Fri. Sep 6, 2019

Poster Place

Poster Session | Postharvest/Food Technology and Process Engineering

[6-1130-P] Postharvest/Food Technology and Process Engineering (6th)

11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-18] **Optimization and Evaluating of Pomegranate Peel Extract by Micro Wet Milling Using Response Surface Methodology**

*Rasool Khan Amini[1], Yutaka Kitamura[2], Mito Kokawa[2], M. Z. Islam[2] (1. Graduate School of Life and Environmental Sciences, University of Tsukuba(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tennoda, Tsukuba, Ibaraki 305-8572, Japan(Japan))

11:30 AM - 12:30 PM

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

*nutthachai pongprasert[1], Varit Srilaong[1,2], Songsin Photchanachai[1,2], Panida Boonyaritthongchai[1,2], Kornkanok Aryusuk[3] (1. Postharvest Technology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10140(Thailand), 2. Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400,Thailand(Thailand), 3. Biochemical Technology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10140(Thailand))

11:30 AM - 12:30 PM

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

*Tatsuya Oshima[1], Kodai Kato[1], Satoshi Iwamoto[1], Teppei Imaizumi[1] (1. Gifu University(Japan))

11:30 AM - 12:30 PM

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

*JIA FANG[1], Yutaka KITAMURA[1], Mito KOKAWA[1], Kazunobu KAJIHARA[2], Kozi KAWAKAMI[2], Hidenori MIZUNO[2] (1. Tsukuba Univ.(Japan), 2. Satake Corporation(Japan))

11:30 AM - 12:30 PM

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

*Yuri Koshima[1], Yutaka Kitamura[1], Mito Kokawa[1], Thais M.F.S. Vieira[2], Juliana Antunes Gavalão[2], Luis Felipe de Freitas Fabricio[2], Md Zohurul Islam[1] (1. University of Tsukuba(Japan), 2. University of Sao Paulo(Brazil))

11:30 AM - 12:30 PM

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

*Yoshiki Muramatsu[1], Masanori Hashiguchi[2], Eiichiro Sakaguchi[1], Shotaro Kawakami[1] (1. Tokyo University of Agriculture(Japan), 2. Keisoku Engineering System Co., Ltd.(Japan))

11:30 AM - 12:30 PM

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

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

11:30 AM - 12:30 PM

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

*Daisuke Kurata[1], Takahiro Orikasa[2,3], Shoji Koide[2] (1. Graduate School of Arts and Sciences, Iwate University.(Japan), 2. Faculty of Agriculture, Iwate University.(Japan), 3. Agri-Innovation Center, Iwate University.(Japan))

11:30 AM - 12:30 PM

[6-1130-P-26] **The effect of molecular hydrogen on the**

shelf life of banana

*Naoya Fujino[1], Teruo Wada[1] （1. Osaka Prefecture University(Japan)）

11:30 AM - 12:30 PM

[6-1130-P-27] **The Potential of Biogas Production from Caribbean Seaweed Biomass**

*Yuhendra AP[1], Mohamed Farghali[1], Takaki Yamashiro[2], Ryuichi Sakai[3], Kazutaka Umetsu[1] （1. Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine(Japan), 2. Tokachi Agri Works(Japan), 3. Graduate School of Fisheries Sciences, Hokkaido University(Japan)）

11:30 AM - 12:30 PM

[6-1130-P-28] **Study on the Characteristics of Micro Wet Milling and Spray Drying of Sea-buckthorn (*Hippophae rhamnoides*)**

*ODGEREL Ulziibat[1], Md.ZOHURUL ISLAM[1], KITAMURA Yutaka[2], KOKAWA Mito[2], ODBAYAR Tseyen-Oidov[3], SOLONGO Ganbold[3] （1. Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan(Japan), 3. School of Industrial Technology, Department of Food Engineering, Main Campus of MUST, Baga Toiruu 34, Sukhbaabatar District, Ulaanbaatar, Mongolia(Mongolia)）

11:30 AM - 12:30 PM

[6-1130-P-29] **Combined Effect of Pre-treatment and Vacuum Packaging for Maintaining the Quality of Peeled Shallot (*Allium ascalonicum* L.)**

*Phanida Renumarn[1], Kranert Kilian Joachim [4], Natthaya Choosuk[1], Chanthima Phungamngoen[2], Kasama Chareekhot[3] （1. Department of Innovation and Product Development Technology, Faculty of Agro-Industry, King Mongkut’s University of Technology North Bangkok(Thailand), 2. Department of Agro-Industry Technology and Management, Faculty of Agro-Industry, King Mongkut’s University of Technology North Bangkok(Thailand), 3. Department of Food Science and Technology, Faculty of Technology, Udon Thani Rajabhat University(Thailand), 4. Food Science -Technology and Economics, University of Applied Sciences Bremerhaven(Germany)）

11:30 AM - 12:30 PM

[6-1130-P-30] **High pressure processing of ‘Nanglae’ pineapple juice: Quality preservation and shelf life extension**

Nuntawan Chuensombat[1], Natthakan Rungraeng[1], Sutthiwal Setha[1,2], *Phunsiri Suthiluk[1,2] （1. School of Agro-Industry, Mae Fah luang University, Chiang Rai, THAILAND(Thailand), 2. Research Group of Postharvest Technology, School of Agro-Industry, Mae Fah Luang University, Chaing Rai, THAILAND(Thailand)）

11:30 AM - 12:30 PM

Poster Session | Food Function/Nutrition

[6-1130-P] Functional/Wellness Foods &Nutrition (6th)

11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-01] **Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds**

*Pairote Wongputtisin[1], Narin Lahsom[1] （1. Program in Biotechnology, Faculty of Science, Maejo university, Chiang mai, Thailand (Thailand)）

11:30 AM - 12:30 PM

[6-1130-P-02] **Effect of Sucrose and Glucose on Coffee Kombucha Carbonation**

*Chutamas Maneewong[1], Thittaya Choompoosee[1] （1. Department of Biotechnology, Faculty of Science, Maejo University, San Sai, Chiang Mai 50290(Thailand)）

11:30 AM - 12:30 PM

[6-1130-P-03] **Evaluation of Total Anthocyanins and Antioxidant Activity of Thai Rice Cultivars for Phenotypic Selection in Rice Breeding**

*Chotipa Sakulsingharoj[1], Lalita Na Rachasima[1], Anongnad Richinda[1], Pairote Wongputtisin[2], Rungthip Kawaree[2], Saengtong Pongjaroenkit[1], Varaporn Sangtong[1] （1. Program in Genetics, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand), 2. Program in

Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand))
11:30 AM - 12:30 PM

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

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

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

[6-1130-P-07] **Nutritional and Functional Properties of Yoghurt Drink with Philippine Gac (*Momordica cochinchinensis* Spreng.) and Bignay (*Antidesma bunius*) Fruits**
Rowie Joy Gonzales Bucks[1], *Ara Fatima Cuvinar Algar[1], Ryan Rodrigo Paner Tayobong[2] (1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines), 2. Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines))
11:30 AM - 12:30 PM

[6-1130-P-08] **Effect of Extracting Conditions on Plant Extract Colors and Stability of Antioxidant Properties during *in vitro* Gastrointestinal Digestion**
*Rattika Aeka[1], Titikan Liangpanth[1], Rungarun Sasanatayart[1] (1. School of Agro-Industry, Mae Fah Luang University(Thailand))
11:30 AM - 12:30 PM

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

[6-1130-P-10] **Changes in the Growth and Antioxidant Components of Komina with Different Red and Blue Light Emitting Diode (LED) Irradiation Ratios**
Kanako Niiya[1], *Takahiro Saito[2], Masatsugu Tamura[2], San Woo Bang[2] (1. Utsunomiya University Graduate School(Japan), 2. Utsunomiya Univ.(Japan))
11:30 AM - 12:30 PM

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

[6-1130-P] Other Categories (6th)

11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-11] **Temporal Source Strength Estimation of Sweet Pepper for Crop Management and LED Supplementation Efficiency Improvement**
*Masaaki Takahashi[1], So Kaneko[1], Osamu Koike[1], Hiroki Umeda[2], Yasunaga Iwasaki[3] (1. Miyagi Prefectural Agriculture and Horticulture Research Center(Japan), 2. Graduate School of Bioresource Sciences, Nihon University(Japan), 3. National Agriculture and Food Research Organization(Japan))
11:30 AM - 12:30 PM

[6-1130-P-12] **Study on Analysis of Loads Effect on Path-Tracking Accuracy of an Autonomous Tractor during Plow Tillage**
*YEONSOO KIM[1,2], YONGJOO KIM[2], HYOGEOL KIM[1], YOUNGJOO KIM[1], SANGDAE LEE[1] (1. KITECH(Korea), 2. Chungnam Univ.(Korea))
11:30 AM - 12:30 PM

[6-1130-P-13] **Classification of Sugarcane Variety using Image Processing and Multivariate Analysis**
*KITTIPON APARATANA[1], Hiroo Takaragawa[1,2], Yoshinari Izumikawa[1,2], Eizo Taira[1] (1. Faculty

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

11:30 AM - 12:30 PM

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

*Misaki Mito[1], Takuya Aoki[1], Koichi Mizutani[2], Keiichi Zempo[2], Naoto Wakatsuki[2], Yuka Maeda[2], Nobuhiro Takemae[3], Takehiko Saito[3] (1. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan))

11:30 AM - 12:30 PM

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

*Akifumi Goto[1], Misaki Mito[1], Tadashi Ebihara[2], Koichi Mizutani[2], Naoto Wakatsuki[2], Nobuhiro Takemae[3], Takehiko Saito[3] (1. Graduate School of Systems and information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan))

11:30 AM - 12:30 PM

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

*Yasuhiko Nishijima[1], Koichi Mizutani[1,2], Tadashi Ebihara[1,2], Naoto Wakatsuki[1,2], Kenji Kubota[3], Hiroyuki Uga[4] (1. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, Division of Engineering Interaction Technologies, University of Tsukuba(Japan), 3. Agriculture Research Center, National Agriculture and Food Research Organization(Japan), 4. Saitama Prefecture Agriculture Research Center(Japan))

11:30 AM - 12:30 PM

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

*Songsin Photchanachai[1], Nipada Ranmeechai[1,2], Chalinee Sungkajorn[1,2], Anantaporn Phankhaek[1,2], Kornkanok Aryusuk[1], Varit Srilaong[1,2], Panida Boonyarithongchai[1,2], Nutthachai Pongprasert[1,2] (1. School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok(Thailand), 2. Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok(Thailand))

11:30 AM - 12:30 PM

Poster Session | Postharvest/Food Technology and Process Engineering

[6-1130-P] Postharvest/Food Technology and Process Engineering (6th)

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

[6-1130-P-18] Optimization and Evaluating of Pomegranate Peel Extract by Micro Wet Milling Using Response Surface Methodology

*Rasool Khan Amini[1], Yutaka Kitamura[2], Mito Kokawa[2], M. Z. Islam[2] (1. Graduate School of Life and Environmental Sciences, University of Tsukuba(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tennoda, Tsukuba, Ibaraki 305-8572, Japan(Japan))

11:30 AM - 12:30 PM

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

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*Yuri Koshima[1], Yutaka Kitamura[1], Mito Kokawa[1], Thais M.F.S. Vieira[2], Juliana Antunes Gavalão[2], Luis Felipe de Freitas Fabricio[2], Md Zohurul Islam[1] (1. University of Tsukuba(Japan), 2. University of Sao Paulo(Brazil))

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*Yoshiki Muramatsu[1], Masanori Hashiguchi[2], Eiichiro Sakaguchi[1], Shotaro Kawakami[1] (1. Tokyo University of Agriculture(Japan), 2. Keisoku Engineering System Co., Ltd.(Japan))

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

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

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11:30 AM - 12:30 PM

[6-1130-P-26] The effect of molecular hydrogen on the shelf life of banana

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[6-1130-P-27] The Potential of Biogas Production from Caribbean Seaweed Biomass

*Yuhendra AP[1], Mohamed Farghali[1], Takaki Yamashiro[2], Ryuichi Sakai[3], Kazutaka Umetsu[1] （1. Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine(Japan), 2. Tokachi Agri Works(Japan), 3. Graduate School of Fisheries Sciences, Hokkaido University(Japan)）

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11:30 AM - 12:30 PM

[6-1130-P-30] High pressure processing of ‘Nanglae’ pineapple juice: Quality preservation and shelf life extension

Nuntawan Chuensombat[1], Natthakan Rungraeng[1], Sutthiwal Setha[1,2], *Phunsiri Suthiluk[1,2]

（1. School of Agro-Industry, Mae Fah luang University, Chiang Rai, THAILAND(Thailand), 2. Research Group of Postharvest Technology, School of Agro-Industry, Mae Fah Luang University, Chaing Rai, THAILAND(Thailand)）
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*Rasool Khan Amini[1], Yutaka Kitamura[2], Mito Kokawa[2], M. Z. Islam[2] (1. Graduate School of Life and Environmental Sciences, University of Tsukuba(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tennoda, Tsukuba, Ibaraki 305-8572, Japan(Japan))

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

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

Optimization and Evaluating of Pomegranate Peel Extract by Micro Wet Milling Using Response Surface Methodology

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

Pomegranate peel extract, Antioxidant, MWM, phenolics, RSM

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

*nutthachai pongprasert[1], Varit Srilaong[1,2], Songsin Photchanachai[1,2], Panida Boonyaritthongchai[1,2], Kornkanok Aryusuk[3] (1. Postharvest Technology Program, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bangkok 10140(Thailand), 2. Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400,Thailand(Thailand), 3. Biochemical Technology Program, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bangkok 10140(Thailand))

Keywords: Palm oil based wax, 'Khai' banana, Senescence spot, Ripening

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

The Effect of Palm Oil Based Wax Coating on Delaying of Ripening and Reduce Senescence Spot of 'Khai' Banana

Nutthachai Pongprasert[1,2] Varit Srilaong[1,2] Songsin Photchanachai[1,2] Panida Boonyaritthongchai[1,2] Kornkanok Aryusuk[3]

[1] Postharvest Technology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10140
[2] Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400, Thailand
[3] Biochemical Technology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10140

*Corresponding author: nutthachai.pon@kmutt.ac.th

ABSTRACT

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

Keywords: Palm oil based wax, Khai banana, Senescence spot, Ripening

1. INTRODUCTION

Banana is a quite popular tropical fruit, especially in commercial local trade. It contains a lot of nutrients and minerals which are very beneficial for health. Banana is a climacteric fruit which has a short shelf-life at ambient temperature. The short shelf life of bananas is attributed to a rapid senescence process leading to deterioration in visual appearances of the fruit peel. Senescent spotting of the banana peel is a physiological postharvest disorder. Initially, some very small brownish spots are found locally. Subsequently, such spots are observed all over the peel, and their number, intensity of browning, and size increase. The spots may then overlap to form larger patches, they become dark brown or even black, and form sunken pits on the surface. Little is known both about the origin of this disorder and about its physiological mechanism (Ketsa, 1996, 2000).

Several postharvest technologies, including low temperature storage, control atmosphere storage, and surface coating of fruit have been investigated to delay fruit ripening (Ahmed & Palta, 2016; Deng et.,all.,2017) Recently, there have been many researches on edible coatings and films to diminish crop losses and maintain the quality of fresh fruit for a longer period. Edible coating is one of the methods of extending postharvest shelf-life. Many edible coating techniques to extend the shelf life and prolong freshness of fruits have been developed using polyethylene wax emulsion, bee wax, carnuba, candelilla, chitosan, and paraffin (Po-Jung, 2007; Lozel S., 2010; Shahidi, F., 1999) Natural materials that used to produce edible coatings can be divided into three categories including Hydrocolloids, polysaccharides, proteins , lipids, fatty acids, waxes, and composites (Navarro-Tarazaga, et al.,2008). Among lipids, waxes are the most attractive and promising coating materials for fruits and vegetables. The wax-based coatings are known to have a

good barrier capacity against moisture transfer. In addition, it has been used to reduce respiration, improve appearance of fruits and vegetables by generating a shiny skin (Morillon, et al, 2002) In this work, the palm oil wax ester (POWE) was prepared and used as POWE-based coating emulsion to maintain the quality and reduce senescence spot of 'Khai' banana.

2. MATERIALS AND METHODS

Banana fruit of the cultivar 'Khai' (*Musa acuminata*, AA Group) fruits at mature green and ripening stage were obtained from a wholesale market in Bangkok, THAILAND. Fingers were selected and separated from the bunch, sorted to eliminate damaged and shriveled fruit. Selected fruits were randomized and used for the experiment. After sorting, banana fruit were stored at 25°C for 8 days. Bananas were randomly sampled for determination of percentage of weight loss, pulp firmness, ethylene production, respiration and senescence spotting.

Percentage of weight loss was determined by weighing the banana on a digital balance. It was reported as percentage loss in weight based on the original mass.

Firmness of banana was determined using a Texture Analyzer, TA-XT2i (Stable Micro Systems, Surrey, UK). A load cell of 5 kg and a 5 mm cylindrical plunger at a constant penetration speed of 2 mm/min was used. Each fruit was penetrated at 3 different locations.

Senescence of peel spotting was determined using a scale of 1- 6, where 1 means no spotting and 6 severe spotting. Score 1: peel yellow without spots; score 2: the surface is a little darker yellow, some brownish spots occur, small as the point of a needle; score 3: spots found around 20% of peel surface area. Score 4: spots found around 30% of peel surface area. Score 5: spots found around 40% of peel surface area. Score 6: spots found more than 40% of peel surface area size, spots has further increased, they sometimes overlap into larger patches spots are darker, or are even black, and form sunken pits on the surface.

For statistical analysis, the ANOVA procedure was used, and mean separation was analyzed by Least significant difference (LSD) ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

During postharvest period, fruit and vegetables are susceptible to water loss, which reduces the quality and value of fresh produce (Cosme Silva et al., 2017). Coating with an palm oil based wax can significantly reduce weight loss both mature green and ripening stage banana. Water losses was continuous throughout the storage period, with the control showed a higher in weight loss than the palm oil based wax coated fruit (Fig.1) Nawab et al. (2017) observed a similar reduction in weight loss in tomato fruit coated with a starch-based film. Kerdchoechuen et. al.,2011) also studied the starch films coating on citrus fruit, reporting that coated fruit showed 4.8–7.7% less weight loss relative to the control. As water play an important role in fruit shelf life and quality, less loss of water is critical and our findings showed that palm oil based wax is effective to minimize the water loss for banana during storage.

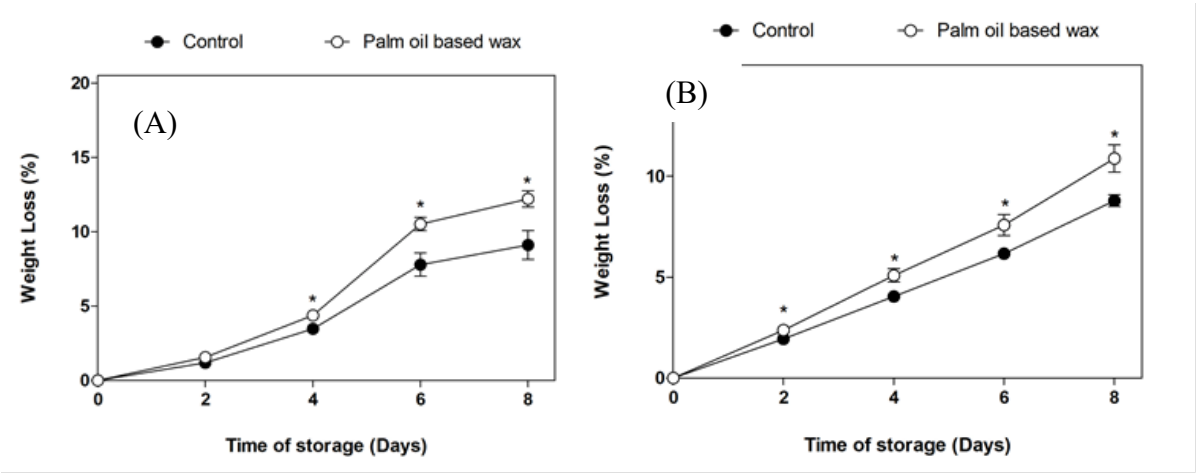

Figure 1. Changes in weight loss (%) of 'Khai' banana at mature green stage (A) and ripening stage (B) after coated with palm oil based wax stored at 25°C for 8 days. The vertical bars represent the standard error of means for five replicates.

A continuously loss in the fruit firmness was observed in both the control and coated fruit during ripening. Firmness loss, expressed as fruit softening, are related to dehydration and loss of integrity in cell wall structures during the course of fruit ripening (Deng etal.,2017). The higher firmness on the 0 day indicates the compact tissues and firm nature of banana fruit (Fig. 2A,B). The treated ripening stage fruit showed a lower rate of loss of firmness compared to the control through out the storage time to day 8 (Fig.2B). For mature green stage fruit, both control and treated fruit undergoes rapid fruit softening within the first two days of ripening and remained relatively constant in the subsequent sampling times (Fig.2A). Better retention of firmness in case of coated fruit indicated that palm oil based wax was effective in slowing down the metabolic and enzymatic activities in fruit, resulted into the slower degradation of pulp tissues**.**

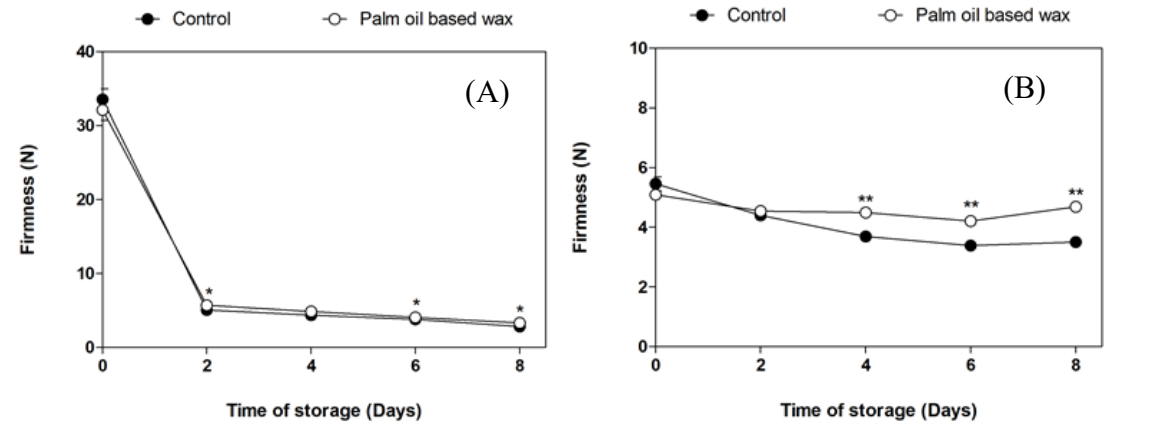

Figure 2. Changes in firmness (N) of 'Khai' banana at mature green stage (A) and ripening stage (B) after coated with palm oil based wax stored at 25°C for 8 days. The vertical bars represent the standard error of means for five replicates.

Ripening in climacteric fruit such as banana is characterised by a significant and rapid increase in respiration rate which is accompanied by intensive metabolic change (Wills & Golding,2016). Respiration rate and endogenous ethylene production rates tracked similarly during the storage period, decreasing over the first 2 days, then increase further till day 8 of storage (Fig.3). Respiration rate in the control fruit was significantly and consistently greater than the coated fruit across the entirety of the storage period. Importantly, the maximum respiration rate in the

treated fruit was maintained below the minimum value observed for the control across the entire storage period.

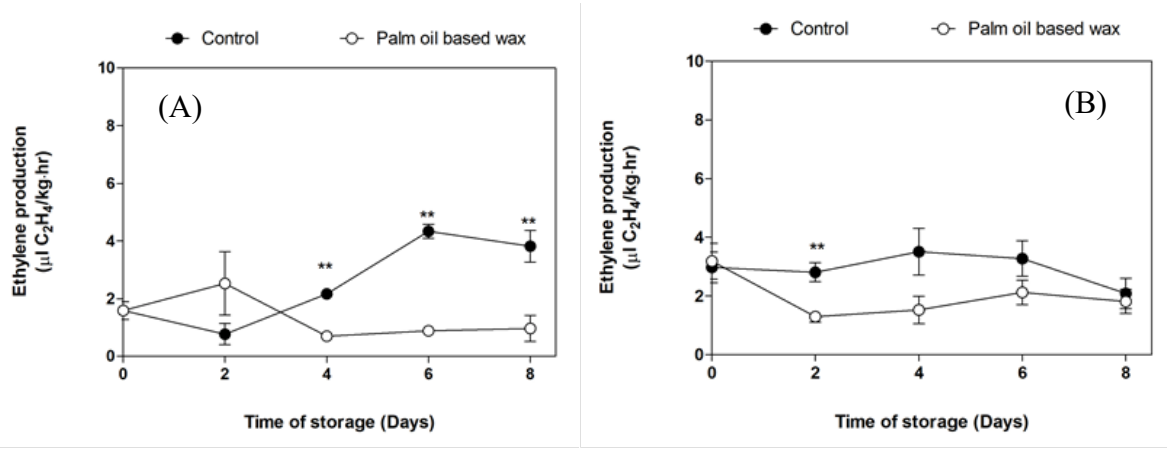

Figure 3. Changes in ethylene production of 'Khai' banana at mature green stage (A) and ripening stage (B) after coated with palm oil based wax stored at 25°C for 8 days. The vertical bars represent the standard error of means for five replicates.

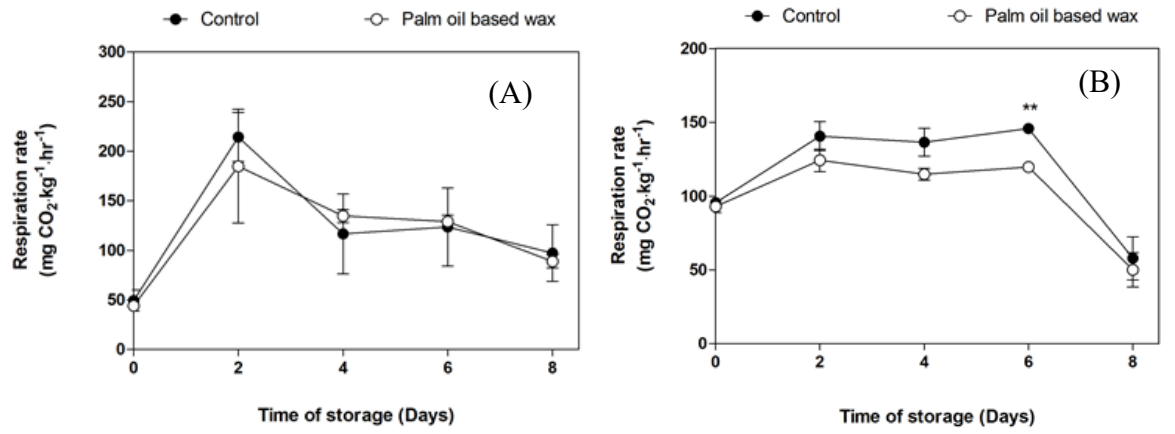

Figure 4. Changes in respiration rate of 'Khai' banana at mature green stage (A) and ripening stage (B) after coated with palm oil based wax stored at 25°C for 8 days. The vertical bars represent the standard error of means for five replicates.

The symptoms of senescence spotting incidence increased during storage period both mature green or ripening stage. However, banana at ripening stage exhibited faster senescence spotting than that of mature green stage banana. Mature green banana showed a rapid increase in senescence spotting at day 6 of storage while ripening stage banana showed the obvious symptom at day 4 of storage. Palm oil based wax was effective to reduce the senescence spotting of banana during storage especially banana fruits at ripening stage. Previously study shown that a lower than atmospheric oxygen level (5 kPa) inhibited peel spotting in 'Sucrier' banana (Choehom et al., 2004). This suggested that the spot-ting required rather elevated oxygen concentrations. The reduction of oxygen level after coated with palm oil based wax may reduced the senescence spotting of 'Khai' banana.

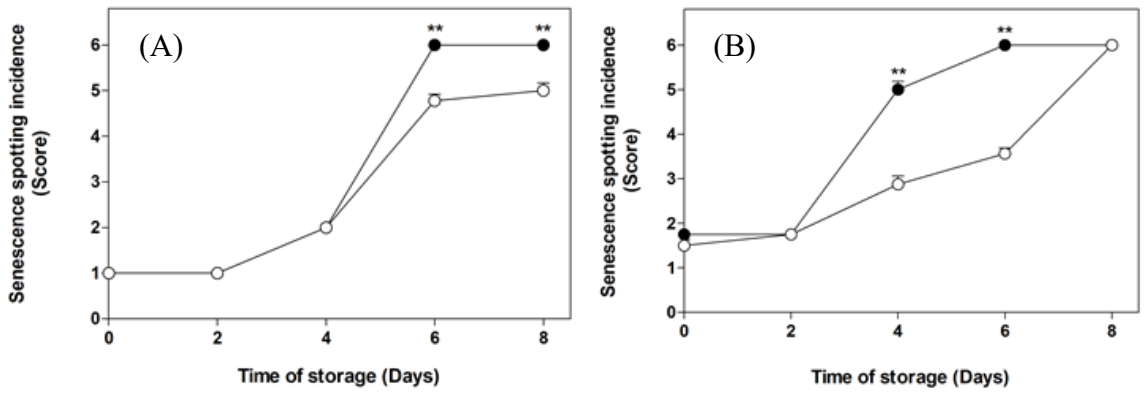

Figure 5. Changes in senescence spotting incidence of 'Khai' banana at mature green stage (A) and ripening stage (B) after coated with palm oil based wax stored at 25°C for 8 days. The vertical bars represent the standard error of means for five replicates.

4. CONCLUSION

These results can be concluded that coating with palm oil based wax has the potential to delay the ripening and maintained the quality as well as reduce the senescence spotting of 'Khai' banana fruit both at mature green and ripening stage.

ACKNOWLEDGMENT

This work was supported by a grant from National Research Council of Thailand (NRCT) Grant No. 61000146

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

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

*Tatsuya Oshima[1], Kodai Kato[1], Satoshi Iwamoto[1], Teppei Imaizumi[1] (1. Gifu University(Japan))

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

‘Fuyu’ persimmon is popular in Japan and distributed not only as fresh fruits but also dried ones. Although fruits and vegetables are often dried by hot air, length of the drying time is considered as a big problem. To improve drying efficiency, blanching treatment is sometimes applied prior to drying. However, for ‘Fuyu’ persimmon, effects of these processes on drying characteristics and quality-related components are not clarified sufficiently. Among components in fruits, pectic substances are known to contribute to texture formation. Additionally, functional aspects of the substances, such as intestinal regulating function and prebiotic effect, also attract attention. Thus, we investigated drying characteristics and changes in pectin states during several drying treatments including blanching. Persimmon fruits (cv ‘Fuyu’) harvested in Gifu city was used in this study. The persimmon flesh was cut into cylinder (20.5 mm diameter and 10 mm height) using a cork borer and a knife. The initial moisture content of the sample was 5.06 (dry basis). In this study, we prepared blanched and non-blanched samples. For the blanched one, the persimmon samples were immersed in hot water at 95 degree for 2 min, then immediately cooled in iced water for 2 min. Both of the blanched and the non-blanched samples were dried in a forced hot air oven controlled at 40, 50, 60 and 70 degree. During the drying, sample weight was weighed at every hour, and converted to moisture content. A model was fitted to measured value and rate constant k (h^{-1}) of the drying process was calculated for each temperature. Next, alcohol insoluble solid (AIS) of the samples, which dried at 40 and 60 degree until 2.0 and 0.3 (d.b.), was prepared to extract pectin. Pectin fractions were sequentially extracted from AIS with distilled water, 0.05 M CDTA solution and 0.05 M Na_2CO_3 + 20 mM $NaBH_4$ solution, and water-soluble pectin (WSP) fraction, chelator-soluble pectin (CSP) fraction and diluted alkali-soluble pectin (DASP) fraction were collected, respectively. Galacturonic acid content in pectin fractions were determined using carbazole sulfuric acid method. Also, atomic force microscopy (AFM) observation was performed. Extracted pectin fractions were diluted 200 times with distilled water. A 3 μl of the resulting solution was dropped onto freshly cleaved mica, then it were dried overnight at room temperature. An AFM5400L (Hitachi High Technologies) was used for imaging in the dynamic force mode. A silicon cantilever with nominal spring constant of 15 N m^{-1} and resonant frequency of 110-150 kHz was used. The scanning area was set at 2 µm × 2 µm in the XY plane, and the scanning resolution was 512 × 512 points. AFM images were morphologically analyzed using the SPIP software. Regardless of whether it was blanched sample or not in the drying process, the exponential model could be fitted (R^2=0.9962 - 0.9996). Comparing the obtained rate constants, blanched samples had high value, which indicating blanching is effective to improve the drying process. In addition, we prepared dried samples having 2.0 and 0.3 of moisture content (d.b.) and evaluated state of pectin in these samples. After the blanching, the ratio of WSP amount in total pectin obviously decreased, and the ratio of CSP and DASP increased. However, the ratio of WSP increased with drying in all samples. At 0.3 of moisture content (d.b.), Overall, the blanching treatment indicated greater effect on pectin composition than the drying process. In AFM images of pectin nanostructure, short chain and granule like objects were appeared in WSP. Also, lager structures were observed in CSP. The result in this study shown that drying treatment and blanching pretreatment changed pectin composition and structure. Thus, we assumed that texture and functional properties of dried products will be modified by selected conditions.

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

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

*JIA FANG[1], Yutaka KITAMURA[1], Mito KOKAWA[1], Kazunobu KAJIHARA[2], Kozi KAWAKAMI[2], Hidenori MIZUNO[2]
（1. Tsukuba Univ.(Japan), 2. Satake Corporation(Japan)）

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

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

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

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

*Yuri Koshima[1], Yutaka Kitamura[1], Mito Kokawa[1], Thais M.F.S. Vieira[2], Juliana Antunes Gavalão[2], Luis Felipe de Freitas Fabricio[2], Md Zohurul Islam[1] （1. University of Tsukuba(Japan), 2. University of Sao Paulo(Brazil)）

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

Coffee is the most consumed food product in the world. Among them, coffee beans which are evaluated as 80 or more points in the sensory evaluation of Specialty Coffee Association of America (SCAA) is called specialty coffee. And specialty coffee has unique flavor characteristics and high traceability of the value chain. Specialty coffee consumption is increasing in recent years. Flavor is the most important criteria for coffee quality evaluation, and also one of the major motivations for consumer preferences. The storage period of specialty coffee is relatively short compared to commodity coffee, but some commercial products are stored for a period longer than the recommendation of specialists. Many studies have conducted on aromatic components such as phenol in coffee, but there are still few findings on lipid oxidation. Roasting induces transformation on chemical and physical composition in coffee beans. During storage, further chemical and physical changes that affect the quality of brew may occur. Along with this change, the

acceptability of consumers also changes. In this experiment, the quality change of coffee due to oxidation of lipid is clarified. Catuai Amarelo coffee cultivars from Alta Mogiana, SP, Brazil was used. The sample was harvested on August 2018 then processed according to natural method. Green bean, light roasted bean, medium roasted bean and dark roasted bean were stored up to 85 days then analyzed for chemical components of lipid oxidation. Hydroperoxide content as a primary oxidation compounds evolution during storage were monitored by conjugated dienes and trienes determination by spectrophotometric method. Free fatty acids (FFA) as a secondary oxidation compounds were evaluated by American Oil Chemists' Society (AOCS) method. The sensory evaluation was conducted according to the sensory test protocol of SCAA. The production of fatty acid, which is the final product of lipid oxidation, stabilized after transient increase. On the other hand, the sensory evaluation score decreased overall. A weak correlation was found between fatty acid content and sensory score, with a correlation coefficient of R^2=0.39.

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

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

*Yoshiki Muramatsu[1], Masanori Hashiguchi[2], Eiichiro Sakaguchi[1], Shotaro Kawakami[1] (1. Tokyo University of Agriculture(Japan), 2. Keisoku Engineering System Co., Ltd.(Japan))

Keywords: Thermal diffusivity, Heat transfer simulation, Finite element method, Agricultural products, Inverse problem

The thermal diffusivity is an important thermophysical property needed in modeling and computations of transient heat transfer in basic food processing. In addition, the prediction of nutritional and microbial changes occurring in food during thermal processing requires knowledge of thermal diffusivity of foods. The measurement methods of thermal diffusivity are classified into direct measurement, to which the present work belongs, and indirect measurement. The thermal diffusivity can be obtained from the experimentally determined values of thermal conductivity, specific heat, and density in the indirect measurement. This indirect measurement requires much time and experimentation. Some direct measurement methods need expensive and/or special devices. In addition, it is frequently necessary to do the complicated calculation procedures to determine thermal diffusivity under direct measurement methods. Therefore, it would be useful to easily determine thermal diffusivity with simple and inexpensive devices. The thermal diffusivity of some agricultural products and foods have been measured by Dickerson method. The calculation in the Dickerson method is based on the analytical solution of the heat conduction equation. Several kinds of the analytical solution of the heat conduction equation have been used to estimate the thermal diffusivity of the agricultural product based on the temperature profiles of the material. However, the geometry and/or the boundary conditions are strictly limited for those methods. The objectives of this study were to propose a new determination method of thermal diffusivity and to estimate the thermal diffusivity of some agricultural products using that new method. Thermal diffusivities of three kinds of vegetable (burdock, carrot, and radish) were estimated using an inverse technique. The burdock and radish were cut into a cylinder (diameter (D) 20 and height (H) 100 mm). The carrot was created three kinds of geometry: cylinder (D = 20, H = 100 mm), cylinder (D = 20, H = 20 mm), and disk (D = 40, H = 10 mm). Each sample was fitted with a needle-type thermocouple to measure the center temperature. The samples were heated in a water bath at 90° C. The rotational axisymmetric 2-dimensional transient heat conduction problem for radial coordinates and the 3-dimensional transient heat conduction problem for cartesian coordinates were numerically solved by a finite element method using the commercial finite element software: COMSOL Multiphysics®. The thermal diffusivity of each sample was determined by an ordinary nonlinear least squares method using the

MATLAB® which is a programming platform designed specifically for engineers and scientists, and a numerical optimization technique using COMSOL Multiphysics®, respectively. The thermal diffusivity values of the samples ranged from 1.1×10^{-7} to 1.5×10^{-7} m^2/s by the ordinary least squares method. A significant difference was not statistically recognized among the values of thermal diffusivity of all sample sizes and shapes for the carrot. Also, between the rotational axisymmetric 2-dimensional analysis and the 3-dimensional analysis, there was no significant difference for all samples. The advantages of this method are that the device and the estimation method are simple, inexpensive, rapid, and can apply to various shapes of a sample and the dimension. The results obtained in this study will be useful in the design of equipment and in calculations for the thermal processing of vegetables.

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

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

*Natthakan Rungraeng[1,2], Supaluck Kraithong[1] （1. School of Agro-Industry, Mae Fah Luang University, Chiang Rai, Thailand 57100(Thailand), 2. Unit of Innovative Food Packaging and Biomaterials, Mae Fah Luang University, Chiang Rai, Thailand 57100(Thailand)）
Keywords: Acidic extraction, Waste utilization, Pectin properties, Raspberry jam

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

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

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

*Daisuke Kurata[1], Takahiro Orikasa[2,3], Shoji Koide[2] （1. Graduate School of Arts and Sciences, Iwate University.(Japan), 2. Faculty of Agriculture, Iwate University.(Japan), 3. Agri-Innovation Center, Iwate University.(Japan)）

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

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

Poster Session | Postharvest/Food Technology and Process Engineering
[6-1130-P] Postharvest/Food Technology and Process Engineering (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-26] The effect of molecular hydrogen on the shelf life of banana

*Naoya Fujino[1], Teruo Wada[1] (1. Osaka Prefecture University(Japan))

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

Molecular hydrogen has been known to have the ability to eliminate reactive oxygen species. It is thought that the hydrogen can inhibit oxidation of biological membranes by reactive oxygen especially because it can dissolve more in lipids than in water. The aim of this study was to improve the shelf life of fruit and vegetable using molecular hydrogen.
Commercially available banana was used for the experiments.
First, hydrogen water was mist-sprayed to banana. hydrogen water was made in the polyethylene terephthalate bottle containing deionized water with hydrogen generated from the mixture of aluminum, calcium oxide and water. Two test chambers (W: L: H = 0.5: 0.4: 0.9 m) was used in this experiment. the mist was sprayed from the bottom of the chamber 0.18 m below the banana, and ventilation fan fixed on the roof of the chamber was operated during mist-spraying. Deionized water or hydrogen water (hydrogen concentration was approx. 3 mg L^{-1}) was mist-sprayed for 10 min every hour during the storage at 25 ˚C. The mist-spray of hydrogen water decreased the appearance of brown spots on the skin of banana. However, saturated humidity in the box sometimes progress the decay of stem end of fruit. And then, when the relative humidity in the test chamber was kept to 95% or less with ventilation fan operated by monitoring the humidity, it was shown that the progress of decay with high humidity during storage could be suppressed.
Next, hydrogen gas was treated to banana. Hydrogen gas was generated by hydrogen generator. Half green colored banana was kept in the air- tight box and stored at 5 ˚C. Ambient air containing 0 or 4% of hydrogen gas was filled in the box. The air in the box was exchanged with the same gases every 3 days. When banana fruits were stored with 4% of hydrogen gas, the skin browning by chilling injury was suppressed. Moreover, even if banana fruits were kept in 4% of hydrogen gas for only 24 h and then kept in the air without hydrogen, the skin browning was suppressed. When banana fruits were pretreated with 0 (control), 1, 2, 4, 10, 50 % of hydrogen gas for 24 h in the box and then the fruits were taken out of the box and put in perforated polypropylene bag without hydrogen gas and stored at room temperature, the appearance of brown spot on the skin was significantly suppressed under 2 or 4% of hydrogen concentration, comparing with control.
From our results, it was suggested that the treatments of molecular hydrogen could suppress the skin browning and prolong the shelf life of banana fruit during storage at both of room temperature and low temperature.

Effect of Molecular Hydrogen on the Shelf Life of Banana

Naoya Fujino, Teruo Wada

Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Japan

*Corresponding author: wadoo@bioinfo.osakafu-u.ac.jp

ABSTRACT

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

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

1. INTRODUCTION

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

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

In our previous study, when some fruits and vegetables were treated with hydrogen water, shelf life of them tended to increase, but long time wetting of hydrogen water on the surface of fruits and vegetables promoted deterioration of them.

The aim of this study was to improve the shelf life of banana using molecular hydrogen. As the methods which molecular hydrogen was treated to the fruits without long time wetting of hydrogen dip, two methods as intermittent misting of hydrogen water and treatment of hydrogen gas were examined.

2. MATERIALS AND METHODS

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

2.1.1 Treatment without Humidity Control

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

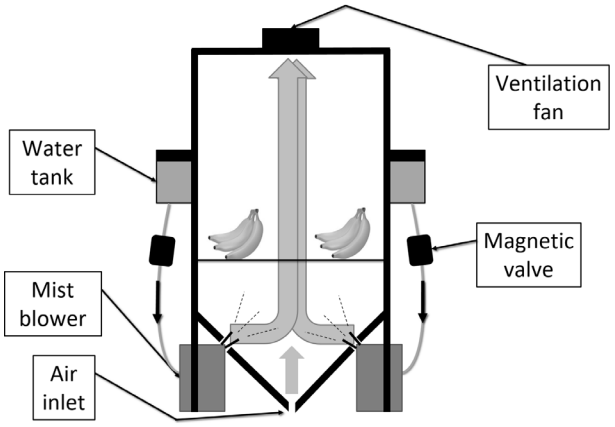

Figure 1. Schematic diagram of the chamber used in mist treatment of this study.

2.1.2 Treatment with Humidity Control

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

2.2 Effect of Hydrogen Gas Treatment

2.2.1 Effect on Chilling Injury

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

2.2.2 Effect on Shelf Life of Banana Fruit at Room Temperature

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

2.3 Methods of Measurements

2.3.1 Fruit Firmness

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

2.3.2 Percentage of Area of brown spots on skin

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

2.3.3 Evaluation of Degree of Chilling Injury

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

3. RESULTS AND DISCUSSION

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

3.1.1 Treatment without Humidity Control

The mist-spray of hydrogen water seemed to decrease the occurrence of brown spots on the skin of fruit (Figure 2). While, decay from the stem end of fruit was observed in both of deionized water and hydrogen water treatments. It was thought that mostly saturated humidity in the chamber might progress the decay. The packing of OPP film increased the occurrence of brown spots and progress the decay. It might be also caused by higher humidity in the film. In the film, drops of dew might appear on the surface of the fruits.

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

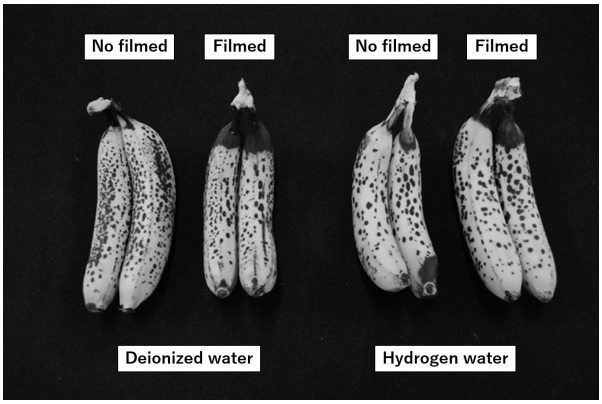

Figure 2. Appearance of banana fruits mist-sprayed without humidity control on 15 days after the start of experiment.

3.1.2 Treatment with Humidity Control

The humidity control could suppress the decay from the stem end of fruits (Data not shown).

The brown spots area on the skin of fruit had no significant difference between the kind of misted water in filmed fruits, but it was significantly lower in the hydrogen water treatment than the deionized water treatment in no-filmed fruits (Figure 3). The brown spots area of the no-filmed fruit treated with hydrogen water was lowest than that in any treatments.

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

In this experiment, it was suggested that misting of hydrogen could suppress occurrence of brown spot of skin of banana fruit during the preservation at room temperature. In addition, it was shown that the

humidity control delayed the occurrence of rot and brown spots from the stem end of the banana fruit. Further research was needed in order to defined appropriate condition of misting of hydrogen water for more effective treatment.

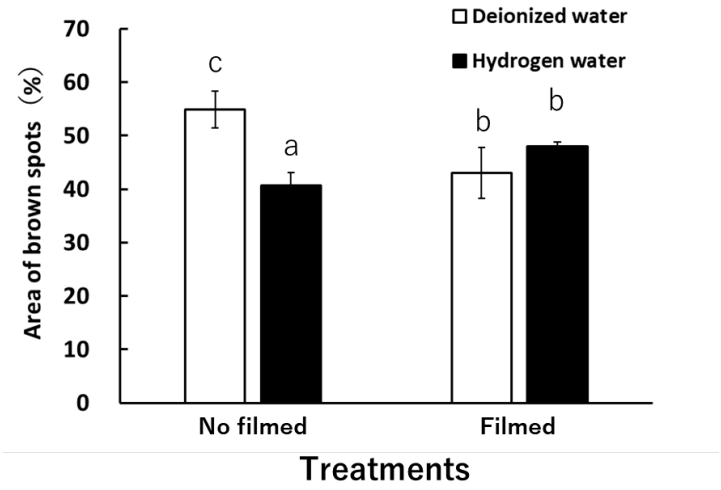

Figure 3. Effect of misting of hydrogen water on the percentage of area of brown spots on the skin of banana fruits after 15 days preservation. Same letter indicates no significant difference by Tukey's HSD test (P = 0.05). Vertical bars indicate s.e. (n = 3).

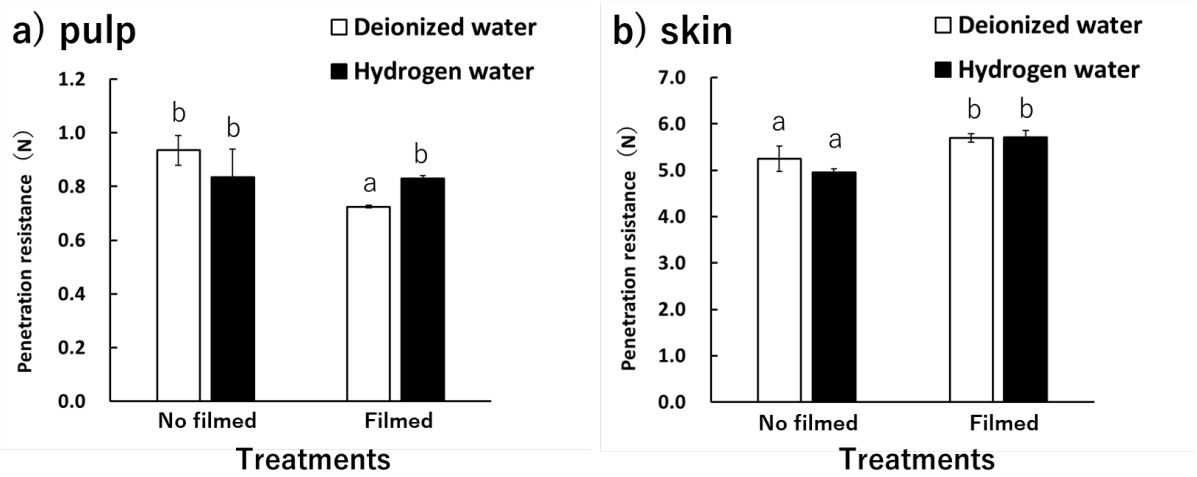

Figure 4. Effect of misting of hydrogen water on penetration resistance of pulp (a) and skin (b) of banana fruit after 15 days preservation. Same letter in each figure indicates no significant difference by Tukey's HSD test (P = 0.05). Vertical bars indicate s.e. (n = 6).

3.2 Effect of Hydrogen Gas Treatment

3.2.1 Effect on Chilling Injury

On 8 days after the start of experiment, browning of skin was suppressed by both of pre and continuous treatment of hydrogen gas, compared to control. On 15 days, the browning of the skin developed in control, but the development of browning was significantly suppressed in both of hydrogen gas treatments (Figure 5).

L* value of the skin was significantly high in both of hydrogen treatments than control (Figure 6). There was no significant difference between the two hydrogen gas treatments. L* value indicates brightness. Higher L* value means brighter skin color. From this result, it was shown that hydrogen gas treatments could suppress the browning of skin caused by chilling injury, even if the hydrogen gas was treated only for first 24 h during preservation.

The penetration resistance of skin after 15 days preservation at 5°C tended to increase by hydrogen gas treatments, and it was significantly high in continuous treatment of hydrogen, compared with control (Figure 7).

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

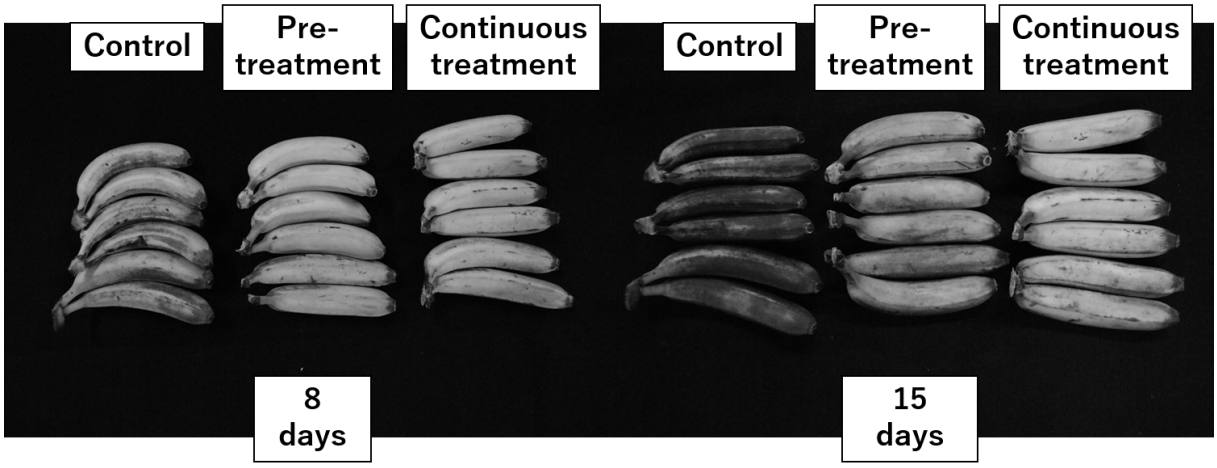

Figure 5. Appearance of banana fruits treated with hydrogen gas on 8 (left) and 15 (right) days after the start of experiment. The fruits were stored at 5°C.

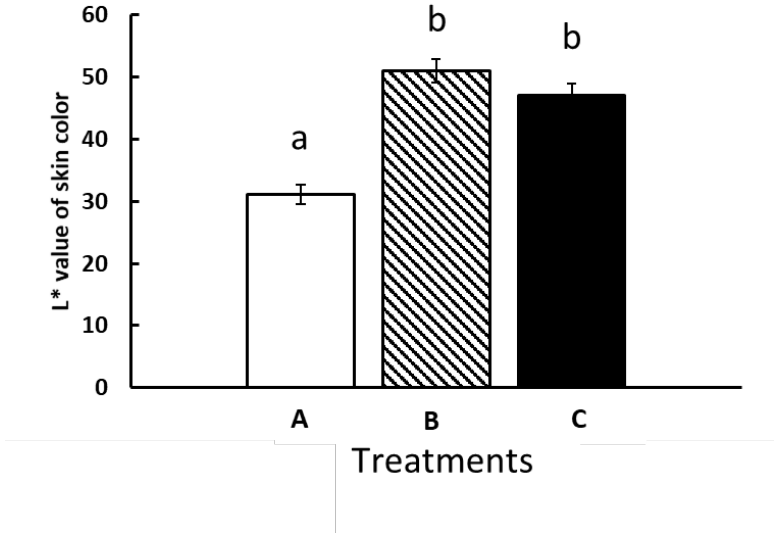

Figure 6. Effect of hydrogen gas treatments (A: Control, B: Pretreatment with hydrogen gas, C: Continuous treatment with hydrogen gas) on the L* value of skin color of banana fruit after 15 days preservation at 5°C. Same letter indicates no significant difference by Tukey's HSD test (P = 0.05). Vertical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

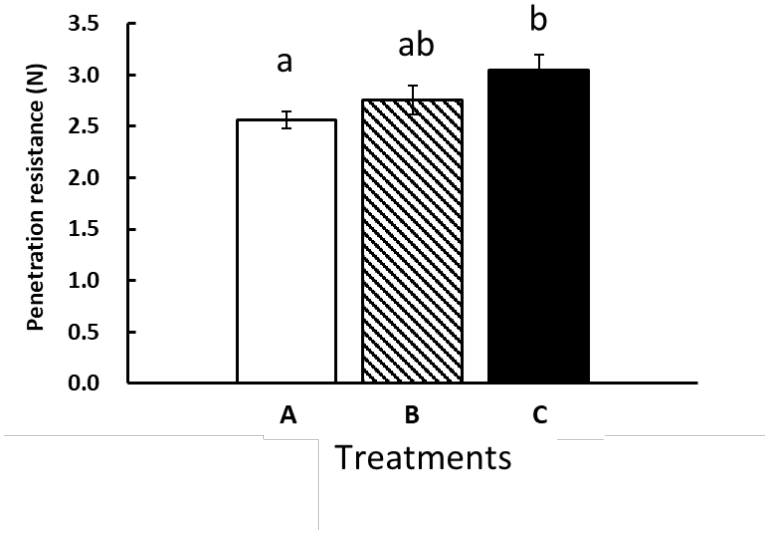

Figure 7. Effect of hydrogen gas treatments (A: Control, B: Pretreatment with hydrogen gas, C: Continuous treatment with hydrogen gas) on the penetration resistance of skin of banana fruit after 15 days preservation at 5°C. Same letter indicates no significant difference by Tukey's HSD test (P = 0.05). Vertical bars indicate s.e. (n = 6).

3.2.2 Effect on Shelf Life of Banana Fruit at Room Temperature

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

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

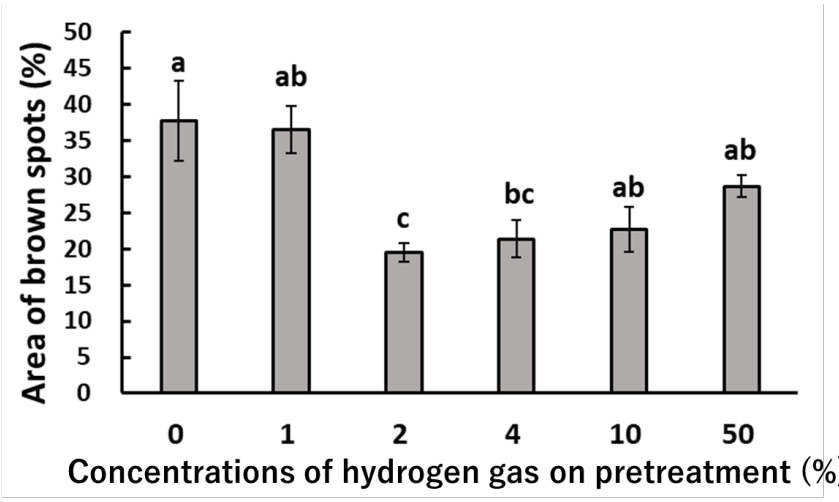

Figure 8. Effect of pretreatment of hydrogen gas on the percentage of area of brown spots of banana fruit after 11 days preservation at 25°C. Same letter indicates no significant difference by Tukey's HSD test (P = 0.05). Vertical bars indicate s.e. (n = 3).

4. CONCLUSION

From these results, it was suggested that the occurrence of brown spots on banana fruits could be suppressed by mist-spray of molecular hydrogen without of progress of decay if the relative humidity kept below 95%. In addition, it was suggested that the occurrence of chilling injury and brown spots of banana fruit could be suppressed by treatment of hydrogen gas. It may be possible to prolong the shelf

life of banana fruits by the treatment with molecular hydrogen. These phenomena might be caused by suppressing the oxidation of cell membrane in the skin of banana fruit by molecular hydrogen. Further research will be needed to make clear the mechanism of effects of molecular hydrogen.

ACKNOWLEDGMENT

A part of this study was financially supported by Industrial Technology Agency of Co-Creation (ITAC). Development of the chambers used in mist treatment was cooperated with Konishi Seiko Co., Ltd. We are pleased to acknowledge the cooperation of them.

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Poster Session | Postharvest/Food Technology and Process Engineering
[6-1130-P] Postharvest/Food Technology and Process Engineering (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-27] The Potential of Biogas Production from Caribbean Seaweed Biomass

*Yuhendra AP[1], Mohamed Farghali[1], Takaki Yamashiro[2], Ryuichi Sakai[3], Kazutaka Umetsu[1] (1. Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine(Japan), 2. Tokachi Agri Works(Japan), 3. Graduate School of Fisheries Sciences, Hokkaido University(Japan))
Keywords: Saint Lucia, Seaweed, Sargassum Fulvellum, Anaerobic digestion, Biogas

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

The Potential of Biogas Production from Caribbean Seaweed Biomass

Yuhendra AP[1**], Mohamed Farghali[1], Takaki Yamashiro[2], Ryuichi Sakai[3], Kazutaka Umetsu[1*]
[1] Department of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Japan
[2] Tokachi Agri Works
[3] Graduate School of Fisheries Sciences, Hokkaido University
* Corresponding author: umetsu@obihiro.ac.jp

ABSTRACT

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

1. INTRODUCTION

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

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

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

In a long time, history, seaweed just only used for food and cosmetics product. Seaweed is not much ogled as a substitute for renewable energy. Recently, after many researchers have examined the content of seaweed can be used as alternative energy. Seaweed biomass as third-generation feedstock is used as green energy for biofuels and biogas. In particular, seaweed biomass is not quite used as a food source on a global scale, like palm oil or corn as the first-generation for renewable energy.

All types of organic waste can be processed to produce biogas such as biomass waste, human waste, animal waste can be used as energy through the anaerobic digestion process. This process is a great opportunity to produce alternative energy so that it will reduce the impact of using fossil fuels. Besides,

making biogas can reduce a variety of plant organic waste and animal waste so that it has economic value.

Anaerobic digestion may be a series of biological processes during which microorganisms break down perishable material within the absence of element. One of the top products is biogas, which is combusted to generate electricity and heat, or can be processed into renewable natural gas and transportation fuels. A range of anaerobic digestion technologies is dynamical stock manure, municipal waste product solids, food waste, high strength industrial waste product and residuals, fats, oils and grease (FOG), and varied different organic waste streams into biogas, twenty-four hours every day, seven days per week.

2. MATERIALS AND METHODS

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

2.1 Gas Produce

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

2.2. Chemical Characteristic and Composition

The amount and composition were determined daily. Substrate samples are taken before and after experimentation to determine total solid, volatile solid, volatile fatty acid and pH. Volatile solid (VS) is the weight loss after a sample is ignited (heated to dryness at 550 EC). The total solid were determined by drying the samples at 105 °C for 24 h. The solid content was calculated from the difference between weights before and after drying. The dried matter was heated at 550 °C for 4 h, and organic matter volatile solid content was calculated from the loss on ignition. Methane (CH_4) was measurement with GC (Suraju. et al. 2018; Marildo. et, al. 2018; Nayak, A. et, al. 2018).

3. RESULTS AND DISCUSSION

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

3.1 Gas Produce

Gas production after 10 days anaerobic digestion showed that increased.

3.2 Chemical Characteristic and Composition

Gas composition was measurement every day. In table 3, we can see about the gas component for six days. Methane gas higher at 1st days and 2nd days.

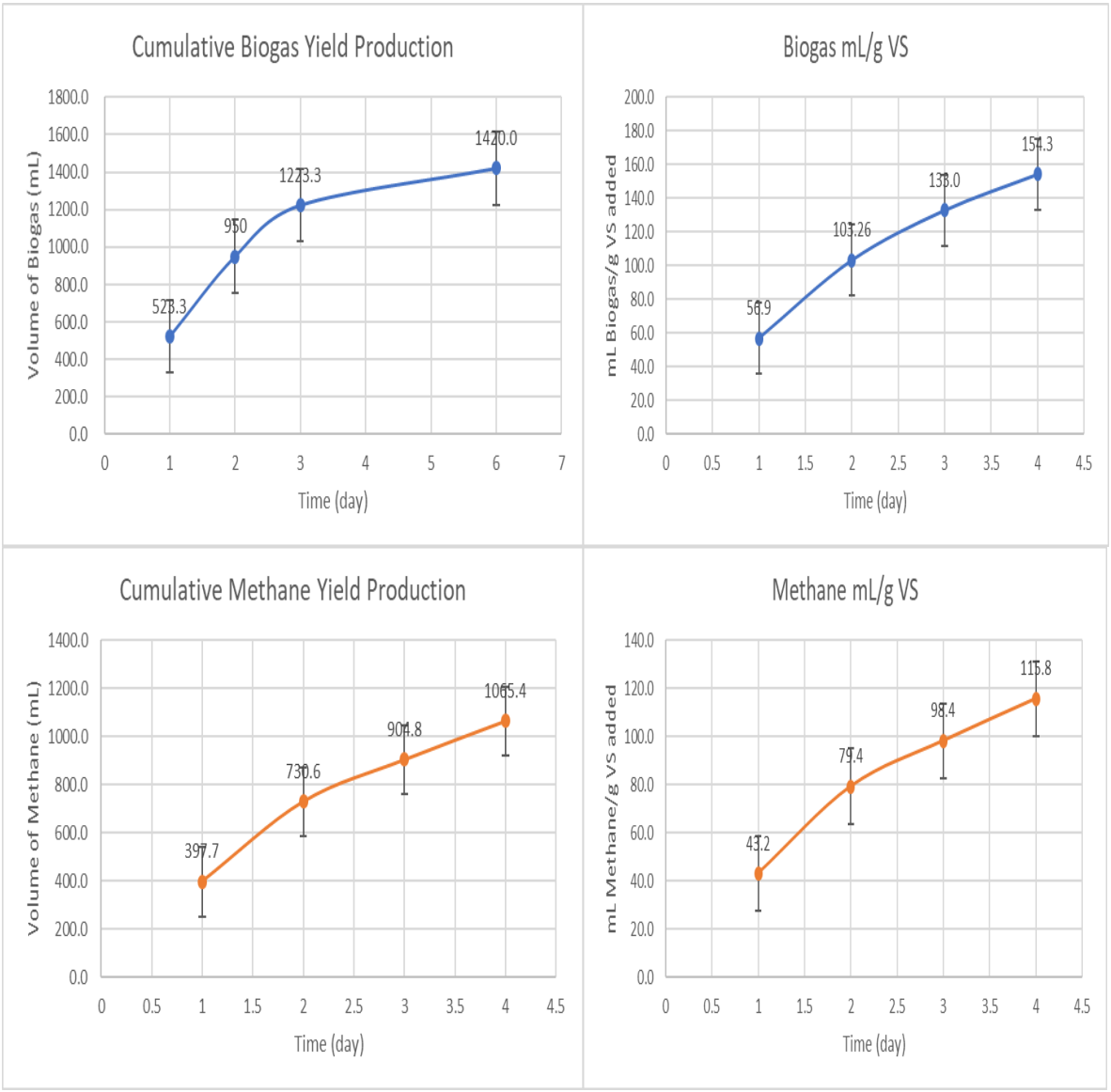

Figure 1. Cumulative biogas vs methane yield production, and biogas mL/gVS vs methane mL/gVS

Table 1. Chemical Characteristic Dried seaweed biomass.

	VS (%)	TS (%)	Ash (%)
Dried Biomass	46.14	81.19	35.05

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

Table 2. Chemical Characteristic after Anaerobic digestion.

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

Table 3. Gas Composition.

Time (day)	Gas composition		
	Biogas (mL)	Methane (%)	Carbon Dioxide (%)
1	523.3	76	24
2	426.7	78.0	22.0
3	273.3	63.7	36.3
6	196.7	81.7	18.3

4. CONCLUSION

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

ACKNOWLEDGMENT

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

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Poster Session | Postharvest/Food Technology and Process Engineering
[6-1130-P] Postharvest/Food Technology and Process Engineering (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-28] Study on the Characteristics of Micro Wet Milling and Spray Drying of Sea-buckthorn (*Hippophae rhamnoides*)

*ODGEREL Ulziibat[1], Md.ZOHURUL ISLAM[1], KITAMURA Yutaka[2], KOKAWA Mito[2], ODBAYAR Tseyen-Oidov[3], SOLONGO Ganbold[3] （1. Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan(Japan), 2. Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan(Japan), 3. School of Industrial Technology, Department of Food Engineering, Main Campus of MUST, Baga Toiruu 34, Sukhbaabatar District, Ulaanbaatar, Mongolia(Mongolia)）
Keywords: Sea-buckthorn juice, Micro wet milling, Particle size, Spray drying

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

Study on the Characteristics of Micro Wet Milling and Spray Drying of Sea-buckthorn (*Hippophae rhamnoides*)

Ulziibat ODGEREL[1], Md. Zohurul ISLAM[1], Yutaka KITAMURA[2], Mito KOKAWA[2], Tseye-Oidov ODBAYAR[3] and Ganbold SOLONGO[3]

[1]Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan
[2]Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan
[3]School of Industrial Technology, Department of Food Engineering, Main Campus of MUST, Ulaanbaatar, Mongolia

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

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

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

[6-1130-P-29] Combined Effect of Pre-treatment and Vacuum Packaging for Maintaining the Quality of Peeled Shallot (*Allium ascalonicum* L.)

*Phanida Renumarn[1], Kranert Kilian Joachim [4], Natthaya Choosuk[1], Chanthima Phungamngoen[2], Kasama Chareekhot[3] （1. Department of Innovation and Product Development Technology, Faculty of Agro-Industry, King Mongkut’s University of Technology North Bangkok(Thailand), 2. Department of Agro-Industry Technology and Management, Faculty of Agro-Industry, King Mongkut’s University of Technology North Bangkok(Thailand), 3. Department of Food Science and Technology, Faculty of Technology, Udon Thani Rajabhat University(Thailand), 4. Food Science -Technology and Economics, University of Applied Sciences Bremerhaven(Germany)）

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

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

Poster Session | Postharvest/Food Technology and Process Engineering
[6-1130-P] Postharvest/Food Technology and Process Engineering (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-30] High pressure processing of ‘Nanglae’ pineapple juice: Quality preservation and shelf life extension

Nuntawan Chuensombat[1], Natthakan Rungraeng[1], Sutthiwal Setha[1,2], *Phunsiri Suthiluk[1,2] (1. School of Agro-Industry, Mae Fah luang University, Chiang Rai, THAILAND(Thailand), 2. Research Group of Postharvest Technology, School of Agro-Industry, Mae Fah Luang University, Chaing Rai, THAILAND(Thailand))
Keywords: Bioactive compounds, Fruit juice, High hydrostatic pressure

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

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

Nuntawan Chuensombat[1], Natthakan Rungraeng[1], Sutthiwal Setha[1,2] and Phunsiri Suthiluk[1,2*]

[1]School of Agro-Industry, Mae Fah Luang University, Chiang Rai 57100, Thailand
[2]Research Group of Postharvest Technology, Mae Fah Luang University, Chiang Rai 57100, Thailand

Abstract

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Keywords: Bioactive compounds, fruit juice, high hydrostatic pressure

Poster Session | Food Function/Nutrition

[6-1130-P] Functional/Wellness Foods &Nutrition (6th)

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

[6-1130-P-01] Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds

*Pairote Wongputtisin[1], Narin Lahsom[1] （1. Program in Biotechnology, Faculty of Science, Maejo university, Chiang mai, Thailand (Thailand)）

11:30 AM - 12:30 PM

[6-1130-P-02] Effect of Sucrose and Glucose on Coffee Kombucha Carbonation

*Chutamas Maneewong[1], Thittaya Choompoosee[1] （1. Department of Biotechnology, Faculty of Science, Maejo University, San Sai, Chiang Mai 50290(Thailand)）

11:30 AM - 12:30 PM

[6-1130-P-03] Evaluation of Total Anthocyanins and Antioxidant Activity of Thai Rice Cultivars for Phenotypic Selection in Rice Breeding

*Chotipa Sakulsingharoj[1], Lalita Na Rachasima[1], Anongnad Richinda[1], Pairote Wongputtisin[2], Rungthip Kawaree[2], Saengtong Pongjaroenkit[1], Varaporn Sangtong[1] （1. Program in Genetics, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand), 2. Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand)）

11:30 AM - 12:30 PM

[6-1130-P-04] Investigation of some biological activities of local shallot (*Allium ascalonicum* Linn.) extract from Thailand

*Premruethai Phansaard[1], Pairote Wongputtisin[1] （1. Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand)）

11:30 AM - 12:30 PM

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

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

11:30 AM - 12:30 PM

[6-1130-P-06] Process optimization for antioxidant extraction from seed of soybean cultivar Chiang mai60

*Arpatsara Seekoompa[1], Pairote Wongputtisin[1], Piyanuch Niamsup[1] （1. Program in Biotechnology, Faculty of science, Maejo University, Chiang mai(Thailand)）

11:30 AM - 12:30 PM

[6-1130-P-07] Nutritional and Functional Properties of Yoghurt Drink with Philippine Gac (*Momordica cochinchinensis* Spreng.) and Bignay (*Antidesma bunius*) Fruits

Rowie Joy Gonzales Bucks[1], *Ara Fatima Cuvinar Algar[1], Ryan Rodrigo Paner Tayobong[2] （1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines), 2. Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines)）

11:30 AM - 12:30 PM

[6-1130-P-08] **Effect of Extracting Conditions on Plant Extract Colors and Stability of Antioxidant Properties during *in vitro* Gastrointestinal Digestion**

*Rattika Aeka[1], Titikan Liangpanth[1], Rungarun Sasanatayart[1] （1. School of Agro-Industry, Mae Fah Luang University(Thailand)）

11:30 AM - 12:30 PM

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

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

11:30 AM - 12:30 PM

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

Kanako Niiya[1], *Takahiro Saito[2], Masatsugu Tamura[2], San Woo Bang[2] （1. Utsunomiya University Graduate School(Japan), 2. Utsunomiya Univ.(Japan)）

11:30 AM - 12:30 PM

Poster Session | Food Function/Nutrition

[6-1130-P] Functional/Wellness Foods &Nutrition (6th)

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

[6-1130-P-01] Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds

*Pairote Wongputtisin[1], Narin Lahsom[1] (1. Program in Biotechnology, Faculty of Science, Maejo university, Chiang mai, Thailand (Thailand))

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

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

Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds

Pairote Wongputtisin* and Narin Lahsom
Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai, Thailand 50290

*corresponding author: pairotewong@gmail.com

ABSTRACT

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

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

1. INTRODUCTION

Raffinose family oligosaccharides (RFOs) are oligosaccharides widely found in leguminous seeds. They are α-galactosyl derivative of sucrose linked with α(1→6) bond. The major member of RFOs are raffinose, stachyose and verbascose, which their chemical structures are shown in Figure 1. Biosynthesis of raffinose in legume seeds proceeds by transferring of galactosyl residue (donor) from galactinol (*O*-α-D-galactopyranosyl-(1→1)-L-*myo*-inositol) to sucrose (acceptor) by the action of raffinose synthase. Subsequently, stachyose synthase transfers another one or two galactosyl residue from galactinol to raffinose molecule, resulting of stachyose and verbascose, respectively (Peterbauer et al., 2002; Karner et al., 2004). The RFOs content in various leguminous seeds; i.e. soybean, lupin, chickpea, mung bean, pigeon pea, jack bean, lentil and groundnut has been reported (Muzquiz et al., 1999; Kadlec, 2001; Martinez-Villaluenga et al., 2005; Giannoccaro et al., 2006; Xiaoli et al., 2008; Kumar et al., 2010). In case of groundnut (*Arachis hypogaea* L.), variation of RFOs in different cultivars was reported by other research groups. However, those of local groundnut cultivars in Thailand have not been investigated yet.

These sugars play an important role in seed by involving in defense mechanism of some abiotic stresses; low temperature, drought, high salinity and oxidative stress (ElSayed et al., 2014). However, these oligosaccharides have been reported as an effective prebiotic substance for human and animal too. The term "prebiotics" was firstly introduced by Gibson and Roberfroid in 1995 and presently, its definition has been modified, for example "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Gibson et al., 2004)" and "live micro-organisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002)". According to these concepts, non-digestible oligosaccharides (NDOs) such as fructooligosaccharide (FOS), galactooligosaccharide (GOS), isomaltooligosaccharide (IMO), xylooligosaccharide (XOS), human milk oligosaccharide (HMO) and raffinose family of oligosaccharides (RFO) are accepted as prebiotic (Ziemer and Gibson, 1998; Chow, 2002; Mussato and Mancilha, 2007).

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

2. METHODOLOGIES

2.1 Groundnut seeds

Seeds of three groundnut cultivars; Tainan 9, Khonkean 5 and Khonkean 6, were kindly obtained from Field Crop Research Center, Thailand, and stored in vacuumed plastic bag at 4 ºC.

2.2Microorganisms

All tested bacteria were from the Thailand Institute of Scientific and Technological Research (TISTR). There are totally three probiotic strains; including *Lactobacillus plantarum* TISTR541, *L. lactis* TISTR464 and *L. acidophilus* TISTR1338. The normal flora and pathogenic strains used in this study were *Escherichia coli* TISTR887 and *Salmonella enterica* serovar Typhimurium TISTR292, respectively. Probiotics were maintained on MRS agar, while *E. coli* and *S.* Typhimurium were maintained on nutrient agar.

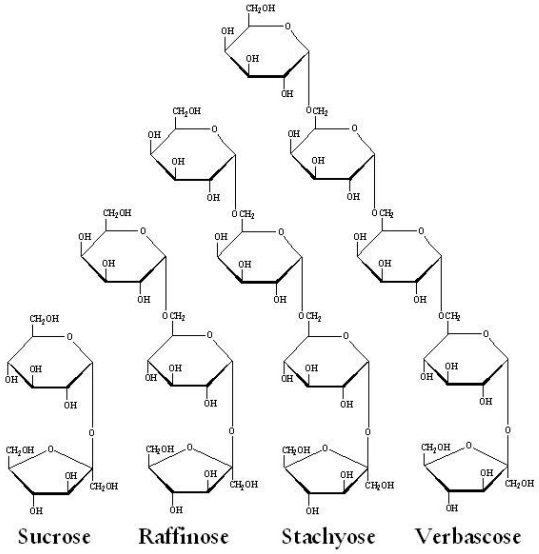

Figure 1. The chemical structures of raffinose family of oligosaccharides (RFOs)

2.3 RFOs-rich extract preparation

The crude extract containing low molecular weight sugar (LMWS) and rich of RFOs was prepared from ground and dried seeds according to the modified method of Xiaoli et al. (2008). Ground seed was defatted using hexane and mixed with 50% (v/v) ethanol with the ratio of 3 g : 50 ml. The mixture was continuously shaken for 1 hr at 30 ^{o}C and then filtered through filter paper (Whatmann® No. 1). The obtained filtrate was subsequently centrifuged at 8,000 rpm for 10 min at 4^{o}C to remove the remaining particles. Supernatant was concentrated using rotary vacuum evaporator (Buchi®) under the temperature below 50^{O}C.

2.4 Analysis of sugars

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

2.5 Primary prebiotic properties of RFOs-rich extracts

Growth stimulation of individual bacteria by groundnut sugar extracts were investigated. The extract was supplemented in basal medium (g/ L: 0.3 K_2HPO_4, 0.1 KH_2PO_4, 1.0 yeast extract, 1.0 peptone, 0.2 $MgSO_4$, and 2.5 $(NH_4)_2SO_4$, pH 7.0) as a carbon source at a concentration of 1% (w/v). Approximately 10^8 CFU of 24 hr-old inoculum of tested bacterium was transferred to 100 ml sterilized basal medium and statically incubated in anaerobic jar for 24 hr and at 37^{o}C. Viable cell (CFU/ml) of probiotics, *Sal.* Typhimurium and *E. coli* was enumerated on De Man, Rogosa and Sharpe agar (MRS) (Himedia®), *Salmonella – Shigella* agar (SS agar) (Himedia®) and Eosin methylene blue agar (EMB agar) (Himedia®); respectively.

The growth dynamic of each bacterium in defined-mixed culture was also studied. Total 10^8 CFU of 3 probiotic strains (~3.3 x 10^7 CFU for each strain), 10^8 CFU of *S.* Typhimurium and 10^8 CFU of *E. coli* were transferred as a mixed inoculum to 100 ml basal medium supplemented with groundnut sugar extract. The culture conditions were as described in previous experiment. The bacterial population were monitored

at 0, 12 and 24 hr of cultivation. In both experiment, basal media with glucose as a carbon source and without carbon source were used as control treatments.

2.6 Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1 Sugar analysis

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

3.2 Prebiotic properties of ethanolic extract from groundnut seeds

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

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

Soluble sugar content	Cultivars		
(g/ 100 g dry seed)	Tainan 9	Khonkean 5	Khonkean 6
total sugar	3.63±0.46	4.00±0.30	2.82±0.47
reducing sugar	1.71±0.24	0.56±0.15	0.48±0.13
degree of polymerization	2.2	7.0	5.8
glucose	0.16±0.04	0.09±0.07	trace
sucrose	1.48±0.05	1.32±0.33	0.88±0.17
raffinose	0.01±0.02	0.04±0.04	trace
stachyose	0.56±0.034	1.17±0.17	0.39±0.079
verbascose	trace	0.05±0.05	trace

Table 2. comparison of LMWSs detected in some leguminous seeds

Legumes	Content (g/ 100g seed)				
	Sucrose	Raffinose	Stachyose	Verbascose	References
Groundnut					
60 Spanish cultivars	2.44 – 7.61	0.17 – 1.56 (total RFOs)			Bishi et al. (2013)
40 Indian cultivars	2.61 - 6.50	0.01 - 0.12	0.11 - 0.67	0.00 - 0.07	Bishi et al. (2014)
30 Spanish cultivars	2.79 – 5.33	0.02 – 0.06	0.35 – 0.79	No report	Mahatma et al. (2016)
30 Virginia cultivars	3.85 – 6.90	0.04 – 0.16	0.46 – 1.03	No report	Mahatma et al. (2016)
3 Thai cultivars	0.81 – 1.48	0.00 – 0.04	0.39 – 1.17	0.00 – 0.05	This study
Soybean					
Chiang mai60	1.32	0.67	14.53	0.16	Wongputtisin et al.(2015)
V95-7456	4.96	0.64	3.77	No report	Saldivar et al. (2010)
Vine pea	No report	0.29	0.14	0.13	Ekvall et al. (2007)
Lupin	16.2	19.0	54.0	10.8	Muzquiz *et al*. (1999)
Chick pea	2.56	0.89	2.38	0.42	Xiaoli *et al*. (2008)

The change in population of total probiotics, *S*. Typhimurium and *E. coli* in defined-mixed culture experiment were illustrated in Figure 3. It was found that survivability of all bacteria declined along with cultivation time in broth without carbon source, while growth of total probiotic and *E. coli* increased non-significantly and that of *S*. Typhimurium was not significantly changed when glucose was used as carbon source. The interesting results were found in treatment of Tainan9 extract addition. Sugar extract of this cultivar was able to promote growth of total probiotics, resulting in decreasing of *E. coli* and *S*. Typhimurium survivals markedly. Sugar extracts of Khonkean5 and Khonkean6 also gradually enhanced total probiotic growth but not obviously different. However, inhibitory effect on growth of *E. coli* and *S*. Typhimurium still could be observed. The growth pattern of probiotic strains and *S*. Typhimurium in media with groundnut sugar extracts were consistent with the results from single culture study.

From all results above, it was clear that sugar extract from groundnuts could stimulate all tested probiotic strains but not for *E. coli* and pathogenic *S.* Typhimurium. This characteristic is considered as an important primary property prior accepted as prebiotic substance. Probiotic growths could be from both RFOs, which were major sugars in the extract, and the other LMWSs; i.e. glucose and sucrose. To utilize RFOs, bacterial cell required α-galactosidase to hydrolyze α linkage and raffinose delivery system into cell. Mechanisms of RFO utilization in Bifidobacterium and Lactobacilli probiotics were also reported by Hachem et al. (2012). Glycoside hydrolase family 36 (GH36) α-galatosidase encoding genes, sugar transport systems of the glycoside – pentoside – hexuronide cation symporter family (GPH), sugar phosphotransferase systems (PTSs) or ATP-binding cassette systems (ABCs) are key factors. Schmid and Schmitt (1976) reported that *E. coli* cells lack of raffinose delivering system. Moreover, there have been no report on the activity of α-galactosidase in *S*. Typhimurium and *E. coli*, while that was reported in three Lactobacilli used in this study (Donkor et al., 2007; Sumarna, 2008; LeBlanc et al., 2004; Fredslund et al., 2011; Silvestroni et al., 2002; Jeong et al., 2008). Thus, there was high possibility that growth of *E. coli* and *S.* Typhimurium observed in this work were from LMWSs not from RFOs. The expected results were obtained in media added by groundnut sugar extracts. Promoted probiotic population subsequently exhibited the inhibitory effect on *E. coli* and *S*. Typhimurium growth. The mechanisms involved might be commonly explained that lactic acid bacteria produce various inhibitors, for example, organic acids (lactate and acetate), short chain fatty acids, hydrogen peroxide and bacteriocins (lactacin B, lactacin F and acidocin CH5, nisin and lactocin S (Parada et al., 2007; Vrese and Schrezenmeir, 2008; Zhou et al., 2010; Gao et al., 2019).

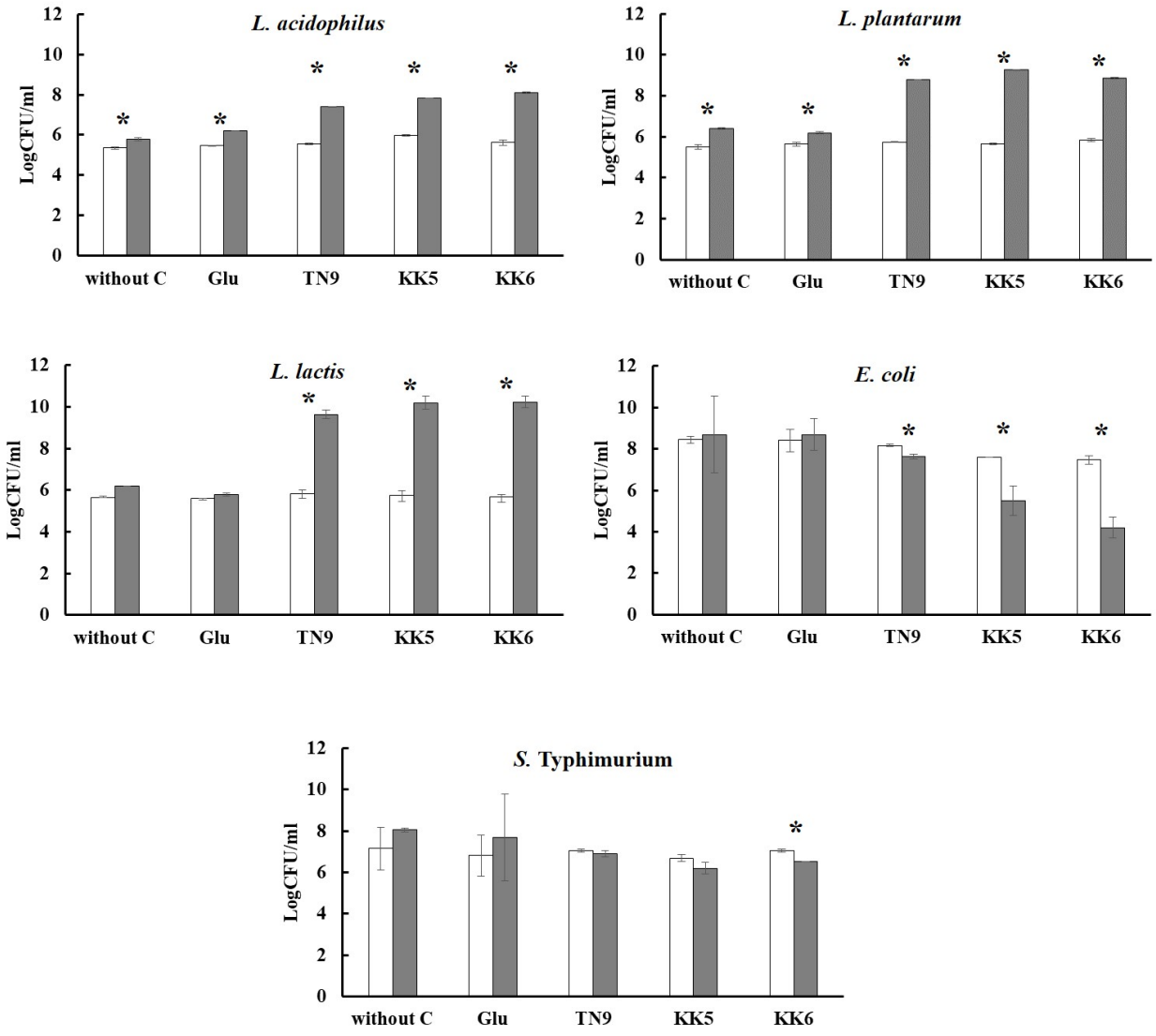

Figure 2. Growth of single tested strains in basal medium supplemented with different carbon source when cultivating for 0 hour (□) and 24 hours (■). The (*) in each experiment indicates significant difference at $p<0.05$.

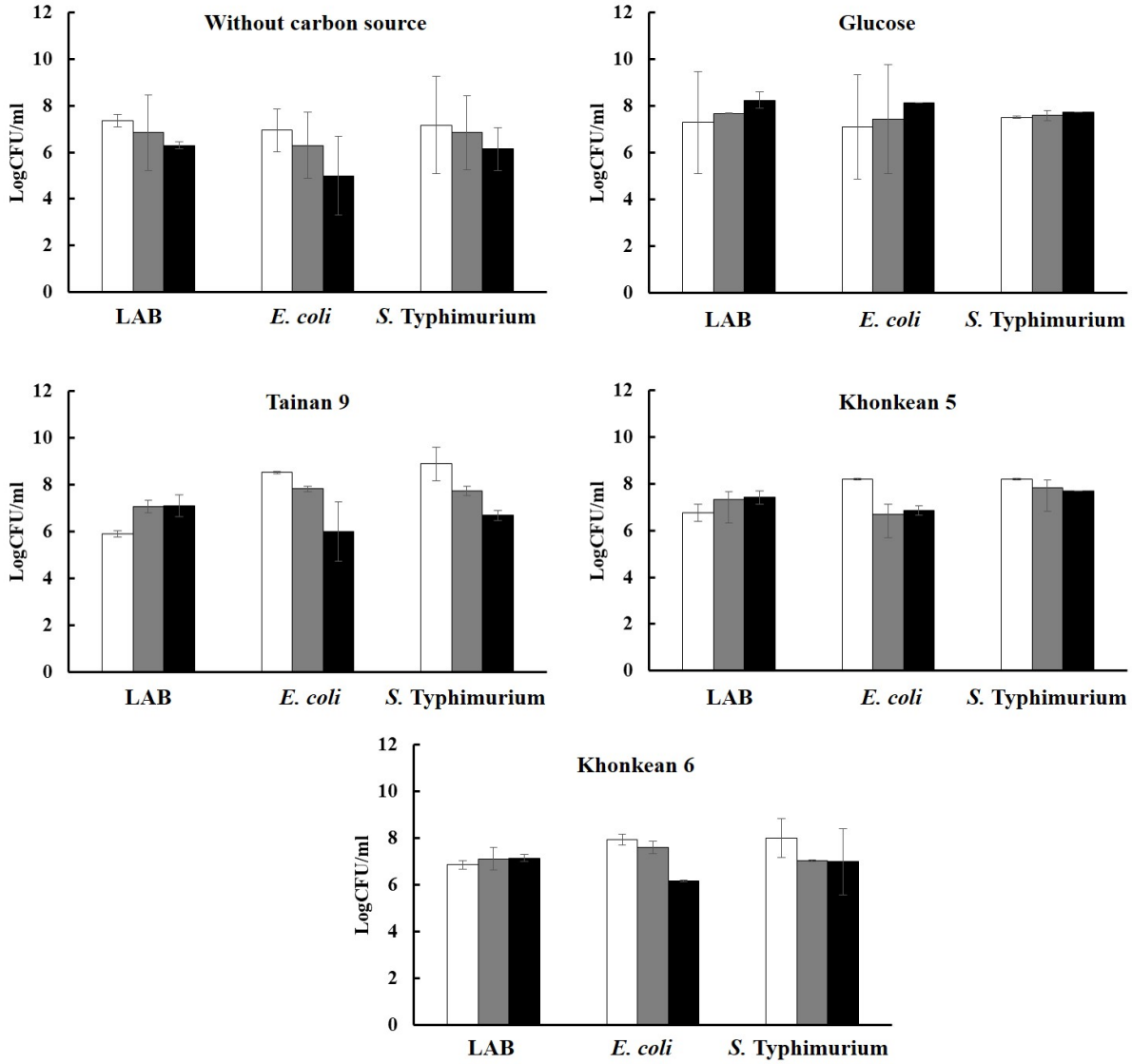

Figure 3. Dynamic of the bacterial population in defined-mixed culture supplemented with different carbon source after 0 hour (□), 12 hours (▒) and 24 hours (■) of cultivation

4. CONCLUSION

From all of the results above, the extract prepared from groundnut seeds cultivar Tainan9, Khonkean5 and Khonkean6 showed a potential to be source of an effective prebiotic substance and preliminary exhibited the prebiotic properties by promote growth of probiotic strains; resulting in inhibition of pathogenic growths. Thus consuming of groundnut seed may help to improve the bacterial balance in gastrointestinal tract and receiving of many advantages from grown probiotics. Moreover, synbiotic food containing groundnut RFOs and selective probiotics can be manufactured and promoted as functional foods.

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Poster Session | Food Function/Nutrition
[6-1130-P] Functional/Wellness Foods &Nutrition (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-02] Effect of Sucrose and Glucose on Coffee Kombucha Carbonation

*Chutamas Maneewong[1], Thittaya Choompoosee[1] （1. Department of Biotechnology, Faculty of Science, Maejo University, San Sai, Chiang Mai 50290(Thailand)）

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

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

Effect of Sucrose and Glucose on Coffee Kombucha Carbonation

Chutamas Maneewong[1*] and Thittaya Choompoosee[1]

[1] Department of Biotechnology, Faculty of Science, Maejo University, San Sai, Chiang Mai, Thailand, 50290

*Corresponding author: chutamas_m@yahoo.com and chutamas@mju.ac.th

ABSTRACT

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

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1. INTRODUCTION

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

Coffee is one of the most popular beverages worldwide. There are different kinds of coffee beverages, coffee kombucha is fermented coffee with SCOBY. The coffee kombucha generally use 5-10 % (v/v) sucrose as a substrate for fermentation, these can produce acid but low gas formation. Thus, the objective of this study was to enhance carbonation in coffee kombucha by comparing sugars such as sucrose and glucose with different concentration for increasing gas formation. Sensory evaluation of the coffee kombucha was also tested.

2. MATERIALS AND METHODS

2.1 Materials

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

SCOBY (Symbiotic Culture of Bacteria and Yeast) was obtained from Jib-Kefir shop, Bangkok, Thailand

2.2 Methods

2.2.1 Preparation of coffee

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

2.2.2 Coffee kombucha fermentation

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

2.2.3 Sugars for kombucha fermentation

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

2.3 Analysis

2.3.1 Acidity

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

2.3.2 Number of microorganism

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

2.3.3 Sensory Evaluation

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

3. RESULTS AND DISCUSSION

3.1 Sugars for gas and acid formation in coffee kombucha

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

Acidity of coffee kombucha, the initial pH of the coffee kombucha containing 5% glucose + 5% sucrose was 5.41, and it dropped to 3.16 during the fermentation period (Figure 1). In the coffee kombucha containing 5% glucose + 5% sucrose, the acid concentration continuously increased from 0.71 % (v/v) to 7.44% (v/v) at day 14 of fermentation. However, high acid content (12.08 % v/v) was found in the kombucha making from 10% sucrose, pH dropped from

5.20 to 2.09 at day 14 of fermentation. The 5% glucose + 5% sucrose could produce highest gas formation because yeast could survive at pH 3.2-3.7 (second fermentation) and produce higher CO_2. The other treatments, the pH value during second fermentation was lower than 3.0, which was much lower than the pH for optimum growth of yeasts (Chen and Liu, 2000), resulting to low gas production. Malbaša et al. (2008) reported kombucha converted sucrose to glucose and fructose, and further to ethanol, acetic acid, lactic acid, and a large number of other compounds.

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

Sugar content	Gas formation level	Gas formation
5% sucrose,	+	
7% sucrose,	+	
10% sucrose,	++	
5% glucose	++	
5% glucose + 5% sucrose	+++	

Gas formation level: low (+), medium (++), high (+++)

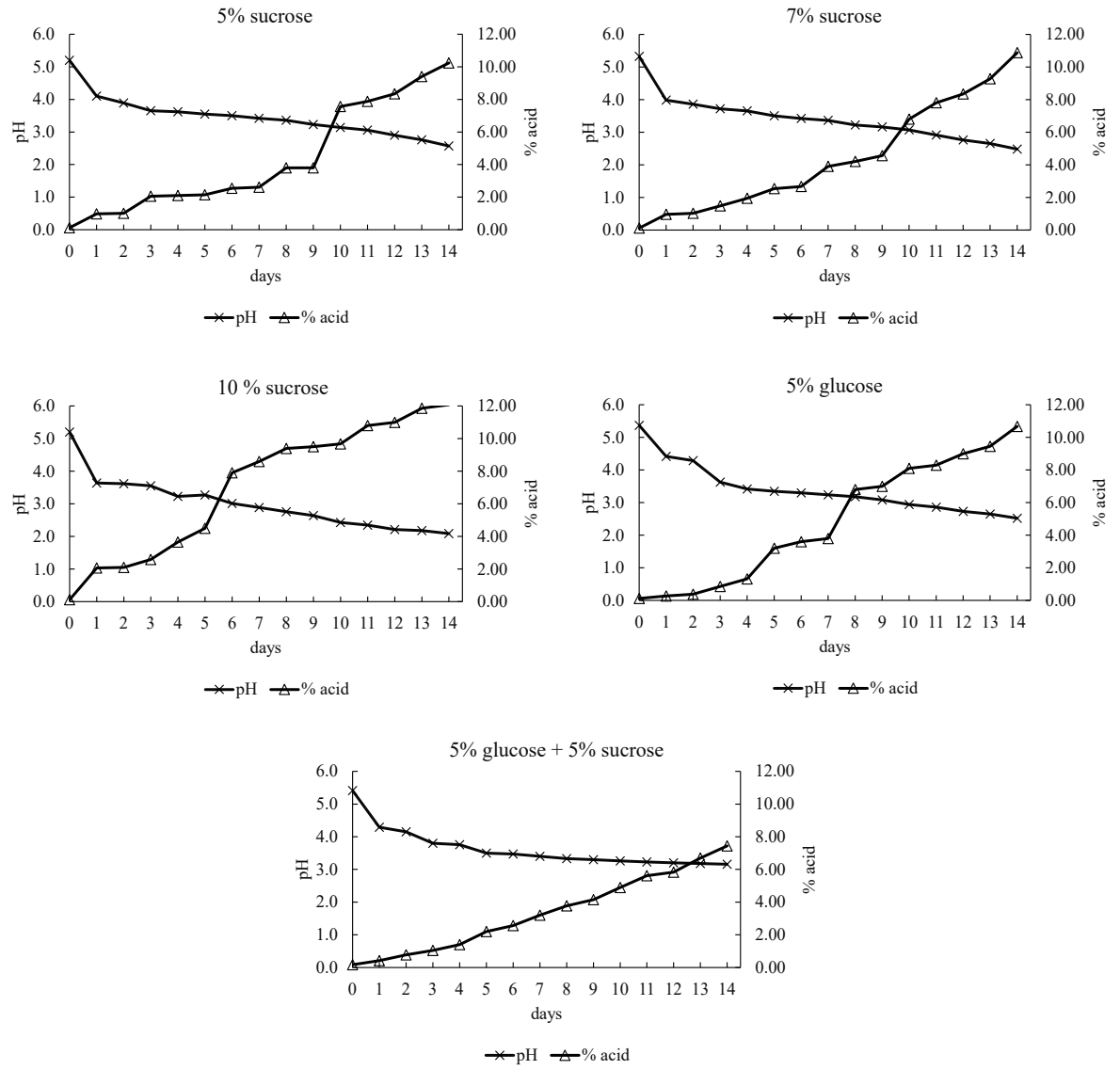

Figure 1. Changes of pH and acid concentration during 14 days of coffee kombucha fermentation compared with 5% sucrose, 7% sucrose, 10% sucrose, 5% glucose and 5% glucose + 5% sucrose

3.2 Number of Microorganism

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

gluconic acid and ethanol to produce acetic acid. The pH value of kombucha beverage decreased due to the production of organic acids during fermentation (Sievers et al., 1995)

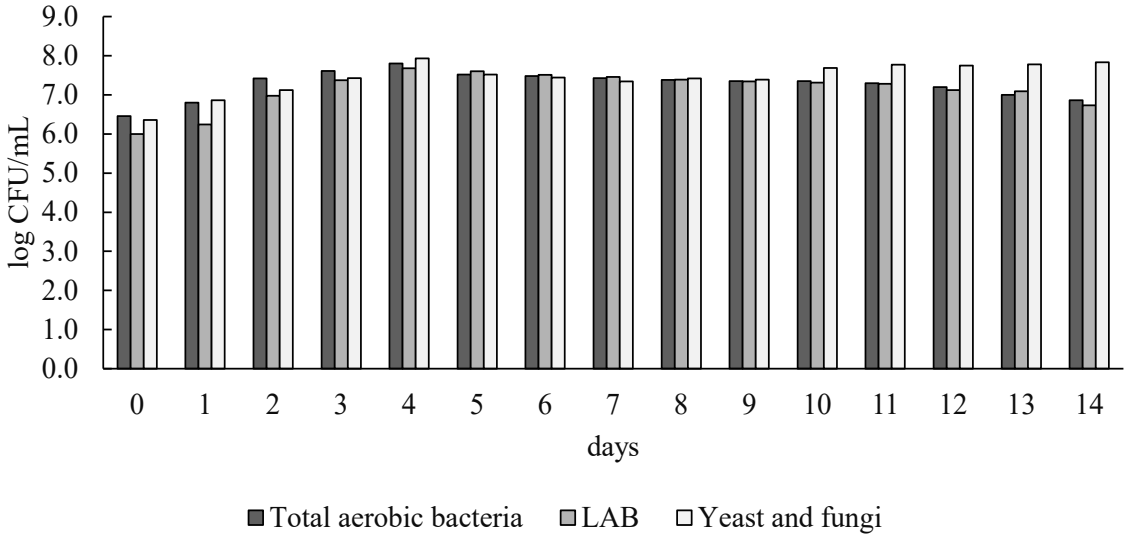

Figure 2. Number of total aerobic bacteria, lactic acid bacteria (LAB) and yeast and fungi during coffee kombucha fermentation

3.6. Sensory characteristics of coffee kombucha

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

Table 2. Sensory evaluation of coffee kombucha

Characteristics	Score
coffee smell	6.10 ± 0.48
sweetness	5.10 ± 0.92
sourness	7.00 ± 0.83
sparkling taste	6.67 ± 0.92
mouthfeel	7.16 ± 0.63
overall acceptance	6.96 ± 0.49

4. CONCLUSION

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

ACKNOWLEDGMENT

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

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Poster Session | Food Function/Nutrition

[6-1130-P] Functional/Wellness Foods &Nutrition (6th)

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

[6-1130-P-03] Evaluation of Total Anthocyanins and Antioxidant Activity of Thai Rice Cultivars for Phenotypic Selection in Rice Breeding

*Chotipa Sakulsingharoj[1], Lalita Na Rachasima[1], Anongnad Richinda[1], Pairote Wongputtisin[2], Rungthip Kawaree[2], Saengtong Pongjaroenkit[1], Varaporn Sangtong[1] (1. Program in Genetics, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand), 2. Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand))

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

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

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

Chotipa Sakulsingharoj[1*], Lalita Na Rachasima[1], Anongnad Richinda[1], Pairote Wongputtisin[2], Rungthip Kawaree[2], Saengtong Pongjaroenkit[1] and Varaporn Sangtong[1]

[1]Program in Genetics, Faculty of Science, Maejo University, Chiang Mai, Thailand
[2]Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand

*Corresponding author: chotipa.cs@gmail.com

ABSTRACT

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

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

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereals, serving as a staple food consumed by people in many countries, especially in Asia (Liu et al., 2017; Pang et al., 2018). Pigmented rice has been popular as a healthy food because it contains more nutrients beneficial for human health (Alves et al., 2016; Maulani et al., 2019). Rice can be classified by grain colors which are white, brown, red and black. Black rice has high accumulation of anthocyanins in its pericarp tissues (Reddy et al., 1994; Goufo and Trindade, 2014). Recently, black rice has received more attention from consumers and rice breeders since it contains several nutrients and antioxidant compounds (Lim and Ha, 2013; Rahman et al., 2016).

Anthocyanins which belong to a major class of water-soluble flavonoids, are the primary pigments in colored rice grains (Abdel-Aal et al., 2006). Several health benefits of anthocyains as health-promoting substances due to their antioxidant activity have been recognized (Nam et al., 2006). They include anti-inflammatory activity, anti-cancer activity, prevention of cardiovascular diseases and obesity, control of diabetes, mitigation of oxidative stress, vision improvement and anti-microbial activity (He and Giusti, 2010; Kruger et al., 2014; Sompong et al., 2011).

Extraction of anthocyanins has been conducted using different extracting solvents, including water, methanol, and ethanol, combined with some acids such as citric acid, hydrochloric acid, and acetic acid (Halee et al, 2018; Jansom et al., 2016). In rice, extraction of anthocyanins with acidified

methanol/ethanol followed by spectrophotometric measurement has been used extensively (Chin et al., 2016; Jansom et al., 2016; Jiamyangyuen et al., 2017; Na Rachasima et al., 2017). DPPH (2,2-Diphenyl-1-picryhydrazyl) assay is commonly used to evaluate antioxidant activity of foods and plant extracts because it is simple, rapid, inexpensive and reproducible. There are factors that may influence the reaction in DPPH method such as extracting solvents, sample concentration, pH, reaction time, different antioxidant standard and assay conditions (Ferri et al., 2013; Mishra et al, 2012).

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

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

2. MATERIALS AND METHODS

2.1 Plant materials

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

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

2.2 Extraction of rice seeds with various solvent types

The mature rice seeds were dehulled and grounded into fine powder. Seed powder of 100 mg from HN black rice cultivar were extracted by 1 ml of six different solvent types which were water, 50% methanol, 80% methanol, 1% HCl in water (V/V), 1% HCl in 50% methanol (V/V) and 1% HCl in 80% methanol (V/V). The seed extracts were mixed by vortexing and incubated at room

temperature for 30 min. The supernatants were collected by centrifugation at 12,000 rpm for 10 min. Each extraction was performed with three replicates. Each extract from different solvents types was diluted using the same extracting solvent type with the sample extract / solvent volume ratio at 1/4. The diluted extracts with different solvent types were subjected to measurement of total anthocyanin contents and antioxidant activity. The optimal solvents which were found to be 80 % methanol and 1% HCl in 80% methanol were selected and used to extract the seeds of eight rice cultivars.

2.3 Determination of total anthocyanin contents

One hundred milligrams of mature seeds of eight rice cultivars, including four white rice (T65, Kit, RDMJU2, and PTT1) and four black rice (KNO, KY, HN and MLD) were grounded into fine powder followed by the extraction with 1 ml of two solvent types which were 1% HCl in 80% methanol and 80 % methanol. The seed extracts were mixed by vortexing and incubated at room temperature for 30 min. The supernatants were collected by centrifugation at 12,000 rpm for 10 min. Each extraction was performed with three replicates. Each extract from different solvents types was diluted using the same extracting solvent type with the sample extract / solvent volume ratio at 1/6. The rice seed extracts were used for determination of total anthocyanin contents by the method modified from Chin et al. (2016). The absorbance was measured at 530 and 675 nm using microplate reader (SPECTROstar® Nano, Germany). The anthocyanin contents were determined as follow: monomeric anthocyanin = (A x MW x DF x 1000) / ε x 1 (Na Rachasima et al., 2017). Three replicates were analyzed for each sample.

2.4 Determination of antioxidant activity by DPPH assay

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

2.5 Statistical analysis

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

3. RESULTS AND DISCUSSION

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

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

μmol/gDW, followed by the extracts with 1 % HCl in 50% methanol and 1% HCl in water which exhibited the total anthocyanins of 1.36 and 0.49 μmol/gDW, respectively.

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

Solvent types	Total anthocyanin contents (μmol/gDW)	Antioxidant activity / TEAC (μmol/gDW)
water	0.17 ± 0.01^{c}	7.72 ± 0.01^{d}
50% methanol	0.20 ± 0.02^{c}	11.32 ± 0.33^{b}
80% methanol	0.12 ± 0.01^{c}	9.23 ± 0.03^{c}
1% HCl in water	0.49 ± 0.01^{b}	13.70 ± 0.03^{a}
1% HCl in 50% methanol	1.36 ± 0.04^{a}	13.64 ± 0.13^{a}
1% HCl in 80% methanol	1.41 ± 0.10^{a}	13.94 ± 0.06^{a}

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

The antioxidant activities of the extracts by 1% HCl in 80% methanol, 1% HCl in 50% methanol and 1% HCl in water were higher than those of water and methanol. The extract with 1% HCl in 80% methanol gave the highest value of antioxidant activity of 13.94 μmol/gDW (Table 1). The values of antioxidant activity using 1% HCl with water and 1% HCl in 50% methanol were 13.70 and 13.64 μmol/gDW, respectively, and showed no significant differences ($P\geq0.01$). The previous report showed that the solvents of ethanol or methanol acidified with 1% HCl were optimal for the extraction of rice seeds for anthocyanin content determination (Chin et al., 2016; Krongsuksirichai et al., 2016). In this study, we found that 1% HCl in 80% methanol gave highest values of anthocyanin content and antioxidant activity; therefore, we selected this solvent type for the seed extraction of eight rice cultivars for further studies.

3.2 Total anthocyanin contents and antioxidant activities of eight rice cultivars

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

The antioxidant activity by DPPH-radical scavenging activity assay of the extracts by 1%HCl in 80% methanol was evaluated. The results showed that the antioxidant activity of black rice were higher than white rice. The black rice cv. MLD had highest TEAC against DPPH of 17.37 μmol/gDW. This result was consistent with highest amount of anthocyanin contents (1.374 μmol/gDW) and darkest black color in it pericarp (Figure 2 and 1). Other black rice cv. HN, KY and KNO had the antioxidant activity of 17.09, 16.42 and 16.71 μmol/gDW, respectively. The white rice cv. T65, Kit, RDMJU2 and PTT1 had not significantly different values of TEAC which were 3.71, 3.69, 3.70 and 3.70 μmol/gDW, respectively ($P\geq0.01$). Although black rice cultivars cv. HN, KY and KNO with significantly different anthocyanin contents ($P<0.01$) corresponding to their pericarp colors, the values

of TEAC could not be clearly distinguished among these black rice (Figure 2). Therefore, it might be difficult to evaluate relative antioxidant activities among different black rice cultivars for selection of parental lines and progeny lines derived from the crosses between white and black rice in our breeding programs.

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

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

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

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

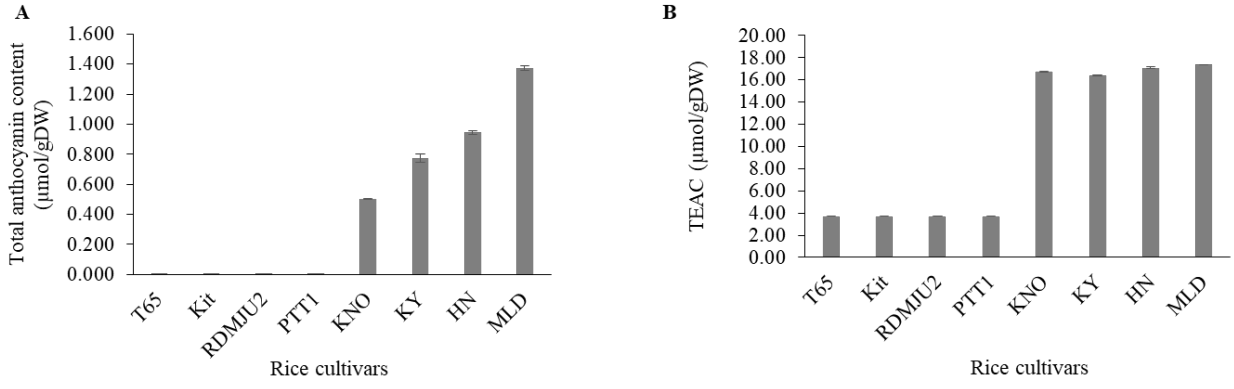

Figure 2 Assessments of total anthocyanin contents (a) and antioxidant activity against DPPH (b) of the rice seeds extracts with 1%HCl in 80% methanol. White rice cultivars were T65, Kit, RDMJU2 and PPT1. Black rice cultivars were KNO, KY, HN and MLD. All the values were represented as mean ± SD.

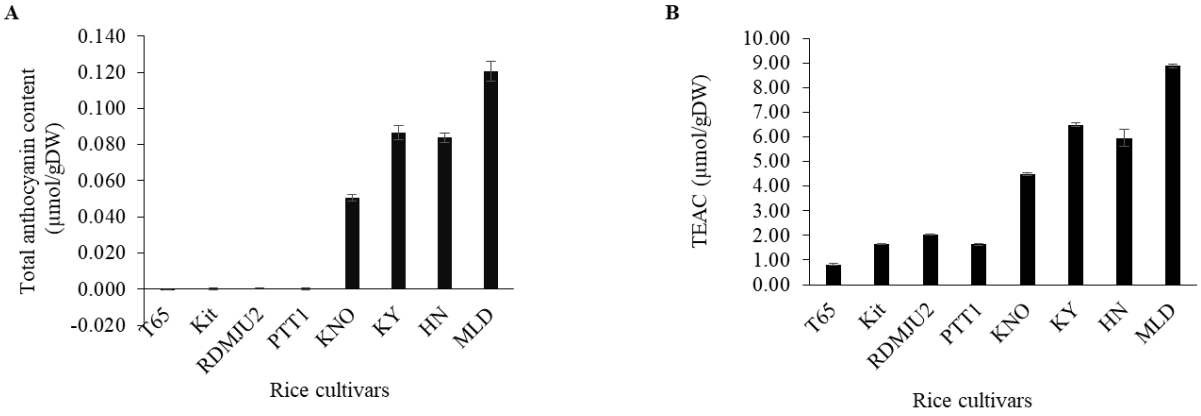

Figure 3 Assessments of total anthocyanin contents (a) and antioxidant activity against DPPH (b) of the rice seeds extracts with 80% methanol. White rice cultivars were T65, Kit, RDMJU2 and PPT1. Black rice cultivars were KNO, KY, HN and MLD. All the values were represented as mean ± SD.

3.3 The optimal incubation time for antioxidant activity in DPPH assays

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

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

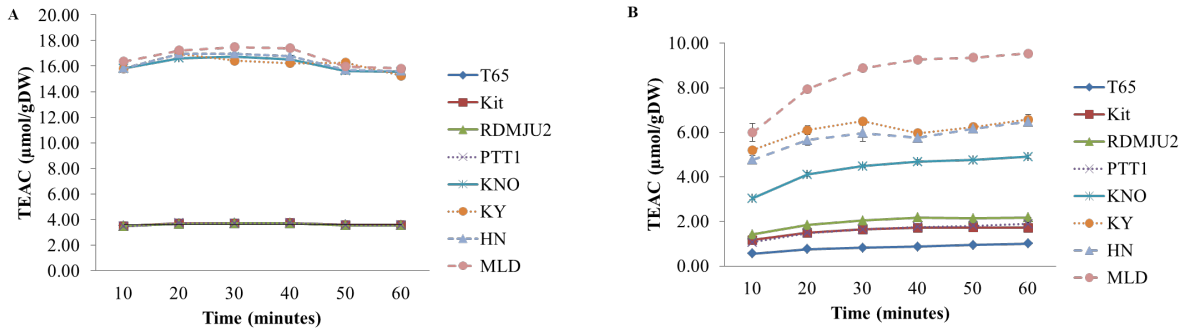

Figure 4 Assessment of incubation time in antioxidant activity by DPPH assay of extracts with 1% HCl in 80% methanol (a) and 80% methanol (b). White rice cultivars were T65, Kit, RDMJU2 and PPT1. Black rice cultivars were KNO, KY, HN and MLD. All the values were represented as mean ± SD.

The results revealed that white rice also had antioxidant activity but much lower than black rice. RDMJU2 which is glutinous rice cultivar made from rice breeders at Maejo University, Chiang Mai, Thailand showed highest antioxidant activity among white rice. In addition, RDMJU2 has high yield and good agronomic characteristics, including semi-dwarf and non-photoperiod sensitivity. The black rice, MLD which is landrace rice in the northeastern region of Thailand gave highest anthocyanin contents and antioxidant activity, consistent with the previous report (Kongkachuichai and Charoensiri, 2010). Hence, both RDMJU2 and MLD would be good sources for use as parental lines in improvement of rice with high quality traits and increased nutritional values.

4. CONCLUSION

Extracting solvents and assay conditions could affect the measurement of total anthocyanin contents and antioxidant activity. The results demonstrated that the appropriate solvent for rice seed extraction to analyze total anthocyanin contents was 1 % HCl in 80 % methanol followed by the spectrophotometric measurement. For antioxidant activity, the extraction with 80 % methanol could be suitable for DPPH assay, because the acidity of 1 % HCl might interfere with the reactions. These extraction solvents and the methods for determination of anthocyanins and antioxidant activity will be applied for screening and selection of rice cultivars with high anthocyanins and antioxidant activity to be used as parental lines and for selection of progeny lines in rice breeding program. In rice breeding, it is needed to determine the correlation between genotype and phenotype of rice populations such as F_2 progeny. The simple method for evaluating phenotypes of relative total anthocyanins and antioxidant activity of many lines will be very useful, less time-consuming and less costs. This study also suggested that RDMJU2 (white rice) and MLD (black rice) would be good candidates for use in rice breeding to provide health-promoting foods.

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Poster Session | Food Function/Nutrition
[6-1130-P] Functional/Wellness Foods &Nutrition (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-04] Investigation of some biological activities of local shallot (*Allium ascalonicum* Linn.) extract from Thailand

*Premruethai Phansaard[1], Pairote Wongputtisin[1] (1. Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand(Thailand))

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

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

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

Premruethai Phansaard and Pairote Wongputtisin*
Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai, Thailand

*Corresponding author: pairotewong@gmail.com

ABSTRACT

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

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

1. INTRODUCTION

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

2. MATERIALS AND METHODS

2.1 Shallot and shallot extract preparation

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

2.2 Sugar content analysis

Reducing sugar and total sugar of the extracts were determined by DNS and phenol-sulfuric acid method; respectively. Size of sugar, in term of an average degree of polymerization (DP) was calculated by the ratio between total sugar and reducing sugar content (Wongputtisin et al., 2012). The distribution of individual sugars in shallot extract was investigated by thin layer chromatography (TLC). The aluminum sheet coated by siliga gel (Merck©) was used as stationary phase and mobile phase was a mixture of butanol: ethanol: water (5:3:2). The sugar bands were visualized by dipping in 5% (v/v) H_2SO_4 in methanol and heating at 150°C in hot air oven.

2.3 Antioxidant activity

To generate $ABTS^{\bullet+}$, the protocol according to Re et al. (1999) was used. Five ml of 14 mM ABTS (0.385 g ABTS in 50 ml deionized water) and 5 ml potassium persulfate (0.066 g potassium persulfate in 50 ml deionized water) were mixed together and stand in the dark for 12-16 h before use. To determine scavenging activity of FCSBM extract, 10 µl of extract was added to 990 µl of $ABTS^{\bullet+}$ solution (adjusted the absorbance at 734 nm to 0.700±0.020 before used) and recorded the decreasing of A_{734} every 1 min until stable. The standard antioxidants used in this study were α-tocopherol (Merck®), ascorbic acid (Fisher Chemicals®), butylated hydroxyanisole (BHA, Fluka®). The percent of scavenging activity at 1 min of reaction can be calculated by the formula:

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

A_{734} at 0 min

2.4 Antibacterial activity

The antibacterial activity of crude and partial purified shallot extract against *Salmonella enterica* serovar Typhimurium TISTR292, *Escherichia coli* and *Staphyrococcus aureus* were studied. The extracts were sterilized using filtration through Sartorius Minisart® syringe filter (0.2 µm). Firstly, gel diffusion assay method was carried out by transferring of 20 µl extract into agar wells which were prior spread with 24 h- old pathogen suspension, subsequently further incubating at 37ºC for 24 h and recording the clear zone around wells.

Minimum inhibitory concentration (MIC) of the extracts against those pathogens was tested. The sterile extract was diluted by nutrient broth (NB) with 2-fold serial dilution (total volume at 5 ml in test tube), then inoculated by approximately 10^8 CFU of pathogen. The tubes were incubated at 37ºC for 24 h The minimum concentration of extract that did not show visible growth was recorded as MIC value. The control treatment diluted by sterile distil water was carried out in parallel. Ten µl of culture broth from those test tubes with no visible growth were spread on *Salmonella – Shigella* agar (SS agar) (Himedia®) and eosin methylene blue agar (EMB agar) (Himedia®) and nutrient agar (NA) and incubated at 37ºC for 24 h for cell enumeration of *S.* Typhimurium, *E. coli* and *S. aureus*, respectively. Minimum concentration of extract with no viable cell was considered as minimum bactericidal concentration (MBC) value.

3. RESULTS AND DISCUSSION

3.1 Shallot extract

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

Table 1. Total sugar, reducing sugar and degree of polymerization of shallot extracts

Extracts	Total sugar (mg/ml)	Reducing sugar (mg/ml)	DP
Crude extract	167.54±2.55^{ab}	7.19±0.04^{i}	23.31^{g}
Partial purified extracts			
1 cycle	191.15±0.70 ^{ab}	3.59±0.06^{h}	53.29^{fg}
2 cycles	190.49±17.62 ^{ab}	2.27±0.02^{g}	84.04^{f}
3 cycles	193.55±2.95^{a}	1.50±0.03^{f}	129.03^{e}
4 cycles	172.38±15.88 ^{ab}	1.13±0.03^{e}	152.10^{de}
5 cycles	166.61±18.45 ^{ab}	0.94±0.02^{d}	177.25^{cd}
6 cycles	158.20±20.29^{b}	0.83±0.01^{c}	189.84^{c}
7 cycles	166.80±23.53 ^{ab}	0.71±0.03^{b}	233.84^{b}
8 cycles	164.64±10.36 ^{ab}	0.58±0.02^{a}	283.87^{a}

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

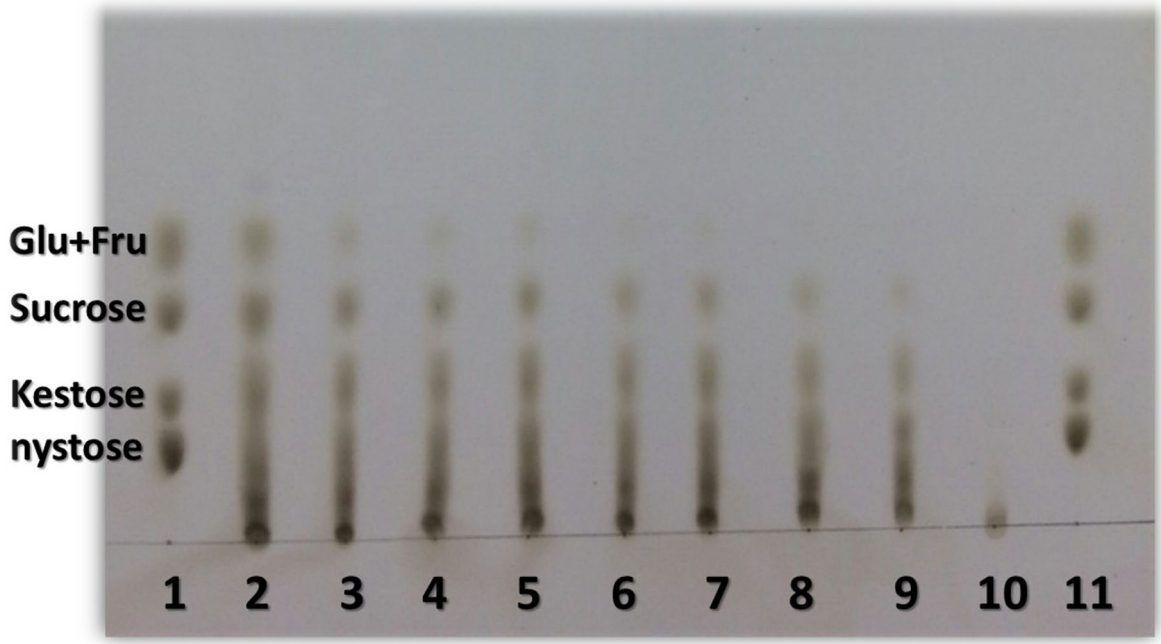

Figure 1. TLC chromatogram of sugar composition in crude and partial purified shallot extracts (Lane 1, 11: Standard sugars; Lane 2: crude extract; Lane 3: purified with 1 cycle; Lane 4: purified with 2 cycles; Lane 5: purified with 3 cycles; Lane 6: purified with 4 cycles; Lane 7: purified with 5 cycles; Lane 8: purified with 6 cycles; Lane 9: purified with 8 cycles; Lane 10: inulin)

3.2 ABTS radical scavenging activity

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

Table 2. ABTS scavenging activity of crude and purified extracts

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

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

3.3 Antibacterial activity

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

Table 3. MIC value of crude extract for inhibiting the growth of *Escherichia coli, Salmonella* Typhimurium TISTR292 and *Staphylococcus aureus*

Dilution factor	The growth of bacteria		
	E. coli	*S.* Typhimurium TISTR292	*S. aureus*
10:0	+	-	-
9:1	+	-	+
8:2	++	-	++
7:3	++	-	++
6:4	++	+	++
5:5	++	++	++
4:6	++	++	++
3:7	++	++	++
2:8	++	++	++
1:9	++	++	++
Positive control	++	++	++

"-" = Clear solution, "+" = Medium turbidity and "++" = Very turbidity

4. CONCLUSION

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

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Poster Session | Food Function/Nutrition
[6-1130-P] Functional/Wellness Foods &Nutrition (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

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

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

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

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

Probiotic characterization of thermotolerant *Lactobacillus johnsonii* isolated from broiler intestine

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

*Corresponding author: pairotewong@gmail.com

ABSTRACT

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

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

1. INTRODUCTION

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

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

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

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

2. MATERIALS AND METHODS

2.1 Isolation of thermotolerant LAB and identification.

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

2.2 Characterization of probiotic properties

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

2.2.1 Hemolytic activity

Hemolytic activity of the isolates was tested by inoculation on blood agar (7% (v/v) sheep blood) and incubation at 37°C for 24 h (Pieniza et al., 2014). The isolates which did not exhibit lyse zone around their colonies were considered as non-hemolysis (γ-hemolysis). In case of hemolytic isolate, there were considered and classified into 2 types, green-hued zone (α-hemolysis) and clear lysed zone (β-hemolysis) production.

2.2.2 Acid and bile tolerant ability

The test of resistance under acid condition was carried out *in vitro* according to Rajam et al. (2012). Simulated gastric juice was prepared by 0.5% (w/v) pepsin in phosphate-buffered saline (PBS), pH 2.5. One ml of cell suspension (10^8 CFU) was transferred into 9 ml of simulated gastric juice, mixed well and incubated anaerobically at 37 ºC for 3 h. The interval sampling during incubation for viable cell enumeration on MRS agar was done. The bile tolerance assay was tested according to Yamano et al. (2006) with modifications. One ml of cell suspension (10^8 CFU) were transferred to MRS broth supplemented by 0.3% (w/v) oxgall bile (Sigma) and subsequently incubated anaerobically at 37 °C for 3 h. Cell suspension was taken interval and enumerated for the survival cells on MRS agar.

2.2.3 Autoaggregation Assay

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

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

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

2.2.4 Antibiotic susceptibility

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

2.2.5 Antimicrobial activity

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

3. RESULTS AND DISCUSSION

3.1 Identification of thermotolerant LAB

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

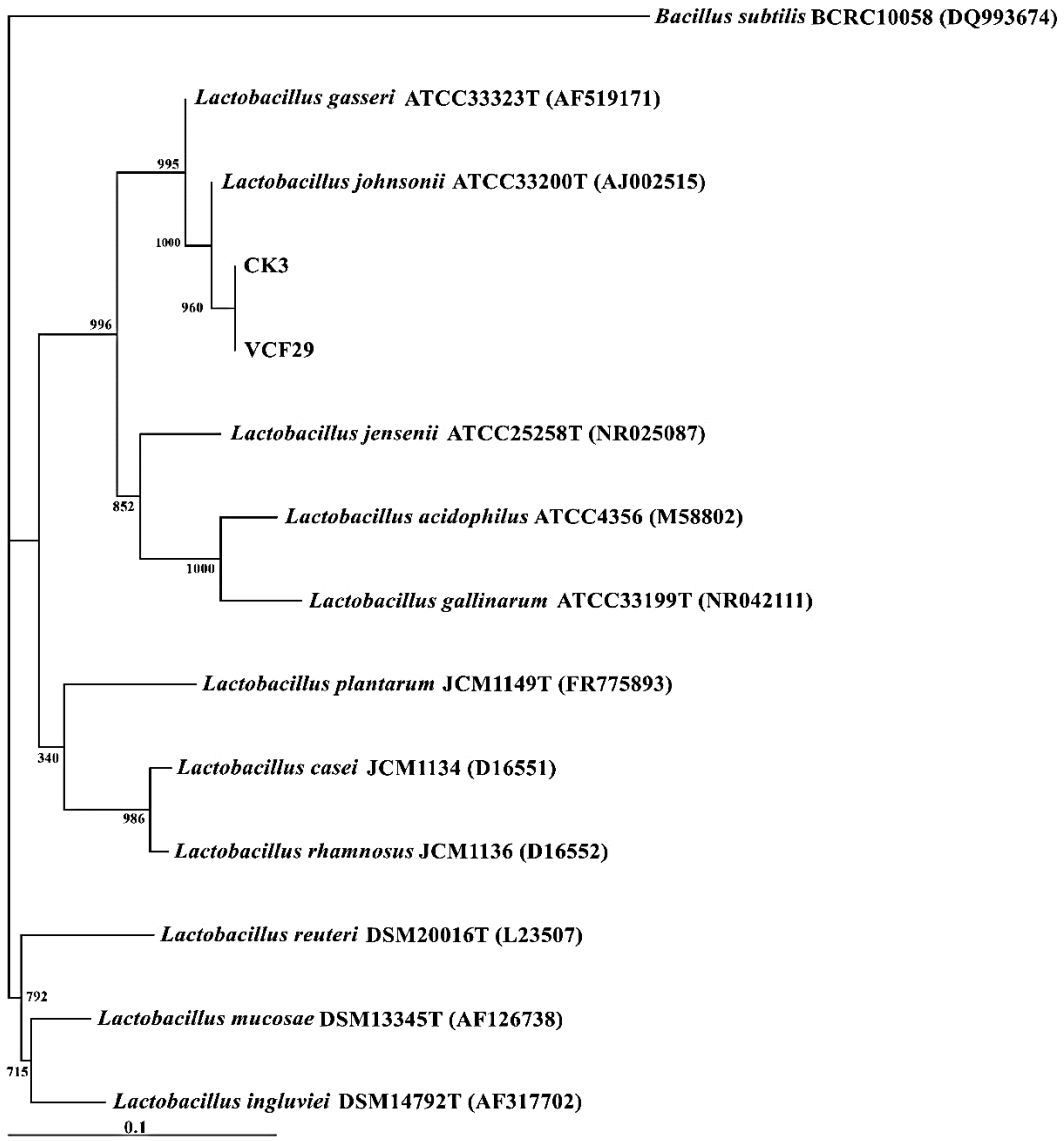

Figure 1. Phylogenetic trees constructed using the neighbor-joining method with the full-length 16S rRNA gene sequences from the isolated thermotolerant LAB strains

3.2 Characterization of probiotic properties

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

We found that all isolates including reference strains were non-hemolytic bacteria (γ-hemolysis), since they did not exhibit any effect on blood agar plates after 48 h of incubation. It might be indicated that they were not harmful strains or rarely cause illness. Probiotics must survive from the extreme conditions in GI tract of animals, especially high acidity in stomach and bile in the upper small intestine. Figure 2 and 3 show the survival ability of the selected thermotolerant isolates. After 3 h incubation in simulated gastric juice (0.5% pepsin, pH 2.5), we found that VCF29 could survive in this condition similar to the reference strain of JCM1022. Even the survivability of other two strains were lower, but about 65-80% of their survival rate were obtained (Figure 2). CK3 was the lowest survivability strain under gastric condition. This strain also

exhibited lower survivability in the simulated bile (0.3% bile acid) but only slightly lower than other strains (Figure 3). Interestingly, they could resist to bile with the survival rate higher than 90%. Effective probiotics must possess these characteristic to guarantee the number of viable probiotic cells reach to the colon. Survival of probiotic along the GI tract depends on not only these particular characteristics, but also because of the feed matrix (composition of feed ingested) and competition of microbiota in the intestine. The results from this experiment were consistent to the study of Aiba et al. (2015) and Yamano et al. (2006). A commercial *L. johnsonii* could survive when incubated at pH 1.0, 1.5 or 2.0 at 37 °C up to 120 min. Moreover, *L. johnsonii* La1 showed the great survivability after 15 h incubation in 0.1% bile acids and the simulated gastric juice among all tested bacteria. In case of bile resistance of Lactobacilli and Bifidobacteria, multi mechanisms involve in detoxification of bile; i.e. bile salt hydrolase production, active efflux of bile acids/salts and changing in the composition of cell membrane and cell wall (Ruiz et al., 2013).

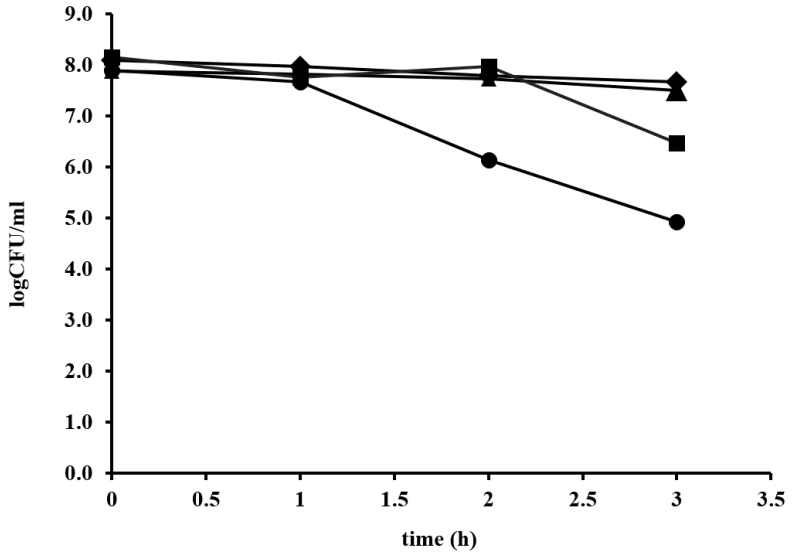

Figure 2. Survivability of thermotolerant *L. johnsonii* CK3 (●) and *L. johnsonii* VCF29 (♦) in simulated gastric juice comparing to the reference strains of *L. johnsonii* JCM1022 (▲) and *L. johnsonii* JCM8791 (■)

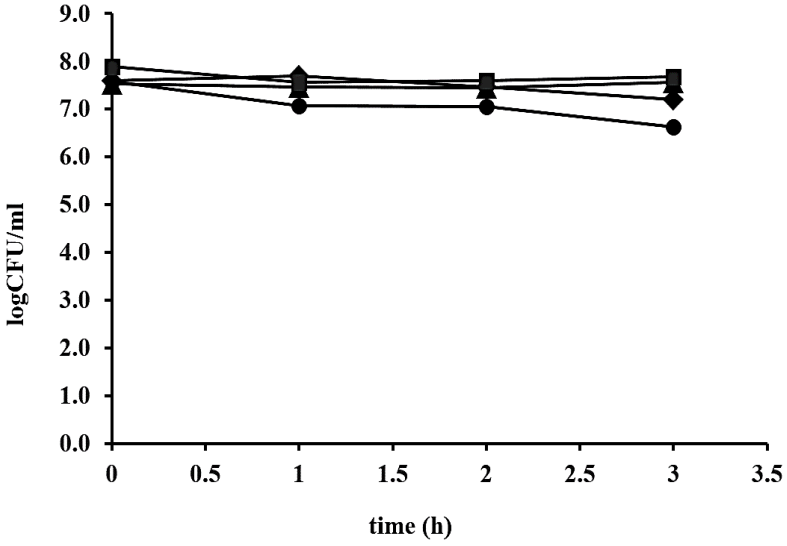

Figure 3. Survivability of thermotolerant *L. johnsonii* CK3 (●) and *L. johnsonii* VCF29 (♦) in bile comparing to the reference strains of *L. johnsonii* JCM1022 (▲) and *L. johnsonii* JCM8791 (■)

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

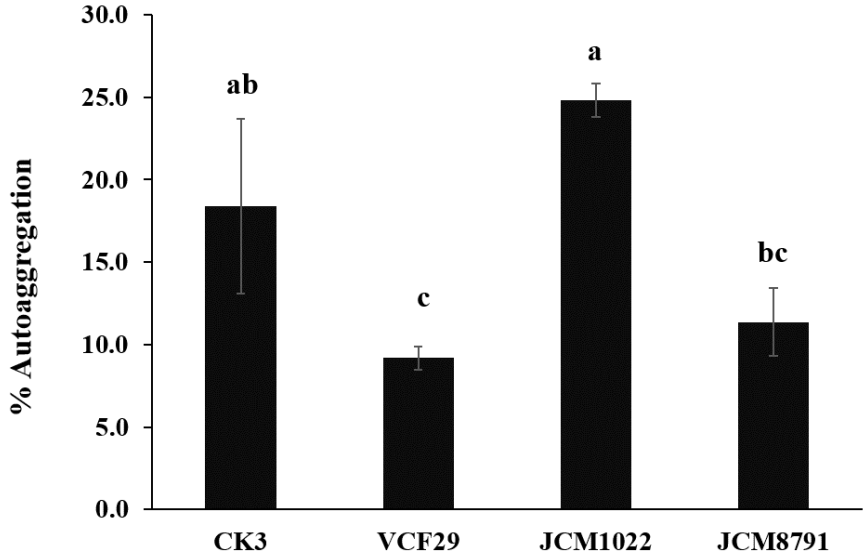

Figure 4. Autoaggregation percentages of the isolated thermotolerant LAB comparing to reference strains of *L. johnsonii*

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

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

Table 1. Antibiotic resistances of thermotolerant LAB depending upon various antibiotic

Antibiotics disc	CK3	VCF29	JCM1022	JCM8791
Cefoxitin	S	S	S	S
Tetracycline	S	R	S	R
Chloramphenicol	S	S	S	S
Erythromycin	R	S	S	S
Clindamycin	S	S	S	R
Vancomycin	S	S	S	S
Ampicillin	S	S	S	S
Ceftriaxone	S	S	S	S

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

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

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

Strains	clear zone (mm)			
	S. aureus	*P. vulgaris*	*S.* Typhimurium	*E. coli*
CK3	4.0±0.0	6.0±0.0	5.0±0.0	4.0±0.0
VCF29	10.3±0.6	6.0±0.0	5.0±0.0	5.0±0.0
JCM1022	6.7±1.2	7.0±0.0	7.0±0.0	6.3±2.3
JCM8791	10.0±1.7	7.0±0.0	7.0±0.0	6.7±0.6

4. CONCLUSION

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

ACKNOWLEDGMENT

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Poster Session | Food Function/Nutrition
[6-1130-P] Functional/Wellness Foods &Nutrition (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-06] Process optimization for antioxidant extraction from seed of soybean cultivar Chiang mai60

*Arpatsara Seekoompa[1], Pairote Wongputtisin[1], Piyanuch Niamsup[1] (1. Program in Biotechnology, Faculty of science, Maejo University, Chiang mai(Thailand))

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

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

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

Arpatsara Seekoompa, Pairote Wongputtisin*, Piyanuch Niamsup
Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai, Thailand

*Corresponding author: pairotewong@gmail.com

ABSTRACT

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

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

1. INTRODUCTION

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

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

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

2. MATERIALS AND METHODS

2.1 Raw material

Soybean seed, cultivar Chiang mai60, was kindly obtained from Chiang mai Field Crop Research Center, Chiang mai, Thailand. The seed was grind into fine powder by electric grinder and then dried at 55ºC for 12 h soybean powder was kept under -20ºC.

2.2 Optimization for process of antioxidant extraction

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

Table 1. The range and level of each setting variables in the central composite design

Variables	Parameter	Range and levels		
		-1	0	1
A	Ratio of water to powder (ml : g)	10.00	20.00	30.00
B	Temperature (ºC)	30.00	45.00	60.00
C	Time (h)	2.00	4.00	6.00

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

Treatments	Variables		
	Ratio of water to soybean powder (ml : g)	Temperature (°C)	Time (h)
1	10.00	30.00	2.00
2	30.00	30.00	2.00
3	10.00	60.00	2.00
4	30.00	60.00	2.00
5	10.00	30.00	6.00
6	30.00	30.00	6.00
7	10.00	60.00	6.00
8	30.00	60.00	6.00
9	3.18	45.00	4.00
10	36.82	45.00	4.00
11	20.00	19.80	4.00
12	20.00	70.20	4.00
13	20.00	45.00	0.64
14	20.00	45.00	7.36
15	20.00	45.00	4.00
16	20.00	45.00	4.00
17	20.00	45.00	4.00
18	20.00	45.00	4.00
19	20.00	45.00	4.00
20	20.00	45.00	4.00

2.3 ABTS inhibition activity assay

Antioxidant activity of the extracts were determined by measurement of free radical scavenging activity using ABTS inhibition assay. Briefly, ABTS cation radical ($ABTS^{\bullet+}$) solution was diluted with DI water to obtain an absorbance of 0.700 at 734 nm. 10 µl of extract was added to 990 µl of $ABTS^{\bullet+}$ solution, mixed well and recorded the decreasing of A_{734} every 1 min until stable. Percent inhibition was calculated using the formula,

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

2.4 Isoflavones determination by HPLC

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

3. RESULTS AND DISCUSSIO

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

model according to lack of fit ($p<0.0001$). The estimated coefficient of three factors and their interaction were also analyzed. We found only the ratio between water and powder was highly significant ($p<0.001$) to antioxidant extraction with negative effect. Extraction time was another negative factor to antioxidant extraction from Chiang mai60 powder but not significant. The interactions between time and other two factors were negative but slightly influenced to antioxidant activity ($P>0.05$). Subsequently, the quadratic model for prediction of antioxidant extraction from the powder of Chiang mai60 seed was simulated by the software as follow:

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

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

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

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

Table 3. The actual and predicted antioxidant activity results optimization for process of antioxidant extraction by the central composite design

Treatments	Ratio of water to soybean powder (ml : g)	Temperature (°C)	Time (h)	ABTS scavenging activity (%)	
				Actual	Predicted
1	10.00	30.00	2.00	37.37	30.52
2	30.00	30.00	2.00	16.41	38.34
3	10.00	60.00	2.00	41.48	18.02
4	30.00	60.00	2.00	17.08	13.74
5	10.00	30.00	6.00	38.93	35.29
6	30.00	30.00	6.00	12.98	15.75
7	10.00	60.00	6.00	36.90	23.38
8	30.00	60.00	6.00	14.83	14.25
9	3.18	45.00	4.00	85.55	22.88
10	36.82	45.00	4.00	10.96	45.26
11	20.00	19.80	4.00	21.84	12.94
12	20.00	70.20	4.00	22.33	14.03
13	20.00	45.00	0.64	22.20	18.42
14	20.00	45.00	7.36	22.98	16.20
15	20.00	45.00	4.00	23.58	15.74
16	20.00	45.00	4.00	21.98	15.06
17	20.00	45.00	4.00	23.06	20.31
18	20.00	45.00	4.00	23.56	19.23
19	20.00	45.00	4.00	21.76	20.40
20	20.00	45.00	4.00	23.41	17.41

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

Source	SS	DF	MS	F-value	Significant value (p-value)
Model	4544.58	9	504.95	11.33	0.0004
Residual	445.77	10	44.58		
Lack of fit	442.44	5	88.49	132.89	<0.0001
Pure error	3.33	5	0.67		
Total	4990.35	19			
R^2= 0.9107					

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

Table 5. Coefficient estimates by the regression model

Independent variables (parameter)	Coefficient	Standard error	Significant value (p-value)
Intercept	23.04	2.72	
A-ratio	-16.02	1.81	<0.0001
B-temp	0.40	1.81	0.8304
C-time	-0.54	1.81	0.7707
AB	0.055	2.36	0.9819
AC	-0.33	2.36	0.8908
BC	-0.62	2.36	0.7982

*Statistically significant at 95% of confidence level.

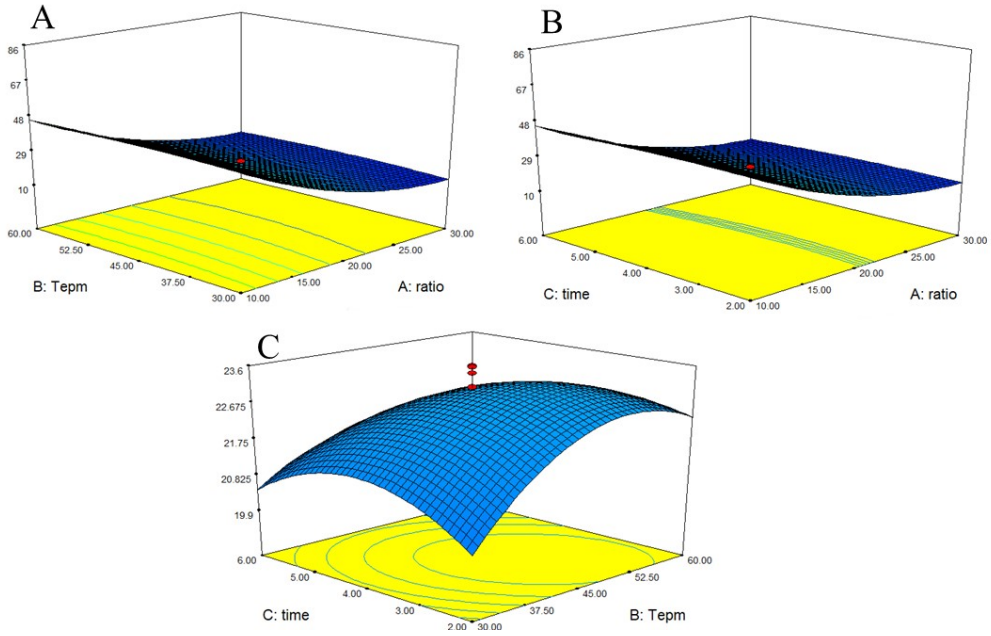

Figure 1. The response surface graphs exhibiting the antioxidant activity of the extract obtained as the function of three factors (A: ratio of water to soybean powder, B: extraction temperature and C: extraction time)

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

Trt	Isoflavones content (mg/g)								
	Glucosides			Aglycones			Total glucosides	Total aglycones	Total Isoflavones
	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein			
1	0.04	0.02	0.11	0.02	0.01	0.01	0.16	0.07	0.23
2	0.06	0.02	0.16	0.04	0.01	0.10	0.23	0.15	0.38
3	0.09	0.03	0.17	0.02	0.01	0.05	0.29	0.08	0.37
4	0.09	0.02	0.21	0.03	0.00	0.09	0.32	0.12	0.44
5	0.02	0.01	0.07	0.03	0.01	0.06	0.10	0.09	0.19
6	0.04	0.01	0.10	0.04	0.01	0.11	0.15	0.15	0.30
7	0.09	0.03	0.15	0.02	0.01	0.06	0.26	0.09	0.35
8	0.09	0.02	0.20	0.03	0.01	0.11	0.32	0.14	0.46
9	0.05	0.02	0.17	0.07	0.02	0.20	0.24	0.30	0.54
10	0.01	0.00	0.07	0.04	0.00	0.14	0.08	0.18	0.27
11	0.05	0.02	0.12	0.02	0.00	0.08	0.18	0.10	0.28
12	0.10	0.03	0.19	0.02	0.00	0.09	0.32	0.12	0.44
13	0.05	0.02	0.14	0.03	0.01	0.09	0.20	0.13	0.33
14	0.02	0.00	0.06	0.04	0.01	0.12	0.08	0.16	0.24
15	0.02	0.00	0.09	0.04	0.01	0.10	0.11	0.15	0.26
16	0.00	0.00	0.08	0.04	0.01	0.09	0.08	0.13	0.21
17	0.02	0.01	0.09	0.04	0.01	0.10	0.12	0.15	0.27
18	0.02	0.00	0.08	0.04	0.01	0.09	0.10	0.14	0.24
19	0.02	0.00	0.07	0.04	0.00	0.10	0.10	0.14	0.23
20	0.02	0.00	0.07	0.04	0.00	0.10	0.10	0.14	0.23

4. CONCLUSION

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

ACKNOWLEDGMENT

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

[6-1130-P-07] Nutritional and Functional Properties of Yoghurt Drink with Philippine Gac (*Momordica cochinchinensis* Spreng.) and Bignay (*Antidesma bunius*) Fruits

Rowie Joy Gonzales Bucks[1], *Ara Fatima Cuvinar Algar[1], Ryan Rodrigo Paner Tayobong[2] (1. Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines), 2. Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Banos(Philippines))

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

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

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

[6-1130-P-08] Effect of Extracting Conditions on Plant Extract Colors and Stability of Antioxidant Properties during *in vitro* Gastrointestinal Digestion

*Rattika Aeka[1], Titikan Liangpanth[1], Rungarun Sasanatayart[1] (1. School of Agro-Industry, Mae Fah Luang University(Thailand))

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

Natural pigments extracted from plants provide distinctive color and exert antioxidant effects that are far more superior than synthetic colorants. Synthetic colorants tend to be undesirable by consumers, due to the harmful effects on human health, including allergic reactions, mutagenicity and potential carcinogenicity. As a result, there is a worldwide trend toward the natural colorants, in particular in food applications. In this study, four major types of plant pigment including anthocyanins from butterfly pea flower (*Clitoria ternatea L*

.), betalains from dragon fruit peel (*Hylocereus undatus*), carotenoids from turmeric rhizome (*Curcuma longa*) and chlorophylls from pandan leaf (*Pandanus amaryllifolius*) were extracted under different conditions. Three types of solvent (water, 70% w/w acedic acetone and 50% w/w aqueous ethanol) and three mechanical extraction methods (shaking 25˚ C for 24 h, sonication at 25˚ C for 1 h and sonication at 65˚ C for 1 h) were compared. Results showed that 50% w/w aqueous ethanol was the most effective solvent for extraction of carotenoid form turmeric and chlorophyll from pandan leaf whereas, water was the most effective solvent for extraction of betalain form dragon fruits peel and anthocyanin form butterfly pea flower. Between mechanical extractions, sonication was better than shaking in extracting the require pigments (carotenoids, betalains, anthocyanins and chlorophylls), total phenolics and total flavonoid from selected plants. Overall, sonication at 25˚ C was better than sonication at 65˚ C in obtaining plant extract with high antioxidant activities based on FRAP and DPPH with the reduced energy consumption. Therefore, color extracted with sonication at 25˚ C was used for testing the stability upon *in vitro* gastrointestinal digestion. Results showed that pigment compounds and related antioxidant activities of all four color extracts become less stable along digestion. Results revealed that the stability of each pigments and their related antioxidant during *in vitro* digestion from high to low were anthocyanins, carotenoids, chlorophylls and betalains, respectively. Data of this study supports the extension use of natural colorants as substitutes for synthetic dyes in food applications. However, the effect of food processing parameters including pH, heat and ingredients must be taken into accounted.

Poster Session | Food Function/Nutrition
[6-1130-P] Functional/Wellness Foods &Nutrition (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

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

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

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

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

pH Adjustment and Thermal Treatments Affect Plant Extract Colors and Antioxidant Activities during *in vitro* Digestion

Baifah Sangarun[1], Titikan Liangpanth[1] and Rungarun Sasanatayart1*

[1]Program of Postharvest Technology and Logistics, School of Agro-Industry, Mae Fah Luang University, Chiang Rai 57100, Thailand

*Corresponding author: rungarun.s@ mfu.ac.th

ABSTRACT

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

Keywords: Anthocyanins Betalains Carotenoids Chlorophylls Antioxidant pH Heat treatments *in vitro* gastrointestinal digestion

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

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

Kanako Niiya[1], *Takahiro Saito[2], Masatsugu Tamura[2], San Woo Bang[2] （1. Utsunomiya University Graduate School(Japan), 2. Utsunomiya Univ.(Japan)）

Keywords: Antioxidant, Growth, Komina, Radiation ratio

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

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

[6-1130-P] Other Categories (6th)

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

[6-1130-P-11] Temporal Source Strength Estimation of Sweet Pepper for Crop Management and LED Supplementation Efficiency Improvement

*Masaaki Takahashi[1], So Kaneko[1], Osamu Koike[1], Hiroki Umeda[2], Yasunaga Iwasaki[3] （1. Miyagi Prefectural Agriculture and Horticulture Research Center(Japan), 2. Graduate School of Bioresource Sciences, Nihon University(Japan), 3. National Agriculture and Food Research Organization(Japan)）

11:30 AM - 12:30 PM

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

*YEONSOO KIM[1,2], YONGJOO KIM[2], HYOGEOL KIM[1], YOUNGJOO KIM[1], SANGDAE LEE[1] （1. KITECH(Korea), 2. Chungnam Univ.(Korea)）

11:30 AM - 12:30 PM

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

*KITTIPON APARATANA[1], Hiroo Takaragawa[1,2], Yoshinari Izumikawa[1,2], Eizo Taira[1] （1. Faculty of agriculture, University of the Ryukyus, Okinawa 903-0213(Japan), 2. The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima 890-0065(Japan)）

11:30 AM - 12:30 PM

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

*Misaki Mito[1], Takuya Aoki[1], Koichi Mizutani[2], Keiichi Zempo[2], Naoto Wakatsuki[2], Yuka Maeda[2], Nobuhiro Takemae[3], Takehiko Saito[3] （1. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan)）

11:30 AM - 12:30 PM

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

*Akifumi Goto[1], Misaki Mito[1], Tadashi Ebihara[2], Koichi Mizutani[2], Naoto Wakatsuki[2], Nobuhiro Takemae[3], Takehiko Saito[3] （1. Graduate School of Systems and information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan)）

11:30 AM - 12:30 PM

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

*Yasuhiko Nishijima[1], Koichi Mizutani[1,2], Tadashi Ebihara[1,2], Naoto Wakatsuki[1,2], Kenji Kubota[3], Hiroyuki Uga[4] （1. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, Division of Engineering Interaction Technologies, University of Tsukuba(Japan), 3. Agriculture Research Center, National Agriculture and Food Research Organization(Japan), 4.

Saitama Prefecture Agriculture Research Center(Japan))
11:30 AM - 12:30 PM

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

*Songsin Photchanachai[1], Nipada Ranmeechai[1,2], Chalinee Sungkajorn[1,2], Anantaporn Phankhaek[1,2], Kornkanok Aryusuk[1], Varit Srilaong[1,2], Panida Boonyarithongchai[1,2], Nutthachai Pongprasert[1,2] (1. School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok(Thailand), 2. Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok(Thailand))
11:30 AM - 12:30 PM

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

[6-1130-P-11] Temporal Source Strength Estimation of Sweet Pepper for Crop Management and LED Supplementation Efficiency Improvement

*Masaaki Takahashi[1], So Kaneko[1], Osamu Koike[1], Hiroki Umeda[2], Yasunaga Iwasaki[3] (1. Miyagi Prefectural Agriculture and Horticulture Research Center(Japan), 2. Graduate School of Bioresource Sciences, Nihon University(Japan), 3. National Agriculture and Food Research Organization(Japan))

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

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

Poster Session | Others (including the category of JSAM and SASJ)
[6-1130-P] Other Categories (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

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

*YEONSOO KIM[1,2], YONGJOO KIM[2], HYOGEOL KIM[1], YOUNGJOO KIM[1], SANGDAE LEE[1] （1. KITECH(Korea), 2. Chungnam Univ.(Korea)）

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

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

Study on Analysis of Loads Effect on Path-Tracking Accuracy of an Autonomous Tractor during Plow Tillage

Yeon-Soo Kim[1, 2], Hyo-Geol Kim[1], Young-Joo Kim[1], Yong-Joo Kim[2], Sang-Dae Lee[1*]

[1] Convergence Agricultural Machinery Group, Korea Institute of Industrial Technology (KITECH), Republic of Korea

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

*Corresponding author: sdlee96@kitech.re.kr

ABSTRACT

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

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

1. INTRODUCTION

The agricultural production and labor shortage issues are constantly increasing due to rural aging (Celik et al., 2018). Agricultural machinery automation is one of the most effective solution of improving agricultural challenges such as food, operating cost, working environment, and so on (Zhang and Pierce, 2013). The global agricultural machinery market is expected to grow at a CAGR (Compound Annual Growth Rate) of 6.6% from US $ 140.7 billion in 2014 to US $ 193.5 billion in 2019 (KREI, 2018). The global autonomous tractors market is expected to grow at a CAGR of 24.8%, reaching 12,508 Units in 2019 and 60,901 Units by 2025. The growth of the autonomous tractor market is expected to lead the government or several primary manufactures as a part of the adoption of new technologies to improve the working efficiency and productivity of crop yields (Li et al., 2019; MarketsandMarkets, 2018). The tractor is main product of agricultural machinery due to various uses as agricultural power source (Kim et al, 2018). Especially, the most important performance evaluation factor of

autonomous tractor is lateral error distance (McCall and Trivedi, 2005). Therefore, the importance of developing autonomous tractor equipped with accurate path-tracking technology for securing competitiveness of the global market is increasing. In addition, the research on the performance evaluation method of path-tracking accuracy has been actively carried out.

In order to improve the convenience of farmers and crop productivity, many studies have been conducted various autonomous agricultural machinery. Some studies related to the autonomous agricultural machinery have been carried out in relation to path-tracking performance considering lateral error and heading angle on off-road surface without tillage operation. The study has been conducted on methods for estimating a utility and agricultural vehicle's dynamic parameters using a RTK-GPS and Inertial Measurement Unit (IMU). The results showed that the measured data using a RTK-GPS and IMU can be used to estimate the tire sideslip and the tire cornering stiffness under different soil conditions (Ospina and Noguchi, 2016; Ospina and Noguchi, 2018). Liu et al, (2019) studied the image processing based UAV (Unmanaged Aerial Vehicle) used for spraying pesticides and herbicide. The results show that the proposed algorithm has more accurate path-tracking performance than DGPS based UAV. Yin et al, (2018) developed an autonomous navigation system using sensor fusion algorithm that automatically guided a rice transplanter working along predetermined paths including steering, stop, going forward and reverse. The results showed that path-tracking were robustly executed in terms of following straight paths. Rahman et al, (2019) developed an optimum harvesting area of a convex and concave polygon for path planning of robot combine harvester. The results show that this developed algorithm estimates the optimum harvesting and reduces crop losses. It is also calculated based on the corner vertices minimizes the total operation time. In another study, the leader follower system was developed using two autonomous tractors for agricultural operation (Zhang and Noguchi, 2017). This experiment results showed the two autonomous tractor can work safely to complete the operation, and the system's efficiency improved by 95.1% compared with using a single tractor. In another study, an adaptive turning algorithm for a four-wheel autonomous tractor was developed using navigation sensors consisted of an inertial measurement unit and a real-time kinematic global positioning system (Wang and Noguchi, 2018). The results showed that the time consumption and turning trajectory were decreased by 17% and 21%, respectively, compared to a conventional turning algorithm. There have been many studies on the automatic steering system of agricultural machinery. These performance evaluation method only using the posture and position information which are logged on the IMU and GPS in the path-tracking performance evaluation has been performed. This method is a performance evaluation method widely used in the automotive field. Generally, an autonomous commercial vehicles drive on a standard road surface such as asphalt without disturbance. However, an agriculture tractor have a relatively high load due to tire slip, sudden change in attitude angle, soil resistance, etc., depending on the correlation between soil and attachment implement during tillage operation (Wong, 2008). This does not take into account the error on the GPS sensor, and therefore it is not an accurate performance evaluation method. In addition, performance evaluation was performed by simple driving without tillage operation which can be used as a representative use purpose of actual farm machinery. The performance evaluation method of the path-tracking accuracy of an autonomous tractor under no tillage operation condition is did not consider the effect of the work load according to the terramechanics factors between soil and the agricultural tractor with attached implement. The load of the agricultural machinery is an important indicator of farming characteristics, and performance evaluation of agricultural machinery through load analysis is essential (Nahmgung, 2001). Therefore, it is necessary to study the new performance evaluation method from the viewpoint of work load which is generated on tractor's main part under tillage operation conditions.

To improve the quality of the tractor, it is necessary to analyze the tractor working load during operation. This is because the work load characteristics are affected by various factors such as soil properties, operation type, traveling speed (gear selection), tillage implement shape, and tillage depth. Load analysis of agricultural tractor during field operation is important in achieving the optimum design of tractors.

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

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

2. MATERIALS AND METHODS

2.1 Agricultural tractor and implement

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

Table 1. Specification of the 78-kW agricultural tractor.

Item		Specification
Length × Width × Height (mm)		4225 × 2140 × 2830
Weight (kg)		3985
Engine	Rated power (kW)	78 @ 2300 rpm
	Max. torque (Nm)	430 @ 1400 rpm
Transmission	Main	4 stages and power shift (High, Low)
	Sub	4 stages

2.2 Lateral error measurement system

The lateral error measurement system was built to measure the lateral error distance and the traveling speed of tractor. First, the lateral error distance was measured using an electric tacheometer (iX series, TOPCON, Tokyo, Japan), a GNSS receiver (GCX3, TOPCON, Tokyo, Japan), and a prism attached to the tractor cabins at the center of gravity position. The electric tacheometer was used to evaluate the accuracy of the steering system of the autonomous tractor.

Second, the traveling speed of the tractor, which is the basic measuring element of tillage operation, was measured using RTK–GPS (Span CPT, NovAtel Inc., Calgary, Canada) and an antenna attached to the tractor's center of gravity. In addition, the RTK base station was installed to ensure stable RTK–GPS status. The precisely measured traveling speed with RTK–GPS was used as a factor to determine the longitudinal slip ratio, which is the basic factor for evaluating the working performance of a tractor. The detailed lateral error measurement system configuration is shown in Figure 1.

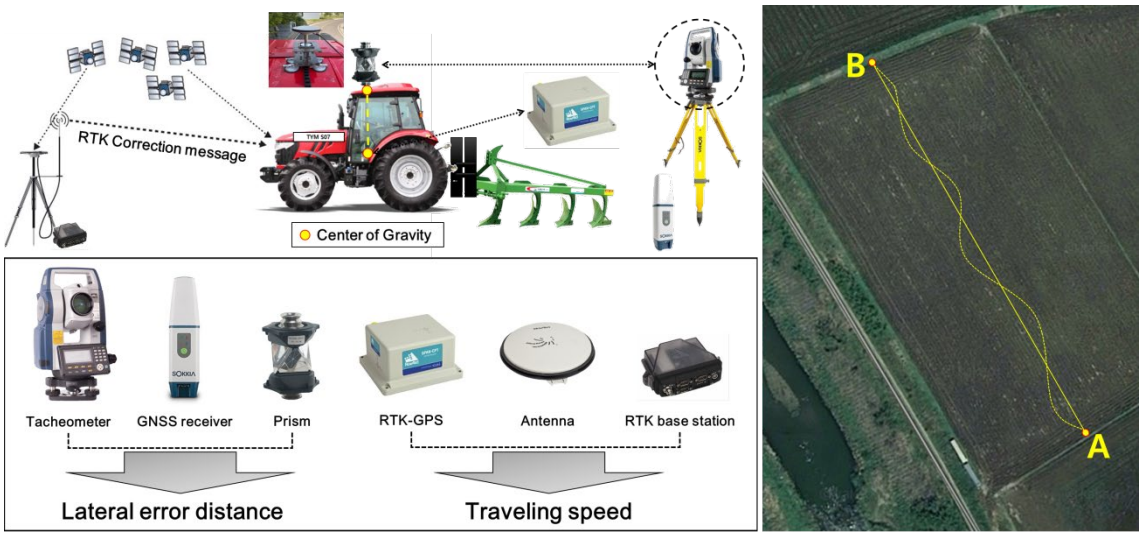

Figure 1. Configuration of lateral error distance system.

2.3 Load measurement system

The load measurement system was configured to measure the draft force and wheel torque off agricultural tractor. The detailed configuration of load measurement system is shown in Figure 2. In this experiment, the torque meter (PCM16, MANNER, Spaichingen, Germany) and the telemetric proximity sensor (PRDCML30–25DN, Autonics, Busan, Korea) were used to measure both the torque and the rotational speed of the agricultural tractor. The torque meter was installed on each of the four axle shafts. One antenna was provided for each torque meter. The axle torque data measured at the torque meter were amplified by the amplifier in the torque meter and transmitted to the antenna, and the data transmitted to the antenna were transmitted to the meter along the cable line. The nominal load of the torque meter was 20 kNm, the maximum load was 400%, and the sensor was of a strain gage type. The sampling rate was 4 kS/s, the maximum axle rotation speed was 4000 rpm. The operating temperature was -25 to 125 °C. The 6–component load cell (UU–T2, DACELL, Cheongju, Korea) was installed between the moldboard plow and tractor body rear side for measuring draft force. The 6-component load cell consisted of three load cells measuring the horizontal force and three load cells measuring the vertical force. In this experiment, only three load cells were used to measure the horizontal component of the implement draft.

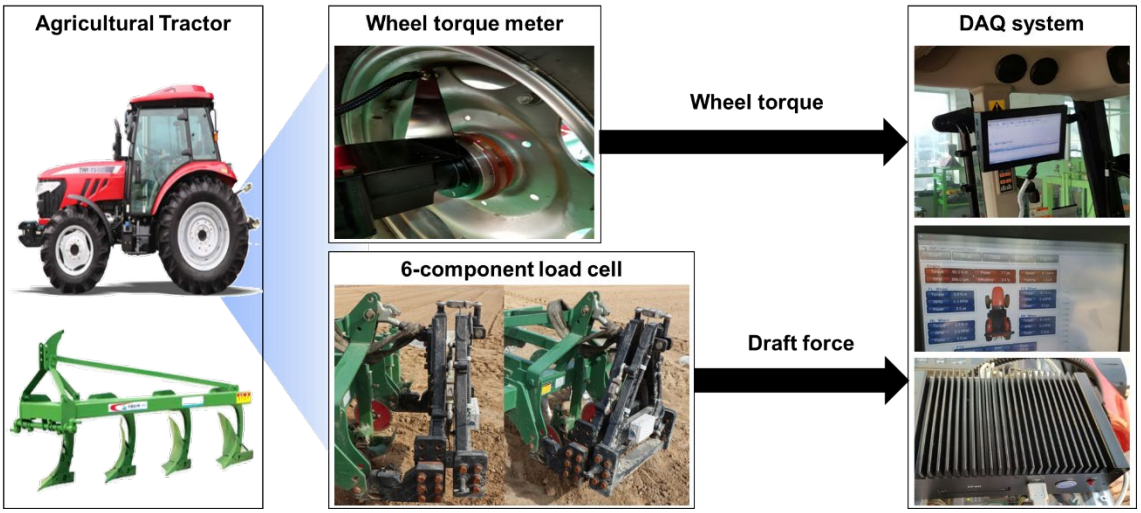

Figure 2. Configuration of load measurement system.

2.4 Measurement of soil physical properties

Tractor tillage operations have a major impact on crop growth and crop yields. The plow layer is the target tillage depth section that is cultivated annually or periodically through an agricultural tractor. The thickness of the plow layer is usually 5–25 cm, and this layer is often greatly worked on in relation to tillage operation, fertilizer, irrigation, and crops. The physical properties of soil such as the cone index, moisture content, and soil classification affect the interactions between the soils and the agricultural machine. The measurement process of soil properties is shown in Figure 3. In order to analyze the lateral error distance of autonomous tractor according to work load during tillage operation, the soil physical properties (cone index, moisture content, and soil classification) of the test site were confirmed using a cone penetrometer (FieldScout SC 900, Spectrum Technologies, Aurora, USA) and a soil sensor (FieldScout TDR 350, Spectrum Technologies, Aurora, USA). The measured soil properties such as cone index (CI), the moisture content (MC), and soil classification by particle size were analyzed following USDA standard methods.

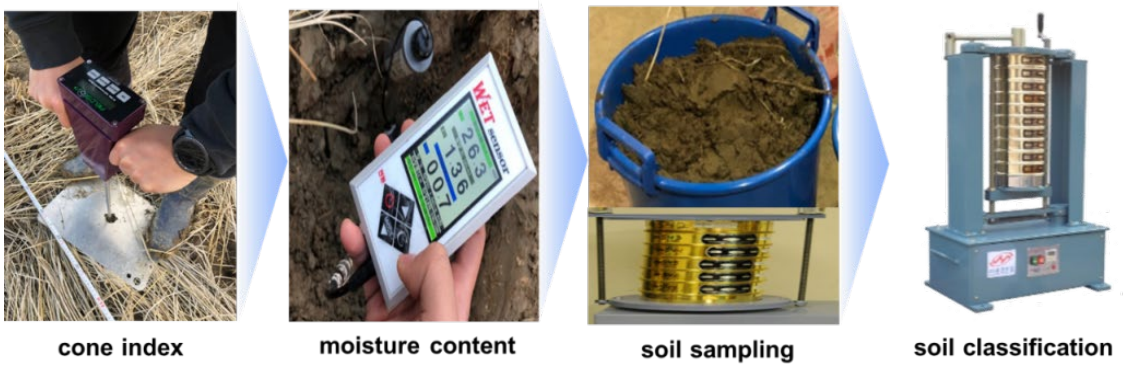

Figure 3. Test procedure of soil physical properties measurement.

2.5 Test procedure

The field experiments were conducted in Kumam–ri, Songsan–myeon, Chungcheongnam–do, Korea. The test site is 100×100 (m) in size and is located at latitude and longitude coordinates 36°55’48” N and 126°37’59” E, respectively. The field test was conducted in four–wheel mode and at three gear stages (M2-high, M3-Low, M3-High), which are commonly used to perform moldboard plow operation. The moldboard plow operation was carried out at the lowest tractor 3-point hitch to perform the under top hardpan section of the plow layer.

3. RESULTS AND DISCUSSION

3.1 Soil physical properties

The main analysis results of soil properties are as follows. The average moisture content of the test site was 38.6%, the mean CI was 2407 kPa, and the mean formation depth of hardpan was 12.5-25 cm. A soil particle size analysis revealed loamy sand in all soil layers. High cone index values indicate high soil compaction, which is a major problem when managing poorly drained soils. A soil hardpan layer with high soil compaction resulting from excessive and improper use of agricultural machinery leads to lower soil porosity and air permeability interferes with the growth of crop roots, and poor drainage. Therefore, during moldboard plow operation, the minimum tillage depth should be where soil compaction is increased. Thus, tillage operations should promote crop growth and ensure porosity and air permeability. In general, the results of plow tillage tend to show irregular tillage depths. Based on an analysis of the test results of the cone index, there is a point at a certain depth where an instantaneous slope occurs. The occurrence of an instantaneous slope implies that a rigid plate is located, and this depth is called the top hardpan. Owing to this reason, the target tillage depth must be set considering the hardpan layer, which indicates the distance from the depth of the top hardpan to the depth of the peak cone index. The cone index was measured using a cone penetrometer and detailed analysis results are shown in Figure 4.

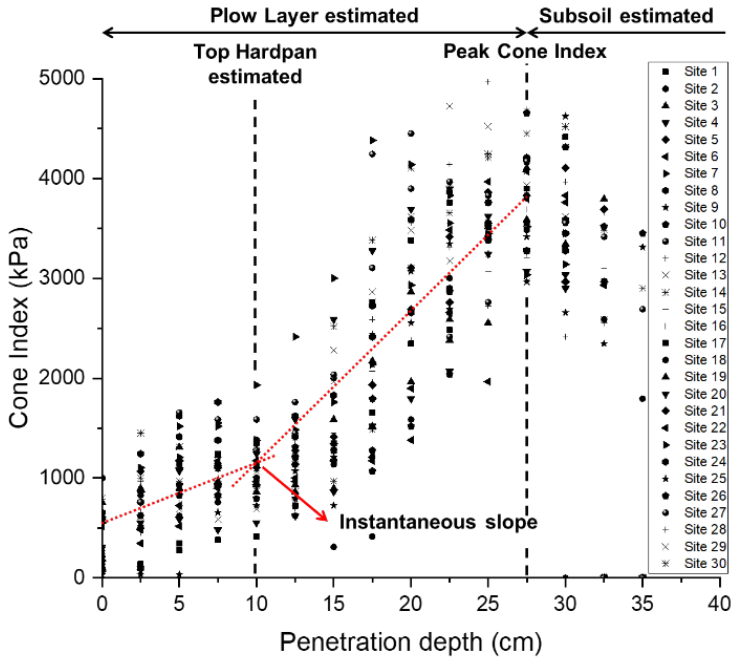

Figure 4. Test results of the cone index using a penetrometer.

3.2 Lateral error distance

The average the draft force were 30.39, 32.57, and 32.79 kW at each gear selection. The draft force increased 2.18 kN when gear shift from M2-High to M3-Low, and increased 0.22 kN when gear shift from M3-Low to M3-High. Almost same torque values were shown in M2-High and M3-Low gear selection. The average front wheel torque were 3267.34, 3693.32, and 3852.37 kW at each gear selection. The average front wheel torque increased 65.98 Nm when gear shift from M2-High to M3-Low, and increased 159.05 Nm when gear shift from M3-Low to M3-High. The front axle load showed a tendency to increase as the number of gears selection. The average the rear wheel torque were 6080.12, 6934.53, and 6727.92 Nm at each gear

selection. The rear axle load, which is the most affected factor according to the traveling speed of the agricultural tractor, the tillage depth of the attachment workstation, and the operation type, was found to be the largest in M3-Low gear selection, not M3-High. This is judged to have resulted in a relatively large slip rate at the M3-High gear selection, resulting in a torque loss. The overall data tends to be similar to the traveling speed data as the number of gears selection. However, if the draft force values were similar, the lateral error was greatest in the M2-Low gear selection with a large rear wheel torque. Based on these results, the lateral error of the autonomous tractor is judged to be most affected by the rear wheel torque rather than the draft force and front wheel torque. The detailed overall test results of the lateral error distance according to work load are listed in Table 2.

Table 2. Lateral error distance according to draft force and wheel torque.

Gear selection	Tillage depth (cm)			
	Draft force (kN)	Front wheel (Nm)	Rear wheel (Nm)	Lateral error (cm)
M2-High	30.39	3627.34	6080.12	4.59
M3-Low	32.57	3693.32	6934.53	7.21
M3-High	32.79	3852.37	6727.92	5.88

4. CONCLUSION

A lateral error measurement system and load measurement system are proposed here for measuring tractor work load and lateral error distance during moldboard plow operation. This system configuration allows for precise measurement of lateral error distance, which was previously difficult to measure, and shows the effect of work load on path-following performance of autonomous tractor. The conclusions of this study are as follows.

The work load has a great effect on the lateral error distance of autonomous tractor during moldboard plow operation. In particular, the rear axle load was found to have a significant effect on lateral error compared to draft force and front axle load. Therefore, the influence of work load should be considered when analyzing the lateral error distance of autonomous tractor in an actual paddy field.

In conclusion, the effect of work load on the lateral error distance of an autonomous tractor during moldboard plow operation was confirmed with the measurement system configuration presented in this paper. These findings can be used in future research on the path-following performance of autonomous agricultural machinery.

ACKNOWLEDGMENT

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Poster Session | Others (including the category of JSAM and SASJ)
[6-1130-P] Other Categories (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

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

*KITTIPON APARATANA[1], Hiroo Takaragawa[1,2], Yoshinari Izumikawa[1,2], Eizo Taira[1] (1. Faculty of agriculture, University of the Ryukyus, Okinawa 903-0213(Japan), 2. The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima 890-0065(Japan))

Keywords: Sugarcane, Variety classification, PCA, PLS-DA, SVM-C

Sugarcane variety classification is essential for data collecting and learning for the breeder. It is difficult for a farmer to identify a sugarcane variety without specialist help. In this research, three Japanese sugarcane varieties (Ni15, Ni22, and Ni27) from six areas in the south of Japan were classified according to full pixel and color features of the sugarcane bud. The 54 images of sugarcane bud were acquired from the sugarcane field using a mobile phone's digital camera, equipped with a fixed distance accessory. To develop classification models, two types of data; The full pixel data and color feature data from images were investigated for input to the model. The full pixel and color features were subjected to Principal component analysis (PCA) to describe the sugarcane bud samples. Then, the samples were classified into varieties by performing partial least squares discriminant analysis (PLS-DA) and support vector machine classification (SVM-C). The results of the full pixel show that the pooled classification rates (averaged three classification rate) by PLS-DA and SVM-C were 79.6% and 84.5%, respectively, while the pooled classification rates by PLS-DA and SVM-C of the color features were 75.9% and 74.1%, respectively. Therefore, these results show that the size and color spaces of sugarcane buds could be the keys to classifying sugarcane varieties and that the best way of classifying Japanese sugarcane (Ni15, Ni22, and Ni27) was the SVM-C method using full pixel of sugarcane bud.

Classification of Sugarcane Variety using Image Processing and Multivariate Analysis

Kittipon Aparatana[1*], Hiroo Takaragawa[1, 2], Yoshinari Izumikawa[1, 2] and Eizo Taira[1]

[1]Department of Agriculture, University of the Ryukyus, Japan
[2]United Graduate School of Agricultural Sciences, Kagoshima University, Japan

*Corresponding author: kittipon.aparatana@gmail.com

ABSTRACT

Sugarcane variety classification is essential for data collecting and learning for the breeder. It is difficult for a farmer to identify a sugarcane variety without specialist help. In this research, three Japanese sugarcane varieties (Ni15, Ni22, and Ni27) from six areas in the south of Japan were classified according to full pixel and color features of the sugarcane bud. The 54 images of sugarcane bud were acquired from the sugarcane field using a mobile phone's digital camera, equipped with a fixed distance accessory. To develop classification models, two types of data; The full pixel data and color feature data from images were investigated for input to the model. The full pixel and color features were subjected to Principal component analysis (PCA) to describe the sugarcane bud samples. Then, the samples were classified into varieties by performing partial least squares discriminant analysis (PLS-DA) and support vector machine classification (SVM-C). The results of the full pixel show that the pooled classification rates (averaged three classification rate) by PLS-DA and SVM-C were 79.6% and 84.5%, respectively, while the pooled classification rates by PLS-DA and SVM-C of the color features were 75.9% and 74.1%, respectively. Therefore, these results show that the size and color spaces of sugarcane buds could be the keys to classifying sugarcane varieties and that the best way of classifying Japanese sugarcane (Ni15, Ni22, and Ni27) was the SVM-C method using full pixel of sugarcane bud.

Keywords: Sugarcane, Variety classification, PCA, PLS-DA, SVM-C.

1. INTRODUCTION

Sugarcane is a critical economic crop in the world and Japan. Currently, in Japan, sugarcane is generally planted in the southern part during the summer or spring and then harvested in winter, and production has increased from 2015 to 2018 (Okinawa Prefectural, 2018). However, severe droughts and tropical storms (typhoons) frequently occur from July to September, which causes severe damage to sugarcane yield and sugar content through the loss of green leaves, lodging, and broken stalks (Takagi et al., 2005; Terauchi et al., 2012). Any delays in planting and ratooning due to planting and harvest conflicts will consistently affect the next season's harvest (Terauchi et al., 2012). Moreover, the loss of sugarcane continues for many reasons, including rotting, disease, parasitism, and harvesting (Kawanobe et al., 2017; Sharma and Tamta, 2015; Shinzato et al., 2015).

Sugarcane variety databases are key indices to learning and improving sugarcane variety for high yielding, high sucrose content, high biomass (Matsuoka, 2006), and high durability of a natural disaster. Generally, several sugarcane factories in Japan obtain data of sugarcane variety through inquiries with farmers and with the experience of a specialist. The sugarcane variety classification method nowadays is inconvenient, and it is difficult for a farmer to identify a sugarcane variety without specialist help, which can affect the quality of the database. Sugarcane variety classification mostly uses pictorial identification techniques based on bud shape, dewlap shape, leaf shape, etc. (Gravois et al., 2018; Takaragawa et al., 2019). Nevertheless, these techniques need a long time to correct the data and need the experience to be identified. Thus, there is a need for new tools or methods that could work faster, be more accurate, and be more convenient to use to identify sugarcane variety.

With recent advancements in computer technology, the image can extract much information from image data, such as many types of color space and intensity of color, with the image processing technique, which was widely used for detection or identification in the agriculture and food industry because it is fast, accurate, and cost-efficient (Chen et al., 2010; Khan and Yadav, 2017; Moshashai et

al., 2008). However, sugarcane variety classification using image processing has not been researched yet.
Therefore, the current research focuses on classifying Japanese sugarcane varieties using image processing. This research aims to use full pixels and color features of sugarcane bud images to describe and separate sugarcane varieties using multivariate analysis methods.

2. MATERIALS AND METHODS

Matlab R2018a (version: 9.4.0.813654, The Math Works, Natick, MA, USA, 2017) with the PLS toolbox (Eigenvector Research, Inc., Manson, WA, USA, 2017) was used for data processing and analysis.

2.1 Sugarcane samples

In this research, as shown in Figure 1, three Japanese sugarcane varieties (Ni15, Ni22, and Ni27) from six areas in Southern Japan (Minami island, Ishigaki island, Miyako island, Okinawa Nanbu, and two difference crops in University of the Ryukyus) were selected as sugarcane variety samples for classification according to full pixel image and color features. The image of 54 sugarcane bud samples (18 samples per variety) was acquired from the sugarcane field using a mobile phone's digital camera (iPhone SE, Apple Inc, USA) equipped with a fixed distance accessory. The first dimensions of the images were 3024 x 4032 pixels in JPG-format.

(a) (b) (c)

Figure 1. The sample image of sugarcane variety samples; (a) Ni15, (b) Ni22, and (c) Ni27.

2.2 Image processing

As shown in Figure 2, the images were then cropped on the bud area and their sizes reduced to 100 x 100 pixels in order to diminish the time and load of the analysis process. The acquired image is generally displayed in three-dimensional RGB color space. However, RGB color space is not perceptually uniform, and the proximity of colors does not indicate a color similarity. Color space transformations make for an effective means of distinguishing color images. The classification performance could be improved by weighting each color component differently. For this research, The RGB color space was evaluated as normalized RGB, YCbCr, and HSV color spaces.
The normalized RGB can be obtained from Eq. (1); in order to remove the brightness from the RGB color space, one can normalize the values of red, green, and blue.

$$\begin{aligned} r &= R/(R+G+B) \\ g &= G/(R+G+B) \\ b &= B/(R+G+B) \end{aligned} \quad (1)$$

The YCbCr can be obtained from Eq. (2) (Umbaugh, 2005).

$$\begin{aligned} Y &= 0.299R-0.587G+0.114B \\ Cb &= -0.1687-0.3313G+0.500B+128 \\ Cr &= 0.500R-0.4187G+0.0813B+128 \end{aligned} \quad (2)$$

As such, the Y element represents the luminance component, and the Cb and Cr elements represent two chrominance components.
The 12 color spaces were then extracted to 24 color features by computing the mean and standard deviation of color spaces. Subsequently, two types of data, the full pixel data and color feature data from images, were investigated for input to description analysis and classification analysis.

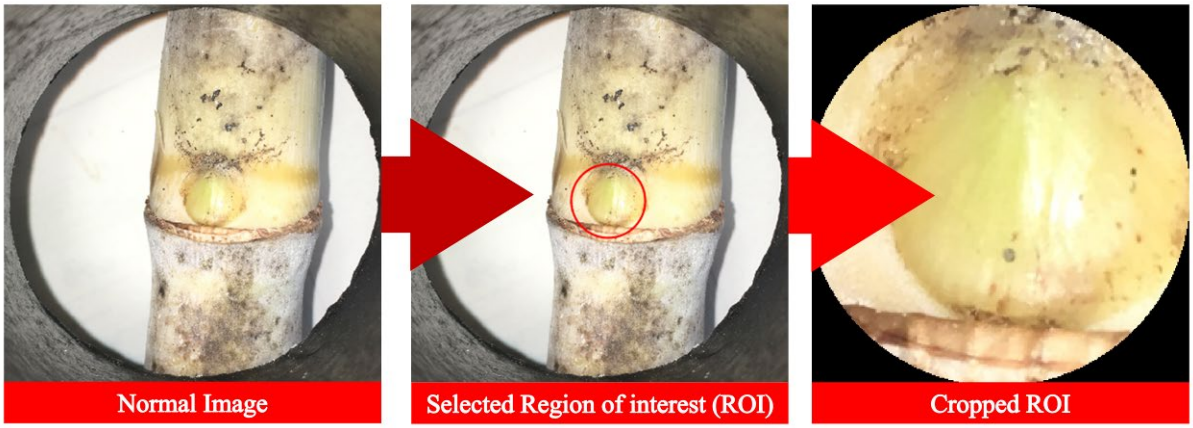


Figure 2. The example of sugarcane bud sample processing

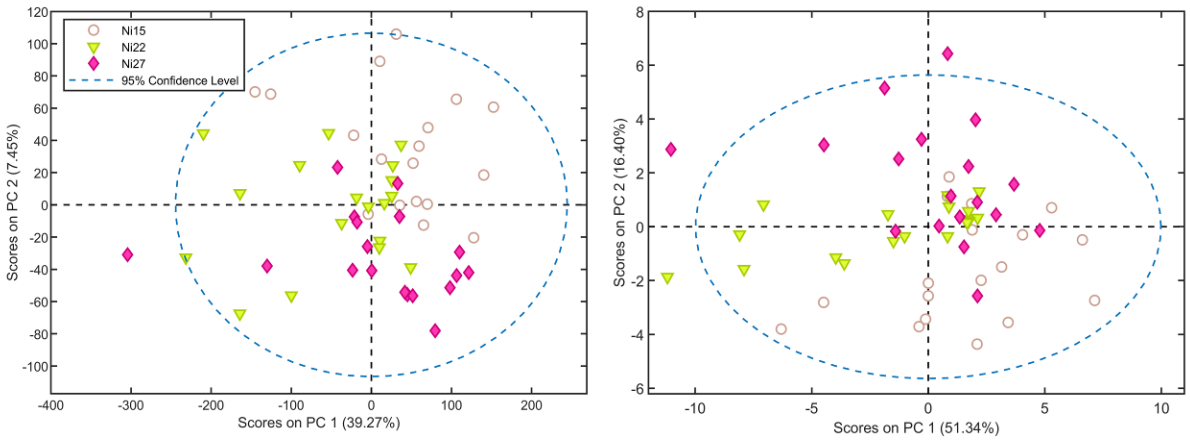
2.3 Multivariate analysis

The multivariate analysis techniques were objectives for description, classification, and prediction analysis. There are many types of multivariate data analysis techniques to choose from nowadays. The principal component analysis (PCA) is one of the frequently used methods for data description and explorative data structure modeling (Esbensen, 2000) and it's also one of the most critical and influential ways to decompose complex data (Bro and Smilde, 2014). Moreover, PCA could be used on a digital image for the benefit of learning and reducing size, as it enables locating the highest variance in data (Ng, 2017). The same goes for partial least squares discriminant analysis (PLS-DA) (Amigo et al., 2009) and support vector machine (SVM-C) (Zhang, 2012), which are the dominant methods for classifying data. Thus, this research chose PCA to describe the sugarcane bud samples and both PLS-DA and SVM-C for classifying sugarcane varieties.

3. RESULTS AND DISCUSSION

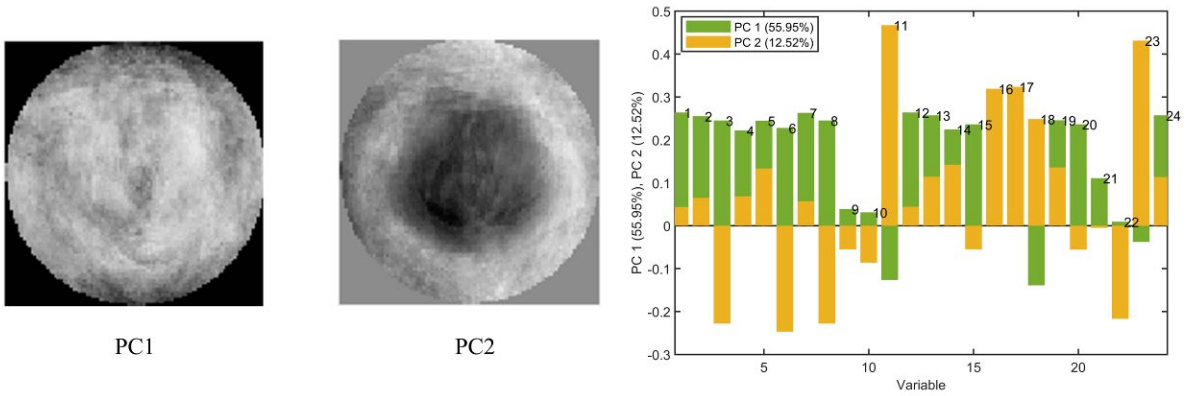
3.1 Principal component analysis results

Fifty-four of the sugarcane variety samples with two types of data (full pixel and 24 color features) were divided into three variety classes, resulting in 18 samples per variety. Then, a PCA analysis using the scores was undertaken to create a scattering plot of principal components 1 and 2, as shown in Figure 3. The sugarcane variety Ni15 and Ni27 were distinguished, but Ni22 overlapped a little with the other two varieties per the implementation of the first and second principal components in the two types of data. After recreating an image from full pixel loadings of principal component analysis, figure 4 (a) shows the first principal component was related to the lightness of the image; the second was related to the size of the sugarcane bud in the case of a full pixel by recreating an image from the loading of principal. Figure 4 (b) show in the color space of the sugarcane buds, the first principal component loading was mainly related to the mean of RGB, normalized RGB, Y, Cb, and V; the standard deviation of RGB, Y, Cb, and V. The second principal component loading mainly related to the mean of B, normalized b, Cb and S; the standard deviation of normalized RGB, H, and S.



(a) (b)

Figure 3. Score plots of principal component analysis for modeling samples (18 samples of sugarcane variety Ni15 in circle mark, Ni22 in triangle mark, and Ni27 in theta mark, respectively); (a) full pixel (b) color features.



(a) (b)

Figure 4. Loadings of principal component analysis; (a) full pixel (b) color features (where 1, 2, and 3 means mean of RGB. 4, 5, and 6 means mean of normalized RGB. 7, 8, and 9 means mean of YCbCr. 10, 11, and 12 means mean of HSV. 13, 14, and 15 means standard deviation of RGB. 16, 17, and 18 means standard deviation of normalized RGB. 19, 20, and 21 means standard deviation of YCbCr. 22, 23, and 24 means standard deviation of HSV.)

3.2 Classification of PLS-DA and SVM-V

Two types of data (full-pixel and 24-color features) and a two-class (model variety and other varieties) PLS-DA and SVM-C model were developed for variety classification. The 54 samples (18 model variety and 36 other variety) were used to create the PLS-DA and SVM-C model with Venetian blind cross-validation to determine the number of factors and evaluate the classification rate. The results presented in Table 1 reveal that the variety of Ni15 classification rates results of the full pixel by PLS-DA and SVM-C were 83.3% and 87.0%, respectively; the Ni22 classification rates by PLS-DA and SVM-C of color spaces were 74.1% and 83.3%, respectively; the Ni27 classification rates by PLS-DA and SVM-C of color spaces were 81.5% and 83.3%, respectively. Moreover, the results of the color features show that the sugarcane variety Ni15 classification rates by PLS-DA and SVM-C were 88.9% and 85.2%, respectively; the Ni22 classification rates by PLS-DA and SVM-C of color spaces were 66.7% and 72.2%, respectively; the Ni27 classification rates by PLS-DA and SVM-C of color spaces were 72.2% and 64.8%, respectively.

Table 1. Classification results of PLS-DA and SVM-C using cross-validation.

Classification methods		Classification rates	
		PLS-DA	SVM-C
Data types	Variety	%	%
Full pixel	Ni15	83.3	87.0
	Ni22	74.1	83.3
	Ni27	81.5	83.3
Color features	Ni15	88.9	85.2
	Ni22	66.7	72.2
	Ni27	72.2	64.8

4. CONCLUSION

The three Japanese sugarcane varieties (Ni15, Ni27, and Ni22) were mostly distinguished by the implementation of first and second principal components in the full pixel and color features. The samples were classified into varieties by performing a partial least squares discriminant analysis (PLS-DA) and a support vector machine classification (SVM-C). The results of the full pixel set show that the pooled classification rates by PLS-DA and SVM-C were 79.6% and 84.5%, respectively. Meanwhile, the pooled classification rates by PLS-DA and SVM-C of the color features set were 75.9% and 74.1%, respectively. However, this research could not correctly classify the sugarcane variety because the input images had various factors that might have affected the results, such as sunlight, a damaged sugarcane bud, the age of the cane, and fertility in the field. These results therefore show that the size and color spaces of sugarcane buds could be the keys to classifying sugarcane varieties. Moreover, the best way to classify Japanese sugarcane (Ni15, Ni22, and Ni27) was the SVM-C method using a full pixel of sugarcane bud.

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

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

*Misaki Mito[1], Takuya Aoki[1], Koichi Mizutani[2], Keiichi Zempo[2], Naoto Wakatsuki[2], Yuka Maeda[2], Nobuhiro Takemae[3], Takehiko Saito[3] （1. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan)）
Keywords: Swine, Influenza, Automatic measurement, Sneezing

Swine influenza spread quickly because of respiratory infection. In dual infection with other diseases, infection symptom will be severe (Reeth et al., 1996). Furthermore, when the virus mutated, human pandemic occurred in 2009 (WHO, 2009). Hence, a lot of previous researches measured relationships between influenza virus titers and infection symptoms (Takemae et al., 2018); These results found influenza infection induced increasing sneezing. If we can detect influenza by sneezing, we can detect disease earlier than an antibody test (Mengeling, 1995) that is a general method. Although previous researches measured only once a day and 1-hour monitoring, we do not know what infection experiment’ s factors induced increasing sneezes. As for examples factors, there are a virus, human stimulus, eating meal and others. The purpose of this paper is discussing relationships between the number of sneezes and factors in swine influenza infection experiment. Because of this, we measure the number of sneezes around the clock during 2-week infection experiment using automatic sneeze detector. In the experiment, we use 3 virus groups and 1 healthy control group, and there are 4 pigs in each group. Regarding automatic sneeze detector, it performs feature extraction from acoustic signals for dimension reduction, and classify sneeze or not based on support vector machine (Mito et al., 2018). As a result, we can observe some relationships between increasing sneezes and factor. The number of sneezing increase after meal supply and infection check timing in each group. As regards this result, we guess these factors have stimuli to pig. Specifically, entering meal into a nasal cavity in a miss, and collecting mucous membranes from a nasal cavity. In addition, we observed increasing the number of sneezes at night, before virus titers cannot detect in 3 virus groups. That means, measuring the number of sneezes at night, we may be able to detect infection influenza or not. Moreover, the previous method cannot measure that time. Consequently, we could measure and discuss the relationship between the number of sneezes and factors in swine influenza infection experiment by a measurement around the clock automatically.

Poster Session | Others (including the category of JSAM and SASJ)
[6-1130-P] Other Categories (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

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

*Akifumi Goto[1], Misaki Mito[1], Tadashi Ebihara[2], Koichi Mizutani[2], Naoto Wakatsuki[2], Nobuhiro Takemae[3], Takehiko Saito[3] (1. Graduate School of Systems and information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, University of Tsukuba(Japan), 3. National Institute of Animal Health, National Agriculture and Food Research Organization(Japan))

Keywords: swine sneezing, respiratory disease, monitoring system, wireless, sound source localization

The recent increase in breeding density due to intensive management of swine leads to an expanding risk of highly infectious respiratory infections. In particular, Porcine Reproductive and Respiratory Syndrome (PRRS) is the main factor inhibiting production in swine farming. Thus, early detection of PRRS is an essential issue in the management of group-housed livestock. In order to achieve early detection, our research group developed a system to detect PRRS automatically. The developed system utilizes a relationship that a frequency of cough and sneezing in swine increases as it is infected by disease, and monitors the sounds in a pig house using multiple microphones to localize the sneezing swine. However, the wiring to connect microphones has been a barrier to deploy a system in pig houses.In this study, we developed a monitoring system using wireless microphones to make the system deployment more flexible. On deploying the wireless monitoring system to a large space, the degradation of the communication quality affects detection of sneezing sound and sound source localization. Therefore, we examined a relationship between an installation position of the wireless microphones and the localization accuracy. Specifically, sound source localization was performed using developed wireless microphones and sound source that emits an actual sneezing sound of swine by changing two parameters: the source-microphone distance (l), and the microphone-receiver distance (d). The obtained results suggest that the measurement error increases as the source-microphone distance (l) increases, while measurement error did not change although the microphone-receiver distance (d) increases. The first result indicates that the localization accuracy was enough (within 0.4 m) when (l) is 4 m or less, and the second result indicates that the wireless microphones can be deployed in a large space. We also deployed the proposed wireless acoustic wave sensor in a pig house to perform a two-week swine influenza infection experiment. In this experiment, the source-microphone distance (l), and the microphone-receiver distance (d) were set as 2 m and 3 m, respectively. We found that the proposed sensor works for two weeks and can localize the sneezing swine within an accuracy of 0.2 m.

Sound Source Localization in Pig Houses Using Wireless Microphone Array and Its Accuracy by Microphone Arrangements

Akifumi Goto[1], Misaki Mito[1], Tadashi Ebihara[2*], Koichi Mizutani[2], Naoto Wakatsuki[2], Nobuhiro Takemae[3] and Takehiko Saito[3]

[1]Graduate School of Systems and Information Engineering, University of Tsukuba

[2] Faculty of Engineering, Information and Systems, University of Tsukuba

[3]National Institute of Animal Health, National Agriculture and Food Reseach Organization

*Tadashi Ebihara : ebihara@iit.tsukuba.ac.jp

ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is a main factor inhibiting production of swine farming and, early detection of PRRS is an essential issue in the management of group-housed livestock. To achieve early detection, our research group developed a system to detect PRRS automatically, which detects cough and sneezing of swine acoustically using wired microphones. However, the wiring becomes a barrier to deploy a system in a pig house smoothly. In this study, we develop a monitoring system using wireless microphones to make the system deployment more flexible. When deploying the wireless monitoring system to a large space, the degradation of the communication quality affects detection of sneezing sound and sound source localization. Therefore, we analyzed a relationship between an installation position of the wireless microphones and the localization accuracy. To evaluate the proposed system, we performed experiments both in laboratory and pig house. As results, we found that the proposed wireless system reduces the load of workers much for system deployment, Furthermore, the proposed system achieves enough quality of sound source localization, while ensuring the system flexibility.

Keywords: swine sneezing, respiratory disease, monitoring system, wireless, sound source localization

1. INTRODUCTION

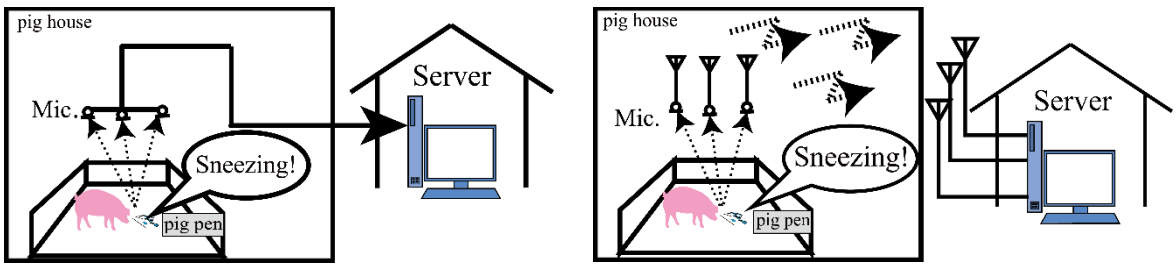
Recent increase in breeding density of swine due to intensive management leads to an expending risk of highly infectious respiratory infections (Frost *et al*., 1997). Among them, porcine reproductive and respiratory syndrome (PRRS) is an important swine disease worldwide, since it prevents production in swine farming resulting in the highest economic impact in swine industry (Shimizu *et al*., 1994). Therefore, early detection of PRRS is an important issue in pig farming. To detect PRRS in early stage, several techniques, such as antibody testing (Scott *et al*., 1997), PCR testing (Duinhof *et al*., 2011), and monitoring weight gain (Destajo *et al*., 2007) have been proposed. However, these techniques require high-cost reagents, laboratory equipment, or human resources that become a barrier to utilize these technologies in a commercial pig farm.

On the other hand, it was found that the acoustical information can also become an important indicator of PRRS. Specifically, it has been reported that the increase of frequency of sneezing and cough of swine increase as the swine is infected by PRRS (Shimizu *et al*., 1994 and Exadaktylos *et al*., 2008). Hence, methods of sound source localization of cough sounds (Silva

et al., 2008) and sneezing sounds (Kawagishi *et al.*, 2014) have been proposed to gather information about the health of swine automatically. A procedure of sound source (sneezing swine) localization is as follows; (1) several microphones are deployed in a pig house, and the internal sound of the pig house is continuously monitored, (2) when a pig sneezes, a sneezing sound is recorded by microphones, (3) the system detects the sneezing sound and calculates time-difference-of-arrivals (TDoAs) of multiple signals by calculating cross-correlation function between receiving signals, and (4) localizes the sound source from direction-of-arrival (angle-of-arrival) using TDoAs and position of the microphones.

Although sound source localization in pig houses has been found to become a viable alternative that achieves early detection of PRRS, there exists a margin for improvement especially in the transmission of acoustic signals recorded by the microphones. In the existing system [Fig. 1(a)], the microphones are connected to the system by wire, which becomes a barrier to deploy the system in pig house that requires human resources and time (wires of length 5–10 m should be placed near a ceiling of the pig house to avoid damages by swine and daily work). If we can remove such wiring by transmitting acoustic signals using radio wave [Fig. 1(b)], we can make the sound source localization system more flexible. However, the quality of the sound source localization would be affected by the quality of wireless radio transmission. Hence, in this study, we design a sound source localization system using wireless microphones and evaluate the quality of the sound source localization by changing two parameters; the source-microphone distance (l) and the microphone-receiver distance (d). Furthermore, we deploy the system in a pig house and perform monitoring for two weeks.

The remaining of this paper is as follows. Section 2 overviews the existing sound source localization system and the proposed (wireless) system. Section 3 evaluates the quality of the proposed sound source localization system in a laboratory. Section 4 evaluates the performance of the proposed system in a pig house. Section 5 concludes this work.



(a) (b)

Figure 1. Outline of acoustic monitoring system of swine; (a) existing and (b) proposed (wireless) system.

2. OVERVIEW OF SOUND SOURCE MONITORING SYSTEM

2.1 Existing (wired) sound source monitoring system

Figure 2 shows the existing (wired) sound source monitoring system. As shown in the figure, we set K microphones (K: positive integer and K=3 in the figure) at relative position of (x_k, y_k) (k = 0, 1,…, K-1). A relative position of the sound source is set as (x_s, y_s). When the sound source emits the sound (sneezing sound), the sound propagates and recorded by the microphones [the recorded sound at microphone #k is defined as $r_k(t)$]. The server judges whether $r_k(t)$ contains a sneezing sound or not by comparing the recorded signal and template (sample of sneezing sound) in the frequency domain. If the sneezing sound is detected, the

server calculates cross-correlation functions between $r_k(t)$ and $r_m(t)$ (m = 0, 1, …, K-1 and $m \neq k$), $s_{km}(t)$, where

$$s_{km}(t) = \sum r_k(n) r_m(t-n). \quad (1)$$

Then the server calculates time-difference of TDoAs, u_{km}, by measuring the peak shift of $s_{km}(t)$. Finally, the server finds (x_s, y_s) that satisfies the following simultaneous equation for all m and k.

$$\sqrt{(x-x_m)^2+(y-y_m)^2} + cu_{km} = \sqrt{(x-x_k)^2+(y-y_k)^2}. \quad (2)$$

Note that the above equation represents a hyperbolic curve determined by (x_k, y_k), (x_m, y_m) and u_{km}, as shown in Fig. 3, and c is a sound velocity.

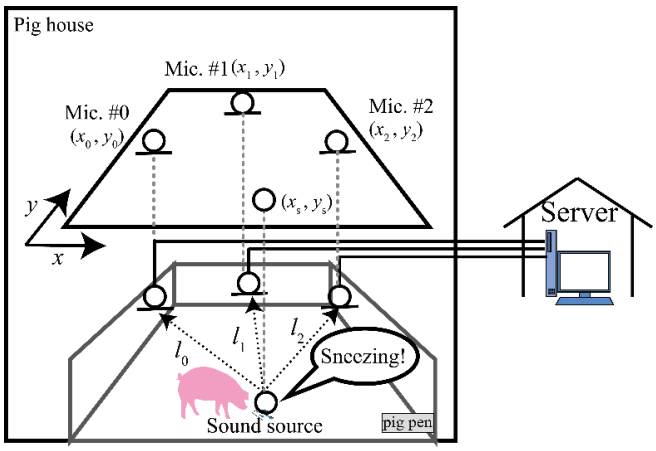


Figure 2. Existing (wired) sound source monitoring system.

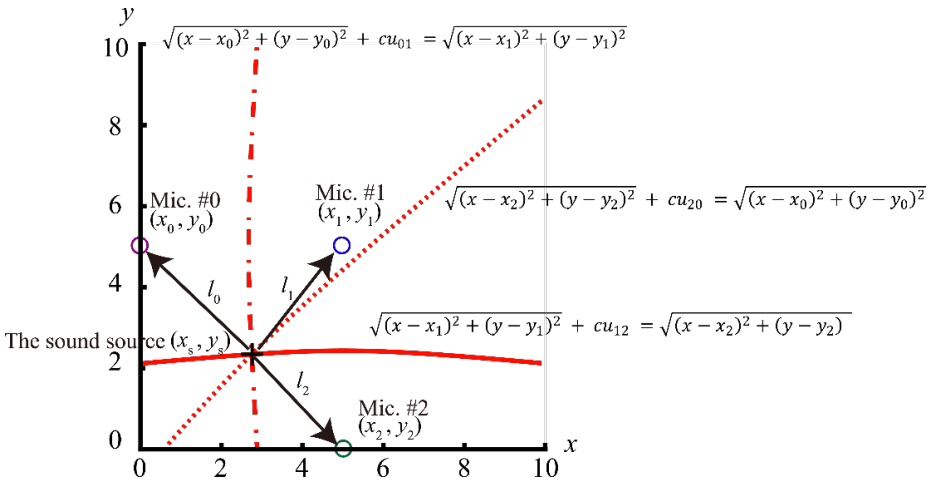


Figure 3. Relationship among (x_s, y_s), (x_m, y_m), (x_k, y_k), and cu_{km} when K = 3.

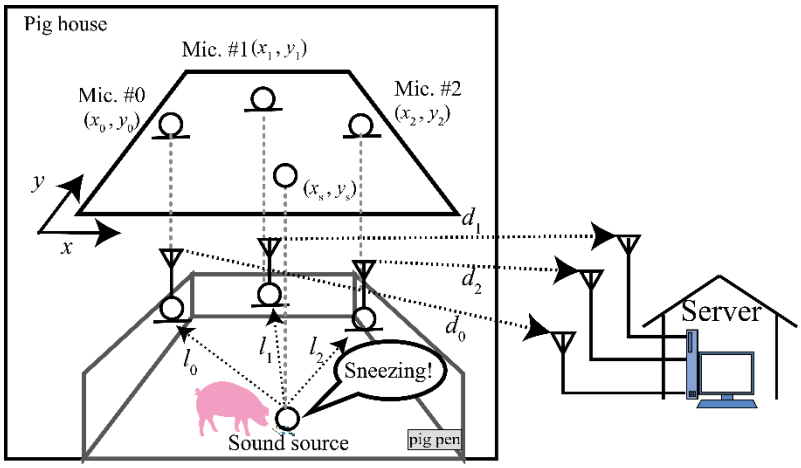


Figure 4. Proposed (wireless) sound source monitoring system.

2.2 Proposed (wireless) sound source monitoring system

In this paper, we design a sound source localization system using wireless microphones, as shown in Fig. 1(b) and Fig. 4. When the sound source emits the sound, the sound propagates and recorded by the microphones. The radio transmitter #k that is connected to the microphone #k modulates the radio frequency of f_k by the recorded sound (frequency modulation) and emits as the radio signal. The radio receiver #k that is connected to the server receives and demodulates the signal from the transmitter #k and the server obtains $r_k(t)$. Note that the radio frequency f_k should be independent to avoid signal interference. A procedure of the sound source localization is the same to that of the existing system. However, in this system, the quality of the sound source localization is affected by two noise sources (environmental noise and transmission noise), as shown in Fig. 4. If the distance between sound source and microphone #k, l_k, becomes large, the system can observe wide area in exchange for the signal-to-noise ratio of $r_k(t)$. Furthermore, if the distance between microphone #k and the server, d_k, becomes large, the system can cover a large pig house in exchange for the signal-to-noise ratio (SNR) of $r_k(t)$. Hence, the quality of the sound source localization of the proposed system should be evaluated by changing two parameters; the source-microphone distance (l_k) and the microphone-server distance (d_k).

3. PERFORMANCE EVALUATION OF THE PROPOSED SYSTEM IN LABORATORY

3.1 Experimental environment

We evaluate the performance of the proposed system in a laboratory. Figure 5 shows the experimental environment. As shown in the figure, the experiment is performed in a room whose size is $7.68 \times 7.35 \times 3.44$ (m^3). We set three microphones with a radio transmitter (88-108MHz, diymore) at a height of 1.5 m from the floor. The carrier frequency of each transmitter is 95, 88, and 101 (MHz), respectively. We also put three radio receivers (RAD-P088S, AudioComn) that are connected to the analog-to-digital converter (USB-6221, National Instruments). The signal processing is performed on a server (ThinkPad X250, Lenovo). Furthermore, we set a speaker (S-300HR, TEAC) on the floor as the sound source. As emitting sound, we use a recorded sound of swine sneezing whose sound pressure level is the same of the swine (2.1 Pa).

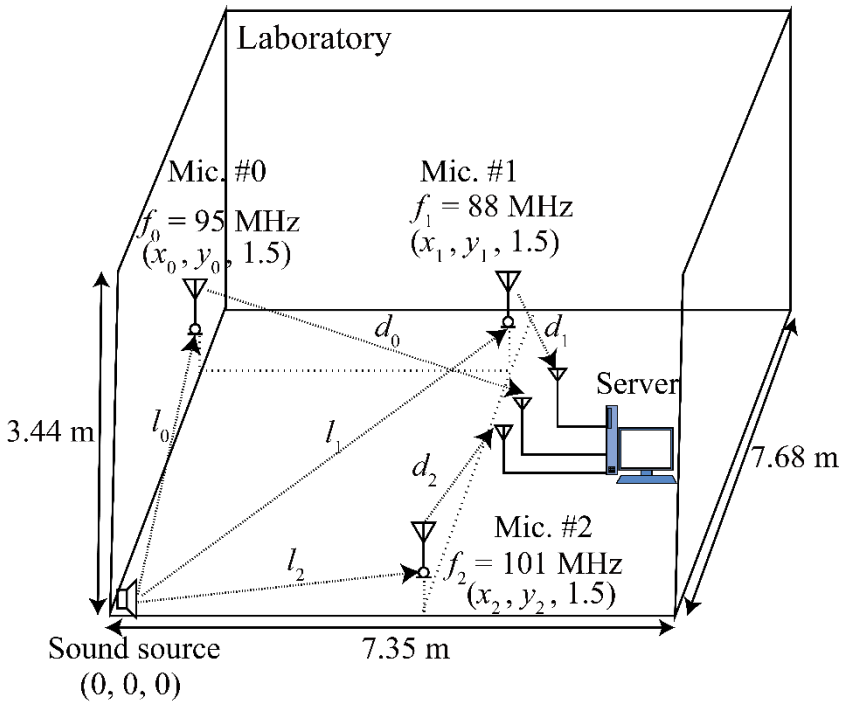


Figure 5. Experimental environment (laboratory).

Table I. Parameters of l_k and d_k used in experiment in laboratory.

		l_0 (m)	l_1 (m)	l_2 (m)	d_0 (m)	d_1 (m)	d_2 (m)
Experiment I	(i)	1.0	1.4	1.0	2.0	2.0	2.0
	(ii)	2.0	2.8	2.0			
	(iii)	3.0	4.2	3.0			
	(iv)	4.0	5.6	4.0			
	(v)	5.0	7.0	5.0			
Experiment II	(i)	1.0	1.4	1.0	1.0	1.0	1.0
	(ii)				2.0	2.0	2.0
	(iii)				3.0	3.0	3.0
	(iv)				4.0	4.0	4.0
	(v)				5.0	5.0	5.0

In this experiment, we evaluate the quality of the sound source localization of the proposed system by changing the source-microphone distance (l_k) and the microphone-server distance (d_k). At first, the sound source localization is performed by changing l_k with a specific value of d_k (Experiment I). Then the sound source localization is performed by changing d_k with a specific value of l_k (Experiment II). Table I shows the parameter combinations of l_k and d_k used in the experiment. During the experiment, we also measure the SNR of $r_k(t)$, as well as the quality of the sound source localization (localization error).

3.2 Experimental results and discussions

Figure 6 and Table II show the experimental results. Figures 6(a) shows a relationship between sound source localization error and source-microphone distance (l_k). Figures 6(b) shows a relationship between sound source localization error and microphone-server distance (d_k). Table II shows a relationship between SNR and source-microphone distance (l_k) and microphone-server distance (d_k).

From this experiment, we found that the distance between sound source and microphone is a main factor that affects the quality of the sound source localization. Specifically, the localization error increases as the source-microphone distance (l_k) increases, while the localization error does not change much even if the microphone-server distance (d_k) increases [Fig. 6(a)]. Furthermore, the SNR decreases as the source-microphone distance (l_k) increases, while that does not change much even if the microphone-server distance (d_k) increases [Table II].

Next, we focus on the value of the localization error. In previous studies, it was found that the localization error should be less than 0.4 m to detect a sneezing swine individual from a group of pigs in a pig pen (Kawagishi *et al.*, 2014). From Fig. 6, we found that the source-microphone distance (l_k) should not over 3 m while the microphone-server distance (d_k) can be set flexible within 5 m.

Consequently, we found that the quality of the sound source localization would not be affected by the quality of wireless radio transmission.

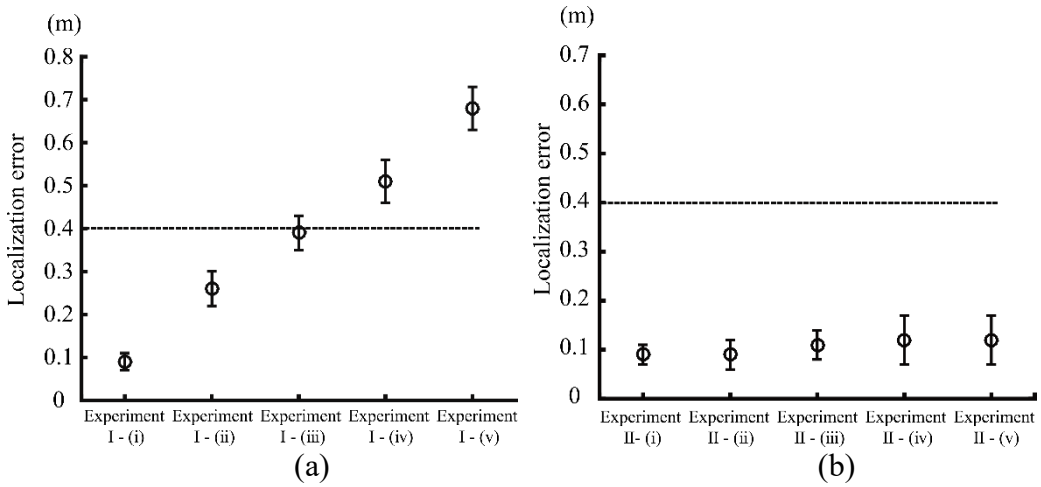


Figure 6. Experimental results obtained in laboratory; sound source localization error obtained in (a) Experiment I and (b) Experiment II.

Table II. SNR of $r_k(t)$ obtained in Experiment I and II.

	Experiment I					Experiment II				
	(i)	(ii)	(iii)	(iv)	(v)	(i)	(ii)	(iii)	(iv)	(v)
SNR (dB)	28.1	26.0	23.8	23.1	22.27	28.1	28.0	28.3	28.5	29.3

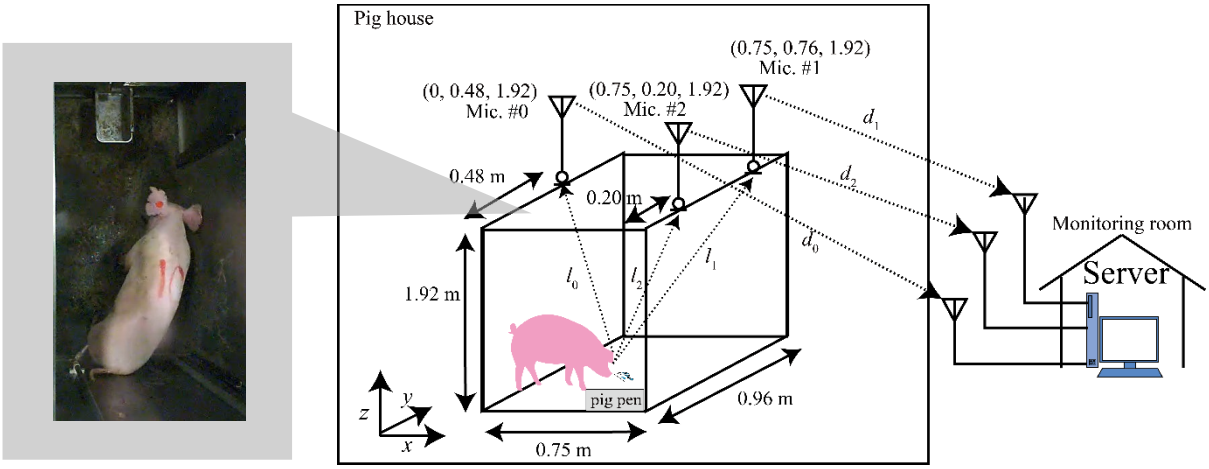


Figure 7. Experimental environment (pig house).

4. PERFORMANCE EVALUATION OF THE PROPOSED SYTEM IN PIG HOUSE

4.1 Experimental environment

We evaluate the performance of the proposed system in a pig house. Figure 7 shows the experimental environment. As shown in the figure, the experiment is performed in a pig house of National Institute of Animal Health, National Agriculture and Food Research Organization whose size is 1.35×3.45×2.05 (m^3). As well as Section 3, we set three microphones with a radio transmitter at a height of 1.92 m from the floor. The microphone-server distance (d_0, d_1, d_2) was set to 2–3 m, the source-microphone distance (l_0, l_1, l_2) is set as 2.1–2.2 m. Note that l_k and d_k satisfy the values that achieve localization error of less than 0.4 m in Section 3. The carrier frequency of each transmitter is the same to that used in preliminary experiment. We also put three radio receivers (RAD-P088S, AudioComn) that are connected to the analog-to-digital converter (USB-6221, National Instruments) on an adjacent monitoring room. The signal processing is performed on a server (i5-4690 CPU, RAM 16GB). Different from the preliminary experiment, the sound source is a weaned piglet (8 week old) (Takemae *et al*., 2018).

In this experiment, we deploy the proposed system and existing (wired) system, while measuring the length of time for system deployment. Furthermore, we evaluate the quality of the sound source localization of the proposed system for two weeks. We also evaluate the quality of the sound source localization of the existing system as reference.

4.2 Experimental results and discussions

The length of time for proposed system deployment was approximately 30. Min. by one worker, while that for existing system deployment was approximately 120 min. by three workers. We found that the proposed wireless system is much easier than the existing system, since there is no need to install long cables in a pig house.

During the experiment, both the proposed system and existing system work successfully for two weeks. During the experiment, both system detected the swine sneezing 10 times. Figure 8 shows an example of sound source localization result by the proposed system [Fig. 8(a)] and existing system [Fig. 8(b)]. From this figure, we found that the proposed system and existing system achieve localization error of 0.2 and 0.25 m, respectively. This means that the sound source localization system using wireless microphones achieves almost the same quality of that using wired microphones, while ensuring the system flexibility.

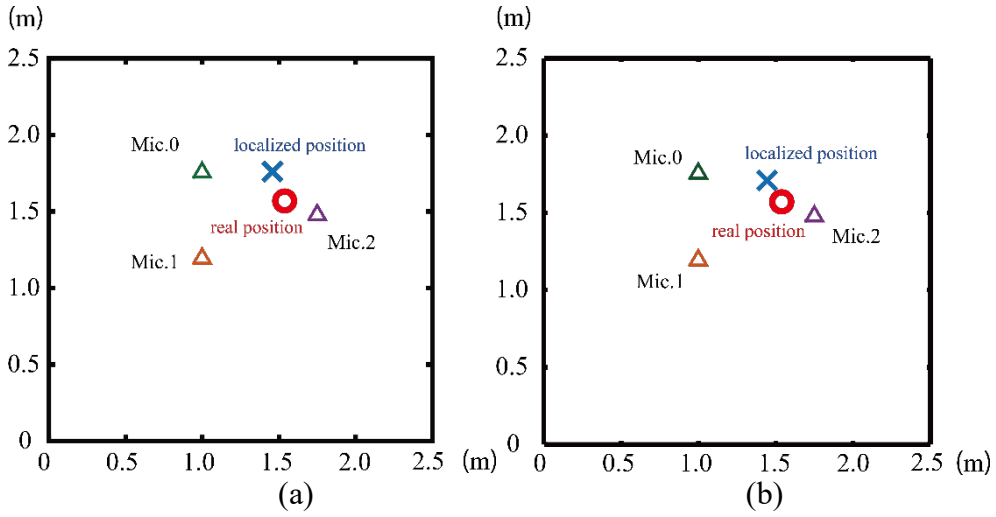


Figure 8. Example of sound source localization result obtained in pig house; localization error of (a) proposed system (wired) and (b) existing system (wireless).

5. CONCLUSIONS

In this paper, we develop a monitoring system using wireless microphones to make the system deployment more flexible. When deploying the wireless monitoring system to a large space, the degradation of the communication quality affects detection of sneezing sound and sound source localization. Therefore, we analyzed a relationship between an installation position of the wireless microphones and the localization accuracy. To evaluate the proposed system, we experiment both in laboratory and pig house. In the experiment in the laboratory, we evaluate the quality of the sound source localization of the proposed system by changing the source-microphone distance (l_k) and the microphone-server distance (d_k). From the result of the experiment, we found that the distance between sound source and microphone is a main factor that affects the quality of the sound source localization. In the experiment in a pig house, we deploy the proposed system and existing (wired) system, while measuring the length of time for system deployment. We also evaluate the quality of the sound source localization of the existing system as reference. The length of time for proposed system deployment was approximately 30. min. by one worker, while that for existing system deployment was approximately 120 min. by three workers. We found that the proposed wireless system is much easier than the existing system, since there is no need to install long cables in a pig house. From figure 8, we found that the proposed system and existing system achieve localization error of 0.2 and 0.25 m, respectively. This means that the sound source localization system using wireless microphones achieves almost the same quality of that using wired microphones, while ensuring the system flexibility.

ACKNOWLEDGMENT

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

[6-1130-P] Other Categories (6th)

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

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

*Yasuhiko Nishijima[1], Koichi Mizutani[1,2], Tadashi Ebihara[1,2], Naoto Wakatsuki[1,2], Kenji Kubota[3], Hiroyuki Uga[4]
（1. Graduate School of Systems and Information Engineering, University of Tsukuba(Japan), 2. Faculty of Engineering, Information and Systems, Division of Engineering Interaction Technologies, University of Tsukuba(Japan), 3. Agriculture Research Center, National Agriculture and Food Research Organization(Japan), 4. Saitama Prefecture Agriculture Research Center(Japan)）

Keywords: whitefly , vibrational sensitibity, mating behavior

Whiteflies are major pests that damage a wide variety of plants such as tomatoes and cucumbers. Whiteflies suppress the growth of plants and reduce crop quality by acquisition feeding. Furthermore, they carry various viruses such as tomato yellow leaf curl virus (TYLCV) and cucurbit chlorotic yellows virus (CCYV). For this reason, control of whiteflies is an urgent matter for farming. The current control method is spraying pesticides. However, whiteflies acquire pesticide resistance early because it performs a generation cycle within one month. For example, *Bemisia tabaci* (biotype Q) has high resistance to most pesticides. Hence, the development of a new technology to control whiteflies is required. Focusing on the behavior of whiteflies, it has been reported that they communicate using leaf substrate-borne vibrations by oscillating their abdomens in their courtship behavior. Besides, our research group has clarified that their courtship behavior can be controlled by applying the artificial vibration of 200-1500 (Hz). However, the effective amplitude of artificial vibration has not been clarified yet. Hence, in this paper, we clarify the vibration sensitivity of whiteflies by experiments. The experimental condition is as follows. *Bemisia tabaci* (biotype Q1) (five males and five females) were released in a rectangular plastic case of approximate size 60×60×100 (mm^3). The case has a hole with a diameter of 41 mm at its top and is covered with perilla leaf. The leaf was vibrated with various amplitudes (vibrational amplitude: 1.0, 0.6, and 0.3 μm), and the number of courtship behavior was measured by analyzing video recorded by the camera (FDR-AX45/SONY). The experiment was performed twice (each is for 1.5 hours) at each amplitude in an anechoic chamber. During this experiment, the temperature was 27-31 ˚C, and the humidity was 27-39 %. From experiments, we found that ratio of the number of mating behavior to that of courtship behavior is small (0 %) when vibration amplitude is 1.0 µm, although that is large (about 30 %) when vibration amplitude is 0.6 µm or less. Hence, we found that the sufficient amplitude of artificial vibration is about 1.0 µm. This result can be expected to contribute to the development of novel whitefly control technology.

Behavioral Study of Vibrational Sensitivity in Whitefly

Yasuhiko Nishijima[1], Koichi Mizutani[1,2*], Tadashi Ebihara[1,2*], Naoto Wakatsuki[1,2], Kenji Kubota[3], Hiroyuki Uga[4]

[1] Graduate School of Systems and Information Engineering, University of Tsukuba

[2] Faculty of Engineering, Information and Systems, University of Tsukuba

[3] Agriculture Research Centre, National Agriculture and Food Research Organization

[4] Saitama Prefecture Agriculture Research Centre

*mizutani@iit.tsukuba.ac.jp

ABSTRACT

Whiteflies are major pests that damage a wide variety of plants such as tomatoes and cucumbers. The current control method is spraying pesticides. However, whiteflies acquire pesticide resistance early because it performs a generation cycle within one month. Hence, the development of a new technology to control whiteflies is required. Focusing on the behavior of whiteflies, our research group clarified that their courtship behavior can be controlled by applying the artificial vibration of 200-1500 (Hz). However, the effective amplitude of artificial vibration has not been clarified yet. Hence, in this paper, we clarify the vibration sensitivity of whiteflies by experiments. Specifically, *Bemisia tabaci* (biotype Q1) were released on a perilla leaf in a rectangular plastic case. The leaf was vibrated with various amplitudes, and the number of mating behavior was measured by analyzing video recorded. As a result, we found that the sufficient amplitude of artificial vibration is about 1.0 μm.

Keywords: Whitefly, *Bemisia tabaci*, Mating behavior, Vibration sensitivity

1. INTRODUCTION

Whiteflies (*e.g.*, *Bemisia tabaci* and *Trialeurodes vaporariorum*) are major pests that damage a wide variety of plants such as tomatoes and cucumbers (Azab *et al*., 1970; Zhang *et al*., 2005; Martin *et al.*, 2007). Whiteflies suppress the growth of plants and reduce crop quality by acquisition feeding and emitting a honeydew to the plants (Matsui, 1992; Nelson, 2008). Furthermore, they carry various viruses such as tomato yellow leaf curl virus (TYLCV) and cucurbit chlorotic yellows virus (CCYV). TYLCV and CCYV are one of the most well-known tomato and cucumber infecting begomoviruses transmitted by *Bemisia tabaci*, and they cause severe economic loss worldwide (Navot *et al.*, 1991; Czosnek *et al.*, 1997; Jones, 2003). For this reason, control of whiteflies is an urgent matter for farming.

To control whiteflies, various tactics –such as physical barriers (*e.g.*, insect screen), chemical controls and biological controls (Matsuura *et al*., 2005; Kodandaram, 2018; Nomikou, 2001, 2002, 2010)– have been considered. Focusing on physical barriers, it has been clarified that an insect screen with mesh size of 0.4 mm can efficiently reduce the entrance of whiteflies, in exchange for increasing difficulty regulating temperature of the greenhouse due to limited air flow (Mihara *et al*., 2005). Focusing on chemical controls, numbers of chemical pesticides have been proposed, However, whiteflies quickly develop resistance to chemical pesticides (Wardlow *et al.*, 1972) and *Bemisia tabaci* (biotype Q) has high resistance to popular pesticides now (Horowitz *et al*., 2005). Focusing on biological controls, *Nesidiocoris tenuis* and *Typhlodromips swirskii* have been found to become a biopesticide of whitefly. However, *Nesidiocoris tenuis* has a risk to damage the plants (Nakaishi, 2013), and *Typhlodromips swirskii* is not livable on the plants that discharge sticky secretions such as tomatoes (Sakamoto, 2012). Hence, current control techniques have both advantages and disadvantages, and innovative combination of control techniques are necessary to achieve effective pest management. Furthermore, development of a new technology to control whiteflies can contribute to broaden pest management.

Focusing on the behavior of whiteflies, Kanmiya (1996) has reported that they communicate using leaf substrate-borne vibrations by oscillating abdomens in their courtship behavior. Furthermore, the communication signal of whiteflies has been found to be unique to each species and biotype (Kanmiya

et al., 2002; Nakabayashi *et al.*, 2017). This means that the communication of whitely play an important role in mating behavior, considering the fact that hybrid of different species or biotype remains rare (Matsuura, 2010). Hence, we may control the mating behavior of whiteflies by applying artificial vibration on the leaf. As preliminary study, we have clarified that their courtship behavior can be controlled by applying the artificial vibration of 200-1500 (Hz) (Nishijima *et al*., 2019). However, the effective amplitude of artificial vibration has not been clarified yet. Hence, in this paper, we clarify the vibration sensitivity of whiteflies by experiments.

2. MATERIALS AND METHODS

In this study, we put whiteflies on a perilla leaf, vibrate the leaf at specific amplitude, and observe the behavior of whiteflies. The test was performed in a laboratory with the temperature and humidity of 25-31°C and 27-39%, respectively. We used adult whiteflies (*Bemisia tabaci*, biotype Q1) of 5 pairs (5 males and 5 females, collected from a colony) for the test. The whiteflies were put on the underside of a perilla leaf, and the leaf was put on a rectangular plastic case of size 60×60×100 (mm^3), as shown in Figure 1. The leaf was set to cover a hole of the case (diameter: 41 mm) so that the whiteflies can be monitored from the bottom of the case using a video recorder (FDR-AX45/SONY). Also, a polypropylene sheet was placed between the leaf and the case to keep the whitefly within a field view of the camera. To capture the behavior of the whiteflies clearly, the underside of the leaf was illuminated by a desk light. The leaf was vibrated artificially, and its vibration was monitored by a laser Doppler vibrometer (LDV) (AT2300 and AT3700, Graphteck). During the test, the leaf was vibrated artificially (vibrational direction: vertical for surface of leaf), and a behavior of the whiteflies was monitored for 1.5 hours. The test was conducted by changing the vibrational amplitude [maximum amplitude of 1.0, 0.6, 0.3 and 0 (μm)], and the test was repeated twice at each amplitude. We then analyze the video and count the number of "mating success" and "mating failure". Note that the mating behavior of whiteflies consists of three steps; (1) searching a female, (2) forming a pair (close contact with female) and (3) mounting (males overlap his hips with hers and shack his wings rapidly) (Kanmiya, 1998). Hence, we define the following labels could count each occurrence;

(a) Mating success: mounting is clearly observed after pair forming.
(b) Mating failure: mounting is not observed (they get a divorce after pair forming).
(c) Unknown: mounting is not clearly observed after pair forming (*e.g.*, they form a pair continuously.

Figure 2 shows a flowchart to perform labeling from the video, where *Np, Ns, Nf, Nu* are the number of pair forming, mating success, mating failure, and unknown, respectively, and "Ratio of *Ns*" represents *Ns* per *Np* (%).

3. RESULTS AND DISCUSSIONS

The experimental results are shown in Table 1. From the table, we couuld observe pair forming (*Np*) in each trial. However, it was found that the ratio of successful mating (ratio of *Ns* in the table) becomes 0 when the vibrational amplitude of the leaf is 1.0 μm, while the ratio of successful mating increases 28-35 (%) when the ampltude is 0-0.6 μm. This means that the effective amplitude of the artificial vibration to control the mating behavior of whiteflies is more than 1.0 μm.

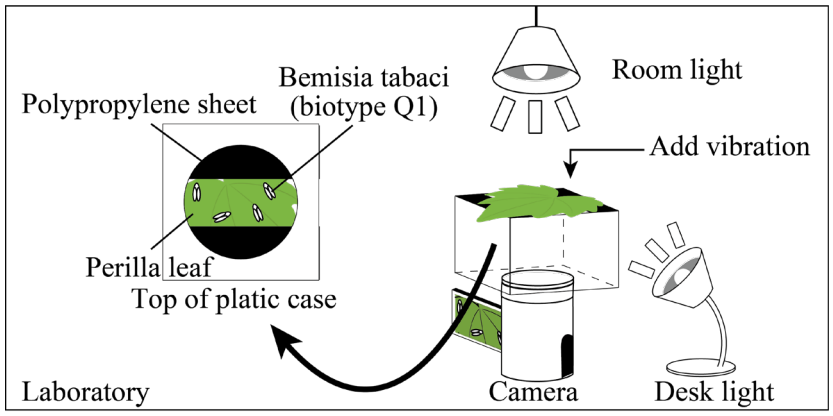

Figure 1. Overview of experimental system

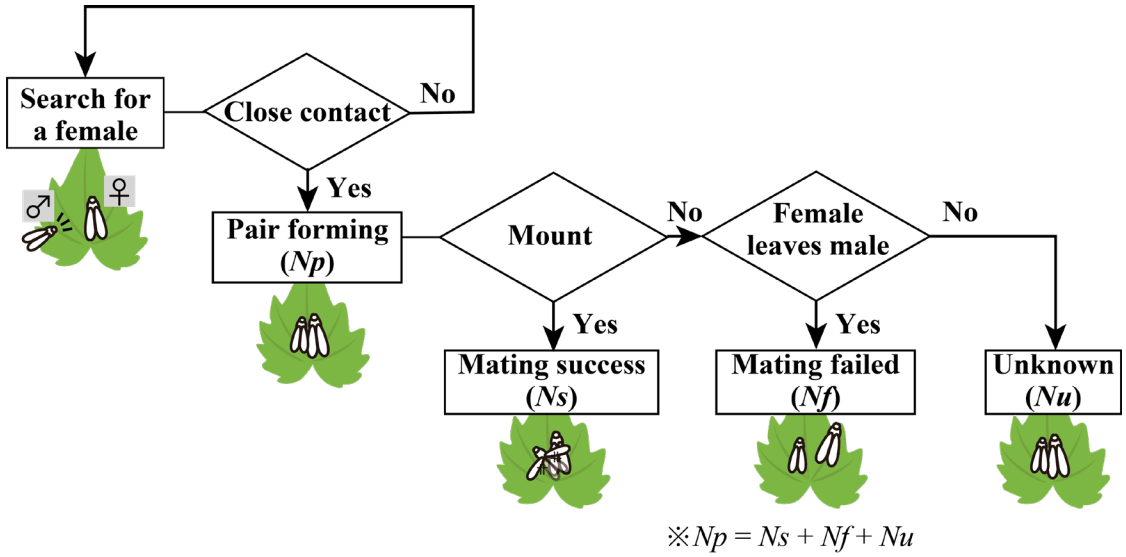

Figure 2. Determination method of mating behavior

Table 1. Numbers of pair forming and Mating under various amplitudes

Amplitude	Trials	*Np*	*Ns*	*Nf*	*Nu*	Ratio of *Ns* (%)
1.0 μm	①	11	0	9	2	0
	②	10	0	8	2	0
0.6 μm	①	3	1	1	1	33
	②	7	2	3	2	28
0.3 μm	①	19	6	12	1	31
	②	14	5	8	1	35
0 μm	①	3	1	1	1	30
	②	6	2	2	2	30

4. CONCLUSIONS

Control of whiteflies that cause severe economic loss is an urgent matter for farming. Focusing on the behavior of whiteflies, our research group clarified that their courtship behavior can be controlled by applying the artificial vibration of 200-1500 (Hz). However, the effective amplitude of artificial vibration has not been clarified yet. Hence, in this paper, we clarify the vibration sensitivity of whiteflies by experiments. The whiteflies (*Bemisia tabaci*, biotype Q1) of 5 pairs (5 males and 5 females, collected from a colony) were put on the underside of a perilla leaf. Also, the leaf was vibrated artificially (vibrational direction: vertical for surface of leaf), and a behavior of the whiteflies was monitored for 1.5 hours. The test was conducted by changing the vibrational amplitude [maximum amplitude of 1.0, 0.6, 0.3 and 0 (μm)], and the test was repeated twice at each amplitude. As a result, it was found that the ratio of successful mating becomes 0 when the vibrational amplitude of the leaf is 1.0 μm, while the ratio of successful mating increases 28-35 (%) when the ampltude is 0-0.6 μm. Hence, the effective amplitude of the artificial vibration to control the mating behavior of whiteflies is at least 1.0 μm. This result can be expected to contribute to the development of novel whitefly control technology.

ACKNOWLEDGMENT

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Poster Session | Others (including the category of JSAM and SASJ)
[6-1130-P] Other Categories (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

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

*Songsin Photchanachai[1], Nipada Ranmeechai[1,2], Chalinee Sungkajorn[1,2], Anantaporn Phankhaek[1,2], Kornkanok Aryusuk[1], Varit Srilaong[1,2], Panida Boonyarithongchai[1,2], Nutthachai Pongprasert[1,2] (1. School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok(Thailand), 2. Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok(Thailand))
Keywords: Palm oil based wax, Cucumber seeds, Coating material

Colouring the seeds enhances physical appearance which is necessary for commercial purposes. The colouring agents are synthetic chemical dyes and film coating polymers. Palm oil wax is a by-product of palm oil industry. It is used to prepare palm oil-based wax as a coating material. Therefore, this research aimed to study the effects of palm oil-based wax as an alternative synthetic coating material. There were three formulas of the palm oil-based wax designated as A, B and C. These were used as coating on cucumber seeds using the top-spray fluidized bed coating technique. The experimental conditions were carried out through atomization air pressure of 150 kPa, inlet air velocity of 2 mm/sec, inlet air temperatures at 40˚C, spray rate of coating solution of 125 mL/min, spraying time for 2 min, and drying after spraying for 15 min. The surface appearance and uniformity of palm oil based wax coating were evaluated under the stereomicroscope. Moisture content, germination percentage, days to emergence (DTE), germination index and free fatty acid content were also determined. Results showed no difference in the appearance and uniformity of the three formulas of palm oil based wax coating on seed coat surface. The moisture content and free fatty acid of the coated seeds increased, while germination percentage and germination index were lower than the control. Moreover, the formula A, consisted of 99.51% wax ester with the carbon chain lengths of 32-34 atoms, obtained similar seed quality with the control. Therefore, the properties of formula A palm oil based wax coating could be improved to minimize the impact on cucumber seed quality. Further studies can be done on the experimental conditions used during the application of the coating material.

Application of Palm Oil Based Wax as a Coating Material on the Quality of Cucumber Seed

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Abstract

Colouring the seeds enhances physical appearance which is necessary for commercial purposes. The colouring agents are synthetic chemical dyes and film coating polymers. Palm oil wax is a by-product of palm oil industry. It is used to prepare palm oil-based wax as a coating material. Therefore, this research aimed to study the effects of palm oil-based wax as an alternative synthetic coating material. There were three formulas of the palm oil-based wax designated as A, B and C. These were used as coating on cucumber seeds using the top-spray fluidized bed coating technique. The experimental conditions were carried out through atomization air pressure of 150 kPa, inlet air velocity of 2 mm/sec, inlet air temperatures at 40°C, spray rate of coating solution of 125 mL/min, spraying time for 2 min, and drying after spraying for 15 min. The surface appearance and uniformity of palm oil based wax coating were evaluated under the stereomicroscope. Moisture content, germination percentage, days to emergence (DTE), germination index and free fatty acid content were also determined. Results showed no difference in the appearance and uniformity of the three formulas of palm oil based wax coating on seed coat surface. The moisture content and free fatty acid of the coated seeds increased, while germination percentage and germination index were lower than the control. Moreover, the formula A, consisted of 99.51% wax ester with the carbon chain lengths of 32-34 atoms, obtained similar seed quality with the control. Therefore, the properties of formula A palm oil based wax coating could be improved to minimize the impact on cucumber seed quality. Further studies can be done on the experimental conditions used during the application of the coating material.

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