[5-1350-A] Public Symposium
Robust Agricultural and Food Production for SDGs (Sustainable Development Goals)
Chair: Hiroshi Shimizu, (Kyoto University), Eriko Yasunaga (University of Tokyo, Japan), Yukiharu Ogawa (Chiba University, Japan), Shuso Kawamura (Hokkaido University, Japan), Naoshi Kondo (Kyoto University, Japan)
1:50 PM - 5:20 PM Hall A (Main Hall)

[5-1350-A-OR] Opening Remark
Mayumi Ishizuka (Council Member of Science Council of Japan (SCJ), Hokkaido University)
1:50 PM - 2:00 PM

[5-1350-A-01] Approach Toward SDGs by Science Council of Japan
*Miyoko Watanabe¹,² (1. Science Council of Japan (Japan), 2. Japan Science and Technology Agency (Japan))
2:00 PM - 2:40 PM

*Umezuruike Linus Opara¹ (1. Stellenbosch University (South Africa))
2:40 PM - 3:20 PM

*Charles Boliko¹ (1. Food and Agriculture Organization of the United Nations (Japan))
3:40 PM - 4:20 PM

[5-1350-A-04] Community-Based Digital Farming Approaches
*Sakae Shibusawa¹ (1. Tokyo University of Agriculture and Technology (Japan))
4:20 PM - 5:00 PM

[5-1350-A-CR] Closing remark
Announcement of The XX CIGR World Congress 2022 in Kyoto
Noboru Noguchi (Member of SCJ, Hokkaido University)
5:00 PM - 5:20 PM
Keynote Lecture 5th
Chair: Olaniyi A. Fawole (Stellenbosch University, South Africa)
9:00 AM - 10:15 AM  Hall A (Main Hall)

  *Amauri Rosenthal¹ (1. Embrapa Food Technology (Brazil))
  9:00 AM - 9:30 AM

  *Anthony Mutukumira¹ (1. Massey University (New Zealand))
  9:30 AM - 10:00 AM
**Oral Session | Food Safety**

[5-1015-A] **Food Safety (2)**  
Chair: Ubonrat Siripatrawan (Chulalongkorn University, Thailand)  
10:15 AM - 11:30 AM  Hall A (Main Hall)

**[5-1015-A-04] Cinnamon Oil Nanoemulsion as a Natural Microbial Decontaminant of Chilled Fish Flesh**  
Piyanan Chuesiang$^{1,2}$, Romanee Sanguandeekul$^1$, Ubonrat Siripatrawan$^{1,2}$  
(1. Chulalongkorn University, Department of Food Technology, Faculty of Science (Thailand), 2. The Novel Technology for Food Packaging & Control of Shelf Life Research Group, Chulalongkorn University (Thailand))  
10:15 AM - 10:30 AM

**[5-1015-A-05] Responsiveness to Food Safety Emergencies in Eswatini following the Outbreak of listeriosis in South Africa**  
*Tendekayi Henry Gadaga$^1$, Anthony N Mutukumira$^2$  
(1. University of Eswatini (Swaziland), 2. Massey University (New Zealand))  
11:15 AM - 11:30 AM

**Room C**

**Oral Session | Postharvest/Food Technology and Process Engineering**

[5-1015-C] **Postharvest/Food Technology and Process Engineering (5)**  
Chair: Akindele Folarin Alonge (University of Uyo, Nigeria)  
10:15 AM - 11:30 AM  Room C (3rd room)

**[5-1015-C-01] THE EFFECT OF DRYING METHODS ON THE QUALITY OF TIGER NUT (Cyperus esculentus lativum)**  
*Akindele Folarin ALONGE$^1$, Edikan Ufot GILBERT$^1$  
(1. University of Uyo (Nigeria))  
10:15 AM - 10:30 AM

**[5-1015-C-02] Optimization and Storage Stability Evaluation of Antioxidant Extracts From Batangas Cherry (Terminalia microcarpa Decne)**  
*Dennis Marvin Opena Santiago$^1$, Shekayna Eunice Balmes Pacia$^1$, Jake Lloyd Cabrera Peña$^{1,2}$, Claire Solis Zubia$^1$, Sheba Mae Magbanua Duque$^1$  
(1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Banos, College, Laguna 4031 Philippines (Philippines), 2. Department of Science and Technology CALABARZON Region, Regional Science and Technology Center Complex, Jamboree Road, Timugan, Los Banos, Laguna 4030 Philippines (Philippines))  
10:30 AM - 10:45 AM

**[5-1015-C-03] Effects of Pre-drying treatment and Drying-air Temperature on Moisture Ratio and Effective Moisture Diffusivity of Tomato (Nigerian Local and Foreign Varieties)**  
*Obafemi Ibitayo Obajemihi$^1$, Joshua Olanrewaju$^1$  
(1. Federal University of Technology Akure (Nigeria), 2. University of Ibadan (Nigeria))  
11:00 AM - 11:15 AM

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Thu. Sep 5, 2019 Oral Session

**[5-1015-C-04] Extending the Shelf-life of Upland Water Spinach (Ipomoea aquatica) Using Trimming, Modified Atmosphere Packaging (MAP) and Low-Temperature Storage**
*Ana Mithuzela Espigol*, Josephine Agravante
(1. Postharvest Horticulture Training and Research Center (PThRC), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), Laguna, Philippines; 2. Postharvest Horticulture Training and Research Center, University of Agriculture, Benin City, Edo State, Nigeria)
10:45 AM - 11:00 AM

**[5-1015-C-05] Investigation of Cowpea Variety and Storage Methods on Cowpea Beetle Infestation**
*VICTORIA ADA ABODENYI*, YAHAYA MOBMI MUSA, ABDULLAH MUHAMMED BAKO
(1. Agricultural Engineering, Federal Polytechnic, Bauchi(Nigeria); 2. Federal polytechnic, Bauchi(Nigeria))
11:00 AM - 11:15 AM

**Room D**

Oral Session | Others (including the category of JSAM and SASJ)

[5-1015-D] Other Categories (2)
Chair:Tri Yuliana(Universitas Padjadjaran, Indonesia)
10:15 AM - 11:30 AM Room D (4th room)

[5-1015-D-01] Screening and Enzyme Activity of Cellulose-Producing Bacteria Isolated from Kemiri Sunan (Reutealis trisperma (Blanco) Airy Shaw) and Empty Fruit Bunches of Palm Oil
*Tri Yuliana*, Efri Mardawati, Souvia Rahimah, Emilda Ayu Febrianty, Agus Try Hartono
(1. Univ. Padjadjaran, Indonesia; 2. Universitas Gadjah Mada, Department of Agro-industrial Technology(Indonesia))
10:15 AM - 10:30 AM

[5-1015-D-02] Development of a Cloud-based Internet of things Monitoring System for Fish Activity and Water Quality in Aquaponics
*Chien Lee*, Yu-Jen Wang
(1. Department of Mechanical and Electromechanical Engineering, National Sun Yat-sen University(Taiwan))
10:30 AM - 10:45 AM

[5-1015-D-03] EFFECT OF DIFFERENT MODES OF PLANTING AND WEEDING ON MACHINE FIELD CAPACITY AND YIELD OF A MIXED CROPPING SMALL HOLDER FARM
Folasayo Titilola Fayose, Adesoji Mathew Olanikan, Babatope Albert Alabadan, Anthony Ayodele Fajinmi, Kayode Ogunleye, Olanrewaju Omoju, Olufemi Aladejebi, Oluwaseun Ilesanmi
(1. Federal University Oye Ekiti(Nigeria))
10:45 AM - 11:00 AM

[5-1015-D-04] Development of Agro-industrial Worker Trust Assessment System for Sustainable Ergonomic Program in Food Small and Medium-sized Enterprises
*Mirwan Ushada*, Nur Achmad Sulistyio Putro, Titis Wijayanto, Fitri Trapsilawati, Nafis Khuriyati
(1. Universitas Gadjah Mada, Department of Agro-industrial Technology(Indonesia); 2. Universitas Gadjah Mada, Department of Computer Science and Electronics(Indonesia))
11:00 AM - 11:15 AM

[5-1015-D-05] ASSESSING LAND USE TYPES IMPACT ON SOIL ORGANIC CARBON IN SOUTH WEST, NIGERIA
*OLORUNWA ERIC OMOMUNI*, ADESOJI MATTHEW OLANIYAN
(1. FEDERAL UNIVERSITY OYE-EKITI(Nigeria))
11:15 AM - 11:30 AM
Detection of Outliers in Pre-processing of Datasets for Recognition of Classifiers Using Partial Least Squares Discriminant Analysis
*Miki Fujii, Ryozo Noguchi, Tofael Ahamed
Takuma Genkawa (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

Effects of Heating under Pasteurization Conditions on Mechanical and Electrical Properties of Mung Bean Sprout
*Hayato Ogino, Haruki Ando, Satoshi Iwamoto, Tepej Imaizumi (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 TSAI, Pin-Chih Fang, Yi-Tzu Shen, Yung-Huei Chang, Han-Chun Hsu, Suming Chen (1. Department of Bio-Industrial Mechatronics Engineering, National Taiwan University(Taiwan))
11:30 AM - 12:30 PM

Development of dumpling rich in barley flour with gluten added
*Masatsugu Tamura, Naoya Takahashi, Takahiro Saito, Satomi Akutsu, Yoshihiro Hoshi, Takemi Okamoto
(1. Utsunomiya Univ.(Japan), 2. Tochigi Industrial Promotion Center(Japan), 3. Industrial Technology Center of Tochigi Pref.(Japan))
11:30 AM - 12:30 PM

Development of Blueberry Wine with High Content of Polyphenol
*Hongpu Wang, Yutaka Kitamura, Mito Kokawa (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

Properties of Rice Starch-Based Film Incorporated with Zinc Oxide Nanoparticles
*KHALISHAH RAHMA SAFIRA, *SAROAT RAWDKUEN (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
in hot air drying
*Koya Yamakage¹, Takahiro Yamada¹, Takahiro Orikasa²³, Katsuyuki Takahashi²⁴, Shoji Koide³, Koichi Takaki²⁴, 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 Farghali¹², Maejima Mayumi³, Kuramoto Syo¹, Aoki Satoshi⁴, Yasui Seiichi⁵, Sayoko Takashima¹, Hijiri Ono¹, Yuhendra AP¹, Takaki Yamashiro⁵, Moustafa M. Ahmed⁶, Saber Kotb⁶, 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 Andriamanohiarisoamanana¹⁴, Yoshiteru Takeuchi⁵, 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, *Lorraine 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 Norii¹, Yuya Muchizuki¹, Takashi Ishii², Keiko Shinozaka³, Takao Miyamoto ⁴, Eiichi Inoue ¹ (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

[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 Shinozaka³, Takao Miyamoto ⁴, Eiichi Inoue ¹ (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

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

[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
[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
*Ikko Ihara*¹, *Homi Takato*¹, *John K Schueller*², *Gen Yoshida*¹, *Kazutaka Umetsu*¹, *Hitomi Yamaguchi*² (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-21] Screening and Identification of Endophytic Bacteria from Thai Organic Rice for Plant Growth Promotion
*Somkid Deejing*¹ (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 Win*¹, *Kikuhito Kawasue*¹, *Hsu Lai Wai*¹, *Kumiko Yoshida*² (1. University of Miyazaki(Japan), 2. KOYO Plant Service(Japan))
11:30 AM - 12:30 PM
[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
Public Symposium

[5-1350-A] Public Symposium

Robust Agricultural and Food Production for SDGs
(Sustainable Development Goals)

Chair: Hiroshi Shimizu, (Kyoto University), Eriko Yasunaga(University of Tokyo, Japan), Yukiharu Ogawa(Chiba University, Japan), Shuso Kawamura(Hokkaido University, Japan), Naoshi Kondo(Kyoto University, Japan)

Thu. Sep 5, 2019 1:50 PM - 5:20 PM  Hall A (Main Hall)

[5-1350-A-OR] Opening Remark
Mayumi Ishizuka （Council Member of Science Council of Japan (SCJ), Hokkaido University）
1:50 PM - 2:00 PM

[5-1350-A-01] Approach Toward SDGs by Science Council of Japan
*Miyoko Watanabe1,2 （1. Science Council of Japan(Japan), 2. Japan Science and Technology Agency(Japan))
2:00 PM - 2:40 PM

*Umezuruike Linus Opara1 （1. Stellenbosch University(South Africa))
2:40 PM - 3:20 PM

*Charles Boliko1 （1. Food and Agriculture Organization of the United Nations(Japan))
3:40 PM - 4:20 PM

[5-1350-A-04] Community-Based Digital Farming Approaches
*Sakae Shibusawa1 （1. Tokyo University of Agriculture and Technology(Japan))
4:20 PM - 5:00 PM

[5-1350-A-CR] Closing remark
Announcement of The XX CIGR World Congress 2022 in Kyoto
Noboru Noguchi （Member of SCJ, Hokkaido University）
5:00 PM - 5:20 PM
[5-1350-A-OR] Opening Remark
Mayumi Ishizuka (Council Member of Science Council of Japan (SCJ), Hokkaido University)

[5-1350-A-01] Approach Toward SDGs by Science Council of Japan
*Miyoko Watanabe¹,² (1. Science Council of Japan(Japan), 2. Japan Science and Technology Agency(Japan))

*Umezuruike Linus Opara¹ (1. Stellenbosch University(South Africa))

*Charles Boliko¹ (1. Food and Agriculture Organization of the United Nations(Japan))

[5-1350-A-04] Community-Based Digital Farming Approaches
*Sakae Shibusawa¹ (1. Tokyo University of Agriculture and Technology(Japan))

[5-1350-A-CR] Closing remark
Announcement of The XX CIGR World Congress 2022 in Kyoto
Noboru Noguchi (Member of SCJ, Hokkaido University)
[5-0900-A] **Keynote Lecture 5th**
Chair: Olaniyi A. Fawole (Stellenbosch University, South Africa)
Thu. Sep 5, 2019 9:00 AM - 10:15 AM  Hall A (Main Hall)

**[5-0900-A-01] Microbiological Risk of Nonthermal Food Preservation Technologies: outputs from High Hydrostatic Pressure studies and state of art**
*Amauri Rosenthal¹ (1. Embrapa Food Technology (Brazil))
9:00 AM - 9:30 AM

**[5-0900-A-02] Microbial Safety of Traditionally Fermented Foods in East and South Asia**
*Anthony Mutukumira¹ (1. Massey University (New Zealand))
9:30 AM - 10:00 AM

*Amauri Rosenthal¹ (1. Embrapa Food Technology(Brazil))

Keywords: Microbiological risk, Nonthermal, Hydrostatic pressure

Nonthermal emerging technologies have been industrially applied or studied for food preservation as an alternative thermal processes for obtaining products with better nutritional and sensory attributes. Several issues may be considered for designing the process to avoid microbiological risks, such as food composition and other characteristics, baroresistant variability of target microorganisms, sub lethal injuries and recovery capacity, storage conditions, inactivation and growth kinetics after microbial recovery. Furthermore, the matter turns even more complex when involving hurdle technologies by combining other technologies with high pressure for food preservation. This presentation discusses the main aspects to be considered in process design and validation based on different studies and commercial examples with different food products.


*Anthony Mutukumira¹ (1. Massey University(New Zealand))

Dr Tony Mutukumira gained his Doctor Scientarium Degree (PhD) in Food Science and Technology at The Norwegian University of Life Sciences in Norway, Ås, where he worked on the development of lactic fermented milks using novel cultures. His current research includes food safety, food preservations and food fermentations with a special interest in the development of fermented functional foods. Tony also has particular interest in preservation using emerging technologies such as HPP and UV. He is, however, passionate about using natural food preservatives. Tony is a Fellow of the New Zealand Institute of Food Science and Technology and several other professional bodies which include The South African Association of Food Science and Technology (SAAFoST), South African Society of Dairy Science and Technology (SASDST), and The International Union of Food Science and Technology (IUFoST) Committee on Distance Education. Tony is presently the Secretary of the Food Safety Working Group of the International Commission of Agricultural and Biosystems Engineering (CIGR). Tony is a scientific reviewer to several international peer-reviewed journals which include the International Journal of Food Microbiology, Food Science and Technology International, Journal of Natural and Mathematical Sciences, and Food Pathogens and Diseases. Tony has published more than 70 papers in scientific journals in addition to presenting and chairing sessions at several international conferences.

Keywords: Food safety, Traditional fermentation, Asian foods and beverages

Traditionally fermented food and beverages of East and South Asia play a dominant role in the culture and heritage of the region. For centuries, Asians have been practicing traditional food fermentations generating a wide diversity of products with unique attributes. Typical indigenous fermented products in East and South Asia include cereals and legumes, fruits and vegetables, milk, meat, fish and sea-foods, condiments and beverages. All these products are renowned for their appealing sensory profiles and are considered
nutritious, thus contributing to food security. Their specific recipes and sensitive preparation methods are highly depended on the indigenous knowledge of the native communities which is transmitted through generations with little, if any documentation. Traditional fermentation generally involves the use of an undefined microflora which naturally developed as the dominant starter culture through traditional fermentation techniques such as back-slopping and repeated use of fermenting vessels. Each fermented food is characterised by a group of distinct microflora and typical examples of the most common microorganisms used are lactic acid bacteria, yeasts and moulds. The mode of action of traditional fermentation ensure the safety of fermented foods through synthesis of numerous antimicrobial compounds, and removal or destruction of harmful substances. However, improper handling, low quality raw materials, incorrect processing conditions, poor hygiene and sanitation enable pathogens and their toxic metabolites to impart a potential risk on food safety. Synergistic interactions among beneficial microflora, antagonistic effects on undesirable microbiota and the utilisation of certain natural antimicrobial ingredients in food preparation contribute to safeguard the safety of the products further. The paper provides a background to the traditional fermented foods in East and South Asia, associated microbial hazards and assuring microbial safety.
Oral Session | Food Safety

[5-1015-A] Food Safety (2)
Chair: Ubonrat Siripatrawan (Chulalongkorn University, Thailand)
Thu. Sep 5, 2019 10:15 AM - 11:30 AM Hall A (Main Hall)

[5-1015-A-04] Cinnamon Oil Nanoemulsion as a Natural Microbial Decontaminant of Chilled Fish Flesh
Piyanan Chuesiang\textsuperscript{1,2}, Romanee Sanguandeekul\textsuperscript{1}, Ubonrat Siripatrawan\textsuperscript{1,2} (1. Chulalongkorn University, Department of Food Technology, Faculty of Science (Thailand), 2. The Novel Technology for Food Packaging & Control of Shelf Life Research Group, Chulalongkorn University (Thailand))
10:15 AM - 10:30 AM

Abdullah Iqbal\textsuperscript{1,2}, Mizuki Tsuta\textsuperscript{1} (1. Food Research Institute, National Agriculture and Food Research Organization 2-1-12 Kan-nondai, Tsukuba, Ibaraki 305-8642 Japan (Japan), 2. Dept. of Food Technology & Rural Industries, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh (Bangladesh))
10:30 AM - 10:45 AM

[5-1015-A-03] Preservation of sardine and scallop by high hydrostatic pressure: safety and quality aspects
Amauri Rosenthal\textsuperscript{1}, Rosiane Costa Bonfim\textsuperscript{1,2}, Fabiano Alves Oliveira\textsuperscript{3}, Ronoel Luiz de Oliveira Godoy\textsuperscript{1}, Carlos Adam Conte Junior\textsuperscript{4}, Eduardo Henrique Miranda Walter\textsuperscript{1} (1. Embrapa (Brazil), 2. Federal Rural University of Rio de Janeiro (Brazil), 3. Cefet Valença (Brazil), 4. Federal Fluminense University (Brazil))
10:45 AM - 11:00 AM

Okwunna Maryjane Umego\textsuperscript{1}, Habeeb Adedotun Alabi\textsuperscript{2}, Yahaya Mijinyawa\textsuperscript{2} (1. Federal University Oye Ekiti (Nigeria), 2. University of Ibadan (Nigeria))
11:00 AM - 11:15 AM

[5-1015-A-05] Responsiveness to Food Safety Emergencies in Eswatini following the Outbreak of listeriosis in South Africa
Tendekayi Henry Gadaga\textsuperscript{1}, Anthony N Mutukumira\textsuperscript{2} (1. University of Eswatini (Swaziland), 2. Massey University (New Zealand))
11:15 AM - 11:30 AM
Cinnamon Oil Nanoemulsion as a Natural Microbial Decontaminant of Chilled Fish Flesh

Piyanan Chuesiang¹,², Romanee Sanguandeekul¹, *Ubonrat Siripatrawan¹,² (1. Chulalongkorn University, Department of Food Technology, Faculty of Science(Thailand), 2. The Novel Technology for Food Packaging &Control of Shelf Life Research Group, Chulalongkorn University(Thailand))

Keywords: Essential oil, Nanoemulsion, Phase inversion temperature, Antimicrobial, Cell morphology

Economic losses caused by foodborne pathogen and spoilage are a driving force to apply food preservatives in perishable food products. However, the increasing awareness in recent years of the health risks for chemical preservatives added to the increasing demands of consumers for natural antimicrobial agents. This study aimed to develop cinnamon (Cinnamomum verum) essential oil nanoemulsion (CEO-NE) as a natural fledgling microbial decontaminant of a chilled fish product. The optimum CEO-NE formulation contained cinnamon essential oil with medium chain triglyceride (MCT) = 10 wt%, a non-ionic surfactant (Tween 80) =15 wt%, and deionized water 75 wt%. The CEO-NE was fabricated using a low energy Phase Inversion Temperature (PIT) method. Sea bass fish flesh was used to represent a seafood product. The fish flesh was artificially contaminated with Escherichia coli (ATCC 25922) prior to dipping into the CEO-NE solution at its minimum inhibitory concentration (MIC) determined from the previous experiments. The samples were stored at 4 C. The growth of E. coli and total viable counts of the CEO-NE treated samples was examined in comparison to those treated with bulk CEO and untreated (control) samples. The results showed that CEO-NE effectively inhibited E. coli and total aerobic bacteria better than bulk CEO. The bacterial cell morphological deformation by the CEO-NE was evidenced by field emission scanning electron microscopy (FE-SEM). The antimicrobial activity of the CEO-NE against E. coli was attributed to its ability to disrupt bacterial cell wall structures and promote expulsion of internal cellular material. The results suggest that the encapsulation of cinnamon oil in nanoemulsion enhanced its bactericidal activity against the targeted foodborne microorganism. The developed CEO-NE has potential to be used as natural antimicrobial agent for ensuring food safety of fish flesh or other seafood products.

*Abdullah Iqbal\(^1,2\), Mizuki Tsuta\(^1\) (1. Food Research Institute, National Agriculture and Food Research Organization 2-1-12 Kan-nondai, Tsukuba, Ibaraki 305-8642 Japan (Japan), 2. Dept. of Food Technology & Rural Industries, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh(Bangladesh))

Keywords: Honey, chemometrics, classification, geographic origin

The Front-face fluorescence spectroscopy was applied in this study for the classification of honey based on geographical origin. Honey samples (Robinia pseudoacacia and Blended floral source) of different origin (i.e., China, Hungary and Japan etc) used in this study were collected from their production sites. Before the fluorescence measurement, the samples were put in shaking water bath at 60°C for 30 min with 100 rpm shaking speed. Then after stirring to obtain the homogeneity, the honey samples were diluted to 100 times with the addition of 20% (v/v) ethanol solution. The front-face fluorescence excitation-emission matrices were then recorded from 200nm to 800nm (at an interval of 1 nm) whereas excitation spectra were recorded between 200nm to 500nm (with an interval of 5nm). With the application of necessary pre-processing (i.e., normalization, mean centering, autoscaling and/or combination thereof) and digital smoothing polynomial filters (i.e., Savitzky-Golay smoothing filters) for smoothing out the noisy signals, the rayleigh scattering rays were removed from the spectra. The chemometric analysis were then applied to the spectral data using principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) for classification of the honey samples. A reasonable sensitivity (ranging from 0.90 to 1.000) and specificity (ranging from 0.795 to 1.000) for class predictions was obtained from the PLS-DA model. The results showed that front-face fluorescence spectroscopy has potential for the discrimination of Robinia pseudoacacia honey based on geographical origin. But it is not possible to discriminate the blended samples based on geographical origin.
Application of Fluorescence Spectroscopy for the Classification of Honey Based on Geographical Origin

Abdullah Iqbal¹,²*, Mizuki Tsuta¹

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ABSTRACT

The Front-face fluorescence spectroscopy was applied in this study for the classification of honey based on geographical origin. Honey samples (Robinia pseudoacacia and Blended floral source) of different origin (i.e., China, Hungary and Japan etc) used in this study were collected from their production sites. Before the fluorescence measurement, the samples were put in shaking water bath at 60°C for 30 min with 100 rpm shaking speed. Then after stirring to obtain the homogeneity, the honey samples were diluted to 100 times with the addition of 20% (v/v) ethanol solution. The front-face fluorescence excitation-emission matrices were then recorded from 200nm to 800nm (at an interval of 1 nm) whereas excitation spectra were recorded between 200nm to 500nm (with an interval of 5nm). With the application of necessary pre-processing (i.e., normalization, mean centering, autoscaling and/or combination thereof) and digital smoothing polynomial filters (i.e., Savitzky-Golay smoothing filters) for smoothing out the noisy signals, the rayleigh scattering rays were removed from the spectra. The chemometric analysis were then applied to the spectral data using principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) for classification of the honey samples. A reasonable sensitivity (ranging from 0.90 to 1.000) and specificity (ranging from 0.795 to 1.000) for class predictions was obtained from the PLS-DA model. The results showed that front-face fluorescence spectroscopy has potential for the discrimination of Robinia pseudoacacia honey based on geographical origin. But it is not possible to discriminate the blended samples based on geographical origin.

Keywords:
Honey, chemometrics, classification, geographic origin, PCA, PLS-DA

1. INTRODUCTION

Honey is a healthy natural, pure and nutritious food produced by honeybee containing 60–80% of carbohydrates, 17–20% of water, 0.3–0.8% of proteins, 0.2% of minerals and minor quantities of amino acids, phenols, pigments, vitamins, volatile substances, and others (Ball, 2007, Bogdanov et al., 2008, Khan et al., 2017).

Traditionally, honey has been used by human being not only as a nutritious substance but also as a therapeutic product due to its antioxidative components, such as polyphenols, amino and organic acids, enzymes and proteins (Oryan et al., 2016). These components are highly dependent on the floral source, the geographical region of production and external factors associated with environmental conditions, processing and storage methods (Alzahrani et al., 2012). Regional and/or geographical characteristics of honey in terms of the composition varies depending on the climate, altitude and other environmental factors etc (Salonen et al., 2017). Therefore, the geographical origin play an important role in the overall quality and authenticity of honey which is essential to be considered for quality point of view.

Recently, authenticity of foodstuffs became a major issue for the consumers and producers worldwide (Petróczi et al., 2010). In case of honey, authenticity is related to both geographical and floral source determinations as well as detection of unwanted substances, like syrups or sugars. Geographical
origins are economically important and therefore, subjected to frauds, leading to false or doubtful labelling. During the last two decades, several researchers attempted to characterize the botanical and geographical origin of honey by exploiting different analytical techniques (Anklam, 1998), such as FTIR (Wang et al., 2010), FT-Raman spectroscopy (Corvucci et al., 2015), mid-infrared spectroscopy (Ruoff et al., 2006), near-infrared spectroscopy (Woodcock et al., 2007), and fluorescence spectroscopy (Lenhardt et al., 2015, Mehretie et al., 2018). In many cases, several analytical methods are simultaneously essential for a reliable authentication of geographical origin of honeys which is time-consuming, laborious, costly and requires vast technical skill. Therefore, there is a need for new methods which can provide a rapid and reproducible authentication of the geographical origin of honey. As a result, the determination of botanical and geographical origin of honey is of increasing interest worldwide. The use of excitation-emission matrix (EEM) seems to be a promising approach as it has been successfully applied for different products. Fluorescence spectroscopy provides information on the fluorescent molecules’ presence and the environment of honey produced like other biological samples. Hence, fluorescence spectroscopy seems to be effective for classification of honey based on geographical origin. Therefore, the aim of the present research is to classify honey collected from different geographical origins using front-face fluorescence spectroscopy.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation for Measurement
A total of 23 honey samples (Robinia pseudoacacia) and 49 samples (Blended) produced in different countries (i.e., China, Hungary and Japan, Canada, Argentina and Myanmar) were used in this study. The honey samples were collected from their production sites and stored at 4°C until analysis. Before the fluorescence measurement, the samples were put in shaking water bath at 60°C (for liquefaction) for 30 min with 100 rpm shaking speed. Then after stirring to obtain the homogeneity, the honey samples were diluted to 100 times with the addition of 20% (v/v) ethanol solution. Then 3ml of diluted samples were pipetted into quartz cuvette and placed in the sample holder. The excitation-emission matrices were then recorded with the fluorescence spectrometer (F–7000, Hitachi High–Technologies Corporation, Tokyo, Japan), from 200nm to 800nm (at an interval of 1 nm), whereas excitation spectra were recorded between 200nm to 500nm (with an interval of 5nm). The spectra were then converted into ASCII files to be further analyzed using MATLAB®2019a (The MathWorks, Inc., Natick, MA).

2.2 Processing of Spectra and Multivariate Analysis
With the application of necessary pre-processing (i.e., normalization, mean centering, autoscaling and/or combination thereof) and digital smoothing polynomial filters (i.e., Savitzky-Golay smoothing filters) for smoothing out the noisy signals, the Rayleigh scattering rays were removed from the spectra using a script coded and designed on MATLAB®2019a (The MathWorks, Inc., Natick, MA). The chemometric analysis such as Principal component analysis (PCA) was used to eliminate the spectral collinearity, random noise, and to reduce the dimensionality of variables. It was also applied in visualizing the data set as well as exploring the variations among the sample classes. The data sets in PCA are not correlated with each other and the score plot of the PCA has been used to explain the variations or similarities among the samples (Rahman et al., 2016). Subsequently, the partial least squares-discriminant analysis (PLS-DA) was used for classification of the honey samples. PLS-DA is a classification technique used for building linear discriminant analysis transforming the observed data into a set of intermediate linear latent variables which are then used for predicting the dependent variables. To select the number of PLS variables included in the model, Venetian blind cross-validation was used in this investigation. PCA and PLS-DA of the samples were computed by using the PLS toolbox for MATLAB (Eigenvector Research Inc., Wenatchee, WA, USA).

3. RESULTS AND DISCUSSION

3.1 Spectral information
The excitation-emission matrix (EEM) for typical geographical samples after removing Rayleigh’s scattering rays from the spectra are shown in Figure 1(a). It is seen that all the honey samples irrespective of geographical origin, shows three peaks in the contour plot of EEM. The peak exists at around excitation of 230 nm and emission of 340 may be responsible for the fluorescence of aromatic
amino acids (Karoui et al., 2007) present in the honey. The excitation/emission wavelengths corresponding to the peak 280/340 nm, common to all three samples may be due to the presence of flavonoids (such as apigenin, chrysin, kaempferol, pinocembrin), although it is tough to identify the flavonoids responsible for such peaks (Lenhardt et al., 2015). Another fluorescence peak corresponding to excitation wavelength range of 320-340 nm and emission wavelength range of 400-460 nm could be related to the Maillard reaction products such as hydroxymethylfurfural and furosine (Lenhardt et al., 2015). The contour plot of spectra for the *Robinia pseudoacacia* samples generally indicates that the considered honey samples may have similar components although they are originated or produced in different countries. However, the variation in peaks may be due to the concentration of different components.

The spectral behavior of blended honey samples is bit complex and there are distinct differences among the samples. Even the samples blended in the same geographical location (i.e., samples from same country), the different blended samples gave different fluorescence signatures as shown in figure 1(b). This may be due to the ingredient of the blended components and their concentration as well as other parameters associated with the blending process which cannot be explained unknown during the investigation.

![Figure 1. Excitation-emission spectra of different honey: (a) Robinia pseudoacacia, (b) Blended](image)

**3.2. Chemometric analysis**

**3.2.1 Principal component analysis (PCA)**

PCA is applied for both types of honey samples (*Robinia pseudoacacia* and blended) to reduce multidimensionality to two dimensions and the results are shown in Figure 2. From the PC scores it is seen that for the pseudoacacia samples (Figure 2.a), the PC1 explained 63.80% of the total variance in the data set while PC2 explained 17.46% and remaining 18.74% of data variance belongs to the other dimensionality. From the score plot, the honey samples are not completely separated into different classes or groups. It is seen from the plot that they are very close to each other (as it is mentioned earlier that showing similar peaks). The similar but more prominent behavior has been observed for
the blended sample as shown in Figure 2(b), although the PC1 explained 61.23% of the total variance in the data set while PC2 explained 18.33% whereas remaining 20.44% of data variance belongs to the other PCs.

Figure 2. Two dimensional PCA score plots of honey*: (a) Robinia pseudoacacia (b) Blended (*CN=sample from China, HU=sample from Hungary, JP= sample from Japan, CA=sample from Canada, MM=sample from Myanmar)

3.2.2 Partial least squares-discriminant analysis (PLS-DA)
The PLS-DA classification model was developed to classify different honey samples based on geographical origin and four parameters such as sensitivity, specificity for calibration (Cal), and cross-validation (CV) were considered as the indicator of the robustness of the model (Table 1).

Table 1. Sensitivity, specificity, and classification error of PLS DA models.

<table>
<thead>
<tr>
<th>Parameters*</th>
<th>Robinia pseudoacacia</th>
<th>Blended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (Cal)</td>
<td>1.000</td>
<td>0.900</td>
</tr>
<tr>
<td>Sensitivity (CV)</td>
<td>1.000</td>
<td>0.900</td>
</tr>
<tr>
<td>Specificity (Cal)</td>
<td>1.000</td>
<td>0.872</td>
</tr>
<tr>
<td>Specificity (CV)</td>
<td>0.944</td>
<td>0.795</td>
</tr>
</tbody>
</table>

*Cal refers to calibration set, and CV refers to the cross-validation results

The best model was built with 5 and 4 latent variables for pseudoacacia and blended samples, respectively. The models showed reasonable sensitivity (from 0.900 to 1.00 for both Cal and CV) and specificity (0.795 to 1.00 for Cal and 0944 for CV) for the classification of honey samples for both the Pseudoacacia and blended samples (Table-1). The confusion table (CV) for both types of honey samples are shown in Table 2.

Table 2. Confusion table for honey samples

<table>
<thead>
<tr>
<th>Predicted as...</th>
<th>Actual Class (Robinia pseudoacacia)</th>
<th>Predicted as...</th>
<th>Actual Class (Blended)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CN (5)</td>
<td>HU (14)</td>
<td>JP (4)</td>
</tr>
<tr>
<td>China (CN)</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hungary (HU)</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Japan (JP)</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

From table-2, it is seen that Robinia pseudoacacia samples can be classified based on geographical origin. But for the blended honey, samples only from Argentina and Myanmar, has been classified as the origin they belong. Remaining samples are mixed with other classes which seems to be like that group(s) as described in section 3.1.
4. CONCLUSION

This study was conducted to demonstrate the potential application of excitation-emission matrix (EEM) patterns for the classification of complex matrix of honey samples from different geographical origin. The front face fluorescence measurement used in this study revealed that it is not possible to obtain complete classification of honey based on geographic origin by Front-face fluorescence spectroscopy. Although the classification accuracy for pseudoacacia samples was reasonable but not up to the level for the blended samples to conclude with a sentence ‘complete classification’ is possible! However, further studies need to be carried out with the modification of existing Front-face fluorescence spectroscopy with more samples to make a conclusion about the potential for the classification of honey based on geographic origin.

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REFERENCES


Preservation of sardine and scallop by high hydrostatic pressure: safety and quality aspects

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Keywords: Sardine, Scallop, High Hydrostatic Pressure, Preservation, Shelf life

High hydrostatic pressure (HHP) has been a successful novel technology for preservation of different foods, including seafood and fishes. However, safety and quality aspects have to be considered for designing a proper process aiming at optimizing the quality and assuring the safety of the products. Some studies have been carried out for comparing quality and safety aspects of sardine and "Lion Paw" scallop muscle processed by HHP. Therefore, sardine fillets and scallops muscle were treated by 300 MPa to 400 MPa for 0 to 15 min. and compared regarding microbiology, TBARS, N-TVB formation and nucleotide degradation along refrigerated (4-5°C) shelf-life. In the case of sardines, HHP did not completely cease N-TVB formation and nucleotide degradation, but minimized the development of those processes, especially at higher pressure levels and holding times. Regarding scallops, HHP decreased the count of mesophilic and psychotrophic microorganisms below the legislation standard requirements. However, proper caution should be taken mainly considering specific pathogenic microorganisms. As expected, HHP accelerated lipid oxidation in the case of scallops, resulting in increase of TBARS, but did not exceed the standard limit of 2 mg/kg. Nucleotide degradation followed different patterns considering the different metabolisms and specificities of the muscle fibers. These results indicate that HHP can significantly increase the refrigerated storage time for sardine and scallop but intrinsic and extrinsic factors and characteristics may influence the safety and quality aspects.
Yams are scarce during non-harvest seasons and the prices are exorbitant with majority of the population unable to buy. This situation motivated the interest for this research to assess the handling of yam and the temporal storage practices among traders in order to identify and have good understanding of the various activities pertaining to the yam markets. Visits to the markets, interview with the traders and measurement of the storage temperature and relative humidity were carried out to obtain data for the assessment of handling and temporal storage of yams in the markets. Five activities were identified pertaining to yams in the markets, namely: arrival of yams in vehicles, unloading of the yams, display of the yams for sale, packaging and loading of sold yams, and lastly temporal storage of the unsold tubers. The assessment of the handling of yam tubers in each of the above mentioned activities revealed that; the handling operations are rudimentary and results in bruising, breakage and exposure of tubers to adverse environmental conditions thereby causing substantial losses. The assessment of the temporal storage structures for yams in the markets showed that; there are two types of storage structures for yams in the markets, these are: the open shed and the market stalls. The storage environment, the design and construction materials of these storage structures are not effective for yam, thereby contributing to losses. These findings revealed that the open shed and market stall rooms used by yam wholesalers in Bodija and Bere-Mapo markets are ineffective for yam storage because the storage environment within these structures as influenced by the design and construction materials cannot allow for effective storage of yams. The problems associated with these structures in percentage are roof leakage 34.8% and 11.4%, rodent and pest attacks 82.6% and 11.4%, and adverse environmental conditions 91.3% and 85.7% for open sheds and stalls respectively. It is recommended that the materials of construction and design of these structures be modified to make them more effective.
Assessment of the Handling and Temporary Storage Methods of Yams in Market Places in Ibadan

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ABSTRACT

Yams are scarce during non-harvest seasons and the prices are exorbitant with majority of the population unable to buy. This situation motivated the interest for this research to assess the handling of yam and the temporal storage practices among traders in order to identify and have good understanding of the various activities pertaining to the yam markets. Visits to the markets, interview with the traders and measurement of the storage temperature and relative humidity was carried out to obtain data for the assessment of handling and temporal storage of yams in the markets. Five activities were identified pertaining to yams in the markets, namely: arrival of yams in vehicles, unloading of the yams, display of the yams for sale, packaging and loading of sold yams, and lastly temporal storage of the unsold tubers. The assessment of the handling of yam tubers in each of the above mentioned activities revealed that; the handling operations are rudimentary and results in bruising, breakage and exposure of tubers to adverse environmental conditions thereby causing substantial losses. The assessment of the temporal storage structures for yams in the markets showed that; there are two types of storage structures for yams in the markets, these are: the open shed and the market stalls. The storage environment, the design and construction materials of these storage structures are not effective for yam, thereby contributing to losses. These findings revealed that the open shed and market stall rooms used by yam wholesalers in Bodija and Bere-Mapo markets are ineffective for yam storage because the storage environment within these structures as influenced by the design and construction materials cannot allow for effective storage of yams. The problems associated with these structures in percentage are roof leakage 34.8% and 11.4%, rodent and pest attacks 82.6% and 11.4%, and adverse environmental
1. INTRODUCTION

Nigeria is the largest producer of yams in the world, annually producing about 31 million tonnes. Nigeria produced 60% of the world’s yams in 2010, and is the largest contributor in Africa’s “Yam Belt,” a yam production area that comprises Nigeria, Ghana, Benin, Côte d’Ivoire, Central African Republic, Cameroon, and Togo. Yams have had the second highest production level of any food crop in Nigeria in the past 50 years after cassava (Bergh et al., 2012). Yam losses are one of the greatest problems facing yam production in Nigeria and are of concern to everyone, from the research scientists to the extension workers, marketers in the field to the farmers on the farm and to the government policy formulators. The post-harvest handling and storage practices for yams in Nigeria presents a dismal picture and are mostly comprised of traditional techniques practiced by growers, traders and the processor resulting in considerable deterioration of physical and nutritional qualities of harvested crop (Oni and Obiakor, 2002). Interest in the reduction of post-harvest losses is not new. Mrema and Rolle (2002) reported that after the mid-1970s food crisis, the United Nations brought post-harvest storage losses into international focus in 1975 when it declared that “further reduction of post-harvest food losses in developing countries should be undertaken as a matter of priority”. In underdeveloped and developed tropical countries, both quantitative and qualitative losses of agricultural products occur at all stages in the post-harvest chain, from harvesting, through handling, storage, processing, packaging, transportation and marketing until crops are delivered to the final consumers.

Ibadan North local Government is a big urban center with a population of over 350,000 inhabitants, according to the 2006 Nigerian census (NPC, 2006). The town is home to two major urban yam markets in Oyo State, that is, Bodija and Bere-Mapo yam markets. According to the Natural Resources Institute (NRI, 2012) report, diverse challenges constrain yam farmers and marketers’ ability to fully exploit the potential of yams and yam products in the southwest, these includes, high cost of inputs and labour, lack of credit, limited access to proper secure storage facilities, high transportation costs and ineffective handling practices. The yam traders in Ibadan North are no exception to the above mentioned challenges.
Despite the elaborated agricultural programs, Ibadan is still unable to provide an all year round supply of yams within the purchasing power of majority of the people. Besides economic factors, the supply of food in the local government is limited by losses due to wastage and spoilage. Though no one knows how much yams is lost between harvest and consumption, but post-harvest management complements efforts to enhance food security through improved farm level productivity, thus tending to benefit producers, and more specifically, the rural farmers.

Post-harvest management reduces post-harvest losses thus, generates income, improves product quality and safety, and contributes to food and nutritional security. It is against this background that an analysis of the post-harvest management strategies like handling and temporary storage by yam traders is deemed important. This work assessed the handling and temporary storage of yams in markets in Ibadan North local government.

2. METHODOLOGY

2.1 Study Area

This study was carried out in Ibadan North Local Government area. The city of Ibadan is located approximately on longitude 3°5’ E of the Greenwich Meridian and latitude 7°23’ N of the Equator. Economic activities undertaken by people in the Local Government Area include trading, public service and agriculture. Ibadan North Local Government has a land area of 145.58km² and a population of 306,795 people (NPC, 2006). The study area experiences a tropical type of climate. It has a mean annual temperature of about 32° C. The relative humidity can be as high as 95% and a total of about 1250 mm as mean annual rainfall.

2.2 Methodology

The markets were visited for physical observation of the activities taking place, particularly among the yam wholesalers. Also, the yam storage structures were assessed. Temperature and relative humidity of the storage structures and the ambient environment were measured once every other day for a period of one month (August 19th to September 16th, 2016). A dry bulb and wet bulb thermometer with psychrometric chart was used to achieve this. A questionnaire was designed to obtain information on some of the questions regarding yam storage among yam wholesalers in Bodija and Bere-Mapo markets. The data collected was analyzed using the Microsoft Excel 2010 to obtain statistics of frequencies and percentages of the data.

3. RESULTS AND DISCUSSIONS
3.1 Arrival of Yams to the Markets
Yams are transported from the farms or small local district markets in rural areas to the large urban markets of Bodija and Bere-Mapo in big lorries, buses, open pick-up vans, and trucks. It was observed that, yams are stacked one upon another like timber, without any packaging material. Yams that are in contact with the edges of the vehicle sustained abrasions and cuts, those at the bottom are subjected to compressive loads due to the weight of the overburdening yams lead to internal injury or damage of the yams at the bottom. Depending on the degree of injury on the yam, the level of periderm formation might be affected. When the periderm is not formed the yam cannot heal the bruised part, thus, the storage life is reduced.

3.2 Unloading Operation
This operation is carried out manually by the market labourers. The labourers unload the vehicles either by using metal pans to pack the yams from the vehicle or by throwing the yams from the vehicle to other labourers standing on the ground who then place it on the ground gently. Unloading by throwing if not done carefully can lead to breakage (figure 1). In the case of unloading with the metal pan, there is risk of compression damage due to the force acting on individual tubers. It was also observed that the labourers carry plenty yams at a time and get fatigue under the weight. Thus, instead of gently putting down the tubers they drop it by pouring. This results in tuber bruises and breakages.

Figure 1: Unloading operation by throwig

3.4 Packaging and Loading of Sold Yams
Sometimes the market labourers are contracted to carry the yams to the vehicle. The practice of loading yams in the vehicle by pouring before arranging as shown in figure 2 is damaging to the yams. Some yams break and others get bruised and as a result such tubers do not take long to spoil. As recommended by Ayoub and Lennox (2013), packaging materials such as telescopic fiberboard cartons with paper wrapping or excelsior should be used. This reduces bruising and damage due to heat from the tuber respiration and breakage and internal injury caused by compression of tubers from the weight of the overlying tubers.

![Figure 2: Loading operation by pouring](image)

3.5 Temporal Storage of Yams in Markets

The markets are not used for long storage of yams, however, there is yam storage in the markets on a temporary basis. This is because tubers are usually supplied in very large quantities and the supplies are usually not exhausted in a few days. Sometimes it takes over a month before some traders are able to exhaust their supplies. Thus, there is storage of the produce while the stock lasts.

3.5.1 Types of Yam Storage Structures
There are only two types of yam storage structures in Bodija and Bere-Mapo markets in Ibadan North local government, Oyo State. In Bodija yam market, wholesalers use open sheds (figure 3) to store yams, while those in Bere-Mapo yam market, use stalls as structures for yam storage (figure 4). These structures can best be described as improvised yam storage structures as the design and types of construction materials are not in tandem with any known design criteria or principle for yam storage structures. However, traders have been using these structures for years for the storage of yams. These structures were assessed to see how they vary from known traditional and modern yam storage structures, and what improvements they need to become effective in storing yams.

3.5.2 Storage Environment of the Structures

The average daily ambient temperatures and the temperatures inside the open shed in Bodija and the market store rooms in Bere-Mapo yam markets are presented in figures 5 and 6. While the temperatures in the open shed storage structures varied from 25.3°C to 30.3°C with an average value of 28.03°C and an average ambient temperature of 28.67°C, those within the stall rooms varied from 30.1°C to 33.8°C with an average value of 33.21°C, and for the ambient temperatures, the range is from 26.0°C to 33.0°C with an average value of 29°C. The temperatures within the open shed was generally equal to that of the ambient conditions, but lower than those obtained in the market stall rooms for all periods throughout the study.
period. These average temperature values are higher than the storage temperature of 13\(^\circ\)C to 15\(^\circ\)C for yam recommended by the National Agriculture Research Institute, 2004.

The ambient relative humidity ranged from 76\% to 82\% with an average value of 79.73\%, while for the open shed, it varied from 79\% to 83\% with an average value of 80.10\%, and for the stall rooms it varied from 82\% to 88\% with an average value of 85.15\%. Although, there were a few overlaps, the ambient relative humidity was equal to those in the open shed structure but lower than those within the market store rooms (figures 7 and 8). However, these average relative humidity are lower than the recommended value of 90\% to 95\% by the National Agriculture Research Institute, 2004.

The variation in the environmental conditions within the market stall rooms and the open shed structure is attributed to the lack of ventilation in the store rooms, material of construction and the arrangement of the yams in the store rooms. This observation is due to the fact that within the stall room storage structures, the respiration of the tubers of yam increased the internal temperatures of the structures which is not the case under the open shed environment.

Figure 5: Average Daily Temperatures of Yam Storage Structures in Bodija
**Figure 6:** Average Daily Temperatures of Yam Storage Structures in Bodija

**Figure 7:** Average Daily Relative Humidity of Yam Storage Structures in Bodija

**Figure 8:** Average Daily Relative Humidity of Yam Storage Structures in Bere-Mapo
3.5.3 Storage Structure Construction Materials

The materials of construction are wood, sand and cement blocks walls, the roof is corrugated zinc or aluminum roofing sheets and ventilation is inadequate because only one opening, which is the door, is fitted on the store. These construction materials are not very good insulators of heat. The roofing sheets for example, easily conducts solar heat and transmits it easily into the room as no ceiling is provided and the height is low (2.3 meters). The arrangement of the yams in the stalls by heaping does not permit maximum air circulation between the tubers as compared to the arrangement in the open sheds. This is detrimental to the tubers because, during respiration of yams, oxygen is used and CO₂, water and heat are produced. Since there is no proper air circulation to transport the heat and water away from the tubers, the heat causes rise in temperature and water increase the moisture in the air, which is the relative humidity. Physiological activities like respiration and sprouting of the tubers are promoted by high temperature of the storage environment which results in a steady loss of carbohydrate in the form of carbon dioxide and water, making the yams to lose weight, size and market value.

3.5.4 Problems Associated with the Temporal Storage Structures

The problems associated with yam storage structures in Bodija and Bere-Mapo yam Markets are presented in Table 1. The results indicate that environmental factors constitute a major problem in both markets. While for Bodija market, 91.3% of the respondents said storage environment conditions within the open shed was not favorable for yam storage, for Bere-Mapo market, 85.7% attributed yam storage losses to adverse environmental conditions within the store rooms. Decay was very high in tubers heaped on floor as a result of direct contact with the soil on the bare ground. Presence of rot pathogen in soil on the storage area serves as a source that initiates decay. Poor air circulation within the heaped yam aid in the build-up of heat and increase humidity as a result of respiration. Hence induces spore germination and growth of pathogens.

Another major problem is the incidence of pest and rodents attacks which is particularly high (82.6%) in the open shed, but low in the market stall rooms with 11.4%. This high variation is attributed to the fact that in the market stalls, the rooms are fumigated and the doors are closed and rat poisons are used in preventing rodents and other pests from damaging the stored yams. However, within the open sheds, fumigation is not effective because the structure has no enclosure. Rat poisons are used against rodent attacks, but soon afterwards,
another set of rodents migrate from the nearby refuse dump sites and bushes to attack the stored yams since they are kept in the open space.

Other storage problems identified are roof leakage and storage space. While in Bodija 34.8% of sheds assessed had the problem of roof leakage, those within Bere-Mapo is 11.4%. Roof leakage allows direct sun rays and rain water to impact on the stored tubers. The continuous heating and wetting of the tubers result in breaking yam dormancy period sooner than necessary. Once dormancy period is over, sprouting sets in. Sprouting of stored yams is not desired because it affects the nutrition and size of the tuber. It can also result to decay of the yam and after its viability. Also, roof leakage increases the relative humidity within the storage environment and in combination with high temperature, encourages mold growth and insect activity.

**Table 1:** Problems Associated with Yam Storage Structure in Bodija and Bere-Mapo Markets.

<table>
<thead>
<tr>
<th>Problems</th>
<th>Frequency(n=23)</th>
<th>Bodija</th>
<th>Frequency(n=35)</th>
<th>Mapo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leakages</td>
<td>8</td>
<td>34.8%</td>
<td>5</td>
<td>14.3%</td>
</tr>
<tr>
<td>Collapse</td>
<td>1</td>
<td>4.3%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rodents Attack</td>
<td>19</td>
<td>82.6%</td>
<td>7</td>
<td>20%</td>
</tr>
<tr>
<td>Environmental</td>
<td>21</td>
<td>91.3%</td>
<td>30</td>
<td>85.7%</td>
</tr>
</tbody>
</table>

4. CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

The assessment of the handling of yam tubers in each of the above mentioned activities reveals that; the handling operations are rudimentary and results in bruising, breakage and damage of the yams causing substantial losses.

Furthermore, this study reveals that, there are only two types of temporal storage structures for yams in Bodija and Bere-Mapo yam markets, that is, the open shed and market stalls structures respectively. The assessment of these methods of yam storage structures shows that they are not efficient methods for yam storage because, the design and types of construction materials used cannot significantly moderate the storage temperature and relative humidity of the stored yams. Also, the design does not provide the means for protecting the stored yam
tubers from rodents and other insects attack. Roof leakage, rodents attack, collapse, and harsh environmental conditions were some of the problems with these temporal storage structures.

4.2 Recommendations

Proper handling of yams should be adopted both by farmers and wholesalers to minimize losses. The stall room used for yam storage should be well ventilated in order to enhance the exchange of air between the enclosure and the surroundings thereby eliminating the enzymatic action and micro-organism activities which result in the rapid spoilage of stored produce. Markets storage structures should be modified by adopting the design and types of construction materials recommended by Nigerian Stored Produce Research Institute (NISPRI).

REFERENCES


for Cooperating Agencies under the CCF-I Framework on Post-Harvest Food Loss Prevention, April 18-19, Ibadan, pp1-10.
Responsiveness to Food Safety Emergencies in Eswatini following the Outbreak of listeriosis in South Africa

*Tendekayi Henry Gadaga¹, Anthony N Mutukumira² (1. University of Eswatini(Swaziland), 2. Massey University(New Zealand))

Keywords: food safety, listeriosis, Eswatini, food control, pathogens

The FAO defines food safety as the absence, or safe, acceptable levels of hazards in food that may harm the health of consumers. Microbiological hazards pose a disproportionate threat to human health in all countries, more so in developing countries due to inadequate resources and fragmented food control systems. The food control system in Eswatini is administered by different departments. The Ministry of Health is responsible for the administration of the Public Health Act; Ministry of Agriculture, the Dairy act, and the Ministry of Trade, Industry and Commerce, standards, including food standards. A 2013 Food Safety Bill that aims at coordinating food control activities under a single food control authority has yet to be finalised. The outbreak of listeriosis in South Africa in 2017 revealed the importance of an effective food control system in Eswatini. Under the current food control system, the country runs the risk food poisoning outbreaks that may be difficult to control. Like other countries in southern Africa, Eswatini depends on South africa for substantial amounts of its food requirements, including cereals, fruits, vegetables, and meat products. Brands of ready-to-eat cold meat products that were implicated in the listeriosis outbreak in South Africa are also marketed in Eswatini. As a strategy to prevent the spread of the outbreak in Eswatini, the Ministry of Health embarked on a consumer awareness campaign and initiated a recall of affected products. The country had no capacity to test the products to verify presence of Listeria monocytogenes, thereby highlighting the need to strengthen the food control system. This paper reviews the state of the food control system in Eswatini and assesses the readiness of the country to respond to food safety emergencies using the listeriosis outbreak in South Africa as a case study.
POSTHARVEST/FOOD TECHNOLOGY AND PROCESS ENGINEERING (5)

Chair: Akindele Folarin Alonge (University of Uyo, Nigeria)
Thu. Sep 5, 2019 10:15 AM - 11:30 AM Room C (3rd room)

[5-1015-C-01] THE EFFECT OF DRYING METHODS ON THE QUALITY OF TIGER NUT (Cyperus esculentus lativum)
* Akindele Folarin ALONGE1, Edikan Ufot GILBERT (1. University of Uyo(Nigeria))
10:15 AM - 10:30 AM

[5-1015-C-02] Optimization and Storage Stability Evaluation of Antioxidant Extracts From Batangas Cherry (Terminalia microcarpa Decne)
*Dennis Marvin Opena Santiago1, Shekayna Eunice Balmes Pacia1, Jake Lloyd Cabrera Peña1,2, Claire Solis Zubia1, Sheba Mae Magbanua Duque1 (1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Banos, College, Laguna 4031 Philippines, 2. Department of Science and Technology CALABARZON Region, Regional Science and Technology Center Complex, Jamboree Road, Timugan, Los Banos, Laguna 4030 Philippines)
10:30 AM - 10:45 AM

[5-1015-C-03] Effects of Pre-drying treatment and Drying-air Temperature on Moisture Ratio and Effective Moisture Diffusivity of Tomato (Nigerian Local and Foreign Varieties)
*Obafemi Ibitayo Obajemihi1, Joshua Olanrewaju Olaoye2, Mayowa Saheed Sanusi1 (1. Food Engineering Department, University of Ilorin(Nigeria), 2. Agricultural and Biosystems Engineering, University of Ilorin(Nigeria))
10:45 AM - 11:00 AM

[5-1015-C-04] Extending the Shelf-life of Upland Water Spinach (Ipomoea aquatica) Using Trimming, Modified Atmosphere Packaging (MAP) and Low-Temperature Storage
*Ana Mithuzela Espigol1, Josephine Agravante1 (1. Postharvest Horticulture Training and Research Center (PTHRC), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), Laguna, Philippines)
11:00 AM - 11:15 AM

[5-1015-C-05] Investigation of Cowpea Variety and Storage Methods on Cowpea Beetle Infestation
*VICTORIA ADA ABODENYI1, YAHAYA MOBMI MUSA2, ABDULLAH MUHAMMED BAKO3 (1. Agricultural Engineering, Federal Polytechnic, Bauchi(Nigeria), 2. Federal polytechnic, Bauchi(Nigeria), 3. (Nigeria))
11:15 AM - 11:30 AM
This study aimed at evaluating the effect of different drying methods on the quality of tiger nut (Cyperus esculentus lativum). Three drying methods: sun drying, oven drying and microwave-oven drying were employed. Analysis of proximate, minerals, anti-nutrient and anti-oxidant composition of fresh (control) and dried tiger nut were carried out using the official method of analysis by the association of analytical chemist (AOAC, 2010). Fresh tiger nut tubers were divided into four portions. Three of the four portions were dried to constant weight using sun, oven and micro-wave drying methods respectively. The fourth portion of the sample was not dried but serves as the control. Result showed that the proximate composition of fresh and dried tiger nut sample for moisture content ranged from (5-45%), Protein (1.04-3.50%), Ash (0.05-0.51%), fibre (3.69-5.04%), fat (23.48-24.11%). For the dried samples, microwave oven drying had the lowest moisture (5.0%), oven had the highest fibre (3.80%), oven had the highest ash (0.48%), oven had the highest fat (24.11%), and oven had the highest protein (3.50%) contents contents. These values were significantly different from (p<0.05) the control. The minerals composition of the fresh and dried tiger nut ranged from Calcium (1.97mg/g-2.41mg/g), Potassium (2.29mg/g-3.83mg/g), Magnesium (1.03mg/g-5.33mg/g), and Zinc (5.09mg/g-8.11mg/g). Anti-nutrients of dried tiger nut were significantly reduced among other drying methods when compared with the control; anti-nutrient of the fresh and dried tiger nut range from Hydrogen cyanide (HCN) (0.012mg/g-0.401mg/g), Oxalate (0.016mg/g-0.084mg/g), Phytate (0.022mg/g-0.062mg/g), Tannin (Ta) (0.029mg/-0.0364mg/g). Anti-oxidant of the fresh and dried tiger nut ranged from 1, 1-diphenyl-2-picryllydrazyl (DPPH) (0.577%-2.23%), Cupric ion reducing capacity assay (CUPRAC) (0.52%-0.44%), Ferric ion reducing anti-oxidant power assay (FRAP) (0.40%-0.68%). At the end of this study, Oven drying maintained high nutritional content among the drying methods. Microwave oven drying method had the highest retention of its mineral composition when compared with the control. Sun drying had the lowest anti-nutrient among the drying methods. Microwave oven drying was effective in its anti-oxidant activity with reference to the control.
The Effect of Drying Methods on the Quality of Tiger nut
(Cyperus esculentus lativum)

1Akindele Folarin ALONGE 1Edikan GILBERT
2Department of Agricultural and Food Engineering, University of Uyo, Uyo, Nigeria

*Corresponding author: akindelealonge@uniuyo.edu.ng

ABSTRACT

This project aimed at evaluating the effect of different drying methods on the quality of tiger nut (Cyperus esculentus lativum). Three drying methods: sun drying, oven drying and microwave-oven drying were employed. Analysis of proximate, minerals, anti-nutrient and anti-oxidant composition of fresh (control) and dried tiger nut were carried out using the official method of analysis by the association of analytical chemist (AOAC, 2010). Fresh tiger nut tubers were divided into four portions. Three of the four portions were dried to constant weights using sun, oven and micro-wave drying methods respectively. The fourth portion of the sample was not dried but serves as the control. Result showed that the proximate composition of fresh and dried tiger nut sample for Moisture content ranged from (5-45%), Protein (1.04-3.50%), Ash (0.05-0.51%), fibre (3.69-5.04%), fat (23.48-24.11%). for the dried samples, microwave oven drying had the lowest moisture (5.0%), oven had the highest fibre (3.80%), oven had the highest ash (0.48%), oven had the highest fat (24.11%), and oven had the highest protein (3.50%) contents. These values were significantly different from (p<0.05) the control. The minerals composition of the fresh and dried tiger nut ranged from calcium (1.97mg/g-2.41mg/g), Potassium (2.29mg/g-3.83mg/g), Magnesium (1.03mg/g-5.33mg/g), and Zinc (5.09mg/g-8.11mg/g). Anti-nutrients of dried tiger nut were significantly reduced among other drying methods when compared with the control; anti-nutrient of the fresh and dried tiger nut range from hydrogen cyanide (HCN) (0.012mg/g-0.401mg/g), oxalate (0.016mg/g-0.084mg/g), Phytate (0.022mg/g-0.062mg/g), Tannin (Ta) (0.029mg/-0.0364mg/g). anti-oxidant of the fresh and dried tiger nut ranged from 1, 1-diphenyl-2-picyrylhydrazyl (DPPH) (0.577%-2.23%), Cupric ion reducing capacity assay (CUPRAC) (0.52%-0.44%), Ferric ion reducing anti-oxidant power assay (FRAP) (0.40%-0.68%). At the end of this study, oven drying maintained high nutritional content among the drying methods. Microwave oven drying method had the highest retention of its mineral composition when compared with the control. Sun drying had the lowest anti-nutrient among the drying methods. Microwave oven drying was effective in its anti-oxidant activity with reference to the control.

Keywords: Drying, Tiger nuts, Sun drying, Oven drying, Microwave drying, Drying rates, Drying methods

1. INTRODUCTION

Tiger nut “Cyperus esculentus lativum” is an underutilized tuber of family Cyperaceae, it produces rhizomes from the base of the tuber that is spherical (Devries and Feuke, 1999). It is a tuber that grows freely and is consumed widely in Nigeria and other parts of West Africa.

Tiger nuts exist in varieties (black, brown and yellow which are cultivated. Among these, the yellow variety is preferred over others because of its inherent properties such as large size, attractive color and flesher nature. It yield more milk upon extraction, contains lower fat and higher protein (Okafor and Okolo, 2003). Tiger nut tubers appear long or round in shape with a dimension of 8mm to 16mm, the smaller size however, are not used for human consumption. Recently, there is awareness for increased consumption of tiger nut (Belewu and Abodunrin, 2006; Belewu, 2007). When hydrated, it is slightly harder (nut texture), but with a rather more intense and concentrated taste. The cultivation time is April to November.

Tiger nut, a tuber with sweet and nutty taste can be consumed raw, roasted, dried or as tiger nut milk or oil (Rita,2009). It can be stored and rehydrated by soaking without losing the crop texture.
which ensures acceptable sensory quality (Tucson, 2003). Drying of agricultural products helps to reduce the moisture content to a level that halts or control microbial growth and to reduce deteriorative chemical reaction in order to extend the shelf life of food (Mujumdar and Law, 2010).

In most agricultural based economies like Nigeria, large quantities of food products are dried to improve shelf life, reduce packaging costs, lower weights, enhance appearance, retain original flavor and most importantly maintain nutritional quality (Baysal et al., 2003; Demir et al., 2007; Simal et al., 2000; Ertekin and Yaldiz, 2004).

Sun, oven and microwave oven drying are common drying methods for agricultural crops. These drying methods have been reported to affect the nutrient composition of food in various ways. It can either increase the concentration of some nutrients by making them more available or decrease the concentration of some nutrients (Hassan et al., 2007; Morris et al., 2004; Ladan et al., 1997). Therefore, this project seeks to investigate the effect of different drying methods like sun drying, oven drying, and microwave oven drying on the quality of tiger nuts.

2. MATERIALS AND METHODS

SAMPLES COLLECTION AND PREPARATIONS

Fresh Tiger nuts were purchased from Itam Main Market, Uyo, Akwa Ibom State, Nigeria. The tubers were thoroughly screened to remove the bad ones and stones. They were washed, air dried and divided into four portions. Three of the four portions were dried to constant weight using sun, oven and micro-wave drying methods respectively. The fourth portion was not dried but was used as fresh sample which served as the control.

**Control:** Hundred grammes of fresh tiger nut sample were kept as the control to be compared with the dried tiger nut samples.

**Sun Drying:** Hundred grammes of the samples were kept in the sun between 10:30 am to 3:30 pm daily and were dried to constant weight (22.690 g) for 60 hours.

**Oven Drying:** Hundred grammes of the samples was also placed in an electric oven and dried to constant weight (27.328 g) at 65°C for 20 hours.

**Microwave Oven Drying:** Hundred grammes of the samples were dried using a microwave oven to constant weight (28.120 g) for at 50°C for 15 minutes.

2.1 Materials and Equipment

For a successful execution of this research work, Sulphuric acid (H₂SO₄), copper sulphate (CUSO₄), sodium sulphate (Na₂SO₄), boric acid (H₃BO₃), hexane (C₆H₁₄), sodium hydroxide (NaOH) would be used in carrying out the proximate analysis of the samples. Equipment to be used include: Kjeldahl (soxhlet) apparatus, water bath, electric oven (model PVHB-90G2HA), fume cupboard, desiccators, crucibles, Buckner funnel, measuring scale, muffle furnace (by Uhlg, Kern, U.S.A), sifter, JENWAY 6100 Spectrophotometer, Pearson Gallenkamp Flame analyzer, Buch Model 205 Atomic Absorption Spectrophotometer, electric oven, micro wave oven (Westpoint Microwave oven dryer), Digital thermometer, Weighing Balance, conical flask (250ml), volumetric flask, reflux device, acid burette, filtration device etc were used in this study.

2.2 Proximate Analysis of Tiger Nuts

The proximate components of the fresh, dried tubers of tiger nuts were using the standard methods of Analysis of Association of Official Chemists (AOAC), 2010. Crude protein, crude lipid, carbohydrate, Moisture, and Ash contents in the samples was analyzed. The methods are described below. The same procedures were carried out on all samples.

2.3 Mineral Content Analyses of Tiger Nuts

The minerals to be analysed would be Potassium, Calcium, Magnesium and Zinc. Potassium would be determined using Gallenkamp Flame analyzer, while calcium, magnesium and zinc would be determined using the atomic absorption spectrophotometer (model Unicam 900, Buck Scientific). The digest solutions of the samples were prepared by weighing 1 grams of each of the powdered plant samples, these were digested with aqua regia at 130°C using electric hotplate for 30 minute. The
filtered was made up to 100ml after filtration using 100ml volumetric flask. Standard solutions of the metal to be analyzed were prepared. The atomic absorption spectrophotometer (model Unicam 900, Buck Scientific) was set with power on for ten minutes. The standard minerals solutions were injected to calibrate the AAS using acetylene gas. An aliquot of ash solutions were injected and the concentrations obtained from the AAS.

Two grammes (2 g) of each tiger nut sample would be heated gently over a Bunsen burner flame until most of the organic matter was destroyed. This will be further heated strongly in a muffle furnace for several hours until white-grey ash was obtained. The ash material was cooled. About 20 ml of distilled water and 10 ml of the dilute hydrochloric acid was added to the ashen material. This mixture would be boiled, filtered into a 250 ml volumetric flask, washed thoroughly with hot water, cooled and made up to volume.

2.4 Anti-nutrients Analysis

2.4.1 Hydrogen Cyanide

Extraction of hydrogen cyanide was done using Wang and filled method. The sample (2g) was ground into paste and dissolved in distilled water (50ml) using a conical corked flask. The extract was allowed to stay overnight and the filtered solution was used for the cyanide determination. Alkaline picrate 4ml was added to 1m of the filtrate in a corked test tube and incubated in water bath for 15minutes. Reddish colour developed and the absorbance was taken using a spectrometer at 490nm (AOAC, 1984). Also, the absorbance of the blank containing only 1ml distilled water and 4ml alkaline picrate solution was taken and the extrapolation of the cyanide content from the cyanide standard curve.

Concentration of hydrogen cyanide is thus as follows:

\[
\text{Concentration} = \frac{\text{absorbance test} \times \text{conc.std} \times 100}{\text{absorbance of std} \times \text{weight of sample} \times 1}
\]

2.4.2 Determination of Oxalate by Titration Method

The oxalate content of the sample was determined using titration method. It involves three general steps which include digestion, precipitation and KMnO₄ titration.

**Digestion:** 5g of the sample was introduced into a 250ml beaker suspended in 95ml of distilled water and 5ml 6N HCl was added to the beaker. The mixture was heated on a water bath at 50°C for 2 hours. The digestion was filtered and diluted with distilled water to 126ml.

**Precipitation:** 50ml of the filtrate was placed in a 100ml beaker and drops of methyl red indicator was added which evaporated on eating to 250ml in volume. The sample was filtered to remove the precipitate containing ferrous irons. The filtrates were again treated with 5ml NH₄OH and heated to 90°C and 10mm of 5% CaCl solution was added and stirred constantly as heat was applied and allowed to cool overnight at 5°C. The solution was then centrifuged (filtered) at 2500rpm for 5 minutes. The supernatant was decanted and he precipitate were obtained which was washed into a beaker with H₂SO₄ (10ml of 20% v/v) and diluted with 125ml of distilled water.

**Titration:** the 125ml aliquot solution was heated near boiling point (90°C) and was titrated against 0.05N standardized KMnO₄ solution to a faint pink color which persists for 10seconds. The calcium oxalate content is calculated using the formular 0.05N KMnO₄ = 2.2g Oxalate.

2.4.3 Determination of Phytate

The phytate content of the tiger nut was determined by Maga method. Two (2g) grammes each finely ground flour sample was soak in 20ml of 0.2N HCl and filtered. After filtration, 0.5ml of the filtrate was mixed with 1ml ferric ammonium sulphate solution in a test tube, boiled for 30min in a water bath, cooled in ice for 15minute and centrifuged at 3000× g for 15 minutes. One millitre of the supernatant was mixed with 1.5ml of 2,2-pyridine solution and the absorbance measured in a spectrophotometer at 519nm. The concentration of phytic acid was obtained by extrapolation from a standard curve using standard phytic acid solution.
2.4.4 Determination of Tannin Content
For Tannin determination, 10ml 70% aqueous acetone was added to 2g of finely ground sample in a bottle and properly covered. The bottle was put in an ice bath shaker for 2h at 30oC. The solution was then centrifuged and the supernatant stored in ice. From the supernatant, 0.2ml was pipetted into 0.8ml of distilled water. Standard tannic acid solution was prepared. Folin reagent (0.5ml) was added to both sample and standard followed by 2.5ml 20% Na2CO3. The solution was vortexed and allowed to incubate for 40minute at room temperature after which the absorbance was read at 725nm. The concentration of tannin in the sample was estimated from the standard tannic acid curve.

2.5 Antioxidants Analysis

2.5.1 DPPH Radical Scavenging Assay: The free radical scavenging capacity of the extracts from different plant samples were estimated according to Baraca, 2003 with slight modification using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical which has an absorption maximum at 515nm. A solution of the radical is prepared by dissolving 2.4mg DPPH in 100ml methanol. A test solution100-500Nl was added to 3.95ml (4ml) of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30min in the dark. Absorbance of the reaction mixture was measured at 515nm spectrophotometric absorbance of the DPPH radical without antioxidant i.e. blank was also measured. All the determinations were performed in duplicates. The capacity to scavenge the DPPH radical was calculated using the following equation:

\[
DPPH \text{ Scavenged} (\%) = \left( \frac{AB - AA}{AB} \right) \times 100
\]

Where AB = Absorbance of Blank
AA is absorbance of the antioxidant at t = 30minutes

2.5.2 Ferric Ion Reducing Antioxidant Power Assay (Frap)
Ferric ion reducing power was measured according to the method of Oyaizu with a slightest modification.

Procedure: Hydroalcoholic extract of the sample in different concentration ranging from 100nl to 500nl were mixed with a 2.5mM phosphate buffer and 2.5ml, 1%, w/v potassium ferric cyanide, and then the mixture was I incubated at 50⁰c for 30minutes. Afterward, 2.5ml of 10%, w/v trichloroacetic acid and 0.5ml 0.1%, w/v ferric chloride were added to the mixture, which was kept aside for 10min. finally, the absorbance was measured at 700nm. Ascorbic acid was used as positive reference standard. All assays were run in duplicates and averaged.

2.5.3 Cupric Ion Reducing Capacity Assay (Cuprac)
Cupric ion reducing capacity was measured in accordance to the method of Apal.

Procedure
1ml, 10mM cupric chloride, 1ml 7.5mM neocuproine and 1ml, 1M ammonium acetate buffer of PH 7 solutions were to test tubes containing 2ml of distilled water. Hydroalcoholic extract of the sample in different concentration ranging from 100nl to 500nl were added to each test tube separately. These mixtures were incubated for half an hour at room temperature and measured against blank at 450nm. Ascorbic acid was used as positive reference standard. All methods were repeated in duplicates in order to get a mean value.

2.6 Statistical Analysis
The experiments were conducted in duplicates. The mean and standard deviation of the result data from the experiment will be calculated and analyze using single factor ANOVA in the Statistical Package for Social Science (SPSS, 2017) Software (SPSS version 20 for windows). The Duncan’s New Multiple Range Test (DNMRT) and Ordinary Least Significant Difference (LSD) were also used to determine the significant difference between mean values (Spiegiel et al., 2008).


3. RESULTS AND DISCUSSIONS

3.1 Drying Rate Curve

Below are the drying rate curves showing different drying methods with different drying rates. Figure 4.1 shows the drying rate curve for sun drying. Here, there was a rapid increase in drying rate from 0 - 14.44 gH₂O/hr between 0-5 hours. A rapid decrease in drying rate from 14.44 - 0.177gH₂O/hr between 5 - 45 hours of drying time, and a minimum of 0.114 gH₂O/hr constant drying rate was found between 45-60 hours of drying time. Figure 4.2 shows the drying rate curve for oven drying. Here, there was a rapid increase in drying rate from 0-1 hour with a maximum corresponding drying rate of 66.311gH₂O/hr. Between 1-12 hours of drying time, there was a rapid decrease in drying rate from 66.311 - 0.851gH₂O/hr and between 12-20 hours of drying, the drying rate decreased from 0.851 gH₂O/hr to a constant value of 0.237 gH₂O/hr. Figure 4.3 shows the drying rate curve for microwave oven drying. Here, there was a rapid increase in drying rate from 0 - 11.058 gH₂O/min between 0-5 minutes and decreased from 11.058 - 0.662gH₂O/min between 5-15 minutes of drying time. Different drying methods had varying energy output and usage and these had different impact on the samples; and also affect the quality of product differently. Generally, the drying rate decreased as the drying time increased. For sun drying, the drying rate was low and it took about 60 hours to dry to a bone dry weight of 22.690 grams. For oven drying, the drying rate was faster compared with the sun drying, and it took about 20 hours to dry to a bone dry weight of 27.328 grams. Microwave oven had the highest drying rate, which took about 15 minutes to dry to a bone dry weight of 28.120 grams. Microwave oven had the highest drying rate among other drying methods.

![Sun drying](image)

Figure 3.1: Drying rate curve for sun drying.
3.2 Effect of drying methods on the proximate compositions of tiger nut

### Table 3.2A: Effect of different drying methods on proximate composition of tiger nut

<table>
<thead>
<tr>
<th>Drying Method</th>
<th>Moisture Content (%)</th>
<th>Crude Fibre (%)</th>
<th>Ash Content</th>
<th>Crude Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>45.005 ± 0.00707</td>
<td>5.0495 ± 0.07000</td>
<td>0.5105 ± 0.00071*</td>
<td>23.4830 ± 0.00141*</td>
</tr>
<tr>
<td>Microwave</td>
<td>5.0005 ± 0.00711</td>
<td>3.6985 ± 0.00071b</td>
<td>0.0495 ± 0.00071p</td>
<td>24.1075 ± 0.00071*</td>
</tr>
<tr>
<td>Oven</td>
<td>10.0050 ± 0.00707a</td>
<td>3.8050 ± 0.00707*</td>
<td>0.4805 ± 0.00071*</td>
<td>24.1150 ± 0.00424*</td>
</tr>
<tr>
<td>Sun</td>
<td>10.0010 ± 0.00141a</td>
<td>3.6910 ± 0.00141b</td>
<td>.0505 ± 0.00071p</td>
<td>24.0340 ± 0.00141*</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation from duplicate analyses.

Values with asterisk (*) showed significance difference in their mean at 5% level. Values with same alphabet in the same column did not differ in their mean at 5% level of significance.

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**Figure 3.2:** Drying rate curve for Oven drying.

**Figure 3.3:** Drying rate curve for Microwave oven drying.
Table 3.2B: Effect of different drying methods on proximate composition of tiger nut

<table>
<thead>
<tr>
<th>Drying Method</th>
<th>Crude protein (%)</th>
<th>Total Carbohydrate (%)</th>
<th>Caloric value (Kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>1.7600±0.01414*</td>
<td>69.1965 ± 0.08697*</td>
<td>495.1750±0.27577*</td>
</tr>
<tr>
<td>Microwave</td>
<td>1.7005±1.7005*</td>
<td>70.4435 ± 0.00212*</td>
<td>505.5455±0.00354b</td>
</tr>
<tr>
<td>Oven</td>
<td>3.5035 ± 0.00495*</td>
<td>70.0955 ± 0.00778*</td>
<td>503.4310 ± 0.04950*</td>
</tr>
<tr>
<td>Sun</td>
<td>1.0400 ± 0.01414*</td>
<td>71.1835 ± 0.01020*</td>
<td>505.2000±0.00424ab</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation from duplicate analyses

Values with asterisk (*) showed significance difference in their mean at 5 % level of significance. Values with same alphabet in the same column did not differ in their mean.

Tables 3.2A and B present the effect of different drying methods on the proximate composition of fresh and dried tiger nuts per 100 grams. The moisture content of tiger nut ranged from 45% in the fresh sample (control) to 5.0% in the microwave oven; only oven and sun drying methods did not produce significant difference in their mean values, since p [0.474] > 0.05. Reduction in the moisture content as observed in this study decreases the perishability of tiger nut, adds value and also extends the shelf life, thereby making it available throughout the year, similar to the report of Demirel and Turhan (2003) and Emperatriz et al. (2008). The tiger nut samples were samples were significantly different (p<0.05) in fibre content except for microwave and sun drying methods which did not produce significant difference in their mean values of fibre content since p [0.842] > 0.05. The fresh tiger nut sample had higher (5.04%) fibre content than the dried samples as compared with (Okorie and Nwanekesi, 2014). The reduction observed in the dried sample might be due to the fact that drying softens cellulose, and encourage loss of indigestible plant components, causing the cells to separate easily and making the nut easier to digest (Cameron, 1983). Loss of soluble fibre by hydrolysis, enzymatic degradation and decomposition caused fibre to reduce (Morris et al., 2004). Ash content was highest in the fresh sample (0.51%) and it was lowest in the microwave dried tiger nut (0.04%) but only microwave and sun drying methods did not produce significant difference in their mean values, since p [0.230] > 0.05, this is as a result of their moisture content and leaching of it minerals during drying as reported by Ogunlade et al. (2015). There was a significant increase in lipid content as fresh sample had the lowest lipid content (23.4%) but highest in the oven dried sample (24.1%). Increased in the lipid content of dried tiger nuts is attributed to concentration of fat due to moisture loss (Ndubuisi, 2009). Protein content decreases significantly when compared with the fresh sample, this is in line with the report by Miroslawa et al.(1997) that heat application caused the unzipping of the hydrophobic force leading to partial or complete disruption of the primary, secondary tertiary or quaternary structure of protein molecules thereby leading to the protein content of the dried sample. Crude protein was lowest in the sun dried sample (1.04%) when compared with that of the fresh sample (1.76%). There was an increased in the carbohydrate contents when compared with the fresh sample (69.19%), this may be attributed to moisture loss which leads to concentration of nutrient (Ndubuisi, 2009); Total carbohydrates were highest in the sun dried tiger nuts (71.1%) but lowest in the oven dried samples (70.09%). Caloric value increased significantly among the drying methods with reference to the control. Microwave dried tiger nut had the highest caloric value (505.5 KJ) and lowest in the fresh sample (495.17%); Only microwave and sun drying methods did not produce significant difference their mean values since their p [0.069] > 0.05.

3.3 Effect of Drying Methods on the Minerals Composition of Tiger Nut

Table 3.3: Mineral composition of fresh and dried tiger nuts (mg/100g)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calcium</th>
<th>Potassium</th>
<th>Magnesium</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>2.4150±0.00141*</td>
<td>3.8305±0.0071*</td>
<td>5.3305±0.7566*</td>
<td>8.1165±0.0071*</td>
</tr>
<tr>
<td>Microwave</td>
<td>2.0845±0.00071a</td>
<td>2.3050±0.00141*</td>
<td>2.0320±0.141*</td>
<td>6.0080±0.00141*</td>
</tr>
<tr>
<td>Oven</td>
<td>2.0845±0.00071a</td>
<td>2.2960±0.00141b</td>
<td>1.2090±0.000*</td>
<td>5.1210±0.00141*</td>
</tr>
<tr>
<td>Sun</td>
<td>1.9740±0.00141*</td>
<td>2.2960±0.00141b</td>
<td>1.0375±0.00071*</td>
<td>5.0920±0.00141*</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation from duplicate analyses

Values with asterisk (*) showed significance difference in their mean at 5 % level of significance. Values with same alphabet in the same column did not differ in their mean.
Table 3.3 presents the effects of different drying methods on the mineral composition of tiger nuts. The mineral composition of the dried tiger nuts was reduced when compared with the fresh sample. The fresh sample indicates high calcium content (2.41mg) but lowest in sun dried sample (1.97mg) when compared with the other drying methods. Calcium content of the microwave oven and the oven dried tiger nut were not significantly different with their means at 5% level of significance. Potassium content was highest in the fresh sample (3.83mg) but lowest in oven and sun dried tiger nuts (2.29mg) which were not significantly different with their mean. There was a significant difference among Magnesium content of tiger nuts with its content highest in the fresh sample (5.33mg) but lowest in the sun dried samples (1.03mg). Fresh tiger nut had the highest Zinc composition (8.11mg) but lowest in the sun dried tiger nuts (5.09mg). The decrease in the mineral content of tiger nuts after drying, suggest that the presence of anti-nutritional factors such as oxalate and phytate in this tuber made these minerals unavailable by reacting with them, this is similar to the report of Akpan and Umoh (2004). Microwave oven drying had the highest mineral retention when compared with other drying methods with reference to the control sample.

### 3.4 Effects of drying methods on the anti nutrient composition of tiger nuts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HCN</th>
<th>Oxalate</th>
<th>Phytate</th>
<th>Tannin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0.0122±0.00007*</td>
<td>0.084±0.00141*</td>
<td>0.0622±0.0014*</td>
<td>0.0292±0.00021*</td>
</tr>
<tr>
<td>Microwave</td>
<td>0.0226±0.00078*</td>
<td>0.141±0.00141*</td>
<td>0.0482±0.0014*</td>
<td>0.314±0.00014*</td>
</tr>
<tr>
<td>Oven</td>
<td>0.0279±0.00007*</td>
<td>0.0435±0.00212*</td>
<td>0.0227±0.0028*</td>
<td>0.0358±0.0000*</td>
</tr>
<tr>
<td>Sun</td>
<td>0.4011±0.0007*</td>
<td>0.0160±0.00141*</td>
<td>0.0227±0.00028*</td>
<td>0.0364±0.00035*</td>
</tr>
</tbody>
</table>

Values with asterisk (*) show significance difference in their means at 5% level of significance.

Values with same alphabet in the same column did not differ in their mean.

Table 3.4 presents the effects of drying methods on the anti nutrient composition of fresh and tiger nut. It shows that drying methods had a significant reducing effect on anti-nutrient compositions except for Hydrogen cyanide and Tannin. Reduction in anti-nutrient of the sample was observed mostly in sun dried sample; this is as a result of the evaporation of toxic chemicals from tiger nut samples during sun drying into the atmosphere. Oxalate content was reduce significantly when compared with the fresh sample, it was highest in the microwave sample (0.141mg) and lowest in the sun sample (0.016mg). Phytate was highest in the fresh sample (0.0622mg) and lowest in the oven and sun dried samples (0.0227mg). Hydrogen Cyanide content of the tiger nut were increase and significantly different with their means at 5% level of significance. It was lowest in the fresh sample (0.0122mg) and highest in the sun dried samples (0.4011mg). Tannin indicates a high content in microwave oven sample (0.314mg) but low in the fresh sample (0.029mg).

### 3.5 EFFECT OF DRYING METHODS ON ANTI OXIDANTS OF FRESH AND DRIED TIGER NUTS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DPPH</th>
<th>Cuprac</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>2.2315±0.00212*</td>
<td>0.0520±0.0000a</td>
<td>0.4040±0.00283*</td>
</tr>
<tr>
<td>Microwave</td>
<td>1.0400±0.00141*</td>
<td>0.4450±0.0000*</td>
<td>0.6820±0.00141*</td>
</tr>
<tr>
<td>Oven</td>
<td>0.9710±0.00283*</td>
<td>0.2890±0.0000*</td>
<td>0.4065±0.00212*</td>
</tr>
<tr>
<td>Sun</td>
<td>0.5775±0.000212*</td>
<td>0.0530±0.00141*</td>
<td>0.5880±0.00141*</td>
</tr>
</tbody>
</table>

Values with asterisk (*) show significance difference in their means.

Values with same alphabet in the same column did not differ in their mean.
Table 3.5 highlights the effect of drying methods on the antioxidant activity of fresh and dried tiger nuts. Their antioxidant activity was significantly different at 0.05 significant level among their mean. DPPH was found to be highest in the fresh sample (2.23mg) but lowest in the sun dried tiger nuts (0.577mg). Cupric ion reducing capacity assay CUPRAC was highest in the microwave sample (0.445mg) but lowest in the fresh sample (0.052mg); only sun drying method and fresh did not produce significant difference their mean values since p [0.184] > 0.05. Ferric ion reducing antioxidant power assay (FRAP) was highest in the microwave sample (0.682mg) but lowest in the fresh sample (0.404mg). Microwave dried sample was highest in its anti-oxidants activity to neutralize the toxicity of anti-nutrients in tiger nuts.

4. CONCLUSION

Preservation of food by drying is a common practice in different parts of the world and it is used to extend the shelf life of food. Drying allows food to be preserved by removing the moisture in the food, in order to prevent the growth of microorganisms that cause deterioration (Mukhtar, 2009). It ensures their availability all year round, reduce post harvest losses and achieve food security. In this study, drying methods used includes: sun drying, oven drying and microwave oven used were capable of preserving the nutrients in the food crops without total loss of any nutrient. The following conclusions were deduced:

a. Oven and microwave drying were observed to be more hygienic and faster than the sun drying. However, Microwave drying had the highest drying rate than oven drying and it also gave the lowest moisture content in this study, suggesting a higher capacity to prevent microbial growth and decay in the dried samples, thus, confers a greater increase in shelf life on the dried samples.

b. There were decrease in fiber, ash and protein contents of dried samples, using all the drying methods while fat, carbohydrate and energy value were increased.

c. The drying methods had a reducing effect on the minerals composition of the dried tiger nuts when compared with the fresh sample, though microwave samples had the highest retentions among the other drying methods.

d. This study showed that drying method reduced the anti-nutrients in tiger nut (Cyperus esculentus lativum) when compared with the fresh sample.

e. There was a significant difference in the anti-oxidant activity of the tiger nut tubers. Cupric ion reducing capacity (CUPRAC) and ferric ion reducing antioxidant power (FRAP) increased with all the drying methods.

f. The drying time affected the anti-oxidant activity of the product

g. At the end of this study, it was observed that oven drying had the best nutritional composition, microwave dried tiger nuts had the highest anti-oxidant activity and minerals composition. Sun drying had the lowest anti nutrient composition on tiger nuts as a result of decomposition of these anti nutrients into the soil and escape into the atmosphere during sun drying process.

ACKNOWLEDGMENT

I am most grateful to God Almighty, the sole provider of knowledge, wisdom, love, mercy and grace, for his protection throughout the period of the project.

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I appreciate my parents; Mr and Mrs. Ufot Gilbert for their unquantifiable love, support and guidance during this period. I wish to thank my wonderful siblings who provide unending support and inspiration.

I am grateful to my friends and colleagues for their individual and collective contributions towards the success of this work. Your continuous encouragements were indeed helpful. May God bless you all in Jesus name. Amen.
REFERENCES


Effects of extraction parameters, including temperature, solvent to sample (S/S) ratio and ethanol concentration on % 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were optimized using Box-Behnken design (BBD) of experiment. Moreover, effects of pH (3.5 to 9), and storage condition (5 and 25 °C) on the stability and antimicrobial activity of antioxidant extracts from Batangas Cherry were determined. Result showed that the optimum condition for extraction antioxidants from Batangas Cherry was at 80°C, 10mL g⁻¹ S/S and 51.66% ethanol. Batangas cherry extracts exposed at pH8 and 9 showed significant decrease in antioxidant and antimicrobial activities. On the other hand, storage at 5°C better retained the antioxidant and antimicrobial activities of Batangas Cherry extracts. The baseline data in this research is important on maximizing the potential of Batangas as source of functional ingredient for food processing.
Effects of Pre-drying treatment and Drying-air Temperature on Moisture Ratio and Effective Moisture Diffusivity of Tomato (Nigerian Local and Foreign Varieties)

*Obafemi Ibitayo Obajemihi¹, Joshua Olanrewaju Olaoye², Mayowa Saheed Sanusi¹ (1. Food Engineering Department, University of Ilorin (Nigeria), 2. Agricultural and Biosystems Engineering, University of Ilorin (Nigeria))

Keywords: Hausa, Tiwantiwa, Honey and Sugar, Tomato

Tomato is a crop that is highly perishable and there are huge postharvest losses incurred annually in Nigeria. Drying of the fruit is important and suitable for developing economies. However, the heat employed during drying of tomato can influence its quality adversely, as a result it is important to use pre-drying treatments prior to drying operation. Therefore, the aim of this research was focussed on studying the effects of pre-drying treatment and drying-air temperature on moisture ratio (MR) and effective moisture diffusivity of tomato. Three varieties (Hausa, Tiwantiwa and Roma VFN) of fresh tomato were obtained from local farmers in Ilorin province, Kwara state of Nigeria. The samples were sorted and cleaned under running water, and were sliced at different thicknesses (5 mm, 7.5 mm and 10 mm), deseeded and blanched in hot distilled water at 90oC. The samples were further pre-treated using different chemical and osmotic solutions (2% ethyl acetate, 1% MgCl2 .6H2O and 0.5% Na2S2O5, 0.5% NaCl and 40oBx of honey and sugar) and Control (Non-pretreated). Samples were drained for 10 minutes after pre-drying treatment and were dried at different temperatures (45, 55 and 65oC) in an automated forced convection cabinet dryer (FCCD) instrumented for the purpose of this experiment. Weight loss of the samples were recorded at different intervals (15 - 60 min) on the trays per stage with the aid of a weight reduction sensing mechanism attached through the rear of the dryer. The drying process was stopped (through a computer system connected to the dryer) when the samples had reached their final moisture content <5% (db). The data obtained from the drying process were used to compute the samples moisture ratio and effective moisture diffusivity and were analyzed using regression and analysis of variance (ANOVA) with Design expert v. 6.0.6 statistical tool at p <0.05. The results obtained show that samples lowest MR were obtained under these conditions; processed Hausa variety, 10 mm thickness, ethyl acetate pre-drying treatment and dried at 55oC while highest effective moisture diffusivity were obtained under these conditions processed Hausa variety, 5 mm thickness, honey and sugar pre-drying treatment and dried at 65oC in a FCCD. It was therefore concluded that processed Hausa variety was more preferable to other varieties used as it promotes low MR and high effective moisture diffusivity during the drying process. This will help reduce energy consumption associated with drying process.
Effects of Pre-drying Treatment and Drying-air Temperature on Moisture Ratio and Effective Moisture Diffusivity of Tomato (Nigerian Local and Foreign Varieties)

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ABSTRACT
Tomato is a crop that is highly perishable and there are huge postharvest losses incurred annually in Nigeria. Drying of the fruit is important and suitable for developing economies. However, the heat employed during drying of tomato can influence its quality adversely, as a result it is important to use pretreatments prior to drying operation. Therefore, the aim of this research was focused on studying the effects of pretreatment and drying-air temperature on moisture ratio (MR) and effective moisture diffusivity of tomato. Three varieties (Hausa, Tiwantiwa and Roma VFN) of fresh tomato were obtained from local farmers in Ilorin province, Kwara state of Nigeria, the samples were sorted and cleaned under running water, and were sliced at different thicknesses (5 mm, 7.5 mm and 10 mm), deseeded and blanched in hot distilled water at 90°C. The samples were further pretreated using different chemical and osmotic solutions (2% Ethyl acetate, 1% MgCl2∙6H2O and 0.5% Na2S2O5, 0.5% NaCl and 40°Bx of Honey and Sugar) and Control (Non-pretreated). Samples were drained for 10 minutes after pre-drying treatment and were dried at different temperatures (45, 55 and 65°C) in an automated forced convection cabinet dryer (FCCD) designed for the purpose of this experiment. Moisture loss of the samples were recorded at different intervals (15 - 60 min) on the trays per stage with the aid of a weight loss sensing mechanism attached through the rear of the dryer. The drying process was stopped (through a computer system connected to the dryer) when the samples had reached their final moisture content < 5% (db). The data obtained from the drying process were used to compute the samples moisture ratio and effective moisture diffusivity and were analyzed using regression and analysis of variance (ANOVA) with Design Expert V 6.0.6 statistical tool at p < 0.05. The results obtained show that samples lowest MR were obtained under these conditions; processed Hausa variety, 10 mm thickness, Ethyl acetate pre-drying treatment and dried at 55°C while highest effective moisture diffusivity were obtained under these conditions processed Hausa variety, 5 mm thickness, Honey and Sugar pre-drying treatment and dried at 65°C in a FCCD. It was therefore concluded that processed Hausa variety is more preferable to other varieties used as it promotes low MR and high effective moisture diffusivity during the drying process. This will help reduce energy consumption associated with drying process.

Keywords: Honey and Sugar Hausa Tiwantiwa Slice thickness Tomato

1. INTRODUCTION
Tomato is one of the major vegetable crops cultivated in Nigeria and has been known to be highly perishable (Onifade et al., 2013; Idah and Obajemihi, 2014). Drying of fruits and vegetables such as tomato is gaining popularity in Nigeria, where post-harvest loss of farm produce is on increase on yearly basis, due to poor post-harvest handling techniques; as a result, Nigerians have spent a whooping sum of $1bn annually on imported tomato products (UNEP, 2016). Drying is a heat and mass transfer phenomenon and has saved more than 20% of crops that are perishable in the world; by extending their shelf lives and ensuring food security (Sohail et al., 2011). Drying is important and most times indispensable in the formulation of functional food products (Trivedi et al., 2011). Drying
of tomato products usually occur in the falling rate period as the moisture content tends to decrease with time. It is important to study and understand mass transport mechanisms such as moisture ratio (MR) and effective moisture diffusivity ($D_{eff}$) responsible for drying of tomato and what to be done during pre-drying and drying processes to favour them. Moisture ratio is the ratio of the instantaneous moisture content to that of the fruit’s initial moisture content. Effective moisture diffusivity is an internal transport phenomena and it is the rate at which moisture is moved from the center of the fruit to its surface where it will be evaporated (Onwude et al., 2016). $D_{eff}$ is a function of drying-air temperature and samples’ MR and was seen as an important mass transport mechanism when it comes to studying drying processes involving fruit and vegetable (Onwude et al., 2016). Zogzas et al. (1996), stated that increase in temperature brings about increase in effective diffusivity but changes with respect to moisture content. When the temperature of food is high, the water molecules in it are bounded loosely to food matrix than at low temperature, therefore more energy is required to remove moisture at lower temperatures compared with high temperatures. Also food structure and void fraction present can significantly affect moisture diffusivity and hence reported that at low porosity, value of effective diffusivity of moisture is majorly by liquid diffusion which is different from that obtainable for granular or porous material, moisture movement is mainly by vapour diffusion through the void or empty spaces. $D_{eff}$ and velocity of moisture movement within the material are relatively related while drying rate is the rate at which moisture vaporizes to the surrounding air or a change of moisture to vapour by evaporation which depends largely on the pressure difference existing between the food material and surrounding air as a result of temperature difference (So’bah et al., 2017). Pre-drying treatment of fruits and vegetables has been known to favour or disfavour their drying rates which is a function of both internal mass transport mechanisms and external heat (Mauro et al., 2005). It helps retain food sensory and nutritional qualities; as previous researches have shown that the effects of drying-air conditions, most especially drying-air temperature have adversely affected the quality attributes of tomato if not properly controlled. Therefore, it becomes imperative to investigate the effects of pre-drying treatment and drying-air temperature on moisture ratio and effective moisture diffusivity of tomato.

2. MATERIALS AND METHODS

2.1 Raw Material
Fresh tomato samples of three different varieties were obtained from local growers in Oteh area, a suburb of Ilorin Kwara State of Nigeria. The samples were sorted visually according to their ripeness, firmness and size. Samples were thoroughly washed under tap water, sliced using a stainless steel knife, deseeded with a needle and blanched in hot water for 1 min at 90°C to minimize browning and enzymatic reaction during drying process.

2.2 Pre-drying treatment Process
Sliced samples were divided into five parts and were subjected to further pre-drying treatments following the mechanical and thermal pre-drying treatments used on them initially. These other methods include treatment in osmotic and chemical solutions. The first, second, third and fourth parts were treated in a mixture of honey and sugar solution at 40°Bx concentration (honey: sugar: water ratio 2: 1: 1.9) for 10 min which was prepared using a refractometer (Model: M10481, by ABBE MARK II, USA), 2% ethyl acetate solution for 1 min, 0.5g/100ml NaCl solution for 10 min and 1% MgCl2:6H2O and 0.5% Na2S2O5 for 10 min respectively. And the fifth part served as the control sample which was immersed in distilled water at room temperature for 10 min. Each sample weighed 250 g with an electronic balance (Model: WH-B06, sensitivity ± 0.01g by WEIHENG, China) before pre-drying treatment. After pre-drying treatment samples were drained and bloated with absorbent paper.

2.3 Drying Procedure
After pre-drying treatments of the samples they were dried in an automated forced convection cabinet dryer (FCCD) designed for the purpose of this research at different drying-air temperatures (45, 55 and 65°C), the dryer was run to attain the desired temperature before loading the samples on its labelled
trays. The trays were rested on load cells which were linked to a microcontroller which was in turn connected to a computer system which has a software with Arduino programme used in monitoring and controlling the dryer. The dryer was also equipped with a thermo-hygrometer sensor used in sensing the drying-air temperature and humidity and three (3) solid state relays which were used for switching on and off the two (2) heaters (3.6 kW) and a centrifugal fan (2m/s). The dryer was pre-selected to take record of every 5 min as the drying experiment progressed. The measurements recorded were the instantaneous weight on each of the four (4) trays, the drying-air temperature and humidity. This dryer totally eliminates the drudgery, time and energy wastages associated with previous drying experiments when samples were brought out to measure. The FCCD is shown in Figure 1.

![3D View of the Automated FCCD](image)

**Figure 1. 3D View of the Automated FCCD**

### 2.4 Determination of output Parameters

#### 2.4.1 Instantaneous Moisture Content (Mt)

The instantaneous moisture content ($M_t$) of tomato at any given time ($t$) during the drying experiment was estimated using Equation 1;

$$M_t = \frac{(M_i + t) - W_t - 1}{W_o}$$  \hspace{1cm} (1)

where;

- $M_i$ = Instantaneous m.c. (% wb)
- $M_o$ = Initial m. c. (% wb)
- $W_t$ = Weight of product at any time, $t$ during drying (g)
- $W_o$ = Initial weight of the sample (g)

#### 2.4.2 Moisture Ratio (MR)

Moisture ratio was calculated as expressed in Equation 2;

$$MR = \frac{M_t - M_o}{N_o - M_t}$$  \hspace{1cm} (2)
where;
\( M_t \) = m.c. of the tomato samples at any time \( t \) (\% db)
\( M_e \) = Equilibrum m.c. of the tomato samples (\% db)
\( M_0 \) = Initial m.c. of the samples before drying (\% db)

2.4.3 Effective Moisture Diffusivity

The effective moisture diffusivity (\( D_{eff} \)) was estimated using the “simplified mathematical Fick’s second diffusion model”. The solution of Fick’s second law in slab geometry, having the following assumptions; that moisture migration is strictly dependent on diffusion, shrinkage is negligible, diffusion coefficients are constant and temperature which was the diffusion model was simplified to linear equation by Crank (1975) as it is expressed in Equation 3.

\[
MB = \frac{M}{M_0} = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} \exp \left[ -\frac{(2n-1)^2 \pi^2 D t}{4L^2} \right]
\]

where,
\( MB \) = Moisture Ratio
\( M \) = Moisture content at any time (kg water/kg dry matter)
\( M_0 \) = Initial moisture content (kg water/kg dry matter)
\( n \) = 1, 2, 3, ..., the number of terms taken into consideration
\( t \) = time of drying in seconds
\( D_{eff} \) = Effective moisture diffusivity (m²/s)
\( L \) = thickness of the slice (m)

Equation 4 was used since the drying process involved a long term drying due to high moisture content present in tomato

\[
MB = \frac{3}{\pi^2} \exp \left[ \frac{3 \pi^2 D}{4L^2} t \right]
\]

The slope (\( K_o \)) of the graph was estimated by plotting \( ln(MR) \) against time (\( t \)) as presented in Equation 5;

\[
K_o = \frac{\pi^2 D}{4L^2}
\]

2.5 Design of Experiment

The experiment was designed using the Box-behnken design (BBD) of response surface framework of Design Expert Software V 6.0.6 (US, Stat-Ease Inc.) resulting in 68 runs. The experimental input parameters were sample variety (Hausa, Tiwantiwa and Roma VFN), slice thickness (5.0 mm, 7.5 mm and 10 mm), pre-drying treatment (Ethyl acetate, MgCl₂·6H₂O and Na₂S₂O₅, NaCl and Honey and Sugar) and drying-air temperature (45, 55 and 65°C).

2.56 Statistical Analysis

In this experiment statistical analysis of responses were done using quadratic model interface of the Design Expert software with alpha to exit 0.050 and regression coefficients were obtained.

3. RESULTS AND DISCUSSION

3.1 Effects of Input Parameters on Moisture Ratio and Effective Moisture Diffusivity
3.1.1 Effect of Drying-air Temperature, Slice Thickness, Variety and Pre-drying Treatments on Moisture Ratio

The effect of drying-air temperature on the moisture ratio (MR) of tomato samples is shown in Figure 2a, it shows that samples subjected to 65°C had average MR of 70.19%, 55°C had 69.13%, while those subjected to 45°C had the highest average MR of 77.54%. This result agrees with the findings of Yousefi et al. (2013) on the drying of papaya slices at 40, 50 and 60°C. The highest MR in samples dried at 45°C can be attributed to the slowest rate at which...
Table 1: Analysis of Variance for Moisture Ratio of Tomato Slices

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>DF</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2.32</td>
<td>21</td>
<td>0.11</td>
<td>11.95</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>A</td>
<td>0.061</td>
<td>1</td>
<td>0.061</td>
<td>6.60</td>
<td>0.0137</td>
</tr>
<tr>
<td>B</td>
<td>2.979E-005</td>
<td>1</td>
<td>2.979E-005</td>
<td>3.217E-003</td>
<td>0.9550</td>
</tr>
<tr>
<td>C</td>
<td>0.90</td>
<td>1</td>
<td>0.90</td>
<td>97.05</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>D</td>
<td>0.21</td>
<td>3</td>
<td>0.071</td>
<td>7.63</td>
<td>0.0003</td>
</tr>
<tr>
<td>A²</td>
<td>9.739E-003</td>
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<td>Cor Total</td>
<td>2.73</td>
<td>65</td>
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</tbody>
</table>

LOF-Lack of Fit; Significance Level (p≥0.05)

moisture was diffusing out of the samples in which more moisture was retained in the samples compared with those dried at 55°C and 65°C. Moisture gets more excited at higher drying-air temperature and diffuses. ANOVA Table 1, revealed that the effect of drying-air temperature (D) was significant on the MR of samples at significance level of p ≤ 0.05.

The effect of slice thickness on MR is shown in Figure 2 b. It is seen that the MR of samples sliced at 5.0 mm, 7.5 mm and 10 mm were 71.59 %, 71.89% and 71.29% respectively. This values were seen to be very close and the ANOVA Table 2 shows that the effect of slice thickness (B) was not statistically significant (p ≤ 0.05) on the MR.

The effect of samples’ variety on the MR of tomato is shown in Figure 2 c, it was found that Hausa variety had an average MR of 45.27%, Tiwantiwa variety had 84.25% and Roma VFN variety had 76.03%, these show that Tiwantiwa variety has the highest MR and Hausa variety has the lowest. This can be attributed to the high initial m.c. of Tiwantiwa variety which was 95.82% and low moisture content of Hausa variety which was 85.03%. While that of Roma VFN variety had initial moisture content of 93.76%. The difference in MR of the samples might be attributed to the microstructural characteristics of the tomato varieties as the cells of Tiwantiwa variety might be less porous and able to retain more moisture compared with others. The ANOVA Table 1 further reveals it that statistically the individual effect of tomato variety (C), its quadratic effect (C²) and the interactive effect CD between variety and pre-drying treatment were highly significant statistically at p ≤ 0.05 on the MR.

The effect of samples’ pre-drying treatment on MR of treated tomato samples is shown in Figure 2 d samples pre-treated with ethyl acetate solution had 65.34% MR, those with MgCl₂·6H₂O and Na₂S₂O₅ solution had 80.21%, while those with NaCl solution had 66.95%, those pre-treated with mixture of honey and sugar solution had 74.11% and control samples had an average MR of 74.09%. This
observation would result from the hydrophilic property of MgCl₂·6H₂O which makes it highly hygroscopic in nature and therefore samples pre-treated in it has the highest MR. Ethyl acetate easily vaporizes into the air and not hygroscopic in nature and therefore was seen to have the least MR among others. As further shown by the ANOVA Table 1 statistically the effect of pre-drying treatment was significant on the MR of treated samples (p ≤ 0.05).

3.1.2 Effects of Drying-air Temperature, Slice Thickness, Variety and Pre-drying Treatments on Effective Moisture Diffusivity

The effect of drying-air temperature on the effective moisture diffusivity (D\text{eff}) of tomato samples is shown in Figure 3 a, it was seen that samples subjected to 65°C had highest D\text{eff} of 1.69 x 10^{-8} m²/s, 55°C had 1.20 x 10^{-8} m²/s, while those subjected to 45°C had the lowest D\text{eff} of 5.99 x 10^{-9} m²/s, this agrees with the findings of Yilmaz et al. (2017), that increase in air temperature led to increase in D\text{eff}. Results with this trend had been reported earlier by Jaiyeoba and Raji (2012) who had worked on the estimation of D\text{eff} of Tomato and found that D\text{eff} increases with increase in air temperature and also found that the D\text{eff} of tomato was within 10^{-8} m²/s. The highest D\text{eff} observed in samples dried at 65°C can be attributed to the high level of drying-air temperature used which contains more heat energy required to activate the movement of water from the internal part of the products to their surface for drying to occur. As reported by Mewa et al. (2018), that water activity increases with increase in temperature which results in increase in D\text{eff} of beef during drying. Analysis of Variance (ANOVA) Table 2, shows that the effect of drying-air temperature was highly significant on the D\text{eff} of samples at p ≤ 0.05, therefore the findings of Yilmaz et al. (2017) was replicated that effect of drying-air temperature was significant on the D\text{eff} of pomegranate fruit leather.

Figure 3 a: Effect of Air-temp. on Effective Moisture Diffusivity
Figure 3 b: Effect of Slice Thickness on Eff. Moisture Diffusivity
Effect of slice thickness on $D_{eff}$ is found in Figure 3 b. The $D_{eff}$ of tomato samples sliced at 5 mm, 7.5 mm and 10 mm were 1.25, 1.00 and 1.07 x $10^{-8}$ m²/s respectively. These values were seen to be quite close and the ANOVA Table 2 shows that the effect of slice thickness was not significant statistically at $p \leq 0.05$ on the $D_{eff}$. However, this is not in agreement to the report by Yilmaz et al. (2017) that increase in slice thickness results in increase in effective moisture diffusivity and states that slice thickness was statically significant on $D_{eff}$.

The effect of samples’ variety on the $D_{eff}$ of tomato is shown in Figure 3 c, it was found that Hausa variety had an average $D_{eff}$ of 6.73 x $10^{-9}$ m²/s, Tiwantiwa variety had 1.78 x $10^{-9}$ m²/s and Roma VFN variety had 1.07 x $10^{-9}$ m²/s.

Table 2: Analysis of Variance for Effective Moisture Diffusivity of Tomato Slices

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>DF</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Prob.&gt;F</th>
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<td>21</td>
<td>1.956E-016</td>
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<tr>
<td>A</td>
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<td>25.63</td>
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<tr>
<td>B</td>
<td>1.575E-017</td>
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<td>1.575E-017</td>
<td>0.39</td>
<td>0.5369</td>
</tr>
<tr>
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<td>1.574E-016</td>
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<td>1.574E-016</td>
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<td>1.433E-017</td>
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<td>16</td>
<td>4.000E-017</td>
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</tr>
</tbody>
</table>

LOF-Lack of Fit; Significance level ($p \geq 0.05$)
variety had 3.02 x 10^{-9} \text{m}^2/\text{s}, these show that Hausa variety had the highest $D_{\text{eff}}$ and Tiwantiwa variety had the lowest. These show that the samples with the highest moisture content tend to have lower effective moisture diffusivity than those with the lowest moisture content which have higher effective moisture diffusivity. Tiwantiwa variety had the highest initial moisture content of 95.82% and MR while Hausa variety had lowest initial moisture content of 85.03% and lowest MR. Roma VFN variety has initial moisture content of 93.76% and average MR. This was in agreement with the findings Sharma and Prasad (2004) in which it was found that $D_{\text{eff}}$ is a function of samples’ m.c. which increases gradually with decrease in m.c. The reason for this was that as m.c. decreased vapour phase diffusivity increased provided the pores were kept opened. The ANOVA Table 2 further reveals it that statically the individual effect of tomato variety (C) and its quadratic effect ($C^2$) were highly significant ($p \leq 0.05$) on the $D_{\text{eff}}$.

The effect of samples’ pre-drying treatment on $D_{\text{eff}}$ of treated tomato samples is shown in Figure 3 d, samples pre-treated with ethyl acetate solution had $1.31 \times 10^{-8}$ m$^2$/s $D_{\text{eff}}$, those with MgCl$_2$$\cdot$6H$_2$O and Na$_2$S$_2$O$_5$ solution had $9.76 \times 10^{-9}$ m$^2$/s, while those with NaCl solution had $1.23 \times 10^{-8}$ m$^2$/s, those pre-treated with mixture of honey and sugar solution had $1.24 \times 10^{-8}$ m$^2$/s and control had $8.425 \times 10^{-9}$ m$^2$/s. Samples pre-treated with ethyl acetate solution had the highest effective moisture diffusivity while those pre-treated in MgCl$_2$$\cdot$6H$_2$O and Na$_2$S$_2$O$_5$ solution had the lowest. From Figure 2 d it can be seen that samples pre-treated in ethyl acetate had the lowest MR while those pre-treated in MgCl$_2$$\cdot$6H$_2$O and Na$_2$S$_2$O$_5$ solution had the highest MR. Therefore, the higher the MR the lower the effective moisture diffusivity and vice versa. This claim had also been found by Sharma and Prasad (2004) and Darvishi et al. (2016). As further shown by the ANOVA Table 2 statistically the effect of samples’ pre-drying treatment was not significant on the $D_{\text{eff}}$ of treated samples ($p \leq 0.05$).

4. CONCLUSION

1. Moisture ratio of tomato at any stage during the drying process reduces with increase in drying-air temperature but effective moisture diffusivity increases with increase in drying-air temperature
2. The moisture ratio of tomato was dependent on its variety which can be linked to its initial moisture content, also Pre-drying treatment of samples had strong influence on samples’ MR and effective moisture diffusivity which can either increase or decrease it.
3. Samples’ lowest MR were obtained under these conditions; processed Hausa variety, 10 mm thickness, Ethyl acetate pre-drying treatment and dried at 55°C while highest effective moisture diffusivity was obtained under these conditions processed Hausa variety, 5 mm thickness, Honey and Sugar pre-drying treatment and dried at 65°C in a FCCD.
4. Processed Hausa variety is more preferable to other varieties of tomato used; as it promotes low MR and high effective moisture diffusivity during the drying process. This will help reduced energy consumed during drying of tomato and will save processing time.

REFERENCES


Extending the Shelf-life of Upland Water Spinach (*Ipomoea aquatica*) Using Trimming, Modified Atmosphere Packaging (MAP) and Low-Temperature Storage

*Ana Mithuzela Espigol¹, Josephine Agravante¹ (1. Postharvest Horticulture Training and Research Center (PTHRC), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), Laguna, Philippines)

Keywords: upland water spinach, modified atmosphere packaging, low temperature storage, postharvest, leafy vegetable

Upland Water Spinach (UWS) is highly perishable in nature and have a short shelf life (2 days). Small-scale farmers, traders, and restaurant owners sought simple, low-cost techniques to prolong its shelf-life. In this study, the effects of trimming of roots, modified atmosphere packaging (MAP) using polyethylene bag with 1 pinprick, and low temperature storage (20±0.5°C and 10±0.5°C ) on the shelf life of UWS were evaluated based on its visual quality, yellowing, wilting, disease incidence and shelf-life. Results showed that at room temperature storage (29±1.0°C), packed UWS had a higher shelf life (3 days) compared to the unpacked ones (2 days), regardless of the presence of roots. At 20±0.5°C storage, unpacked UWS without roots had a longer shelf life (3 days) than those with roots (2 days). Packed UWS at 20±0.5°C, regardless of the presence of roots, had a longer shelf life (4 days) as compared to the unpacked ones. At 10±0.5°C storage, unpacked UWS had a shelf life of 3 days. Among all treatments, packing UWS without roots in PEB with 1 pinprick in combination with storage at 10±0.5°C extends the shelf life to 5 days, with notable delay in occurrence and reduction of the extent of wilting and yellowing. This practice can be used by small-scale farmers, traders, and restaurant owners to reduce daily procurement costs incurred for transportation, hauling and manpower.
Extending the Shelf-life of Upland Water Spinach (*Ipomoea aquatica*) Using Trimming, Modified Atmosphere Packaging (MAP) and Low-Temperature Storage

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ABSTRACT

Upland Water Spinach (UWS) is highly perishable in nature and have a short shelf life (2 days). Small-scale farmers, traders, and restaurant owners sought simple, low-cost techniques to prolong its shelf-life. In this study, the effects of trimming of roots, modified atmosphere packaging (MAP) using polyethylene bag with 1 pinprick, and low temperature storage (20±0.5°C and 10±0.5°C) on the shelf life of UWS were evaluated based on its visual quality, yellowing, wilting, disease incidence and shelf-life. Results showed that at room temperature storage (29±1.0°C), packed UWS had a higher shelf life (3days) compared to the unpacked ones (2 days), regardless of the presence of roots. At 20±0.5°C storage, unpacked UWS without roots had a longer shelf life (3days) than those with roots (2days). Packed UWS at 20±0.5°C, regardless of the presence of roots, had a longer shelf life (4 days) as compared to the unpacked ones. At 10±0.5°C storage, unpacked UWS had a shelf life of 3 days. Among all treatments, packing UWS without roots in PEB with 1 pinprick in combination with storage at 10±0.5°C extends the shelf life to 5 days, with notable delay in occurrence and reduction of the extent of wilting and yellowing. This practice can be used by small-scale farmers, traders, and restaurant owners to reduce daily procurement costs incurred for transportation, hauling and manpower.

Keywords:
Upland water spinach
Modified atmosphere packaging
Low temperature storage
Postharvest
Kangkong

1. INTRODUCTION

Upland Water Spinach, also known as upland *kangkong* in the Philippines, is a leafy vegetable that grows rapidly (~25-30 days), is easily cultivated, and can thrive in most soil types (Goebel et al., 2010) and various seasons throughout the year (Science and Development Network, 2013). In developing countries like Philippines, the importance of UWS has been recognized due to its availability in the market at a remarkably low price (Prasad et al., 2008). Young leaves, petioles and stems used as viand, cooked alone or with meat or fish, while vines are used as fodder for cattle and pigs. More attention is continuously drawn to UWS due to its high nutritional value (protein, fiber, calcium, magnesium, iron, vitamins A, C, and E, folic acid, and phenolic compounds) and better appearance than low land water spinach (Dua et al., 2015), as both market and consumers are driven towards healthier food choices.
UWS is highly perishable in nature and have a short shelf life (2 days). Effective yet simple and low-cost techniques in prolonging the shelf-life of UWS is sought by farmers, traders, as well as restaurants. Farmers and traders believe that trimming the roots of UWS will hasten its deterioration hence, selling UWS with roots has been their common practice. However, the roots can add to the bulk of vegetable handled and transported, may be source of contaminants in which concerns on safety and quality may arise, and may cause fraudulent addition to weight and price that can be burdensome for customers. This theory in trimming of roots was tested in this study.

Various techniques on modified atmosphere packaging (MAP), appropriate storage temperatures, and minimal processing were explored to maintain quality and reduce losses in leafy vegetables as recommended in published literatures such as Kitinoja and Kader (2002), Cantwell and Suslow (2006), Kanlarayat (2007), and Acedo (2010). However, the use of these techniques has not been reported for UWS in the Philippines. Hence, these techniques were studied to match the needs of concerned UWS small-scale farmers, traders and restaurant owners.

This study aims to prolong the shelf-life of upland water spinach using simple and low-cost techniques by determining the effect of trimming the roots, MAP using polyethylene bags with pinprick, and low temperature storage (20±0.5°C and 10±0.5°C) on the shelf-life of UWS.

2. MATERIALS AND METHODS

2.1. Plant Materials
Freshly harvested 30 days old UWS with roots were obtained from a nearby vegetable farm in UPLB. These were placed in clean 20-kg capacity plastic crates and hauled immediately to the Postharvest Horticulture Training and Research Center (PHTRC) laboratory.

2.2. Sample Preparation
Damage-free UWS plants with tender leaves and stems were selected from the harvest pool. In the packing house, these were washed thoroughly using tap water and sanitized using 100 ppm hypochlorite solution (Suslow, 2000) and then drained. Samples were then air-dried in the minimal processing laboratory (operating temperature: 25°C).

2.3. Treatment
Completely dried UWS were distributed for each treatment as stipulated in Table 1. Packed samples were tape-sealed in 0.02mm polyethylene bag (PEB) with 1 pinprick. Treatments were selected based on best practices taken from preliminary studies.

<table>
<thead>
<tr>
<th>Table 1. Postharvest techniques to prolong the shelf-life of UWS.</th>
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<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
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</tr>
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<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
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</table>
Permeating the package with a pinprick-sized air passage creates a naturally induced modified atmosphere for the product, providing protection for water loss while allowing enough respiration to occur through the hole. Modified atmosphere packaging (MAP) is retains freshness and extends shelf life of fresh produce by inhibiting moisture loss and slow down respiration thereby maintaining its color, reducing loss due to product respiratory heat, and maintaining the natural fresh taste of produce (Acedo, 2010).

Unpacked samples were bundled using rubber bands and placed uncovered on clean trays, simulating storage practices of UWS in Filipino households. Packed and unpacked UWS were placed in temperature simulations of room temperature (29±1.0ºC), open-type display chiller temperature (20±0.5ºC) and door-type display chiller temperature (10±0.5ºC). These storage temperatures were chosen as these are used in local restaurants that sell fresh UWS and offer UWS in their menu. Each replicate weighs 250±0.50 grams and there are 10 replicates for each temperature studied.

2.4. Data Collection and Analysis

Being a leafy vegetable, the quality of upland water spinach is mainly based on appearance that can be discerned by the human senses such as freshness, shape, size, maturity, color, turgidity, freedom from defects such as rot, physical damage, yellowing, or wilting (Acedo, 2010). In this study, these parameters were scored using Visual Quality Rating (VQR) to consider all visual factors that may affect the physical appearance of commodities. Shelf-life was determined by the number of days wherein the samples are edible.

Visual quality, yellowing, wilting and disease incidence were evaluated daily using indices developed by PHTRC-UPLB (Table 2, 3). Samples were evaluated daily for these parameters until it surpassed the limit of marketability (VQR=3). All data obtained were subjected to statistical analyses using SAS V9.0.

<table>
<thead>
<tr>
<th>Visual Quality Rating</th>
<th>Description</th>
</tr>
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<tr>
<td>9,8</td>
<td>Excellent, field fresh</td>
</tr>
<tr>
<td>7,6</td>
<td>Very good, trace defects</td>
</tr>
<tr>
<td>5,4</td>
<td>Good, defects minor</td>
</tr>
<tr>
<td>3</td>
<td>Fair, defects moderate, limit of marketability</td>
</tr>
<tr>
<td>2</td>
<td>Poor, defects serious, limit of edibility</td>
</tr>
<tr>
<td>1</td>
<td>Non-edible under usual condition</td>
</tr>
</tbody>
</table>

Table 2. Visual quality rating for fruits and vegetables (Horticulture 109.1 Laboratory Manual, PHTRC-UPLB).

<table>
<thead>
<tr>
<th>Index</th>
<th>Wilting</th>
<th>Yellowing</th>
<th>Disease Incidence</th>
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</thead>
<tbody>
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<td>1</td>
<td>None</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>Trace, &lt;10% leaves wilted (mostly tips and edges)</td>
<td>Slight (up to 20% leaves discolored)</td>
<td>Slight (up to 20% leaves infected)</td>
</tr>
<tr>
<td>3</td>
<td>Slight, 10-25% leaves wilted</td>
<td>Moderate (21-40% leaves discolored)</td>
<td>Moderate (21-40% leaves infected)</td>
</tr>
</tbody>
</table>

Table 3. Indices for wilting, yellowing, and disease incidence (Horticulture 109.1 Laboratory Manual, PHTRC-UPLB).
3. RESULTS AND DISCUSSION

3.1. Visual Quality Rating (VQR) and Shelf-life

VQR for UWS were shown in Figure 1.a-c. Regardless of the type of packaging, trimming of roots did not affect VQR at 29±1.0°C. PEB-packed UWS remained marketable until day 2, which shows that packaging in PEB with 1 pinprick extends the shelf life by 1 day at 29±1.0°C (Table 4). On the other hand, rapid deterioration on visual quality can be observed in unpacked UWS at 29±1.0°C from day 1 to day 2.

In samples stored at 20±0.5°C, PEB-packed UWS were marketable until the fourth day of storage, regardless of the presence of roots while unpacked samples with roots had increased its shelf-life by 1 day. It can be noted that the shelf-life of PEB-packed samples, regardless of the presence of roots, also increased by one day at 20±0.5°C.

At 10±0.5°C, PEB-packed samples were marketable up to 5 days of storage while unpacked samples lasted for 3 days. The rapid decline in VQR of unpacked samples on the second storage day is notable.

The presence of roots did not have effects on the VQR and shelf-life of packed samples (Figure 1.a and b). This can be attributed to the protection provided by the packaging which inhibits water loss thereby retaining freshness of the leaves (Acedo, 2010). However, retail packs and bundles with trimmed roots are fuller in terms of useful portions (young leaves and stems) which may provide more value for the price of each pack and benefit consumers.

With the base UWS shelf-life of 2 days (control), storage in 20±0.5°C and 10±0.5°C in conjunction with PEB-packing with one pinprick increased the shelf-life of UWS by 1 day and 2 days, respectively. Among all samples in different storage temperatures, PEB-packed UWS without roots and stored at 10±0.5°C had the superior VQR and remained marketable up to 5 days of storage.
Figure 1.a-c. Visual quality rating for UWS stored at 29±1.0°C, 20±0.5°C and 10±0.5°C (Packed in polyethylene bag with one pinprick = PEB, unpacked = UNP; with roots = WR; without / no roots = NR; VQR scores: 9,8= Excellent, field fresh, 7,6= Good, defects minor, 5,4= Fair, defects moderate, limit of marketability; 3= Poor, defects serious, 2=Limit of edibility, 1=non-edible under usual condition; N=100).

Table 4. Shelf-life of UWS stored at 29±1.0°C, 20±0.5°C and 10±0.5°C (1 Packed in polyethylene bag with one pinprick = PEB, unpacked = UNP; 2 with roots = WR; without / no roots = NR; N=100).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Packaging</th>
<th>Presence of Roots</th>
<th>Storage Temperature</th>
<th>Shelf-life ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PEB</td>
<td>WR</td>
<td>29±1.0°C</td>
<td>3c</td>
</tr>
<tr>
<td>2</td>
<td>PEB</td>
<td>NR</td>
<td>29±1.0°C</td>
<td>3c</td>
</tr>
<tr>
<td>3</td>
<td>UNP</td>
<td>WR</td>
<td>29±1.0°C</td>
<td>2d</td>
</tr>
<tr>
<td>4</td>
<td>UNP</td>
<td>NR</td>
<td>29±1.0°C</td>
<td>2d</td>
</tr>
<tr>
<td>5</td>
<td>PEB</td>
<td>WR</td>
<td>20±0.5°C</td>
<td>4b</td>
</tr>
<tr>
<td>6</td>
<td>PEB</td>
<td>NR</td>
<td>20±0.5°C</td>
<td>4b</td>
</tr>
<tr>
<td>7</td>
<td>UNP</td>
<td>WR</td>
<td>20±0.5°C</td>
<td>2d</td>
</tr>
<tr>
<td>8</td>
<td>UNP</td>
<td>NR</td>
<td>20±0.5°C</td>
<td>3c</td>
</tr>
<tr>
<td>9</td>
<td>PEB</td>
<td>NR</td>
<td>10±0.5°C</td>
<td>5a</td>
</tr>
<tr>
<td>10</td>
<td>UNP</td>
<td>NR</td>
<td>10±0.5°C</td>
<td>3c</td>
</tr>
</tbody>
</table>

¹Shelf-life values followed by similar letters are significantly different from each other.

3.2.  Yellowing
Yellowing was observed on all samples at the second day of storage except for the PEB with roots at 20±0.5°C and samples at 10±0.5°C (Figure 2.a-c.). Packed samples exhibited discoloration which can be attributed to exposure to its own ethylene. While MAP slows down respiration and protects the leaves from moisture loss, ethylene produced during senescence can build up inside the packaging with prolonged storage. This may cause yellowing, epinasty (leaf curving) and abscission (Cantwell and Suslow, 2006).

At 10±0.5°C, yellowing was delayed until the third day of storage. This can be attributed to the effect of cold storage which slows down the rates of physiological changes that the commodity undergoes, thereby reducing its effects such as discoloration (Kitinoja and Kader, 2002).

No significant trends were observed on the response of UWS to MAP and presence of roots in relation to yellowing.

![Figure 2.a-c. Yellowing in UWS stored at 29±1.0°C, 20±0.5°C and 10±0.5°C (Packed in polyethylene bag with one pinprick = PEB, unpacked = UNP; with roots = WR; without / no roots = NR; Yellowing Index:1=absent, 2=Slight or 20%, 3= Moderate or 21-40%, 4=Severe or >40% of leaves discolored; N=100).](image)

### 3.3. Wilting

Unpacked UWS had a significantly higher rate of wilting compared to the PEB-packed with 1 pinprick UWS in all storage temperatures (Figure 3.a-c.). This exhibits the effectiveness of MAP to decrease rates of moisture loss (Kitinoja and Kader, 2003).
Wilting was observed in PEB-packed samples only on the third day of storage in 29±1.0°C and 20±0.5°C. For the PEB-packed UWS in 10±0.5°C, the incidence of wilting was delayed up to 4 days. On the other hand, no significant trends were observed on the response of UWS to presence of roots in relation to wilting.

It is important to reduce the incidence of wilting because it promotes degradation of nutritional components (e.g. vitamins and minerals) and imposes stress (i.e. water stress) that increases respiration and ethylene production. This should be prevented to maximize the health benefits of the vegetable. According to Kanlayanarat (2007), 5-10% in fresh weight make leafy vegetables appear wilted and unusable.

![Figure 3.a-c. Wilting in UWS stored at 29±1.0°C, 20±0.5°C and 10±0.5°C (Packed in polyethylene bag with one pinprick = PEB, unpacked = UNP; with roots = WR; without / no roots = NR; Wilting Index: 1=none, 2=trace or <10%, 3=slight or 10-25%, 4=moderate or 25-50%, 5=severe or >50% wilted leaves; N=100).](image)

### 3.4. Disease Incidence

Disease incidence was not observed on all samples (data not shown). This can be attributed to the systematic preparation of samples including washing with 100ppm hypochlorite solution and proper air drying.

According to the Philippine National Standards (Bureau of Agriculture and Fisheries Standards, 2016), washing with sodium hypochlorite solution is allowable for organically produced vegetables. The most effective concentration at the safe range is 100ppm (Suslow, 2000). Application of sanitizing agents may
help minimize the risk of a variety of biological hazards or contaminants such as *Salmonella* sp., *Escherichia coli*, *Listeria* sp., and mycotoxins that pose food safety concerns leading to outbreaks (Herman et al., 2015). At the same time, sanitation practices also reduce risk for bacterial soft rot, commonly caused by *Erwinia carotovora* (Tournas, 2005). This causes decay especially in packed leafy vegetables, since the packaging promotes build up of moisture that is promotes bacterial growth.

4. **CONCLUSION**

Regardless if packed or not, trimming of roots does not affect visual quality and shelf life of UWS at 29±1.0°C and 20±0.5°C except for the unpacked UWS. Trimming the roots and storing UWS unpacked at 20±0.5°C extends the shelf life by 1 day.

Wilting was significantly delayed in samples packed in PEB with 1 pinprick compared to the unpacked ones in all storage temperatures. Packing in PEB with 1 pinprick extends the shelf life by 1 day at 29±1.0°C (with or without roots), 2 days at 20±0.5°C (without roots), 1 day at 20±0.5°C (with roots) and 4 days at 10±0.5°C (without roots).

Yellowing was delayed for 1, 2 and 3 days in 29±1.0°C, 20±0.5°C and 10±0.5°C, respectively. This shows the effectiveness of low temperature storage in delaying the incidence of yellowing. On the other hand, no significant trends were observed on the response of UWS to MAP and presence of roots in relation to yellowing.

Disease incidence was not observed on all samples. This can be attributed to the systematic preparation of samples including washing with 100ppm hypochlorite solution and proper air drying.

Given that the preparation of leaves in this experiment was followed, packing UWS without roots in PEB with 1 pinprick in combination with storage at 10±0.5°C extends the shelf life to 5 days, with notable delay in occurrence and reduction of the extent of wilting and yellowing.

**ACKNOWLEDGEMENT**

This research is a portion of the project, “*Increasing Marketability and Availability of Safe and Quality Vegetables and Herbs: Implementation of Best Postharvest Handling Practices and Packaging Technologies*” funded by the Department of Agriculture – Bureau of Agricultural Research (DA-BAR) Philippines. The support of DA-BAR is greatly acknowledged.

**REFERENCES**


Investigation of Cowpea Variety and Storage Methods on Cowpea Beetle Infestation

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Keywords: cowpea beetle, polyethylene, Hessian bags, phostoxin, Aluminum bins

Investigation of effect of variety and storage methods on cowpea beetle (callosobruchus maculatus) infestation was carried out with the main aim of providing suitable, safe and affordable methods of storing various varieties of cowpea devoid of infestation. Three varieties of cowpea which are White, Brown and Black varieties were used. Various storage methods which include Plastics, Polyethylene, Hessian bags and Aluminum Bins of 10 kg capacity each were used in storing the cowpea. Twenty (20) cowpea beetle were introduced into the stored cowpea. Storage chemicals (Protoxin and Atelic dust) were put into the various stored cowpea at the same time of introducing the chemicals. The period of storage was four months. Completely randomized design (CRD) with four treatment and three replications were used for the experiment. Data collected include number of dead beetles, number of live beetle and number and percentage of damaged seed. The data were analyzed using analysis of variance (ANOVA). The result showed that Phostoxin and Atelic dust are toxic to the beetle in all the storage methods used leading to high mortality of the beetle though with less significant difference in the Hessian bag storage method. The result also showed that there is a strong significant difference among the treatment on White and Black varieties and no significant difference among the treatment on the Brown variety in terms of cowpea beetle damage. It was also found that Plastics and Polyethylene method of storage impaired respiration of the beetle leading to high mortality than in the Aluminum bin and the Hessian bag storage methods. Plastics and Polyethylene are therefore recommended for cheaper and environmentally safer for storage of cowpea. Also, the percentage of damage in the Black and White varieties were negligible and the period of storage with less infestation was longer than in the Brown variety.

Key words: cowpea beetle, polyethylene, Hessian bags, phostoxin, Aluminum bins.
1.0 Introduction

Cowpea (vignaunguiculata) (L) walp is a warm weather crop that is well adapted to drier region of the tropics like Nigeria where other food legumes do not thrive well. It is one of the most economically and nutritionally important indigenous African grain legumes produced throughout the tropical and subtropical areas of the world (Golob et. al., 1999). Nigeria is its largest producer and consumer, accounting for about 45 percent of its world production (Degri, 2008), while Africa account for about 75% (Brternburg et.al., 1995). Cowpea seed pods are consumed in fresh form as green vegetables in some African countries, while the rest of the cowpea plant serves as a nutritious fodder for livestock and also as a source of cash income when sold to farmers who use them as livestock feed. Cowpea seeds are also a rich source of minerals and obtains (Adeduntan et.al., 1998). Cowpea is sometimes called poor man meat or vegetable meat due to its high protein content. Cowpea grain contain 23.4% protein, 1.8 % fat and 60.3 % carbohydrates and also a good source of vitamins and phosphorus (Adediran and Akinneye. 2004).

In spite of the great value of cowpea particularly in Nigeria, their availability and utilization have been impaired due to the seed damage by insect pest particularly the larvae of cowpea beetle (callosobuchusmaculatus) (Ofuya and Lale, 2001). Attack by insect pest species begins in the field and continues in storage causing substantial damage to store grain legumes as the pest rapidly increase. It has been reported that both quantitative and qualitative losses arising from physical, chemical and biological factors e. g fungi, rodents birds and insect occur during storage of grains (Emeasor et.al., 2007). Callusobruchusmaculatus. Up to 100% infestation of cowpea can occur after three to six months storage (Maina, 2011).

Majority of farmers in Northern Nigeria and some other countries, including the Sudan, (Baribusta et. al, 2010) use local or indigenous storage facilities to forestall the menace of these insect pest they use storage insecticide where available and affordable like the banned and highly restricted lindens (gammalin A) and the acceptable are like Aluminum or Atelic EC for storing their legume grains against cowpea beets, termites, rats and disease pathogens (Degri,2007).

Some local plants have been studied to show they have effect against the activity of insect pest. They include; NeemAzadiracta (A.juss), Nicotine (Nicotiniaspp), pyrethrum chrysanthemceneraefolium), Rotenme (Derriselliptica) (C.P.F, 1987). Sadim apple “Locally name Usher” (Calotropisprocera (J.), Sesame (Sesamumindicum L.), Garlic (AllumSativum L.) and (Lantana Camara), (Mueller et. al., 1995). They were all found to lower fecundity per female and adult emergence (Singh et’al, 1996). But the availability and side effects of these are also a major concern to farmers. Hermetic storage technology has emerge as a potent alternative to other method of storage that protects commodities from insect and moulds have been developed and applied and they abound in type and the PICS (Purdue Improved Cowpea Storage) which
was founded by the Bill and Melinda Gates foundation, is just one of these. The goal of the project is to have 50% of farm-stored cowpea in hermetic storage without insecticide in west and central Africa (Murdock et al., 2003). This is still on-going.

From the forgoing, some methods of cowpea beetle control abound but not without so many limitations, they are not cheap and some are also hazardous to health. Application of storage chemicals are sometimes not done properly by the local farmer which can lead to food poisoning. Larger quantity of cowpea are sold off immediately after harvest by the local farmers because of lack of adequate storage methods and fear of infestation by cowpea beetles thereby selling at a lower price compared to cost of production. This makes the produce scarce after the period of harvest.

This research was carried out to investigate the effect of variety and storage methods on the control of the cowpea beetle *Callosobruchus maculates* (f) (*coleopteran: Bruchide*) on stored cowpea. Effect of various storage methods on the control of cowpea beetle was also investigated as well as the variety that responds well to the various storage methods.

### 2.0 Materials and Methods

#### 2.1 Sample collection and preparation

The following materials were used for the research, three varieties of cowpea: white variety (Kanannado), brown (Ife brown) and black (Akidi) variety. Insect pest cowpea beetle *callosobruchus maculates*, was used as the insect pest, which were introduced to each treatment at same level. The seed scanner also known as dianophoscope was used to scan the cowpea seed in order to detect the effect of insect damage from each treatment. The storage methods used in this research are polyethylene (hermetic), storage bins which are made of aluminum, plastic containers and hessian bags. The storage chemicals that were used are phostoxin and atelic dust. These chemicals were chosen because they are mostly used by farmers in Bauchi State and in the wrong proportion and application. All the experimental materials were purchased from a local grain market in Bauchi State, Nigeria.

#### 2.2 Methods

##### 2.2.1 Cleaning and Determination of Moisture content

The purchased cowpea were cleaned to remove debris and all other foreign materials, this was done by hand picking, sorting and using winnower. Moisture content of each of the cowpea variety were determined using standard methods as used by Abodenyi et. al., 2018. This was to
ensure that the sample were at the safe storage moisture content to minimize spoilage during storage period.

2.2.2 Experimental procedures

2 kg of each variety were put in nine Polyethylene bags, the first three had phostoxine tablets introduced into it, and the next three had the atelic dust of 2 gm introduced into them, the last three served as control with no treatment. Each of the storage samples had Twenty (20) cowpea beetles introduced into them. These methods were repeated for the Aluminum storage bins, the Plastic containers and the Hessian bags for each variety. After introduction of the storage pest, the samples were agitated for one minute each to allow even spread of the pest and storage chemical (Ebiamadon et al., 2011)

The experimental set up were laid out in a completely randomized design with three replicates kept in the post-harvest laboratory of the department of agricultural bio-environmental engineering of federal polytechnic, Bauchi, Nigeria at 31± 2 °C and a relative humidity of 65±5 for a period of 90 days

2.3 Data Collection and Statistical Analysis

The rate of infestation was determined for each variety after 90 days of infestation with the pest, the following data were collected.

1. Number of live and dead insects: this was counted manually and recorded from each treatment.
2. Percentage damage grains. The number of grains with holes and grains without roles in all the treatments in each variety: this was done by pouring the seed on a seed scanner to detect the damage seeds in each treatment, and manually counting the number of grains with holes and those without holes. The holes on the grain was used as an indicator of damage. Percentage grain damage was determined using the following formula.

\[
\text{percentage damage} \, (\%) = \frac{\text{number of damage grains}}{\text{total number of grain sampled}} \times 100
\]

Minitab statistical software was used in the analysis of variance (ANOVA) to determine the variation in results of all the experiments under the various independent variables and their interaction at 95% level. Descriptive statistics such as percentage was also used in presenting the data.
3.0 RESULTS

The results obtained are as presented in the tables below for the three varieties of cowpea.

Table 1: Mean Effect of Cowpea Beetle Mortality on White Variety at 90 Days after Infestation

<table>
<thead>
<tr>
<th>Storage methods</th>
<th>Phostoxine</th>
<th></th>
<th>Atelic</th>
<th></th>
<th>Control</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Numb of live beetles</td>
<td>Numb of dead beetles</td>
<td>Percentag e mortality (%)</td>
<td>Numb of live beetles</td>
<td>Numb of dead beetles</td>
<td>Percentag e mortality (%)</td>
</tr>
<tr>
<td>Polyethylene Bags</td>
<td>1</td>
<td>19</td>
<td>95</td>
<td>3</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td>Aluminum Bins</td>
<td>4</td>
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<td>80</td>
<td>5</td>
<td>15</td>
<td>75</td>
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<tr>
<td>Hessian Bags</td>
<td>7</td>
<td>13</td>
<td>65</td>
<td>10</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Plastic containers</td>
<td>0</td>
<td>20</td>
<td>100</td>
<td>3</td>
<td>17</td>
<td>85</td>
</tr>
</tbody>
</table>
### Table 2: Mean Effect of Cowpea Beetle Mortality on Brown Variety at 90 Days after Infestation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage methods</th>
<th>Phostoxine</th>
<th></th>
<th></th>
<th>Atelic</th>
<th></th>
<th></th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of live beetles</td>
<td>Number of dead beetles</td>
<td>Percentage mortality (%)</td>
<td>Number of live beetles</td>
<td>Number of dead beetles</td>
<td>Percentage mortality (%)</td>
<td>Number of live beetles</td>
<td>Number of dead beetles</td>
<td>Percentage mortality (%)</td>
<td></td>
</tr>
<tr>
<td>Polyethylene Bags</td>
<td>3</td>
<td>17</td>
<td>85</td>
<td>5</td>
<td>15</td>
<td>75</td>
<td>9</td>
<td>11</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminum Bins</td>
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<td>14</td>
<td>70</td>
<td>6</td>
<td>14</td>
<td>70</td>
<td>15</td>
<td>5</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hessian Bags</td>
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<td>11</td>
<td>55</td>
<td>11</td>
<td>9</td>
<td>45</td>
<td>20</td>
<td>0</td>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>Plastic containers</td>
<td>3</td>
<td>17</td>
<td>85</td>
<td>4</td>
<td>16</td>
<td>80</td>
<td>9</td>
<td>11</td>
<td>55</td>
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</tr>
</tbody>
</table>

### Table 3: Mean Effect of Cowpea Beetle Mortality on Black Variety at 90 Days after Infestation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage methods</th>
<th>Phostoxine</th>
<th></th>
<th></th>
<th>Atelic</th>
<th></th>
<th></th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of live beetles</td>
<td>Number of dead beetles</td>
<td>Percentage mortality (%)</td>
<td>Number of live beetles</td>
<td>Number of dead beetles</td>
<td>Percentage mortality (%)</td>
<td>Number of live beetles</td>
<td>Number of dead beetles</td>
<td>Percentage mortality (%)</td>
<td></td>
</tr>
<tr>
<td>Polyethylene Bags</td>
<td>0</td>
<td>20</td>
<td>100</td>
<td>1</td>
<td>19</td>
<td>95</td>
<td>7</td>
<td>13</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminum Bins</td>
<td>3</td>
<td>17</td>
<td>85</td>
<td>5</td>
<td>15</td>
<td>75</td>
<td>11</td>
<td>9</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hessian Bags</td>
<td>Plastic containers</td>
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<td>13</td>
<td>65</td>
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<td>12</td>
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<td>90</td>
<td>7</td>
<td>13</td>
<td>65</td>
<td></td>
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</tr>
</tbody>
</table>
Table 4: Mean Percentage (%) of Damaged Cowpea at 90 Days after Infestation

<table>
<thead>
<tr>
<th>Storage methods</th>
<th>White variety</th>
<th>Brown variety</th>
<th>Black variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phostoxine</td>
<td>Ateleic</td>
<td>Control</td>
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<tr>
<td>Polyethylene Bags</td>
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<tr>
<td>Aluminum Bins</td>
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<td>Hessian Bags</td>
<td>50</td>
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<td>90</td>
</tr>
<tr>
<td>Plastic containers</td>
<td>9</td>
<td>10</td>
<td>35</td>
</tr>
</tbody>
</table>

3.1 Discussion

3.1.1 Cowpea Beetle Mortality on the various varieties of cowpea

The control treatment was generally less effective than the phostoxine and atelic dust at 90 days of storage and infestation of the cowpea. From tables 1, 2 and 3 all the storage methods were effective against the insect with significantly varying degree of efficiencies. Cowpea beetle mortality was significantly affected on the white variety more especially on the polyethylene storage and plastic containers with 95% and 100% mortality respectively. The Atelic showed mortality rate of 85% for both polyethylene and plastic containers while the control treatment has a value of 65% and 70% for both the polyethylene and plastic containers respectively. The Aluminum bin showed 80% mortality on phostoxine combination, 75% for the atelic and 50% for the control treatment. The reduction of oxygen during the 90 days of storage after the infestation reduced the insect count drastically especially in the polyethylene bags and the plastic containers. This cannot be said of the Hessian bags because they are porous and allowed the
thrusting of the storage pest in all the treatments. This result agrees with the findings of (Ebiamadon et al., 2011) which researched the effectiveness of different botanical pesticides on control of *C. maculatus* at 30 and 90 days of infestation.

The mortality of cowpea beetle on the Brown variety, cowpea beetle mortality was significantly affected by the storage chemicals and the storage methods. Polyethylene together with phostoxine and plastic containers showed high mortality of 85%. Atelic with polyethylene and plastic containers has mortality rate of 75% and 80% respectively. The control treatment indicated mortality of 55%, this result agrees with PICS project (Villers, et al., 2008) which used the Hermetic storage methods by keeping away oxygen from the pest they were able to record 50% mortality. The Hessian bags showed 0% mortality for the control treatment.

Cowpea beetle mortality on the Black variety was significantly high after the 90 days infestation and storage for all the treatments and storage methods except for the Hessian bags that indicated 65% for phostoxine, 60% for atelic and 0% for the control treatment.

### 3.1.2 Cowpea Damage at 90 Days after Infestation

Table four shows the degree of damage on the three varieties of cowpea after 90 days of infestation. The Hessian bag recorded the highest percentage of damage on all the storage methods and treatments for the three varieties. This can be attributed to the fact that the Hessian bag is porous that allowed intake of oxygen that allowed the survival of the storage pest. The White and black variety recorded less damage form the beetle from all the storage methods and treatments, this could be as a result of the high protein content of Brown beans, storage pest tend to fist more on highly protein food (AOAC, 2010)

### 4.0 Conclusion

From the above results, it can be concluded that the black variety is less susceptible to cowpea infestation when stored in a polyethylene bag as well as in an airtight plastic container.
References


[5-1015-D] Other Categories (2)
Chair: Tri Yuliana (Universitas Padjadjaran, Indonesia)
Thu. Sep 5, 2019 10:15 AM - 11:30 AM Room D (4th room)

[5-1015-D-01] Screening and Enzyme Activity of Cellulose-Producing Bacteria Isolated from Kemiri Sunan (*Reutealis trisperma* (Blanco) Airy Shaw) and Empty Fruit Bunches of Palm Oil
*Tri Yuliana¹, Efri Mardawati¹, Souvia Rahimah¹, Emilda Ayu Febrianty¹, Agus Try Hartono¹ (1. Univ. Padjadjaran, Indonesia(Indonesia))
10:15 AM - 10:30 AM

[5-1015-D-02] Development of a Cloud-based Internet of things Monitoring System for Fish Activity and Water Quality in Aquaponics
*Chien Lee¹, Yu-Jen Wang¹ (1. Department of Mechanical and Electromechanical Engineering, National Sun Yat-sen University(Taiwan))
10:30 AM - 10:45 AM

[5-1015-D-03] EFFECT OF DIFFERENT MODES OF PLANTING AND WEEDING ON MACHINE FIELD CAPACITY AND YIELD OF A MIXED CROPPING SMALL HOLDER FARM
Folasayo Titilola Fayose¹, Adesoji Mathew Olaniyan¹, *Babatope Albert Alabadan¹, Anthony Ayodele Fajinmi¹, Kayode Ogunleye¹, Olanrewaju Omoju¹, Olufemi Aladejebi¹, Oluwaseun Ilesanmi¹ (1. Federal University Oye Ekiti(Nigeria))
10:45 AM - 11:00 AM

[5-1015-D-04] Development of Agro-industrial Worker Trust Assessment System for Sustainable Ergonomic Program in Food Small and Medium-sized Enterprises
*Mirwan Ushada¹, Nur Achmad Sulistyo Putro², Titis Wijayanto³, Fitri Trapsilawati³, Nafis Khuriyati¹ (1. Universitas Gadjah Mada, Department of Agro-industrial Technology(Indonesia), 2. Universitas Gadjah Mada, Department of Computer Science and Electronics(Indonesia), 3. Universitas Gadjah Mada, Department of Mechanical and Industrial Engineering(Indonesia))
11:00 AM - 11:15 AM

[5-1015-D-05] ASSESSING LAND USE TYPES IMPACT ON SOIL ORGANIC CARBON IN SOUTH WEST, NIGERIA
*OLORUNWA ERIC OMOFUNMI¹, ADESOJI MATTHEW OLANIYAN¹ (1. FEDERAL UNIVERSITY OYE-EKITI(Nigeria))
11:15 AM - 11:30 AM
Biocatalyst technology is needed for the industry to improve performance of production. Cellulase enzymes has an important role in biocatalyst technology, especially in pulp industry. Cellulase is produced by certain types of microbes. The selection of cellulase-producing bacteria from Trisperma shell and empty fruit bunches from oil palm were carried out in order to produce cellulase which can be used for the pulp industry. Effectiveness test of cellulase-producing bacteria from Trisperma shell and palm bunches were also carried out using the liquid phase fermentation method. The result shows isolat K2 gave the widest clear zone with a value of 77.19% ± 0.00835 in BSM-CMC-CR media. OD value was calculated within 8 hours, 24 hours, 32 hours, and 48 hours in NB media. The result shows at 32 hours, the K3 isolate gave the highest absorbance with the value of 0.9163. Test of enzyme activity shown the K3 isolate had a highest enzyme activity with its value of 43.2 x 10-5 U/mL at 48 hours. The result of gram negative bacteria staining was assumed that the bacteria was Pseudomonas sp.
EFFECT OF DIFFERENT MODES OF PLANTING AND WEEDING ON MACHINE FIELD CAPACITY AND YIELD OF A MIXED CROPPING SMALL HOLDER FARM

Folasayo Titilola Fayose¹, Adesoji Mathew Olaniyan¹, Babatope Albert Alabadan¹, Anthony Ayodele Fajinmi¹, Kayode Ogunleye², Olanrewaju Omoju³, Olufemi Aladejebi³, Oluwaseun Ilesanmi³ (¹. Federal University Oye Ekiti(Nigeria))

Keywords: planting, weeding, field capacity, yield

Nigeria has great potential for cultivation of a wide variety of crops as its soil and climatic conditions are suitable for crop cultivation. However, growing crops with human labour (planting, weeding) has been the common practice. After an initial conventional tillage, labour saving mechanical jab and rotary planters, reciprocating weeder and manual methods were used to establish a mixed cropping one hectare farm of maize and cassava under rain-fed conditions. The effects of these treatments were studied using the following parameters: field capacity of planting, weeding and yield of crops. The highest field capacity among the planting modes was that of rotary planting with 1.53 ha/hr while, 0.44 ha/hr and 0.24 ha/hr were obtained for jab and manual planting respectively. A field capacity of 0.012 ha/hr was obtained for mechanical weeding as against 0.0036 ha/hr with manual weeding. The yields of the maize stover are as follows: Manual planting 6.9 tonnes/ha, Rotary planting 11.5 tonnes/ha, Jab planting 3.9 tonnes/ha while that of the average ear weight are 15.42 tonnes/ha for rotary planting, 10.33 tonnes/ha for manual planting and 5.83 tonnes/ha for jab planting. The effect of the use of chemical weeding reduced the yield of cassava roots to 60 ton/ha as against 81 ton/ha for manual/mechanical weeding. Further investigation is ongoing to substantiate the facts. However, these observations are in agreement with the fact that mechanical manipulation of the soil by way of planting and weeding loosen the soil between rows, thus increasing air and water intake capacity, thereby increasing yield.

Development of Agro-industrial Worker Trust Assessment System for Sustainable Ergonomic Program in Food Small and Medium-sized Enterprises

Mirwan Ushada¹, Nur Achmad Sulistyoo Putro², Titis Wijayanto³, Fitri Trapsilawati³, Nafis Khuriyati¹ (¹. Universitas Gadjah Mada, Department of Agro-industrial Technology(Indonesia), 2. Universitas Gadjah Mada, Department of Computer Science and Electronics(Indonesia), 3. Universitas Gadjah Mada, Department of Mechanical and Industrial Engineering(Indonesia))

Keywords: Bird swarm algorithm, Collective trust, Environmental ergonomics, Individual trust, Kansei Engineering

Ergonomic program has not yet fully gained the worker trust in food Small Medium-sized Enterprises (SMEs) due to the gap between ergonomics and financial amenities. The tangible financial amenities as wages, incentives, and insurance have been more attractive than the intangible ergonomics program in the form of a comfortable workplace environment (Environmental ergonomics), efficient work methods and optimum workload. Trust could be defined as an abstractive (Kansei) human factor which is characterized by uncertainty and vulnerability to support their individual and collective decision. Trust influence the
The attractiveness of ergonomic program to worker as individual and worker union as the collective. The abstractive communication between 1 (one) individual worker and other partners in same union is possible to be simulated in an artificial bird swarm algorithm. Kansei engineering was selected to model the individual trust due to the reliability for modeling the abstractive human factors. Artificial swarm intelligence was selected to simulate the collective trust due to capability to model non-linear of human factors. The research goal was to develop an agro-industrial worker trust assessment system for sustainable ergonomic program in food SMEs. The research objective was: 1) To predict the worker individual trust using Kansei Engineering; 2) To simulate the worker collective trust using bird swarm algorithm. The system is expected to assist the SME’s management for developing trust evidence-based ergonomic policy. Generally, the system is expected to support the Sustainable Development Goals numbers 3 (Good health & well-being) and number 9 (Industry, innovation and infrastructure). The system was tested on the database of worker human factors in Food SMEs. The inputs of the system was extracted from database as: 1) Workload; 2) Workplace temperature; 3) Relative humidity; 4) Light intensity; 5) Incentive. The output was individual and collective trust. The agro-industrial worker trust assessment system consists of 7 sub-systems. In the Sub-system 1, measurement is carried out to obtain the worker mood states, heart rate and workplace environment parameters. In Sub-system 2, the manager obtain measurement result in Sub-system 1 as the input to determine integrated workload and workplace temperature set point. If the workload indicated the normal status, then the workplace temperature is set. If the workload status indicated under or over load, then the system provides feedback for the manager to evaluate the existing ergonomic program. In Sub-system 3, the temperature was set in an air conditioner to create the comfortable workplace environment (Environmental ergonomics). In Sub-system 4, the work incentive is determined based on integrated workload (Sub-system 2) and environmental ergonomics (Sub-system 3). The individual trust index is determined in Sub-system 5. If the index indicated the status of trust, the system proceeds the status to the Sub-system 6. If the index indicated distrust, the system provides feedback to the manager to evaluate the existing ergonomic program. The Sub-system 6 processes the individual trust in Sub-system 5 using the Bird Swarm Algorithm in Kansei Engineering (BISAKE). The algorithm simulated the worker union to behave like a bird swarm in determining whether an individual trust is satisfied or not against their mentality constraints of prior knowledge, familiarity, agreement and preference. Finally, in the Sub-system 7, the collective trust was validated. The simulation result indicated that worker trust index could be assessed based on workload status, a percentage of incentive and workplace environmental cost per month. Furthermore, this assessment could make the trust data more manageable to store, retrieve and enable interchange in big data system for sustainable ergonomic program in food SMEs.

11:15 AM - 11:30 AM  (Thu. Sep 5, 2019 10:15 AM - 11:30 AM  Room D)

[5-1015-D-05] ASSESSING LAND USE TYPES IMPACT ON SOIL ORGANIC CARBON IN SOUTH WEST, NIGERIA

*OLORUNWA ERIC OMOFUNMI¹, ADESOJI MATTHEW OLANIYAN¹ (1. FEDERAL UNIVERSITY OYE-EKITI(Nigeria))

Keywords: Federal University Oye Ekiti (Ikole campus), land use type, Soil organic carbon, Soil properties

The amount of soil organic carbon (SOC) stored in a particular soil is influenced by several factors including climate, vegetation type, land management, soil properties and current and last land use. The impacts of land use types on soil organic carbon were assessed. Four land use types were used in the study. Sampled soils were taken at depth of 0 - 45 cm and at intervals of 15 cm. The soil samples were examined in accordance with the standard methods described by the American Public Health Association (APHA). The data were
analyzed using descriptive statistics. The results showed the mean soil organic carbon content was higher under oil palm plantation land [D] compared with other land use types at 0 - 15 cm soil depth (22.87g/kg) which was 1.5, 2.6 and 53.3 % more than in the Faculty of Agriculture Teaching and Research farm land [A], the cashew plantation land [B] and the Agricultural and Bioresources experimental farm land [C] respectively. This could be attributed to the greater inputs of vegetation (litter fall) and reduced decomposition of organic matter. Similarly, the lowest soil organic carbon content under land use type C could be due to reduced inputs of organic matter and frequent tillage which encouraged oxidation of organic matter. The finding indicated that the means of SOC in land use types were not significantly different (p = 0.05) except in the land use type C. It is concluded that land use types have influenced on soil organic carbon
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
Detection of Outliers in Pre-processing of Datasets for Recognition of Classifiers Using Partial Least Squares Discriminant Analysis

*Miki Fujii\(^1\), Ryozo Noguchi\(^2\), Tofael Ahamed\(^2\), Takuma Genkawa\(^3\) (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))

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 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] Postharvest/Food Technology and Process Engineering (5th)  
Thu. Sep 5, 2019 11:30 AM - 12:30 PM  Poster Place (Entrance Hall)

[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, ², ³ (1. Utsunomiya Univ.(Japan), 2. Tochigi Industrial Promotion Center(Japan), 3. Industrial Technology Center of Tochigi Pref.(Japan))

[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, ² (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))

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

[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 (1. Gifu University(Japan))

[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 (1. Department of Bio-Industrial Mechatronics Engineering, National Taiwan University(Taiwan))

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

[5-1130-P-07] Properties of Rice Starch-Based Film Incorporated with Zinc Oxide Nanoparticles  
KHALISHAH RAHMA SAFIRA¹, ², *SAROAT RAWDKUEN²  
¹, ² (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))
Effect of pulsed electric field treatment on drying rate and quality changes of spinach in hot air drying
*Koya Yamakage¹, Takahiro Yamada¹, Takahiro Orikasa²,³, Katsuyuki Takahashi²,⁴, Shoji Koide³, Koichi Takaki²,⁴, 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))

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, Hijiri 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))

Anaerobic Digestion of Bean Sprouts Waste
*Yuki Yamamoto¹, Yuki Mizuya², Takaki Yamashiro³, Fetra J Andriamanohiarisoamanana¹,⁴, 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))

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))

Temporal Transition of Spatial Dependence of Weeds in Grassland
*Katsuyuki Tanaka¹, Ayako Oide¹, Hideo Minagawa¹ (1. Kitasato University(Japan))

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))
[5-1130-P-01] Development of dumpling rich in barley flour with gluten added

*Masatsugu Tamura¹, Naoya Takahashi¹, Takahiro Saito¹, Satomi Akatsu², Yoshihiro Hoshi³, Takemi Okamoto³
(1. Utsunomiya Univ.(Japan), 2. Tochigi Industrial Promotion Center(Japan), 3. Industrial Technology Center of Tochigi Pref.(Japan))

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 barley dumpling skins with added 10% gluten and 65%, 70% and 75% moisture (1.82–2.28 N). The barley 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.

[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))

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.

**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.

**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.

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

[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 (1,2,2,1) 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% @ pH 5.9). Moreover, significant decrease in TSS (24.99, 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.
Properties of Rice Starch-Based Film Incorporated with Zinc Oxide Nanoparticles

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

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/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 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 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 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 ºC for 2 h in a water bath. Following the heating, the reaction mixture was left standing overnight (12 h) at 24 ºC and filtered through Whatman filter number one. The precipitate result was kept in a hot air oven at 60 ºC 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 20º-80º 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 ºC, and then sonicated in an ultrasonic bath (Marconi model, Unique...
USC 35 kHz) for 30 min at 60 °C. 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 °C. 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 60° 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 °C 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 °C for 1 h, and then sonicated in an ultrasonic bath (Marconi model, Unique USC 35 kHz) at 60 °C 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 °C and held for 45 min to allow gelatinization. Upon completion of starch gelatinization, the film-forming solution (FFS) was cooled to 45 °C. 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 °C 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 °C. 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:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL*, Δa*, and Δb* 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:

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 m/m² Pa s as follows:

$$WVP = (WVTR \times L)/\Delta p$$

where L was the thickness of the film (m) and Δp 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 2Θ, 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.](image1)

![Figure 2. SEM micrograph of synthesized ZnO-NPs with magnification 20000x.](image2)
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 (\textit{S. aureus}) and Gram-negative (\textit{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 \textit{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>\textit{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 (\textit{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 Zn$^{2+}$ 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 nanoparticles 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 10^8 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 starch 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 ± 2.13</td>
<td>5.00 ± 0.44</td>
<td>76.62 ± 5.68</td>
<td>11.95 ± 1.48</td>
<td>27.12 ± 1.33</td>
<td>0.06 ± 0.01b</td>
</tr>
<tr>
<td>Rice starch/ZnO-NPs</td>
<td>69.50 ± 3.32</td>
<td>9.14 ± 0.78</td>
<td>37.18 ± 2.61</td>
<td>12.55 ± 1.12</td>
<td>19.22 ± 0.39</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 ± 0.83a</td>
<td>-1.17 ± 0.15a</td>
<td>2.63 ± 0.35a</td>
<td>6.37 ± 0.83b</td>
</tr>
<tr>
<td>Rice starch/ZnO-NPs</td>
<td>89.73 ± 0.06a</td>
<td>-0.90 ± 0.21a</td>
<td>2.11 ± 0.04a</td>
<td>3.38 ± 0.04a</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>Rice starch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice starch/ZnO-NPs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T200</td>
<td>0.07</td>
<td>22.57</td>
</tr>
<tr>
<td>T280</td>
<td>0.07</td>
<td>34.77</td>
</tr>
<tr>
<td>T350</td>
<td>0.07</td>
<td>34.77</td>
</tr>
<tr>
<td>T400</td>
<td>0.07</td>
<td>34.77</td>
</tr>
<tr>
<td>T400</td>
<td>0.07</td>
<td>34.77</td>
</tr>
<tr>
<td>T600</td>
<td>0.07</td>
<td>34.77</td>
</tr>
<tr>
<td>T700</td>
<td>0.07</td>
<td>34.77</td>
</tr>
<tr>
<td>T800</td>
<td>0.07</td>
<td>34.77</td>
</tr>
<tr>
<td>Rice starch/ZnO-NPs</td>
<td>0.03</td>
<td>3.28</td>
</tr>
<tr>
<td>T200</td>
<td>0.03</td>
<td>4.07</td>
</tr>
<tr>
<td>T280</td>
<td>0.03</td>
<td>14.05</td>
</tr>
<tr>
<td>T350</td>
<td>0.03</td>
<td>24.56</td>
</tr>
<tr>
<td>T400</td>
<td>0.03</td>
<td>30.83</td>
</tr>
<tr>
<td>T500</td>
<td>0.03</td>
<td>35.49</td>
</tr>
<tr>
<td>T600</td>
<td>0.03</td>
<td>39.26</td>
</tr>
<tr>
<td>T700</td>
<td>0.03</td>
<td>2.64 ± 0.01a</td>
</tr>
<tr>
<td>T800</td>
<td>0.03</td>
<td>2.64 ± 0.01a</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>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>Rice starch</td>
<td>0.00 ± 0.00(^a)</td>
<td>0.00 ± 0.00(^a)</td>
<td></td>
</tr>
<tr>
<td>Rice starch/ZnO-NPs</td>
<td>4.50 ± 0.71(^b)</td>
<td>2.50 ± 0.71(^b)</td>
<td></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 swollen
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.](image)

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), Kanmani 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.

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REFERENCES


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

*Yuki Yamamoto¹, Yuki Mizuya², Takaki Yamashiro³, Fetra J Andriamanohiarisoamanana¹⁴, Yoshiteru Takeuchi⁵, 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))

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 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 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
Yuki Yamamoto1, Yuki Mizuya2, Takaki Yamashiro3, Freta J Andriamanohiarisoamanana1,4, Yoshiteru Takeuchi5, Kazutaka Umetsu1*

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2 Obihiro University of Agriculture and Veterinary Medicine,
3 Tokachi Agri Works,
4 Graduate School of Agricultural Science, Kobe University,
5 Biomass Research

*umetsu@obihiro.ac.jp

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 waste, therefore, the 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 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.

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 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 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 of 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 digester 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 1 Experimental 2-1 design, TS, VS, HRT |
| materials | TS(%) | VS(%) | HRT(d) |
| RUN A | Bean sprouts 200g | 15.93 | 15.31 | 50 |
| RUN B | Bean sprouts 200g + Return digestate 500g | 3.26 | 3.05 | 15 |

| Table 2 Experimental 2-2 design, TS, VS, HRT |
| materials | TS(%) | VS(%) | HRT(d) |
| RUN A | Bean sprouts 100 g + Return digestate 300 g (acid fermentation) | 3.89 | 3.17 | 25 |
| RUN B | Bean sprouts 100 g + Return digestate 300 g (no acid fermentation) | 5.13 | 4.53 | 25 |
### Table 3 Experimental 2-3 design, TS, VS, HRT, organic loading

<table>
<thead>
<tr>
<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>RUN A Bean sprouts 75 g + Return digestate</td>
<td>6.09</td>
<td>5.38</td>
<td>44</td>
<td>2.75</td>
</tr>
<tr>
<td>150 g + Trace elements 0.12 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RUN B Bean sprouts 100 g + Return digestate</td>
<td>5.58</td>
<td>4.68</td>
<td>33</td>
<td>4.26</td>
</tr>
<tr>
<td>200 g + Trace elements 0.16 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RUN C Bean sprouts 150 g + Return digestate</td>
<td>5.11</td>
<td>4.41</td>
<td>22</td>
<td>9.02</td>
</tr>
<tr>
<td>300 g + Trace elements 0.24 g</td>
<td></td>
<td></td>
<td></td>
<td></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.

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 6 Amount of biogas and methane concentration per input VS

<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.

![Figure 3 VFA](image)

**Figure 3 VFA**

### 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.

![Figure 4 Biogas production per input VS](image)

**Figure 4 Biogas production per input VS**

#### 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 7 Organic matter decomposition rate

<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 0.16g</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 elements0.24g</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 (acetric 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. Aranzado, *Loraine C. Bainto*, Dennis Marvin O. Santiago (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

Katsuyuki Tanaka, Ayako Oide*, Hideo Minagawa

Division of Environmental Bioscience, Kitasato University, Japan

*Corresponding author: oideayak@vmas.kitasato-u.ac.jp

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 ≤ R <0.2 m), middle (0.2 (R <0.4 m), large (R ≥ 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.


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 ($\gamma$) at the point of semi-variance ($\gamma$) becomes constant is called the sill. Range is the spatial distance when the semi-variance ($\gamma$) 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.

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>
<td></td>
<td></td>
</tr>
</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>3</td>
<td>0.460</td>
<td>983</td>
<td>-3.75</td>
<td>1.84e-04</td>
</tr>
<tr>
<td>G3</td>
<td>3</td>
<td>0.682</td>
<td>862</td>
<td>-10.62</td>
<td>7.51e-25</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

**[5-1130-P-13] RNA-Seq analysis of the transcriptome and genes expression profile during the browning of Lotus Root (Nelumbo nucifera)**

*Kanjana Worarad*¹, Haruka Norii¹, Yuya Muchizuki¹, Takashi Ishii², Keiko Shinohara³, Takao Miyamoto ⁴, Eiichi Inoue ³ (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 Imaizumi³ (1. Gifu Graduate School of Applied Biological Sciences and Faculty of Applied Biological Sciences(Japan))
11:30 AM - 12:30 PM
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))

Keywords: blended fruit wine, carbonation, antioxidant, sensory

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.

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))

Keywords: Pesticide, Spider mite, Fatty acid

Spider mite is one of the pests that infest many crops. Pesticides to prevent spider mites are less effective drugs due to the development of drug resistance by spider mites. Thus, new drugs are needed. The control of indoor environmental pollutants by fatty acid was examined for the purpose of creating a new safer control agent. In the process, it became clear that isopalmitic acid, which is a hyperbranched fatty acid, shows high acaricidal activity against house dust mite. Therefore, we decided to investigate the pest control effect of isopalmitic acid on the spider mites. The sample used isopalmitic acid (isoC16). As test ticks, the black spider mite (Tetranychus urticae) was used. It was fed with pea leaves. An acaricidal test was conducted against the spider mite. Pea leaves cut to a size of 2 cm × 2 cm were placed on damp filter paper. Ten female adults of Tetranychus urticae were placed on it. The sample was sprayed to 20 mg/cm² using a spray. After 24 h, lethality determination was performed under a stereomicroscope. The repellent effect was tested. Pea leaves were prepared in the same manner as the acaricidal test. Half of the leaf pieces were treated with the sample. One half was treated with ion-exchanged water to which 0.01% Tween 80 was added. Ten female adults of Tetranychus urticae were placed at the center of each disc. Under a microscope, the number of adult females was determined after 24 h and the number of eggs was determined after 72 h. The sustainability was tested. Pea leaf pieces were sprayed with the sample as in the acaricidal test. Sample Inoculation Five female
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*.
was small (1363±212 um) while the value of control was large (906±39.6 μ m). In addition, object surface density of control (0.00548 ± 0.0000283 um⁻¹) was higher than C30 (0.00420 ± 0.000769 um⁻¹). These results shown that C30 constructed with larger pores in comparison with control. About structure thickness, C30 indicated large value (127±81.7 μ m) more than control (94.5±20.6 μ m), 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.
[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**  
*Ikko Ihara*\(^1\), *Homi Takato*\(^1\), *John K Schueller*\(^2\), *Gen Yoshida*\(^1\), *Kazutaka Umetsu*\(^3\), *Hitomi Yamaguchi*\(^2\) (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
Key Process Variables Affecting the Formation of Chlormequat Compounds During Baking of Cereal Products

*Adam Ekielski* (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 chlormequat 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 miticidal test as samples. They were obtained from FUJIFILM Wako Pure Chemical Corporation. 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. The black cloth (45 mm×45 mm) was fixed on a petri dish with double sided tape, 30 adult mites were placed on the cloth. 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 hexadecanoic 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 miticidal 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.

Keywords: Tyrophagus putrescentiae, linear fatty acid, acaricidal test

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**Improvement of the Cleanability of Milk Soil on a Highly Smooth Surface of Stainless Steel Tubing**

*Ikko Ihara*¹, Homi Takato¹, John K Schueller², Gen Yoshida¹, Kazutaka Umetsu³, Hitomi Yamaguchi² (1. Kobe University(Japan), 2. University of Florida(United States of America), 3. Obihiro University of Agriculture and Veterinary Medicine(Japan))

Keywords: milk soil, surface roughness, cleanability

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] Other Categories (5th)
Thu. Sep 5, 2019 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 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 Deejing* and Witchayaporn Pawong

1,2 Program in Biotechnology, Faculty of Science, Maejo University, Thailand

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ABSTRACT
Endophytic bacteria are able to colonize in plant tissues without causing harm fulness 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 acetice 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 climate 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. Petcharot 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>Number of endophytic bacteria (isolates)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Stems</td>
</tr>
<tr>
<td>PCA</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>PVK</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>TSA</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>ISP2</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>32</td>
<td>9</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. Petcharat 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 CEO: flat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRSPCA2</td>
<td>CC: yellow CF: circular</td>
<td>Gram negative Rods, single</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CM: entire 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

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>abEU169201.1</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 antifungal 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). Susilowatia 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 amyloliquenficiens* promote roots dry weight. *Pseudomonas putida* was found to promote root and shoot growth and protect the plants against the phytotoxic effects of phanethrene which environmental contaminants such as polycyclic aromatic hydrocarbons (Khan et al., 2014). Joshi et al. (2018) reported endophytic bacteria *Enterobacter* sp., *Pseudomonas* sp. and *Azospirillium* sp. that isolated from *Ocimum sanctum* and *Aloe vera* roots could produced enzymes urease, pectinase, cellulase, catalase, lipase, casienase, gelatinase and chitinase. In recent years, co-inoculation of
Endophytic microorganisms are playing a key role for improving nutrient availability in sustainable agriculture production systems. 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 the 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 used 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
Authors are grateful to Office of the National Research Council of Thailand (The Thailand Research, and the facility provided from Program in Biotechnology, Faculty of Science, Maejo University.

REFERENCES


[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))
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.

[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))
Keywords: Power tiller, Hexagonal Wheel, Subsoiler, Oscillatory motion
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 \(T_{\text{min}}, T_{\text{a}}, T_{\text{max}}\) 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
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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2Fukushima Research Station, National Agriculture and Food Research Organization, Japan
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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 worried 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 sensors 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 (T_{min}, T_{a}, 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.
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 decreased compared to that near north wall and that near soil surface, because south wall had extremely heated as shown 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.

Figure. 6 Time dependent change data of net radiation around pot from Feb.4 to Mar. 4 in 2019.
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.

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
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</td>
<td>0.7</td>
<td>m</td>
</tr>
<tr>
<td>body and front wheel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance between center of gravity of tractor</td>
<td>0.64</td>
<td>m</td>
</tr>
<tr>
<td>body and rear wheel</td>
<td></td>
<td></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 1a: Road surface with a slope](image1a)
![Figure 1b: Road surface without a slope](image1b)

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
We thank Prof. Shrini Upadhyaya and Prof. Heinz Bernhardt for their kind support. This work was supported by JSPS Grant-in-Aid No. 19J11183 and 19H00959.

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 hyperspectral 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°92'42.0224"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 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))

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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²Dept. of Biosystems Engineering, Gyeongsang National University, Republic of Korea

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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

![Dynamometer 3D Modeling](image)

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.

<table>
<thead>
<tr>
<th>Model name</th>
<th>CAS S-Beam Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rated capacity (kgf)</td>
<td>2000</td>
</tr>
<tr>
<td>Accuracy rating</td>
<td>D3 / C3</td>
</tr>
<tr>
<td>Combined error (%)</td>
<td>≤0.03 / ≤0.02</td>
</tr>
<tr>
<td>Creep (half hour, %)</td>
<td>≤0.03 / ≤0.017</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.

![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).

\[
\text{Traction Force } P_T = F_a + F_b + F_c \tag{1}
\]
Vertical Force \( P_V = F_d \sin \theta + F_e \sin \theta \) \hspace{1cm} (2)

Lateral Force \( P_H = F_d \cos \theta - F_e \cos \theta - F_l \) \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.

![Figure 3. 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.

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

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

\[
\text{Lateral Moment } M_H = \zeta(F_b + F_c) - (F_a \varepsilon) \hspace{1cm} (6)
\]

### 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>Top</th>
<th>Bottom Left</th>
<th>Bottom Right</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traction Force</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>1500</td>
</tr>
<tr>
<td>Vertical Force</td>
<td>500</td>
<td>250</td>
<td>250</td>
<td>1000</td>
</tr>
<tr>
<td>Lateral Force</td>
<td>250</td>
<td>125</td>
<td>125</td>
<td>500</td>
</tr>
<tr>
<td>Traction Moment</td>
<td>500</td>
<td>-500</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vertical Moment</td>
<td>500</td>
<td>-500</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lateral Moment</td>
<td>500</td>
<td>-500</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
3. RESULTS AND DISCUSSION

![Figure 7. Measured Force and Moment.](image)

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>
<thead>
<tr>
<th>Applied Force</th>
<th>Measured Force</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

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))

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 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.

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 since 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-plated 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 homogenous 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’ GGTTACCTTGTTACGACTT 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, Gluconococcus, Gluconacetobacter, Ameyamaea, Tanticharoenia, Asaia, Swaminathania, Kozakia, Neosasaia, Granulibacter, 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, 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 pibulans, which have been isolated from idli, dosa and dhokla, varieties of steamed blend 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%), Saccharomycopsis fibuligera (22.2%), Torulaspora 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
starch.

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>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. Δ-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>D-glucose</td>
</tr>
<tr>
<td>D-fructose</td>
<td>+</td>
</tr>
<tr>
<td>D-xylene</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)

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 γ-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. aurantiacus* 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 γ pyrones from D-glucose. It was also the only isolate that did not ferment D-sucrose, exactly the same as its homologous species *A. lovaninensis* (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. lovaninensis* 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.

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Keywords: broiler production, layer, egg production, LCA

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.