The 1st International Conference on Sustainable Agriculture and Aquaculture For Well Being and Food Security

e-Proceedings

PISAI
Participatory and Integrative Support for Agricultural Initiative

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THAILAND

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Message from Chairman of Organizing Committee and Coordinator of PISAI Project

(**P**articipatory and **I**ntegrative **S**upport for **A**gricultural **I**nitiative- the Erasmus+ Capacity Building in Higher Education Project under the European Union)

The First International Conference on Sustainable Agriculture and Aquaculture materialized due to four main factors. The first factor is the PISAI project commitment to organise the conference as an arena for students to present their work to a wide audience, get feedback from professors, and exchange with friends. The second factor is the PISAI project consortium, which have been working together since 2017 on a collaborative Double Degree Master's Programme, whose students are presenting their theses at this event. The third factor is the Discipline of Excellence in Sustainable Aquaculture, the collaboration of three PSU faculties offering aquaculture programmes, with strong support from Aquatic Science Alumni Association and commitment to furthering their contribution to sustainable aquaculture by having their students present up-to-date research finds, as well as organise a special seminar on sustainable aquaculture. The last important factor is the professors, researchers and students from different institutions nationally and internationally who participated in the conference. Without their participation, the meeting would have been incomplete.

For these reasons, I would like to thank PISAI consortium members for their input and continuous contributions to the PISAI project and this conference. Appreciation is extended to scientific committees who donated valuable time reviewing the papers, and to the invited speakers for their precious time providing excellent presentations. I would lastly like to take this opportunity to express my sincere gratitude to our colleagues and friends, both in Thailand and overseas, for their contributions to the event, either in-person or on-line, especially considering the obstacles posed by the COVID19 pandemic.

Asst. Prof. Dr. Chutima Tantikitti
Faculty of Natural Resources
Prince of Songkla University

The 1st International Conference on Sustainable Agriculture and Aquaculture (ICSAA 2021)

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Overall Schedule

Program for International Conference on Sustainable Agriculture and Aquaculture for Well Being and Food Security

10 – 12 Jan 2021

Venue: Learning Resource Center, Prince of Songkla University

Date	Activity		
Sunday 10 January 2021			
14.30 – 17.30	**Registration of participants** - Arrival of participants - Registration/Fee payment/Setting-up posters		
Monday 11 January 2021			
	Morning		
08.30 – 12.00	**Registration of participants** - Registration/Fee payment - Conference document - Setting-up posters	**Aquaculture Session** 08.30 – 09.30	**Registration**
		10.15 – 11.45	**Scientific Sessions** ***Oral Presentations*** - Aquatic Animal Nutrition **(LRC2)** - Aquatic Animal Breeding **(Training Room)** - Aquatic Animal Health and Disease **(Meeting Room)**
12.00 – 13.30	***Lunch***		
	Afternoon		
13.30 – 14.00	**Opening sessions (LRC 1)** - Welcome Address by PSU President - **Opening Speech by Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation**		
14.00 – 14.30	**Keynote speech (LRC 1)** **- Dr. Sumet Tantivejkul, Secretary General of the Chaipattana Foundation** *The Chaipattana Foundation, established for His Majesty the King's principle of sustainable development, aims for promoting and supporting sustainable development and self-reliance of farmers and their livelihoods.* *"*Chaipattana*" the name bestowed upon the Foundation by His Majesty the King, suitably means "*Victory of Development.*"* **- EU Ambassador to Thailand**		
14.30 – 15.00	***Coffee break***	14.30-14.40 ***Coffee break***	
15.00 – 15.30	**Keynote speakers (LRC 1)** - **Dr. Budsara Limnirankul** *Chiang Mai University* *"Enhancing sustainable agriculture and food system for rural livelihood in Northern Thailand"* - EU partner representative **Dr. Hanna Tuomisto** *University of Helsinki, Finland* *"Towards Sustainable Food Systems*"	**Aquaculture Session** 14.40-15.55 **Scientific Sessions** - ***Oral Presentations*** - Aquatic Animal Nutrition **(LRC2)** - Aquaculture System **(Training Room)** - Eco-Management **(Meeting Room)**	
15.30 – 17.00	**Scientific Sessions** - ***Poster Presentations*** 1) Agriculture 2) Agricultural System	15.55-17.00 **Scientific Sessions** - ***Poster Presentations***	

Program for International Conference on Sustainable Agriculture and Aquaculture for Well Being and Food Security (cont.)

10 – 12 Jan 2021

Date	Activity	
Tuesday 12 January 2021		
	Morning	
09.00-10.30	**Agriculture Session**	
	Scientific Sessions - ***Oral Presentations*** 1) Agriculture - Plant Science **(LRC 2)** - Animal Science **(Training Room)** 2) Agricultural System **(Meeting Room)**	
10.30-10.45	***Coffee break***	
10.45-12.00	**Scientific Sessions -** ***Oral Presentations*** 1) Agriculture - Plant Science **(LRC 2)** - Pest Management **(Training Room)** 2) Agricultural System **(Meeting Room)**	
12.00–13.00	***Lunch***	
	Afternoon	
13.00-14.45	**Scientific Sessions** - ***Oral Presentations*** 1) Agriculture - Plant Science **(LRC 2)** - Pest Management **(Training Room)**	14.00-14.45 **Scientific Sessions** - ***Oral Presentations*** - Agricultural System **(Meeting Room)**
14.45-15.00	***Coffee break***	
15.00-15.45	**Scientific Sessions -** ***Oral Presentations*** 1) Agriculture - Technology **(Training Room)**	15.00-15.45 **Special Seminar** **(Meeting Room)**
16.00–16.30	**Student Presentation Award Session**	
16.30–17.00	***Closing Ceremony***	

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Agriculture– Plant Science

AG-PS-01

Chitosan and Sodium Alginate - Double Coatings Integrated with Sweet-Flag Extract Affecting the Postharvest Quality of 'Nam Dok Mai' Mango

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Abstract

'Nam Dok Mai' mango, a champion fruit of Thailand's potential fruits, is exported in global markets. The fruit conducts rapid fruit ripening, and the ripe fruit is susceptible to disease infections, particularly anthracnose, mainly caused by *Colletotrichum gloeosporioides*. Various concentrations of sweet-flag extract were *in vitro* examined for inhibiting *C. gloeosporioides* growth. Sweet-flag extracts at above 1,000 mg·L^{-1} extract completely inhibited the growth of *C. gloeosporioides* inoculated on potato dextrose agar. Double coatings of 'Nam Dok Mai' mango fruit with 0.5% chitosan in the first layer and then ended up with 0.1% sodium alginate (SA) subsequently experimented. One thousand mg·L^{-1} sweet-flag extract was mixed in 0.5% chitosan coating to investigate fruit's fungal growth inhibition. Double layers of 0.5% chitosan + 1,000 mg·L^{-1} sweet-flag extract and 0.1% SA coated on 'Nam Dok Mai' mango slightly delayed the peel colour changes but did not affect fruit firmness, soluble solids, and titratable acidity contents. Although the double layer coating did not decrease disease incidences of coated mango, compared to control, it significantly reduced 'Nam Dok Mai' mango fruit disease severity during 25°C incubation.

Keywords: *Layer by layer coating, Mango, Acorus calamus L., Retailing condition*

1. Introduction

'Nam Dok Mai' mango is one of Thailand's potential fruits, sold both in domestic and worldwide markets. Apart from rapid fruit ripening, the ripe 'Nam Dok Mai' mango fruit is susceptible to disease infections, in particular anthracnose. Mango anthracnose disease is mainly caused by *Colletotrichum gloeosporioides* (Dinh, Chongwungse, Pongam, & Sangchote, 2003).

Recently, individual coatings have been applied to many fruits at postharvest during retails. Chitosan coating, an effective coating, has been introduced to maintain the postharvest quality of fresh fruits (Vangnai, Wongs-Aree, Nimitkeatkai, & Kanlayanarat, 2006; Soe Win, Mejunpet, Buanong, Kanlayanarat, & Wongs-Aree, 2015; Jongsri, Wangsomboondee, Rojsitthisak, & Seraypheap, 2016). However, ordinary coating methods could not completely protect the whole fruit adequately. To overcome the problem, we were interested in using double coating by two different coating materials. In the present study, coating by double layers or layer by layer was applied to mango fruit using different materials' polarities. One was chitosan, which is held on positive charges (+). Chitosan is a polysaccharide extracted from crab and prawn. It is safe and can be biodegradable (Shiekh, Malik, Al-Thabaiti, & Shiekh, 2013). Chitosan exhibits an anti-microbial property by directly reacting to the opposing cell wall of microorganisms, resulting in misfunction of the cells (Jongsri et al., 2016; Jana & Jana, 2020). The other coating material was sodium alginate, which is held on negative charges (-). Sodium alginate extracted from brown algae is a natural polysaccharide that could be transformed into a gel when dissolving in water. The alginate-based edible coatings coating on fruit can be a barrier of gaseous transmission between the fruit and the storage atmosphere (Kulig, Korzycka, Jarmoluk, & Marycz, 2016; Parredit, Müller, & Schmid, 2018). Thus, a modified coating technique using ionic linkage between 2 different chitosan and sodium alginate charges applied on mango was introduced. The ionic association could be added of cohesion forced between the material and the surface (Poverenov et al., 2014)

The most useful application and general use for the anti-microbial property in plants are to use bactericide and fungicide. In postharvest of fruit, fungicides are commercially used to extend the shelf/storage life. However, apart from chemical-remaining residues in fruit, which results in human health, resist chemical actions have been increasingly in the ecosystem. As a result, plant extract, an alternative technique with eco-friendly, has now been progressively studied. Sweet-flag, or 'Waan-Nam' in Thai (*Acorus calamus* Linn.), belonging to the Araceae Family), is a well-known medicinal plant used in ayurvedic medicine (Pandy, Jose, & Subhash, 2009). The extract has been reported to conduct fungicide activity to many plant pathogenic fungi (Thaenthanee, Sukprasert, Daosukho, Saijit, & Rodprasert, 2014; Muangthip et al., 2015; Dethoup, Songkumarn, Sirirak, & Kijjoa, 2019). In the present study, extracts from dried sweet-flag were subsequently added into the chitosan layer to investigate disease growth and quality changes of 'Nam Dok Mai, No#4' mango during retailing.

2. Materials and Methods

2.1 Preparation of Materials

2.1.1 Preparation of mango fruit materials

'Nam Dok Mai, No#4' fruit at the mature green stage of 110-120 days after anthesis were collected from a commercial orchard in Chachoengsao province, Thailand. The fruit was sorted for uniformity of size (average 420 g), colour, and free from defects. The peduncle was then re-cut and left for 1 cm. The sorted fruit was washed in 200 mg·L^{-1} sodium hypochlorite for 5 min.

2.1.2 Preparation of coating materials

High molecular weight chitosan (food grade) was prepared at 0.5% by dissolving with 1.0% lactic acid while stirring for 24 h. On the other hand, sodium alginate (SA) (food grade) was dissolved in distilled water while stirring and heating at 60-70°C for 2-3 h until completely dissolved. The volume was adjusted to obtain 0.1% SA.

2.1.3 Preparation of sweet-flag extracts

Dried sweet-flag ('Waan-Nam') leaves were bought from a market in Bangkok. The dried material was submerged in 95% ethanol in the ratio of 1:10 (w/v) for 5 days at ambient. The solution was filtrated by a Whatman No.1 paper and then evaporated at 40°C with 50-80 mbar by a rotary evaporator. The pure extract was weighted and kept at -20°C until used.

2.1.4 Preparation of a Colletotrichum gloeosporioides' isolate

A cutting (0.5 x 0.5 cm) of mango tissues at normal connected with anthracnose disease tissue was collected from decay mango fruit. The cut tissue was dipped in 10% Clorox for 3 min and then washed up with distilled water 3 times. The example was laid on potato dextrose agar (PDA) and incubated at ambient for 3-5 days. Mycelium of the colonies' edge (Figure 1A) was taken and put onto a new PDA for 7 days (Figure 1B). The *Colletotrichum gloeosporioides* culture was used for *in vitro* inhibition of plant extract.

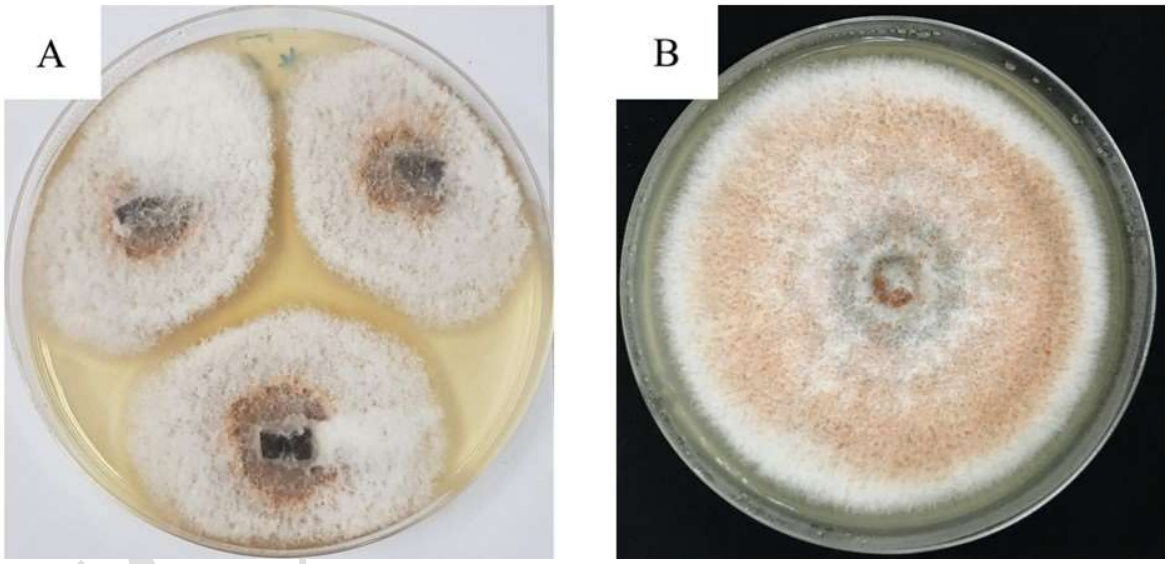

Figure 1 *Colletrotichum gloeosporioides* isolated from Anthracnose decay mango and cultured on PDA at 25°C for 5 days (A) and further isolation of the pure fungus on PDA at 25°C for 7 days

2.2 Experimental Designs

2.2.1 Effect of sweet-flag *extract on in vitro inhibition of C. gloeosporioides*

Disease inhibition was in vitro studied on PDA comprised of different concentrations of the sweet-flag extract. Seven mm diameter of *C. gloeosporioides* culture from 2.1.4 was inoculated on PDA containing 1000, 2000, 3000, 4000 mg·L^{-1} sweet-flag extract. PDA plates containing distilled water, 500 mg·L^{-1} Prochloraz, 5% Dimethyl Sulfoxide (DMSO), and 1.0% chitosan were set to be controls. All treatments comprised 4 replications. PDA plates were incubated at room temperature (28-30°C) for two weeks. The diameter of mycelium growth was measured daily.

2.2.2 Effect of sweet-flag *extract combined with double coatings on postharvest diseases and quality of 'Nam Dok Mai' mango*

Mango fruit in 2.1.1 were then separated into 4 treatments of (1) non-coated control; (2) 0.5% chitosan (the 1st layer) and 0.1% sodium alginate (SA) (the 2nd layer) coated fruit; (3) 0.5% chitosan + 500 mg·L^{-1} Prochloraz® (the 1st layer) and 0.1% SA (the 2nd layer) coated fruit; and (4) 0.5% chitosan + 1,000 mg·L^{-1} sweet-flag extract (the 1st layer) and 0.1% SA (the 2nd layer) coated fruit. Each treatment comprised six replications (one fruit per replication). All fruit were incubated at 25°C, 65-70% RH as a retailing condition.

2.3 Evaluation parameters

2.3.1 Changes in fruit respiration and ethylene production rates

Mango fruit was kept and sealed in an airtight 900 mL container at 25°C for 1 h. The respiration was measure in mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$ using a LI7000 CO_2/H_2O analyzer (USA). For ethylene production, 1 mL of the headspace was withdrawn and injected into the injector of an 8 A Shimadzu Gas chromatography - FID detector (Japan), equipped with a 20 m Carbowax column. The injector and column oven were set to 120°C, whereas the detector was set to 150°C. The rate was reported in μL $C_2H_4 \cdot kg^{-1} \cdot h^{-1}$.

2.3.2 Changes in peel colour

Peel colour changes at the stem end, middle, and blossom end were detected using a CR-400 Konica Minolta (Japan) and reported in CIELAB values of L* value, hue angle, and ΔE.

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

When 1 was the initial values
2 was the current values

2.3.3 Changes in flesh firmness

The fruit was peeled off, and measure the firmness through P/5 probe (5 mm diameter) using a Texture analyzer (TA-TX Plus, Stable Micro, England). The condition was set to penetrate for 10 mm with 2 $mm \cdot sec^{-1}$ constant force. The peak of maximum strength was reported in Newtons (N).

2.3.4 Changes in soluble solids and titratable acidity contents

Fruit juice was measured for the soluble solids contents using an ATAGO digital refractometer, PAL-1 #3810 model. The titratable acidity content was detected by titration. One mL of the juice was mixed with 9 mL of distilled water. The mixture was added with several drops of phenolphthalein and then titrated by 0.1 N NaOH. The volume of NaOH at the terminating point was calculated.

$$\text{Titratable acidity (\%)} = \frac{\text{volume of NaOH(ml)} \times \text{concentration of NaOH(N)} \times 0.064 \times 100}{\text{volume of sample (ml)}}$$

When 0.064 is the milliequivalent of citric acid

2.3.5. Evaluation of fruit disease incidence

The disease incident score of fruit during incubation was recorded. The scores were given as 1-5 of disease appearance on fruit referred to Figure 2. Fruit replication for this evidence was ten fruit each.

Figure 2 Disease severity scores; 1 = healthy fruit, 2 = appeared disease spots <10% of the peel area; 3 = appeared disease 10-20% of the peel area; 4 = appeared disease spots 21-40% of the peel area; 5 = appeared disease spots >50% of the peel area

2.4 Statistical Analysis

The experiments were managed as a completely randomized design. All data measured were subjected to analysis of variance. Mean comparison was performed by Duncan Multiple Range Test at $p \leq 0.05$ using SPSS software version 19.0 for MS-Windows.

3. Results and Discussion

3.1 Effect of sweet-flag extract on *in vitro* inhibition of *C. gloeosporioides*

Sweet-flag extract at ≥ 1000 mg·L^{-1} completely *in vitro* inhibited *Colletotrichum gloeosporioides*, as same as 500 mg·L^{-1} Prochloraz, a commercial fungicide (Figure 3A, B). The distilled water control plate exhibited full of fungal growth since day 9, whereas 1.0% chitosan slightly inhibited the growth (Figure 3B). DMSO at 5%, which was the solvent dissolving the sweet-flag extract, was approximately 50% inhibition of the fungus. The result was consistent with Bhasabutra (1997), who reported 82.5% *in vitro* inhibition of the vegetative growth of *C. gloeosporioides* by 1000 mg·L^{-1} sweet-flag crude extract. The property of sweet-flag inhibiting some plant pathogen, including *Colletrotichum* spp. had been previously reported (Thaenthanee et al., 2014; Dethoup et al., 2019). β-Asarone, a key bioactive compound in sweet-flag, was reported to interfere with ergosterol in fungi' cell membrane (Venkatesan, Karuppiah, Arumugam, & Balamuthu, 2019).

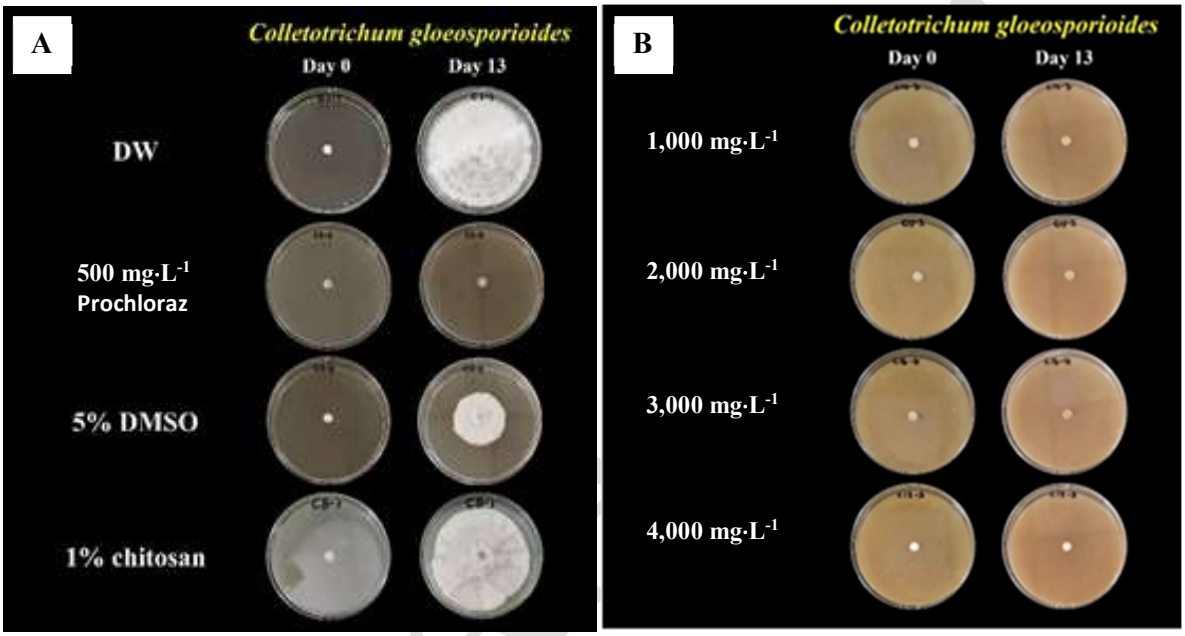

Figure 3 Visual appearance of *Colletrotichum gloeosporioides*' growth on PDA combined with control solutions (A) and sweet-flag extracts at various concentrations (B) on day 0 and day 13 at ambient temperature

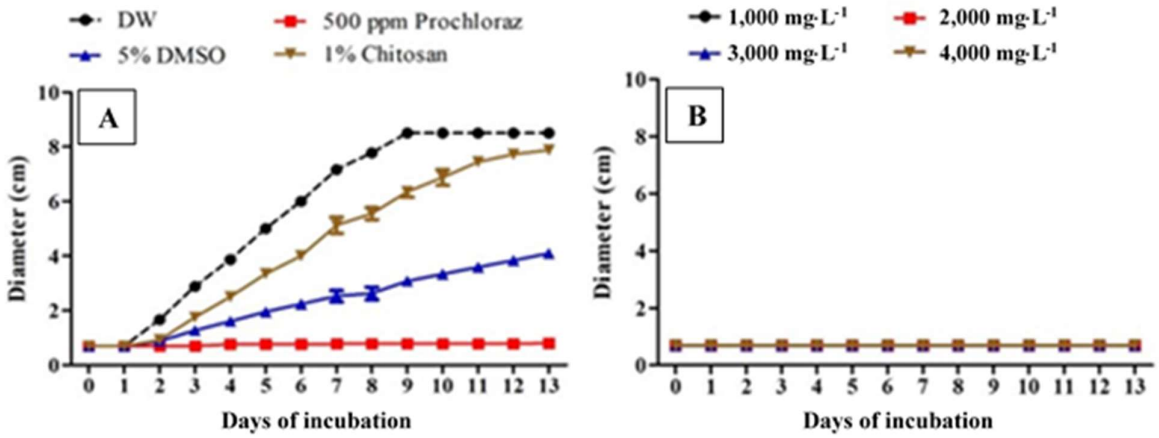

Figure 4 *Colletrotichum gloeosporioides*' growth daily on PDA combined with control solutions (A) and sweet-flag extracts at various concentrations (B) during incubation at ambient temperature

3.2 Effect of sweet-flag extract combined with double coatings on postharvest diseases and quality of 'Nam Dok Mai' mango

3.2.1 Changes in physico-chemical attributes

The climacteric peaks of all treatments appeared on day 6 of incubation (Figure 5A). Interestingly, all double-coated fruit had higher respiration rates compared to noncoated control. There was some evidence that chitosan-based coating induced respiratory metabolisms in fruits (Soe Win et al., 2015; Nguyen, Boonyaritthongchai, Buanong, Supapvanich, & Wongs-Aree, 2020). Each treatment's ethylene production rate sharply increased until day 6 and then remained stable (Figure 5B). The ethylene production rates were not significant between treatments during retailing.

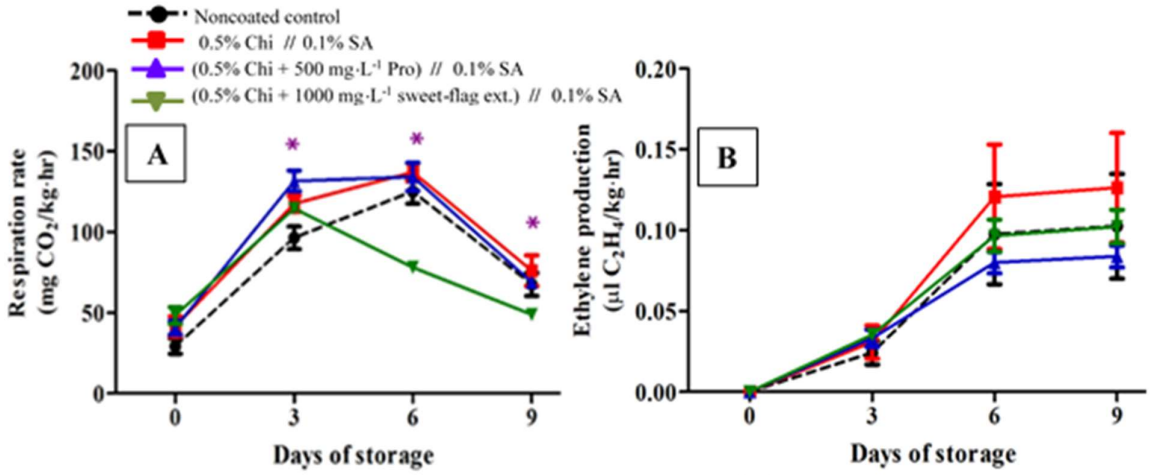

Figure 5 Changes in respiration (A) and ethylene production (B) rates of 'Nam Dok Mai' mango fruit double-coated with 0.5% chitosan (± Prochloraz or sweet-flag extract) and 0.1% sodium alginate, compared to noncoated control and stored at 25°C.

All double coating treatments delayed the peel colour changes but did not affect the increasing soluble solids and texture softening of flesh during nine storage days. The peel L* (Figure 6A) and ΔE (colour changes ratio) (Figure 6C) of noncoated mango were higher than the coated fruit, while the hue angle was lower. The peel of noncoated control turned brighter yellow than coated fruit. On the other hand, although soluble solid contents were lower (Figure 7A) and titratable acidity contents were higher (Figure 7B) in double-coated fruit, there were no significant differences between all treatments. Furthermore, the firmness dramatically reduced after incubation without difference between treatments (Figure 7C). These pieces of evidence suggest that mango fruit ripened after day 6. Double coatings based on chitosan and sodium alginate did not affect the changes in flesh attributes such as firmness, soluble solids, and titratable acidity contents but slightly delayed the peel colour changes.

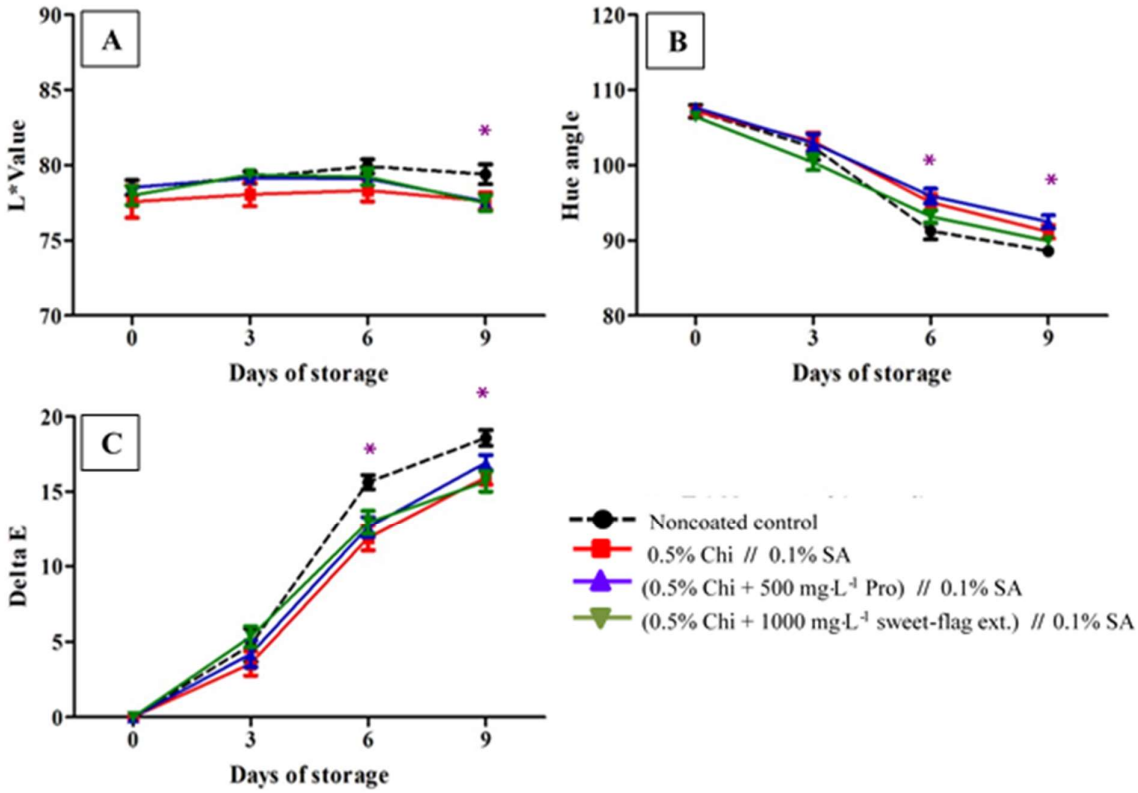

Figure 6 Changes in peel colour of L* (A), hue angle (B), and ΔE of 'Nam Dok Mai' mango fruit double-coated with 0.5% chitosan (± Prochloraz or sweet-flag extract) and 0.1% sodium alginate, compared to noncoated control and stored at 25°C.

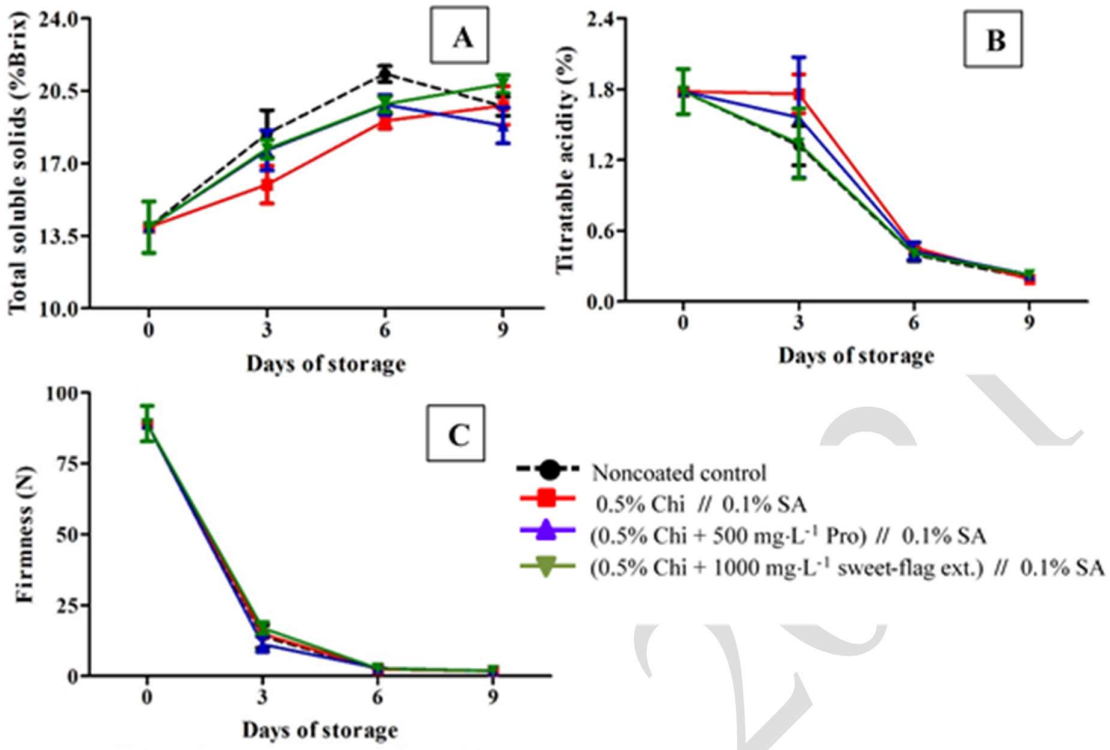

Figure 7 Changes in flesh soluble solids (A), titratable acidity (B), and firmness of 'Nam Dok Mai' mango fruit double-coated with 0.5% chitosan (± Prochloraz or sweet-flag extract) and 0.1% sodium alginate, compared to noncoated control and stored at 25°C.

3.2.2 Disease inhibition on fruit

in vitro disease incidences of mango fruit during incubation at 25°C are shown in Figure 8 and 9. Noncoated, 0.5% Chitosan // 0.1% SA, and (0.5% Chitosan + 1000 mg·L^{-1} sweet-flag extract) // 0.1% SA coated fruit conducted approximately 50% decay on day 6 and 100% on day 9 (Figure 9). Fruit coated with chitosan + 500 mg·L^{-1} Procloraz in the first layer had 40% on day 6 and then remained constant. However, double coatings of 0.5% chitosan + 1,000 mg·L^{-1} sweet-flag extract and 0.1% SA significantly reduced the disease severity on fruit during 12 days of incubation (Figure 8). Although there were reports of sweet-flag extracts inhibiting *in vitro* plant fungi (Thaenthanee et al., 2014; Dethoup et al., 2019), the extracts' efficiency was reduced on *in vivo* tests. In the present study, chitosan + 1,000 mg·L^{-1} sweet-flag extract did not reduce the number of decay fruit but the severity. Increasing the concentration of sweet-flag extract in the chitosan layer showed a better reduction. However, above 5,000 mg·L^{-1} caused mango peel injury (data not shown). Further experiments about mango fruit preparation and/or modification of integrated coating materials could be subsequently explored to obtain more evidence of the plant extracts' functions.

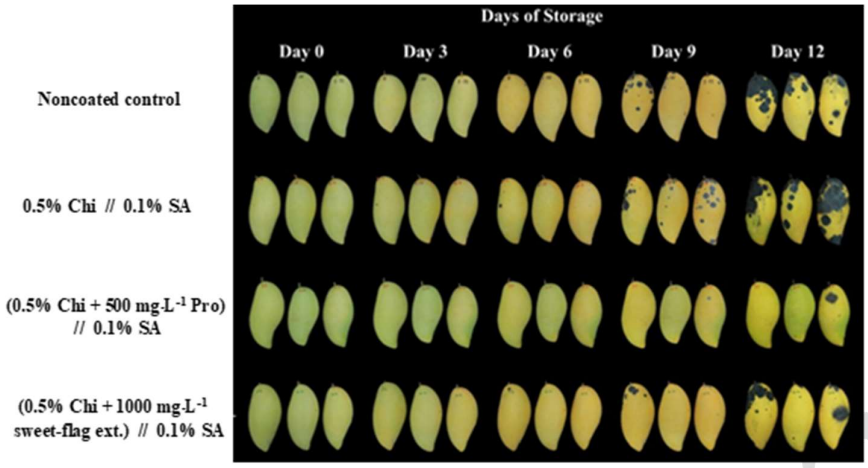

Figure 8 Visual appearance of 'Nam Dok Mai' mango fruit double-coated with 0.5% chitosan (± Prochloraz or sweet-flag extract) and 0.1% sodium alginate, compared to noncoated control and stored at 25°C

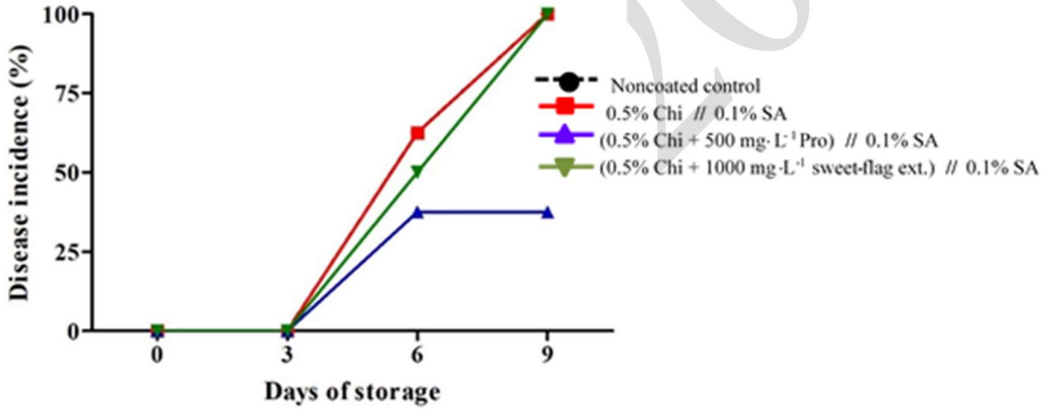

Figure 9 Disease incidence of 'Nam Dok Mai' mango fruit double-coated with 0.5% chitosan (± Prochloraz or sweet-flag extract) and 0.1% sodium alginate, compared to noncoated control and stored at 25°C.

4. Conclusions

One thousand mg·L^{-1} sweet-flag extract was enough to *in vitro* inhibit the mycelium growth of *Colletotrichum gloeosporioides* completely. Double layers of 0.5% chitosan + 1,000 mg·L^{-1} sweet-flag extract and 0.1% SA coated on 'Nam Dok Mai' mango slightly delayed the peel colour changes and significantly reduced 'Nam Dok Mai' mango fruit disease severity at 25°C incubation.

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AG-PS-02

Tolerance Evaluation of 10 Tian Corn Inbred Lines under Temporary Waterlogging Condition

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Abstract

Tian corn is an important crop at the community economy level in Thailand's central region, a flood plain with clay soil with poor drainage. This research's objective was to evaluate ten inbred lines of tian corn for tolerance to temporary waterlogging stress. It was carried out using a split plot in a completely randomized design. The main plots were 15 days of waterlogging versus regular watering and the subplots being 12 genotypes, comprising ten sixth - generation (S6) inbred lines and two open- pollinated check varieties. The results showed that waterlogging showed significant adverse effects on leaf greenness, chlorophyll content, leaf area, and shoot dry weight. The check varieties TAY 60 and TBK had the best waterlogging tolerance, better than all the inbred lines tested. Of the inbred lines, RSTi#8 had the best waterlogging tolerance. The parameters of leaf greenness and chlorophyll content were found to be poor indicators of waterlogging tolerance for tian corn**.**

Keywords: *Tolerance, inbred lines, waterlogging stress, waxy corn, Zea may L.*

1. Introduction

Tian corn is a type of waxy corn that is important at the community economy level. It is widely grown in several provinces in the central region. Farmers like to grow it because it has a short growing season and can be harvested early. The ears are small at only 10-15 cm long and 2-3 cm wide with 8-12 kernel rows, so they are suitable for snacking. The flavor is good, with soft, chewy and slightly sweet kernels (Boonlertnirun, Suvannasara & Boonlertnirun, 2012). Tian corn is a type of waxy corn. When it is dry, the kernels are opaque whitish (Simla, 2013). However, in the central region when farmers grow tian corn during the rainy season, they often experience flooding because most of the soil is clay soil with poor drainage. When the plants are in flooded conditions the roots cannot obtain the oxygen they need and cannot transport water and nutrients to the rest of the plant. The stomata close to reduce transpiration (Sdoodee, Chanaweerawan & Pongkeaw, 2004), then causes photosynthesis to slow down, and the plant growth is inhibited, resulting in reduced yield (Zhu, Li& Shi, 2016). Duan et al. (2018) reported that waterlogging markedly decreased seedling emergence, shoot dry weight, stomatal conductance, chlorophyll content and net photosynthetic rate. Wheat grain yield was significantly and positively correlated with leaf area at the milk-ripe stage, and net photosynthetic rate in flag leaf. Moreover, waterlogging did not significantly affect flag leaf area (Ding et al., 2020). Tian et al. (2019) found that adverse effects of waterlogging on spring maize growth varied with the duration of waterlogging and the growth stage. The most apparent effect of waterlogging stress occurred at the V3 stage, followed by the V6 and VT stages. Corn yield decreased, meanwhile, the height of corn and the day of tasseling and silking were respectively inhibited and delayed for seedling and jointing stage water logging. The susceptibility factors in four phases of corn are ranked, and the susceptibility order of different corn stage is seedling>jointing>tasseling-silking>maturity. (Kuang, Xianjiang, Xiuqing & Yafeng, 2012).

The objective of this research was to evaluate 10 S6 inbred lines of tian corn for their ability to withstand temporary waterlogging to select superior varieties of tian corn to recommend to farmers in the central flood plains.

2. Materials and Methods

2.1. Experimental design

Split plot in Completely randomized design was utilized with two main plots (normal and waterlogging conditions) and twelve tian corn lines (10 S6 inbred lines and two open pollinated lines) as subplot (Table 1).

Entry	code	pedigree
1	RSTi#1	INS/TBK//TBK-13-1-3-1-1
2	RSTi# 2	INS/TBK//TBK-17-1-1-1-1
3	RSTi#3	INS/TBK//TBK-26-1-2-1-1
4	RSTi#4	INS/TBK//TBK-31-2-3-1-1
5	RSTi#5	TBK-11-1-1-1-1-1
6	RSTi#6	TBK-16-1-1-1-1-1
7	RSTi#7	TKKU1-20-2-1-1--11
8	RSTi#8	TKKU1-5-2-1-1-1-1
9	RSTi#9	TSW-20-2-1-1-1-1
10	RSTi#10	TSW-20-3-1-1-1-1
11	TAY	Tian Ayutthaya 60
12	TBK	Tian BanKhoa

Table 1 Pedigrees of 10 S6 inbred lines and two open pollinated lines (check varieties).

2.2. Growing conditions

Three corn seeds were sown in a container in 4-inch pots filled with clay soil. They were watered with a watering can once a day. After ten days, excess seedlings were pulled out to one plant per pot then 2 g of urea (46-0-0) fertilizer was added to each pot. When the corn plants were 14 days old they were divided into 2 groups with 10 plants of each genotype for one replication. The first group was treated as regular watering and the second group was put in waterlogging condition by placing the pots in a 0.60 x 1.00 meter plastic tub 25 cm tall filled with water to a level of 3 cm above the rims of the pots. The water level was maintained at the same level for 15 days.

The experiments were conducted at the greenhouse and plant physiology lab of the Plant Science department, Faculty of Agricultural Technology and Agro-industry, Rajamangala University of Technology Suvarnabhumi.

2.3. Data collection

2.3.1. Leaf greenness was measured in spad units with a SPAD 502 chlorophyll meter. Five plants were randomly sampled for each genotype and the top fully expanded leaf was measured 15 days after waterlogging.

2.3.2. Total chlorophyll was measured in 5 sample plants from each genotype at the end of the experiment. A section of a leaf, not including midrib, was cut from the center of each leaf and chopped into small pieces to obtain 0.1 g (W) of leaf tissue from which chlorophyll was extracted by soaking in 10 ml (V) of dimethylformamide and incubating in the dark for 24 h. The resulting liquid was filtered and light absorbance was measured by spectrophotometer (Libra S50) at wavelengths D654 and D663 nm. Chlorophyll content was calculated in mg/g

$$\text{Total chlorophyll (mg/g)} = [\,20.2\,(D645) + 8.02\,(D663)\,] \times [\,V/1000\,W\,)]$$

2.3.3. Leaf area in square centimeters was measured in 5 sample plants from each genotype at the end of the experiment. The plants were cut off at the plant base near the soil surface and only green leaves were measured using a Win Dias 3 model leaf area measurement system from Delta-T Devices.

2.3.4. Shoot dry weight was measured by taking the samples from the leaf area measurement above and drying them in a hot air oven at 75° C for 72 h and weighing them on a balance in grams to 2 decimal points.

2.3.5. To investigate aerenchyma in roots, completed true roots (primary root) were cross -sectioned with a razor and stained by immersing in methylene blue, then placed on a slide with one drop of distilled water and a cover slip was added. The slides were viewed under a light microscope and photographs were taken.

2.3.6. Waterlogging differential value (WDV) = (Xn-Xw)/Xn

Xn = characters measured in Normal irrigation

Xw = characters measured in Waterlogging condition

2.3.7. Waterlogged tolerance index (WTI) = (Xw/Xn)/(Yw/Yn)

Xn = characters of each genotype measured in Normal irrigation

Xw = characters of each genotype measured in Waterlogging condition

Yn = character average of all genotypes measured in Normal irrigation

Yw = character average of all genotypes measured Waterlogging condition

2.4. Data analysis

STAR software (IRRI, 2014) was used to analyze the variance, test the significance of differences with F-test and compare means by Least Significant Difference (LSD).

3. Results and Discussion

3.1. Leaf greenness

There were statistically significant differences in leaf greenness among the different genotypes of tian corn tested. The leaf greenness values under waterlogged conditions significantly decreased in every genotypes. RSTi #4 had the highest leaf greenness at 36.88 spad units, and a different value of 0.15. These values were significantly different from the other inbred genotypes but not significantly different from TAY 60, one of the check varieties (leaf greenness 34.49 spad units, different value 0.22). RSTi#6 had the lowest leaf greenness (27.43 spad units) and a different value of 0.49 (Table 2 and Figure 1). If leaf greenness is considered an indicator of waterlogging tolerance, then RSTi #4 had the best waterlogging tolerance.

Genotype (G)	Leaf greenness (Spad unit)		Different value	(G) average	Chlorophyll content (mg/g. fresh wt.)		Different value	(G) average
	Condition (C)				condition			
	Waterlogging	Normal			Waterlogging	Normal		
RSTi # 1	22.32	34.58	0.35	28.45	1.39	2.07	0.33	1.73
RSTi # 2	22.62	36.70	0.38	29.66	1.63	2.75	0.41	2.19
RSTi # 3	27.98	35.54	0.21	31.76	1.48	2.11	0.30	1.795
RSTi # 4	33.94	39.82	0.15	36.88	1.37	2.28	0.40	1.825
RSTi # 5	22.78	32.52	0.30	27.65	1.32	2.03	0.35	1.675
RSTi # 6	18.58	36.28	0.49	27.43	1.81	2.61	0.31	2.21
RSTi # 7	19.56	40.32	0.51	29.94	1.87	2.97	0.37	2.42
RSTi # 8	21.82	34.88	0.37	28.35	1.64	2.08	0.21	1.86
RSTi # 9	28.50	34.82	0.18	31.66	1.24	2.17	0.43	1.705
RSTi # 10	24.94	37.48	0.33	31.21	1.26	2.46	0.49	1.86
TAY 60	30.16	38.82	0.22	34.49	1.30	2.31	0.44	1.805
TBK	27.34	34.92	0.22	31.13	1.45	2.37	0.39	1.91
C average	25.05	36.39			1.48	2.35		
LSD.0.5 (G)	3.90				0.37			
LSD.0.5 (C)	6.40				0.59			
LSD.0.5(G)X (C)	6.40				0.59			
CV (G) (%)	9.82				11.19			
CV. (C) (%)	12.87				17.68			

Table 2 Leaf greenness and chlorophyll content of 12 tian corn seedling genotypes grown in normal and waterlogging conditions.

3.2. Chlorophyll content

Chlorophyll content of tian corn dropped under waterlogged conditions, and there were statistically significant differences in response to waterlogging as seen through chlorophyll content among the different genotypes. RSTi#8 had the lowest difference value at 0.21 and a chlorophyll content of 1.64 mg/g. RSTi#7 had the highest chlorophyll content at 1.87 mg/g, followed by RSTi#6 at 1.81 mg/g. RSTi#9 had the lowest chlorophyll content at 1.24 mg/g and a different value of 0.43 (Table 2 and Figure 1).

3.3. Leaf area

There were statistically significant differences in response to waterlogging in leaf area among the different genotypes of tian corn tested. Leaf area decreased under waterlogging conditions in every genotypes. The genotype with the largest leaf area was the check variety TAY 60 at 2,891.5 cm2. These were significantly different from all other genotypes and the difference value was 0.64. RSTi#5 had the lowest leaf area at 1,273.5 cm2 and the different value was 0.90. If leaf area is taken as an indicator of waterlogging tolerance, then TAY 60 had the best waterlogging tolerance (Table 3 and Figure 1).

Genotype (G)	Leaf area (cm^2) Condition (C)		Different value	(G) average	Shoot dry weight (g) condition		Different value	(G) average
	Waterlogging	Normal			Waterlogging	Normal		
RSTi # 1	263	3175	0.92	1719.0	0.82	3.64	0.77	2.23
RSTi # 2	205	3618	0.94	1911.5	0.47	3.78	0.88	2.12
RSTi # 3	338	3210	0.89	1774.0	1.19	3.26	0.63	2.22
RSTi # 4	364	2822	0.87	1593.0	0.78	2.89	0.73	1.83
RSTi # 5	230	2317	0.90	1273.5	0.39	3.35	0.88	1.87
RSTi # 6	280	3381	0.92	1830.5	0.52	3.13	0.83	1.82
RSTi # 7	200	3570	0.94	1885.0	1.02	3.51	0.71	2.26
RSTi # 8	752	3403	0.78	2077.5	1.33	4.03	0.67	2.68
RSTi # 9	336	3554	0.91	1945.0	0.78	3.18	0.75	1.98
RSTi # 10	297	3067	0.90	1682.0	0.73	3.09	0.76	1.91
TAY 60	1526	4257	0.64	2891.5	2.99	4.94	0.39	3.96
TBK	889	3712	0.76	2300.5	1.63	2.85	0.43	2.24
(C)average	457	3341			1.05	3.48		
LSD.0.5 (G)	256				0.58			
LSD.0.5 (C)	724				0.96			
LSD.0.5(G)X (C)	724				0.96			
CV (G) (%)	22.11				25.50			
CV. (C) (%)	22.03				20.07			

Table 3. Leaf area and shoot dry weight of 12 tian corn seedling genotypes grown in normal and waterlogging conditions

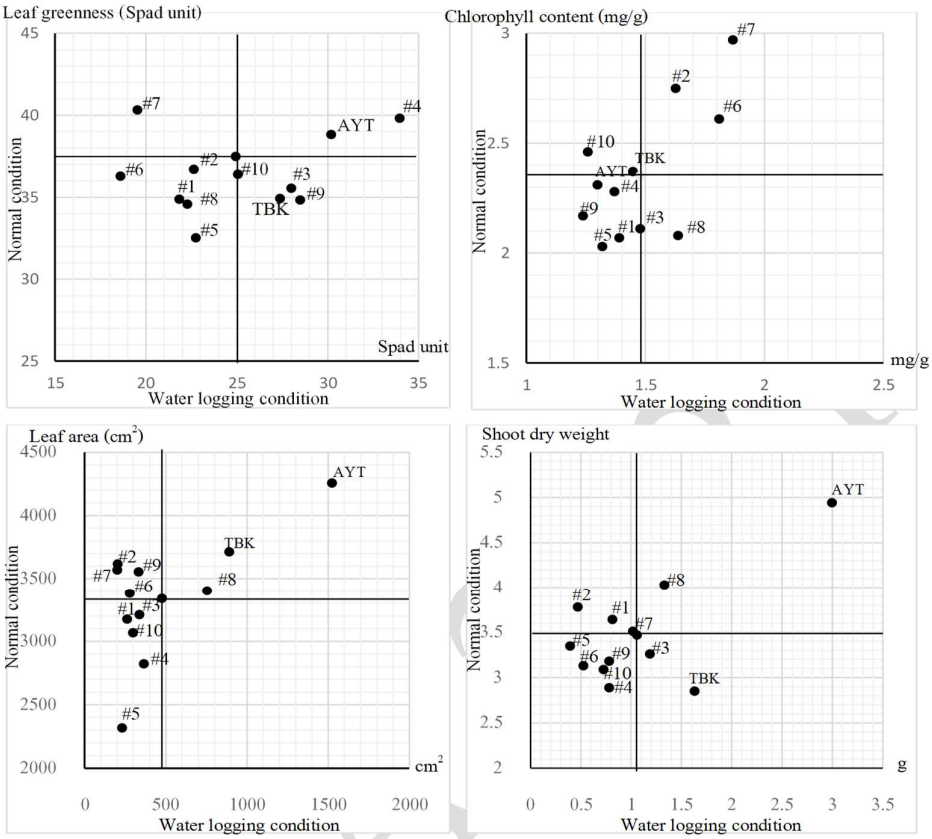


Figure 1 Leaf greenness, chlorophyll content, leaf area and shoot dry weight of 12 tian corn genotypes under normal and waterlogging conditions

3.4. Shoot dry weight

Shoot dry weight of all tian corn genotypes significantly decreased under waterlogging conditions compared to normal conditions. The genotype with the greatest vatue of shoot dry weight was the check variety TAY 60 at 3.97 g/plant. It was significantly different from all other genotypes and the difference value was 0.39. RSTi#6 had the lowest shoot dry weight at 1.825 g/plant and the different value was 0.83. If shoot dry weight is taken as an indicator of waterlogging tolerance, then TAY 60 had the best waterlogging tolerance (Table 3).

3.5. Waterlogging tolerance index

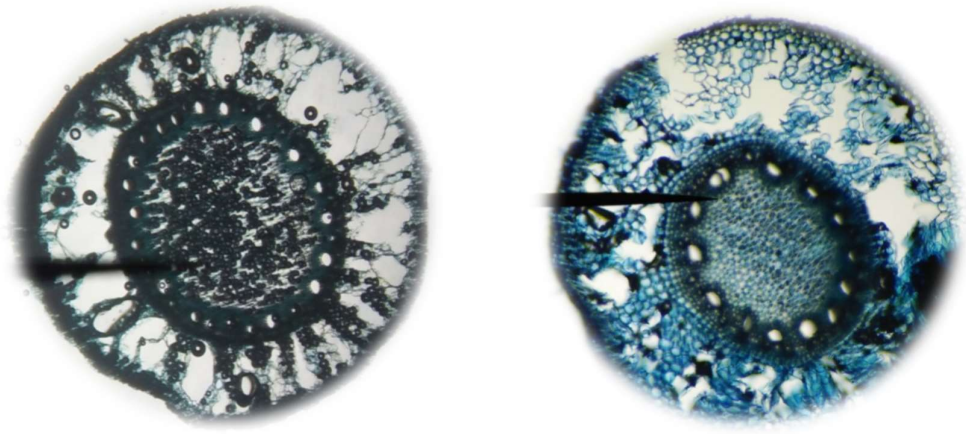
Different tian corn genotypes had different waterlogging tolerance index values for the different parameters measured. Considering leaf greenness and chlorophyll content, RSTi#4 and RSTi#8 had the highest indexes, but considering leaf area and shoot dry weight, the open- pollinated check variety TAY 60 had the highest waterlogging tolerance index. Of all 10 inbred lines being evaluated, RSTi#8 had the best waterlogging tolerance index in several parameters, so it can be considered quite tolerant of short- term waterlogging (Table 4).

Genotype (G)	Waterlogging tolerance index			
	Leaf greenness (spad unit)	Chlorophyll content (mg/g. fresh wt.)	Leaf area (cm^2)	Shoot dry weight (g)
RSTi # 1	0.94	1.03	0.58	0.74
RSTi # 2	0.90	0.91	0.40	0.41
RSTi # 3	1.14	1.07	0.74	1.20
RSTi # 4	1.24	0.92	0.91	0.89
RSTi # 5	1.02	1.00	0.70	0.38
RSTi # 6	0.74	1.06	0.58	0.55
RSTi # 7	0.70	0.96	0.40	0.96
RSTi # 8	0.91	1.21	1.56	1.09
RSTi # 9	1.19	0.88	0.67	0.81
RSTi # 10	0.97	0.78	0.68	0.78
TAY 60	1.13	0.86	2.53	1.99
TBK	1.14	0.94	1.69	1.88

Table 4 Waterlogging tolerance index value of each characteristic of 12 tian corn seedling genotypes

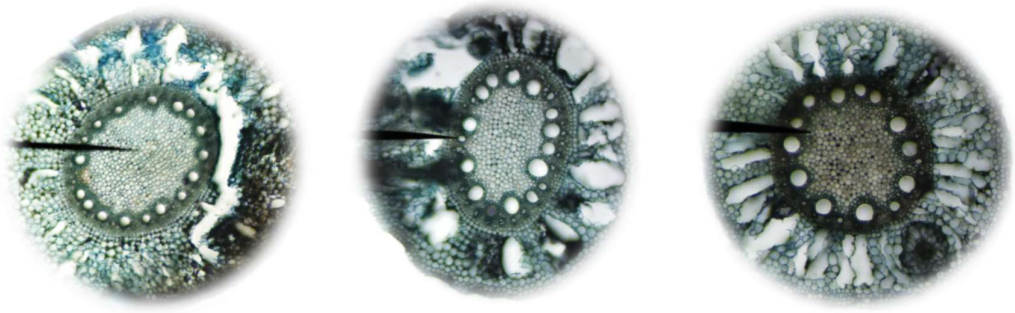
3.6. Aerenchyma formation

When root cross- sections were microscopically analyzed for aerenchyma formation after 15 days of waterlogging, the check varieties TAY 60 and TBK tended to have a quite obvious aerenchyma formation when compared to the other genotypes (Figure 2), which means that they adapted well to waterlogging conditions. Most of the inbred lines that were evaluated had little aerenchyma formation. However, those lines with higher waterlogging index values and different low values in various parameters, such as RSTi#8, RSTi#4 and RSTi#3, indicating better tolerance to waterlogging, did show evidence of some aerenchyma formation (Figure 3).



TAY60 TBK

Figure 2 Aerenchyma formation of TAY 60 and TBK under waterlogging condition.



RSTi#3 RSTi#4 RSTi#8

Figure 3. Aerenchyma formation of RSTi#3, RSTi#4 and RSTi#8 under waterlogging condition

TAY 60 is an open- pollinated variety of tian corn notable for its fast growth in the early seedling establishment phase. This experiment achieved a larger leaf area and dry weight than the other lines tested, even under waterlogged conditions. TAY 60's waterlogging tolerance index came out higher than all the inbred lines that we evaluated. It may likely be due to inbreeding depression because the inbred lines were self- pollinated for 6 generations. The degree of genetic diversity in the parents of an inbred line affects the severity of inbreeding depression. There are generally observed in growth-related traits in corn. Zhu et al. (2016) reported that waterlogging causes plant weight to drop and stimulates more aerial roots. The excess water affects root respiration and accelerates root aging. When the root respiration rate decreases, root tissues form aerenchyma, helping the plants survive flooded conditions. Sundgren, Uhlen, Lillemo, Briese and Wojciechowski (2018) reported that seminal roots developed faster in the seedling establishment phase and more nodal roots during the waterlogging treatment; they concluded that anatomical root traits, such as a narrow stele and aerenchyma may contribute to improving waterlogging tolerance. Boonlertnirun, Meechoui and Sarobol (2010) revealed that susceptible field corn genotype, 30B80(Pioneer) was poorly adapted to generate aerenchyma under hypoxia (temporary waterlogging). Zhang, Liu and Dong (2020) suggested that waterlogging tolerance improvement could be made by exploiting genotype × management × environment interactions. Waterlogging markedly decreased seedling emergence and inhibited the shoot dry weight, and chlorophyll content (Duan et al., 2018). Shin et al. (2016) reported that when plants are subjected to waterlogging during the seedling establishment phase it will cause a decrease in the chlorophyll concentration in the leaves and cause premature aging in the leaves, so this can be a good indicator of excess soil moisture. Yong-zhong et al. (2010) revealed that the second leaf stage (V2) was the most susceptible stage, whereas the dry weight of both shoots and roots of maize (Zea mays L.) were significantly reduced at 6 d time-point of waterlogging.

4. Conclusions

1. Every genotype of tian corn had reduced chlorophyll content, reduced leaf greenness, reduced shoot dry weight, and reduced leaf area under waterlogged conditions, and those genotypes that were more tolerant of waterlogging started to form aerenchyma in their roots to enable oxygen exchange.

2. Tests of waterlogging stress on tian corn seedling showed that the open- pollinated check variety TAY 60, followed by the check variety TBK, had the best waterlogging tolerance. Among the inbred lines being evaluated, RSTi#8 showed the best waterlogging tolerance.

3. Leaf greenness and chlorophyll content are not accurate indicators of waterlogging tolerance for tian corn..

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Yield and Nutritional Composition of Sweet Potato Shoots Genotypes with Varying Fleshed Colors and Various Application Fertilizer

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Abstract

This study aimed to investigate the food potential of 5 sweet potato shoots genotypes (Kapi, Pakchong, Carrot, Okud, and Japanese yellow) include various application fertilizers. The tips yield was the highest in Pakchong and also exhibited high carbohydrate and antioxidant activity. Japanese yellow leaf had a greater amount of fat, fiber, and ash (5.5, 11.09, and 12.9 g/100 g DW, respectively) than the others accompanied by the highest content of antioxidants and antioxidant activity which was related to phenolic content (0.71 mg GAE/100 g FW [DPPH], and 0.83 mg GAE/100 g FW [FRAP]). The fertilizer application based on soil testing can increase tips yield, some nutritional composition (fat, moisture content), and antioxidants (phenolic, anthocyanin) of the Pakchong leaf. While, conventional application fertilizer also increased the Pakchong leaf area, leaf greenness, chlorophyll content, and carbohydrate. Besides, the Pakchong leaf had the greatest protein and antioxidants although without fertilizer. These findings suggest that Pakchong shoot could be produced and consumed as a green vegetable by high shoot yield and nutritional composition attributes.

Keywords: *sweet potato shoots, sweet potato leaf, nutritional composition, antioxidants, antioxidant activity.*

1. Introduction

Sweet potato (*Ipomoea batatas* L.) is the most important food crop after rice, wheat, potato, maize, and cassava (Shekhar, Mishra, Buragohain, Chakraborty, &
Chakraborty, 2015). China is the main sweet potato producer in Asia. The young shoot of sweet potato is consumed as nutrient-dense and health-promoting green leafy vegetables, especially in Southeast Asia (Johnson & Pace, 2010). Because sweet potato shoots can be harvested several times a year, their annual yield is much higher than other green vegetables. Recent studies have shown that sweet potato leaves are a good source of minerals (K, P, Ca, Mg, Fe, Mn, Cu), dietary fibers, and dietary antioxidants (Johnson & Pace, 2010; Sun, Mu, Xi, Zhang, & Chen, 2014a). The concentration of sweet potato leaf polyphenols was 7–9 times as much as those of grape seeds (Xi, Mu, & Sun, 2015). Sweet potato leaf has a much higher content of polyphenols and antioxidants that are beneficial to the human body than that of storage roots (Islam et al., 2002).

In Thailand, several varieties of sweet potato tubers are produced. Some varieties can be used to produce a young shoots for distribution as vegetables. Vegetable growers grew sweet potatoes in the same way as vegetable cultivation and applied the rich nitrogen fertilizer by two times of potassium and phosphorus. Therefore, the objective of this study was to investigate the potential of genotypes varying with fleshed colors and type of application fertilizer for sweet potato shoot production.

2. Materials and Methods

2.1. Plant materials

The studies consisted of two experiments using Randomized Complete Block Design (RCBD) with 4 replications. Experiment 1; comparison of sweet potato cultivars for shoot production, The vine of five varieties of sweet potato, (Kapi, Pakchong, Carrot, Okud, and Japanese yellow), 30 cm long sizes were obtained from a local farm then cut into 20 cm length and planted 5 cm deep and about 15 cm apart. After two months, harvest young shoots every 15 days for 90 days. Experiment 2; effects of varieties and fertilizer type and application on yield and nutritional composition of sweet potato shoot. The two varieties of sweet potato (Pachong and Japanese yellow) which were selected from the previous experiment were cultivated and compared the 3 types of fertilizer application, (i) conventional application fertilizer (CAF), (according to the recommendations for green vegetable cultivation, N:P:K=150: 75: 75 kg/ha), (ii) fertilizer application based on soil testing (FABS), (N:P:K=125: 31: 31 kg/ha), and (iii) without fertilizer (WF). The soil testing was performed by quantified the organic matter using Walkley and Black method, available phosphorus (P_2O_5) by Bray II and the analysis of potassium by the ammonium acetate extraction and atomic absorption spectrophotometer.

2.2 Young shoot yield and Leaf area per plant

Yield of sweet potato shoots were determined by the number of young shoots per plant, shoot fresh weight per plant, and young shoot fresh weight. The leaf area per plant was assessed by leaf area meter (S NO: A30969).

2.3. Leaves greenness and chlorophyll contents

The leaves greenness of the fully expanded leaf was measured by a chlorophyll meter (SPAD 502).

The chlorophyll contents were determined by Moran & Porath (1980) with slight modification. The weighed samples, having been cleaned and cut into small pieces, were extracted with N,N-dimethyl formamide (DMF). The 0.1 g of sample was mixed with 7 ml DMF in the test tube. After incubated at room temperature for 24 h, the mixture was filtered through filter paper and adjusted volume to 10 ml. The extracted sample was read at an absorbance of 663 and 645 nm. The amounts of chlorophyll were calculated according to the following formulas:

mg total chlorophyll/g fresh leaf wt = $[20.2(D_{645})+8.02(D_{663})]\times[V/(1000\times W)]$

D = OD of chlorophyll in that wavelength.
V = the final volume of the extraction chemical
W = leaf sample weight

2.4. Proximate composition analysis

Moisture content was measured following official methods of the Association of Official Analytical Chemists (AOAC, 2005). Briefly, triplicates of sweet potato leaf samples were oven-dried at 103°C till constant weight and the percentage of moisture content was calculated.

Crude fat, crude protein, and ash were determined by AOAC methods (Association of Analytical Chemists, 2000) Crude fat content was determined according to AOAC method 960.39. Crude protein was assessed by the micro-Kjeldahl method with nitrogen to a protein conversion factor of 6.25 (AOAC method 976.05)

Carbohydrate content (g/100 g DW) was calculated by subtracting the sum of percent ash, crude fat, crude protein, and crude fiber contents from 100.

2.5. Vitamin C content

A sample consisting of 10 g of sweet potato leaves, previously ground, was placed in a 100 mL 2% hydrochloric acid solution. After stirring, and sedimentation, it was filtered. A 10-mL aliquot was added to a beaker containing 30 mL of distilled water, 5 mL of 1% potassium iodate, and a 1mL solution of starch. It was then titrated with potassium iodate using the method described in Elgailani et al. (2017). The ascorbic acid concentration was expressed as mg/100 g sample fresh weight.

2.6. *Anthocyanin content*

The anthocyanin content of samples was determined using the pH differential method (Giusti and Wrolstad, 2005). The extracts were prepared by mixing the crushed leaf (5 g) with 20 mL ethanol 95% (v/v) acidified with HCl (0.1%, v/v) for 2 min. The obtained mixture was kept at room temperature in the dark for 16 h and then filtered using Whatman No. 3 filter paper. The filtrate extracts were diluted 15-fold with 0.025 M potassium chloride buffer (pH = 1.0) or 0.4 M sodium acetate buffer (pH = 4.5). A UV visible spectrophotometer and 1-cm path length disposable glass cells were used for spectral measurements at 520 and 700 nm against distilled water as blank. Pigment content was calculated as milligrams cyanidin-3-glucoside per 100 g sample fresh weight.

2.7. Total polyphenol content

Sweet potato leaf sample was extracted with acetone solvent (Wu et al., 2006; Mahattanatawee et al., 2006). Briefly, 20 g of leaf sample was homogenized with 80 ml of 80% acetone for 15 min and the mixture was filtered through filter paper (No.1, Whatman Inc.) then kept in dark and cool condition for further analysis of total phenolic compound content and antioxidant activity (DPPH).

Polyphenol content was measured by the Folin Ciocalteu method (Lim et al., 2007). The results were calculated based on standard curve for gallic acid and expressed as milligrams gallic acid equivalents (GAE) per 100-gram fresh weight.

2.8. DPPH free radical scavenging activity

1,1-diphenyl-2-picryl hydroxyl (DPPH) was used to determine the antioxidant activity of the sweet potato leaf extract. This assay was performed using protocols by Wu et al. (2006) with slight modification. The sample extracts were diluted to different concentrations. A 4 ml of 0.6 mM DPPH in 80% ethanol was mixed with 1 ml of sample and incubated for 30 min at room temperature in the dark. The mixture absorbance was measured at 517 nm. The remaining DPPH at the completion of the reaction was determined and quantified as the DPPH radical scavenging activity using the gallic acid standard curve. The DPPH was expressed as mg GAE/100 g FW.

2.9. Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed by a slight modification method of Benzie & Strain, (1996). The extracts for FRAP assay were obtained from sweet potato leaf by mixing crushed samples (~20 g) with 20 mL ethanol 95% (v/v) acidified with HCl

(0.1%, v/v) for 60 min followed by the filtering of solution. The residue was re-extracted twice and the obtained extracts were combined and diluted to the volume of 50 mL with ethanol acidified with HCl (0.1%).

The stock solutions included 300 mM acetate buffer, 10 mM TPTZ [2,4,6-tris (2-pyridyl)-s-triazine] solution in 40 mM HCl, and 20 mM $FeCl_3$ solution. Acetate buffer (25 mL) and TPTZ (2.5 mL) were mixed, and 2.5 mL $FeCl_3$ was added. The sample extract (150 µL) was added to 2850 µL of the FRAP solution and kept for 30 min in the dark. The absorbance was measured at 593 nm. The antioxidant capacity was calculated using the standard curve of standard solutions (ferrous sulfate at various concentrations from 0.1 to 1.0 mM). The antioxidant capacities of the samples were expressed as µM of Fe (II)/100 g of sample fresh weight.

2.10. Statistical analysis

The statistical significance of differences between variants was determined with an analysis of variance (ANOVA) at $p < 0.05$. for comparing mean values. The least significant differences (LSD) or Tukey's Honest Significant Difference (HSD) test was used for comparing group means. Analysis was performed using the STAR program (Statistical Tool for Agricultural research, IRRI). The correlation coefficients (r) of antioxidants and antioxidant activities data were also performed.

3. Results and Discussion

3.1 Yield of sweet potato shoots

3.1.1 Yield of shoots of five sweet potato varieties

The five varieties of sweet potato, namely Kapi, Pakchong, Carrots, Okud, and Japanese yellow were cultivated to produce the young shoots. The number and fresh weight of shoots per plant of Pakchong were significantly greater than four others (Table 1). But the highest tip fresh weight was obtained from Carrot. These findings suggest that Pakchong produced high numbers of shoots that were smaller in size than Carrot. The yield of sweet potato shoot vary by genotypes and cultivation method. Islam, Rabbani, Adam, & Sorker (2019) reported that the average tips of 27 accessions yield were between 4.9-38.3 g fresh weight.

Table 1 Number of young shoot per plant, fresh weight per plant, and young shoot fresh weight of five sweet potato varieties.

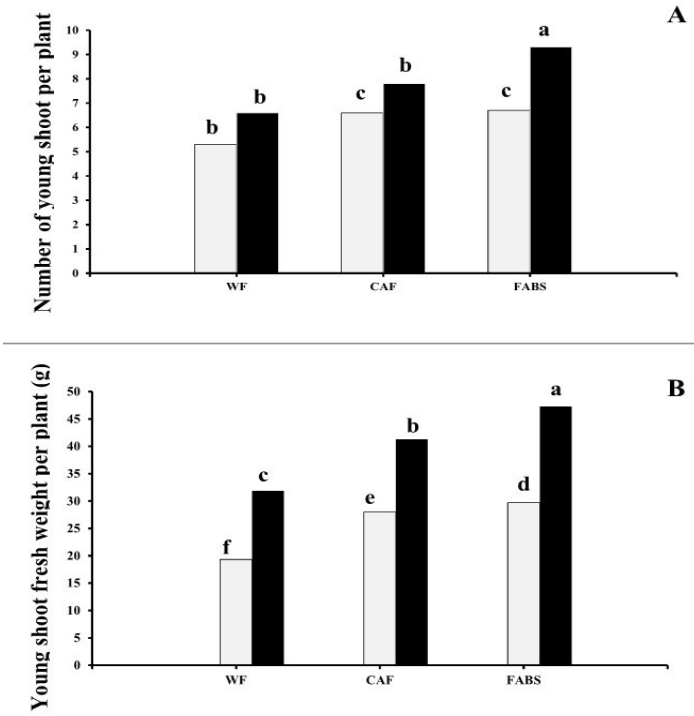
Varieties	Number of young shoot per plant	Fresh weight per plant (g)	Young shoot fresh weight (g)
Kapi	7.1^{b}	45.8^{b}	6.5^{b}
Pakchong	10^{a}	60.4^{a}	6.0^{c}
Carrot	6.5^{c}	45.1^{b}	6.9^{a}
Okud	6.6^{c}	40.0^{c}	6.1^{c}
Japanese yellow	7.1^{b}	36.3^{d}	5.1^{d}

Different letters in the same columns indicate the values are significantly different ($p < 0.05$).

3.1.2 Yield of shoots of two sweet potato varieties with various type of fertilizer application

The number of shoot and shoot fresh weight per plant of Pakchong with fertilizer application based on soil testing (FABS) were significantly greater than the others. Whereas, the lowest shoot numbers were obtained from both Pakchong and Japanese yellow without fertilizer (WF) (Figure1). The shoots fresh weight were increased by FABS even had lower amount of N: P: K than conventional application fertilizer (CAF). This consistent with the range of fertilizer requirement that depends on the varieties, soil type, and climatic condition (Hartemink, Johnstonb, O'Sullivanc, & Poloma, 2000)

Figure 1 Number of young shoot per plant of two sweet potato varieties (A) and Young shoot fresh weight per plant of two sweet potato varieties (B). Data are Japanese yellow (•) and Pakchong (•) with different fertilizer application as follow; without fertilizer (WF), conventional application fertilizer (CAF), and fertilizer application based on soil testing (FABS). Different letter on top of the bar indicate the data are significantly different ($p \leq 0.05$)



3.2 Proximate composition

3.2.1 Proximate composition of leaves of five sweet potato varieties

The proximate composition of leaves of five sweet potato varieties in Table 2 (A), (B) showed the moisture content ranged between 80.16 and 85.24 g/100 g FW that different from those reported by Sun, Mu, Xi, Zhang, & Chen (2014b). The moisture contents obtained in their study ranged between 84.09 and 88.92 g/100 g FW.

There were significantly different in protein content among the varieties ($p \leq 0.05$). Kapi had the highest protein content, whereas Pakchong was the lowest one Table 2 A). Our results were like those reported by Sun et al., (2014b) who reported the crude protein content of 40 sweet potato cultivar leaves in China. The crude protein content was highest in Shi No,5 (31.08 ± 0.09 g/100 g DW), whereas Shangshu No. 19 (spring) had the lowest crude protein content (16.69 ± 0.0909 g/100 g DW). The protein content of sweet potato leaves may depend on genotype.

The fat content of leaves of five sweet potato varieties was not different excepted the Okud which had the lowest with a significant difference in fat content (Table 2 A). The fat content in leaves was higher than that of sweet potato storage root but lower than sweet potato stems (Ishida, Suzuno, Sugiyama, Innami, Tadokoro, & Maekawa, 2000). Fat is involved in the maintenance of body temperature and cell function. In addition, fats are required for the digestion, absorption, and transport of vitamins A, D, E, and K (Sun et al., 2014b).

Fiber content varied among the five sweet potato varieties (Table 2 B), Japanese yellow had higher fiber content. In this study, the fiber of sweet potato leaves was lower than the nutrient reference value for fiber (25 g/day). Many factors contribute to the differences in fiber content including genotype, maturity, and nutritional composition for the cultivation system.

Pachong had significantly different greater carbohydrate content than other varieties whereas Japanese yellow and Kapi had the greatest ash content.

Table 2 (A) Moisture protein, and fat contents of leaves of five sweet potato varieties (g/100 g DW). (B) Fiber, carbohydrate, and ash contents of leaves of five sweet potato varieties (g/100 g DW).

A

Varieties	Moisture	Protein	Fat
Kapi	82.89±0.00^{b}	23.26 ± 0.03^{a}	5.00 ± 0.00^{a}
Pakchong	85.24±0.00^{a}	16.6 ± 0.03^{d}	5.50 ± 0.01^{a}
Carrot	81.78±0.00^{c}	20.80 ± 0.05^{b}	5.50 ± 0.00^{a}
Okud	82.96±0.02^{b}	21.00 ± 0.07^{b}	2.00 ± 0.01^{b}
Japanese yellow	80.16±0.06^{d}	19.27 ± 0.02^{c}	5.50 ± 0.00^{a}

B

Varieties	Fiber	Carbohydrate	Ash
Kapi	10.83 ± 0.09^{b}	47.39 ± 0.54^{d}	13.55±0.22^{a}
Pakchong	10.81 ± 0.11^{b}	56.97 ± 0.85^{a}	10.12±0.40^{d}
Carrot	10.94 ± 0.02^{b}	51.20 ± 0.30^{c}	11.56±0.23^{b}
Okud	10.88 ± 0.02^{b}	55.06 ± 0.28^{b}	11.06±0.12^{c}
Japanese yellow	11.09 ± 0.16^{a}	51.27 ± 0.56^{c}	12.90±0.32^{a}

Data are means ± SD. Different letters in the same columns indicate the values are significantly different ($p < 0.05$).

3.2.2 Proximate composition of leaves of two sweet potato varieties with various type of fertilizer application

The selected varieties from the previous experiment were Pakchong and Japanese yellow. Pakchong with fertilizer application based on soil testing (FABS) was the greatest in carbohydrate and fiber while even without fertilizer the highest content of protein and fiber were obtained. The greatest fat and fiber content was derived from Japanese yellow without fertilizer (WF). Additionally, Japanese yellow with conventional application fertilizer (CAF) had given significantly greater moisture content (Table 3).

Table 3 Carbohydrate, protein, fat, fiber, ash, (g/100 g DW) and moisture (g/100 g FW) contents of two sweet potato varieties with various types of fertilizer application.

Nutritional contents	Japanese yellow			Pakchong		
	WF	FABS	CAF	WF	FABS	CAF
Carbohydrate	48.27^{c}	59.36^{a}	60.57^{a}	43.28^{d}	58.72^{a}	53.88^{b}
Protein	23.79^{b}	18.90^{c}	16.11^{d}	33.06^{a}	17.01^{cd}	25.44^{b}
Fat	4.4^{a}	4.1^{b}	4.0^{b}	3.9^{b}	4.0^{b}	4.0^{b}
Fiber	13.24^{a}	9.24^{b}	10.52^{a}	9.16^{b}	10.28^{a}	6.08^{c}
Ash	10.30^{ns}	8.40^{ns}	8.80^{ns}	10.60^{ns}	10.00^{ns}	10.6^{ns}
Moisture	82.84^{c}	84.67^{b}	86.10^{a}	80.50^{d}	84.43^{b}	82.65^{c}

Different letters in the same rows indicate the values are significantly different ($p < 0.05$).

^{ns} The values are nonsignificant different
Fertilizer application types as follow:
WF; Without fertilizer.
FABS; Fertilizer application based on soil testing.
CAF; Conventional fertilizer application.

3.3 Leaf area, leaf greenness, and chlorophyll content

There were significant differences between sweet potato varieties and fertilizer application type. The leaf area/plant of Pachong with fertilizer application based on soil testing (FABS) was greater (1,393.57 cm^2) than without fertilizer (WF) (665.90 cm^2). Both Pakchong and Japanese yellow with conventional application fertilizer (CAF), FASB, or WF were not difference in greenness and higher than did Japanese yellow. Chlorophyll content was the highest (44.40 mg/100 g FW) in Japanese yellow variety (Table 4). Chlorophylls found at different concentrations in many edible plants can be associated with certain protective effects in diets rich in green vegetables (Fahey et al., 2005). Furthermore, chlorophyll is present in those plants at much higher content than other phytochemicals, similar to reported by Zikalala (2014) on baby spinach. Additionally, chlorophyll content in sweet potato leaves that reported in previous studies was different in a range such as in Li et al. (2017) study, their report showed different chlorophyll contents in leaves of 14 Korean sweet potato cultivars that higher than in other leaves of Chinese sweet potato varieties.

Table 4 Leaf area per plant (cm^2), leaf greenness (spad unit), and chlorophyll content (mg/100 g FW) of two sweet potato varieties with various types of fertilizer application.

	Japanese yellow			Pakchong		
	WF	FABS	CAF	WF	FABS	CAF
Leaf area / plant	451.33^{d}	631.00b^{c}	516.70^{c}	665.29^{b}	1,393.57^{a}	692.68^{b}
Leaf greenness	33.13^{b}	36.62^{a}	36.70^{a}	38.26^{a}	38.51^{a}	39.89^{a}
Chlorophyll content	44.40^{a}	41.10^{b}	40.00^{b}	39.90^{b}	40.00^{b}	40.00^{b}

Different letters in the same rows indicate the values are significantly different ($p < 0.05$).
Fertilizer application types as follow:
WF : Without fertilizer.
FABS : Fertilizer application based on soil testing.
CAF: Conventional fertilizer application

3.4 Vitamin C content

The measurements of vitamin C content in sweet potato leaves showed the level of the largest amount in Japanese yellow leaves (Table 5) while, Okud and Carrot leaves were fewer. Many reports of vitamin C content in sweet potato leaves were different level such Dinu et al study, (2018) recorded the vitamin C content level of two sweet potato cultivars (5.96 and 3.56 mg/100 g FW) which lower than those of Ishiguro et al. report (2004). The studies revealed that vitamin C content in sweet potato leaves depends on genotypes.

Table 5 Vitamin C (µg/g FW) and total phenolic contents (mg GAE/100 g FW) of leaves of five sweet potato varieties.

Varieties	VitaminC	Total phenolic content
Kapi	33.67 ± 1.53^{b}	389.56 ± 6.53^{c}
Pakchong	30.54 ± 0.58^{c}	489.89 ± 2.50^{a}
Carrot	27.00 ± 0.00^{cd}	458.69 ± 12.35^{b}
Okud	26.00 ± 1.00^{d}	343.24 ± 4.08^{e}
Japanese yellow	36.00 ± 1.73^{a}	372.97 ± 9.82^{d}

Different letters in the same columns indicate the values are significantly different ($p < 0.05$).

3.4.1 Total phenolic content of sweet potato leaves

Sweet potato leaves are a source of polyphenolic compounds. The content of the total phenolic compound was significantly different ($p \leq 0.05$) among the leaves of five varieties. The Pakchong leaves contained the largest amount of total phenolic content with an amount significantly greater than in other varieties (489.89 mg/100 g) followed by Carrot, Kapi Okud, and Japanese yellow varieties with values of 458.69, 389.56, 372.97, and 343.24 mg/100 g respectively (table 5). Sweet potato leaves contain more total phenolic compounds than any other commercial vegetables, including sweet potato storage roots and potato tubers. These bioactive compounds have multiple actions, including antioxidation, antimutagenicity, and anticarcinogenesis. On the other hand, sweet potato leaves contain more polyphenols than any other commercial vegetables, such as spinach (Dinu, Soare, Babeanu, & Hoza, 2018). A study of three sweet potato cultivars, claimed that the phenols from the leaves of this species were approximately 16 times higher than those found in the entire root (Truong et al., 2007)

The total phenolics content of two sweet potato varieties, with various fertilizer application types, were significantly different from each other. Pakchong with fertilizer application based on soil testing (FABS) contained the highest phenolic content followed by the conventional application fertilizer (CAF) and without fertilizer (WF) type of fertilizer application (table 6). Whilst, Japanese sweet potato with CAF had the lowest total phenolics content.

Table 6 Total phenolic content (mg GAE/100 g FW) and anthocyanin content (mg/100 g FW) of two sweet potato varieties with various various types of fertilizer application.

	Japanese yellow			Pakchong		
	WF	FABS	CAF	WF	FABS	CAF
Total phenolic content	451.33^{d}	631.00b^{c}	516.70^{c}	665.29^{b}	1,393.57^{a}	692.68^{b}
Anthocyanin content	33.13^{b}	36.62^{a}	36.70^{a}	38.26^{a}	38.51^{a}	39.89^{a}

Different letters in the same rows indicate the values are significantly different ($p < 0.05$).

Fertilizer application types as follow:
WF : Without fertilizer.
FABS : Fertilizer application based on soil testing.
CAF: Conventional fertilizer application.

3.5 Anthocyanin content of two sweet potato varieties with various type of fertilizer application

The one essential phytochemical in the flavonoid group is anthocyanin. This provide the antioxidant property of plant. The purple-fleshed storage root of sweet potato was rich in anthocyanin content but the reports of anthocyanin in sweet potato leaves were little. In this study, we determined anthocyanin content in leaves of two sweet potato varieties (Pakchong and Japanese yellow) with three types of fertilizer application; conventional application fertilizer (CAF), fertilizer application based on soil testing (FABS), and without fertilizer (WF). The data in Table 6 showed that the difference of anthocyanin content was not significant among Pakchong and Japanese yellow varieties with CAF and FABS accepted in Japanese yellow without fertilizer (WF). The results were consistent with the difference of total anthocyanin content in leaves of three sweet potato varieties (Islam et al., 2002) and the increase of anthocyanin content in grape after foliar nitrogen application at 69 mg N/plant from veraison to preharvest (Cheng et al., 2020). These indicated that the anthocyanin content in sweet potato leaves may influent by genotypes and the right amount of nitrogen fertilizer

3.6 DPPH and FRAP of five sweet potato varieties

The antioxidant activity of sweet potato leaves was measured by DPPH and FRAP methods. The highest DPPH and FRAP were found in Pakchong leaves whereas the lowest was Japanese yellow (Table 7). In this study, a highly positive correlation between DPPH and total phenolic content was found, and also between FRAP and total phenolic content (Table 8) while, no correlation was observed between vitamin C and DPPH or FRAP. The result was consistent with previous research which indicates that DPPH radical scavenging is positively correlated with total phenolic content (Huang, Chen, Hou, Lin, & Lin, 2004). Similar to Liao, Lai, Yuan, Hsu & Chan (2011) who reported that water extract of sweet potato leaves has high total phenolic content and potent activity, in several antioxidative assays. Additionally, Ghasemzadeh, Omidvar, & Jaafar (2012) reported the highly correlation of antioxidant activity and total phenolics compounds in 6 varieties of sweet potato leaves while no-correlation was observed between total flavonoid content and antioxidant activity. This is further confirmed that the sweet potato leaves which contained high total phenolic compounds are good for healthy or functional food.

Table 7 DPPH (mg GAE/100 g FW) and FRAP ((μM of Fe (II)/100 g FW) of leaves of five sweet potato varieties.

Varieties	DPPH	FRAP
Kapi	136.27 ± 0.41^{b}	130.86 ± 0.14^{c}
Pakchong	145.91 ± 0.03^{a}	140.53 ± 0.03^{a}
Carrot	135.37 ± 0.18^{b}	132.66 ± 0.04^{b}
Okud	134.76 ± 0.03^{c}	129.03 ± 0.10^{d}
Japanese yellow	127.74 ± 0.12^{d}	123.73 ± 0.23^{e}

Different letters in the same columns indicate the values are significantly different ($p < 0.05$).

Table 8 Correlation coefficients (r) between total phenolic compounds and antioxidant activities (DPPH, FRAP) of leaves of five sweet potato varieties.

Specification	Total phenolic content	DPPH	FRAP
Total phenolic content	-	0.71	0.83
DPPH	0.71	-	0.98
FRAP	0.83	0.98	-

4. Conclusions

In conclusion, the healthy food potential was found in the Pakchong variety by higher shoots yield, nutritional composition attributes (carbohydrate, fat, fiber, and moisture). In addition, the Pakchong tips contain more nutritional compositions such as carbohydrates and fiber when cultivated by fertilizer application based on soil testing. In terms of phytochemical content in sweet potato leaf, Pakchong leaves provided the highest total phenolics content especially by fertilizer application based on soil testing. A highly correlation between total phenolic compound and antioxidant activity in Pakchong leaf extract was found. These results indicate that sweet potato leaves of Pakchong variety could be consumed as leafy vegetables in an attempt to be a suitable source of natural antioxidants.

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Genetic Diversity of Native Pumpkin Accessions in Nan Province with Genotyping-by-Sequencing Technology

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Abstract

Pumpkin (*Cucurbita moschata*) is an important local vegetable in Nan Province. However, its selling and intake still remain low. These local pumpkins still have diverse morphology and have not been investigated species identification accuracy. In this study, genotyping by sequencing (GBS) was applied to reveal polymorphisms in 33 pumpkin accessions collected from three sampling areas. The genetic relationship among pumpkin genotypes was assessed using single-nucleotide polymorphisms (SNPs) originated from genotyping-by-sequencing. A total of 473,195 SNPs was generated with mapping rate of each sample ranges from 80.22% to 87.69%. Phylogenetic analysis showed that the genotyping of each pumpkin accession from the three sampling areas in Nan Province are not duplicated, but they are related on some levels. Still, they are not in the same accessions. These results will help further exploitation and understanding of phylogenetic relationships of local pumpkin in Nan Province and will serve as a reference for pumpkin breeding improvements.

Keywords: *Local Pumpkin, Nan Province, Genetic Diversity, Genotyping-By-Sequencing*

1. Introduction

Communities in many areas of Nan province commonly grow corns for livestock feed, which are the main source of their income. Chemical fertilizers are used and create a huge effect on the wellness of community members. Consequently, many communities are finding an alternative crop to solve this problem. Pumpkin is one of the important native vegetables in Nan Province. Some communities initiated the transformation of monoculture to the integrated farming system. They grow pumpkins for distributing to Bangkok markets. However, these native pumpkins are difficult to identify morphologically because the origin and morphological characteristics of many local cultivars have been poorly documented. Moreover, the pumpkins are unsystematically produced in Nan Province and cultivar identification using genetic resources has not yet been done.

Genotyping-by-sequencing (GBS) has been successfully applied to large scale genotyping in plants, which is poor in morphological differentiation and low in genetic divergence among different species. This method is useful to phylogenetic studies, which not only generate many genome wide phylogenetic information locis in absence of a reference genome, but also facilitate the laboratory protocols, reducing cost and efficiency. GBS has been widely used in genetic diversity (Bhattacharjee et al., 2020) and phylogenetic analysis (Loureiro, Engstrom, & Lim, 2020), In this study, we analyzed the genetic diversity of 33 pumpkin accessions with GBS technology, aiming at collecting useful information for further conservation and breeding of pumpkin accessions for the business benefits of local communities.

2. Materials and Methods

Plant materials

A total of 33 pumpkin accessions collected from three sampling areas in Nan Province were used in this study. Our collections were restricted to local varieties with three different sea level locations including alluvial plains (Muang Chang Sub-district, Phu Piang District), plateau (Du Phong Sub-district, Santi Suk District) and highland (Bo Kluea Tai Sub-district, Bo Kluea District) as shown in Figure 1. Morphological observations were used to reveal the great variations existing in pumpkins. Most of the accessions that were assessed exhibited high variability with respect to fruit characters. Morphological characters of pumpkin appear dominantly on the fruit than on leaves, flowers, and seeds.

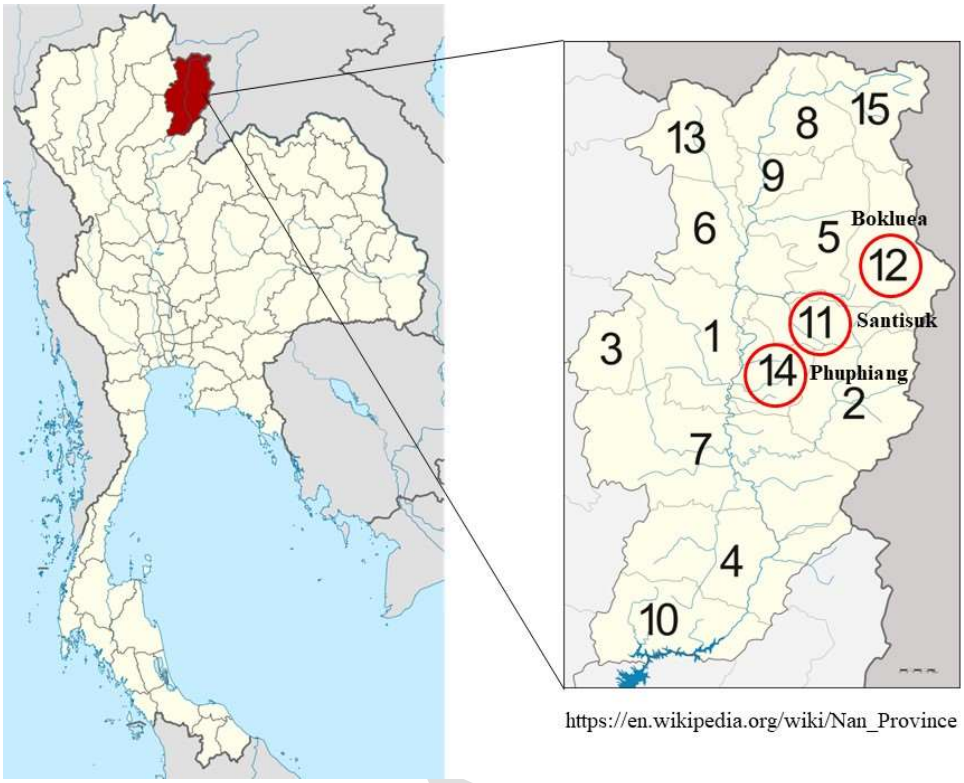

Figure 1 Collection sites of the exploration of pumpkin in Nan Province

DNA extraction and GBS library preparation.

The total genomic DNA was isolated from young leaf tissue using the DNeasy Plant Mini Kit (Qiagen, USA) following the manufacturer's instructions. The purity of DNA was determined by using a NanoDrop 8000 spectrophotometer and the ratio of the readings at 260 and 280 nm (A260/A280). Then, the needed volume of DNA stocks was diluted to obtain 50 ng/µl concentration of working DNA. The GBS libraries for each sample were generated from 50 µl of genomic DNA using the restriction enzyme Mse I and EcoR I following the GBS protocol as previously described (Elshire et al., 2011).

Sequencing and GBS data processing

GBS libraries were sequenced on the Illumina HiSeq 2000 platform at the Beijing Genomics Institute (BGI, Shenzen, China), with the read length of 144 bp at each end. The effective sequencing data was aligned with the reference sequence through BWA (Li, & Durbin, 2009) software and the mapping rate and coverage was counted according to the alignment results. The BAM files were handled by SAMTOOLS (Li et al., 2009). Single nucleotide polymorphism (SNP) refers to a variation in a single nucleotide which may occur at some specific position in the genome, including transition and transversion of a single nucleotide. We detected the individual SNP variations using SAMtools (Li et al., 2009).

ANNOVAR (Wang, Li, & Hakonarson, 2010) is a widely used software in variation annotation with multiple capabilities, including gene-based annotation, region-based annotation, filter-based annotation as well as other functionalities. We use ANNOVAR to do annotation of detected SNPs.

Phylogenetic Analysis

Phylogenetic trees using the unweighted pair group method with arithmetic mean (UPGMA) and neighbor-joining (NJ) algorithms with the MEGA X program (Kumar et al., 2018).

3. Results

Sequencing Evaluation

Totally 10.89G raw data were sequenced from this run, with 10.889G clean data generated after filtering low-quality data. The raw data production for each sample ranged from 173.044 M to 454.375 M, indicating the sufficient amount of data production. As the Q20 and Q30 reached 95.28% and 88.47%, respectively, the sequencing quality could meet the proper analysis requirements. The GC content of 39.49% to 41.53% are also in the normal distribution range, fulfilling the quality standard (Table1). The library construction and sequencing procedures are successful and highly reliable.

Sample	Raw Base (bp)	Clean Base (bp)	Effective Rate (%)	Error Rate (%)	Q20 (%)	Q30 (%)	GC Content (%)
PK_2	278,396,640	278,379,936	99.99	0.03	95.88	90.38	40.06
Y3	285,544,224	285,536,736	100	0.03	95.73	89.59	40.8
Y7	397,965,888	397,948,032	100	0.03	95.97	90.39	40.7
M15	385,176,096	385,149,024	99.99	0.03	95.92	90.3	40.7
M1	354,250,656	354,230,784	99.99	0.03	95.78	89.88	40.2
M8	413,923,968	413,890,272	99.99	0.03	95.95	90.23	40.38
PK_3	325,647,360	325,629,216	99.99	0.03	96	90.37	40.61
Y16	285,214,176	285,194,304	99.99	0.03	96.03	90.44	40.86
PK_5	292,446,144	292,437,216	100	0.03	95.65	89.4	40.78
Y10	329,727,744	329,708,160	99.99	0.03	95.9	90.42	40.48
PK_1	361,545,696	361,528,992	100	0.03	95.98	90.55	40.33
PKC_7	325,376,928	325,360,512	99.99	0.03	95.76	89.68	40.75
M2	386,760,960	386,744,544	100	0.03	95.91	90.2	40.37
Y4	255,122,784	255,113,280	100	0.03	95.74	89.56	40.94
Y13	304,031,808	304,012,224	99.99	0.03	95.44	88.85	41.14
Y17	454,375,296	454,340,736	99.99	0.03	95.73	89.49	40.8
Y1	370,690,848	370,669,248	99.99	0.03	95.92	90.04	39.84
M5	347,577,696	347,562,720	100	0.03	95.92	90.28	40.12
PK_4	317,340,288	317,327,040	100	0.03	96.03	90.5	40.73
PKC_1	343,077,984	343,061,280	100	0.03	95.28	88.47	40.53
M20	362,214,144	362,187,936	99.99	0.03	95.97	90.39	40.72
M9_S	327,284,640	327,267,936	99.99	0.03	95.94	90.45	40.52
Y2	173,043,648	173,036,160	100	0.03	95.78	89.64	40.32
PKC_3	327,509,280	327,492,288	99.99	0.03	95.91	90.15	41.53
M7	403,763,040	403,737,408	99.99	0.03	96.09	90.66	39.49
Y14	277,687,872	277,665,696	99.99	0.03	95.77	89.76	40.8
M9	296,688,672	296,679,168	100	0.03	95.47	88.96	40.43
Y5	276,708,096	276,683,040	99.99	0.03	95.89	90.09	40.79
Y12	242,276,832	242,266,176	100	0.03	95.95	90.42	40.66
M12	313,784,928	313,772,256	100	0.03	95.94	90.18	40.72
M3	405,833,760	405,809,280	99.99	0.03	95.89	90.06	40.56
Y6	287,847,360	287,812,512	99.99	0.03	95.91	90.44	40.2
Y8	380,816,928	380,778,624	99.99	0.03	95.9	90.25	40.57

Table 1 Statistics of Sequencing Data

M: Muang Chang Sub-district, Phu Piang District
PK: Du Phong Sub-district, Santi Suk District
Y: Bo Kluea Tai Sub-district, Bo Kluea District

The details for the sequencing data statistics are as follows:

(1) Sample: Sample name.
(2) Raw Base (bp): The output of raw data calculated by the number and length of sequence (in bp).
(3) Clean Base (bp): The valid data output of sequence (in bp) after filtering low quality reads, calculated by the number and length of sequences in clean data.
(4) Effective Rate (%): The ratio of clean data to raw data.
(5) Error Rate (%): Overall error rate of base.
(6) Q20 and Q30 (%): The percentage of bases with higher Phred score than 20 and 30 in total bases.
(7) GC Content (%): The percentage of G and C in total bases.

Sample	Mapped reads	Total reads	Tag number	Tag4 number	Mapping rate(%)	Average depth(X)	Coverage at least 1X(%)	Coverage at least 4X(%)
PK_2	1572569	1813234	344040	105707	86.73	6.04	12.68	5.19
Y3	1618695	1862176	334346	108549	86.92	6.51	12.01	5.23
Y7	2287562	2622890	365287	138140	87.22	8.64	12.75	6.48
M15	2211396	2541558	345095	133326	87.01	8.8	12.07	6.24
M1	2021414	2329042	316374	126490	86.79	8.69	11.17	5.86
M8	2253685	2691202	341239	138752	83.74	9.07	11.96	6.44
PK_3	1847768	2123008	343809	118098	87.04	7.25	12.33	5.67
Y16	1638960	1869034	318472	102264	87.69	6.86	11.57	4.92
PK_5	1652237	1910100	326345	105384	86.5	6.79	11.74	5.09
Y10	1875591	2169866	356273	124939	86.44	7.11	12.72	5.95
PK_1	2014341	2349708	371809	129925	85.73	7.34	13.26	6.21
PKC_7	1797851	2133476	299237	108005	84.27	8.14	10.61	5.09
M2	2193818	2538616	325134	137379	86.42	9.3	11.36	6.34
Y4	1460037	1681422	307494	96685	86.83	6.27	11.25	4.68
Y13	1726501	1986536	326065	106639	86.91	7.16	11.6	5.15
Y17	2600231	2994114	375670	143528	86.84	9.73	12.78	6.7
Y1	2106048	2422488	318032	130816	86.94	9.13	11.13	6.06
M5	1972935	2276770	310940	129019	86.65	8.67	10.96	5.98
PK_4	1802310	2095366	332385	117063	86.01	7.24	12	5.6
PKC_1	1921072	2248282	317709	116196	85.45	8.32	11.02	5.41
M20	2050096	2369208	328342	129699	86.53	8.51	11.59	6.07
M9_S	1867808	2154430	303179	119818	86.7	8.27	10.92	5.61
Y2	986419	1138816	258877	72049	86.62	4.83	9.87	3.53
PKC_3	1724435	2149656	303558	108513	80.22	7.68	10.77	5.08
M7	2304223	2636594	327195	138130	87.39	9.72	11.52	6.39
Y14	1568930	1804712	309655	101470	86.94	6.69	11.33	4.87
M9	1690308	1945416	298313	117799	86.89	7.64	10.66	5.53
Y5	1583021	1822812	323098	108870	86.84	6.55	11.67	5.21
Y12	1379239	1591932	317691	95479	86.64	5.67	11.79	4.65
M12	1805462	2074296	297741	112811	87.04	8.08	10.81	5.33
M3	2332928	2682696	340964	137156	86.96	9.42	11.92	6.37
Y6	1637930	1880218	329970	110736	87.11	6.57	12.09	5.33
Y8	2180123	2506208	360913	132407	86.99	8.32	12.66	6.27

Mapping Statistics with Reference Genome and Tag Summary

The mapping rates of samples reflect the similarity between each sample and the reference genome. The depth and coverage are indicators of the evenness and homology with the reference genome. With Re-sequencing, tag-related statistics are also calculated for the current 279,691,401 bp reference genome, the mapping rate of each sample ranges from 80.22% to 87.69%. The average depth on the reference genome (without Ns) is in 4.83X to 9.73X range, while the more than 1X coverage exceeds 9.87% (Table2). This result is in the qualified normal range and may serve in the subsequent variation detection and related analyses.

Table 2 Statistics of mapping rate, depth and coverage, as well as tag-related statistics

The details for mapping statistics are as follows:

(1) Sample: Sample names.

(2) Mapped reads: The number of clean reads mapped to the reference assembly, including both single-end reads and reads in pairs.

(3) Total reads: Total number of effective reads in clean data.

(4) Tag number: The number of unique tags (enzyme cutting fragment).
(5) Tag4 number: Number of tags with depth larger than 4.
(6) Mapping rate: The ratio of the reference genome assembly mapped reads to the total sequenced clean reads.
(7) Average depth: The average depth of mapped reads at each site, calculated by the total number of bases in the mapped reads dividing by size of the covered genome.
(8) Coverage at least 1X: The percentage of the assembled genome with more than one read at each site.
(9) Coverage at least 4X: The percentage of the assembled genome with ≥4X coverage at each site.

SNP Mutation Frequency

Take the T:A>C:G mutations as an example, this category includes mutations from T to C and A to G. When T>C mutation appears on either of the double-strand, the A>G mutation will be found in the same position of the other chain. Therefore, the T>C and A>G mutations are classified into one category. Accordingly, the whole-genome SNP mutations could be classified into six categories. The frequency of each type is shown in Figure 2.

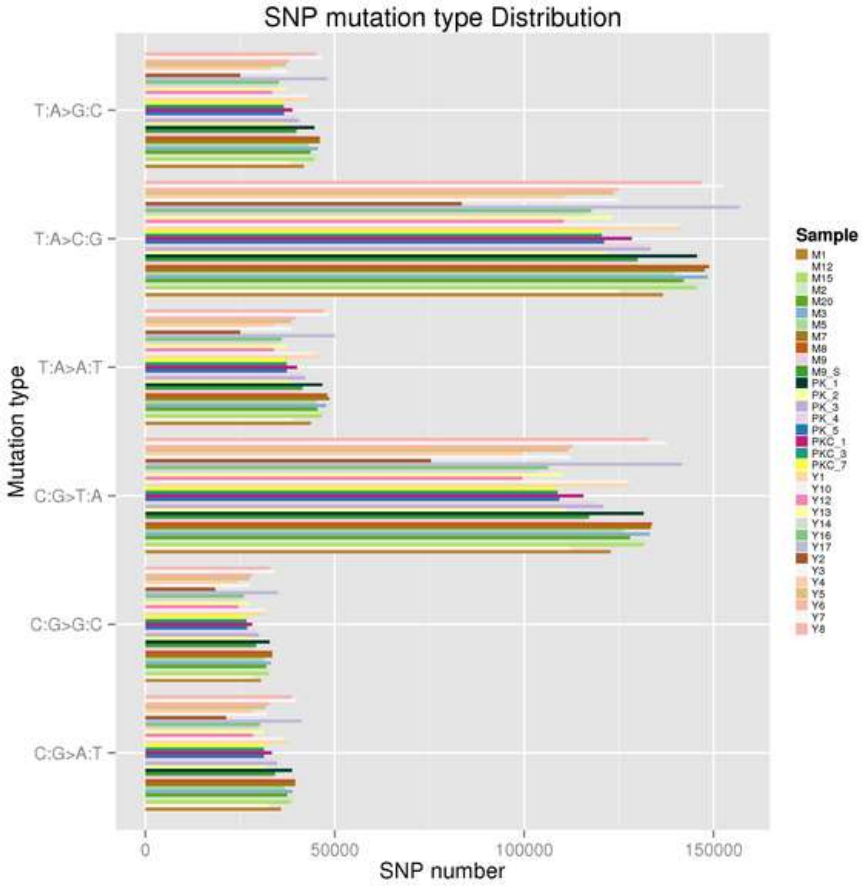

Figure 2 Frequency of SNP mutations. The x-axis represents the number of the SNPs, and y-axis indicates the mutation types

Phylogenetic Relationships

Phylogenetic analysis was performed to resolve genetic relationships in a complex of the 33 pumpkin accessions in three sampling areas in Nan. Phylogenetic tree based on the filtered SNPs revealed indistinct relationships among three areas. The results showed that each genotyping of pumpkin accession from the three sampling areas in Nan Province are not duplicated, but they are related on some levels. Still, they are not in the same accessions. Y2 pumpkin from Baan Yod Doi Wattana, Bo Kluea district is an origin of most native pumpkins in the three sampling areas (Figure3).

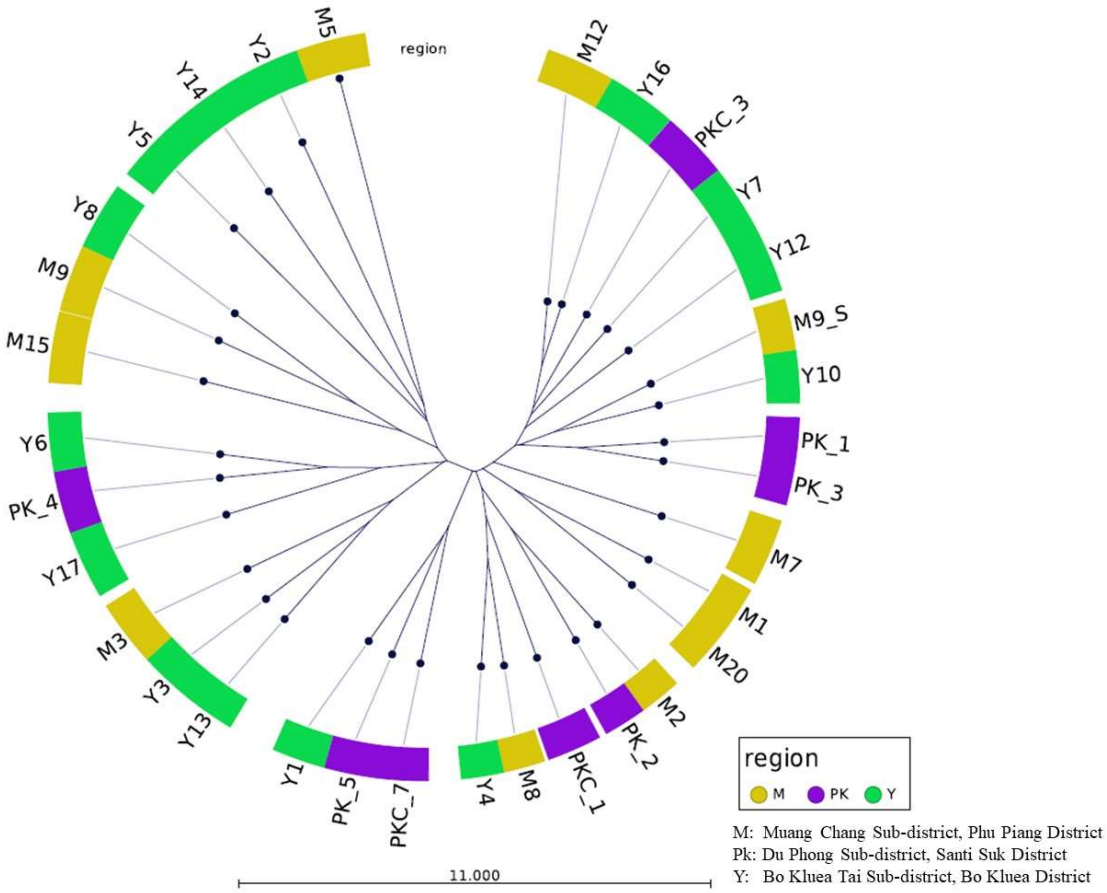

Figure 3 Phylogenetic analysis of 33 pumpkin accessions generated through the UPGMA method and neighbor-joining (NJ) algorithms with the MEGA X program

4. Discussion

Local pumpkin in Nan Province presents widely morphological and genetic variations in three sampling areas with different sea level locations. Climate and planting system can influence crop responses and morphological characters. Genetic identification and phylogenetic relationships of pumpkin landraces is a prerequisite for investigation of ambiguous cultivars. In this study, the phylogeny and evolution of the pumpkins were explained by using GBS data. The relationships among accessions are shown in the UPGMA tree, which indicates the relationship between the primary geographical origins of the accessions. This was ascribed to their adaptation to diverse growing conditions, and due to genetic adjustment to many agro-ecological conditions for many years.

5. Conclusions

In this study, we investigated the genetic variation of 33 local pumpkin accessions in Nan province in three sampling areas by using advanced sequencing techniques such as GBS coupled with statistical analysis for assessing genetic diversity and genetic relationship. Clean reads of 33 pumpkin accessions were mapped to the pumpkin reference genome and SNP call. Phylogenetic analysis of the filtered SNPs showed unclear relationships among accessions from three sampling areas. However, they were still closely related in some accessions. The results of this study will benefit the conservation of pumpkin species. Moreover, it will help classify the species to serve consumer needs.

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Yield and Quality Evaluation Trials of Purple Sweet Waxy Corn Hybrids

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Abstract

Purple sweet waxy corn is a very nutritious food. Developing a new hybrid with good eating quality and high yield could help boost farmers' income. In this research we crossed a sweet waxy corn containing the sugary gene with a purple waxy corn as the base population, then selected inbred lines. The fourth generation of selfed plants segregated into 2 groups: 30 lines of white sweet waxy corn and 5 lines of purple sweet waxy corn. These were crossed and 50 hybrid lines were selected and grown in field trials in 2 locations (Ayutthaya and Chachoengsao) with 8 commercial varieties. The experiment was done in 2 replications in RCB in each location. The hybrid L15/T3 gave the highest yield, ear size was appropriate and the eating quality was good, with high sugar content and medium anthocyanin content. The hybrids L15/T4, L16/T1, and L19/T4 also gave high yield but their sugar was only medium.

Keywords: *Waxy corn, Purple corn, Sugary gene, F_1 hybrid.*

1. Introduction

Nowadays most consumers are interested in health foods and the market value of health foods is continuously rising. Corn is a natural food that is a good source of several nutrients including ferulic acid and antioxidants in the carotenoid group, such as zeaxanthin, lutein, cryptoxanthin and beta carotene (Cherian, 2010). Waxy corn is a type of corn that is widely grown in Southeast Asia. It is commonly consumed boiled or steamed when the kernels are still slightly immature (Ketthaisonga, Suriharna, Tangwongchaic, & Lertrat, 2013). The immature kernels are soft, sticky and a little sweet because almost all the carbohydrate in the endosperm is in the form of amylopectin, which is a highly branched large polysaccharide molecule (Hanyu, 2012). The high amylopectin content in waxy corn is a trait conferred by a mutation of the waxy gene (*wx*) which is a recessive gene on Chromosome 9 (Coe & Neuffer, 1988). Waxy corn is an easily-accessible source of nutrition for rural people (Chander, Meng, Zhang, Yan, & Li., 2008). There is a good opportunity to expand exports of waxy corn to the European and American markets in the form of frozen whole ears or canned kernels, which is consistent with growth trends in the seed market in Asia (Ketthaisonga, Suriharna, Tangwongchaic, & Lertrat, 2013). Developing a new variety of purple sweet waxy corn will create added value for fresh corn products because the purple kernels are more nutritious since they contain anthocyanins. The anthocyanins most commonly found in corn are cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside (Yang & Zhai, 2010). When fresh, purple corn contains 118.92 ± 14.97 mg 100 g^{-1} anthocyanin, and after it is steamed for 10 minutes the anthocyanin content decreases somewhat to 96.82 mg 100 g^{-1} (Lago et al., 2014). Purple corn thus has more antioxidant activity than either white corn or yellow corn (Khampas, Lertrat, Lomthaisong, & Suriharn., 2013).

Inheritance of the purple kernel color trait in tropical corn is controlled by the joint action of 2 dominant genes, Booster1 (*B1*) and Purple Plant1 (*Pl1*), that cause cyanidin-3-glucoside to be synthesized in cells in the pericarp (Lago et al., 2014). The purple kernel phenotype is present in plants that are either dominant homozygous or heterozygous for those genes. A purple sweet waxy corn hybrid can thus be developed using an inbred white sweet waxy corn as one of the parent lines, and the hybrid seeds will be heterozygous for those genes. The objective of this research to evaluate the quality of hybrids from crossing white sweet waxy corn with a pure line purple sweet waxy corn is to select a suitable purple sweet waxy corn hybrid that has high anthocyanin content and is well adapted to the growing conditions in the central and eastern regions of Thailand. It can contribute to the increased production and use of high quality hybrid corn seed.

2. Materials and Methods

Plant materials

Corn varieties came from Rajamangala University of Technology Suvarnabhumi's waxy corn and tian corn breeding project and Khaohinsorn Royal Development Project Study Centre, consisting of:

1. Chat-ngern (CNG) – a sweet waxy corn with white kernels with sweetness that comes from the sugary gene (susu)
2. Fancy 111 (FC111) – a purple waxy corn
3. Second generation hybrids of (CNG (F2)) and FC111 × CNG (FC111/CNG (F2))

4. Eight check varieties for comparison, consisting of commercial hybrid seed from 5 seed companies: 1) Fancy 111 (Pacific Seeds), 2) Sweet White 25 (East West Seeds), 3) Ruby Waxy (East West Seeds), 4) Anchan (Jia Tai), 5) Niow Muang Wahn 2 (Jia Tai), 6) Sweet Berry (Seedline), 7) Sweet Purple (Seedline), and 8) Rachinee Tabtim Siam (Sweet Seeds).

Methods

Developing inbred lines by the standard method

The second-generation hybrids of CNG (F2) and FC111/CNG (F2) were grown out, selected and selfed ear to row, using spacing of 75 cm between rows and 25 cm between plants in order to give the plants enough space to clearly display their traits. The best candidates were selected from the best performing rows based on agronomic characteristics, trueness to breed, having silk emergence and pollen shed dates close together, and no evidence of diseases seen on the leaves. The selected plants were selfed. When the ears were dry, the best ears and kernels with the desired traits were selected and grown out, selfed, then selected from and grown out again for 4 generations to obtain 2 groups of S4 plants: CNG-S4 lines of white sweet waxy corn and FC111/CNG-S4 lines of purple sweet waxy corn.

Crossing to obtain hybrids

Thirty of the CNG-S4 white sweet waxy corn lines and 5 of the FC111/CNG-S4 purple sweet waxy corn lines were selected. These 2 groups were crossed to obtain 150 first generation 2019PSWCH hybrid lines. Fifty of those lines that produced enough seeds for the field trial were selected for the quality evaluation field trial the next season.

Preliminary productivity tests of hybrids

The 50 selected 2019PSWCH purple sweet waxy corn hybrids were grown in comparison with 8 check varieties: 1) Fancy 111, 2) Sweet White 25, 3) Ruby Waxy, 4) Anchan, 5) Niow Muang Wahn 2, 6) Sweet Berry, 7) Sweet Purple, and 8) Rachinee Tabtim Siam. Random complete block (RCB) design was used with 2 repetitions at 2 locations: the Rajamangala University of Technology Suvarnabhumi test field in Ayutthaya Province (GPS:13.72264,100.52931) and the Khaohinsorn Royal Development Project Study Centre in Chachoengsao Province (GPS:13.745821,101.508751). For each repetition, 1 test line or check variety was planted per row with 75 cm between rows and 20 cm between plants, one plant per hole. Before planting 15-15-15 fertilizer was applied at the rate of 312.5 kg h^{-1}, and 20 days after emergence, urea was added at the rate of 156.25 kg h^{-1}. The pre-emergence herbicide alachlor was applied to control weeds. Irrigation was by a sprinkler system.

Data collection for the field trials

1. For yield data, consisting of weight of husked ears and unhusked ears, ears were harvested from the plants in the rows, 20 days after pollination, and weight was calculated as tons per hectare.

2. For the agronomic characteristics of plant height and height of first ear, 10 plants were randomly sampled from the rows, 2 weeks after pollination, and time of tasseling and time of silk emergence were recorded as days from the planting date until 50% of the plants in the rows were flowering.

3. Ear size (length and width of unhusked ear and number of kernel rows) was measured from 10 randomly sampled fresh ears harvested from the rows.

4. Eating quality – the samples from number 3 above were further analyzed as follows.

4.1 Total soluble solids (%TSS) – liquid was squeezed from kernels at the center of the ear and measured by hand refractometer.

4.2 Reduced sugars were measured by the method of AOAC (2000).

4.3 Percent amylase and amylopectin were measured by the Iodine calorimetric method (Juliano, 1971).

4.4 Anthocyanin was measured by the pH differential method of AOAC (2005).

3. Results and Discussion

Yield

Yield, as measured by weight of husked ears and weight of unhusked ears, was influenced by environmental factors. Mean weight of husked ears and weight of unhusked ears of all the tested lines and check varieties at the Chachoengsao station were 8.56 and 5.88 t ha^{-1}, while at the Ayutthaya station the means were 8.06 and 5.5 t ha^{-1}, which was a statistically significant difference ($P<0.01$) (Table 1). This was most likely due to differences in the type and fertility of the soil and differences in temperature, rainfall and humidity at the 2 trial sites. Environmental factors influence corn growth and corn yield (Magari & Kang, 1993). The pedigree of the 2019PSWCH hybrid lines being evaluated came from Chat-ngern or CNG parentage, which was a variety developed by the Khaohinsorn Royal Development Study Center in Chachoengsao, so the hybrids were better adapted to climatic conditions in Chachoengsao. The mean weight of husked ears and weight of unhusked ears of all the tested hybrid lines were less than the check varieties at both trial sites. The mean weight of husked ears and weight of unhusked ears of all the tested hybrid lines across both trial locations was 7.69 and 5.19 t ha^{-1}, while the means of all 8 check varieties were 12.06 and 8.5 t ha^{-1}, respectively. In this research the interaction between genetics and environment was found to be not statistically significant ($P>0.05$) Analyzing the interaction between genetic and environmental factors can help researchers understand the unknown components of factors that influence the phenotype of different genotypes in different environments (Williams II, 2017). In the case of this research, unexplained factors had a low level of influence, so plants with the same genotype reacted very similarly to environmental factors. Our ranking of the performance of the hybrid lines came out about the same at both trial sites, so the mean across both trial sites for each criterion can be used to evaluate the traits of the different hybrids. As for yield, there were 8 hybrid lines that achieved

mean weight of husked ears higher than 1.5 tons per rai (the unit of land measurement used in Thailand) or 9.38 t ha^{-1}, and mean weight of unhusked ears of higher than 1 ton per rai or 6.25 t ha^{-1}: L6/T3, L15/T2, L15/T3, L15/T4, L16/T1, L16/T3, L19/T3,

and L19/T4 with mean weight of husked ears of 9.44, 10.63, 12.56, 10.13, 9.94, 11.00, 9.88 and 9.81 t ha^{-1}, and mean weight of unhusked ears of 6.31, 6.88, 8.31, 7.06, 6.81, 8.00, 6.44, and 6.94 t ha^{-1}, respectively. By comparison, the check variety FC111 yielded the highest weight of husked ears and unhusked ears of 17.13 and 12.13 t ha^{-1}, followed by ANC at 12.88 and 8.19 t ha^{-1}, respectively (Table 1).

No.	line	Weight of husked ear (t ha^{-1})			Weight of unhusked ear (t ha^{-1})		
		Cha.[1/]	Ay.	mean	Cha.	Ay.	Mean
Test hybrids							
27	L15/T3	11.81	13.38	12.56	7.88	8.75	8.31
31	L16/T3	9.75	12.25	11.00	7.44	8.56	8.00
26	L15/T2	9.81	11.38	10.63	6.38	7.31	6.88
28	L15/T4	10.81	9.50	10.13	7.56	6.56	7.06
30	L16/T1	10.69	9.19	9.94	6.94	6.69	6.81
33	L19/T3	11.00	8.75	9.88	7.44	5.44	6.44
34	L19/T4	9.94	9.69	9.81	6.94	7.13	7.00
10	L6/T3	8.44	10.50	9.44	5.69	6.94	6.31
29	L15/T5	9.81	9.06	9.44	6.88	5.38	6.13
19	L12/T2	9.50	9.06	9.31	6.56	8.31	7.44
Check varieties							
54	FC111	17.56	16.75	17.13	12.38	11.88	12.13
55	SWW	10.38	11.81	11.06	7.75	8.75	8.25
56	NTT	8.50	12.31	10.38	6.50	8.13	7.31
57	ANC	13.94	11.81	12.88	8.88	7.50	8.19
58	RTS	11.69	12.63	12.19	7.94	10.31	9.13
59	NMW	13.06	11.88	12.44	9.19	8.81	9.00
60	SPP	8.06	9.31	8.69	6.00	7.25	6.63
61	SWB	11.81	11.06	11.44	7.44	7.63	7.56
Minimum		2.75	3.31	3.25	1.25	1.31	1.31
Maximum		17.56	16.75	17.13	12.38	11.88	12.13
Mean of all		8.56	8.06	8.31	5.88	5.50	5.69
Mean of hybrids		8.06	7.31	7.69	5.50	4.88	5.19
Mean of checks		11.88	12.19	12.06	8.25	8.75	8.50
	Location.	-	-	**	-	-	**
F test	Genotype	**	**	**	**	**	**
	Loc x Gen	-	-	ns	-	-	ns
$LSD_{.05}$ (Gen)		0.66	0.64	0.92	0.49	0.54	0.73
CV (%)		24.00	24.70	23.63	26.14	30.88	27.49

[1/] Cha. = Chachoengsao Ay. = Ayutthaya

Table 1 Yield of the top 10 best performing 2019PSWCH purple sweet waxy corn hybrids at 2 trial sites in December 2019 – March 2020.

Agronomic Traits

Environmental factors affected the growth of corn plants, so there were statistically significant differences in plant height, height of first ear, and tasseling date between the 2 trial locations ($P<0.05$). However, the interaction between genetic and environmental factors was not statistically significant ($P>0.05$). Overall, the plants at the Ayutthaya site were taller and tasseled sooner than those at the Chachoengsao site (data not shown). The test hybrid lines had mean plant height of 147 cm. and height of first ear of 64 cm., which is less than the mean of the 8 check varieties at 162 and 72 cm., respectively. Most of the test hybrid lines that were identified as high-yield did have plant height and ear height that was higher than the overall mean for all groups (Table 2). As for tasseling date and silk emergence date, the means for the test hybrids were 41 and 43 days from planting, while the means for the group of check varieties were a little later at 43 and 45 days after planting. In most cases the date of 50% tasseling and the date of 50% silk emergence were 1-2 days apart, so there would be no problem with pollination and kernel formation.

No.	Line	Height (cm.)		Flowering date		Ear size (cm.)		No. of kernel rows
		plant	ear	tassel	silk	width	length	
Hybrids								
27	L15/T3	159	75	43	45	4.2	17.3	13.1
31	L16/T3	153	72	40	42	4.3	15.2	13.6
26	L15/T2	144	55	40	42	4.1	16.0	12.9
28	L15/T4	164	68	41	42	4.1	15.6	11.9
30	L16/T1	169	82	41	43	4.2	14.8	13
33	L19/T3	154	83	40	42	4.0	15.5	13.7
34	L19/T4	153	69	41	43	4.0	15.4	12.6
10	L6/T3	154	55	41	42	3.9	14.1	12.5
29	L15/T5	149	62	42	44	4.1	15.3	13.6
19	L12/T2	158	63	42	43	4.2	14.7	13
Check varieties								
54	FC111	159	67	42	44	4.5	20.2	16.8
55	SWW	155	63	42	44	4.1	16.2	11.9
56	NTT	155	66	43	45	4.2	17.3	13.2
57	ANC	155	73	44	46	4.2	17.2	13.2
58	RTS	164	77	43	44	4.3	17.5	13.9
59	NMW	163	80	43	45	4.4	18.2	14.2
60	SPP	163	71	42	44	4.2	15.8	12.8
61	SWB	180	77	44	46	4.3	16.2	13.7
Minimum		122	48	40	41	3.6	9.1	10.0
Maximum		180	89	44	46	4.5	20.2	16.8
Mean of all		150	65	41	43	4.0	14.3	12.7
Mean of hybrids		147	64	41	43	3.9	13.8	12.5
Mean of checks		162	72	43	45	4.3	17.3	13.7
F test	Loc.	**	*	*	ns	ns	**	ns
	Gen.	**	**	**	**	**	**	**
	Loc x Gen	ns	ns	ns	*	ns	ns	**
$LSD_{0.05}$		54.7	36	3.8	2.8	0.65	5.29	1.32
CV (%)		12.48	18.83	3.11	3.30	5.65	12.6	5.25

Table 2 Agronomic traits and ear size of the top 10 best performing 2019PSWCH purple sweet waxy corn hybrids at 2 trial sites in December 2019 – March 2020.

Ear size

Environment had a highly statistically significant effect on ear length ($P<0.01$). The corn plants grown at the site in Chachoengsao had longer ears than those grown at the site in Ayutthaya (data not shown). However, environment did not have a statistically significant effect on either ear width or number of kernel rows ($P>0.05$) (Table 2). There was no statistically significant interaction between genetics and environment ($P>0.05$). Thus, for the criteria of ear width and ear length we could consider the mean value from both trial sites. The check varieties all had larger ears than the test hybrids. FC111 had the largest ear size with width of 4.5 cm, length of 20.2 cm and number of kernel rows of 16.8 rows, followed by NMW with width of 4.4 cm, length of 18.2 cm and 14.2 kernel rows.

The 10 highest yielding hybrid lines (L6/T3, L12/T2, L15/T2, L15/T3, L15/T4, L15/T5, L16/T1, L16/T3, L19/T3, and L19/T4) that gave the highest yields in both trial locations had acceptable ear size with ear width of 3.9-4.3 cm and ear length of 14.1-17.3 cm. L15/T3 had the largest ears at 4.1 cm wide by 17.3 cm long, and L15/T3 also had the highest yield by weight.

Chemical constituent quality

Most of the 2019PSWCH hybrids had total soluble solids (TSS) of higher than 10° brix (Table 3), while almost all the check varieties had TSS of less than 10° brix. The hybrids got their sweetness from the sugary gene, so they contained a large amount of phytoglycogen. Phytoglycogen is a water soluble carbohydrate that makes corn taste soft and creamy (Szymaneka, Tanasa, & Kassar., 2015). The check varieties RTS and SWW get their sweetness from the shunken2 gene, so they had the highest total sugar contents at 316.29 and 296.29 mg/g, respectively. Sugar content in the kernels is directly related to the perceived sweetness of sweet corn (Azanza, Juvik, & Klein., 1994). Sugar content in the test hybrids differed to a statistically significant degree ($P<0.01$). The hybrid lines L15/T3, L15/T2 and L12/T2 had total sugar content that was significantly higher than the other hybrids at 281.39, 286.43 and 278.32 mg/g, respectively. They also had high TSS and were deemed to have good eating quality. The most important characteristics that determine eating quality are softness and sweetness (Szymaneka, Tanasa, & Kassar, 2015). When harvested 20 days after silk emergence, the majority of hybrid lines had purple pericarps due to anthocyanin content, and the amount of anthocyanin content differed to a statistically significant degree among different hybrids lines ($P<0.01$). The line L15/T3 was a high yielding hybrid (weight of husked ears greater than 9.38 t ha^{-1} and weight of unhusked ears greater than 6.25 t ha^{-1}) with appropriate ear size, and it also had high total sugar content at 281.39 mg/g and medium anthocyanin content at 230.32 mg Cyanidin/100g. The hybrid lines L6/T3, L15/T4, L16/T1, L16/T3, L19/T3, and L19/T4 were also high yielding and had medium total sugar content levels and anthocyanin content levels. The hybrid lines L15/T2 and L12/T2 had high sugar content but

their pericarps were not purple colored. This might be because some of the T2 plants were not dominant homozygous for the genes involved in anthocyanin synthesis.

No.	Line	%TSS (°brix)	TAC (mg Cyanidin/100g)	Total sugar content (mg/g)	% Amylopectin (Fresh Kernel)
Hybrids					
27	L15/T3	16.20±0.00^{a}	230.32±0.38^{e}	281.39±0.74 ^{a}	73.70±0.99^{b}
31	L16/T3	9.06±0.11^{d}	222.17±0.23^{e}	220.14±1.03^{e}	74.10±0.97^{b}
26	L15/T2	15.93±0.00^{b}	-	286.43±0.44 ^{a}	73.00±0.81^{b}
28	L15/T4	15.86±0.32^{b}	245.45±0.96^{e}	162.90±0.82^{i}	86.50±1.05^{a}
30	L16/T1	11.00±0.03^{c}	281.23±0.47^{d}	225.98±0.92^{e}	76.69±1.35^{a}
33	L19/T3	13.20±0.00^{c}	295.35±0.67^{c}	165.98±0.99^{i}	86.00±0.96^{a}
34	L19/T4	13.20±0.65^{c}	197.32±0.49^{f}	168.97±0.53^{i}	85.45±0.34^{a}
10	L6/T3	13.00±0.11^{c}	254.67±0.32^{e}	190.48±0.75^{g}	78.90±0.11^{a}
29	L15/T5	12.46±0.00^{c}	283.12±0.67^{d}	209.10±0.73^{f}	75.40±0.94^{a}
19	L12/T2	14.86±0.04^{b}	-	278.32±0.91a^{a}	73.10±0.35^{b}
Check varieties					
	FC111	6.26±0.43^{e}	187.35±0.95 ^{f}	203.23±0.76^{e}	86.75±0.44^{a}
	SWW	7.06±0.94^{e}	-	296.45±0.45a^{a}	81.51±0.35^{a}
	NTT	3.93±0.94^{e}	301.32±0.23 ^{b}	156.37±0.79^{i}	79.87±0.42^{a}
	ANC	8.93±0.08^{d}	253.34±0.65 ^{d}	185.34±0.35^{h}	76.18±0.34^{ab}
	RTS	6.93±0.10^{e}	300.32±0.76 ^{b}	316.29±0.81a^{a}	72.23±0.58^{b}
	NMW	10.13±0.43^{d}	289.54±0.32 ^{c}	181.54±0.49^{h}	82.30±0.31^{a}
	SPP	4.73±0.21^{e}	-	168.43±0.73^{i}	76.54±0.45^{ab}
	SWB	4.26±0.03^{e}	295.87±0.45 ^{b}	149.18±0.95^{i}	75.04±0.46^{ab}
Different superscripts indicate statistically significant differences					

Table 3 Chemical qualities of the top 10 best performing 2019PSWCH purple sweet waxy corn hybrids at 2 trial sites in December 2019 – March 2020.

4. Conclusions

Pure lines of a group of white sweet waxy corn and a group of purple sweet waxy corn developed by 4 generations of selfing were crossed in this experiment to generate 50 hybrids for testing in field trials in Ayutthaya and Chachoengsao. The hybrid line L15/T3 gave the highest yield at both locations. It has an appropriate ear size, high sugar content, purple pericarp and a medium level of anthocyanin. The hybrid lines L6/T3, L15/T4, L16/T1, L16/T3, L19/T3, and L19/T4 were also high yielding but had slightly lower sugar content and anthocyanin levels that were comparable to the check varieties. The parent plants that were the progenitors of the above hybrids have been selected and are being selfed to the sixth generation to increase homozygosity. They will be used in the continuing purple sweet waxy corn breeding program.

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Agriculture– Animal Science

AG-AS 01

Effect of Perilla Seed Meal for Soybean Meal Replacement in Diets on Carcass Characteristic of Crossbred Pigs

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Abstract

Perilla seed meal (PSM) is high protein content. It could be used as an alternative protein source for soybean meal (SM) replacement. This study was to evaluate the effect of PSM replacement SM on carcass characteristics of a crossbred pig. Twenty-one crossbred pigs ((Meishan × Duroc) × (Thai native × Pietran)); 70 kg, approximately) were randomly divided into three treatments (7 pigs per treatment) receiving diets containing 0%, 25%, and 50% PSM replaced SM (T1: 0% PSM, T2: 25% PSM, and T3: 50% PSM, respectively) for 12 weeks. At the end of the experiment, all pigs were slaughtered for carcass characteristics measurement. The PSM replacement for SM in all treatments had no different impacts on slaughter weight, percentage of carcass, carcass length, hot carcass weight, and chill carcass weight (P>0.05). But *longisimus dorsi* from T2 and T3 were significantly higher than T1 (P<0.05). These results indicate that PSM replaced SM in crossbred pigs' diets can increase the pork loin.

Keywords: *Soybean meal, Perilla seed meal, crossbred pig, carcass characteristic*

1. Introduction

Soybean meal (SM) is one of the most commonly used protein sources in the pig diet. It is a by-product of oil extraction from whole soybeans. SM content 39.6%–47.4% crude protein (CP) and 0.74%–2.46% ether extract (EE) (Son, Park, & Kim, 2017). Nowadays, the demand of SM for animal nutrition is increase. Then, the price of SM is rising. The pig industry leads to an increase in production costs. Therefore, there is a search for alternative protein sources to replace SM. Perilla seed meal (*Perilla frutescens* Linn.) or PSM is obtained as a by-product after oil extraction from perilla seeds that interesting to use to replace SM as a protein source in pig diet. PSM contained 43.2% of CP, 1.08% EE, and 4,240 kcal/kg gross energy (GE) (Son et al., 2017), which could be used in animal nutrition. Previous studies have reported that perilla and by-products could supplement diet formulations without adverse effects on animals' health and production. Hadi and Sudiyono (2019) also reported ducks that received 5% PSM in the diet increase average daily gain and meat fat content. Oh et al. (2020) also reported that the broiler received 2% PSM in a diet that improves growth performance, meat quality, and fatty acids composition of thigh meat. However, there was a limited study on the use of PSM in pig diets on pigs' carcass characteristics. This study objective was to determine the effects of the replacement of SM with PSM in the diet on carcass characteristics of crossbred pigs.

2. Materials and methods

Animal care and use and location

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Agricultural Animals in Research and Teaching. All animal protocols were approved by the Animal Care and Use Committee Chiang Mai University (2562/AG-0004) prior to the experiment. This experiment was conducted at the Mea Hia Agriculture Resource Demonstrative and Training Center, Faculty of Agriculture, Chiang Mai University, Chaing Mai, Thailand; the sample was analyzed in the Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University.

Experimental design

Twenty-one barrow crossbred pigs ((Meishan × Duroc) × (Thai native × Pietran)) with initial body weight (BW) 73.9 ± 3.3 kg, approximately were randomly divided into three dietary treatments. The experimental diets were formulated using PSM replace SM (T1=0% PSM, T2= 25% PSM, and T3= 50% PSM), following the National Research Council (NRC) nutrient requirement for pigs. The ingredients and composition of the experimental diets are presented in Table 1. All pigs were in the same rearing conditions, in open-sided pens with feeders and nipple drinkers, concrete floor. The experiment period was 12 weeks. Before the experiment starts, all pigs were dewormed and received the vaccination. The freshwater and feed were available *ad libitum* to pigs throughout the experiment.

Items	PSM replace SM		
	T1	T2	T3
Ingredient (%)			
Maize	31.00	28.50	27.00
Broken rice	31.00	27.22	27.00
Rice bran	9.92	15.00	15.00
Perilla meal	0.00	5.17	10.34
Soybean meal (44%)	20.68	15.51	10.34
Fish meal (58%)	4.80	6.00	7.72
Dicalcium phosphate	2.00	2.00	2.00
Salt	0.25	0.25	0.25
Premix	0.35	0.35	0.35
Total	100	100	100
Chemical composition			
Dry matter, %	89.99	89.59	89.76
Crude protein, %	20.40	20.33	20.10
Ether Extract, %	3.97	4.31	4.58
Ash, %	4.37	6.51	6.78
Crude fiber, %	22.94	25.49	28.43
Gross energy, cal/g	4,056	4,400	4,668
Digestible energy, cal/g	4,013	3,972	3,950
Metabolizable energy, cal/g	3,999	3,958	3,936
Net energy, cal/g	3,037	3,001	2,983

Table 1 T1= 0% PSM; T= 25% PSM; T3= 50%PSM (PSM = Perilla seed meal; SM = Soybean meal)
Data for the chemical composition represent the mean of triplicates.

Sampling and measurements

At the end of the experiment period, all pigs were fasting for 18 h. Then the pigs were slaughtered after obtaining slaughter weight. The slaughtering procedure included stunning with an electric stunning, bleeding, scalding, de-hairing, and evisceration. Thereupon evisceration, organs of the digestive system were collected and emptied. The carcasses were splitting carcass. Half-carcass was weighed to express the hot carcass weight. The carcasses were refrigerated at 4°C for 24 h. The carcasses weight was re-weighed to determine the weight of the cooled carcass (carcass weight minus the weight of the head, foot, and tail). The carcass length was measured from the first rib to the end of the pubic bone. Back fat thickness was measured using a vernier caliper according to Jiang et al. (2012), and the average measurements at three points: the first rib, last rib, and last lumbar vertebra were recorded. The carcasses were dressed according to USDA style (Jaturasitha, 2007) into four carcass cuts (shoulder, hide leg, loin, and belly). The bone, pork, fat, and skin were physically dissected to calculate the pork ratio.

Statistical analysis

The statistical analyses were performed using SPSS for Windows version 23.0 (SPSS Inc., Chicago, IL, USA); $P \leq 0.05$ was considered significant. Analysis of variance (ANOVA) was used to evaluate the effects of different PSM levels in the diet on carcass characteristics of crossbred pigs. Differences between groups were tested using Duncan's test.

3. Results and Discussion

The carcass characteristics of pigs in all experimental diets are shown in Table 2. The replacement SM with PSM in pig diets showed no difference in statistics (P>0.05) on the slaughter weight, carcass percentage, chilled carcass weight, slaughtering, hot carcass weight, chill carcass weight, carcass length, backfat thickness, and total edible non-carcass percentage. According to McDonnell et al. (2010), replacing soya bean meal with rapeseed meal in pig diets did not affect carcass characteristics. On the other hand, Prandini et al. (2011) presented that replacing SM with faba bean in heavy pig diets was affected by increased live weight (135.7 kg) and carcass weight (143.3kg).

Items	T1	T2	T3	SEM	P-value
Slaughter weight (kg)	108.36	104.54	106.08	1.103	0.860
Carcass percentage (%)	75.78	76.88	76.13	0.469	0.634
Hot Carcass weight, kg	81.43	80.36	80.75	0.945	0.902
Chilled carcass weight, kg	80.21	78.78	78.92	0.940	0.803
Carcass length, cm	93.57	93.00	92.00	0.684	0.673
Back fat thickness, cm	3.51	3.72	3.54	0.129	0.787
Total edible non-carcass (%)	23.92	24.66	23.78	0.451	0.709
Primal cutting (%)					
Shoulder	34.42	33.38	32.38	0.536	0.337
Hide leg	28.69^{b}	29.84^{ab}	30.38^{a}	0.263	0.022
Belly	16.11	15.03	14.09	0.446	0.203
Loin	16.15^{b}	17.96^{a}	17.35^{a}	0.310	0.019
Parts carcass (%)					
Pork	48.29	49.67	48.99	1.132	0.893
Fat and skin	32.98	31.09	29.19	1.207	0.484
Bone	19.24	15.88	15.93	0.856	0.200
Slaughter weight (%)					
Pork	35.97	37.46	36.45	0.863	0.784
Fat and skin	24.72	23.61	21.74	1.047	0.545
Bone	14.48	11.97	11.86	0.707	0.253

Table 2. Effect of replacement SM with PSM in the diet on carcasses characteristic of crossbred pigs

Interestingly, among the parameters, we found that in the hide legs percentage in T3 (30.38%) was significantly higher (P<0.05) than T1 (28.69%). We assumed that might affect the percentage of shoulder, belly, percentage of fat and skin and bone of parts carcass and slaughter weight were tended decrease (P>0.05) when the percentage of PSM increased in diets. Moreover, the *longissimus dorsi* (LD) percentage in T2 and T3 significantly higher (P<0.05) than in T1 (17.96% and 17.35% vs. 16.15%, respectively). This result was higher than the reported of Okrouhlá et al. (2012), Who reported that replacing soybean meal with rapeseed meal in pig diet had an LD percentage was a range from 11.99 to 12.21%. Also, Glinoubol et al. (2015) reported the LD of Pietrain × Native pigs was 16.4% and Native × Pietrain pigs were 16.6%.

4. Conclusion

In conclusion, the present study results showed that the replacement SM with PSM in the pig diets was no effect on the slaughter weight, carcass percentage, chilled carcass weight, slaughtering, hot carcass weight, chill carcass weight, carcass length, backfat thickness and total edible non-carcass percentage (P>0.05), but it was affected to the hide legs and LD (P<0.05). In the further study, it has to study on a large scale to confirm the influence of PSM in pig diet effect on pig carcass also the productive performance, health, and meat quality of the pig.

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AG-AS 02

Production of Nutrient Enrichment Cassava Pulp by Ruminal Microbes Fermentation

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Abstract

Cassava pulp can be used as a raw material for cattle feedstuff but it is restricted by the limited of the protein content. The use of ruminal microbes as inoculum might be one strategy to improve cassava pulp's nutrient because this consortium can ferment several carbohydrates to produce short-chain fatty acid (SCFA) and synthesize single-cell protein. Nitrogen plays an important role in ruminal microbes growth and microbial protein production. The objective of this study was to evaluate the influence of the ruminal microbes and ammonium chloride (NH_4Cl) levels on the lactic acid, SCFA profiles and chemical composition of inoculated cassava pulp. The cassava pulp was mixed with 10:1 ratio of 0.85% sodium chloride solution (Control; CON) and rumen fluid (RF) with various levels (0, 0.25, 0.5% DM) of ammonium chloride (NH_4Cl) and made airtight for fermentation period of 0, 7, and 14 days at ambient temperature. The cassava pulp in RF treatment had higher the lactic acid and SCFA concentration compared with CON treatment. There was a significant increase ($P < 0.001$) in the true protein content of cassava pulp in RF treatment after 7 and 14 days of fermentation while, the true protein content of cassava pulp in CON treatment was stable throughout the fermentation process. However, the effect of NH_4Cl on SCFA and true protein content were not observed in this experiment. The ruminal microbes can be used as the inoculum to enhance the lactic acid, SCFA concentration and improve true protein content of cassava pulp.

Keywords: cassava pulp, cattle, ruminal microbes

1. Introduction

Thailand is the world's largest cassava starch exporter (Buddhakulsomsiri, Parthanadee, & Pannakkong, 2018). Cassava pulp is the main by-product of cassava starch manufacturing and more than 5.15 million tons were produced annually (Ghimire, Sen, & Annachhatre, 2015). These pulps are easily perishable and causing environmental pollution (Napasirth, Napasirth, Sulinthone, Phommachanh, & Cai, 2015) then, the cassava pulp is classified as waste by the department of industrial works. The main current practice of factories is to sell to the agricultural sector as a raw material for cattle feedstuff due to containing high amounts of fiber and starch (Kamphayae, Kumagai, Butcha, Ritruechai, & Udchachon, 2017) but it is restricted by the limited of the protein content. Fermentation is one technique to enhance the short-chain fatty acid (SCFA) and improve the protein content of the material (Adejuwon, Osundahunsi, Akinola, Oluwamukomi, & Mwanza, 2021; Filannino *et al.*, 2020). Cassava pulp has great potential to use as a carbon source for microbes fermentation without any pretreatment (Pason *et al.*, 2020). The use of ruminal microbial as inoculum might be one strategy to improve the nutrient of cassava pulp because this consortium can ferment several carbohydrates to produce short-chain fatty acid (SCFA) and synthesize single-cell protein (Hackmann & Firkins, 2015). Nitrogen also plays an important role in ruminal microbes growth and microbial protein production (Lu, Xu, Shen, Tian, & Shen, 2019). Ammonium chloride (NH_4Cl) is soluble non-protein nitrogen source, has been widely used in livestock production (Wang *et al.*, 2018). The aim of this study was to evaluate the influence of the ruminal microbes and NH_4Cl levels supplementation on the lactic acid, SCFA profiles and chemical composition of fermented cassava pulp.

2. Materials and Methods

Animals and preparation of rumen inoculums: The trial was carried out using one fistulated White Lamphun cattle with 345 kg body weight for rumen fluid donor. The animal was placed on individual pens with free choice of clean freshwater and mineral blocks. The cattle was fed corn silage (*ad libitum*) given twice a day, at 08:00 and 16:00. The cassava pulp was offered in diet (0.5 kg DM per day) for 4 weeks before the rumen fluid collection. The rumen fluid was collected for used as the inoculum, then filtered through four layers of cheesecloth into pre-warmed thermo flasks (anaerobes condition).

Fermentation procedure: Cassava pulp was treated according to 2×3 factorial arrangement in a completely randomized design. Factor A was the fermentation including, 1). fermentation without any inoculum added and 2). fermentation with ruminal microbes inoculum, and factor B were NH_4Cl levels supplementation. For each treatment, three replicates were used. The cassava pulp was mixed with 10:1 ratio of 0.85% Sodium chloride (NaCl) solution (Control; CON) and rumen fluid (RF) with

various levels (0, 0.25, 0.5% DM) of ammonium chloride (NH_4Cl) supplementation then, made airtight for fermentation period of 0, 7, and 14 days at ambient temperature.

Sampling and analyses: Fifty grams of each sample was homogenized with 100 ml. of distilled water and stored overnight at 4°C then, the homogenate was filtered through 0.22 µm syringe filter nylon membrane, and the filtrate was used for pH, lactic acid and SCFA determination. The pH levels was directly measured by using an Oakton WD-35419-03 series pH meter 700 Benchtop (Oakton Inst, Vernon Hills, USA). The lactic acid concentration was determined using a YSI 2500 Glucose/Lactate Analyzer (YSI Inc., Ohio, USA). SCFA concentrations including, acetic acid (C2), propionic acid (C3) and butyric acid (C4) were determined by the gas chromatography technique using Shimadzu GC2010 plus, AOC-20i auto-injector (Shimadzu Corp., Kyoto, Japan). The remaining samples were oven-dried at 60°C for 48 hr. Dried samples were ground pass through a 1 mm. screen for the subsequent analyses of chemical composition including, crude protein (CP), ether extract (EE), non-fiber carbohydrate (NFC), and inorganic matter (Ash) following the proximate analysis method (AOAC, 2000). Non-protein nitrogen (NPN) and true protein (TP) content were analyzed according to Licitra, Hernandez, & Van Soest (1996). Analyses of fiber content were carried out as described by Van Soest, Robertson, & Lewis (1991).

Statistical analysis: All obtained data were subjected to analysis of variance by the general linear models (GLM) of IBM SPSS Statistics (version 25) according to the following model : $Yijk = \mu + Ti + Nj + TNij + Dk + eijk$ where Yij is the dependent, continuous variable; µ is the overall mean; Ti is the fixed effect of the inoculation, Nj is the fixed effect of NH_4Cl levels, TNij is the fixed effect of the inoculation by the NH4Cl levels, Dk is the fixed effect of the fermentation period and eij is the residual error. The comparison between the fermentation period was tested by a completely randomized design according to the following model: $Yij = \mu + Ti + eij$ where Yij is the dependent, continuous variable; µ is the overall mean; Ti is the fixed effect of the fermentation period and eij is the residual error. Significant differences among treatment means were assessed by Duncan's new multiple range test (DMRT) and the significance level was set at $p < 0.05$ (Steel & Torrie, 1986).

3. Results and Discussion

Lactic acid, SCFA profiles and pH levels

Changes in the lactic acid, SCFA and pH levels of fermented cassava pulp are shown in Table 1. Lactic acid and SCFA concentration of the fermented samples on day 7 and day 14 were higher than the initial period (day 0). Cassava pulp inoculated with rumen fluid (RF treatment) result in a higher lactic acid and SCFA concentration when compared to the CON treatment. This result related with the study of Rattanachomsri, Tanapongpipat, Eurwilaichitr, & Champreda (2009) who reported that cassava pulp is the source of fermentable carbohydrate composed mainly of starch and fibrous material then, the increasing in the organic acid (lactic acid and SCFA) of the fermented cassava pulp could be attributed to the ability of the microbial complex to hydrolyzed and conversion of fermentable carbohydrates to lactic acid and SCFA (Chumpawadee & Soychuta, 2009; Kim, Lee, & Kim, 2005). pH levels of all samples were continuously decreased following the accumulated organic acid.

Table 1. Lactic acid concentration, short chain fatty acid profiles and pH levels of fermented cassava pulp

Items	Control (CON)			Rumen fluid (RF)			SEM	*P*-value		
	0% NH_4Cl	0.25% NH_4Cl	0.5% NH_4Cl	0% NH_4Cl	0.25% NH_4Cl	0.5% NH_4Cl		R	N	R*N
Lactic acid (% DM)										
Day 0	0.08^{C}	0.09^{C}	0.08^{C}	0.08^{C}	0.10^{C}	0.08^{C}				
Day 7	17.54^{B}	18.22^{B}	18.39^{B}	59.73^{A}	63.22^{A}	65.46^{A}	1.412	***	NS	NS
Day 14	33.56^{A}	36.96^{A}	44.80^{A}	51.18^{B}	55.32^{B}	60.20^{B}				
SEM	4.850	5.363	6.501	9.389	9.934	10.490				
P-value	***	***	***	***	***	***				
Acetic acid (% DM)										
Day 0	35.61^{B}	36.37^{B}	36.91^{B}	36.63^{C}	36.37^{C}	38.27^{C}				
Day 7	38.85^{B}	39.15^{B}	41.93^{AB}	46.88^{B}	44.56^{B}	43.06^{B}	0.602	*	NS	NS
Day 14	46.35^{A}	44.42^{A}	46.38^{A}	48.07^{A}	49.87^{A}	48.42^{A}				
SEM	1.687	1.954	1.815	2.039	2.168	1.595				
P-value	**	*	**	*	**	**				
Propionic acid (% DM)										
Day 0	17.35^{C}	18.98^{C}	18.93^{C}	17.69^{C}	20.00^{C}	19.27^{C}				
Day 7	23.43^{B}	23.32^{B}	22.84^{B}	23.96^{B}	23.98^{B}	23.96^{B}	0.220	***	NS	NS
Day 14	28.36^{A}	30.59^{A}	30.56^{A}	33.31^{A}	34.30^{A}	33.72^{A}				
SEM	1.693	1.753	1.732	2.328	2.163	2.106				
P-value	**	***	***	***	***	***				
Butyric acid (% DM)										
Day 0	ND	ND	ND	ND	ND	ND				
Day 7	3.40	3.38	3.43	4.28	4.62	4.53	0.036	***	NS	NS
Day 14	2.50	2.89	2.58	3.84	3.57	3.99				
SEM	0.209	0.118	0.214	0.131	0.240	0.128				
P-value	**	**	*	*	**	**				
pH levels										
Day 0	3.47^{A}	3.48^{A}	3.47^{A}	3.47^{A}	3.48^{A}	3.47^{A}				
Day 7	3.32^{B}	3.32^{B}	3.31^{B}	3.21^{B}	3.22^{B}	3.19^{B}	0.006	***	*	NS
Day 14	3.23^{C}	3.07^{C}	3.02^{C}	3.16^{C}	3.14^{C}	3.11^{C}				
SEM	0.036	0.059	0.066	0.048	0.051	0.055				

CON = cassava pulp ferment without any inoculum; RF = cassava pulp ferment with ruminal microbes inoculation; R = Effect of ruminal microbes; N = Effect of ammonium chloride supplementation; ND = Not detected; ^{A,B,C} = Values on the same column with different superscripts differ significantly ($P \leq 0.05$); NS = non-significant; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

Chemical composition

The chemical composition of fermented cassava pulp presented in Table 2. The TP of cassava pulp inoculated with rumen fluid (RF treatment) was significantly increased on day 7 and day 14 of fermentation. Conversely, the NPN and the NFC content were significantly decreased but there was no significant ($P>0.05$) for the fiber content and EE. The reason for the increasing of TP content and the reducing of NPN and NFC content in RF treatment could be attributed to the secretion of some extracellular enzymes such as amylases by the microbial complex to hydrolyzed starch into glucose for used as a carbon source (Oboh, 2006) and the ability of ruminal microbes to use of NPN as a nitrogen source (Pathak, 2008) for growth and synthesize microbial biomass rich in protein. There was no significant difference was observed in the true protein content of CON treatment throughout the incubation.

Table 2. Chemical composition of fermented cassava pulp

Items	Control (CON)			Rumen fluid (RF)			SEM	*P*-value		
	0% NH_4Cl	0.25% NH_4Cl	0.5% NH_4Cl	0% NH_4Cl	0.25% NH_4Cl	0.5% NH_4Cl		R	N	R*N
Organic matter (OM), g/Kg DM										
Day 0	965.57	966.36	967.22	963.96	961.40	961.69				
Day 7	963.85	965.49	963.88	960.56	960.05	958.20	0.240	***	NS	NS
Day 14	964.20	968.30	966.30	960.09	960.93	959.68				
SEM	0.302	0.829	0.683	0.779	0.285	0.893				
P-value	NS	NS	NS	NS	NS	NS				
Crude protein (CP), g/Kg DM										
Day 0	28.10	54.93	77.72	33.15	58.67	79.33				
Day 7	27.97	54.14	77.57	33.21	58.20	78.96	0.076	***	***	NS
Day 14	28.06	54.37	77.58	33.57	58.67	79.33				
SEM	0.128	0.205	0.143	0.080	0.287	0.241				
P-value	NS	NS	NS	NS	NS	NS				
True protein (TP), g/Kg DM										
Day 0	24.08	23.99	24.20	26.87^{B}	26.98^{C}	27.07^{C}				
Day 7	24.23	24.37	24.42	27.25^{B}	28.01^{B}	28.39^{B}	0.105	***	NS	NS
Day 14	24.33	24.50	24.32	29.81^{A}	30.67^{A}	30.78^{A}				
SEM	0.061	0.108	0.078	0.467	0.558	0.546				
P-value	NS	NS	NS	***	***	***				
Non protein nitrogen (NPN), g/Kg DM										
Day 0	4.02	30.94	53.51	6.27^{A}	31.69^{A}	52.25^{A}				
Day 7	3.73	29.76	53.14	5.95^{A}	30.19^{A}	50.56^{B}	0.115	*	***	NS
Day 14	3.73	29.87	53.07	3.75^{B}	28.00^{B}	48.54^{C}				
SEM	0.146	0.256	0.099	0.402	0.588	0.571				
P-value	NS	NS	NS	***	**	**				
Non-fiber carbohydrate (NFC), g/Kg DM										
Day 0	441.32	436.64	439.70	436.40^{A}	421.63^{A}	431.26^{A}				
Day 7	439.54	422.46	427.82	416.83^{B}	418.77^{A}	408.07^{B}	1.653	***	NS	NS
Day 14	417.86	428.37	436.62	389.19^{C}	398.37^{B}	398.13^{B}				
SEM	5.251	3.882	3.078	7.299	4.641	5.822				
P-value	NS	NS	NS	**	*	*				
Hemicellulose, g/Kg DM										
Day 0	141.88	141.95	131.96	143.88	149.65	142.78				
Day 7	136.88	149.61	150.61	152.18	156.16	156.47	1.213	NS	NS	NS
Day 14	146.36	148.83	147.94	168.95	159.13	161.45				
SEM	3.383	1.934	4.733	4.956	3.752	3.098				
P-value	NS	NS	NS	NS	NS	NS				
Cellulose, g/Kg DM										
Day 0	243.69	251.93	255.84	242.28	250.01	253.52				
Day 7	257.97	258.06	247.67	253.77	245.87	251.45	1.200	NS	NS	NS
Day 14	264.47	252.94	247.78	255.16	261.83	261.33				
SEM	5.435	2.743	4.479	4.689	3.325	3.632				
P-value	NS	NS	NS	NS	NS	NS				
Lignin, g/Kg DM										
Day 0	42.90	41.01	43.59	43.99	40.54	41.11				
Day 7	37.95	39.09	43.44	40.76	40.71	43.42	0.577	NS	NS	NS
Day 14	42.16	41.28	37.06	45.11	40.66	39.24				
SEM	1.314	0.916	0.917	1.139	0.614	1.339				
P-value	NS	NS	NS	NS	NS	NS				
Ether extract (EE), g/Kg DM										
Day 0	66.32	65.70	66.16	65.88	66.22	67.57				
Day 7	65.88	66.22	67.57	61.69	65.64	66.10	0.452	NS	NS	NS
Day 14	92.97	67.12	67.04	65.40	64.63	62.95				
SEM	1.095	1.279	1.055	1.186	0.832	1.206				
P-value	NS	NS	NS	NS	NS	NS				

CON = cassava pulp ferment without any inoculum; RF = cassava pulp ferment with ruminal microbes inoculation; R = Effect of ruminal microbes; N = Effect of ammonium chloride supplementation; ^{A,B,C} = Values on the same column with different superscripts differ significantly ($P \leq 0.05$); NS = non-significant; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

4. Conclusions

The current study indicated that ruminal microbes can be used as the inoculum to enhance the short-chain fatty acid concentration and improve true protein content of cassava pulp. However the influence of NH_4Cl supplementation was not observed on lactic acid concentration, SCFA profiles and true protein content of fermented cassava pulp.

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AG-AS 03

An Opportunity for Dairy Goat Farming in Thailand

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Abstract

The market for goat milk in Thailand was rather limited until the year 2015 when it started experiencing growth. This increase in the amount of milk consumption from 2015 to 2019 was probably related to the growth in middle-class consumers as well as the increasing awareness of the health benefits of goat milk. In addition, the growth of the goat milk market may also be related to growth in Thailand's senior population, who have a strong desire for good quality food. This resulted in a promoting future for dairy goat farming in Thailand. However, most Thai dairy goat farms are faced with a low quality and quantity of milk, a lack of GAP standard for farming, and a lack of GMP standard for milk processing. Furthermore, most farmers lack appropriate production and marketing knowledge, and are not innovative. Although raising dairy goats holds opportunity as Thai people's demand for goat milk increases, but it will become necessary for Thai dairy goat farmers to improve their production systems including breeding and genetics, milk processing, and marketing systems.

Keywords: *Dairy goat farming, opportunity, Thailand*

1. Introduction

Goats are an alternative livestock in Thailand. This is because the goat population is only about 0.16% (832,533 heads) of the total livestock population (505,848,025 heads), as compared to chickens, ducks, swine, and beef cattle, which make up approximately 90.0% (455,637,640 heads), 6.2% (31,086,208 heads), 2.2 (11,289,185 heads), and 1.2% (5,871,807 heads), respectively (Department of Livestock Development (DLD), 2020). The small goat population reflects its importance in Thai livestock industry, in comparison with poultry, swine and cattle. This is probably because goat products have not been a common feature in Thai meals. Moreover, the mistaken belief of Thai consumers that goat meat and milk have a "goaty smell" may be another reason that goat products are less important than other animal products (Wattanachant, 2008). Raising meat goats is more common than raising dairy goats -96.5% vs. 3.5% (DLD, 2020). This is partly related to the increasing number of meat goats exported to Laos and China over five years. This is why between 2015 and 2019 the number of meat goats increased 129.1% (350,851 to 803,768 heads) while the number of dairy goats decreased 2.25% (29,426 to 28,765 heads) (Figure 1). Raising dairy goats is neither as popular as raising meat goats, nor is the milk as accepted by consumers as cow milk. Goat milk has proven to have a higher nutritional value than cow milk, close to human breast milk. This has resulted in increased interest in the consumption of goat milk, with the price of raw goat milk being about 3.3 to 4.4 times higher than that of raw cow milk. As a result, the number of dairy goats has slowly increased (17%) since the year 2015. Therefore, raising dairy goats has high potential for Thai farmers. This paper discusses the opportunities for raising dairy goats in Thailand.

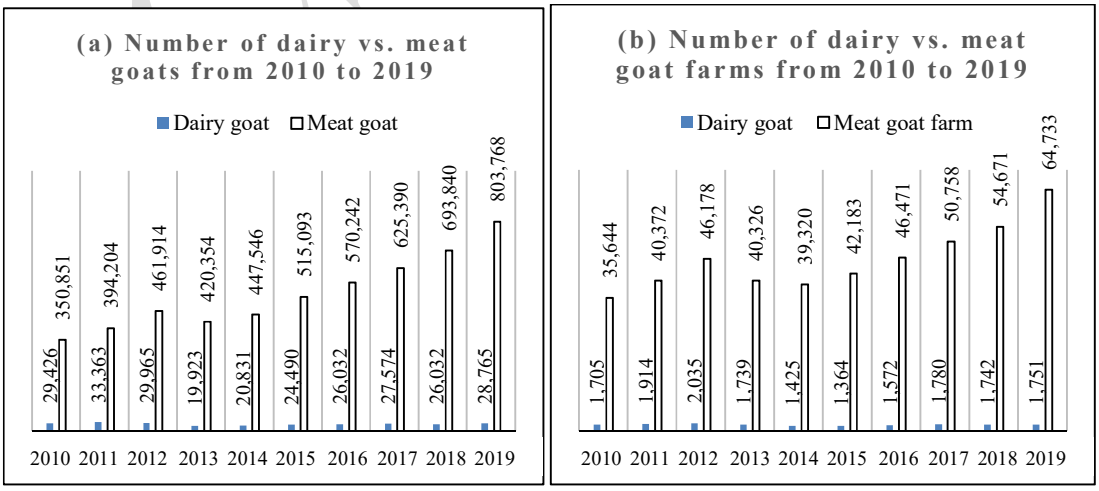

Figure 1. Numbers of dairy and meat goats (a) and farms (b) in Thailand from 2010 to 2019
Source: Adapted from DLD (2020)

2. Distribution of dairy goats and capacity of milk production

As can be seen in Table 1, there were 28,765 dairy goats raised in the year 2019. The largest distribution of dairy goats was in the Central region (54.6%; 15,700 heads), followed by the South (30.1%; 8,664 heads), Northeast (10.2%; 2,927 heads), and North (5.1%; 1,474 heads), respectively. When considering the number of female dairy goats by region, the Central region had the highest number of female goats (10,456 heads; 50.2%) followed by the South (6,798 heads; 32.6%) and Northeast (2,383 heads; 11.4%), while North had the lowest number of females (1,204 heads; 5.8%).

In terms of the changing of the female dairy goat population in between the year 2015 to 2019, information from Table 1 showed that the Northeast had the largest increase in female populations (206.3%) followed by the South (20.6%) and the North (20.4), while the number has declined in the Central region (-4.4%). The increasing number of the female goats in the South, North, and Northeast regions may illustrate the growing demand for goat milk in each region. However, when consider the growing number of farmers in each region between 2015 to 2019, all regions had about 28.4% increased, while the Northern having the highest number of farmers (188.3%), followed by the Northeast (91.1%), the Central (58.9%), and the South had only 4.8% increased.

When considering the capacity of farms to produce milk, according to the number of female goats per farm, the Northeast had the highest milk production capacity (22.3), followed by the North (18.8), Center (13.9), and South (8.2), respectively. This means that the North, Northeast and Central regions have more opportunities to expand milk production capacity than the Southern region. This is possibly related to the large number of farms in the Southern region. However, when estimating the milk yield among regions (Table 1), it can be seen that the Central region is able to produce the highest milk yield (12,547 liters per day, or 2509 tons per 200 days) followed by the South (8,158 liters per day, or 1,631 tons per 200 days), while the North and Northeast produced 4,304 liters per day, or 860 tons per 200 days.

Table 1. Number of dairy goats and farms in Thailand by region in 2010, 2015, and 2019

Regions	Sex	2010			2015			2019			Milk production (litres/ day)**
		Goats	Farms	F/F*	Goats	Farms	F/F*	Goat	Farms	F/F*	
North	F	2,595	133	19.5	1,000	22.2	29.5	1,204	64	18.8	1,445
	M	934			327			270			
	Total	3,529			1,327			1,474			
Northeast	F	924	103	9.1	778	56	13.8	2,383	107	22.3	2,860
	M	420			358			544			
	Total	1,344			1,136			2,927			
Central	F	14,247	434	32.8	10,941	474	23.1	10,456	753	13.9	12,547
	M	3,350			2,669			5,244			
	Total	17,597			13,610			15,700			
South	F	4,864	1,035	4.7	5,639	789	7.1	6,798	827	8.2	8,158
	M	2,092			2,778			1,866			
	Total	6,956			8,417			8,664			
Total	F	22,630	1,705	13.3	18,358	1,364	18	20,841	1,751	11.9	25,009
	M	6,826			6,132			7,924			
	Total	29,456			24,490			28,765			

* F/F: number of female goats ÷ number of farms = capacity of milk production
** estimated milk production by number of female dairy goats * 2 liters of milking * 60% of milk production
Source: Adapted from DLD (2020)

3. Breeds of goat

Because most goats in Thailand are native goats, which are more suitable for meat than for milk (Wattanachant, 2008; Nakavisut & Anothaisinthawee, 2014), they are not suitable for commercial milk production. Thus, seven exotic dairy breeds such Saanen, Alpine, Toggenburg, Anglo-Nubian, LaMacha, Laoshan, and Shami or Damascus were imported for upgrading the capacity of milk production. Saanen is the most popular dairy breed because it was crossbred to produce more milk, and because it is not too large.

Thepparat (2012) reported on the performance of Thai dairy goats, illustrating that the overall milk production from DLD farms was in the range of 0.79 to 1.38 liters per day. This was very low when compared to private farms that could produce about 2 to 3 liter for a lactation period of 200 days (Nakavisut & Anothaisinthawee, 2014). Unpublished data from the Prince of

Songkla University's (PSU) Small Ruminant Research and Development Center reported that the overall milk production per day of Shami goats was between 1.8 to 2.7 liters for a lactation period of 200 days, while 75% of Saanen goats intensively raised at

Division of Animal Production and Innovation, Faculty of Natural Resources, PSU, produced about 0.9 to 1.4 liters per day in a 200-day period. It is clear that Thailand's goat milk production is too low and needs to be improved. In particular, the average milk yield per day should increase from 0.8-1.4 to 2.0-2.5 liters, and the lactation period should increase from 150-200 days to 200-240 days. To increase milk production potential, not only the genetics of Thai dairy goats should be improved, but farmers must increase their knowledge of goat husbandry, namely raising, nutrition, and health care.

4. Ability of farmers

Most dairy goat farms in Thailand are small scales and their management systems are not in accordance with GAP guidelines. This is possibly due to lack of farm development budget and lack of farm management knowledge. The amount of milk produced per farm is small and is not economically viable. In addition, although most farmers are able to process a primary pasteurize milk by themselves, most farmers' processing systems are not yet compliant with GMP standards. Therefore, famers must be trained and educated, along with appropriate technology and innovation adjustments to increase milk production.

5. Production systems

The systems of dairy goat production depend on many factors such as budget, herd size, availability of grass and fodder, feed supplies, and the market. The most popular system of dairy goat farming is the intensive type. This system does not require a large area to accommodate a large number of goats, and the management is easily monitored. However, since goat milk is getting more attention from Thai consumers, dairy goat farmers need to start following GAP principles. In addition, they must also comply with policies of the Ministry of Agriculture and Cooperative that focus on smart and green production systems.

6. Health and diseases

Internal parasite, particularly, barber pole worm (*Haemonchus*), bankrupt worm (*Trichostrongylus*), and brown stomach worm (*Ostertogia*), and infectious diseases such as Melioidosis, CAE, Brucellosis, Enterotoxemia, Pneumonia, and caseous lymphadenitis are the major cause of health problems for Thai goat industry (Wattanachant, 2008; Wattanachant, 2019; Chumek, Aocharoen, & Thongnoon, 2007; Tantaswasdi, Wattanavijarn, & Pinyochon, 1985; Thongnoon, Chumek, & Aocharoen, 2005). To ensure that goats do not carry any zoonotic and infectious diseases, the DLD requires all dairy goat farms be tested for Brucellosis, Melioidosis, and CAE. The DLD has also established Veterinary Research and Development Centers across the country to provide farmers with free disease diagnosis and other health care services for goats and other livestock. Furthermore, the DLD has issued a requirement that all goat farms have a GAP assessment request. Therefore, this is one of the opportunities that Thai dairy goat farmers can benefit from.

7. Marketing

Goat milk can be considered as a functional food that benefits human health. It is a richer source of protein, calcium, fats, vitamins, and other essential nutrients than cow milk. As the present generation of Thais are more interested in health, especially with the growing number of elderly people, goat milk has gained more attention. Therefore, although the price of goat milk is about 3.3 to 4.4 times more expensive than cow milk (50 to 80.-baht vs 18 baht per liter), the market still demands it. To expand the market, improving the quality and image of milk, especially the goaty smell, are necessary, while to GAP and principles are also needed to implement.

In addition to milk, the main product from raising dairy goats, the male is a by-product that can be raised for meat. The supply of goat meat from raising meat goats does not meet the demand from Thai consumers, thus fattening male dairy goats should be considered as an option. Results from many studies show that young male dairy goat fattening can produce a good carcass and meat percentage (Dhanda, Taylor, Murray, Pegg, & Shand, 2003; Horcada *et al,* 2012). Nevertheless, the public needs to become more aware in order to create greater acceptance of goat products.

8. Conclusion

Raising dairy goats in Thailand has a bright future as Thai people's demand for goat milk increases. This may be due to the high nutritional value of goat milk - close to breast milk, increased purchasing potential of the middle class, and the already high price of raw goat milk is an incentive to raise. However, there are major weaknesses that need to be addressed: (1) the unavailability of suitable dairy goat genetics; (2) dairy goat farmers' lack of knowledge and innovation; (3) the inadequacy of milk processing plants; (4) the inadequate promotion of consumption of goat milk products; (5) inadequacy of milk products in the market; and (6) the mistaken attitude about the goaty smell in milk. Nevertheless, the opportunities for Thai dairy goat farming are due to: (1) government agencies and educational institutions being ready to support the farmers; (2) an abundance of green vegetation and feedstuffs around the country for goats; (3) the milk market's upward trend due to an increasing number of Thai senior and middle-class consumers. Most of them are interested in health and wellness, and are ready to pay.

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AG-AS 04

Screening and Selection of Lactic Acid Bacteria from Ensiled Total Mixed Ration at Different Ensiling Time

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Abstract

Ensiled total mixed ration is the feed preservation. Lactic acid bacteria are a common species in silage and has an important role in ensiling process. (Muck, 2010). The accumulation of lactic acid leads to reduction of pH. The low pH condition inhibits the growth and nutrient utilization of desirable microorganism which results in reducing loss of nutrient. (McDonald et al., 1991) The quality of silage could be improved by lactic acid bacteria inoculants while the period of ensiling process associates with species of Lactic acid bacteria. the objective of this study is to screen the best performance of lactic acid bacteria for inoculated in ensiled total mixed ration from different ensiling period. Total mixed ration was prepared and collected at 3, 7, 14 and 21 day of ensiled. The colony were selected for 30 colony of each fermentation collection period. The lactic acid bacteria were tested including by pH measurement, thermotolerant test, lactic acid and acetic acid production and growth curve. The nucleotide sequences of lactic acid bacteria were compared with Gen Bank data base using BLAST algorithm. eighty-four colony can reduce pH value under 4.2. Thirty-three colony can grow at 45°C. Thirty-three colony were measured lactic acid and acetic acid production. Fifteen lactic acid bacteria that can produce highest Lactic acid were identified and compared in NCBI database. Lactic acid bacteria can identify species as *Lactobacillus plantarum, Pediococcus acidilactici, Lactobacillus paracasei and Pediococcus pentosaceus. Pediococcus pentosaceus* has a growth rate rapidly increased compared with other species at 3, 6 and peak at 12 hr. of incubation. The best performance of lactic acid bacteria in this study is *Pediococcus pentosaceous*. This stain rapidly reduces pH value lower than 4.2. Thermotolerant and grow at high temperature condition while high lactic acid production and growth rate.

Keywords: *About 5 keywords should be provided.*

1. Introduction

Ensiled total mixed ration is the feed preservation. Lactic acid bacteria is a common species in silage and has an important role in ensiling process. (Muck, 2010) Lactic acid bacteria can change water-soluble carbohydrate to lactic acid. The accumulation of lactic acid leads to reduction of pH. The low pH condition inhibits the growth and nutrient utilization of desirable microorganism which results in reducing loss of nutrient. (McDonald, Henderson & Heron, 1991) Natural fermentation of silage can cause loss of nutrient by epiphytic bacteria. The quality of silage could be improved by lactic acid bacteria inoculants, consequently lactic acid production occurs more quickly and loss of nutrients during ensilage can be reduced. (Widyastuti, 2008)

In previous study, Lactobacillus fermentum (*L. fermentum*) and *Streptococcus bovis* (*S. bovis*) were found in the untreated materials, *Leuconostoc pseudomesenteroides* (*L. pseudomesenteroides*) in 14-day silage and *Lactobacillus plantarum* (*L. plantarum*) in all silages. *Pediococcus acidilactici* (*P. acidilactici*), *Lactobacillus paracasei* (*L. paracasei*), and *Lactobacillus brevis* (*L. brevis*) formed more than 90% of the isolates in 56-day silage. (Li et al., 2016) The period of ensiling process associates with species of Lactic acid bacteria. Therefore, the objective of this study is to screen the best performance of lactic acid bacteria for inoculated in ensiled total mixed ration from different ensiling period.

2. Materials and Methods

TMR preparation

Total mixed ration was prepared from ingredient in Table 1. Protein 16% and TDN 67%

Ingredient	Volume (%)
Molasses	**2.00**
Rice bran	**4.50**
Corn mill	**1.50**
Soybean meal	**5.00**
Palm meal	**15.60**
Urea	**0.40**
Dicalcium phosphate	**0.50**

Ingredient	Volume (%)
Premix	**0.50**
Fresh Napier grass	**60.00**
Maize hulk	**10.00**
Total	**100.00**

Table 1 The ingredient of total mixed ration

Screening of lactic acid bacteria

The sample of ensiled total mixed ration were collected at 3, 7, 14 and 21 day of ensiled. The sample were mixed with distilled water (1:9) for Ten-fold serial dilution as 10^{-1},10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} ,10^{-6} ,10^{-7} and 10^{-8}, then spread on MRS agar and incubated at 37°C for 48 h. The colony were picked up from different per day of each ensiling time and storage in -20°C.

Characterization of lactic acid bacteria

pH measurement

Lactic acid bacteria were cultured in MRS broth and incubated at 37 °C for 18 h. The MRS broth were measured pH by pH meter. Lactic acid bacteria which pH lower than 4.2 were selected to heat tolerant analysis.

Thermotolerant

Lactic acid bacteria were inoculated in MRS broth and incubated 37 °C for 18 h. The MRS broth were serial dilution and spread on MRS agar. The MRS agar were incubated at 45°C. Lactic acid bacteria which can growth at 45°C were selected to measurement of lactic acid and acetic production. (De Baere et al., 2013)

Lactic and acetic acid production

Lactic acid bacteria were inoculated in MRS broth and incubated at 37 °C for 18 h. The broths were extracted supernatant for measurement of lactic acid and acetic by high performance liquid chromatography. The high-performance liquid chromatography was run by Restek 150x4.5mm column used condition: Mobile phase: 97 % H_3PO_4, 3 % Acetonitrile; Temperature: 25 Degree, Injection volume 5 uL. UV 210 nm. Flow rate was used 1 mL/min. (De Baere et al., 2013)

Growth Curve

Lactic acid bacteria were inoculated in MRS broth and incubated at 37 °C for 24 h. The broth were measured Optical density (OD) by spectrophotometer at 0, 3, 6, 9, 12, 15, 18 and 24 h. The OD were plotted for growth curve.

Identification of Species

The lactic acid bacteria were cultured in MRS broth at 37°C for 18 h. The DNA of lactic acid bacteria were extracted by commercial kits (Biofact, Korea). Following the manufacturer's manual protocol. The DNA were amplified by polymerase chain reaction using 16s rRNA Primer 27f (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492r (5′-GGT TAC CTT GTT ACG ACT T-3′). PCR amplifications were achieved using the following program: pre-denaturation at 95°C for 10 min, then 30 cycles of denaturation at 95°C for 30 s, annealing for 1 min and extension at 72°C for 1.5 min. The last cycle was followed by a 7 min extension at 72°C. (Hou et al., 2018) The PCR product were nucleotide sequenced. The nucleotide sequencing result were compared with Gen Bank data base using BLAST algorithm via https://blast.ncbi.nlm.nih.gov/Blast.cgi

3. Results and Discussion

From one hundred twenty colony of lactic acid bacteria found eighty-four colony reduce pH value under 4.2. For the thermotolerant found thirty-four colony can grow at 45°C. Thirty-three colony were measured lactic acid and acetic acid production. The characterization of 34 lactic acid bacteria shows in Table 2.

Fifteen lactic acid bacteria that can produce highest lactic acid were identified and compared in NCBI database found 4 colony can identified species in Table 3. The four lactic acid bacteria are dominant species that can found in silage fermentation.

Four lactic acid bacteria were measured growth rate. The result showed in Figure 1. *Pediococcus pentosaceus* has a growth rate rapidly increased compared with other species at 3, 6 and peak at 12 hr. of incubation According to the studied of Fitzsimons et al. (1992) and Cai, Kumai, Ogawa, Benno & Nakase, (1999) that reported the lactic acid bacteria in Pediococcus species can grow rapidly more Lactobacillus species at initial stage of incubation. All species into stationary phase in 15 hours of incubation. The growth curves were in the line with generally bacteria growth curve

	Characterization			
Sample	pH	Grow at 45°C	Lactic acid production (g/L)	Acetic acid production (g/L)
T3.2	3.97	✓	10.62	3.15
T3.6	3.92	✓	12.42	3.89
T3.8	4.00	✓	12.11	3.93
T3.10	3.96	✓	12.49	3.66
T3.11	4.00	✓	12.12	3.59
T3.12	3.90	✓	12.5	3.69
T3.14	4.03	✓	12.12	3.93
T3.18	4.06	✓	16.41	3.97
T3.19	3.98	✓	15.91	3.62
T3.20	3.99	✓	14.3	3.26
T3.22	3.94	✓	13.3	3.18
T3.25	3.91	✓	14.82	2.93
T3.28	3.99	✓	13.62	4.22
T3.29	4.10	✓	12.04	4.08
T3.30	3.85	✓	16.58	3.61
T7.1	4.09	✓	13.69	4.00
T7.2	3.86	✓	16.70	3.45
T7.9	3.94	✓	14.67	4.33
T7.13	3.99	✓	14.99	4.01
T7.25	4.00	✓	15.03	3.25
T7.27	3.99	✓	16.91	3.83
T7.28	3.89	✓	17.01	3.52
T7.3	3.96	✓	17.71	4.02
T14.1	4.08	✓	18.27	4.27
T14.2	4.01	✓	18.21	4.31
T14.6	3.97	✓	17.09	4.06
T14.8	3.97	✓	18.19	4.52
T14.10	4.00	✓	17.43	4.12
T14.12	3.87	✓	16.84	3.99
T21.14	4.04	✓	15.48	4.39
T21.17	4.21	✓	16.47	3.98
T21.21	3.91	✓	13.3	4.51
T21.24	4.09	✓	14.44	4.03

Table 2. The characterization of lactic acid bacteria that can reduce pH lower 4.2, thermotolerant and lactic and acetic production.

Isolates	Identification					
	AC	Genus	Species	Strain	Bit Score	Similarity (%)
3.18	KX346613.1	*Lactobacillus*	*plantarum*	c55	1079	94
3.19	KM062019.1	*Pediococcus*	*acidilactici*	JFP1	1258	99
14.2	MF423812.1	*Lactobacillu*	*paracasei*	CAU5144	996	94
14.6	MF424863.1	*Pediococcus*	*pentosaceus*	CAU2471	182	80

Table 3. Show the nucleotide of lactic acid bacteria compare with NCBI database.

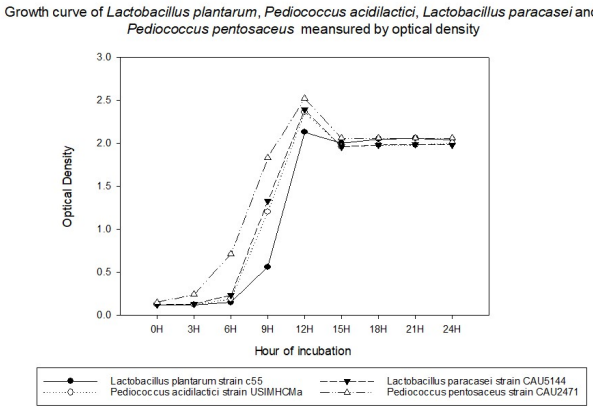

Figure 1. Show growth curve of selected lactic acid bacteria

4. Conclusions

The best performance of lactic acid bacteria is *Pediococcus pentosaceous*. This stain rapidly reduces pH value lower than 4.2. Thermotolerant and grow at high temperature condition. *Pediococcus pentosaceous is* high lactic acid production and growth rate.

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Agricultural Technology

AG-TE 01

Development on Propagation of Water Onion by Temporary Immersion Bioreactor System (TIBs)

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Abstract

Water onion is an endangered aquatic plant and thrive in a particular habitat. This research aimed to develop propagated by temporary immersion bioreactor system (TIBs) in 2017-2019. The result showed explants cultured by TIBs with sucrose and naphthalene acetic acid (NAA) has induced new shoots after 6 months after culturing. The highest of new shoots was 19 shoots/bulb in liquid Murashige and Skoog (MS) medium supplemented with 60 gl^{-1} sucrose and 0.1 mgl^{-1} NAA and feeding 2 minutes for 48 times per days. For rooting, the cultured in liquid MS medium supplemented with 30 gl^{-1} sucrose and 0.05 mg l^{-1} NAA has highest number as 2.5 roots per plant.

Keywords: *crinum thaianum, micro propagation, temporary immersion bioreactor system*

1. Introduction

Water onion (*Crinum thaianum*) is aquatic plant belonging to family *Amaryllidaceae*. It is a biennial plant which natural grows in running fresh water habitat (Schulze, 1972). In Thailand, Water onion, now confine to isolate patches on a few river and steam in south part as Ranong and Phang Nga Province which has been classified status as a likely endangered plant of Thailand (Santisuk *et al.*, 2006). Besides, the International Union for Conservation of Nature (IUCN) has placed the water onion in a risk of endangered species list in 2011. A major threat to the habitat is the dredging of river and stream for removal of sediment and rock for construction including by diversion of the stream for agriculture purpose or land use changes (Soonthornnawaphat *et al.*, 2011).

The micro propagation as tissue culture method as allows a large turnover of plants in a short time with a little space. It may also help with preservation of the wild plants that are collected for propagation material (Pongchawee *et al.*, 2010) Also produce to solve the problem of shortage in aquatic plant. For the water onion, published reported was used twin-scale bulb division, cultured on solid MS medium could induce tubers only added BA. (Pipatcharoenchai and Pradissan, 2008). On the other hand, Chomchuen *et al*. (2012) were cut lengthwise into 8 parts and cultured in agar media with or without MS showed not difference in number of bulblets.

At the present time, the temporary immersion bioreactor system (TIBs) uses in many types of commercial plant tissue culture industries; ex. banana, tomato, carrot, rubber tree, citrus, pineapples. In addition, the aforementioned systems are developed for Robusta coffee (Kasinkaseampong *et al.*, 2008) and Pathumma (*curcuma alismatifolia*) propagation (Topoonyanont *et al.*, 2005). The advantages are get more plant quantity, reduce costs and also labor. The objective of this study was developed suitable medium for water onion propagate in TIBs which against them from extinction in the wild.

2. Materials and Methods

Plant Materials

Fresh bulbs of Water onion from Ranong Province were cut upper part and root. They were shaken in 70% alcohol for 15 min fallowed by in 15% Clorox solution together with 2 drops of Tween-20 for further 20 min fallowed by 10% clorox solution for 15 min and then washing with sterile distilled water 3 times in laminar air flow station. They were trimmed the parts and cut in 4 lengthwise and cultured in aseptic condition, on Murashige and Skoog (MS) solid medium contain with 2 mgl^{-1} BA and 15% (v/v) coconut water, 30 g mgl^{-1} sucrose, 7 gl^{-1} agar and 0.1% Plant Preservative Mixture (Figure 1). Increase the number and size of bulbs, those cut in 2 lengthwise and cultured on MS with 0.02 mgl^{-1} NAA, respectively. The pH of the medium was adjusted to 5.8.

The cultures were placed under the illumination of cool-white fluorescent tubes of about 37 µmol $m^{-2}s^{-1}$ for 16 h/day photoperiod, 25+2° C.

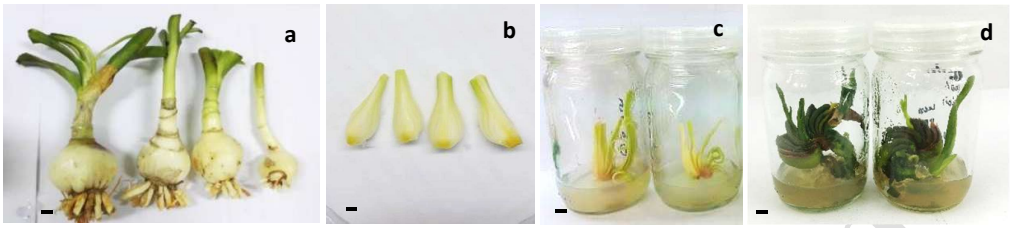

Figure 1. Preparing plant material: fresh bulbs of Water onion (a), four lengthwise cuts (b), cultured on MS with 2 mgl^{-1} BA and 15% (v/v) coconut water, 30 gl^{-1} sucrose, 7 gl^{-1} agar and 0.1% PPM (c) and plantlets (d)

Effect of sucrose and NAA on multiplication in Temporary Immersion Bioreactor system (TIBs).

The plantlets were cut in half and cultured in Liquid medium MS with BA of 6 mgl^{-1} which consist of 4 kind of supplemented (treatment) as 30 g L^{-1} sucrose, 60 g L^{-1} sucrose, 60 gl^{-1} sucrose and 0.05 mgl^{-1} NAA, 60 g L^{-1} sucrose and 0.1 mgl^{-1} NAA, respectively. Also combined with medium feeding system as 2 minutes for 6 times per day and 2 minutes for 48 times per day. The other one is Liquid medium system MS with BA of 6 mgl^{-1} with air feeding 2 minutes for 6 times per day. These experiment was taken for 6 months under the illumination of cool-white fluorescent tubes of about 37 µmol $m^{-2}s^{-1}$ for 16 h/day photoperiod, 25+2 °C. Number of new shoots were observed and recorded.

Effect of NAA on root induction in Temporary Immersion Bioreactor system (TIBs).

The plantlets from the first experiment were cultured on MS without plant growth regulator and MS supplemented with 3 concentrations of NAA (0.02, 0.05 and 0.1 mgl^{-1}) for 3 months under the same conditions as described above. Number of roots per plantlet and root length were observed and recorded each month from 3 months after culturing.

3. Results and discussion

Effect of sucrose and NAA on multiplication

The new shoots from all treatment were occurring 6 months after culturing. The result showed a new shoot increasing with increased concentration of sucrose, particularly when supplemented with NAA (Table 1). The liquid medium MS supplemented with BA of 6 mgl^{-1} which consist 60 gl^{-1} sucrose and 0.1 mgl^{-1} NAA gave the highest new shoots fallowed by with 60 gl^{-1} sucrose and 0.05 mgl^{-1} NAA and with 60 gl^{-1} sucrose, respectively. Which Shou *et al*. (2008) reported that the maximum number of shoots was induced from lotus bud explants on MS medium containing agar, sucrose, and BA added with NAA similar to Noraini *et al*. (2014). In addition, Jala (2012) reported shoot tips of *Curcuma longa* L. were gave the highest average number of new shoots when cultured on MS medium supplemented with NAA and BA.

The liquid medium system with air feeding 2 minutes for 6 times/day was classified as high capacity in their average shoot induce (22.8 shoots) fallowed by TIBs with medium feeding 2 minutes for 48 times/day (19.0 shoots).

Table 1 Number of new shoots (average) after 6 months of culturing

Liquid Medium MS with BA of 6 mgl^{-1}	TIBs with medium feeding 2 minutes for 6 times/day	TIBs with medium feeding 2 minutes for 48 times/day[1/]	Liquid medium system with air feeding 2 minutes for 6 times/day
30 gl^{-1} sucrose	6.0	4.0 c	4.3 c
60 gl^{-1} sucrose	6.8	5.0 c	7.0 c
60 gl^{-1} sucrose and 0.05 mgl^{-1} NAA	7.8	8.0 b	17.0 b
60 gl^{-1} sucrose and 0.1 mgl^{-1} NAA	7.0	19.0 a	22.8 a
C.V. (%)	30.7	18.1	18.0

[1/] The averages in the same column that follow with the same letter were not statistical difference at 95% confidence level by DMRT

Effect of NAA on root induction

The results showed after 3 months of incubation, the plantlets in all media can produced roots. NAA-added Medias have a plump root appearance while the MS formula without NAA have thin roots (Figure 2). Those cultured on MS supplemented with 0.1 mg L^{-1} NAA gave highest average number of roots per plantlet and root length, 5.8 and 9.3, respectively (Table 2).

Table 2. Number of roots per plantlet and root length of water onion plantlet from TIBs after 3 months of culturing on MS supplemented with NAA

MS supplemented with NAA	Number of roots per plantlet 1/	root length (cm) 1/
MS without NAA (control)	2.5 b	2.3 b
MS with 0.02 mg L^{-1} NAA	2.8 b	2.9 b
MS with 0.05 mg L^{-1} NAA	3.5 b	4.4 b
MS with 0.1 mg L^{-1} NAA	5.8 a	9.3 a
C.V. (%)	35.9	55.8

1/ The averages in the same column that follow with the same letter were not statistical difference at 95% confidence level by DMRT

MS supplemented with NAA	Number of roots per plantlet 1/	root length (cm)
MS without NAA (control)	2.5 b	2.3 b
MS with 0.02 mgl^{-1} NAA	2.8 b	2.9 b
MS with 0.05 mgl^{-1} NAA	3.5 b	4.4 b
MS with 0.1 mgl^{-1} NAA	5.8 a	9.3 a
C.V. (%)	35.9	55.8

1/ The averages in the same column that follow with the same letter were not statistical difference at 95% confidence level by DMRT

Figure 2. Root cultured of water onion plantlet from TIBs after 3 months of culturing on MS supplemented without NAA (a), with 0.02 mgl^{-1} (b), 0.05 mgl^{-1} (c) and 0.1 mgl^{-1} (d) NAA (bar = 1 cm)

4. Conclusion

The study found that sucrose content, BA and NAA concentrations, had an effect on the germination rate of new shoots. In addition, cultivation of plant tissue in Temporary Immersion Bioreactor system (TIBs) resulted in the growth of new shoots than in solid or semi-liquid media. The MS with BA, NAA and 60 gl^{-1} sucrose was found more germination than those without NAA and 30 gl^{-1} sucrose. The media with the highest germination rate was MS with 6 mgl^{-1} BA, 0.1 mgl^{-1} NAA and 60 gl^{-1} sucrose. The transient sinking liquid feeding system (TIB) was fed for 2 minutes 48 times a day, the germination rate of 19 new shoots per head. However, the new shoots that germinate are very small. And takes up to 6 months to germinate. For rooting, the highest average numbers of roots were 5.8 roots per plant when cultured in liquid MS medium supplemented with 0.1 mgl^{-1} NAA and 30 gl^{-1} sucrose with TIBs.

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Agricultural System

AgS 01

Study on Supply Chain Model of Coconut Production in Prachuap Khiri Khan, Chumphon and Surat Thani Provinces

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Abstract

Prachuap Khiri Khan, Chumphon and Surat Thani provinces are important coconut product areas of Thailand. The areas, however, currently have problems resulting in imbalance between production and demand, e.g. planting area decrease, pest infestation, and improper management. Therefore, this research aims to study the coconut model' supply chain in Prachuap Khiri Khan, Chumphon and Surat Thani provinces. The data were collected by interviewing three sample groups regarding farmers, product collectors, and fresh coconut milk shops from October 2018 to August 2019. This study was found that the supply chain of coconut production of the three provinces has the same model in which four levels consisting of farmers (upstream), product collectors and processing (midstream), and customers (downstream). Beginning with the farmers (upstream), the average age of them is over 60 years. Most of their coconut trees are in tall-stem tribes and the average age is over 50 years. The average quantity of yield is lower than 1,000 coconuts/rai/year. The next level is product collectors or merchants. They collect and manage coconuts in various commercial mature coconut fruits before delivery to customers, such as semi-dehusked mature coconut, dehusked mature coconut except for the perianth area, deshell coconut, kernel, copra, and fresh coconut milk, which depend on the area. The next level is processing, namely fresh coconut milk shop, deshell coconut factory, coconut processing factory, extract coconut oil factory, and coconut products from processing group. The end level is the customers. Furthermore, this study was found that the farmers lacked knowledge about proper management. Some coconuts sent to the processing factory were controlled by the factory regarding the price, quantity, and quality grading. However, for the coconuts sent to various regions for fresh coconut milk shops, the product collectors can control the price by themselves. Household processing of farmers lacked knowledge about the development of products and marketing for the customer's demand. This study gave manageable guidelines for proposal policy to the relevant sectors to increase farmer's competitive and productive efficiency in order to increase their income-generating. There are two recommendations 1) develop the coconut production system for quantity and quality throughout the year to comply with GAP standards and 2) create networking among stakeholders in the supply chain, to support each other for a collaborative management approach.

Keywords: *Coconut, Supply chain*

1. Introduction

Supply chain is the processes that work together starting from the process of production, purchasing, procurement, moving, transportation, storage, distribution, delivery to the consumer, including the use of information technology to support various processes to be able to coordinate with each other smoothly (Agricultural Economics, 2015) Study of the supply chain in each of agricultural product type is important to obtain information of those involved in the production system. Analysis of gathered information will lead to suggestions for ways to develop and solve problems in the supply chain and achieve more efficient supply chain management.

Coconuts are one of the most important crops in Thailand. Thai people use them to cook both sweet and savory dishes in their daily life. They are used as an raw material in various industries such as concentrated coconut milk industry. Thailand is considered a leading producer and exporter of coconut milk in global market. In 2016-2018, the export value of Thailand was 10,928 - 13,932 million baht (Agricultural Economics, 2019).

Aside from coconut milk production, there are also other coconut industry such coconut oil and desiccated coconut. Moreover, coconuts are also a staple in the food for domestic consumption and coconut oil is also used as composition in cosmetic industries. Therefore, coconut is considered an economic crop that Thailand has the potential to produce in which 60% of the country's coconut cultivated area locates in Prachuap Khiri Khan, Chumphon and Surat Thani provinces. However, the producing area was continued declined annually, for instant, the planted area of 2007 was 1.59 million rai and 2018 was 828,614 rai (Agricultural Economics, 2019). Currently, coconut production is facing with coconut pests and poor garden management that resulting in decreased yield. However, the demand for consumption continues increase for both domestic and international market. As a result, coconut as raw material was insufficient for processing industry and prices went up resulting in continued imported from abroad. The import value of mature coconut in 2016-2017 was 1.8-4.6 billion baht (Agricultural Economics, 2019).

However, domestic coconut prices have fluctuated throughout the year, especially from 2010 to 2018 which the average price of coconuts was 5, 10, 5, 7, 9, 8, 11, 13 and 7 baht, respectively (Agricultural Economics, 2019) As a result, farmers and those involved in the production of coconut products have an unstable income and insecure in household economic. This is because there is an imbalance between production quantity and market demand. In addition, importation is a major factor affecting the coconut price mechanism in Thailand. However, at present, there is still a lack of data linking the whole supply chain system of coconut production from farm to consumer/end user. Therefore, it is necessary to study information on the production, marketing and stakeholder in the production of coconut products. The research aims to study the patterns of the coconut supply chain in key production sites by the interview of sample groups which are the coconut farmers, collectors, small processing entrepreneurs and fresh coconut milk shop owners in Prachuap Khiri Khan, Chumphon and Surat Thani provinces. Collected data will be analyzed for the cause of problems or the critical point. The result would be the recommendation to institutions for the management policy or guidelines for improve efficiency and sustainability of coconut production systems. This may support farmers and stakeholders involved in coconut production to have more stable incomes and also increase their market competitiveness in the future.

2. Materials and Methods

The secondary data related to coconut growers and the purchasing houses was collected in Prachuap Khiri Khan, Chumphon, and Surat Thani Province. While the questionnaire was tested and improve then use for collecting data from farmers, collectors and also the fresh coconut milk shop owners. The number of samples was determined following the Yamane sample size cited by66 Office of Agricultural Economics (no date) which covered 320 coconut farmers, 65 buyers of produce and 35 fresh coconut milk shops. A simple random sampling was conducted without specific samples and no any appointments in target area. The study was conducted between October 2018 and September 2019 and descriptive data were analyzed by preparing data tables or graphs to describe the issues studied, including quantitative analysis by using statistical parameter, mean and percentage, to support descriptive analysis.

3. Results and Discussion

- Coconut production

General information more than 46 percent of the interviewed farmers in the three provinces were 60 years old and over 60 percent had a primary education 17 percent of farmers are between 30-50 years old and 23 percent of them has higher education than primary school. This is an opportunity to improve and develop coconut production. For the knowledge of coconut production, more than 50 percent received from their family. However, at present the knowledge from government institution are accessible. Moreover, various government projects had more training activities to educate farmers in those 3 provinces.

In all 3 provinces, the farmers own the land in Prachuap Khiri Khan and Chumphon Provinces, most farmers have less than 10 rai of coconut plantation area, accounting for 55.70 and 80.17 percent respectively. In Surat Thani Province, 48.48 percent of farmers have a coconut plantation area in the range of 10-20 rai. (Table 1). Coconut cultivation - more than 54 percent of the coconut plantation areas of the 3 provinces are flat areas which are suitable areas for planting coconut. According to Department of Agriculture, the area at an altitude not more than 500 above sea level is still suitable for coconut (Department of Agriculture, 2016). Besides, other factors in determining the suitability of the coconut cultivation area are the rainfall of at least 1,500 mm per year, average temperature at 27 degrees Celsius, relative humidity at 70 percent up and average amount of sunlight at 5 hours per day (Department of Agriculture, 2019). In addition, in Surat Thani province, there are 39.39 percent of the lowland planted areas, which are sediments being suitable for growing coconut. While more than 51 percent of Prachuap Kiri Khan and Chumphon are sandy soils and 48.48 percent of sandy clay in Surat Thani (Table 1). According to Tippaya (2016), the suitable soil for coconut cultivation should have property of well holding water and drain well. The study shows that most coconut cultivation areas are sandy and sandy soils which has poor water holding. Therefore, improving the soil by adding organic matter is recommended for increasing production efficiency. The data showed that age of coconut in Prachuap Khiri Khan Province spread across all ages from less than 30 years to more than 50 years. In Chumphon and Surat Thani Provinces, most of the coconuts are older than 50 years, accounting for 40 and 66 percent, respectively (Table 1). However, from the interview data, it was found that coconuts older than 50 years still have normal yield which is consistent with the report of Agricultural Economics (2020). According to the study, it was found that, in 2017-2018, coconut palm had yield at 900-1,000 fruits/rai/year, but encounter difficulties in harvesting. Without good care and management, it can easily become prone to damage. From figure 1, it can be observed that in Surat Thani Province, coconut palms age is older than 50 years, thus, it has a risk for yield decrease if the farmer provide just poor agricultural inputs, especially chemical and organic fertilizers, and improper management.

List	percent		
	Prachuap Khiri Khan N=176	**Chumphon** N=118	**Surat Thani** N=33
Planting and production			
1.Planted area (rai)			
<10	55.70	80.17	42.42
10-20	33.50	15.52	48.48
> 20	10.80	4.31	9.09
2. Area			
Lowland	0.00	0.00	39.39
Flat	100.00	93.22	54.55
Slope	0.00	6.78	6.06
3. Soil texture			
Clay	2.89	0.00	21.21
Loam	1.72	11.02	0.00
Sandy loam	33.53	35.59	0.00
Loamy Sand	53.76	51.69	30.30
Laterite	0.00	1.69	0.00
Sandy clay	8.09	0.00	48.48
4. Cultivation period (years)			
<30	29.00	19.50	0.00
31-40	22.70	9.30	18.18
41-50	20.50	30.50	15.15
> 50	27.80	40.70	66.67
5. Standard certification			
None	90.90	93.10	51.52
GAP	8.52	6.90	21.21
Organic	0.58	0.00	27.27
6. Varieties			
Local Thai Tall	99.43	99.14	100.00
Chumphon Hybrid no. 2	0.57	0.86	0.00
7. Planting system			
Monocrop	94.32	83.90	71.88
Intercrop	5.68	16.10	28.12
8. Yield (nuts/rai/year)			
< 1,000	75.43	41.38	36.36
1,000-1,200	16.00	37.07	60.61
> 1,200	8.57	32.76	3.03

Table 1 Coconut production of farmer in Prachuap Khiri Khan, Chumphon and Surat Thani provinces.

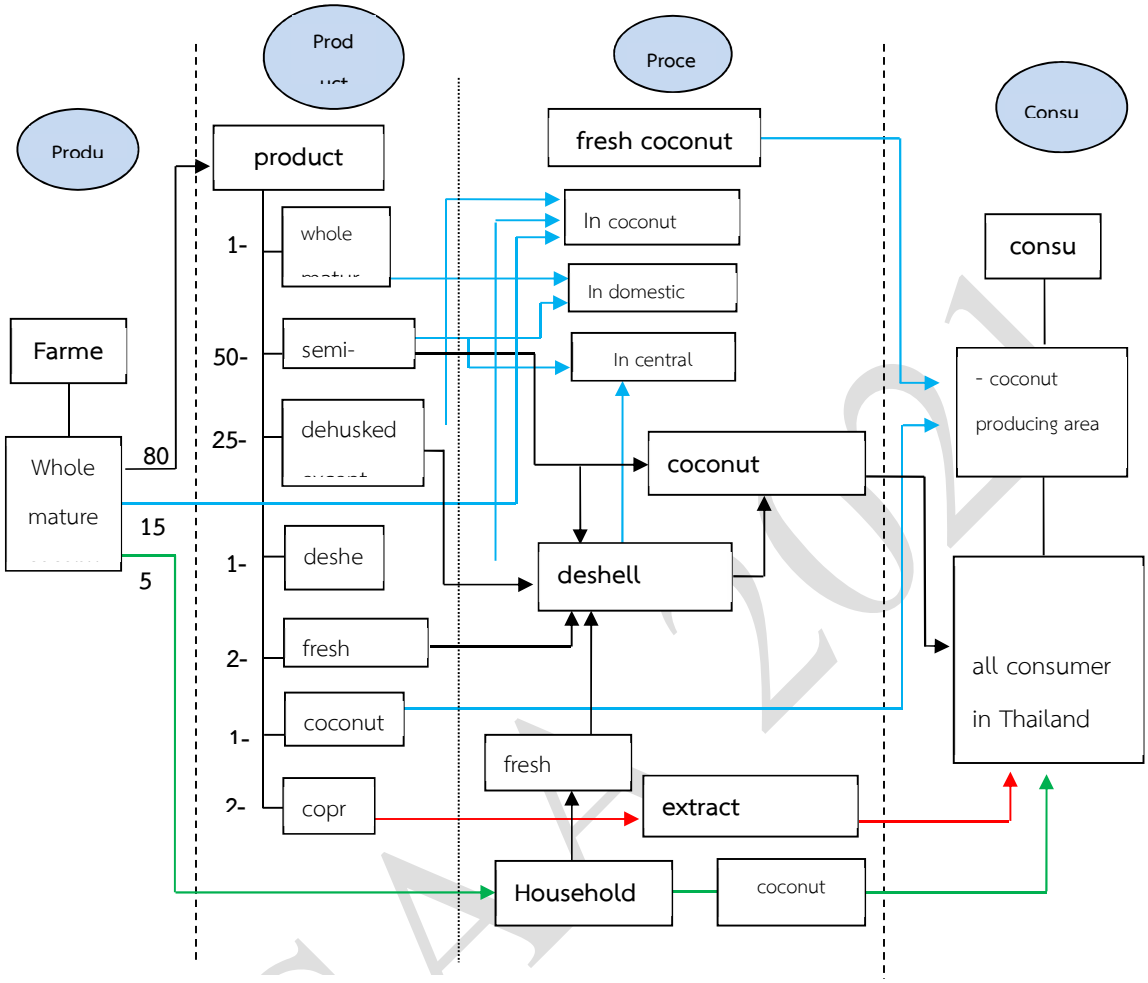

Figure 1 The supply chain diagram of coconut in Prachuap Khiri Khan, Chumphon and Surat Thani provinces

Considering coconut varieties, most farmers in the 3 provinces plant more than 90 percent of Thai Local Tall variety which selecting varieties by themselves. This is consistent with the report of the Department of Agriculture (2020). According to this study, more than 41 percent of farmers in Prachuap Khiri Khan and Chumphon Provinces satisfy with Thai Local Tall variety because it's good characteristics of consistent yields, big fruit size, disease and insect resistance and good drought tolerance. However, the hybrid coconuts recommended by the Department of Agriculture have high productivity in quantity and quality but not easy for accessible. Therefore, the relevant government agencies should provide farmers with knowledge of good hybrid coconuts in order to make decisions on production to suit their area, while the Local Thai Tall are still accepted by farmers. In case farmer want to produce seedling by themselves, the knowledge about the quality coconut seedling production should be provided. In addition, the farmer group should be support to produce high quality coconut seedling. This is also to conserve the Thai coconut species to remain in the area as well.

Coconut planting systems in the three provinces, most of which more than 70 percent of them have mono-cropping system. However, the decision to have intercrop in the coconut plantation must consider various factors especially the market demand, suitable plant species especially growing perennial crop as intercrop. Some coconut farmers already planted this intercropping system but still lacking appropriate academic information for management. Therefore, intercropping system should be demonstrate to cover coconut producing area. This system would be alternative coconut production and farmer would have additional income.

Farm management - Coconut farmers in 3 provinces have similar on application of fertilizer which most farmers practice does not comply with the recommendations of the Department of Agriculture (2016) that recommends applying formula 13-13-21 at rates of 4 kg/tree/year, divided into 2 times per year, and manure 50 kg/tree/year. The result showed that more than 66 percent has applied fertilizers once a year, more than 56 percent have chemical fertilizers in formula 15-15-15. In Chumphon and

Surat Thani Provinces, farmers applied fertilizer at the rate of 1 kg/tree/year (93 - 100 percent) while the famers in Prachuap Khiri Khan applied chemical fertilizer at the rate of 2 kg/tree/year (54.55 percent).

There are three main types of coconut pests namely coconut black-headed caterpillar, coconut hispine beetle and rhinoceros beetle. The study found that more than 40 percent of coconut plantation areas in the three provinces were destroyed by all of the major coconut insect pests. There are 66, 36 and 42 percent of farm interviewed has coconut palms destroyed by coconut black-headed caterpillar, and 55, 40 and 32 percent has coconut palms destroyed by coconut hispine beetle and 28, 24 and 25 percent has coconut palms destroyed by rhinoceros beetle infestation in Prachuap Khiri Khan, Chumphon and Surat Thani Provinces, respectively. Therefore, there is an urgent need to activate the farmers to aware on coconut pest management and transfer technology is needed.

Seventy-five percent of coconut yields in Prachuap Khiri Khan Province had an average yield of less than 1,000 nuts/rai/year (Table 1). The yield was lower than the potential of the cultivar. From the study of Tippaya (2016), Local Thai Tall variety has an average yield of 1,000-1,200 nuts/rai/year. Considering the factors from this study related to the productivity, especially the rainfall in 2016-2018 in Prachuap Khiri Khan Province, the average rainfall is 1,362 millimeters/year (Thai Meteorological Department, 2020), However, the rainfall of suitable coconut planting area is at least 1,500 millimeters/year (Department of Agriculture, 2016). The reproductive organs of coconut (from flowers to mature nut) are more sensitive to water stress, therefore, the principal deleterious effect of the rainfall of coconut is on fruit set, which is the main yield determining factor (Ranasinghe, no date).

In addition, Prachuap Khiri Khan province is also classified as a moderately appropriate area (S2) for growing coconuts (Suthara et al, 2016).

Although pest infestation during the past 1-2 years is another factor affecting productivity. More than 60 percent of Chumphon and Surat Thani provinces had an optimal yield of more than 1,000 nut/rai/year (Table 1), despite the destruction of the coconuts by pests as well. This higher production due to the conditions of suitable planting area where the average rainfall is 2,125 and 1,630 millimeters/year in Chumphon and Surat Thani Provinces, respectively. Besides, the areas in the two provinces were classified as both moderate (S2) and very suitable (S1) for growing coconuts (Suthara et al, 2016). On the other hand, in the Prachuap Khiri Khan area, there is probably increase productivity by good management according to academic advice.

- **Coconut marketing**

Coconut sales model of farmers in all 3 provinces, the farmer normally sells mature coconut to local merchants. Most of harvested coconut were graded, which had 2 grades namely large and small size. 85 percent of the merchants come to buy at the farm and select the size by themselves. While 17 percent of sales in Prachuap Khiri Khan was in the form of fresh coconut meat. There are many factory suppliers come to buy this coconut product in this province. Therefore, planting of coconut hybrid should be advised to farmers in order to utilize for coconut meat for sale due to the hybrid considered to give higher ratio of meat in the fruit component. In addition, famer grouping should be advised to produce fresh coconut meat for sale because the margin is a bit higher than selling whole mature coconut.

Since GAP farm certification was advised for export commodities. But most of the three provinces, more than 50 percent of which are not GAP certified. This is due to the fact that most farmers have not given importance and lack of incentives for standardized coconut production. However, future agricultural market trends in both domestic and world markets, there is an increase in the consumption of products that have been certified as a production standard. While the government sector already has a policy to educate agriculture in the area. There is also a GAP and organic certification agency in the area. It is a readiness to increase opportunities and competitiveness of farmers in the future.

Coconut entrepreneurs

Most of coconut buyers (68 percent) were older than 51 years old and a 49 percent of them had primary education. The other had secondary level at 42 percent. All of coconut buyers operate as a household level and has experience in their business operation for more than 10 years. This makes them capable of analyzing the market situation of coconut. There is also a network for trading with farmers and collectors or factory supplier who purchases the products for processing.

- **Purchasing coconut products**

The majority of coconut buyers in the 3 provinces are local merchants, 81 percent of them purchase from farmers in which 65 percent of them buy less than 120,000 nuts annually. The nut was prepared in various forms as follows:

1. Semi-dehusked form was the most produced (50-60 percent) and distributed to different parts of the supply chain, divided into 3 main parts: 1) fresh coconut milk shops in different regions, 2) primary coconut processing factory who produce white coconut meat to supply coconut milk plant and 3) coconut processing factory.
2. Dehusked except perianth area (25-30 percent) were sent to the factory to make white coconut meat. There was some of this product delivered to the local fresh coconut milk shop in local district because this kind of peeling has a short shelf life.
3. Deshell coconut meat (1-2 percent) was produced only in Surat Thani province which sent to fresh coconut milk shops in the local area only.
4. White coconut meat (2-3 percent) was produced only in Prachuap Khiri Khan and Chumphon provinces which sent to the primary coconut processing factory located in both provinces.
5. Copra (2-3 percent) was produced from broken and germinated coconuts by the coconut collector. It was delivered to extract coconut oil factory.
6. Fresh coconut milk (1-2 percent) was sold daily to local consumers in the area within the district.

Coconut warehouse business operators who buys mature coconuts will distribute their product, mainly in semi-dehusked form, by pick-up trucks to processing factory and to coconut milk shops in other region. The loss from transportation is 3-5 percent.

- **Production and marketing of fresh coconut milk shops**

All interviewed fresh coconut milk shops are located in a fresh market in the coconut production area. Most of them buy coconuts directly from farmers because the quality of coconut milk is fresh.

Seventy-one percent of fresh coconut milk shops in Prachuap and Chumphon Provinces purchase coconuts directly from farmers. For Surat Thani province, 50 percent of the shop bought mature coconuts from farmers and the rest bought from the local coconut collecting house (primary processing factory) in the form of semi-dehusked, dehusked except perianth area and deshell. They has reduced-step management compared with Prachuap Khiri Khan and Chumphon Provinces and have less by-product (husk, water and shell) left over. Their customers who buy fresh coconut milk in large quantities are restaurants, dessert shops and hotels.

The amount of purchasing and sales depends on the time or season such as period of semester break would less than during school opening period, and also during festivals or holidays, the coconut milk sales are higher than normal period. Most of the shop in 3 provinces (52%) had purchasing capacity and operate at less than 200 nuts/day. And more than 68% sale their product as fresh coconut milk only.

- **Supply Chain Model of Coconut production**

From studying was founded the stakeholders in supply chain of coconut production of 3 provinces has the same model which there are 4 levels consisting of farmers (upstream), product collectors (midstream) processing (midstream), and customers (downstream).

1. Producer (upstream) are farmers.

There are 3 main activities: 1) Procurement of agricultural inputs such as seedling, fertilizer, pesticides, herbicides. 2) Farm management activities such as fertilizing, pest control, weed removal. These activities resulting in to quantity and quality of products and 3) Harvesting management activities. These activities are managed by collectors/merchants but the wage for harvesting was paid by coconut farm owner.

For the production cost of 3 provinces was calculated from coconut farm have fruiting palm and without pest infestation. The production cost per nut was classified into 2 types of management. The production cost of proper management farm and improper management farm was 6.24 and 3.84 bath/coconut respectively, in which the cost is similar to the study of Thaila-ong et al. (2014) found that the production cost of farmer in Chumphon provinces average was 3.05 bath/nut or 3,049.41 bath/rai. And the study of Office of Agricultural Economics (2018) found that the production cost average was 3.02 bath/nut or 3,170.19 bath/rai which 85% of farmers have improper management. However, yield of proper management average is 1,100 – 1,500 nuts/rai/year, which trends to be higher than improper management farm. Therefore, the relationship of production cost and yield trends could help coconut farmers to decide for farm management to increase or decrease production cost for sustainable income generating.

2. Product collectors (midstream) there are 3 activities as follows.

1) coconut purchasing, which 80% of them come to buy coconuts at farmer's plantation and the coconut were harvested and graded by them. The large and medium size were group into big size nut and the rest are will be in small size group in which the merchant normally counted 2 nuts as one nut. 2) Raw material preparation - there are 6 types of commercial mature coconut fruits such as semi-dehuske mature coconut, dehusked mature coconut except the perianth area, deshell coconut, kernel, copra and fresh coconut milk which depends on demand from each the area. And 3) Transportation - the products were transported from farmer's plantation to their collecting house and distribute to the customer later on.

The report of Agricultural Economics (2018) indicated that product collectors had market disparity at 2.37 bath/coconut but in this study found that they had market disparity are 3 bath/coconut, which profited 1 bath/coconut. The collector bought at farm gate price at average of 5 baht/nut and after dehusked, they sold at 8 baht/nut,

3. Processor (midstream) as follows

1) Fresh coconut milk shop which processed to grated coconut and fresh coconut milk, and sale to main customer such as restaurant and dessert shop. The shops in 3 provinces usually buys whole mature coconuts from farmer and from the collectors inform of semi-dehuske mature coconut, dehusked mature coconut except the perianth area, deshell coconut. The shop in other area usually buy whole mature coconuts from collectors. While the shop in central region buys semi-dehusked mature coconut and deshell coconut (coconut meat) from deshell coconut factory nearby area. The Agricultural Economics (2018) reported that the fresh coconut milk processing could add value to coconut with profited 15.04 bath/nut.

2) Deshell coconut factory which processed to fresh white coconut meat, which were sent to the instant coconut milk factory. They bought semi-dehuske mature coconut, dehusked mature coconut except the perianth area from product collectors.

3) Coconut processing factory which produced to instant coconut milk which were sent to consumer both in Thailand and other countries. Most of raw material are fresh coconut meat from deshell coconut factory and some of material are semi-dehuske mature coconut from product collectors.

4) Home scale processing which some of farmers are primary processor such as fresh coconut meat processing in Prachuap Khiri Khan and Chumphon Provinces. They process mature nut at home and do whole sale to middleman or individual sale direct to customer. Moreover, some of them also process the mature nut to other product such as virgin coconut oil, soap, shampoo etc., which were distributed to consumer.

5) Extract coconut oil factory which processed to coconut oil for consumption. They bought copra from product collectors for raw material.

4. Customers (downstream)

There are 4 products from coconut for consumption in Thailand, namely fresh coconut milk, instant coconut milk, extract coconut oil and other products. The instant coconut milk in box and extracted oil were distributed for domestic sale.

4. Conclusions

The supply chain of coconut model in Prachuap Khiri Khan, Chumphon and Surat Thani Provinces has the same model which 4 levels consisting of farmers (upstream), product collectors (midstream), processor (midstream), and customers (downstream). The result from information gathered showed that 1) farmers (upstream) planted and preferred Local Thai Tall variety, however, most of coconut population is over 50 years old. Farmers didn't follow recommendation of DOA for application of fertilizer. Therefore, there is potential to improve coconut productivity by new planting or replanting and motivating farmers to follow DOA recommendation. 2) Coconut collectors (midstream) - Merchants/Collectors can earn from primary processing with increase 2-2.50 bath/nut for production cost. 3) Processor (midstream) can earn from value added, different processing activity and product. Therefore, there is a room to link them for business matching and linkages because they utilize raw material from each other. 4) Customers (downstream) there are 4 product types from coconut for consumption in Thailand, namely fresh coconut milk, instant coconut milk, extract coconut oil and other products. Since the study aim to utilized the information and come out with suggestions for development and achieve more efficient supply chain management. There are suggestions as follows:

1. The farmer should be trained for giving them more information and knowledge on new planting, rehabilitation, hybrid varieties, good agricultural practices (GAP) certification and organic standard for coconut production for sustainable production and increasing of market channels.

2. The assembly of farmer should be supported, which they can do brainstorming and knowledge exchanging for production development

3. Dissemination of research results especially on intercropping system should be continued for expanding to farmer cover on coconut producing area resulting in more income generating in limit planting area.

4. Minimize using chemical fertilizer and increasing production efficiency should be researched.

5. The government agencies should mobilize to the coconut strategic plan, tightening coconut network among all stakeholders for sharing and understanding the situation and threat of coconut industry and better effective supply chain management, not only rely on coconut importation but also support coconut production in country. This will elevate all stakeholders having sustainable livelihood.

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AgS 02

The Competitiveness of Thailand Food Valley 1st year: Agroindustry Networking in Case of Aromatic Coconut Product

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Abstract

This article aims to portray how to enhance the agroindustry networking development under the Thailand Food Valley (TFV) project in the Western area. This project aimed to launch the pilot network to build up a communication channels among consumers and producers. Analyzed by Diamond Model, it was found that the key strengths in the production factor were the special coconut variety and specific planting area, which gave unique yield in sweetness and aroma. However, output in each year depended on several factors and there was no formal agency to certify product quality. In demand conditions, it gained more popularity in foreign markets due to increased trends in health and wellness although consumer awareness in its nutrition was needed to raise. Enhancing the competitiveness of the aromatic coconut producer network could be in fields of high value and quality production development, organic production, and national agency to support for continuous improvement.

Keywords: *Aromatic coconut, Diamond model, Value chain, Network.*

1. Introduction

It has been well known that Thailand can supply sufficient food for Thai people consumption and export to other countries thanks to high biodiversity and abundant resources. However, there are many rooms for improvement and many factors affecting all stages of the production chain, most notably those associated with downstream, midstream, and upstream industries. The National Food Committee Act 2008, which became effective on 9 February 2008, was thus set up a national committee to work for national food management and promote cooperation and the integration of budgetary and other resources. A major part of the Committee's work goes for the proposing food quality standards, food safety, food security and food education policies and strategies, including an emergency plan and a food alert system (Thai National Food Committee, 2012). To serve the strategic framework for food management in Thailand, it is important to realize current situation. This leads to the objective of this paper which is to portray how to enhance the agroindustry networking development to support sustainable growth of Thai food industry. The paper was developed from the Thailand Food Valley (TFV) project in Western area with the support from the Department of Industrial Promotion (DIP), Ministry of Industry.

To explore across key players in the supply chain network, it is recommended by Thailand Food Committee that there are many areas to review, starting from fundamental issues, good practice, and result. Error! Reference source not found. shows the concept of the agricultural production chain which requires proper land use, water management, genetic resources of plants and animals, prevention and control of plant and animal diseases, good agricultural practices (GAPs), good hygienic practices, and good manufacturing practices.

Although there are several agricultural products that present uniqueness and Thainess, this paper chooses the aromatic coconut within the lower central region. This is because the study wants to explore findings in detail and draw insightful recommendations to the related government agencies. In addition, the aromatic coconut producers in this region are in all stages of production, for example, coconut plant suppliers, coconut farmers, coconut collector, coconut factory, and community enterprises, as well as the producers in other industries who consume or use the aromatic coconut parts to process to other products. Therefore, the producers in this area can well present good agricultural and manufacturing practices, which other agricultural sectors can adapt to raise standards for food manufacturing and operational efficiency. The results of this paper are not only serving the objectives of the Thailand Food Valley (TFV) project and the strategies of the National Food Committee Act 2008, they are also aligned with the goals of the 20-Years National Strategy (National Strategy Secretariat Office, 2018) in improving people's capabilities in business management, administration, along with developing transformational leadership at community and local levels, which will enhance people's learning abilities from within. Also, this paper could provide suggestions related the country's operations towards the Sustainable Development Goals (United Nations, 2020), such as Goal 8 decent work and economic growth, Goal 9 industry, innovation and infrastructure, Goal 10 reduced inequalities, and Goal 11 sustainable cities and communities.

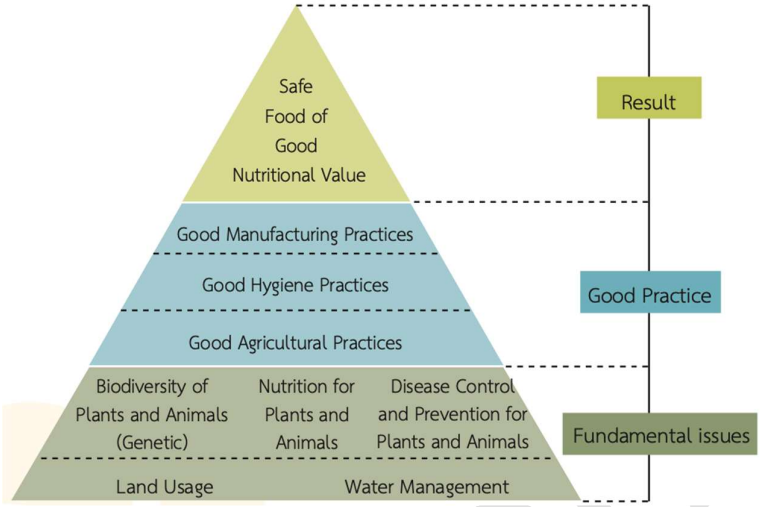

Figure 1 Food Production Chain

Source: Thai National Food Committee (2012)

2. Materials and Methods

To analyze competitiveness of the aromatic coconut producer network (TFVcoconut) under Thailand Food Valley project, the Diamond Model was employed to explore insights all producers in the supply chain network. It should be noted that the supply chain network has become well integrated into modern business decision making processes at leading edge companies. They are used to rigorously analyze and make the best decision in response to both short-term events and long-term strategy, such as capacity expansion or mergers and acquisitions. These analytical approaches and technologies have recently been extended to enable businesses to analyze not just their own operations, but the sum of multiple supply chains in the competitive marketplace. (Kosansky, 2016). The selected area to study is the lower central region, including Samut Songkram, Samut Sakhorn, Nakhon Pathom, Ratchaburi, Supan Buri, Petchaburi, Kanchanaburi, and Prachuab Khiri Khan. After researching the potential aromatic coconut producers, it is found that most of the producers are in Samut Sakhorn province. Such producers are really variety presenting upstream, midstream, and downstream industries characteristics, including coconut plant suppliers, coconut farmers, coconut collector, and community enterprises, local company, coconut processing company, distributor, and exporter.

To create system to support the aromatic coconut stakeholders, the triple helix model was adapted in this paper. Galvao et al. (2019) explained that the model sought to explain the regional economic development through research systems, social context, and innovation policies (Etzkowitz and Klofsten, 2005; Ritala and Huizingh, 2014; Park et al., 2015). It is also known as the university–industry–government interaction model which has been evolved over time (Leydesdorff, 2012). In this project, King Mongut's University of Technology Thonburi worked as the network facilitator from the university side, Department of Industrial Promotion served as policy support from the government side, and the aromatic producers in Samut Sakhon province and related areas were selected as a case study. The coconut networking was developed by various activities, for example, focus group, interviews, team-building workshop, industrial visit, and activities enhancing business opportunities and matching. Insights of the aromatic coconut situation could be summarized by using the Diamond Model, which covers details from all groups of producers and stages in the agricultural production chain. Key aspects in the Diamond Model by Porter (1990) includes factor conditions or endowments, related and supporting industries, demand condition, strategy, structure and rivalry, and other factors from roles of the government and chance (The result was consistent with Bhasabutra (1997), who reported 82.5% *in* vitro inhibition of the vegetative growth of *C.* gloeosporioides by 1000 mg·L-1 sweet-flag crude extract. The property of sweet-flag inhibiting some plant pathogen, including *Colletrotichum* spp. had been previously reported (Thaenthanee et al., 2014; Dethoup et al., 2019). β-Asarone, a key bioactive compound in sweet-flag, was reported to interfere with ergosterol in fungi' cell membrane (Venkatesan, Karuppiah, Arumugam, & Balamuthu, 2019).).

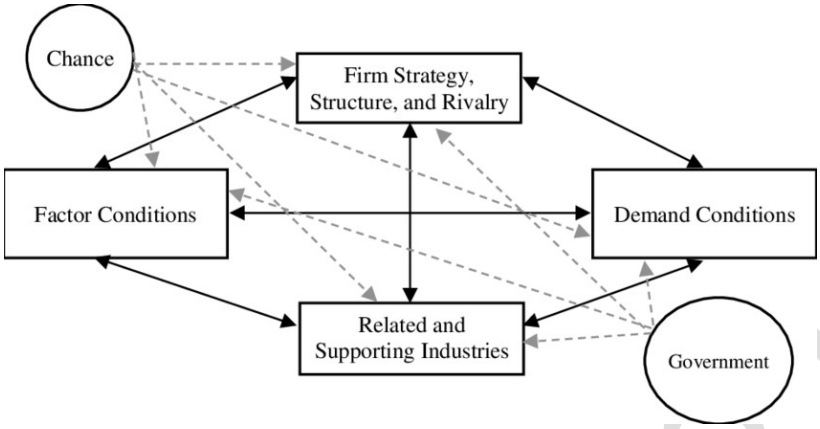

Figure 2 Porter's Diamond Model

Source: Porter (1990)

3. Results and Discussion

The results could be categorized by components in the Diamond Model and presented by Table 1

(1) Factor condition (endowments)	**(2) Related and supported**
(-) Lack of the ability to control production capacity during some periods (-) Mutation due to too much species planted nearby (-) Agricultural pests (+) Customer awareness of the Uniqueness of Thai aromatic coconut compared with other countries' producers	(-) High logistics costs and too expensive aromatic coconut during some periods (+) High technology and innovation in production process, packaging, and marketing channels (+) Investment supported by government for big firms (+) Producer network and cooperation
(3) Demand condition	**(4) Strategy, structure and rivalry**
(-) Need of technology and innovation in quality control (+) High global demand (+) Good story about Thainess which can support Thai aromatic coconut	(-) High degree of competition (-) Lack of long-term strategic plan and supporting agencies, such as, research and price monitoring or control (-) Limited capacity of organic products (+) Good support measures to protect product uniqueness by government by promoting producers to have the Geographical Indicator or GI
(5) Other factors (roles of government and chance)	
(+) An increasing number of studies and research of the aromatic coconut (+) Good infrastructure supports, such as, logistics system (+) Trends in green and good health	

Table 1 Key Results following the Diamond Model Framework
Note: (+) and (-) are used to present negative and positive factors, respectively.

Exploring details of different types of the aromatic coconut producers, it was found that strengths, weaknesses, opportunities, and threats of upstream, midstream, and downstream producers are as in Table 2.

UPSTREAM	MIDSTREAM	DOWNSTREAM
Strengths		
- Uniqueness - Every part of the aromatic coconut can be used - Low Glycemic Index	- Organic - Expertise of the producers - Low Glycemic Index - Every part of the aromatic coconut can be used	- Every part of the aromatic coconut can be used - Low Glycemic Index
Weaknesses		
- High logistics costs - Difficulty in quality control - Agricultural pests - Lack of financial support, skilled labor, and collector - No price controls - Mutation - High harvesting costs	- High logistics costs - Difficulty in quality control - Agricultural pests - Lack of financial support, skilled labor, and collector - No price controls - Storage technology - Low food processing technology	- High logistics costs - Difficulty in quality control - Agricultural pests - No price controls - Marketing channels
Opportunities		
- Unique species - Government supports - Ease of access to capital	- Variety of processed products - Government supports - Ease of access to capital	- Variety of processed products - Government supports - Ease of access to capital - Healthy trends and the aromatic nutrition - High demand from global markets
Threats		
- More expensive cost of coconut compared with other countries - Lack of knowledge in handling the agricultural pests - Insufficient storage area - Insufficient labor	- More expensive cost of coconut compared with other countries - Lack of knowledge in handling the agricultural pests - Insufficient storage area - Insufficient labor - More price competition	- Logistics limitation (long periods) - Customers lack knowledge about the aromatic coconut - Insufficient labor - High competition form Viet Nam and Indonesia

Table 2 Key Results following the Diamond Model Framework

From the Diamond Model and SWOT (strengths, weaknesses, opportunities, and threats) results, this paper recommends strategic areas for development in six aspects, including agricultural management, production process, product development, pricing strategy, marketing channels, and promotion. Details of the recommendation are as follows.

(1) Agricultural management
- Establish sustainable organic crop standards
- The Legislation or the Act to control the ownership of the area (due to the problem of foreign ownership)
- Create the standard of real coconut sugar
- Providing knowledge transfer to farmers such as storage, processing, and marketing.

(2) Production process
- Promoting Thai product for both domestic and international standards such as GMP, FDA, etc.
- Improving quality control process to solve the problem of instability of the product
- Creating technology and innovation in production such as safety system
- Quality control in production to meet

(3) Product development
- Using technology to sort out raw materials
- Developing new products to market such as healthy drinks
- Creating storage standards and supporting storage areas
- Introducing a standard for separating between the real coconut sugar and the mixed by having product certification label or QR Code
- Supporting the production innovation in the aromatic coconut by universities or government agencies

(4) Pricing strategy
- Setting standard price system of various types of coconut such as fragrant coconut, mature coconut, fresh coconut, and coconut sugar
- Establishing the Coconut Association to be a focal point of sharing business practices and knowledge sharing
- Supporting value creation process of the product, for example, new markets, packaging, product shelf life

(5) Marketing channels
- Promoting new marketing channels such as electronic platforms both domestic and
- Building and developing a strong network to increase bargaining power to customers in other countries
- Building an easy platform for foreigners to access such as government platform, language translation, etc.
- Building a knowledge sharing system to update producers market trends

(6) Promotion
- Providing benefits and knowledge or story about coconuts to add high value to Thai products
- Promoting through various media such as social media and create awareness of the value of organic coconut nutrition
- Organizing events to communicate stories or give knowledge about products producer association or government agencies

This project created an online platform for all types of the producers to share business ideas, events, and opportunities to improve quality of the products. It is found that bringing them together could raise the awareness of product development and competitiveness enhancement. They well realized how importance of the product development, marketing trends, especially business cooperation across all stages of the supply chain. Since the existing competitiveness advantage has been gradually reduced, an active business network would be a next step to follow to overcome an intense competition from foreign countries. Once the producers take it into consideration, it would be easy for the university to introduce research and innovation for product improvement, and the government to implement any policy measures. Even though the close participation across university, industry, and government had been only six months, the network has been continuing because of the communication channel among consumers and producers via website http://tfvcoconut.com.

4. Conclusions

This research demonstrates the agroindustry networking development under the Thailand Food Valley (TFV) project with the case study of the aromatic coconut producers. Thailand has been obtaining key strengths in the production factors, for example, coconut variety, specific planting area, and unique yield in sweetness and aroma. However, such competitive advantage has been gradually reduced, business cooperation from various types of produces is the next approach for sustainable agriculture. The project brought the aromatic coconut producers from upstream, midstream, and downstream industries to share ideas, business practices, opportunities, and obstacles during current situation. It was found that Thai aromatic coconut gained more popularity in foreign markets due to increased trends in health and wellness. With respect to the supporting industry, there was a huge number of foreign direct investment to the packaging and exporting industry, which implied great market opportunities in the future. Concerning market rivalry, key strengths were found in the network, including clear vision and mission, assistance within the network, and support by the government, specifically the strategic plan, the budget, and the Geographical Indicator (GI) registration promotion. Therefore, enhancing the competitiveness of the aromatic coconut producer network could be in fields of high value and quality production, especially rooms from the university's and the government's participation to help improve capacity building of the industry. This could be good practices for other industries to apply so as to overcome a dynamic business environment.

Acknowledgment

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AgS 03

Development of Chinese Cabbage Production Processes for Distribution in "The Green Market" of Songkhla Province: The Agri – Market, Faculty of Natural Resources, Prince of Songkla University, Thailand

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Abstract

This study aims to study the problems or obstacles of the Chinese Cabbage production process and factors affecting consumer demand for Chinese cabbage leading to the development of the Chinese Cabbage production process for distribution in the Agri-Market. Study the quantitative research with 138 people consume Chinese Cabbage and the qualitative research with 10 farmers who registered as distributors in the Market. The quantitative analyzed by descriptive and Inferential statistics were used stepwise multiple regression analysis. The results showed, the disease and pest are the main problem of production process. The consumer demand path was focused on the confidence of the place to purchase and was followed by the product. In terms of consumer demand Chinese Cabbage based on the marketing mix (4Ps), found the motivation factor that affects more than another factor. This contributes to the development of Chinese Cabbage to confidence and meet consumer demand for safety and sustainability.

Keywords: *Chinese Cabbage, Consumer Demand, Marketing Mix (4Ps)*

1. Introduction

Chinese Cabbages are among the most popular cruciferous plants with both leaves and flowers. It is popularly grown in Asia, including Thailand. Which can be grown in any region grow and bloom all year round (Department of Agricultural Extension, 2019) Chinese Cabbage important for the economy in the country that available both in the country and exported to foreign distribution (National Bureau of Agricultural Commodity and Food Standards, 2018) Although Chinese Cabbage can be cultivated in all regions. But the quality of Cantonese green lettuce may differ in different cultivated areas with different environments, as well as pests that influence the growth (Sirima, 1991) However, nowadays, chemical residues are still being detected in vegetables sold in retail and fresh markets. Which randomly detects residues in vegetables that exceed the standard (up to 40.00%) In particular, Chinese Cabbage was most likely to be found to exceed the standard residue level (83.33%) In addition, the certified vegetables were randomized to 26 percent of the contamination. (Pesticide Alarm Network, 2019). Besides, it was found that the distribution of agricultural produce that has been certified for production and safety standards is an important factor in building credibility and affecting the choice of organic vegetables, especially the consumer consume healthy food. (Bangkok Biz News, 2014) The Agri-Market, Prince of Songkla University (PSU.), although there is a system for selecting farmers that have been certified organic vegetables able to control and monitor the output to meet the market safety standards on a regular basis. However, it is still unable to control the quality in terms of size, taste, color or traces of insects and disease. Therefore, production processes must be developed to meet consumer satisfaction. Therefore, if known consumer behavior is influencing the decision to buy organic vegetables in the agricultural safety market, Songkhla province will create motivation and confidence in the development of farmers' organic vegetable production processes in accordance with consumer needs and provide sustainable development.

Objective

1. Study on the production process and the problems or obstacles in the production Chinese Cabbage
2. Study of socio-economic factors and consumer opinions on demand for Chinese Cabbage
3. Study of factors that affects consumers' demand for Chinese Cabbage
4. Study the guidelines for the Development of Chinese Cabbage Production Processes for Distribution in The Agri-Market, PSU

2. Materials and Methods

The research location is the Agri-Market, Faculty of Natural Resources, PSU that conducting both quantitative and qualitative studies. The quantitative data were collected from 138 consumers of Chinese Cabbage in the Agri-Market with a structured interview. The qualitative data were collected from 10 producers and distributors of Chinese Cabbage using a semi-structured interview. The quantitative data analyzed was used descriptive statistics such as percentage (Percentage), frequency distribution (Mean), and standard deviation (S.D.) and Inferential statistics were used to analyze the relationship between independent and dependent variables, including stepwise multiple regression analysis. The qualitative data section was analyzed by content analysis.

3. Results

1. The results of the study of personal factors of Chinese Cabbage producers for The Agri – Market, Faculty of Natural Resources Prince of Songkla University found that the most of farmers is male (60.00%) and aged 50-59 years with an average of 12 years of cultivation experience. The farmers earn an average of 23,777.78 baht. They have an average planting area of 1.44 rai, 50.00% flat land, 37.50% sandy loamy soil, 62.50% certified organic standards. Most farmers plant seedlings before planting (75.00%) spaced 15-20 centimeters per plant. Watering in the morning and evening for 5 minutes per time and the result of the study on the production process and the problems or obstacles in the production Chinese Cabbage can be displayed in figure 1 as follows

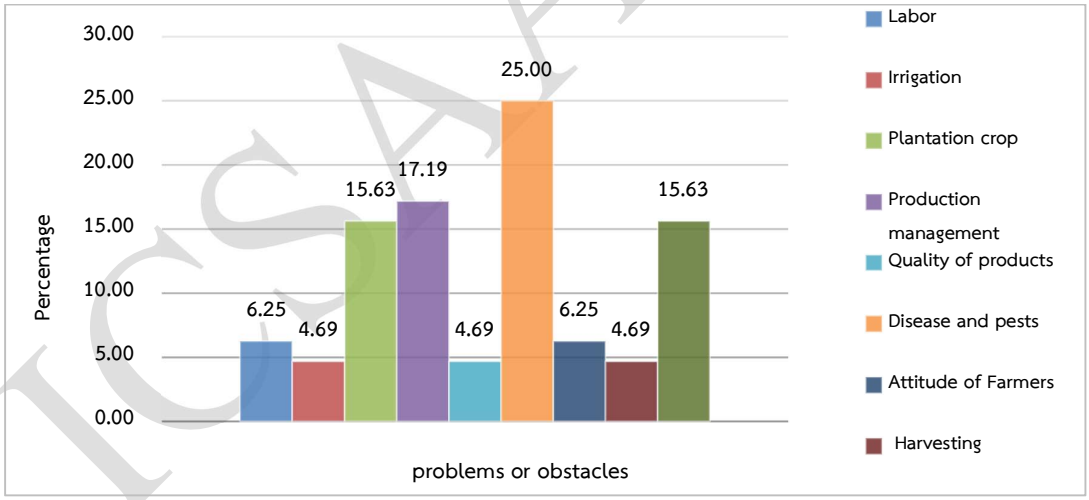

Figure 1 The results of the study of problems or obstacles in the production of Chinese Cabbage of farmers sold in the Agri-Market, Faculty of Natural Resources. Prince of Songkla University

From Figure 1 shows the results of the study on the main problems affecting the production of Chinese Cabbage sold in The Agri-Market, Faculty of Natural Resources, Prince of Songkla University found that the main problems affecting the production of Chinese Cabbage are diseases and pests (25.00%) that the most common diseases or pests are Damping-off (50.00%) and Spodoptera litura (62.50%), Followed by the problem of production management (17.19%) that the most farmers thought that fertilizers were the main factor affecting the production process and 87.50% of the soil condition and the water system and the time to pay attention (75.00%), Problems in arable plantation crop and lack of Government support (15.63%), Labor problems and

negative attitudes of producers (6.25%) and the main problems least affecting the production of Chinese Cabbage were lack of water source, quality of products and harvesting.

2. The results of the study of socio-economic factors and consumer opinions on demand for Chinese Cabbage found that the most of them were female (77.50%), status single (56.50%), working-age about 30-39 years (36.20%), the level of education is bachelor's degree (65.90%), 29.70% of universities/university professors, They have an average monthly income of 29,512.41 baht, the frequency of buying vegetables in this market is 2-3 times a week (31.90%), the cost of purchasing Chinese Cabbage is 41.97 baht per time.

The results of the study of consumer opinions on-demand Chinese Cabbage (Table 1). It was found that most of the marketing mix was very important to demand, when considered individually, it was found that the distribution location had an extremely important on demand ($\bar{X}$=4.32), followed by the product, price and promotion that very important to the demand ($\bar{X}$=4.03, 3.97 and 3.92)

Marketing Mix	Mean	standard deviation	Consumer demand level
1. Product	4.03	0.66	Very important
2. Price	3.97	0.87	Very important
3. Place	4.32	0.73	Extremely important
4. Promotion	3.92	0.89	Very important
Total	**4.05**	**0.69**	Very important

Table 1 Mean, Standard Deviation and the level of demand for Chinese Cabbage to the consumer marketing mix

Level of measurement
Mean 1.00-1.79 This means that is not at all important for consumer demand
Mean 1.80-2.59 This means that is slightly important for consumer demand
Mean 2.60-3.39 This means that is Moderately important for consumer demand
Mean 3.40-4.19 This means that is Very important for consumer demand
Mean 4.20-5.00 This means that is Extremely important for consumer demand

	Model	Equation function	Standardized Equation Function	Std. Error of the Estimate	Significant	Adjust R^2
1	Product	Y_1= 4.213-0.58$X_{Motivation}$ +0.347X_{Age}	Z_1= 0.225$ZX_{Motivation}$- 0.201ZX_{Age}	0.63195	0.002	0.073
2	Price	Y_2= 3.643+0.028$X_{Motivation}$	Z_2= 0.207$ZX_{Motivation}$	0.85315	0.015	0.036
3	Place	Y_3=3.930+0.023$X_{Motivation}$	Z_3= 0.298$ZX_{Motivation}$	0.69929	0.000	0.082
4	Promotion	Y_4= 3.544+0.081$X_{Motivation}$	Z_4= 0.234$ZX_{Motivation}$	0.86530	0.006	0.048
5	Marketing Mix (4Ps)	Y_{Total}= 3.723+0.73$X_{Motivation}$	Z_{Total}= 0.271$ZX_{Motivation}$	0.66688	0.001	0.066

Table 2 The results of the study factors that affect consumers' demand for Chinese Cabbage

Overall analysis (Table 2), It can be explained from the simulated equation 5, that found the incentives for the purchase of Chinese Cabbage. It affects consumers' demand for Chinese Cabbage in all four aspects of the marketing mix, overall at 6.60 percent (Adjust R^2 = 0.066) statistically significant at 0.05. Then, when analyzing the standard equation, it was found that the motivation for the purchase of Chinese Cabbage. It affects consumers' demand for Chinese Cabbage in all four aspects of the marketing mix, overall more than any other factor (β=0.271$ZX_{Motivation}$)

4. Discussion and Conclusions

The results of the study of marketing mix influencing the level of consumer demand for Chinese Cabbage in the Agri-Market, Faculty of Natural Resources, Prince of Songkla University, it was found that the marketing mix consisted of the product, the price, the place of distribution and sales promotion was very important to consumer demand for choosing Chinese Cabbage. The distribution location had the extremely important on demand that consumers want the market to be spacious, clean and ventilated. This is consistent with the current situation of the epidemic COVID 19 that has to reduce congestion, keep a distance between each other and maintain strict cleanliness. Followed by the product aspect, it had very important on demand. Most consumers want vegetables to be of good quality and fresh every day. In accordance with the research of Warunee (2011), it was found that consumers in Bangkok Province attach extremely important products and distribution location factors for deciding on organic vegetables. The price aspect affects the demand to a large extent. The consumers want the shops to clearly show the price of each type of product and easy to see. This is consistent with the current epidemic situation, which needs to reduces the proximity and talks such as asking the price of the product in order to reduce the diffusion of saliva. The last aspect is sale promotion that the important effects to demand. They want to reorganize their products to make it easier to choose/buy and according to hygiene principles to avoid the build-up of pathogens such as Set the vegetables in batches, Separate types of vegetables. In the field of farmers' production processes, most of the farmers who grow organic will have production certification standards. Most of them encountered disease and insect problems that affect the quality of vegetables and made it impossible to control the quality of vegetables. In addition, farmers also found problems in the production management process that affected the quality of vegetables. They believe that fertilizers are the main factor affecting cultivation quality, followed by the soil conditions, water systems, and caring for them, respectively. Bangkok Biz News (2014) certified cultivation sites will affect consumers' purchasing of organic vegetables that prefer healthy food.

Finally, to find ways to develop the production process of Chinese Cabbage for distribution in the green market in Songkhla province, the researcher is of the opinion that new cultivation knowledge and techniques should be promoted for farmers to develop production processes, which in addition to being safe from the use of chemicals. It can also control the quality of vegetables. For example, using Smart Farm technology, which can control factors such as light, humidity, pH, temperature and nutrients that affect the quality of vegetables in terms of size, taste, color, pests and chemicals to meet the same standards. To build confidence and meet consumer demand for sustainable, safe food consumption.

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Aquaculture

AQ 01

Some Bological Aspects of Mature Female Sepat Siam, *Trichogaster pectoralis* (Regan,1910) for Breeding

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Abstract

The sexual maturity of female Sepat Siam, *Trichogaster pectoralis* was studied by determining and gonadosomatic index (GSI). It was found that the size at sexual maturity of female Sepat Siam was 17.31±0.55cm (Mean±SD,n=20) in average total length and 73.78±7.18g (Mean±SD,n=20) in average body weight. fecundity and 20 of them were randomly for measurements The expansion percentage of abdominal increases average 85.31±25.04% in maturity fish (Mean±SD,n=20).

Newly hatched larvae of Sepat Siam were produced by the chemical injection (Suprefact and Motilium). The sexually mature fishes were cultured in Sement pound (water volume 50 ton) with the ratio of male and female brooders 1:1. The fertilization rate, time of hatching and hatching rate experiments were carried out using a 15-liter aquarium (water volume 10 liters) containing 500 eggs. The type of eggs were floating and rounded and yellow color. The fertilized eggs had a diameter of 822±65.71mm (Mean±SD,n=10,000) with a diameter can divide ratio diameter of eggs into 5 groups as follows. The group 1 (500- 600) µm, the group2 (601-700) µm, the group3 (701-800) µm, the group4 (801-900), µm and group5 (901-1000) µm, which there were 0.10%, 1.63%, 47.56%, 40.28%, 10.43%, respectively. The fecundity was 17,140±1,017ova/fish and gonadosomatic index (GSI) 4.50±0.95%(Mean±SD,n=20). The average fertilization rate was 89.49±0.5% (Mean±SD,n=1,500). The average hatching rate was 86.07±0.33% in maturity fish (Mean±SD, n=1,500). The time of hatching average 22.08±0.01hr (Mean±SD,n=1,500) at water temperature 27.0-30.5°C. The spawning ratio was 60.09±45.97% in maturity fish (Mean±SD,n=20).

Keywords: *Fecundity; GSI; Spawning ratio; Sepat Siam, Trichogaster pectoralis (Regan, 1910)*

1.Introduction

The Sepat Siam, *Trichogaster pectoralis* is a native species of freshwater fish an economic value to another of Thailand (Chumti, 2005), originally started farming in a big pond or culture in rice paddies (Information Science and Technology, 2013) A survey of research and analysis of fisheries (2013) found this species from aquaculture, had Productivity 26,700 tons. 1.581743 billion baht, and great demand by aquaculture. The expansion of aquaculture. And management breeder hatchery. In the case of a breeder is a major factor leading to the production of this species. The need for a proper management. By breeding quality. Which contains the information necessary for the cultivation of fish, including the amount of fecundity. Reproductive index (Amornsakun *et al*., 2004), an increase in abdominal width bisexual. The diameter of the eggs Breeding (Amornsakun *et al*., 2004) to make arrangements for broodstock fish breeding can be implemented.

2. Materials and Methods

Principle of the *T. pectoralis* was studed in female broodstock. The experiments were using complete randomized design method, and data analyzed using PC+ program. The sexual maturity *of T. pectoralis* twenty samples was studied by determining its fecundity, diameter of eggs,GSI and expansion percentage of abdominal increases. Fecundity estimation was made by using a gravitric method. Diameter of eggs was measured using ocular microscope. GSI was calculated by using the formula (weight of ovary/ weight of body)×100 (Amornsakun *et al*., 2004). The abdominal increases in maturity fish using the percentage.

2.1 Fecundity experiment

The fecundity was using female broodstock twenty fish were measure body weight and total length. Ripped ovaries taken out, weighed sampling 1% the weight of ovary. Fecundity estimation was made using a gravimetric method (Amornsakun *et al*., 2004).

2.2 Diameter of eggs

The Diameter of eggs was measured 10,000 eggs using ocular microscope (Amornsakun *et al*., 2004). The data analyze by average value group size range and diameter of the eggs.

2.3 Gonadosomatic index (GSI)

The gonadosomatic index was using female broodstock twenty fish were measure body weight, total length and ovary weight was calculated by using the formula (weight of ovary/ weight of body)×100 (Amornsakun *et al*., 2004).

2.4 The abdominal increases in maturity fish

The abdominal increases in maturity fish was using female broodstock twenty fish were measure thickness of abdominal and body thick, was calculated by percentage.

2.5 Spawning ratio

Newly hatched larvae of *T. pectoralis* were produced by induced spawning using chemical injection (Suprefact and Motilium). The injection was using Suprefact 15 µg/kg and Motilium 5 mg/kg. For male and female brooders, the injection was done once. The sexually mature fish were cultured in fiber-glass tank (water volume 300 liters) with stocking density of 10 fishes/m^2. The fertilization rate, hatching out and hatching rate experiments were carried out using a 15-liter aquarium (water volume 10 liters) containing 1,000 eggs, and observation of the amount of fertilized eggs at 5 hr after incubation. The fertilization rate was calculated by (number of fertilization eggs/number of eggs)×100. The time required for the appearance of the first newlyhatched larvae, which would signal hatching out, was recorded. All newly-hatched larvae were collected using a dropper. The hatching rate was calculated by (number of newly-hatched/number of eggs)×100 The procedure was carried out with three replications**.**

The spawning ratio was using female broodstock twenty fish were measure body weight and total length. Ripped ovaries taken out, weighed sampling 1% the weight of ovary. After breeding estimation was made using a gravimetric method (Amornsakun *et al*., 2004).

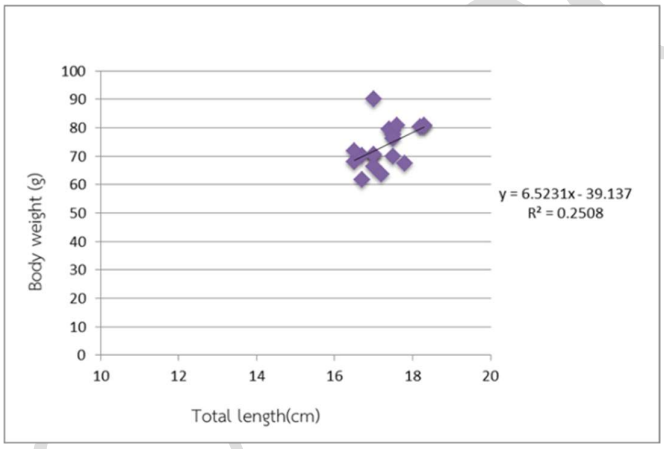

Figure1. Body weight – total length relationship of matured female Sepat Siam, *Trichogaster pectoralis*.

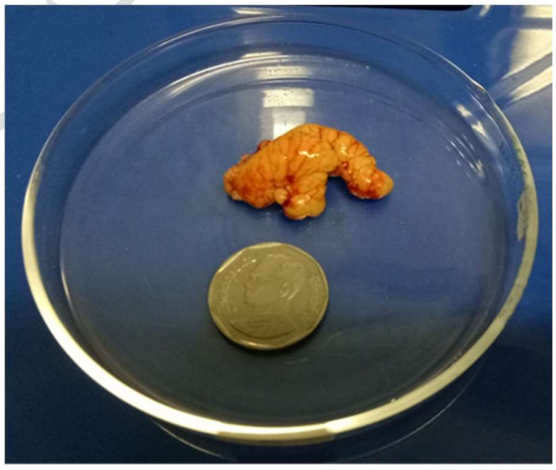

Figure2. Ovary of Sepat Siam, *Trichogaster pectoralis*.

Table 1. Body weight(g) total length(cm) fecundity(egg) gonadosomatic index (GSI,%) and ovary weight(g) of matured Sepat Siam, *Trichogaster pectoralis*.

Sample	Body weight (g.)	Total Length (cm.)	Fecundity (egg)	GSI (%)	Ovary weight (g.)
1	77.48	17.50	16366.67	2.87	2.22
2	79.02	17.50	18133.33	3.47	2.74
3	79.41	17.40	17000.00	3.07	2.44
4	63.67	17.20	15366.67	5.53	3.52
5	70.40	17.00	16633.33	4.12	2.90
6	66.45	17.00	16866.67	4.39	2.92
7	80.70	18.30	17466.67	4.68	3.78
8	68.15	16.50	18600.00	3.11	2.12
9	70.45	17.00	16000.00	2.91	2.05
10	70.38	17.00	18466.67	4.86	3.42
11	80.92	17.60	17866.67	4.75	3.84
12	61.73	16.70	15533.33	5.80	3.58
13	80.93	18.30	17166.67	4.88	3.95
14	67.34	17.80	15666.67	5.82	3.92
15	70.25	16.70	18200.00	4.91	3.45
16	76.25	17.50	16233.33	5.17	3.94
17	80.27	18.20	18266.67	4.90	3.93
18	69.83	17.50	17600.00	4.95	3.46
19	90.17	17.00	17700.00	4.40	3.97
20	71.75	16.50	17666.67	5.39	3.87
Mean	73.78	17.31	17140.00	4.50	3.30
SD	7.18	0.55	1017.66	0.95	0.67
Min	73.78	17.31	15366.67	2.87	2.05
Max	7.18	0.55	18600.00	5.82	3.97

3. Results and Discussion

The sexual maturity of female Sepat Siam was 17.31±0.55cm (Mean±SD, n=20) in average total length and 73.78±7.18g (Mean±SD, n=20) in average body weight. The fecundity was 17,140±1,017ova/fish, and average ovary weight 3.30±0.67g (Mean±SD, n=20) The relationship between total length (TL) and body weight (BW) could be represented by the linear regression as (Figure1): TL= 6.2531-39.317, R^2 = 0.2508, n=20 Mean while the relationship between body weight and total length. The average gonadosomatic index (GSI) was 4.50% (n=20). As in Table 1 and Figure2. The type of eggs were floating and rounded are yellow color in bubble nest as Figure3.The distribution of the fish egg's diameter could be categorized into five groups i.e. group 1 (0.10%,), group2(1.63%), group3 (47.56%), group4 (40.28%) and group5 (10.43%), With values of 500-600 µm, 601-700µm, 701-800µm, 801-900µm and 901-1000µm. The fertilized eggs had a diameter of 822±65.71µm (Mean±SD, n=10,000) (Table2). By observing the egg's diameter of group 3, 4and5, lead us to conclude that the fish is ready for spawning. The expansion percentage of abdominal increases average 85.31±25.04% in maturity fish (Mean±SD, n=20)).

Figure 3. Eggs of Sepat Siam, *Trichogaster pectoralis* in bubble nest.

Table2. Diameter of egg(µm), fecundity(egg) and percentage of egg (%) of matured Sepat Siam, *Trichogaster pectoralis*. (n=10,000)

Diameter of egg (µm)	Fecundity (egg)	percentage of egg (%)
500 - 600	10	0.1
601 - 700	163	1.63
701 - 800	4756	47.56
801 - 900	4028	40.28
901 - 1,000	1043	10.43

The average fertilization rate was 89.49±0.5%(Mean±SD,n=1,500). The average hatching rate was 86.07±0.33% in maturity fish (Mean±SD, n=1,500). The time of hatching average 22.08±0.01hr (Mean±SD,n=1,500) at water temperature 27.0-30.5°C. The spawning ratio was 60.09±45.97% in maturity fish (Mean±SD,n=20).

The Sepat Siam, *Trichogaster pectoralis* is a commercially important species for freshwater fish. The type of eggs were floating and rounded are yellow color. The sexual maturity of female fish size was 17.31cm in average total length, with 73.78 g in average body weight, and the average fecundity was 17,140 egg/fish. The relatively low fecundity indicates that the natural behavior of the spawner is to take care of the newly hatched larvae by constructing a buble nest for spawning (Shinnabuh, 1974). Comeparatively, the fecundity of the *T. pectoralis* was lesser than other species such as Gray-eel catfish, *Plotosus canius* which was reported to have 18,421 egg/fish, and sexually matured size was at 18.07 cm in total length and 94.20 g in average body weight (Amornsakun *et al*., 2004). In addition Amornsakun *et al*. (2005) reported the size of sexually matured female Climbing perch, *Anabas testudineus*, was 15.20 cm in total length and 61.10 g in body weight and Snake head fish, *Channa striatus* has medium fecundity the size at sexual maturity female fish was26.45cm in average total length and 167.4g in average body weight and fecundity was 10,279 ova/fish. Fecundity varies with different species depending on age, length-weight, environmental (Amornsakun *et al*., 2005, 2011). The fertilized eggs of the Sepat Siam (822 µm in diameter) are bigger than the snake head fish (588 µm in diameter) but similar the climbing perch (830 µm in diameter) and Siamese gourami (908 µm in diameter) (Amornsakun *et al*., 2004, 2005and 2011) The female of sepat siam in this study was mature and gonadosomatic index (GSI) was found to be 4.50%, similar snake head fish 5.07% (Amornsakun *et al*., 2011), and giant gouramy was found 2.32% (Amornsakun *et al*., 2014) less than the other fishes. The gonadosomatic index (GSI) of mature freshwater fishes were reported as 8-10% (Tarnchalanukit *et al*., 1892).

4. Conclusions

It was concluded that size at sexual maturity of female sepat siam was 17.31 cm in average total length and 73.78 g in average body weight. The fecundity was 17,140 ova/fish, and average ovary weight 3.30 g. The average gonadosomatic index (GSI) was 4.50 %. The type of eggs were floating and rounded are yellow color. The fertilized eggs had a diameter of 822 µm. The expansion percentage of abdominal increases average 85.31 % in maturity fish. The average fertilization rate was 89.49 %. The average hatching rate was 86.07 % in maturity fish. The time of hatching average 22.08 hr at water temperature 27.0-30.5°C. The spawning ratio was 60.09 % in maturity fish.

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AQ 02

Isolation and Screening of Lactic Acid Bacteria (LAB) for Antagonizing *Vibrio parahaemolyticus* (AHPND strains) in White Shrimp (*Litopenaeus vannamei*)

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Abstract

Shrimp farming in Thailand has been very problematical due to *Vibrio parahaemolyticus*, which causes acute hepatopancreatic necrosis disease (AHPND). To effectively overcome this problem, efficacious lactic acid bacteria (LAB) candidates were isolated from shrimp farms near coastal areas. Fifty strains of LAB were screened for their ability to control pathogenic *V. parahaemolyticus* (AHPND strains). LAB strain of TBPV1 exhibiting highest reduction of *V. parahaemolyticus* was identified as *Enterococcus faecalis* TBPV1 based on the nucleotide sequence of its 16S rDNA. Co-cultivation of *V. parahaemolyticus* and *E. faecalis* TBPV1 showed complete reduction of *V. parahaemolyticus* at 12 h under aerobic condition, whereas *E. faecalis* TBPV1 increased from 5.29 to 9.47 Log CFU/mL. Additionally, *E. faecalis* TBPV1 could produce extracellular enzyme for utilization protein. The result from this study indicated the strong potential for the application of *E. faecalis* TBPV1 for the control of pathogenic *V. parahaemolyticus* and also as a probiotic for Pacific white shrimp.

Keywords: *LAB; Vibrio parahaemolyticus; AHPND; Litopenaeus vannamei*

1. Introduction

White shrimp (*Litopenaeus vannamei*) is one of the most important commercial species of shrimp in the world, and the production of aquacultured *L. vannamei* accounts for > 60% that of all farm-raised shrimp (Anderson *et al.*, 2017). However, in recent years vibriosis has been implicated as being one of the major causes of bacterial infections in shrimp aquaculture (Joshi *et al.*, 2014; Thitamadee *et al.*, 2016). Acute hepatopancreatic necrosis disease (AHPND), also known as early mortality syndrome (EMS), is one of the emerging shrimp diseases. This disease has decreased shrimp production and caused serious economic losses in these affected countries (Joshi *et al.,* 2014). One causative agent for AHPND is *Vibrio parahaemolyticus*, harboring toxin genes named $pirA^{vp}$ and $pirB^{vp}$ (Tran *et al.*, 2013; Lee *et al.*, 2015). To prevent and control diseases, antibiotics are considered the standard treatment for AHPND/EMS. Unfortunately, the imprudent use of antibiotics has resulted in the emergence of antibiotic resistant pathogens in aquaculture environments making the antibiotic treatment ineffective and this type of incident has been reported from all areas of aquaculture (Wang *et al*., 2018; Zuo *et al*., 2019).

Probiotics as live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Das *et al.*, 2017). There are several types of microorganisms used as probiotics in aquatic animals, including *Bacillus* sp., *Pseudomonas* sp., *Arthrobacter* sp., and Lactic bacteria, etc. (Ajitha *et al*., 2004; Chiu *et al*., 2007; Chythanya *et al*., 2002). The mechanisms of probiotic bacteria are beneficial actions include growth performance, disease resistance, increasing immune response, enzymatic contribution to digestion and can improve water quality (Akhter *et al*., 2015). The probiotic bacteria reduced foodborne pathogenic bacteria *in vivo* through many different processes, including antibacterial secretion, competition for nutrients and adhesion site, host immune modulation and stimulation. The antimicrobial activity against pathogenic bacteria is an important criterion required for probiotic selection (Luis-Villasenor *et al*., 2013; Wongsasak *et al*., 2015). However, the ability of probiotic to survive and dominate in shrimp gut environment is extremely crucial for the antagonistic effect. Therefore, isolation and selection of Lactic acid bacteria (LAB) from shrimp gut could be a potential approach to obtain the well adapted probiotic used as an effective bio-control agent for shrimp with high level of inhibition against *V. parahaemolyticus* (AHPND strain).

In this study, LAB inhibitory to *V. parahaemolyticus* were isolated and selected from digestive tract of wild shrimp and cultured shrimp, and competition of LAB to *V. parahaemolyticus* was evaluated both *in vitro*. This is another way to prevent disease and can reduce the amount of antibiotic and chemical consumption.

2. Materials and methods

2.1. Experimental samples, bacteria, media and reagents

Wild shrimp and cultured shrimp were obtained from fish markets in Pattani province, Thailand. De Man, Rogosa, and Sharpe (MRS), Thiosulfate Citrate Bile Salt Sucrose (TCBS) Agar, bromocresol purple, typhan blue and catalase. All media were supplemented with 1.5% NaCl.

Vibrio parahaemolyticus previously isolated from diseased shrimp was obtained from aquatic animal health research center, Songkla province, Thailand.*V. parahaemolyticus* was cultivated and maintained in Tryptic Soy Broth (TSB).

2.2. Isolation of LAB from digestive tracts of wild shrimp

LAB strains were isolated from digestive tracts of wild shrimp and cultured shrimp from fish markets in Pattani province, Thailand. Shrimp surface was sterilized with 70% ethanol. Thereafter, the intestinal tract of shrimps was removed and placed in test tubes containing 9 mL of sterilized 0.85% NaCl solution. Shrimp intestine was crushed by means of a stomacher to form a homogenate solution which was left for 5 min to settle down. The suspension was then diluted with sterilized 0.85% NaCl solution at 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions, respectively. 0.1 mL of each dilution was spread on MRS agar supplemented with 1.5% NaCl and incubated at 37 °C for 48 h. The difference colonies were later picked and purified by re-streaking on MRS agar containing 1.5% NaCl. The isolates were then selected based on their Gram positive and catalase-negative characteristics (Axelsson, 1993).

2.3. Screening of antibacterial producing LAB

LAB strains were grown in MRS broth supplemented with 1.5% NaCl (w/v) incubated at 37 °C for 48 h. The LAB supernatant (10 µL) was dropped on MRS agar supplemented with 1.5% NaCl and incubated at 37 °C for 48 h. The plates were thereafter overlaid with 10 mL BHI agar (0.7% agar) seeded with an overnight culture of *V. parahaemolyticus* at the final concentration of 10^6 CFU/mL. After 12-18 h of incubation, the clear zone around colonies were measured using a vernier caliper (Kongnum and Hongpattarakere, 2012).

2.3.1 Determination of antibacterial activity against *V. parahaemolyticus* (AHPND) by agar well diffusion method

LAB strains were cultivated in 5 mL MRS broth at 37 °C for 24 h. The LAB supernatant was sterilized by filtering through a 0.22 µm Millipore filter (Sartorius, Goettingen, Germany). The sterile aliquot (80 µL) was placed in a 7 mm-diameter agar well punched in a BHI agar, in which *V. parahaemolyticus* (10^6 CFU/mL) were seeded previously. After 12-18 h of incubation, the clear zone around each well was measured using a vernier caliper (Kongnum and Hongpattarakere, 2012).

2.4. Determination for producing digestive enzymes

2.4.1 Proteolytic activity

The culture broth of selected LAB was dropped onto a 2% skim milk MRS agar medium as a carbon source and a 1.5% (w / v) sodium chloride concentration and then incubated at 37 ° C for 24 h. The diameter of the halos formed by the proteolytic strains was measure in millimeters (Landeta *et al*., 2013).

2.4.2 Amylolytic activity

The culture broth of selected LAB was dropped onto a 2% soluble starch MRS agar medium as a carbon source and a 1.5% (w / v) sodium chloride concentration and then incubated at 37 ° C for 24 h. Test for starch digestion by placing iodine flakes on the lid of the agar plate. For the evaporation of iodine into contact with the culture medium to be tested if the starch is digested, the color of the agar will turn blue (Landeta *et al*., 2013).

2.4.3 Lipolytic activity

The culture broth of selected LAB was dropped onto a 2% tributyrin MRS agar medium as a carbon source and a 1.5% (w / v) sodium chloride concentration and then incubated at 37 ° C for 24 h. The diameter of the halos formed by the lipolytic strains was measure in millimeters (Landeta *et al*., 2013).

2.5. Analysis of 16S rDNA sequences for identification of the selected LAB

Bacterial DNA was extracted and the 16S rDNA gene was amplified by PCR using universal primer. DNA sequencing PCR product was purify using PCR purification kit. Nucleotide sequencing was carried out with an automated DNA sequencer by Ward Medic Ltd., Thailand and comparing the data of the sequence nucleotides obtained with a database in GenBank. (Kanjan and Hongpattarakere, 2017).

2.6. Competitiveness of *Enterococcus faecalis* TBPV1 in co-cultivation with *V. parahaemolyticus* (AHPND) *in vitro*

Overnight cultures of *E. faecalis* TBPV1 and *V. parahaemolyticus* were co-inoculated into the vial to achieve a final concentration of 10^6 CFU/mL of each. The viable pathogenic bacteria and LAB were counted at 0, 6, 12, 18, 24, 48 and 72 h of incubation by culturing on TCBS and MRS agar, respectively. It was ensured that LAB was not able to grow on TCBS by directly inoculating on the plate as a control (Kongnum and Hongpattarakere, 2012).

2.7. Statistical analysis

SPSS software of 22.0 version was used to compare the mean between different treatments and one-way analysis of variance (ANOVA) used to analyze the data. Duncan's multiple range test was used to determine the significant difference among treatments at a 0.05 significance level.

3. Results and discussion

3.1. Isolation and purification of LAB strain from digestive tracts of wild shrimp and cultured shrimp

A total of 50 LAB strains were isolated from digestive tract of wild shrimp and cultured shrimp in Pattani province, Thailand. The study revealed that gut of shrimp were good sources for LAB inhabitation. This is in good agreement with Dempsey *et al.* (1989) proposed that the number of LAB in digestive tracts of shrimp were $7.5x10^6$ -$2.6x10^6$ CFU/mL. Most LAB isolates obtained in this work were coccoid shape and all isolates were catalase-negative (Table 1). Coccoid LAB such as *Streptococcus*, *Lactococcus* and *Enterococcus* are very common and prevalent LAB in the digestive tract of aquatic animals than those in rearing seawater (Kongnum and Hongpattarakere, 2012). LAB inhibitory against were isolated in greater number from wild shrimp than cultured shrimp indicating high prevalence and diversity of LAB in the wild shrimp (Moss *et al.*, 2006).

Table 1. Characteristics of LAB isolated from digestive tract of wild shrimp and cultured shrimp in Pattani province, Thailand

Sample	Total Isolates	Colony Shape	Cell Shape	Gram-Staining	Spore-Forming	Catalase
digestive tract of wild shrimp	16	Opaque, Creamy, Smooth round	Rod shape	+	-	-
digestive tract of cultured shrimp	34	Opaque, Creamy, Smooth round	Rod shape	+	-	-

3.2. Antimicrobial activity of LAB against *V. parahaemolyticus* (AHPND)

The total of 50 isolates of LAB were tested for antibacterial activity against *V. parahaemolyticus* by agar spot assay and agar-well diffusion method. Among these, six isolates, including BFPM9, BFPM12, TBPV1, TBPV8, TBPV9 and TBPV10, were found to inhibit the growth of *V. parahaemolyticus* with inhibition zone ranging from 18.0 to 29.0 mm (Table 2 and Figure1) by agar spot assay. In the agar-well diffusion method, the strain of TBPV1 showed the highest inhibition zones greater than 8 mm against *V. parahaemolyticus* (Table 3). Similarly, Vázquez *et al.* (2005) reported that lactic and acetic acids but not the bacteriocins produced from probiotics (LAB isolated from fermented foods) were responsible for the inhibition against fish pathogens such as *Carnobacterium piscicola*, *Vibrio alginolyticus*, *V. pelagius* and *V. splendidus*. Also, Truc *et al.*, (2019) reported that the supplementation of three LAB strains including, *L. plantarum, L. fermentum* and *P. pentosaceus*, can reduce the mortality of shrimp with challenging *V. parahaemolyticus* AHPND strain.

Table 2. Diameter of inhibition zones by LAB isolates against *V. parahaemolyticus* by agar spot assay

Isolates	Inhibition zones (mm)
BFPM9	21.50 ± 0.65^{b}
BFPM12	29.00 ± 0.41^{a}
TBPV1	19.75 ± 0.25^{c}
TBPV8	19.50 ± 0.29^{c}
TBPV9	20.00 ± 0.00^{c}
TBPV10	18.75 ± 0.63^{c}

Data (mean ± SE) with different superscripted letters (a-c) significantly differ ($P < 0.05$) among treatments. Shows inhibition of zones 18 to 29 mm.

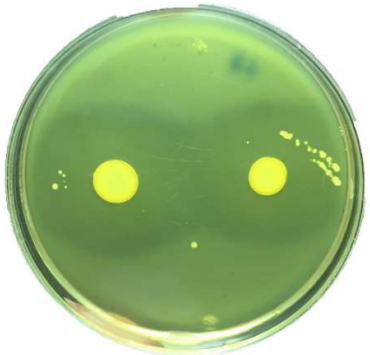

Figure 1. Inhibition zones of *E. faecalis* TBPV1 against *V. parahaemolyticus* by agar spot assay

Table 3. Diameter of inhibition zones by LAB isolates against *V. parahaemolyticus* by agar well diffusion method

Isolates	Inhibition zones (mm)
BFPM9	6.25±0.25^{b}
BFPM12	6.75±0.25^{b}
TBPV1	8.00±0.00^{a}
TBPV8	6.00±0.00^{b}
TBPV9	6.25±0.25^{b}
TBPV10	6.00±0.00^{b}

Data (mean ± SE) with different superscripted letters (a-b) significantly differ ($P < 0.05$) among treatments.

3.3. Determination of digestive enzymes

The six LAB strains were tested for digestive enzymes. All isolates exhibited protease activity with the clear zone diameter greater than 10 mm. The isolate of TBPV1 showed the highest activity, whereas only 2–11 mm zones were observed from the others. However, all isolates could not produce amylase and lipase activities (Table 4). It is widely believed that the higher activity of digestive enzymes in the digestive system can increase digestive capacity and host growth efficiency (Suzer *et al.*, 2008). Proteases, lipase and amylase are effective enzymes in a wide variety of metabolism for proteins, lipids, and carbohydrates, respectively (Liu *et al*., 2009).

Table 4. Extracellular enzyme production test of 6 candidate probiotic isolates

Isolates	Protease (mm)	Lipase (mm)	Amylase (mm)
BFPM9	10.75±0.25^{b}	-	-
BFPM12	7.75±0.25^{e}	-	-
TBPV1	11.25±0.25^{a}	-	-
TBPV8	9.00±0.00^{d}	-	-
TBPV9	9.75±0.25b^{c}	-	-
TBPV10	9.25±0.25^{cd}	-	-

Zone of enzyme activity was shown as the Data (mean ± SE) with different superscripted letters (a-g) significantly differ ($P < 0.05$) among treatments, - indicate no zone of enzyme activity.

3.4. Identification of LAB isolates by 16S rRNA gene sequencing

Regarding 16S rDNA nucleotide sequences, LAB strains of TBPV1 based on the nucleotide sequence of its 16S rDNA showed >99 % homology to *Enterococcus faecalis* (GenBank accession number CP005942.1). Enterococci are normal populations of the human and animal gastrointestinal tract. These bacteria are everywhere and are used in the food industry as probiotics (Franz *et al.*, 2003). Some strains of enterococci produce multiple bacteria simultaneously, which gives enterococci an advantage in competition with other microorganisms for colonization and control (Hanchi *et al.,* 2018). Probiotic *Enterococcus* species such as *E. faecalis, E. faecium, E. lactis,* and *E. hirae* has been reported (Adnan *et al*., 2017). These bacteria were found to be resistant to the harsh conditions of the digestive tract, acidity and bile salts (Baccouri *et al.,* 2019).

3.5. Inhibitory activity of LAB against *V. parahaemolyticus* in co-cultivation

The co-cultured test of *E. faecalis* TBPV1 and *V. parahaemolyticus* showed that *E. faecalis* TBPV1 was found to completely inhibit *V. parahaemolyticus* at 12 h, whereas *E. faecalis* TBPV1 increased from 5.29 to 9.47 Log CFU/mL (Figure.2), in which the inhibitory activity occurs as a result of organic acids that *E. faecalis* TBPV1 produced. Experiments show that lactic bacteria are more competitive than *V. parahaemolyticus*. This may be the result of lactic bacteria producing inhibitors *V. parahaemolyticus*. The inhibitors produced by lactic bacteria are lactic acid, hydrogenperoxide, diacetyl, reuterin, reutericyclin, pyroglutamic acid and bacteriocin. These inhibitors are effective in inhibition Gram-positive and Gram-negative pathogenic bacteria (Ouwehand and Vesterlund, 2004). In addition, studied the inhibition of *V. harveyi* by co-culture experiment with *L. plantarum* MRO3.12 was found to inhibit the growth of *V. harveyi* was completely at 24 h (Kongnum and Hongpattarakere, 2012). Therefore, the application of lactic bacteria as probiotics in the treatment of aquatic animal disease may be very possible.

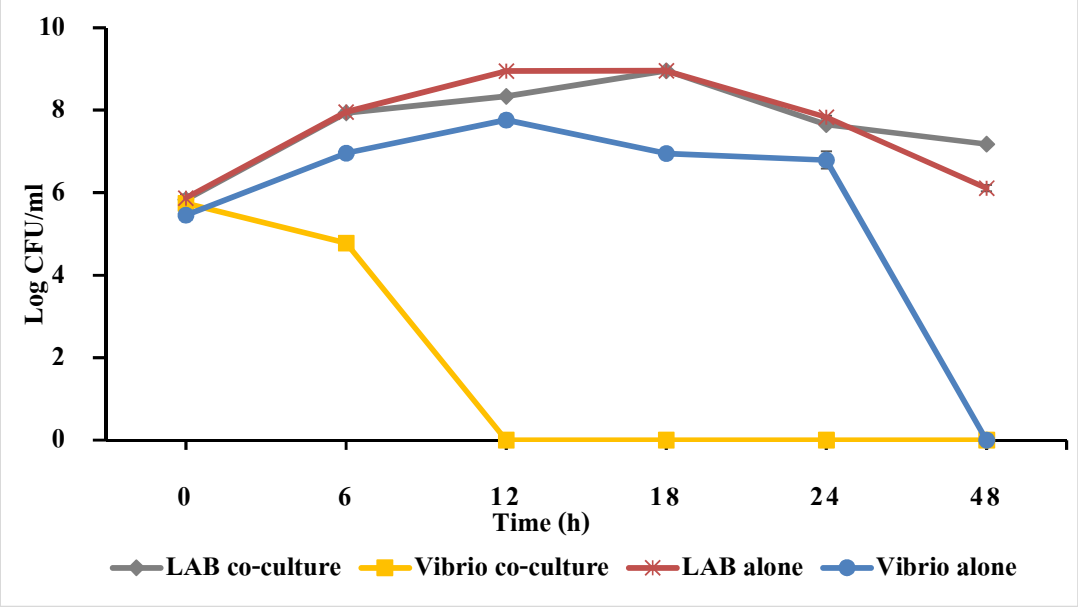

Figure 2. Growth of *V. parahaemolyticus* and *E. faecalis* TBPV1 in co-culture system as 48 h

4. Conclusions

The results of this study illustrated that *E. faecalis* TBPV1 isolated from digestive tract of cultured shrimp has a potential for use as antibacterial substance in *V. parahaemolyticus* (AHPND strain) control during Pacific white shrimp cultivation. This strain also produced extracellular protease enzyme that could promote the growth performance of shrimp cultivation. This strain therefore can be effectively applied for controlling AHPND/EMS in the aquaculture. This strategy would decrease each antibiotic level introduced into the environment, thereby reducing the toxicity to consumer as well as the development of resistant pathogenic strains.

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AQ 03

Physical Pretreatments of Food Waste and Its Possible Potential as Diet for Juvenile Striped Catfish (*Pangasianodon hypophthalmus*)

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Abstract

Food waste (FW) is a neglected source of inexpensive and readily available nutrients for use as feedstuff. In the present study, FW was pretreated by various physical pretreatment methods (boiling, autoclaving, microwave irradiation and hot air oven drying) as compared to non-pretreated FW. Significant changes in proximate chemical composition (crude protein, crude lipid, crude fiber and nitrogen-free extract) were observed between pretreated and non-pretreated FW and varied between pretreatment methods ($P < 0.05$), while ash content and available energy did not differ across all groups. Physicochemical properties in aspects to enhance enzymatic hydrolysis (pH, turbidity, relative crystallinity, thermal properties and microstructure) indicate the suitable pretreatment methods of autoclaving and microwave irradiation. The both pretreatment protocols also provided superior *in vitro* digestibility of protein and carbohydrate using digestive enzymes extracted from juvenile striped catfish (*Pangasianodon hypophthalmus*), followed by boiling pretreatment. These findings indicate that autoclaving and microwave irradiation, followed by boiling, were the suitable pretreatment methods for improving FW quality in striped catfish aquafeed production.

Keywords: *autoclaving, boiling, chemical composition, in vitro digestibility, microwave irradiation, physicochemical property*

1. Introduction

Aquafeed is one of the major factors contributing to aquaculture, often amounts to more than 60% of aquaculture production costs **(**Rana, Siriwardena, & Hasan, 2009). While commercial diet tends to be expensive, an approach to reduce the cost of feedstuff is replacing or supplementing expensive commercial diets with locally available feedstuffs. Generally, most of substitute raw materials are plant products or agricultural by-products. These sources might contain growth inhibitors and anti-nutritional factors, and lack some essential amino acids (Nasser, Abiad, Babikian, Monzer, & Saoud, 2018). At present, researching various raw materials that is an inexpensive and has readily available nutrient for use as feedstuff is necessary.

Food waste (FW) is a neglected source of nutrients obtained from cooked and plate wastes. Globally, 1/3 of all food produced or approximately 1.3 billion tons of food per year will spoil and classify as FW (FAO, 2011). Researchers have shown that waste disposal by incineration methods may not be suitable due to FW has high moisture content and low energy (Zhuang, Wu, Wang, Wu, & Chen, 2008), while landfill methods often generate large amounts of methane which can cause pollution problems, and also distribute various pathogens into water source, affecting people's living hygiene (Mirabella, Castellani, & Sala, 2014). Since FW has a relatively high nutritional content (Khalid, Naseer, Shahid, & Shah, 2019; Wong, Mo, Choi, Cheng, & Man, 2016), so that it is reasonable to reuse FW as raw material for feedstuff.

For improving FW quality, supplementation of enzymes, prebiotics, probiotics, vitamin-mineral premix and medicinal herbs has been used for enhancing digestion and feed utilization in animals (Wong et al., 2016). However, these methods require substantial chemicals, parts of plant, or components or cells of microorganism, leading to increase in production cost of the diet. Recently, physical pretreatment methods have been used to improve chemical composition and physicochemical properties of feedstuffs in aquafeed production (Sansuwan et al., 2017; Thongprajukaew, 2014; Thongprajukaew, Rodjaroen, Tantikitti, & Kovitvadhi, 2015). The convenient methods, such as boiling, autoclaving, microwave irradiation, and hot air oven drying, can be applied for a number of feedstuffs (Alajaji & El-Adawy, 2006; Chumwaengwapee, Soontornchai, & Thongprajukaew, 2013; Deka & Sit, 2016; Rehman & Shah, 2005). These methods significantly alter physicochemical properties for enhancing enzymatic hydrolysis *in vitro*, such as pH, water solubility, relative crystallinity, thermal properties and microstructure (Chumwaengwapee et al., 2013; Chung et al., 2010; Thongprajukaew, 2014).

Therefore, the present study aimed to investigate the suitable pretreatment method for improving FW quality. The proximate chemical composition and physiocochemical properties in aspects to enhance enzymatic hydrolysis were used as criteria. Nutrient availability was accessed through *in vitro* digestibility based on digestive enzymes extracted from commercially important fish, striped catfish (*Pangasianodon hypophthalmus*). Findings from the current study could be used as practical guideline for preparing FW in striped catfish aquafeed production.

2. Materials and Methods

2.1 FW sampling

FW was freshly collected from three cafeterias at Prince of Songkla University (09.00 to 10.00 h.) for four consecutive weeks. The samples (n = 3) were mixed, packed in polyethylene bags and kept at –20°C until use. Classified FW from the current study contained cereal (55.82% of fresh weight), animal by-products (24.74%), vegetables (18.13%) and mixed FW (1.31%).

2.2 Pretreatment of FW

The FW samples from each replicate were equally divided into five portions prior to pretreatment. Non-pretreated FW was used as a control. Boiling and autoclaving pretreatments were conducted by placing100 g of FW in 600 mL beaker, mixed with distilled water (1: 0.5 *w*/*v*) and then boiled at 100°C for 5 min (Alajaji& El-Adawy, 2006), or autoclaved at 121°C for 10 min (Rehman & Shah, 2005). Microwave-irradiated samples were prepared by mixing 100 g of FW as described above and then irradiated at 800W for 5 min (Chumwaengwapee et al., 2013). For hot air oven drying, 100 g of FW were dried at 60°C for 24 h (Deka & Sit, 2016). After pretreatment, all FW samples were dried with a freeze dryer (Delta 2-24 LSC; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 24 h, ground, and then kept in a desiccator for later analysis of chemical composition, physicochemical properties and *in vitro* digestibility.

2.3 Proximate chemical composition

Moisture, crude protein, crude lipid, crude fiber and ash contents were analyzed according to standard methods of AOAC (2005). Nitrogen-free extract (NFE, %) and gross energy (GE, kcal kg^{-1}) were calculated from 100 – (moisture + crude protein + crude lipid + ash + crude fiber) and from (crude protein × 5.6) + (crude lipid × 9.44) + (crude fiber × 4.1) + (NFE × 4.1), respectively. All the chemical analyses were performed in triplicate and are reported on % dry matter basis.

2.4 Physicochemical properties

The pH was determined according to the method of Sokhey and Chinnaswamy (1993). One gram of freeze-dried FW was mixed with 25 mL of distilled water. The mixture was shaken with orbital shaker at 200 rpm for 10 min, and then the pH was determined by a pH meter.

The turbidity of freeze-dried FW was analyzed according to the method as described by Thongprajukaew et al. (2015). An aqueous suspension (1% *w*/*v*) of FW samples was kept at 90°C for 1 h under 100 rpm agitation. The suspension was stored at 4°C for 48 h. The light transmittance of the supernatant was spectrophotometrically measured at 640 nm against distilled water.

Relative crystallinity of freeze-dried FW was determined with x-ray diffractometer (X' Pert MPD; Philips, Amsterdam, Netherlands), operated at 40 kV voltage and 40 mA current. The diffractograms were recorded for 4 to 35° (2θ), with a scanning rate of 2° min^{-1}. Relative crystallinity was calculated from the ratio of peak area to total area by using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA).

Thermal properties of freeze-dried FW were determined with a differential scanning calorimeter (DSC7; Perkin Elmer, Waltham, MA, USA). Three milligrams of samples were placed in an aluminum pan, sealed, allowed to equilibrate at room temperature for 1 h, and then heated from 40 to 400°C at a rate of 10°C min^{-1}. The transition enthalpy (ΔH) was automatically detected from the thermograms.

Microstructure of freeze-dried FW was studied by using scanning electron microscopy (Quanta 400; FEI, Brno, Czech Republic). The samples were mounted with double-sided adhesive tape on an aluminum stub and coated with gold. The photography was taken at 10,000× and 25,000× magnifications and accelerating voltage was set at 20 kV.

2.5 *In vitro* digestibility

The preparation and euthanasia of fish for *in vitro* digestibility study conformed to the "Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes", National Research Council, Thailand (Application No. U1-06514-2560), and were approved by Institutional Animal Care and Use Committees (Project Code 2562-01-022). The juvenile striped catfish (2.12–3.37 g body weight) were collected from a farm in Songkhla province of Thailand. The fish were sacrificed by chilling in ice. The digestive enzymes were extracted by homogenizing fish intestine with 0.2 M Na_2HPO_4-NaH_2PO_4 buffer pH 8 (1: 4 *w*/*v*) using a tissue micro-homogenizer (THP-220; Omni International, Kennesaw, GA, USA) for 30 seconds. The homogenate was centrifuged at 15,000 ×*g* for 30 min at 4°C. The supernatant was collected after removing lipid layer. The crude enzyme extracts were dialyzed with 50 mM Na_2HPO_4-NaH_2PO_4 buffer pH 8 for 24 h and then kept at –20°C until use for *in vitro* digestibility.

The *in vitro* digestibility reaction was performed according to the method as described by Thongprajukaew, Kovitvadhi, Kovitvadhi, Somsueb, and Rungruangsak-Torrissen (2011). The reaction mixture contained 5 mg of freeze-dried FW, 10 mL of 50 mM Na_2HPO_4-NaH_2PO_4 buffer (pH 8), 50 µL of 0.5% chloramphenicol, and 125 µL of dialyzed crude enzyme extracts. The reaction was performed at 30°C and 200 rpm agitation for 24 h, and was terminated by boiling at 100°C for 10 min. The digested solution was used for determining protein and carbohydrate digestibilities, as described by Rungruangsak-Torrissen et al. (2002)

and Areekijseree et al. (2006), respectively. The reaction mixture of protein digestibility assay contained 100 µL of digested solution, 1 mL of 50 mM Na_2HPO_4-NaH_2PO_4buffer (pH 8) and 0.5 mL of 0.1% trinitrobenzenesulphonic acid. These mixtures were incubated in the dark at 60°C for 1 h, followed by adding 0.5 mL of 1 M HCl. The protein digestibility was spectrophotometrically measured at 420 nm and compared with linear ranges of *DL*-alanine standard curve. The reaction mixture of carbohydrate digestibility assay contained 1 mL of digested solution and 0.5 mL of 1% dinitrosalicylic acid. These mixtures were boiled at 100°C for 5 min. The carbohydrate digestibility was spectrophotometrically measured at 540 nm against linear ranges of maltose standard curve.

2.6 Statistical analysis

The experiment setup was followed completely randomized design. Data are reported as mean ± SEM from triplicate observations. Statistical analysis is performed using SPSS Version 17 (SPSS Inc., Chicago, USA). One-Way ANOVA was used to compare means among treatments. Duncan's multiple range test for mean comparison was tested at 0.05 significance level.

3. Results and Discussion

3.1 Chemical composition of non-pretreated and pretreated FW

Physical pretreatment methods had significant effects on proximate chemical compositions ($P < 0.05$), except for ash content and GE (Table 1). Significantly decreased crude protein in boiled, autoclaved and hot air oven dried FWs might be possible due to the denaturation of protein (Sakla, Ghali, El Farra, & Rizk, 1988) or the partial loss of amino acids after pretreatment. These can cause significant change in the protein content, as detected by the Kjeldahl method. However, protein content was still maintained in microwave-irradiated FW. Modification of the molecular properties of protein by forming covalent cross-linkages or by conversion to higher molecular weight aggregates would recover the amount of protein as observed in non-pretreated group (Sadeghi & Shawrang, 2007). For crude lipid, relatively high amount was observed across all groups, and significantly decreased lipid content was influenced by all physical pretreatment methods. The oxidation of unsaturated fatty acids during cooking, depending on time and temperature of processing, may cause loss of lipids in pretreated FW (Stewart, Raghavan, Orsat, & Golden, 2003; Malheiro et al., 2009).

Table 1 The proximate chemical composition of non-pretreated and pretreated FW. Data were calculated from triplicate determinations and are expressed on % of dry weight basis.

Composition	Control	Boling	Autoclaving	Microwave irradiation	Hot air oven
Crude protein	22.18 ± 0.02^a	21.22 ± 0.06^b	21.28 ± 0.05^b	22.04 ± 0.06^a	20.34 ± 0.04^c
Crude lipid	16.75 ± 0.21^a	15.85 ± 0.04^b	15.75 ± 0.17^b	14.45 ± 0.02^d	14.92 ± 0.07^c
Crude fiber	1.29 ± 0.01^a	1.11 ± 0.04^b	1.21 ± 0.03^a	1.14 ± 0.05^b	1.09 ± 0.04^b
Ash	3.76 ± 0.05	3.64 ± 0.04	3.56 ± 0.05	3.81 ± 0.03	3.50 ± 0.13
NFE	56.02 ± 4.36^c	58.18 ± 8.35^b	58.20 ± 4.17^b	58.56 ± 4.19^b	60.15 ± 3.97^a
GE (kcal kg^{-1})	517.30 ± 46.83	511.54 ± 48.31	511.43 ± 48.20	504.60 ± 48.18	505.83 ± 49.74

NFE, nitrogen-free extract; GE, gross energy.
Data are expressed as mean ± SEM ($n = 3$).
Differences between means were tested with Duncan's multiple range test.
Different superscripts in the same row indicate a significant difference ($P < 0.05$).

Physical pretreatments can disrupt the main cell wall constituents (Sansuwan et al., 2017), so that crude fiber content decreased in boiled, microwave-irradiated and hot air oven dried FWs. On the other hand, similar content was observed in autoclaved FW relative to non-pretreated group. The formation of protein-fiber complexes after pretreatment might contribute such effect (Bressani, 1993). Significant changes in crude fiber, as well as other constituents above, lead to increase in amount of NFE in all pretreated groups (Thongprajukaew et al., 2015). This desirable characteristic would improve the utilization of NFE in FW.

3.2 Physicochemical properties of non-pretreated and pretreated FW

Significant changes in physicochemical properties varied by pretreatment methods (Table 2). Statistically increased pH was observed in all pretreated groups. Such effect is postulated by the release of hydroxyl groups from lignocellulosic degradation after physical pretreatment (Thongprajukaew et al., 2013). This finding is in agreement with the decrease of crude fiber contents in some pretreated groups. Significantly increased turbidity was observed in all pretreated groups, except for hot air oven dried FW. Thisrelates to some phenomena in physicochemical changes of NFE, such as granule swelling, granule remnants, leached amylose, and amylopectin chain length (Jacobson, Obanni, & BeMiller, 1997). These elements dissolve in solution after pretreatment and reflect or scatter light significantly (Perera & Hoover, 1999).

Table 2 Physicochemical properties of non-pretreated and pretreated FW. Data were obtained by triplicate observations.

Physicochemical properties	Control	Boling	Autoclaving	Microwave irradiation	Hot air oven
pH	5.18 ± 0.01^{c}	5.31 ± 0.01^{a}	5.24 ± 0.01^{b}	5.26 ± 0.01^{b}	4.01 ± 0.00^{d}
Turbidity (A_{640})	0.032 ± 0.002^{d}	0.065 ± 0.003^{b}	0.046 ± 0.004^{c}	0.095 ± 0.002^{a}	0.036 ± 0.004^{d}
X-ray diffractometer					
RC (%)*	4.38	3.57	3.35	3.52	5.19
A (%)	95.62	96.43	96.65	96.48	94.81
RC/A	0.05	0.04	0.03	0.03	0.05
Differential scanning calorimeter					
ΔH Peak I (J g^{-1})	25.57 ± 15.31^{b}	86.58 ± 19.27^{a}	24.85 ± 0.86^{b}	32.95 ± 6.67^{b}	11.57 ± 8.16^{b}
ΔH Peak II (J g^{-1})	55.77 ± 35.34^{ab}	56.55 ± 4.59^{ab}	38.78 ± 2.91^{ab}	89.99 ± 4.39^{a}	18.92 ± 0.03^{b}
ΣΔH (J g^{-1})	81.35 ± 20.04^{bc}	143.15 ± 14.67^{a}	63.63 ± 2.05^{cd}	122.94 ± 11.07^{ab}	30.49 ± 8.02^{d}

RC, relative crystallinity; A, amorphous; RC/A, relative crystallinity/amorphous
Data are expressed as mean ± SEM ($n = 3$).
Differences between means were tested with Duncan's multiple range test.
Different superscripts in the same row indicate a significant difference ($P < 0.05$).

Relative crystallinity, determined by x-ray diffractometer, obstructs the capacity of animals to digest feedstuffs. In the current study, decreased crystallinity of FW pretreated by boiling, autoclaving and microwave irradiation indicates significant improvement in feedstuff quality by expansion of amorphous region (Table 2). Similar results were found for enthalpic response (ΔH) of FW determined by DSC. The presence of peaks I at low temperature (42.3 to 125.2°C) indicates the thermal response of available nutrients (mainly protein, lipid and NFE), while peak II at high temperature (276.0 to 348.1°C) relates to amount of unavailable nutrients (mainly crude fiber) (Jualaong et al., 2020). therefore, FW quality was improved by boiling, autoclaving or microwave irradiation, as indicated by increased ΔH of peak I or ΔH of peak II. However, negative effects from relative crystallinity and thermograms were observed in FW pretreated by hot air oven.

Microstructure can be used as criteria for assessing feedstuff quality (Chumwaengwapee et al., 2013; Thongprajukaew et al., 2012, 2015). In the current study, general morphology and surface roughness were similar in FW pretreated by boiling, autoclaving and microwave irradiation (Figure 1). The fusion of gelatinized compartments was most prominent in these treatments, whereas it was nearly absent in non-pretreated and hot air oven-dried FW. Since FW contained high amount of moisture, NFE and crude protein, so that the hydrothermal pretreatment methods can efficiently alter physicochemical properties via gelatinization and denaturation processes, relative to hot air oven drying method.

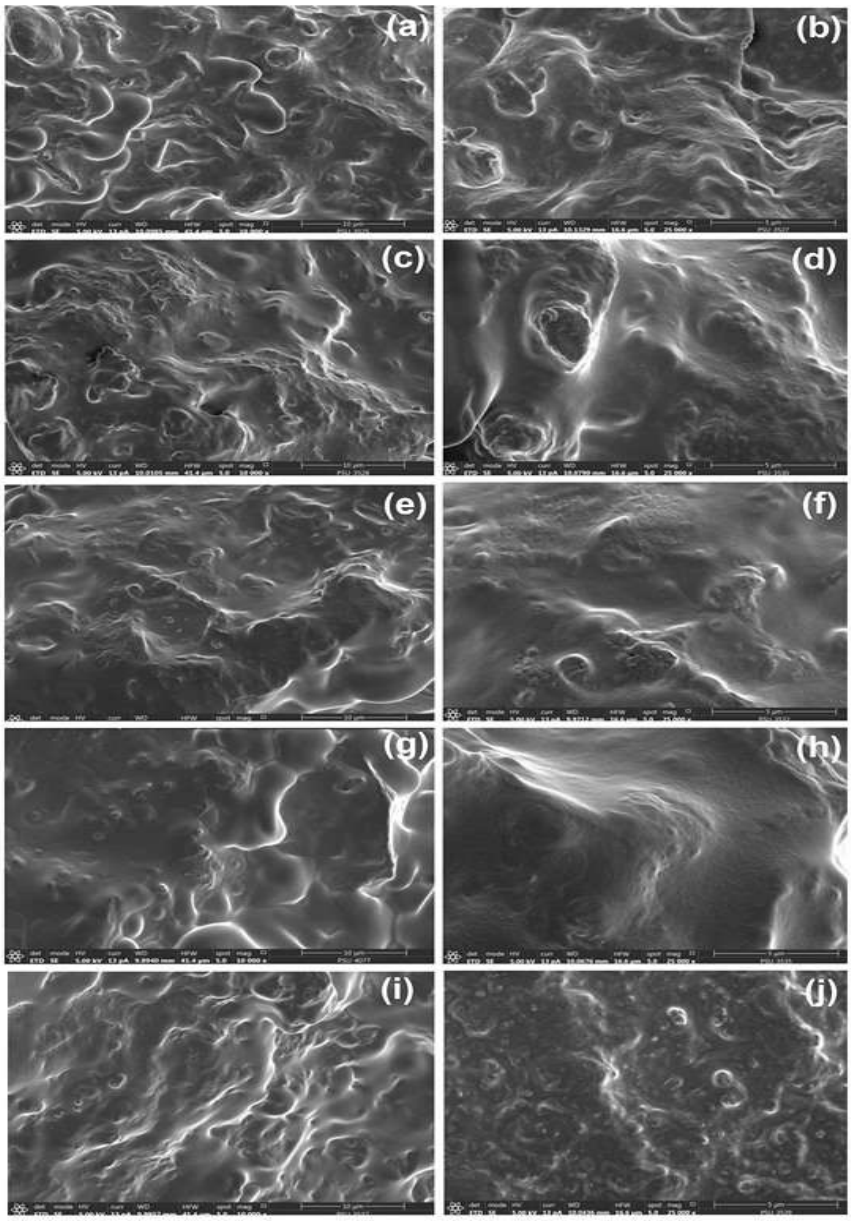

Figure 1 Microstructure of non-pretreated FW (a and b) and pretreated FW by boiling (c and d), autoclaving (e and f), microwave irradiation (g and h) and hot air oven drying (I and j). The photographs were taken at 10,000× (left panel) and 25,000× (right panel) magnifications.

3.3 *In vitro* digestibility using digestive enzymes from striped catfish

Based on growth and feeding trials, raw FW or FW supplemented with amino acids or vitamin-mineral premix could be used for rearing various fish species (Al-Ruqaie, 2007; Bake, Endo, Satoh, Sadiku, & Takeuchi, 2013; Cheng, Mo, Lam, Choi, & Wong, 2015; Choi, Lam, Mo, & Wong, 2016; Mo et al., 2014; Nasser et al., 2018). Pretreatment by boiling has been suggested by Sugiura, Yamatani, Watahara, and Onodera (2009) for safety purpose, while information on nutrient utilization in reared animals has not been focused. In the current study, the highest *in vitro* digestibility of protein was obtained in FW pretreated by microwave irradiation and autoclaving, followed by boiling, while carbohydrate digestibility also improved in these three treatments (Figure 2). These match well with physicochemical changes of FW after pretreatments. These indicate that higher amount of pretreated FW might be included in fish diet rather than the use of raw FW as previously reported. The use of these three methods for improving digestibility have been reported for some raw materials, such as pigmented rice (Thuengtung, Matsushita, & Ogawa,

2019), cowpea (Torres, Peters, & Montoy, 2019), pigeon pea flour (Sun, Ohanenye, Ahmed, & Udenigwe, 2020) and fish bone powder (Nawaz et al., 2020), as well as for feed mixture for fish (Sansuwan et al., 2017; Thongprajukaew et al., 2011, 2015).

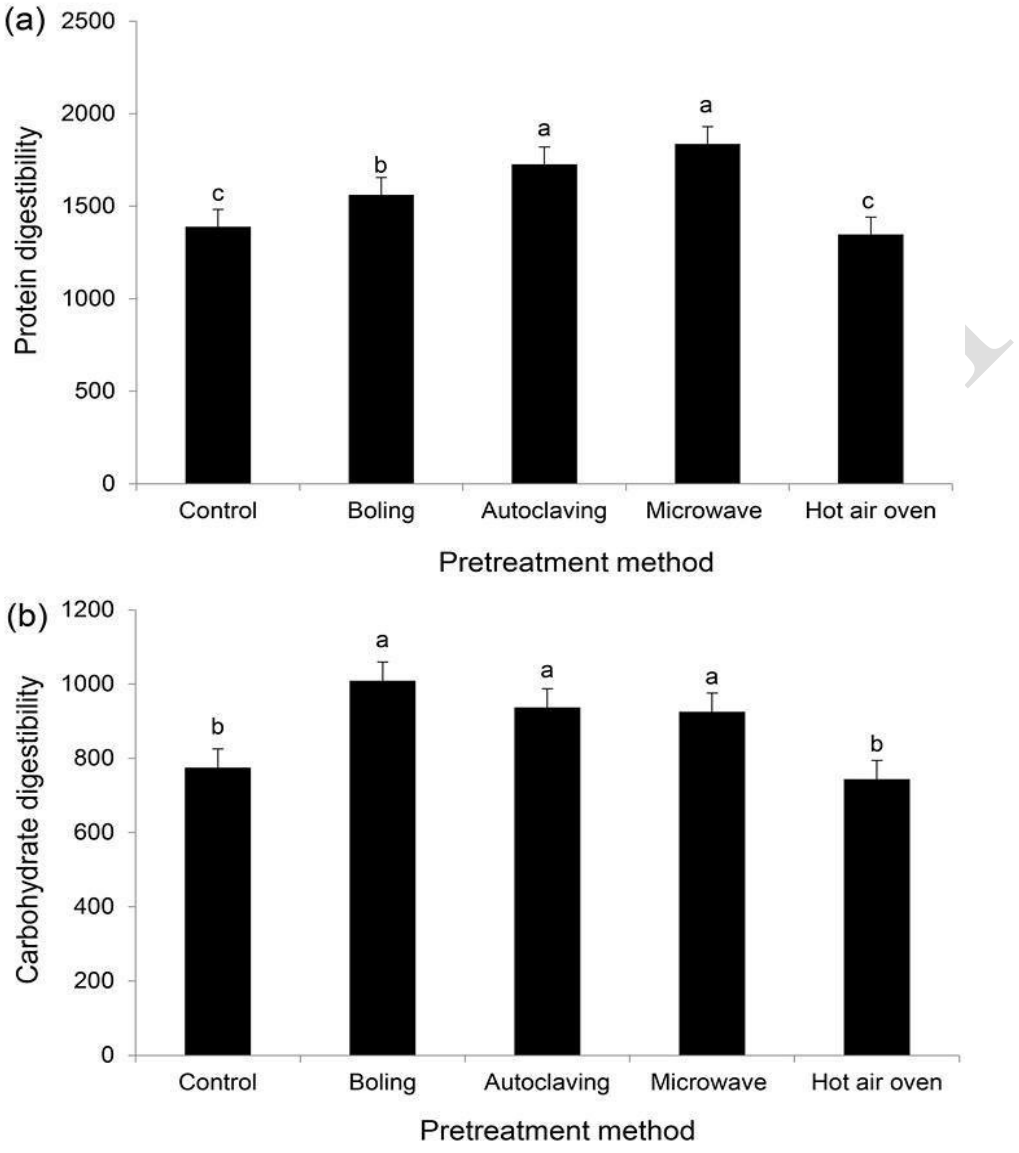

Figure 2 *In vitro* digestibility of protein (a, µmol *DL*-alanine equivalent g FW^{-1}) and carbohydrate (b, µmol maltose g FW^{-1}) of non-pretreated and pretreated FW, using digestive enzyme extracts from striped catfish. Data are expressed as mean ± SEM from triplicate determinations. The values with different superscripts are significantly different ($P < 0.05$).

4. Conclusions

Physical pretreatments of food waste had significant effects on chemical composition, physicochemical properties in aspects to enhance enzymatic hydrolysis, and *in vitro* digestibility based on digestive enzymes extracted from striped catfish. Findings from the current study indicate that autoclaving and microwave irradiation, followed by boiling, are suitable pretreatment methods. These processes could be used to prepare food waste for stripe catfish aquafeed production. Practically, the pretreated food waste (without drying) can be incorporated in the diet preparation which reduces the amount of additional water required for pelleting process. Optimization of the conditions for these hydrothermal pretreatments might significantly improve the food waste quality.

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AQ 04

Role of Pom-nang Seaweed, *Gracilaria* spp. on the Growth and Survival of Juvenile Mud Crab, *Scylla paramamosain*

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Abstract

Effect of diets with different level of Pom-nang seaweed powder (PSP) supplementations and the usage of Pom-nang seaweed as shelter for growth and survival of *Scylla paramamosain* crablets were conducted using crablets with an initial body weight of 0.02 g. Two factors including five dietary treatments (PSP0, PSP2, PSP4, PSP6, and control; mysid shrimp) and two shelter treatments (with and without shelter) were designed in this study. It found that different diets had significant effects on WG, SGR, PER ($P<0.05$) but not for FCR and survival rate of the crabs ($P>0.05$) (Table 2). However, crabs living with seaweeds as shelter had significantly different WG, SGR, PER, FCR and survival rate compared to those without seaweeds as shelter $P<0.05$). There were interaction effects of the combined factor on WG, SGR and PER ($P<0.05$). Shrimp was the most appropriate food for nursing crablets *S. paramamosain* and formulated diets supplemented with seaweeds is a potential feed to replace shrimp. The use of *Gracilaria* as shelter is essential for nursing crablets as indicated by all growth performances and survival rate. Additionally, the combination of seaweed as shelter and the formulated diets with 4% seaweed had the highest values of all growth performances and survival rate compared to others.

Keywords: *Juvenile Mud Crab, Gracilaria spp., Growth Rate, Survival Rate, Crablet*

1. Introduction

Mud crabs are commercially important species in Thailand and highly accepted as one of the main sources of food for human consumption (Kornthong et al., 2019). Several provinces in Thailand such as Chantaburi, Trat, Samutsongkhram, Ranong, and Trang have been cultured mud crab for more than two decades (Areekijseree, Chuen-Im, & Panyarachun, 2010). With high demand both in local and international markets, they are one of the main target species required for aquaculture. However, in most of the culturing practice, the juvenile mud crabs were currently collected from wild. Production from hatchery cannot sustain the demand from farming industry. In Thailand, the production of mud crabs decreased during 2012 – 2017 from around 2,900 to 400 tons and the total value decreased from 424.2 to 74.9 million Baht (DOF, 2019).

Nursing of crablets, *Scylla paramamosain*, is a crucial stage for the success of mud crab culture. However, there is a paucity of knowledge and information on the crablets rearing especially in Thailand. Factors that promoting optimum growth and survival are essential, but the information is very limited on especially appropriate food for nursing crablets. Naturally, mud crabs consume marine detritus, mangrove plants, seagrass, mollusks, crustaceans and fish (FAO, 2011).

Macroalgae has been used to develop low-cost feed for aquatic animal and supplements in aquaculture due to their nutritional value and to replace animal ingredients (Niu et al., 2015). It has been supplemented as a source of nutrient food materials, water quality improvement and habitat for aquatic animal including mud crab. Trino, Millamena, and Keenan (1999) used seaweeds such as *Gracilariopsis* and bamboo as shelters for mud crab *Scylla serrata* in grow-out ponds to provide substrates or shelters, adequate feed and reduce stocking density in nursery systems. This could lead to reduce cannibalism of mud crabs (Ut, Vay, Nghia, & Hanh, 2007). *Gracillaria* or Pom-nang seaweed has long been cultured in Thailand and used to promote the success of shrimp and crab culture (Nguyen, 2015). Normally, the main components of Pom-nang seaweed including carbohydrates, protein, and polysaccharides play an important role in growth of crustaceans (Holme, Zeng, & Soutgate, 2009). Many studies indicated that seaweed supplementation contributed to an improvement of growth, feed utilization, carcass quality and immune response of cultured fish or shrimp. However, total replacement of fish meal by seaweeds showed negative impact on growth and feed efficiency of shrimp (Yu et al., 2016). It is postulated that partial supplementation of seaweed to crab diet may promote growth rate, survival rate of mud crab, especially during juvenile stages.

Therefore, study on diets with different Pom-nang seaweed level supplemented, and Pom-nang seaweed used as shelter to determine growth rate and survival rate of crablets is crucial and has potential to indirectly develop mud crab industry. This study is aimed to assess impact of diets with different level of seaweed supplemented and using seaweed as shelter on growth and survival of juvenile mud crab. The knowledge obtained from this study will contribute to an advancement of nursing technique for *S. paramamosain* and lead to commercial production of the juvenile mud crab.

2. Materials and Methods

2.1 Diet preparation

Pom-nang seaweeds (*Gracilaria* spp.) were obtained from Muang Pattani district, Pattani province. They were washed and dried by sunlight before finely grounded by using a laboratory mill. Feed formulations consisted of fish meal, squid meal, soybean meal, shrimp head meal, wheat flour, rice bran, fish oil, vitamin, mineral, and varying concentration of Pom-nang seaweed powder. The Pom-nang seaweed powder (PSP) was supplemented at the level of 0%, 2%, 4% and 6% (corresponding to PSP0, PSP2, PSP4, and PSP6). The ingredients were then mixed, and 1 mm feed pellets were produced using a pelletizer. Proximate analysis was done for all four diets. Total protein concentration for all diet formula is in Table 1.

2.2 Crab acclimatization

Crab megalopa (*Scylla paramamosain*) from hatchery was acclimated into 8 tanks (2×3×0.2 m^3) at density of 100 megalopae/m^2, 25 psu of salinity and temperature of 27 ℃. They were fed with on-grown *Artemia* and an artificial diet until reaching to juvenile mud crab at instar 2 crablet (C2). The fully intact and active crablets were used in the experiment. The crablets were gently dried using tissue paper and were individually weighed for an initial body weight (IBW) by electronic balance (accuracy to 0.0001 g). The carapace width was measured (0.1 mm) by vernier calipers (Ruscoe, Shelley, & Williams, 2004).

2.3 Experimental design

Juveniles *S. paramamosain* at instar 2 crablets were individually stocked in 950 ml plastic containers (116 mm diameter × 146 mm deep) inside an environmentally controlled room at salinity of 25 psu and temperature of 27 ℃. Crablets with an initial body weight of 0.02 g were distributed randomly into plastic containers. All containers were supplemented with sand substrate (Mirera & Moksnes, 2013).

A two-factor factorial design was used in the experiment (dietary diets × Pom-nang seaweed as a shelter) with five dietary treatments (PSP0, PSP2, PSP4, PSP6, and control; mysid shrimp) and two sheltered treatments (with and without seaweed as a shelter). Three replicates for each treatment were conducted, 10 crablets per replicate. Altogether 300 individual crabs were used in this study.

Crablets were fed with the experimental diets at 8% body weight for each treatment (Zheng et al., 2018), twice a day (08:00 and 16:00 hour). Three hours after each meal, the uneaten feed was siphoned out (Unnikrishnan & Paulraj, 2010). They were grown for 28 days before all crabs were measured individually for carapace width, carapace length and weighed for final body weight (FBW).

2.4 Water quality management and analysis

Approximately, ten percent of water were exchanged daily. Daily water parameters including temperature, alkalinity and pH were recorded at 09:00. Salinity was measured with a refractometer. Dissolved oxygen, temperature and pH were measured using dissolved oxygen meter (YSI PRO 2030). Alkalinity was measured every three days in each tank using titration method. Ammonia was measured every three days in each tank using phenol hypochlorite method.

2.5 Growth experiment

An initial average body weight and carapace size of the crabs were measured at the beginning of the experiment. A weekly monitoring of average body weight, carapace size and survival rate of the crabs were recorded. Several attributes were calculated as follows (Yu et al., 2016).

Percent weight gain (WG %)

$$WG = 100 \times \frac{(Final\ body\ weight\ (g) - Initial\ body\ weight\ (g)}{Initial\ body\ weight\ (g)}$$

Feed conversion ratio (FCR)

$$FCR = \frac{Dry\ voluntary\ feed\ intake}{Total\ final\ body\ weight - Total\ initial\ body\ weight}$$

Specific growth ratio (SGR %/d)

$$SGR = 100 \times \frac{ln\ Final\ body\ weight\ (g) - ln\ Initial\ body\ weight\ (g)}{Experimental\ duration\ in\ days}$$

Protein efficiency ratio (PER)

$$PER = 100 \times \frac{Total\ final\ body\ weight - Total\ initial\ body\ weight}{Total\ amount\ of\ the\ feed \times Protein\ content\ in\ the\ feed}$$

Survival rate

$$Survival\ (\%) = 100 \times \frac{Final\ amount\ of\ crabs}{Initial\ amount\ of\ crabs}$$

2.6 Statistical analysis

To determine the effects of Pom-nang seaweed as supplementary diet and as shelter on survival rate, WG, FCR, SGR and PER, a two-way analysis of variance (ANOVA) were used. Once the significance was found, Duncan' s multiple range test was applied to identify the difference of each treatment.

3. Results and Discussion

The mean initial weight (±S.E.) of the crablets was 0.022 ± 0.003g. Formulation of feed in this experiment was done by using PSP supplemented in diet. There was no significant level of protein concentration for all dietary treatments ($P<0.05$) with an average of 45% protein concentration (Table 1). However, the control diet had significantly higher protein, 65%, compared to the dietary diets. Water parameters during experimental period including temperature, salinity, pH, ammonia nitrogen, and dissolved oxygen were 27 to 29 °C, 25 to 26 psu, 7.78 to 8.16, <0.05 mg/L, and > 6.5 mg /L, respectively.

Ingredient (%)	**PSP0**	**PSP2**	**PSP4**	**PSP6**
Fish meal	35	35	35	35
Pom-nang seaweed powder	0	2	4	6
Squid meal	15	15	15	15
Soybean meal	12	11.5	11	10.5
Shrimp head meal	10	10	10	10
Wheat flour	19.9	18.4	16.9	15.4
Rice bran	1.1	1.1	1.1	1.1
Fish oil	6	6	6	6
Mineral	0.5	0.5	0.5	0.5
Vitamin	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**
Proximate analysis (%dry matter)				
Crude protein	44.06±1.97	45.46±1.05	46.50±0.56	44.92±0.45
Crude lipid	9.48±0.29	9.53±0.38	9.53±0.24	9.58±0.34
Ash	11.51±0.03683	10.70±0.19	11.02±0.01	11.30±0.11

Table 1 Values of proximate analysis of nutritional values (%) of experimental diets with different levels of Pom-nang seaweed powder (PSP) inclusion in feed. Mysid shrimp; control diet, PSP0; PSP2, PSP4 and PSP6. Values with different superscript letters within a same column are significantly different ($P<0.05$).

It was found that different diets had significant effects on WG, SGR, PER ($P<0.05$) but not for FCR and survival rate of the crabs ($P>0.05$) (Table 2). However, crabs living with seaweeds as shelter had significantly different WG, SGR, PER, FCR

and survival rate compared to those without seaweeds as shelter P<0.05). There were interaction effects of the combined factor on WG, SGR and PER (P<0.05). Results from Duncan's multiple range test on the comparison among different dietary treatments found that the WG of the control feed, shrimp, were significantly higher than those of other diets (P<0.05) (Figure 1). There was no difference among other dietary diets (P>0.05). Similar result was also found for SGR which the control feed, shrimp, had significantly higher than those of other diets (P<0.05) but no difference among dietary diets (*P*>0.05) (Figure 2). For PER, different effects from different dietary diets were detected as shown in Figure 3. Average values for percent weight gain (WG), specific growth ratio (SGR), protein efficiency ratio (PER), food conversion ratio (FCR) and survival rate (SR) of crablets nursing with different diets and shelter are shown in table 3.

Source	WG	SGR	PER	FCR	SR
PSP	1527316.527*	9.479*	0.844*	0.494	153.333
Shelter	985522.589*	8.309*	1.327*	4.304*	853.333*
PSP* Shelter	328372.924*	2.955*	0.176*	0.155	136.667

Table 2 Values of mean square for percent weight gain (WG), specific growth ratio (SGR), protein efficiency ratio (PER), food conversion ratio (FCR) and survival rate (SR) by using two-way ANOVA. *within a same column are significantly different (*P*<0.05).

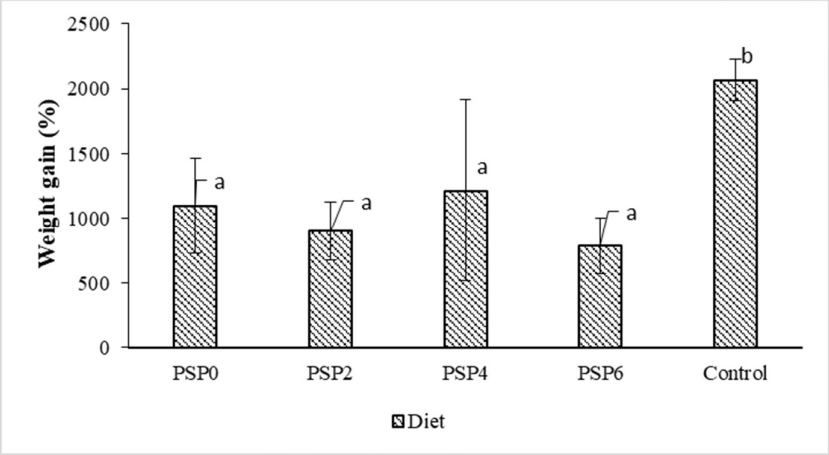

Figure 1 Effect of diets with different Pom-nang seaweed powder (PSP) level supplemented, and Pom-nang seaweed used as shelter on weight gain of juvenile *S. paramamosain*. Values with different letters on the tops of bars are significantly different (*P*<0.05).

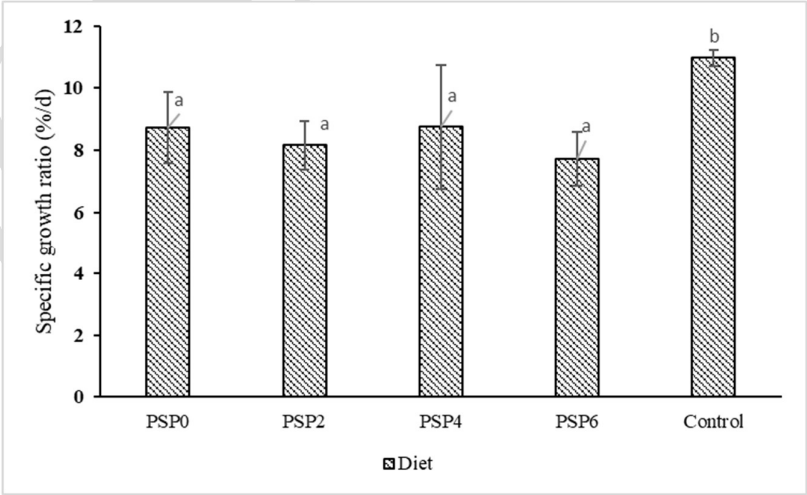

Figure 2 Effect of diets with different Pom-nang seaweed powder (PSP) level supplemented, and Pom-nang seaweed used as shelter on specific growth ratio juvenile *S. paramamosain*. Values with different letters on the tops of bars are significantly different (*P*<0.05).

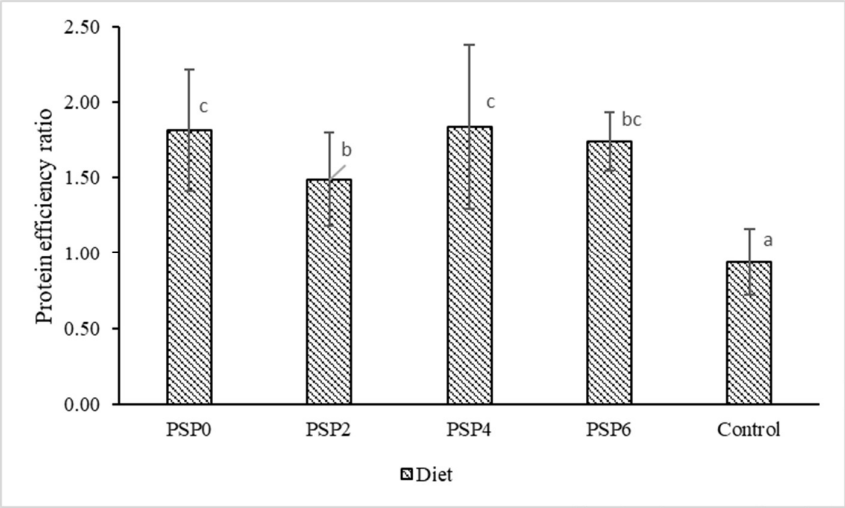

Figure 3 Effect of diets with different Pom-nang seaweed powder (PSP) level supplemented, and Pom-nang seaweed used as shelter on Protein efficiency ratio juvenile *S. paramamosain***.** Values with different letters on the tops of bars are significantly different (P<0.05).

It is evident from this study that nursing crablets with seaweeds as shelter helps to improve nursing performance based on higher values of several parameters including WG, SGR, PER, FCR and survival rate. Therefore, the use of seaweed is therefore essential in nursing crablets. The assumption is that seaweed will help to reduce stress from external factors such as light and self-defending mechanism leading to more effective feeding activity. This finding is in agreement with Ut et al. (2007) who found that survival rate of crablet with shelter was higher than without shelter (sand substrate alone) reared for 15 days from first crab instar.

Table 3 Percent weight gain (WG), specific growth ratio (SGR), protein efficiency ratio (PER), food conversion ratio (FCR) and survival rate (SR) of crablets nursing with different diets and shelter.

Diet	Shelter	WG	SGR	PER	FCR	Survival rate
PSP0	with	1345.40±290.38	9.49±0.77	2.09±0.07	1.19±0.05	80.00±17.32
	without	851.56±270.26	7.96±0.95	1.53±0.41	1.69±0.51	83.33±15.28
	Total	1098.50±368.95	8.72±1.14	1.81±0.40	1.44±0.42	81.67±14.72
PSP2	with	810.91±163.81	7.85±0.62	1.68±0.18	1.76±0.41	76.67±15.28
	without	991.85±273.04	8.46±0.92	1.29±0.31	2.11±0.71	66.67±5.77
	Total	901.38±224.44	8.16±0.78	1.49±0.31	1.94±0.55	71.67±11.69
PSP4	with	1755.10±535.17	10.33±1.07	2.30±0.21	0.94±0.21	73.33±5.77
	without	674.16±236.95	7.18±1.19	1.38±0.22	2.02±0.47	63.33±5.77
	Total	1214.60±698.23	8.76±2.00	1.84±0.54	1.48±0.68	68.33±7.53
PSP6	with	894.26±232.49	8.14±0.83	1.76±0.15	1.44±0.17	83.33±11.55
	without	680.72±161.29	7.28±0.79	1.72±0.26	2.45±0.53	70.00±10.00
	Total	787.49±213.79	7.71±0.86	1.74±0.19	1.94±0.65	76.67±12.11
control	with	2168.10±80.80	11.15±0.13	1.04±0.27	1.62±0.53	86.67±23.09
	without	1963.10±154.87	10.80±0.26	0.84±0.13	2.48±0.25	63.33±5.77
	Total	2065.60±157.55	10.98±0.26	0.94±0.22	2.05±0.60	75.00±19.75

Table 3 Percent weight gain (WG), specific growth ratio (SGR), protein efficiency ratio (PER), food conversion ratio (FCR) and survival rate (SR) of crablets nursing with different diets and shelter.

Shrimp was the most effective diets for nursing crablets compared to the formulated diets based on WG, SGR and PER. However, there is no difference in terms of FCR and survival rate between shrimp as feed and other formulated feed. It is thus suggested that using of formulated feed is possible in nursing crablets for mud crab industry. Compared to other studies on mud crab, Zheng et al. (2018) found that WG of juvenile *S. paramamosain* increased from 2,606% to 3,264% in 56-day trial with an initial body weight about 0.04 g which was higher than WG of juvenile *S. paramamosain* in this experiment (416.07% to 2279.43%). A similar SGR with Ruscoe, Shelley, and Williams., (2004) found that SGR of *S. serrata* from 10.35 to 12.68% day^{-1} in 18-day trial with an initial body weight about 0.02 g.

The use of seaweed as feed supplement had been done in many shrimp studies. Yu et al. (2016) suggested that juvenile *Litopenaeus vannamei* had the highest values of final mean weight (6.52±0.20 g), WG (2343.7± 73.43%), SGR (5.72±0.04% day^{-1}), PER (1.32±0.02) and FCR (1.59±0.02) when shrimp fed with diet supplemented with *Gracilaria lemaneiformis* 3% in 56-day trial with an initial body weight about 0.27±0.00 g. Niu et al. (2015) revealed that the highest final mean weight (2.33±0.08 g), WG (243.09±8.11%) and SGR (2.20±0.04% day^{-1}) when shrimp *L. vannamei* fed with diet supplement with wakame at 2% inclusion level in 56-day trial with an initial body weight about 0.68±0.01 g. It was also found in this study that among formulated diets, the crablets fed with the diets supplemented with 4% of seaweed (PSP4) combined with seaweed as shelter had the highest final mean weight (0.42±0.052 g), WG (1,755.06±535.17%) SGR (10.32±1.07% day^{-1}) and PER (2.30±0.21) than those of crablest fed with PSP0, PSP2 and PSP6. This value indicates that the optimum supplement rate of Pom-nang seaweed (*Gracilaria* spp.) in the diet for crablets shall be 4% for 28-day trial with the carb juvenile of an initial body weight about 0.02±0.00 g.

4. Conclusions

It is concluded that shrimp is the most appropriate food for nursing crablets *S. paramamosain* and formulated diets supplemented with seaweeds has a potential to replace shrimp although some growth performances from the formulated diets are lower than those of shrimp. The use of *Gracilaria* as shelter is essential for nursing crablets as indicated by all growth performances and survival rate. Additionally, the combination of seaweed as shelter and the formulated diets with 4% seaweed had the highest values of all growth performances and survival rate compared to others.

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AQ 05

Preliminary Study on The Culture of Seagrapes (*Caulerpa lentillifera*) in Semi-enclosed and Enclosed Area in Pulau Pinang, Malaysia

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Abstract

A preliminary study was done on the culture *Caulerpa lentillifera* in two different conditions in a hatchery system situated at the coastal area of Pulau Pinang, Malaysia. Growth of *C. lentillifera* cultured in an enclosed and semi-enclosed area were evaluated. Two parameters of temperature and light intensity at the two areas were recorded through-out the 30 days of the experiment and analyzed. Tanks in enclosed area received an average total daily light intensity of 4,521.39±7,819.31 lx, as compared to only 2,778.04±5,134.95 lx in the semi-enclosed area. All seagrapes cultured in tanks at the enclosed area showed positive growth with an average increase of 30.3±0.7 g in weight after three weeks of culturing. Seagrapes in tanks at semi-enclosed area showed only 2.9±5.7 g increase in average weight. Seawater temperature in both culture areas showed within tolerable range for growth of *C. lentillifera.* However, light intensity received in all the tanks in both cultured areas exceeds previously known optimum level for growth. This showed that *C. lentillifera* may have complex adaptation for growth in different light intensity conditions.

Keywords: *Seaweed, water quality, indoor culture, temperature, light intensity.*

1. Introduction

The green seaweed *Caulerpa lentillifera* is widely distributed in the tropical and subtropical areas especially in the Indo-Pacific region. Due to its abundance, *C. lentillifera* has been utilized as viable food in the Asian region. In fact, studies have shown that they are very nutritious especially in polyunsaturated fatty acids (PUFA) (Saito, Xue, Yamashiro, Moromizoto, & Itabashi, 2010), multiple essential amino acids, mineral, vitamins and dietary fibers (Matanjun, Matanjum, Mohamed, Mustapha, & Muhammad, 2009). In addition, there are also many other potentials in terms of bioactive components such phenolic compounds, polysaccharides and other biological components which can be beneficial for pharmaceutical in this species of seaweed (Chen et al., 2019). Apart from being utilized as food, seagrapes are also used as a biological treatment for industrial wastewater (Apiratikul & Pavasant, 2008) and aquaculture effluents (Paul & De Nys, 2008).

The demand for the seaweed *C. lentillifera* in the region is on the rise due to its popularity as food as well as health food supplement. Seagrapes have been cultured commonly in brackish water ponds especially in the Philippines (Trono, 1988). Recently, some studies have been done on different culture methods (Rabia, 2016) as well as to off bottom culture in open sea (Tanduyam, Gonzaga & Bensig, 2013). Currently, this species of seaweed is also cultured in Vietnam, Indonesia and Malaysia. In Malaysia, the supply of this seaweed is dependent from the culture outdoor in the open sea mainly in Borneo Island of Sabah. A preliminary study was done on the culture *C. lentillifera* in two different conditions of semi-enclosed and enclosed hatchery system situated at the coastal area of Pulau Pinang.

2. Methods

Seagrapes of the species *C. lentillifera*, with initial weight of about 21–29 g were used to evaluate the growth by weight cultured in two different separate areas with different conditions: 1) semi-enclosed area and 2) enclosed area. Both areas received different amount of sunlight intensity as well as ambience temperature. Tanks in the enclosed area are situated in a building with translucent roofing. Tanks in semi-enclosed area are situated outside of a building but were shaded with roofing. Three separate round tanks (1,000 litre capacity) were used in each area with five replicates each tank in each conditions. A flow-through system of filtered seawater was supplied to each tanks with continuous flow and depth of immersion of seagrapes can be negligible as both condition have no significant effect on the growth of seagrapes based on Nguyen, Le, Nguyen, Thach & Nguyen (2020). The salinity of the flow-through seawater ranges of 27–31 ppt, well within the salinity range for *C. lentillifera* to grow (Guo, Yao, Sun, & Duan, 2015). The temperature of sea water and light intensity were recorded hourly for each tank using HOBO Pendant® UA 002-64 Temperature/Light Data Logger for the duration of one month. An average hourly temperature and light intensity per day were calculated. The wet weight of seagrapes were measured in weigh weekly for three weeks. Data were analysed using IBM SPSS Statistics Version 27.

3. Results and Discussion

Initial average weight of seagrapes in the enclosed area and semi-enclosed area were 21.8±0.53 g and 22.3±0.7 g respectively. All seagrapes cultured in tanks at the enclosed area showed positive growth with the fastest growth recorded with final average weight of 64.4±5.7 g after three weeks of culturing in one of the tanks; an equivalent to weight increase of 42.6±5.7 g over three weeks. Unlike in tanks located in the enclosed area, the growth in the semi-enclosed area recorded average positive growth to 30.3±0.7 g on the first week, and recorded a decline for the rest of the experimental period (Figure 1) ending with average weight of 27.4±13.2 g; an equivalent weight increase of only 2.9±5.7 g from initial weight over three weeks. The average temperature of all the tanks in enclosed area was 29.57±0.58 °C with maximum temperature recorded at 32.17 °C and minimum temperature of 27.07 °C (Figure 2). The average temperature of all the tanks in semi-enclosed area was significantly lower ($P<0.05$) than enclosed area recording at 28.96±0.39 °C with maximum temperature recorded at 31.33 °C and minimum temperature of 26.78 °C. At the enclosed area, the maximum light intensity received goes up to 74,400.5 lx whereas in the semi-enclosed area was 82,667.2 lx (Figure 3). An average total daily light intensity received by tanks in the enclosed were 4,521.39±7,819.31 lx, as compared to only 2,778.04±5,134.95 lx in the semi-enclosed area. The t-test determined that the average total light received by the two area were significantly different ($P<0.05$).

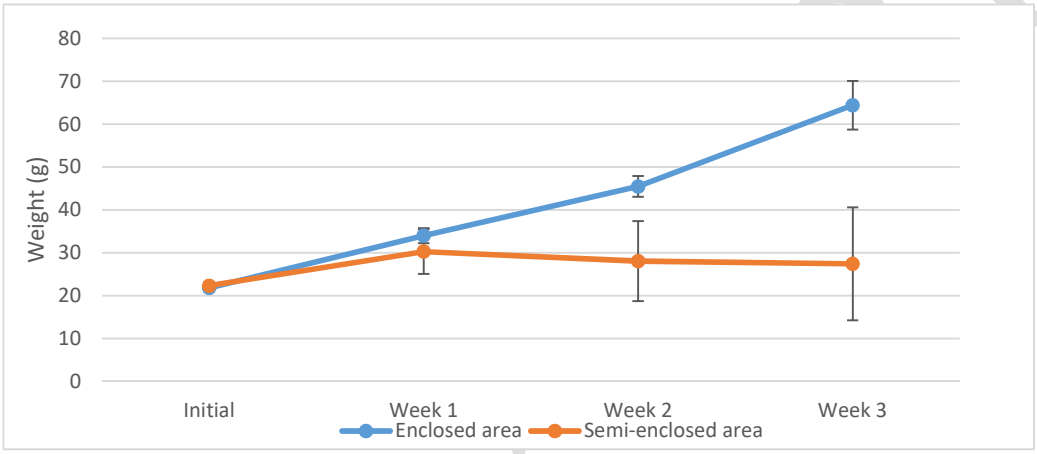

Figure 1 Average growth of seagrapes by wet weight (g) of both enclosed and semi-enclosed area measured for a period of three weeks.

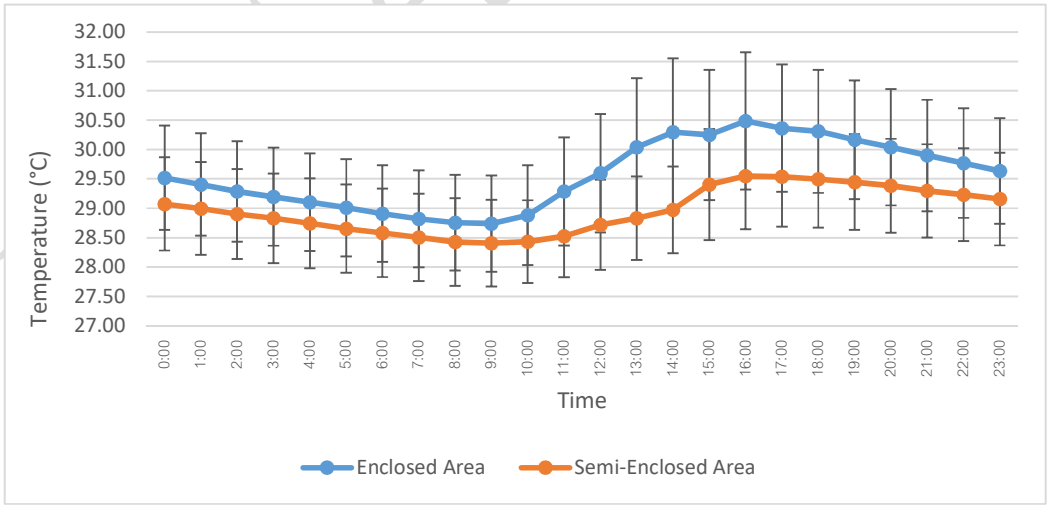

Figure 2 Average daily temperature of seawater in culture tanks of both enclosed and semi-enclosed area measured for 30 days.

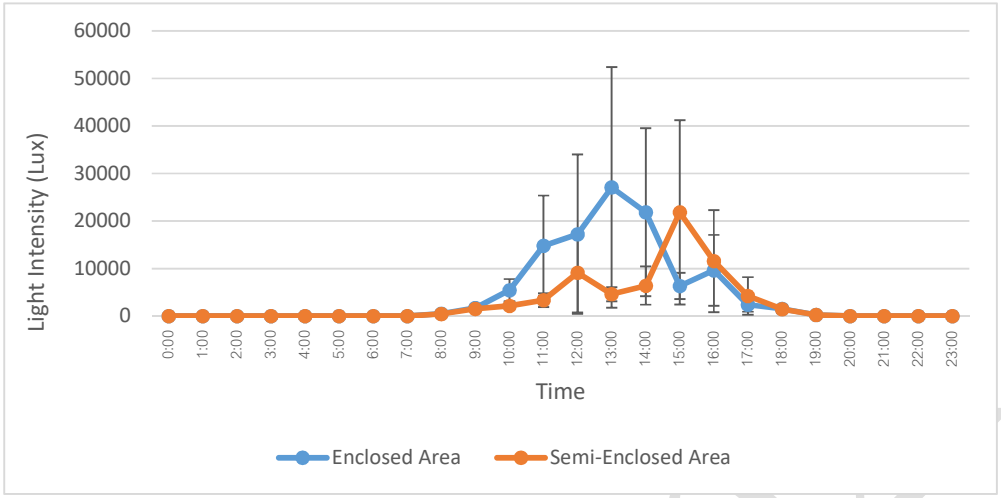

Figure 3 Light intensity in culture tanks of both enclosed and semi-enclosed area measured for 30 days.

The optimal temperatures for the growth of different species *Caulerpa* spp. varies as the distribution of this genus occurs both in the temperate, subtropical and the tropical areas. *Caulerpa paspaloides* can grow within the temperature range of 15 to 39 °C (O'Neil and Prince, 1988), *Caulerpa taxifolia* in the range of 15–17.5 °C and cannot survive below 10 °C (Komatsu, Meinesx, & Buckles, 1997), *Caulerpa prolifera* grows fast at 23–26 °C (Friedlander, Kosov, Keret, & Dawes, 2006), and *C. lentillifera* in Guo, Yao, Sun, and Duan (2014) grows best in 27.5 °C. Since *C. lentillifera* is a tropical species, the minimum and maximum temperature that allows them to grow ranges from 20–30 °C with 27.5 °C being the optimum temperature for maximum growth rate (Guo et al., 2014). Contrary to the observation done by Guo et al. (2014), the growth performances (in terms of weight increase) in this study were found at tanks in the enclosed area with daily average temperature in the range of 28.74–30.49 °C as compared to tanks in semi-enclosed area with daily average temperature in the range of 28.41–29.55 °C. This shows that different strains or locality of *C. lentillifera* may have different temperature tolerance and optimum growth temperature. Furthermore, the seawater temperature where *C. lentillifera* were cultured in both of the conditions were allowed to fluctuate according to the ambient temperature as compared to Guo et al. (2014) where their *C. lentillifera* were experimented in a constant temperature.

Guo et al. (2014) also subsequently determined the amount of irradiance that was favourable for the growth of *C. lentillifera* and found that amount of 40 µmol photons m^{-2} s^{-1} (about 2,960 lx) was ideal amount of light with 100 µmol photons m^{-2} s^{-1} (about 7,400 lx) showed lowered growth rate. Similar observation was done by Stuthmann, Springer, and Kunzmann (2020) with *C. lentillifera* cultured in Vietnam. As this study was done in areas exposed to natural sunlight, the amount of light intensity is dependent on the weather changes as well as affected by natural photoperiods. The amount of both enclosed and semi-enclosed area in this study have maximum light intensity of 74,400.5 lx and 82,667.2 lx, which were well beyond the total irradiance of 7,400 lx which according to Guo et al. (2013) and Stuthmann et al. (2020) will reduce the growth of *C. lentillifera.* On the contrary, both areas recorded positive growth with higher growth rate in the enclosed area which received higher light intensity. This shows that the *C. lentillifera* have the ability to adapt to continue growing in areas with high irradiance which also indicated by Stuthmann et al. (2020) where *C. lentillifera* in their experiment showed long-term photoacclimation in irradiance of more than 100 µmol photons m^{-2} s^{-1} (about 7,400 lx). Similar observation was shown in the *Caulerpa racemosa* that demonstrated changes in their photosynthetic apparatus and pigment composition as method of photoacclimation to continue growing in different irradiance conditions (Peterson, 1972; Raniello, Lorenti, Burnet, & Buia, 2004).

4. Conclusion

The preliminary data shows that the amount of maximum light intensity and higher temperature favors the growth of seagrapes. Further study will be conducted to ascertain the optimum total amount of light intensity require for the growth of *C. lentillifera*. This study only elucidated the effects of light intensity and temperature to the growth of seagrapes in Penang, Malaysia. Future study will include the effects of different concentration of chemicals (Nitrate, Nitrite, and Phosophorus) in the seawater to the growth of seagrapes in the same area.

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