SCM during thermal processing and can be used as a volatile flavour enhancement technology for the development of protein nutrition food with good flavour from low-value fish.

Keywords: silver carp; glycation; heating time; total amino acids; volatile flavor; odor activity value (OAV)

Effects of Black Lemon Water Extracts on Anti-inflammation in RAW264.7 Macrophages and Adipogenesis in 3T3-L1 Preadipocytes.

Kuei-Tse Tseng[1], Yi-Jun Chen[2], Zwe-Ling Kong[3]

1, Department of Food Science, National Taiwan Ocean University, Beining Rd 2, Keelung, Taiwan

2, Department of Food Science, National Taiwan Ocean University, Beining Rd 2, Keelung, Taiwan

3, Department of Food Science, National Taiwan Ocean University, Beining Rd 2, Keelung, Taiwan

[Objective]

To explore the anti-inflammatory properties of black lemon on RAW264.7 macrophages and its effect on the differentiation of pre-adipocytes.

[Methods]

Black lemon which formed after Maillard reaction and the raw lemon that has not been treated by the Maillard reaction are cut into pieces, then 500g lemon or black lemon boiled in 1L distilled water for 20 min. Filter after cooling, and then freeze dry to obtain the Black lemon extracts (BLE) and Lemon extracts (LE). The samples were analyzed for phytochemical composition and antioxidant capacity. Moreover, using these samples to give mouse macrophages RAW264.7 that induce inflammation with LPS and 3T3-L1 pre-adipocytes for cell experiments to analyze the anti-inflammatory ability of BLE and its effect on fat production.

[Results]

The results showed that the total polyphenols and total flavonoids of BLE are only slightly higher than LE. The free radical scavenging rate of BLE by DPPH and ABTS methods are slightly higher than LE, and the chelating ability of ferrous ion is significantly higher than LE. Both LE and BLE have a tendency to gradually inhibit the inflammatory response cytokine as the dose increases, and BLE has the better effect. The results of the Western blot method showed that the expression of fat metabolism-related proteins increased with the increase in dose, and the expression of pAMPK, pPPARα, pHSLs563, and pHSLs565 in BLE was more obvious than that of LE.

[Conlusion]

This experiment has proved that Black Lemon extracts have better antioxidant capacity and anti-inflammatory response than raw lemons, and have the ability to increase fat metabolism, and may further promote the browning of white fat, thereby increasing energy metabolism to achieve the effect of inhibiting obesity.

Keywords: Black lemon, Maillard reaction, antioxidant, RAW264.7, anti-inflammatory, obesity, 3T3-L1, white fat browning.

Myosin in longitudinal retractor muscle of sea cucumber

Kunihiko Konno*, Xiuping Dong

National Engineering Research Center of Seafood, Dalian Polytechnic University, Dalian 116034, PR China
* *konno@fish.hokudai.ac.jp*

[Objective]

Sea cucubmer (SC) is an important sea food in Asian contiries, espedcially in Japan and China. Although there are many reports on the properties of body wall, edible part, there is practically no report on the myosin of the longitudinal retracter muscle attached on the inner body wall, which is used for slow moving on sea floor. Biochemical properties and denaturation profile of the myosin was studiedwith myofibrils (Mf).

[Methods]

The sample used was captured near Dalian. Mf was prepared from the muscle and suspended in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5). Electron microscopic observation, ATPase activities, chymotryptic digestion were employed to characterize the myosin. Myosin denaturation in heated Mf was analysed by measuring ATPase activity, salt-solubility (0.5 M NaCl, with or without 1 mM Mg-ATP), and chymotryptic digestion (0.5 M NaCl, 1 mM $CaCl_2$ at 20C using 1/500 (w/w) chymotrypsin).

[Results]

1. Electron microscopic image and low activities of three types of ATPase of Mf showed the muscle is slow muscle.
2. Chymotrypsin did not cleave the myosin at S-1/rod (with EDTA), but cleaved at HMM/LMM when dissolved in salt. Different structure at head/tail junction was proved.
3. Ca-ATPase inactivation was detected at raised temperatures such as 37.5C or 40C. It was proved that the myosin is very stable compared with the myosin in cold water fish species.
4. However, quick loss of salt-solubility upon heating was characteristic which was well explained by quick denaturation of rod confirmed by chymotryptic digestion.

[Conlusion]

Although sea cucumber used was from cold water environment, its myosin was significantly stable judged by ATPase inactivation. Well established rule that cold water species contains unstable myosin cannot be applied to SC.

Keywords: sea cucumber, myosin, stability, chymotryptic digestion

Immunostimulating Activity of the Extracts of Marine Resources

Kustiariyah Tarman[1,2]*, Wini Trilaksani[1], Iriani Setyaningsih[1], Mega Safithri[3], Joko Santoso[1], Uju[1]

[1]Deaprtment of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, IPB University
[2]Center for Coastal and Marine Resources Studies, IPB University
[3]Department of Biochemistry, Faculty of Mathemathics and Natural Resources, IPB University
* kustiaz@apps.ipb.ac.id

[Objective]

Marine resources such as spirulina, seaweed, sea cucumber, mangrove and virgin fish oil are rich of bioactive compounds and potentially used for healthy purposes. The bioactive compounds of these marine resources in increasing immunity are interesting to be more explored. The objective of the study was to observe the bioactive contents of the marine resources (spirulina, *Sargassum* sp., *Stichopus* sp., *Rhizophora* sp. and virgin fish oil) and to study their potential in increasing the immunity.

[Methods]

Marine resources used in this study including spirulina, *Sargassum* sp., *Stichopus* sp., and *Rhizophora* sp. were extracted using ethanol. The bioactive contents of the extracts was qualitatively observed using phytochemical test and the effect of the samples in increasing immunity was studied using MTT assay with white blood cells to measure the proliferation of the tested cells.

[Results]

1. The extracts of spirulina, *Sargassum* sp., *Stichopus* sp., and *Rhizophora* sp. contained saponins, flavonoids and steroids.
2. Virgin fish oil contained saponins and steroids.
3. Virgin fish oil, and spirulina, *Sargassum* sp., and *Rhizophora* sp. extracts at concentration of 20 ppm were potentially increased the proliferation of lymphocyte cells.
4. Spirulina extract showed the strongest effect in stimulating the the proliferation of lymphocyte cells.

[Conlusion]

Marine resources including spirulina, *Sargassum* sp., *Stichopus* sp., *Rhizophora* sp. and virgin fish oil contained various bioactive compounds. Microalga *Spirulina platensis* extract showed the strongest effect in stimulating the proliferation of lymphocyte cells.

Keywords: immunity, lymphocyte cell, phytochemical, *Rhizophora, Sargassum,* virgin fish oil

The use of seaweed, *Kappaphycus alvarezii*, in Indonesian Food and Beverages

La Ode M. Aslan*[1], Andi Besse Patadjai[2], Asnani Asnan[2]

1, Department of Aquaculture, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232, Indonesia
2, Department of Fisheries Processing Technology, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232, Indonesia
* *laodemaslan@uho.ac.id*

[Objective]

Indonesia is the largest producer of seaweed, *Kappaphycus alvarezii*, in the world. This seaweed species has a variety of nutrients that are needed for health as it is high in several nutrients such as carbohydrate, fiber, protein, lipid, and minerals. . Seaweeds have long been consumed in a variety of dishes such as raw salads, soups, cookies, meals, and beverages, particularly in the Indonesian coastal areas. One of the efforts to optimize the utilization of this seaweed is to apply the utilization of this seaweed through processed food and beverage products that could commonly consumed by the community.

The objectives of this paper are to describe the process of making processed food and beverage products containing seaweed and to promote the creation of new dishes of food and beverages.

[Methods]

After harvest, the whole part of seaweed thalli of *K. alvarezii* is washed thoroughly with seawater and dried 2-3 days in the sun to make dried seaweed. After that, the dried seaweed is washed with freshwater 3 times. The seaweed that has been cleaned with fresh water then drained until it is used to produce food and drinks from seaweed.

[Results]

The results showed that 25 different varieties of foods and drinks were produced using the seaweed. In addition, all products were well consumed by people living in urban area of SE Sulawesi province .

[Conlusion]

It is concluded that Seaweed has great potential to be a source of nutrients that are suitable for daily consumption.

Keywords: seaweed, *K. alvarezii*, food, beverages, Indonesia

Astaxanthin reduces colon damages caused by dextran sulfate sodium-induced colitis in BALB/c mice

Lanzhu Yang[1], Jing Li[1], Jingya Yang*[1]

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

yanglanzhuy@163.com

[Objective]

Astaxanthin is a xanthophyll carotenoid, mainly found in marine organisms such as *Haematococcus pluvialis* and shrimp. Due to its strong antioxidant property, anti-inflammatory and immune modulation, astaxanthin widely used for the prevention and treatment of various diseases, such as skin disease, cardiovascular diseases, obesity and diabetes. This study investigated the effects of astaxanthin in BALB/c mice with dextran sulfate sodium (DSS)-induced colitis.

[Methods]

A total of 40 BALB/c mice were divided randomly into 4 groups, namely normal group, model group, astaxanthin-treated group and positive group. Astaxanthin was mixed with a normal rodent diet (0.04%), and the BALB/c mice were first given 3% w/v DSS for 7 days and then astaxanthin for 21 days. Subsequently, the mice were sacrificed after an overnight fast. The effects of astaxanthin were determined by the disease activity index (DAI), colon length, as well as histological evaluations and biochemical parameters.

[Results]

Treatment with astaxanthin significantly decreased the DAI and ameliorated the inflammation-associated pathological damage in colon length, as well as the histopathological features of DSS-induced colitis. Astaxanthin also suppressed expression of proinflammatory cytokines in serum, including interleukin (IL)-1β, IL-6, and tumor necrosis factor-α. Additionally, as compared with model group, astaxanthin could significantly increase in colon total superoxide dismutase and glutathione peroxidase activities, and the decrease in colon malonaldehyde and hydrogen peroxide levels.

[Conlusion]

In conclusion, these findings indicated that astaxanthin could be of significant advantage in suppressing the colonic injury induced by DSS.

Keywords: astaxanthin, colitis, oxidative stress, proinflammatory cytokines

Microwave assisted optimization of chitosan extraction from *Portunus trituberculatus* shell

Li Hu[1],Yinzhe Jin*[1], Ningping Tao[2], Shanggui Deng[2]

1, College of Food Science and Technology, Shanghai Ocean University, Engineering Research Center of Food Thermal-processing Technology, National Experimental Teaching Demonstration Center for Food Science and Engineering, Shanghai 201306, China

* *yzjin@shou.edu.cn*

[Objective]

The objective of this study is to optimize the extraction process of chitosan from *Portunus trituberculatus* shell by physical (microwave, ultrasonic) assisted traditional chemical methods chemical method.

[Methods]

The deacetylation degree and Fourier transform infrared spectroscopy (FTIR) chitosan were studied by microwave method (250W, 5min), ultrasonic method (40KHz, 1h) and microwave combined with ultrasonic method under the condition of sodium hydroxide concentration of 50% and solid-liquid ratio of 1:25. Under the condition of microwave power of 250W, the X-ray diffraction (XRD) and scanning electron microscope (SEM) images of chitosan and standard chitosan obtained at 3min, 5min and 7min were compared. The deacetylation degrees of chitosan and commercial chitosan under microwave 1, 2 and 3 times were compared, and the effect of microwave times on FTIR was analyzed.

[Results]

1. There was no significant difference in deacetylation degree among microwave method (72.54%), ultrasonic method (71.41%) and microwave combined ultrasonic method (72.80%), and the microwave effect was better than ultrasonic method.
2. The three methods have peaks in amide region Ⅰ, amide region Ⅱ and amide region Ⅲ, which show the unique structure of chitosan.
3. The coincidence between XRD and commercial chitosan was high after 5 minutes of microwave. After 7 min, the SEM structure of chitosan was destroyed and the surface roughness increased.
4. After three times of microwave treatment, the degree of deacetylation of chitosan (78.15%) was similar to that of commercial chitosan (78.89%), and the coincidence between FTIR of chitosan and FTIR of commercial chitosan was the highest.

[Conlusion]

It is concluded that microwave-assisted chemical method can significantly shorten the time required for chitosan extraction and obtain products with similar quality to commercial chitosan, which provides a reference for the efficient extraction of high-quality chitosan.

Keywords: chitosan, chemical method, physical assistance, deacetylation, characterization

Research progress of glycosaminoglycans from marine organism

lingcui Li[1], Zhenqing Zhang[2], Peipei Wang[1*]

1. College of Food Science and Technology, Shanghai Ocean University, Shanghai, 201306， China;
2. Jiangsu Key Laboratory of Neuropsychiatric Diseases and College of Pharmaceutical Sciences, Soochow University, Suzhou, Jiangsu 215123, China
[*]ppwang@shou.edu.cn

[Objective]

Compared with terrestrial ecosystems, marine ecosystems have great biodiversity. They provide abundant resources for the discovery of new GAGs, with considerable structural diversity and novel biological activities. A large amount of treated marine biological waste provides a valuable emerging resource for large-scale separation and preparation of GAGs, which has great potential in future therapeutic applications.

[Methods]

Glycosaminoglycans (GAGs), also known as acid mucopolysaccharides, are a kind of linear polysaccharides formed by polymerization of repeating units of hexuronic acid and hexosamine disaccharide, and widely present in a variety of animal tissues. According to the structure, GAG is mainly divided into Heparin (HP), Heparan Sulphate (HS), Hyaluronic Acid (HA), Chondroitin Sulfate (CS), Dermatan Sulfate (DS), Keratan Sulphate (KS), and so on. GAGs have been reported to have a wide variety of biological activities, such as anticoagulation, antivirus, anticancer, immunomodulation and neuroprotection et al., which are closely related to the disaccharide units in its fine structure, the degree of sulfate substitution and the substitution position, and molecular weight.

[Results]

For example, chondroitin sulfate (CS), especially chondroitin sulfate E (CSE), is mainly obtained from marine organisms and has potential antiviral activity. A unique fucosylated chondroitin sulfate (FCS) isolated from sea cucumbers, has been reported to have anticoagulant, antiviral, antitumor and other biological activities, which structural type has not been found in mammals. Many GAGs-like glycans, which are also isolated from by-products of the food industry such as shrimp head and squid skin, have anti-inflammatory and anticoagulant activities, and have low hemorrhagic toxicity and side effects. In addition, compared to mammalian-derived GAGs, marine-derived GAGs are not contaminated by mammalian pathogens or prions.

[Conclusion]

Therefore, Researches on the source distribution, structural characteristics, biological activity and potential application value of marine biological GAGs could lay a foundation for the deep processing and high-value utilization of marine organisms, and provide scientific and material basis for the development of innovative marine carbohydrate-based drugs and new functional foods.

Keywords: Glycosaminoglycan; Source Distribution; Structural Characteristics; Biological Activity

Differentiation of three commercial tuna species through Q-Exactive Orbitrap mass spectrometry based lipidomics and chemometrics

Lingping Hu, Yaqin Hu*

College of Biosystems Engineering and Food Science, National-Local Joint Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang Key Laboratory for Agro-Food Processing, Fuli Institute of Food Science, Zhejiang University, Hangzhou 310058, China
** 1004346262@qq.com*

[Objective]

Mislabeling and adulteration of tuna occur due to the disappearance of morphological characteristics during processing. The authenticity of tuna has now being focused in the seafood supply chain. In this study, the lipid profiles of 3 commercial tuna species (skipjack tuna, bigeye tuna and yellowfin tuna) were investigated via ultra-high performance liquid chromatography couple with Q-Exactive Orbitrap mass spectrometry (UPLC-Q-Exactive Orbitrap MS) based lipidomics. Lipid biomarkers discovery was carried out by chemometrics. Verification by real samples showed that lipid biomarkers were effective for discrimination of tuna species.

[Methods]

Fifteen tuna individuals of each species were randomly divided into 3 groups. The middle section of flesh near the dorsal fin of tuna specimens was ground into fine powder in a liquid nitrogen bath respectively. The total lipids extraction was employed with the Folch method with slight modification. Lipids separation was performed on a 100×2.1 mm, 130 Å, 1.7 μm ACQUITY UPLC CSH C18 Column (Waters, Milford, MA, USA) in a Thermo Scientific Dionex UltiMate 3000 UPLCs coupled with a Thermo Scientific Q-Exactive Orbitrap mass spectrometer in both the ESI+ and ESI− modes. The open-source software MS-DIAL was used for identification and quantitation of lipid molecular species. Pairwise comparisons of the OPLS-DA models among three tuna species were performed for biomarker discovery. The ability of discovered lipid biomarkers for discrimination of three tuna species was evaluated by hierarchical cluster analysis (HCA). To confirm the validity of the lipid biomarkers for discrimination of tuna species, 15 real tuna samples which did not used for model building were applied for verification.

[Results]

1. Total of 212 and 227 lipid molecular species were identified from positive ion mode and negative ion mode respectively.
2. A total of 27 potential lipid biomarkers were obtained by 3 comparisons of OPLS-DA models.
3. HCA result showed that only using the screened lipid biomarkers can provide a discrimination ability equivalent to the complex OPLS-DA modeling described above.
4. Verification of real sample showed that our strategy was effective in discriminating tuna species and could be further applied for discrimination of other products containing lipids.

Effect of self-assembled type II collagen fibrils on morphology and growth of pre-chondrogenic ATDC5 cells

Linyan Shi*[1], Kazuhiro Ura[2], Yasuaki Takagi[2]

1, Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate, Hokkaido 041-8611, Japan

2, Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate, Hokkaido 041-8611, Japan

**shilinyan88@outlook.com*

[Objective]

The study aimed to prove that the type II collagen from sturgeon by-product notochord is suitable as a biomaterial to construct cartilage tissue engineering (CTE) scaffolds that offer natural habitat mimicking the environment of chondrocytes inside the human body. As regenerative medical scaffolds using type II collagen from aquatic animals are scarce, the method reported here is innovative and new to science.

[Methods]

Construction of type II collagen fibrils

Type II collagen was extracted and purified from the notochord of Bester sturgeon. The method to coat type II collagen fibrils onto the cell-culture coverslip (flip-contact method) was developed. The type II collagen solution concentration, the Na-phosphate buffer (PB) concentration for fibril formation, the time of fibril-formation, and the incubation temperature were screened step by step. The optimum coating conditions were finally determined as follows: 8 mg/ml collagen concentration, 35 μl/coverslip, 30 mM PB (pH 7.6), 48 h fibril-formation period followed by 48 h crosslinking at 12 °C. As a control, type II collagen molecules were coated using a conventional collagen-coating method.

ATDC5 cell culture and time-lapse observations

The morphological responses of pre-chondrogenic ATDC5 cells to the initial contact to type II collagen fibril-coated surface in the growth medium (GM) were studied using a time-lapse video system. After 20 h, the medium changed to differentiation medium (DM), and responses of ATDC5 cells were further observed for 12 h.

[Results]

In GM, the cells moved more intensely when attached to the molecules and gradually spread pseudopods in diverse directions; thus, cells showed flat polygonal or tapered morphology. In contrast, most cells on the fibrils stretched the two pseudopodia to counter directions; therefore, cells were long and tapered. In DM, cell proliferation continued on the fibrils but completely disappeared after 12 h on the molecules. Cells on the fibrils continued to be dynamic with active pseudopodia movement, while those on the molecules stopped moving without visible pseudopodia and became round after 12 h.

[Conclusion]

It is concluded that the type II fibrils had positive effects on the proliferation and activity of ATDC5 pre-chondrocytes, suggesting that type II fibrils are the promised material applying for the scaffolds of CTE.

Keywords: sturgeon, type II collagen fibrils, cartilage tissue engineering

Effects of partial replacement of NaCl by potassium salts on physicochemical properties of preserved tilapia fillets

Lunan Jing, Wenzheng Shi

College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
* *wzshi@shou.edu.cn*

[Objective]

To evaluate the effects of partial replacement of sodium chloride by potassium chloride on the physical and chemical properties of preserved tilapia fillets, and to provide theoretical basis for the further development of low-sodium preserved tilapia fillets.

[Methods]

Fresh tilapia fillets were used as raw materials, sodium chloride was replaced by potassium chloride with different mole replacement ratios, and tilapia fillets in the control group were replaced by sodium chloride with KCl at 10%, 30%, 50% and 70%, respectively, and the tilapia fillets with 100% NaCl content were used as research objects. The salt content for curing was 6% of the weight of tilapia fillets, and the ratio of solid to liquid was 1: 3. Water content, salt content, pH, color, texture and sensory characteristics of tilapia fillets were determined by curing at 4℃ for 4 h.

[Results]

The results showed that Compared with the control group, the content of sodium chloride was significantly increased with the increase of replacement ratio of potassium chloride ($P < 0.05$); There was no significant difference in water content ($P > 0.05$). Hardness was increased, and the difference was significant ($P > 0.05$). When the replacement ratio of potassium chloride was less than 50%, there was no significant effect on the physical and chemical properties of low-salt salted tilapia fillets ($P > 0.05$).

[Conlusion]

Therefore, potassium chloride partial replacement of sodium chloride in the appropriate replacement ratio, on the basis of maintaining the original physical and chemical properties, can reduce the use of sodium salt.

Keywords: potassium salts, tilapia fillets, the physical and chemical properties

Optimization of Gelatin Extraction Conditions from Pangasius Skin and Its Utilization as a Hard Capsule Material

Mala Nurilmala*[1], Hani Adytia Pratiwi[1], Roni Nugraha[1], Yoshihiro Ochiai[3]

1, Aquatic Product Technology Departement, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, Indonesia
2, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
3, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
* *mnurilmala@apps.ipb.ac.id*

[Objective]

Gelatin is a natural polymer made from collagen hydrolysis and is often utilized as a raw material for hard capsules. The aim of this research was to characterize and optimize gelatin production from pangasius (*Pangasianodon hypophthalmus*) skin using response surface methodology (RSM) and to determine the characteristics of the hard capsule.

[Methods] Gelatin and the hard capsule were prepared from the pangasius skin as previously reported (Nurilmala et al., 2020). The Central Composite Design (CCD) consisted of the two factors (NaCl concentration and temperature) with the eight responses (yield, moisture content, ash content, pH, gel strength, viscosity, and setting point) was performed on the capsules obtained.

[Results]

The result showed an optimum response at 0.337% citric acid (w/v) and 7.432 h of extraction time. The validation process obtained the yield of 19.08±0.08%, moisture content of 10.24±0.23%, ash content of 0.58±0.01%, pH of 4.47±0.10, gel strength of 147.08±0.92 bloom, viscosity of 55.50±0.71 mps, and setting point of 18.50±0.00°C.

[Conclusion]

The characteristics of the obtained hard capsules from gelation of pangasius skin including dimensions, moisture content, ash content, pH, and disintegration time met the requirement for the standard of the commercially available capsules.

Keywords: gelatin, RSM, skin, hard capsules

[Reference]

Nurilmala et al., Fisheries Science (2020) 86:917–924.

Effects of Drying Methods on Physico-chemical, Microbiological and Sensory Properties of Torpedo Scad (*Megalaspis cordyla*)

Md. Golam Rasul*[1], Chunhong Yuan[2], A.K.M. Azad Shah[1]

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

*rasul@bsmrau.edu.bd

Abstract

Normally fish are unhygienically sun dried which causes the considerable reduction of quality and safety of the product. So, the effects of drying methods on physico-chemical, microbiological, and sensory properties of *Megalaspis cordyla* were investigated. Fish were dried using traditional (without pre-treatment), improved (treated with 5% salt solution), improved-TC (treated with 5% salt solution and rubbed with chili and turmeric powder) and solar tunnel (treated with 5% salt solution) drying methods. Results showed the sensory evaluation revealed that solar-dried products showed comparatively better quality than the products produced by other drying methods. Rehydration ability of solar dried products was comparatively higher than other dried products. Moisture content of dried *M. cordyla* was ranged from 16.28% to 21.30%. However, no significant ($p > 0.05$) variation was found in protein, lipid and ash content on dry matter basis. Significantly ($p < 0.05$) the lowest peroxide value, acid value and carbonyl value were observed in solar dried products. In contrast, comparatively higher amount of PUFAs were found in solar dried products followed by improved, improved-TC and traditionally produced dried fish. The total aerobic plate count of dried *M. cordyla* varied between 2.04 log cfu/g and 5.71 log cfu/g. Results of this study suggested that the dried fish produced by solar tunnel drying method showed comparatively better quality than other drying methods for the consumer's safety.

Keywords: *Megalaspis cordyla*, drying methods, sensory properties, chemical composition, fatty acid, lipid oxidation.

Effects of chitosan coating combined with hypotaurine on the quality of shrimps (*Litopenaeus Vannamei*) during cold storage

Meiyu Chen[1,2], Lingping Hu[1,2], Zhiheng Hu[1,2], Yaqi Zhou[1,2], Gaoshang Li[1,2], Yaoxian Chin[1], Yaqin Hu[1,*]

1 College of Food Science and Technology, Hainan Tropical Ocean University, Yucai Rd 1#, Sanya 210027, China
2 College of Biosystem Engineering and Food Science, Zhejiang University, Yuhang Tang Rd 866#, Hangzhou 310058, China
* *1004346262@qq.com*

[Objective]

Chitosan is a widely-used biomaterial for seafood preservation. Previous studies have also shown that hypotaurine delayed the formation of melanosis of shrimps during cold storage. The objective of this study was to verify whether or not chitosan coating combined with hypotaurine is an ideal alternative method to traditional treatments of sulfates, and exhibited synergistic effects on the quality of shrimps (*Litopenaeus vannamei)* during cold storage.

[Methods]

Shrimps (*Litopenaeus Vannamei*) were randomly divided into 5 different groups for further treatments, including the control group, hypotaurine treatment (HPT) group, chitosan treatment (CTS) group, hypotaurine combined with chitosan treatment (HPT-CTS) group, and sodium metabisulfite treatment (SMS) group. The concentration of the soaking solutions, i.e., HPT, CTS, HPT-CTS and SMS, were 2% (w/w), 1%, 2%+1%, and 1.5%, respectively. Shrimps were immersed into each solution at a ratio of 1:2 (solution to shrimp) for 30 min at 4 °C and drained naturally at room temperature. Subsequently, shrimps with different treatments were packaged in sterile polyethylene bags and stored at 4 °C for further 10-day experiments. Quality changes of total plate counts (TPC), colour differences, pH, TVB-N and TBA values, degrees of melanosis, textural properties, and sensory properties were carefully evaluated.

[Results]

Results revealed that chitosan coating combined with hypotaurine exhibited excellent performances and synergistic effects on inhibiting microbial growth and delaying quality deterioration, in terms of the changes of colour differences, texture, and sensory scores, as well as the degree of melanosis, protein degradation, and lipid oxidation, compared to the control group, HPT group, CTS group, and SMS group.

[Conclusion]

In conclusion, chitosan coating combined with hypotaurine was a more effective method for shrimp preservation during cold storage, with significant enhancements on safety-quality properties and shelf-life.

Keywords: *Litopenaeus Vannamei*; chitosan coating; hypotaurine; chilled storage; quality evaluation

Active-intelligent food packaging nanofibers containing double indicators

Mengxia Duan[1,3], Shan Yu[1], Jishuai Sun[1], Haixin Jiang[1], Jianbo Zhao[1], Cailing Tong[1], Yaqin Hu[4*], Jie Pang[1,2,3*], Chunhua Wu[1,2,3*]

1. College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China;
2. Engineering Research Centre of Fujian-Taiwan Special Marine Food Processing and Nutrition, Ministry of Education, Fuzhou, Fujian, 350002, China;
3. Key Laboratory of Marine Biotechnology of Fujian Province, Institute of Oceanology, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China;
4. College of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, China

**yqhu@hntou.edu.cn (Y. Hu); *pang3721941@163.com (J. Pang); *chwu0283@163.com (C. Wu).*

[Objective]

Research on active-intelligent food packaging is one of the important measures to ensure food safety. Although there are many reports on the active-intelligent food packaging, there are few reports on the active-intelligent food packaging loaded with double indicators, which is used to convey information about food quality and extend food shelf life. The active-intelligent food packaging containing double indicators is to improve the antioxidant and antibacterial capacity of food packaging and the sensitivity of food quality monitoring.

[Methods]

Curcumin (CR) purchased from Aladdin Reagents Co., Ltd. (Shanghai, China). Anthocyanins (ATH) purchased from Xian Huilin Biological Technology Co., Ltd. (China). Pullulan (30%, w/v) and chitin nanofibers (0.25%, w/v) were dissolved in 1% acetic acid solution. The solution was separated into four parts with the same volume. Different proportions of colorants were added to the four solutions. The solution without the colorant was called "PCN," and the solutions with 0.2% (w/v) CR or 3.8% (w/v) ATH were named "PCN/CR" and "PCN/ATH," respectively. The solution with both 0.2% (w/v) CR and 3.8% (w/v) ATH was called "PCN/CR/ATH." The four solutions were stirred in a water bath at room temperature for 12 h and then allowed to stand for 6 h to obtain the spinning solution. The nanofibers were then prepared using electrospinning technology. The electrospinning equipment used in this study was uniaxial blend electrospinning with a set voltage of 26 kV, a receiving distance of 15 cm, and a jet speed of 0.1 mm/min.

[Results]

1. Scanning electron microscopy results indicated that CR and ATH could be well embedded in the PCN nanofiber-forming matrix and had only effect on the nanofiber diameter.
2. The DPPH free radical scavenging rate of the PCN/CR/ATH nanofiber was 61.72%± 1.73%, which was higher than the other nanofibers. It had been verified that the double indicators nanofiber had higher antioxidant activity than the single indicator nanofibers.
3. The antibacterial activity of the nanofiber loaded with double indicators against *E. coli* and *S. aureus* was similar to that of the nanofiber loaded with CR but higher than that of the nanofiber loaded with ATH.
4. The color change of the PCN/CR/ATH nanofiber at different pH values is more obvious than that of the nanofibers loaded with single indicator. PCN/CR/ATH nanofiber can be more sensitive to monitor the changes of food environment.

[Conclusion]

The nanofiber loaded with CR and ATH indicators had higher antioxidant and antibacterial abilities than the nanofibers loaded with single indicator, so it was more beneficial to extend the shelf life of food. In addition, the above-mentioned double indicators nanofiber was more sensitive to the changes of the food environment, which can convey food quality information more effectively.

Keywords: active-intelligent packaging, nanofiber, double indicators

Progress in the study of functional and flavour properties of fish protein enzymatic products

Mingyu Yin[1], Yinci Xi*[1], Xichang Wang*[1]

1, College of Food Science and Technology, Shanghai Ocean University, Hucheng Rd 999#, Shanghai 201306, China

** yin214300841@163.com.*

[Objective]

Based on the utilization of fish protein resources and maximization of added value, a systematic review of domestic and international research on the preparation of fish protein enzymes hydrolysis (FPEH) using enzymatic technology is presented.

[Methods]

From the perspective of food ingredients, firstly, the sources and preparation of FPEH are outlined, including fish species, preparation process and proteolytic parameters; secondly, the solubility, emulsification, foaming, water-holding and oil-holding properties of FPEH are analysed around the functional properties of food ingredients; then, the taste and odour properties of FPEH are further explored with respect to their flavour properties.

[Results]

The good functional food properties of FPEH have determined that it has great potential for use in food ingredients. However, while it brings good flavour, it also has undesirable bitterness and fishy taste, which needs to be further explored and optimised in terms of pre-treatment, enzymatic conditions and subsequent flavour enhancement techniques to provide good properties as a nutritional base, functional base and flavouring base.

[Conlusion]

It is concluded that more research should be carried out in the future to optimise the optimum process parameters with the functional properties and flavour characteristics of the food as the target. For some raw materials with a heavy fishy flavour, this can be combined with an effective pre-treatment to control the problem at source

Keywords: fish protein enzymic hydolysates, food functional properties, flavor profiles, taste, odour

Optimisation of enzymatic hydrolysis conditions for yellowfin tuna rest raw materials using alcalase enzyme

Musiige Denis[1], Hung Duc Pham[2], Minh Van Nguyen*[3]
1, Tonnet Agro Engineering Company Limited, Plot 699 Gayaza Road Kyebando behind Fish Factory P.O.BOX35048, Kampala, Uganda
2, Aquaculture Institute, Nha Trang University, 02 Nguyen Dinh Chieu, Nha Trang, Vietnam
3, Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu, Nha Trang, Vietnam
* *minhnv@ntu.edu.vn*

[Objective]

It is estimated that the rest raw materials from the tuna canning industry is about 450,000 tons anually. Therefore, these materials are potential sources for protein hydrolysates. The objective of this study is to optimize enzymatic hydrolysis conditions for the production of fish protein hydrolysates from yellowfin tuna rest raw materials.

[Methods]

Head and viscera from Yellowfin tuna (*Thunnus albacares*) were obtained from the local tuna processing company. Heads and viscera were separately hydrolysed using Alcalase with activity of 2.4 AU/g which is a bacterial endoproteinase from a strain of *Bacillus licheniformis*. Hydrolysis conditions (viz. temperature, time, and enzyme to substrate level) were optimized by a complete composite design (CCD) of a response surface methodology (RSM) using JMP software (version 15, SAS, Cary, NC). The degree of hydrolysis (DH) and protein solubility were carried out as dependent variables of experiments.

[Results]

The results showed that the regression coefficients observed during both experimental and validation runs were close to 1.0, showing the validity of prediction models. All the hydrolysis conditions had a significant effect ($p<0.05$) on both the degree of hydrolysis and solubility for both viscera and head. A hydrolysis time of 6.7 h, temperature of 53.4°C and an enzyme to substrate level of 0.88% (v/w), were found to be the optimum conditions to obtain a higher degree of hydrolysis of 66% and solubility 71.0% for visceral hydrolysis. The optimal conditions for head hydrolysis were found to be 7 h for hydrolysis time, 55°C for hydrolysis temperature and 0.82% (v/w) enzyme to substrate level yielding a higher degree of hydrolysis of 28% and solubility 89.1%.

[Conlusion]

The amino acid composition of both, the viscera and head protein hydrolysates prepared using the optimized conditions revealed that the protein hydrolysates were similar to FAO/WHO reference protein. Therefore, the protein hydrolysates would have the potential for application as an ingredient in balanced fish diets for fingerlings.

Keywords: yellowfin tuna, alcalase protease, hydrolysis optimization, degree of hydrolysis

Growth stimulation effect of chitosan on in vitro culture of mokara orchid

Minh-Thu Pham-Thi[1*], Trang Ngoc Thien Nguyen[1], Dinh Kim Huynh[1]

1, Institute of Biotechnology and Environment, Nha Trang university, 2 Nguyen Dinh Chieu street, Nha Trang city, Khanh Hoa province, Vietnam
** thuptm@ntu.edu.vn*

[Objective]

Mokara orchid is a tropical species with colorful and long-lasting flowers. Its vitality and endurance makes mokara an intensively planted orchid in Southest Asia and especially Vietnam for the last several decades. Thus, it has been widely propagated by both traditional and tissue culture methods. However, despite the rapid multiplication, the micropropagated mokara shoot and plantlet grow very slowly. On the other hand, it is well-known that chitosan might work as a plant growth promoter both *in vivo* and *in vitro*. Thus, chitosan was used in this study to examine its ability in enhancing the *in vitro* growth of mokara shoot.

[Methods]

Chitosans extracted from shimp shells and squid pens with 3 molecular weight ranges (Mw <10, 30-50 and 80-100 kDa, coded as Mw10, Mw30 and Mw80; respectively) and 3 deacetylation degrees (72-75, 82-85 and 92-95%, coded as D70, D80 and D90; respectively) were used in the experiments. They were added to the shoot elongation medium to select suitable extraction origin, molecular weight and deacetylation degree at a concentration of 20 ppm. The most suitable chitosan was then further examined different concentrations from 5 to 320 ppm. Height of shoot, formation of new shoot, new leaf and new root were recorded to compare between treatments.

[Results]

As the result, chitosan originated from shrimp shell with Mw30 and D80 was the most suitable for the purpose of enhancing shoot development. Among the examined concentrations, the application of this kind of chitosan at 20 ppm resulted in highest shoot multiplication (1,67 shoots), shoot height increase (17,2 mm for main shoot) and new leaf formation (5,8 leaves for main shoot) after 2 months. The formation of new roots were not different between treatments.

[Conlusion]

Thus, chitosan could be used as a growth stimulator for mokara *in vitro* growth. More observation should be done in a longer time and *in vivo* conditions.

Keywords: chitosan, mokara orchid, growth, *in vitro*

Photoinactivation of bacteriophage MS2 in oyster-derived matrices by microencapsulated rose bengal

Mohamad Eshaghi Gorji, Dan Li*
Department of Food Science & Technology, Faculty of Science, National University of Singapore, Singapore *
fstlda@nus.edu.sg

[Objective]

Bivalve molluscan shellfish such as oysters are filter feeders and could potentially bio-accumulate viral particles from large quantities of water. Photoinactivation provides a cold-sterilization option against the viral contamination as the photosensitizers could lead to oxidative damage and death of viruses.

[Methods]

In this study, Rose Bengal (RB), a strong photosensitiser, was encapsulated with alginate and subsequently coated with chitosan. Then, photoinactivation of bacteriophage MS2 was tested *in vitro* (oysters' tissues) and *in vivo* (live oyster) using encapsulated RB.

[Results]

An extra coating of chitosan effectively prevented the release of RB from the microbeads in seawater, and more importantly, successfully enhanced the selectivity of the photoinactivation via the electrostatic forces between chitosan and virus particles in oyster-derived matrices tested both in vitro and in vivo.

[Conlusion]

This study demonstrated a new strategy in delivering comprehensively formulated biochemical sanitizers in bivalve shellfish through their natural filter feeding activity and thereby enhancing the mitigation efficiency of foodborne virus contamination.

Keywords: MS2, virus, photosensitiser, encapsulation, oyster

Syntheses and evaluation of C*oumarin* devirates as novel G protein–coupled receptor inhibitors and activators

Na Li*[1] ,Wenhui Wu[1], Ruihua Guo[1]

1. college of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
**ln16658@163.com*

[Objective]

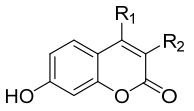
It's generally accepted that coumarin and its derivatives have a wide range of biological activities, such as anti-cancer and anti-inflammatory. The objective of this study is to improve inhibitory and activation potency on GPCRs of coumarin derivatives through structural modification.

[Methods]

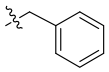
25 coumarin derivatives were synthesized *via* phenolic *O*-acylation/*O*-alkylation (nucleophilic acyl/alkyl substitution) or Pechmann reaction in our work. The synthesized compounds were fully characterized by NMR and MS spectroscopy.All the pharmacological activities were evaluated in G protein–coupled receptors (GPCRS) by double antibody sandwich ELISA (DAS–ELISA) *in vitro*.

[Results]

The results showed that derivatives possessed different bioactivities on GPCRs ranging from inhibitors to activators. 7,8-dihydro derivatives **13–17** with different substitutes at C–4 position had week activation potency (33.86%-47.10%). 7,8-dihydro derivatives **8** showed inhibitory activity on GPCRs with inhibition rate of 32.73%. For 7-hydro derivatives **1**–**12** with different substitution patterns/groups in C-3 or C-4, derivatives **3**–**5**, **10** and **11** showed activation potency on GPCRs with an EC_{50} value of 2.40, 0.18, 0.08, 0.15 and 0.54 nM, respectively. Derivatives 7 possessed inhibitory activity on GPCRs with an IC_{50} value of 5.03 nM.



	R_1	R_2
3	$-CH_3$	
4	$-CH_3$	$-CH_3$
5	$-CF_3$	-H

	R_1	R_2
7	$-CH_3$	
10		-H
11		-H

[Conclusion]

It is concluded that the most derivatives possessed moderate activation potency on GPCRs. Among them **3 - 5, 10** and **11** showed remarkable activation potency on GPCRs .GPCRs are important drug targets for the treatment of metabolic and nervous system related diseases. Therefore, coumarin derivatives synthesized in this paper are expected to be used as targets for the treatment of GPCRs metabolic related diseases.

Keywords: coumarin, G protein-coupled receptors, synthesis, structure-activity relationships

Effect of *Ulva nitidum* hydrolysated polysaccharide sulfate on High-Fat Diet Induced Obese Rats after Developing Osteoarthritis Caused by Ligamentous/ Meniscal Injury

Nai-Lian Wu[1], Yi-Chia, Lin[1], Zwe-Ling Kong[1*]
1, Department of Food Science, National Taiwan Ocean University, Keelung City 20224, Taiwan (R.O.C.)
[*]*kongzl@mail.ntou.edu.tw*

[Objective]

The objective of this study is to explore *Ulva nitidum* sulfated polysaccharides mitigated Osteoarthritis (OA) caused by high-fat diet-induced obesity (OB) and anterior cruciate ligament and meniscus injury (ACLT + MMX).

[Methods]

In vitro, *Ulva nitidum* sulfated polysaccharides (UE) and *Ulva nitidum* sulfated polysaccharides hydrolyzed by enzymes (UH) were respectively added to LPS-induced RAW264.7 to explore cell viability and nitric oxide (NO) release. Besides, MIA-induced SW1353 adds UH and UE respectively to analyze OA-related factors. *In vivo*, investigating whether the UH has the potential to reduce the inflammation of the knee joint in rats and improve the course of OA under the condition of high-fat diet-induced obesity and ACLT + MMX.

[Results]

The results showed that UE and UH can inhibit LPS-induced RAW264.7 cell apoptosis and reduce NO production and UH show a lower NO production level in cells than UE. UH and UE also inhibited the expression of OA-related factors in human chondrocytes SW-1353 induced by MIA with UH have a greater inhibition in the cells. *In vivo study*, OBOA rats model was induced by high-fat diet and meniscus meniscectomy and anterior cruciate ligament transection. UH significantly reduces the levels of adiponectin, leptin, TG, TC, MMP-3, CTX-II, pro-inflammatory cytokines, and increases the levels of antioxidant enzymes in serum of OA rats. Furthermore, treated with UH (100 mg/kg b.w.) shows less proteoglycan loss in OBOA rats.

[Conlusion]

It is concluded that UH could contribute to the treatment of OA and reduce the pain of OA patients.

Keywords: *Ulva nitidum*, Hydrolysate, Seaweed, Sulfated polysaccharide, High-fat diet, Osteoarthritis, Inflammation

FGFC1 Selectively Inhibits Erlotinib-Resistant Non-small Cell Lung Cancer via Elevation of ROS Mediated by the EGFR/PI3K/Akt/mTOR pathway

Namin Duan*[1], Bing Zhang[1], Jingwen Feng[1], Xiaohui Hu[1], Wenhui Wu[1] , Ning Liu[1],

1, college of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
* *naminduan@163.com*

[Objective]

FGFC1 (Fungi fibrinolytic compound 1), a type of bisindole alkaloid from a metabolite of the rare marine fungi *Starchbotrys longispora.* FG216, has exhibited good fibrinolytic activity and anti-inflammatory activity. However, the potent efficacy of FGFC1 in human cancers requires further study. Herein, we demonstrated that FGFC1 selectively suppressed the growth of NSCLC cells with *EGFR* mutation.

[Methods]

The cell counting kit-8 assay was used to determine relative cell viability; flow cytometry was used to evaluate apoptosis and ROS Measurement; real-time PCR and Western blotting analysis were performed to measure the expression of apoptosis-related genes in NSCLC cells; wound healing and Transwell invasion assays were used to measure the ability of migration and invasion; Western blotting was performed to measure the expression of kinase proteins involved in the EGFR/PI3K/Akt/mTOR signaling pathway, exploring the influence of FGFC1 on this signaling pathway.

[Results]

The results showed that FGFC1 treatment significantly induced the apoptosis of H1975 erlotinib-resistant NSCLC cells in a dose-dependent manner, which was proved to be mediated by mitochondrial dysfunction and elevated accumulation of intracellular reactive oxygen species (ROS). Scavenging ROS not only alleviated FGFC1-induced apoptosis but also relieved the decrease of phospho-Akt. We further confirmed that FGFC1 significantly decreased the phosphorylation of protein EGFR, PI3K, Akt, and mTOR in H1975 cells. Notably, PI3K inhibitor (LY294002) could promote the accumulation of ROS and the expression levels of apoptosis-related proteins induced by FGFC1. Molecular dynamics simulations indicated that FGFC1 inhibited EGFR and its downstream EGFR/PI3K/Akt/mTOR signaling pathway through directly binding to EGFR and showed a much higher binding affinity to $EGFR^{T790M/L858R}$ than $EGFR^{WT}$. Additionally, FGFC1 treatment also inhibited the migration and invasion of H1975 cells.

[Conlusion]

It is concluded that FGFC1 effectively inhibited tumor growth in the nude mice xenograft model of NSCLC. Taken together, our results indicate that FGFC1 may be a potential candidate for erlotinib-resistant NSCLC therapy.

Keywords: FGFC1, Non-small cell lung cancer, Erlotinib-resistant, Mitochondrial dysfunction, ROS, EGFR/PI3K/Akt/mTOR pathway

NUTRITIONAL AND FUNCTIONAL PROPERTIES OF PROTEIN HYDROLYSATE FROM WHITE LEG SHRIMP HEAD

Nguyen Thi My Huong

Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu, Nha Trang, Vietnam
huongntm@ntu.edu.vn

[Objective]

The objective of this study is to assess nutritional and functional properties of shrimp head protein hydrolysates such as solubility, foaming capacity and emulsifying capacity.

[Methods]

White leg shrimp heads from industrial processing were hydrolyzed by Protamex enzyme 0.5% (w/w) with a water/material ratio of 1:1 at the temperature of 50°C and different hydrolysis times (1h, 2h, 3h and 4h) to obtain four shrimp head protein hydrolysates.

[Results]

1. The degree of hydrolysis of the protein hydrolysates from shrimp heads increased with the increase in hydrolysis time. The degrees of hydrolysis after 1h, 2h, 3 h and 4h of hydrolysis were 17.35%, 25.76%, 29.87% and 31.74%, respectively.
2. The protein hydrolysate from white leg shrimp head after 4 h of hydrolysis had 74.32% protein, 0.85% lipid and 11.18% ash.
3. The shrimp head protein hydrolysate was found to have high nutritional value with the ratio of essential amino acids to total amino acids of 41.13%. The amino acids with high contents were glutamic, aspartic, glycine, leucine, lysine and valine.
4. The solubility of shrimp head protein hydrolysates increased when hydrolysis time increased from 1h to 4h. The protein hydrolysate with hydrolysis time of 4h had the highest solubility (95.85%). Foaming capacity and emulsifying capacity of shrimp head protein hydrolysates decreased with increasing hydrolysis time. The shrimp head protein hydrolysates had foaming capacity of 15.83%, - 26.34% and emulsifying capacity of 13.37 - 23.45 ml/g.

[Conlusion]

It is concluded that with high nutritional value and important functional properties, the shrimp head protein hydrolysates could be used as a protein source in food systems and were a potential ingredient for food industry.

Keywords: Functional property, nutritional property, protein hydrolysate, white leg shrimp head.

Effects of Different Collagen on Osteoarthritis in Male Rats Induced by Anterior Cruciate Ligament Transection and Medial Meniscectomy

Pei-Yu Wang[1], Jerrell Felim[1], Zwe-Ling Kong[1]
[1]Department of Food Science, National Taiwan Ocean University, Taiwan

Abstract

Osteoarthritis (OA) is arthritis characterized by degeneration of the articular cartilage and joint dysfunction. Pharmacological and non-pharmacological strategies have been used to manage these diseases. Due to its various therapeutic properties, marine collagen has received much attention in pharmacological applications. As a result, the goal of this study was to compare jellyfish collagen, collagen peptide, other sources of marine collagen, and glycine for the treatment of OA. In a cell experiment, RAW 264.7 cells were used, and they were inflamed with Lipopolysaccharide (LPS).

[Methods]

An anterior cruciate ligament transection with medial meniscectomy operation (ACLT + MMx) was used in an animal experiment to generate osteoarthritis symptoms in rats. Before surgery, male Sprague-Dawley rats were fed a chow-fat diet for two weeks for domestication. Rats were treated with samples for six weeks by oral administration then euthanized to evaluate proinflammatory cytokines expression in blood plasma.

[Results]

Rats with treated groups result in lower levels of matrix metalloproteinase COX-2, MMP-13, and CTX-II and less cartilage breakdown. Collagen and glycine have protective properties, while collagen peptides have cartilage repair effects, but less protective effects. Due to protection and cartilage regeneration in the knee, jellyfish collagen peptide at a dose of 5 mg/kg b.w. has the most promise for OA treatment.

[Conlusion]

The NO inhibition predicted by collagen's -OH and -NH2 groups, which bind to NO radicals and glycine, can cause morphological changes in cells and suppress NO production. An anterior cruciate ligament transection with medial meniscectomy operation (ACLT + MMx) was used in an animal experiment to generate osteoarthritis symptoms in rats. Before surgery, male Sprague-Dawley rats were fed a chow-fat diet for two weeks for domestication. Rats were treated with samples for six weeks by oral administration then euthanized to evaluate proinflammatory cytokines expression in blood plasma.

Keywords: Osteoarthritis; Collagen peptide; Jellyfish collagen; Glycine

Inhibition of hydrogen peroxide induced injuring on human skin fibroblast by protease hydrolysates from the carcass of Symplectoteuthis oualaniensis

Pengjie Tang [1], Bailie Li [1], Wenhui Wu[1,] *
1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China;
15722869010@163.com (Pengjie Tang)

[Objective]

This study was to isolated and purify antioxidant peptides from protease hydrolysates from the carcass of Symplectoteuthis oualaniensis (PHCSO) and further evaluate PHCSO and PHCSO-2 (< 10 kDa) separated by ultrafiltration cytoprotective effects against H_2O_2-induced oxidative stress in human skin fibroblast (HSF) cells.

[Methods]

Preparation of PHCSO, Sephadex G-50 gel separation of PHCSO-2, DPPH radical scavenging activity, Hydroxyl radical scavenging activity, HSF cells culture, Effect of PHCSO on cell viability of HSF cells, Establishment of H_2O_2-induced oxidative damage model of HSF cells, PHCSO protects HSF cells from oxidative damage induced by H_2O_2, ROS fluorescence staining, and Effect of PSCH on intracellular SOD activity, CAT activity, GSH and MDA level and cells secretes the inflammatory factors IL-1, IL-6 and TNF-α level.

[Results]

The results showed that the cell proliferation of PHCSO and PHCSO-2 both can protect HSF cells from being injured by hydrogen peroxide (H_2O_2). Moreover, ROS and inflammatory factors detection indices, including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA), including interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) indicated that PHCSO-2 could improve cellular ability to scavenge free radical and decrease the levels of inflammatory factors. This work explored the antioxidant properties of PHCSO and the inhibition of H2O2 induced injuries on HSF cells by PHCSO and PHCSO-2, which may further evaluate the application of PHCSO and PHCSO-2 on cosmetics.

[Conlusion]

In this work, the H_2O_2-induced HSF cell injury model was used to detect the cytotoxicity of PHCSO and PHCSO-2 and the protective effect on HSF cell injury. It is found that both PHCSO and PHCSO-2 have protective effects on HSF cell damage, and PHCSO-2 has a better protective effect on HSF cell damage than PHCSO. In summary, it is found that PHCSO-2 exhibits significant resistance to both oxidation and inflammation caused by HSF cells under H2O2 damage, and can effectively protect the skin from H2O2 damage. The ability of PHCSO-2 to resist H2O2 damage gives it a broad application prospect. This article can provide a certain theoretical basis for its development and use in the cosmetics or pharmaceutical fields.

Keywords: Protease hydrolysate; Human skin fibroblast; Hydrogen peroxide; ROS Enzyme activity; Inflammatory cytokines

Polyphenols extracted from *Enteromorpha clathrata* alleviates inflammation in lipopolysaccharide-induced RAW 264.7 cells by inhibiting the MAPKs/NF-κB signaling pathways

Ping Huang [a], Jingxia Hong [a], Jie Mi [a], Bolun Sun [a], Jinjie Zhang [a,b], Chao Li [a,b,*], Wenge Yang [a,b,*]

[a] College of Food and Pharmaceutical Sciences, Ningbo University, 315211, China
[b] Key Laboratory of Animal Protein Food Deep Processing Technology of Zhejiang Province, Ningbo University, Ningbo 315211, China
** lichao@nbu.edu.cn; yangwenge@nbu.edu.cn*

[Objective]

Enteromorpha clathrata (*E. clathrata*) is a common edible seaweed along the southeast coast of China, and has benefits for human health. Although there are many reports on the biological functions of *E. clathrata* derived components, especially on polysaccharides, research on the bioactive activity of polyphenols extracted from *E. clathrata* is scarce. The present study was aimed to evaluate the therapeutic potential of ECPs as anti-inflammatory agents using a series of cell-based bioassays.

[Methods]

E. clathrata was collected from Ningbo, China. The polyphenol-enriched fraction was extracted from *E. clathrata* with ethyl acetate (ECPs), and six individual polyphenols were isolated from ECPs via high-speed counter-current chromatography coupled with high-performance liquid chromatography. The anti-inflammatory effects of ECPs on LPS-induced RAW 264.7 macrophages were examined, by monitoring the activation of MAPKs and NF-κB signaling pathways and subsequent production of inflammation-associated mediators at both gene and protein levels, using various techniques including quantitative real-time PCR, enzyme-linked immunosorbent assay (ELISA), western blot, and flow cytometry.

[Results]

1. ECPs and the three individual polyphenols, including (-)-epicatechin, epigallocatechin-3-*O*-gallate - and (-)-epicatechin-3-*O*-gallate, showed *in vitro* anti-inflammatory activities by inhibiting LPS-induced production of NO and its upstream enzyme iNOS, the pro-inflammatory cytokines (IL-6, IL-1β and TNF-α), as well as the phagocytotic capacity, without cytotoxicity.
2. The mechanism study further revealed that these anti-inflammatory properties were, at least partly, attributed to the suppressed activation of nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK) signaling pathways.

[Conlusion]

These findings indicated for the first time the correlation between the anti-inflammatory activity of ECPs and NF-κB and MAPK signaling pathways, suggesting that polyphenol-enriched organic fraction of *E. clathrata* could be potential candidate as therapeutic agent for treating inflammatory diseases.

Keywords: *Enteromorpha clathrata*; polyphenol; anti-inflammatory activity; MAPKs; NF-κB

Comparison of Volatile Odorants in Different Edible Parts of Female Portunus trituberculatus Fed by Formulated Diets and Mixture Diets

Qin Chen[1], Wenzheng Shi*[1]

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
Chenqin_0703@163.com

[Objective]

The objective of this study is to explore the differences in volatile order components of female *Portunus trituberculatus* under different feeding modes and analyze the substitutability of the two feeding conditions.

[Methods]

Taking female *Portunus trituberculatus* as the research object, headspace solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) was used to study the different performance of female *Portunus trituberculatus* cultured with compound feed and traditional bait.

[Results]

The results showed that 35 volatile substances were detected in female *Portunus trituberculatus* cultured with compound feed, 24 types of body flesh, 23 types of hepatopancreas, and 29 types of gonads were detected in the traditional diet cultured *Portunus trituberculatus*. 39 kinds of volatile substances, 17 kinds of flesh, 20 kinds of hepatopancreas, 18 kinds of gonads were detected in traditional group. The main volatile components of the two feeding modes include nonanal, sunflower aldehyde, undecyl aldehyde, dodecyl aldehyde, trimethylamine and so on.

[Conclusion]

It is concluded that from the perspective of smell, compound feed feeding can replace traditional bait feeding.

Key words: *Portunus trituberculatus*, compound feed, traditional bait, volatile constituents, GC－MS

Variations in quality properties and oxidative stability of lightly salted fish meat during processing and storage

Qingqing Jiang*[1,2], Yaqin Hu[3], Wenzheng Shi[1], Xichang Wang[1], Emiko Okazaki[2]

1,Department of Food Science and Technology, Shanghai Engineering Research Center of Aquatic Product Processing & Preservation, Shanghai Ocean University, Shanghai 201306, China
2,Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan
3,College of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, China
*qqjiang@shou.edu.cn

[Objective]

The objective of the present study was to clarify the changes in the quality properties and the interrelationship among discoloration, myoglobin transformation, and lipid oxidation in lightly salted fish meat during storage.

[Methods]

The quality properties (water-holding capacity, color, and texture) were evaluated after thawing immediately at specified intervals of frozen storage, and the meat was kept at –80°C before the physicochemical analyses.

[Results]

The bright and vivid color became totally brown after 4 w at –20°C and 8 w at –30°C, where the a^*/b^* value decreased from 1.35 to 0.38 and 0.58, respectively ($p < 0.05$). Discoloration and lipid oxidation occurred concurrently in lightly salted tuna meat, and it was speculated that the oxidation of oxymyoglobin to metmyoglobin exacerbated lipid oxidation and vice versa. Storage at –40°C or lower temperatures effectively suppressed the discoloration and maintained the high water-holding capacity and unique textural properties of lightly salted tuna meat. It was attributed to the reduction in the conformational changes and molecular interactions among meat proteins, protecting myoglobin from oxidative damages during frozen storage.

[Conclusion]

Considering the quality maintenance and energy savings, storage at –40°C was appropriate for lightly salted tuna meat.

Keywords: quality attributes, discoloration, oxidative stability, lightly salted fish meat

Quality-determination-period handling in less organized local shrimp supply chains

QU Qinkun, LIU Junrong*, LIU Yuepeng, TIAN Yuanyong, XU Tanye
College of Food Science and Engineering, Dalian Ocean University, Dalian 116023, China
quqinkun@163.com

[Objective]

To reconstruct less organized local shrimp supply chains for the scattered shrimp-farming operations areas, local pond-farmed (*Litopenaeus vannamei*) was selected as a case study. A post-harvest handling center (PHHC) around production area was designed with operations of collection and distrbuation.

[Methods]

Shrimp supply chain was divided into two stages, from pond-farmed to PHHC as the quality-determination-period (QDP), and from PHHC to markets as the post quality-determination-period (P-QDP). The products in the PHHC were divided into two groups: the rewatered live product group and the freshness-locked product group, and then entered the storage and transportation link. The live product group was kept alive for 60 hours, while the freshness-locked group was iced cold storage for 132 hours. The detection indexes included muscle whiteness, pH, glycogen, ATP related compound, protein solubility and SDH (succinate dehydrogenase). In addition, glucose served as an indicator of fitness of live shrimp.

[Results]

(1)The recoverability of QDP-treatment to live product. After waterless transportation, the pH and ATP of live products muscle increased slightly, while the glucose and glycogen contents decreased slightly. The results of glucose, pH, glycogen and ATP related compound analysis after rewater showed recovery. During the subsequent storage and transportation of live products, the live products showed a certain stability at the initial stage, and then decreased rapidly after 36 hours and a high mortality was observed. (2)Delayed effect of fresh-locked treatment during QDP. The glycogen and ATP of the iced collection decreased somewhat during the sales process, but the decline rate was slow during the first 112 h, and the pH value always remained below 7.98, presenting a 5 d shelf life of the freshness-locked products. It was worth noting that the indexes of freshness-locked were all higher than those of live products, indicating that the perishable period disposal can effectively implement freshness-locking and delay the quality decline rate of fresh products during storage.

[Conclusion]

The results showed that *Litopenaeus vannamei* had a QDP after waterless, and had a delayed effect on the P-QDP after waterless. The establishment of post-harvest handling center can effectively implement quality control in the QDP and actively implement product design, thus changing the passive status of small scattered aquaculture industry after fishing. On the one hand, it will improve the economic income of fishermen and provide high-quality prawn products for the market.

Key words: *Litopenaeus vannamei*; quality determination period; post-harvest handling center; live products; freshness-locked products; storage stability

Extraction and characterization of chitin and chitosan from white shrimp (*Penaeus vannamei*) shell waste via microwave and ultrasound technology

Qiqi Dong[1], Yinzhe Jin[*1], Ningping Tao[2], Shanggui Deng[2]

Engineering Research Center of Food Thermal-processing, Technology, college of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China **yzjin@shou.edu.cn*

[Objective]

White shrimp (*Penaeus vannamei*) is one of the main shrimp types cultured in China. The byproducts from shrimp processing account for about 40%–60% of whole shrimp, which cause resource waste. Chitosan is an extremely valuable polysaccharide and usually obtained from marine animal byproduct. Microwave, as a mild and efficient technology, has been applied in the preparation of chitosan.

[Methods]

The chitin was prepared through a fast, easy and efficient method, by using a conventional heating in the three step of the extraction: demineralization, deproteinization and decoloration. Chitosan extracted by microwave (250W, 5min), ultrasound (40KHz, 400W, 1h, 40 °C) and ultrasound-microwave were compared. The impact of microwave power (150 W, 250W, 350W), and duration (3min, 5min, 7min), microwave heating times (1, 2, 3) on chitosan crystallinity (CrI) and degree of deacetylation (DDA) were investigated. The chitosan was characterized through Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray Diffractometry (XRD), Differential Scanning Calorimetry (DSC).

[Results]

1. Chitosan extracted by microwave, ultrasound and ultrasound-microwave with a degree of deacetylation of 71.37%, 70.70% and 72.16%. The results showed that ultrasound had little effect on improving the deacetylation degree of chitosan.
2. The result show that the degree of deacetylation increased with increasing microwave heating times. A degree of deacetylation of 76.73% was achieved after heating chitin with 50% NaOH solution in a microwave for 5min at 250-watt power. The deacetylation degree of the standard chitosan was 78.89% (marked DD=95%) by the same method.
3. Microwave heating has reduced enormously the time of heating from 20-40min to 5min.

[Conlusion]

The microwave technique can be very useful for synthesizing good functional properties chitosan with rapid and clean chemistry.

Keywords: chitosan, rapid extraction, deacetylation degree, ultrasound irradiation, microwave heating

Effect of Short-term Rearing on the energetic-related metabolites in Scallop adductor muscle during refrigerated storage

Riko Sato*[1], Yuanyong Tian[2], Kuniaki Sasaki[3], Misaki Komuro[1], Chunhong Yuan*[1]
1, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka-shi, 020-8550, Japan
2, College of Food Science and Technology, Dalian Ocean University, Dalian 116023, China
3, Electron Microscope Laboratory, Iwate University, 3-18-8 Ueda, Morioka-shi, 020-8550, Japan
* g0120029@iwate-u.ac.jp

[Background and Objective] According to the FY2020 Fisheries White Paper, the item with the highest percentage of Japan's marine products exported is scallop. It's known that scallops are in season in summer because they have the highest edible portion ratio and high glycogen content. In the COVID-19 period, the percentage of consumers eating at home and purchasing marine products is increasing. However, the problem of quality deterioration arises during distribution. At present, there is a lot of knowledge on the refrigeration changes of scallops, but little knowledge on the refrigeration changes of glycogen. In this study, we quantitatively and qualitatively evaluated the changes in glycogen content of scallops over time during refrigeration by biochemical analysis and Scanning Electron Microscopy (SEM) image analysis.

[Samples and Methods] In this study, two types of scallops from Iwate at September with and without rearing were used. The samples were stored in a refrigerator at 4°C, both peeled and shelled, and the experiments were conducted on days 0 to 3. The other samples were frozen at -60°C, thawed in ice water, and stored in a refrigerator at 4°C, then the experiments were conducted same as fresh one. The pH value was measured with a pH meter (D-71, Horiba Co., Ltd.) after adding 10 ml of 20 mM sodium monoiodoacetate solution to 1 g of adductor muscle and homogenizing at 8,000 rpm for 30 s and 12,000 rpm for 30 s under ice-cold conditions. The glycogen content was extracted by the thermal alkaline extraction-ethanol precipitation method and quantified by the anthrone sulfate method. The samples (2 mm square sections) were pre-fixed with 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide, dehydrated in ethanol, embedded in epoxy resin, thinly sliced at 200 nm, strained with uranium acetate and lead, and observed for changes in glycogen content by SEM (JSM-7800F PRIME, JEOL). Statistical analysis was performed by t-test for significant difference test ($p<0.05$).

[Results and Conclusion] For peeled samples, the difference in pH values was significant among samples with or without rearing. But the difference for shelled samples was not clear due to many surviving samples. It was the first time to detect the changes in glycogen content visualized by SEM images, and they were found that glycogen granules were also present in the scallop myocytes. The biochemical analysis as well as the SEM images showed that the glycogen content decreased during refrigerated storage. These results suggested that the pH values and the glycogen content of scallops decreased during refrigerated storage, rearing could have effect on the scallops to remain high glycogen content.

Keywords: scallop, rearing, refrigerated storage, glycogen, SEM, pH

A new method for determining the denaturation temperature of collagen

Xin Chen, Zhiwen Guo, Junjie Zhang, Rui Duan*

College of Food Science and Engineering, Jiangsu Ocean University, 59 Cangwu Rd., Lianyungang 222005, China
* *duanrui516@jou.edu.cn*

[Objective]

The denaturation temperature (Td) of collagen has been determined using several methods, such as circular dichroism, fourier transform infrared spectroscopy, and differential scanning calorimetry, etc. Such methods need specific equipment or mass samples, which means higher inspection costs. In this study, Sirius red was employed to determine the Tds of collagen from calf tendon, silver carp skin, frog and salmon skins (38.2 ℃, 32.6 ℃, 33.8 ℃, and 15.6 ℃, respectively) to build up a new method which does not require special equipment and mass samples.

[Methods]

The 100 μL collagen solutions (1 mg/mL) were accurately placed in 1.5 mL centrifuge tubes, which were bathed in Eppendorf ThermoStatTM C at different temperatures (5-60 ℃) for 5 min to denature the collagen. Then the Sirius Red staining solution (1 mL) was added to 100 μL treated collagen solution. The mixture was reacted at room temperature for 30 min and then centrifuged at 12,000 r/min for 20 min. After centrifugation the supernatant was discarded, and 1 mL of 0.5 M acetic acid was added to the precipitate and thoroughly oscillated for 1 min. Then the mixture was centrifuged again. Next, the precipitate was fully dissolved with 1 mL of 1 M NaOH. The Abs of the solution was measured at 550 nm by spectrophotometer. The above operations were repeated three times, and the Abs rate(ΔA) was calculated using the following formula:

$$\Delta A = \frac{A_m}{A_N} \times 100\%$$

where ΔA is the Abs rate, Am is the average of measured Abs, and AN is the average Abs measured with unheated collagen (natural collagen).

[Results]

The results showed that A linear relationship was found between Abs rate and heating temperature. The denaturation temperatures of collagen from calf tendon and skins of silver carp, frog and salmon were obtained as 38.2 ℃, 32.6 ℃, 33.8 ℃, and 15.6 ℃, respectively.

[Conlusion]

It is concluded that the Tds of collagen from mammal, fish, and amphibian were determined with the Sirius Red method to build up a new denaturation temperature measurement method which does not require special equipment and mass samples. Through ANOVA and t-test, no significant difference was observed between this method and the viscosity method.

Keywords: collagen; denaturation temperature; new method; Sirius Red

Effect of preparation factors on fish oil-loaded emulsions stabilized by natural silk fibroin

Shudan Huang[1]*, Yulu Zheng[1], Ye Zi[1], Jiawei Peng[1], Jian Zhong[1]

1, Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China

* *hsd18768144348@163.com*

[Objective]

It’s generally accepted that silk fibroin(SF) has superior bio-compatibility and bio-degradability, and self-assembles at fluid interfaces, which can making SF a potential new good macromolecular surfactant with potential applications in novel biocompatible emulsion. The objective of this study is to study the .effect of preparation factors on fish oil-loaded emulsions stabilized by natural silk fibroin.

[Methods]

The obtained SF were redispersed in ultrapure water to obtain solutions with designated concentrations. Then, fish oil was added into the solution. The solutions were mechanical sheared by a T 10 basic ULTRA-TURRAX® homogenizer.

[Results]

The results showed that the concentration of SF solution, homogenizing time, homogenizing speed, and the ratio of SF/oil can effect the stabilization of the emulsions.

[Conlusion]

It is concluded that fish oil-loaded emulsions have the potential to reduce the disadvantages of fish oils. It would be also beneficial to basic understanding of the formation of SF nanoparticle-based Pickering emulsions.

Keywords: silk fibroin, fish oils, pickering emulsion, preparation factors

Molecular Mechanisms Involved in Changes of Thermally Induced Gel Properties of Fish Meat Paste during Two-Step Heating Procedure

Shugo Watabe

School of Marine Biosciences, Kitasato University, Minami, Sagamihara, Kanagawa 252-0373, Japan
swatabe@kitasato-u.ac.jp

The production of the surimi-based products is one of the most promising candidate technologies to enhance the value addition of underutilized fish in the countries, where the surimi-based products are popular and routinely consumed. The two-step heating procedure, consisting of pre-heating at around 40ºC and subsequent main-heating at 80 - 90ºC, is commonly practiced in Japan to prepare surimi-based products and to improve their quality at the industrial level. While soft gels are formed (suwari in Japanese) at pre-heating, elastic gels characteristic to sumiri-based products are formed by subsequent main-heating (honkanetsu in Japanse). Hence, we analyzed changes in rheological properties of thermally induced gels of Japanese codling during the two-step heating procedure, first pre-heating at 0 - 70ºC with 5 or 10˚C intervals and subsequent secondary main heating at 85ºC, in association with protein insolubilization and myosin polymerization. Changes in breaking strength and breaking strain rate showed roughly three phases associated with pre-heating temperatures of 0 – 25˚C, 30 – 50˚C and 55 – 70˚C. The most prominent change was observed for pre-heating gels on pre-heating from 30ºC to 50ºC, where myosin polymerization rate was high. The increase of pre-heating temperature from 0 to 25ºC was accompanied by the gradual decrease of protein solubilization and rapid increase of myosin polymerization for pre-heating gels, suggesting that gel formation occurred even at low pre-heating temperatures. NH_4Cl, one of inhibitors against transglutaminase, reduced such myosin polymerization in meat paste. Meanwhile, dynamic viscoelasticity measurement indicated that structural changes in myosin other than polymerization also participate in gel formation during the two-step heating procedure. We conclude that these changes unrelated to myosin polymerization are possibly due to changes in interaction of myosin with actin.

Keywords: actin, fish meat paste, myosin, surimi-based products, two-step heating

Preventive Effects of Dietary Sea Cucumber and its Enzymatic Hydrolysate against Ultraviolet A-induced Skin Photoaging

Shuyu Liu[1], Yi Lu[2], Masahiro Kurono[2], Yoshiharu Matahira[2], Yuki Manabe[1], Tatsuya Sugawara[1]

1, Graduate School of Agriculture, Kyoto University, Kyoto 606, Japan
2, Sunsho Pharmaceutical Co., Ltd., Shizuoka, Japan
*sugawara.tatsuya.6v@kyoto-u.ac.jp

[Objective]

Chronic exposure to UV radiation promotes skin photoaging which is clinically characterized by dryness, laxity, and wrinkling. Recent studies have suggested that collagen hydrolysates from aquatic products exerted beneficial effects on photoaging. Sea cucumber (*Stichopus japonicus*) (SC), a marine organism whose body wall contains a high level of collagen, has therefore aroused our interest in the present study. Additionally, SC is considered as a potential nutraceutical owing to its bioactivities including antioxidant, antitumor, and anticancer activities. This study examined the effects of SC and its enzymatic hydrolysate (SCH) on UVA-induced skin dysfunctions and wrinkle formation *in vivo* using hairless mice.

[Methods]

Hairless Hos:HR-1 mice in experimental groups were exposed to UVA irradiation at a dose of 20 J/cm^2 five times weekly for 10 weeks, fed with a diet of AIN-93G containing 5% SC or SCH powder. Transepidermal water loss (TEWL) and the water content of the stratum corneum in the dorsal skin of mice were measured, and dorsal skin was replicated using silicone for analysis of wrinkle formation, under isoflurane anesthesia on the last day of 4, 8, 9, and 10 weeks. After the completion of irradiation, the mice were sacrificed, and dorsal skin specimens were collected for morphological analysis as well as mRNA expression. Meanwhile, the content of epidermal natural moisturizing factors (NMFs) was quantified by HPLC analysis.

[Results]

UVA significantly induced TEWL and wrinkle formation, while these skin dysfunctions were significantly mitigated upon oral administration of SC and SCH. Additionally, SC and SCH mitigated the UVA-induced downregulation of epidermal NMFs and upregulation of *Aqp3*, *Mmp13*, *Tnfa*, and *Il6* mRNA levels in the mouse skin.

[Conclusion]

Taken together, it is concluded that dietary SC and SCH exert anti-photoaging effects by modulating filaggrin synthesis and desquamation in the epidermis and regulating the NF-κB pathway in the skin. Our research indicates that both SC and SCH have potential applications in nutricosmetics for photoaging.

Keywords: sea cucumber, anti-photoaging, collagen hydrolysate, skin barrier function, hairless mice

Use of plant polyphenols/extracts in fish/shellfish for quality improvement and shelf-life extension

Soottawat Benjakul
International Center of Excellence in Seafood Science and Innovation
Faculty of Agro-Industry
Prince of Songkla University, Hat Yai, Thailand
* *soottawat.b@psu.ac.th*

Fish and shellfish are popular due to their nutritive value and delicacy. However, they are perishable with the limited shelf-life. To maintain the quality, the additives, especially antimicrobial agent, have been applied. Because of safety concern, the natural additive, particularly plant polyphenols or plant extracts have gained increasing interest as the promising safe additives. Apart from antimicrobial activity, polyphenols have varying activities such as anti-melanosis, antioxidant, etc. depending on their size, reactive groups and structure. They can be extracted from leaves or wood or bark. Extraction efficacy can be enhanced with the aid of ultrasonication. For leaves, dechlorophyllization must be implemented and sedimentation process, a green process, is strongly recommended to avoid the discoloration of treated samples. Polyphenols or plant extract could be used to retard the melanosis of shrimp during the refrigerated storage. Inhibition of polyphenoloxidase, which induces the melanosis in crustacean, is depending upon the type of polyphenols and inhibition kinetic is varied. Owing to their antimicrobial activity, microbial growth could be retarded in shrimp during the extended storage, In addition, plant polyphenols or plant extract can be used as gel strengthening agent via inducing the cross-linking of myofibrillar proteins via both hydrogen bond or covalent bond. This could improve the quality of mince or surimi gel, especially when used in conjunction with high pressure. Since polyphenols or plant extracts have been known as the excellent source of antioxidants, they can be used to prevent oxidation of products rich in polyunsaturated fatty acids. It showed high efficacy in lower lipid oxidation when used in combination with modified atmosphere packaging (MAP). In general, the effectiveness of polyphenols or plant extract is dose-dependent. However, to exploit plant polyphenols or plant extract in fish and shellfish and their products, the appropriate use, such prior dechlorophyllization, optimal level, etc. must be taken into consideration. Thus, they can be a promising safe additive in fish and shellfish instead of synthetic counterpart.

Keywords: plant polyphenols, plant extract, melanosis, quality, antioxidant, gelation, lipid oxidation, shelf-life

The freshness-locked mechanism of cooling disposal during quality determination period of cultured *Scophthalmus maximus*

Tanye Xu, Yuanyong Tian, Junrong Liu*

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

* *xutanye1199@126.com*

[Objective]

Scophthalmus maximus is one of the dominant economic fishes in mariculture in China and has high nutritional value. Previous studies have found that there is a quality determination period after post-harvesting, and the way of disposal at this stage is closely related to the fish quality. The purpose of this study was to elucidate the molecular mechanism of fish quality changes after spinal cord destruction of turbot during quality determination period from the perspective of muscle metabolism, and to provide theoretical basis for quality management of cultured fish after post-harvesting.

[Methods]

Cultured turbot with good vigour was selected and killed by spinal cord cutting. And then exsanguinated in the -1.7℃ and normal temperature seawater. After removing gills, gutting, and cleaning, the packaged products were refrigerated at 4℃ for 7 days covered by crushed ice in bubble chamber. The turbot samples bleeding at normal temperature seawater were used as control group, and the turbot samples bleeding at chilled seawater were used as treatment group. A non-target metabolomics technique combined physicochemical indexes were applied to investigate the muscles metabolism of fish.

[Results]

The results showed that the initial pH of the treatment group was the same as that of the control group. The pH of the treatment group was decreased to 6.60 at 3 d during storage, while that of the control group decreased to 6.62 after 1 d. The K values of muscles in control group was higher than that in treatment group. Metabolomics analysis showed that the two groups were distinguished well in the OPLS-DA model. Eleven endogenous metabolites were significantly changed, including 5-aminopentanoic acid, dihydroxyacetone phosphate, glycerol 3-phosphate, L-saccharopine, L-phenylalanine, and decanoyl-L-carnitine were up-regulated and pyruvaldehyde, D-ribose, D-lyxose, 2-amino-2-methyl-1,3-propanediol, L-anserine were down-regulated. Pathway analysis showed that cooling disposal affected the glycerolipid metabolism in muscle.

[Conlusion]

The results showed that the cooling disposal during quality determination period could achieve the purpose of freshness-locked and had delayed effect. In addition, metabolomics techniques can be used to monitor muscle metabolic changes of fish in early stage.

Keywords: *Scophthalmus maximus*; spinal cord cutting; cooling disposal; freshness-lock; muscle metabolism

Effects of ascorbic acid and sodium citrate treatments on the lipid stability and quality of snakehead fish (*Channa striata*) fillets during refrigerated storage

Tatenda Allen Chawafambira[1], Ngan Thi Nguyen[2], Minh Van Nguyen*[2]
1, Beast Kraal Farm Plot number 32, Mutare, Zimbabwe
2, Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu, Nha Trang, Vietnam
* *minhnv@ntu.edu.vn; allenchawaz@gmail.com*

[Objective]

Lipid oxidation is one of the major deteriorative reactions taking place in fish muscle during processing and storage, affecting the physicochemical properties and limiting the shelf-life of fishery products. The objective of this study was to investigate the effects of ascorbic acid (AA) and sodium citrate (SC) treatments on the lipid stability, sensory quality, and physicochemical properties of snakehead fish fillets during refrigerated storage at 2-4°C.

[Methods]

Snakehead fish (*Channa striata*) samples used in this research were bought from the local fish farm in Nha Trang City with an average weight of 700-800 g. Fish were rested for 2 h before bleeding and filleting. The fillets were divided into four groups: control group, 0.25% AA treated group, 0.50% AA treated group and 0.80% SC treated group. Lipid stability was assessed by determinations of free fatty acid (FFA), phospholipid content (PL), hydroperoxide value (PV), and thiobarbituric acid-reactive substances (TBARS). Sensory quality of the fish samples was carried out by using QIM and Torry schemes. The Colour of the flesh side, cooking yield and shear strength were also determined.

[Results]

The results indicated that ascorbic acid and sodium citrate treatments significantly retarded lipid hydrolysis and lipid oxidation progress in the fish muscle, resulting in lower FFA, PV, TBARS values and higher PL content of the treated samples. The samples treated with ascorbic acid and sodium citrate had significantly higher whiteness values and lower yellowish (b^*) values compared to the untreated samples throughout the storage period. Higher cooking yield and shear strength values were observed in the treated samples. Based on the QIM and Torry scores, the shelf lives of the untreated, 0.80% sodium citrate treated, 0.25% ascorbic acid treated and 0.50% ascorbic acid treated samples were of 10 days, 11 days, 13 days, and 14 days, respectively.

[Conlusion]

Ascorbic acid treatments (i.e. 0.25% and 0.50%) demonstrated a greater lipid antioxidative activity compared to sodium citrate treatment (0.8%). The development of lipid hydrolysis and oxidation was in high correlation with the sensory quality and physicochemical properties of snakehead fish fillets. Ascorbic acid and sodium citrate treatments resulted in the prolonged shelf life of snakehead fish fillets during refrigerated storage.

Keywords: snakehead fish, ascorbic acid, sodium citrate, lipid oxidation, colour, sensory quality

Recovery of hydroxyapatite, chitosan and protein hydrolysate from blue crab shells *(Portunus pelagicus)*

Thi Hoang Quyen Tran*, Vinh Thinh Phan

Department of Chemical Engineering, Faculty of Food Technology, Nha Trang University, 2 Nguyen Dinh Chieu, Nha Trang, Khanh Hoa 57000, Vietnam
** quyentth@ntu.edu.vn*

[Objective]

It's generally accepted that the blue crab shell is an available resource, a natural composite and it consists of highly mineralized chitin–protein fibers. The objective of this study is to recover simultaneously chitin, calcium carbonate and protein hydrolysate from blue crab shells and convert them to valuable products: chitosan and nano-sized hydroxyapatite (HA).

[Methods]

The liquid after 5 % NaOH treatment of minced crab shells (1:10, w/v) in 24 h was collected as protein hydrolysate. Protein determination was performed by using Kjeldahl method. The shells were then treated in 5 % HCl in 6 h to release calcium carbonate as HA precursor. HA preparation was carried out using hydrothermal method in an autoclave at 200 °C in 24 h with $(NH_4)_2HPO_4$ 0.2 M and obtained above $CaCO_3$. After that, the powder was sintered at 900 °C in 6 h, at the heating rate of 5 °C/min to improve HA crystallinity. The morphology and particle surface of HA were characterized by scanning electron microscope (SEM) and transmission electron microscopy (TEM). The crystallinity of HA was examined by X-ray diffraction (XRD). The chemical groups were determined by fourier transform infrared spectroscopy (FTIR). Chitosan was assayed for degree of deacetylation, viscosity, solubility and molecular weight by Mark-Houwink formula.

[Results]

The results showed that 16.4% chitin fibrils wrapped with 22.3% protein and a very high degree of mineralization (55.9% of typically calcium carbonate as HA precursor) was found in the blue crab shells. The simultaneous recovery yield was approximately 94.6%. HA particles were in nanosized size with the width of 20–40 nm and the length of 40–100 nm. Based on the XRD pattern, the sharp peaks corresponding to planes which are attributed to HA structure were noted. Three strongest peaks of XRD with a very high intensity corresponding crystalline HA with 2θ at around 31,99° were well observed. The vibrations of functional groups presenting in HA (PO_4^{3-}, OH^- and CO_3^{2-}) were detected by FTIR. The prepared chitosan had viscosity 624 cps, molecular weight 1170.4 kDa, degree of deacetylation 91.5 % and solubility (1 wt.% in 1 % acetic acid).

[Conclusion]

It is concluded that the recovery of protein hydrolysate, calcium carbonate as HA precursor and chitin from blue crab (*Portunus pelagicus*) shells was done. From these products, nano-sized HA and high molecular weight chitosan were successfully obtained.

Risk Assessment to nitrate of Khanh Hoa population due to raw vegetable consumption

Thuan Anh Nguyen*[1]
1, Faculty of Food technology, Nha Trang University, Nha Trang, Vietnam
*anhnt@ntu.edu.vn

Objective
The objective of this study is to estimate the dietary intake of nitrate due to the raw vegetable consumption of the population in Khanh Hoa province, Vietnam.
Methods
The nitrate concentrations in the raw vegetable consumed popularly by the Khanh Hoa population were investigated by molecular absorption spectrometric method. The dietary intake of nitrate was determined by a total diet study.
Results
The results showed that the average nitrate concentration in raw vegetable is equal to 201,8 ± 13,1 mg/kg. The nitrate intake was estimated for six subpopulation groups: men and women aged 18–29, 30–54, and ≥55. The dietary intakes of nitrate by the Khanh Hoa population are currently well below the acceptable daily intake (ADI) of nitrate.
Conlusion
It is concluded that no risk exists concerning the levels of exposure of Khanh Hoa consumers to nitrat due to raw vegetable consumption.

Keywords: Risk Assessment, nitrate, raw vegetable, Khanh Hoa province, Vietnam

Progress on Keep-alive Transportation of Shrimp and Crab

Tong XIAO [1], Hong-li WANG [1], Xi-chang WANG[1]

1, college of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
** 13337217515@163.com (First Author)*

Abstract: Live shrimp and crab occupy an important position in the aquatic market. In order to reduce transport injury and improving transport survival rate and meet the needs of consumers for live shrimp and crab, the keep-alive transportation of shrimp and crab is particularly important. The effects of temperature, oxygen, water quality and other factors on shrimp and crab during live transportation were introduced in this paper. It also lists the behavioral and physiological indicators to evaluate the vitality and health of shrimp and crab and summarizes the methods commonly used at home and abroad to keep shrimp and crab alive for transportation, such as lowering temperature, increasing oxygen, purifying water, using chemical anesthesia and so on. Finally, the future research and development of the keep-alive transportation of shrimp and crab are prospected. These will certainly provide a reference for the research on the keep-alive transportation of shrimp and crab.

Keywords: shrimp; crab; keep-alive transportation; survival rate; stress response; vitality; health

Effect of iota carrageenan on the syneresis properties of mixed gels of iota and kappa carrageenan in presence of potassium ions

Viet Bui Tran Nu Thanh*[1], Taco Nicolai[2]

1, Food Technology Faculty, Nha Trang University, Vietnam
2, Le Mans Université, IMMM UMR-CNRS 6283, Polymères, Colloïdes et Interfaces, 72085, Le Mans, cedex 9, France
* *thanhviet@ntu.edu.vn*

[Objective]

The purpose of this study is to observe the syneresis properties of pure κ- carrageenan (KC) and mixture of κ- and ι- carrageenan in presence of potassium ions via the rheological properties and microstructure.

[Methods]

In this study, the rheological measurements were determined using a rheometer (ARG2, TA Instruments) in combination with a cone and plate or plate – plate geometry. The storage modulus (G')/ loss modulus (G") were determined as a function of the frequency and the temperature using plate – plate geometry (diameter 40 mm, gap 1mm). The temperature was controlled by a Neslab system and the geometry was covered with paraffin oil to prevent water evaporation. The microstructure was used Confocal Laser Scanning Microscopy (CLSM). The κ- and ι- carrageenan were visualized separately with a CLSM by using different fluorescent labelling. The κ-carrageenan labelled with FITC, and the ι- carrageenan labelled with rhodamine B. CLSM observations were made with a Zeiss LSM800 (Germany). The images of 512 x 512 pixels were produced at different zooms with objective 63. The solutions were inserted between a concave slide and a coverslip and hermetically sealed. The incident light was emitted by a laser beam at 543 and/or 488 nm.

[Results]

The results showed that for KC solution, increasing the KCl between 5 and 30 mM increased the elastic modulus (Gel) and the gelling temperature (Tg). However, further increase of the KCl concentration led to a sharp decrease of the elasticity, Tg and increase the syneresis ratios. On the other hand, adding KCl between 5 and 70 mM to mixed gels not only enhanced of Tg and Gel, but aslo reduced the syneresis in the gels. In addition, the elastic modulus of the mixtures at low temperatures was much higher than the sum of those of the pure systems within the same conditions.

In parallel, the influence KCl on the structure of pure and mixed gels was studied by confocal laser scanning microscopy (CLSM). In the mixtures, KC and IC could be distinguished because they were covalently labeled with different fluorescent dyes. The structure of KC and IC in the mixtures was found to be different to each other indicating that the two polysaccharides were not fully co-located. The results will be used to better understand the observed synergy in KC/IC gels.

[Conclusion]

It is concluded that the syneresis of κ-carrageenan network is lower in the mixed gels than in the corresponding individual gels. The syneresis ratios of the mixed gels aslo depend on the presence of ι-carrageenan concentrations.

Keywords: syneresis, microstructure, rheological properties, carrageenan

Effects of phenolic acid grafted chitosan on moisture state and protein properties of vacuum packaged sea bass (*Lateolabrax japonicus*) during refrigerated storage

Weiqing Lan[1,2,3], Xin Yang[1] Jing XIE[1,2,3*]

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai, China
2, Shanghai Aquatic Products Processing and Storage Engineering Technology Research Center, Shanghai, China
3, National Experimental Teaching Demonstration Center for Food Science and Engineering, Shanghai Ocean University, Shanghai, China
**** jxie@shou.edu.cn***

[Objective]

In this study, the effects of chitosan-grafted-phenolic acid on moisture state and protein properties of vacuum packaged sea bass (*Lateolabrax japonicus*) have been evaluated.

[Methods]

Samples were removed the head and bone and cut into fillets, then they were divided into five groups, which treated by deionized water (CK), 1% chitosan (CS), 1% CS-grafted-protocatechuic acid (CS-g-PA) copolymer and 1% CS-grafted-gallic acid (CS-g-GA) copolymer for 10 min, respectively. Then drained it on a pre-sterilized metal mesh at 4°C for 5 min, using vacuum packaged and stored at 4°C. Moisture state (drip loss, cooking loss, Aw, Low-field nuclear magnetic resonance (LF-NMR), magnetic resonance imaging (MRI)) and protein properties (TCA-soluble peptides, protein solubility, SDS-PAGE, Myofibril fragmentation index (MFI), surface hydrophobicity, Fluorescence spectroscopy analysis, differential scanning calorimetry (DSC), Myofibril microstructure) were used to analyze the effect of chitosan graft copolymer in sea bass.

[Results]

The results showed that 1% CS-g-PA and 1% CS-g-GA could reduce the decline of drip loss, cooking loss and Aw in vacuum packaged sea bass during refrigerated storage. Moreover, the results of LF-NMR analysis further showed that CS-phenolic acid graft copolymer treatment could effectively delay water migration. Compared with CK and CS groups, graft copolymer groups could delay the oxidative degradation of protein, including decreased the growth of TCA-soluble peptide, and inhibited the decrease of protein solubility. The results of SDS-PAGE further showed that graft copolymer treatment inhibited the protein degradation. The results of MFI, surface hydrophobicity, IFI and SEM showed that graft copolymer treatment could maintain the structural stability of samples. At the same time, DSC analysis showed that graft copolymer treatment could better maintain the protein stability.

[Conclusion]

1% CS-g-PA copolymer and 1% CS-g-GA copolymer combined with vacuum packaging could maintain the quality of vacuum packaged sea bass from water loss and protein degradation during refrigerated storage.

Keywords: chitosan-grafted-phenolic; moisture state; protein properties; sea bass

Seasonal variation affects the gonad index and protein content related protein levels as revealed by iTRAQ

Wen-Hui Shang[1,2], Jia-Run Han[1], Jia-Nan Yan[1], Yi-Nan Du[1], Yun-Sheng Xu[2], Chang-Feng Xue[2], Tie-Tao Zhang[2], Hai-Tao Wu[1,3,*] and Bei-Wei Zhu[1,3,*]

[1] School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China
[2] College of Food Science and Engineering, Hainan Tropical Ocean University, Hainan 572022, China
[3] National Engineering Research Center of Seafood, Dalian 116034, China
* *wht205@163.com (H.-T.W.); zhubeiwei@163.com (B.-W.Z.)*

[Objective]

Sea urchin *Strongylocentrotus nudus* (*S. nudus*) is an economically important species in northwestern Pacific countries, such as China, Japan, and Korea. Many studies focused on the composition and property of sea urchin gonads. However, seasonal variation in the gonad protein profile of *S. nudus* has not yet been reported. In the present study, the relationship between the gonad index (GI) and protein content variation during the fishing season was investigated by iTRAQ-based quantitative proteomics.

[Methods]

Fresh *S. nudus* was attained near Dalian. Wet body and gonad mass of *S. nudus* gonads were weighted to calculate GI values. The Kjeldahl method was used to determine the protein content. Protein profile of the extracted proteins was obtained by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A series of experiments were conducted to identify differentially expressed proteins (DEPs), including trypsin digestion and iTRAQ labelling, peptide fractionation by strong cation exchange (SCX) chromatography separation, high pressure liquid chromatography (HPLC) coupled with mass spectrometer (MS) detection, and database search and iTRAQ quantification analysis.

[Results]

1. The transforming growth factor-beta-induced protein ig-h3 (TGFBI) at about 37 kDa from July was enhanced comparing to other groups.
2. A total of 174 differentially expressed DEPs were identified.
3. Seven of the DEPs showed significant correlations with both the GI and protein content.
4. The 6PGD, IDH, multifunctional protein ADE2 isoform X3, and ALDH were shown to interact with each other and might play key roles in changing the condition factor of *S. nudus* gonads.

[Conlusion]

The mechanism of *S. nudus* gonads that changes the condition factor was revealed by screening, as well as the characteristics of the 10 DEPs that had a correlation with GI and protein content.

Keywords: *Strongylocentrotus nudus*, seasonal variation, gonad index, condition factor

Enhanced anti-inflammatory activity of glycated salmon myofibrillar protein with reducing sugars containing carboxyl group

Wenzhao Li*, Ga-Hyun Joe, Hiroki Saeki

Laboratory of Marine Food Science and Technology, Faculty of Fisheries Sciences, Hokkaido University, Minato 3, Hakodate, Hokkaido 041-8611, Japan
**leewz930206@gmail.com*

[Objective]
Myofibrillar protein (Mf) from spawned-out chum salmon, glycated with alginate oligosaccharide through Maillard reaction, gained strong anti-inflammatory activity, suggesting that glycation with alginate oligosaccharide is one of the advanced methods for developing food-functional material from chum salmon protein. Considering that alginate oligosaccharide is a copolymer linked by guluronic acid and mannuronic acid possessing one carboxyl group in each molecule, this study investigated the role of carboxyl group in reducing sugar for enhancing the anti-inflammatory activity of myofibrillar protein (Mf) from spawned-out chum salmon through Maillard Reaction.

[Methods]
Mf was mixed with sorbitol and various reducing sugars: alginate oligosaccharide, glucose, glucuronic acid, galactose, galacturonic acid; each lyophilized Mf-sugar mixture was incubation at 60 ℃ and 35 % relative humidity for 12 h to conjugate the reducing sugars with Mf via the Maillard reaction. SDS-PAGE, available lysine content, and UV-absorbance were verified to confirm the glycation. After digested with pepsin and trypsin, the anti-inflammatory activity of the glycated Mf was evaluating the inhibitory effect on the secretions of TNF-α and IL-6 in LPS-stimulated RAW264.7 macrophages.

[Results]
(1) The results showed that Mf glycated with glucuronic acids and galacturonic acid markedly suppressed the TNF-α and IL-6 secretions in RAW264.7 macrophages stimulated with high concentration of LPS (200 ng/mL), as the same level as that of glycation with alginate oligosaccharide. On the contrary, (2) no enhancement was observed in glycated Mf with glucose and galactose. In addition, (3) the numbers of lysine loss to gain the strongest TNF-α inhibitory effect in the glycated Mf with glucuronic acid and galacturonic acid were 5.7 times and 5.3 times higher than glycated Mf with alginate oligosaccharide (Degree of polymerization=6), respectively.

[Conclusion]
The current study indicates that the existence of carboxyl group in reducing sugar is an important factor for enhancing anti-inflammatory activity of Mf through the Maillard reaction and the enhancement may be related to the amount of introduced carboxyl group.
Keywords: fish myofibrillar protein, anti-inflammatory activity, glycation, Maillard reaction, carboxyl group

Effect of methanol extract from walnut shell on serum pro-inflammatory factors in mice with superficial second degree scald

Xiaojing Sun[1], Wenhui Wu[1], Ruihua Guo[1].

1, college of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
* *Valeriesun52@163.com*

[Objective]

It's generally accepted that walnut shell is one by-product in walnut industry with many activities such as antioxidant and lowering blood fat. The objective of this study is and investigate the effect of methanol extract of walnut shell on the treatment of scald, and improve the utilization rate of walnut.

[Methods]

1. After scalding, 54 experimental mice were randomly divided into 6 groups as follows:experimental group A (EGA, 0.01 g/mL walnut shell methanol extract), experimental group B (EGB, 0.1 g/mL walnut shell methanol extract), experiment group C (EGC, 1 g/mL walnut shell methanol extract), control group (CG, 75% ethanol), positive control group (PCG, Jingwanhong ointment) and blank group (BG, not treated).
2. Three randomly selected mice in each group were sacrificed under diethyl ether anesthesia at 3rd, 7th and 14th day.
3. Serum was taken, TNF-α, IL-1 and endotoxin were assayed to investigate the degree of inflammation caused by bacterial infection after scalding skin tissue.

[Results]

Wound observation showed that the wound returned to normal after 14 days of treatment. ELISA findings that the IL-1 level in serum exhibited significant difference between EGC and CG ($p < 0.05$) on the 14th day and the TNF concentration showed significant difference on the 7th day ($p < 0.05$).

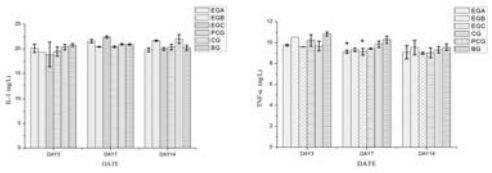


Figure 1 The variation of IL-1, TNF-α concentration (ng/L) within post-burn 3 d to 14 d for all groups.

[Conlusion]

It is concluded that the methanol extract of walnut shell can promot the healing of secondary scald. The methanol extract of walnut shell promoted the healing of secondary scald in mice by inhibiting the production of endotoxin and the expression of IL-1 and TNF-α.

Keywords: walnut shell, methanol extract, serum pro-inflammatory factors, tumor necrosis factor-alpha, interleukin-1

Separation and Preparation of Japanese Eel Skin Collagen by Different Methods and Characterization of Its Structural Characteristics

Xiaomin Li[1], Chenyu Yang[2], Yinzhe Jin*[1], Yun Deng[2]

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
2,Engineering Research Center of Food Thermal-processing Technology, National Experimental Teaching Demonstration Center for Food Science and Engineering
* yzjin@shou.edu.cn

[Objective]

Japanese eel is an important export aquatic product in China. In this study, eel skin collagen was extracted from eel skin by acid method and pepsin method, and its structural characteristics were characterized, To provide basic data for the follow-up study and use of eel skin collagen.

[Methods]

Firstly, acid soluble collagen (ASC) and enzyme soluble collagen (PSC) in eel skin were extracted and purified by acid method and pepsin method respectively. Secondly, the structure of collagen was characterized by SDS - PAGE, Fourier transform infrared spectroscopy (FTIR), ultraviolet spectrophotometry (UV), differential scanning calorimetry (DSC), amino acid analysis and scanning electron microscope.

[Results]

1. The extraction rates of ASC and PSC were 4.9% and 8.2% (dry basis content) respectively.
2. SDS - PAGE showed that both collagen was type I collagen. UV analysis determined the best wavelength of ASC and PSC, and FTIR analysis confirmed the triple helix structure of collagen.
3. The contents of glycine, alanine, arginine and aspartic acid in ASC and PSC account for 62% and 64% of the total amino acids,The total amino acid content of PSC was 1.2 times that of ASC.
4. According to the analysis of microstructure by SEM, it is found that both ASC and PSC are sheet structures with smooth surface, but PSC is smoother and more uniform than ASC, and there are fewer holes.
5. DSC showed that the denaturation temperatures of the two proteins were 38.35 ℃ and 37.59 ℃, and there was no significant difference in thermal stability, which may be due to the synergistic effect of molecular weight and hydrogen bond. In terms of solubility and water retention, PSC > ASC.

[Conlusion]

The eel skin collagen extracted by enzyme method has more potential application value than that extracted by acid method.

Keywords: Japanese eel skin, Acid soluble collagen, Enzyme soluble collagen, Structural characteristics

Biosynthesis Gene Cluster Mining and Analysis of Secondary Metabolites of a rare fungus from South China Sea, in *Paraconiothyrium cyclothyrioides* 1-I2

Xiaoqing Tian[1,2], Yanan Lu[2],Liyan Ma[2], ShaChen[1,2], Chongbin Li[1,2], Chenqi Fan [2] ,Wenhui Wu[1*]

1, College of Food Science and Technology, Shanghai Ocean University, Huchenghuan Rd 999#, Nanhui New City, Shanghai, 201306, China
2, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Jungong Rd 300#, Shanghai, 200090, China
* *tianxq@ecsf.ac.cn*

[Objective]

Paraconiothyrium fungi, which has no genome information has been reported so far, was for the first time isolated from water samples of the South China Sea. In this paper, the gene cluster and secondary metabolites of this strain were studied.

[Methods]

The whole genome sequences of *Paraconiothyrium cyclothyrioides* 1-I2 was analysed by using antiSMASH and PacBio. Rice is used as a medium for large-scale fermentation for this fungus. Secondary Metabolites were separated and purified by column chromatography and semi-preparative high performance liquid chromatography (HPLC), and identified by a variety of spectroscopy methods.

[Results]

The genome sequence results showed 23 scaffolds, 41 gene clusters encoding secondary metabolite biosynthesis. The 41 gene clusters contained 5 types, T1PKS, NRPS, NRPS-like，indole, NRPS/ T1PKS. 7 of 41 gene clusters are 100% similar to known gene clusters, which involved in the synthesis of dimethylcoprogen, melanin, fusarin, pyranonigrin E, mellein, and ACT toxins. 8 secondary metabolites were isolated and identified from the fungi as polyketides (1-5), alkaloids (6, 7), peptides (8). 2 compounds are new ones (1, 3). Compounds 2-4 showed same isochroman-1,3-dione core., and the mellein predicted by the biosynthetic gene cluster has been isolated to the corresponding compounds(2-4).

[Conlusion]

The prediction of biosynthetic gene clusters will lay a foundation for the preparation and functional research of secondary metabolites of *Paraconiothyrium cyclothyrioides* 1-I2.

Keywords: *Paraconiothyrium cyclothyrioides* 1-I2, Secondary Metabolite, Biosynthesis Gene Cluster Mining

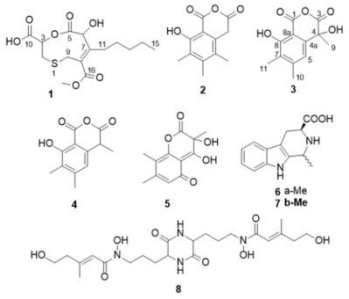


Fig. 1 Structures of compounds 1-8

Shewanella baltica has highly spoilage potential in fish, mainly via nitrogen and nucleotide pathways.

Xiaowei Lou, Hongshun Yang*

Department of Food Science and Technology, National University of Singapore, Singapore 117542, Singapore
* *fstynghs@nus.edu.sg*

[Objective]

To improve food security in Singapore, golden pomfret (*Trachinotus blochii*) was successfully spawned. However, it is highly perishable mainly due to microorganisms. *Shewanella* spp. are specific spoilage organisms (SSOs) in low-temperature stored fish. This study investigates the spoilage mechanism of *Shewanella baltica* in fish, thus providing information for controlling seafood spoilage during chilled storage.

[Methods]

Sterile fish sticks and broths were prepared and inoculated with three different strains (ABa4, ABe2, and BBe1) of *S. baltica*, respectively. Fish models were stored at 4 °C for 10 d. Metabolites of fish models were extracted and tested on day 0, 4 and 10. The populations of *S. baltica* and TVB-N were tested every two days.

[Results]

Totally 39 metabolites in two fish models were identified, which were involved in four main metabolic pathways: peptide and amino acid, nitrogen, nucleotide, and carbohydrate pathways. In fish sticks, proteins were hydrolysed to increase amino acids (up to 230%) by *S. baltica*, especially by strain ABa4. In both fish sticks and broths, *S. baltica* induced the formations of biogenic amines from amino acids and trimethylamine-N-oxide (TMAO), and the degradations of adenine nucleotides to form inosine and hypoxanthine (2- to 4-fold increment). In addition, sugars and lactate were consumed by *S. baltica*, accompanied with the productions of acetate and succinate. Strain BBe1 showed higher TVB-N and capability of biogenic amine production and nucleotide degradation.

[Conlusion]

S. baltica has highly spoilage potential and activity to decompose nutrients in fish, mainly via nitrogen and nucleotide pathways. In both sticks and broths, *S. baltica* induced the formations of biogenic amines, and the degradations of nucleotides. For amino acids, *S. baltica* increased their contents in fish sticks but decreased them in fish broths.

Keywords: fish spoilage; NMR; metabolomics; *Shewanella baltica*

Isolation and antimicrobial activities of fungus DSF059 derived from Deep-sea sediment

Xiaoxiong Xu*, Huimin Feng

Hainan Tropical Ocean University, Sanya 572000, PR China

* *xuxiaoxiong@hntou.edu.cn*

[Objective]

In order to explore fungi resource from deep-sea sediment and detect their potential bioactivities with respect to antimicrobial activity, four media including GPY medium, MEA medium, Martin medium and the Fungi NO.Ⅱ medium, were used to isolate cultured fungi. After purification, phylogenetic analysis and biodiversity analysis were performed. After liquid fermentation of each deep-sea derived fungus, each secondary metabolite ant its ethyl acetate extraction were both were carried out biological activity assay.

[Methods]

Four deep-sea sediments were sampled from 2595 to 6000 meters depth underwater. After treatment with air-drying and crushing, samples were put on four isolation media with spread plate method and stamp method, respectively. All the plates were incubated in 28℃. All the stains were purified in PDA medium with 40% seawater. Genomic DNA was extracted by CTAB method. Primers ITS 1 and ITS 4 were used in ITS gene amplification. Phylogenetic analysis was operated by software BioEdit 7.0 and MEGA 7.0. YPM medium (mannitol 0.8g, peptone 0.4g, yeast extract 0.4g, pure seawater 200 mL, pH 6.5-7.0) was the fermentation medium for secondary metabolite. Plate confrontation method was used to screen antagonistic effects on food-borne pathogenic bacteria.

[Results]

1. One hundred and sixty-one strains of fungi were successfully isolated from 4 deep-sea sediments, which were identified to 44 genera bias on their ITS gene sequences analysis .
2. Strain DSF059 is isolated from the deep sea sediments sample DS03 with Martin medium. Strain DSF059 was identified as a potential new taxon of genus *Penicillium*.
3. The fermentation products of strain DSF059 and its ethyl acetate extraction are both showing stable antagonistic effects on *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

[Conlusion]

Strain DSF059 isolated from deep-sea sediment may be a new taxon in genus *Penicillium* and its secondary metabolite showed stable antibacterial effects on *S. aureus* and *P. aeruginosa.*

Keywords: deep-sea fungi, DSF 059, phylogenetic analysis, antibacterial

Response mechanisms of foodborne *Staphylococcus aureus* to acid-heat cross adaptation on Membrane fatty acid composition and fluidity

Chen Xin[1], Yaqin Hu[2]*

[1]National Engineering Laboratory of Intelligent Food Technology and Equipment, Key Laboratory for Agro-Products Postharvest Handling of Ministry of Agriculture, Key Laboratory for Agro-Products Nutritional Evaluation of Ministry of Agriculture, Zhejiang Key Laboratory for Agro-Food Processing, Fuli Institute of Food Science, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China ; [2]College of Food Science and Technology, Hainan Tropical Ocean University, Sanya, China, 572022

[Objective]

Staphylococcus aureus (*S. aureus*) is a well-known foodborne pathogenic bacterium, making a severe menace to human wellness.Acid and heat are the most common adversity encountered by microorganisms in food. However, *S. aureus* in weak acid environment will produce acid resistance mechanism, and further enhance its resistance to heat stress, resulting in cross adaptation. Cell membrane is the first barrier for bacteria to resist external stress. Under the phenomenon of acid-heat cross adaptation, the fatty acid composition and fluidity of cell membrane will change accordingly, which plays a coping mechanism. In this case, this study focused on the response mechanism of fatty acid composition and fluidity of cell membrane to acid-heat cross adaptation, so as to provide a theoretical basis for ensuring microbial safety in food processing.

[Methods]

Different S. aureus strains were treated with Acetic acid, citric acid and lactic acid, three common organic acids in foods with pH = 4 for 48 hours and then subjected to 60 degree heat stress. To study the fatty acids composition, the bacteria were saponified, methylated, extracted and alkaline washed,then determined by LC. Membrane fluidity of S. aureus, response to different treatments, was assessed by the fluorescence anisotropy analysis with the fluorescent probe 1,6-dephenyl-1,3,5-hexatriene (DPH).

[Results]

S.aureus after organic acid stress can resist subsequent heat stress by reducing cell membrane fluidity. The change of fluidity of bacterial cell membrane is due to the change of fatty acid composition of cell membrane. Acid tolerant *S. aureus* can reduce cell membrane fluidity by reducing the proportion of anteiso / iso fatty acids, which is related to that anteiso C15:0 is the largest proportion of fatty acids in *S. aureus* cell membrane. Under heat stress,the fluidity of cell membrane futher decreased because of the increase of the content of straight chain fatty acids, so as to resist high temperature stress. The increase of straight chain fatty acids was mainly due to the increase of palmitic acid (C16:0) and stearic acid (C18:0).

Keywords: cross adaption;foodborne pathgen; response mechanism; cell membrance

Comparison of Nutrient Composition and Fatty acids of Muscles of Grass Carp, *Ctenopharyngodan idellus*

Xinru Fang[1], Wenzheng Shi[*1]

1, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China
**wzshi@shou.edu.cn*

[Objective]

The aim of this study was to investigate the differences of fatty acid composition among different parts of grass carp and to provide references for determining the edible quality and improving the economic value of grass carp.

[Methods]

The fatty acids composition of the dorsal meat, belly meat and red meat of fresh grass carp was determined by gas chromatography.

[Results]

The results of fatty acids composition showed that different parts of fresh grass carp meat are all rich in unsaturated fatty acids. 29, 24 and 31 kinds of fatty acids were detected in the dorsal meat, belly meat and red meat, respectively. In the total fat, the fatty acids composition of the three parts was basically the same, and the three most abundant fatty acids were oleic acid (18.85%-39.83%), palmitic acid (10.93%-22.22%) and linoleic acid (7.16%-18.24%). The oleic acid content in red meat (39.83%) was significantly higher than that in dorsal meat (27.69%), and dorsal meat was significantly higher than that in belly meat (18.85%) ($P < 0.01$); in terms of palmitic and linoleic acid contents, red meat was also significantly higher than dorsal and belly meat, but there was no significant difference between dorsal and belly meat ($P < 0.05$).

[Conclusion]

Therefore, all parts of grass carp have excellent fatty acids composition and distribution for human nutrition, and the fatty acid composition of red meat has the value of exploitation and utilization, which can provide reference for the subsequent volatile flavor research and control technology of grass carp.

Keywords: grass carp, muscle, fatty acids

Multi-frequency ultrasound：A potential method to improve the effects of surface decontamination and structural characteristics on large yellow croaker (*Pseudosciaena crocea*) during refrigerated storage

Xinyu Zhao[1], Weiqing Lan[1,2,3,*], Yuting Zhai[1], Jing Xie[1,2,3*]

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306
2, Shanghai Aquatic Products Processing and Storage Engineering Technology Research Center, Shanghai, 201306
3. National Experimental Teaching Demonstration Center for Food Science and Engineering (Shanghai Ocean University), Shanghai 201306)
*wqlan@shou.edu.cn(W.Q. LAN); jxie@shou.edu.cn (J.XIE)

[Objective]

The effects of multi-frequency ultrasound on surface decontamination and structural characteristics of large yellow croaker (*Pseudosciaena crocea*) during refrigerated storage were evaluated.

[Methods]

After the samples were processed, they were treated with 20 kHz single-frequency ultrasound (SUS), 20/28 kHz dual-frequency ultrasound (DUS) and 20/28/40 kHz triple-frequency ultrasound (TUS) for 10 min respectively. Then, samples were dried, put in PE bags and stored at 4 °C. Different indexes, such as microbial indicators (total viable count (TVC) and *psychrophilic* bacteria count (PBC)) , total volatile base nitrogen (TVB-N), pH, texture profile analysis (TPA), water holding capacity (WHC), which also combined with Low-field nuclear magnetic resonance (LF-NMR) and magnetic resonance imaging (MRI), myofibrillar fragmentation index (MFI), intrinsic fluorescence intensity (IFI), atomic force microscope (AFM), were used to analyze the effects with different ultrasound treatments comprehensively.

[Results]

Multi-frequency ultrasound retarded the growth of microorganisms. The bacteriostatic effect was positively correlated with the increase of ultrasound frequencies. However, compared with TUS treatment, DUS treatment had higher WHC and texture characteristics, inhibited the rise of pH and TVB-N. Through the results of MFI, IFI and AFM, multi-frequency ultrasound could effectively stabilize the myofibrillar protein structure of refrigerated large yellow croaker, which could maintain better texture characteristics. Among them, DUS had the best effects.

[Conclusion]

Multi-frequency ultrasound treatment could inhibit the growth of microorganisms and improve the structural characteristics of large yellow croaker during refrigerated storage. Moreover, multi-frequency ultrasound is a promising auxiliary method for improving the quality of aquatic products during refrigerated storage.

Keywords: multi-frequency ultrasound; large yellow croaker; surface decontamination; structural characteristics

Elucidating antimicrobial mechanism of nisin and grape seed extract against *Listeria monocytogenes* on shrimp through NMR-based metabolomics

Xue Zhao[1], Hongshun Yang[1,*]

[1] Department of Food Science and Technology, National University of Singapore, 117542, Singapore
fstynghs@nus.edu.sg

[Objective]

1. Evaluate the *in vitro* and *in vivo* antilisterial effects of nisin and grape seed extract (GSE);
2. Examine the effects of nisin and GSE on cell membrane integrity of *L. monocytogenes*;
3. Assess the metabolic response of *L. monocytogenes* under stresses of nisin and GSE.

[Methods]

L. monocytogenes SSA184 was grown in broth or cooked shrimp and treated by nisin (2000 IU/mL) and GSE (1%, w/v) alone or in combination. Cell membrane integrity of *L. monocytogenes* was determined by confocal laser scanning microscopy (CLSM) analysis and leakage of protein and nucleic acid. Metabolites of *L. monocytogenes* were extracted by cold methanol with three freeze-thaw cycles and analysed by NMR.

[Results]

The combination of nisin and GSE resulted in an enhanced and lasting antilisterial effect (1.79 CFU/g reduction), as compared with single nisin or GSE interventions. The cell structure was damaged and the permeability of cell membrane of *L. monocytogenes* was changed after an initial effect of nisin; this was confirmednby the leakage of biomacromolecules (protein and nucleic acid). Significant decreases in threonine, cysteine, ATP, NADP, adenine were observed based on NMR results, whereas a few of metabolites such as lactic acid and γ-aminobutyric acid (GABA) increased after the combination of nisin and GSE treatment. Those metabolites that contributed to the discrimination between the control group and the combination group were identified and 9 metabolites (e.g., lactic acid, leucine, glucose-6-phosphate, glutamate and pyruvate) with VIP value > 1 were selected biomarkers for the characterisation of *L. monocytogenes* on shrimp in response to combined nisin and GSE. Pathway analysis based on the selected differential metabolites further manifested that the combination treatment inhibited the survival of *L. monocytogenes* by blocking the TCA cycle, amino acid biosynthesis and energy-producing pathway. Due to the interaction between shrimp matrix and nisin, as well as the high protein concentration and slightly acidic condition of shrimp, the metabolic response of *L. monocytogenes* on shrimp was more complicated. GABA shunt and protein degradation from shrimp collectively compensated the unbalanced glycolysis and altered amino acid metabolism in *L. monocytogenes* on shrimp, leading to an enhanced resistant to the combined treatment as compared with the bacteria grown in broth.

Keywords: Nisin; Plant extract; Omics; *Listeria monocytogenes*; Antimicrobial mechanism

Antibacterial mechanism of ε-polylysine hydrochloride against *Shewanella putrefaciens*

Weiqing Lan[1,2], Xuening Chen[1], Haojie Feng[1] , Jing Xie[1,2*]

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

[Objective]

The objective of this research is to study the antibacterial mechanism of ε-polylysine hydrochloride (ε-PLH) against *Shewanella putrefaciens*.

[Methods]

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of ε-PLH against *Shewanella putrefaciens* were measured. The antibacterial mechanism of ε-PLH with different concentrations against *Shewanella putrefaciens* was evaluated by growth curve, electrical conductivity, propidium iodide (PI) intake, alkaline phosphatase (AKPase), lactate dehydrogenase (LDHase) and adenosine triphosphatase (ATPase) activity, which also combined with scanning electron microscope (SEM).

[Results]

The MIC and MBC of ε-PLH against *Shewanella putrefaciens* were 1.0 mg/mL and 2.0 mg/mL respectively. The growth of bacteria was inhibited, the extracellular AKPase and LDHase activities were significantly increased, while the ATPase activity in the cell membrane was greatly decreased. The results of PI intake and electrical conductivity were positive correlated with the concentration of ε-PLH. The results of SEM showed that after PLH with MIC treatment, the bacteria appeared depression, hole and other phenomena, the appearance had changed. After PLH with 2MIC treatment, the cell wall and cell membrane were seriously damaged, the contents leaked, and the cell had been seriously deformed.

[Conclusion]

ε-PLH could affect the normal growth of *Shewanella putrefaciens*, damage the cell wall and leak the contents, and lead to the death of cells due to their inability to carry out normal growth and metabolism finally. Therefore, ε-PLH had a wide application prospect in the field of seafood, and the antibacterial mechanism would provide a theoretical basis for the application of ε-PLH in the storage of aquatic products.

Keywords: ε-polylysine hydrochloride, *Shewanella putrefaciens*, antibacterial mechanism

Study on biochemical and sensorial changes of fresh and frozen-thawed scallop adductor muscle as raw materials for sashimi during cold storage

Yabin Niu[1] Shiliang Dong[1] Naoto Shimakage[2] Huamao Wei[3] Kefeng Yu[4] Chunhong Yuan*[2] Koichi Takaki[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 Sanriku Fisheries Research Center, Iwate University, Heta 3-75-1, Kamaishi, Iwate 026-0001, Japan
5 Faculty of Science and Engineering, Iwate University, Ueda 4-3-5, Morioka, Iwate 020-8551, Japan
*chyuan@iwate-u.ac.jp

[Objective]

Yesso scallop (*Mizuhopecten yessoensis*) is one of the major marine products in Japan. Adductor muscle is the main edible part of scallops, which has great popularity in making sushi and sashimi used both fresh and frozen-thawed scallop adductor muscle as raw material. With the progress of globalization, sushi and sashimi have become very popular worldwide. In order to cope with long-distance transportation and prolong the shelf life of perishable food materials, freezing is regarded as a good way, even with inevitable quality loss. There are some previous studies focused on the quality changes of scallops after the freezing and thawing process. However, there are rare information about the quality changes of scallop adductor muscle after the freeze-thaw process from the perspective of protein, even though proteins are basic components of the scallop adductor muscle. In this study, the quality changes of frozen-thawed scallop adductor muscle were discussed from the viewpoint of myofibrillar protein. It is generally known that quality change or loss of freshness for aquatic products is due to a complex combination of biochemical and physical processes. The objective of this study is to differentiate the biochemical and sensorial changes of fresh and frozen-thawed scallop adductor muscle during cold storage at 4℃, for studying quality maintenance of frozen scallop adductor muscle.

[Methods]

All the fan-shaped pieces of scallop adductor muscle were divided into two groups, including the fresh group (soaked in sterilized seawater for 20 min) and the frozen-thawed group (frozen at -60℃→ thawed in ice water→ soaked in sterilized seawater for 20 min). After soaking, all the samples were placed at 4℃, and the analytical measurements were taken on day 0, 1, 2, 4 during storage as follows, determination of Ca^{2+}-ATPase activity, Mg^{2+}-ATPase activity, ATP-related compounds and pH value. Triangle test and quality index method (QIM) were used to confirm the changes in the sensory characteristics of fresh and frozen-thawed scallop adductor muscle. The microstructure of myofibrils was examined using an optical microscope with a 400 magnification. Microbial analysis was used to detect frozen-thawed scallop adductor muscle on day 4.

[Results]

The results of sensory evaluation showed that fresh and frozen-thawed scallop adductor muscle (day 0) can be distinguished and frozen-thawed scallop adductor muscle can be happily accepted by panelists on day 0~1. The length of myofibrils in the fresh group was 116.06 ± 19.87 μm, much longer than that in the frozen-thawed group. Sarcomere was hardly identified and there were a large number of small masses in the frozen-thawed group. There are no significant differences ($p > 0.05$) in Ca^{2+}-ATPase activity between the fresh and frozen-thawed scallop adductor muscle during storage for four days while there are significant differences ($p < 0.05$) in Mg^{2+}-ATPase activity between fresh and frozen-thawed scallop adductor muscle at the initial days of cold storage (day 0~1). The ATP content of frozen-thawed scallop adductor muscle decreased remarkably to 0.79 ± 0.02 μmol/g after thawing immediately, compared with 6.70 ± 0.13 μmol/g of ATP content in the fresh group. Meanwhile, pH value after thawing dropped to 6.67 ± 0.02 immediately, while the pH value of fresh scallop adductor muscle was 7.06 ± 0.08.

[Conclusion]

The sensorial differences between fresh and frozen-thawed scallop adductor muscle as raw materials for sashimi demonstrated by the triangle test, which can be also reflected in the results of ATP-related compounds and pH value. The myofibrillar protein which constitutes myofibrils could change due to freezing and thawing, even though the head region of myosin remained stable. Freezing can be regarded as an available method for long-distance transportation, as long as the scallop adductor muscles are handled properly and eaten in time after thawing.

Keywords: Sashimi, Sensorial differences, Quality index method, Myofibrillar protein, ATPase activity

Identification of MT1-MMP in yellowtail muscle for utilization of low quality meat as *surimi* products

Yan-Rong Jiang[1], Asami Yoshida*[1], Yi-Li Gao[1], Xiao-Mi Sun[1], Hina Noguchi[2], Kairi Yamada[3], Erika Noguchi[3], Koichi Kuwahara[3], Kiyoshi Osatomi[1]

1, Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki 852-8521, Japan
2, Faculty of Fisheries, Nagasaki University, Nagasaki, 852-8521, Japan
3, Nagasaki Prefectural Institute of Fisheries, Nagasaki 851-2213, Japan
* bb53420002@ms.nagasaki-u.ac.jp; y-asami@nagasaki-u.ac.jp

[Objective]

After spawning, the deterioration of yellowtail (*Seriola quinqueradiata*) meat have resulted in a reduction of commercial value, so we expect the utilization as a raw material of *surimi*-based products. However, even though the water-soluble proteinases in yellowtail muscle is removed by washing, a decrease of breaking force and deformation of *surimi* gel (*modori* phenomenon) still happened at 50-70°C. In our laboratory, it has found that *modori* phenomenon can be prevented by inhibiting insoluble metalloproteinase and as a candidate of the insoluble metalloproteinase, MT1-MMP (Membrane type 1-matrix metalloproteinase) mRNA has been detected in jack mackerel. Thus, the objective of this study is to identify MT1-MMP in yellowtail muscle and investigate its expression level in low and high quality meat.

[Methods]

Total RNA was extracted from the yellowtail muscle by ISOGEN II and synthesized cDNA. The degenerated primers used for RT-PCR were designed based on the MT1-MMP gene sequences of zebrafish, yellowtail amberjack and pufferfish. The synthesized cDNA was used as a template, and Ampli*Taq* Gold 360 DNA polymerase were used for amplification of MT1-MMP. TA cloning was performed with pGEM T-Easy Vector and sequence of each of 3-5 independent positive clones were verified. The mRNA expression levels were evaluated by RT-PCR.

[Results]

The results showed that the two isoforms of MT1-MMP cDNA were successfully identified in yellowtail muscle. MT1-MMPa cDNA sequence was consisted of a 3,325 bp nucleotide sequence encoding 538 amino acids (ORF 1,619 bp). MT1-MMPb cDNA sequence was consisted of a 2,443 bp nucleotide sequence encoding 536 amino acids (ORF 1,612 bp). The two isoforms consisted of a peptidase M10 domain, hemopexin I-IV domain and transmembrane domain, which were conserved among the species we compared. Based on these sequence data, the specific primers were designed for RT-PCR and the mRNA expression analysis of both isoforms in low and high quality meat is now in progress.

[Conclusion]

Two isoforms of MT1-MMP genes were firstly identified in yellowtail muscle.

Keywords: yellowtail muscle, *surimi*, *modori* phenomenon, MT1-MMP

Myosin in longitudinal retractor muscle of sea cucumber

Yao Xian Chin[1]*, Ye Mi[1], Wan Xiu Cao[1], Phaik Eem Lim[2], Chang Hu Xue[1] and Qing Juan Tang[1]

1 Human Health Research Laboratory, College of Food Science and Engineering, Ocean University of China, Qingdao 266003, Shandong, China
2 Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur 50603, Malaysia

chinyx1@163.com

[Objective]

Kappaphycus alvarezii is a commercially important red alga widely cultivated for carrageenan production. It is also a traditional food that received lack of research attention due to the overwhelming focus on the controversial safety of its main polysaccharides, the widely-used hydrocolloid, κ-carrageenan. This study investigates the anti-obesity properties of *Kappaphycus alvarezii* as three separate components (whole Kappaphycus, κ-carrageenan, and sans-carrageenan fraction) on obese C57BL/6J mice when supplemented to high-fat diet (HFD).

[Methods]

The sample used was harvested in coastal areas near Sabah, Malaysia. Carrageenan was extracted using hot water extraction and ethanol (95% v/v) precipitation at 1:3 ratio. Whole *Kappaphycus*, carrageenan and sans-carrageenan fraction were respectively supplemented (5% w/w) in HFD of obese C57BL/6J mice. The animal phenotype, biochemical analysis of serum, adipocytes size, fecal short-chained fatty acids (SCFAs) profile, gut microbiota changes and gene expressions in lipid metabolism were measured after 10 weeks.

[Results]

1. All treatment diets successfully reduced the effects of the HFD.
2. Both carrageenan and sans-carrageenan fraction were more effective compared to whole Kappaphycus.
3. Carrageenan and sans-carrageenan fraction influenced the production of SCFAs and altered the gut microbiota in mice, partially restored some bacteria associated with leanness.
4. Carrageenan and sans-carrageenan might have different anti-obesity mechanism by affecting distinct genes involved in lipid metabolism.

[Conlusion]

The seaweed *Kappaphycus alvarezii* has anti-obesity effects that are relayed though both carrageenan and sans-carrageenan fraction, possibly via different mechanisms.

Keywords: seaweed, *Kappaphycus alvarezii*, carrageenan, obesity, gut microbiota, lipid metabolism

Effect of octenyl succinic anhydride modified bovine bone gelatin and interaction with surfactants on the oil/water interface of fish oil-loaded emulsion

Ye Zi[1], Jiamin Xu[1], Mengyang Yang[1], Xichang Wang[1], Jian Zhong*[1]

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
* jzhong@hntou.edu.cn

[Objective]

The Octenyl succinic anhydride (OSA) modified bovine bone gelatin (BBG) and four surfactants (Span 80, soybean lecithin (SL), Tween 80, SDS were used to stabilize the emulsion, and to explore the effects of OSA modification on bovine bone gelatin and its interaction mechanism with the four surfactants in the emulsion interface.

[Methods]

Fourier infrared spectroscopy for modified and undecorated gelatin, SDS-PAGE gel electrophoresis analysis and texture analysis to explore the effects of OSA modification on bovine gelatin. At the same time, the emulsion is prepared by gelatin/surfactant, and the interaction mechanism of the emulsion interface is explored by microscope observation, particle size analysis, emulsion analysis and emulsion analysis.

[Results]

The results showed that the bovine bone gelatin modified by OSA and the four surfactants were synergetic (Span 80 and SL) or competitive (Tween 80, SDS) adsorption behavior at pH 6. The bovine gelatin/surfactant acts as a synergy (SDS, Tween 80) or competition (Span 80, SL) adsorption at pH 6.

[Conlusion]

This shows that OSA modified bovine bone gelatin changed its interaction with surfactants at the emulsion interface.

Keywords: Octenyl succinic anhydride; Emulsion; Competitive adsorption; Synergetic adsorption; Surfactant

稳定鱼油乳液辛烯基琥珀酸酐改性的牛骨明胶与表面活性剂在油水界面相互作用的影响

The quality improvement of threadfin bream (*Nemipterus virgatus*) *surimi*-gel with soy protein as a natural food additive

Yi-Li Gao[1], Asami Yoshida*[1], Shinnosuke Maeda[2], Jin-Yang Liu[3], Yan-Rong Jiang[1], Xiao-Mi Sun[1], Chao-Ping Chen[4], Yajun Wang[5], Tetsuhiko Okajima[4] and Kiyoshi Osatomi[1]

1, Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki, 852-8521, Japan
2, Faculty of Fisheries, Nagasaki University, Nagasaki, 852-8521, Japan
3, China National Research Institute of Food and Fermentation Industries, Beijing, 100015, China
4, Wilmar Japan Co., Ltd., Tokyo, 104-0061, Japan
5, Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd., Shanghai, 200137, China
* *loootutugaoyili@163.com; y-asami@nagasaki-u.ac.jp;*

[Objective]

Threadfin bream (*Nemipterus virgatus*) is one of the most important commercial fish species in Japan and Southeast Asia, and it has been widely used as a raw material for *surimi*-based products. Previously, we found that the leakage of a visceral novel trypsin (SSP; sarcoplasmic serine proteinase) into the muscle can induce *modori* phenomenon in threadfin bream. The *modori*-inducing proteinase also has a high possibility of remaining in commercial *surimi* products due to insufficient washing. While, soy protein containing soybean trypsin inhibitor (SBTI) can supress the *modori*-inducing proteinase. The objective of this study is to evaluate soy protein as a natural food additive, which contribute to improve *surimi*-based products of threadfin bream.

[Methods]

The commercial soy protein was obtained from Wilmar Japan Co., Ltd. The autolytic activity of threadfin bream *surimi* was measured at 60°C for 60 min at pH 7.5 using SDS-PAGE. The *surimi*-gel was prepared using commercial *surimi* with or without soy protein and incubated at several temperatures for gel forming. The soy protein inhibitory effects were evaluated by the gel strength of 15-mm thickness *surimi*-gel, using a rheometer.

[Results]

Autolytic activity of threadfin bream *surimi* was suppressed by SBTI, which proved that SSP was remained in the commercial threadfin bream *surimi*. And the addition of soy protein also showed inhibitory effects on autolytic activities of threadfin bream *surimi*. Besides, the addition of soy protein increased breaking strength of *surimi*-gel, even if it was incubated at 60°C, *modori*-inducing condition.

[Conclusion]

It is concluded that SSP is remained in the commercial threadfin bream *surimi*, which can be suppressed by soy protein. Therefore, soy protein can be regarded as a good natural food additive to improve *surimi*-based products of threadfin bream.

Keywords: *surimi*-gel, *modori* phenomenon, threadfin bream, soybean additives

AI-2/Lux-S quorum sensing of Lactic acid bacteria SS-128 endows refrigerated *Litopenaeus vannmei* better texture: Mechanism exploration by multiple proteomics

Yuan Li, Zunying Liu*, Mingyong Zeng*
College of Food Science and Engineering, Ocean University of China, Qingdao 266003, China
liyuan@ouc.edu.cn

[Objective]

Litopenaeus vannamei is one of the most representative aquatic products with high demand of quality, and its texture maintenance has become a virtual issue. The quorum sensing (QS) system of biocontrol lactic acid bacteria is an essential target for delaying storage corruption. This study aims to clarify how biocontrol lactic acid bacteria *Lactobacillus plantarum* SS-128 help maintain the texture of *Litopenaeus vannamei* during storage by manipulating the metabolism of spoilage bacteria metabolism.

[Methods]

The *Lactobacillus plantarum* and *luxS*-mutant of SS-128（Δ*luxS*/SS-128) was constructed in the Ocean University of China. The *Lactobacillus plantarum* SS-128 and *Shewanella baltica* was incubated into *Litopenaeus vannamei*. The proteomics of *Litopenaeus vannamei* was conducted by TMT proteomics, and the analysis for surface bacteria was conducted by Label free proteomics.

[Results]

1. The inoculation of *Shewanella baltica* accelerated the deterioration of *Litopenaeus vannamei*. The inoculation of lactic acid bacteria SS-128 ameliorate the process.
2. The texture of *Litopenaeus vannamei* in lactic acid bacteria SS-128-inoculated group indicated a tardier decreasing trend, corresponded to the better sensory score.
3. Myosin heavy chain, skeletal muscle actin 6, and beta-actin were protected by AI-2/Lux-S quorum sensing of lactic acid bacteria SS-128 during storage.
4. The amount of up-regulated protein concerning catalytic activity of *Lactobacillus plantarum* Δ*luxS*/SS-128 compared to the ordinary strain was 89, those proteins were potential reasons for quicker decomposition in *Lactobacillus plantarum* Δ*luxS*/SS-128-inoculated *Litopenaeus vannamei.*

[Conclusion]

Lactobacillus plantarum SS-128 holds the ability to maintain the texture of polluted *Litopenaeus vannamei.* AI-2/Lux-S quorum sensing system plays a vital role in the texture maintaining process, by inhibit the expression of specific protease, eliminating their deterioration on texture-related proteins

Keywords: Biocontrol lactic acid, quorum sensing, proteomics, *Litopenaeus vannmei*

Effect of carp (*Cyprinus carpio*) scale hydrolysates on the quality of Malaysian longarm shrimps (*Macrobrachium rosenbergii*) during freeze-thaw cycles

Yubin Ying[1], Yaqin Hu[2]*
[1]National Engineering Laboratory of Intelligent Food Technology and Equipment, Key Laboratory for Agro-Products Postharvest Handling of Ministry of Agriculture, Key Laboratory for Agro-Products Nutritional Evaluation of Ministry of Agriculture, Zhejiang Key Laboratory for Agro-Food Processing, Fuli Institute of Food Science, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China
[2*] College of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, China
* *1004346262@qq.com*

[Objective]

Malaysian longarm shrimp (*Macrobrachium rosenbergii*) is one of the important freshwater economic shrimps in the world. Frozen storage is a common and effective means of shrimp preservation, but there must be freeze-thaw cycles in shrimp freezing storage, and the quality of shrimps is obviously damaged by freeze-thaw cycles. Cryoprotectant can effectively inhibit the quality deterioration of shrimps during freeze-thaw cycles, but the traditional cryoprotectants have many problems, such as high sweetness, adverse to human health and so on. In this case, In this case, the study focused on the effect of carp (Cyprinus carpio) scale hydrolysate on the quality of Malaysian longarm shrimps during freeze-thaw cycles, and provided a basis for carp scale hydrolysate as a new efficient and healthy cryoprotectant.

[Methods]

Carp scales were hydrolyzed by alkaline protease to prepare fish scale hydrolysates. The shelled Malaysian longarm shrimps were soaked and vacuum soaked in fish scale hydrolysate as the experimental group, which were recorded as SSH and VSSH respectively. The untreated shrimp meat was used as the blank control, and the shrimp meat was soaked in sodium tripolyphosphate as the positive control. Every group was put into - 25 ℃ for quick freezing, and five freeze-thaw cycles were carried out after freezing. After each freeze-thaw cycle, quality indexes of shrimp meat were measured, such as change of water content, color, pH, hardness, salt soluble protein content, sulfhydryl content, TBA and so on.

[Results]

1. Compared with fresh shrimps, sodium tripolyphosphate soaking increased the L value of shrimp meat and reduced the shear force, while there was no difference in the color and shear force of shrimp meat in VSSH and SSH.
2. The inhibition effect of VSSH on the quality deterioration of shrimp during freeze-thaw cycles was better than that of SSH, which proved that vacuum immersion could effectively improve the protective effect of fish scale hydrolysate on frozen shrimp meat.
3. VSSH effectively inhibited drip loss, internal water migration, protein denaturation and structural damage of shrimps during freeze-thaw cycles. The effect of VSSH was not as good as the positive control in the short-term freeze-thaw cycle, but the effect was better than the positive control after the third freeze-thaw cycle.

[Conclusion]

. The combination of carp scale hydrolysates and vacuum impregnation effectively inhibited the quality deterioration of Malaysian longarm shrimp during freeze-thaw cycles. And the effect was better in long-term freeze-thaw cycle, compared with sodium tripolyphosphate..

Keywords: *Macrobrachium rosenbergii*, carp scale hydrolysates, quality, freeze-thaw circles

Improvement of water-holding capacity and textural properties of frozen tilapia (*Oreochromis Niloticus*) fillets by light salting

Yufan Du[1], Qingqing Jiang[*1], Wenzheng Shi[1], Zhihe Wang[1]

1,Department of Food Science and Technology, Shanghai Engineering Research Center of Aquatic Product Processing & Preservation, Shanghai Ocean University, Shanghai 201306, China
[*]*qqjiang@shou.edu.cn*

[Objective]

The objective of the present study was to investigate the quality changes of lightly salted tilapia fillets prepared by different salting conditions (6%-2 h, 6%-4 h, 9%-1 h, 12%-0.5 h) during frozen storage.

[Methods]

Changes in the thawing loss, centrifuging loss, water-binding capacity, textural properties, and color characteristics were analyzed to reveal the effects of light salting on the quality of frozen tilapia fillets. Moreover, the mechanism was studied from the aspects of lipid oxidation, protein denaturation and microstructure changes.

[Results]

Lightly salted tilapia fillets were characterized by higher water-holding capability, springiness and cohesiveness after thawing due to the higher recovery ability of microstructure compared with the control group. After 28 d of frozen storage, the samples obtained by brining with 9% NaCl solution for 1 h had the highest water holding capability, springiness and cohesiveness. However, in the later period of frozen storage, the degree of lipid oxidation of lightly salted tilapia fillets increased.

[Conclusion]

It was concluded that the water-holding capacity and textural properties of frozen tilapia fillets were improved by light salting. However, lightly salting before freezing reduced the oxidative stability of tilapia fillets during frozen storage, which has become one of the key issues to be resolved.

Keywords: water-holding capacity, texture, lipid oxidation, lightly salting

Ameliorative Effects of *Eucheuma cottonii* Extract on Osteoarthritis Induced by Meniscal/Anterior Cruciate Ligament Injury in Obese Male Rats

Yu-Hsuan Kao[1], Sabri Sudirman[2], Zwe-Ling Kong[1*]
[1]Department of Food Science, National Taiwan Ocean University, Keelung 20224, Taiwan.
aabbccdd1234603@gmail.com

[Objective]

It's generally accepted that seaweeds have many therapeutic properties, including the anti-inflammation and antioxidant effects. The objective of this study is to investigate the ameliorative effects of red seaweed *Eucheuma cottonii* extract (ECE) on osteoarthritis (OA) model in obese rat.

[Methods]

OA rats were fed a 40% high-fat diet for 12 weeks, then operated the surgery by anterior cruciate ligament tear and meniscus injury on the right knee joint. ECEs were applied to fed by using oral gavage. Moreover, after 5 weeks of treatments, rats were sacrificed and the sample analysis was performed.

[Results]

The results showed that treatment with ECE for 5 weeks decreased the body weight, triglyceride, and total cholesterol (TC) levels, and the TC/high-density lipoprotein-cholesterol ratio in obese rats. ECE can also reduce the pain caused by OA and decrease the loss of articular cartilage proteoglycan. ECE accurately inhibited the expression of pro-inflammatory factors, such as tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), leptin, prostaglandin (PG-E2) and matrix metalloproteinases in the blood plasma of obese OA rats by interfering with the nuclear factor-kappa B (NF-κB) and extracellular signal regulated kinase, (ERK1/2) pathway.

[Conlusion]

It is concluded that dietary polysaccharide from ECE suppressed OA development in obese rats, suggesting its potential efficacy against OA.

Keywords: osteoarthritis, *Eucheuma cottonii*, anti-inflammatory, seaweed, obesity

Fish oil emulsion and millimeter capsules prepared from sodium alginate / Span: effects of span type on physicochemical stability

Zheng Yulu[1], Xu Jiamin[1], Yang Lili[1], Huang Shudan[1], Wang Xichang[1], Zhong Jian[1*]

1 College of Food Science & Technology, Shanghai Ocean University, National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, Shanghai 201306, China
18026569473@163.com

[Objective]

to study the effects of different Span on the stability of sodium alginate stabilized fish oil emulsion and the prepared millimeter capsule.

[Methods]

different types of Span were added into alginate aqueous solution to prepare sodium alginate stabilized fish oil emulsion. Macroscopical photography and optical microscopy were used to observe emulsion, and the effects of different Span on sodium alginate emulsions and their granules were compared by particle size, loading rate, peroxide value, and in vitro digestion.

[Results]

the alginate emulsion added with 3H had good stability, while in 20h, the stability of Span 40 was the best. In addition, the sodium alginate concentration remained the same. This means that the water content and loading rate remained the same. The results of accelerated test confirmed that fish oil leakage would occur within 48 hours, resulting in oxidation and yellowing on the particle surface. The peroxide value of Span 20 was the highest, which could reach 45mmol/kg. In addition, the capsule also showed the specificity and sustained-release behaviour of fish oil in the small intestine phase of gastrointestinal and small intestine models in vitro. The in vitro digestion trend of sodium alginate microspheres containing Span 20 and Span 40 was like that of non-added capsule, and the final free fatty acid release reached about 45%, while the addition of Span 40 and Span 60 could significantly improve the free fatty acid release effect, and finally reached about 65%.

[Conlusion]

Different Span can have different effects on alginate fish oil emulsion and can also affect the release of substances contained in alginate microspheres. These works provide useful information for the study of surfactant series in the study of natural macromolecule sodium alginate.

Keywords: Emulsion; Fish oil; Sodium alginate; Span; In vitro digestion

Partial purification and biochemical properties of enzyme related to melanosis of *Euphausia Pacifica*

Yumeng Lin[1], Kefeng Yu[2], Huamao Wei[3], Shiliang Dong[2], Tetsuro Yamashita[1], Chunhong Yuan*[1]
1 Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan
2 United Graduate School of Agricultural Sciences, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan
3 College of Food and Pharmaceutical Sciences, Ningbo University, Ningbo, Zhejiang 315211, China

[Background and Purpose]
Isada krill, *Euphausia pacifica* Hansen (Isada), is the dominant specie of euphausiid in the North Pacific Ocean. There is a commercial fishery for this species off the Sanriku and Joban coasts of north-eastern Japan from February to April, and the annual harvesting amount is about 10,000 tons in Iwate coast which accounting for about 50 % of the national harvesting amount. Compared to shrimps, the blackening (melanosis) occurred more quickly in Isada, and its freshness deteriorated within a few hours after landing. Therefore, the isada is majorly used for low-value applications such as fish meal and animal feed rather than edible food for human. On the other hand, some health functional ingredients such as 8-HEPE and EPA have been discovered in Isada in recent years, and the effective use of Isada as a food has received widespread attention. However, there are few studies on the enzymes responsible for melanosis from Isada. In this study, we investigated the partial purification of the enzymes responsible for melanosis and their biochemical properties.

[Materials and methods]
Fresh Isada was obtained from Kamaishi Bay of Iwate Prefecture in February 2021 and stored at -60 ℃ after they were transported to lab. The frozen Isada was freeze-dried into powder for subsequent analysis. One gram Isada powder was stirred with 10 ml of 0.1 M Tris-HCl buffer (pH 7.2) containing 0.5 M NaCl and 0.2 % Brij35 for 1.5 h at 0 ℃ and the supernatant was obtained after centrifugation at 12,000 g for 30 min. The supernatant was fractionated by 30-60% ammonium sulfate, and the partial purification of enzyme was conducted by using Sephadex G-100 and Superdex 200 3/100g. The enzyme activity was determined with 0.1 M pyrocatechol at 25℃. The partial purification enzyme was characterized by determining the optimum pH, optimum temperature, pH stability, temperature stability. Furthermore, the band of enzyme obtained by SDS-PAGE was treated with trypsin and analyzed by a liquid chromatograph mass spectrometer (LC-MS).

[Results and Discussion]
(1) The highest activity of enzyme was shown at 25 ℃, and the optimum pH was around 6.5. (2) Under the heat treatment from 15 ℃ to 60 ℃ for 30 minutes at each temperature, the high stability of enzyme was shown at 15-30 ℃ and the enzyme activity decreased with temperature in the range of 30-60 ℃. (3) The enzyme activity was promoted by Cu^{2+}. (4) the purified enzyme of Isada was around 70 kDa, showing a close relationship with the tyrosinase enzyme of *Litopenaeus vannamei* around 73 kDa by LC-MS.

Key words: Isada krill, melanosis, pH stability, thermal stability

Effect of slightly acid electrolyzed water ice on microbiota composition and quality of shrimp (*Litopenaeus Vannamei*)

Yun He [1], Hongshun Yang [1, *]

[1]Department of Food Science and Technology, National University of Singapore, Science Drive 2, Singapore 117542, Singapore
* *fstynghs@nus.edu.sg*

[Objective]

Ice is helpful for the preservation of perishable seafood but not much effective in killing bacteria. The aim of present study was to investigate the effects of generated slightly acid electrolyzed water (SAEW) ice on quality and microbial change of shrimp during ice storage.

[Methods]

A portable sanitizing unit was developed consisting of an electrochemical cell, made up RuO_2-IrO_2/TiO_2 electrodes, with 1% (w/v) NaCl solution and 4 mM $NaHCO_3$ as electrolytes. The shrimps were placed in tap water (TW) ice, NaCl ice and SAEW ice for 7-day storage. Bacterial counts were enumerated by standard plate count agar. The microbiota community was studied by 16S rDNA-based Illumina sequencing. The polyphenol oxidase (PPO) and acid phosphatase (ACP) were evaluated by test kits. K-value, color, pH and TVB-N values were determined to test the quality of the shrimp. Analysis of variance (ANOVA) was performed for mean comparisons with significant difference being set at $P < 0.05$.

[Results]

The SAEW ice (FAC: 34 mg/L; pH: 6.24; ORP: 836.4 mV) generated by water yielded from the sanitizing unit exhibited inhibitory activity toward PPO and ACP with 55.3 % and 61.9 % inhibition rate at day 7, respectively, along with less discoloration and the lowest K-value. Besides, SAEW ice controlled well aerobic mesophilic and psychrotrophic bacteria (2.36 and 0.43 log CFU/g reduction, respectively), which exhibited better antibacterial activity compared with other two groups. 16s rDNA high-throughput sequencing analysis elucidated the growth of major spoilage genus presented in TW ice treated shrimp (*Shewanella*, *Vibrio* and *Aeromonas*) and in NaCl ice treated shrimp (*Psychrobacter*) was inhibited in SAEW ice treated shrimps, which further led to lowest increase of pH and TVB-N during storage. Overall, the SAEW ice exhibited promising preservative effect on the shrimp.

[Conclusion]

It is concluded that the slightly acid electrolyzed water ice generated by developed portable sanitizing unit is effective in controlling seafood spoilage and preserve the quality.

Keywords: slightly acid electrolyzed water, quality, spoilage bacteria, freshness

Enzymatic Hydrolysis Process of Silver Carp Protein

Yuting Wang[1], Wenzheng Shi*[1,2]

1, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
2, Shanghai Engineering Research Center of Aquatic Product Processing and Preservation, Shanghai Ocean University, Shanghai 201306, China
* *wzshi@shou.edu.cn*

[Objective]

It's generally accepted that silver carp is a rich low-value fish in China. It has rich nutritional value, large yield and high protein content. It is easy to be absorbed by the human body. The objective of this study is to provide a scientific basis for the preparation of fish flavor base material, and play a positive role in the deep processing of silver carp.

[Methods]

Taking the protein recovery rate as the index, the utilization degree of four common proteases (flavor protease, papain, trypsin and alkaline protease) on silver carp meat protein was compared. Two proteases were selected for complex enzymatic hydrolysis of silver carp meat. The effects of protease complex ratio, enzyme addition, pH value, enzymatic hydrolysis time and enzymatic hydrolysis temperature on the enzymatic hydrolysis products of silver carp were compared.

[Results]

The results showed that the optimum hydrolysis conditions are: compound ratio is 1:3(papain: alkaline protease),the dosage of compound enzyme is 4 000 U/g, the initial pH is 6, hydrolyzed for 4 h at 55℃. Under the optimal enzymatic hydrolysis conditions, the protein recovery of silver carp is 87.48%.

[Conlusion]

It is concluded that the recovery of silver carp protein can be improved by double enzyme hydrolysis.

Keywords: silver carp, enzymatic hydrolysis, papain, alkaline protease

Ultrasonication induced nano-emulsification of thyme essential oil: optimization and antibacterial mechanism against *Escherichia coli*

Zhehao Yang [a], Qiao He [a], Balarabe B. Ismail [a], Yaqin Hu[b *], Mingming Guo[a, **]

a College of Biosystems Engineering and Food Science, Fuli Institute of Food Science, Zhejiang University, Hangzhou, 310058, China
b College of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, China
* yqhu@hntou.edu.cn; ** mingguo@zju.edu.cn

[Objective]

Essential oil nanoemulsions are homogeneous dispersion systems with excellent dispersion characteristics and antibacterial activity. However, few reports have simultaneously elucidated the effects of fabrication parameters on dispersion characteristics and antibacterial properties. In this regard, different parameters are selected to prepare essential oil nanoemulsions and then their antibacterial mechanism against *E. coli* are discussed.

[Methods]

Response surface methodology is conducted to optimize the fabrication of thyme essential oil nanoemulsions and the significance of the optimal models are also analysed. Sonication time, power together with emulsifier types (Tween 80, SDS and CPC) and concentrations are selected as variables. Moreover, cell integrity, morphological changes and interaction analysis are conducted to study the antibacterial mechanism of thyme essential nanoemulsions.

[Results]

1. Optimized conditions of ultrasonication induced thyme essential oil nanoemulsions (TEON) were obtained.
2. Optimized TEON with Tween 80 as emulsifier (TEON-T80) exhibited excellent storage stability.
3. Optimized TEON with CPC as emulsifier (TEON-CPC) showed the strongest antibacterial activity on *E. coli*.
4. TEON-CPC showed bactericidal effect through strong electrostatic interaction.

[Conlusion]

Optimal ultrasonication and emulsifier concentration conditions for the preparation of three different TEON with enhanced dispersion characteristics and antibacterial activity were obtained by response surface methodology. Despite the need to optimize further when expanding its application in the food industry, TEON-CPC was suggested as the most optimal nanoemulsions considering its superior antibacterial efficacy.

Keywords: Thyme essential oil, Ultrasonication, Nanoemulsions, Response Surface Methodology, Antibacterial mechanism, *Escherichia coli* O157:H7

Bacteriostasis research of citral nanoemulsion against *Shewanella putrefaciens* by invitro culture observation and gene expression analysis

Zhiheng Hu[1,2],Yaqin Hu[2*]

[1]College of Biosystems Engineering and Food Science, National-Local Joint Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang Key Laboratory for Agro-Food Processing, Integrated Research Base of Southern Fruit and Vegetable Preservation Technology, Fuli Institute of Food Science, Zhejiang University, Hangzhou 310058

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

[Objective]

The spoilage organisms control in aquatic products is vital for the maintaining of quality and edible safety. *Shewanella putrefaciens* is one of the common specific spoilage organisms in aquatic products and citral is an active antibacterial substance for microorganism control. Till now, the researches about the influence and antibacterial mechanism of citral to *Shewanella putrefaciens* were seldom reported. For a comprehensive analysis of bacteriostasis, in addition to invitro culture observation, the bacteriostasis can also be indicated by gene analysis such as transcriptome analysis and PCR in a more accurate way and few studies have focused on the gene expression change of *Shewanella putrefaciens* treated by citral. Hence, the investigation of bacteriostasis of citral against *Shewanella putrefaciens* can reveal a potential application in spoilage microorganism control in aquatic products.

[Methods]

In order to ensure the stability and good water solubility of citral, citral was prepared into nanoemulsion by ultrasonic treatment and applied to *Shewanella putrefaciens*. The bacteriostasis effects were measured by invitro culture observation and gene expression analysis. The invitro culture observation included the strain variation of growth, biochemical structure and quorum sensing (QS) system (luxS/AI-2). The gene expression analysis were performed by transcriptome analysis when the gene expression situations of genetic function system, biological structure formation, spoilage ability, and QS system were compared with the *Shewanella putrefaciens* treated by citral nanoemulsion and not be treated. Meanwhile, the PCR analysis was performed for the verification of gene expression results from transcriptome analysis.

[Results]

1.The invitro culture observation results indicated the significant inhibition effects of citral nanoemulsion to the strain's growth, biological structure formation and the activity of QS signal molecule AI-2;

2.The gene expression analysis more accurately verified the bacteriostasis of citral nanoemulsion when the majority genes were down-regulated included various properties expression and metabolic pathway;

3.The existence of several QS systems were verified by gene analysis included a relatively complete AI-2/luxS QS system and other QS systems like AHLs QS system and DKPs QS system;

4.The relative fluorescence PCR further verified the down-regulation of gene expression in strain treated by citral nanoemulsion.

[Conlusion]

Citral nanoemulsion had a significant bacteriostasis against *Shewanella putrefaciens* in terms of strain growth, biological structure destruction and gene expression down-regulation, revealed that the citral nanoemulsion was of great use to the inhibition of spoilage organisms and had the potential to be a new bacteriostat, QS inhibitor and preservative in aquatic products.

Keywords: citral; bacteriostasis; quorum sensing system; gene expression analysis

A green, healthy and safe strategy for the preparation of surimi products rich in highly unsaturated fatty acids with myofibrillar protein emulsion gels

Zhisheng Pei *, Huibo Wang , Chuan Li, Yaqin Hu

Hainan Provincial Engineering Research Centre of Aquatic Resources Efficient Utilization in the South China Sea, School of Food Science and Engineering, Hainan University, Haikou 570228, China.
School of Food Science and Engineering, Hainan Tropical Ocean University, Sanya 572022, China.
53663540@qq.com

[Objective]

The development of green, healthy and safe surimi products rich in highly unsaturated fatty acids (UFAs) has remained a great challenge in food processing. In this study, tilapia (Oreochromis mossambicus) myofibrillar protein (MP) was used to prepare a stable emulsion gel and surimi products rich in highly UFAs.

[Methods]

Fresh, skinned tilapia fillets purchased from Golden Spring Foods Co. Ltd. (Haikou, China) were used to extract MP. SDS-PAGE, raman spectrometry, surface hydrophobicity and electron microscopic observation were performed to characterize the MP. CLSM, particle size, dynamic rheological properties, and stability evaluation were determined to characterize the emulsion gel. Surimi was analysed by measuring TPA and gel strength, whiteness, water holding capacity, cooking loss, LF-NMR, MRI, SEM and observation of MP and oil distribution.

[Results]

1. The optimal storage modulus (G′) of the emulsion gel was obtained with an MP concentration of 1.5 wt% (0.6 mol/L NaCl, pH 7.0) and oil fraction ø = 0.68.
2. The coexistence of multiple emulsions (W/O/W) and single emulsions (O/W) in the emulsion gel was shown by confocal laser scanning microscopy (CLSM).
3. The addition of the emulsion gel enabled the development of stable surimi products with more than 35 wt% UFAs and decreased the strength ($p < 0.05$) but significantly increased the whiteness of the surimi gel ($p < 0.05$).
4. Double emulsification with both surimi protein and emulsion gel increased contents of UFAs in the surimi gel.

[Conlusion]

The use of MP emulsion gel could significantly improve the quality of freshwater surimi products without the need for exogenous substances, providing a novel and safe preparation method for surimi products rich in UFAs that are softer for consumption by children and the elderly.

Keywords: myofibrillar protein; emulsion gel; unsaturated fatty acid; surimi; gel properties

Effect of oxygenated seawater on the 4℃ cold storage of scallop (*Mizuhopecten yessoensis*)adductor muscle

Zhongzhuan Yin[1], Yuanyong Tian[1*], Yan Cai[1], Chunhong Yuan[2], Junrong Liu[1] and Tanye Xu[1]
1, College of Food Engineering, Dalian Ocean University, Dalian, 116023, Liaoning, China
2,Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate, 020-8550, Japan

[Objective]

The adductor muscle of *Mizuhopecten yessoensis* appears "hardening" due to muscle contraction during 4℃ cold storage, which leads to nutritional loss and texture quality deterioration. In order to reduce the adverse effects caused by hardening. In this study, it was attempted to preserve raw scallop adductor muscles to keep better freshness and reduce the cost of transportation.

[Methods]

During chilled storage, the change in the height of the adductor muscle was monitored by time-lapse photography. And biochemical indexes (ATP and related compounds, glycogen, mitochondrial membrane potential) and microstructure (muscle fiber, sarcomere) of adductor muscle were analyzed.

[Results]

- At +4℃, the hardening of Mizuhopecten yessoensis adductor muscle mainly occurred after 96 h, hardening due to muscle fiber changes rather than sarcomere changes.
- Because the sarcoplasmic reticulum was broken during hardening, in which Ca^{2+} was released ,Ca^{2+}-ATPase was activated.
- After 120 h cold storage, ATP still exists in the adductor muscles.
- Within oxygenated seawater of adductor muscle, ATP catabolism slows down, hardening is delayed, mitochondrial inactivation is alleviated.

[Conlusion]

These results suggest that the oxygenated seawater package could delay the hardening of the adductor muscle and extend the storage time of the adductor muscle.

Key words：Adductor muscle；*Mizuhopecten yessoensis*; Chilled storage; Freshness; Hardening

Actin denaturation in silver carp myofibrils affected by the stability of myosin bound

Zhuolin Wang[1], Chunhong Yuan[1], Yuanyong Tian[2], Shanzhen Zhao[3]

1, United Graduate School of Agricultural Sciences, Iwate University, Morioka 0208550, Japan
2, College of Food Science and Technology, Dalian Ocean University, Dalian 116023, China
3, Shanghai Customs, Shanghai 200135, China
*u3221010@iwate-u.ac.jp

[Objective]

It's generally accepted that the thermal stability of myosin is related to the environmental temperature in which the species live. We have reported that summer (S) silver carp myosin was 20 times more stable than winter (W) one according to the Ca^{2+}-ATPase inactivation rate. The objective of this study is to find out the difference in thermal stability of silver carp actin between S and W and the effect of myosin bound.

[Methods]

Silver carp myofibrils (Mf) in S and W was heated with or without 2 mM magnesium pyrophosphate (Mg-PPi) at different temperature (T), then digested by 1/200 (w/w) chymotrypsin (0.1 M NaCl, 20 °C, 60 min). W ATPase-inactive Mf was digested by chymotrypsin and dialysis in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5) to remove fragmented myosin S-1, named WDM, then heated at 45 °C with or without Mg-PPi. S myosin was added into that WDM, and heated at 45 °C. Actin denaturation was detected by measuring the decrease in the staining intensity of actin bands on SDS-PAGE using Image-J software.

[Results]

When heated without 2 mM Mg-PPi, under the condition of which myosin and actin were binding together in Mf, denaturation rate of W actin was 7, 4, 3 times faster than S one at 42, 45, 48 °C, respectively. When heated Mf at different temperature for 30 mins, the 50% denaturation T was 43 °C and 36 °C for S and W actin respectively. After the addition of Mg-PPi, there was no difference in the stability of actin in S and W at 42 °C. The denaturation rates for S and W actin were 8 and 3 times faster than the ones heated without PPi. To detect the effect of myosin bound, Mf was heated at 43 °C for S and 35 °C for W to inactivate Mf ATPase completely. Then it was heated again at 48 °C for S and 45 °C for W. The result showed S actin was still more stable than W one, which means ATPase-inactive myosin also protected actin by binding with them during heating. As Mg-PPi couldn`t separate denatured myosin from actin, the thermal stability of actin in S and W kept unchanged irrelevant to the addition of Mg-PPi. Moreover, the addition of Mg-PPi didn`t promote actin denaturation but actin became unstable after losing the protection from denatured myosin S-1 when heated. The addition of S myosin into WDM stabled actin obviously, which showed myosin bound suppressed actin denaturation.

[Conclusion]

It is concluded that S actin and W actin has the same thermal stability. Myosin bound can stable actin. The different thermal stability of S and W actin in Mf comes from binding with myosin.

Keywords: actin thermal stability, Mg-PPi, chymotryptic digestion, SDS-PAGE