Detection of Outliers in Pre-processing of Datasets for Recognition of Classifiers Using Partial Least Squares Discriminant Analysis
*Miki Fujii¹, Ryozo Noguchi², Tofael Ahamed², Takuma Genkawa³ (1. Graduate School of Life and Environmental Sciences, University of Tsukuba(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba(Japan), 3. Food Research Institute, NARO(Japan))
11:30 AM - 12:30 PM

Development of dumpling rich in barley flour with gluten added
*Masatsugu Tamura¹, Naoya Takahashi¹, Takahiro Saito¹, Satomi Akutsu², Yoshihiro Hoshi², Takemi Okamoto³ (1. Utsunomiya Univ.(Japan), 2. Tochigi Industrial Promotion Center(Japan), 3. Industrial Technology Center of Tochigi Pref.(Japan))
11:30 AM - 12:30 PM

Palm Oil based Wax Coating Maintained Postharvest Quality of Thai Lime cv. Paan Pichit#1
*Varit Srilaong¹, Nutthachai Pongprasert¹, Songsin Photchanachai¹, Panida Boonyaritthongchai¹, Kornkanok Aryusuk² (1. Division of Postharvest Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi(Thailand), 2. Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi(Thailand))
11:30 AM - 12:30 PM

Development of Blueberry Wine with High Content of Polyphenol
*Hongpu Wang¹, Yutaka Kitamura², Mito Kokawa² (1. Graduate school of Life and Environmental Sciences, Tsukuba Univ.(Japan), 2. Faculty of Life and Environmental Sciences, Tsukuba Univ.(Japan))
11:30 AM - 12:30 PM

Effects of Heating under Pasteurization Conditions on Mechanical and Electrical Properties of Mung Bean Sprout
*Hayato Ogino¹, Haruki Ando¹, Satoshi Iwamoto¹, Tepppei Imaizumi¹ (1. Gifu University(Japan))
11:30 AM - 12:30 PM

Study on Non-Destructive Measurements to Predict Sugar Content of Melons Using a DLP Based Miniature Spectrometer
*Chao-Yin TSAI¹, Pin-Chih Fang¹, Yi-Tzu Shen¹, Yung-Huei Chang¹, Han-Chun Hsu¹, Suming Chen¹ (1. Department of Bio-Industrial Mechatronics Engineering, National Taiwan University(Taiwan))
11:30 AM - 12:30 PM

Effect of Lactic acid bacteria fermentation on the microbial diversity, physico-chemical properties, and organic acid profile of pindang damulag, a fermented carabeef
*Michael Angelo Santos Esteban¹, Lotis Mopera¹, Maria Cynthia Oliveros¹, Erlinda Dizon¹ (1. University of the Philippines Los Banos(Philippines))
11:30 AM - 12:30 PM

Properties of Rice Starch-Based Film Incorporated with Zinc Oxide Nanoparticles
KHALISHAH RAHMA SAFIRA¹,², *SAROAT RAWDKUEN² (1. Department of Food Science and Technology, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University(Indonesia), 2. Unit of Innovative Food Packaging and Biomaterials, School of Agro-Industry, Mae Fah Luang University(Thailand))
11:30 AM - 12:30 PM

Effect of pulsed electric field treatment on drying rate and quality changes of spinach
**Anaerobic Digestion of Bean Sprouts Waste**

*Yuki Yamamoto¹, Yuki Mizuya², Takaki Yamashiro³, Fetra J Andriamanohanirisoamana⁴, Yoshiteru Takeuchi⁵, Kazutaka Umetsu¹*  
¹Graduate School of Obihiro University of Agriculture and Veterinary Medicine(Japan), ²Obihiro University of Agriculture and Veterinary Medicine(Japan), ³Obihiro University of Agriculture and Veterinary Medicine(Japan), ⁴Tokachi Agri Works(Japan), ⁵Biomass Research(Japan)

11:30 AM - 12:30 PM

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**Optimization of Orange-Fleshed Sweet Potato (Ipomoea batatas var. Kinerot) Flour Processing for Carotenoid Retention**

*Kiko Kuroda¹, Tatsuya Oshima¹, Teppei Imaizumi¹*  
¹Gifu Graduate School of Applied Bioscience(Japan)

11:30 AM - 12:30 PM

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**Prospects of Biogas Production From The Manure of Dairy Cattle Fed on Iron-supplemented Ration**

*Mohamed Farghali¹,², Maejima Mayumi³, Kuramoto Syo¹, Aoki Satoshi⁴, Yasui Seichiro⁵, Sayoko Takashima¹, Hijiri Ono¹, Yuhendra AP¹, Takaki Yamashiro³, Moustafa M. Ahmed², Saber Kotb², Masahiro Iwasaki¹, Kazutaka Umetsu¹*  
¹Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine(Japan), ²Department of Animal and Poultry Hygiene & Environmental Sanitation, Faculty of Veterinary Medicine, Assiut University(Egypt), ³Maezawa Engineering service Inc.(Japan), ⁴Maezawa Industries Inc.(Japan), ⁵Hokkaido Air Water Inc.(Japan), ⁶Tokachi Agri Works(Japan)

11:30 AM - 12:30 PM

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**Effect of Blending at Different Stages of Winemaking on the Quality of Mixed Fruit Wine**

*Claire Solis Zubia¹, Erlinda Ignacio Dizon¹*  
¹University of the Philippines Los Banos(Philippines)

11:30 AM - 12:30 PM

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**Pest Control of Tetranychus urticae by Branched Fatty Acids**

*Mai Nagano¹, Akitaka Teshima³, Toshinari Koda², Hiroshi Morita¹*  
¹The University of Kitakyushu(Japan), ²Nissan Chemical corporation(Japan)

11:30 AM - 12:30 PM

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**Evaluation of Quality and Structural Properties of Bread Containing Edible Cricket**

*Kiko Kuroda¹, Tatsuya Oshima³, Teppei Imaizumi¹*  
¹Gifu Graduate School of Applied Bioscience(Japan)

11:30 AM - 12:30 PM

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[5-1130-P] Food Safety (5th)
11:30 AM - 12:30 PM  Poster Place (Entrance Hall)

[5-1130-P-18] Key Process Variables Affecting the Formation of Chloromequat Compounds During Baking of Cereal Products
*Adam Ekielski1 (1. Warsaw University of Life Sciences(Poland))
11:30 AM - 12:30 PM

[5-1130-P-19] Acaricidal effects of Linear fatty acids against Tyrophagus putrescentiae
*Kosuke Matsuoka1, Toshinari Koda2, Hiroshi Morita1 (1. The University of Kitakyushu(Japan),
2. Nissan Chemical Corporation(Japan))
11:30 AM - 12:30 PM

[5-1130-P-20] Improvement of the Cleanability of Milk Soil on a Highly Smooth Surface of Stainless Steel Tubing
*Ikko Ihara1, Homi Takato1, John K Schueller2, Gen Yoshida1, Kazutaka Umetu1, Hitomi Yamaguchi2 (1. Kobe University(Japan), 2.
University of Florida(United States of America), 3. Obihiro University of Agriculture and Veterinary Medicine(Japan))
11:30 AM - 12:30 PM

[5-1130-P] Other Categories (5th)
11:30 AM - 12:30 PM  Poster Place (Entrance Hall)

[5-1130-P-21] Screening and Identification of Endophytic Bacteria from Thai Organic Rice for Plant Growth Promotion
*Somkid Deejing1, Witchayaporn Pawong1 (1. Program in biotechnology, Faculty of Science,
Maejo University, Sansai, Chiang Mai(Thailand))
11:30 AM - 12:30 PM

[5-1130-P-22] Data Extraction for Pig Weight Prediction Model
*Khin Dagon Win1, Kikuhito Kawasue1, Hsu Lai Wai1, Kumiko Yoshida2 (1. University of
Miyazak(Japan), 2. KOYO Plant Service(Japan))
11:30 AM - 12:30 PM

[5-1130-P-23] Power Tiller’s Wheel Structure and its Oscillatory Effects on Subsoiling Operation
*Oyetayo Olukorede Oyebode1, Koichi Shoji2 (1. Graduate School of Agricultural Science,
Kobe University(Japan))
11:30 AM - 12:30 PM

[5-1130-P-24] Proposal of temperature control technology in pot cultivation for the citrus fruits
*Ryuta IBUKI1, Yoshimichi Yamashita2, Sachie Horii3, Norihiro Hoshi2, Madoka Chiba1 (1.
Miyagi University(Japan), 2. National Agriculture and Food Research Organization(Japan))
11:30 AM - 12:30 PM

[5-1130-P-25] Investigation by Driving Simulation of Tractor Overturning Accidents Caused by Steering Instability
*Masahisa Watanabe1, Kenshi Sakai1 (1. Tokyo University of Agriculture and Technology(Japan))
11:30 AM - 12:30 PM

[5-1130-P-26] Classification of Salinity Damaged Spring Potato (Solanum tuberosum) using Hyperspectral Imagery based on Decision Tree Classifier
*KyungSuk Kang1, Sae Rom Jun1, Si Hyeong Jang1, Jun Woo Park1, Hye Young Song1, Ye Seong Kang1, Chan Seok Ryu1, Su Hwan Lee1 (1. GNU(Korea), 2. RDA(Korea))
11:30 AM - 12:30 PM

[5-1130-P-27] Classification for Fire Blight Disease Infection Area using Vegetation Index and Background Segmentation based on Multispectral Image
*Jun-woo Park1, Chan-seok Ryu1, Ye-seong Kang1, Sae-Rom Jean1, Si-Hyeong Jang1, Hye-Young Song1, Kyung-Suk Kang1 (1. GNU(Korea))
11:30 AM - 12:30 PM

[5-1130-P-28] The Static Load Test for Tractor Attached Three-Point Hitch Type Dynamometer
*Hyo-Geol Kim1, Sung-Bo Shim2, Yeon-Soon Kim1, Young-Joo Kim1, Sang-Dae Lee1 (1. Korea Institute of Industrial Technology(Korea), 2.
Gyeongsang National University(Korea))
11:30 AM - 12:30 PM
Isolation and Identification of Acetic Acid Bacteria from Philippine Fermented Rice Cake Batters by 16S rRNA Gene Sequence Analysis
Audrey Mae Villamin Orillaza¹, Honey Bhabes R Iñigo¹, *Baby Richard Ragudo Navarro¹ (1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Baños(Philippines))

*Tatsuo Hishinuma³, Tetsuya Hoshino¹, Atsuo Ikeguchi¹, (1.Utsunomiya Univ.(Japan))

*Rasool Khan Amini¹, Yutaka Kitamura², Mito Kokawa³, M. Z. Islam² (1. Graduate School of Life and Environmental Sciences, University of Tsukuba(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tennoda, Tsukuba, Ibaraki 305-8572, Japan(Japan))

11:30 AM - 12:30 PM

[6-1130-P-19] The Effect of Palm Oil Based Wax Coating on Delaying of Ripening and Reduce Senescence Spot of ‘Khai’ Banana

*nutthachai pongprasert¹, Varit Srlaong¹,², Songsin Photchanachai¹,², Panida Boonyaritthongchai¹,², Kornkanok Aryusuk³ (1. Postharvest Technology Program, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bangkok 10140(Thailand), 2. Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400, Thailand(Thailand), 3. Biochemical Technology Program, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bangkok 10140(Thailand))

11:30 AM - 12:30 PM

[6-1130-P-20] Effects of Blanching Pretreatment on Drying Characteristics and Pectic States of Dried ‘Fuyu’ Persimmon

*Tatsuya Oshima¹, Kodai Kato¹, Satoshi Iwamoto¹, Teppei Imaizumi¹ (1. Gifu University(Japan))

11:30 AM - 12:30 PM

[6-1130-P-21] Beverage Process Using By-product Water of the Production of Wash-free Rice as Raw Material and the Continuous Process of Lactic Acid Fermentation

*JIA FANG¹, Yutaka KITAMURA¹, Mito KOKAWA¹, Kazunobu KAJIHARA², Kozi KAWAKAMI², Hidenori MIZUNO³ (1. Tsukuba Univ.(Japan), 2. Satake Corporation(Japan))

11:30 AM - 12:30 PM

[6-1130-P-22] Effect of roasting and storage on chemical compounds and sensory score of specialty coffee

*Yuri Koshima¹, Yutaka Kitamura¹, Mito Kokawa¹, Thais M.F.S. Vieira², Juliana Antunes Gavalão², Luis Felipe de Freitas Fabricio², Md Zohurul Islam³ (1. University of Tsukuba(Japan), 2. University of Sao Paulo(Brazil))

11:30 AM - 12:30 PM

[6-1130-P-23] Inverse Method Using Heat Transfer Simulation to Estimate Thermal Diffusivity of Agricultural Products

*Yoshiki Muramatsu¹, Masanori Hashiguchi², Eiichiro Sakaguchi¹, Shotaro Kawakami¹ (1. Tokyo University of Agriculture(Japan), 2. Keisoku Engineering System Co., Ltd.(Japan))

11:30 AM - 12:30 PM

[6-1130-P-24] Effect of Acid Type and Concentration on Properties of Pectin Extracted from Unripe Cavendish Banana Peel and Its Application in Raspberry Jam

*Natthakan Rungraeng¹,², Supaluck Kraithong¹ (1. School of Agro-Industry, Mae Fah Luang University, Chiang Rai, Thailand 57100(Thailand), 2. Unit of Innovative Food Packaging and Biomaterials, Mae Fah Luang University, Chiang Rai, Thailand 57100(Thailand))

11:30 AM - 12:30 PM

[6-1130-P-25] Evaluation of color and flavor for shiitake mushroom dried using vacuum microwave treatment

*Daisuke Kurata¹, Takahiro Orikasa², Shoji Koide² (1. Graduate School of Arts and Sciences, Iwate University.(Japan), 2. Faculty of Agriculture, Iwate University.(Japan), 3. Agri-Innovation Center, Iwate University.(Japan))

11:30 AM - 12:30 PM

[6-1130-P-26] The effect of molecular hydrogen on the
shelf life of banana
*Naoya Fujino¹, Teruo Wada¹ (1. Osaka Prefecture University(Japan))
11:30 AM - 12:30 PM

[6-1130-P-27] The Potential of Biogas Production from Caribbean Seaweed Biomass
*Yuhendra AP¹, Mohamed Farghali¹, Takaki Yamashiro², Ruyichi Sakai³, Kazutaka Umetsu¹ (1. Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine(Japan), 2. Tokachi Agri Works(Japan), 3. Graduate School of Fisheries Sciences, Hokkaido University(Japan))
11:30 AM - 12:30 PM

Study on the Characteristics of Micro Wet Milling and Spray Drying of Sea-buckthorn (Hippophae rhamnoides)
*ODGEREL Ulziibat¹, Md.ZOHURUL ISLAM¹, KITAMURA Yutaka², KOKAWA Mito², ODBAYAR Tseyen-Oidov³, SOLONGO Ganbold³ (1. Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan(Japan), 3. School of Industrial Technology, Department of Food Engineering, Main Campus of MUST, Baga Tioruu 34, Sukhbaabarart District, Ulaanbaatar, Mongolia(Mongolia))
11:30 AM - 12:30 PM

Combined Effect of Pre-treatment and Vacuum Packaging for Maintaining the Quality of Peeled Shallot (Allium ascalonicum L.)
*Phanida Renumarn¹, Kranert Kilian Joachim ⁴, Natthaya Choosuk¹, Chanthima Phungamngeo², Kasama Chareekhott¹ (1. Department of Innovation and Product Development Technology, Faculty of Agro-Industry, King Mongkut’s University of Technology North Bangkok(Thailand), 2. Department of Agro-Industry Technology and Management, Faculty of Agro-Industry, King Mongkut’s University of Technology North Bangkok(Thailand), 3. Department of Food Science and Technology, Faculty of Technology, Udon Thani Rajabhat University(Thailand), 4. Food Science -Technology and Economics, University of Applied Sciences Bremerhaven(Germany))
11:30 AM - 12:30 PM

High pressure processing of ‘ Nanglae’ pineapple juice: Quality preservation and shelf life extension
Nuntawan Chensombat¹, Natthakan Rungraeng¹, Sutthiwal Sethà¹,², *Phunsiri Suthilik¹,² (1. School of Agro-Industry, Mae Fah luang University, Chiang Rai, THAILAND(Thailand), 2. Research Group of Postharvest Technology, School of Agro-Industry, Mae Fah Luang University, Chaing Rai, THAILAND(Thailand))
11:30 AM - 12:30 PM

Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds
*Pairote Wongputtisin¹, Narin Lahsom¹ (1. Program in Biotechnology, Faculty of Science, Maejo university, Chiang mai, Thailand(Thailand))
11:30 AM - 12:30 PM

Effect of Sucrose and Glucose on Coffee Kombucha Carbonation
*Chutamas Maneewong¹, Thittaya Choompoosée¹ (1. Department of Biotechnology, Faculty of Science, Maejo University, San Sai, Chiang Mai 50290(Thailand))
11:30 AM - 12:30 PM

Evaluation of Total Anthocyanins and Antioxidant Activity of Thai Rice Cultivars for Phenotypic Selection in Rice Breeding
*Chotipa Sakulsingharoj¹, Lalita Na Rachasima¹, Anongnad Richinda¹, Pairote Wongputtisin², Rungthip Kawaree², Saengtong Pongjaroenkit¹, Varaporn Sangtong¹ (1. Program in Genetics, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand), 2. Program in
Investigation of some biological activities of local shallot (*Allium ascalonicum* Linn.) extract from Thailand
*Premruethai Phansaard¹, Pairote Wongputtisin¹ (1. Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand))
11:30 AM - 12:30 PM

Probiotic characterization of thermotolerant *Lactobacillus johnsonii* isolated from broiler intestine
*Rutaimas Wongpanti¹, Pairote Wongputtisin¹, Piyanuch Niamsup¹ (1. Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai(Thailand))
11:30 AM - 12:30 PM

Process optimization for antioxidant extraction from seed of soybean cultivar Chiang mai60
*Arpatsara Seekoompa¹, Pairote Wongputtisin¹, Piyanuch Niamsup¹ (1. Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai(Thailand))
11:30 AM - 12:30 PM

Nutritional and Functional Properties of Yoghurt Drink with Philippine Gac (*Momordica cochinchinensis* Spreng.) and Bignay (*Antidesma bunius*) Fruits
Rowie Joy Gonzales Bucks¹, *Ara Fatima Cuvinar Algar², Ryan Rodrigo Paner Tayobong² (1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines), 2. Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines))
11:30 AM - 12:30 PM

Effect of Extracting Conditions on Plant Extract Colors and Stability of Antioxidant Properties during *in vitro* Gastrointestinal Digestion
*Rattika Aeka¹, Titikan Liangpanth¹, Rungarun Sasananayart¹ (1. School of Agro-Industry, Mae Fah Luang University(Thailand))
11:30 AM - 12:30 PM

Temporal Source Strength Estimation of Sweet Pepper for Crop Management and LED Supplementation Efficiency Improvement
*Masaaki Takahashi¹, So Kaneko¹, Osamu Koike¹, Hiroki Umeda², Yasunaga Iwasaki³ (1. Miyagi Prefectural Agriculture and Horticulture Research Center(Japan), 2. Graduate School of Bioresource Sciences, Nihon University(Japan), 3. National Agriculture and Food Research Organization(Japan))
11:30 AM - 12:30 PM

Study on Analysis of Loads Effect on Path-Tracking Accuracy of an Autonomous Tractor during Plow Tillage
*YEONSOO KIM¹,², YONGJOO KIM², HYOGEOL KIM¹, YOUNGJOO KIM¹, SANGDAE LEE¹ (1. KITECH(Korea), 2. Chungnam Univ.(Korea))
11:30 AM - 12:30 PM

Classification of Sugarcane Variety using Image Processing and Multivariate Analysis
*KITTIPON APARATANA¹, Hiroo Takaragawa¹,², Yoshinari Izumikawa¹,², Eizo Taira¹ (1. Faculty
Relationships between the Number of Sneezes and Swine Influenza Infection Experiment Factors

*Misaki Mito¹, Takuya Aoki¹, Koichi Mizutani², Keiichi Zempo², Naoto Wakatsuki², Yuka Maeda², Nobuhiro Takemae³, Takehiko Saito³

(1. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan))

11:30 AM - 12:30 PM

[6-1130-P-15] Sound Source Localization in Pig Houses Using Wireless Microphone Array and Its Accuracy by Microphone Arrangements

*Akifumi Goto¹, Misaki Mito¹, Tadashi Ebihara², Koichi Mizutani², Naoto Wakatsuki², Nobuhiro Takemae³, Takehiko Saito³

(1. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan))

11:30 AM - 12:30 PM

[6-1130-P-16] Behavioral Study of Vibrational Sensitivity in Whitefly

*Yasuhiko Nishijima¹, Koichi Mizutani¹, Tadashi Ebihara¹, Naoto Wakatsuki¹, Kenji Kubota³, Hiroyuki Uga⁷

(1. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, Division of Engineering Interaction Technologies, University of Tsukuba(Japan), 3. Agriculture Research Center, National Agriculture and Food Research Organization(Japan), 4. Saitama Prefecture Agriculture Research Center(Japan))

11:30 AM - 12:30 PM

[6-1130-P-17] Application of Palm Oil Based Wax as a Coating Material on the Quality of Cucumber Seed

*Songsin Photchanachai¹, Nipada Ranmeechai¹, Chalinee Sungkajorn¹, Anantaporn Phankhaek¹, Kornkanok Aryusuk¹, Varit Srilaong¹, Panida Boonyaritthongchai¹, Nutthachai Pongprasert¹ (1. School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok(Thailand), 2. Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok(Thailand))

11:30 AM - 12:30 PM
Detection of Outliers in Pre-processing of Datasets for Recognition of Classifiers Using Partial Least Squares Discriminant Analysis

*Miki Fujii\textsuperscript{1}, Ryozo Noguchi\textsuperscript{2}, Tofael Ahamed\textsuperscript{2}, Takuma Genkawa\textsuperscript{3} (1. Graduate School of Life and Environmental Sciences, University of Tsukuba(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba(Japan), 3. Food Research Institute, NARO(Japan))

11:30 AM - 12:30 PM
Detection of Outliers in Pre-processing of Datasets for Recognition of Classifiers Using Partial Least Squares Discriminant Analysis

*Miki Fujii¹, Ryozo Noguchi², Tofael Ahamed², Takuma Genkawa³ (1. Graduate School of Life and Environmental Sciences, University of Tsukuba(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba(Japan), 3. Food Research Institute, NARO(Japan))

Keywords: Pre-Processing, Dataset for Recognition of Classifiers, Machine Learning, Multivariate Analysis

In recent years, smart agriculture has received increasing attention in Japan. Image recognition is used to confirm the growth of vegetables and to determine the proper harvest timing. In machine learning, the choice of images used for the data set affects the accuracy rate of recognition of classifiers. Generally, collected data sets are pre-processed by analysts according to their experience and knowledge. Among them, there are images that could be outliers that adversely affect the accuracy rate. In this study, pre-processing was performed to datasets with objective indicators using partial least squares discriminant analysis (PLS-DA), which is one of the multivariate analyses. In datasets, 300 images of lemon and 300 images of strawberry were used. All images were 75x75 pixels in size. In first test, recognition of classifiers was performed on this dataset by Support Vector Machine (SVM). Of all the data, 75% was set as training data and 25% was randomly set as test data. The rate at which images are correctly classified is defined as the accuracy rate. Also, the images of the dataset were resized from 2x2 pixels to 64x64 pixels, and the same verification was performed. Verification was performed 100 times at each pixel condition. The outliers were detected by PLS-DA before recognition of classifiers by SVM. The objective variable of the data of the lemon images were set to 1, and data of strawberry images were set to 0. The threshold value was determined to be 0.5. In the model of PLS-DA, data of lemon images whose predicted values showed a value of 0.5 or more and data of strawberry images whose predicted values showed 0.5 or less were detected as outliers. Data detected as outliers were removed from the dataset and then image recognition was performed in the same flow as the first test. First test was conducted and noted that SVM had 91.6% ~ 96.5% accuracy rates in each pixel images. It means recognition of classifiers was performed almost accurately. Focusing on the increase in the number of pixels, the accuracy rate continued to improve up to 8x8 pixels images and stayed about 96% after that. At 2x2 pixels images, its standard deviation shows 7.6% (maximum accuracy rate: 98.0%, minimum accuracy rate: 51.7%) and its coefficient of variation shows 0.083. On the other hand, 4x4 pixels and more pixels images showed 1.4 ~ 1.8% standard deviation and less than 0.009 coefficient of variation. Comparing these two, the accuracy rate varied widely for each test when using 2x2 pixels images for testing. Second test was conducted and noted that PLS-DA for preprocessing and performed SVM had more than 99% accuracy regardless of the number of pixels. Images detected as outliers were less than 6% (4 images ~ 17 images) in each pixel image. The t test between the first test and the second test showed that the accuracy rate was significantly improved in all pixel conditions. And the coefficient of variation in each pixel images showed less than 0.009. In particular, in the 2x2 pixels images, the value of the coefficient of variation decreased significantly. This means that it proved removal of outliers can suppress variation in accuracy rate. From the above, by detection of outliers to remove from dataset using PLS-DA, it proved that the accuracy rate of recognition of classifiers could be significantly improved from 96% to 99%, and the variation in accuracy rate values could also be suppressed. In the machine-learning algorithm for training and testing, the developed outlier detection method can be implemented to increase the accuracy of validation.
[5-1130-P-01] Development of dumpling rich in barley flour with gluten added
*Masatsugu TamURA¹, Naoya Takahashi¹, Takahiro Saito¹, Satomi Akutsu², Yoshihiro Hoshi³, Takemi Okamoto³ (1. Utsunomiya Univ.(Japan), 2. Tochigi Industrial Promotion Center(Japan), 3. Industrial Technology Center of Tochigi Pref.(Japan))
11:30 AM - 12:30 PM

[5-1130-P-02] Palm Oil based Wax Coating Maintained Postharvest Quality of Thai Lime cv. Paan Pichit #1
*Varit Srilaong¹, Nutthachai Pongprasert¹, Songsin Photchanachai¹, Panida Boonyaritthongchai¹, Kornkanok Aryusuk² (1. Division of Postharvest Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi(Thailand), 2. Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi(Thailand))
11:30 AM - 12:30 PM

[5-1130-P-03] Development of Blueberry Wine with High Content of Polyphenol
*Hongpu Wang¹, Yutaka Kitamura², Mito Kokawa² (1. Graduate school of Life and Environmental Sciences, Tsukuba Univ.(Japan), 2. Faculty of Life and Environmental Sciences, Tsukuba Univ.(Japan))
11:30 AM - 12:30 PM

[5-1130-P-04] Effects of Heating under Pasteurization Conditions on Mechanical and Electrical Properties of Mung Bean Sprout
*Hayato Ogino¹, Haruki Ando¹, Satoshi Iwamoto¹, Teppei Imaizumi¹ (1. Gifu University(Japan))
11:30 AM - 12:30 PM

[5-1130-P-05] Study on Non-Destructive Measurements to Predict Sugar Content of Melons Using a DLP Based Miniature Spectrometer
*Chao-Yin TSAI¹, Pin-Chih Fang¹, Yi-Tzu Shen¹, Yung-Huei Chang¹, Han-Chun Hsu¹, Suming Chen¹ (1. Department of Bio-Industrial Mechatronics Engineering, National Taiwan University(Taiwan))
11:30 AM - 12:30 PM

[5-1130-P-06] Effect of Lactic acid bacteria fermentation on the microbial diversity, physico-chemical properties, and organic acid profile of pindang damulag, a fermented carabeef
*Michael Angelo Santos Esteban¹, Lotis Mopera¹, Maria Cynthia Oliveros¹, Erlinda Dizon¹ (1. University of the Philippines Los Banos(Philippines))
11:30 AM - 12:30 PM

[5-1130-P-07] Properties of Rice Starch-Based Film Incorporated with Zinc Oxide Nanoparticles
KHALISHAH RAHMA SAFIRA¹,², *SAROAT RAWDKUEN² (1. Department of Food Science and Technology, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University(Indonesia), 2. Unit of Innovative Food Packaging and Biomaterials, School of Agro-Industry, Mae Fah Luang University(Thailand))
11:30 AM - 12:30 PM
11:30 AM - 12:30 PM

[5-1130-P-08] Effect of pulsed electric field treatment on drying rate and quality changes of spinach in hot air drying
*Koya Yamakage¹, Takahiro Yamada¹, Takahiro Orikasa², Katsuyuki Takahashi², Shoji Koide³, Koichi Takaki⁴, Hitoshi Aoki⁵, Junichi Kamagata⁵ (1. Graduate School of Arts and Science, Iwate University(Japan), 2. Agri-Innovation Center, Iwate University(Japan), 3. Faculty of Agriculture, Iwate University(Japan), 4. Faculty of Science and Engineering, Iwate University(Japan), 5. Nichirei Foods Inc.(Japan))

11:30 AM - 12:30 PM

[5-1130-P-09] Prospects of Biogas Production From The Manure of Dairy Cattle Fed on Iron-supplemented Ration
*Mohamed Farghali¹,2, Maejima Mayumi³, Kuramoto Syo³, Aoki Satoshi⁴, Yasui Seiichi⁵, Sayoko Takashima¹, Hijiro Ono¹, Yuhendra AP¹, Takaki Yamashiro⁶, Moustafa M. Ahmed², Saber Kotb², Masahiro Iwasaki¹, Kazutaka Umetsu¹ (1. Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine(Japan), 2. Department of Animal and Poultry Hygiene &Environmental Sanitation, Faculty of Veterinary Medicine, Assiut University(Egypt), 3. Maezawa Engineering service Inc.(Japan), 4. Maezawa Industries Inc.(Japan), 5. Hokkaido Air Water Inc.(Japan), 6. Tokachi Agri Works(Japan))

11:30 AM - 12:30 PM

[5-1130-P-10] Anaerobic Digestion of Bean Sprouts Waste
*Yuki Yamamoto¹, Yuki Mizuya², Takaki Yamashiro³, Fetra J Andriamahaniarisoamanana¹,4, Yoshiteru Takeuchi², Kazutaka Umetsu¹ (1. Graduate school of Obihiro University of Agriculture and Veterinary Medicine(Japan), 2. Obihiro University of Agriculture and Veterinary Medicine(Japan), 3. Tokachi Agri Works(Japan), 4. Graduate School of Agricultural Science, Kobe University(Japan), 5. Biomass Research(Japan))

11:30 AM - 12:30 PM

James Ryan D. Aranzado¹, *Loraine C. Bainto¹, Dennis Marvin O. Santiago¹ (1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Baños(Philippines))

11:30 AM - 12:30 PM

[5-1130-P-12] Temporal Transition of Spatial Dependence of Weeds in Grassland
*Katsuyuki Tanaka¹, Ayako Oide³, Hideo Minagawa¹ (1. Kitasato University(Japan))

11:30 AM - 12:30 PM

[5-1130-P-13] RNA-Seq analysis of the transcriptome and genes expression profile during the browning of Lotus Root (Nelumbo nucifera)
*Kanjana Worarad¹, Haruka Norii¹, Yuya Muchizuki¹, Takashi Ishii², Keiko Shinohara³, Takao Miyamoto⁴, Eiichi Inoue¹ (1. Ibaraki University(Japan), 2. Ibaraki Agricultural Center, Horticultural Research Institute (Japan), 3. Tokushima Agriculture, Forestry and Fisheries Technology Support Center(Japan), 4. Renkon3kyodai Co.Ltd(Japan))

11:30 AM - 12:30 PM
Development of dumpling rich in barley flour with gluten added

*Masatsugu Tamura¹, Naoya Takahashi¹, Takahiro Saito¹, Satomi Akutsu², Yoshihiro Hoshi³, Takemi Okamoto³
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Keywords: Barley, Dumpling, β-glucan, Total polyphenol, Texture

This study aimed to develop dumplings with high barley content, by the incorporation of less than 10% gluten. To 100 g of barley flour, 5% and 10% gluten, and 50%, 60%, 65%, 70% and 75% water, respectively, were added. The mixture was kneaded, left for 3 hours to allow dough development, cut to form raw barley dumpling skin, and then baked for analysis of color, texture, β-glucan content and total polyphenol content. Cooked barley dumplings with mincemeat filling were prepared for sensory evaluation. In addition, wheat dumplings were also prepared and examined, for comparison. The barley dumpling skin had significantly lower L* and higher a* when compared with wheat dumpling skin. No significant difference in firmness was observed between baked wheat dumpling skins (2.07 N) and burley dumpling skins with added 10% gluten and 65%, 70% and 75% moisture (1.82–2.28 N). The burley dumpling skin with 10% gluten and 70% moisture, used to prepare the meat dumplings, displayed the closest texture to that of the baked wheat dumpling skin. A higher β-glucan content (2.2% vs. 0.2% dry basis) and total polyphenol content (183.2 vs. 86.4 mg gallic acid equivalents/g dry weight) were provided by baked barley dumpling than the baked wheat dumpling. The sensory test revealed no difference between baked barley and wheat dumplings, except for appearance. The proposed method provides barley dumpling with high functional components and palatability.

Palm Oil based Wax Coating Maintained Postharvest Quality of Thai Lime cv. Paan Pichit#1

*Varit Srilaong¹, Nutthachai Pongprasert¹, Songsin Photchanachai¹, Panida Boonyariththongchai¹, Kornkanok Aryusuk²
(1. Division of Postharvest Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi(Thailand), 2. Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi(Thailand))

Keywords: Coating, Palm oil wax, Lime, Postharvest, Quality

Immature green lime fruit cv. Pann Pichit#1 is widely consumption in Thailand as an ingredient of Thai’s dish. Most of consumers prefer to have immature green lime due to its enriches with special aromatic compound, taste and flavor. Thus, to maintain the green color of lime fruit is very important for retarding quality losses. Peel yellowing is one of a major problem in lime fruit during postharvest period which lead to reduction of fruit qualities thus the inhibiting or delaying of chlorophyll breakdown is needed. There are several kind of postharvest technology to prolong storage life and maintain green color of fresh produces and one of them is coating technique by using natural based wax. According to Thailand produces a lot of palm oil and a byproduct from palm oil industry, palm oil wax, has potential to use as a wax based to form coating material. Thus, this research aimed to use palm oil based wax coating for maintaining quality of immature green lime cv. Pann Pichit#1. Lime fruit were harvested from commercial orchard and coated with palm oil based wax (PW) and commercial wax (CW), and then stored at 13°C. Uncoated fruit was set as a control.
Changes of lime fruit qualities including fresh weight loss, browning spot, chlorophyll content, ascorbic acid content and acetaldehyde content were investigated at 5 days interval. The results found that lime fruit coated with PW showed the lowest water loss compared with that of CW coated and the control, respectively. The percentage of peel browning spot occurrence was also reduced in the fruit coated with PW while the application of CW induced a browning spot to higher level than the control. This result was concomitant with the incidence of peel browning. Lime fruit coated with both PW and CW delayed the chlorophyll breakdown in the same trend while the continuously degradation of chlorophyll was observed in the control. There was no consistent change of ascorbic acid content in all treatments, anyway the content was slightly change from the initial until the end of storage. The accumulation of acetaldehyde in lime juice was initially observed on day 10 in all treatments and then declined throughout the end of storage with slightly difference among the treatments. From the results indicated that PW has potential to apply with immature green lime fruit during postharvest period. In addition, the use of byproduct from palm oil industry for formulating a coating material will support the zero waste policy and also added a value of byproduct.

11:30 AM - 12:30 PM (Thu. Sep 5, 2019 11:30 AM - 12:30 PM Poster Place)

[5-1130-P-03] Development of Blueberry Wine with High Content of Polyphenol

*Hongpu Wang¹, Yutaka Kitamura², Mito Kokawa² (1. Graduate school of Life and Environmental Sciences, Tsukuba Univ.(Japan), 2. Faculty of Life and Environmental Sciences, Tsukuba Univ.(Japan))

Keywords: blueberry wine, micro wet milling, alcohol production, polyphenol, anthocyanin, antioxidant activity

Rabbit-eye blueberry (*Vaccinium virgatum*) is suitable to be produced into wine because of high content of sugar and phenolic compounds. However, to obtain clear wine, pomace is produced after wine processing. It is a kind of by-product, including skin, seeds and some pulps, which contains most of bioactive compounds. In the research, micro wet milling technology (MWM) was used to improve reserved content of bioactive compounds such as polyphenol in the final product and increase taste of wine. Rabbit-eye blueberry (harvested from Ibaraki, Japan) was used to ferment wine by wine yeast. The soluble solids content was enriched up to 21° Brix before fermentation to obtain a potential alcohol level of approximately 12%vol. Fermentation was conducted to finish after 35 days when soluble solids content reached a constant level (between 6-7° Brix). MWM was used to decrease particle size into micro scale after fermentation. Blueberry wine were evaluated for total phenolic content (TPC) using Folin-Ciocalteu method, total anthocyanin content (TAC) using pH differential method, antioxidant activity using the radical scavenging capacity (DPPH) method and some physicochemical properties such as pH, ° brix. The total polyphenol content and antioxidant activity were increased by MWM compared with conventional processing, which means it is possible to produce blueberry wine with high content of polyphenol and increase utilization of pomace by MWM.

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[5-1130-P-04] Effects of Heating under Pasteurization Conditions on Mechanical and Electrical Properties of Mung Bean
Sprout
*Hayato Ogino¹, Haruki Ando¹, Satoshi Iwamoto¹, Teppei Imaizumi¹ (1. Gifu University(Japan))
Keywords: impedance, pasteurization condition, electrical property, mechanical property, mung bean sprout

Heat pasteurization using hot water is easy to introduce in small-scale facilities. However, heat treatments often degrade tissue structure and decrease texture of vegetables. Although cell membrane structure, which makes turgor pressure, is one of the most important factors to determine vegetable texture, heat-resisting properties of the structure is not well clarified yet. To date, impedance measurement has been used to evaluate cell membrane state. In this study, we mainly investigated such an electrical properties of mung bean sprout heated under pasteurization conditions, and evaluated relationships with its quality. Mung bean sprout is used in this study. A beaker containing 300 mL of distilled water was controlled at temperatures of 50, 60, 65 and 70 degree in a water bath. After measuring weight of mung bean sprout, it was put into a net and immersed in the beaker for 0 - 60 sec. Then, the sprout was immediately cooled in iced water for 30 sec. For the heated mung bean sprout, mechanical and electrical properties were measured. In order to measure the mechanical properties, a creepmeter (TPU-2D, Yamaden Co., Ltd.), equipped with a wedge-shaped plunger or a knife-shaped plunger, was used. The wedge-shaped and the knife-shaped ones were moved at 1 mm/sec for compression test and 10 mm/sec for shear test, respectively. For the impedance measurement, two needle electrodes (diameter: 0.25 mm) connected to a LCR tester (IM3536, HIOKI) were inserted into the sample. In this study, equivalent circuit analysis was conducted on the measured impedance values, and cell membrane capacitance, intracellular resistance and extracellular resistance were obtained. In addition, cell membrane structure was observed by using a confocal laser scanning microscope. In this study, two kinds of mechanical properties were evaluated for heated sprouts. While the compression force of the sample did not change a lot, significant differences were appeared on the shear force especially at 65 degree. In impedance measurement, measured values showed an arc when resistance and reactance were plotted on vertical and horizontal axis, respectively. Top coordinate of the arc decreased as the heating temperature risen. Additionally, an equivalent circuit model was well fitted to the measured values. The cell membrane capacitance decreased by the heating. Also, the extracellular resistance showed a decreasing tendency at heating above 60 degree. These changes seemed to relate with cell membrane damage which observed by the confocal laser scanning microscope. Consequently, our study indicated that impedance measurement was a good way to estimate texture and tissue structure of mung bean sprout. These findings will contribute to quality control of vegetables during heat processings.

[5-1130-P-05] Study on Non-Destructive Measurements to Predict Sugar Content of Melons Using a DLP Based Miniature Spectrometer
*Chao-Yin TSAI¹, Pin-Chih Fang¹, Yi-Tzu Shen¹, Yung-Huei Chang¹, Han-Chun Hsu¹, Suming Chen¹ (1. Department of Bio-Industrial Mechatronics Engineering, National Taiwan University(Taiwan))
Keywords: Digital Light Processing, Micromirror, Spectrometer, Near Infrared

Spectrometers based on digital light processing (DLP) design replace the traditional linear array detector with a micromirror array for wavelength selection. It has the advantages of lower cost and higher performance through the use of a larger and cheaper single element detector. In this study, a commercially available DLP based spectrometer and mobile phone were used. The former was used as a measurement tool; the latter
was used as a control panel with APP. Spectra and sugar content of 151 samples were measured at the different parts of eight melons. Peel and flesh measuring modes had been conducted and one laboratory spectrophotometer (Model: NIRS 6500) was also used to measure the spectra of two modes together with DLP based spectrometer. WinISI spectral analysis software was used to build a cross validation model with MPLSR method. The best DLP spectrometer’s model of SEC, RSQ, SECV, 1-VR for peel and flesh modes were 0.598, 0.786, 0.735, 0.681 when mathematic treatment was done in (1,2,1) model and 0.614, 0.781, 0.745, 0.677 when mathematic treatment was done in (1,12,12,1) model, respectively. The best NIRS 6500 spectrophotometer’s model of SEC, RSQ, SECV, 1-VR for peel and flesh modes were 0.544, 0.823, 0.702, 0.705 when mathematic treatment was done in (1,4,4,1) model and 0.413, 0.898, 0.512, 0.841 when mathematic treatment was done in (1,10,10,1) model, respectively. Observing the two apparatuses model’s result, the performance of DLP spectrometer is worse when compared with NIRS6500 spectrophotometer; but it is enough for industrial applications.

11:30 AM - 12:30 PM (Thu. Sep 5, 2019 11:30 AM - 12:30 PM Poster Place)

[5-1130-P-06] Effect of Lactic acid bacteria fermentation on the microbial diversity, physico-chemical properties, and organic acid profile of pindang damulag, a fermented carabeef

*Michael Angelo Santos Esteban¹, Lotis Mopera¹, Maria Cynthia Oliveros¹, Erlinda Dizon¹ (1. University of the Philippines Los Banos(Philippines))

Keywords: carabeef, fermentation, lactic acid bacteria, pindang damulag

Fermented carabeef or pindang damulag is a native traditional food from Pampanga, Philippines. It is produced through the action of naturally growing lactic acid bacteria (LAB) via fermentation for 1 week at room temperature. The study aimed to determine the changes on the microbial diversity, physico-chemical properties, and organic acid profile of pindang damulag brought by the lactic acid bacteria fermentation. Procurement and processing of pindang damulag was based on the method of known makers from Pampanga, Philippines, but minor revisions were made to address the food safety concerns of the researcher. During fermentation, all targeted groups of microorganisms (fungi, common bacteria, coliforms, acid producing bacteria and LAB) grew significantly until the 3rd day. After day 3, only acid producing bacteria and LAB grew significantly. There was also a significant decrease in total soluble solids (TSS) from day 0 to day 3 (30.31 – 28.17° Brix), while titratable acidity (TA) and pH were found to be statistically constant (3.5% @ pH 5.97 – 3.6% @ pH5.9). Moreover, significant decrease in TSS (24.89, 22.76, and 20.53° Brix) and pH (5.60, 4.93, and 4.53) were observed, while TA increased significantly (4.5, 5.6, 6.6%) during days 5, 7, and 9. Moisture content, on the other hand, increased significantly from day 0 to day 1 (64.5759 ± 1.5085 – 66.1952 ± 1.2023) but remained statistically constant until day 9 (65.6447 ± 0.8445). The L* value also decreased significantly from day 0 to day 1 (26.99 – 23.48) but increased significantly at day 3 and day 7 (28.60 – 34.12). The a* value increased significantly from day 0 to day 1 (10.92 – 16.90) but remained statistically constant until day 9 (17.77). While b* value remained statistically constant throughout fermentation. After the culture dependent phenotypic tests, some LAB isolates were found to be heterofermentative, which also reflected on the predominance of other organic acids such as citric acid (448.70 mg/100g), acetic acid (1724 mg/100g) other than the lactic acid (4440 mg/100g) alone. Therefore, LAB was found to have a major role in the food safety, food quality and overall profile of pindang damulag.
This study aimed to develop rice starch based antimicrobial film as an active food packaging with zinc oxide nanoparticles (ZnO-NPs) incorporation. ZnO-NPs were synthesized by hydrothermal method and their formation was confirmed by using XRD analysis. The synthesized ZnO-NPs showed an average size of <100 nm with spherical shape under the scanning electron microscope (SEM). The nanoparticles were studied against two foodborne pathogens bacteria; *Staphylococcus aureus* and *Escherichia coli* at different concentrations (0 – 5 %, w/v) and found effective against both microorganisms. The ZnO-NPs (3%, w/w) was selected for the incorporation into rice starch-based (5%) antimicrobial film with sorbitol as plasticizer via solution casting method. Physical, mechanical, chemical, and antimicrobial properties of the films were examined. Presence and distribution of nanoparticles in the film were confirmed with SEM and FTIR. Incorporation of zinc oxide nanoparticles significantly decreased (p<0.05) the transparency (2.64 ±0.01), solubility (19.22 ±0.39%), WVP (0.04 ±0.00 x 10^{-10} g m/m^2 Pa s), and elongation at break (37.18 ±2.61%), while increased the lightness (89.73 ±0.06) and tensile strength (9.14 ±0.78 MPa) of the film were observed (p<0.05). The rice starch/ZnO-NPs nanocomposite films showed antibacterial activity against *S. aureus* and *E. coli*. These results suggest that rice starch/ZnO-NPs nanocomposite film can be used as active packaging materials.
Rice Starch-Based Film Incorporated with Zinc Oxide Nanoparticles

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ABSTRACT

This study aimed to develop rice starch-based antimicrobial film with zinc oxide nanoparticles (ZnO-NPs) incorporation. ZnO-NPs were synthesized by hydrothermal method and their formation was confirmed by using XRD analysis. The synthesized ZnO-NPs showed an average size of ≤ 100 nm with spherical shape under the scanning electron microscope (SEM). The nanoparticles were studied against two foodborne pathogens bacteria; Staphylococcus aureus and Escherichia coli at different concentrations (1, 3, and 5 %, w/v) and found effective against both microorganisms. The ZnO-NPs (3 %, w/w) was selected for the incorporation into rice starch-based (5 %) antimicrobial film with sorbitol as plasticizer via solution casting method. Physical, mechanical, chemical and antimicrobial properties of the films were examined. Presence and distribution of nanoparticles in the film were confirmed with SEM and FTIR. Incorporation of ZnO-NPs significantly decreased (p<0.05) the transparency (2.64 ± 0.01), solubility (19.22 ± 0.39 %), WVP (0.04 ± 0.00 x 10⁻¹⁰ g m²/m² Pa s), and elongation at break (37.18 ± 2.61 %), while increased the lightness (89.73 ± 0.06) and tensile strength (9.14 ± 0.78 MPa ) of the film were observed (p<0.05). The rice starch/ZnO-NPs film showed antibacterial activity against tested bacteria. These results suggest that rice starch/ZnO-NPs film can be used as an active packaging material.

Keywords: Antimicrobial packaging, Nanocomposite film, Nanoparticles, Rice starch, Zinc oxide

1. INTRODUCTION

Recently, there is an increasing concern for the microbial safety of food products. Food deterioration is mainly caused due to microbial activity. Growth of spoilage microorganisms, spoilage bacteria, mold, and yeast can reduce the quality of food products during storage. It will shorten the shelf life of the foods and lead to food waste and economic losses. Besides, the contamination of pathogenic microorganism in the foods can cause foodborne illnesses. The Center for Disease and Control and Prevention has estimated there are 48 million people are sick, 128000 are hospitalized and 3000 die every year due to foodborne illnesses (CDC, 2018).

Food packaging is essential for maintaining quality and providing the safety of food products. The current trend of the food packaging system is concerning about developing more innovative approaches to inhibit pathogenic microbial activities in foods (Sung et al., 2013). One of the packaging technology that has been developed is active biodegradable packaging. According to Kaewprachu and Rawdkuen (2016), active packaging is a system in which the product, the package, and the environment interact in a positive way to extend shelf life or improve microbial safety or sensory properties while maintaining the quality of food products. Antimicrobial packaging is the type of active packaging which can kill or inhibit the growth of microorganism by releasing the antimicrobial agents from the food packaging system.

The incorporation of inorganic materials in nanoscale is a great opportunity to use as antimicrobial agents due to their high surface area, thus it can present strong antibacterial activity (Espitia et al., 2012). Zinc oxide nanoparticles (ZnO-NPs) is one of the metallic oxide nanoparticles that have been explored to incorporate into food packaging materials as an antimicrobial agent. They have better stability compared to organic agents and exhibit antibacterial activity against Gram-positive and Gram-negative bacteria as well as fungi (Espitia et al., 2012; Kanmani and Rhim, 2014). Moreover,
ZnO is currently listed as generally recognized as safe (GRAS) material by the Food and Drug Administration and is used as a food additive (U.S. Food and Drug Administration, 2018). The renewable biopolymer can be used as a carrier of active antimicrobial agents. It can be obtained from local sources such as polysaccharides, proteins, and lipids. Among the variety of polysaccharides have been used, starch is one of the most abundant natural polysaccharide raw material, inexpensive, renewable, and non-toxic (Jiménez et al., 2012; Kotharangannagari and Krishnan, 2016). Rice starch is an attractive raw material because its major components, such as amylose and amylopectin can act as barriers in packaging materials (Phattaraporn et al., 2011) and they have been used to produce biodegradable films to partially or entirely replace plastic polymers (Detduangchan et al., 2014). Unfortunately, films prepared from rice starch products have disadvantages including low mechanical properties and lack of efficient barrier against high polarity compounds due to the highly hydrophilic character of rice starch polymers (Wittaya, 2012). Besides its antimicrobial activity, the incorporation of nanoparticles into biopolymer films can be a new alternative technique for improving the film properties.

Previous work had been done about the incorporation of ZnO-NPs in the food active packaging system. Suyatma et al., (2014) reported that the use of ZnO-NPs as Nano-filler could increase functional properties of pectin film in view of tensile strength, water vapor barrier, and antimicrobial capacity. Therefore, this study aimed to develop rice starch based antimicrobial film as an active food packaging with zinc oxide nanoparticles incorporation because only rice starch film doesn’t inherent antimicrobial activity and it susceptible to microbial growth. Furthermore, this study was to investigate the characteristics of rice starch film with ZnO-NPs incorporation.

2. MATERIALS AND METHODS

2.1 Materials

All chemicals were obtained from Scientific and Technological Instruments Center Store, Mae Fah Luang University (Chiang Rai, Thailand). Sodium hydroxide and zinc chloride were used for the preparation of ZnO nanoparticles. Rice starch and liquid sorbitol were used for film preparation were obtained from the Food Packaging Laboratory, Mae Fah Luang University (Chiang Rai, Thailand). Nutrient broth (NB) and Mueller-Hinton agar (MHA) were used for the antimicrobial assay. Foodborne pathogenic microorganisms, Staphylococcus aureus TISTR 746 and Escherichia coli TISTR 527 were obtained from culture collection center (Mae Fah Luang University, Chiang Rai, Thailand). All chemicals and solvent used were analytical grade.

2.2 Preparation and Characterization of Zinc Oxide Nanoparticles

The ZnO nanoparticles were prepared by hydrothermal synthesis according to Akbar and Anal (2014). Aqueous solutions (100 mL) with a molar concentration of 0.2 M and zinc chloride solution 0.1 M were prepared. Sodium hydroxide (0.2 M, 100 mL) solution was added dropwise to aqueous zinc chloride (0.1 M, 100 mL) solution under constant stirring (100 rpm). The mixture solution was heated at 60 °C for 2 h in a water bath. Following the heating, the reaction mixture was left standing overnight (12 h) at 24 °C and filtered through Whatman filter number one. The precipitate result was kept in a hot air oven at 60 °C for 48 h to ensure the complete formation of ZnO nanoparticles. The powdered nanoparticles will be used for further experiments. X-ray diffraction (XRD) patterns were observed in the range of 2θ values from 20°-80° with PANalytical X’Pert Pro MPD, X-ray diffractometer. Morphology and size of nanoparticles were observed under scanning electron microscope SEM (LEO, 1450 VP) with magnification range 5000-20000x, resolution 200 Å and an acceleration voltage of 20 kV. Samples were coated with gold before observation.

2.3 The Antimicrobial Activity of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles solution were prepared following Nafchi et al. (2012) with slight modification. ZnO nanoparticles were dispersed in distilled water at different concentrations (1, 3, and 5 %; w/v), stirred for 1 h at 60 °C, and then sonicated in an ultrasonic bath (Marconi model, Unique
USC 35 kHz) for 30 min at 60 ºC. The solution was used for further studies of antimicrobial activity against target foodborne pathogens *S. aureus* and *E. coli* with the disk diffusion method. A disk diffusion method was used following Shahverdi et al. (2007) with modifications. The filter paper disk was cut into 6 mm-diameter disks. Each paper disk was further immersed in the freshly prepared ZnO-NPs solution at different concentrations (1 %, 3 %, and 5 %; w/v). The disks were removed and dried, followed by sterilized under UV for 30 min. A single colony of each test strain was grown overnight in nutrient broth medium on a rotary shaker (200 rpm) at 37 ºC. The inocula were prepared by diluting the overnight cultures with 0.85 % NaCl to a 0.5 McFarland turbidity (approximately 1.5 x 10^8 CFU/mL). A sterile cotton swab was used to inoculate the surface of Mueller Hinton agar plate rotating the plate every 60º to ensure homogeneous growth. The prepared disks containing different amounts of ZnO-NPs were placed on Mueller Hinton agar plates. The plates were then incubated at 37 ºC for 24 h. After that, the plates were examined for the zone of inhibition of the film discs. Inhibition zone (diameter) of the disc was calculated in mm as follows:

\[ \text{Inhibition zone} = \text{diameter of inhibition area} - \text{diameter of disc area} \]

All samples were performed in duplicate. The best antimicrobial activity of the ZnO nanoparticles concentration will be selected for further film development.

### 2.4 Preparation of Rice Starch/ZnO-NPs Nanocomposite Film

The nanocomposite films were prepared by using solution casting method according to Nafchi et al. (2012) with modifications. ZnO nanoparticles were dispersed in distilled water at 3 % (w/w of rice starch) stirred at 60 ºC for 1 h, and then sonicated in an ultrasonic bath (Marconi model, Unique USC 35 kHz) at 60 ºC for 30 min. The solution was used to prepare the aqueous starch dispersion at 5 % (w/v). Sorbitol at 30 % (w/w of rice starch) was added as plasticizers in accordance with Laohakunjit and Noomhorm (2004). Starch nanocomposites were heated to 75±5 ºC and held for 45 min to allow gelatinization. Upon completion of starch gelatinization, the film-forming solution (FFS) was cooled to 45 ºC. A portion (4.04±0.02 g) of the FFS was cast on onto a rimmed silicone resin plate (50×50 mm) and then evaporated at room temperature for 24 h before dried with a ventilated oven environmental chamber at 25±0.5 ºC and 50±5 % relative humidity (RH) for another 24 h. The obtained dried films were manually peeled. Control films were prepared similarly but without the addition of nanoparticles.

### 2.5 Characterization of Rice Starch-based Nanocomposite Film

#### 2.5.1 Morphological Observation and FT-IR

The morphological features and nanoparticles distribution pattern of the ZnO nanoparticles loaded films will be characterized by using SEM. According to Suyatma et al. (2014), film specimens were scratched on the top surface before being mounted on an aluminum stub and were covered with double-sided carbon tape then sputter coated with gold to enhance surface conductivity. Samples will be viewed in SEM at 20 kV with 5000x magnification on the surface.

FTIR spectra of the films were analyzed following Nafchi et al. (2012). FTIR spectra of the films were recorded using an attenuated total reflection (ATR) method in FTIR Spectrum GX (Perkin Elmer). The thin films were applied directly onto the ZnSe ATR cell. The spectrum was recorded at wave number 650-4000 cm\(^{-1}\). For each spectrum, 64 consecutive scans at 4 cm\(^{-1}\) resolutions were averaged to reduce spectral noise.

#### 2.5.2 Thickness and Mechanical Properties of The Films

The thickness of the film samples was measured using a hand-held micrometer (Dial Thickness Gauge 7301, Mitutoyo Corporation, Kanagawa, Japan). Nine random measurements were taken from each film sample of the ten film samples were used for thickness determination and the average values were used as the film thickness.

The mechanical properties of the film samples were measured according to Rawdkuen et al. (2012). Prior to testing the mechanical properties, the films were conditioned for 48 h at 50±5 % RH at 25 ºC.

The tensile strength (TS) and elongation at break (EAB) were determined by using a Universal Testing
Machine (Instron, 5566). Ten samples (20×50 mm) with an initial grip length of 30 mm were used for testing. The cross-head speed was set at 30 mm/min with 100 N load.

2.5.3 Surface Color and Transparency
Surface color of the film samples was measured following Kanmani and Rhim (2014) using a Chroma meter (Hunter Lab MiniScan EZ) with a white color plate (L* = 93.09, a* = -1.07, and b* = 2.40) as a standard background for color measurement. The CIE color values (L*, a*, and b*) were determined by the average of five readings from each film sample. The total color difference (ΔE) was calculated as follows:

\[ \Delta E = \left[ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{0.5} \]

where \( \Delta L^* \), \( \Delta a^* \), and \( \Delta b^* \) were the difference between the color of the standard plate and film samples, respectively.

The ultraviolet and visible light barrier properties of the films were measured according to Rawdkuen et al. (2012) at selected wavelengths between 200 and 800 nm by using a UV-Vis spectrophotometer. The film transparency was calculated by the following equation:

\[ \text{Transparency} = \log T_{600}/x \]

where \( T_{600} \) was the fractional transmittance at 600 nm, and \( x \) is the film thickness (mm). This experiment was performed in triplicate.

2.5.4 Moisture Content (MC)
Moisture content (MC) of the films was determined following Shankar et al. (2016) with slight modification. Each film was cut into 2×2 cm and dried at 105 °C for 24 h using hot air oven. The weight loss of each film was measured as MC and expressed as percent MC based on the initial weight of the film. This experiment was performed in triplicate.

2.5.5 Film Solubility
The film solubility was determined according to the method of Rawdkuen et al. (2012) with slight modification. The dried film samples were weighed and placed in a 50 mL centrifuge tube containing 10 mL of distilled water. The mixture was shaken at a speed of 250 rpm using a shaker for 24 h. The un-dissolved debris was then removed by centrifugation at 3000 rpm for 20 min. The pellet was dried at 105 °C for 24 h and weighed. The weight of the solubilized dry matter was calculated by subtracting its difference from the initial weight of the dry matter. It was then expressed as a percentage of the total weight. This experiment was performed in triplicate.

2.5.6 Water Vapor Permeability (WVP)
The film water vapor permeability (WVP) was measured following Shankar et al. (2016) with slight modification. The films were sealed onto a permeation cup containing silica gel (0 % RH) and sealed to prevent the leakage of water vapor. The cups were then placed in a humidity chamber controlled at 50 % RH and 25 °C. The weight loss of the cup was measured every hour for 8 h to determine the water vapor transmission rate (WVTR) (g/m²s) of the film, then the WVP of the film was calculated in g/m²Pa s as follows:

\[ \text{WVP} = \frac{(\text{WVTR} \times L)}{\Delta p} \]

where \( L \) was the thickness of the film (m) and \( \Delta p \) was partial water vapor pressure difference (Pa) across the film.

2.5.7 Antimicrobial Activity of Films
The evaluation of the antimicrobial activity of the rice starch film containing zinc oxide nanoparticles was carried out by using two test microorganisms: S. aureus (Gram-positive, TISTR 746) and E. coli (Gram-negative, TISTR 527). The rice starch film was also tested as a control. Antimicrobial activity tests were carried out by using the agar disk diffusion method according to Ramos et al. (2012) with modifications. Disks cut from films (5 mm) were sterilized under UV for 30 min. A single colony of each test strain was grown overnight in nutrient broth medium on a rotary shaker (200 rpm) at 37 °C. The inocula were prepared by diluting the overnight cultures with 0.85 % NaCl to a 0.5 McFarland turbidity (approximately 1.5 x 10⁸ CFU/mL). A sterile cotton swab was used to inoculate the surface.
of Mueller Hinton agar plate rotating the plate every 60° to ensure homogeneous growth. The prepared disks were placed on Mueller–Hinton agar plate. The petri dishes were then incubated at 37 °C for 24 h. Sterile water and antibiotic were used as negative and positive control, respectively. The antimicrobial activity of each material was evaluated by observing the growth inhibition zone and measuring the diameter (mm) by a ruler. Tests were carried out in duplicate. Inhibition zone (diameter) of the discs was calculated in mm as follows:

\[
\text{Inhibition zone} = \text{diameter of inhibition area} - \text{diameter of film area}
\]

### 2.6 Statistical Analysis

Data were expressed as means ± standard deviation. The data were also subjected to analysis of variance (ANOVA) and Duncan’s multiple range tests using SPSS 25.0 for Windows. The significance level of \( p < 0.05 \) was considered significantly different.

### 3. RESULTS AND DISCUSSION

#### 3.1 Preparation and Characterization of Zinc Oxide Nanoparticles

XRD analysis was used to determine the structural characterization of the nanoparticles. The ZnO nanoparticles formation was confirmed by XRD. XRD patterns of synthesized ZnO nanoparticles is presented in Figure 1. The XRD spectra showed sharp diffraction peaks at 31.92°, 34.56°, 36.44°, 47.70°, 56.78°, 62.99°, 66.49°, 68.12°, 69.14°, 72.67°, and 77.07° of 2\( \theta \), corresponds to (100), (002), (101), (102), (103), (200), (112), (201), (004), and (202) crystal planes. All diffraction peaks indicate the ZnO wurtzite hexagonal structure found in the standard reference data (ICSD 029272, 01-075-0576, Zincite).

![Figure 1. XRD spectra of synthesized ZnO-NPs.](image)

![Figure 2. SEM micrograph of synthesized ZnO-NPs with magnification 20000x.](image)
The particle size measurement and morphology of the nanoparticles were observed under SEM (Figure 2) at magnification 20000x. Single nanoparticles indicated with the arrows sign in the figure. The synthesized ZnO-NPs showed an average size (diameter) of 79.25 nm with a spherical shape. Akbar and Anal (2014) reported that the particle size of ZnO-NPs synthesis using hydrothermal method was around 50 nm with a spherical shape. The size and shape of nanoparticles depend on several factors, such as the type of precursor and the solvent used as well as chemical and physical conditions (pH, temperature) in the reaction (Espitia et al., 2012).

3.2 Antimicrobial Activity of Zinc Oxide Nanoparticles

The preliminary test of ZnO nanoparticles antimicrobial activity were tested against the Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria. The paper disk containing different concentration of ZnO nanoparticles showed a clear zone against the target bacteria. The clear zone of ZnO nanoparticles against the target bacteria is illustrated in Figure 3. Its antimicrobial properties are associated to several mechanisms including the release of antimicrobial ions (Zn\(^2\+\)), the interaction of nanoparticles with microorganisms, subsequently damaging the integrity of bacterial cell and the formation of ROS by the effect of light radiation (Espitia et al. 2012). Li et al. (2011) found that the toxicity of Nano-ZnO was mainly attributed to the released Zn\(^2\+\) ions. Akbar and Anal (2014) observed that the nanoparticles have a high impact on the cell surface integrity, which responsible to make the cell wall porous, and the target bacterial cells with ruptured bodies are clearly noted in the electron micrograph.

![Figure 3. Zone of inhibition of ZnO-NPs loaded paper disk against the target bacteria on Mueller Hinton agar plate.](image)

Particle size and shape may affect its antimicrobial activity. Song et al. (2010) found that 10–30 nm spherical ZnO particles were slightly highly toxic than three rod-like ZnO particles. Nair et al. (2009) also found that antibacterial activity toward E. coli increased as the particle size decreased because the smaller sized particles would be expected to have a higher surface charge because of the increased surface area per unit volume.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>1% ZnO NPs</td>
<td>1.50 ± 0.71(^a)</td>
</tr>
<tr>
<td>3% ZnO NPs</td>
<td>3.00 ± 0.00(^b)</td>
</tr>
<tr>
<td>5% ZnO NPs</td>
<td>4.00 ± 0.00(^b)</td>
</tr>
</tbody>
</table>

Results were represented as means of replicates ± standard deviation. Values with different superscripts in the column are significantly different (p<0.05).
The higher concentrations of ZnO nanoparticles showed higher antibacterial activity against the target bacteria (Table 1). According to Li et al. (2011), the Zn²⁺ ions concentration increased with the increasing concentration of ZnO-NPs, thus it resulted in higher toxicity towards tested bacteria. Akbar and Anal (2014) reported that the antimicrobial effect of the ZnO-NPs increased with the increase of ZnO-NPs concentration because nanocarrier particles are responsible to make the cell wall porous. However, the 1 % concentration of ZnO-NPs was not effective to inhibit the growth of *E. coli* (approximately 1.5 x 10⁸ CFU/mL) after 24 h of incubation. This result might be attributed to *E. coli* can excrete large amounts of extracellular polymer substances during growth to resist toxicity, thus *E. coli* could still survive with low concentrations of ZnO-NPs (Li et al. 2011). There was no significant difference in the inhibition zone of *S. aureus* at 3 % and 5 % ZnO-NPs concentration. A similar result also found by Pamuji (2014), the incorporation of 3 % ZnO-NPs has a significant effect on antibacterial properties of tapioca starch film against *E. coli*, *B. cereus*, and *S. aureus*. Therefore, the 3 % of zinc oxide nanoparticles is the optimum concentration to inhibit the growth of tested bacteria and it was selected to further use in active film development.

3.3 Characterization of Rice Starch-based Nanocomposite Film

3.3.1 Morphological Observation and FT-IR

FT-IR analysis was performed to examine the interactions between rice starch polymer and ZnO NPs as shown in Figure 4. A broad absorption band of rice starch film 3263.27 cm⁻¹ was attributed to the stretching of hydroxyl (O-H) groups (Li et al. 2011). The peak at 2923.83 cm⁻¹ was the C-H stretching, while the peak at 1367.28 cm⁻¹ was the O-H of water (Bourtoom and Chinnan, 2008). According to Kizil et al. (2002), water adsorbed in the amorphous region of starches could be identified as a broad infrared band with a peak at 1637 cm⁻¹, as a result of the vibration of water molecules adsorbed in the nanocrystalline region of the starch. The IR peaks for rice starch at 1076.72 and 1015.46 cm⁻¹ were assigned to the anhydroglucose ring of the O–C stretch (Matmin et al., 2018), whereas the band obtained at 994.91 cm⁻¹ was attributed to the vibrations originating from the C-O-C of α-1,4 glycosidic linkages (Kizil et al., 2002). Other vibrational bands in the fingerprint region, at 667.47, 704.36 and 759.83 cm⁻¹, were due to the skeletal mode vibrations of the pyranose ring in the glucose unit (Matmin et al., 2018).

![Figure 4. FTIR spectra of rice starch and rice starch film incorporated with 3% ZnO-NPs.](image)

No new functional group was added after the ZnO-NPs incorporation. It indicated an only physical interaction between the ZnO- N and the film matrix occurs (Nafchi et al., 2012). However, some of the peaks were shifted to higher and lower wave number with ZnO-NPs incorporation may be due to certain interactions between ZnO NPs and biopolymer matrix (Anitha et al., 2013). The presence of ZnO nanoparticles in the film was observed under SEM, illustrated in Figure 5. The nanoparticles in the rice starchy film indicated with the arrows sign in the figure. The neat rice starch film was smooth and had a compact surface, while rice starch/ZnO-NPs films showed rough surface
and the ZnO nanoparticles were distributed through the film surface. Similar surface morphologies of nanocomposite films with ZnO-NPs incorporation such as agar/ZnO-NPS, carrageenan/ZnO-NPs, CMC/ZnO-NPs (Kanmani and Rhim, 2014), and gelatin/ZnO-NPs (Shankar et al., 2016).

Figure 5. SEM micrograph of rice starch film and rice starch film incorporated with ZnO-NPs on the surface with magnification 20000x.

3.3.2 Thickness and Mechanical Properties of The Films
The thickness and mechanical properties of the films are shown in Table 2. The thickness of neat rice starch films was 70.00 µm, which was not significantly (p>0.05) changed after blending with ZnO nanoparticles. A contrary result was found by Kanmani and Rhim (2014) who reported that the thickness of various biopolymer films increased with the addition of ZnO NPs.

Table 2. Thickness, tensile properties, moisture content, film solubility, and water vapor permeability of rice starch film and rice starch film incorporated with ZnO-NPs.

<table>
<thead>
<tr>
<th>Films</th>
<th>Thickness (µm)</th>
<th>TS (MPa)</th>
<th>EAB (%)</th>
<th>MC (%)</th>
<th>Film solubility (%)</th>
<th>WVP (x 10^{-10} g m/ m² Pa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice starch</td>
<td>70.00 ± 2.13a</td>
<td>5.00 ± 0.44a</td>
<td>76.62 ± 5.68b</td>
<td>11.95 ± 1.48a</td>
<td>27.12 ± 1.33b</td>
<td>0.06 ± 0.01b</td>
</tr>
<tr>
<td>Rice starch/ZnO-NPs</td>
<td>69.50 ± 3.32a</td>
<td>9.14 ± 0.78b</td>
<td>37.18 ± 2.61a</td>
<td>12.55 ± 1.12a</td>
<td>19.22 ± 0.39a</td>
<td>0.04 ± 0.00a</td>
</tr>
</tbody>
</table>

Results were represented as means of replicates ± standard deviation. Values with different superscripts in the column are significantly different (p<0.05).

The mechanical properties (TS and EAB) of rice starch film greatly influenced after incorporation with ZnO-NPs (p<0.05). The TS of the rice starch film increased from 5.00 MPa to 9.14 MPa after ZnO-NPs incorporation. In contrast, the EAB decreased from 76.62 % to 37.18 % after ZnO-NPs incorporation. It was expected to improve the tensile strength of rice starch films by incorporating ZnO-NPs. Tensile strength (TS) is a measure of film integrity and elongation at break (EAB) is a quantitative representation of the ability to stretch of the films. A Similar result was found by Suyatma et al. (2014) who reported that the incorporation of ZnO-NPs into pectin films would raise TS and slightly reduce EAB. The increase in mechanical strength of the rice starch/ZnO-NPs composite film might be due to the interaction formed by the hydrogen bond between ZnO-NPs and rice starch. The mechanical properties of the films are closely related to the distribution and density of the intra and intermolecular interactions between the polymer chains in the film matrix (Shankar et al., 2016).

3.3.3 Surface Color and Transparency
The color characteristics of the films are summarized in Table 3. Apparently, the neat rice starch films were translucent with a whitish tint (Figure. 6). However, the rice starch/ZnO-NPs film was changed appearance to milky white and more opaque. It indicated the formation of ZnO nanoparticles (Shankar et al., 2014). The lightness (Hunter L-value) of rice starch film was 86.73, but it increased significantly (p<0.05) after incorporation with ZnO-NPs. Hunter a and b values (indicating greenness-
redness and blueness-yellowness, respectively) of rice starch/ZnO-NPs film were not significantly different (p>0.05). Consequently, the total color difference (ΔE) of rice starch/ZnO-NPs film (3.38) decreased compared with the neat rice starch film (6.37).

Table 3. Color parameters of rice starch film and rice starch film incorporated with ZnO-NPs.

<table>
<thead>
<tr>
<th>Films</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice starch</td>
<td>86.73 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.17 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.63 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.37 ± 0.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rice starch/ZnO-NPs</td>
<td>89.73 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.90 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.38 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results were represented as means of replicates ± standard deviation. Values with different superscripts in the column are significantly different (p<0.05).

Figure 6. Appearance of rice starch film and rice starch film incorporated with ZnO-NPs.

Optical properties of films are an important attribute that influences its appearance, marketability, and their suitability for various applications (Rawdkuen et al., 2012). Light transmission in UV (200–280 nm) and visible ranges (350–800 nm), as well as the transparency of the film samples, are shown in Table 4. Generally, all films exhibited lower light transmission in the UV range than in the visible range. The light transmission of the film was decreased significantly by the formation of nanocomposite with ZnO (p<0.05). It indicated that the ZnO-NPs in the film matrices prevented the passage of UV light. This result was consistent with Kanmani and Rhim (2014) who observed low transmissions of light in the UV range of various biopolymer incorporated with ZnO-NPs. For film transparency, there were significant differences between treatments and the control were observed (p<0.05). This result was also confirmed by the surface morphology with different backgrounds of the films in Figure 6. The higher transparency value indicated that the film was less transparent. It was found that incorporating ZnO-NPs into the rice starch-based film affected the transparency of the resulting films. Based on the optical properties of the film, the application of rice starch film incorporated with ZnO-NPs may be limited to certain food products (e.g. meatball).

Table 4. Light transmission and transparency value of rice starch film and rice starch film incorporated with ZnO-NPs.

<table>
<thead>
<tr>
<th>Films</th>
<th>% Transmittance</th>
<th>Transparency value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T&lt;sub&gt;200&lt;/sub&gt;</td>
<td>T&lt;sub&gt;280&lt;/sub&gt;</td>
</tr>
<tr>
<td>Rice starch</td>
<td>0.07</td>
<td>22.57</td>
</tr>
<tr>
<td>Rice starch/ZnO-NPs</td>
<td>0.03</td>
<td>3.28</td>
</tr>
</tbody>
</table>

Results were represented as means of replicates ± standard deviation. Values with different superscripts in the column are significantly different (p<0.05).

3.3.4 Moisture Content (MC)

The moisture content of the films is shown in Table 2. The rice starch/ZnO-NPs film exhibited slightly higher MC (12.55 %) compared with the control films (11.95 %), however, they were not significantly different (p>0.05). It indicated there was no significant change to the total solid of the films after incorporation with ZnO-NPs. The difference in moisture content may be caused by the drying process of the film. A similar result was found by Kanmani and Rhim (2014), who reported that moisture content of various biopolymer films slightly increased with the addition of ZnO-NPs.
3.3.5 Film Solubility

The solubility of the rice starch incorporated with ZnO-NPs in term of water solubility is shown in Table 2. The control film showed the higher film solubility (27.12 %), while decreased significantly (p<0.05) in the film incorporated with ZnO-NPs (19.22 %). A similar result was found by Nafchi et al. (2012), who incorporated ZnO nano-rods to sago starch film significantly decreased the solubility of the biocomposites. This result may be attributed to the interactions between ZnO and starch in the biopolymer film structure. Furthermore, it can be caused by the hydrophobic nature of ZnO-NPs. Nafchi et al. (2012) reported that increasing the ZnO-NPs content of films increased the hydrophobicity of the films may be due to the formation of more hydrogen bonds the ZnO-NPs and the matrix components.

3.3.6 Water vapor permeability (WVP)

The results of WVP studies are presented in Table 2. The incorporation of ZnO NPs into the rice starch film significantly decreased (p<0.05) the WVP of the rice starch films. The WVP results indicated that the water vapor barrier property of the nanocomposite films was improved compared with the control films. The increased water vapor barrier property may be attributed to the water vapor impermeable nanoparticles and the formation of a tortuous path for passage of water molecules by ZnO NPs addition in the polymer matrix since ZnO could disperse well in the matrix (Yu et al., 2009). Due to their small size, the nanoparticles might enhance the water vapor resistance of the films because they can increase the compactness of the films and they can prevent the formation of intermolecular hydrogen bonding amongst starch molecules which can reduce the water vapor diffusion through the film (Shi et al., 2013). The significant decrease in WVP after the incorporation of ZnO-NPs may be attributed to the greater water resistance of ZnO-NPs compared with the pure rice starch film (Nafchi et al., 2012). Kanmani and Rhim (2014) found that the WVP of incorporation of ZnO NPs into the various polymer films clearly decreased the WVP. Nafchi et al. (2012) also found that the incorporation of ZnO-N into sago starch film decreased the WVP of the film.

3.3.7 Antimicrobial Activity of Films

The ZnO nanoparticles incorporation into rice starch film showed a clear zone against the target bacteria (Table 5). The clear zone of active films against the target bacteria is illustrated in Figure. 7. Rice starch films used as a control (without ZnO nanoparticles) showed no clear zone against the tested bacteria. Results indicate that the antimicrobial activity of rice starch/ZnO-NPs film should be attributed to the ZnO-NPs because the control film didn’t show antibacterial activity against tested bacteria. The inactivation of bacteria by ZnO involves mainly direct interaction between ZnO nanoparticles and the surface of cells, affecting the permeability of the membrane, allowing the internalization of nanoparticles and inducing oxidative stress in bacterial cells, resulting in the inhibition of cell growth (Espitia et al., 2012).

<table>
<thead>
<tr>
<th>Films</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>Rice starch</td>
<td>0.00 ± 0.00a</td>
</tr>
<tr>
<td>Rice starch/ZnO-NPs</td>
<td>4.50 ± 0.71b</td>
</tr>
</tbody>
</table>

Results were represented as means of replicates ± standard deviation. Values with different superscripts in the column are significantly different (p<0.05).

Tankhiwale and Bajpai (2012) found that petri plate supplemented with ZnO-loaded SCP film shows a clear zone of inhibition around the film which indicates that ZnO nanoparticles must have diffused away from the film, thus causing bacterial cell death and forming a clear zone of inhibition around the film. Li et al. (2011) were observed the cytoplasmic membranes deformed, wherein some cells swelled.
and the intracellular substances leaked out under Zn stress, thus Zn$^{2+}$ ions dissolved from ZnO were considered as the primary cause for ZnO ecotoxicity.

Figure 7. Antimicrobial activity of the film against *S. aureus* and *E. coli*.

The observed inhibition zone of the ZnO-NPs loaded film showed that *S. aureus* inhibition zone was larger than *E. coli* inhibition zone. It indicates rice starch/ZnO-NPs film had higher antibacterial activity against Gram-positive *S. aureus* than Gram-negative *E. coli*. A similar result also found by Anitha et al. (2013), Banoee et al. (2010), Kammani and Rhim (2014), and Li et al. (2009). This result may be attributed to the different structure and thickness of the membrane cell wall between *S. aureus* and *E. coli*. The Gram-positive *S. aureus* is composed of multi-layers of peptidoglycan which has plenty of pores that could render them more susceptible to the intracellular transduction by the nanoparticles leading to cell disruption, while the cell wall of Gram-negative *E. coli* is relatively thin mainly consisting of peptidoglycan and an outer layer of lipopolysaccharide, lipoprotein, and phospholipids, which would be less prone to the attack of the nanoparticles (Anitha et al. 2013).

4. CONCLUSION

The ZnO-NPs were successfully obtained through hydrothermal synthesis with an average size of 79.25 nm and spherical shape. The synthesized ZnO-NPs showed antimicrobial activity against tested bacteria (*S. aureus* and *E. coli*). The optimum concentration to inhibit the growth of tested bacteria was 3% and it was used to develop antimicrobial nanocomposite films. ZnO-NPs were successfully incorporated into rice starch film through a solution casting method. The ZnO-NPs were distributed on the surface of the nanocomposite film. Significant changes in color, transparency, mechanical properties, solubility, and water vapor permeability were observed. Incorporation of ZnO NPs into rice starch film showed an antimicrobial effect against *S. aureus* and *E. coli*. Based on these results, rice starch/ZnO-NPs nanocomposite film had the potential to be used as biodegradable antimicrobial packaging. Nevertheless, further studies such as an application part for the real foods are needed to analyze their potential performance.

ACKNOWLEDGMENTS

The authors warmly thank School of Agro-Industry, Mae Fah Luang University for financial support.

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Pamuji, M.W. (2014). Development of bionanocomposite film based from cassava starch and nanoparticle ZnO with glycerol as plasticizer.


**Effect of pulsed electric field treatment on drying rate and quality changes of spinach in hot air drying**

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Keywords: pulsed electric field, spinach, drying rate, L-ascorbic acid, potassium

Although hot air drying is a commonly method for vegetable preservation, it has various disadvantages, including a slow drying rate. To increase the drying rate, hot water (HW) pretreatment is often applied during dried vegetable production using hot air. However, HW pretreatment can result in the elution of water-soluble components. Therefore, we examined the application of pulsed electric field (PEF) technology before drying as a waterless treatment to overcome the disadvantages of HW pretreatment. We measured the moisture content and quality changes in spinach (residual ratios of L-ascorbic acid (L-AsA) and potassium (K)) after drying with PEF, HW and control (CONT) treatments. The drying rates were faster for PEF and HW than for CONT. The residual ratios of L-AsA and K were higher for PEF than for HW. Our results indicated that PEF was more effective than HW as a pretreatment method before drying with respect to the drying rate and the maintenance of water-soluble components. This pretreatment approach has potentially applications for the productions of high-quality dried vegetables.

**Prospects of Biogas Production From The Manure of Dairy Cattle Fed on Iron-supplemented Ration**

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Keywords: iron supplement, animal feed, biogas, manure, anaerobic digestion

Anaerobic digestion (AD) is a promising bio-technology for energy recovery from organic wastes. This study provides a novel method for the enhanced AD of dairy manure (DM) without pre/post-treatment by the direct supplementation of special natural ash from soil called Mineraso (MS) to the feed of lactating Holstein dairy cattle (HDC). MS is chiefly composed of approximately 84.8% of iron hydroxide. MS was supplemented at rates of 0 (F1), 25 (F2), and 50 (F3) g/head of HDC/day for two months. Thereafter, the manure of each group of HDC was collected and examined for iron concentrations prior to the batch AD experiments. The results revealed that the amounts of iron excreted in manure were reduced by 63.64% and 68.42%, respectively. Interestingly, the supplementation of MS at concentrations of 25 and 50 g/head of HDC improved biogas yields from DM by 21.90% and 40.05%, respectively than the control (no MS supplementation). Additionally, the equivalent dosages of MS improved methane yield by 25.87% and 46.51%, respectively. The highest cumulative production of biogas and CH₄ was 1.11 and 0.63 L/gVS removed, respectively, which was achieved by F3 supplement, while the corresponding values in the case of...
F1 were 0.79 and 0.43 L/gVS removed. Therefore, the supplementation of animals with iron-containing MS might represent a sustainable and practical approach to enhancing CH$_4$ yields.
Anaerobic Digestion of Bean Sprouts Waste

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Keywords: bean sprouts, anaerobic digestion, biogas , acid fermentation, elements addition

Wastes from food represents a critical issue globally. Bean sprouts, which are a familiar diet in Japan, are directly linked to the problem. In Ibaraki, a prefecture of Japan, around 20% of the whole bean sprouts are disposed as a waste, therefore, their use as substrates for the Anaerobic digestion (AD) is of great challenge. Therefore, this study was considered to explore the potential of batch and continuous fermentation on the AD of bean sprouts wastes. In batch experiment, the biogas yields of boiled bean sprouts after 20 days were 2.4-times higher than raw bean sprouts. The continuous mesophilic experiments (38 °C) were conducted in three different experiments. The first experiment proposed a long period stable process after 30 days, with higher biogas yields from the mixtures of bean sprouts and return digestate than the use of bean sprout alone. The second experiment aimed to explore the impact of acid fermentation on the AD process, while the third experiment was involved the addition of trace element and different organic loading rates of bean sprouts. The results showed that acid fermentation enhanced biogas yield after 50 days by 1.5 time than no acid fermentation digester. Additionally, in third experiment, the B digester with 100g bean sprout, 200g return digestate, and 0.16g of iron, cobalt and nickel additives was produced higher organic decomposition rate of 71.02 % than the corresponding A digester (with 75g, 150g, and 0.12g, respectively) and C digesters (with 150g, 300g, and 0.24g, respectively). Therefore, the AD of bean sprouts wastes might represent a hygienic approach for their disposal with the advantage of large amounts of CH₄ production, especially when using a mixture of bean sprouts and a return digestate as a substrate. Additionally, acid fermentation, appropriate organic loading rate, and trace elements additions improved biogas production.
Anaerobic Digestion of Bean Sprouts Waste
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ABSTRACT
Wastes from food represents a critical issue globally. Bean sprouts, which are a familiar diet in Japan, are directly linked to the problem. In Ibaraki, a prefecture of Japan, around 20% of the whole bean sprouts are disposed as a waste, therefore, their use as substrates for the Anaerobic digestion (AD) is of great challenge. Therefore, this study was considered to explore the potential of batch and continuous fermentation on the AD of bean sprouts wastes. In batch experiment, the biogas yields of boiled bean sprouts after 20 days were 2.4-times higher than raw bean sprouts. The continuous mesophilic experiments (38°C) were conducted in three different experiments. The first experiment proposed a long period stable process after 30 days, with higher biogas yields from the mixtures of bean sprouts and return digestate than the use of bean sprout alone. The second experiment aimed to explore the impact of acid fermentation on the AD process, while the third experiment was involved the addition of trace element and different organic lading rates of bean sprouts. The results showed that acid fermentation enhanced biogas yield after 50 days by 1.5 time than no acid fermentation digester. Additionally, in third experiment, the B digester with 100g bean sprout, 200g return digestate, and 0.16g of iron, cobalt and nickel additives was produced higher organic decomposition rate of 71.02 % than the corresponding A digester (with 75g, 150g, and 0.12g, respectively) and C digester (with 150g, 300g, and 0.24g, respectively). Therefore, the AD of bean sprouts wastes might represent a hygienic approach for their disposal with the advantage of large amounts of CH4 production, especially when using a mixture of bean sprouts and a return digestate as a substrate. Additionally, acid fermentation, appropriate organic loading rate, and trace elements additions improved biogas production.

Keywords: Bean sprouts, Anaerobic Digestion, Biogas, Acid Fermentation, Elements Addition

1. INTRODUCTION
Methane fermentation, also referred to as anaerobic fermentation is a decomposition reaction of organic matter that proceeds under anaerobic conditions. The organic matter is decomposed by microorganisms belonging to the methanogen group to generate methane (CH4) gas from hydrogen and carbon dioxide gas (Paritosh et al., 2017).
In recent years, from the standpoint of environmental protection view, CH4 fermentation has been positioned as the main method of manure treatment, and in Japan efforts are also being made from both aspects of effective utilization of livestock manure and utilization of methane energy as natural bio-energy source. In addition, large amounts of waste biomasses such as sewage sludge, garbage and livestock excrement can be used as materials to be digested, with a global environmental protection. By the action of anaerobic bacteria, energy recovery from biomass organic matter to CH4 leads to saving of electricity, reduce the use of fossil fuel and reduce of CO2 and other greenhouse gases emissions. Moreover, the digestate which is a methane fermentation residue has the advantage to be used as a fertilizer to substitute the chemicals one to offer a safe and high-quality crop growth (Tatsuya Noike et al. 2009).
Currently, about 1.3 billion tons of food, which is one-third of the world's food production, is discarded every year, especially in industrialized countries (FAO, 2011). In Japan, about 17 million
 tons of food waste are discharged annually. Among them, 5 to 8 million tons of originally eaten food is discarded as food loss each a year.

Japan's food loss is about twice the amount of food aid to worldwide. This is comparable to Japan's rice production, and corresponds to the domestic supply of food for Namibia, Liberia, and the Democratic Republic of Congo, to which Japan has provided ODA assistance. About one-fourth of the food before the expiration date is discarded as a food waste (WFP, FAOSTAT” Food balance sheets” 2009)

Bean sprouts are familiar foods to Japanese food culture since ancient times. They are characterized by their potential nutrients such as starch, fat and protein, which stored in seeds, also their cells and tissues are made to grow while releasing energy at the stage of bean sprouting (Hedges and Lister, 2006.). In addition to the nutrients inherent in seeds, it is considered a special vegetable that produces new nutrients. Bean sprouts have been produced and consumed in large numbers in the past 25 years. Because stable production is possible regardless of the weather, the production and consumption of bean sprouts increase to compensate for the shortage of vegetables when the amount of supply of other vegetables decreases due to irregular weather (Bean sprout producers association, 2017).

However, up to 20% of the total production of bean sprouts in Ibaraki prefecture are not be sold and discarded as a waste. Therefore, in this study, we thought it could be effectively used as a material for anaerobic digestion to produce methane. In this context, batch and continuous experiments were conducted aimed to verify whether the bean waste was effective as methane fermentation material. In batch fermentation tests, raw and boiled bean sprouts were used as materials. In the continuous experiment, three study items were set up. The first is to explore the potential of continuous methane fermentation on bean sprouts as a substrate, the second is to examine the effectiveness of acid fermentation, and the third is to investigate the impacts of trace element addition on HRT and appropriate organic substance loading. HRT refers to the number of days of hydraulic retention time for which the substrate is in the fermenter. The organic load represents the weight of organic entering the fermenter. HRT and organic matter load are factors that determine the volume of the digester.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Bean sprouts
Raw bean sprouts that collect from Ibaraki prefecture and boiled bean sprouts were used as a substrate for digestion. In the batch experiment, three runs of raw bean sprouts, boiled bean sprouts, and inoculum, which were collected from an active food processing biogas plant were set up. In the continuous experiment, ground bean sprouts were used based on the results of the batch experiment. The TS% and VS% of raw and boiled bean sprouts were 11.89, 11.22 and 11.22, 10.71, respectively.

2.1.2 Return digestate
The discharged digestate from the fermenter in the continuous experiment was mixed with bean sprouts substrate and used as the input material for the next day, and acid fermentation was performed until the input.

2.1.3 Trace elements
It is considered to be a substance necessary for the activity of microorganisms involved in methane fermentation. Among them, iron, cobalt and nickel were added at rates of 0.16, 0.12, and 0.24 g, respectively. In order to facilitate mixing, the three elements were made into an aqueous solution and mixed immediately before feeding into the fermenter.

2.1.4 Inoculum
Inoculum was collected from a food factory in Shihoro-cho, Hokkaido. The TS% and VS% of inoculum was 1.57 and 1.05, respectively.
2.2 Methods

2.2.1 Batch experiment

In this experiment, 700 g of materials were added into a 1L polyethylene digester. The fermentation conditions were operated in mesophilic temperature at 38 °C. The experimental period was setup to 20 days, and stirring was performed manually once a day. Measurement of biogas volume and biogas component were carried out daily, TS% and VS% of materials, pH samples were measured before and after fermentation.

2.2.2 Continuous experiment

In this study, continuous experiments were performed as following: experiment 2-1, to investigate the methane fermentation using bean sprouts as material, experiment 2-2, to examine the effectiveness of acid fermentation, and experiment 2-3, to determine the effect of HRT, trace element input, and the appropriate organic load rate. In Experiment 2-1, RUN A was used only bean sprouts as a material and RUN B, which used bean sprouts and return digest as a material, were setup. In Experiment 2-2, RUN A using bean sprouts from acid fermenter digester and a return digest solution as a material, and RUN B mixed with bean sprouts and a return digester on the day without acid fermentation were set. In Experiment 2-3, RUN A, B, and C, which used bean sprouts and the digestate and different amounts trace elements as materials, were set. The details of experimental design were shown in table 1, 2 and 3. In each experiment, one 10 L stainless fermenter was used per RUN. The fermenter was sealed and kept anaerobic, and placed in a water bath at 38 °C mesophilic temperature. The input materials were stirred manually at least 1 min per day. The biogas volume was measured daily before the input of materials, and the biogas was collected once a week in a gas bag to measure the methane concentration. The weight and pH of the excreted digestate were measured daily, while total solid (TS%) and the volatile organic solid (VS%) were measured once a week (the measurement method will be described later). In the continuous experiment 2-2 A, the materials were exposed to acid fermentation before use. The material was put in 1L of polybin for mesophilic fermentation at 38°C., and acid fermentation was carried out for the input material from the next day onwards.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Experimental 2-1 design, TS, VS, HRT</th>
<th>materials</th>
<th>TS(%)</th>
<th>VS(%)</th>
<th>HRT(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUN A</td>
<td>Bean sprouts 200g</td>
<td>15.93</td>
<td>15.31</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>RUN B</td>
<td>Bean sprouts 200g + Return digestate 500g</td>
<td>3.26</td>
<td>3.05</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Experimental 2-2 design, TS, VS, HRT</th>
<th>materials</th>
<th>TS(%)</th>
<th>VS(%)</th>
<th>HRT(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUN A</td>
<td>Bean sprouts 100 g + Return digestate 300 g (acid fermentation)</td>
<td>3.89</td>
<td>3.17</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>RUN B</td>
<td>Bean sprouts 100 g + Return digestate 300 g (no acid fermentation)</td>
<td>5.13</td>
<td>4.53</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>
Table 3 Experimental 2-3 design, TS, VS, HRT, organic loading

<table>
<thead>
<tr>
<th>RUN</th>
<th>Materials</th>
<th>TS (%)</th>
<th>VS (%)</th>
<th>HRT (d)</th>
<th>Organic loading (g-VS/L/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Bean sprouts 75 g + Return digestate 150 g + Trace elements 0.12 g</td>
<td>6.09</td>
<td>5.38</td>
<td>44</td>
<td>2.75</td>
</tr>
<tr>
<td>B</td>
<td>Bean sprouts 100 g + Return digestate 200 g + Trace elements 0.16 g</td>
<td>5.58</td>
<td>4.68</td>
<td>33</td>
<td>4.26</td>
</tr>
<tr>
<td>C</td>
<td>Bean sprouts 150 g + Return digestate 300 g + Trace elements 0.24 g</td>
<td>5.11</td>
<td>4.41</td>
<td>22</td>
<td>9.02</td>
</tr>
</tbody>
</table>

2.3 Parameter analysis

2.3.1 Biogas volume and composition

Biogas was collected in gas bags and its volume was measured by using wet gas flow meter (Shinagawa meter). The biogas was sampled with a gas bag, and the content ratio of H2, O2, N2, CO2, CH4 in the biogas was analyzed with a gas chromatograph (SHIMAZU GC-14A). Before and after the batch test, the total solids (TS%), volatile solids, (VS%), and pH, in each biodigester were determined. Standard process (section 2540G) was followed to calculate the TS and VS contents (APHA, 2005). The pH was considered using a Horiba (D-55) pH meter.

2.3.2 Volatile fatty acid (mg/L)

It analyzed by the high-performance liquid chromatograph (HPLC: Shimadzu LC-10AD). An ion exclusion column was used and the column temperature was 45 °C. The mobile phase was a 5 mM aqueous solution of p-toluene sulfonic acid at a flow rate of 0.8 mL/min. The buffer phase was a mixture of 20 mM Bis-Tris and 100 μM ethylenediaminetetraacetic acid in an aqueous solution of the mobile phase at a flow rate of 0.8 mL / min. For sample pretreatment, we added 6 mL of 10% tungstic acid and 6 mL of 2/3N sulfuric acid to 3 g of each sample, homogenized (10000 rpm, 5 min), centrifuged (10000 rpm, 20 min), then the collected supernatant was filtered with a membrane filter. A mixed solution of 1000 mg / L, 500 mg / L and 250 mg / L of formic acid, acetic acid, propionic acid and butyric acid, respectively, was used as a standard substance.

3. RESULTS AND DISCUSSION

3.1 Batch experiment

3.1.1 Cumulative biogas volume

As shown in figure 1, in batch raw bean sprouts experiment (RUN II), the biogas was generated up to 2 days after the start of the test, but no more gas was generated thereafter. However, in batch boiled bean sprouts experiment (RUN III), it was found that RUN III generated gas until 14 days after the start of the test, and generated about 2.4 times the gas volume of RUN II.
3.1.2 Biogas component
In RUN II, the generation of gas ceased two days after the start of the test, so the generation of methane and carbon dioxide was insufficient. RUN III, fermentation was performed smoothly by 14 days after the start of the test, and methane and carbon dioxide were accordingly produced, and the methane concentration was about 1.6 times that of RUN II (Table 4)

Table 4 Biogas components

<table>
<thead>
<tr>
<th></th>
<th>CH$_4$(%)</th>
<th>CO$_2$(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUN II</td>
<td>32.89</td>
<td>35.37</td>
</tr>
<tr>
<td>RUN III</td>
<td>54.72</td>
<td>23.39</td>
</tr>
</tbody>
</table>

3.1.3 Organic matter decomposition rate
The decomposition rate of organic matter was significantly higher in RUN III than RUN II. As the factor, it is considered that the fermentation period is longer RUN III, and the fermentation was performed smoothly (Table 5).

Table 5 Organic matter decomposition rate

<table>
<thead>
<tr>
<th></th>
<th>Before (VS%)</th>
<th>After (VS%)</th>
<th>Organic matter decomposition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUN II</td>
<td>2.26</td>
<td>1.43</td>
<td>36.73</td>
</tr>
<tr>
<td>RUN III</td>
<td>2.83</td>
<td>0.93</td>
<td>67.14</td>
</tr>
</tbody>
</table>

3.2 Continuous experiment
3.2.1 effect of continuous methane fermentation from bean sprouts as a material
Seven days after the start of the experiment in RUN A, the amount of gas decreased sharply and the generation of gas stopped. In addition, the optimum pH of the methane fermenter in methanogenesis is supposed to be 6.5 to 8.2, but when the amount of gas decreases, the pH shows a very low value of
4.39. Here too, it was determined that it was difficult to continue the fermentation, using only the bean sprouts as a material. On the other hand, RUN B which used bean sprouts and return digester as a material continued to generate gas until 25 days as shown in figure 2.

![Figure 2 Daily change of amount of biogas](image)

**Figure 2 Daily change of amount of biogas**

### 3.2.2 The effectiveness of acid fermentation

#### 3.2.2.1 Amount of biogas and methane concentration per input VS

The acid fermentation RUN A produced more biogas than the RUN B, which did not undergo acid fermentation as shown in table 6. Moreover, since the average methane concentration was higher in RUN A than RUN B, it was found that an increase in methane concentration can be expected by performing acid fermentation.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Average amount of biogas per input VS (L/g-VS/d)</th>
<th>Average CH₄ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Bean sprouts + Return digestate (acid fermentation)</td>
<td>0.65 (±0.032)</td>
<td>69.31 (±1.336)</td>
</tr>
<tr>
<td>B Bean sprouts + Return digestate (no acid fermentation)</td>
<td>0.43 (±0.010)</td>
<td>63.18 (±1.592)</td>
</tr>
</tbody>
</table>

#### 3.2.2.2 pH

Through the experiment, the average value of pH for each test area was 7.74 for RUN A and 7.62 for RUN B, and no difference was found in the input materials. Moreover, both were in the range of optimal pH.

#### 3.2.2.3 Organic matter decomposition rate

In the organic matter decomposition rate, RUN B was 1.6 times higher than RUN A. This can be attributed to that the material which carried out acid fermentation is used in RUN A; therefore, it is thought that their organic matter is decomposed at the stage of acid fermentation.

#### 3.2.2.4 VFA

Since the acid fermentation is performed, the volatile organic acid concentration of the material in RUN A is high. Therefore, by performing methane fermentation, volatile organic acids were decomposed, and a reduction of approximately 40.50% was observed. On the other hand, RUN B, the
volatile organic acid was not decomposed well and an increase of about 27.13% after digestion as observed in figure 3.

3.2.3 Impacts of trace element input HRT, and appropriate organic substance load
3.2.3.1 Biogas production per input VS
RUN A, B and C, to which trace elements were added, were higher and stable than biogas produced from RUN B of experiment 2-1 (without the addition of trace elements) as presented in figure 4.

3.2.3.2 pH
The average value of the digestate pH for each RUN was 7.64 for RUN A, 7.65 for RUN B, 7.78 for RUN C, and no difference was found in the input materials. Also, all were within the optimum pH range.
### 3.2.3.3 Organic matter decomposition rate

As shown in table 7, RUN B showed the highest organic matter decomposition rate. From this, it was found that HRT around 33 days, organic load 4.26g-VS/L/d is suitable for continuous methane fermentation of bean sprout waste for 10L fermenter.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Organic matter decomposition rate (%)</th>
<th>HRT (d)</th>
<th>Organic loading (g-VS/L/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Bean sprouts 75 g + Return digestate 150 g + Trace elements 0.12 g</td>
<td>62.94</td>
<td>44</td>
<td>2.75</td>
</tr>
<tr>
<td>B Bean sprouts 100 g + Return digestate 200 g + Trace elements 0.16 g</td>
<td>71.02</td>
<td>33</td>
<td>4.26</td>
</tr>
<tr>
<td>C Bean sprouts 150 g + Return digestate 300 g + Trace elements 0.24 g</td>
<td>59.06</td>
<td>22</td>
<td>9.02</td>
</tr>
</tbody>
</table>

### 3.2.3.4 VFA

Organic matter decomposition rate was observed in all RUN. Decrease of 77.07% in RUN A, 78.94% in RUN B and 82.90% in RUN C was observed.

### 4. CONCLUSION

It was found that after boiling, the sprout produces about 2.4 times the amount of gas than fresh sprout. This is thought to have a positive effect on gas production as it was hydrolyzed the nutrients such as starch, fat and protein stored in seeds by the heat action.

In continuous fermentation, it is recommended to use a mixture of bean sprout and return digest as a raw material than single bean sprout for effective AD process. Additionally, by performing acid fermentation, more biogas is generated, and this due to the action of acid-fermented to decompose the protein (acetic acid) contained in the bean sprout, and in turn this is used as the input material.

Therefore, it is thought that not only acid fermentation is appropriate for bean sprouts but also for methane fermentation of other food wastes to enhance their output gas production potential.

Biogas production was sustained by the addition of trace elements, but it was found that the decomposition rate of organic matter was lower when the trace elements amount was too small or too large. In addition, it has become clear that it is necessary to set the HRT (hydraulic retention days) and the organic substance load appropriate for the volume of the fermenter.

Based on the above results and considerations, it is thought that methane fermentation using bean sprouts is possible. In addition, we think that there is need to consider whether it can be applied to food waste including other garbage.

### ACKNOWLEDGMENT

We deeply grateful to bean sprout manufacturing plant in Ibaraki prefecture for providing the research material for this experiment.

### REFERENCES


Bean sprout producer’s association, 2017 [http://www.moyashi.or.jp/nutrition/]

Optimization of Orange-Fleshed Sweet Potato (*Ipomoea batatas* var. Kinerot) Flour Processing for Carotenoid Retention

James Ryan D. Aranzado¹, *Loraine C. Bainto¹, Dennis Marvin O. Santiago¹ (¹ Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Baños(Philippines))

Keywords: orange-fleshed sweet potato, response surface methodology, carotenoid retention, flour processing

Orange-fleshed sweet potato (OFSP) is a rich source of carotenoids which upon body intake, is converted to Vitamin A. This raw material is commonly processed into popular food ingredients such as flour, however, the conversion process renders carotenoids susceptible to degradation. To maximize the retention of carotenoids in OFSP, optimized processing conditions must be determined using appropriate tool. In the study, response surface methodology was used to optimize the different process parameters involved in the production of sweet potato flour which will yield the desired level of identified responses related to its carotenoid content. Factor levels of processing conditions including slab thickness (ST), blanching time (Bt), blanching temperature (BT), and drying temperature (DT) were varied to determine their effect on selected responses namely vitamin A value, $L^a*b^*$ color values, and antioxidant activity. The optimized values obtained for the independent variables were 1.55 mm, 1.46 minutes, 100° C, and 50° C for ST, Bt, BT, and DT, respectively. Sweet potato flour produced under these conditions displayed maximized Vitamin A value (3810.09 IU per gram), $a^*$ (16.04) and $b^*$ (38.42) values, and antioxidant activity (81.19% DPPH inhibition) with minimized $L^*$ value (78.93). These experimental values were within the predicted interval of the responses which proves the applicability of the model.
Temporal Transition of Spatial Dependence of Weeds in Grassland

*Katsuyuki Tanaka¹, Ayako Oide¹, Hideo Minagawa¹ (1. Kitasato University (Japan))

Keywords: Spatial Modeling, Rumex obtusifolius.L, Grassland

Grasslands with high yield have a large percentage of grass as the main component and a low percentage of weeds and bare land. Especially, Broad−Leaved Bock (*Rumex obtusifolius.L*) has high seed productivity and regeneration ability and is recognized as a highly harmful weed. In order to control the amount, it is necessary to grasp the growing point. In this study, we clarified changes in spatial dependence by examining the spatial modeling by using the time-series distribution survey data from 2015 to 2018.
Temporal Transition of Spatial Dependence of Weeds in Grassland

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Division of Environmental Bioscience, Kitasato University, Japan

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ABSTRACT
Grassland with high yield are characterized as the high composition of grass and low composition of weeds and bare land. Among the weed, especially, Broad-Leaved Bock (*Rumex obtusifolius*.L) has high seed productivity and regeneration ability and is recognized as a highly harmful weed. In order to control the amount of Broad-Leaved Bock, it is necessary to grasp the growing point effectively.

In this study, we clarified the temporal changes in spatial dependence of weeds occurrence by examining the semi-variogram model to multi temporal vegetation survey dataset, observed from 2015 to 2018. As the result, the significant trend of spatial dependence was indicated. From these results, this study proposed the practical and effective strategy for weeding in grassland, that is, preferential spot weeding to large individual or the cluster controlling for the dense crowded area within the range of space dependency.

Keywords: Forage corn UAV, Precision Agriculture Remote sensing

1. INTRODUCTION
Grassland with high yield are characterized as the high composition of grass and low composition of weeds and bare land. To rid of these noxious weeds from grassland, it's growing spot is need to be identified first. However, the growing spots of these noxious weeds is not constant, and often appears in completely different place after harvesting. Therefore, the field manager needs to identify these weeds one by one on site to proceed the weeding works, which requires a great deal of labor and time. There are many studies aimed at efficient weed control, focusing on weed detection(Ayumi Nakatsubo et.al, 2013), but few studies focus on the dynamics of weeds expansion inside grassland. Therefore, this study aims to clarify the change of spatial dependence in weed occurrence in the grassland using semi-variance analysis, which is a method of spatial statistics. In this study, the *Rumex obtusifolius*.L which has especially high seed productivity and regeneration ability are targeted among several weeds which appears in grassland.

2. MATERIALS AND METHODS
2.1 Study Site
The study site is established in the second field of Field Science Center (FSC) Towada Farm, Kitasato University Faculty of Veterinary Medicine. 50m survey zone was established in the both north-south and north-south directions, respectively, and divided by a 2 m square mesh, providing a total of 2500 small sections.

2.2 Vegetation Survey
The distributing position of *Rumex obtusifolius*.L (hereinafter, referred to as RO) in settled test site was identified using quadrats divided into 1.0m x 1.0m. Table 1 shows the survey dates for each fiscal year. According to the weighted scoring method depends on the diameter (R) of the equivalent circle including the tip of the leaf, each sampling point have been divided into three categories, that is, small (0 \(\leq R <0.2\) m), middle (0.2 (R <0.4 m), large (R \(\geq 0.4\) m), and is scored 1,3,5 respectively. Then the scores were counted by each section which is consists of 0.5 m square mesh to standardize.

1
Table 1. Date of survey in each fiscal year

<table>
<thead>
<tr>
<th>Fiscal year</th>
<th>First harvest</th>
<th>Second harvest</th>
<th>Third harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>30-April</td>
<td>7-July</td>
<td>3-September</td>
</tr>
<tr>
<td>2016</td>
<td>30-April</td>
<td>7-July</td>
<td>9-September</td>
</tr>
<tr>
<td>2017</td>
<td>26-April</td>
<td>5-July</td>
<td>15-September</td>
</tr>
<tr>
<td>2018</td>
<td>21-May</td>
<td>16-July</td>
<td>17-November</td>
</tr>
</tbody>
</table>

2.3 Semi-Variance Analysis
The spatial dependence was examined by applying semi-variance analysis to the standardized score for each small mesh. Semi-variogram was calculated by using the definition of two-dimensional analysis. After that, a sphere model was applied for the semi-variogram to find three semi-variogram parameters, which is nugget, sill and range. Nugget is the parameter which shows the variation that appears even when the distance between the two becomes zero. The value of the semi-variance (γ) at the point of semi-variance (γ) becomes constant is called the sill. Range is the spatial distance when the semi-variance (γ) becomes constant. The range shows the limit of space dependence. Therefore, in this study, the spatial dependence of RO is indicated as the semi-variance parameter of range.

![General semi-variogram model](image)

2.4 Statistic Test
In order to check if there is spatial dependence within the range, each section have been categorized to 3 groups by the centered score as shown in Table 2. Then each group were examined by the following two examination methods. Firstly, the significant difference between the mean of expected values of weighted scores within 2 spatial distances segments, that is, 3 m from the center, 3 m to 10 m from the center, was examined by a t-test. Second, when the score of the center in the 3m range is different, the significant difference in the mean value of each expected t-value was examined by the t-test. For examining these two statistical tests, the 500 section sample data was randomly extracted from total 2,500 sections.

Table 2. 3 Groups categorized by the centered score

<table>
<thead>
<tr>
<th>Group</th>
<th>Centered score</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0</td>
</tr>
<tr>
<td>G2</td>
<td>1–2</td>
</tr>
<tr>
<td>G3</td>
<td>3–12</td>
</tr>
</tbody>
</table>

3. RESULTS AND DISCUSSION
3.1 Distribution Plot
The result of vegetation survey in each fiscal year have been plotted to the map by categorizing to three types by the diameter size. Figure 2 shows an example from the survey result of 3rd crop in 2018.
3.2 Temporal Change of Spatial Dependence

Figure 3 shows the temporal change of spatial dependence (range) over the past 4 years. The a, b, c in the figure represent the harvest timing of each year, and the numerical values indicate the range values. Range increased from the first to the second crop, and decreased from the third to the first crop of following year. On the other hand, in the period of second to third crop, the both trend of increase and decrease was observed. The average value of the range from 2015 to 2018 was 3.51 and the standard deviation was 1.11, and it was found that the range was not constant but varied for each grass of each year (coefficient of variation = 0.316)
3.3 Statistic Test
Table 3 shows the results of first statistic test. As a result of t-test (significance level of 5%), the expected score of peripheral value showed the significant difference between within and outside the range in all score groups except for G1.

<table>
<thead>
<tr>
<th>Center</th>
<th>Peripheral range (m)</th>
<th>Mean</th>
<th>Degree of Freedom</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>3</td>
<td>0.392</td>
<td>822</td>
<td>-1.77</td>
<td>0.0775</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.567</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>3</td>
<td>0.460</td>
<td>683</td>
<td>4.88</td>
<td>1.34e-06</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.662</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>3</td>
<td>0.682</td>
<td>693</td>
<td>5.87</td>
<td>6.76e-09</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.535</td>
<td></td>
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</tbody>
</table>

Table 4 shows the results of second statistic test. There were significant differences in the expected values among the central scores within the 3m range. Therefore, in within range, the smaller the central score, the smaller the peripheral score was observed, and vice versa, the larger the central score, the larger the peripheral score was observed. Therefore, the space dependence was confirmed within the range value.

<table>
<thead>
<tr>
<th>Center</th>
<th>Peripheral range (m)</th>
<th>Mean</th>
<th>Degree of Freedom</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>3</td>
<td>0.392</td>
<td>922</td>
<td>-7.00</td>
<td>5.05e-12</td>
</tr>
<tr>
<td>G2</td>
<td>10</td>
<td>0.567</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>3</td>
<td>0.567</td>
<td>983</td>
<td>-3.75</td>
<td>1.84e-04</td>
</tr>
<tr>
<td>G3</td>
<td>10</td>
<td>0.682</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>3</td>
<td>0.392</td>
<td>862</td>
<td>-10.62</td>
<td>7.51e-25</td>
</tr>
<tr>
<td>G3</td>
<td>10</td>
<td>0.682</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. CONCLUSION
The temporal changes of the range value showed the increasing trend in the period which is from the first crop to the second crop, and the decreasing trend in the period which is from the third crop to the first crop of the following year regularity. The results of t-test showed that within the range where spatial dependency was observed, the total amount of RO tended to increase in the vicinity of the point where the amount of RO is large.
From these results, this study proposed the practical and effective strategy for weeding RO in grassland, that is, preferential spot weeding to large individual or the cluster controlling for the dense crowded area within the range of space dependency.

REFERENCES
RNA-Seq analysis of the transcriptome and genes expression profile during the browning of Lotus Root (*Nelumbo nucifera*)

Kanjana Worarad, Haruka Norii, Yuya Muchizuki, Takashi Ishii, Keiko Shinohara, Takao Miyamoto, Eiichi Inoue (1. Ibaraki University(Japan), 2. Ibaraki Agricultural Center, Horticultural Research Institute (Japan), 3. Tokushima Agriculture, Forestry and Fisheries Technology Support Center(Japan), 4. Renkon3kyodai Co,Ltd(Japan))

Keywords: Browning disorder, Metabolic pathways, RNA sequencing, Transcriptomics, Postharvest physiology

Lotus root (*Nelumbo nucifera*) has been widely cultivated in Japan. There is crisp texture, white color and enriched with a source of nutritional components. The consumption/production of fresh-cut lotus root has continuously increased as more consumers demand convenient and ready-to-eat foods. However, it is well known that the processing, storage and transportation of fresh-cut fruits and vegetables promotes a faster physiological deterioration, mainly browning and reduces the value of a product. This study aimed to clarify the functions of unigenes and browning associated metabolic pathway of intact lotus root during long-term storage using RNA-sequencing techniques. Lotus peel from the main cultivar in Ibaraki prefecture ‘Kanasumi No.34’ after harvest (AH), and unpacked (UP), and packed with water (PW) after storage under 5°C for 6 hr. were collected. Over 200 million short single-end reads were mapped onto the *Nelumbo nucifera* consensus coding sequence set, and differences in the expression profiles between AH, UP, and PW tissues were assessed to identify candidate genes associated with internal browning in a tissue-specific manner. Based on Swiss-Prot, TrEMBL, KEGG mapping pathway and GO ontology databases, genes involved in phenylpropanoid biosynthesis, tyrosine metabolism, and lipid metabolism were significantly upregulated in the UP and PW when compared with AH. The expression levels of several of them will be confirmed using qRT-PCR. Additionally, the gene expression data presented in this study will help elucidate the molecular mechanism of browning development in lotus root at long-term storage. Base on this study, including phenylpropanoid biosynthesis-related genes, lipid-related genes (related to membrane alterations, and fatty acid degradation), for browning development in lotus root is proposed, which may be relevant for future studies towards improving the postharvest life of lotus root.
[5-1130-P-15] **Effect of Blending at Different Stages of Winemaking on the Quality of Mixed Fruit Wine**  
*Claire Solis Zubia¹, Erlinda Ignacio Dizon¹ (1. University of the Philippines Los Banos(Philippines))  
11:30 AM - 12:30 PM

[5-1130-P-16] **Pest Control of *Tetranychus urticae* by Branched Fatty Acids**  
*Mai Nagano¹, Akitaka Teshima¹, Toshinari Koda², Hiroshi Morita¹ (1. The University of Kitakyushu(Japan), 2. Nissan Chemical corporation(Japan))  
11:30 AM - 12:30 PM

[5-1130-P-17] **Evaluation of Quality and Structural Properties of Bread Containing Edible Cricket**  
*Kiko Kuroda¹, Tatsuya Oshima¹, Teppei Imaizumi¹ (1. Gifu Graduate School of Applied Biological Sciences and Faculty of Applied Biological Sciences(Japan))  
11:30 AM - 12:30 PM
From a prior study on determination of best formulation for a multi-flavored fruit wine product, another study was conducted to determine the effect of blending at different stages of winemaking on the quality of mixed fruit wine. Using the optimized formulation of 50% mango, 25% pineapple and 25% passion fruit as components of the blend, three treatments were used: (1) blending of individually prepared mango, pineapple and passion fruit musts before fermentation, (2) blending of individually fermented mango, pineapple and passion fruit wines before aging, and (3) blending of individually aged mango, pineapple and passion fruit wines before bottling. Resulting products were evaluated and compared in terms of their physico-chemical and sensory properties. It was found out that blending of individually prepared mango, pineapple and passion fruit musts prior to fermentation, produced wine with the greatest alcohol content (13.37%) and total phenolic content (378 mg/mL GAE). It also achieved lowest acidity and highest pH level. By employing DPPH radical scavenging assay, the said sample was also observed to exhibit the highest antioxidant activity with 69% inhibition compared to samples from the two other treatments. The obtained wine products were carbonated and bottled and then subjected to sensory evaluation by quality scoring. Sample produced from blending of individually prepared musts scored highest in terms of bitterness, clarity and overall acceptability. It was also perceived to be the least sour and to have the most intense yellow color.
adults of *Tetranychus urticae* were inoculated on days 0, 1, 3 and 5 of sample inoculation. After 24 h, the lethality of adult females was determined under a microscope. As a result of the acaricidal test, it was found that when the concentration of isoC16 was 1%, the acaricidal effect was 50% or more. As a result of the repulsion test, no significant difference was observed in the population of the spider mite on the treated area and the non-treated area in isoC16. As a result of the sustainability test, isoC16 showed an adjusted mortality rate of 50% or less at 0-5 days after treatment. For this reason, it became clear that isoC16 is low in sustainability. The corrected mortality rate was less than 50% even after 0 days of sample processing. Therefore, it was shown that in order for isoC16 to exert its pest control effect, it is necessary for the drug to be in direct contact with *Tetranychus urticae*.

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**Evaluation of Quality and Structural Properties of Bread Containing Edible Cricket**

*Kiko Kuroda¹, Tatsuya Oshima¹, Teppei Imaizumi¹ (1. Gifu Graduate School of Applied Biological Sciences and Faculty of Applied Biological Sciences(Japan))

**Keywords:** edible insect, cricket, bread, micro X-ray CT, structure

In the near future, it is predicated that we will be suffered from food shortage by climate change and population growth. Animal protein is estimated especially shortage, due to need more energy for production than any other nutrients. To overcome this problem, various solutions are suggested, and edible insects are one of the effective approaches. To reduce consumers’ discomfort, insect should be mixed with processed food like bread. However, effects of insect addition on food quality have not been sufficiently clarified. In this study, we baked bread containing cricket powder, then evaluated physical and chemical quality. Bread sample ware baked using a bread machine (BK-B67, CCP Co., Ltd). After setting ingredients, the machine performs mixing, kneading, fermentation and baking automatically. In this study, normal bread (control) was made with 250 g of wheat flour and other ingredients (180 mL of water, 10 g of butter, 17 g of sugar, 5 g of salt, 6 g of skim milk and 2.8 g of dried east). For making bread containing cricket, 10 to 50 % of the flour weight was replaced with cricket powder, and named C10, C20, C30, C40 and C50, respectively. First, hardness of each bread was measured by AACC method with a little modification. The bread sample was cut into slices each having 25 mm thickness, then a slice obtained from middle part of the loaf was used. A cross section of the slice was compressed using a creep meter (TPU-2DL, YAMADEN Co., Ltd) equipped with a disk-shaped plunger (20 mm diameter). The plunger was moved at 1 mm/sec. The compressive force at 25 % of deformation was defined as hardness. Second, structural properties of the bread sample (control, C10, C30) ware evaluated. Loaf volume of each bread was measured with the rapeseed replacement method. Additionally, the internal structure of the bread sample (control, C30) was analyzed by using an X-ray micro CT (SKYSCAN1172, Brucker Co., Ltd). A cube (10 mm) was obtained from central part of each bread. The flaming condition was X-ray power settings of 100 kV, 100 μ A, four-flame averaging and a rotation step of 0.7 °. For image processing and analysis, the skyscan software, CT-Analyser was used and microstructural parameters ware obtained. Although the hardness of control was 0.488±0.0749 N, that of the cricket bread indicated higher values (0.565±0.182 - 6.12±1.27 N). The value increased with the amount of the cricket powder. Considering the actual use for bread making, hardness of the cricket bread should be similar to normal bread. Thus, in the subsequent experiments, we focused on the bread made with 30 % or less of cricket powder. Loaf volume of the bread was 1800.8, 1481.6 and 1255.3 mL for control, C10 and C30, respectively. It was implied bread rising was inhibited due to adding cricket powder and it contributed to increase hardness. According to the result of X-ray micro CT, structure separation of the cricket bread (C30)
was small (1363±212 μm) while the value of control was large (906±39.6 μm). In addition, object surface density of control (0.00548 ± 0.0000283 μm⁻¹) was higher than C30 (0.00420 ± 0.000769 μm⁻¹). These results shown that C30 constructed with larger pores in comparison with control. About structure thickness, C30 indicated large value (127±81.7 μm) more than control (94.5±20.6 μm), although the standard deviation was large. Therefore, C30 has partial thick structure in contrast to control, it agreed with the result of measuring volume or hardness experiments.
**Poster Session | Food Safety**

**[5-1130-P] Food Safety (5th)**
Thu. Sep 5, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

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**[5-1130-P-18]** **Key Process Variables Affecting the Formation of Chlormequat Compounds During Baking of Cereal Products**

*Adam Ekielski*¹ (1. Warsaw University of Life Sciences(Poland))
11:30 AM - 12:30 PM

**[5-1130-P-19]** **Acaricidal effects of Linear fatty acids against *Tyrophagus putrescentiae***

*Kosuke Matsuoka*¹, *Toshinari Koda*², *Hiroshi Morita*¹ (1. The University of Kitakyushu(Japan), 2. Nissan Chemical Corporation(Japan))
11:30 AM - 12:30 PM

**[5-1130-P-20]** **Improvement of the Cleanability of Milk Soil on a Highly Smooth Surface of Stainless Steel Tubing**

11:30 AM - 12:30 PM
Key Process Variables Affecting the Formation of Chlormequat Compounds During Baking of Cereal Products

*Adam Ekielski*\(^1\) (1. Warsaw University of Life Sciences(Poland))

Keywords: chlormequat formation, baking process, cereals

The aim of this work was to examine the effect of temperature and time chlormequat pesticides formation during the bread baking process. The flour and other dough addition used for the study were of the ecological type and verified by us to be free of any quaternary ammonium pesticides. Plant growth regulators are widely used in agricultural food production, mainly in the production of cereals, where they are used to shorten and strengthen the stem. Among the plant growth regulators, chlormequat is by far the most common. Residues of plant growth regulators must be expected in food products due to their extensive use. Permissible level of chlormequat is regulated at 0.02 mg/kg in citrus fruits up to 0.05 mg/kg in nuts. Chlormequat is not considered to pose any risk to human health so long as the residues are below the legal maximum residue levels. However, there is general concern that they may impair human fertility due to the detrimental effects of chlormequat on certain aspects of animal reproduction. Some reports clearly suggest that chlormequat may have serious adverse effects on animal reproduction, even at doses below the Acceptable Daily Intake for humans. Probably due these reasons, chlormequat is not approved for use in the UK. In previous studies, the possibility of formation of chlormequat compounds in brewing malt has been observed, and current studies have confirmed the possibility of formation of chlormequat compounds in the baking of cereal products. The paper presents the results of investigations of chlormequat content in baking products obtained in different production parameters. There are some published papers about mepiquat formation during food thermal processing. Considering the structural similarity chlormequat and mepiquat (Quarternary ammonium nature) and closer resemblance to methylating agents commonly found, it has been hypothesized with high probable that chlormequat formation can take similiar route. Mepiquat is generated under Maillard conditions via transmethylation reactions involving the nucleophile piperidine (formed by cyclisation of free lysine in the presence of reducing sugars) and a methyl donor (trigonelline, choline, glycine). Nevertheless, there is no obvious clue about the possible formation of chlormequat in such conditions. We have studied the effect of processing parameters (temperature and time, dough humidity etc.) and dough components share (type of flour, malt, dried milk), on the quantity of chlormeqat formed during the baking process. The experiment was prepared by using response surface and PCA (Principal Component Analysis) methods. It was found that the key factor determining the amount of the chloroquat compound produced during baking was temperature, which may suggest that the formation of chloromequat is correlated with Maillard's reactions. In our experiment, chlormequat was detected at temperatures above 165 °C, but when the malt contain in the baking dough was reduced (from 4% to 1%), chlormequat was not observed in bakery products.
Tyrophagus putrescentiae is one of the cause of allergies and acarasis because they breed in various places in the room such as food, bedding and carpets. *Tyrophagus putrescentiae* also cause pollution of the food. Previous study has shown that 2-hexyldecanoic acid that is a branched higher fatty acid has an acaricidal effect against *Tyrophagus putrescentiae*. However, it is necessary to search for samples that has higher acaricidal effects. This study focused on linear fatty acids. We performed acaricidal test using hexadecanoic acid that has same number of carbons with 2-hexyldecanoic acid. After that, we performed same test using hexanoic acid, octanoic acid and decanoic acid that have carbons less than hexadecanoic acid. *Tyrophagus putrescentiae* were obtained from Earth Chemical Co., Ltd., and maintained in our laboratory without exposure to any acaricides. Hexanoic acid (C6), octanoic acid (C8), decanoic acid (C10) and hexadecanoic acid (C16) were used to the mitidical test as samples. Ethanol was used as the dilution solvent. In the acaricidal test, Tyrophagus putrescentiae was placed on a black cloth (45 mm × 45 mm) and samples were sprayed. Ethanol was used as the dilution solvent. After that, samples of linear fatty acids (undiluted solution and 350mM) were dropped on the cloth and feed of insects were placed on the cloth. The petri dish was placed into a plastic container containing saturated saline solution. The temperature and humidity inside the container were kept at 25 °C and 75 %. After 24 h, mortality was determined by observation using a microscope. As a result, the mortality of hexanoic acid, octanoic acid and decanoic acid were over 70 %. However, the mortality of hexadecenoic acid was 0 %. These results were suggested that the carbon number of linear fatty acids was related to the mortality of mites. As a problem, linear fatty acids have unpleasant smell. It is necessary to discover compounds that have mitidical effect and not smelling. In addition, it is thought that we remove the smell of linear fatty acids by masking agents as a possible solution.

Stainless steel tubing is widely used for process equipment in milk processing industries. The presence of milk soils on internal surfaces of stainless steel tubing may cause deterioration in quality and food poisoning. Frequent cleaning of the equipment surface is needed to avoid contamination, however, it may cause an increase environmental impacts, linked to the consumption of water, detergent and energy. Surface roughness is one factor affecting the attachment and removal of food soils. EHEDG (European Hygienic Engineering & Design Group) recommends that large areas of food product contact surface should have a surface finish of 0.8 m Rₐ. In this work, we studied cleanability of milk soil on a highly-smooth internal surface with 0.01 m Rₐ of stainless tubing. The highly-smoothed stainless tube was prepared by magnetic abrasive finishing (MAF), which is an internal finishing process by the application of a magnetic field of permanent magnets. Three different levels of surface roughness of stainless steel tubings were tested to evaluate the
cleanability of milk soil. On the deposition test, whole milk at 44° C was circulated in a tested loops connected with the tested stainless tubings. After the deposition process, deionized water at different temperatures was flushed into the tested loops to clean milk deposition on the internal surface of stainless steel tubings. To evaluate the cleanability of the milk deposition in the tubings, we measured amounts of milk residues and residual proteins on the internal surface of the tubings. The data showed that the smoother surface had a tendency to improve the cleanability of milk soil and milk protein at 45° C of cleaning solution. When the temperature is raised from 20 to 45° C, the cleanability of milk soil was improved. However, when the temperature was raised from 45 to 50° C, almost no change was observed. At 35, 45, and 50° C, smoothing of the surface showed a tendency to improve detachment of milk soil. The cleaning solution temperature affected the removal of milk soil. The relationship between surface roughness and detachment of milk soil was clearly observed, when the cleaning solution temperature was at 45° C.
[5-1130-P-21] **Screening and Identification of Endophytic Bacteria from Thai Organic Rice for Plant Growth Promotion**  
*Somkid Deejing¹, Witchayaporn Pawong¹ (1. Program in biotechnology, Faculty of Science, Maejo University, Sansai, Chiang Mai(Thailand))
11:30 AM - 12:30 PM

[5-1130-P-22] **Data Extraction for Pig Weight Prediction Model**  
*Khin Dagon Win¹, Kikuhito Kawasue¹, Hsu Lai Wai¹, Kumiko Yoshida² (1. University of Miyazaki(Japan), 2. KOYO Plant Service(Japan))
11:30 AM - 12:30 PM

[5-1130-P-23] **Power Tiller’s Wheel Structure and its Oscillatory Effects on Subsoiling Operation**  
*Oyetayo Olukorede Oyebode¹, Koichi Shoji¹ (1. Graduate School of Agricultural Science, Kobe University(Japan))
11:30 AM - 12:30 PM

[5-1130-P-24] **Proposal of temperature control technology in pot cultivation for the citrus fruits**  
*Ryuta IBUKI¹, Yoshimichi Yamashita², Sachie Horii², Norihiro Hoshi², Madoka Chiba¹ (1. Miyagi University(Japan), 2. National Agriculture and Food Research Organization(Japan))
11:30 AM - 12:30 PM

[5-1130-P-25] **Investigation by Driving Simulation of Tractor Overturning Accidents Caused by Steering Instability**  
*Masahisa Watanabe¹, Kenshi Sakai¹ (1. Tokyo University of Agriculture and Technology(Japan))
11:30 AM - 12:30 PM

[5-1130-P-26] **Classification of Salinity Damaged Spring Potato (Solanum tuberosum) using Hyperspectral Imagery based on Decision Tree Classifier**  
*KyungSuk Kang¹, Sae Rom Jun¹, Si Hyeong Jang¹, Jun Woo Park¹, Hye Young Song¹, Ye Seong Kang¹, Chan Seok Ryu¹, Su Hwan Lee² (1. GNU(Korea), 2. RDA(Korea))
11:30 AM - 12:30 PM

[5-1130-P-27] **Classification for Fire Blight Disease Infection Area using Vegetation Index and Background Segmentation based on Multispectral Image**  
*Jun-woo Park¹, Chan-seok Ryu¹, Ye-seong Kang¹, Sae-Rom Jean¹, Si-Hyeong Jang¹, Hye-Young Song¹, Kyung-Suk Kang¹ (1. GNU(Korea))
11:30 AM - 12:30 PM

[5-1130-P-28] **The Static Load Test for Tractor Attached Three-Point Hitch Type Dynamometer**  
*Hyo-Geol Kim¹, Sung-Bo Shim², Yeon-Soo Kim¹, Young-Joo Kim¹, Sang-Dae Lee¹ (1. Korea Institute of Industrial Technology(Korea), 2. Gyeongsang National University(Korea))
[5-1130-P-29] **Isolation and Identification of Acetic Acid Bacteria from Philippine Fermented Rice Cake Batters by 16S rRNA Gene Sequence Analysis**

Audrey Mae Villamin Orillaza¹, Honey Bhabes R Iñigo¹, Baby Richard Ragudo Navarro¹

(1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Baños(Philippines))

11:30 AM - 12:30 PM


*Tatsuo Hishinuma¹, Tetsuya Hoshino¹, Atsuo Ikeguchi¹, (1.Utsunomiya Univ.(Japan))

11:30 AM - 12:30 PM
Screening and Identification of Endophytic Bacteria from Thai Organic Rice for Plant Growth Promotion

*Somkid Deejing¹, Witchayaporn Pawong¹ (1. Program in biotechnology, Faculty of Science, Maejo University, Sansai, Chiang Mai(Thailand))

Keywords: Endophytic bacteria, Indole acetic acid, Organic agriculture, Bacterial characteristics

Endophytic bacteria are able to colonize in plant tissues without causing harmfulness subsequently, sharing and exchanging beneficial metabolites to plant hosts. Plant growth can be promoted by these bacteria via their phytohormones i.e. indole acetic acid (IAA) and/or enhancement of nutrient availability. IAA is associated with plant cell division, cell elongation and lateral root formation. The population of endophytic bacteria are more diverse in crops planted following to organic practice. Thus, organic crops are interesting sources for endophytic isolation for further agricultural application as plant growth promoter. The aims of this present work were to isolate and identify promising endophytic bacteria from various part of rice with respect to their IAA production. Rice tissue samples were collected from five-year-old organic farm in Chiang Mai, Thailand. Bacteria were cultured on Plate count agar (PCA), Pikovskaya’s medium (PVK), Tryptic soy agar (TSA) and International Streptomyces project (ISP₂). The results showed that 53 bacterial isolates were obtained and further screened for IAA production in medium containing 0.2 % tryptophan. The IAA producing bacteria were RRSPCA and LRSPCA2 which produced at 20.93 and 7.12 mg/L, respectively. They were identified as *Pseudomonas* sp. and *Chryseobacterium kwangyangense*, respectively, based on 16s rRNA gene sequencing at 100 % similarity. These endophytic bacteria in this study could be applied for enhancing a plant growth, resulted a plant yield. Moreover, their bioactive compounds could be used for biotechnological applications. Therefore, the endophytic bacteria will contribute to organic agriculture for more environmentally sustainable in the future.
Screening and Identification of Endophytic Bacteria from Thai Organic Rice for Plant Growth Promotion

Somkid Deeijing1* and Witchayaporn Pawong2
1,2 Program in Biotechnology, Faculty of Science, Maejo University, Thailand
*Corresponding author: kittydeeijing@gmail.com, somkid_d@mju.ac.th

ABSTRACT
Endophytic bacteria are able to colonize in plant tissues without causing harmfulness subsequently, sharing and exchanging beneficial metabolites to plant hosts. Plant growth can be promoted by these bacteria via their phytohormones i.e. indole acetic acid (IAA) and/or enhancement of nutrient availability. IAA is associated with plant cell division, cell elongation and lateral root formation. The population of endophytic bacteria are more diverse in crops planted following to organic practice. Thus, organic crops are interesting sources for endophytic isolation for further agricultural application as plant growth promoter. The aims of this present work were to isolate and identify promising endophytic bacteria from various part of rice with respect to their IAA production. Rice tissue samples were collected from five-year-old organic farm in Chiang Mai, Thailand. Bacteria were cultured on Plate count agar (PCA), Pikovskaya’s medium (PVK), Tryptic soy agar (TSA) and International Streptomyces project (ISP2). The results showed that 53 bacterial isolates were obtained and further screened for IAA production in medium containing 0.2 % tryptophan. The IAA producing bacteria were RRSPCA and LRSPCA2 which produced at 20.93 and 7.12 mg/L, respectively. They were identified as Pseudomonas sp. and Chryseobacterium kwangyangense, respectively, based on 16s rRNA gene sequencing at 100 % similarity. These endophytic bacteria in this study could be applied for enhancing a plant growth, resulted a plant yield. Moreover, their bioactive compounds could be used for biotechnological applications. Therefore, the endophytic bacteria will contribute to organic agriculture for more environmentally sustainable in the future.

Keywords: Endophytic bacteria, Indole acetic acid, Organic agriculture, Bacterial characteristics

1. INTRODUCTION
Thailand is one of major rice producer and exporter in the world. In 2011, export value of Thai Rice was 210,527 million baht (Nara et al., 2014). Organic rice derived from organic farming which uses fertilizers from organic substances and pesticides made from natural ingredients instead of chemical pesticides and chemical fertilizers. Therefore, organic rice is better for our health and environment safety make sustainable agriculture. Increasing environmental damage and human population pressure are two important problems indicating that global food production may soon become insufficient to feed all of the world’s people (Etesami et al., 2015). Climate change, increases in temperatures, extreme temperatures, droughts, and rainfall intensity are abiotic stress that effected on rice production. The organic farming management system which application of endophytic bacteria offer a promising alternative and reduce health and environmental problems. Endophytic bacteria are bacteria that live within various parts of plants such as seeds, roots, stems, leaves result in benefit of their host plants by increasing nutrient uptake, producing biologically active phytohormones and suppressing pathogens by production of antibiotics, siderophores, and fungal cell wall-lysing enzymes including enhancement of the tolerance respond to abiotic stresses (Hameeda et al., 2008). Among these, indole acetic acid (IAA) is one of the most vital hormones which involed in lateral and adventitious root formation (Idris et al., 2007), increasing shoot growth, tillering and root elongation (Yang et al., 1993). IAA producing bacteria play a major role as plant growth promoter that were used as biofertilizer for enhancement of rice growth and yield (Etesami et al., 2015). The commonly found bacterial endophytic genera are Pseudomonas, Bacillus, Burkholderia, Stenotrophomonas, Micrococcus, Pantoea and Microbacterium etc. (Romero et al. 2014). Phetcharat and Duangpang (2012) found that the percentage of endophytic of IAA producing bacteria, ACC deaminase, and siderophore higher than rhizosphere bacteria (Prakamhan et al., 2009). Ji et al. (2014) isolated and characterized plant growth promoting endophytic bacteria from Korean rice. They obtained 576 isolates endophytic bacteria from the leaves, stems, and roots of 10 rice cultivars and identified
through 16S rDNA sequence analysis belong to *Penibacillus* sp., *Microbacterium* sp., *Bacillus* sp., and *Klebsiella* sp. Ten isolates have shown higher IAA producing activity, 6 isolates with high siderophore producing activity and 4 isolates high phosphate-solubilizing activity. Population density of endophytic diazotrophic bacteria (EDB) was highest in the rice landrace root tissues at nursery stage. Indole-3-acetic acid (IAA) production (0.85–16.66 μg/mL) was found in 21 strains tested. More than 80 % (18 isolates) of the isolates solubilized phosphate, while only 28.57 % (six isolates) of selected strains produced siderophore (Rangjaroen et al., 2014). Blanco and Lugtenberg (2014) reported the biotechnological applications of endophytic bacteria can promote plant growth, for example by the production of hormones or by making nutrients (such as nitrogen, phosphate and ferric ions) available to the plant. In addition, endophytes can also promote plant growth indirectly, for example by suppression of plant diseases, by inactivating environmental pollutants, and by alleviating stresses of the plant caused by excess of the hormone ethylene, by heavy metals, by draught and by salinated soil. Some endophytic bacteria can produce nanoparticles which have numerous applications. They concluded that endophytes are much more efficient in their application of active compounds and their metabolite.

The organic farming increases the crop productivity while sustaining the ecosystems. Health is also a consideration in organic farming practices. It is conceivable that the application of endophytic bacteria could be an advantage since they are present in a much more protected environment than rhizosphere bacteria and likely to be less vulnerable to changing environmental conditions. Therefore, the objectives of this study were to isolate, screening and identify of IAA producing endophytic bacteria from Thai organic red jasmine rice tissues in Sansai, Chiang Mai, Thailand for application to organic rice production system and help plant under climage change, including biotechnological application in the future.

2. MATERIALS AND METHODS

2.1 Isolation of endophytic bacteria from organic red jasmine rice tissue

Roots, stems, and leaves of rice were collected during growth stage from organic red jasmine rice farming in Sansai, Chiang Mai, Thailand. Tissue of rice samples were dipped in 70% ethanol for 2 min, then in 4% sodium hypochlorite for 15 min and finally rinsed eight times with sterile distilled water. After that, the sterilized pieces were put onto Plate count agar (PCA); (g/L) tryptone (5), yeast extract (2.5), glucose (1.0), agar (15) and distilled water (1 L); Pikovskaya ‘s medium (PVK); (g/L) glucose (10), Ca₃(PO₄)₂ (5), (NH₄)₂SO₄ (0.5), KCl (0.2), MgSO₄.7H₂O (0.1), agar (15) distilled water (1 L); Tryptic soy agar (TSA); (g/L) tryptone (15), soytone (5), NaCl (5), agar (15) and distilled water (1 L), and International Streptomyces project (ISP2); (g/L) malt extract (10), yeast extract (4), glucose (4) agar (15) and distilled water (1 L). Culture medium plate were incubated at 37 °C for 24-48 h., while on ISP2 medium was incubated at 37 °C for 7-14 days. Surface sterility test was performed for each of sample to ensure the elimination of surface microorganism. The soaking water from sterilized rice tissues were plated on Nutrient agar (NA) (g/L); beef extract (3), peptone (5), agar (15) and distilled water (1 L) by using pour plate technique. Endophytic bacterial strains growing on selective media plates were isolated, purified and were preserved on agar slants for further studies.

2.2 Preliminary screening of IAA producing endophytic bacteria

Preliminary screening of IAA production test was evaluated by growing the isolates bacteria in tryptone containing (g/L) tryptone (5) and distilled water (1 L) and then incubated by shaking 130 rpm at ambient temperature for 48 h. After incubation, Kovac’s reagent was added to culture medium. Development of cherry red colour at the top layer in the form of ring indicated the positive test while its absence indicated the negative test. The isolates bacteria that positive test in primary screening test were selected for further study.

2.3 Quantitative analysis of IAA production of endophytic bacteria

Production of IAA was measured the quantitative analysis by culturing bacteria in Nutrient broth (NB) containing (g/L) beef extract (3), peptone (5) and distilled water (1 L) supplemented with 0.2 % L-tryptophan as precursor of IAA and then incubated 130 rpm on shaker at ambient temperature for 48 h. After incubation, the culture was centrifuged at 10,000 rpm for 20 min to collect the supernatant. Then, Salkowski coloring reagent (1 ml of 0.5 M FeCl₃ in 49 ml of 35% perchloric acid (HClO₄) and the supernatant were mixed and left in the dark for 25 min. After the reaction, the absorbance of the
mixtures was estimated at 530 nm. The IAA concentration in the culture was estimated based on the IAA standard curve. Endophytic bacteria which high IAA production was selected for identification.

2.4 Identification of selected endophytic bacteria

The selected bacteria was identified by studying the cultural, morphological and biochemical characteristics. Cultural characteristics of selected endophytic bacteria was streaked on Nutrient agar plates and then observed colonies such as shape, elevation, margin, colour and pigment after incubation at 37 °C for 24-48 h. Morphological characteristics was examined by Gram’s staining and observed under bright field microscope. Biochemical and physiological characteristics of endophytic bacteria were studied. Catalase test was done by adding 2% hydrogen peroxide solution to the culture on a slide. The release of free oxygen bubbles indicated a positive result. Oxidase test was determined by dipping the filter paper strip in 1% N, N, N,N-tetramethylene p-phenylene diamine dihydrochloride and then transferred the endophytic bacteria to filter paper strip. In a positive reaction, the reagent was oxidized to give intense blue violet colour within 5 min. Carbohydrate utilization test was also examined in culture broth with bromocresol purple as indicator and supplemented with different sources of carbohydrate (glucose, fructose, galactose, lactose, maltose, mannitol, xylitol and sucrose). Pure culture of selected endophytic bacteria was inoculated and incubated at 37 °C for 24 h. A positive test was represented by development of yellow colour due to acid production and bubbles trapped within the durham tube indicated the gas production.

The identification of selected IAA producing endophytic bacteria was examined by using 16S rRNA gene sequencing. Amplification of the 16S rRNA gene was performed with 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) universal primers. Sequencing of bases was undertaken by First BASE Laboratories, Malaysia. The sequence data were compared with NCBI GenBank and the similarities were determined by the Basic Local Alignment Search Tool (BLAST) software algorithm.

3. RESULTS AND DISCUSSION

3.1 Isolation of endophytic bacteria from organic red jasmine rice tissue

Endophytic bacteria were isolated from tissue of five-year-old organic red jasmine rice in Chiang Mai, Thailand on PCA, PVK, TSA and ISP2 medium. Total fifty-three isolates of endophytic bacteria of organic rice (isolates); roots (32); stems (9), and leaves (12) were obtained (Table 1). The results in this study found that endophytic bacteria was highest in roots. Our results are in agreement with Mano et al. (2008) found that the most number of endophytic bacteria was greatest in the rice roots. Ma et al. (2013) observed that the bacterial diversity in roots reed Phragmites australis was significantly higher than in the leaves. Petcharat and Duangpang (2012) isolated endophytic bacteria from various rice tissue of different three types of rice farm; 1 year, 3 years organic rice, and conventional rice farms in Thailand. They found that seventy-one isolates of endophytic bacteria were screened using PDA and TSA medium. The majority of strains isolated from root tissues were totally 26 isolates, exclusively collected from 3 years organic rice farm. Previous researches have reported that endophytic bacteria from root and stem of rice tissues of diverse varieties grown in different soil types (Stoltzfus et al., 1997). Huang (1986) described that endophytic have been considered to originate from the outside environment and enter the plant through stomata, lenticles, wounds, areas of emergence of lateral roots and germinating radicles. The capability of endophytic bacteria ascending migration from root to leaf of the rice seedlings was probably due to its ability in producing the plant-cell wall degrading enzymes endopolygalacturonase and endoglucanase. These enzymes play an important role in promoting colonization and ascending migration of endophytes from roots to leaves of the plant hosts. (Tharek et al., 2011). The root exudates produced by rice plants promote the interaction between endophytic bacteria and root tissues (Jiménez et al., 2003).

3.2 Screening of IAA producing endophytic bacteria

In this study, 53 isolates endophytic bacteria were screened among which two isolates showed positive test. Among all endophytic bacteria isolates, the isolates RRSPCA from roots and LRSPCA2 from leaves of organic red jasmine rice showed red color reaction with Kovac’s reagent indicating their ability to produce IAA. These isolates were selected for further investigated the quantitative of IAA production.
Table 1 Isolation of endophytic bacteria from organic rice on various kinds of culture media

<table>
<thead>
<tr>
<th>Culture media</th>
<th>Roots</th>
<th>Stems</th>
<th>Leaves</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>PVK</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>TSA</td>
<td>11</td>
<td>4</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>ISP2</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>32</td>
<td>9</td>
<td>12</td>
<td>53</td>
</tr>
</tbody>
</table>

3.3 Quantitative analysis of IAA production of endophytic bacteria

The result of quantitative analysis of IAA production of isolates RRSPCA and LRSPCA2 were 20.93 and 7.12 mg/L, respectively (Table 2). Among the endophytic bacteria, active isolates RRSPCA and LRSPCA2 showed positive reactions to Salkowski’s reagent with a pinkish or a deep red coloration (Fett et al. 1987). These positive reactions of test bacteria indicate their capacity of metabolizing L-tryptophane to IAA or some analogous compounds of IAA. Bacteria RRSPCA and LRSPCA2 could produce IAA 20.93 and 7.12 mg/L, respectively. Petcharit and Duangpang (2012) reported that endophytic bacteria Bacillus sp. which isolated from Thai organic rice produced IAA 14.58 μg/ml. Bandara et al. (2006) found that endophytic bacteria isolated from rice also produced IAA with variable quantity. Moreover, Hung et al. (2004) found that endophytic bacteria from soybean produced IAA over than 25 Pg/ml and endophytic bacteria R7 from rice could produce IAA 120.55 ppm (Sev et al., 2016). Therefore, in the present study bacteria RRSPCA and LRSPCA2 were further identified.

Table 2 IAA production of endophytic bacteria isolated from organic rice

<table>
<thead>
<tr>
<th>Bacterial code</th>
<th>Source</th>
<th>IAA content (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRSPCA</td>
<td>Rice roots</td>
<td>20.93</td>
</tr>
<tr>
<td>LRSPCA2</td>
<td>Rice leaves</td>
<td>7.12</td>
</tr>
</tbody>
</table>

3.4 Identification of selected endophytic bacteria

Selected isolates endophytic bacteria RRSPCA and LRSPCA2 were examined cultural, morphological and biochemical characteristics. It was found that bacterial RRSPA had creamy white colony, circular, entire, flat colony and LRSPCA2 had yellow pigmented colony, circular, entire and raise colony on Nutrient agar. Bacteria RRSPCA and LRSPCA2 colonies were shown in Figure 1A and 1B, respectively. Both selected bacteria RRSPCA and LRSPCA2 were gram-negative, rods shape and appeared in single cell. Catalase and oxidase of both isolates were positive. The types of carbohydrates which are utilized by these bacteria can serve as a diagnostic tool for the identification of bacteria. Isolate RRSPCA fermented only glucose whereas LRSPCA2 not fermented various kinds of sugar in this test. The characteristic of those bacterium is given in Table 3.

In order to identify RRSPCA and LRSPCA2, these isolates were subjected to 16S rRNA amplification and sequencing. The sequence analyses revealed that two selected bacteria belong to Pseudomonas sp. and Chryseobacterium kwangyangense at 100% similarity, respectively. The similarities with the closest type strain are shown in Table 4. Barrios et al. (2018) studied bacterial microbiota of rice roots by 16S rRNA-based taxonomic profiling of endophytic and rhizospheric diversity. They found that IAA producing endophytic bacteria from rice root were Bacillus sp., Rhizobium sp., Delftia sp., Serratia sp., Aeromonas sp. and Pseudomonas sp. Pseudomonas sp. has been reported to be among the most abundant members of the rice endophytic bacteria (Mano et al., 2008; Sessitsch et al., 2012.).

There are many application and benefit of endophytic bacteria such as promote plant and act as biocontrol agents producing a range of natural products that could be harnessed for potential use in medicine, agriculture or industry including biotechnological applications. Devi et al. (2017) found that endophytic Pseudomonas aeruginosa isolated from leaves of Achyranthes aspera had plant growth
Table 3 Characteristics of selected endophytic bacteria RRSPCA and LRSPCA2

<table>
<thead>
<tr>
<th>Bacterial code</th>
<th>Cultural characteristic</th>
<th>Morphological characteristic</th>
<th>Biochemical and physiological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Catalase</td>
</tr>
<tr>
<td>RRSPCA</td>
<td>CC: creamy white</td>
<td>Gram negative Rods, single</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CF: circular</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CM: entire</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CE: flat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRSPCA2</td>
<td>CC: yellow</td>
<td>Gram negative Rods, single</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CF: circular</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CM: entire</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CE: raised</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Remark: CC = colony color, CF = colony form, CM = colony margin, CE = colony elevation, F = fructose, S = sucrose, M = maltose, G = glucose, Ga = galactose, X = xylitol, L = lactose, M = manitol

![Image A) RRSPCA](image1.png) ![Image B) LRSPCA2](image2.png)

Figure 1 Colony of selected IAA producing endophytic bacteria on Nutrient agar for 24 h.

Table 4 Identification of selected endophytic bacteria by 16S rRNA genes sequencing

<table>
<thead>
<tr>
<th>Bacterial code</th>
<th>Bacteria</th>
<th>Accession number</th>
<th>Query Cover</th>
<th>Identities</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRSPCA</td>
<td><em>Pseudomonas</em> sp.</td>
<td>abKU312801.1</td>
<td>100%</td>
<td>801/801 (100%)</td>
</tr>
<tr>
<td>LRSPCA2</td>
<td><em>Chryseobacterium kwangyangense</em></td>
<td>abEU196201.1</td>
<td>100%</td>
<td>800/800 (100%)</td>
</tr>
</tbody>
</table>

stimulating attributes including siderophore and indole acetic acid release, inorganic phosphate solubilization, along with nitrogenase, ammonification, and protease activities. It also exhibited anti-fungal property against *Rhizoctonia solani*. The enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase expressing endophyte *Pseudomonas* sp. enhances NaCl stress tolerance by reducing stress-related ethylene production, resulting in improved growth, photosynthetic performance, and ionic balance in tomato plants (Win et al., 2018). Susilowati et al. (2018) found that IAA producing bacteria *Pseudomonas fragi*, *Bacillus cereus* and *Rhizobium* can promote plant height, while *Bacillus aerius*, *Pseudomonas fragi* and *Bacillus cereus* promote dry weight of rice grain, and *Bacillus amyloliqufaciens* promote roots dry weight. *Pseudomonas putida* was found to promote root and shoot growth, while protecting the plants against the phytotoxic effects of phanathrene which environmental contaminants such as polycyclic aromatic hydrocarbons (Khan et al., 2014). Joshi et al. (2018) reported endophytic bacteria *Enterobacter* sp., *Pseudomonas* sp. and *Azospirillum* sp. that isolated from *Ocimum sanctum* and *Aloe vera* roots could produce enzymes urease, pectinase, cellulase, catalase, lipase, casienase, gelatinase and chitinase. In recent years, co-inoculation of
Endophytic microorganisms are playing key role for improving nutrient availability in sustainable agriculture production system. Jeong et al. (2016) suggested that the combination of several plant growth promoting bacteria could be more effective than individual strains as a horticultural product. Lally et al. (2017) reported application of endophytic Pseudomonas fluorescens and a bacterial consortium to Brassica napus can increase plant height and biomass under greenhouse and field conditions. They demonstrated that significant increases in crop height, stem/leaf, and pod biomass, particularly, in the case of the consortium inoculated treatment. Pragash et al. (2009) reported that Chryseobacterium aquaticum produces an antifungal protease, plant growth promoting enzymes such as ACC deaminase and phosphatase. Bacteria could be applied for plant growth promotion and biocontrol of fungal diseases. The synergistic interaction between ACC deaminase and both plant and IAA producing bacteria promoted plant growth, protect plants against flooding, drought, salt, flower wilting, metals, organic contaminants, and both bacterial and fungal pathogens (Glick, 2014). Radzki et al. (2013) reported that siderophores from strain Chryseobacterium sp. C138 are effective in supplying Fe to iron-starved tomato plants by the roots. Naik et al. (2009) found that colonization rates of endophytic microorganisms from rice Oryza sativa tissues were 40.3% in roots and 25.83% in leaves during winter season, 20.15% in roots and 8.66% in leaves during summer season. Chaetomium globosum, P. chrysogenum and Streptomyces sp. are suitable candidates for extraction biologically active compounds. Moreover, endophytic microorganisms have antagonistic properties against fungal pathogens. Domenech et al. (2006) reported the combination of bacteria Bacillus subtilis (a growth-promoting agent), B. amyloliquefaciens (an inducer of systemic resistance) and chitosan, B. licheniformis, Pseudomonas fluorescens and Chryseobacterium balustinum with BioControl LS213. They found that bacteria would have a synergistic effect on growth promotion and biocontrol on tomato and pepper against Fusarium wilt and Rhizoctonia damping off.

The combination of microorganisms gives better results probably due to the different mechanisms used. The selected IAA producing endophytic bacteria in this study might be use as environmentally friendly biofertilizers in microbial consortium and applied to organic agriculture for sustainable agriculture similar to previous report. There are many opinions on what an ideal agricultural system. Many would also agree that organic agriculture system should be maintained and improved human health, be economically and spiritually beneficial to both producers and consumers, actively preserve and protect the environment, be self-contained and regenerative, and produce enough food for world’s population (Higa, 1991).

4. CONCLUSION

Fifty-three isolates of IAA endophytic bacteria were obtained. Two endophytic bacteria RRSPCA and LRSPCA2 produced IAA production in medium containing 0.2 % tryptophan at 20.93 and 7.12 mg/L, respectively. These endophytic bacteria identified as Pseudomonas sp. and Chryseobacterium kwangyangense, respectively, based on 16s rRNA gene sequencing. The results can be used selected IAA producing endophytic bacteria for production some bioactive compound which high value added of biotechnologically including has potentially lead to making organic farming more environmentally sustainable in the future.

ACKNOWLEDGMENT

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REFERENCES


**Data Extraction for Pig Weight Prediction Model**

*Khin Dagon Win¹, Kikuhito Kawasue¹, Hsu Lai Wai¹, Kumiko Yoshida² (1. University of Miyazaki(Japan), 2. KOYO Plant Service(Japan))

**Keywords:** Weight estimation, Machine learning, 3D information, Random Forest, Multiple slits

Recently, automatic pig sorting systems have been popular to manage pigs in some pig farms. This systems automatically select pigs with appropriate weight for delivery. Normally, the pigs with over 115 kg are delivered in Japan. Therefore, this weight estimation system is essential to determine the maturity of pigs for shipment. A load cell is generally used in automatic sorting systems. However, it takes over 20 seconds to measure weight to detect stable weight. Sawdust is often used in pig house, but it can be attached to load cell and can lead to mechanic errors. Therefore, the use of load cell becomes big challenges to apply in actual pig farms.

To overcome problems of load cell, we have developed an automatic pig weight measurement system using a camera. This system is composed on a camera, multiple slits and random dots projector. The camera with band-pass filter captures the pig image which enters into the system without influence on external luminous. Random dots and multiple slits are simultaneously projected to the pig body. Random dots projector is used to detect the location of pigs in the system and multiple slits projector is used to measure 3-dimensional shapes of pig body. Random dots projector is simultaneously projected to cover the whole surface of multiple slits. This measurement device is set up at the top of the system to detect back shape of pig body because the back shape can hold the definite growth conditions of pigs without being influenced by their daily nourishment levels. The image processing based weight estimation system consists of 3 steps: Extraction of pig from capture image, Quantitative analysis of the pig size from extracted image, Weight estimation from pig size using machine learning algorithm. Sawdust is often used in pig house. Moreover, those sawdust can be attached to a pig body. These attached sawdust can be influenced on extraction process of pig from captured images. In our system, Fast Fourier Transform (FFT) is applied to extract the pigs without being influenced by the surface situations of pig body. FFT detects the displacement of random dots to judge of existence of pigs in measurement area. 2-dimensional pig size information can be established with silhouette pig image. Furthermore, 3-dimensional pig size information is also considered to observe more specific growth conditions of pigs. For 3-dimensional information, it is needed to process slits image which are projected on pig body. Each slit location is detected to perform in the triangulations and 3D information such as length, girth and height are calculated. The adequate selection from 2D & 3D information to estimate the pig weight is important and difficult process for our system. Therefore, Random Forest algorithm is utilized in our system. Random Forest randomly selects the samples from datasets and splits the data into several trees according to their features importance. The estimated weights are resulted by majority voting of its several trees. This method is adequate for pig weight estimation on practical conditions. The experimental results show the usefulness of our pig weight estimation system for automatic sorting system.
The path followed by a subsoiler attached to a hexagonal wheeled power tiller was studied. Many researchers have reported a significant reduction in draft force and an improved tillage quality when the performance of oscillated tillage tools was compared with rigidly fixed tillage tools. However, these improvements usually come with drastically increased engine power use and fuel consumption. Developing the oscillatory motion without significantly increasing the engine power use is therefore the focus of this research. A model subsoiler was fabricated and attached to a power tiller. The tiller wheels were replaced with 200 mm regular hexagonal wheels made of perforated steel and having a width of 200 mm. To have an understanding of the workings of the subsoiler, the path followed by the tip of the subsoiler was measured at two speeds of 0.037 m/s and 0.140 m/s. An ultrasonic sensor which was rigidly fixed above but independent of the power tiller was used to measure the vertical displacements made by the subsoiler as it travels in the soil bin. A graph of the height of the subsoiler versus time was thus plotted. The results show that the path followed by the subsoiler as it travels laterally at both speeds was sinusoidal or oscillatory in the vertical direction. The amplitudes for both speeds were approximately the same, but the frequency increased with increase in speed. It was also observed that the tip of the subsoiler moved downward through uncut soil suggesting that the effort at reducing power consumption with the investigated wheel configuration may not be as successful as expected.
Proposal of temperature control technology in pot cultivation for the citrus fruits

*Ryuta IBUKI¹, Yoshimichi Yamashita², Sachie Horii², Norihiro Hoshi², Madoka Chiba¹ (1. Miyagi University(Japan), 2. National Agriculture and Food Research Organization(Japan))

Keywords: pot cultivation, thermal management

Disaster area of Fukushima restarted farming mainly by the large-scale rice production corporation by farmland accumulation and the flower farmer using pipe house, which have little concern about reputational damage. For effective use of pipe house, there is a need for new crops that can be grown in pipe house at times other than floriculture and rice seedlings. With this situation as the background, we focused on pot cultivation. It has been considered to cultivate ‘citrus fruits’, which is cultivated in warmer regions under meteorological conditions by cultivation using pots, outdoors in summer and in a pipe house in winter. In addition to alleviating the northern limit of temperature-based cultivation, we are exploring new thermal management techniques for the pot cultivation environment. A difference was observed in the condition depending on the presence or absence of the whole covering sheet on the ‘citrus fruits’ (e.g. ‘Citrus sphaerocarpa’, etc.) in pot placed in the pipe house from 2017 to 2018, and the plant growth was good at the tree with the covering. Then, from 2018 to 2019, we investigated the thermal effect of the covering.

‘Citrus sudachi’ grown in pots (diameter 385 mm, depth 310 mm, black soil and pumice in the bottom of pots) was wintered, and the temperature and heat transfer conditions in the cultivation environment were compared for the presence or absence of the covering. The leaf surface temperature with an infrared radiation thermometer and the 10 cm depth soil temperature with a T-type thermocouple were examined during the winter (February 4 to March 4, 2019). The lowest, average and the highest (T_{min}, T_{avr}, T_{max}) were surveyed, with leaf temperatures of (-7 °C, 7 °C, 38 °C) in the covering tree, (-6 °C, 8 °C, 41 °C) in the control area, with the soil temperature (1 °C, 11 °C, 29 °C) under the covering tree, and (1 °C, 12 °C, 33 °C) in the control. From this, it was found that the cover texture contributes to the suppression of the high temperature of 3 to 4 °C during the day rather than the heat retention effect at night. In addition, we also investigated the time-dependent change of the temperature distribution of the soil in the pot placed in the pipe house from February 4 to February 26, 2019. The soil temperatures in pot at the inner side of the south sidewall, the center and the inner side of the north wall were measured at intervals of 10 minutes using a T-type thermocouple for a depth of 2 cm, 10 cm and 20 cm. The inner side of the south wall surface is the hottest and the maximum value on a fine day is extremely high, showing 50 to 60 °C. On the other hand, the daily maximum value of the pot center 10 cm deep showed a value 20 to 30 °C lower than that of the south side wall surface. Also, the time to reach the maximum temperature at the point showed a delay of about 3 hours as compared with the wall surface. During the period, the soil temperature changes at the center of the pot is delayed while the air temperature goes up with the sunrise during the daytime. According to Konakahara(1975), due to strong winds and physiological changes in the tree, low land temperatures in the land-planted ‘Citrus Unshiu’ inhibit water supply from the roots, and the amount of transpiration exceeds water supply, resulting in poor water balance in the tree. The balance tends to occur, the decrease of the water content in the leaves becomes remarkable, and quantitatively the effect starts to be seen at the soil temperature of 10 °C or less, and the effect becomes remarkable at that of 5 °C or less. In the measurement, the time when the central soil temperature exceeded 5 °C was after 11:00, and the time exceeding 10 °C was after noon. On the other hand, the temperature difference between the air and the center of the pot was

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maximum in the morning and was 20 to 30 °C. This is consistent with the case of Konakahara, and suggests the need to manage the ground temperature and temperature difference, taking into consideration the high temperature of the daytime inside the house even in winter. Based on these results, we considered that more sophisticated control of temperature distribution and heat transfer in the pot throughout the year will contribute to the improvement of productivity in pot cultivation. For example, the water content of the soil, which affects the thermal conductivity, is considered to have a large effect, and measurements were performed to understand the change in the water content in the pot. This work was conducted under “A Scheme to Revitalize Agriculture and Fisheries in Disaster Area through Deploying Highly Advanced Technology” by the Ministry of Agriculture, Forestry and Fisheries, Japan.
Proposal of Temperature Control Technology in Pot Cultivation for the Citrus Fruits

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ABSTRACT

In this study we investigated thermal environment condition of the pot cultivation in pipe house during winter for future development of novel thermal management system on pot cultivation. Disaster area of Fukushima restarted farming and the flower farmer using pipe house, which have little concern about reputational damage. For effective use of pipe house, there is a need for new crops that can be grown in pipe house. Cultivation using pots was planned and effective thermal management was start to be considered. A whole covering that is used as a simple method to protect plants from cold damage was evaluated its heat retention effect by temperature measurement of leaf and cultivating soil of ‘Citrus sudachi’ in the winter during 2018 to 2019. The lowest, average and the highest (Tmin, Ta, Tmax) were surveyed, with leaf temperatures of (-7 °C, 7 °C, 38 °C) in the covering tree, (-6 °C, 8 °C, 41 °C) in the control area, with the soil temperature (1 °C, 11 °C, 29 °C) under the covering tree, and (1 °C, 12 °C, 33 °C) in the control. Also, temperature, heat flux, net radiation and moisture distribution in and around pot soil was measured. Temperature difference of cold soil and hot air in the pipe house of early morning was observed and warned about water balance in plant. Heat flux decreasing in soil near south wall was observed and it was considered to be influenced by moisture content of soil. Although plastic pots are lighter and more durable than pottery pots, we consider it necessary to devise thermal management. In the winter months, it is necessary to warm the soil in the morning to maintain a healthy water balance of the plants. Oppositely, it is necessary to have a device that does not overheat the soil during winter daytime or summer season.

Keywords: Pot cultivation, Thermal management

1. INTRODUCTION

Disaster area of Fukushima restarted farming mainly by the large-scale rice production corporation by farmland accumulation and the flower farmer using pipe house, which have little concern about reputational damage. For effective use of pipe house, there is a need for new crops that can be grown in pipe house at times other than floriculture and rice seedlings. With this situation as the background, we focused on pot cultivation. It has been considered to cultivate ‘citrus fruits’, which is cultivated in warmer regions under meteorological conditions by cultivation using pots, outdoors in summer and in a pipe house in winter. In addition to alleviating the northern limit of temperature-based cultivation, we are exploring new thermal management techniques for the pot cultivation environment. In this study we investigated thermal environment condition of the pot cultivation in pipe house during winter for future development of novel thermal management system on pot cultivation.

2. MATERIALS AND METHODS

Firstly measurement of heat retention effect of the whole covering on pot cultivating ‘Citrus sudachi’ was carried out. Then measurement of pot soil circumstances, temperature and water distribution was carried out to grasp heat transfer in pot.
2.1 Experiment on Heat Retention Effect of Covering
From 2017 to 2018, we considered optimizing the cultivation environment of ‘citrus fruits’ (e.g. ‘Citrus sphaerocarpa’, etc.) in the pipe house by using a whole covering that is used as a simple method to protect plants from cold damage. Breathable polypropylene sheet is generally used as the covering. A difference was observed in the condition depending on the presence or absence of the whole covering sheet on the ‘citrus fruits’ in pot placed in the pipe house, and the plant growth was good at the tree with the covering. Then, from 2018 to 2019, we investigated the thermal effect of the covering. ‘Citrus sudachi’ grown in pots (diameter 385 mm, depth 310 mm, black soil and pumice in the bottom of pots) was wintered, and the temperature and heat transfer conditions in the cultivation environment were compared for the presence or absence of the covering. The leaf surface temperature with an infrared radiation thermometer and the 10 cm depth soil temperature with a T-type thermocouple were examined during the winter during February 4 to March 4 in 2019. Air temperature was measured with forced convection.

2.2 Experiment on Temperature and Water Distribution in Pot Soil
Because the temperature distribution in the pot is affected by solar radiation, the change in temperature due to the azimuth is not uniform. Iizuka (1956) was measured the time-dependent change of temperature distribution about the soil in the some types of pottery pot. Since pottery pots are heavy in workability, we tested using a practical plastic pot. Okamoto and Yanagawa (2013) told that unlike ground planting, roots grow in a limited space, we must be aware of the growth conditions such as nutrients, moisture, and temperature, which are the environment of the rhizosphere. They measured soil temperature under flowers cultivated condition with some types of pot including plastic pot and reported about directional soil temperature difference near pot surface. The pot wall surface and the soil surface is irradiated with solar radiation and the amount of evaporation of water is larger than that in the deep part. The thermal conductivity of water is higher than the thermal conductivity of air, and the dried soil with reduced water content has lower thermal conductivity (Datta, 2002). Since the thermal conductivity of the soil is related to the warming of the soil and the heat retention at night, it is important information in temperature control of the pot to grasp the state.

2.2.1 Pot Soil Temperature Measurement
The temporal change of the temperature distribution of black soil in the plastic pots in the pipe house was also investigated from February 4 to February 26, 2019. Figure 1 show the measurement setup. The temperatures at the inner side of the south sidewall (TC3, 6 and 9), the center (TC2, 5 and 8) and the inner side of the north wall (TC1, 4 and 7) were measured at intervals of 10 minutes using a T-type thermocouple for a depth of 2 cm, 10 cm and 20 cm.
2.2.2 Measurement on Heat Flux and Net Radiation
Two net radiation sensors (CPR-NR-LITE, Kipp & Zonen) were placed on soil surface and southern side of the pot wall and also three heat flux sensor were placed in the pot soil as shown in fig. 1. Pyranometer (PCM-01N, PLEDE) was used to measure solar irradiation.

2.2.3 Measurement on Water Content Distribution and Time Dependent Variation
The water content of the soil affects the thermal conductivity was assumed to have a large effect in our experiment and measurements were carried out to understand the water content distribution in the pot and its time dependent change. Several pots of soil were placed in the pipe house. The each pots were collected different few days and the moisture content was measured by vertical layer and location. Also, black soil with different water content was prepared in a beaker, and thermal conductivity was measured for each soil by the thermal probe method. Thermal properties analyzer, DECAGON KD-2, was used for measurement. We compared water content and thermal conductivity then we considered about heat transfer in pot.

3. RESULTS AND DISCUSSION
Heat retention effect of covering was considered from results of leaf temperatures and soil temperatures. Soil temperatures were more influenced than leaf temperatures. Then thermal circumstances of pot soil was measured in detail.

3.1 Test of Covering on Heat Retention Effect
Figure. 2 shows the average temperature, the minimum temperature, and the maximum temperature obtained from the time-dependent change data of leaf surface temperature and soil temperature in the pot which had grown "Citrus sudachi" from 2018 to 2019 over winter. The lowest, average and the highest (Tmin, Tn, Tmax) were surveyed, with leaf temperatures of (-7 °C, 7 °C, 38 °C) in the covering tree, (-6 °C, 8 °C, 41 °C) in the control area, with the soil temperature (1 °C, 11 °C, 29 °C) under the covering tree, and (1 °C, 12 °C, 33 °C) in the control. From this, it was found that the cover texture contributes to the suppression of the high temperature of 3 to 4 °C during the day rather than the heat retention effect at night.
Figure 2 Leaf temperature of "Citrus sudachi" and growing Pot soil temperature of minimum, average and maximum in the measurement period from February 4 to March 4, 2019, which compares the presence or absence of a covering.

3.2 Test of Pot Soil Circumstance
Circumstance of the pot soil, temperature distribution, heat flux, net radiation and water content were measured.

3.2.1 Soil Temperature Variation
Figure 3 shows the change over time in the temperature distribution of middle depth when only the soil was put in the pot for the entire measurement period. The inner side of the south wall surface is the hottest and the maximum value on a fine day is extremely high, showing 50 to 60 °C. It is concerned that such high temperatures near the walls would affect root damage. Figure 4 shows representative day data of temperatures when only soil was put in the pot and the time-dependent change in temperature distribution was measured. South side wall had maximum temperature in the pot. Okamoto et al. reported the higher temperature trend at the south wall and west wall. The daily maximum value of the pot center 10 cm deep showed a value 20 to 30 °C lower than that of the south side wall surface. Also, the time to reach the maximum temperature at the point showed a delay of about 3 hours as compared with the wall surface. During the period, the soil temperature changes at the center of the pot is delayed while the air temperature goes up with the sunrise during the daytime. According to Konakahara (1975), due to strong winds and physiological changes in the tree, low land temperatures in the land-planted ‘Citrus Unshiu’ inhibit water supply from the roots, and the amount of transpiration exceeds water supply, resulting in poor water balance in the tree. The balance tends to occur, the decrease of the water content in the leaves becomes remarkable, and quantitatively the effect starts to be seen at the soil temperature of 10 °C or less, and the effect becomes remarkable at that of 5 °C or less. In the measurement, the time when the central soil temperature exceeded 5 °C was after 11:00, and the time exceeding 10 °C was after noon. On the other hand, the temperature difference between the air and the center of the pot was maximum in the morning and was 20 to 30 °C. This is consistent with the case of Konakahara, and suggests the need to manage the ground temperature and temperature difference, taking into consideration the high temperature of the daytime inside the house even in winter. Based on these results, we considered that more sophisticated control of temperature distribution and heat transfer in the pot throughout the year will contribute to the improvement of productivity in pot cultivation.
Figure. 3 Time dependent change data of temperature in the middle depth of the pot soil.

Figure. 4 Time dependent change in temperatures distribution when only soil was put in the pot on Feb 14, 2019.
3.2.2 Heat Flux and Net Radiation around Pot

Figure 5 shows time dependent change data of solar irradiation and heat flux in pot soil at three points, soil surface, south wall and north wall. Compared to time dependent change of solar irradiation, that of heat flux at south wall showed tendency to decrease day by day. It could be assumed that soil touching with south wall was dried and thermal conductivity near south wall was degreased compared to that near north wall and that near soil surface, because south wall had extremely heated as show in figure 4.

Figure 6 shows time dependent change data of net radiation around pot. Intensity of net radiation of pot side was 25% smaller at daytime of fine day and 75% larger at night than that of pot top.

![Figure 5 Time dependent change data of solar irradiation and heat flux in pot soil from Feb.4 to Mar. 4 in 2019.](image1)

![Figure 6 Time dependent change data of net radiation around pot from Feb.4 to Mar. 4 in 2019.](image2)
3.2.3 Moisture Content in Pot Soil

Figure 7 shows time dependent water content in pot soil. Uniform water content in pot was measured before Feb. 13. Figure 8 shows water content vs thermal conductivity of black soil between 5% and 35% of water content. Large difference of thermal conductivity was measured between 25% and 30% of water content. Compared to Figure 6, significant change of thermal conductivity relating on heat transfer might be happen after Feb. 18. However, heat flux at south wall showed significant decrease on Feb. 7. Therefore, the water content of soil we measured was not directly influenced the heat flux at south wall in Fig. 5. We considered that pot soil was dried by inner wall surface of the pot and thermal resistance on this boundary was increased on early stage of the measurement. Although plastic pots are lighter and more durable than pottery pots, we consider it necessary to devise thermal management. In the winter months, it is necessary to warm the soil in the morning to maintain a healthy water balance of the plants. Oppositely, it is necessary to have a device that does not overheat the soil during winter daytime or summer season.

Figure 7 Time dependent change of water content distribution in middle layer of pot soil

Figure 8 Water content vs thermal conductivity of black soil

4. CONCLUSION

Initial test measurements were carried out on the thermal management of citrus grown in pots.
The effect of the whole covering on pot cultivated ‘citrus fruits’ in pipe house had an effect on suppressing temperature rise during the daytime. Temperature difference between pot soil and air, lower temperature of soil and higher temperature of air, was observed in winter morning which causes unbalance of transpiration and water supply from roots was concerned.

ACKNOWLEDGMENT
This work was conducted under "A Scheme to Revitalize Agriculture and Fisheries in Disaster Area through Deploying Highly Advanced Technology" by the Ministry of Agriculture, Forestry and Fisheries, Japan.

REFERENCES
Investigation by Driving Simulation of Tractor Overturning Accidents Caused by Steering Instability

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Keywords: Tractor, Farm accident, Driving simulator, Overturning, Lateral slippage

Overturning tractors are the leading cause of fatalities on farms. Steering instability contributes significantly to the tractor overturning. This study investigated tractor overturning accidents caused by the steering instability using a driving simulator. The general commercial driving simulator CarSim® (Mechanical Simulation Cooperation, MI, USA) was used. Tractor operations on steep passage slopes were simulated to mimic conditions present for a real accident case reported in Japan. Simulations were performed on roads with and without slopes. The tractor overturned only when on the road with the steep slope. The decrease in the vertical force on the front wheel caused the steering instability and the tractor to overturn. The steering instability caused understeer which prevents the operator from being able to control the tractor properly. Subsequently, the tractor overturned in the simulation. The tractor driving simulator was capable of reproducing the steering instability which can lead to the overturning accident.
Investigation by Driving Simulation of Tractor Overturning Accidents Caused by Steering Instability

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ABSTRACT

Overturning tractors are the leading cause of fatalities on farms. Steering instability contributes significantly to the tractor overturning. This study investigated tractor overturning accidents caused by the steering instability using a driving simulator. The general commercial driving simulator CarSim® (Mechanical Simulation Cooperation, MI, USA) was used. Tractor operations on steep passage slopes were simulated to mimic conditions present for a real accident case reported in Japan. Simulations were performed on roads with and without slopes. The tractor overturned only when on the road with the steep slope. The decrease in the vertical force on the front wheel caused the steering instability and the tractor to overturn. The steering instability caused understeer which prevents the operator from being able to control the tractor properly. Subsequently, the tractor overturned in the simulation. The tractor driving simulator was capable of reproducing the steering instability which can lead to the overturning accident.

Keywords: Tractor Farm accident Driving simulator Overturning Steering instability

1. INTRODUCTION

There are approximately 400 fatal farm accidents each year in Japan. Accidents involving agricultural tractors are a major contributor to farm fatalities. In 2016, 115 of the total 312 fatal farm accidents were tractor-related (Ministry of Agriculture, Fishery, and Forestry, 2018). More specifically, the tractor overturning is the leading cause of fatalities with 53 cases in 2016.

In Japan, small tractors specially designed for paddy fields are used in harsh environments such as rough farm roads, steep passage slopes, and narrow inclined side paths. This dangerous terrain can lead to a decrease in the vertical force on the front wheel. In some cases, this can result in separation of the front wheel from the underlying ground. This phenomenon causes vertical bouncing and lateral slippage of the tractor, both of which can lead to steering instability and overturning. The impact dynamics induced by the bouncing dramatically deteriorate tractor stability (Sakai, 1999; Sakai et al, 2000; Watanabe & Sakai, 2019a). If in addition to the bouncing slippage of the wheels occurs, the operator will not be able to maintain full control of the tractor. Consequently, the quality of the tractor posture dramatically decreases.

Several studies have contributed to the development of the tractor driving simulator and its application to farm safety and automation research (Gonzalez et al., 2017; Han et al., 2019; Watanabe & Sakai, 2019b). The tractor driving simulator is a strong platform for accident prevention research. The aim of the present paper is to apply the tractor driving simulator to investigation of overturning accidents induced by steering instability. A general driving simulator called CarSim® (Mechanical Simulation Cooperation, MI, USA) was used as a platform for the tractor driving simulator. Simulations of tractor operation on steep passage slopes were conducted. A real accident case reported in Japan was used as the basis for these simulations.
2. MATERIALS AND METHODS
The configuration of the tractor driving simulator is presented. CarSim® 2016 version was employed for the driving simulator. Vehicle and road configuration can be input by the user. Table 1 shows the tractor parameters used.

Table 1 Tractor parameter specification.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of tractor body</td>
<td>788</td>
<td>kg</td>
</tr>
<tr>
<td>Mass of wheels</td>
<td>200</td>
<td>kg</td>
</tr>
<tr>
<td>Pitch moment of inertia</td>
<td>700</td>
<td>kg m²</td>
</tr>
<tr>
<td>Distance between center of gravity of tractor body and front wheel</td>
<td>0.7</td>
<td>m</td>
</tr>
<tr>
<td>Distance between center of gravity of tractor body and rear wheel</td>
<td>0.64</td>
<td>m</td>
</tr>
</tbody>
</table>

The road surface of the steep passage slope (on which the real accident case occurred) was recreated in the tractor driving simulator. According to the survey conducted by the Japanese Association of Rural Medicine, the tractor overturning accident happened on a steep passage slope of 19° gradient and 0.7 m in height (JARM, 2013). The tractor moved onto the passage slope from the farm field to the farm road and tried to turn right on the road to move into another farm field. However, the tractor was not able to turn and fell from the road. The road surface and scenario were configured in the driving simulator. To investigate the influence of the steep slope on the steering instability, two different types of the road surface were compared. Namely, with slope and without slope. Figure 1a and b shows the road surface with slope and without slope, respectively.

Figure 1 (a) Road surface with a slope; (b) Road surface without a slope.

Figure 2 shows the road profile of the slope.
3. RESULTS AND DISCUSSION

The velocity of the tractor was set to 4.3 m/s in the simulation. The tractor was ran on the road with slope and without slope. Figure 3 shows the tractor trajectories on the road in each simulation.

The tractor remained in contact with the road during the whole simulation when the tractor ran on the road without slope. In contrast, the tractor ran off the road and then overturned when the tractor ran on
the road with slope. To visualize the numerical results, Figure 4 and 5 show the animation of the driving simulation for the simulation without slope and with slope, respectively.

Figure 4 Animation of the tractor operation on the road without slope. (a) Tractor moved onto the corner; (b) Tractor ran on the corner; (c) Tractor was on the edge of the road; (d) Tractor continued to run without overturning.
Figure 5 Animation of the tractor operation on the road with slope. (a) Tractor moved onto the slope; (b) Tractor ran on the slope; (c) The wheels went off the road; (d) Tractor overturning occurred.

Figure 6a and b show the vertical force on the front wheel and the cornering force on the front wheel, and the road elevation and the steering angle of the operator, respectively.
Figure 6 (a) The vertical force and the cornering force on the front wheel; (b) Road elevation and the steering angle of the operator.

When the front wheel of the tractor moved onto the slope, vibrations were induced and the vertical force on the front wheel decreased to zero as the road elevation increased. This caused the cornering force to be zero. Consequently, the operator cannot maintain control of the tractor and steering instability occurred. The steering instability caused understeer of the tractor and overturning. The results indicated that the tractor driving simulator could reproduce the steering instability which can lead to overturning.

4. CONCLUSION
The simulations of the tractor operations on the steep passage slope were conducted using the tractor driving simulator. Tractor overturning occurred in the simulation due to the steering instability. Future research will investigate how to avoid overturning by steering and develop accident prevention control for the overturning.

ACKNOWLEDGMENT
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REFERENCES


Classification of Salinity Damaged Spring Potato (Solanum tuberosum) using Hyperspectral Imagery based on Decision Tree Classifier

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Keywords: Hyperspectral imagery, Potato, Salinity, Decision tree, Classification accuracy

Salinity which is detected on reclaimed land is a major obstacle factor to crop growth. Currently, salinity is determined by experts directly examining the salinity of water and soil on farmland suspected of salinity. However, if salinity can be identified in real time and non-destructive way on the vast landfills, it can quickly respond to salinity to ensure stable cultivation. Accordingly, the objective of this paper is to verify the possibility of saline determination of non-destructively spring potatoes (Solanum tuberosum) through decision tree classifier using hyperspectral imagery of spring potatoes. In each vegetative period (VP), root formative period (RFP) and root growing period (RGP), the potatoes deal with treatment of normal watering, no-watering(drought) and salinity watering. The hyperspectral imagery of the treated potatoes was acquired at every midday. Individual potatoes canopies in hyperspectral imagery were extracted by a spectral imagery processing software (ENVI 4.7, Exeils Visual Information Solution Inc., USA). Reflectance data in the extracted canopies areas was used to classify each treatment. Calculated classification accuracy was evaluated by overall accuracy (OA) and kappa coefficient (KC). As a result, in all growth stage and treatment, the Rpart shows the highest classification accuracy. In particular, the classification accuracy was the highest between treatments OA 93.3% and KC 87.3% in the RFP that highly absorbs the moisture, and the lowest below OA 90.5% and KC 82.7% in the VP. As a classification of normal, drought and salinity using hypersepectral imagery, it showed that the possibility of salinity is different with spring potatoes in all the growth stage and it is also judged that these results can be applied as important basic results for further research to qualify and quantify salinity.

Classification for Fire Blight Disease Infection Area using Vegetation Index and Background Segmentation based on Multispectral Image

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Keywords: Multispectral image, Point Cloud, Fire Blight, Vegetation index, Pear tree

Fire Blight (FB) is a bacterial virus called erwinia amylovora. The disease enters the flower or wounded area of the fruit tree, turning leaves and branches brown or black, and dies within one year. Leaves and branches dead by natural wind or pruning also fall into the orchard soil and become brown, similar to FB infection. In the aerial image for the FB discrimination of a wide orchard, there are naturally cut leaf and branches in addition to the desired FB area, which interferes with the FB discrimination.

In this study, we used the digital surface model (DSM) and vegetation index to remove unwanted areas and try to classify the FB infection area. The study area will be located on orchard A at Dokjeong-ri, Ipjang-myeon Cheonan-si, Chungcheongnam-do, Republic of Korea (36°22'42.0224"N, 127°22'70.6734"E) on June 7,
2018, and on June 20, it will be an orchard B at the National Institute of Horticultural & Herbal Science Pear Research Institute, Naju, Jeollanam-do, Republic of Korea (35°01'27.9912”N, 126°44'53.0412”E). Study equipment Unmanned aerial vehicles (UAVs) equipped with multispectral image sensors were used to acquire pear infection and non-infection multispectral images from two orchards. The acquired images were removed by using DSM generated by using the point cloud technique of Drone mapping software (Pix4D 4.3.31, Pix4D SA, Swiss) and GIS software (ArcGIS 10.5.1, Esri, USA), and the images were matched. The images were classified by FB area using vegetation index maps converted to spectral image software (ENVI 5.3, Exelis Visual Information, USA). Drone mapping software and GIS software were used to remove the background height of 100cm from the surface considering the FB area. As a result, an area of about 2,780 m² has been reduced to about 778 m². The area of the FB-infected area was estimated using the histogram and reflection values for the FB-infected and non-infected areas in the background-removed image. When histograms were used, the area of expected FB infection area was 142m² when Otsu's method was used at the NIR wavelength. When using the reflection values, a significant difference was found in the histograms of the red-red edge region and the red-NIR region, and only the overlapping regions were extracted by dividing the regions by Otsu’s method. As a result, the estimated area of FB infection was reduced to 71m². As a result, removing the 100-cm-high background and then slinging certain areas of the reflection value could reduce the area of the FB-infected area the most.
The Static Load Test for Tractor Attached Three-Point Hitch Type Dynamometer

Hyo-Geol Kim, Sung-Bo Shim, Yeon-Soo Kim, Young-Joo Kim, Sang-Dae Lee (1. Korea Institute of Industrial Technology(Korea), 2. Gyeongsang National University(Korea))

Keywords: Static load test, Three-point hitch, Tractor dynamometer, Traction force, Six-component force

Due to the mechanization of agriculture and the aging of the countryside, the use of tractors and tractor machines is increasing. The tractor generates a force between the tractor and the implement, which depends on the soil properties and moisture content. The tractor travels and generates traction force, generates vertical force for maintains the position of the implement, and creates lateral forces by rolling and soil surfaces. It also interacts with the soil and causes moment in the same direction. These forces act as stresses on the tractor and the implement, causing fatigue damage and fatigue failure on the frames and components. Accurately measuring the force generated during tractor operation can predict vulnerable parts and residual life of the tractor and machine. In this study, we developed a three-point hitch type dynamometer that can accurately measure these forces, and formulated a formula for calculating the force with the geometry of the load cell attached to the three-point hitch type dynamometer. The developed dynamometer measures six components force with a single axis load cell combination and measures the PTO torque with a strain gage and telemetry system. In addition, a static load test was conducted to verify the validity of the dynamometer. Static load tests showed an accuracy of 97% or more over the entire range, from 98.9% in the traction force direction, 99.2% in the vertical force direction and 97.4% in the lateral force direction. The accuracy of the traction direction moment was 98.2%, the vertical direction moment was 97.3%, and the lateral direction moment was 96.8%, which is more than 96% accurate in all moment sections. Therefore, the formula used in the experiment is more than 96% accurate, and the reliability of the dynamometer is more than 96%. In future studies, we will establish and verify the improved formula considering the transportation pitch caused by the three-point hitch moving.
The Static Load Test for Tractor Attached Three-Point Hitch Type Dynamometer

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ABSTRACT
Due to the mechanization of agriculture and the aging of the countryside, the use of tractors and tractor machines is increasing. The tractor generates a force between the tractor and the implement, which depends on the soil properties and moisture content. The tractor travels and generates traction force, generates vertical force for maintains the position of the implement, and creates lateral forces by rolling and soil surfaces. It also interacts with the soil and causes moment in the same direction. These forces act as stresses on the tractor and the implement, causing fatigue damage and fatigue failure on the frames and components. Accurately measuring the force generated during tractor operation can predict vulnerable parts and residual life of the tractor and machine. In this study, we developed a three-point hitch type dynamometer that can accurately measure these forces, and formulated a formula for calculating the force with the geometry of the load cell attached to the three-point hitch type dynamometer. The developed dynamometer measures six components force with a single axis load cell combination and measures the PTO torque with a strain gage and telemetry system. In addition, a static load test was conducted to verify the validity of the dynamometer. Static load tests showed an accuracy of 97% or more over the entire range, from 98.9% in the traction force direction, 99.2% in the vertical force direction and 97.4% in the lateral force direction. The accuracy of the traction direction moment was 98.2%, the vertical direction moment was 97.3%, and the lateral direction moment was 96.8%, which is more than 96% accurate in all moment sections. Therefore, the formula used in the experiment is more than 96% accurate, and the reliability of the dynamometer is more than 96%. In future studies, we will establish and verify the improved formula considering the transportation pitch caused by the three-point hitch moving.

Keywords: Static load test, Three-point hitch, Tractor dynamometer, Traction force, Six-component force

1. INTRODUCTION
Recently, as agriculture becomes mechanized and agriculture workforce ages, the use of tractors and tractor attaching implement is increasing. When a tractor is working, a force is generated between the tractor and the implement, and this force acts as a stress on the tractor and the implement. Therefore, these forces affect the fatigue and residual life of the tractor and the implement parts, and the reliability and durability of the tractor and the implement can be evaluated if these forces can be accurately measured. The Wismer-Luth et al. (1974) and Brixius (1987) model are used to predict the traction force and are the ASABE standard test method, but only the traction force is calculated and no other forces are obtained. Because it is also a predictive model, it is more inaccurate than the measured value. Therefore, the most accurate value can be obtained by directly attaching the dynamometer. Al-Jalil et al. (2001) developed an inverted U-shaped dynamometer mounted on a three-point hitch using a strain gauge. Kim
et al. (2017) performed a static load test of a dynamometer, and predicted the residual life of the combined implement. However, only two directions force and one direction moment among six directions were tested and no static load test was performed for three directions. By using a three-point hitch-mounted dynamometer, you can get the most realistic data. However, because the dynamometer is made up of six single-axis load cells, you can get more inaccurate data if the formula is not accurate or the calibration is not accurate. In this study, we formulated a formula to derive three directional forces and moments using the geometric elements of the load cell combination. In addition, a static load test was carried out using a hydraulic actuator and a surface plate to apply force to six directions and confirm that they match the formula.

2. MATERIALS AND METHODS
2.1 Three-Point Hitch Type Dynamometer

The dynamometer is based on the Category I tractor specified in ISO Standard 730-1. Three tractor connection points are hard points, and three implement connection points are soft points. The dynamometer is connected via six single-axis load cells, and the load cell is connected to the rod end with limited spherical joint constraints at both ends. The upper hitch point connection of the implement can be adjusted by the up and down hinge hole and the lower hitch point connection is adjustable by the left-right hinge hole. The center of the dynamometer has a space for the universal joint for power connection. The dynamometer configuration is shown in Fig. 1 and the load cell specifications used in dynamometer configuration are shown in Table 1.

![Dynamometer 3D Modeling](image)

**Figure 1. Dynamometer 3D Modeling.**

<table>
<thead>
<tr>
<th>Table 1. Specification of Load Cell.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model name</strong></td>
</tr>
<tr>
<td>Rated capacity (kgf)</td>
</tr>
<tr>
<td>Accuracy rating</td>
</tr>
<tr>
<td>Combined error (%)</td>
</tr>
<tr>
<td>Creep (half hour, %)</td>
</tr>
<tr>
<td>Proper input voltage (V)</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Maximum input voltage (V)</td>
</tr>
<tr>
<td>Temperature compensation range (°C)</td>
</tr>
<tr>
<td>Allowable temperature range (°C)</td>
</tr>
<tr>
<td>Materials and Painting</td>
</tr>
</tbody>
</table>

The three-point hitch-type dynamometer is equipped with a telemetry system using a strain gauge and a wireless transmit-receive. The strain measured at the strain gage is transmitted at the transmitter in the form of strain ratio, and the receiver interlocks with the DAQ (Data Acquisition) system and converts it into torque. The strain gage and transmitter are attached to the universal joint for power connection of the tractor and the implement. The system can measure the torque of implement that is operating with PTO (Power Take Off) power.

### 2.2 Dynamometer Component Force-Moment Equation

#### 2.2.1 Component Force Equation

The force components in three directions are defined as shown in Fig 2. The traction force is defined as the sum of the pulling force direction load cell \( F_a, F_b, F_c \). The vertical force is defined as the sum of the vertical component force of \( F_d \) and \( F_e \). The lateral force is defined as the sum of the lateral component of \( F_d \) and \( F_e \) and \( F_f \). The six load cells mounted on the dynamometer measure the force in each direction. \( F_a, F_b, \) and \( F_c \) detect only force in the traction direction, and \( F_d \) and \( F_e \) detect both vertical and lateral forces. \( F_f \) detects only lateral force. The angle \( \theta \) that determines the vertical-lateral force is determined by the load cell mounting angle.

\[
Traction \ Force \ P_T = F_a + F_b + F_c \quad (1)
\]

![Figure 2. Force Diagram of Dynamometer](image)

(a) : Traction Force, (b) : Vertical Force, (c) : Lateral Force.

Therefore, when the center of a triangle is defined as a reference coordinate, the force in three directions is as shown in eq. (1), (2), (3).
Vertical Force $P_V = F_d \sin \theta + F_e \sin \theta$  \hspace{1cm} (2)

Lateral Force $P_H = F_d \cos \theta - F_e \cos \theta - F_f$  \hspace{1cm} (3)

### 2.2.2 Moment Force Equation

The Moment forces in three directions are defined as shown in Fig 3. The moment force is calculated by the moment balance equation when looking at the dynamometer in the 3-axis direction. The traction direction moment is calculated as the moment balance equation when viewed from the front view of the dynamometer. The vertical force moment is calculated as the moment balance equation when viewed from the top view. The lateral force moment is calculated as the moment balance equation when viewed from the side view.

![Moment Force Diagram of Dynamometer](image)

The length of the moment arm is determined by the geometry of the dynamometer. The beta value is the same on both sides, and the delta value is also the same. Moment balance equation formulated using force and moment arm length is shown in Eq. (4), (5) and (6). The moment force is positive in the clockwise direction and negative in the counterclockwise direction.

Traction Moment $M_T = (F_d \sin \theta \beta) + (F_d \cos \theta \alpha) - (F_e \sin \theta \beta) - (F_e \cos \theta \alpha) + (F_f \gamma)$  \hspace{1cm} (4)

Vertical Moment $M_V = (F_b \delta) - (F_c \delta)$  \hspace{1cm} (5)

Lateral Moment $M_H = \zeta(F_b + F_c) - (F_a \epsilon)$  \hspace{1cm} (6)

### 2.3 Data Collection System
Fig 4 shows the data collection diagram of six load cells. Six load cells are connected to the data acquisition device through the Wheatstone bridge, and the data acquisition device matches the IP with the PC and Ethernet cable and collects the data.

Fig 5 shows the data collection diagram of PTO torque telemetry. The strain gauge is connected to the telemetry transmitter through the normal wire, and the transmitter and the receiver are connected by BLUETOOTH. The receiver is connected to the DAQ by the I/O cable, and finally the strain is converted to torque in the program.

2.4 Test Method
1. Fix the dynamometer hard point (tractor connection side) to the jig and place it on the surface plate.
2. Apply force as shown in Table 2. At this time, the force is set to be a peak at 90 seconds.
3. Each value calculated by the formula is measured and compared with the value calculated by the actual force.

Table 2. Hydraulic Actuator Force Magnitude

<table>
<thead>
<tr>
<th></th>
<th>Applied Force (kgf)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Top</td>
</tr>
<tr>
<td>Traction Force</td>
<td>500</td>
</tr>
<tr>
<td>Vertical Force</td>
<td>500</td>
</tr>
<tr>
<td>Lateral Force</td>
<td>250</td>
</tr>
<tr>
<td>Traction Moment</td>
<td>500</td>
</tr>
<tr>
<td>Vertical Moment</td>
<td>500</td>
</tr>
<tr>
<td>Lateral Moment</td>
<td>500</td>
</tr>
</tbody>
</table>
3. RESULTS AND DISCUSSION

Figure 6 and Table 3 show the measured forces when the force of Table 2 is applied. The force is multiplied by the gravity acceleration g to represent the Newton unit system. The moment force is calculated by multiplying the vertical distance between the hydraulic actuator and the center of the dynamometer.

Table 3. Applied Force and Measured Force.

<table>
<thead>
<tr>
<th>Applied Force (kN)</th>
<th>Measured Force (kN)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traction Force</td>
<td>14.709</td>
<td>14.551</td>
</tr>
<tr>
<td>Lateral Force</td>
<td>4.903</td>
<td>5.032</td>
</tr>
<tr>
<td>Traction Moment</td>
<td>3.912</td>
<td>3.983</td>
</tr>
<tr>
<td>Vertical Moment</td>
<td>3.912</td>
<td>3.807</td>
</tr>
<tr>
<td>Lateral Moment</td>
<td>2.535</td>
<td>2.619</td>
</tr>
</tbody>
</table>

As a result of the experiment, the accuracy of 96% or more was obtained in all the sections and satisfactory reliability was secured. The small errors in the component force tests are expected to be due to manufacturing error and misalignment when joining the surface plates. The small error of the moment force test is expected to be the measurement error of the manufacturing error and the moment arm length. The maximum accuracy of 99.2% and the minimum accuracy of 96.8% were calculated for the whole test period, and the dynamometer is considered reliable even considering the error rate.

4. CONCLUSION

This study was conducted to verify the reliability of three-point hitch type dynamometer and static test was conducted to verify reliability. The conclusion of this study is as follows.

1) Since the dynamometer is composed of a single axis load cell combination, the three direction force of traction force - vertical force - lateral force is calculated by the component force equation.
2) Likewise, the traction moment - vertical moment - lateral moment is also calculated by the moment balance equation.

3) The forces calculated by the component force equation are compared with the forces actually applied by the hydraulic actuator. As a result, the accuracy of 99.2% and 96.8% was obtained.

4) A small amount of error is expected to be due to manufacturing errors, surface plate mounting error, moment arm length measurement error, and the dynamometer is considered reliable.

ACKNOWLEDGMENT
This work was supported by the Technology Innovation Program (or Industrial Strategic Technology Development Program (KM190022, Development of an autonomous sprayer suitable for atypical road surface of an actual orchard) funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea)

REFERENCES
Isolation and Identification of Acetic Acid Bacteria from Philippine Fermented Rice Cake Batters by 16S rRNA Gene Sequence Analysis

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Keywords: Acetic acid bacteria, fermented rice cake, 16S rRNA sequence analysis, phylogenetics

As part of our work to study the microflora of Philippine traditional fermented foods, batters from fermented rice cakes, or puto in the vernacular, from different parts of the Philippines were sampled and used for the isolation, screening and purification of acetic acid bacteria (AAB) by culture-based methods. Pure AAB isolates were then identified by DNA-based methods [i.e., cetyl trimethylammonium bromide (CTAB) DNA extraction, polymerase chain reaction (PCR), and 16S rRNA gene sequence analysis], DNA base composition determination, phenotypic characterization, and phylogenetic analysis. Six isolates were obtained from three types of rice cake batter: puto Calasiao, puto Lanson, and puto Boac batters. The AAB isolates were identified to belong to the genera Acetobacter at 94-99% homology with DNA base compositions ranging from 54.40-55.74 mol% GC content. The isolates were Gram-negative, catalase-positive rods that oxidize ethanol to acetic acid and grow in mannitol agar and in most sugars. None of them were cellulose producer or motile. 02CPPu1-2 produced a water-soluble brown pigment in glucose-yeast extract-peptone (GYP) medium and 24BMTa2-3 yielded γ-pyrones from D-glucose. From the phylogenetic tree deduced from the 16S rRNA gene sequence analysis results, the isolates clearly formed an independent clade distinct from the type strains of other genera of acetic acid bacteria. The puto Lanson and puto Boac batter isolates were closely related to A. pasteurianus and A. lovaniensis, respectively. On the other hand, the puto Calasiao isolates were associated with none of the type species of AAB. Overall, our data suggest that the fermented rice cake batter isolates comprise a possibly new species of acetic acid bacteria under the genus Acetobacter. This is very interesting considering that all the isolates were sourced from batters of only traditionally fermented rice cakes. DNA-DNA hybridization and detailed phenotypic characterization are recommended to verify this new species possibility, which may be linked the difference in geographical location, raw material and processing technique employed in traditional rice cake making in the Philippines.
Isolation and Identification of Acetic Acid Bacteria Isolates from Philippine Fermented Rice Cake Batters by 16S rRNA Gene Sequence Analysis

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ABSTRACT

As part of our work to study the microflora of Philippine traditional fermented foods, batters from fermented rice cakes, or puto in the vernacular, from different parts of the Philippines were sampled and used for the isolation, screening and purification of acetic acid bacteria (AAB) by culture-based methods. Pure AAB isolates were then identified by DNA-based methods [i.e., cetyl trimethylammonium bromide (CTAB) DNA extraction, polymerase chain reaction (PCR), and 16S rRNA gene sequence analysis], DNA base composition determination, phenotypic characterization, and phylogenetic analysis. Six isolates were obtained from three types of rice cake batter: puto Calasiao, puto Lanson, and puto Boac batters. The AAB isolates were identified to belong to the genera Acetobacter at 94-99% homology with DNA base compositions ranging from 54.40-55.74 mol% GC content. The isolates were Gram-negative, catalase-positive rods that oxidize ethanol to acetic acid and grow in mannitol agar and in most sugars. None of them were cellulose producer or motile. 02CPPu1-2 produced a water-soluble brown pigment in glucose-yeast extract-peptone (GYP) medium and 24BMTa2-3 yielded γ-pyrones from D-glucose. From the phylogenetic tree deduced from the 16S rRNA gene sequence analysis results, the isolates clearly formed an independent clade distinct from the type strains of other genera of acetic acid bacteria. The puto Lanson and puto Boac batter isolates were closely related to A. pasteurianus and A. lovaniensis, respectively. On the other hand, the puto Calasiao isolates were associated with none of the type species of AAB. Overall, our data suggest that the fermented rice cake batter isolates comprise a possibly newspecies of acetic acid bacteria under the genus Acetobacter. This is very interesting considering that all the isolates were sourced from batters of only traditionally fermented rice cakes. DNA-DNA hybridization and detailed phenotypic characterization are recommended to verify this new species possibility, which may be linked the difference in geographical location, raw material and processing technique employed in traditional rice cake making in the Philippines.

Keywords: Acetic acid bacteria, fermented rice cake, puto, 16S rRNA sequence analysis, CTAB method, DNA extraction, phylogenetics

1. INTRODUCTION

The Philippines, a tropical country in Southeast Asia, is recognized as one of the centers of microbial diversity. This is in part due to the various traditional methods of food preservation in the country. An archipelago of 7107 islands, it has different food preservation methods that vary among islands, and hence a wide array of fermented foods (Sanchez, 2008) consumed throughout the archipelago, which are part and parcel of our culture (Banaay et al., 2013). However, it is common knowledge that the market of traditional Philippine fermented foods has always remained local, for instance, domestic for bagoong, patis, alamang, and suka, regional for some such as buro and tuba, and even provincial for some such as etag, pindang, bahalina, and native longanisa (i.e., longanisang Vigan and Lucban). In fact, a few of our traditional fermented foods such as sabeng and tengba never reach the market, their production only linked to festivities that celebrate the richness or sanctity of our indigenous cultures.
This is unfortunate considering that the top 5 most consumed food items in the US are all fermented foods, namely, beer, bread, cheese, wine and fermented meat in descending order. Moreover, with the trend in globalization, a few Asian fermented food products have already seeped through the changing Western culinary landscape, with soy sauce now considered the most used condiment not only in Asia but also in the world, while a few such as kimchi and fish sauce are slowly finding their niche in international cuisines. Thus said, there is clearly an explosive market that awaits other fermented foods in the future with the increasing population and intensifying food insecurity across the globe. It is therefore vital that active up-to-date research on traditional Philippine fermented foods be carefully carried out with the purpose of making them at par, in terms of quality and product image and design, with fermented foods of other countries that have reached global commercialization. Note that, in recent years, traditional fermented foods have become increasingly relevant not only because of their guaranteed safety, high nutritional quality or unique sensory profile, but also because of their potential huge market contribution, as proved by the global probiotic market projected to reach USD 46.6 billion by 2020, with Europe as the largest and Asia-Pacific region as the fastest-growing markets (Elegado et al., 2016).

Currently, there is a dearth of solid statistics concerning our traditional fermented foods, most likely linked to the limited market and consumer research on them. Nonetheless, it is unequivocal that our traditional fermented foods can stand up to those of other countries in terms of flavor, nutritional value and health benefits. It is only the lack of consistent quality and use of non-standardized and unhygienic manufacturing processes that have relegated our food products to their local status and inferior image. In addition, since most of the traditional food fermentation industries in the Philippines are rural, seasonal, labor-intensive, informal, and capital-deficient, their supply remains much too limited to establish a huge market such that their market and ultimately their consumption remain confined only to places where they are produced. Also, most producers of our traditional fermented foods are local farmers, fisherfolks, and housewives who are poor and capital-deficient, which logically dictates the choice of the least expensive methods of production (even if these methods are non-GMP-compliant and non-HACCP-certified) as well as understandably highlight the need for easy money turn-over (vending “unripe” products), which often result in compromised product qualities and products that do not reach their full bloom. Lastly, there is extremely limited scientific and technological knowledge about our traditional fermented food products, particularly about their microbial and biochemical aspects because of the lack of research institutes passionate for, dedicated to, and fully equipped for research and development of our traditional fermented food products. (Only large private food industries are technologically equipped for food science research, most of which however neither prioritize nor sense the importance of our traditional food products.) This is in large contrast to the comprehensive research knowledge on wine and cheese in France, on balsamic vinegar in Italy, on soy sauce in Japan, and on kimchi in Korea, which perhaps explain why these fermented food products, unlike ours, command global respect, as these products are continuously being researched and polished to perfection. These might as well be the reasons behind the meager research on Philippine traditional fermented food products, as well as behind the insignificant contribution of the fermentation food industry to our gross domestic products compared with that of the agricultural industry. But the crux of the matter is this: without research on our fermented foods, there will be no improvement in them; without improvement, there will be no increase in their market share; without an increase in market share, there will be no research attention on them. Therefore, it is crucial that extensive research on Philippine fermented foods be carefully performed if such food products are to infiltrate the global food market. This could be started through the use of genotypic methods side by side with biomolecular analyses in conducting an in-depth accurate analysis of the fermenting microflora (including unculturable microorganisms) of these fermented foods.

Thus, as part of our comprehensive research on Philippine fermented foods, in this study, we isolated, screened, purified and identified acetic acid bacteria (AAB) from batters of traditionally fermented rice cakes by culture-based methods and molecular methods. We focused on acetic acid bacteria in previous works, lactic acid bacteria (e.g., Leuconostoc mesenteroides, Streptococcus faecalis, and Lactobacillus plantarum) and yeasts (e.g. Saccharomyces cerevisiae) have already been isolated from fermented rice cake batters; these microorganisms are expected as they are commonly associated with cereal fermentation (Kelly, Asmundson, Harrison, & Huang, 1995; Sanchez, 1999; Tamang et al., 2016; Uchimura, Garcia, & Flores, 1984). We consider it interesting to determine the proliferation of
other fermentative microorganisms such as acetic acid bacteria (AAB) in rice cake fermentation. Our objectives were to isolate, identify and characterize AAB from different Philippine traditional fermented rice cakes.

2. MATERIALS AND METHODS

2.1 Sampling
Fermenting batters from local fermented rice cakes were obtained on site or purchased from local producers and processed for AAB isolation by inoculating a loopful of each batter onto glucose-yeast extract-peptone (GYP) slants within 12 h of collection and incubating it at room temperature. The slants were kept in an ice box once growth had been observed.

2.2 Isolation, screening, purification, and storage
In the laboratory, 5 mL of sterile physiological saline solution (PSS) was added to each GYP slant with growth, and the cell culture was suspended by aseptically scraping it using a wire loop and then vortexing the mixture. Appropriate dilutions of the suspension were then spread-plate on GYP agar plates with CaCO₃ and then incubated at 30°C for 18-48 h. Colonies that formed a zone of clearing on the GYP agar plates were then individually transferred onto GYP slants and incubated as described above. The cultures were again suspended in PSS, and appropriate dilutions of the suspension were then streaked on GYP agar plates with CaCO₃ as acid production indicator. The plates were then incubated at 30°C for 18-48 h. After incubation, colonies with a zone of clearing were picked up and again transferred onto GYP slants. Resuspension and replating were repeated several times until visual and microscopic examinations of colonies and cells of each isolate showed homogeneous morphological characteristics. The pure isolates were then subjected to Gram staining by the Hucker method (Hucker & Conn, 1923) and to catalase test using the method of MacFaddin (2000). Only pure isolates from GYP agar that were Gram-negative and catalase-positive were presumed to be AAB and stored in glycerol solution.

2.3. DNA extraction
DNA was extracted from each AAB isolate using a modified cetyl trimethylammonium bromide (CTAB) DNA extraction protocol (Wilson, 1987). 5 mL of 24-h GYP broth culture at 30°C was centrifuged at 12,000 rpm for 45 s at room temperature. The cell pellet obtained was suspended in 200 µL of Tris-EDTA (TE) buffer (pH 8), to which 25 µL of 10% sodium dodecyl sulfate (SDS) and 5 µL of 25 mg mL⁻¹ proteinase K were added. The mixture was then incubated with gentle shaking at 37°C for 1 h. The resulting viscous lysate was added with 45 µL of 5 M sodium chloride (NaCl) and 40 µL of CTAB solution (10% CTAB in 0.7 M NaCl), and then with an equal volume of chloroform:isoamyl alcohol (24:1); this was left to stand for 10 min, centrifuged at 12,000 rpm for 10 min at room temperature, added with an equal volume of cold isopropanol, and mixed gently. The resulting mixture was centrifuged at 8,000 rpm for 5 min at 4°C, and the supernatant was decanted to obtain a DNA pellet, which was then washed with 1 mL of 70% ethanol by centrifugation at 12,000 rpm for 5 min at 4°C. The supernatant was discarded, and the remaining precipitate was air-dried for 5-10 min and redissolved in 100 µL of TE buffer. This DNA solution was subjected to spectrophotometry and agarose gel electrophoresis to confirm its purity and quality, respectively.

2.4. Polymerase chain reaction (PCR) amplification
PCR amplification was done based on the optimized protocol of Dalmacio et al. (2011), in which the V1-V8 region of the 16S rRNA gene was amplified using universal primers: 8F (5’ AGAGTTTGATCCTGGCTCAG 3’) and 1492R (5’ GGTACCTTGTTACGACTT 3’). The PCR reaction mixture (1x TE buffer, 0.5 U Taq polymerase, 0.3 µM each of the bacterial 8f and 1492r primers, 1.5 mM MgCl₂, 0.2 mM dNTP, ≥ 30 ng/µL template DNA, and nanopure water) was subjected to an optimized amplification program as follows: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, and elongation at 72°C for 1.75 min, and final elongation at 72°C for 5 min. The PCR products were subjected to electrophoresis on 1.0% (w/v) agarose gel with 0.5x Tris-acetate EDTA (TAE) buffer and visualized using ethidium bromide for confirmation of the desired length of 1.5 kb.

2.5. 16S rRNA gene sequencing
The amplified DNAs of the AAB isolates were sent to First Base Laboratories in Malaysia for 16S rRNA gene sequencing using the same primers mentioned above and determined of their identity
and % homology to type strains of different species of acetic acid bacteria using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.6. Determination of DNA base composition
DNA base composition expressed as mol% GC content was determined using an online GC calculator (http://www.endmemo.com/bio/gc.php).

2.7. Phenotypic characterization
Cell form was determined by growing AAB isolates on GYP agar. Unless otherwise stated, the isolates were incubated at 30°C for 18-48 h. The oxidation of ethanol to acetic acid as indicated by a zone of clearing after 2-3 days of incubation, and catalase production as indicated by evolution of gas were tested in GYP agar with CaCO₃. Motility was also determined by growing the isolates in soft GYP agar stabs. The formation of cellulose and a water-soluble brown pigment was examined by visual observation in GYP broth and agar cultures, respectively. The production of dark brown γ-pyrones from D-glucose and D-fructose was determined by adding FeCl₃ to 11-day broth cultures. Growth in mannitol agar and various sugars (i.e., D-glucose, D-fructose, D-xyllose, D-sucrose, D-galactose, D-sorbitol, D-maltose, and D-starch) in broth cultures was also determined.

2.8. Phylogenetic analysis
DNA sequences of the AAB isolates and type species of the 14 valid AAB genera (i.e., Acetobacter, Gluconobacter, Gluconoacetobacter, Ameyamaea, Tanticharoenia, Asaia, Swaminathania, Kozakia, Neosasaia, Granuliibacter, Acidimonas, Komagataeibacter, Saccharibacter, and Neokomagataea) (Mamlouk & Gullo, 2013) were subjected to multiple sequence alignment using CLUSTAL W and the neighbor-joining method (Saitou & Nei, 1987) with 1000 bootstrapping replicates (Felsenstein, 1985) to construct the phylogenetic tree (Nei & Kumar, 2000) using Mega X software (Kumar, Stecher, Li, Knyaz, & Tamura 2018).

3. RESULTS AND DISCUSSION

3.1. Sampling and isolation, screening and purification of AAB isolates
Samples of batter from four types of local fermented rice cakes (i.e., puto Calasiao from Calasiao, Pangasinan; puto Lumban from Lumban, Laguna; puto Lanson from Irosin, Sorsogon; and puto Boac from Boac, Marinduque) procured from their towns of production were used in AAB isolation. Initially, seven isolates with acid production ability in GYP agar were isolated. This number was whittled down to six aerobic, acid-producing, Gram-negative, catalase-positive, ellipsoidal to rod-shaped isolates after preliminary characterization and purification. The six isolates were sourced from puto Calasiao, puto Lanson, and puto Boac batters; no isolates were obtained from the puto Lumban batter.

The predominant microorganisms in fermented rice cakes include LAB and yeasts such as Leuconostoc mesenteroides, Streptococcus faecalis, Lactobacillus delbrueckii, Lactobacillus fermenti, Lactobacillus lactis, Pediococcus cerevisiae, Geotrichum candidum, Torulopsis holmii, Torulopsis candida and Trichospora pulbans, which have been isolated from idli, dosa and dhokla, varieties of steamed bread of rice and black gram (Phaseolus mungo) in India (Blandino et al., 2003), as well as Lactobacillus casei, Lactobacillus brevis, Leuconostoc mesenteroides and Saccharomyces cerevisiae, which are found in jeung-pyun, a sponge-like bread in Korea (Park et al., 2017). However, AAB have been shown to be in symbiotic relationship with LAB and yeasts in jiaozi, a traditional steamed bread in China. Li et al. (2016) have identified Acetobacter tropicalis (22.8%), together with Saccharomyces cerevisiae (42.9%), Pediococcus pentosaceus (38.6%), Wicherhamomyces anomalus (27.0%), Lactobacillus plantarum (24.3%), Saccharomyces fibuliger (22.2%), Torulopsis delbrueckii (7.9%), Enterococcus durans (5.7%), Bacillus cereus (2.9%), and Enterococcus faecium (1.4%) in jiaozi by combined culture-based method and PCR-DGGE analysis. One possible reason for AAB seemingly being the minor microflora in fermented rice cakes is their late proliferation in the fermenting batter, growing only after yeasts and LAB have already taken hold during fermentation. Thus, their growth is hindered by the predominance of these earlier colonizers of the fermenting batter, such that they only exist in very small numbers. At such small numbers, they are not generally isolated by traditional culture-based methods using common growth media, being classified as ‘VBNC’.

Another possible reason for the low AAB load in puto batter is linked to the inherent nature of AAB. AAB generally thrive in the natural environment (e.g., soil, herbs, fruit, and flowers) and a wide variety of fermentation substrates (Crotti et al., 2010) that are good sources of simple sugars, not
3.2. Molecular identification of AAB isolates

Through the alignment of their DNA nucleotide sequences to sequences in the BLAST database, the six potential AAB isolates were all confirmed to share 94-99% nucleotide sequence homologies to known species of acetic acid bacteria belonging to the genus *Acetobacter*. The four isolates of *puto* Calasiao showed >94% homologies with the following AAB species indicated: 02CPPu1-2 with *Acetobacter orientalis* (at 94% homology), 02CPPu2-1 with *Acetobacter persici* (at 95%) and both 02CPPu2-2 and 02CPPu3-1 with *Acetobacter malorum* (at 98 and 99%, respectively). The isolates from *puto* Lanson (12ISPu1-1) and *putong* Boac, on the other hand, were found to have 97 and 99% homologies with *Acetobacter pasteurianus* and *Acetobacter lovaniensis*, respectively.

The DNA base contents of the six AAB isolates ranged from 54.40 to 55.74 mol% GC content (Table 1), which fit the DNA base content range of the genus *Acetobacter*.

### Table 1. Phenotypic characteristics and DNA base composition of AAB isolates from local fermented cake batter.

<table>
<thead>
<tr>
<th>Test</th>
<th>Isolate Code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>02CPPu1-2</td>
</tr>
<tr>
<td>A. Cell form</td>
<td>short rods</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Oxidation of ethanol to acetic acid</td>
<td>+</td>
</tr>
<tr>
<td>C. Catalase test</td>
<td>+</td>
</tr>
<tr>
<td>D. Cellulose production</td>
<td>-</td>
</tr>
<tr>
<td>E. Formation of brown soluble pigment</td>
<td>+</td>
</tr>
<tr>
<td>F. Motility test</td>
<td>-</td>
</tr>
<tr>
<td>G. γ-pyrones from sugars</td>
<td></td>
</tr>
<tr>
<td>D-glucose</td>
<td>-</td>
</tr>
<tr>
<td>D-fructose</td>
<td>-</td>
</tr>
<tr>
<td>H. Growth in MYPA</td>
<td>+</td>
</tr>
<tr>
<td>I. Growth in sugars</td>
<td></td>
</tr>
<tr>
<td>D-glucose</td>
<td>+</td>
</tr>
<tr>
<td>D-fructose</td>
<td>+</td>
</tr>
<tr>
<td>D-xylose</td>
<td>+</td>
</tr>
<tr>
<td>D-sucrose</td>
<td>+</td>
</tr>
<tr>
<td>D-galactose</td>
<td>-</td>
</tr>
<tr>
<td>D-sorbitol</td>
<td>+</td>
</tr>
<tr>
<td>D-maltose</td>
<td>+</td>
</tr>
<tr>
<td>D-starch</td>
<td>+</td>
</tr>
<tr>
<td>J. G+C content (mol%)</td>
<td>55.03</td>
</tr>
</tbody>
</table>

Positive, +; negative, -, weak, (+)

In this study, the AAB isolates from the batters of three of the rice cakes sampled, namely, *puto* Calasiao, *puto* Lanson, and *puto* Boac, were found to belong to the genus *Acetobacter*, differently from those of the *puto* Lumban batter. According to Raspor and Goranovic (2008), *Acetobacter* strains...
prefer alcohol-enriched environments, which explain the presence of *Acetobacter* in the fermented rice cake batter samples. Note that all the batter samples were obtained in the late fermentation stage prior to steaming or baking, and hence fermentation by yeasts and/or lactic acid bacteria is almost complete, making the conditions in the fermenting batter supportive of AAB growth, that is, rich in alcohol as a result of the alcoholic fermentation by yeasts and with a slightly acidic pH of approximately 5.0 as a result the addition of lye, sugar and other flavoring ingredients, which bumped up the low batter pH of approximately 3.5 caused by lactic acid production by LAB. Furthermore, *Acetobacter* species have an optimum growth pH range of 5-6.5 (although they can grow even as low as pH 3-4) at 28-30 °C (Mamlouk & Gullo, 2013), the very same conditions present in local fermented rice cake batter.

### 3.3. Phylogenetic relationship among AAB isolates

From the phylogenetic tree (Fig. 1) constructed based on the alignment of 905 bp 16S rRNA gene sequences, all the AAB isolates from the local fermented rice cake batter samples distinctly clustered with the type strains of all known *Acetobacter* species, separate from the other type species of other AAB genera. Moreover, our isolates formed an independent clade together with *A. pasteurianus* and *A. lovaniensis*. 12ISPu1-1 and 24BMTa2-3 from *puto* Lanson and *puto* Boac batters corroborated their BLAST database homologies to *A. pasteurianus* and *A. lovaniensis*, respectively. On the other hand, all four isolates from *puto* Calasiao batter interestingly formed a tight-knit clade not associated with the AAB species to which they had high % homologies based on BLAST alignment results.

![Fig. 1. Phylogenetic relationships of AAB isolates from 16S rRNA gene sequence clustering. The tree was made based on an alignment of 905 bp of 16S rRNA gene sequences and constructed by neighbor-joining method. Numbers indicate the bootstrap percentage values derived from 1000 replications. Sequences used in this study are represented in sample codes. *Lactobacillus fermentum* strain 1 (GenBank accession number FJ462686.1) is used as an outgroup.](image-url)

Interestingly, despite the differences in preparation method, ingredients and geographic location of *puto* Calasiao, *puto* Lanson, and *puto* Boac, all the AAB species isolated from all three fermented rice cakes were of the same genus, *Acetobacter*. This suggests that similar microorganisms are at work in the fermentation of our local fermented rice cakes, regardless of type. This is evident in the deduced phylogenetic tree of the six isolates together with the type strains of all valid *Acetobacter* species and the type species of the other 13 valid AAB genera. Despite the homology data obtained from the nucleotide sequence alignment with the BLAST database suggesting the wide variety of species (i.e., *A. malorum*, *A. persici*, *A. tropicalis*, *A. pasteurianus*, and *A. lovaniensis*) responsible in rice cake
fermentation, results of the phylogenetic analysis indicate otherwise. All our six isolates formed a highly distinct clade, with the four isolates from *puto* Calasiao forming a clade that is entirely separate from all known valid *Acetobacter* species. Therefore, this strongly suggests that the four aforementioned isolates constitute a new species in the genus *Acetobacter*. Thus, it is important that DNA-DNA hybridization of the *puto* Calasiao isolates with all valid *Acetobacter* species as well as quinone analysis be conducted to confirm this possibility. If confirmed, it will be highly interesting to study why a highly specific AAB microflora is associated with Philippine rice cake fermentation. This could likely lead to hitherto unknown fermentation mechanisms by AAB that utilizes starch as substrate.

### 3.4. Phenotypic characteristics relevant to the acetification by AAB species

As shown in Table 1, all the AAB isolates are Gram-negative, catalase-positive rods. They oxidize ethanol to acetic acid. They also grow in mannitol agar and in most of the sugar sources, particularly starch. None of them are cellulose producer or motile. 02CPPu1-2 produces a brown water-soluble pigment in GYP medium and 24BMTa2-3 yields \(\gamma\)-pyrones from D-glucose.

Majority of the phenotypic characteristics of the isolates were reflective of the species indicated in the BLAST homology search. Their growth in mannitol agar confirmed their identity as *Acetobacter* utilizing mannitol as an energy source. 02CPPu1-2 was noted to produce a brown water-soluble pigment, similarly to a few *Acetobacter* species such as *A. polyoxogenes* isolated from vinegar broth (Entani et al., 1985) and *A. aurantius* now under genus *Frateuria* isolated from golden-rayed lily (*Lilium auratum* Lindl.) (Swings et al., 1980), as well as to *Gluconacetobacter liquefaciens* (Navarro and Komagata, 1997). 12ISPu1-1 was observed to ferment all representative sugars in the study but its homologous species *A. pasteurianus* prefers only glucose, mannitol and ethanol as carbon sources (Konig et al., 2009). 24BMTa2-3 produced \(\gamma\) pyrones from D-glucose. It was also the only isolate that did not ferment D-sucrose, exactly the same as its homologous species *A. lovaniensis* (Konig et al., 2009), unequivocally confirming its identity. Furthermore, note that all the isolates fermented D-starch, the major component of rice-based products. This characteristic is not typical of *Acetobacter* species (Sievers & Swings, 2015), a possible indication of the unique fermentation mechanism conducted by these rice cake batter isolates. More importantly, this ability to grow on starch provides a strong support to the possibility not just of a new species but perhaps also of a new genus.

AAB isolates are generally associated with dough acidification, which favors LAB growth, as well as with the production of enzymes and exopolysaccharides (such as levan) resulting in the hydrolysis of biochemical compounds and in the formation of the structural network of bread in the absence of gluten proteins in rice flour, respectively (Korakli et al., 2001; Tieking et al., 2003). However, the exact role of our isolates in fermented rice cake fermentation remains to be elucidated, what with the yet to be confirmed identity of the *puto* Calasiao. Further analyses (e.g., DNA-DNA hybridization, as mentioned earlier, and detailed phenotypic characterization). Likewise, microbial succession analysis using PCR-DGGE must be conducted to determine the fermentation mechanism wherein the VBNC state of AAB can also be resolved.

### 4. CONCLUSION

From this study, the similarity in the fermenting microflora isolated from batters of various Philippine fermented rice cakes in Luzon was highlighted. Although a wide variety of AAB species were identified by BLAST search analysis, namely, *A. malorum*, *A. orientalis*, *A. persici*, *A. pasteurianus* and *A. lovaniensis* from fermented rice cake batters from Pangasinan, Sorsogon, and Marinduque, results of phylogenetic analysis, indicated otherwise. The deduced phylogenetic tree showed that the isolates from the *puto* Calasiao batter formed a tight-knit clade completely separate from all known species of *Acetobacter* and the other 13 genera of AAB. This points to a hitherto undiscovered group of starch-fermenting *Acetobacter* strains that may perhaps constitute a new *Acetobacter* species, at least, if not an altogether novel genus in the family Acetobacteraceae.

**ACKNOWLEDGMENT**
We thank the UP OVPAA Balik Scientist Program for the financial support, and the UPLB Institute of Animal Science for granting us access to the equipment and facility used in the study.

REFERENCES


The increase of greenhouse gas is a cause of global warming, and the possible contributing to cause climate change locally and globally. The reduction of greenhouse gas emissions is an issue to be solved worldwide. In Japan, the aim of reducing greenhouse gas emissions was 26% by 2030 comparison with 2013. In 2015, agriculture contributed 34.8 Mt-CO₂e (2.5%) of Japan’s greenhouse gas emissions. Contributions for greenhouse gas emissions from agriculture are from livestock, soil and manure sources (methane and nitrous oxide). Furthermore, CO₂ emission from energy and material use at agricultural practices was contributed environmental impact. Furthermore, CO₂ emission from energy and material used at agricultural practices is contributed to environmental impact. Therefore, the evaluation of environmental aspects of agricultural production systems by life cycle thinking was required to reveal direct impacts as well as indirect impacts for considering mitigation measures. Several studies have been reported GHG emissions from beef production systems and pork production systems by the LCA in Japan. However, life cycle GHG emissions from broiler meat production systems and egg production systems have not been reported. The aim of this study was to assess life cycle GHG emissions from poultry farming systems of a broiler meat production system and an egg production system.

The system boundary and process model of poultry farming system of broiler meat and egg production include the feed production process, livestock management process and manure treatment process. The functional unit was defined as 1 kg of broiler meat and 1 kg of an egg at the evaluation of broiler meat and egg production system respectively.

The amount of agricultural material consumption data of the process model of the poultry farming system for life cycle inventory analysis were collected from statistical based data and reports. Most of the background data, such as GHG emission from fuel combustion and indirect GHG emission at agricultural materials, for inventory analysis were used the values from the database of the IDEA ver.2.2, which mostly represents Japanese production. The indirect GHG emissions associated with animal husbandry equipment, machinery, and poultry houses production processes were excluded from the system boundary. The GHG emissions were evaluated using GWP100 (CO₂:1, CH₄:34, N₂O: 298). The objective broiler production system utilized three of the two-story windowless poultry house and handled 4000 hundred birds (broilers) annually. The egg production system used two of windowless poultry house with two steps floor type for handled 630 hundred birds (layers) and produced 1100t eggs annually. The GHG emissions from the broiler meat production system was estimated to 3.12 [kg-CO₂e/kg-broiler meat]. The GHG emissions from the feed production process, fuel consumption of warming at the poultry management process and the manure treatment process were contributed to total GHG emissions from broiler meat production systems. The GHG emissions from the egg production system was 2.86 [kg-CO₂e/kg-egg]. The processes contributed to GHG emissions from egg production systems were the feed production process and the manure treatment process.
*Rasool Khan Amini¹, Yutaka Kitamura², Mito Kokawa², M. Z. Islam² (1. Graduate School of Life and Environmental Sciences, University of Tsukuba(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tennoda, Tsukuba, Ibaraki 305-8572, Japan(Japan))
11:30 AM - 12:30 PM

[6-1130-P-19] The Effect of Palm Oil Based Wax Coating on Delaying of Ripening and Reduce Senescence Spot of ‘ Khai’ Banana
*nutthachai pongprasert¹, Varit Srilaong¹,², Songsin Photchanachai¹,², Panida Boonyaritthongchai¹,², Kornkanok Aryusuk³ (1. Postharvest Technology Program, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bangkok 10140(Thailand), 2. Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400, Thailand(Thailand), 3. Biochemical Technology Program, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bangkok 10140(Thailand))
11:30 AM - 12:30 PM

[6-1130-P-20] Effects of Blanching Pretreatment on Drying Characteristics and Pectic States of Dried ‘ Fuyu’ Persimmon
*Tatsuya Oshima¹, Kodai Kato¹, Satoshi Iwamoto¹, Teppei Imaizumi¹ (1. Gifu University(Japan))
11:30 AM - 12:30 PM

[6-1130-P-21] Beverage Process Using By-product Water of the Production of Wash-free Rice as Raw Material and the Continuous Process of Lactic Acid Fermentation
*JIA FANG¹, Yutaka KITAMURA¹, Mito KOKAWA¹, Kazunobu KAJIHARA², Kozi KAWAKAMI², Hidenori MIZUNO² (1. Tsukuba Univ.(Japan), 2. Satake Corporation(Japan))
11:30 AM - 12:30 PM

[6-1130-P-22] Effect of roasting and storage on chemical compounds and sensory score of specialty coffee
*Yuri Koshima¹, Yutaka Kitamura¹, Mito Kokawa¹, Thais M.F.S Vieira², Juliana Antunes Gavalão², Luis Felipe de Freitas Fabricio², Md Zohurul Islam¹ (1. University of Tsukuba(Japan), 2. University of Sao Paulo(Brazil))
11:30 AM - 12:30 PM

[6-1130-P-23] Inverse Method Using Heat Transfer Simulation to Estimate Thermal Diffusivity of Agricultural Products
*Yoshiki Muramatsu¹, Masanori Hashiguchi², Eiichiro Sakaguchi¹, Shotaro Kawakami¹ (1. Tokyo University of Agriculture(Japan), 2. Keisoku Engineering System Co., Ltd.(Japan))
11:30 AM - 12:30 PM

[6-1130-P-24] Effect of Acid Type and Concentration on Properties of Pectin Extracted from Unripe Cavendish Banana Peel and Its Application in
Raspberry Jam
*Natthakan Rungraeng¹,², Supaluck Kraithong¹ (1. School of Agro-Industry, Mae Fah Luang University, Chiang Rai, Thailand 57100(Thailand), 2. Unit of Innovative Food Packaging and Biomaterials, Mae Fah Luang University, Chiang Rai, Thailand 57100(Thailand))
11:30 AM - 12:30 PM

[6-1130-P-25] Evaluation of color and flavor for shiitake mushroom dried using vacuum microwave treatment
*Daisuke Kurata¹, Takahiro Orikasa²,³, Shoji Koide² (1. Graduate School of Arts and Sciences, Iwate University.(Japan), 2. Faculty of Agriculture, Iwate University.(Japan), 3. Agri-Innovation Center, Iwate University.(Japan))
11:30 AM - 12:30 PM

[6-1130-P-26] The effect of molecular hydrogen on the shelf life of banana
*Naoya Fujino¹, Teruo Wada¹ (1. Osaka Prefecture University(Japan))
11:30 AM - 12:30 PM

[6-1130-P-27] The Potential of Biogas Production from Caribbean Seaweed Biomass
*Yuhendra AP¹, Mohamed Farghali¹, Takaki Yamashiro², Ryuichi Sakai³, Kazutaka Umetsu¹ (1. Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine(Japan), 2. Tokachi Agri Works(Japan), 3. Graduate School of Fisheries Sciences, Hokkaido University(Japan))
11:30 AM - 12:30 PM

[6-1130-P-28] Study on the Characteristics of Micro Wet Milling and Spray Drying of Sea-buckthorn (Hippophae rhamnoides)
*ODGEREL Ulziibat¹, Md.ZOHURUL ISLAM¹, KITAMURA Yutaka², KOKAWA Mito², ODBAYAR Tseyen-Oidov³, SOLONGO Ganbold³ (1. Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan(Japan), 3. School of Industrial Technology, Department of Food Engineering, Main Campus of MUST, Bag Toiruu 34, Sukhbaabatar District, Ulaanbaatar, Mongolia(Mongolia))
11:30 AM - 12:30 PM

[6-1130-P-29] Combined Effect of Pre-treatment and Vacuum Packaging for Maintaining the Quality of Peeled Shallot (Allium ascalonicum L.)
*Phanida Renumarn¹, Kranert Kilian Joachim ⁴, Natthaya Choosuk¹, Chanthima Phungamngoen², Kasama Chaareekhot³ (1. Department of Innovation and Product Development Technology, Faculty of Agro-Industry, King Mongkut’s University of Technology North Bangkok(Thailand), 2. Department of Agro-Industry Technology and Management, Faculty of Agro-Industry, King Mongkut’s University of Technology North Bangkok(Thailand), 3. Department of Food Science and Technology, Faculty of Technology, Udon Thani Rajabhat University(Thailand), 4. Food Science -Technology and Economics, University of Applied Sciences Bremerhaven(Germany))
11:30 AM - 12:30 PM

[6-1130-P-30] High pressure processing of ‘Nanglae’ pineapple juice: Quality preservation and shelf life extension
Nuntawan Chuensombat¹, Natthakan Rungraeng¹, Sutthiwal Setha¹,², *Phunsiri Suthiluk¹,²
11:30 AM - 12:30 PM
(1. School of Agro-Industry, Mae Fah Luang University, Chiang Rai, THAILAND(Thailand),
2. Research Group of Postharvest Technology, School of Agro-Industry, Mae Fah Luang University, Chaing Rai, THAILAND(Thailand))
11:30 AM - 12:30 PM
Optimization and Evaluating of Pomegranate Peel Extract by Micro Wet Milling Using Response Surface Methodology

*Rasool Khan Amini¹, Yutaka Kitamura², Mito Kokawa², M. Z. Islam² (1. Graduate School of Life and Environmental Sciences, University of Tsukuba(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tennoda, Tsukuba, Ibaraki 305-8572, Japan(Japan))

Keywords: Pomegranate peel extract, Antioxidant, Micro wet milling, phenolics, Response surface methodology

Recently, studies on Pomegranate peel discarded as waste has increased due to high phenolics and antioxidant content as well as its antimicrobial activities. In this study, the extraction method for Pomegranate Peel Extract (PPE) was developed and optimized using the Micro Wet Milling System (MWM). Response Surface Methodology (RSM) was used to determine the optimum condition for Phenolics and their Antioxidant and Antimicrobial activities. The effects of solid to solvent ratio ($X_1$: 10-30%), Ethanol and water ratio ($X_2$: 40-80%), feeding rate ($X_3$: 10-20 mL/min) and rotational speed ($X_4$: 20-50 rpm) on Particle Size, Antioxidant activities, Total Phenolic Content (TPC), Catechin content, Gallic Acid and Punicalagin were optimized using RSM. Scanning Electron Microscope (SEM) will be used to study the pomegranate peel cell structure disruption with MWM. Results suggest that Micro Wet Milling (MWM) can produce smaller (minimum 9m) Particle Size and can be used as a new method for pomegranate peel phenolic extraction. The solid to solvent ratio, ethanol percentage, feeding rate, and rotational speed has significant effects on the phenolics content as well as catechin content antioxidant activities. Further study will be conducted for optimization of phenolics and antimicrobial activities of pomegranate peel extract.
The Effect of Palm Oil Based Wax Coating on Delaying of Ripening and Reduce Senescence Spot of ‘Khai’ Banana

*nutchachai pongprasert*, Varit Srilaong\(^1,2\), Songsin Photchanachai\(^1,2\), Panida Boonyaritthongchaj\(^1,2\), Kornkanok Aryusuk\(^3\)  
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Keywords: Palm oil based wax, 'Khai' banana, Senescence spot, Ripening

The objective of this research was to study the effect of palm oil based wax coating on delaying of ripening and reduce senescence spot of ‘Khai’ banana. Banana fruits at mature green stage and ripening stage were coated with palm oil based wax. After coating, banana fruits were storage at 25°C, 70–75% relative humidity, for 8 days. while uncoated fruits served as a control. The result found that coatings of palm oil based wax delayed the changes in the weight loss percentage, and softening both mature green and ripening stage fruits compared to uncoated ones. The coated banana fruits also showed the lower ethylene production and respiration rate as compared to the control. In additions, the coatings of palm oil based wax reduced senescence spots incidence compared to the non-coated fruits. These results can be concluded that coating with palm oil based wax has the potential to delay the ripening and maintained the quality as well as reduce the senescence spotting of ‘Khai’ banana fruit.
Palm Oil based Wax Coating Maintained Postharvest Quality of Thai Lime cv. Paan Pichit#1

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ABSTRACT

Immature green lime fruit cv. Pann Pichit#1 is widely consumption in Thailand as an ingredient of Thai’s dish. Most of consumers prefer to have immature green lime due to its enriches with special aromatic compound, taste and flavor. Thus, to maintain the green color of lime fruit is very important for retarding quality losses. Peel yellowing is one of a major problem in lime fruit during postharvest period which lead to reduction of fruit qualities thus the inhibiting or delaying of chlorophyll breakdown is needed. There are several kind of postharvest technology to prolong storage life and maintain green color of fresh produces and one of them is coating technique by using natural based wax. According to Thailand produces a lot of palm oil and a byproduct from palm oil industry, palm oil wax, has potential to use as a wax based to form coating material. Thus, this research aimed to use palm oil based wax coating for maintaining quality of immature green lime cv. Paan Pichit#1. Lime fruit were harvested from commercial orchard and coated with palm oil based wax (PW) and commercial wax (CW), and then stored at 13°C. Uncoated fruit was set as a control. Changes of lime fruit qualities including fresh weight loss, browning spot, chlorophyll content, ascorbic acid content and acetaldehyde content were investigated at 5 days interval. The results found that lime fruit coated with PW showed the lowest water loss compared with that of CW coated and the control, respectively. The percentage of peel browning spot occurrence was also reduced in the fruit coated with PW while the application of CW induced a browning spot to higher level than the control. This result was concomitant with the incidence of peel browning. Lime fruit coated with both PW and CW delayed the chlorophyll breakdown in the same trend while the continuously degradation of chlorophyll was observed in the control. There was no consistent change of ascorbic acid content in all treatments, anyway the content was slightly change from the initial until the end of storage. The accumulation of acetaldehyde in lime juice was initially observed on day 10 in all treatments and then declined throughout the end of storage with slightly difference among the treatments. From the results indicated that PW has potential to apply with immature green lime fruit during postharvest period. In addition, the use of byproduct from palm oil industry for formulating a coating material will support the zero waste policy and also added a value of byproduct.

Keywords: Coating, Palm oil wax, Lime, Postharvest, Quality

1. INTRODUCTION

Lime (Citrus aurantifolia Swingle) is considered as a unique fruit endowed with high flavor and acidity often used to accent the flavor of various Thai foods and beverages. Nutritionally, lime fruit are an excellent source of vitamin C, dietary fiber and contain numerous other nutrients in small quantities. The green peel of the fruit is a very important indicator of quality in terms of a potential to attract premium prices in the market especially in Thailand (Pranamornkith et al., 2010). During postharvest period (storage, transportation and retailing), the fruit is highly predisposed to postharvest yellowing as a result of chlorophyll degradation (Srilaong et al., 2011). The loss in the green color affects the quality attributes as well as the market value of lime. It is therefore important to investigate techniques that delay chlorophyll catabolism and how it is suppressed. In addition, the desiccation of lime fruit
due to transpiration during postharvest period is another problem. This leads to unacceptable external quality. From the above mentioned, it is really need a postharvest technique to overcome yellowing and wilting problems in lime fruit.

Literature gleaned from several authors elucidates that many postharvest techniques can delay senescence and retard quality losses of horticultural products. One of them is the application of coating material to maintain postharvest life of fresh produces. Natural materials that used to produce coating materials can be divided into three categories including hydrocolloids (polysaccharides, proteins), lipids (fatty acids, waxes), and composites (Navarro-Tarazaga, et al., 2008). Among lipids, waxes are the most attractive and promising coating materials for fruits and vegetables. The wax-based coatings are known to have a good barrier property against moisture transfer. In addition, it has been used to reduce respiration, improve appearance of fruits and vegetables by generating a shiny skin (Morillon, et al, 2002). Carnauba and candelilla are among the plant waxes that commonly used as a component in coating materials (Puttalingamma, 2014). However, these waxes are very expensive. Thus, an alternative wax based is becoming more intensive focused. In Thailand, wax is widely used in citrus with a purpose of prolong storage life and also for shiny appearance to attract the consumer. In each year, Thailand imported a wax for citrus around 500-700 tons which cost 60-84 million baht/year. It is quite big amount of investment in citrus business. Recently, many researchers try to formulate a wax from palm oil by using a byproduct from palm oil industry and found a potential to use for coating of seed (Pinkrajay et al., 2019). However, until now no report studies the effect of palm oil based wax on prolonging a storage life of fresh produces. Thus, the objective of this research was to investigate the effect of palm oil based wax coating on quality changes of lime fruit in comparison with a commercial coating solution during storage at low temperature. In addition, this research aimed to promote the zero waste concept by using a byproduct from palm oil industry to do value added product.

2. MATERIALS AND METHODS

2.1 Lime fruit preparation
Immature green lime fruit were harvested from commercial orchard in Samutprakarn province, Thailand and then transported to laboratory within 30 min. Fruit were selected for uniformity of color, size and shape, and also free from diseases and defects. The selected lime fruit were washed with running tap water and dipped in 150 ppm sodium hypochlorite solution for 5 min, and then air-dried at 25°C.

2.2 Coating application
Fruit prepared from 2.1 were divided into 3 groups and each group has 200 fruits. Then, the lime fruit from each group were coated with commercial wax (CW) and palm oil based wax (PW), respectively. All coated fruit were subjected to air-dry at 25°C. The control group was uncoated fruit. The CW concentration was prepared according to the recommendation for citrus as mentioned on label. The PW was prepared by using byproduct from palm oil industry and the formulation was developed by Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bangkok, Thailand. The mixture and protocol of PW preparation could not inform in this report according to it will be applied for patent. All of lime fruit were stored in a cold room at 13°C (90-95%RH). The experimental design was completely randomized design (CRD) with 5 replications. LSD was analyzed for significant difference among treatment.

2.3 Analytical parameters

2.3.1 Weight loss
Individual weight loss in 24 fruits were registered and expressed as the percentage loss of initial weight.

2.3.2 Browning severity and percentage
Browning severity was evaluated in 24 fruits per treatment. The different degrees of browning severity were rated as 1 = none, 2 = 0.1-5% browning severity on lime surface, 3 = 5.1-10% browning severity on lime surface, 4 = 10.1-15% browning severity on lime surface and 5 = browning severity on lime surface more than 15.1%. Results were converted to an average index (1–5). The fruit was calculated for the total fruit (n = 24) per treatment at each storage time as:

\[
\text{% Browning} = \left( \frac{\text{number of fruit browning}}{\text{total number of fruit}} \right) \times 100
\]

2.3.3 Total chlorophyll content
Total chlorophyll content was determined by the method of Inskeep and Bloom (1985). 1 gram of flavedo tissue from lime peel were added with 20 ml N,N-Dimethylformamide. Then incubated in dark condition at 4ºC for 24 hour. The chlorophyll extract was measured as chlorophyll $a$, $b$ and total chlorophyll content in a UV-visible spectrophotometer, at 664 and 647 nm.

2.3.4 Ascorbic acid content

Ascorbic acid content was determined according the method of Kapur et al. (2012) with some modifications. Two ml of juice was taken into 10 ml of 5% meta-phosphoric acid then mixed and filtrated with whatman #01 filter paper and clear sample was taken. 0.2 ml of 0.02% indophenol solution was added with 0.4 ml of sample extract and incubated 2-3 min until it became a stable reddish-pink color. After that, 0.4 ml of 2% thiourea and 0.2 ml of 2% DNP solution were added and then incubated 1 hour at 50ºC in a hot water bath. Then, 1 ml of 85% sulfuric acid was added and then incubated at room temperature for 30 min. The absorbance was determined at 540 nm using visible spectrophotometer (SP-830 plus, Metertech). A standard curve was prepared using standard ascorbic acid with concentrations of 20, 40, 60, 80, 100 mg/L

2.3.5 Acetaldehyde content

Acetaldehyde content was determined by Fuggate et al. (2010) with some modifications. 10 ml of lime juice added in 18 ml container (Precision Scientific, Chicago, IL, USA) and then incubated in a water bath at 50°C for 15 min. One-milliliter headspace samples were injected into a GC-2014 Shimadzu equipped with 80/100 Am mesh Porapack Q column. Chromatographic parameters were: detector: FID; helium as gas carrier; column temperature: 200°C; injector port temperature: 120°C.

3. RESULTS AND DISCUSSION

The quality of lime fruit with and without coating treatments stored at 13ºC for 30 days was observed and showed results as in following:

3.1 Weight loss

Fresh weight loss of lime fruit in all treatments increased with a progress of storage time (Fig. 1). Fruit without coating (control) showed the highest weight loss and reached a level of 5% on day 25 which started to observe skin wilting. This agree with the previous reported about a level of water loss at higher than 5% induces external quality loss in fresh produces (Wills et al., 1998). While the coated fruit showed lower fresh weight loss compared with the control. Fruit coated with PW has significantly lower water loss compared with that of coated with CW throughout storage and found the percentage of weight loss was lower than the control about 50%. The results indicated that PW coating could reduce transpiration of lime fruit due to the coating technique can generate modified atmosphere condition in the fruit and led to decrease of respiration rate. It showed a similar result of the study in tomato fruit that found reduction of respiration rate in cellulose based coated fruit (Tosati et al., 2015).

![Figure 1. Weight loss of lime fruit coated with commercial wax (CW) and palm oil based wax (PW) compared with the uncoated control during storage at 13ºC for 30 days. The error bar indicates ±SE (n=5).](image-url)
3.2 Browning spot

3.2.1 Browning spot severity
Browning on the peel of lime fruit was found from day 15 in all treatments and the severity was increased with the progress of storage time (Fig. 2). The severity of browning spot was lowered in lime fruit coated with PW compared with that of other treatments. Interestingly, the browning severity of the CW coated fruit was induced and reached higher level than that of other treatments from day 15 until the end of storage. This might be due to a toxicity of the commercial wax which has adverse effect on lime fruit appearance. The results were concomitant with the percentage of browning spot in Table 1. The browning spot is a sign of senescence on the peel of lime fruit after storage for a certain time. This may relate with the percentage of water loss, a higher water loss in the control and CW coated fruit showed higher browning severity than the PW coated. PW is classified in a group of edible coating material thus it is not harmful to fruit itself and also consumer (Navarro-Tarazaga, et al., 2008).

Figure 2. Browning spot severity on the peel of lime fruit coated with commercial wax (CW) and palm oil based wax (PW) compared with the uncoated control during storage at 13°C for 30 days. The error bar indicates ±SE (n=5).

3.2.2 Percentage of peel browning spot
The percentage of peel browning spot of lime fruit was exhibited in the same trend with the severity of browning spot (Table 1). The highest percentage of brown spot was recorded in lime fruit coated with CW, while those coated PW alleviated the percentage of browning until day 20 of storage and then increased to a similar level with that of control. From the results, it seems PW coating has ability to delay browning disorder on fruit peel. The limitation of gas transmission (oxygen) from PW film might play an important role to reduce the oxidative process of phenolic compounds by polyphenol oxidase enzyme to form brown pigment. As in the browning mechanism needs oxygen for oxidation of phenol (Massantini and Mencarelli, 2007).

Table 1. Percentage of browning spot on the peel of lime fruit coated with commercial wax (CW) and palm oil based wax (PW) compared with the uncoated control during storage at 13°C for 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of peel browning spot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
</tr>
<tr>
<td>Commercial Wax</td>
<td>0.00</td>
</tr>
<tr>
<td>Palm Oil Wax</td>
<td>0.00</td>
</tr>
</tbody>
</table>
3.3 Total chlorophyll content

Total chlorophyll content in the peel of lime fruit in all treatments declined from 30-40 mg/100 gFW to less than 10 mg/100 gFW at the end of storage period (Fig. 3). However, the application of PW delayed the reduction of total chlorophyll content in lime during the first 10 days of storage and thereafter it showed the same trend of change as in the fruit coated with CW. Lime fruit coated with both CW and PW maintained higher total chlorophyll content from day 15 to day 30 of storage compared with the control. The catabolism of chlorophyll could initiate by oxidative process and also exogenous ethylene (Kaewsuksaeng, 2011). As above mentioned, coating material can control gas permeability through the fruit thus it limits the oxygen for oxidation and also protect the fruit from exogenous ethylene. In addition, coating technique also retards the respiration process and also delay ethylene production in the fruit thus minimizes the degradation of chlorophyll.

Figure 3. Total chlorophyll content in the peel of lime fruit coated with commercial wax (CW) and palm oil based wax (PW) compared with the uncoated control during storage at 13°C for 30 days. The error bar indicates ±SE (n=5).

3.4 Ascorbic acid content

Ascorbic acid content in lime juice was slightly change from the initial until the end of storage especially in the control and CW coated fruit which showed the same trend during storage (Fig. 4). However, the content in lime fruit coated with PW was temporary increased at day 5 and decreased thereafter to similar level with that of other treatments, and then increased again on day 25 and declined at the end of storage. Normally the change of ascorbic acid content in fresh produce is related with the percentage of water loss (Lee and Kader, 2000). From the results of this experiment, a bit higher ascorbic acid content in PW coated fruit might be a response of lower water loss.

Figure 4. Ascorbic acid content in the juice of lime fruit coated with commercial wax (CW) and palm oil based wax (PW) compared with the uncoated control during storage at 13°C for 30 days. The error bar indicates ±SE (n=5).
3.5 Acetaldehyde content
The acetaldehyde content in coated fruit is one of indicator to inform fruit quality and anaerobic respiration process which led to fermentation. The acetaldehyde content in lime juice from all treatments was not significant difference from the initial until day 10 (Fig. 5). A sharp increase of the acetaldehyde content on day 10 to level of 2.5 ppm was detected in all treatments, this might be the adaptation of lime fruit to low temperature storage. The previous study reported that low temperature induces the alcohol dehydrogenase enzyme activity in rice as a defense mechanism to stress condition and led to accumulation of alcohol (Kato-Noguchi, 2007). However, it could not detect any off-flavor by smelling in all treatments until from day 10 until the end of storage. This indicates that both of CW and PW were not induced anaerobic respiration process in lime fruit during a month storage.

Figure 5. Acetaldehyde content in the juice of lime fruit coated with commercial wax (CW) and palm oil based wax (PW) compared with the uncoated control during storage at 13°C for 30 days. The error bar indicates ±SE (n=5).

4. CONCLUSION
The application of palm oil based wax coating has potential to maintain postharvest quality of immature green lime fruit through reduction of water loss, chlorophyll breakdown and browning disorder. A proper commercial wax must be concerned for coating of immature green lime fruit to avoid a toxic component that may induce brown spot development on fruit surface.

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REFERENCES


Effects of Blanching Pretreatment on Drying Characteristics and Pectic States of Dried ‘Fuyu’ Persimmon

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Keywords: pectin, atomic force microscope, drying, blanching, persimmon

‘Fuyu’ persimmon is popular in Japan and distributed not only as fresh fruits but also dried ones. Although fruits and vegetables are often dried by hot air, length of the drying time is considered as a big problem. To improve drying efficiency, blanching treatment is sometimes applied prior to drying. However, for ‘Fuyu’ persimmon, effects of these processes on drying characteristics and quality-related components are not clarified sufficiently. Among components in fruits, pectic substances are known to contribute to texture formation. Additionally, functional aspects of the substances, such as intestinal regulating function and prebiotic effect, also attract attention. Thus, we investigated drying characteristics and changes in pectin states during several drying treatments including blanching. Persimmon fruits (cv ‘Fuyu’) harvested in Gifu city was used in this study. The persimmon flesh was cut into cylinder (20.5 mm diameter and 10 mm height) using a cork borer and a knife. The initial moisture content of the sample was 5.06 (dry basis). In this study, we prepared blanched and non-blanched samples. For the blanched one, the persimmon samples were immersed in hot water at 95 degree for 2 min, then immediately cooled in iced water for 2 min. Both of the blanched and the non-blanched samples were dried in a forced hot air oven controlled at 40, 50, 60, and 70 degree. During the drying, sample weight was weighed at every hour, and converted to moisture content. A model was fitted to measured value and rate constant $k$ (h$^{-1}$) of the drying process was calculated for each temperature. Next, alcohol insoluble solid (AIS) of the samples, which dried at 40 and 60 degree until 2.0 and 0.3 (d.b.), was prepared to extract pectin. Pectin fractions were sequentially extracted from AIS with distilled water, 0.05 M CDTA solution and 0.05 M Na$_2$CO$_3$ + 20 mM NaBH$_4$ solution, and water-soluble pectin (WSP) fraction, chelator-soluble pectin (CSP) fraction and diluted alkali-soluble pectin (DASP) fraction were collected, respectively. Galacturonic acid content in pectin fractions were determined using carbazole sulfuric acid method. Also, atomic force microscopy (AFM) observation was performed. Extracted pectin fractions were diluted 200 times with distilled water. A 3 µ l of the resulting solution was dropped onto freshly cleaved mica, then it were dried overnight at room temperature. An AFM5400L (Hitachi High Technologies) was used for imaging in the dynamic force mode. A silicon cantilever with nominal spring constant of 15 N m$^{-1}$ and resonant frequency of 110 - 150 kHz was used. The scanning area was set at 2 m × 2 m in the XY plane, and the scanning resolution was 512 × 512 points. AFM images were morphologically analyzed using the SPIP software. Regardless of whether it was blanched sample or not in the drying process, the exponential model could be fitted ($R^2$=0.9962 - 0.9996). Comparing the obtained rate constants, blanched samples had high value, which indicating blanching is effective to improve the drying process. In addition, we prepared dried samples having 2.0 and 0.3 of moisture content (d.b.) and evaluated state of pectin in these samples. After the blanching, the ratio of WSP amount in total pectin obviously decreased, and the ratio of CSP and DASP increased. However, the ratio of WSP increased with drying in all samples. At 0.3 of moisture content (d.b.), Overall, the blanching treatment indicated greater effect on pectin composition than the drying process. In AFM images of pectin nanostructure, short chain and granule like objects were appeared in WSP. Also, lager structures were observed in CSP. The result in this study shown that drying treatment and blanching pretreatment changed pectin composition and structure. Thus, we assumed that texture and functional properties of dried products will be modified by selected conditions.
Beverage Process Using By-product Water of the Production of Wash-free Rice as Raw Material and the Continuous Process of Lactic Acid Fermentation

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Keywords: Fermented beverage, Wash-free Rice, By-product Water, Lactic Acid Bacteria, Response Surface Methodology

Wash-free Rice (MUSENMAI) is a new type of rice product developed in Japan. It does not require washing before cooking, due to the separation of “skin bran” in advance during the processing of Wash-free rice, which is demonstrated that may influence the taste of cooked rice. One way to produce Wash-free rice is washing by small amount of water then drying by hot air. Through this process, By-product Water will be produced, which has high nutritional value (protein, carbohydrate, dietary fiber and lipid) and mainly used to produce liquid feed for pig raising nowadays. In order to improve the utilization rate and added value of this potential raw material, this study focuses on using lactic acid bacteria to study the applicability of By-product Water for the development of a fermented drink. Fermentation characteristics in Wash-free Rice substrate by selected lactic acid bacteria starter culture were preliminary identified. The effects of fermentation temperature, inoculation amount of starter culture, the type of starter culture and initial glucose content before fermentation will be optimized by Response Surface Methodology in order to obtain the optimal preparation process of fermented beverage. And finally a complete assessment of product will be provided, including major constituents, physico-chemical characteristics and sensory characteristics.

Effect of roasting and storage on chemical compounds and sensory score of specialty coffee

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Keywords: lipid oxidation, specialty coffee, coffee roasting, shelf life, sensory evaluation

Coffee is the most consumed food product in the world. Among them, coffee beans which are evaluated as 80 or more points in the sensory evaluation of Specialty Coffee Association of America (SCAA) is called specialty coffee. And specialty coffee has unique flavor characteristics and high traceability of the value chain. Specialty coffee consumption is increasing in recent years. Flavor is the most important criteria for coffee quality evaluation, and also one of the major motivations for consumer preferences. The storage period of specialty coffee is relatively short compared to commodity coffee, but some commercial products are stored for a period longer than the recommendation of specialists. Many studies have conducted on aromatic components such as phenol in coffee, but there are still few findings on lipid oxidation. Roasting induces transformation on chemical and physical composition in coffee beans. During storage, further chemical and physical changes that affect the quality of brew may occur. Along with this change, the
acceptability of consumers also changes. In this experiment, the quality change of coffee due to oxidation of lipid is clarified. Catuai Amarelo coffee cultivars from Alta Mogiana, SP, Brazil was used. The sample was harvested on August 2018 then processed according to natural method. Green bean, light roasted bean, medium roasted bean and dark roasted bean were stored up to 85 days then analyzed for chemical components of lipid oxidation. Hydroperoxide content as a primary oxidation compounds evolution during storage were monitored by conjugated dienes and trienes determination by spectrophotometric method. Free fatty acids (FFA) as a secondary oxidation compounds were evaluated by American Oil Chemists’ Society (AOCS) method. The sensory evaluation was conducted according to the sensory test protocol of SCAA. The production of fatty acid, which is the final product of lipid oxidation, stabilized after transient increase. On the other hand, the sensory evaluation score decreased overall. A weak correlation was found between fatty acid content and sensory score, with a correlation coefficient of $R^2=0.39$.
MATLAB®, which is a programming platform designed specifically for engineers and scientists, and a numerical optimization technique using COMSOL Multiphysics®, respectively. The thermal diffusivity values of the samples ranged from $1.1 \times 10^{-7}$ to $1.5 \times 10^{-7}$ m$^2$/s by the ordinary least squares method. A significant difference was not statistically recognized among the values of thermal diffusivity of all sample sizes and shapes for the carrot. Also, between the rotational axisymmetric 2-dimensional analysis and the 3-dimensional analysis, there was no significant difference for all samples. The advantages of this method are that the device and the estimation method are simple, inexpensive, rapid, and can apply to various shapes of a sample and the dimension. The results obtained in this study will be useful in the design of equipment and in calculations for the thermal processing of vegetables.

**[6-1130-P-24] Effect of Acid Type and Concentration on Properties of Pectin Extracted from Unripe Cavendish Banana Peel and Its Application in Raspberry Jam**

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Keywords: Acidic extraction, Waste utilization, Pectin properties, Raspberry jam

This work was aimed at evaluating the properties of pectin from unripe cavendish banana peel using different acidic extractions. Hydrochoric (HCl), citric, and malic acid solutions at various pH values (1.5, 2.0, and 2.5) were used in this study. The physical properties of a raspberry jam added with the obtained pectins were also investigated. The extraction yield, galacturonic acid content, degrees of esterification (DE) and methylation (DM) of the samples were quantified and compared. The results showed that most of the pectins were low methoxyl types. The highest pectin yield was obtained using extraction with citric at pH 2.0. It was found that the citric extraction also gave the highest percentages of DE (50.27%) and DM (59.57%) at pH 1.5 (p<0.05). Extraction with HCl showed to give higher galacturonic acid content to the extracted pectin (p<0.05). Additionally, the use of this acid at pH 1.5 also provided the highest gel hardness (30.26 g) (p<0.05). For food application, it was observed that most of the pectins significantly decreased raspberry jam hardness along with decreasing lightness and redness when compared with the control (no pectin added) (p<0.05). It was observed that only a pectin extracted with HCl at pH 1.5 increased the jam hardness (p<0.05). Therefore, the developed extraction process can be further used to utilize agricultural waste (banana peel) as a food ingredient.

**[6-1130-P-25] Evaluation of color and flavor for shiitake mushroom dried using vacuum microwave treatment**

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Keywords: vacuum microwave, dried shiitake mushroom, color, flavor

We evaluated the color and flavor of the mushrooms dried using vacuum microwave drying (VMD) treatment. The shiitake mushrooms were subjected to microwave treatments at different levels of power (25 W/g dry matter, 50 W/g dry matter, and 75 W/g dry matter) and absolute pressures (3 kPa, 10 kPa, and 20 kPa). The shiitake mushrooms treated at 3 kPa and 10 kPa showed the more desired yellow and bright colors, however, those treated at 20 kPa displayed dark colors including brown and black indicating quality degradation. Moreover, the total color difference ($\Delta E$) of the VMD samples was greater than 10, implying a marked difference in the color of the VMD samples compared to their original condition. However, the $\Delta E$ of samples treated at 3 kPa was lower than that of those treated with hot air drying (HAD). On sensory evaluation, the sample treated at 3 kPa and 25 W/g dry matter, received the highest score and was greater than that of all items evaluated, including the samples which received HAD treatment. Together, these results indicate that application of VMD treatment is a more effective method for producing dried shiitake mushrooms than HAD in terms of color and flavor.
The effect of molecular hydrogen on the shelf life of banana

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Keywords: browning, chilling injury, hydrogen gas, hydrogen water

Molecular hydrogen has been known to have the ability to eliminate reactive oxygen species. It is thought that the hydrogen can inhibit oxidation of biological membranes by reactive oxygen especially because it can dissolve more in lipids than in water. The aim of this study was to improve the shelf life of fruit and vegetable using molecular hydrogen.

Commercially available banana was used for the experiments. First, hydrogen water was mist-sprayed to banana. Hydrogen water was made in the polyethylene terephthalate bottle containing deionized water with hydrogen generated from the mixture of aluminum, calcium oxide and water. Two test chambers (W: L: H = 0.5: 0.4: 0.9 m) was used in this experiment. The mist was sprayed from the bottom of the chamber 0.18 m below the banana, and ventilation fan fixed on the roof of the chamber was operated during mist-spraying. Deionized water or hydrogen water (hydrogen concentration was approx. 3 mg L⁻¹) was mist-sprayed for 10 min every hour during the storage at 25 °C. The mist-spray of hydrogen water decreased the appearance of brown spots on the skin of banana. However, saturated humidity in the box sometimes progress the decay of stem end of fruit. And then, when the relative humidity in the test chamber was kept to 95% or less with ventilation fan operated by monitoring the humidity, it was shown that the progress of decay with high humidity during storage could be suppressed.

Next, hydrogen gas was treated to banana. Hydrogen gas was generated by hydrogen generator. Half green colored banana was kept in the airtight box and stored at 5 °C. Ambient air containing 0 or 4% of hydrogen gas was filled in the box. The air in the box was exchanged with the same gases every 3 days. When banana fruits were stored with 4% of hydrogen gas, the skin browning by chilling injury was suppressed. Moreover, even if banana fruits were kept in 4% of hydrogen gas for only 24 h and then kept in the air without hydrogen, the skin browning was suppressed. When banana fruits were pretreated with 0 (control), 1, 2, 4, 10, 50 % of hydrogen gas for 24 h in the box and then the fruits were taken out of the box and put in perforated polypropylene bag without hydrogen gas and stored at room temperature, the appearance of brown spot on the skin was significantly suppressed under 2 or 4% of hydrogen concentration, comparing with control.

From our results, it was suggested that the treatments of molecular hydrogen could suppress the skin browning and prolong the shelf life of banana fruit during storage at both of room temperature and low temperature.
Effect of Molecular Hydrogen on the Shelf Life of Banana

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ABSTRACT

Molecular hydrogen has been known to have the ability to eliminate reactive oxygen species. It is thought that the hydrogen can inhibit oxidation of biological membranes by reactive oxygen especially because it can dissolve more in lipids than in water. The aim of this study was to improve the shelf life of banana using molecular hydrogen. Commercially available banana was used for the experiments. First, hydrogen water was mist-sprayed to banana in the box. Deionized water or hydrogen water (hydrogen concentration was approx. 3 mg L⁻¹) was mist-sprayed to the fruits for 10 min every hour during the storage at 25 °C. During the misting, the air in the box was ventilated by fun. The mist-spray of hydrogen water decreased the occurrence of brown spots on the skin of banana. However, mostly saturated humidity in the box sometimes progress the decay of stem end of fruit. When the relative humidity in the test chamber was kept to 95% or less with ventilation fan operated by monitoring the humidity, it was shown that the progress of decay with high humidity during storage could be suppressed. Next, hydrogen gas was treated to banana. Half green colored banana was kept in the air-tight box and stored at 5 °C. Ambient air containing 0 or 4% of hydrogen gas was filled in the box. The air in the box was exchanged with the same gases every 3 days. When banana fruits were stored with 4% of hydrogen gas, the skin browning by chilling injury was suppressed. Moreover, even if banana fruits were kept in 4% of hydrogen gas for only 24 h and then kept in the air without hydrogen, the skin browning was suppressed. When banana fruits were pretreated with 0 (control), 1, 2, 4, 10, 50 % of hydrogen gas for 24 h in the box, and then the fruits were taken out of the box, putting in perforated polypropylene bag without hydrogen gas and stored at room temperature, the occurrence of brown spot on the skin was significantly suppressed under 2 or 4% of hydrogen concentration, comparing with control. From our results, it was suggested that the treatments of molecular hydrogen might suppress the skin browning and prolong the shelf life of banana fruit during storage at both of room temperature and low temperature.

Keywords: Browning, Brown spot, Chilling injury, Hydrogen gas, Hydrogen water

1. INTRODUCTION

Bananas (Musa spp.) are sensitive to low temperatures and chilling injury is caused by it. The major symptoms of chilling injury include browning of the skin and poor ripening. When the banana fruits were stored at not only low temperature but also room temperature, the brown spots, which affecting the shelf life of banana, occur on the skin. It is thought that the browning of the skin is the result of the oxidation of o-diphenols by polyphenol oxidase (PPO).

Molecular hydrogen is known to have the ability to eliminate reactive oxygen species (Ohsawa et al., 2007). It was thought that the hydrogen could inhibit oxidation of biological membranes by reactive oxygen especially because it dissolved more in lipids than in water (Iuchi et al., 2016). The therapeutic effects of molecular hydrogen on neurological outcomes after cardiac arrest (Hayashida et al., 2012; 2014), Parkinson’s disease (Ito et al., 2012), etc. were reported in animal models. Also, in plants, enhancement of salt tolerance of Arabidopsis (Xie et al., 2014), delay of postharvest ripening of kiwifruit (Hu et al., 2014) by hydrogen water were reported.

In our previous study, when some fruits and vegetables were treated with hydrogen water, shelf life of them tended to increase, but long time wetting of hydrogen water on the surface of fruits and vegetables promoted deterioration of them. The aim of this study was to improve the shelf life of banana using molecular hydrogen. As the methods which molecular hydrogen was treated to the fruits without long time wetting of hydrogen dip, two methods as intermittent misting of hydrogen water and treatment of hydrogen gas were examined.
2. MATERIALS AND METHODS

2.1 Effect of Misting of Hydrogen Water on Shelf Life of Banana Fruit

2.1.1 Treatment without Humidity Control
The green chip colored banana (*Musa* AAA group, Cavendish subgroup, cv. Cavendish) fruits purchased at market were used. Bananas were prepared for packaging with or without perforated orientated polypropylene (OPP) film. Two test chambers (W: L: H = 0.5: 0.4: 0.9 m) described as Figure 1 was used in this experiment. The fruits were put on 18 cm above mist blower in each test chamber. The temperature was set to 25°C. Deionized water or hydrogen water was mist-sprayed for 10 min every hour. During the misting, the air in the box was ventilated by fan in order to make airflow. Hydrogen water was made in the polyethylene terephthalate bottle containing deionized water with hydrogen generated from the mixture of aluminum, calcium oxide and water. On 15 days after the start of experiment, the change in appearance was observed.

![Figure 1. Schematic diagram of the chamber used in mist treatment of this study.](image)

2.1.2 Treatment with Humidity Control
Material and treatments were same as 2.1.1. Humidity sensor (CH S-UPS, TDK Corp.) was put in the chambers. Output of the sensors was monitored by programable logic controller (Smart Relay FL1C-H12RCE, IDE Corp.) through the operation amp. The ventilation fan was operated during the mist-spraying and the time when the relative humidity in the chamber was over 95%. On 15 days after the start of experiment, the change in appearance was observed, and the percentage of area of brown spots on skin and the firmness of skin and pulp of the fruits were measured.

2.2 Effect of Hydrogen Gas Treatment

2.2.1 Effect on Chilling Injury
The half green colored banana fruits purchased at market were used. The banana fruits were placed in the air-tight box (W: L: H = 0.29 m: 0.23 m: 0.08 m). In control, the box was filled with ambient air. In treatments, the box was filled with ambient air containing 4% hydrogen gas. Two treatments were designed. When the fruit was kept in treatment condition for only for 24 hours after the start of treatment and then kept in the ambient air without hydrogen gas, it was called as pretreatment. When the fruit was kept in treatment condition continuously, it was called as continuous treatment. Hydrogen gas (99.99%, v/v) was generated by a hydrogen generator (ZK-200, Kenmin Co., Ltd.). The air in the box was exchanged with the same gases every 3 days. The fruits were kept at 5°C. On 15 days after the start of experiment, the change in appearance was observed, and the degree of chilling injury and the firmness of fruit skin were determined.

2.2.2 Effect on Shelf Life of Banana Fruit at Room Temperature
The materials were prepared the same as in Experiment 2.2.1. Pretreatment of banana fruits in the air-tight box with 0 (control), 1, 2, 4, 10, 50% hydrogen gas for 24 hours. Then, fruits were taken out of the box and put in a perforated OPP film and stored at room temperature (25°C). On 11 days after the start of experiment, the change in appearance was observed and the percentage of area of brown spots on skin of the fruit was measured.
2.3 Methods of Measurements

2.3.1 Fruit Firmness
The fruit firmness was evaluated by measuring the penetration resistance of the pulp and skin of the banana. Penetration resistance of banana was measured by creep meter (RE 2-33005C, Yamaden Co., Ltd.) with a cylindrical plunger as a diameter of 3 mm.

2.3.2 Percentage of Area of brown spots on skin
The removed skin of banana was arranged on the plane, and picture was taken by digital camera. The area of brown spot of the skin on the photographic image was determined by Image J (https://imagej.nih.gov/ij/).

2.3.3 Evaluation of Degree of Chilling Injury
The degree of chilling injury was evaluated by measuring the brightness of the skin. The brightness (L* value) of the skin was measured with a color meter (ZE 6000, Nippon Denshoku Kogyo Co., Ltd.).

3. RESULTS AND DISCUSSION

3.1 Effect of Misting of Hydrogen Water on Shelf Life of Banana Fruit

3.1.1 Treatment without Humidity Control
The mist-spray of hydrogen water seemed to decrease the occurrence of brown spots on the skin of fruit (Figure 2). While, decay from the stem end of fruit was observed in both of deionized water and hydrogen water treatments. It was thought that mostly saturated humidity in the chamber might progress the decay. The packing of OPP film increased the occurrence of brown spots and progress the decay. It might be also caused by higher humidity in the film. In the film, drops of dew might appear on the surface of the fruits.

In this experiment without humidity control, it was shown that misting of hydrogen water might decrease the occurrence of brown spots on the skin. However, high humidity in the test chamber, especially in the OPP film, progress the decay of fruits. Therefore, it was thought that the humidity control for avoiding dew condensation on the surface of fruits was necessary.

3.1.2 Treatment with Humidity Control
The humidity control could suppress the decay from the stem end of fruits (Data not shown). The brown spots area on the skin of fruit had no significant difference between the kind of misted water in filmed fruits, but it was significantly lower in the hydrogen water treatment than the deionized water treatment in no-filmed fruits (Figure 3). The brown spots area of the no-filmed fruit treated with hydrogen water was lowest than that in any treatments.

The penetration resistance of pulp tended to be lower in filmed fruits than no-filmed fruits (Figure 4a). In filmed fruits, it became significantly high by misting of hydrogen water. On the other hand, the penetration resistance of skin was significantly low in no-filmed fruits than filmed fruits (Figure 4b). there was no significant difference in the penetrating resistance of skin between the kind of water misted in both of fruits filmed and no-filmed.

In this experiment, it was suggested that misting of hydrogen could suppress occurrence of brown spot of skin of banana fruit during the preservation at room temperature. In addition, it was shown that the
humidity control delayed the occurrence of rot and brown spots from the stem end of the banana fruit. Further research was needed in order to defined appropriate condition of misting of hydrogen water for more effective treatment.

Figure 3. Effect of misting of hydrogen water on the percentage of area of brown spots on the skin of banana fruits after 15 days preservation. Same letter indicates no significant difference by Tukey’s HSD test (P = 0.05). Vertical bars indicate s.e. (n = 3).

Figure 4. Effect of misting of hydrogen water on penetration resistance of pulp (a) and skin (b) of banana fruit after 15 days preservation. Same letter in each figure indicates no significant difference by Tukey’s HSD test (P = 0.05). Vertical bars indicate s.e. (n = 6).

3.2 Effect of Hydrogen Gas Treatment
3.2.1 Effect on Chilling Injury
On 8 days after the start of experiment, browning of skin was suppressed by both of pre and continuous treatment of hydrogen gas, compared to control. On 15 days, the browning of the skin developed in control, but the development of browning was significantly suppressed in both of hydrogen gas treatments (Figure 5). L* value of the skin was significantly high in both of hydrogen treatments than control (Figure 6). There was no significant difference between the two hydrogen gas treatments. L* value indicates brightness. Higher L* value means brighter skin color. From this result, it was shown that hydrogen gas treatments could suppress the browning of skin caused by chilling injury, even if the hydrogen gas was treated only for first 24 h during preservation.
The penetration resistance of skin after 15 days preservation at 5°C tended to increase by hydrogen gas treatments, and it was significantly high in continuous treatment of hydrogen, compared with control (Figure 7).

In this experiment, it was suggested that chilling injury of banana fruits could be alleviated by treatment of hydrogen gas. The reason why the chilling injury was suppressed by hydrogen gas treatments was not clear. Molecular hydrogen has the ability to eliminate reactive oxygen species (Ohsawa et al., 2007), and it was thought that the hydrogen could inhibit oxidation of biological membranes by reactive oxygen (Iuchi et al., 2016). The browning is caused by oxidation of polyphenols (Walker and Ferrar, 1998). It has been thought that low temperature caused the damage of cell membrane such as vacuole and leakage of phenols from the vacuole to cytosol would increase oxidation of phenols by polyphenol oxidase (Nguyen, 2003). Hydrogen might suppress the oxidation of membranes. While, banana is classified to climacteric fruits. Ethylene associates with their maturation (Golding et al., 1998) and the ethylene binds to its receptors and leads maturation (Fluur and Mattoo, 1996; Lelievre et al., 1997). Storage of fruits at chilling temperature altered the physicochemical properties of ethylene effects (Marangoni et al., 1996). It was suggested that hydrogen might affect ethylene effects.

![Figure 5](image1.png)

Figure 5. Appearance of banana fruits treated with hydrogen gas on 8 (left) and 15 (right) days after the start of experiment. The fruits were stored at 5°C.

![Figure 6](image2.png)

Figure 6. Effect of hydrogen gas treatments (A: Control, B: Pretreatment with hydrogen gas, C: Continuous treatment with hydrogen gas) on the L* value of skin color of banana fruit after 15 days preservation at 5°C. Same letter indicates no significant difference by Tukey’s HSD test (P = 0.05). Vertical bars indicate s.e. (n = 6).
3.2.2 Effect on Shelf Life of Banana Fruit at Room Temperature

The occurrence of brown spots was suppressed by hydrogen gas treatment at more than 2% of hydrogen concentration. The difference was significant at the concentrations of 2% and 4%, compared with 0%. However, higher concentration of hydrogen as more than 10% indicated no significance. It is indicated that hydrogen treatment might have appropriate concentration.

In this experiment, it was indicated that pretreatment with hydrogen gas was effective on suppression of occurrence of brown spots on skin of banana fruits.

4. CONCLUSION

From these results, it was suggested that the occurrence of brown spots on banana fruits could be suppressed by mist-spray of molecular hydrogen without of progress of decay if the relative humidity kept below 95%. In addition, it was suggested that the occurrence of chilling injury and brown spots of banana fruit could be suppressed by treatment of hydrogen gas. It may be possible to prolong the shelf
life of banana fruits by the treatment with molecular hydrogen. These phenomena might be caused by suppressing the oxidation of cell membrane in the skin of banana fruit by molecular hydrogen. Further research will be needed to make clear the mechanism of effects of molecular hydrogen.

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The Potential of Biogas Production from Caribbean Seaweed Biomass

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Keywords: Saint Lucia, Seaweed, Sargassum Fulvellum, Anaerobic digestion, Biogas

Sea tourism in Saint Lucia, which is a Caribbean country, represents 65% of its income. However, the seaweed invasion of this Caribbean country caused a brown seaweed blooming and proposed to markedly reduce the income of this country. Therefore, this study aimed to investigate the potential of biogas production from the Sargassum fulvellum, which is one of the most common invaded seaweeds in this country. Sargassum fulvellum seaweeds were used as a substrate for mesophilic (38 °C) batch anaerobic digestion experiments. The result showed that the chemical characteristics of the dried Sargassum fulvellum were 46.11% (Volatile Solid (VS)), 81.19 (Total Solid (TS)), and 35.05% (ash). Additionally, the biogas and methane yields were 154.3 mL/gVS and 115.8% mL/gVS, respectively. In conclusion, the utilization of seaweed biomass in the anaerobic digestion process not only ensures the beach and sea look better to make tourism flourish, but also enhances the income from the biogas production.
The Potential of Biogas Production from Caribbean Seaweed Biomass

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² Tokachi Agri Works  
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ABSTRACT

Sea tourism in Saint Lucia, which is a Caribbean country, represents 65% of its income. However, the seaweed invasion of this Caribbean country caused a brown seaweed blooming and proposed to markedly reduce the income of this country. Therefore, this study aimed to investigate the potential of biogas production form the *Sargassum fulvellum*, which is one of the most common invaded seaweeds in this country. *Sargassum fulvellum* seaweeds were used as a substrate for mesophilic (38 °C) batch anaerobic digestion experiments. The result showed that the chemical characteristics of the dried *Sargassum fulvellum* were 46.11% (Volatile Solid (VS)), 81.19 (Total Solid (TS)), and 35.05% (ash). Additionally, the biogas and methane yields were 154.3 mL/gVS and 115.8% mL/gVS, respectively. In conclusion, the utilization of seaweed biomass in the anaerobic digestion process not only ensures the beach and sea look better to make tourism flourish, but also enhances the income from the biogas production. 

Keywords: Saint Lucia, Seaweed, *Sargassum*, Anaerobic digestion, Biogas.

1. INTRODUCTION

Fossil energy is natural resources that contain hydrocarbon chains. Natural gas, Petroleum, and Coal are types of fossil energy. The increase in energy demand caused by population growth and the depletion of world oil reserves as well as the issue of emissions from fossil fuels which ultimately led to an increase in fuel prices requires an alternative to obtaining energy sources, one of which is biogas using several species of macroalgae. 

In 2011 reported that the first time blooming a brown seaweed of sargassum in the Caribbean Sea. Before 2011, brown seaweed just only found in the Sargasso Sea but after that, brown seaweed also was found in the Caribbean Sea. Brown seaweed in the Caribbean Sea doesn’t correlate with the brown seaweed of the Sargasso Sea. Most of the online news in 2018 such as The Guardian, BBC News, Smarter travel, noonsite.com, The New Republic, etc. explain that seaweed growth occurring in the Caribbean Sea is a big problem. Today, the invasion of seaweed in the Caribbean country such as Saint Lucia, Barbados, Antigua and Barbuda, Puerto Rico, Martinique, Guadeloupe, etc. have affected to tourism, fisheries, economics, environment, and human health. 

Saint Lucia is one of the beautiful island countries in the Caribbean Sea. Saint Lucia may be a constitutional autocracy and a commonwealth. Saint Lucia is one of commonwealth country in the Caribbean Sea and independence in 1979. The head of state is hectometer Queen Elizabeth World Health Organization appoints and is described by a governor-general. Tourism is the main source of income and jobs for 65% of Saint Lucia GPD. Saint Lucia doesn’t have many natural resources, the geothermal just only natural resources for potential energy. 

In a long time, history, seaweed just only used for food and cosmetics product. Seaweed is not much ogled as a substitute for renewable energy. Recently, after many researchers have examined the content of seaweed can be used as alternative energy. Seaweed biomass as third-generation feedstock is used as green energy for biofuels and biogas. In particular, seaweed biomass is not quite used as a food source on a global scale, like palm oil or corn as the first-generation for renewable energy. All types of organic waste can be processed to produce biogas such as biomass waste, human waste, animal waste can be used as energy through the anaerobic digestion process. This process is a great opportunity to produce alternative energy so that it will reduce the impact of using fossil fuels. Besides,
making biogas can reduce a variety of plant organic waste and animal waste so that it has economic value. Anaerobic digestion may be a series of biological processes during which microorganisms break down perishable material within the absence of element. One of the top products is biogas, which is combusted to generate electricity and heat, or can be processed into renewable natural gas and transportation fuels. A range of anaerobic digestion technologies is dynamical stock manure, municipal waste product solids, food waste, high strength industrial waste product and residuals, fats, oils and grease (FOG), and varied different organic waste streams into biogas, twenty-four hours every day, seven days per week.

2. MATERIALS AND METHODS
The seaweed was collected from Caribbean Sea of the Saint Lucia. Dried seaweed biomass was characterizing in terms of total solid (TS), volatile solid (VS), Volatile fatty acid (VFA) and pH. In 20-gram Dried biomass was soaked 180 grams of water for 24 h. After soaked 24 h, wet biomass mixture with inoculums ratio 2:1.

2.1 Gas Produce
Start from here. Produced biogas was collected in a gas bag. The volume of biogas produced was measured by wet gas meters. All gas measurements are expressed at 0 °C and a pressure of one atmosphere. The composition of biogas was determined using a Shimadzu gas chromatograph (GC-14C) (Suraju. et al. 2018; Marildo. et. al. 2018).

2.2. Chemical Characteristic and Composition
The amount and composition were determined daily. Substrate samples are taken before and after experimentation to determine total solid, volatile solid, volatile fatty acid and pH. Volatile solid (VS) is the weight loss after a sample is ignited (heated to dryness at 550 EC). The total solid were determined by drying the samples at 105 °C for 24 h. The solid content was calculated from the difference between weights before and after drying. The dried matter was heated at 550 °C for 4 h, and organic matter volatile solid content was calculated from the loss on ignition. Methane (CH₄) was measurement with GC (Suraju. et al. 2018; Marildo. et. al. 2018; Nayak, A. et. al. 2018).

3. RESULTS AND DISCUSSION
The word algae are used to designate a large, varied, and heterogeneous group of organisms that, at present, don't have a clear-cut, formal taxonomic status. Some scientists have estimated that there might be between one and ten million completely different species, out and away the bulk of that haven't nevertheless been described. similar to plants, algae carry out photosynthesis, using sunlight to produce carbohydrates and energy.

3.1 Gas Produce
Gas production after 10 days anaerobic digestion showed that increased.

3.2 Chemical Characteristic and Composition
Gas composition was measurement every day. In table 3, we can see about the gas component for six days. Methane gas higher at 1st days and 2nd days.
Figure 1. Cumulative biogas vs methane yield production, and biogas mL/gVS vs methane mL/gVS

Table 1. Chemical Characteristic Dried seaweed biomass.

<table>
<thead>
<tr>
<th></th>
<th>VS (%)</th>
<th>TS (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried Biomass</td>
<td>46.14</td>
<td>81.19</td>
<td>35.05</td>
</tr>
</tbody>
</table>

Table 1. showed that dried biomass of the Sargassum contain have TS (81.19%), VS (46.14) and ash (35.05%) it means that seaweed is good for anaerobic digestion process.
Table 2. Chemical Characteristic after Anaerobic digestion.

<table>
<thead>
<tr>
<th>sample</th>
<th>pH</th>
<th>TS (%)</th>
<th>TS mean (%)</th>
<th>VS (%)</th>
<th>VS mean (%)</th>
<th>VS/TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>7.37</td>
<td>2.48</td>
<td>2.43</td>
<td>1.20</td>
<td>1.15</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.43</td>
<td></td>
<td>1.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.36</td>
<td></td>
<td>1.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>7.4</td>
<td>2.58</td>
<td>2.53</td>
<td>1.25</td>
<td>1.06</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.58</td>
<td></td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.42</td>
<td></td>
<td>0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>7.36</td>
<td>2.56</td>
<td>2.50</td>
<td>1.24</td>
<td>1.18</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.55</td>
<td></td>
<td>1.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.38</td>
<td></td>
<td>1.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Gas Composition.

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Biogas (mL)</th>
<th>Methane (%)</th>
<th>Carbon Dioxide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>523.3</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>426.7</td>
<td>78.0</td>
<td>22.0</td>
</tr>
<tr>
<td>3</td>
<td>273.3</td>
<td>63.7</td>
<td>36.3</td>
</tr>
<tr>
<td>6</td>
<td>196.7</td>
<td>81.7</td>
<td>18.3</td>
</tr>
</tbody>
</table>

4. CONCLUSION
In conclusion, the utilization of seaweed biomass in the anaerobic digestion process not only ensures the beach and sea look better to make tourism flourish, but also enhances the income from the biogas production.

ACKNOWLEDGMENT
We would like thank to my supervisor Kazutaka Umetsu who provided insight and expertise that greatly assisted the research.

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Study on the Characteristics of Micro Wet Milling and Spray Drying of Sea-buckthorn (\textit{Hippophae rhamnoides})

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Keywords: Sea-buckthorn juice, Micro wet milling, Particle size, Spray drying

Sea-buckthorn (\textit{Hippophae rhamnoides}) is by far the most widespread of the species in the genus, with the ranges of its eight subspecies extending from the Atlantic coasts of Europe across to Mongolia and China. Sea-buckthorn (SBT) contains different kinds of nutrients and bioactive compounds such as vitamins, carotenoids, flavonoids, polyunsaturated fatty acids, free amino acids, and elemental components. The aim of this study was to produce whole SBT powders by the application of micro-wet milling (MWM) and spray drying (SD) process. MWM was carried out by varying the different feeding rate of the material at 5, 10, 15 mL/min and rotational speed of the milling stone at 10, 20, 30, 40, 50 rpm respectively. Effective MWM was evaluated based on the obtaining minimum particle size of the whole SBT slurry. It was 5.84 m., which was obtained at 5 mL/min and 50 rpm operation. The antioxidant properties of SBT slurry by MWM showed higher than the commercial SBT juice. The conventional SBT juice contained 10% oil and was difficult to spray-dry without making a good emulsion. However, MWM process successfully produced a better emulsion of SBT slurry. Then it was spray-dried to make stable powder with the combination of maltodextrin as a carrier. The drying parameter was set as inlet temperature of 90, 110, 135° C, the outlet temperature of 55, 70, 88° C, feeding rate of 10 mL/min and atomizing pressure of 2.1 kg/cm\textsuperscript{2}. The spray drying successfully produced the whole SBT powder with 65.6% of total recovery (TS base). The obtained powder is going to be analyzed for moisture content, water activity, bulk density, tapped density, particle density, porosity, particle size distributions and microstructure of the particles. Further study will be carried out to apply vacuum spray drying or VSD for the production of whole SBT powder at lower drying temperature and compare with the conventional spray drying. It is expected that combinations of VSD and MWM could be applied industrially for the production of whole SBT powder.
Combined Effect of Pre-treatment and Vacuum Packaging for Maintaining the Quality of Peeled Shallot (*Allium ascalonicum* L.)

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**Keywords:** Pre-treatment, Microbial Quality, Ready to Use, Fresh-cut, Shallot

The effect of combined pre-treatment by heat treatment by hot water (HW) and acidified sodium chlorite (ASC) solution and vacuum packaging for maintaining the quality of minimally processed shallot (*Allium ascalonicum* L.) were evaluated during stored at 5±2 °C. The shallot were blanching in boiled water and cooled down immediately below 20±2 °C by using tap water. After that, the shallot were then peeled with a sharp stainless steel knife and dipped in citric acid pH 4 with 100 ppm of sodium chlorite as acidified sodium chlorite (ASC) solution for 10 min. The samples were place into the polyethylene bags as packaging materials, stored at 5±2°C for 9 days. The samples were dipped in tap water as the control. The microbial population (total bacteria and yeast and mold counts) and antioxidant qualities of minimally processed shallot were investigated and compared with the control. The results of the study revealed that dipping the peeled shallot with either HW combined ASC solution or pre-treatment with tap water could be reduce the microbial loads. The combined treatments had a powerful effect by decreasing the total bacteria and yeasts and molds during storage with the ranges of 0.30-0.71 and 0.38-0.54 log CFU.g⁻¹, respectively, which are lower than in the control samples. In addition, the combined treatments did not effect on weight loss and total phenolic content as compared to the control throughout the storage period. This results of this study suggest that HW combined ASC treatment has the potential to reduce microbial contamination and maintain the antioxidant capacity of peeled shallot.
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**ABSTRACT**

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**Keywords:** Pre-treatment, Microbial Quality, Ready to Use, Fresh-cut, Shallot

1. **INTRODUCTION**

Shallot (*Allium ascalonicum* L.), is a species of plant in the family *Alliaceae*. Shallots are the common name of an edible plant such as bulbous, herbaceous plant, which is similar to the onion (*Allium cepa* L.). Shallots are an important ingredient in food processing due to its healing properties and its distinctive aroma and flavor (Dron et al., 1997) and also, rich of source in antioxidant and antimicrobial properties (Leelarungrayub et al., 2006; Raeisi et al., 2016). The consumer’s demand for “minimally processed”, “lightly processed”, “ready to eat”, and “fresh-cut” fruits and vegetables has popularity increased (Cantwell and Suslow, 2002). The processing of fresh-cut produces requires such as washing, sorting, trimming, peeling, slicing, shredding or chopping does not affect the “fresh-like” quality of fruits and vegetables. However, the shelf life is usually decreased due to undesirable physiological changes, biochemical changes, and an increasing in microbial population. In particular, the peeling and cutting surface of the produces causing microbial contamination, and loss of the quality that makes the products unmarketable due to undesirable appearance. Many technologies are used to extend the shelf life of fresh produce for example, chlorinated water has been widely often used to eliminate food-borne pathogens and spoilage microorganism in fresh-cut produce (Guo et al., 2017, Huang and Chen, 2018) in freshly cut produce. However, the use of chlorinated water is a concern about the environmental and health impacts associated with the formation of halogenated products (Ölmez and Kretzschmar, 2009). Therefore, the alternative methods are used to control undesirable physiological changes and extend the shelf life of fresh-cut produces, especially by control microbial populations throughout the product shelf-life such as heat treatment. Heat treatment such as
hot water (HW) is one method of without using chemical to reduce food-borne pathogens and also to delay senescence (Funamoto et al., 2002) and HW also anti-browning reaction in fresh-cut apple cubes (Zuo et al., 2004). These method have been used successfully for preserving such as rocket leaves (Koukounaras et al., 2009), shredded carrot (Alegria et al., 2010) and fresh-cut broccoli florets (Renumarn et al., 2010; 2013; 2015) and fresh-cut onions (Siddiq et al., 2013). Acidified Sodium chlorite (ASC) is an oxidizing agent, which is effective and efficient alternative disinfectant chlorine (Cruz et al., 2006). According to the Food and Drug Administration (FDA), the application of ASC is able to the generate chlorine dioxide gas for use as an effective antimicrobial agent for disinfecting water and washing of fruits, vegetables and poultry (FDA, 2010). The FDA has allowed ASC use on fresh and fresh-cut produce in the range of 500–1200 ppm for spraying or dipping (FDA, 2000). ASC is an antimicrobial agent and inhibit the browning reaction on minimally processed in variety of fruit and vegetables such as apple, shredded carrot and broccoli florets (Lu et al., 2006, 2007; Ruiz-Cruz et al., 2006; Cruz et al., 2006; Renumarn et al., 2012). Moreover, ASC does not form carcinogenic products when compared with chlorine (Ruiz-Cruz et al., 2006). Therefore, the use of pre-treatment by to improve the treatment efficiency it is necessary to evaluated. The aim of this study was to investigate the influence of HW treatment combined with ASC solution to improve alternative sanitizing methods for minimizing the microbial loads and use of passive packaging (PP) vs. vacuum packaging (VP) to maintain the quality and antioxidant capacity of peeled shallot throughout storage.

2. MATERIALS AND METHODS
2.1 Plant Material and Treatments
The shallot (Allium ascalonicum L.) were purchased from local market in Prachinburi province, Thailand. Shallots were selected by considering the absence of any infected or damaged, as well as color and the uniformity of shape and size, and stored at room temperature (25±2°C) until the time of use. Experimental procedure began by blanching in boiled water (95±2°C) for 30 seconds and then cooled down immediately below 20±2°C by using tap water. After that, the shallot were then peeled and cutting top and stem-end by using a sharp stainless steel knife and then immersed in citric acid pH 4 with 100 ppm of sodium chlorite as acidified sodium chlorite (ASC) solution for 10 min followed by a potable water rinse. The excess water was removed by transfer samples to a sterile tissue paper. The minimally processed shallot that pre-washed with cold tap water for 2 min as described above were used as control. The minimally processed shallot (approximately 150 grams) were packed into polyethylene (PE) bags (150×200 mm, 30 µm thickness) using two different packing methods: passive modified atmosphere packaging (MAP) and vacuum packaging (VP). The packed samples were kept at 5±2°C for 9 days and randomly taken to evaluate changes in quality attributes, total phenolic content, antioxidant properties and microbial populations (total bacteria and yeasts and molds) every three days. Each treatment had three replicates (bags).

2.2 Microbial Populations
To measure the microbial populations of the shallot, a 25 g sample of Peeled Shallot was homogenized with 225 ml of 1% sterile peptone water using a Stomacher (Stomacher® 400 Circulator, England) for 1 min. Ten-fold dilution series were made in sterile peptone water as required for plating on (1) Plate Count Agar, PCA (HiMedia) incubated at 37±2°C for 24±3 h for determining the total bacteria count; (2) Potato dextrose agar (HiMedia) incubated at 28±2°C for 7 days for determining the yeasts and molds count. The microbial populations were expressed as log CFU g⁻¹ FW (colony forming units per gram of fresh weight). All measurements were performed in triplicate.

2.3 Weight Loss (%)
Fresh weight (FW) of each packaged shallot was measured on each sampling day. Weight loss was determined by weighing the shallot before and after the storage period. Weight loss in shallots was determined as the difference in weight of the sample before and after storage and expressed as percentage of weight loss (%)
2.4 Water Activity
The water activity of shallot samples was measured at 25°C by the AOAC 978.18 hygrometric method (AOAC, 1998).

2.5 Determination of Total Phenolic Content
The phenolic content was determined by Folin-Ciocalteau method (Roy et al., 2008) with slight modifications. First, 0.4 ml of sample extract were mixed with 2 ml of 10% Folin-Ciocalteau’s phenol reagent in a flask, vortexed an placed at room temperature for 10 min. Then, changing is in following conditions: A 7% sodium carbonate solution gets used instead of 5% one. Further, the absorbance in a spectrophotometer is detected on a wavelength of 765 nm. After homogenizing the solution out of ethanol with shallot samples in a blender machine and filtering the concentrate, 0.4 ml of this solution is added with 2 ml 10% Folin-Ciocalteau reagent and vortexed. The reaction is proceeded in the next 30 minutes in darkness before vortexing the tube filled with the reactants again. TPC values were determined from a calibration curve prepared with a series of gallic acid standards (0, 20, 40, 60, 80 and 100 mg/L). Results are expressed as mg of gallic acid equivalents/g fresh weight (mg GAE/g FW).

2.6 DPPH Scavenging Activity
The antioxidant capacity of sample extract were assayed by measuring the inhibition 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical of the sample described by Brand-Williams et al. (1995) and Thaipong, et al. (2006) with some modifications. The reduction of DPPH radicals was determined by measuring the absorption at 517 nm. The radical scavenging activity percentage was calculated following equation: DPPH radical scavenging (%) = [(A0 – A1)/A0] × 100, where A0 is the absorbance of the DPPH solution and A1 is the absorbance of the sample.

2.7 Statistical Analysis
Statistical analysis of all data was represented the mean ± standard mean error (SE) of at least three replications. Significant difference among the mean values was calculated using paired t tests with a significance level of, \( p \leq 0.05 \), and correlation test were performed on data using the statistic software SPSS.

3. RESULTS AND DISCUSSION
3.1 Effects of Pre-treatment on Microbial Populations
3.1.1 Total bacteria counts
The pre-treatment with HW and ASC solution (citric acid pH 4 with 100 ppm of sodium chlorite) for 10 min in vacuum packaging (VP) could be reduce the total bacterial and yeast and molds counts during storage at 5±2°C on day 9 (Table 1). The first day after treatment (day 0), total bacteria reduced from 4.30 log CFU/g FW (control) to 3.59 log CFU/g FW, respectively. However, pre-treatment with vacuum packaging showed the greatest control the number of total bacteria of 0.68-0.80 log CFU/g FW reduction during storage, which significantly compared to the control from initial day to day 9 of storage (Table 1). The counts of total bacteria slightly increased for all samples until the end of storage. The lowest amount of total bacteria was treated for HW and ASC with vacuum packaging as 4.57 log CFU/g FW whereas it was 5.00 log CFU/g FW for control with passive packaging at 9 day of storage.

Table 1. Effects of pre-treatment on total bacteria counts of peeled shallots stored at 5±2°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Package</th>
<th>1st day after treatment</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>VP</td>
<td>4.30±0.30^a</td>
<td>4.37±0.07^b</td>
<td>4.45±0.05^b</td>
<td>5.17±0.02^b</td>
</tr>
<tr>
<td>HW+ASC</td>
<td>VP</td>
<td>3.59±0.11^b</td>
<td>3.83±0.20^c</td>
<td>3.95±0.16^c</td>
<td>4.57±0.04^d</td>
</tr>
<tr>
<td>Control</td>
<td>PP</td>
<td>4.30±0.30^a</td>
<td>4.63±0.07^a</td>
<td>4.67±0.06^a</td>
<td>5.25±0.05^a</td>
</tr>
<tr>
<td>HW+ASC</td>
<td>PP</td>
<td>3.59±0.11^b</td>
<td>4.27±0.06^b</td>
<td>4.53±0.02^ab</td>
<td>5.00±0.05^c</td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different at \( p<0.05 \) for each column.

HW, hot water; ASC, acidified sodium chlorite; PP, passive packaging; VP, vacuum packaging.
3.1.2 Yeast and mold counts

The population of yeast and molds between the treatments, shown in Table 2. The peeled shallot for the control treatment had an initial yeast and molds count of 2.62 log CFU/g FW, while this number as 2.16 log CFU/g of pre-treatments in both of passive and vacuum packaging. Moreover, the minimum amount of yeast and molds was treated for HW and ASC with vacuum packaging as 3.35 log CFU/g FW whereas it was 3.94 log CFU/g FW for control with passive packaging at 9 day of storage, similar trend to those found in total bacteria.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Package</th>
<th>Yeast and molds counts (log CFU/g FW)</th>
<th>1st day after treatment</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>VP</td>
<td>2.62±0.15a</td>
<td>2.79±0.28ab</td>
<td>3.23±0.14b</td>
<td>3.91±0.06ab</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>VP</td>
<td>2.16±0.28b</td>
<td>2.30±0.30b</td>
<td>2.69±0.09c</td>
<td>3.35±0.05c</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PP</td>
<td>2.62±0.15a</td>
<td>3.25±0.08a</td>
<td>3.45±0.05a</td>
<td>3.94±0.03a</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>PP</td>
<td>2.16±0.28b</td>
<td>2.42±0.39b</td>
<td>3.43±0.08a</td>
<td>3.84±0.05b</td>
<td></td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different at p<0.05 for each column.

Results of microbial analysis indicated that the vacuum packaging enhanced the reduction of total bacteria and yeast and molds counts of peeled shallot, compared to passive packaging when subjected to the same treatment.

3.2 Weight Loss (%)

Weight loss in all peeled shallot samples were increased during the storage period, shown in Table 3. However, the percentage of weight loss of peeled shallot was no significant difference among peeled shallot samples, which was 0.093-3.627% throughout storage at 5±2°C. The noticeable changes in the weight loss of peeled shallot treated with pre-treatment and the type of packaging during the 9 days of storage are sufficient for their marketability. Weight loss is an important evaluation index for shelf life of fresh produce and leads to retail value of whole producer (Rivera-López et al., 2005). Therefore, the peeled shallot samples would remain marketable for more 9 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Package</th>
<th>Weight loss (%)</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>VP</td>
<td>0.210±0.01</td>
<td>0.173±0.21</td>
<td>0.240±0.21</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>VP</td>
<td>0.093±0.12</td>
<td>0.137±0.24</td>
<td>0.183±0.21</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PP</td>
<td>0.243±0.24</td>
<td>0.363±0.04</td>
<td>0.390±0.34</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>PP</td>
<td>0.280±0.26</td>
<td>0.333±0.18</td>
<td>0.327±0.38</td>
<td></td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different at p<0.05 for each column.

3.3 Water Activity

The water activity in all peeled shallot sample were determined at the beginning and end of storage, shown in Table 4. The water activity in shallots samples, which was 0.966-0.982 throughout storage at 5±2°C.
Table 4. Effects of pre-treatment on water activity ($a_w$) of peeled shallots stored at 5±2°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Package</th>
<th>Water activity ($a_w$)</th>
<th>1st day after treatment</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>VP</td>
<td>0.977±0.004</td>
<td>0.977±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.976±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.981±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>VP</td>
<td>0.966±0.001</td>
<td>0.980±0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.980±0.001&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.973±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PP</td>
<td>0.977±0.004</td>
<td>0.982±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.981±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.980±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>PP</td>
<td>0.966±0.001</td>
<td>0.979±0.001&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.978±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.977±0.001&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different at $p<0.05$ for each column.

HW, hot water; ASC, acidified sodium chlorite; PP, passive packaging; VP, vacuum packaging.

3.4 Total Phenolic Content

The total phenolic content of the control sample with PP and VP packaging were 18.58-25.55 and 18.58-27.50 mg GAE/g FW, respectively (Table 5). The phenolic contents of shallot was similar to those reported in different cultivars for shallot (*Allium oschaninii* L.), from 17.18 mg GAE/g FW (Lu et al., 2011). It was found that there was no significant ($p\geq0.05$) of total phenolic content in HW and ASC treated in peeled shallot with packed in different packaging, whereas VP packaging treatment resulted slightly higher of total phenolic content in peeled shallot as compared to PP packaging (24.14 vs. 20.97 mg GAE/g FW). Siddiq et al. (2013) report that stress (wounding, cutting) can induce increased synthesis of phenolic compounds. In addition, improving the efficiency of postharvest handling and selection of suitable containers can maintain the phenolic content of fresh produce.

Table 5. Effects of pre-treatment on total phenolic content of peeled shallots stored at 5±2°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Package</th>
<th>Total phenolic content (mg GAE/g FW)</th>
<th>1st day after treatment&lt;sup&gt;ns&lt;/sup&gt;</th>
<th>Day 3&lt;sup&gt;ns&lt;/sup&gt;</th>
<th>Day 6&lt;sup&gt;ns&lt;/sup&gt;</th>
<th>Day 9&lt;sup&gt;ns&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>VP</td>
<td>18.58±2.88</td>
<td>22.83±3.84</td>
<td>15.77±2.30</td>
<td>24.50±2.75</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>VP</td>
<td>22.80±1.18</td>
<td>24.18±1.33</td>
<td>13.71±2.02</td>
<td>24.14±1.61</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PP</td>
<td>18.58±2.88</td>
<td>23.26±2.76</td>
<td>15.34±1.23</td>
<td>25.55±2.60</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>PP</td>
<td>22.80±1.18</td>
<td>24.37±0.06</td>
<td>14.03±2.47</td>
<td>20.97±2.99</td>
<td></td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different at $p<0.05$ for each column.

HW, hot water; ASC, acidified sodium chlorite; PP, passive packaging; VP, vacuum packaging.

3.5 DPPH Scavenging Activity

The DPPH scavenging activity are presented in Table 6. The peeled shallot samples of control with PP and VP packaging contained the DPPH scavenging activity of 63.85-97.68%. While, the peeled shallot treated HW+ASC with PP and VP packaging contained the DPPH scavenging activity of 88.71-108.60%. The results showed that the levels of the antioxidant capacity changed depending on the sanitizer of pre-treatment and type of package. Compared to day 9, the peeled shallot treated HW+ASC with PP packaging had a higher content of antioxidant capacity than those treatment.

Table 6. Effects of pre-treatment on DPPH scavenging activity (%) of peeled shallots stored at 5±2°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Package</th>
<th>DPPH Scavenging Activity (%)</th>
<th>1st day after treatment&lt;sup&gt;ns&lt;/sup&gt;</th>
<th>Day 3&lt;sup&gt;ns&lt;/sup&gt;</th>
<th>Day 6&lt;sup&gt;ns&lt;/sup&gt;</th>
<th>Day 9&lt;sup&gt;ns&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>VP</td>
<td>87.94±4.59</td>
<td>97.68±2.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.10±4.67</td>
<td>63.85±10.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>VP</td>
<td>88.71±13.75</td>
<td>100.22±2.57</td>
<td>98.21±3.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.10±7.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PP</td>
<td>87.94±4.59</td>
<td>89.90±2.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.28±13.89</td>
<td>95.80±7.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HW+ASC</td>
<td>PP</td>
<td>88.71±13.75</td>
<td>91.11±1.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>98.87±1.23</td>
<td>106.72±1.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different at $p<0.05$ for each column.

HW, hot water; ASC, acidified sodium chlorite; PP, passive packaging; VP, vacuum packaging.

5
4. CONCLUSION
In conclusion, minimally processed operations, including washing and sanitizing procedures, are critical to ensuring food safety of the final products and moreover, for extended the shelf-life during distribution. ASC treatment at citric acid pH 4 and 100 ppm of sodium chlorite as acidified sodium chlorite (ASC) solution for 10 min had the effect of reducing the number of microorganisms (total bacteria, yeast and molds) and maintaining total phenolic content of minimally processed shallots during storage for 9 days. However, this treatment is not sufficient to reduce the microbial load.

ACKNOWLEDGMENT
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REFERENCES


High pressure processing of ‘Nanglae’ pineapple juice: Quality preservation and shelf life extension

Nuntawan Chuensombat1, Natthakan Rungraeng1, Sutthiwal Setha1,2, *Phunsiri Suthiluk1,2 (1. School of Agro-Industry, Mae Fah Luang University, Chiang Rai, THAILAND(Thailand), 2. Research Group of Postharvest Technology, School of Agro-Industry, Mae Fah Luang University, Chaing Rai, THAILAND(Thailand))

Keywords: Bioactive compounds, Fruit juice, High hydrostatic pressure

Quality changes and shelf life of high pressure processed (HPP) ‘Nanglae’ pineapple juice were compared to fresh and conventional pasteurized (CP) juices during storage at 5±1°C. A hundred percentage of fresh ‘Nanglae’ pineapple juice was pressure processed at 400 or 600 MPa for 5, 10 or 15 min and stored at 5±1°C for up to 60 days. The pasteurized condition of 80°C for 10 min was used as a control. Changes in pH, total soluble solid (TSS), titratable acidity (TA), color (L* and b*), bioactive compounds (Ascorbic acid, total carotenoid and total phenolic compounds), antioxidant activities (DPPH and FRAP assay) and microbiological quality (Aerobic plate count (APC) and yeast and mold count (YM)) were determined every 5 days until the end of storage time. It was found that pH, TSS, TA and color was no significant different (P>0.05) among HPP juice. After treatment, higher ascorbic acid and total carotenoid content was observed in HPP pineapple juice in a range of 4.72-6.09 and 0.38- 0.41 mg/100 ml, respectively while in CP juice was 2.36+0.59 and 0.31+0.01 mg/100 ml. Moreover, total phenolic compounds content in sample treated with 400 and 600 MPa HPP for 5 min was significantly higher than CP sample (45.45+0.49, 47.82+0.35 and 41.00+1.68 mg GAE/100 ml, respectively). The highest FRAP value was also found in sample treated with HPP at 400 and 600 MPa for 5 min as 709.00+7.37 and 692.50+9.01 mol FeSO4/100 ml while there was no significant different (P>0.05) in DPPH value of all samples. In addition, HPP at 600 MPa for 5 min decreases APC and YM to be less than 1.48+0.00 and 1.18+0.00 log CFU/ml which was similar to CP treatment. Shelf life of HPP ‘Nanglae’ pineapple juice was estimated about 60 days at 5±1°C limited by juice precipitation. Therefore HPP could be an alternative to pasteurization for juice production which preserve nutritional value and organoleptic properties as well as maintain quality and safety of product.
[6-1130-P-01] Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds
*Pairote Wongputtisin¹, Narin Lahsom¹ (1. Program in Biotechnology, Faculty of Science, Maejo university, Chiang mai, Thailand (Thailand))
11:30 AM - 12:30 PM

[6-1130-P-02] Effect of Sucrose and Glucose on Coffee Kombucha Carbonation
*Chutamas Maneewong¹, Thittaya Choompoosee¹ (1. Department of Biotechnology, Faculty of Science, Maejo University, San Sai, Chiang Mai 50290(Thailand))
11:30 AM - 12:30 PM

[6-1130-P-03] Evaluation of Total Anthocyanins and Antioxidant Activity of Thai Rice Cultivars for Phenotypic Selection in Rice Breeding
*Chotipa Sakulsingharoj¹, Lalita Na Rachasima¹, Anongnad Richinda¹, Pairote Wongputtisin², Runghip Kawaree², Saengtong Pongjaroenkit¹, Varaporn Sangtong¹ (1. Program in Genetics, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand), 2. Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand))
11:30 AM - 12:30 PM

[6-1130-P-04] Investigation of some biological activities of local shallot (Allium ascalonicum Linn.) extract from Thailand
*Premruethai Phansaard¹, Pairote Wongputtisin¹ (1. Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand))
11:30 AM - 12:30 PM

[6-1130-P-05] Probiotic characterization of thermotolerant Lactobacillus johnsonii isolated from broiler intestine
*Rutaimas Wongpanti¹, Pairote Wongputtisin¹, Piyanuch Niamsup¹ (1. Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai(Thailand))
11:30 AM - 12:30 PM

[6-1130-P-06] Process optimization for antioxidant extraction from seed of soybean cultivar Chiang mai60
*Arpatsara Seekoompa¹, Pairote Wongputtisin¹, Piyanuch Niamsup¹ (1. Program in Biotechnology, Faculty of science, Maejo University, Chiang mai(Thailand))
11:30 AM - 12:30 PM

[6-1130-P-07] Nutritional and Functional Properties of Yoghurt Drink with Philippine Gac (Momordica cochinchinensis Spreng.) and Bignay (Antidesma bunius) Fruits
Rowie Joy Gonzales Bucks¹, *Ara Fatima Cuvinar Algar¹, Ryan Rodrigo Paner Tayobong² (1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines), 2. Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines))
11:30 AM - 12:30 PM
[6-1130-P-08] Effect of Extracting Conditions on Plant Extract Colors and Stability of Antioxidant Properties during in vitro Gastrointestinal Digestion
*Rattika Aeka¹, Titikan Liangpanth¹, Rungarun Sasanaatayart¹ (1. School of Agro-Industry, Mae Fah Luang University(Thailand))
11:30 AM - 12:30 PM

[6-1130-P-09] pH Adjustment and Thermal Treatments Affect Plant Extract Colors and Antioxidant Activities during in vitro Digestion
*Baifah Sangarun¹, Titikan Liangpanth¹, Rungarun Sasanaatayart¹ (1. School of Agro-Industry, Mae Fah Luang University(Thailand))
11:30 AM - 12:30 PM

[6-1130-P-10] Changes in the Growth and Antioxidant Components of Komina with Different Red and Blue Light Emitting Diode (LED) Irradiation Ratios
Kanako Niiya¹, *Takahiro Saito², Masatsugu Tamura², San Woo Bang² (1. Utsunomiya University Graduate School(Japan), 2. Utsunomiya Univ.(Japan))
11:30 AM - 12:30 PM
Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds

*Pairote Wongputtisin¹, Narin Lahsom¹ (¹ Program in Biotechnology, Faculty of Science, Maejo university, Chiang mai, Thailand (Thailand))

Keywords: Groundnut, Arachis hypogaea, Raffinose Family Oligosaccharides, Prebiotic, Probiotic, Functional Food

Raffinose family oligosaccharides (RFOs) have been accepted as an effective prebiotic substance. They can be generally found in various leguminous seeds. Thus, legume seeds can be considered as promising sources of prebiotic ingredient for development of functional foods. The aims of this work were analysis of RFOs composition in local groundnut (Arachis hypogaea L.) of Thailand and primary investigation for their prebiotic potential. In this study, low molecular weight sugars (LMWSs) including RFOs were extracted from seeds of three local groundnut cultivars in Thailand, i.e. Tainan 9, Khonkean 5 and Khonkean 6, using 50% (v/v) ethanol. LMWSs were qualified and quantified by HPLC apparatus and subsequently investigated for their capacity in growth stimulation of some enteric bacteria. The results showed that these cultivars contained LMWSs approximately 28-40 mg/g dry seed and the average size of sugars in term of degree of polymerization (DP) ranged between 2 and 7. These seeds contained low amount of raffinose and verbascose, while high amount of stachyose was found at 3.9-11.7 mg/g dry seed. Growth of probiotic Lactobacillus acidophilus TISTR1338, L. plantarum TISTR541 and L. lactis TISTR1464 were stimulated significantly in basal media containing groundnut LMWSs (p<0.05), while growth of Salmonella enterica serovar Typhimurium TISTR292 and Escherichia coli were not stimulated. Interestingly, growth of S. Typhimurium and E. coli were suppressed when was co-cultured with those Lactobacillus sp. in basal media contained groundnut LMWSs as a carbon source. Thus, it might be concluded that ethanolic sugar extracted from seeds of Tainan 9, Khonkean 5 and Khonkean 6 exhibited the primary properties to be accepted as prebiotic substance.
Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds

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Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai, Thailand 50290

*corresponding author: pairotewong@gmail.com

ABSTRACT
Raffinose family oligosaccharides (RFOs) have been accepted as an effective prebiotic substance. They can be generally found in various leguminous seeds. Thus, legume seeds can be considered as promising sources of prebiotic ingredient for development of functional foods. The aims of this work were analysis of RFOs composition in local groundnut (Arachis hypogaea L.) of Thailand and primary investigation for their prebiotic potential. In this study, low molecular weight sugars (LMWSs) including RFOs were extracted from seeds of three local groundnut cultivars in Thailand, i.e. Tainan 9, Khonkean 5 and Khonkean 6, using 50% (v/v) ethanol. LMWSs were qualified and quantified by HPLC apparatus and subsequently investigated for their capacity in growth stimulation of some enteric bacteria. The results showed that these cultivars contained LMWSs approximately 28-40 mg/g dry seed and the average size of sugars in term of degree of polymerization (DP) ranged between 2 and 7. These seeds contained low amount of raffinose and verbascose, while high amount of stachyose was found at 3.9-11.7 mg/g dry seed. Growth of probiotic Lactobacillus acidophilus TISTR1338, L. plantarum TISTR541 and L. lactis TISTR1464 were stimulated significantly in basal media containing groundnut LMWSs (p<0.05), while growth of Salmonella enterica serovar Typhimurium TISTR292 and Escherichia coli were not stimulated. Interestingly, growth of S. Typhimurium and E. coli were suppressed when was co-cultured with those Lactobacillus sp. in basal media contained groundnut LMWSs as a carbon source. Thus, it might be concluded that ethanolic sugar extracted from seeds of Tainan 9, Khonkean 5 and Khonkean 6 exhibited the primary properties to be accepted as prebiotic substance.

Keywords: Groundnut, Arachis hypogaea, Raffinose Family Oligosaccharides, Prebiotic, Probiotic, Functional Food
1. INTRODUCTION

Raffinose family oligosaccharides (RFOs) are oligosaccharides widely found in leguminous seeds. They are α-galactosyl derivative of sucrose linked with α(1→6) bond. The major member of RFOs are raffinose, stachyose and verbascose, which their chemical structures are shown in Figure 1. Biosynthesis of raffinose in legume seeds proceeds by transferring of galactosyl residue (donor) from galactinol (O-α-D-galactopyranosyl-(1→1)-L-myo-inositol) to sucrose (acceptor) by the action of raffinose synthase. Subsequently, stachyose synthase transfers another one or two galactosyl residue from galactinol to raffinose molecule, resulting of stachyose and verbascose, respectively (Peterbauer et al., 2002; Karner et al., 2004). The RFOs content in various leguminous seeds; i.e. soybean, lupin, chickpea, mung bean, pigeon pea, jack bean, lentil and groundnut has been reported (Muzquiz et al., 1999; Kadlec, 2001; Martinez-Villaluenga et al., 2005; Giannoccaro et al., 2006; Xiaoli et al., 2008; Kumar et al., 2010). In case of groundnut (Arachis hypogaea L.), variation of RFOs in different cultivars was reported by other research groups. However, those of local groundnut cultivars in Thailand have not been investigated yet. These sugars play an important role in seed by involving in defense mechanism of some abiotic stresses; low temperature, drought, high salinity and oxidative stress (ElSayed et al., 2014). However, these oligosaccharides have been reported as an effective prebiotic substance for human and animal too. The term “prebiotics” was firstly introduced by Gibson and Roberfroid in 1995 and presently, its definition has been modified, for example “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Gibson et al., 2004)” and “live micro-organisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002)”. According to these concepts, non-digestible oligosaccharides (NDOs) such as fructooligosaccharide (FOS), galactooligosaccharide (GOS), isomaltooligosaccharide (IMO), xylooligosaccharide (XOS), human milk oligosaccharide (HMO) and raffinose family of oligosaccharides (RFO) are accepted as prebiotic (Ziemer and Gibson, 1998; Chow, 2002; Mussato and Mancilha, 2007).

There were some evident that groundnut originated from South America before spreads to other regions, including Thailand. Groundnuts, cultivar Tainan9, Khonkean5 and Khonkean6 are the examples of popular and widespread groundnuts in Thailand. In this study, RFOs composition in seed of these cultivars were quantified. Subsequently, primary prebiotic properties of seed extract containing RFOs were investigated, with respect to growth stimulation ability to 3 probiotics strains; i.e. Lactobacillus lactis, L. acidophilus and L. plantarum, and also normal flora Escherichia coli and pathogenic Salmonella Typhimurium. The aim of this study was to introduce the prebiotic property of local groundnuts from Thailand, the other functionality apart from consuming as a protein and oil food.

2. METHODOLOGIES

2.1 Groundnut seeds
Seeds of three groundnut cultivars; Tainan9, Khonkean5 and Khonkean6, were kindly obtained from Field Crop Research Center, Thailand, and stored in vacuumed plastic bag at 4 °C.

2.2 Microorganisms
All tested bacteria were from the Thailand Institute of Scientific and Technological Research (TISTR). There are totally three probiotic strains; including Lactobacillus plantarum TISTR541, L. lactis TISTR464 and L. acidophilus TISTR1338. The normal flora and pathogenic strains used in this study were Escherichia coli TISTR887 and Salmonella enterica serovar Typhimurium TISTR292, respectively. Probiotics were maintained on MRS agar, while E. coli and S. Typhimurium were maintained on nutrient agar.
2.3 RFOs-rich extract preparation
The crude extract containing low molecular weight sugar (LMWS) and rich of RFOs was prepared from ground and dried seeds according to the modified method of Xiaoli et al. (2008). Ground seed was defatted using hexane and mixed with 50% (v/v) ethanol with the ratio of 3 g : 50 ml. The mixture was continuously shaken for 1 hr at 30 °C and then filtered through filter paper (Whatmann® No. 1). The obtained filtrate was subsequently centrifuged at 8,000 rpm for 10 min at 4°C to remove the remaining particles. Supernatant was concentrated using rotary vacuum evaporator (Buchi®) under the temperature below 50°C.

2.4 Analysis of sugars
Reducing sugar and total sugar and in the extract was determined by DNS and phenol-sulfuric acid method; respectively. Size of sugar, in term of an average degree of polymerization (DP) was calculated by the ratio between total sugar and reducing sugar content. Quantity of some LMWSs were analyzed using high performance liquid chromatography (HPLC) apparatus, consisting of 5 µm Previal Amino column (Alltech®), series III HPLC pump and Evaporative Light Scattering Detector (ELSD) (Alltech®). The column temperature was controlled at 30±1 °C during analysis. Acetonitrile: deionized water (75: 25) was used as mobile phase at the flow rate of 1.0 ml/ min. The injection volume was 20 µl and all samples were filtered through nylon membrane (VERTICAL®) (0.45 µm) prior injection. HPLC grade of glucose (Fluka®), sucrose (Fluka®), raffinose (MERCK®), stachyose (ALDRICH®) and verbascose (Fluka®) were used as standard sugars.

2.5 Primary prebiotic properties of RFOs-rich extracts
Growth stimulation of individual bacteria by groundnut sugar extracts were investigated. The extract was supplemented in basal medium (g/ L: 0.3 K2HPO4, 0.1 KH2PO4, 1.0 yeast extract, 1.0 peptone, 0.2 MgSO4, and 2.5 (NH4)2SO4, pH 7.0) as a carbon source at a concentration of 1% (w/v). Approximately 108 CFU of 24 hr-old inoculum of tested bacterium was transferred to 100 ml sterilized basal medium and statically incubated in anaerobic jar for 24 hr and at 37°C. Viable cell (CFU/ml) of probiotics, Sal. Typhimurium and E. coli was enumerated on De Man, Rogosa and Sharpe agar (MRS) (Himedia®), Salmonella – Shigella agar (SS agar) (Himedia®) and Eosin methylene blue agar (EMB agar) (Himedia®); respectively. The growth dynamic of each bacterium in defined-mixed culture was also studied. Total 109 CFU of 3 probiotic strains (~3.3 x 107 CFU for each strain), 108 CFU of S. Typhimurium and 108 CFU of E. coli were transferred as a mixed inoculum to 100 ml basal medium supplemented with groundnut sugar extract. The culture conditions were as described in previous experiment. The bacterial population were monitored.
at 0, 12 and 24 hr of cultivation. In both experiment, basal media with glucose as a carbon source and without carbon source were used as control treatments.

2.6 Statistical analysis
All experiments were performed in triplicate. STATISTIX© software version 9 was used to analyze the significant difference between treatments.

3. RESULTS AND DISCUSSION
3.1 Sugar analysis
The ethanolic sugar extract from seed of three groundnut cultivars composed different amount of soluble LMWSs between 2.82-4.00 g/100g dry seed, while soluble reducing sugar contents were between 0.48-1.71 g/100g dry seed. Then, the average size of LMWSs from all cultivars in term of DP were found in the range of short chain oligosaccharides (Table 1). The results from HPLC were also showed that these three groundnut seeds contained low amount of raffinose and verbascose, while high amount of stachyose was found at 0.39-1.17 g/100g dry seed. Moreover, low molecular weight; i.e. glucose and sucrose were also detected (Table 1). Sucrose was found in these groundnuts with remarkably large proportion similar to other groundnut cultivars previously reported as shown in Table 2, correlating to their sweet attributes. Comparing to other leguminous seeds, sucrose and total RFOs composition were not much different (Muzquiz et al., 1999; Ekvall et al., 2007; Xiaoli et al., 2008; Saldivar et al., 2010; Wongputtisin et al., 2015). However, content of these sugars can be variable, depending on genetic and environmental factors, i.e. vegetation time, storage time, temperature and packaging as earlier reported in lupin and soybean seed by Trugo et al. (1988) and Saldivar et al. (2010).

3.2 Prebiotic properties of ethanolic extract from groundnut seeds
The results showed that growth of three probiotic strains were promoted after 24 hr cultivation in broth supplemented with ethanolic extract containing RFOs from groundnut seeds (p<0.05), especially *L. lactis*, as shown in Figure 2. Considering on the basal medium with glucose, the most common monosaccharide for microorganism to utilize, we found lesser growth than using groundnut extract as carbon source. On the other hand, groundnut extracts did not promote growth of *S. Typhimurium* and *E. coli* (Figure 2). We also found the obvious inhibitory effect on growth of *E. coli* by the extracts of Khonkean5 and Khonkean6 (p<0.05).

Table 1. Sugar content in seeds of three cultivars of groundnut, Tainan 9, Khonkean 5 and Khonkean 6

<table>
<thead>
<tr>
<th>Soluble sugar content (g/100 g dry seed)</th>
<th>Cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tainan 9</td>
</tr>
<tr>
<td>total sugar</td>
<td>3.63±0.46</td>
</tr>
<tr>
<td>reducing sugar</td>
<td>1.71±0.24</td>
</tr>
<tr>
<td>degree of polymerization</td>
<td>2.2</td>
</tr>
<tr>
<td>glucose</td>
<td>0.16±0.04</td>
</tr>
<tr>
<td>sucrose</td>
<td>1.48±0.05</td>
</tr>
<tr>
<td>raffinose</td>
<td>0.01±0.02</td>
</tr>
<tr>
<td>stachyose</td>
<td>0.56±0.034</td>
</tr>
<tr>
<td>verbascose</td>
<td>trace</td>
</tr>
</tbody>
</table>

Table 2. comparison of LMWSs detected in some leguminous seeds
### Legumes

<table>
<thead>
<tr>
<th></th>
<th>Sucrose</th>
<th>Raffinose</th>
<th>Stachyose</th>
<th>Verbascose</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundnut</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 Spanish cultivars</td>
<td>2.44 – 7.61</td>
<td>0.17 – 1.56 (total RFOs)</td>
<td></td>
<td></td>
<td>Bishi et al. (2013)</td>
</tr>
<tr>
<td>40 Indian cultivars</td>
<td>2.61 - 6.50</td>
<td>0.01 - 0.12</td>
<td>0.11 - 0.67</td>
<td>0.00 - 0.07</td>
<td>Bishi et al. (2014)</td>
</tr>
<tr>
<td>30 Spanish cultivars</td>
<td>2.79 – 5.33</td>
<td>0.02 – 0.06</td>
<td>0.35 – 0.79</td>
<td>No report</td>
<td>Mahatma et al. (2016)</td>
</tr>
<tr>
<td>30 Virginia cultivars</td>
<td>3.85 – 6.90</td>
<td>0.04 – 0.16</td>
<td>0.46 – 1.03</td>
<td>No report</td>
<td>Mahatma et al. (2016)</td>
</tr>
<tr>
<td>3 Thai cultivars</td>
<td>0.81 – 1.48</td>
<td>0.00 – 0.04</td>
<td>0.39 – 1.17</td>
<td>0.00 – 0.05</td>
<td>This study</td>
</tr>
<tr>
<td>Soybean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiang mai60</td>
<td>1.32</td>
<td>0.67</td>
<td>14.53</td>
<td>0.16</td>
<td>Wongputtisin et al. (2015)</td>
</tr>
<tr>
<td>V95-7456</td>
<td>4.96</td>
<td>0.64</td>
<td>3.77</td>
<td>No report</td>
<td>Saldivar et al. (2010)</td>
</tr>
<tr>
<td>Vine pea</td>
<td>No report</td>
<td>0.29</td>
<td>0.14</td>
<td>0.13</td>
<td>Ekvall et al. (2007)</td>
</tr>
<tr>
<td>Lupin</td>
<td>16.2</td>
<td>19.0</td>
<td>54.0</td>
<td>10.8</td>
<td>Muzquiz et al. (1999)</td>
</tr>
<tr>
<td>Chick pea</td>
<td>2.56</td>
<td>0.89</td>
<td>2.38</td>
<td>0.42</td>
<td>Xiaoli et al. (2008)</td>
</tr>
</tbody>
</table>

The change in population of total probiotics, *S. Typhimurium* and *E. coli* in defined-mixed culture experiment were illustrated in Figure 3. It was found that survivability of all bacteria declined along with cultivation time in broth without carbon source, while growth of total probiotic and *E. coli* increased non-significantly and that of *S. Typhimurium* was not significantly changed when glucose was used as carbon source. The interesting results were found in treatment of Tainan9 extract addition. Sugar extract of this cultivar was able to promote growth of total probiotics, resulting in decreasing of *E. coli* and *S. Typhimurium* survivals markedly. Sugar extracts of Khonkean5 and Khonkean6 also gradually enhanced total probiotic growth but not obviously different. However, inhibitory effect on growth of *E. coli* and *S. Typhimurium* still could be observed. The growth pattern of probiotic strains and *S. Typhimurium* in media with groundnut sugar extracts were consistent with the results from single culture study. From all results above, it was clear that sugar extract from groundnuts could stimulate all tested probiotic strains but not for *E. coli* and pathogenic *S. Typhimurium*. This characteristic is considered as an important primary property prior accepted as prebiotic substance. Probiotic growths could be from both RFOs, which were major sugars in the extract, and the other LMWSs; i.e. glucose and sucrose. To utilize RFOs, bacterial cell required α-galactosidase to hydrolyze α linkage and raffinose delivery system into cell. Mechanisms of RFO utilization in Bifidobacterium and Lactobacilli probiotics were also reported by Hachem et al. (2012). Glycoside hydrolase family 36 (GH36) α-galatosidase encoding genes, sugar transport systems of the glycoside – pentoside – hexuronide cation symporter family (GPH), sugar phosphotransferase systems (PTSs) or ATP-binding cassette systems (ABCs) are key factors. Schmid and Schmitt (1976) reported that *E. coli* cells lack of raffinose delivering system. Moreover, there have been no report on the activity of α-galactosidase in *S. Typhimurium* and *E. coli*, while that was reported in three Lactobacilli used in this study (Donkor et al., 2007; Sumarna, 2008; LeBlanc et al., 2004; Fredslund et al., 2011; Silvestroni et al., 2002; Jeong et al., 2008). Thus, there was high possibility that growth of *E. coli* and *S. Typhimurium* observed in this work were from LMWSs not from RFOs. The expected results were obtained in media added by groundnut sugar extracts. Promoted probiotic population subsequently exhibited the inhibitory effect on *E. coli* and *S. Typhimurium* growth. The mechanisms involved might be commonly explained that lactic acid bacteria produce various inhibitors, for example, organic acids (lactate and acetate), short chain fatty acids, hydrogen peroxide and bacteriocins (lactacin B, lactacin F and acidocin CH5, nisin and lactocin S (Parada et al., 2007; Vrese and Schrezenmeir, 2008; Zhou et al., 2010; Gao et al., 2019).
Figure 2. Growth of single tested strains in basal medium supplemented with different carbon source when cultivating for 0 hour (□) and 24 hours (■). The (*) in each experiment indicates significant difference at p<0.05.
4. CONCLUSION
From all of the results above, the extract prepared from groundnut seeds cultivar Tainan9, Khonkean5 and Khonkean6 showed a potential to be source of an effective prebiotic substance and preliminary exhibited the prebiotic properties by promote growth of probiotic strains; resulting in inhibition of pathogenic growths. Thus consuming of groundnut seed may help to improve the bacterial balance in gastrointestinal tract and receiving of many advantages from grown probiotics. Moreover, synbiotic food containing groundnut RFOs and selective probiotics can be manufactured and promoted as functional foods.
REFERENCES
Giannoccaro, E., Y. Wang, and P. Chen. 2006. Effects of solvent, temperature, time, solvent-to-sample ratio, sample size and defatting on the extraction of soluble sugars in soybean. Journal of Food Science, 71: Published on web.


Effect of Sucrose and Glucose on Coffee Kombucha Carbonation

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Keywords: kombucha, carbonation, fermented beverage, coffee, functional food

Kombucha is a functional food and a traditional carbonated soft drink. Natural carbonation is formed by microorganism during kombucha fermentation. However, coffee kombucha has a lower gas when compared to tea kombucha. The objectives of this study were to investigate effect of sugars on increasing gas formation and evaluate sensory characteristics of the coffee kombucha. The sugars including sucrose, glucose and mixture of sucrose and glucose were studied. The results found that the mixture of sucrose 5 % (w/v) and glucose 5 % (w/v) aerobically fermenting for 4 days, and then continuously fermenting in the closed container (without aeration) for 5 days revealed highest gas production. Acidity of the product was pH 3.14 and total acid 7.44% (v/v). The number of yeast, lactic acid bacteria and total bacteria in the product were 7.8, 6.8 and 6.7 log CFU/ml, respectively. Additionally, sensory characteristics were evaluated, overall acceptance, carbonation and mouthfeel were marked with 6.96 ± 0.49, 6.67±0.92 and 7.16±0.63, respectively.
Effect of Sucrose and Glucose on Coffee Kombucha Carbonation

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ABSTRACT
Kombucha is a functional food and a traditional carbonated soft drink. Natural carbonation is formed by microorganism during kombucha fermentation. However, coffee kombucha has a lower gas when compared to tea kombucha. The objectives of this study were to investigate effect of sugars on increasing gas formation and evaluate sensory characteristics of the coffee kombucha. The sugars including sucrose, glucose and mixture of sucrose and glucose were studied. The results found that the mixture of sucrose 5 % (w/v) and glucose 5 % (w/v) aerobically fermenting for 4 days, and then continuously fermenting in the closed container (without aeration) for 5 days revealed highest gas production. Acidity of the product was pH 3.14 and total acid 7.44% (v/v). The number of yeast, lactic acid bacteria and total bacteria in the product were 7.8, 6.8 and 6.7 log CFU/mL, respectively. Additionally, sensory characteristics were evaluated, overall acceptance, carbonation and mouthfeel were marked with 6.96 ±0.49, 6.67±0.92 and 7.16±0.63, respectively.

Keywords: Kombucha, Carbonation, Fermented beverage, Coffee, Functional food

1. INTRODUCTION
Kombucha is a fermented functional beverage, which has slightly acidic, carbonated and sweet taste. Most common substrate for kombucha fermentation is tea. kombucha is obtained from tea leaves by the fermentation of a symbiotic association of bacteria and yeasts (Chen and Liu, 2000). kombucha tea is prepared by placing the SCOBY (symbiotic culture of bacteria and yeast) into a sugared tea broth for fermentation. The taste of the kombucha changes during fermentation from a pleasantly fruity sour-like sparkling flavor after a few days to a mild vinegar-like taste after a long incubation period (Jayabalan et al., 2014). Yeasts in kombucha hydrolyze sucrose into glucose and fructose by invertase and produce ethanol. Acetic acid bacteria use glucose to produce gluconic acid and ethanol to produce acetic acid. The pH value of kombucha beverage decreases due to the production of organic acids during fermentation (Dutta and Gachhui, 2006). Acetic acid and gluconic acids are major organic acids that are produced from kombucha fermentation. Microorganism in kombucha, acetic acid bacteria: Gluconacetobacter europaeus, Gluconobacter oxydans, G. saccharivorans and Acetobacter peroxydans emerged as dominant species. Yeasts were mainly identified as Dekkera, Hanseniaspora and Zygosaccharomyces during all fermentations (Coton et al., 2017).

Coffee is one of the most popular beverages worldwide. There are different kinds of coffee beverages, coffee kombucha is fermented coffee with SCOBY. The coffee kombucha generally use 5-10 % (v/v) sucrose as a substrate for fermentation, these can produce acid but low gas formation. Thus, the objective of this study was to enhance carbonation in coffee kombucha by comparing sugars such as sucrose and glucose with different concentration for increasing gas formation. Sensory evaluation of the coffee kombucha was also tested.
2. MATERIALS AND METHODS
2.1 Materials
Arabica roasted coffee was provided from Thai Lahu Coffee and Tea Co., Ltd, Chiang Mai, Thailand.
SCUBY (Symbiotic Culture of Bacteria and Yeast) was obtained from Jib-Kefir shop, Bangkok, Thailand.

2.2 Methods
2.2.1 Preparation of coffee
Arabica roasted coffee 40 g was added to boiling water 4 L for 5 min, the ground coffee was removed. Sucrose 200 g was dissolved in the hot coffee and heated at 100 °C for 10 min. The coffee was allowed to cool at room temperature (30 °C) before fermentation.

2.2.2 Coffee kombucha fermentation
The coffee was poured into a wide-mouthed clean vessel. The SCBY was added to the coffee (200 g SCBY/ 4 L coffee) and left to ferment at room temperature. First fermentation, the coffee was fermented for 4 days in the covering jar with cloth (this period required oxygen for obligate aerobic microorganism). To end the first fermentation, the SCBY was removed from the kombucha. The kombucha was poured into the bottles and tightly capped for secondary fermentation for 5 days and then stored at 4 °C for 5 days.

2.2.3 Sugars for kombucha fermentation
Sucrose and/or glucose with different concentration: 5 % sucrose, 7 % sucrose, 10 % sucrose, 5 % glucose and mixture of 5 % glucose and 5 % sucrose were used as carbon sources for coffee kombucha fermentation. The broth samples were analyzed pH, acidity and number of microorganism. Gas formation volume was measured at the end of fermentation.

2.3 Analysis
2.3.1 Acidity
To study acid production during fermentation, acidity was determined by titrating 50 mL of samples against 0.1 N NaOH. The pH of samples were determined by a pH meter.

2.3.2 Number of microorganism
In order to numerate total bacterial counts, liquid samples were serially diluted with normal saline and plated on plate count agar and then incubated for 72 h at 30°C. Lactic acid bacteria (LAB) were enumerated on De Man Rogosa Sharpe (MRS), incubated at 30°C under anaerobic conditions for 72 h. Yeast and fungi were numerated on potato dextrose agar, incubated at 25°C for 72 h.

2.3.3 Sensory Evaluation
Sensory characteristics of the coffee kombucha were tested using 9-Point Hedonic Scale from 30 subjects. Characteristics of the kombucha: coffee smell, sweetness, sourness, sparkling taste, mouthfeel and overall acceptance were evaluated.

3. RESULTS AND DISCUSSION
3.1 Sugars for gas and acid formation in coffee kombucha
Coffee kombucha usually use sucrose as a carbon source for fermentation. For the Arabica roasted coffee, the use of sucrose could produce high content of acid but low gas formation. In this study, sugar types and concentration were investigated for increasing gas formation in coffee kombucha. The result found that high gas production was observed in coffee kombucha producing from 5% glucose + 5% sucrose as shown in Table1.

Acidity of coffee kombucha, the initial pH of the coffee kombucha containing 5% glucose + 5% sucrose was 5.41, and it dropped to 3.16 during the fermentation period (Figure 1). In the coffee kombucha containing 5% glucose + 5% sucrose, the acid concentration continuously increased from 0.71 % (v/v) to 7.44% (v/v) at day 14 of fermentation. However, high acid content (12.08 % v/v) was found in the kombucha making from 10% sucrose, pH dropped from
5.20 to 2.09 at day 14 of fermentation. The 5% glucose + 5% sucrose could produce highest gas formation because yeast could survive at pH 3.2-3.7 (second fermentation) and produce higher CO₂. The other treatments, the pH value during second fermentation was lower than 3.0, which was much lower than the pH for optimum growth of yeasts (Chen and Liu, 2000), resulting to low gas production. Malbaša et al. (2008) reported kombucha converted sucrose to glucose and fructose, and further to ethanol, acetic acid, lactic acid, and a large number of other compounds.

Table 1. Gas formation of coffee kombucha when compared with different concentration of sugars: 5% sucrose, 7% sucrose, 10% sucrose, 5% glucose and 5% glucose + 5% sucrose

<table>
<thead>
<tr>
<th>Sugar content</th>
<th>Gas formation level</th>
<th>Gas formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% sucrose,</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7% sucrose,</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10% sucrose,</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>5% glucose</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>5% glucose + 5% sucrose</td>
<td>+++</td>
<td></td>
</tr>
</tbody>
</table>

Gas formation level: low (+), medium (++) , high (+++)
Figure 1. Changes of pH and acid concentration during 14 days of coffee kombucha fermentation compared with 5% sucrose, 7% sucrose, 10% sucrose, 5% glucose and 5% glucose + 5% sucrose

3.2 Number of Microorganism
Total bacteria, Lactic Acid Bacteria (LAB) and yeast was enumerated from the kombucha with 5% glucose + 5% sucrose as shown in Figure 2. During first fermentation, total bacteria and yeast slightly increased from 6.46 to 7.8 log CFU/mL and 6.36 to 7.93 log CFU/mL, respectively. After day 10 of fermentation (second fermentation), number of microorganism decreased from 7.35 to 6.86 log CFU/mL, however yeast mainly grew in this period. The result related to Chakravorty et al. (2016) that Candida, Lachancea and Kluyveromyces were found in secondary fermentation. The bacterial community in kombucha was dominated by the genera Acetobacter and Gluconacetobacter (Jarrell et al., 2000). Lactic acid bacteria was found both in first and secondary fermentation. Yeasts and bacteria in Kombucha are involved in such metabolic activities that utilize substrates by different and in complementary ways. Yeasts hydrolyze sucrose into glucose and fructose by invertase and produce ethanol via glycolysis, with a preference for fructose as a substrate. Acetic acid bacteria use glucose to produce
gluconic acid and ethanol to produce acetic acid. The pH value of kombucha beverage decreased due to the production of organic acids during fermentation (Sievers et al., 1995).

Figure 2. Number of total aerobic bacteria, lactic acid bacteria (LAB) and yeast and fungi during coffee kombucha fermentation

3.6. Sensory characteristics of coffee kombucha
Sensory scores for coffee smell, sweet, sour, sparkling and mouthfeel of coffee kombucha with 5% glucose + 5% sucrose were showed in Table 2. The Coffee kombucha was sparkling, sour and slightly sweet. Carbonation enhancement could improve sparkling taste of product.

Table 2. Sensory evaluation of coffee kombucha

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>coffee smell</td>
<td>6.10 ± 0.48</td>
</tr>
<tr>
<td>sweetness</td>
<td>5.10 ± 0.92</td>
</tr>
<tr>
<td>sourness</td>
<td>7.00 ± 0.83</td>
</tr>
<tr>
<td>sparkling taste</td>
<td>6.67 ± 0.92</td>
</tr>
<tr>
<td>mouthfeel</td>
<td>7.16 ± 0.63</td>
</tr>
<tr>
<td>overall acceptance</td>
<td>6.96 ± 0.49</td>
</tr>
</tbody>
</table>

4. CONCLUSION
Coffee kombucha usually prepare by 5-10 % sucrose as a carbon source. Low gas formation obtained from these sugar content. The concentration of sugar that could improve carbonation in coffee kombucha were 5% glucose + 5% sucrose. Acidity of product was 7.44 % (v/v) acid with pH 3.16. Aerobic bacteria largely grew in coffee kombucha during first fermentation, whereas yeast was mainly found in secondary fermentation. Moreover, lactic acid bacteria as a probiotic were found in coffee kombucha. Carbonation enhancement could improve sparkling taste of the product.

ACKNOWLEDGMENT
Arabica roasted coffee was kindly provided from Thai Lahu Coffee and Tea Co., Ltd, Chiang Mai, Thailand.

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Evaluation of Total Anthocyanins and Antioxidant Activity of Thai Rice Cultivars for Phenotypic Selection in Rice Breeding

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Keywords: Extraction, Anthocyanins, Antioxidant activity, Thai black rice

Black rice has been gained increasing interest for consumers and rice breeders due to its high nutritional values of anthocyanin contents and antioxidative properties. The objective of this study was to determine the optimal solvents for anthocyanin extraction and quantification of antioxidant activity for selection of Thai rice cultivars with high anthocyanins and antioxidant activity to be used in rice breeding program. The dehulled mature seeds of Thai black rice cv. Hom Nin were extracted by different solvent types and the extracts were evaluated for total anthocyanin contents and antioxidant activity by spectrophotometry and DPPH assay, respectively. The results demonstrated that the extract with 1% HCl in 80% methanol gave the highest total anthocyanins and antioxidant activity. This solvent was subsequently used for extraction of seeds from eight rice cultivars, which consisted of four non-pigmented (white) and four black rice cultivars. It was found that the extracts from black rice cultivars showed no significantly different levels of antioxidant activity, possibly due to interference by hydrochloric acid in DPPH assay. Therefore, 80% methanol was used for anthocyanin extraction of rice cultivars. The results showed that antioxidant activity had positive correlation with amount of total anthocyanin contents and phenotypic traits of pericarp colors. In this study, Thai black rice cv. Mali Dum (MLD) gave the highest total anthocyanin contents and antioxidant activity which were correlated with coloration of extracted sample and pericarp color. Our study suggested that MLD would be a good source of high anthocyanins and antioxidant activity for use as parental line in rice breeding program for improvement of rice with health promoting properties. Moreover, advanced breeding lines with high anthocyanin contents and antioxidant activity could be identified by methanolic extraction method followed by spectrophotometric measurements and DPPH assay.
Evaluation of Total Anthocyanins and Antioxidant Activity of Thai Rice Cultivars for Phenotypic Selection in Rice Breeding

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Keywords: Extraction, Anthocyanins, Antioxidant activity, Thai black rice

1. INTRODUCTION

Rice (Oryza sativa L.) is one of the most important cereals, serving as a staple food consumed by people in many countries, especially in Asia (Liu et al., 2017; Pang et al., 2018). Pigmented rice has been popular as a healthy food because it contains more nutrients beneficial for human health (Alves et al., 2016; Maulani et al., 2019). Rice can be classified by grain colors which are white, brown, red and black. Black rice has high accumulation of anthocyanins in its pericarp tissues (Reddy et al., 1994; Goufo and Trindade, 2014). Recently, black rice has received more attention from consumers and rice breeders since it contains several nutrients and antioxidant compounds (Lim and Ha, 2013; Rahman et al., 2016). Anthocyanins which belong to a major class of water-soluble flavonoids, are the primary pigments in colored rice grains (Abdel-Aal et al., 2006). Several health benefits of anthocyanins as health-promoting substances due to their antioxidant activity have been recognized (Nam et al., 2006). They include anti-inflammatory activity, anti-cancer activity, prevention of cardiovascular diseases and obesity, control of diabetes, mitigation of oxidative stress, vision improvement and anti-microbial activity (He and Giusti, 2010; Kruger et al., 2014; Sompong et al., 2011).

Extraction of anthocyanins has been conducted using different extracting solvents, including water, methanol, and ethanol, combined with some acids such as citric acid, hydrochloric acid, and acetic acid (Halee et al, 2018; Jansom et al., 2016). In rice, extraction of anthocyanins with acidified
methanol/ethanol followed by spectrophotometric measurement has been used extensively (Chin et al., 2016; Jansom et al., 2016; Jiamyangyuen et al., 2017; Na Rachasima et al., 2017). DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay is commonly used to evaluate antioxidant activity of foods and plant extracts because it is simple, rapid, inexpensive and reproducible. There are factors that may influence the reaction in DPPH method such as extracting solvents, sample concentration, pH, reaction time, different antioxidant standard and assay conditions (Ferri et al., 2013; Mishra et al, 2012).

Due to the increased demand for black rice as health-promoting foods for human, it is important to develop rice varieties with enhanced anthocyanin contents, high yield and other good agronomic characteristics (Rahman et al., 2016). In rice breeding, selection of black rice with high anthocyanins and antioxidant activity is needed to be used as parental lines. The methods to evaluate anthocyanin contents and antioxidant activity are necessary to analyze the phenotypic traits of parental and progeny lines. These methods should be conducted simply and less costly. Moreover, they should be able to distinguish different rice lines in the steps of phenotypic selection.

The objective of this study was to investigate the effects of extracting solvents on anthocyanin contents and antioxidant activity of Hom Nin black rice. The appropriate solvent was subsequently used to evaluate total anthocyanin contents and antioxidant activity against DPPH from different rice cultivars, including white and black rice. The results will provide the simple and reliable method for analyzing phenotypic traits of rice cultivars with high anthocyanins and antioxidant activity for selection of parent and progeny lines in breeding program to improve rice varieties with more nutritional value.

2. MATERIALS AND METHODS

2.1 Plant materials

Mature seeds of eight rice cultivars were used in this study and collected from different sources. Four non-pigmented rice cultivars, which were simply called white rice, were Taichung 65 (T65), Kitaake (Kit), RD-MAEJO 2 (RDMJU 2) and Pathumthani 1 (PTT1). Four pigmented rice cultivars, which were simply called black rice, were Kham Noi (KNO), Kham Yai (KY), Hom Nin (HN) and Mali Dum (MLD) (Figure 1). T65, RDMJU2, PTT1 and HN were kindly provided by Maejo University, Chiang Mai province, Thailand. KNO, KY and MLD were kindly provides by Center of community rice production, Kudchum, Yasothon province, Thailand. Finally, Kit was kindly provided by Prof.Dr.Thomas W. Okita, Institute of Biological Chemistry, Washington State University, USA.

![Figure 1 Phenotypic traits of mature seeds of eight rice cultivars. Non-pigmented rice cultivars, which were simply called white rice, were Taichung 65 (T65), Kitaake (Kit), RD-MAEJO 2 (RDMJU2) and Pathumthani 1 (PTT1). Pigmented rice cultivars, which were simply called black rice, were Kham Noi (KNO), Kham Yai (KY), Hom Nin (HN) and Mali Dum (MLD).](image)

2.2 Extraction of rice seeds with various solvent types

The mature rice seeds were dehulled and grounded into fine powder. Seed powder of 100 mg from HN black rice cultivar were extracted by 1 ml of six different solvent types which were water, 50% methanol, 80% methanol, 1% HCl in water (V/V), 1% HCl in 50% methanol (V/V) and 1% HCl in 80% methanol (V/V). The seed extracts were mixed by vortexing and incubated at room
temperature for 30 min. The supernatants were collected by centrifugation at 12,000 rpm for 10 min. Each extraction was performed with three replicates. Each extract from different solvents types was diluted using the same extracting solvent type with the sample extract / solvent volume ratio at 1/4. The diluted extracts with different solvent types were subjected to measurement of total anthocyanin contents and antioxidant activity. The optimal solvents which were found to be 80 % methanol and 1% HCl in 80% methanol were selected and used to extract the seeds of eight rice cultivars.

2.3 Determination of total anthocyanin contents

One hundred milligrams of mature seeds of eight rice cultivars, including four white rice (T65, Kit, RDMJU2, and PTT1) and four black rice (KNO, KY, HN and MLD) were grounded into fine powder followed by the extraction with 1 ml of two solvent types which were 1% HCl in 80% methanol and 80 % methanol. The seed extracts were mixed by vortexing and incubated at room temperature for 30 min. The supernatants were collected by centrifugation at 12,000 rpm for 10 min. Each extraction was performed with three replicates. Each extract from different solvents types was diluted using the same extracting solvent type with the sample extract / solvent volume ratio at 1/6. The diluted extracts with different solvent types were subjected to measurement of total anthocyanin contents and antioxidant activity. The optimal solvents which were found to be 80 % methanol and 1% HCl in 80% methanol were selected and used to extract the seeds of eight rice cultivars.

2.4 Determination of antioxidant activity by DPPH assay

The anthocyanin extracts of eight rice cultivars with 1% HCl in 80% methanol and 80 % methanol were analyzed for antioxidant activity by DPPH assay. Each extract from different solvents types was diluted using the same extracting solvent type with the sample extract / solvent volume ratio at 1/4. The Trolox equivalent antioxidant capacity (TEAC) assay using Trolox as a standard was used to measure total antioxidant activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH) among seed anthocyanin extracts of eight rice cultivars, according to the described method (Shao et al., 2014; Zhu et al., 2017). The 100 µmol/l DPPH solution was prepared in methanol. The diluted seed extract solution of 20 µl was mixed with 180 µl DPPH solution for the reaction. After incubating the reactions at room temperature for 30 min in the dark, the absorbance at 516 nm was measured by microplate reader (SPECTROstar® Nano, Germany). The antioxidant activity value was calculated as follows: %DPPH inhibition = [(Acontrol - Asample) / Acontrol] x 100. Asample was absorbance value of the extract in DPPH solution and Acontrol was absorbance value of DPPH solution with methanol instead of the extract. The antioxidant activity value was calculated by using different concentration of Trolox standard (10, 15, 20, 25, 50, 75, 100 and 125 mg/l) as a standard curve. The results were expressed as TEAC in µmol Trolox equivalents per gram of powdered rice seeds. Three replicates were analyzed for each sample.

2.5 Statistical analysis

The results were presented as means ± standard deviation (SD) of triplicate determinations. Statistical analysis was performed using R3.6.0 program (http://www.r-project.org). The data were analyzed of variance and the significant differences among means were determined using the Duncan test at a level P<0.01.

3. RESULTS AND DISCUSSION

3.1 The optimal solvents for seed extract of black rice cv. Hom Nin

The black rice cultivar cv. Hom Nin was used to study the appropriate solvent types for rice seed extracts to be used for evaluation of anthocyanin contents and antioxidant activity. The six different solvent types which were water, 50% methanol, 80% methanol, 1% HCl in water (V/V), 1% HCl in 50% methanol (V/V) and 1% HCl in 80% methanol (V/V), were used for the extraction of Hom Nin seeds. The result showed that the extracts exhibited statistically significant differences (P<0.01) in total anthocyanin contents and antioxidant activity by DPPH assay (Table 1). The extracts with solvents containing 1% HCl gave higher total anthocyanin content than the solvents without 1% HCl. The extract with 1% HCl in 80% methanol gave highest anthocyanin contents of 1.41
µmol/gDW, followed by the extracts with 1% HCl in 50% methanol and 1% HCl in water which exhibited the total anthocyanins of 1.36 and 0.49 µmol/gDW, respectively.

Table 1 Total anthocyanin contents and antioxidant activities by DPPH assays of Hom Nin rice powder crude extracts obtained from different extraction solvents.

<table>
<thead>
<tr>
<th>Solvent types</th>
<th>Total anthocyanin contents (µmol/gDW)</th>
<th>Antioxidant activity / TEAC (µmol/gDW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>0.17 ± 0.01</td>
<td>7.72 ± 0.01</td>
</tr>
<tr>
<td>50% methanol</td>
<td>0.20 ± 0.02</td>
<td>11.32 ± 0.33</td>
</tr>
<tr>
<td>80% methanol</td>
<td>0.12 ± 0.01</td>
<td>9.23 ± 0.03</td>
</tr>
<tr>
<td>1% HCl in water</td>
<td>0.49 ± 0.01</td>
<td>13.70 ± 0.03</td>
</tr>
<tr>
<td>1% HCl in 50% methanol</td>
<td>1.36 ± 0.04</td>
<td>13.64 ± 0.13</td>
</tr>
<tr>
<td>1% HCl in 80% methanol</td>
<td>1.41 ± 0.10</td>
<td>13.94 ± 0.06</td>
</tr>
</tbody>
</table>

Mean ± standard deviation of triplicate analyses. Values in each row (small letter) bearing different superscripted letters are statistically different (P<0.01).

The antioxidant activities of the extracts by 1% HCl in 80% methanol, 1% HCl in 50% methanol and 1% HCl in water were higher than those of water and methanol. The extract with 1% HCl in 80% methanol gave the highest value of antioxidant activity of 13.94 µmol/gDW (Table 1). The values of antioxidant activity using 1% HCl with water and 1% HCl in 50% methanol were 13.70 and 13.64 µmol/gDW, respectively, and showed no significant differences (P≥0.01). The previous report showed that the solvents of ethanol or methanol acidified with 1% HCl were optimal for the extraction of rice seeds for anthocyanin content determination (Chin et al., 2016; Krongsusksirichai et al., 2016). In this study, we found that 1% HCl in 80% methanol gave highest values of anthocyanin content and antioxidant activity; therefore, we selected this solvent type for the seed extraction of eight rice cultivars for further studies.

3.2 Total anthocyanin contents and antioxidant activities of eight rice cultivars

The selected solvent of 1%HCl in 80% methanol was used to extract the seed powder of eight rice cultivars. White rice cultivars which were T65, Kit, RDMJU2 and PTT1 as well as black rice cultivars which were KNO, KY, HN and MLD were extracted with 1%HCl in 80% methanol. The extracts were subsequently analyzed for total anthocyanin contents and antioxidant activities. The results showed that the amounts of anthocyanins in black rice were higher than white rice (Figure 2). The level of total anthocyanins of 1.374 µmol/gDW in black rice cv. MLD was highest and consistent with dark black color in it pericarp (Figure 1). Following MLD, there were HN, KY, and KNO which had total anthocyanin contents of 0.945, 0.776, and 0.502 µmol/gDW, respectively, corresponding to their pericarp color intensity. On the other hand, all white rice cultivars which were T65, Kit, RDMJU2 and PTT1, showed little detectable anthocyanin contents of 0.002 µmol/gDW. The results were consistent with the previous study which demonstrated that grain anthocyanin content of black rice was much higher than those of brown and white rice (Rahman et al., 2016).

The antioxidant activity by DPPH-radical scavenging activity assay of the extracts by 1%HCl in 80% methanol was evaluated. The results showed that the antioxidant activity of black rice were higher than white rice. The black rice cv. MLD had highest TEAC against DPPH of 17.37 µmol/gDW. This result was consistent with highest amount of anthocyanin contents (1.374 µmol/gDW) and darkest black color in it pericarp (Figure 2 and 1). Other black rice cv. HN, KY and KNO had the antioxidant activity of 17.09, 16.42 and 16.71 µmol/gDW, respectively. The white rice cv. T65, Kit, RDMJU2 and PTT1 had not significantly different values of TEAC which were 3.71, 3.69, 3.70 and 3.70 µmol/gDW, respectively (P≥0.01). Although black rice cultivars cv. HN, KY and KNO with significantly different anthocyanin contents (P<0.01) corresponding to their pericarp colors, the values
of TEAC could not be clearly distinguished among these black rice (Figure 2). Therefore, it might be difficult to evaluate relative antioxidant activities among different black rice cultivars for selection of parental lines and progeny lines derived from the crosses between white and black rice in our breeding programs.

The previous study showed that the acidity of sample extracts had the effect on DPPH assay, leading to different estimation of their antioxidant activity (Pekal and Pyrzynska, 2015). To determine whether 1% HCl affected the DPPH assay of rice seed extracts, we extracted the rice seeds from eight cultivars with 80% methanol and used for analysis of anthocyanins and antioxidant activity. The results showed that the extract by 80% methanol gave the significantly difference (P<0.01) of total anthocyanin contents among black rice cultivars (Figure 3). The black rice cv. MLD had the highest TEAC of 8.89 µmol/gDW which was consistent with highest total anthocyanins of 0.121 µmol/gDW and darkest color of its pericarp (Figure 3 and 1). The black rice cv. KNO showed the lowest TEAC of 4.48 µmol/gDW which was consistent with lowest total anthocyanins of 0.051 µmol/gDW and less dark color of its pericarp (Figure 1).

The present study indicated that the seed extracts with methanol would be appropriate for antioxidant activity by DPPH method. Several studies reported the factors affecting DPPH assay including reaction time, solvent types, and acidity (Mishra et al., 2012; Pekal and Pyrzynska, 2015). For rice seeds, the extracts with methanol were performed for evaluation of antioxidant activity by TEAC assay (Walter et al., 2013; Huang and Lai, 2016; Jiamyangyuen et al., 2017).

However, the extracts with 80% methanol gave about 10-fold lower amount of total anthocyanin content than those extracted by 1% HCl in 80% methanol. Thus, for the evaluation of total anthocyanins of rice seeds, the extraction with 1% HCl in 80 % methanol might be more appropriate. Several studies on the extraction of black rice seeds for analysis of anthocyanin contents using extraction buffer with acidified methanol have been reported (Chundet et al., 2012; Chin et al., 2016; Jiamyangyuen et al., 2017; Halee et al., 2018).

In this study, the seed extracts with 1% HCl in 80% methanol might be appropriate for evaluation of total anthocyanin contents by spectrophotometry. However, 80 % methanol with no acidity could be suitable for assessment to antioxidant activity by DPPH assay.

![Figure 2 Assessments of total anthocyanin contents (a) and antioxidant activity against DPPH (b) of the rice seeds extracts with 1%HCl in 80% methanol. White rice cultivars were T65, Kit, RDMJU2 and PPT1. Black rice cultivars were KNO, KY, HN and MLD. All the values were represented as mean ± SD.](image-url)
Figure 3 Assessments of total anthocyanin contents (a) and antioxidant activity against DPPH (b) of the rice seeds extracts with 80% methanol. White rice cultivars were T65, Kit, RDMJU2 and PPT1. Black rice cultivars were KNO, KY, HN and MLD. All the values were represented as mean ± SD.

3.3 The optimal incubation time for antioxidant activity in DPPH assays

To determine the appropriate incubation time for evaluation of antioxidant activity of the extracts by DPPH assay, the extracts with 1% HCl in 80% methanol and 80% methanol were measured for antioxidant activity after incubation time of 10, 20, 30, 40, 50 and 60 min. The extracts with 1% HCl in 80% methanol showed that the antioxidant activity of all white rice had no change throughout the time of 10-60 min. Moreover, all black rice showed similar values of antioxidant activity throughout 60 min (Figure 4A). The result demonstrated that the solvent of 1% HCl in 80% methanol might not be suitable for seed extraction for DPPH method probably due to interference of the acidified condition in the assay. The previous study showed that DPPH method for measurement of antioxidant activity of foods and plant extracts required a pH range between 4-8 (Ferri et al., 2013).

On the other hand, the extracts with 80% methanol showed increase in antioxidant activity of all eight rice cultivars when the time increased from 10-30 min (Figure 4B). During incubation period of 10-30 min, the antioxidant activity of all rice cultivars increased at the same pattern and the different values of antioxidant activity among different cultivars could be observed. Thus, the incubation time of reaction at 30 min would be optimal for all rice cultivars to assess antioxidant activity by DPPH method. In addition, at 30 min, the different amounts of antioxidant activity among black rice cultivars could be clearly distinguished (Figure 4B). The result was consistent with several studies on assessment of antioxidant activity by DPPH assay of sample extracts by methanol and incubation time of reaction for 30 min (Ferri et al., 2013; Pekal and Pyrzynska, 2015; Patil et al., 2016; Jiamyangyuen et al., 2017; Halee et al., 2018).

Figure 4 Assessment of incubation time in antioxidant activity by DPPH assay of extracts with 1% HCl in 80% methanol (a) and 80% methanol (b). White rice cultivars were T65, Kit, RDMJU2 and PPT1. Black rice cultivars were KNO, KY, HN and MLD. All the values were represented as mean ± SD.
The results revealed that white rice also had antioxidant activity but much lower than black rice. RDMJU2 which is glutinous rice cultivar made from rice breeders at Maejo University, Chiang Mai, Thailand showed highest antioxidant activity among white rice. In addition, RDMJU2 has high yield and good agronomic characteristics, including semi-dwarf and non-photoperiod sensitivity. The black rice, MLD which is landrace rice in the northeastern region of Thailand gave highest anthocyanin contents and antioxidant activity, consistent with the previous report (Kongkachuichai and Charoensiri, 2010). Hence, both RDMJU2 and MLD would be good sources for use as parental lines in improvement of rice with high quality traits and increased nutritional values.

4. CONCLUSION

Extracting solvents and assay conditions could affect the measurement of total anthocyanin contents and antioxidant activity. The results demonstrated that the appropriate solvent for rice seed extraction to analyze total anthocyanin contents was 1 % HCl in 80 % methanol followed by the spectrophotometric measurement. For antioxidant activity, the extraction with 80 % methanol could be suitable for DPPH assay, because the acidity of 1 % HCl might interfere with the reactions. These extraction solvents and the methods for determination of anthocyanins and antioxidant activity will be applied for screening and selection of rice cultivars with high anthocyanins and antioxidant activity to be used as parental lines and for selection of progeny lines in rice breeding program. In rice breeding, it is needed to determine the correlation between genotype and phenotype of rice populations such as F2 progeny. The simple method for evaluating phenotypes of relative total anthocyanins and antioxidant activity of many lines will be very useful, less time-consuming and less costs. This study also suggested that RDMJU2 (white rice) and MLD (black rice) would be good candidates for use in rice breeding to provide health-promoting foods.

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Investigation of some biological activities of local shallot (Allium ascalonicum Linn.) extract from Thailand

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Keywords: shallot extract, prebiotic, antioxidant activity, antibacterial activity, functional food

Shallot (Allium ascalonicum Linn.) is a good source of several nutrients and phytochemicals. Shallot-based functional foods have been being developed in our research group. The partial purified shallot extract was prepared according to the processes developed in our group for utilizing as functional food ingredient of many food products. The aims of this present work were then to investigate some biological activities including antibacterial activity and antioxidant activity of shallot extract prepared from local cultivar of Srisaket province, Thailand. The results showed that both crude and partial purified extracts were rich in oligosaccharides and polysaccharides, with degree of polymerization (DP) about 23-283. Interestingly, it was found that purification processes used in this study, based on adsorption method, removed some low molecular weight sugars from shallot extract. ABTS radical scavenging assay was used in antioxidant activity test of the extracts. The crude extract exhibited significantly higher ABTS scavenging activity than the purified extract. The results also revealed that ABTS scavenging activity continuously decreased according to number of purification step. The similar results were found in antibacterial test that shallot extract lost the activity after purification processes. However, crude extract could inhibit growth of pathogenic Salmonella Typhimurium and Staphylococcus aureus but not for Escherichia coli in agar diffusion assay. Moreover, the minimum inhibitory concentration (MIC) values of crude extract on S. Typhimurium and S. aureus were 114.66 and 163.80 mg/ml, respectively, and only S. Typhimurium was disinfect by crude extract with the minimum bactericidal concentration (MBC) value at 147.42 mg/ml. It could be concluded that shallot extract possess high potential to be applied in functional food manufacturing. However, crude extract and purified extract might be suitable for different purposes, including prebiotic, antioxidant and antibacterial uses.
Investigation of some biological activities of local shallot
\textit{(Allium ascalonicum Linn.)} extract from Thailand

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ABSTRACT

Shallot \textit{(Allium ascalonicum Linn.)} is a good source of several nutrients and phytochemicals. Shallot-based functional foods have been being developed in our research group. The partial purified shallot extract was prepared according to the processes developed in our group for utilizing as functional food ingredient of many food products. The aims of this present work were then to investigate some biological activities including antibacterial activity and antioxidant activity of shallot extract prepared from local cultivar of Srisaket province, Thailand. The results showed that both crude and partial purified extracts were rich in oligosaccharides and polysaccharides, with degree of polymerization (DP) about 23-283. Interestingly, it was found that purification processes used in this study, based on adsorption method, removed some low molecular weight sugars from shallot extract. ABTS radical scavenging assay was used in antioxidant activity test of the extracts. The crude extract exhibited significantly higher ABTS scavenging activity than the purified extract. The results also revealed that ABTS scavenging activity continuously decreased according to number of purification step. The similar results were found in antibacterial test that shallot extract lost the activity after purification processes. However, crude extract could inhibit growth of pathogenic \textit{Salmonella} Typhimurium and \textit{Staphylococcus} aureus but not for \textit{Escherichia coli} in agar diffusion assay. Moreover, the minimum inhibitory concentration (MIC) values of crude extract on \textit{S.} Typhimurium and \textit{S.} aureus were 114.66 and 163.80 mg/ml, respectively, and only \textit{S.} Typhimurium was disinfected by crude extract with the minimum bactericidal concentration (MBC) value at 147.42 mg/ml. It could be concluded that shallot extract possess high potential to be applied in functional food manufacturing. However, crude extract and purified extract might be suitable for different purposes, including prebiotic, antioxidant and antibacterial uses.

\textbf{Keywords:} shallot extract, prebiotic, antioxidant activity, antibacterial activity, functional food
INTRODUCTION

Shallot or red onion (Allium ascalonicum L.) is a member of the Alliaceae family, is widely cultivated and consumed in many Asian countries. In Thailand, shallot have been cultivated mainly in Chiang mai, Uttaradit and Srisaket provinces. It constitutes important ingredient in many Asian diets and is known for its medicinal properties apart from its nutritional value. Shallot contains both water-soluble nutrients and oil-soluble substances, with 79.8% moisture, 16.8% carbohydrates, 2.5% proteins, 3.2% dietary fibers, and 7.9% sugars (by fresh weight) (Putnika et al., 2019). It is a good source of sugars (oligosaccharides), minerals (Ca and P), vitamins (A, B6 and C) and various functional phytochemicals (organo-sulfur compounds flavonoids and other phenolic compounds) (Brewer, 2011; Ounjaijean et al., 2018). Consequently, this plant exhibits many biological properties, including antibacterial, antiviral, anti-diabetic, antioxidant, and anti-inflammation activities (Sakaewan et al., 2019). Shallot extract inhibit the expression of genes associated with inflammation, including iNOS, TNF-α, IL-1β and IL-6 (Werawattanachai et al., 2015), inhibit proliferation and growth of tumor cell lines (HeLa and MCF-7) (Hamid-Reza et al., 2011). The extract also possess antimicrobial and antioxidant activities (Mnayer et al., 2014) by the action of two main classes of components, organo-sulfur compounds (allyl trisulfide, allyl-cysteine and diallyl sulfide) and flavonoids (quercetin and kamferal) (Brewer, 2011). Moreover, oligosaccharides containing in shallot are promising to be utilized as prebiotic foods (unpublished data). According to the above functional potentials of shallot for consumers, shallot-based functional foods have been being developed in our research group. The partial purified shallot extract was prepared according to the processes developed in our group for utilizing as functional food ingredient of many food products. This shallot extract will be mainly proposed as the functional ingredient for prebiotic, antimicrobial and antioxidant foods. From our previous results (unpublished data), it was interestingly that partial purified shallot extract exhibited prebiotic property greater than original shallot extract. However, the other biological activities have not been yet studied. The aim of this study was subsequently to investigate some biological activities of crude and partial purified shallot extract, including antibacterial activity and antioxidant activity to evaluate their potential prior applying in functional food manufacturing.

MATERIALS AND METHODS

2.1 Shallot and shallot extract preparation

Shallot or red onion or Hom-daeng (in Thai) used in this study was a local cultivar cultivated of Srisaket province, Thailand. The extract was prepared by aqueous extraction of fresh and clean shallot. Shallot extract was then further partial purified through a commercial adsorbent. Crude and partial purified extracts were clarified by centrifugation and stored at -20°C during experiment.

2.2 Sugar content analysis

Reducing sugar and total sugar of the extracts were determined by DNS and phenol-sulfuric acid method; respectively. Size of sugar, in term of an average degree of polymerization (DP) was calculated by the ratio between total sugar and reducing sugar content (Wongputtisin et al., 2012). The distribution of individual sugars in shallot extract was investigated by thin layer chromatography (TLC). The aluminum sheet coated by siliga gel (Merck®) was used as stationary phase and mobile phase was a mixture of butanol: ethanol: water (5:3:2). The sugar bands were visualized by dipping in 5% (v/v) H2SO4 in methanol and heating at 150°C in hot air oven.

2.3 Antioxidant activity

To generate ABTS**, the protocol according to Re et al. (1999) was used. Five ml of 14 mM ABTS (0.385 g ABTS in 50 ml deionized water) and 5 ml potassium persulfate (0.066 g potassium persulfate in 50 ml deionized water) were mixed together and stand in the dark for 12-16 h before use. To determine scavenging activity of FCSBM extract, 10 μl of extract was added to 990 μl of ABTS** solution (adjusted the absorbance at 734 nm to 0.700±0.020 before used) and recorded the decreasing of A734 every 1 min until stable. The standard antioxidants used in this study were α-tocopherol (Merck®), ascorbic acid (Fisher Chemicals®), butylated hydroxyanisole (BHA, Fluka®). The percent of scavenging activity at 1 min of reaction can be calculated by the formula:
The antibacterial activity of crude and partial purified shallot extract against *Salmonella enterica* serovar Typhimurium TISTR292, *Escherichia coli* and *Staphylococcus aureus* were studied. The extracts were sterilized using filtration through Sartorius Minisart® syringe filter (0.2 μm). Firstly, gel diffusion assay method was carried out by transferring of 20 μl extract into agar wells which were prior spread with 24 h-old pathogen suspension, subsequently further incubating at 37°C for 24 h and recording the clear zone around wells.

Minimum inhibitory concentration (MIC) of the extracts against those pathogens was tested. The sterile control treatment diluted by sterile distil water was carried out in parallel. Ten μl of culture broth from those test tubes with no visible growth were spread on *Salmonella – Shigella* agar (SS agar) (Himedia®) and eosin methylene blue agar (EMB agar) (Himedia®) and nutrient agar (NA) and incubated at 37°C for 24 h for cell enumeration of *S. Typhimurium*, *E. coli* and *S. aureus*, respectively. Minimum concentration of extract with no viable cell was considered as minimum bactericidal concentration (MBC) value.

### 3. RESULTS AND DISCUSSION

#### 3.1 Shallot extract

Fresh shallot contained approximately 77.1 ± 0.2% of moisture content (wet basis) and the % yield of shallot extract obtained from electronic juicer was 453 ml/kg of fresh shallot. This extract was further processed for partial purification of FOS following our unique and specific steps based on adsorption strategy as usual. The extract quality in term of sugar content in both crude and partial purified extracts were analyzed for quality confirmation and shown in Table 1 and Figure 1. Increased cycle of elution through absorbent resulted of decreasing of monosaccharides, while the average DP was increased. FOS was the major group of sugar found in these shallot extracts and also in other *Allium* sp. cultivars. According to our unpublished data, prebiotic property of these partial purified extract was greater than that of crude extract. However, the antioxidant and antibacterial activity of these extracts were subsequently investigated as main objectives of this study.

<table>
<thead>
<tr>
<th>Table 1. Total sugar, reducing sugar and degree of polymerization of shallot extracts</th>
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<tbody>
<tr>
<td>Extracts</td>
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<tr>
<td>Crude extract</td>
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<tr>
<td>Partial purified extracts</td>
</tr>
<tr>
<td>1 cycle</td>
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<td>5 cycles</td>
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<tr>
<td>6 cycles</td>
</tr>
<tr>
<td>7 cycles</td>
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<tr>
<td>8 cycles</td>
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Note: different superscript letters mean significant difference at p<0.05.
3.2 ABTS radical scavenging activity

Several antioxidants were naturally found in *Allium* sp., mainly as flavonoids and even the reducing sugars and amino acids. Antioxidant activity of the extracts were assayed in term of %ABTS radical scavenging activity. The results are shown in Table 2. The scavenging activity of crude extract was 50.66±0.22% and slightly increased (p<0.05) about 7% higher than that of crude extract after the first cycle of FOS purification. According to higher concentration of sugar content was obtained after this cycle (Table 1), water moiety might be absorbed on absorbent. Thus, it was possibly that concentration of antioxidants was also increased, even though some were absorbed, resulting of slightly increasing of %scavenging activity. However, further cycles of purification led to continuous decreasing of %scavenging activity. Finally, the activity was lowered about 71% comparing to crude extract after 8 cycles of purification. It was indicated that the crude extract exhibited significantly higher ABTS scavenging activity than the purified extract. During the purification process, it was noticed that color of extract gradually paler along the number of purification steps. Anthocyanins and flavonols, the dominant flavonoid pigments naturally found in *Allium* sp., especially in red onion, might be also removed from the extract similar to monosaccharides. Their polar molecules can be adsorbed on carbonaceous absorbent via Van de Waals force (Li et al., 2017). The dominant anthocyanins and flavonols in red onion are cyanidin and quercitin, respectively (Arifin et al., 1999). They play an important role as antioxidant in plants and several health benefits for consumers (Arifin et al., 1999; Pudzianowska et al., 2012; Mnayer et al., 2014).
Table 2. ABTS scavenging activity of crude and purified extracts

<table>
<thead>
<tr>
<th>Samples</th>
<th>%scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>50.66±0.22b</td>
</tr>
<tr>
<td>Partial purified extracts</td>
<td></td>
</tr>
<tr>
<td>1 cycle</td>
<td>54.51±2.15a</td>
</tr>
<tr>
<td>2 cycles</td>
<td>43.90±0.23c</td>
</tr>
<tr>
<td>3 cycles</td>
<td>31.42±1.16d</td>
</tr>
<tr>
<td>4 cycles</td>
<td>23.14±0.87e</td>
</tr>
<tr>
<td>5 cycles</td>
<td>19.27±0.38f</td>
</tr>
<tr>
<td>6 cycles</td>
<td>16.53±0.45g</td>
</tr>
<tr>
<td>7 cycles</td>
<td>14.66±1.09h</td>
</tr>
<tr>
<td>8 cycles</td>
<td>12.46±0.36i</td>
</tr>
</tbody>
</table>

Note: different superscript letters mean significant difference at p<0.05.

3.3 Antibacterial activity

The antibacterial activity of shallot extracts against some pathogens were tested. The preliminary results by gel diffusion assay showed that crude extracts could inhibit growth of only *S. Typhimurium TISTR292* and *S. aureus*, but the purified extracts could not. Unfortunately, it was clear that shallot extract lost its antibacterial activity during our FOS purification processes similar to its anti-oxidation ability. The antibacterial compounds in *A. ascalonicum* include quercitin, diallyl disulfide, trisulfide, tetrasulfide, and so on (Mnayer et al., 2014; Sharift-Rad et al., 2016; Jaisinghani, 2017). Both flavonoids and sulfide compounds can be adsorbed on carbonaceous absorbent. However, the crude shallot extract was further tested for its minimum concentration to inhibit pathogen growth. The MIC experiment resulted consistently to gel diffusion assay (Table 3). Only growth of *E. coli* was not inhibited. *S. aureus* growth was inhibited by only original concentration of extract (163.80 mg/ml), while the minimum concentration of crude extract for inhibition of *S. Typhimurium* was at the ratio 7:3 (114.66 mg/ml). Thus, it was indicated that MIC values of shallot extract for *S. Typhimurium* and *S. aureus* were 114.66 and 163.80 mg/ml, respectively. Minimum bactericidal concentration (MBC) of the shallot extracts could be determined only in case of *S. Typhimurium* and *S. aureus*. They were tested by enumeration the viable cells in the tube with clear broth. Thus, the tubes with dilution from 7:3 – 10:0 and only 10:0 were tested for *S. Typhimurium* and *S. aureus*, respectively. We found that crude extract could not kill *S. aureus*, while the MBC for *S. Typhimurium* was at the ratio 9:1 (147.42 mg/ml).
Table 3. MIC value of crude extract for inhibiting the growth of *Escherichia coli*, *Salmonella Typhimurium TISTR292* and *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Dilution factor</th>
<th>The growth of bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>10:0</td>
<td>+</td>
</tr>
<tr>
<td>9:1</td>
<td>+</td>
</tr>
<tr>
<td>8:2</td>
<td>++</td>
</tr>
<tr>
<td>7:3</td>
<td>++</td>
</tr>
<tr>
<td>6:4</td>
<td>++</td>
</tr>
<tr>
<td>5:5</td>
<td>++</td>
</tr>
<tr>
<td>4:6</td>
<td>++</td>
</tr>
<tr>
<td>3:7</td>
<td>++</td>
</tr>
<tr>
<td>2:8</td>
<td>++</td>
</tr>
<tr>
<td>1:9</td>
<td>++</td>
</tr>
</tbody>
</table>

Positive control

“-” - Clear solution, “+” - Medium turbidity and “++” - Very turbidity

4. CONCLUSION

Even though the product from purification process of shallot FOS by using commercial absorbent was efficient for using as prebiotic ingredient (previous unpublished data), but the process markedly affected on antioxidant and antibacterial activity of shallot extract. Both biological activities had been gradually declined during purification process. However, crude shallot extract possessed high potential to be applied in functional food manufacturing as antioxidative and antibacterial agents. Thus, it could be concluded that crude extract and purified extract might be suitable for different purposes, including prebiotic, antioxidant and antibacterial uses.

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REFERENCES


**[6-1130-P-05] Probiotic characterization of thermotolerant *Lactobacillus johnsonii* isolated from broiler intestine**

*Rutaimas Wongpanti, Pairote Wongputtisin, Piyanuch Niamsup* (1. Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai(Thailand))

Keywords: Lactobacillus johnsonii, probiotic, broiler gastrointestinal tract, feed supplement

Bacterial community in human and animal gastrointestinal tract (GI) are diverse. In GI tract of healthy hosts, lactic acid bacteria (LAB) can be found as dominant flora. Some strains of LAB have been accepted as probiotic due to the fact that they contribute many health benefits to host. Several probiotics are isolated and applied in functional food and feed products for the specific consumers, including human and animal. Nowadays, thermotolerant probiotics are of interest to industrial application, because of their high heat-resistant ability in food and feed manufacturing. The aims of this study were to isolate thermotolerant LAB from broiler intestine and evaluate their probiotic characteristics for monogastric feed application. Two promising isolates, CK3 and VCF29 were selected and identified by 16S rRNA gene sequencing. Both of them were identified to *Lactobacillus johnsonii* with 100% similarity. *L. johnsonii* CK3 and *L. johnsonii* VCF29 were not haemolytic strains and their percentages of auto-aggregation value were 18.37±5.30 and 9.19±0.71, respectively. Resistibility to acidity at pH 2.5 and 0.3% bile acid of *L. johnsonii* VCF29 (94.68 and 94.73%) were greater than those of *L. johnsonii* CK3 (62.48 and 87.34%). Both strains were susceptible to cefoxitin, chloramphenicol, vancomycin, ampicillin and ceftriaxone. In addition, they exhibited antibacterial activity against pathogenic *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella Typhimurium* and *Escherichia coli*. It might be indicated that *L. johnsonii* CK3 and VCF29 could be good probiotic candidates applied as functional feed supplement for monogastric animal, especially broiler.
Probiotic characterization of thermotolerant *Lactobacillus johnsonii* isolated from broiler intestine

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ABSTRACT

Bacterial community in human and animal gastrointestinal tract (GI) are diverse. In GI tract of healthy hosts, lactic acid bacteria (LAB) can be found as dominant flora. Some strains of LAB have been accepted as probiotic due to the fact that they contribute many health benefits to host. Several probiotics are isolated and applied in functional food and feed products for the specific consumers, including human and animal. Nowadays, thermotolerant probiotics are of interest to industrial application, because of their high heat-resistant ability in food and feed manufacturing. The aims of this study were to isolate thermotolerant LAB from broiler intestine and evaluate their probiotic characteristics for monogastric feed application. Two promising isolates, CK3 and VCF29 were selected and identified by 16S rRNA gene sequencing. Both of them were identified to *Lactobacillus johnsonii* with 100% similarity. *L. johnsonii* CK3 and *L. johnsonii* VCF29 were not haemolytic strains and their percentages of auto-aggregation value were 18.37±5.30 and 9.19±0.71, respectively. Resistibility to acidity at pH 2.5 and 0.3% bile acid of *L. johnsonii* VCF29 (94.68 and 94.73%) were greater than those of *L. johnsonii* CK3 (62.48 and 87.34%). Both strains were susceptible to cefoxitin, chloramphenicol, vancomycin, ampicillin and ceftriaxone. In addition, they exhibited antibacterial activity against pathogenic *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella* Typhimurium and *Escherichia coli*. It might be indicated that *L. johnsonii* CK3 and VCF29 could be good probiotic candidates applied as functional feed supplement for monogastric animal, especially broiler.

Keywords: *Lactobacillus johnsonii*, probiotic, broiler gastrointestinal tract, feed supplement
1. INTRODUCTION
The microbial community of animal gastrointestinal (GI) tracts are complex and diverse, especially in the large intestine. They involve not only in nutritional digestion, but also the synthesis of vitamins, bioconversion of toxic compounds to non-toxic compounds, stimulation of immune system, maintenance of gut peristalsis and intestinal mucosal integrity and prevention of pathogen colonization (Ahasan et al., 2015). Therefore, the strategy in manipulation of microbial ecosystem in GI tract to enhance animal health, productivity and welfare has been introduced by many researcher, meanwhile, study of diversity and role of gut microbiota on animal health have being intensively investigated.

Many species of lactic acid bacteria (LAB) are accepted as probiotic and applied as feed additive in livestock production, since they play important roles on animal health, especially contribute the balance of gut microbiota. FAO and WHO (2001) defined the term “probiotic” as “live microorganisms which when administered in adequate amounts confer a health benefit to the host”. By this definition, probiotics have to be able to tolerate to acid in gastric juice and bile in upper small intestine, susceptible to antibiotics, adhere to epithelial surfaces, exhibit antagonistic activity against pathogens (such as Helicobacter pylori, Salmonella sp., Listeria monocytogenes, Clostridium difficile), anti-mutagenic and anti-carcinogenic properties, and so on (Kumar and Kumar, 2015; García-Hernández et al., 2016). Lactobacilli seem to be the most well-known probiotic potentially used in livestock production. L. reuteri, L. acidophilus, L. animalis, L. fermentum, L. salivarius and L. johnsonii are commonly applied in livestock production. Supplementation of these probiotic to swine and poultry feed gain many benefits. In swine, improvement of colostrum and milk quality, feed conversion ratio, diet digestibility and meat quality, increasing of piglet weight, reducing a risk of diarrhea and limiting constipation were obtained, while the increasing of body weight gain, carcass quality and bone quality and reducing of mortality were found in poultry production.

Nowadays, promising probiotic strains for feed supplement industry have to be considered also about survivability during manufacturing and stability in the product during storage. By this context, thermotolerant probiotic are of interesting. Thermotolerant LAB have been widely used as starter cultures in many food industries such as fermented milk, alcoholic beverages and sourdough, because of their higher heat-resistant ability during manufacturing. Moreover, the strain origin of probiotic must be another criterion for selection prior use. Those isolated from the same animal as the intended use have higher possibility of survival (Gibson and Fuller, 2000).

Previously, diversity of LAB in broiler GI tract was investigated in our Lab and we found some of them were thermotolerant LAB. Promising isolates were subsequently isolated and studied for their possibility applying as probiotic additive for monogastric animal production, especially broiler. The aims of this study were then to isolate and identify thermotolerant LAB from broiler intestine and evaluate their probiotic characteristics for monogastric feed application.

2. MATERIALS AND METHODS
2.1 Isolation of thermotolerant LAB and identification.
Thermotolerant LAB were isolated from broiler feces as previously described (Niamsup et al., 2003). Briefly, the fecal samples were inoculated into glucose/peptone/yeast extract (GPY) broth, incubated anaerobically at either 40, 45 or 50°C for 24 h and spread onto De Man Rogosa and Sharpe agar (MRS). Colonies were selected and maintained on MRS agar. The genomic DNA of the isolates was extracted and purified using a genomic DNA extraction kit (TIANamp Bacteria DNA Kit, China) and used as a template to amplify and sequence 16S rDNA, resulting in species identification.

2.2 Characterization of probiotic properties
Probiotic properties of thermotolerant LAB isolated from previous experiment were characterized as follow.

2.2.1 Hemolytic activity
Hemolytic activity of the isolates was tested by inoculation on blood agar (7% (v/v) sheep blood) and incubation at 37°C for 24 h (Pieniza et al., 2014). The isolates which did not exhibit lyse zone around their colonies were considered as non-hemolysis (γ-hemolysis). In case of hemolytic isolate, there were considered and classified into 2 types, green-hued zone (α-hemolysis) and clear lysed zone (β-hemolysis) production.
2.2.2 Acid and bile tolerant ability
The test of resistance under acid condition was carried out in vitro according to Rajam et al. (2012). Simulated gastric juice was prepared by 0.5% (w/v) pepsin in phosphate-buffered saline (PBS), pH 2.5. One ml of cell suspension (10^8 CFU) was transferred into 9 ml of simulated gastric juice, mixed well and incubated anaerobically at 37 °C for 3 h. The interval sampling during incubation for viable cell enumeration on MRS agar was done. The bile tolerance assay was tested according to Yamano et al. (2006) with modifications. One ml of cell suspension (10^8 CFU) were transferred to MRS broth supplemented by 0.3% (w/v) oxgall bile (Sigma) and subsequently incubated anaerobically at 37 °C for 3 h. Cell suspension was taken interval and enumerated for the survival cells on MRS agar.

2.2.3 Autoaggregation Assay
The isolates were grown anaerobically in MRS broth for 24 h at 37 °C. Cells were harvested by centrifuge at 4500 rpm for 10 min, washed twice and re-suspended in phosphate-buffered saline (PBS) at pH 7.2 to obtain approximately 10^8 CFU/ml. Bacterial cell suspensions and PBS (1:1 mL) were mixed by vortexing for 10 s and incubated at room temperature for 2 h. The optical density of the upper layer was measured at 600 nm (PBS was used as a blank). The auto-aggregation percentage expressed as

\[
(1 - [A_t/A_0]) \times 100
\]

where \( A_t \) represents the absorbance at time \( t = 2 \) h and \( A_0 \) the absorbance at \( t= 0 \) h. (Tarep et al., 2013)

2.2.4 Antibiotic susceptibility
Antibiotic susceptibility of the isolates was tested by the agar diffusion disk method (Gheziel et al., 2019). The commercial antibiotic disc used in this study were of cefoxitin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), clindamycin (2 µg), vancomycin (30 µg), ampicillin (10 µg) and ceftriaxone (30 µg). The 24 h-old inoculum of isolated LAB was spread on MRS agar. Then each antibiotic discs were immediately placed on the surface of agar and incubated at 37 °C for 24 h. The inhibition zone diameters were measured, and susceptibility was expressed in terms of resistant (R) and susceptible (S).

2.2.5 Antimicrobial activity
Some pathogens to monogastric animal were used as tested organism in this study, including *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella Typhimurium* and *Escherichia coli*. The antimicrobial activity test against these pathogens were evaluated using the agar spot test described by Shokryazdan et al. (2014) with modifications. Briefly, two µl of 24 h-old inoculum of each isolated LAB (10^8 CFU/ml) was spotted on MRS agar plates, dried for 30 min at room temperature and then incubated anaerobically at 37 °C for 24 h. After colony development, the agar were overlaid with 10 ml of mixture between 0.7% (w/v) agar and the 24 h-old inoculum of pathogen (adjusted to 10^8 CFU/ml) and incubated aerobically at 37 °C. Inhibition zones around LAB colonies were measured after 18 h of incubation (outer edge of the colony to the outer edge of the clear zone).

3. RESULTS AND DISCUSSION
3.1 Identification of thermotolerant LAB
We found two isolates (CK3 and VCF29) from broiler feces, which could tolerate to 50 °C. They were Gram-positive, rod shape, non-spore forming and catalase negative bacteria. According to 16S rDNA sequencing, CK3 and VCF29 were identified to *Lactobacillus johnsonii* with 100% similarity. In a neighbour-joining dendrogram created based on the sequence of CK3, VCF29 and sequences from the GenBank database, the phylogenetic position of CK3 and VCF29 was determined. The phylogenetic tree showed that the strains form an evolutionary lineage within the radiation of a cluster comprising *Lactobacillus* species and is phylogenetically most closely related to *L. johnsonii*. (Figure 1)
3.2 Characterization of probiotic properties

Two promising isolates, \textit{L. johnsonii} CK3 and \textit{L. johnsonii} VCF29 were selected and characterized for their probiotic properties. Moreover, two standard strains of \textit{L. johnsonii} from the Japan Collection of Microorganisms, RIKEN BioResource Center, Japan, i.e. \textit{L. johnsonii} JCM1022 and \textit{L. johnsonii} JCM8791, were studied for comparison.

We found that all isolates including reference strains were non-hemolytic bacteria (γ-hemolysis), since they did not exhibit any effect on blood agar plates after 48 h of incubation. It might be indicated that they were not harmful strains or rarely cause illness. Probiotics must survive from the extreme conditions in GI tract of animals, especially high acidity in stomach and bile in the upper small intestine. Figure 2 and 3 show the survival ability of the selected thermotolerant isolates. After 3 h incubation in simulated gastric juice (0.5% pepsin, pH 2.5), we found that VCF29 could survive in this condition similar to the reference strain of JCM1022. Even the survivability of other two strains were lower, but about 65-80% of their survival rate were obtained (Figure 2). CK3 was the lowest survivability strain under gastric condition. This strain also
exhibited lower survivability in the simulated bile (0.3% bile acid) but only slightly lower than other strains (Figure 3). Interestingly, they could resist to bile with the survival rate higher than 90%. Effective probiotics must possess these characteristic to guarantee the number of viable probiotic cells reach to the colon. Survival of probiotic along the GI tract depends on not only these particular characteristics, but also because of the feed matrix (composition of feed ingested) and competition of microbiota in the intestine. The results from this experiment were consistent to the study of Aiba et al. (2015) and Yamano et al. (2006). A commercial *L. johnsonii* could survive when incubated at pH 1.0, 1.5 or 2.0 at 37 °C up to 120 min. Moreover, *L. johnsonii* La1 showed the great survivability after 15 h incubation in 0.1% bile acids and the simulated gastric juice among all tested bacteria. In case of bile resistance of Lactobacilli and Bifidobacteria, multi mechanisms involve in detoxification of bile; i.e. bile salt hydrolase production, active efflux of bile acids/salts and changing in the composition of cell membrane and cell wall (Ruiz et al., 2013).

Figure 2. Survivability of thermotolerant *L. johnsonii* CK3 (●) and *L. johnsonii* VCF29 (♦) in simulated gastric juice comparing to the reference strains of *L. johnsonii* JCM1022 (▲) and *L. johnsonii* JCM8791 (■)

Figure 3. Survivability of thermotolerant *L. johnsonii* CK3 (●) and *L. johnsonii* VCF29 (♦) in bile comparing to the reference strains of *L. johnsonii* JCM1022 (▲) and *L. johnsonii* JCM8791 (■)
The ability in adhere to intestinal epithelial cells of these isolates were indirectly tested by the autoaggregation assay. It was found that the autoaggregation percentage values ranged between 9.2% and 24.8% after 2 h incubation (Figure 4). Among the thermotolerant LAB isolates tested, CK3 showed significantly high autoaggregation comparing to VCF29 (p<0.05) and not significantly different to a reference strain of JCM1022, even that of JCM1022 was higher (p>0.05). Autoaggregation of probiotics was considered to be necessary for adhesion to intestinal epithelial cells, then form a barrier preventing a colonization by pathogenic microorganisms. The colonized probiotic cells may also reduce the number of pathogens by reducing the pH of the gut, causing direct antagonism against pathogen (Vesterlund et al., 2005). Thus, CK3 and JCM1022 exhibited higher potential for this purpose. Interestingly, autoaggregation ability of CK3 was slightly higher comparing to other probiotic strains and markedly higher than that of some enteric pathogens in the study of Tareb et al. (2013), although only 2 h incubation was applied in our study. Therefore, CK3 could be accepted as one of the effective competitor in colon colonization.

![Figure 4](image.png)

**Figure 4.** Autoaggregation percentages of the isolated thermotolerant LAB comparing to reference strains of *L. johnsonii*

*Different letters represent significant difference (p<0.05). Duncan’s multiple range test*

The antibiotic resistances of the isolated thermotolerant LAB against eight common antibiotics were determined by the agar diffusion method as shown in Table 1. CK3, VCF29 and JCM8791 were susceptible to almost antibiotics, but only JCM1022 was susceptible to all tested antibiotics. Our thermotolerant CK3 and VCF29 could resist to erythromycin and tetracycline, respectively, while the reference strain of JCM8791 was resistant to tetracycline and clindamycin. The obtained results were in accordance with previously reported data for Lactobacilli and Bifidobacteria. They are generally sensitive to antibiotic erythromycin, tetracycline, chloramphenicol and ampicillin (Georgievaa et al., 2015). Actually, the transferring of antibiotic resistance genes from probiotic to enteric pathogens, either in food matrix or in GI tract, has been concerned as a global issue (Sharma et al., 2017). Thus, non-antibiotic resistance probiotics are of interest for applying in feeds and foods. However, there is an argument on this ability by some researcher. The advantage of antibiotic resistibility of probiotics was introduced, for example they could survive in host GI tract during the treatment by antibiotic in the case of some diseases.
Table 1. Antibiotic resistances of thermotolerant LAB depending upon various antibiotic

<table>
<thead>
<tr>
<th>Antibiotics disc</th>
<th>CK3</th>
<th>VCF29</th>
<th>JCM1022</th>
<th>JCM8791</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

* The inhibition zone diameters were measured, and susceptibility was expressed in terms of resistant (R) and susceptible (S).

Finally, the antibacterial activity of the isolates against those common pathogenic bacteria was studied and the results shown in Table 2. Every *L. johnsonii* could inhibit growth of all tested organisms but different level. Antibacterial activity of CK3 and VCF29 were almost similar, exception with the test of *S. aureus*. VCF29 exhibited strong antibacterial activity against *S. aureus* (zone of inhibition > 6 mm). However, both reference strains showed strong antibacterial ability against all tested pathogens. The antibacterial activity to other enteric bacteria was also reported according to Aiba et al. (2015). They found that *L. johnsonii* No. 1088 inhibited the growth of *H. pylori*, *E. coli* O-157 and *C. difficile*. The possibility of antagonistic activity of probiotics mostly attribute to the production of antimicrobial substances or metabolites such as organic acids, hydrogen peroxide and so on (Pridmore et al., 2008).

Table 2. Antimicrobial activity of the LAB from thermotolerant LAB from broiler intestine

<table>
<thead>
<tr>
<th>Strains</th>
<th><em>S. aureus</em></th>
<th><em>P. vulgaris</em></th>
<th><em>S. Typhimurium</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>CK3</td>
<td>4.0±0.0</td>
<td>6.0±0.0</td>
<td>5.0±0.0</td>
<td>4.0±0.0</td>
</tr>
<tr>
<td>VCF29</td>
<td>10.3±0.6</td>
<td>6.0±0.0</td>
<td>5.0±0.0</td>
<td>5.0±0.0</td>
</tr>
<tr>
<td>JCM1022</td>
<td>6.7±1.2</td>
<td>7.0±0.0</td>
<td>7.0±0.0</td>
<td>6.3±2.3</td>
</tr>
<tr>
<td>JCM8791</td>
<td>10.0±1.7</td>
<td>7.0±0.0</td>
<td>7.0±0.0</td>
<td>6.7±0.6</td>
</tr>
</tbody>
</table>

4. CONCLUSION

Two isolates of thermotolerant LAB, CK3 and VCF29, from broiler feces were identified by 16S rRNA gene sequencing to *L. johnsonii* with 100% similarity. *L. johnsonii* CK3 and *L. johnsonii* VCF29 were not hemolytic strains and able to tolerate in acidic condition of stomach and bile of upper small intestine. According to their percentages of autoaggregation values, there was possibility that both strains could colonize on colon epithelial cells. Both strains were susceptible to common antibiotics (cefotixin, chloramphenicol, vancomycin, ampicillin and ceftriaxone). In addition, they exhibited antibacterial activity against enteric pathogenic *S. aureus*, *P. vulgaris*, *S. Typhimurium* and *E. coli*. Therefore, the thermotolerant *L. johnsonii* CK3 and *L. johnsonii* VCF29 isolated from broilers could be interesting probiotic candidates applied as functional feed supplement for monogastric animal, especially broiler.

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Process optimization for antioxidant extraction from seed of soybean cultivar Chiang mai60

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Keywords: soybean, antioxidant activity, isoflavones, functional food, optimization

Soybean [Glycine max (L.) Merr.] cv. Chiang mai60, a local and popular cultivar of Thailand, plays an important role as source of protein food and phytochemicals contributing many health benefits to consumer. Antioxidant activity is one of the beneficial property obtained from soybean seed. Soy isoflavones, major antioxidants composing in soybean seed, have been isolated and developed into a variety of healthy foods. Therefore, this research aimed to optimize the optimal conditions for antioxidant extraction from seed of Chiang mai 60 for further application in functional food development. Ratio of water to soybean powder, extraction temperature and time were optimized by central composite design (CCD) method, a statistical experimental approach. The results showed that soybean extract with highest ABTS inhibition activity at 85.5% was obtained when the extraction was carried out the ratio of 3.18 ml: 1 g, 45ºC and 4 h (p=0.0004, R-squared = 0.9107). According to HPLC analysis, this soybean extract contained aglycones isoflavones (daidzein, glycitein, genistein) and glucosides isoflavones (daidzin, glycitin, genistin) approximately 0.2985 and 0.2397 mg/g seed, respectively. It might be indicated that seed of soybean cv. Chiang mai60 was one of good source of antioxidant and exhibited a potential to be utilized as ingredient for functional food development.
Process optimization for antioxidant extraction from seed of soybean cultivar Chiang mai60

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ABSTRACT

Soybean [Glycine max (L.) Merr.] cv. Chiang mai60, a local and popular cultivar of Thailand, plays an important role as source of protein food and phytochemicals contributing many health benefits to consumer. Antioxidant activity is one of the beneficial property obtained from soybean seed. Soy isoflavones, major antioxidants composing in soybean seed, have been isolated and developed into a variety of healthy foods. Therefore, this research aimed to optimize the optimal conditions for antioxidant extraction from seed of Chiang mai 60 for further application in functional food development. Ratio of water to soybean powder, extraction temperature and time were optimized by central composite design (CCD) method, a statistical experimental approach. The results showed that soybean extract with highest ABTS inhibition activity at 85.5% was obtained when the extraction was carried out the ratio of 3.18 ml: 1 g, 45ºC and 4 h (p=0.0004, R-squared = 0.9107). According to HPLC analysis, this soybean extract contained aglycones isoflavones (daidzein, glycitein, genistein) and glucosides isoflavones (daidzin, glycitin, genistin) approximately 0.2985 and 0.2397 mg/g seed, respectively. It might be indicated that seed of soybean cv. Chiang mai60 was one of good source of antioxidant and exhibited a potential to be utilized as ingredient for functional food development.

Keywords: soybean, antioxidant activity, isoflavones, functional food, optimization
1. INTRODUCTION
Soybean [*Glycine max* (L.) Merr.] is an important leguminous seed crop in many regions of the world. Its seed is rich of high quality protein, oil, saccharides, fiber, vitamins and many phytochemicals (Obendorf et al., 2008). Therefore, soybeans can be utilized in a variety of uses, mainly as food and feed, both direct consumption and processed into various foods. Soybean has been also an important economic crop in Thailand. Since 1975, the Chiang mai60, Thai soybean cultivar, was bred and developed by the Chiang mai Field Crop Research Center, Thailand. This cultivar has been popular and widespread in northern Thailand because of high productivity, resistance to diseases (rust and mildew disease, etc.) and acclimatization to geographic change.

Apart from utilizing as protein foods, Chiang mai60 was reported as a rich source of raffinose family oligosaccharides (RFOs) (Wongputtisin et al., 2015). These oligosaccharides are accepted as an effective prebiotic in functional food products, contributing to the balance of intestinal microflora. In addition, isoflavones are the group of polyphenolic phytochemicals that are commonly found as large quantity in soybean seed. Natural isoflavones can be classified in to 4 types, i.e. aglycones, glucosides, acetylglucosides and malonylglucosides (Wang et al., 2013). The main functionality of isoflavones is accepted as an antioxidant, resulting of reduce the risk and treatment of several diseases such as antitumor, antimeno/pausal (female) osteoporosis and anti-aging properties, improvement of learning and memory skills of menopausal women, prevention and treatment of heart disease and diabetes, and so on (Wang et al., 2013; Lante et al., 2018). From the benefits of soy isoflavones mentioned above, several isoflavones-based food products have been nowadays developed and commercialized to functional food market.

Our research group has been interested in development of functional food supplement from Chiang mai 60 soybean. However, antioxidant activity and isoflavone content of Chiang mai60 have not been yet investigated. Therefore, the optimization for antioxidant extraction process and quantification of isoflavones content in seed of soybean cultivar Chiang mai60 were aimed in this study for further application in functional food development.

2. MATERIALS AND METHODS
2.1 Raw material
Soybean seed, cultivar Chiang mai60, was kindly obtained from Chiang mai Field Crop Research Center, Chiang mai, Thailand. The seed was grind into fine powder by electric grinder and then dried at 55°C for 12 h soybean powder was kept under -20°C.

2.2 Optimization for process of antioxidant extraction
Three factors (variables), including ratio of water to powder, extraction temperature and extraction time, were optimized for the maximum antioxidant extraction from soybean powder by using statistical experimental design strategy. The central composite design (CCD) method was applied, resulting of established 20 experimental treatments. The range and level of each setting variables are shown in Table 1 and experiments were established as in Table 2. The experiments were carried out and the mixtures were centrifuged at 14,000 rpm, 4°C for 10 min. Supernatants were kept at -80°C during waiting for antioxidant activity determination by ABTS inhibition assay. The obtained data were subjected to regression and graphical analysis using Design Expert® software.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Parameter</th>
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<th>0</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Ratio of water to powder (ml : g)</td>
<td>10.00</td>
<td>20.00</td>
<td>30.00</td>
</tr>
<tr>
<td>B</td>
<td>Temperature (°C)</td>
<td>30.00</td>
<td>45.00</td>
<td>60.00</td>
</tr>
<tr>
<td>C</td>
<td>Time (h)</td>
<td>2.00</td>
<td>4.00</td>
<td>6.00</td>
</tr>
</tbody>
</table>
2.3 ABTS inhibition activity assay
Antioxidant activity of the extracts were determined by measurement of free radical scavenging activity using ABTS inhibition assay. Briefly, ABTS cation radical (ABTS⁺) solution was diluted with DI water to obtain an absorbance of 0.700 at 734 nm. 10 μl of extract was added to 990 μl of ABTS⁺ solution, mixed well and recorded the decreasing of A734 every 1 min until stable. Percent inhibition was calculated using the formula,

\[
\frac{A_{734} \text{ at } 0 \text{ min} - A_{734} \text{ at } 1 \text{ min}}{A_{734} \text{ at } 0 \text{ min}} \times 100
\]

2.4 Isoflavones determination by HPLC
Samples were extracted by 80% methanol (1:1), mixed well and stand overnight at -20°C. The precipitate was removed by centrifugation under 10°C. Soybean isoflavones were analyzed from clear supernatant according to (Lante et al., 2018). The HPLC Ultratechsphere C18 analytical column (size 4.6x250 mm) was used with controlled temperature at 35°C and 10 μl sample injection. The mobile phase was 0.25% (v/v) trifluoroacetic acid (TFA) in water (solvent A) and acetonitrile (ACN) (solvent B). A linear HPLC gradient was used as follow, 15% of solvent B for 6 min, then increased gradually to 30% over 4 min, to 40% over 2 min, to 50% over 1.50 min and 50% over 1.50 min. The duration of the analysis was 15 min at a solvent flow rate of 1.3 ml/min. Standard of aglycone s isoflavones (daidzein, genistein, glycitein) and glycosides forms (daidzin, genistin, glycitin) were obtained from a commercial source Wako Pure Chemical Industries, Ltd., (Osaka, Japan).

3. RESULTS AND DISCUSSION
The antioxidant activity, in term of ABTS inhibition activity, of the established 20 treatments according to CCD experiment were shown in Table 3 with different values. Among these responses, treatment number 9 and 10 exhibited the highest and lowest antioxidant activities, respectively. The analysis of variance (ANOVA) was carried out for the determination of significant factors and to predict the antioxidant activity as a function of these three factors. The analyzing data were shown in Table 4-5. It was found that simulated model was significant at p=0.0004 but not significantly fit to the quadratic

Table 2. The established treatments according to the central composite design experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio of water to soybean powder (ml : g)</td>
</tr>
<tr>
<td>1</td>
<td>10.00</td>
</tr>
<tr>
<td>2</td>
<td>30.00</td>
</tr>
<tr>
<td>3</td>
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<td>20.00</td>
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<tr>
<td>20</td>
<td>20.00</td>
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</tbody>
</table>
model according to lack of fit (p<0.0001). The estimated coefficient of three factors and their interaction were also analyzed. We found only the ratio between water and powder was highly significant (p<0.001) to antioxidant extraction with negative effect. Extraction time was another negative factor to antioxidant extraction from Chiang mai60 powder but not significant. The interactions between time and other two factors were negative but slightly influenced to antioxidant activity (P>0.05). Subsequently, the quadratic model for prediction of antioxidant extraction from the powder of Chiang mai60 seed was simulated by the software as follow:

\[
Y = 23.04 - 16.02A + 0.40B - 0.54C + 0.055AB - 0.33AC - 0.62BC + 8.02A^2 - 1.23B^2 - 1.05C^2
\]

Where
Y = ABTS inhibition activity (%), A = code value of ratio of water to soybean powder
B = code value of extraction temperature, C = code value of extraction time

The response surface graphs of this model were also plotted (Fig 1). The results confirmed the optimal level of extraction time and temperature were around 3-4 h and 45-50°C, while that of ratio of water and powder should be low. Less volume of water, for example around 3:1 as applied in this experiment, was effectively and enough for antioxidant extraction from Chiang mai60 seed. To enhance yield of antioxidant extraction from soybean seed, other strategies can be assisted in the process, for example ultrasonic (Lai et al., 2013) and UV radiation (Lante et al., 2018).

The highest antioxidant activity of treatment 9 correlated to its total isoflavones content which was the highest content among those treatments, meanwhile that of treatment 10 was in the group with low content of total isoflavones as shown in Table 4. Genistin and genistein were the major isoflavones found in seed of Chiang mai60, furthermore, glucosides isoflavones naturally accumulate in soybean seed higher than aglycones isoflavones (Baú and Ida, 2015). But it was noticed that proportions of total aglycones in most of the treatment which exposed to 45°C were higher than those of total glucosides. It was possibly to explain by the activity of endogenous β-glucosidase in soybean seed. The optimal conditions for soybean β-glucosidase were at 45°C and pH 4.5-5.0 (Matsuura and Obata, 1993; Chiou et al., 2010). Glycosidic bonding on glucoside molecules might be hydrolyzed resulting of free aglycones released. Aglycones exhibit greater antioxidant activity than that from glucosides since their smaller molecular size (Baú and Ida, 2015). Interestingly, aglycones content of extract from treatment 9 was also the highest amount. This might be another reason to explain the great antioxidant activity of this treatments.
Table 3. The actual and predicted antioxidant activity results optimization for process of antioxidant extraction by the central composite design

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ratio of water to soybean powder (ml : g)</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>ABTS scavenging activity (%)</th>
<th>Actual</th>
<th>Predicted</th>
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<tr>
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<td>30.00</td>
<td>2.00</td>
<td>16.41</td>
<td>38.34</td>
<td></td>
</tr>
<tr>
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<td>2.00</td>
<td>41.48</td>
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<td>17.08</td>
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<td>38.93</td>
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<td>30.00</td>
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<td>7.36</td>
<td>22.98</td>
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<td>4.00</td>
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<td>4.00</td>
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<td>4.00</td>
<td>23.41</td>
<td>17.41</td>
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</table>

Table 4. Analysis of variance (ANOVA) for the model regression

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F-value</th>
<th>Significant value (p-value)</th>
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</thead>
<tbody>
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<td>Model</td>
<td>4544.58</td>
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<td>504.95</td>
<td>11.33</td>
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<tr>
<td>Residual</td>
<td>445.77</td>
<td>10</td>
<td>44.58</td>
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<td>Lack of fit</td>
<td>442.44</td>
<td>5</td>
<td>88.49</td>
<td>132.89</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pure error</td>
<td>3.33</td>
<td>5</td>
<td>0.67</td>
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<td>Total</td>
<td>4990.35</td>
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<td></td>
</tr>
</tbody>
</table>

R²= 0.9107

SS = sum of squares, DF = degrees of freedom, MS = mean square

Table 5. Coefficient estimates by the regression model

<table>
<thead>
<tr>
<th>Independent variables (parameter)</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>Significant value (p-value)</th>
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</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>23.04</td>
<td>2.72</td>
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<tr>
<td>A-ratio</td>
<td>-16.02</td>
<td>1.81</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B-temp</td>
<td>0.40</td>
<td>1.81</td>
<td>0.8304</td>
</tr>
<tr>
<td>C-time</td>
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<td>1.81</td>
<td>0.7707</td>
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<tr>
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<td>0.9819</td>
</tr>
<tr>
<td>AC</td>
<td>-0.33</td>
<td>2.36</td>
<td>0.8908</td>
</tr>
<tr>
<td>BC</td>
<td>-0.62</td>
<td>2.36</td>
<td>0.7982</td>
</tr>
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</table>

*Statistically significant at 95% of confidence level.
Figure 1. The response surface graphs exhibiting the antioxidant activity of the extract obtained as the function of three factors (A: ratio of water to soybean powder, B: extraction temperature and C: extraction time).

Table 6. The results of soybean extract contained isoflavones by HPLC

<table>
<thead>
<tr>
<th>Trt</th>
<th>Glucosides</th>
<th>Aglycones</th>
<th>Isoflavones content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daidzin</td>
<td>Glycitin</td>
<td>Genistin</td>
</tr>
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<td>0.02</td>
<td>0.11</td>
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<tr>
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<td>0.06</td>
<td>0.02</td>
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<td>0.09</td>
<td>0.03</td>
<td>0.17</td>
</tr>
<tr>
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<td>0.09</td>
<td>0.02</td>
<td>0.21</td>
</tr>
<tr>
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<td>0.02</td>
<td>0.01</td>
<td>0.07</td>
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<td>0.00</td>
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<td>0.08</td>
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<tr>
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</tr>
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</tr>
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<td>20</td>
<td>0.02</td>
<td>0.00</td>
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</tbody>
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4. CONCLUSION

It could be concluded that the optimal processes for antioxidant extraction from seed of soybean cultivar Chiang mai60 could be carried out at 45-50°C, 3-4 h and low ratio between water and soybean powder. Under these conditions, the highest isoflavones content with the highest antioxidant activity were subsequently obtained. It might be indicated that seed of soybean cv. Chiang mai60 was one of good source of potential antioxidants to be utilized as ingredient for functional food development.

ACKNOWLEDGMENT

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Nutritional and Functional Properties of Yoghurt Drink with Philippine Gac (*Momordica cochinchinensis* Spreng.) and Bignay (*Antidesma bunius*) Fruits

Rowie Joy Gonzales Bucks¹, *Ara Fatima Cuvinar Algar¹, Ryan Rodrigo Paner Tayobong² (¹. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines), ² Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines))

**Keywords:** Philippine indigenous fruits, Fortified yoghurt drink, β-carotene, Lycopene, Antioxidant activity

Philippine indigenous fruits, Gac (*Momordica cochinchinensis* Spreng.) and Bignay (*Antidesma bunius*), were added to yogurt drink to increase its nutritional and functional properties. Fresh gac fruit aril was found to have high amounts of lycopene (204.54 μg/g), β-carotene (727.80 μg/g), and antioxidant activity (32.94% scavenging activity) while Bignay berries have high antioxidant property (85.54% scavenging activity). The best formulation, 20g bignay juice with 3.5g gac aril per 100g yogurt drink, was identified through sensory evaluation using quality scoring. The pH, titratable acidity (TA), total soluble solids (TSS), and lactic acid bacterial count of the gac-bignay yogurt drink were determined during a two-week storage period at 4°C. At the same time, the proximate composition, β-carotene, lycopene, and antioxidant activity of the most acceptable formulation were also determined. After the storage period, results showed that the gac-bignay yogurt drink has a pH value of 4.00, TSS of 23° Brix, lactic acid content of 1.00%, and lactic acid bacterial count of 6.75 log CFU/mL. The nutritional composition of the gac-bignay yogurt drink showed no significant difference with the plain yogurt drink in terms of the protein, fiber, and fat contents. However, the gac-bignay yogurt drink was found to have significantly higher β-carotene content (25.92 μg/g), lycopene content (16.56 μg/g), Vitamin A content (4.02 IU/g), and antioxidant activity (3.05% scavenging activity) than the plain yogurt drink. For a serving size of 80mL, it can provide 18% of the daily value required for Vitamin A and this has satisfied the definition for Vitamin A fortification. Thus, the functional properties of a regular probiotic drink has been elevated which can address different diseases such as cardiovascular disease, atherosclerosis, cancer, and neurodegenerative disorders.

Effect of Extracting Conditions on Plant Extract Colors and Stability of Antioxidant Properties during in vitro Gastrointestinal Digestion

*Rattika Aeka¹, Titikan Liangpanth¹, Rungarun Sasanatayart¹ (¹. School of Agro-Industry, Mae Fah Luang University(Thailand))

**Keywords:** Anthocyanins, Carotenoids, Betalains, Chlorophylls, in vitro gastrointestinal digestion, Antioxidant

Natural pigments extracted from plants provide distinctive color and exert antioxidant effects that are far more superior than synthetic colorants. Synthetic colorants tend to be undesirable by consumers, due to the harmful effects on human health, including allergic reactions, mutagenicity and potential carcinogenicity. As a result, there is a worldwide trend toward the natural colorants, in particular in food applications. In this study, four major types of plant pigment including anthocyanins from butterfly pea flower (*Clitoria ternatea* L.)
betalains from dragon fruit peel (*Hylocereus undatus*), carotenoids from turmeric rhizome (*Curcuma longa*) and chlorophylls from pandan leaf (*Pandanus amaryllifolius*) were extracted under different conditions. Three types of solvent (water, 70% w/w acetic acetone and 50% w/w aqueous ethanol) and three mechanical extraction methods (shaking 25° C for 24 h, sonication at 25° C for 1 h and sonication at 65° C for 1 h) were compared. Results showed that 50% w/w aqueous ethanol was the most effective solvent for extraction of carotenoid form turmeric and chlorophyll from pandan leaf whereas, water was the most effective solvent for extraction of betalain form dragon fruits peel and anthocyanin form butterfly pea flower. Between mechanical extractions, sonication was better than shaking in extracting the require pigments (carotenoids, betalains, anthocyanins and chlorophylls), total phenolics and total flavonoid from selected plants. Overall, sonication at 25° C was better than sonication at 65° C in obtaining plant extract with high antioxidant activities based on FRAP and DPPH with the reduced energy consumption. Therefore, color extracted with sonication at 25° C was used for testing the stability upon *in vitro* gastrointestinal digestion. Results showed that pigment compounds and related antioxidant activities of all four color extracts become less stable along digestion. Results revealed that the stability of each pigments and their related antioxidant during *in vitro* digestion from high to low were anthocyanins, carotenoids, chlorophylls and betalains, respectively. Data of this study supports the extension use of natural colorants as substitutes for synthetic dyes in food applications. However, the effect of food processing parameters including pH, heat and ingredients must be taken into accounted.

11:30 AM - 12:30 PM  (Fri. Sep 6, 2019 11:30 AM - 12:30 PM  Poster Place)

**[6-1130-P-09]** **pH Adjustment and Thermal Treatments Affect Plant Extract Colors and Antioxidant Activities during *in vitro* Digestion**

*Baifah Sangarun*, **1** Titikan Liangpanth*, **1** Rungarun Sasanatayart* **1** (1. School of Agro-Industry, Mae Fah Luang University(Thailand))

**Keywords:** Anthocyanins, Carotenoids, Betalains, Chlorophylls, *in vitro* gastrointestinal digestion, Antioxidant

There are restrictions of use for natural pigments because of the low stability and change when adjust pH and applying heat during food processing. In this study, the stability of plant color extract based on pH and heat treatments and the stability of antioxidant activities during *in-vitro* digestions were investigated. Butterfly pea flower and dragon fruit peel was extracted by water whilst, turmeric rhizome and pandan leaves were extracted by 50% w/w aqueous ethanol and subsequently freeze dried into color powders. Each color powder was dissolved in water to concentration of 1.0% w/w and adjusted to pH 1.0-10.0 to observe color and the absorbance measured by spectrophotometry between 400-700 nm. Results showed the change in absorbance at different pH, indicating structural change of pigment compounds and consequently change in color parameters (L*, a*, **b*** and hue values). To investigate effect of pH adjustment and heat treatment, pure color extracts were adjusted to pH 3.0 and 7.0 and subjected to three heat treatments including (1) no heat (control), (2) pasteurization (75°C for 15 min) and (3) sterilization (121°C for 15 min). All samples were measured for color parameters and antioxidant properties were measured in terms of total phenol content (TPC), total flavonoid content (TFC) and antioxidant activities based on FRAP and DPPH assays. Results showed that pH adjustment and heat treatment affected visual color and color parameters, regarding to type of plant pigment and this could limit further food use. Color extracts at pH 3.0 and subjected to pasteurization better retained color, pigment compounds and related antioxidant properties than
sterilization. The exception was for sample coloring with pandan leaves extract which retained the most color after adjusted to pH 7.0 and sterilized. To investigate the stability during \textit{in-vitro} gastrointestinal digestion, all pasteurized plant color extract at pH 3.0 was tested in comparing with the corresponding unheated plant extract. During \textit{in-vitro} gastrointestinal digestion, the greater amount of TPC, TFC and related antioxidant activities based on FRAP and DPPH in pasteurized samples than in unheated samples were observed. Results illustrated the effect of pasteurized heat on increasing bioavailability of the studied bioactive compounds during \textit{in-vitro} digestion. However, along digestion, all bioactive compounds increased slightly from oral phase (G0) to gastric phase (G30) but decreased gradually to the lowest values along intestinal phase (I0-I120). Data of this study supports of extension use and provides the limit use of natural colorants in food applications.

[6-1130-P-10] Changes in the Growth and Antioxidant Components of Komina with Different Red and Blue Light Emitting Diode (LED) Irradiation Ratios

Kanako Niiya\textsuperscript{1}, Takahiro Saito\textsuperscript{2}, Masatsugu Tamura\textsuperscript{2}, San Woo Bang\textsuperscript{2} (1. Utsunomiya University Graduate School(Japan), 2. Utsunomiya Univ.(Japan))

Keywords: Antioxidant, Growth, Komina, Radiation ratio

This study investigated the effects of the ratio of red and blue LED irradiation on the growth and antioxidant components of leafy vegetable Komina. LEDs of red and blue were adjusted to 0, 0.11, 0.43 and 1.0, and used to grow Komina in plant factory. White LED was also utilized. The growth characteristics such as plant height, umber of leaves and fresh weight were evaluated every week after transplanting to the plant plate. With regard to antioxidant properties, the L-AsA content and the total polyphenol content (TPC) were analyzed at the sample plant height of 25cm. Sample plant irrigated with B/R ratio 0 showed that the plant height and the number of leaves were 19.2 cm and 8.0 pieces respectively after 3 weeks of transplantation and the fresh weight was 53.2 g after 4 weeks of transplantation, the largest values among all irradiated samples. Sample plant irrigated with B/R ratio 1.0 was the largest L-AsA (68.4 mg·g\textsuperscript{-1}F.W.) and TPC (9.7 mg·g\textsuperscript{-1}D.W.) respectively. Finally, the growth of Komina was promoted as the B/R ratio decreased, and the antioxidant component was contained more as the B/R ratio increased.
**[6-1130-P-11] Temporal Source Strength Estimation of Sweet Pepper for Crop Management and LED Supplementation Efficiency Improvement**


₁. Miyagi Prefectural Agriculture and Horticulture Research Center(Japan), 2. Graduate School of Bioresource Sciences, Nihon University(Japan), 3. National Agriculture and Food Research Organization(Japan)

11:30 AM - 12:30 PM

**[6-1130-P-12] Study on Analysis of Loads Effect on Path-Tracking Accuracy of an Autonomous Tractor during Plow Tillage**

*YEONSOO KIM*₁, *2, YONGJOO KIM*₂, *HYOGEOL KIM*³, YOUNGJOO KIM*¹, SANGDAE LEE*³

₁. KITECH(Korea), 2. Chungnam Univ.(Korea)

11:30 AM - 12:30 PM

**[6-1130-P-13] Classification of Sugarcane Variety using Image Processing and Multivariate Analysis**

*KITTIPON APARATANA*¹, *Hiroo Takaragawa*₁, *2, Yoshinari Izumikawa*₁, *2, Eizo Taira*¹

₁. Faculty of agriculture, University of the Ryukyus, Okinawa 903-0213(Japan), 2. The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima 890-0065(Japan)

11:30 AM - 12:30 PM

**[6-1130-P-14] Relationships between the Number of Sneezes and Swine Influenza Infection Experiment Factors**


₁. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan)

11:30 AM - 12:30 PM

**[6-1130-P-15] Sound Source Localization in Pig Houses Using Wireless Microphone Array and Its Accuracy by Microphone Arrangements**

*Akifumi Goto*¹, *Misaki Mito*¹, *Tadashi Ebihara*², *Koichi Mizutani*², *Naoto Wakatsuki*², *Nobuhiro Takekuma*³, Takehiko Saito³

₁. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan)

11:30 AM - 12:30 PM

**[6-1130-P-16] Behavioral Study of Vibrational Sensitivity in Whitefly**

*Yasuhiko Nishijima*¹, *Koichi Mizutani*¹, *Tadashi Ebihara*¹, *Naoto Wakatsuki*¹, *Kenji Kubota*², *Hiroyuki Uga*³

₁. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, Division of Engineering Interaction Technologies, University of Tsukuba(Japan), 3. Agriculture Research Center, National Agriculture and Food Research Organization(Japan), 4.
Application of Palm Oil Based Wax as a Coating Material on the Quality of Cucumber Seed

*Songsin Photchanachai\textsuperscript{1}, Nipada Ranmeechai\textsuperscript{1,2}, Chalinee Sungkajorn\textsuperscript{1,2}, Anantaporn Phankhaek\textsuperscript{1,2}, Kornkanok Aryusuk\textsuperscript{1}, Varit Srilangi\textsuperscript{1,2}, Panida Boonyarithongchai\textsuperscript{1,2}, Nutthachai Pongprasert\textsuperscript{1,2} (1. School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok(Thailand), 2. Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok(Thailand))
Temporal Source Strength Estimation of Sweet Pepper for Crop Management and LED Supplementation Efficiency Improvement

*Masaaki Takahashi¹, So Kaneko¹, Osamu Koike¹, Hiroki Umeda², Yasunaga Iwasaki³ (¹. Miyagi Prefectural Agriculture and Horticulture Research Center(Japan), ². Graduate School of Bioresource Sciences, Nihon University(Japan), ³. National Agriculture and Food Research Organization(Japan))

Keywords: sweet pepper, supplemental light, fruit number, yield, source strength, sink strength

The fruit load of sweet pepper (*Capsicum annum* L.) is heavy, and if a sufficient amount of photosynthesis cannot be produced, abscission occurs, and the yield is lowered. When considering photosynthesis, it is important to balance the strength of energy sources and sinks. The source strength is the extent of the supply of assimilates, which depends upon the amount of solar radiation received, leaf area, plant architecture, and photosynthetic characteristics. Since the leaves of sweet peppers are not generally cut, the amount of light in a production facility is important for the generation of high yields. In this study, the amount of light was increased using irradiation by LEDs from above, and the influence of the light intensity on the number of fruits and the yield was measured. We also investigated whether the source strength could be properly evaluated, based on the prediction of the number of fruits. The experiments in sweet peppers were conducted in two plastic houses in Miyagi Prefecture, Japan. Three independent surveys were conducted, with planting times in the middle of July 2017, the end of February 2018, and the end of August 2018. As a result, it was shown that the yield and the number of fruit set were increased in the areas where light was supplemented in the three experiments. By investigating the amount of light received, light utilization efficiency, and fruit distribution rate, it was possible to estimate the number of fruit set. When the source strength was increased by supplementing the LEDs, the predicted number of fruits changed, and the change in the actual number of fruit set showed the same tendency. These results showed that light source strength was properly evaluated. The correct source strength can be quantified more accurately in real time by utilizing such a depth sensor to acquire plant growth information. The most effective way to use LED supplementation involves being able to use additional source strength without waste. Advanced cultivation management methods are made possible by using an estimation of light reception amount by the sensor, and by the adjustment of the light amount by the LED as required. This research was supported by grants from the Project of the NARO Bio-oriented Technology Research Advancement Institution (research program on development of innovative technology).
Study on Analysis of Loads Effect on Path-Tracking Accuracy of an Autonomous Tractor during Plow Tillage

*YEONSOO KIM¹,², YONGJOO KIM², HYOGEOL KIM¹, YOUNGJOO KIM¹, SANGDAE LEE¹ (1. KITECH(Korea), 2. Chungnam Univ.(Korea))

Keywords: Agricultural tractor, Lateral error distance, Wheel axle torque, Draft force

The purpose of this study was to provide guidelines for the basic factor of auto-steering system design considering the measured work load on an autonomous tractor during plow tillage operation. Load of agricultural tractor has been studied intensively, but it is still difficult to analyze the effects of load on the path-following performance of autonomous tractors. The objective of present study was to offer suggestions on measured methods of lateral error distance. The effect of working load such as wheel torque and draft force on the lateral error distance was analyzed. The lateral error distance measurement system consisted of an electric tacheometer, GNSS receiver, and prism. The load measurement system consisted of a wheel torque meter, a telemeter proximity sensor, and 6-component load cells. The field test conducted in a four-wheel mode and an M3-Low gear stage, which are commonly used to perform moldboard plow in Korean paddy fields. The field test was conducted for a 100 m straight line, and the wheel axle torque, draft force, and lateral error distance were simultaneously measured in the same time. Through this field test, the effect of load on the accuracy of path-following performance of agricultural tractor during the plow tillage operation was analyzed. In future study, the field test will be conducted on factors affecting the accuracy of path-following performance among the soil-machine factors. The results of this study can provide useful information to improve the accuracy of path-following performance according to the working load during plow tillage operation.
Study on Analysis of Loads Effect on Path-Tracking Accuracy of an Autonomous Tractor during Plow Tillage
Yeon-Soo Kim1,2, Hyo-Geol Kim1, Young-Joo Kim1, Yong-Joo Kim2, Sang-Dae Lee1*

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2 Department of Biosystems Machinery Engineering, Chungnam National University, Republic of Korea

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ABSTRACT
The purpose of this study was to provide guidelines for the basic factor of auto-steering system design considering the measured work load on an autonomous tractor during moldboard plow operation. Load of agricultural tractor has been studied intensively, but it is still difficult to analyze the effects of load on the path-following performance of autonomous tractors. The objective of present study was to offer suggestions on measured methods of lateral error distance. The effect of working load such as wheel torque and draft force on the lateral error distance was analyzed. The lateral error distance measurement system consisted of an electric tacheometer, GNSS receiver, and prism. The load measurement system consisted of a wheel torque meter, a telemetric proximity sensor, and 6-component load cells. The field test conducted in a four-wheel mode and an M3-Low gear stage, which are commonly used to perform moldboard plow in Korean paddy fields. The field test was conducted for a 100 m straight line, and the wheel axle torque, draft force, and lateral error distance were simultaneously measured in the same time. Through this field test, the effect of load on the accuracy of path-following performance of agricultural tractor during the plow tillage operation was analyzed. In future study, the field test will be conducted on factors affecting the accuracy of path-following performance among the soil-machine factors. The results of this study can provide useful information to improve the accuracy of path-following performance according to the working load during plow tillage operation.

Keywords: Agricultural tractor; Lateral error distance; Draft force; Wheel torque; Moldboard plow operation

1. INTRODUCTION
The agricultural production and labor shortage issues are constantly increasing due to rural aging (Celik et al., 2018). Agricultural machinery automation is one of the most effective solution of improving agricultural challenges such as food, operating cost, working environment, and so on (Zhang and Pierce, 2013). The global agricultural machinery market is expected to grow at a CAGR (Compound Annual Growth Rate) of 6.6% from US $ 140.7 billion in 2014 to US $ 193.5 billion in 2019 (KREI, 2018). The global autonomous tractors market is expected to grow at a CAGR of 24.8%, reaching 12,508 Units in 2019 and 60,901 Units by 2025. The growth of the autonomous tractor market is expected to lead the government or several primary manufactures as a part of the adoption of new technologies to improve the working efficiency and productivity of crop yields (Li et al., 2019; MarketsandMarkets, 2018). The tractor is main product of agricultural machinery due to various uses as agricultural power source (Kim et al, 2018). Especially, the most important performance evaluation factor of
autonomous tractor is lateral error distance (McCall and Trivedi, 2005). Therefore, the importance of developing autonomous tractor equipped with accurate path-tracking technology for securing competitiveness of the global market is increasing. In addition, the research on the performance evaluation method of path-tracking accuracy has been actively carried out. In order to improve the convenience of farmers and crop productivity, many studies have been conducted various autonomous agricultural machinery. Some studies related to the autonomous agricultural machinery have been carried out in relation to path-tracking performance considering lateral error and heading angle on off-road surface without tillage operation. The study has been conducted on methods for estimating a utility and agricultural vehicle’s dynamic parameters using a RTK-GPS and Inertial Measurement Unit (IMU). The results showed that the measured data using a RTK-GPS and IMU can be used to estimate the tire sideslip and the tire cornering stiffness under different soil conditions (Ospina and Noguchi, 2016; Ospina and Noguchi, 2018). Liu et al, (2019) studied the image processing based UAV (Unmanaged Aerial Vehicle) used for spraying pesticides and herbicide. The results show that the proposed algorithm has more accurate path-tracking performance than DGPS based UAV. Yin et al, (2018) developed an autonomous navigation system using sensor fusion algorithm that automatically guided a rice transplanter working along predetermined paths including steering, stop, going forward and reverse. The results showed that path-tracking were robustly executed in terms of following straight paths. Rahman et al, (2019) developed an optimum harvesting area of a convex and concave polygon for path planning of robot combine harvester. The results show that this developed algorithm estimates the optimum harvesting and reduces crop losses. It is also calculated based on the corner vertices minimizes the total operation time. In another study, the leader follower system was developed using two autonomous tractors for agricultural operation (Zhang and Noguchi, 2017). This experiment results showed the two autonomous tractor can work safely to complete the operation, and the system’s efficiency improved by 95.1% compared with using a single tractor. In another study, an adaptive turning algorithm for a four-wheel autonomous tractor was developed using navigation sensors consisted of an inertial measurement unit and a real-time kinematic global positioning system (Wang and Noguchi, 2018). The results showed that the time consumption and turning trajectory were decreased by 17% and 21%, respectively, compared to a conventional turning algorithm. There have been many studies on the automatic steering system of agricultural machinery. These performance evaluation method only using the posture and position information which are logged on the IMU and GPS in the path-tracking performance evaluation has been performed. This method is a performance evaluation method widely used in the automotive field. Generally, an autonomous commercial vehicles drive on a standard road surface such as asphalt without disturbance. However, an agriculture tractor have a relatively high load due to tire slip, sudden change in attitude angle, soil resistance, etc., depending on the correlation between soil and attachment implement during tillage operation (Wong, 2008). This does not take into account the error on the GPS sensor, and therefore it is not an accurate performance evaluation method. In addition, performance evaluation was performed by simple driving without tillage operation which can be used as a representative use purpose of actual farm machinery. The performance evaluation method of the path-tracking accuracy of an autonomous tractor under no tillage operation condition is did not consider the effect of the work load according to the terramechanics factors between soil and the agricultural tractor with attached implement. The load of the agricultural machinery is an important indicator of farming characteristics, and performance evaluation of agricultural machinery through load analysis is essential (Nahmgung, 2001). Therefore, it is necessary to study the new performance evaluation method from the viewpoint of work load which is generated on tractor’s main part under tillage operation conditions.
To improve the quality of the tractor, it is necessary to analyze the tractor working load during operation. This is because the work load characteristics are affected by various factors such as soil properties, operation type, traveling speed (gear selection), tillage implement shape, and tillage depth. Load analysis of agricultural tractor during field operation is important in achieving the optimum design of tractors.

Many studies have been carried out in consideration of soil properties, the type of operation, and the seasonal conditions. Analyzing the above research literature to date related to the work load of agricultural tractor during field operation, it can be confirmed that work load has the greatest influence on field operation. Nevertheless, there has been no consideration of precise lateral error distance methods according to work load. Therefore, it is necessary to develop a improved method of lateral error distance measurement and to analyze accuracy of path-following performance according to the work load.

The purpose of this study was to provide guidelines for the basic factor of auto-steering system design considering the measured work load on an autonomous tractor during moldboard plow operation. The specific objectives were (1) to develop a load measurement system and the path-tracking performance evaluation system, (2) to measure the load of the tractor’s main part and lateral error distance, (3) to analyze the effect of an agricultural tractor's load on path-tracking performance during moldboard plow operation.

2. MATERIALS AND METHODS

2.1 Agricultural tractor and implement

A 78 kW-class agricultural tractor (S07, Tong Yang Moolsan, Gongju, Korea) was used in this field experiment. The tractor had an empty vehicle weight of 3985 kg and dimensions of $4225 \times 2140 \times 2830$ mm (length × width × height) except for attached measurement system. The tractor used for measurement was equipped with a mechanical transmission. The 32 forward and 32 backward traveling speeds of the tractor were determined by the combination of the gear setting according to the operation type. The rated engine power of the tractor at an engine revolution speed of 2300 rpm was 78 kW. In this study, an eight–row moldboard plow (WJSP–8, WOONGJIN MACHINE RY, Kimje, Korea) was used to account for the 78-kW tractor engine power. Moldboard plows are mainly used in Korean rice paddy fields during primary tillage. The moldboard plow is superior to other plow implements in terms of stability; but features relatively large traction resistance. The specifications of the agricultural tractor used in this study are given in Table 1.

<table>
<thead>
<tr>
<th>Item</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length × Width × Height (mm)</td>
<td>$4225 \times 2140 \times 2830$</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3985</td>
</tr>
<tr>
<td>Engine</td>
<td>Rated power (kW)</td>
</tr>
<tr>
<td></td>
<td>Max. torque (Nm)</td>
</tr>
<tr>
<td>Transmission</td>
<td>Main</td>
</tr>
<tr>
<td></td>
<td>Sub</td>
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</tbody>
</table>

2.2 Lateral error measurement system

The lateral error measurement system was built to measure the lateral error distance and the traveling speed of tractor. First, the lateral error distance was measured using an electric tacheometer (IX series, TOPCON, Tokyo, Japan), a GNSS receiver (GCX3, TOPCON, Tokyo, Japan), and a prism attached to the tractor cabins at the center of gravity position. The electric tacheometer was used to evaluate the accuracy of the steering system of the autonomous tractor.
Second, the traveling speed of the tractor, which is the basic measuring element of tillage operation, was measured using RTK–GPS (Span CPT, NovAtel Inc., Calgary, Canada) and an antenna attached to the tractor’s center of gravity. In addition, the RTK base station was installed to ensure stable RTK–GPS status. The precisely measured traveling speed with RTK–GPS was used as a factor to determine the longitudinal slip ratio, which is the basic factor for evaluating the working performance of a tractor. The detailed lateral error measurement system configuration is shown in Figure 1.

![Figure 1. Configuration of lateral error distance system.](image)

### 2.3 Load measurement system

The load measurement system was configured to measure the draft force and wheel torque off agricultural tractor. The detailed configuration of load measurement system is shown in Figure 2. In this experiment, the torque meter (PCM16, MANNER, Spaichingen, Germany) and the telemetric proximity sensor (PRDCML30–25DN, Autonics, Busan, Korea) were used to measure both the torque and the rotational speed of the agricultural tractor. The torque meter was installed on each of the four axle shafts. One antenna was provided for each torque meter. The axle torque data measured at the torque meter were amplified by the amplifier in the torque meter and transmitted to the antenna, and the data transmitted to the antenna were transmitted to the meter along the cable line. The nominal load of the torque meter was 20 kNm, the maximum load was 400%, and the sensor was of a strain gage type. The sampling rate was 4 kS/s, the maximum axle rotation speed was 4000 rpm. The operating temperature was -25 to 125 °C. The 6–component load cell (UU–T2, DACELL, Cheongju, Korea) was installed between the moldboard plow and tractor body rear side for measuring draft force. The 6-component load cell consisted of three load cells measuring the horizontal force and three load cells measuring the vertical force. In this experiment, only three load cells were used to measure the horizontal component of the implement draft.
2.4 Measurement of soil physical properties
Tractor tillage operations have a major impact on crop growth and crop yields. The plow layer is the target tillage depth section that is cultivated annually or periodically through an agricultural tractor. The thickness of the plow layer is usually 5–25 cm, and this layer is often greatly worked on in relation to tillage operation, fertilizer, irrigation, and crops. The physical properties of soil such as the cone index, moisture content, and soil classification affect the interactions between the soils and the agricultural machine. The measurement process of soil properties is shown in Figure 3. In order to analyze the lateral error distance of autonomous tractor according to work load during tillage operation, the soil physical properties (cone index, moisture content, and soil classification) of the test site were confirmed using a cone penetrometer (FieldScout SC 900, Spectrum Technologies, Aurora, USA) and a soil sensor (FieldScout TDR 350, Spectrum Technologies, Aurora, USA). The measured soil properties such as cone index (CI), the moisture content (MC), and soil classification by particle size were analyzed following USDA standard methods.

2.5 Test procedure
The field experiments were conducted in Kumam–ri, Songsan–myeon, Chungcheongnam–do, Korea. The test site is 100×100 (m) in size and is located at latitude and longitude coordinates 36°55’48” N and 126°37’59” E, respectively. The field test was conducted in four–wheel mode and at three gear stages (M2-high, M3-Low, M3-High), which are commonly used to perform moldboard plow operation. The moldboard plow operation was carried out at the lowest tractor 3-point hitch to perform the under top hardpan section of the plow layer.
3. RESULTS AND DISCUSSION

3.1 Soil physical properties
The main analysis results of soil properties are as follows. The average moisture content of the test site was 38.6%, the mean CI was 2407 kPa, and the mean formation depth of hardpan was 12.5-25 cm. A soil particle size analysis revealed loamy sand in all soil layers. High cone index values indicate high soil compaction, which is a major problem when managing poorly drained soils. A soil hardpan layer with high soil compaction resulting from excessive and improper use of agricultural machinery leads to lower soil porosity and air permeability interferes with the growth of crop roots, and poor drainage. Therefore, during moldboard plow operation, the minimum tillage depth should be where soil compaction is increased. Thus, tillage operations should promote crop growth and ensure porosity and air permeability. In general, the results of plow tillage tend to show irregular tillage depths. Based on an analysis of the test results of the cone index, there is a point at a certain depth where an instantaneous slope occurs. The occurrence of an instantaneous slope implies that a rigid plate is located, and this depth is called the top hardpan. Owing to this reason, the target tillage depth must be set considering the hardpan layer, which indicates the distance from the depth of the top hardpan to the depth of the peak cone index. The cone index was measured using a cone penetrometer and detailed analysis results are shown in Figure 4.

![Figure 4](image)

Figure 4. Test results of the cone index using a penetrometer.

3.2 Lateral error distance
The average the draft force were 30.39, 32.57, and 32.79 kW at each gear selection. The draft force increased 2.18 kN when gear shift from M2-High to M3-Low, and increased 0.22 kN when gear shift from M3-Low to M3-High. Almost same torque values were shown in M2-High and M3-Low gear selection. The average front wheel torque were 3267.34, 3693.32, and 3852.37 kW at each gear selection. The average front wheel torque increased 65.98 Nm when gear shift from M2-High to M3-Low, and increased 159.05 Nm when gear shift from M3-Low to M3-High. The front axle load showed a tendency to increase as the number of gears selection. The average the rear wheel torque were 6080.12, 6934.53, and 6727.92 Nm at each gear
selection. The rear axle load, which is the most affected factor according to the traveling speed of the agricultural tractor, the tillage depth of the attachment workstation, and the operation type, was found to be the largest in M3-Low gear selection, not M3-High. This is judged to have resulted in a relatively large slip rate at the M3-High gear selection, resulting in a torque loss. The overall data tends to be similar to the traveling speed data as the number of gears selection. However, if the draft force values were similar, the lateral error was greatest in the M2-Low gear selection with a large rear wheel torque. Based on these results, the lateral error of the autonomous tractor is judged to be most affected by the rear wheel torque rather than the draft force and front wheel torque. The detailed overall test results of the lateral error distance according to work load are listed in Table 2.

<table>
<thead>
<tr>
<th>Gear selection</th>
<th>Tillage depth (cm)</th>
<th>Draft force (kN)</th>
<th>Front wheel (Nm)</th>
<th>Rear wheel (Nm)</th>
<th>Lateral error (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2-High</td>
<td>3</td>
<td>3627.34</td>
<td>6080.12</td>
<td>4.59</td>
<td></td>
</tr>
<tr>
<td>M3-Low</td>
<td>2.5</td>
<td>3693.32</td>
<td>6934.53</td>
<td>7.21</td>
<td></td>
</tr>
<tr>
<td>M3-High</td>
<td>2.7</td>
<td>3852.37</td>
<td>6727.92</td>
<td>5.88</td>
<td></td>
</tr>
</tbody>
</table>

4. CONCLUSION
A lateral error measurement system and load measurement system are proposed here for measuring tractor work load and lateral error distance during moldboard plow operation. This system configuration allows for precise measurement of lateral error distance, which was previously difficult to measure, and shows the effect of work load on path-following performance of autonomous tractor. The conclusions of this study are as follows.

The work load has a great effect on the lateral error distance of autonomous tractor during moldboard plow operation. In particular, the rear axle load was found to have a significant effect on lateral error compared to draft force and front axle load. Therefore, the influence of work load should be considered when analyzing the lateral error distance of autonomous tractor in an actual paddy field.

In conclusion, the effect of work load on the lateral error distance of an autonomous tractor during moldboard plow operation was confirmed with the measurement system configuration presented in this paper. These findings can be used in future research on the path-following performance of autonomous agricultural machinery.

ACKNOWLEDGMENT
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Sugarcane variety classification is essential for data collecting and learning for the breeder. It is difficult for a farmer to identify a sugarcane variety without specialist help. In this research, three Japanese sugarcane varieties (Ni15, Ni22, and Ni27) from six areas in the south of Japan were classified according to full pixel and color features of the sugarcane bud. The 54 images of sugarcane bud were acquired from the sugarcane field using a mobile phone’s digital camera, equipped with a fixed distance accessory. To develop classification models, two types of data; The full pixel data and color feature data from images were investigated for input to the model. The full pixel and color features were subjected to Principal component analysis (PCA) to describe the sugarcane bud samples. Then, the samples were classified into varieties by performing partial least squares discriminant analysis (PLS-DA) and support vector machine classification (SVM-C). The results of the full pixel show that the pooled classification rates (averaged three classification rate) by PLS-DA and SVM-C were 79.6% and 84.5%, respectively, while the pooled classification rates by PLS-DA and SVM-C of the color features were 75.9% and 74.1%, respectively. Therefore, these results show that the size and color spaces of sugarcane buds could be the keys to classifying sugarcane varieties and that the best way of classifying Japanese sugarcane (Ni15, Ni22, and Ni27) was the SVM-C method using full pixel of sugarcane bud.
Classification of Sugarcane Variety using Image Processing and Multivariate Analysis

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ABSTRACT
Sugarcane variety classification is essential for data collecting and learning for the breeder. It is difficult for a farmer to identify a sugarcane variety without specialist help. In this research, three Japanese sugarcane varieties (Ni15, Ni22, and Ni27) from six areas in the south of Japan were classified according to full pixel and color features of the sugarcane bud. The 54 images of sugarcane bud were acquired from the sugarcane field using a mobile phone’s digital camera, equipped with a fixed distance accessory. To develop classification models, two types of data; The full pixel data and color feature data from images were investigated for input to the model. The full pixel and color features were subjected to Principal component analysis (PCA) to describe the sugarcane bud samples. Then, the samples were classified into varieties by performing partial least squares discriminant analysis (PLS-DA) and support vector machine classification (SVM-C). The results of the full pixel show that the pooled classification rates (averaged three classification rate) by PLS-DA and SVM-C were 79.6% and 84.5%, respectively, while the pooled classification rates by PLS-DA and SVM-C of the color features were 75.9% and 74.1%, respectively. Therefore, these results show that the size and color spaces of sugarcane buds could be the keys to classifying sugarcane varieties and that the best way of classifying Japanese sugarcane (Ni15, Ni22, and Ni27) was the SVM-C method using full pixel of sugarcane bud.

Keywords: Sugarcane, Variety classification, PCA, PLS-DA, SVM-C.

1. INTRODUCTION
Sugarcane is a critical economic crop in the world and Japan. Currently, in Japan, sugarcane is generally planted in the southern part during the summer or spring and then harvested in winter, and production has increased from 2015 to 2018 (Okinawa Prefectural, 2018). However, severe droughts and tropical storms (typhoons) frequently occur from July to September, which causes severe damage to sugarcane yield and sugar content through the loss of green leaves, lodging, and broken stalks (Takagi et al., 2005; Terauchi et al., 2012). Any delays in planting and ratooning due to planting and harvest conflicts will consistently affect the next season’s harvest (Terauchi et al., 2012). Moreover, the loss of sugarcane continues for many reasons, including rotting, disease, parasitism, and harvesting (Kawanobe et al., 2017; Sharma and Tamta, 2015; Shinzato et al., 2015).

Sugarcane variety databases are key indices to learning and improving sugarcane variety for high yielding, high sucrose content, high biomass (Matsuoka, 2006), and high durability of a natural disaster. Generally, several sugarcane factories in Japan obtain data of sugarcane variety through inquiries with farmers and with the experience of a specialist. The sugarcane variety classification method nowadays is inconvenient, and it is difficult for a farmer to identify a sugarcane variety without specialist help, which can affect the quality of the database. Sugarcane variety classification mostly uses pictorial identification techniques based on bud shape, dewlap shape, leaf shape, etc. (Gravois et al., 2018; Takaragawa et al., 2019). Nevertheless, these techniques need a long time to correct the data and need the experience to be identified. Thus, there is a need for new tools or methods that could work faster, be more accurate, and be more convenient to use to identify sugarcane variety.

With recent advancements in computer technology, the image can extract much information from image data, such as many types of color space and intensity of color, with the image processing technique, which was widely used for detection or identification in the agriculture and food industry because it is fast, accurate, and cost-efficient (Chen et al., 2010; Khan and Yadav, 2017; Moshashai et
al., 2008). However, sugarcane variety classification using image processing has not been researched yet. Therefore, the current research focuses on classifying Japanese sugarcane varieties using image processing. This research aims to use full pixels and color features of sugarcane bud images to describe and separate sugarcane varieties using multivariate analysis methods.

2. MATERIALS AND METHODS
Matlab R2018a (version: 9.4.0.813654, The Math Works, Natick, MA, USA, 2017) with the PLS toolbox (Eigenvector Research, Inc., Manson, WA, USA, 2017) was used for data processing and analysis.

2.1 Sugarcane samples
In this research, as shown in Figure 1, three Japanese sugarcane varieties (Ni15, Ni22, and Ni27) from six areas in Southern Japan (Minami island, Ishigaki island, Miyako island, Okinawa Nanbu, and two difference crops in University of the Ryukyus) were selected as sugarcane variety samples for classification according to full pixel image and color features. The image of 54 sugarcane bud samples (18 samples per variety) was acquired from the sugarcane field using a mobile phone’s digital camera (iPhone SE, Apple Inc, USA) equipped with a fixed distance accessory. The first dimensions of the images were 3024 x 4032 pixels in JPG-format.

![Figure 1. The sample image of sugarcane variety samples; (a) Ni15, (b) Ni22, and (c) Ni27.](image)

2.2 Image processing
As shown in Figure 2, the images were then cropped on the bud area and their sizes reduced to 100 x 100 pixels in order to diminish the time and load of the analysis process. The acquired image is generally displayed in three-dimensional RGB color space. However, RGB color space is not perceptually uniform, and the proximity of colors does not indicate a color similarity. Color space transformations make for an effective means of distinguishing color images. The classification performance could be improved by weighting each color component differently. For this research, The RGB color space was evaluated as normalized RGB, YCbCr, and HSV color spaces.

The normalized RGB can be obtained from Eq. (1): in order to remove the brightness from the RGB color space, one can normalize the values of red, green, and blue.

\[ r = \frac{R}{R+G+B} \]
\[ g = \frac{G}{R+G+B} \]
\[ b = \frac{B}{R+G+B} \]  

The YCbCr can be obtained from Eq. (2) (Umbaugh, 2005).
\[
\begin{align*}
Y &= 0.299R - 0.587G + 0.114B \\
Cb &= -0.168G - 0.331B + 0.500B + 128 \\
Cr &= 0.500R - 0.418G + 0.0813B + 128
\end{align*}
\]

As such, the Y element represents the luminance component, and the \( C_b \) and \( C_r \) elements represent two chrominance components.

The 12 color spaces were then extracted to 24 color features by computing the mean and standard deviation of color spaces. Subsequently, two types of data, the full pixel data and color feature data from images, were investigated for input to description analysis and classification analysis.

![Figure 2. The example of sugarcane bud sample processing](image)

2.3 Multivariate analysis

The multivariate analysis techniques were objectives for description, classification, and prediction analysis. There are many types of multivariate data analysis techniques to choose from nowadays. The principal component analysis (PCA) is one of the frequently used methods for data description and explorative data structure modeling (Esbensen, 2000) and it’s also one of the most critical and influential ways to decompose complex data (Bro and Smilde, 2014). Moreover, PCA could be used on a digital image for the benefit of learning and reducing size, as it enables locating the highest variance in data (Ng, 2017). The same goes for partial least squares discriminant analysis (PLS-DA) (Amigo et al., 2009) and support vector machine (SVM-C) (Zhang, 2012), which are the dominant methods for classifying data. Thus, this research chose PCA to describe the sugarcane bud samples and both PLS-DA and SVM-C for classifying sugarcane varieties.

3. RESULTS AND DISCUSSION

3.1 Principal component analysis results

Fifty-four of the sugarcane variety samples with two types of data (full pixel and 24 color features) were divided into three variety classes, resulting in 18 samples per variety. Then, a PCA analysis using the scores was undertaken to create a scattering plot of principal components 1 and 2, as shown in Figure 3. The sugarcane variety Ni15 and Ni27 were distinguished, but Ni22 overlapped a little with the other two varieties per the implementation of the first and second principal components in the two types of data. After recreating an image from full pixel loadings of principal component analysis, figure 4 (a) shows the first principal component was related to the lightness of the image; the second was related to the size of the sugarcane bud in the case of a full pixel by recreating an image from the loading of principal. Figure 4 (b) show in the color space of the sugarcane buds, the first principal component loading was mainly related to the mean of RGB, normalized RGB, Y, Cb, and V; the standard deviation of RGB, Y, Cb, and V. The second principal component loading mainly related to the mean of B, normalized b, Cb and S; the standard deviation of normalized RGB, H, and S.
Figure 3. Score plots of principal component analysis for modeling samples (18 samples of sugarcane variety Ni15 in circle mark, Ni22 in triangle mark, and Ni27 in theta mark, respectively); (a) full pixel (b) color features.

Figure 4. Loadings of principal component analysis; (a) full pixel (b) color features (where 1, 2, and 3 means mean of RGB. 4, 5, and 6 means mean of normalized RGB. 7, 8, and 9 means mean of YCbCr. 10, 11, and 12 means mean of HSV. 13, 14, and 15 means standard deviation of RGB. 16, 17, and 18 means standard deviation of normalized RGB. 19, 20, and 21 means standard deviation of YCbCr. 22, 23, and 24 means standard deviation of HSV.)

3.2 Classification of PLS-DA and SVM-V
Two types of data (full-pixel and 24-color features) and a two-class (model variety and other varieties) PLS-DA and SVM-C model were developed for variety classification. The 54 samples (18 model variety and 36 other variety) were used to create the PLS-DA and SVM-C model with Venetian blind cross-validation to determine the number of factors and evaluate the classification rate. The results presented in Table 1 reveal that the variety of Ni15 classification rates results of the full pixel by PLS-DA and SVM-C were 83.3% and 87.0%, respectively; the Ni22 classification rates by PLS-DA and SVM-C of color spaces were 74.1% and 83.3%, respectively; the Ni27 classification rates by PLS-DA and SVM-C of color spaces were 81.5% and 83.3%, respectively. Moreover, the results of the color features show that the sugarcane variety Ni15 classification rates by PLS-DA and SVM-C were 88.9% and 85.2%, respectively; the Ni22 classification rates by PLS-DA and SVM-C of color spaces were 66.7% and 72.2%, respectively; the Ni27 classification rates by PLS-DA and SVM-C of color spaces were 72.2% and 64.8%, respectively.
Table 1. Classification results of PLS-DA and SVM-C using cross-validation.

<table>
<thead>
<tr>
<th>Classification methods</th>
<th>Classification rates</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Data types</td>
<td>Variety</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ni15</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ni22</td>
<td>74.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ni27</td>
<td>81.5</td>
</tr>
<tr>
<td>Full pixel</td>
<td>Color features</td>
<td>Ni15</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ni22</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ni27</td>
<td>72.2</td>
</tr>
<tr>
<td>SVM-C</td>
<td></td>
<td>Ni15</td>
<td>87.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ni22</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ni27</td>
<td>83.3</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The three Japanese sugarcane varieties (Ni15, Ni27, and Ni22) were mostly distinguished by the implementation of first and second principal components in the full pixel and color features. The samples were classified into varieties by performing a partial least squares discriminant analysis (PLS-DA) and a support vector machine classification (SVM-C). The results of the full pixel set show that the pooled classification rates by PLS-DA and SVM-C were 79.6% and 84.5%, respectively. Meanwhile, the pooled classification rates by PLS-DA and SVM-C of the color features set were 75.9% and 74.1%, respectively. However, this research could not correctly classify the sugarcane variety because the input images had various factors that might have affected the results, such as sunlight, a damaged sugarcane bud, the age of the cane, and fertility in the field. These results therefore show that the size and color spaces of sugarcane buds could be the keys to classifying sugarcane varieties. Moreover, the best way to classify Japanese sugarcane (Ni15, Ni22, and Ni27) was the SVM-C method using a full pixel of sugarcane bud.

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Swine influenza spread quickly because of respiratory infection. In dual infection with other diseases, infection symptom will be severe (Reeth et al., 1996). Furthermore, when the virus mutated, human pandemic occurred in 2009 (WHO, 2009). Hence, a lot of previous researches measured relationships between influenza virus titers and infection symptoms (Takemae et al., 2018); These results found influenza infection induced increasing sneezing. If we can detect influenza by sneezing, we can detect disease earlier than an antibody test (Mengeling, 1995) that is a general method. Although previous researches measured only once a day and 1-hour monitoring, we do not know what infection experiment’s factors induced increasing sneezes. As for examples factors, there are a virus, human stimulus, eating meal and others. The purpose of this paper is discussing relationships between the number of sneezes and factors in swine influenza infection experiment. Because of this, we measure the number of sneezes around the clock during 2-week infection experiment using automatic sneeze detector. In the experiment, we use 3 virus groups and 1 healthy control group, and there are 4 pigs in each group. Regarding automatic sneeze detector, it performs feature extraction from acoustic signals for dimension reduction, and classify sneeze or not based on support vector machine (Mito et al., 2018). As a result, we can observe some relationships between increasing sneezes and factor. The number of sneezing increase after meal supply and infection check timing in each group. As regards this result, we guess these factors have stimuli to pig. Specifically, entering meal into a nasal cavity in a miss, and collecting mucous membranes from a nasal cavity. In addition, we observed increasing the number of sneezes at night, before virus titers cannot detect in 3 virus groups. That means, measuring the number of sneezes at night, we may be able to detect infection influenza or not. Moreover, the previous method cannot measure that time. Consequently, we could measure and discuss the relationship between the number of sneezes and factors in swine influenza infection experiment by a measurement around the clock automatically.
Sound Source Localization in Pig Houses Using Wireless Microphone Array and Its Accuracy by Microphone Arrangements

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Keywords: swine sneezing, respiratory disease, monitoring system, wireless, sound source localization

The recent increase in breeding density due to intensive management of swine leads to an expanding risk of highly infectious respiratory infections. In particular, Porcine Reproductive and Respiratory Syndrome (PRRS) is the main factor inhibiting production in swine farming. Thus, early detection of PRRS is an essential issue in the management of group-housed livestock. In order to achieve early detection, our research group developed a system to detect PRRS automatically. The developed system utilizes a relationship that a frequency of cough and sneezing in swine increases as it is infected by disease, and monitors the sounds in a pig house using multiple microphones to localize the sneezing swine. However, the wiring to connect microphones has been a barrier to deploy a system in pig houses. In this study, we developed a monitoring system using wireless microphones to make the system deployment more flexible. On deploying the wireless monitoring system to a large space, the degradation of the communication quality affects detection of sneezing sound and sound source localization. Therefore, we examined a relationship between an installation position of the wireless microphones and the localization accuracy. Specifically, sound source localization was performed using developed wireless microphones and sound source that emits an actual sneezing sound of swine by changing two parameters: the source-microphone distance (l), and the microphone-receiver distance (d). The obtained results suggest that the measurement error increases as the source-microphone distance (l) increases, while measurement error did not change although the microphone-receiver distance (d) increases. The first result indicates that the localization accuracy was enough (within 0.4 m) when (l) is 4 m or less, and the second result indicates that the wireless microphones can be deployed in a large space. We also deployed the proposed wireless acoustic wave sensor in a pig house to perform a two-week swine influenza infection experiment. In this experiment, the source-microphone distance (l), and the microphone-receiver distance (d) were set as 2 m and 3 m, respectively. We found that the proposed sensor works for two weeks and can localize the sneezing swine within an accuracy of 0.2 m.
Sound Source Localization in Pig Houses Using Wireless Microphone Array and Its Accuracy by Microphone Arrangements

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is a main factor inhibiting production of swine farming and, early detection of PRRS is an essential issue in the management of group-housed livestock. To achieve early detection, our research group developed a system to detect PRRS automatically, which detects cough and sneezing of swine acoustically using wired microphones. However, the wiring becomes a barrier to deploy a system in a pig house smoothly. In this study, we develop a monitoring system using wireless microphones to make the system deployment more flexible. When deploying the wireless monitoring system to a large space, the degradation of the communication quality affects detection of sneezing sound and sound source localization. Therefore, we analyzed a relationship between an installation position of the wireless microphones and the localization accuracy. To evaluate the proposed system, we performed experiments both in laboratory and pig house. As results, we found that the proposed wireless system reduces the load of workers much for system deployment, Furthermore, the proposed system achieves enough quality of sound source localization, while ensuring the system flexibility.

Keywords: swine sneezing, respiratory disease, monitoring system, wireless, sound source localization

1. INTRODUCTION

Recent increase in breeding density of swine due to intensive management leads to an expending risk of highly infectious respiratory infections (Frost et al., 1997). Among them, porcine reproductive and respiratory syndrome (PRRS) is an important swine disease worldwide, since it prevents production in swine farming resulting in the highest economic impact in swine industry (Shimizu et al., 1994). Therefore, early detection of PRRS is an important issue in pig farming. To detect PRRS in early stage, several techniques, such as antibody testing (Scott et al., 1997), PCR testing (Duinhof et al., 2011), and monitoring weight gain (Destajo et al., 2007) have been proposed. However, these techniques require high-cost reagents, laboratory equipment, or human resources that become a barrier to utilize these technologies in a commercial pig farm.

On the other hand, it was found that the acoustical information can also become an important indicator of PRRS. Specifically, it has been reported that the increase of frequency of sneezing and cough of swine increase as the swine is infected by PRRS (Shimizu et al., 1994 and Exadaktylos et al., 2008). Hence, methods of sound source localization of cough sounds (Silva
et al., 2008) and sneezing sounds (Kawagishi et al., 2014) have been proposed to gather information about the health of swine automatically. A procedure of sound source (sneezing swine) localization is as follows; (1) several microphones are deployed in a pig house, and the internal sound of the pig house is continuously monitored, (2) when a pig sneezes, a sneezing sound is recorded by microphones, (3) the system detects the sneezing sound and calculates time-difference-of-arrivals (TDoAs) of multiple signals by calculating cross-correlation function between receiving signals, and (4) localizes the sound source from direction-of-arrival (angle-of-arrival) using TDoAs and position of the microphones.

Although sound source localization in pig houses has been found to become a viable alternative that achieves early detection of PRRS, there exists a margin for improvement especially in the transmission of acoustic signals recorded by the microphones. In the existing system [Fig. 1(a)], the microphones are connected to the system by wire, which becomes a barrier to deploy the system in pig house that requires human resources and time (wires of length 5–10 m should be placed near a ceiling of the pig house to avoid damages by swine and daily work). If we can remove such wiring by transmitting acoustic signals using radio wave [Fig. 1(b)], we can make the sound source localization system more flexible. However, the quality of the sound source localization would be affected by the quality of wireless radio transmission. Hence, in this study, we design a sound source localization system using wireless microphones and evaluate the quality of the sound source localization by changing two parameters; the source-microphone distance ($l$) and the microphone-receiver distance ($d$). Furthermore, we deploy the system in a pig house and perform monitoring for two weeks.

The remaining of this paper is as follows. Section 2 overviews the existing sound source localization system and the proposed (wireless) system. Section 3 evaluates the quality of the proposed sound source localization system in a laboratory. Section 4 evaluates the performance of the proposed system in a pig house. Section 5 concludes this work.

![Figure 1](image1.png)

**Figure 1.** Outline of acoustic monitoring system of swine; (a) existing and (b) proposed (wireless) system.

## 2. OVERVIEW OF SOUND SOURCE MONITORING SYSTEM

### 2.1 Existing (wired) sound source monitoring system

Figure 2 shows the existing (wired) sound source monitoring system. As shown in the figure, we set $K$ microphones ($K$: positive integer and $K=3$ in the figure) at relative position of $(x_k, y_k)$ ($k = 0, 1, \ldots, K-1$). A relative position of the sound source is set as $(x_s, y_s)$. When the sound source emits the sound (sneezing sound), the sound propagates and recorded by the microphones [the recorded sound at microphone $k$ is defined as $r_k(t)$]. The server judges whether $r_k(t)$ contains a sneezing sound or not by comparing the recorded signal and template (sample of sneezing sound) in the frequency domain. If the sneezing sound is detected, the
server calculates cross-correlation functions between \( r_k(t) \) and \( r_m(t) \) (\( m = 0, 1, \ldots, K-1 \) and \( m \neq k \)), \( s_{km}(t) \), where
\[
s_{km}(t) = \sum r_k(n)r_m(t-n). \quad (1)
\]
Then the server calculates time-difference of TDoAs, \( u_{km} \), by measuring the peak shift of \( s_{km}(t) \). Finally, the server finds \((x_s, y_s)\) that satisfies the following simultaneous equation for all \( m \) and \( k \).
\[
\sqrt{(x - x_m)^2 + (y - y_m)^2} + c u_{km} = \sqrt{(x - x_k)^2 + (y - y_k)^2}. \quad (2)
\]
Note that the above equation represents a hyperbolic curve determined by \((x_k, y_k)\), \((x_m, y_m)\) and \( u_{km} \), as shown in Fig. 3, and \( c \) is a sound velocity.

Figure 2. Existing (wired) sound source monitoring system.

Figure 3. Relationship among \((x_s, y_s)\), \((x_m, y_m)\), \((x_k, y_k)\), and \( c u_{km} \) when \( K = 3 \).
2.2 Proposed (wireless) sound source monitoring system

In this paper, we design a sound source localization system using wireless microphones, as shown in Fig. 1(b) and Fig. 4. When the sound source emits the sound, the sound propagates and recorded by the microphones. The radio transmitter $#k$ that is connected to the microphone $#k$ modulates the radio frequency of $f_k$ by the recorded sound (frequency modulation) and emits as the radio signal. The radio receiver $#k$ that is connected to the server receives and demodulates the signal from the transmitter $#k$ and the server obtains $r_k(t)$. Note that the radio frequency $f_k$ should be independent to avoid signal interference. A procedure of the sound source localization is the same to that of the existing system. However, in this system, the quality of the sound source localization is affected by two noise sources (environmental noise and transmission noise), as shown in Fig. 4. If the distance between sound source and microphone $#k$, $l_k$, becomes large, the system can observe wide area in exchange for the signal-to-noise ratio of $r_k(t)$. Furthermore, if the distance between microphone $#k$ and the server, $d_k$, becomes large, the system can cover a large pig house in exchange for the signal-to-noise ratio (SNR) of $r_k(t)$. Hence, the quality of the sound source localization of the proposed system should be evaluated by changing two parameters; the source-microphone distance ($l_k$) and the microphone-server distance ($d_k$).

3. PERFORMANCE EVALUATION OF THE PROPOSED SYSTEM IN LABORATORY

3.1 Experimental environment

We evaluate the performance of the proposed system in a laboratory. Figure 5 shows the experimental environment. As shown in the figure, the experiment is performed in a room whose size is $7.68 \times 7.35 \times 3.44$ (m$^3$). We set three microphones with a radio transmitter (88-108MHz, diymore) at a height of 1.5 m from the floor. The carrier frequency of each transmitter is 95, 88, and 101 (MHz), respectively. We also put three radio receivers (RAD-P088S, AudioComm) that are connected to the analog-to-digital converter (USB-6221, National Instruments). The signal processing is performed on a server (ThinkPad X250, Lenovo). Furthermore, we set a speaker (S-300HR, TEAC) on the floor as the sound source. As emitting sound, we use a recorded sound of swine sneezing whose sound pressure level is the same of the swine (2.1 Pa).
In this experiment, we evaluate the quality of the sound source localization of the proposed system by changing the source-microphone distance ($l_k$) and the microphone-server distance ($d_k$). At first, the sound source localization is performed by changing $l_k$ with a specific value of $d_k$ (Experiment I). Then the sound source localization is performed by changing $d_k$ with a specific value of $l_k$ (Experiment II). Table I shows the parameter combinations of $l_k$ and $d_k$ used in the experiment. During the experiment, we also measure the SNR of $r_k(t)$, as well as the quality of the sound source localization (localization error).
3.2 Experimental results and discussions

Figure 6 and Table II show the experimental results. Figures 6(a) shows a relationship between sound source localization error and source-microphone distance \((l_k)\). Figures 6(b) shows a relationship between sound source localization error and microphone-server distance \((d_k)\). Table II shows a relationship between SNR and source-microphone distance \((l_k)\) and microphone-server distance \((d_k)\).

From this experiment, we found that the distance between sound source and microphone is a main factor that affects the quality of the sound source localization. Specifically, the localization error increases as the source-microphone distance \((l_k)\) increases, while the localization error does not change much even if the microphone-server distance \((d_k)\) increases [Fig. 6(a)]. Furthermore, the SNR decreases as the source-microphone distance \((l_k)\) increases, while that does not change much even if the microphone-server distance \((d_k)\) increases [Table II].

Next, we focus on the value of the localization error. In previous studies, it was found that the localization error should be less than 0.4 m to detect a sneezing swine individual from a group of pigs in a pig pen (Kawagishi et al., 2014). From Fig. 6, we found that the source-microphone distance \((l_k)\) should not over 3 m while the microphone-server distance \((d_k)\) can be set flexible within 5 m.

Consequently, we found that the quality of the sound source localization would not be affected by the quality of wireless radio transmission.

![Figure 6](image)

**Figure 6.** Experimental results obtained in laboratory; sound source localization error obtained in (a) Experiment I and (b) Experiment II.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>SNR (dB)</th>
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<tbody>
<tr>
<td>I</td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>28.1</td>
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<tr>
<td>(ii)</td>
<td>26.0</td>
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<tr>
<td>(iii)</td>
<td>23.8</td>
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<td>(iv)</td>
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<td>(v)</td>
<td>22.27</td>
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<td>(iv)</td>
<td>28.5</td>
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<td>(v)</td>
<td>29.3</td>
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</table>

**Table II.** SNR of \(r_k(t)\) obtained in Experiment I and II.
4. PERFORMANCE EVALUATION OF THE PROPOSED SYSTEM IN PIG HOUSE

4.1 Experimental environment

We evaluate the performance of the proposed system in a pig house. Figure 7 shows the experimental environment. As shown in the figure, the experiment is performed in a pig house of National Institute of Animal Health, National Agriculture and Food Research Organization whose size is 1.35×3.45×2.05 (m³). As well as Section 3, we set three microphones with a radio transmitter at a height of 1.92 m from the floor. The microphone-server distance ($d_0$, $d_1$, $d_2$) was set to 2–3 m, the source-microphone distance ($l_0$, $l_1$, $l_2$) is set as 2.1–2.2 m. Note that $l_k$ and $d_k$ satisfy the values that achieve localization error of less than 0.4 m in Section 3. The carrier frequency of each transmitter is the same to that used in preliminary experiment. We also put three radio receivers (RAD-P088S, AudioComm) that are connected to the analog-to-digital converter (USB-6221, National Instruments) on an adjacent monitoring room. The signal processing is performed on a server (i5-4690 CPU, RAM 16GB). Different from the preliminary experiment, the sound source is a weaned piglet (8 week old) (Takemae et al., 2018).

In this experiment, we deploy the proposed system and existing (wired) system, while measuring the length of time for system deployment. Furthermore, we evaluate the quality of the sound source localization of the proposed system for two weeks. We also evaluate the quality of the sound source localization of the existing system as reference.

4.2 Experimental results and discussions

The length of time for proposed system deployment was approximately 30 Min. by one worker, while that for existing system deployment was approximately 120 min. by three workers. We found that the proposed wireless system is much easier than the existing system, since there is no need to install long cables in a pig house.

During the experiment, both the proposed system and existing system work successfully for two weeks. During the experiment, both system detected the swine sneezing 10 times. Figure 8 shows an example of sound source localization result by the proposed system [Fig. 8(a)] and existing system [Fig. 8(b)]. From this figure, we found that the proposed system and existing system achieve localization error of 0.2 and 0.25 m, respectively. This means that the sound source localization system using wireless microphones achieves almost the same quality of that using wired microphones, while ensuring the system flexibility.
5. CONCLUSIONS

In this paper, we develop a monitoring system using wireless microphones to make the system deployment more flexible. When deploying the wireless monitoring system to a large space, the degradation of the communication quality affects detection of sneezing sound and sound source localization. Therefore, we analyzed a relationship between an installation position of the wireless microphones and the localization accuracy. To evaluate the proposed system, we experiment both in laboratory and pig house. In the experiment in the laboratory, we evaluate the quality of the sound source localization of the proposed system by changing the source-microphone distance ($l_s$) and the microphone-server distance ($d_h$). From the result of the experiment, we found that the distance between sound source and microphone is a main factor that affects the quality of the sound source localization. In the experiment in a pig house, we deploy the proposed system and existing (wired) system, while measuring the length of time for system deployment. We also evaluate the quality of the sound source localization of the existing system as reference. The length of time for proposed system deployment was approximately 30 min. by one worker, while that for existing system deployment was approximately 120 min. by three workers. We found that the proposed wireless system is much easier than the existing system, since there is no need to install long cables in a pig house. From figure 8, we found that the proposed system and existing system achieve localization error of 0.2 and 0.25 m, respectively. This means that the sound source localization system using wireless microphones achieves almost the same quality of that using wired microphones, while ensuring the system flexibility.

ACKNOWLEDGMENT

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Behavioral Study of Vibrational Sensitivity in Whitefly

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Keywords: whitefly, vibrational sensitivity, mating behavior

Whiteflies are major pests that damage a wide variety of plants such as tomatoes and cucumbers. Whiteflies suppress the growth of plants and reduce crop quality by acquisition feeding. Furthermore, they carry various viruses such as tomato yellow leaf curl virus (TYLCV) and cucurbit chlorotic yellows virus (CCYV). For this reason, control of whiteflies is an urgent matter for farming. The current control method is spraying pesticides. However, whiteflies acquire pesticide resistance early because it performs a generation cycle within one month. For example, *Bemisia tabaci* (biotype Q) has high resistance to most pesticides. Hence, the development of a new technology to control whiteflies is required. Focusing on the behavior of whiteflies, it has been reported that they communicate using leaf substrate-borne vibrations by oscillating their abdomens in their courtship behavior. Besides, our research group has clarified that their courtship behavior can be controlled by applying the artificial vibration of 200-1500 (Hz). However, the effective amplitude of artificial vibration has not been clarified yet. Hence, in this paper, we clarify the vibration sensitivity of whiteflies by experiments. The experimental condition is as follows. *Bemisia tabaci* (biotype Q1) (five males and five females) were released in a rectangular plastic case of approximate size 60×60×100 (mm³). The case has a hole with a diameter of 41 mm at its top and is covered with perilla leaf. The leaf was vibrated with various amplitudes (vibrational amplitude: 1.0, 0.6, and 0.3 μm), and the number of courtship behavior was measured by analyzing video recorded by the camera (FDR-AX45/SONY). The experiment was performed twice (each is for 1.5 hours) at each amplitude in an anechoic chamber. During this experiment, the temperature was 27-31 °C, and the humidity was 27-39 %. From experiments, we found that ratio of the number of mating behavior to that of courtship behavior is small (0 %) when vibration amplitude is 1.0 m, although that is large (about 30 %) when vibration amplitude is 0.6 m or less. Hence, we found that the sufficient amplitude of artificial vibration is about 1.0 m. This result can be expected to contribute to the development of novel whitefly control technology.
Behavioral Study of Vibrational Sensitivity in Whitefly

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ABSTRACT

Whiteflies are major pests that damage a wide variety of plants such as tomatoes and cucumbers. The current control method is spraying pesticides. However, whiteflies acquire pesticide resistance early because it performs a generation cycle within one month. Hence, the development of a new technology to control whiteflies is required. Focusing on the behavior of whiteflies, our research group clarified that their courtship behavior can be controlled by applying the artificial vibration of 200-1500 (Hz). However, the effective amplitude of artificial vibration has not been clarified yet. Hence, in this paper, we clarify the vibration sensitivity of whiteflies by experiments. Specifically, Bemisia tabaci (biotype Q1) were released on a perilla leaf in a rectangular plastic case. The leaf was vibrated with various amplitudes, and the number of mating behavior was measured by analyzing video recorded. As a result, we found that the sufficient amplitude of artificial vibration is about 1.0 µm.

Keywords: Whitefly, Bemisia tabaci, Mating behavior, Vibration sensitivity

1. INTRODUCTION

Whiteflies (e.g., Bemisia tabaci and Trialeurodes vaporariorum) are major pests that damage a wide variety of plants such as tomatoes and cucumbers (Azab et al., 1970; Zhang et al., 2005; Martin et al., 2007). Whiteflies suppress the growth of plants and reduce crop quality by acquisition feeding and emitting a honeydew to the plants (Matsui, 1992; Nelson, 2008). Furthermore, they carry various viruses such as tomato yellow leaf curl virus (TYLCV) and cucurbit chlorotic yellows virus (CCYV). TYLCV and CCYV are one of the most well-known tomato and cucumber infecting begomoviruses transmitted by Bemisia tabaci, and they cause severe economic loss worldwide (Navot et al., 1991; Czosnek et al., 1997; Jones, 2003). For this reason, control of whiteflies is an urgent matter for farming.

To control whiteflies, various tactics –such as physical barriers (e.g., insect screen), chemical controls and biological controls (Matsuura et al., 2005; Kodandaram, 2018; Nomikou, 2001, 2002, 2010)– have been considered. Focusing on physical barriers, it has been clarified that an insect screen with mesh size of 0.4 mm can efficiently reduce the entrance of whiteflies, in exchange for increasing difficulty regulating temperature of the greenhouse due to limited air flow (Mihara et al., 2005). Focusing on chemical controls, numbers of chemical pesticides have been proposed. However, whiteflies quickly develop resistance to chemical pesticides (Wardlow et al., 1972) and Bemisia tabaci (biotype Q) has high resistance to popular pesticides now (Horowitz et al., 2005). Focusing on biological controls, Nesidiocoris tenuis and Typhlodromips swirskii have been found to become a biopesticide of whitefly. However, Nesidiocoris tenuis has a risk to damage the plants (Nakaishi, 2013), and Typhlodromips swirskii is not livable on the plants that discharge sticky secretions such as tomatoes (Sakamoto, 2012). Hence, current control techniques have both advantages and disadvantages, and innovative combination of control techniques are necessary to achieve effective pest management. Furthermore, development of a new technology to control whiteflies can contribute to broaden pest management.

Focusing on the behavior of whiteflies, Kanmiya (1996) has reported that they communicate using leaf substrate-borne vibrations by oscillating abdomens in their courtship behavior. Furthermore, the communication signal of whiteflies has been found to be unique to each species and biotype (Kanmiya
et al., 2002; Nakabayashi et al., 2017). This means that the communication of whiteflies play an important role in mating behavior, considering the fact that hybrid of different species or biotype remains rare (Matsuura, 2010). Hence, we may control the mating behavior of whiteflies by applying artificial vibration on the leaf. As preliminary study, we have clarified that their courtship behavior can be controlled by applying the artificial vibration of 200-1500 Hz (Nishijima et al., 2019). However, the effective amplitude of artificial vibration has not been clarified yet. Hence, in this paper, we clarify the vibration sensitivity of whiteflies by experiments.

2. MATERIALS AND METHODS

In this study, we put whiteflies on a perilla leaf, vibrate the leaf at specific amplitude, and observe the behavior of whiteflies. The test was performed in a laboratory with the temperature and humidity of 25-31°C and 27-39%, respectively. We used adult whiteflies (Bemisia tabaci, biotype Q1) of 5 pairs (5 males and 5 females, collected from a colony) for the test. The whiteflies were put on the underside of a perilla leaf, and the leaf was put on a rectangular plastic case of size 60×60×100 (mm³), as shown in Figure 1. The leaf was set to cover a hole of the case (diameter: 41 mm) so that the whiteflies can be monitored from the bottom of the case using a video recorder (FDR-AX45/SONY). Also, a polypropylene sheet was placed between the leaf and the case to keep the whitefly within a field view of the camera. To capture the behavior of the whiteflies clearly, the underside of the leaf was illuminated by a desk light. The leaf was vibrated artificially, and its vibration was monitored by a laser Doppler vibrometer (LDV) (AT2300 and AT3700, Graptheck). During the test, the leaf was vibrated artificially (vibrational direction: vertical for surface of leaf), and a behavior of the whiteflies was monitored for 1.5 hours. The test was conducted by changing the vibrational amplitude [maximum amplitude of 1.0, 0.6, 0.3 and 0 (μm)], and the test was repeated twice at each amplitude. We then analyze the video and count the number of “mating success” and “mating failure”. Note that the mating behavior of whiteflies consists of three steps; (1) searching a female, (2) forming a pair (close contact with female) and (3) mounting (males overlap his hips with hers and shake his wings rapidly) (Kanmiya, 1998). Hence, we define the following labels could count each occurrence;

(a) Mating success: mounting is clearly observed after pair forming.
(b) Mating failure: mounting is not observed (they get a divorce after pair forming).
(c) Unknown: mounting is not clearly observed after pair forming (e.g., they form a pair continuously.

Figure 2 shows a flowchart to perform labeling from the video, where $Np$, $Ns$, $Nf$, $Nu$ are the number of pair forming, mating success, mating failure, and unknown, respectively, and “Ratio of Ns” represents $Ns$ per $Np$ (%).

3. RESULTS AND DISCUSSIONS

The experimental results are shown in Table 1. From the table, we could observe pair forming ($Np$) in each trial. However, it was found that the ratio of successful mating (ratio of $Ns$ in the table) becomes 0 when the vibrational amplitude of the leaf is 1.0 μm, while the ratio of successful mating increases 28-35 (%) when the amplitude is 0-0.6 μm. This means that the effective amplitude of the artificial vibration to control the mating behavior of whiteflies is more than 1.0 μm.

![Figure 1. Overview of experimental system](image)
4. CONCLUSIONS
Control of whiteflies that cause severe economic loss is an urgent matter for farming. Focusing on the behavior of whiteflies, our research group clarified that their courtship behavior can be controlled by applying the artificial vibration of 200-1500 (Hz). However, the effective amplitude of artificial vibration has not been clarified yet. Hence, in this paper, we clarify the vibration sensitivity of whiteflies by experiments. The whiteflies (Bemisia tabaci, biotype Q1) of 5 pairs (5 males and 5 females, collected from a colony) were put on the underside of a perilla leaf. Also, the leaf was vibrated artificially (vibrational direction: vertical for surface of leaf), and a behavior of the whiteflies was monitored for 1.5 hours. The test was conducted by changing the vibrational amplitude [maximum amplitude of 1.0, 0.6, 0.3 and 0 (μm)], and the test was repeated twice at each amplitude. As a result, it was found that the ratio of successful mating becomes 0 when the vibrational amplitude of the leaf is 1.0 μm, while the ratio of successful mating increases 28-35 (%) when the amplitude is 0-0.6 μm. Hence, the effective amplitude of the artificial vibration to control the mating behavior of whiteflies is at least 1.0 μm. This result can be expected to contribute to the development of novel whitefly control technology.

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Application of Palm Oil Based Wax as a Coating Material on the Quality of Cucumber Seed

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Keywords: Palm oil based wax, Cucumber seeds, Coating material

Colouring the seeds enhances physical appearance which is necessary for commercial purposes. The colouring agents are synthetic chemical dyes and film coating polymers. Palm oil wax is a by-product of palm oil industry. It is used to prepare palm oil-based wax as a coating material. Therefore, this research aimed to study the effects of palm oil-based wax as an alternative synthetic coating material. There were three formulas of the palm oil-based wax designated as A, B and C. These were used as coating on cucumber seeds using the top-spray fluidized bed coating technique. The experimental conditions were carried out through atomization air pressure of 150 kPa, inlet air velocity of 2 mm/sec, inlet air temperatures at 40° C, spray rate of coating solution of 125 mL/min, spraying time for 2 min, and drying after spraying for 15 min. The surface appearance and uniformity of palm oil based wax coating were evaluated under the stereomicroscope. Moisture content, germination percentage, days to emergence (DTE), germination index and free fatty acid content were also determined. Results showed no difference in the appearance and uniformity of the three formulas of palm oil based wax coating on seed coat surface. The moisture content and free fatty acid of the coated seeds increased, while germination percentage and germination index were lower than the control. Moreover, the formula A, consisted of 99.51% wax ester with the carbon chain lengths of 32-34 atoms, obtained similar seed quality with the control. Therefore, the properties of formula A palm oil based wax coating could be improved to minimize the impact on cucumber seed quality. Further studies can be done on the experimental conditions used during the application of the coating material.