JAD ∗ Volume 18 - Issue 3 (2019) ∗ pISSN 2615-9503 ∗ eISSN 2615-949X

EDITORIAL BOARD OF THE JOURNAL OF AGRICULTURE AND DEVELOPMENT

No.	Full name	Organization	Position
I	**Local members**		
1	Nguyen Hay	Nong Lam University, HCMC, Vietnam	Editor-in-Chief
2	Che Minh Tung	Nong Lam University, HCMC, Vietnam	Deputy Editor-in-Chief
3	Nguyen Dinh Phu	Nong Lam University, HCMC, Vietnam University of California, Irvine, USA	Editor
4	Le Dinh Don	Nong Lam University, HCMC, Vietnam	Editor
5	Le Quoc Tuan	Nong Lam University, HCMC, Vietnam	Editor
6	Nguyen Bach Dang	Nong Lam University, HCMC, Vietnam	Editor
7	Nguyen Huy Bich	Nong Lam University, HCMC, Vietnam	Editor
8	Phan Tai Huan	Nong Lam University, HCMC, Vietnam	Editor
9	Nguyen Phu Hoa	Nong Lam University, HCMC, Vietnam	Editor
10	Vo Thi Tra An	Nong Lam University, HCMC, Vietnam	Editor
11	Tang Thi Kim Hong	Nong Lam University, HCMC, Vietnam	Editor
II	**International members**		
12	To Phuc Tuong	Former expert of IRRI, Vietnam	Editor
13	Peeyush Soni	Asian Institute of Technology, Thailand	Editor
14	Ta-Te Lin	National Taiwan University, Taiwan	Editor
15	Glenn M. Young	University of California, Davis, USA	Editor
16	Soroosh Sorooshian	University of California, Irvine, USA	Editor
17	Katleen Raes	Ghent University, Belgium	Editor
18	Vanessa Louzier	Lyon University, France	Editor
19	Wayne L. Bryden	The University of Queensland, Australia	Editor
20	Jitender Singh	Sardar Vallabhbhai Patel University of Agriculture and Technology, India	Editor
21	Kevin Fitzsimmons	University of Arizona, USA	Editor
22	Cyril Marchand	University of New-Caledonia, France	Editor
23	Koichiro Shiomori	University of Miyazaki, Japan	Editor
24	Kazunari Tsuji	Saga University, Japan	Editor
25	Sreeramanan Subramaniam	Universiti Sains Malaysia, Malaysia	Editor

EDITORIAL SECRETARIAT

No.	Full name	Organization	Position
1	Huynh Tien Dat	Nong Lam University, HCMC, Vietnam	Editorial secretary
2	Truong Quang Binh	Nong Lam University, HCMC, Vietnam	Editorial administrator
3	Huynh Huu Nhan	Nong Lam University, HCMC, Vietnam	Secretary assistant

Contact information:
Nong Lam University
Room 404, Thien Ly Building
Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam
Tel: (84-28)37245670
Email: jad@hcmuaf.edu.vn

The Journal of Agriculture and Development
Volume 18 - Issue 3 (2019)
pISSN 2615-9503
eISSN 2615-949x

CONTENT

Agribusiness

1 Optimizing equipment efficiency: An application of SMED methodology for SMEs
Hien N. Nguyen, & Nhan H. Huynh

Agronomy and Forestry Sciences

10 Optimum condition of manufacturing hybrid particleboard from mixture of cocoa pod husk and bamboo particles
Hong T. K. Tang, Linh D. Nguyen, & Dung T. T. Ho

16 Mould resistance of the bamboo *Thyrostachys siamensis* treated with chitosan
Hong T. K. Tang, & Mai H. Trinh

Animal Sciences, Veterinary Medicine, Aquaculture and Fisheries

21 Use of Marine Sulfated Polysaccharide as an alternative to antibiotics in the diet of broilers
Tung M. Che, Hien T. Le, Vi Q. Tran, Damien Berdeaux, Virgil Meallet, & Michel Guillaume

27 Prevalence, antimicrobial resistance profiles and virulence genes of *Vibrio* spp. isolated from shrimp retails in Ho Chi Minh City (Vietnam)
Trinh N. T. Huynh, An T. T. Vo, Yen P. T. Nguyen, & Cuong V. Nguyen

35 Application of PCR technique in diagnosis of four respiratory pathogenic bacteria in pigs at the slaughterhouse
Han M. Ly, Trinh T. K. Nguyen, Thiep T. X. Dang, & An T. T. Vo

41 Effects of dietary supplementation of β-mannanase on performance and egg quality in laying hens
Tung M. Che, Nhan T. M. Nguyen, & Sarah K. Cervantes-Pahm

Environmental and Natural Resources

48 Ecological planning for the conservation and development of pineapple (*Ananas comosus*) in Tan Phuoc district, Tien Giang province
Mai T. Nguyen, & Thuy T. P. Doan

Food Science and Technology

57 Extraction and encapsulation of lesser galangal (*Alpinia officinarum*) essential oil using microwave pretreatment and spray drying
Tuan T. A. Nguyen, Dat T. Huynh, & Nattakan Rungraeng

64 Effect of different drying methods on the nutritional and physicochemical properties of unpeeled banana flour
Sang Q. Tran, Tuyen C. Kha, & Khanitta Ruttarattanamongkol

Optimizing equipment efficiency: An application of SMED methodology for SMEs

Hien N. Nguyen[1*], **& Nhan H. Huynh**[2]

[1]Faculty of Engineering, Vietnamese-German University, Binh Duong, Vietnam
[2]Scientific Research Management Office, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: April 18, 2019
Revised: May 22, 2019
Accepted: June 06, 2019

Keywords

Overall equipment effectiveness (OEE)
Single minute exchange of dies (SMED)
Small and medium-sized enterprises (SMEs)

***Corresponding author**

Nguyen Ngoc Hien
Email: n.nnhien1990@gmail.com

ABSTRACT

Competitiveness in the era of globalization is tougher than ever before. Most of small medium-sized enterprises, especially in the manufacturing sector, are easily vulnerable due to lack of opportunities and resources to harness the economics of scale as well as business activities in research and development. To drive business competitiveness, the small and medium-sized enterprises (SMEs) must make use of resource efficiency in production processes and optimize the overall equipment effectiveness (OEE). The method of single minute exchange of dies (SMED) appears to be an effective approach, which does not require financial investments but only utilizes the current human resource, to improve and maximize the OEE. The paper describes the step-by-step approach to apply SMED and shows its results in the increase of 18% OEE in a semi-auto cutting machine.

Cited as: Nguyen, H. N., & Huynh, N. H. (2019). Optimizing equipment efficiency: An application of SMED methodology for SMEs. *The Journal of Agriculture and Development* 18(3), 1-9.

1. Introduction

Global trade and state-of-the-art technologies have made the world smaller, which in turn puts any entity in pressure and tough competition in the market place in which SMEs are crucial contributors in the economic development (Matt & Rauch, 2013), but have vulnerable competitive positions (Pius et al., 2006). To take advantages in the global competitive marketplace, the SMEs have struggled for getting flexibility and responsiveness to the changing competitive environment (Wilson, 2010) and making incremental improvements to world glass performance through the implementation of lean production system (Ahmad et al., 2009) in which optimizing and controlling the OEE, one of the most important indicators in the manufacturing sector, play a critical role to manufacturing excellence (Kuznetsov et al., 2018).

One significant reason behind the failure of achieving the best performance of lean initiatives in general and OEE in particular is a lack of an effective implementation methodology and planning (Felix et al., 2018). To capture the point, several methods have been introduced to improve the OEE. One of them was proposed to apply integer programming for finding the optimal point of OEE with the help of simulation software (Marin et al., 2010), the other introduced the fuzzy temporal performance model used to express the performance of OEE across the time line (Laurent et al., 2019). Besides, putting investments in automatic data collection of OEE measurements were also indicated for the data-driven decisions (Richard et al., 2016). Moreover, the DOE, design of experiments, was also used to analyze the impact levels of each OEE component

for problem prioritization, but not showing how the OEE can be improved (Anand & Nandurkar, 2012). However, these approaches are not suitable for SMEs in most cases due to the fact that they are lack of resources and expertise to handle the technical models (Moeuf et al., 2016).The situation is more worse in Viet Nam where more than 80% of labor workforce are high-school graduates who are lack of chance to expose the models as well as lean initiatives (Nguyen & Nguyen, 2017).

The method is named as SMED that is one of the key tools for optimizing the operations (Womack & Johns, 1990) and can be effectively applied to improve the OEE without requiring special technical needs or investments (Eric et al., 2013).

SMED stands for Single Minute Exchange of Dies (Shingo, 1985) and its ultimate objective is to enhance the performance of equipment or machines in terms of time utilization (availability), qualified outputs (quality), capacity utilization (performance) and at the same time meet the requirement of output diversity or small lot-sized production. Regardless of business sizes, the SMED method has been applied in several different processes such as: mold industry, pharmaceutical industry, transformation industry, metallurgical industry, and textile manufacturing (Andreia & Alexandra, 2010).

The application of SMED was also proved to be effective in different industries. For SMED methodology's application, the Electric power controls company was benefited with the reduction in 59% to 90% on average of setup time of studied machines (Domingos et al., 2011), whereas its application in Fogor Press machine shows a very encouraging result in reduction of 70% changeover time and increase in productivity of 6.3% (Suresh Kumar & Syath Abuthakeer, 2012). Moreover, the SMED was also applied in combination with MOST (Maynard's Operation Sequencing Technique) in Aerospace Industry to indicate the improvement of OEE from 84.32% to 88.94% (Puvanasvaran et al., 2013).

Therefore, the SMED methodology is a simple but effective approach that can bring the business results as quick improvements without investment for SMEs. The purpose of this paper is to describe the step-by-step approach to apply the SMED and shows its results in the increase of 18% OEE in an semi-auto machine cutting the sheet of EVA (Etylen-vinyl axetat) into pieces as a typical example.

2. Materials and Methods

2.1. OEE measurement

The Overall Equipment Effectiveness (OEE) is one of the most critical key performance indicators of the Total Productive Maintenance (TPM) that has to be maximized by tacking and minimizing losses as described by the Figure 2.

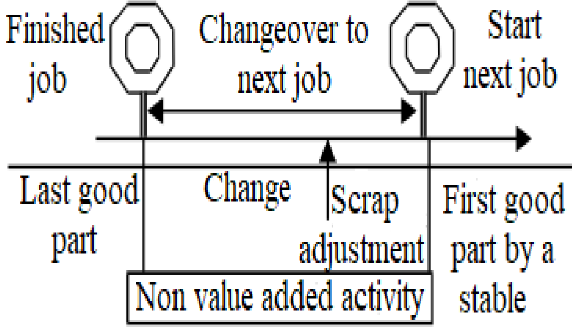


Figure 1. Representation of changeover time (Berna, 2011).

According to the Figure 2, each main component of OEE is responsible to represent for 2 major losses and by qualifying each following component the analyst will know what the most prioritized problem should be solved:

(a) Availability: Availability is a percentage number that indicates how the machine is effectively operated within the planned operating time. It points outs first two of the six big losses, breakdowns, setup/adjustments, changeover time (Figure 1) from one model production to another one.

(b) Performance: Performance efficiency takes into account the unoccupied downtime, such as waiting time due to operator inefficiency or lack of materials, and productivity losses due to machining running below its capacity. The ideal cycle time is needed to calculate the performance efficiency where it is multiplied with the total parts produced divided by the actual operating time.

(c) Quality: The quality rate captures the rejected parts or defectives during production and the losses from initial start-up to process stabilization.

(d) Overall Equipment Effectiveness (OEE): the product of three factors above. It shows how effectiveness (quality) and efficiency (availability

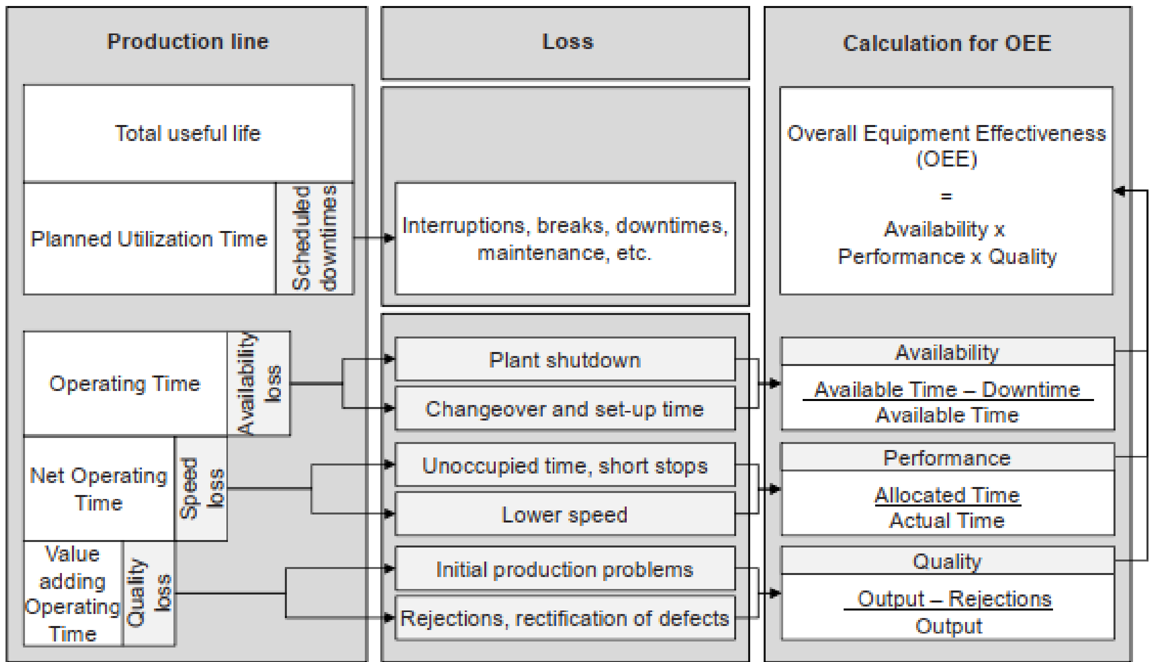


Figure 2. OEE measurement (adapted from Gisela et al., 2013).

and performance) of a machine or workstation are utilized.

2.2. SMED methodology

The classical approach to the SMED was initially proposed by Shingo (1995). It divides the process of changing one production model to another one on an operating machine supervised by the one or more operators into 2 different parts:

(a) Internal activities: Processing steps that can be done only when the machine is shut down, such as attaching and removing cutting dies.

(b) External activities: Processing steps that can be done when the machine is still running, such as preparation of the availability of input materials for the machine.

As illustrated by the Figure 3, the SMED can be done in 3 steps and last step for continuous improvements to drive forward optimization of OEE:

(a) Separating internal and external activities.

(b) Switching internal to external activities as many as possible.

(c) Streamlining all setup activities.

(d) Repeat the 3 steps above for operations excellence.

3. Results and Dicussion

The case study was conducted in a footwear manufacturing in which the semi-auto die cutting machine with traveling head was used to cut the EVA form into soles of slippers. Due to the nature of production of slippers, the machine was required to change the cutting die from one size to another size according to the production plan.

Because of too many changeover times from one size to others, the performance of the machine was affected negatively with low productivity that did not meet the customer output orders. To support for the statement, the OEE data collection was also created as the Figure 4.

In case of SMEs, they are normally lack of financial investment to equip an automatic collection system where the data are synchronized in real-time manner. Therefore, the good starting point for them is to use current equipment like excel and own-design hand-writing book for collecting and storing daily data.

The data collected, OEE and its components should be graphically shown in trends where the

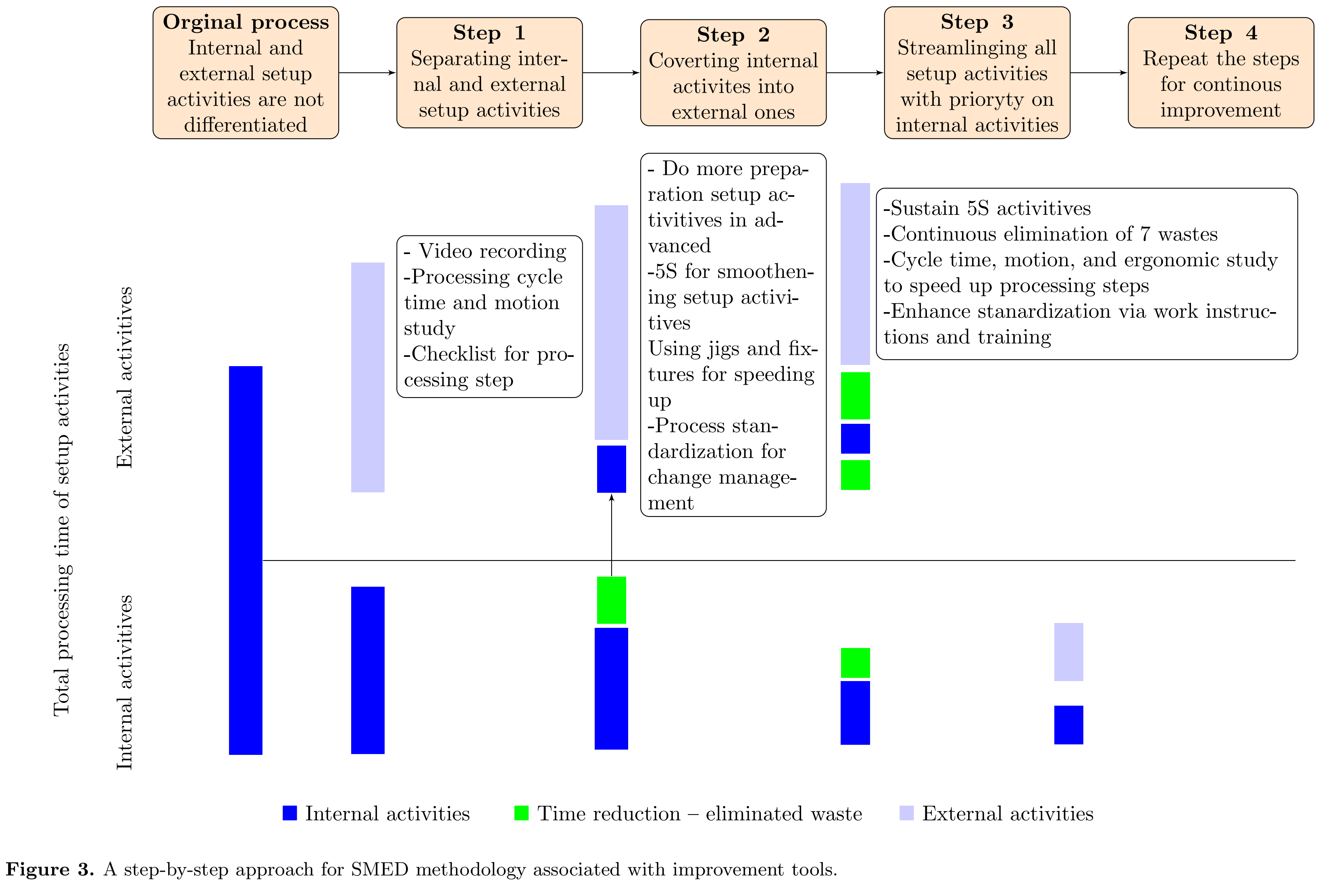


Figure 3. A step-by-step approach for SMED methodology associated with improvement tools.

Table 1. SMED analysis

Semi cutting machine			Before-Kaizen	After-Kaizen	Separating changover activities			Separating changover activities			
Total changover time (min)			7.5	1.9							
Step No.	Changeover processing steps	Associated tools	Cycle time element (s)	Cycle time element (s)	Internal activity (s)	External activity (s)	Waste activity (s)	Improvement actions	Eliminate	Internal → External	Reduce CT
1	Stop the machine	Production order (PO)	5	5	X						
2	Search for Hex key		48				X	6S (design rack for storate)	X		
3	Disassemble the mold	Hek key	9	9	X						
4	Detach the cutting die from the mold	Hek key	31			X		Do it while the machine is running		X	
5	Search for the next cutting die		27				X	6S (design rack for storate)	X		
6	Attach the new die into the mold	PO, Hex key, cutting die	57			X		Do it while the machine is running		X	
7	Search for EVA		80				X	6S (design rack for storate)	X		
8	Attach EVA into the mold	EVA, knife	85			X		Do it while the machine is running		X	
9	Assemble the mold	Hex key	12	12	X						

Table 1. SMED analysis (continue of page 5)

Semi cutting machine			Before-Kaizen	After-Kaizen	Separating changeover activities			Separating changeover activities			
Total changeover time (min)			7.5	1.9							
Step No.	Changeover processing steps	Associated tools	Cycle time element (s)	Cycle time element (s)	Internal activity (s)	External activity (s)	Waste activity (s)	Improvement actions	Eliminate	Internal → External	Reduce CT
10	Set up the machine		75	60	X			Training and standardization			X
11	Get a pair of EVA sheets		6			X		Do it while the machine is running		X	
12	Put the sheets on place	EVA sheets	9	9	X						
13	Run the machine		5	5	X						
Total changeover time				449	100	115	179	155			

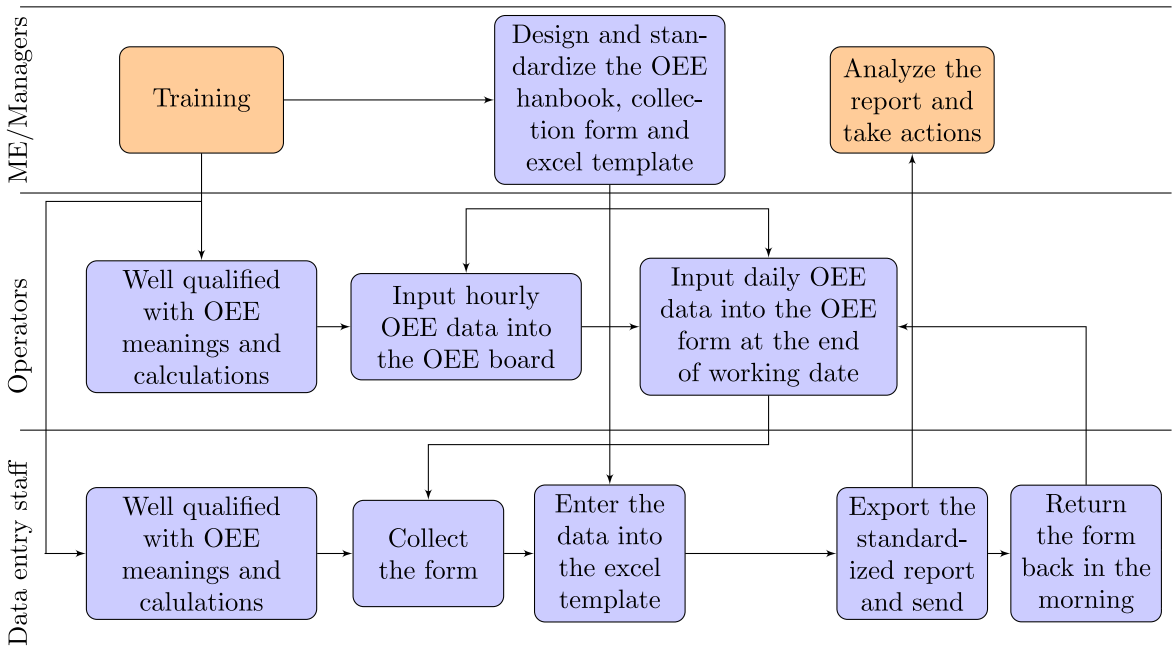


Figure 4. OEE data collection procedure.

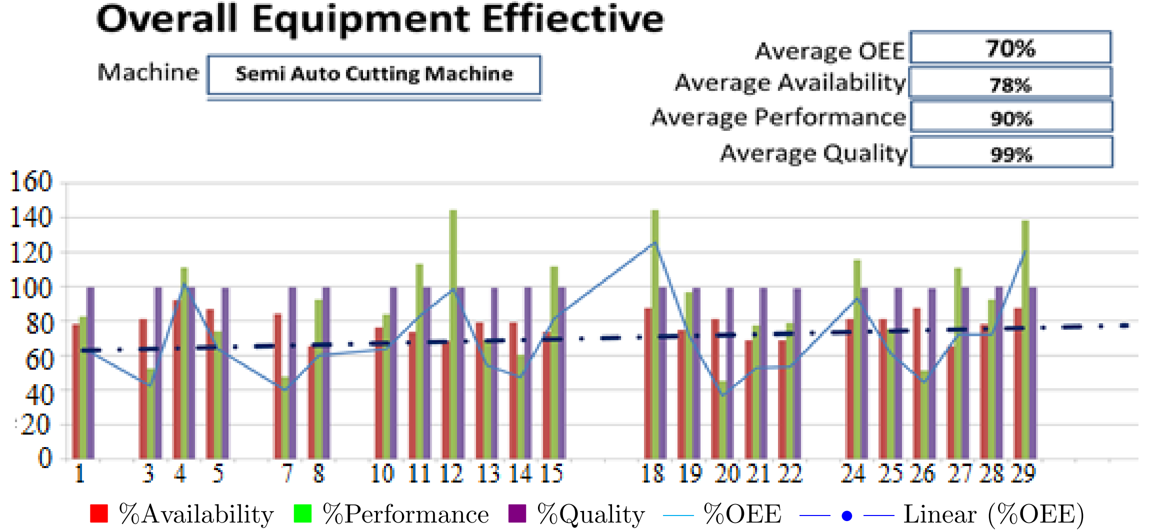


Figure 5. OEE descriptive statistics.

status of performance can be spotted as the following the Figure 5.

As can be seen on the Figure 5, the average availability was only 78%, which means that there was a room of 22% downtime the machine underwent. By breaking further the data of downtime, about 95% of downtime was accounted to changeover time. Hence, by making improvement in 67% reduction changeover time, the availability would be enhanced to 92%, leading to the increase of OEE from 70% to 82%. By doing that way, the analyst can show clear targets, which in turn gets the support from top management to carry out the improvement project.

To tackle the changeover time, the methodology of SMED was applied in the away described as the Figure 3. The result of analysis is indicated as the following Table 1.

The Table 1 shows before-after analysis of SMED on the machine where the highlighted red steps were categorized as waste activities that did not add the value to the process, whereas the yellow ones were internal activities that were converted to external activities while the machine still operated. The highlighted green step was the one whose cycle time was reduced after the operator was trained and the task was standardized in a work instruction. The column of improvement activities is to indicate the actions carried out to reduce the changeover time. For instance, searching activities that are considered as a waste were eliminated by 6S activities, including design a suitable storage of tools where the hex key was always available for the operator without searching for it.

By doing that way, the total changeover time was improved from 7.5 min per cycle to 1.9 min/cycle, which equivalents to 74% reduction in changeover time. The reduction in changeover in turn improved the availability from 78% to 93%, leading to an increase of OEE from 70% to 83%.

4. Conclusions

The paper has shown the most effective and easy-to-implemented methodology that can bring quick performance improvements for SMEs. The case study was also indicated as a comprehensive guidance for the implementation of SMEDs, which is specifically adaptable for SMEs who are lack of resources and expertise in terms manufacturing excellence. The future works after mastering the technique for the SMEs should be the case of digitalization on which the automatic OEE data collection and data analysis are implemented in their factory.

Conflict of interest statement

The authors declare that there is no conflict of interest.

References

Ahmad, N. M. R., Baba, M. D., & Mohd, N. A. R. (2009). A review on lean manufacturing practices in small and medium enterprises. *Seminar 3 - AMReG 09* (1-6). Kajang, Selangor, Malaysia.

Anand, S. R., & Nandurkar, K. N. (2012). Optimizing & anlysing overall equipment effectiveness (OEE) through design of experiements (DOE). *Procedia Engineering* 38, 2973-2980.

Andreia, S., & Alexandra, T. (2010). Improving setup time in a press line – Application of the SMED methodology. *IFAC Proceedings* 43(17), 297-302.

Berna, U. (2011). An application of SMED methodology. *International Journal of Mechanical, Aerospace, Industrial, Mechatronic and Manufacturing Engineering* 5(7), 1194-1197.

Domingos, R., Fernando, B., Rui, S., & Carmo-Silvab, S. (2011). An application of the SMED methodology in an electric power controls company. *Romanian Review Precision Mechanics, Optics and Mechatronics* 40, 115-122.

Eric, C., Rui, S., Sara, B., & Anabela, A. (2013). An industrial application of the SMED methodology and other lean production tools. In Silva Gomes, J. F., & Meguid, S. (Eds.). *Proceedings of the 4th International Conference on Integrity, Reliability and Failure* (1-8). Funchal, Madeira, Portugal: Edições INEGI.

Felix, S., Nguyen, H. N., René, H., & Holger, K. (2018). Implementation of lean production systems in small and medium sized pharmaceutical enterprises. *Procedia Manufacturing* 21, 814-821.

Gisela, L., Johannes, S., Nicole, S., Steven, P., & Christof, L. (2013). Measuring global production effectiveness. *Procedia CIRP* 7, 31-36.

Kuznetsov, A. P., Koriath, H. J., Kalyashina, A. V., & Langer, T. (2018). Equivalence assessment method for the resource efficiency of equipment, technologies, production systems. *Procedia Manufacturing* 21, 525-532.

Laurent, F., Vincent, C., & Lamia, B. (2019). A fuzzy temporal approach to the overall equipment effectiveness measurement. *Computers & Industrial Engineering* 127, 103-115.

Matt, D. T., & Rauch, E. (2013). Implementation of lean production in small sized enterprises. *Procedia CIRP* 12, 420-425.

Marin, M., Luminiţa, D., Paul, C. P., & Ion, C. (2010). A method to optimize the overall equipment effectiveness. *IFAC Proceedings* 43(17), 237-241.

Moeuf, A., Tomayo, S., Lamouri, S., Pellerin, R., & Lelievre, A. (2016). Strengths and weaknesses of small and medium sized enterprises regarding the implementation of lean manufacturing. *IFAC PapersOnLine* 49(12), 71-76.

Nguyen, M. D., & Nguyen, N. D. (2017). Applying lean manufacturing in Vietnamese enterprises: The roadmap to success. *Journal of Economics and Development* 1, 41-48.

Pius, A., Esam, S., Rajkumar, R., & Geoff, N. (2006). Critical success factors for lean implementation within SMEs. *Journal of Manufacturing Technology Management* 17(4), 460-471.

Puvanasvaran, A. P., Mei, C. Z., & Alagendran, V. A. (2013). Overall equipment efficiency improvement using time study in an aerospace industry. *Procedia Engineering* 68, 271-277.

Richard, H., Mukund, S., & Peter, A. (2016). Analysis of critical factors for automatic measurement of OEE. *Procedia CIRP* 57, 128-133.

Shingo, S. (1985). *A revolution in manufacturing: The SMED system*. Oregon, USA: Productivity Press.

Suresh Kumar, B., & Syath Abuthakeer, S. (2012). Implementation of lean tools and techniques in an automotive industry. *Journal of Applied Sciences* 12(10), 1032-1037.

Wilson, L. (2010). *How to implement lean manufacturing*. New York, USA: McGraw-Hill.

Womack, J. P., Jones, D. T., & Roos, D. (1990). *The machine that changed the world*. New York, USA: Macmillan.

Optimum condition of manufacturing hybrid particleboard from mixture of cocoa pod husk and bamboo particles

Hong T. K. Tang[1*], Linh D. Nguyen[1,2], & Dung T. T. Ho[1]

[1]Faculty of Forestry, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Institute of Wood Science, University of Hamburg, Germany

ARTICLE INFO

Research Paper

Received: March 29, 2019
Revised: May 10, 2019
Accepted: May 28, 2019

Keywords

Bamboo
Cocoa pod husk
Particle board
Physical mechanical properties

***Corresponding author**

Tang Thi Kim Hong
Email: tangkimhong@hcmuaf.edu.vn

ABSTRACT

This study was to investigate the feasibility of using cocoa pod husks (CPH) and bamboo in manufacturing hybrid particle board. Three-layer experimental particleboards from mixture of bamboo and CPH participles were manufactured using different surface to core layer ratios (30, 40 and 50%) and various UF ratios for surface layer (6, 8 and 10%) and for core layer (4, 6 and 8%). Modulus of rupture (MOR), internal bond strength (IB) and thickness swelling (TS) properties of the boards were evaluated based on Standard TCVN7756:2007 Test Methods for general purpose used in dry conditions. The results showed that boards in all ratios of surface to core layer investigated could be manufactured using up till 8% UF resin for surface layer and up till 6% UF resin for core layer without falling below the minimum Standard VN7754:2007. The optimal condition was the surface to core layer ratio of 30% used with 9.51% UF resin for surface layer and 7.45% UF resin for core layer obtaining the lowest thickness swelling (TS) 11.13%. The highest values of MOR and IB were 15.25 MPa and 0.45 MPa, respectively. This study demonstrates that cocoa pod husks and bamboo waste can be an alternative raw material source for particleboard production.

Cited as: Tang, H. T. K., Nguyen, L. D., & Ho, D. T. T. (2019). Optimum condition of manufacturing hybrid particleboard from mixture of cocoa pod husk and bamboo particles. *The Journal of Agriculture and Development* 18(3), 10-15.

1. Introduction

The abundance of agricultural residues has stimulated new interests in using agricultural fibres for global panel industries because of their environmental and profit able advantages (Rowell et al., 1997). Selection of agricultural residues have been successfully used in particleboard manufacturing (Ciannamea et al., 2010) and recent advances in the particleboard and recent advances in the particleboard industry show a bright outlook for bio-based particleboards (Bowyer et al., 2001; Pham, 2010). Non-wood plants as well as agro-based residues have been evaluated as raw materials for particleboard manufacture such as bamboo (Hoang, 2002; Nurhazwani et al., 2016), bagasse, corn stalks (Guler et al., 2016), cashew nut shell (Bui et al., 2010), chili pepper stalks (Oh & Yoo, 2011), jatropha shell (Tran, 2012), kenaf (Abdul et al., 2014), sunflower stalks (Guler et al., 2006), walnut shell (Hamidreza et al., 2012), wheat and rice straw (Li et al., 2010), etc. Bamboo has become a main material for the industrial manufacturing of furniture, parquet, and construction in recent years. Vancai (2010) pointed out that the conversion of bamboo into strips had average potential output up to 34.4%. Utilization of biomass byproduct from bamboo processing industry as value added products is an important issue to support the zero

emission concepts.

Cocoa tree is an important and the most widely planted crops in several tropical countries. In Vietnam, Cocoa trees have been planted and growing in abundant numbers recently. In the cocoa industry, Cocoa pod husks (CPH) are treated as by-product of the mature cocoa pod, after obtaining the cocoa beans. In general, CPH accounts for up to 76% of the cocoa pod wet weight. Every ton of dry cocoa been produced will generate ten tons of cocoa pod husk as waste (Cruz et al., 2012). The resource of CPH is readily abundant but does not have marketable value and most of the CPH is discarded as waste or as compost for cocoa farming the ecological impact.

Particleboard made from mixing bamboo and wood as well as agricultural residues provide satisfactory results in terms of strength properties and also address raw material scarcity issues for the particleboard industries (Nurhazwani et al., 2016; De et al., 2017). Our previous study on singer-layer particle board from mixing bamboo and cocoa pod husks has shown that the boards can produced successfully with proper mixing ration of CPH to bamboo and UF resin. In this paper, the producing three-layer particle board is investigated with different ratios of surface and core layers and various ratio of UF resin.

2. Materials and Methods

2.1. Response Surface Methodology (RSM) and Central Composite Design

Central composite design (CCD) using RSM was used in the present study to investigate the effects surface layers ratios and resin ratios on physical and mechanical properties of particle board. Three independent variables, namely, surface layers ratios (%), and urea-formaldehyde (UF) resin ratios (%) for surface and core layers were selected and the response variable names were thickness swelling (TS), Modulus of Rupture (MOR) and Internal Bond (IB). The CCD was conducted using JMP 10.0. A 15-run CCD using RSM was developed and the ranges of the variables are shown in Table 1. Each of the independent variable was coded by five different levels as shown in Table 1, where surface layers ratios (%) and resin ratios (%) for surface and core layers ranged from 30% to 50%, 6 to 10% and 4 to 6%, respectively.

2.2. Manufacturing three-layer particle board

Bamboo waste and CPH were provided from Bamboo Nature Company in Binh Duong and Thanh Dat Cocoa Company in Ba Ria Vung Tau Province. They were chipped using a hacker chipper before the chips were reduced into smaller particles using a knife ring flaker. The particles were sorted using a circulating vibrator screen to separate the particles into various particle sizes retained at 0.5, 1.0, 2.0 mm and 4 mm sieve sizes. Particles of sizes 0.5 to 2.0 mm for the surface layer and particles of sizes 2 to 4 mm for the core layer were used. The particles were dried in an oven maintained at 80°C until moisture content of 6% was reached.

Three-layer particle boards with size of 300 × 300 × 11 mm and a medium density were produced from mixture of 30% CPH and 70% bamboo particles for both surface and core layers. The particle boards were investigated with different ratios of surface to core layers (30, 40 and 50%) and various ratio of UF resin for surface layer (6, 8 and 10%) and for core layer (4, 6 and 8%) as suggested by RSM models (Table 1). The boards were pressed under a temperature of 140oC, pressure of 2.7 MPa for 9 min. Three replications for each run were done, total 45 boards produced.

2.3. Testing the particle boards investigated

The boards were conditioned at ambient temperature and 65% relative humidity until they achieved equilibrium moisture content prior to cutting into test specimens. The samples for testing and the internal bond (IB) and modulus of rupture (MOR) were determined according to procedure Standard TCVN 7756:2007. Thickness swelling (TS) properties of the panels were investigated 24-h soaking test.

3. Results and Discussion

3.1. Properties three-layer particle board investigated

The results of the properties of the particle board investigated are presented in Table 2. The boards in nine experiments (Runs 2-5, Runs 8-10, Run 13 and Run 15) meet the Standard TCVN 7754:2007 required for the modulus of rupture ($\geq$ 12.5 MPa) and the internal bond ($\geq$ 0.28 MPa).

Table 1. The range and levels of the variables

Factor	Variable	Range and level of actual and coded values				
		-α	-1	0	+1	α
X_1	Surface layers ratios (%)	30	30	40	50	50
X_2	Resin ratios for surface layers (%)	6	6	8	10	10
X_3	Resin ratios for core layer (%)	4	4	6	8	8

Table 2. Properties of the particle boards investigated

Run	Surface layers ratios (%)	Resin ratios for surface layers (%)	Resin ratios for core layer (%)	TS[1] (%)	MOR[2] (MPa)	IB[3] (MPa)
1	30	6	4	13.24	13.85	0.26
2	30	6	8	11.51	14.72	0.36
3	30	8	6	11.44	15.01	0.42
4	30	10	4	12.55	14.17	0.35
5	30	10	8	11.41	15.09	0.43
6	40	6	6	12.46	14.10	0.25
7	40	8	4	13.80	13.47	0.27
8	40	8	6	12.33	14.49	0.35
9	40	8	8	12.15	14.83	0.37
10	40	10	6	11.86	14.36	0.36
11	50	6	4	13.92	12.06	0.23
12	50	6	8	13.52	12.22	0.25
13	50	8	6	12.97	13.08	0.34
14	50	10	4	13.82	12.38	0.30
15	50	10	8	12.75	13.14	0.35

[1]TS: Thickness swelling
[2]MOR: Modulus of rupture.
[3]IB: Internal bond.

3.2. Effects of surface to core layers ratio and resin ratios for the layers on properties of particle board

Statistical analysis showed a highly significant effect of the ratio of layers and ratio of UF used in each layer for TS, MOR and IB of the three-layer particle boards tested (Figures 1, 2 and 3).

Thickness swelling (TS): Figure 1 shown that TS is inversely proportional to surface layers ratios and directly proportional to resin ratios for surface and core layer. In which surface layers ratios factors has the greatest influence on TS. When applying surface layers ratios below 31% with resin ratios for surface layers above 9% and resin ratios for core layer 6%, TS has the highest value of 11.41%.

Modulus of Rupture (MOR): In Figure 2, MOR increase as the surface layers ratios decreased with increasing of UF resin for the layers. The MOR has the highest value of 15.09 MPa, when applying surface layers ratios below 32.2% with UF resin for surface above 7.1% and for core layer 6.2%. The board manufactured applying all layer investigated ratios and using up till 8% UF resin for surface layer and up till 6% UF resin for core layer as well as using 30% and 40% surface layer, 6% UF resin for surface layer and 4% UF resin for core layer satisfy the Standard TCVN 7754:2007 (MOR $\geq$ 12.5 MPa).

Internal Bond (IB): Figure 3 shown that IB of the board increase when UF resin for both layers increased and the Surface layer ratios decreased. At the surface layers ratios below 30.9%, using UF above 7.6% for the surface layer and 6.7% for the core layer, the result obtains the highest IB of 0.43 MPa. The board manufactured at all layer ratios and using up till 8% UF resin for surface layer and up till 6% UF resin for core layer as well as using 30% surface layer, 6% UF resin for surface layer and 8% UF resin for core layer and 10% UF resin for surface layer and 4% UF resin for core layer satisfy the Standard TCVN 7754:2007 (IB $\geq$ 0.28 MPa).

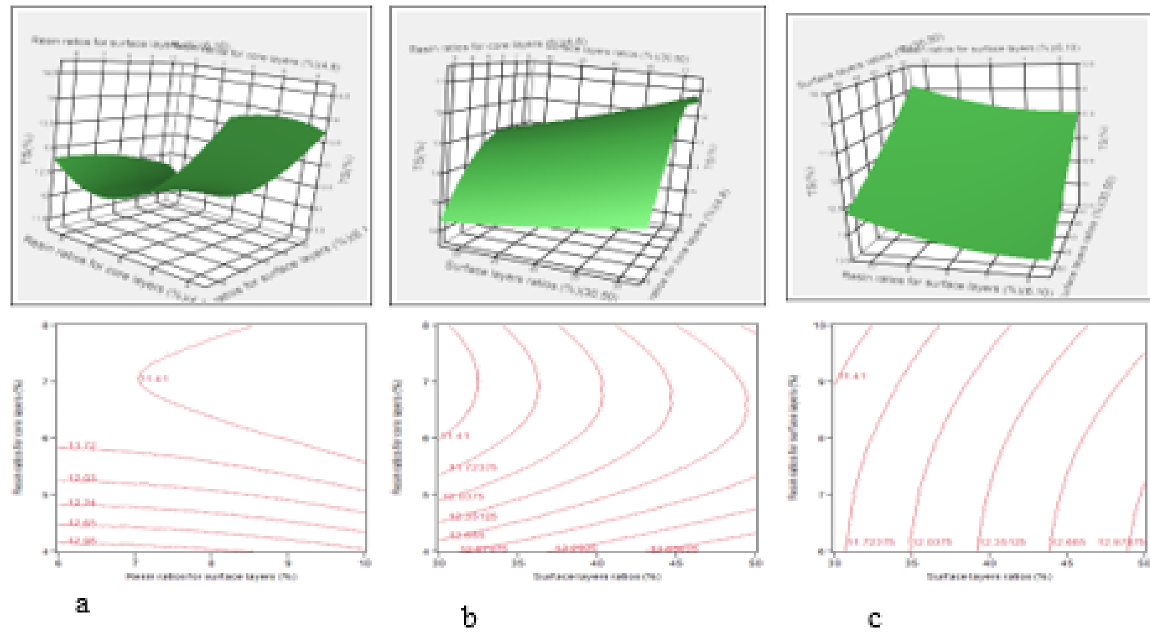


Figure 1. The 3D-surface plots of thickness swelling (TS) as function of (a) Resin ratios for surface layers and resin ratios for core layer (b) Surface layers ratios and resin ratios for core layer (c) Surface layers ratios and resin ratios for surface layers.

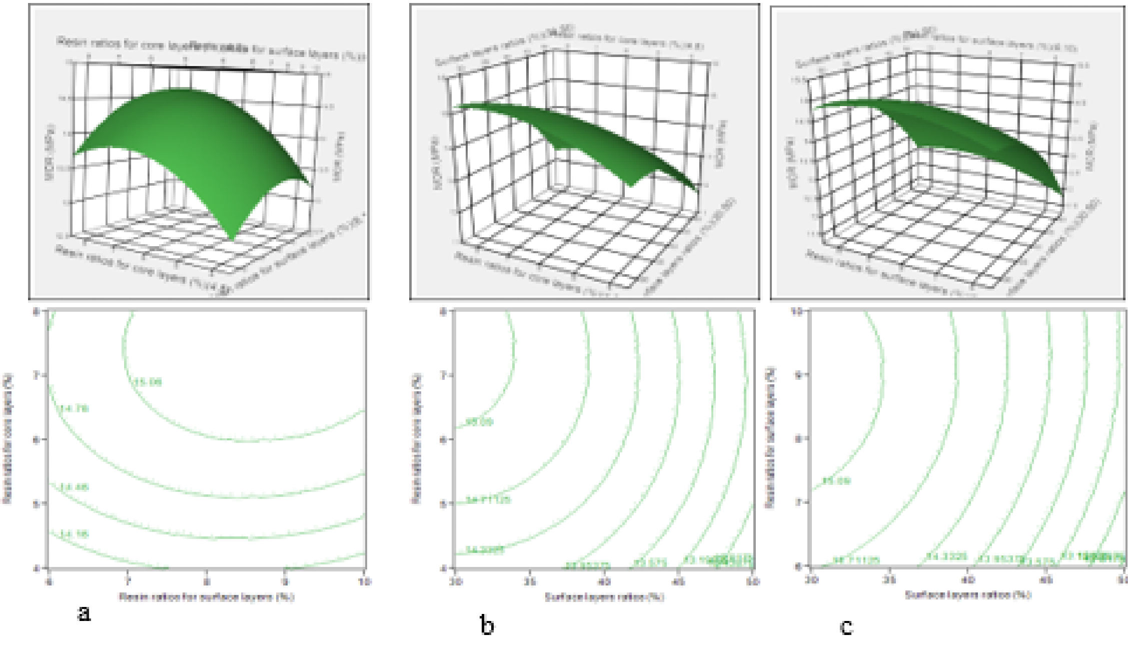


Figure 2. The 3D-surface plots of MOR as function of (a) Resin ratios for surface layers and resin ratios for core layer (b) Surface layers ratios and resin ratios for core layer (c) Surface layers ratios and resin ratios for surface layers.

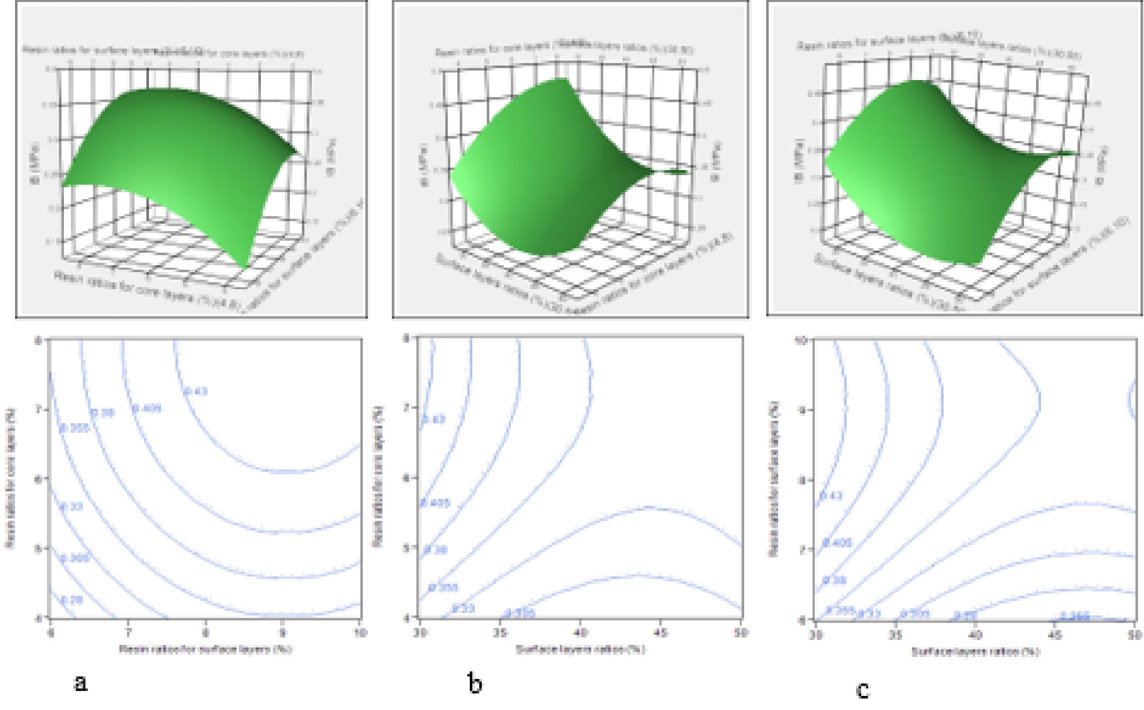


Figure 3. The 3D-surface plots of IB as function of (a) Resin ratios for surface layers and resin ratios for core layers (b) Surface layers ratios and resin ratios for core layers (c) Surface layers ratios and resin ratios for surface layers.

3.3. Regression and Adequacy of the Model and optimal condition

To ensure the fitted model gave a sufficient approximation of the results obtained in the experimental conditions, the adequacy of the model was evaluated. The fit of the model was evaluated using coefficient of multiple regressions (R^2) and adjusted R^2 was used for confirmation of the model adequacy. Based on the analysis, R^2 values of 0.9666, 0.9832 and 0.9769 for the TS, MOR and IB, respectively, indicated high fitness of the model. The adequacy of the model was further proved by high adjusted R^2 of 0.9068, 0.9529 and 0.9354, respectively. Describing the functional relation of the independent variables (X_1: surface layer, X_2: UF resin ratio for surface layer and X_3: UF resin ratio for core layer) and the response variable using regression analysis obtain three models. The final equations in terms of actual factors are shown below:

Y_{TS} (%) $= 18.681 + 0.0683x_1 - 0.113x_2 - 2.4478x_3 + 0.1790x_3^2$

Y_{MOR} (MPa) $= 9.3339 + 0.2524x_1 + 0.1095x_2 + 0.2035x_3 - 0.0044x_1^2$

Y_{IB} (MPa) $= 0.205 - 0.0355x_1 + 0.182x_2 + 0.0175x_3 + 0.0004x_1^2 + 0.01x_2^2$

The optimal condition was computed by the responsive surface response method, resulting shown as Figure 4. The optimal condition is 30% surface layers ratios, 9.51% resin ratios for surface and 7.45% resin ratios core layer obtaining the lowest TS 11.23%, the highest value of MOR and IB is 15.25 MPa and 0.45 MPa, respectively.

4. Conclusions

Results show that it is possible to produce particleboards using mixture of cocoa pod husk particles and bamboo particles using urea formaldehyde resin. The boards manufactured using up till 8% UF resin for surface layer and up till 6% UF resin for core layer meet the Standard TCVN7754:2007 required for the modulus of rupture ($\geq$ 12.5 MPa) and the internal bond ($\geq$ 0.28 MPa). The board has the lowest TS 11.23% and the highest value of MOR 15.25 MPa and IB 0.45

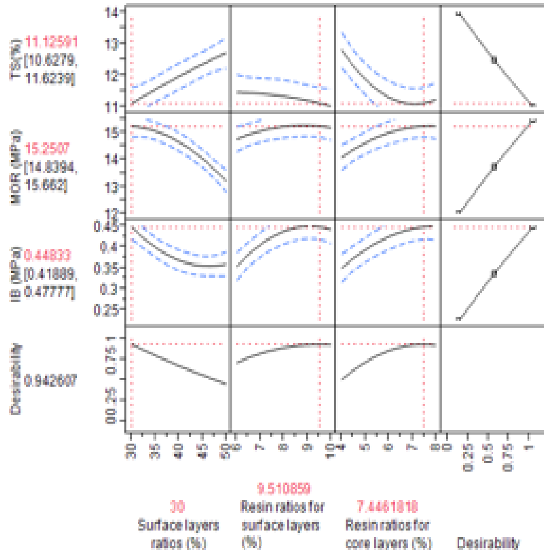


Figure 4. The cross-sectional surface meets the optimum point.

MPa, applying 30% surface layers ratios, 9.5% resin ratios for surface and 7.5% resin ratios core layer. The results of this study notably states that cocoa pod husks and bamboo waste are as an alternative renewable materials and feasible for particle board production.

References

Abdul, H. J., Paridah, M. T., Adrian, C. Y. C., & Zaidon, A. (2014). Effect of Kenaf parts on the performance of single-Layer and three-layer particleboard made from Kenaf and Rubberwood. *BioResources* 9(1), 1401-1416.

Bowyer, J. L., & Stockman, V. E. (2001). Agricultural residues: an exciting bio-based raw material for the global panel industry. *Forest Products Journal* 51(1), 10–21.

Bui, A. V., Nguyen, Q. V., & Pham, M. T. T. (2010). Research on utilizing cashew nut cover and Eucallyptus urophylla chip for common particle board producing. *Vietnam Journal Forest Science* 3, 1383-1387.

Ciannamea, E. M., Stefani, P. M., & Ruseckaite, R. A. (2010). Medium-density particleboards from modified rice husks and soybean protein concentrate-based adhesives. *Bioresource Technology* 101, 818–825.

De, A. A. C., Victor, A., De, A., Elen, A. M. M., Maristela, G., Rafaele, A. M., José, N. G., & Juliana, C. B. (2017). Wood-bamboo particleboard: Mechanical properties. *BioResources* 12(4), 7784-7792.

Guler, C., Bektas, I., & Kala Y. H. (2006). Properties of particleboard from sunflower stalks (*Helianthus annuus* L.) and Calabrian pine (*Pinus brutia* Ten). *Forest Products Journal* 56, 56–60.

Guler, C., Halil I. S., & Sevcan, Y. (2016). The potential for using corn stalks as a raw material for production particleboard with industrial wood chips. *Journal of Wood Research* 61(2), 299-306.

Hamidreza, P. , Abolghasem, K., & Taghi, T. (2012). The potential for using walnut (*Juglans regia*) shell as a raw material for wood-based particleboard manufacturing. *Composites: Part B* 43, 3276–3280.

Hoang, H. T. (2002). *Study on producing board from combining Balcooa bamboo and Rubber wood* (Unpublished doctoral dissertation). Vietnamese Academy of Forest Sciences, Ha Noi, Vietnam.

Li, X., Chi, Z., Winandy, J. E., & Basta, A. H. (2010). Selected properties of particleboard panels manufactured from rice straws of different geometries. *Bioresource Technology* 101, 4662–4666.

Nurhazwani, O., Jawaid, M., Paridah, M. T., Abdul, J. H., & Hamid, S. A. (2016). Hybrid particleboard made from bamboo (*Dendrocalamus asper*) veneer and Rubber wood (*Hevea brasinesis*). *BioResources* 11(1), 306-323.

Pham, N. N. (2010). Study on the production process parameters of particle board from agricultural residues. *Journal of Forestry Science and Technology* 1, 78-82.

Rowell R. M, Anand R., Claufield F. D, & Jacobson E. R. (1997). Utilization of natural fibers in plastic composites, problems and opportunities. *Journal of Thermoplastic Composites Materials* 15(4), 281-300.

Tran, C. V. (2012). Study on producing particle board from Rubber wood and Jatropha shells. *Journal of Forestry Science and Technology* 1, 88-95.

Vancai, L. (2010) Physical and mechanical properties of particleboard from bamboo waste. *World Academy of Science, Engineering and Technology* 40, 566–570.

Oh, Y. S., & Yoo, J. Y. (2011). Properties of particleboard made from chili pepper stalks. *Journal of Tropical Forest Science* 23(4), 473–477.

Mould resistance of the bamboo *Thyrostachys siamensis* treated with chitosan

Hong T. K. Tang[1*], & Mai H. Trinh[2]

[1]Faculty of Forestry, Nong Lam University, Ho Chi Minh City, Vietnam

[2]College of Wood Industry and Interior Design, Vietnam National University of Forestry, Ha Noi, Vietnam

ARTICLE INFO

Research Paper

Received: March 04, 2019
Revised: May 22, 2019
Accepted: June 07, 2019

Keywords

Bamboo
Chitosan
Chitosan-copper complexes
mould-resistance

***Corresponding author**

Tang Thi Kim Hong
Email: tangkimhong@hcmuaf.edu.vn

ABSTRACT

In this study, mould resistance of the bamboo species *Thyrostachys siamensis* treated with chitosan was tested under laboratory and praxis condition. In the laboratory experiment, bamboo specimens were treated with various solutions of both low molecular weight chitosan (LMW) and medium molecular weight chitosan (MMW) at concentrations of 1, 2 and 3% and Chitosan-copper complexes (CC) at concentrations of 2, 4 and 6%. Mould growth on the specimens was evaluated 1, 2, 4 and 8 weeks after they were exposed to the inoculation with a conidia mixture of six moulds isolated from bamboos. In field test, bamboo samples were treated with the effective formulations from the laboratory experiment. Evaluation of mould growth on the samples was done 1, 2, 4 and 8 weeks after exposure at the storage site of Bamboo company, Binh Duong. The results showed that treatments with chitosan (MMW) at the concentration of more than or equal to 3% and Chitosan-copper complexes at the concentration of more than or equal to 4% completely inhibited mould growth on the bamboo *T. siamensis.*

Cited as: Tang, H. T. K., & Trinh, M. H. (2019). Mould resistance of the bamboo *Thyrostachys siamensis* treated with chitosan. *The Journal of Agriculture and Development* 18(3), 16-20.

1. Introduction

Bamboo is one of the important vegetative resources after plantation wood and is a major raw material for the forest product industry. In Vietnam, bamboo has become the main material for industrial manufacturing of round and laminated bamboo furniture and parquet. The bamboo *Thyrsostachys siamensis* with its Vietnamese name "Tam Vong" is one of the most common species growing mainly as a forest and also largely cultivated in the provinces Binh Thuan, Gia Lai, Kong Tum, Lam Dong and Tay Ninh. The culms are the main raw material of many bamboo companies in South Vietnam for furniture for exportation.

Bamboo has low natural durability against fungi and insects compared with wood. In general, several fungi from the groups of moulds, ascomycetes and basidiomycetes colonise the culms of bamboos. Exposed bamboo is especially affected by moulds during storage, processing, transport in containers and its final use (Liese & Tang, 2014). For protection of bamboo against moulds and other fungi, sodium pentachlorophenol had been widely used due to its effectiveness and relative low cost. However, the chemical is banned due to its high toxicity and the public concerns on the environment. Thus, bamboo manufacturers have extreme problems in protecting bamboo for local use and export. Since bamboo countries export large quantities of bamboo culms and utilities in containers, the damage due to mould growth at port arrival has become quite serious. Manufacturers need cost-effective and also environment-friendly treatment

methods for moist bamboo during its susceptible phase.

Chitosan is the de-acytelated form of chitin produced commercially from shellfish. Chitosan can be used in a wide range of industries, due to its high degree of biocompatibility and its ability to offer an environmentally friendly alternative to current applications (Kumar, 2000; Chirkov, 2002). Chitosan-copper complex is produced from chitosan and copper salts. Recently, Chitosan and Chitosan-copper complex have been considered as interesting environmentally friendly material for wood, bamboo preservation. It is used as a potential wood preservative alone or in combination with other biocides for protection of wood and bamboo against fungi and mould (Sun et al., 2007, Gorgija et al., 2014; Gamal et al., 2016). The effectiveness of the chitosan mentioned in these researches led us to investigate mould resistance of the bamboo species *Thyrostachys siamensis* treated with various chitosan solutions of different molecular weight and concentration.

2. Materials and Methods

2.1. Laboratory experiment

Mature 3-year-old bamboo culms of *T. siamensis* were collected from a plantation of Bamboo Nature company in Binh Thuan province. From fresh culms, samples of 60 mm length were taken halfway between the internodes and split lengthwise.

Solutions of both low molecular weight chitosan (LMW) and medium molecular weight chitosan (MMW) and chitosan-Cu (II) complexes (CC) with ratio 1:1 at the concentrations of 1, 2 and 3% were applied.

Three specimens were dipped for 10 min in the respective treatment solution and placed in a small plastic box (12 x 12 x 6 cm). They were exposed to artificial infection with a water-based mixture of conidia of six moulds *A. niger*, *A. flavus*, *A. oryzae*, *Aspergillus* sp., *Paecilomyces variotii*, and *Penicillium* sp. by using a small brush. These six moulds (Figure 1) were isolated from natural growth on bamboos and were identified by DNA-IIS sequencing at the Center of Wood Biology, Hamburg University. The exposure was done in an incubation room at 30°C and 75% RH. The development of mould growth on the surface of the specimens was assessed after 1, 2, 4 and 8 weeks according to the rating method based on the BSI 2005 (Table 1).

2.2. Experiment for field tests

Samples were prepared from *T. siamensis*, as of 1000 mm length. The effective chitosan formulas from the laboratory experiments were applied. The bamboo samples were dipped for 15 min in the treatment solutions, then bundled and placed on supports over wet soil ground. After one day of exposure to natural infection, the samples were covered with a plastic sheet to avoid sunlight and drying. The test was carried out in a raw material storage area in the factory of the Bamboo Nature company, Binh Duong province. The field tests were carried out in three periods, each of 8 weeks during the rainy season. The temperature during exposure was about 28°C and the relative humidity was between 80 and 90%. The development of mould growth on the surface of the samples was assessed after 8 weeks.

3. Results and Discusion

3.1. Laboratory test

Based on the rating system (Table 1) and average mold covering for 3 replicates of each treatment, the results of the laboratory test for *T. siamensis* are presented in Table 2. The samples treated with 3%, chitosan MMW, 4% and 6% Chitosan-copper complexes (CC) did not show any infection, whereas water-treated control samples, chitosan LMW 1% and 2% had the highest infection rate. Medium molecular weight chitosan (MMW) and Chitosan-copper complexes showed better antifungal property than low molecular weight chitosan (LMW). Effect of medium molecular weight chitosan is improved by increasing its concentration from 1% to 3%. This confirms with previous studies of the antifungal effect of chitosan by Larnoy et al. (2006) and Gorgij et al. (2014), and our results are also in agreement with Kobayashi & Furukawa (1995) and Mekahlia & Bouzid (2009) who mentioned that Chitosan-copper complexes prevented mould.

For the laboratory experiments, the specimens were infected only once. Under field conditions with larger samples, bamboo would be exposed to permanent infection pressure from the surrounding air, so that the applied concentrations might not meet those conditions. Therefore, the effec-

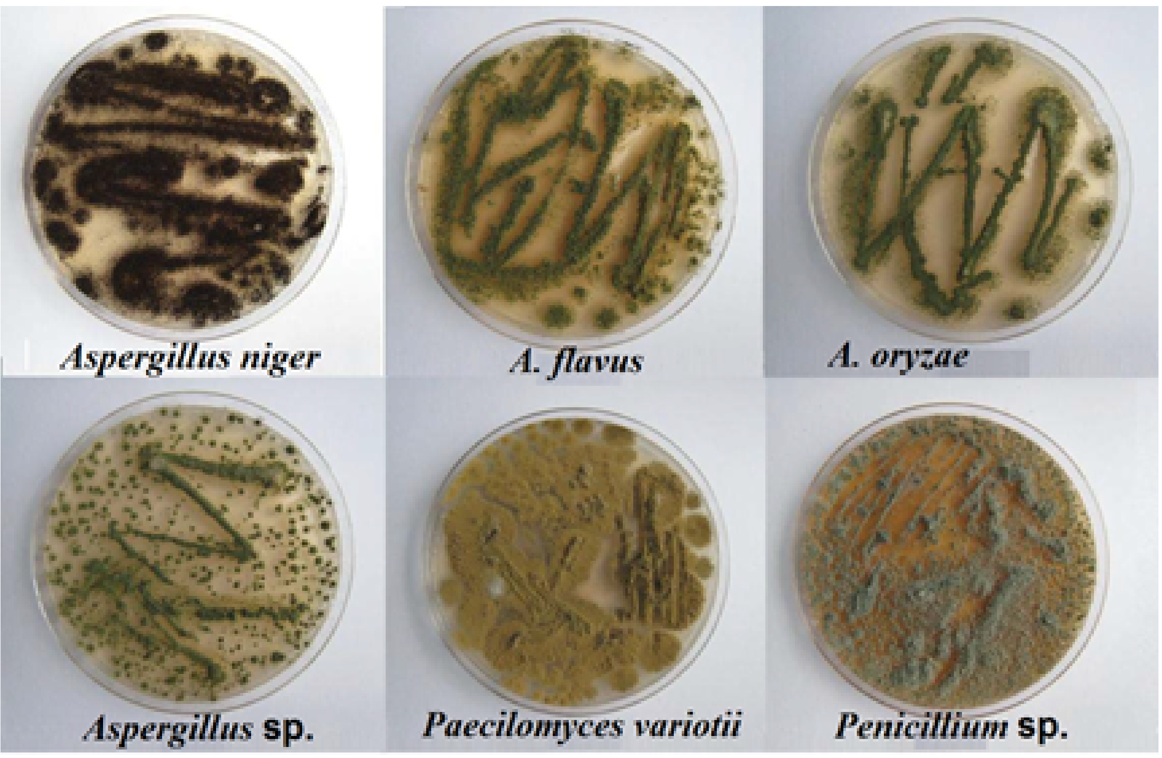


Figure 1. Moulds for testing.

Table 1. Standard method for rating the infection on the surface

Rating	Definition	Samples
1	No growth	
2	Slightly overgrown	
3	Moderately overgrown	
4	Severely overgrown	

Table 2. Infection value of treated bamboo *T. siamensis* in the laboratory test

Treatment solution[1]	Exposure time			
	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks
MMW 1%	0	1	2	3
MMW 2%	0	0	1	1
MMW 3%	0	0	0	0
LMW 1%	1	3	4	4
LMW 2%	0	3	3	4
LMW 3%	0	2	2	2
CC 2%	1	3	4	4
CC 4%	0	0	0	0
CC 6%	0	0	0	0
Control	1	4	4	4

[1]MMW: Medium molecular weight chitosan; LMW: Low molecular weight chitosan; CC: Chitosan-copper complexes.

Table 3. Infection value of treated bamboo *T. siamensis* in the laboratory test

Treatment solution[1]	Test period	Exposure time			
		After 1 week	After 2 weeks	After 3 weeks	After 4 weeks
MMW 2%	I	0	0	2	3
	II	1	3	4	3
	III	0	1	2	3
MMW 3%	I	0	0	0	0
	II	0	0	0	0
	III	0	0	0	0
CC 4%	I	0	0	0	0
	II	0	1	1	1
	III	0	0	0	0
CC 6%	I	0	0	0	0
	II	0	0	0	0
	III	0	0	0	0
Control	I	1	1	3	4
	II	4	4	4	4
	III	2	3	3	4

[1]MMW: Medium molecular weight chitosan; CC: Chitosan-copper complexes.

tive treatment solution of 2% and 3% MMW, 4% and 6% CC were further investigated in field trials.

3.2. Field test

Results of the field experiment are summarized in Table 3. Differences occurred in moulding between exposure periods. In most treatments, the samples from the second period were more quickly overgrown by moulds due to the high relative humidity of about 90%.

Generally, results of this field test are similar to the laboratory experiments with smaller samples. Treatments with 3% medium molecular weight chitosan (MMW), 4% and 6% Chitosan-copper complexes (CC) completely inhibited mould growth on the bamboo *T. siamensis*.

This investigation has shown that treatment of the bamboo *T. siamensis* with medium molecular weight chitosan and Chitosan-copper complexes can be protected from moluding. However, there are significant differences in efficacy of antimould treatments for the bamboo species (Schmidt et al., 2011). A previous study of Sun el al., 2012 indicated that Moso bamboo treated with Chitosan-copper complexes not being effective against moulds such as *Trichoderma viride* and *Aspergillus niger*. Hence, further experiments regarding mould susceptibility of different bamboo species may be of interest.

4. Conclusions

Treatment of the bamboo *T. siamensis* by medium molecular weight chitosan and Chitosan-copper complexes could completely prevent moulding during the exposure period of at least eight weeks, whereas the bamboo *T. siamensis* treated with low molecular weight chitosan was not effective against moulds. Treatments with medium molecular weight chitosan at the concentration of more than or equal to 3% and Chitosan-copper complexes at the concentration of more than or equal to 4% completely inhibited mould growth on the bamboo species.

References

BSI (British Standard Institution). (2005). Wood preservatives-Determination of the preventive effectiveness against sapstain and mould fungi on freshly sawn timber—field test. DD CEN/TS 15082. British Standard Institution, London, UK: British Standard Institution.

Chirkov, S. N. (2002). The antiviral activity of chitosan. *Applied Biochemistry and Microbiology* 3(8), 1–8.

Gamal, R. El., Nikolaivits, E., Zervakis, G. I., Abdel-Maksoud, G., Topakas, E., & Christakopoulos, P. (2016). The use of chitosan in protecting wooden artifacts from damage by mold fungi. *Electronic Journal of Biotechnology* 24, 70-78.

Gorgija, R., Tarmian, A., & Karimic, A. N. (2014). Effect of chitosan on the mold resistance of wood and its surface properties. *International Journal of Lignocellulosic Products* 1, 39-49.

Kobayashi, T., & Furukawa, I. (1995). Wood-preserving effectiveness of chitosan–metal salts against wood decaying fungi. *Journal Antibact Antifung Agents* 23, 343-348.

Kumar, M. N. V. R. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers* 46, 1-27.

Larnoy, E., Eikenes, M., & Militz, H. (2006). Evaluation of factors that have an influence on the fixation of chitosan in wood. *Wood Material Science* 1, 138-145.

Liese, W., & Tang, H. T. K. (2014). Preservation and drying of bamboo. In Liese, W. & Köhl, M. (Eds.). *Bamboo the plant and its uses* (257-298). Hamburg, Germany: Springer.

Mekahlia, S., & Bouzid B. (2009). Chitosan-copper (II) complex as antibacterial agent: synthesis, characterization and coordinating bond-activity correlation study. *Physics Procedia* 2, 1045-1053.

Schmidt, O., Wei, D. S., Liese, W., & Wollenberg, E. (2011). Fungal degradation of bamboo samples. *Holzforschung* 65, 883-888.

Sun, F., Chen, A., & Duan X. (2012). Mould-resistance of bamboo treated with the compound of chitosan-copper complex and organic fungicides. *Journal Wood Science* 58, 51-56.

Sun, F. L., Duan, X. F., Mao, S. F., & Wen, G. F. (2007). Decay resistance of bamboo wood treated with chitosan-metal complexes against the white-rot fungus Coriolous versicolor. *Scientia Silvae Sinica* 43, 82-87.

Use of Marine Sulfated Polysaccharide as an alternative to antibiotics in the diet of broilers

Tung M. Che[1*], Hien T. Le[2], Vi Q. Tran[2], Damien Berdeaux[2], Virgil Meallet[2], & Michel Guillaume[2]

[1]Department of Animal Production, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Olmix Asialand Vietnam, Binh Duong, Vietnam

ARTICLE INFO

Research Paper

Received: May 02, 2019
Revised: May 17, 2019
Accepted: May 24, 2019

Keywords

Algae extract
Broilers
Colistin
Growth performance
Marine sulfated polysaccharide (MSP)

***Corresponding author**

Che Minh Tung
Email: tung.cheminh@hcmuaf.edu.vn

ABSTRACT

The objective of the experiment was to evaluate the efficacy of Marine Sulfated Polysaccharide enhanced by a blend of organic acids (AseaD) as an alternative to colistin in the diet of broilers. A total of two hundred and sixteen one-day-old male chicks (Ross 308, initial body weight: 42.25 ± 0.42 g/bird) were randomly assigned to 1 of 2 treatments each represented with 12 replicate cages of 9 birds. The dietary treatments included (1) basal diet with antibiotic (Control, basal diet + 20 ppm colistin) and (2) basal diet without antibiotic + 0.3% AseaD (AseaD). Birds in the control were fed a basal diet containing colistin from 1 to 28 days of age only. There were no differences in ADG and ADFI between the 2 treatments at any phases or for the overall period ($P > 0.05$). Similarly, no differences in FCR were found during d 1-28 or the overall period ($P > 0.05$). Nevertheless, the FCR of broilers fed AseaD (1.893) was lower than that of broilers fed the control diet (1.991) from 29 to 42 days of age ($P = 0.016$). No differences in the survival rate of birds were found between the 2 treatments ($P > 0.05$). These results confirm the potency of AseaD in broiler diets as a potential alternative to colistin used at a concentration of 20 ppm, with significant benefits and interest during the finishing period when colistin is withdrawn from a diet.

Cited as: Che, T. M., Le, H. T., Tran, V. Q., Berdeaux, D., Meallet, V., & Guillaume, M. (2019). Use of Marine Sulfated Polysaccharide as an alternative to antibiotics in the diet of broilers. *The Journal of Agriculture and Development* 18(3), 21-26.

5. Introduction

Broiler chicks during their early stage of growth are often exposed to multiple potential stressors, such as transportation from hatchery to farm, unfavorable brooding conditions, etc. Besides, their incomplete development of the digestive system and immune functions may make them vulnerable to potentially pathogenic microorganisms leading to depressed growth and high mortality (Adams, 2004; Fascina et al., 2012). Under such conditions, antibiotics are commonly used in poultry diets for the prevention and control of harmful bacteria as well as improvement of growth rate and nutrient digestion. However, the use of antibiotics as growth promoters in animal feeds has been banned in many countries around the world due to a growing public health concern over the evolution of antimicrobial resistance (Castanon, 2007; Singer et al., 2016). Thus, finding alternatives to antibiotics as a feed additive has received much attention from scientists in recent years in

Vietnam and other countries around the world.

It has been shown that organic acids blend and specific seaweed extracts have growth-promoting properties and can be used as substitutes for antibiotics. Panda et al. (2009) showed that 0.4% butyric acid added to a broiler diet improved the FCR while maintaining the growth performance as compared with 0.05% furazolidone. Also, a diet supplemented with a blend of organic acids had a greater growth rate than that supplemented with 0.02% enramycin (Hassan et al., 2010). Beneficial effects of organic acids on the performance of broilers are likely associated with their ability in decreasing enteric pathogens and improving nutrient digestibility (Ghazala et al., 2011; Khan & Iqbal, 2016). However, the effectiveness of organic acids may vary depending on several factors, such as type and dosage of organic acids, buffering capacity of feed ingredients, presence of other antimicrobial compounds and housing conditions. Further, it was shown that marine sulfated polysaccharide (MSP) of *Ulva armoricana* green algae exhibited an antimicrobial activity and stimulated cytokine expression by intestinal epithelial cells (Berri et al., 2016). Tziveleka et al. (2018) also reported that lysozyme complexed with MSP increased its antibacterial activity. Therefore, organic acids combined with MSP would be used as a potential feed additive for improving the health and growth performance of animals, and thereby reducing the use of antibiotics for disease prevention and treatment. The objective of the experiment was to evaluate the efficacy of a Marine Sulfated Polysaccharide enhanced by a blend of organic acids (AseaD) as an alternative to colistin in the diet of broilers.

6. Materials and Methods

6.1. Experimental design, animals and housing

The experiment was conducted using two hundred and sixteen one-day-old male chicks (Ross 308, initial body weight: 42.25 ± 0.42 g/bird. The birds were randomly assigned to 2 dietary treatments in a completely randomized design. The treatments included (1) basal diet with antibiotic (Control) and (2) basal diet without antibiotic + 0.3% AseaD (AseaD). The birds were housed in cages in an open-sided house and each cage measured 1.2 m length x 0.45 m width x 0.4 m height. Each treatment was replicated with 12 cages of 9 birds each. The experiment lasted for 6 weeks.

6.2. Experimental diets and animal feeding

The basal diet was formulated to meet the nutritional requirements of broilers during the experimental period (NRC, 1994). The diets were obtained by adding colistin (20 ppm) or AseaD (0.3%) on top of the basal diet. Birds in the control were fed a basal diet containing colistin from 1 to 28 days of age only. AseaD was included in the AseaD diet and fed throughout the experimental period. AseaD contained a blend of formic acid, citric acid, lactic acid, benzoic acid, sodium butyrate and MSP. This product was provided by Olmix Asialand Co. Ltd, Binh Duong Province, Vietnam. The birds were fed a 3-phase feeding program: phase 1 (1-14 d old), phase 2 (15-28 d old) and phase 3 (29-42 d old). The ingredient composition of the basal diet is presented in Table 4. Diets were in mash form. All birds had free access to water and feed at all times.

6.3. Feed sample analyses

Feed samples were ground to pass through a 1-mm screen before analysis and analyzed according to the standard methods. Diet samples were analyzed for DM (EC 152/2009), CP (AOAC 2001.11), crude fat (TCVN 4331:2001), crude fiber (AOCS Ba-6a-05), ash (EC 152/2009), Ca (AAS08, reference 73/46/EEC), and P (AOAC 965.17). The nutrient analyses were performed by Upscience Vietnam in Binh Duong Province, Vietnam. The analyzed nutrient composition of the basal diet is presented in Table 5.

6.4. Assessment of growth performance and survival rate

The initial body weight of chicks in each cage was recorded at the commencement of the experiment. Subsequent weights of birds and feed disappearance measurements were determined at 14, 28, and 42 days of age. The ADG, ADFI and FCR were calculated on a per-cage basis. The number of dead or removed birds from each cage was recorded daily to calculate the survival rate.

6.5. Statistical analysis

Data were analyzed by an independent Student's t-test to compare the control and treatment groups, using the SAS software (SAS Inst. Inc., Cary, NC). The cage was considered the ex-

Table 4. Ingredient composition of the basal diet (as-fed basis)

Ingredients, g/kg	Days of age		
	1 - 14	15 - 28	29 - 42
Corn, ground	54.70	56.81	59.17
Soybean meal, 46%	35.00	32.80	30.50
Rice bran	4.00	4.00	4.00
Soybean oil	2.40	2.80	3.10
MCP	1.22	1.10	0.96
Limestone	1.70	1.68	1.58
Salt	0.28	0.28	0.28
Vitamin premix[1]	0.10	0.10	0.10
Mineral premix[2]	0.20	0.20	0.20
Phytase	0.02	0.02	0.02
L-Lysine, 78.8%	0.19	0.09	0.00
DL-Methionine, 99%	0.14	0.07	0.04
Pigment	0.05	0.05	0.05

[1]Supplied per kg of feed: vitamin A (10000 IU), vitamin D3 (2000 IU), vitamin E (20 IU), vitamin B2 (5 mg), vitamin B5 (5 mg), vitamin B12 (0.01 mg), niacin (10 mg).
[2]Supplied per kg of feed: Fe (80 mg), Cu (10 mg), Zn (45 mg), Mn (65 mg).

Table 5. Analyzed nutrient composition of the basal diet (as-fed basis)[1]

Items	Days of age		
	1 – 14	15 – 28	29 – 42
ME, kcal/kg[2]	3.000	3.050	3.100
DM, %	88.11	88.45	88.56
Crude protein, %	21.20	20.22	19.28
Crude fat, %	5.32	6.08	7.19
Crude fiber, %	2.54	2.43	2.55
Ash, %	6.25	5.77	5.43
Ca, %	0.99	0.90	0.82
Total phosphorus, %	0.72	0.66	0.62

[1]The analysis was performed by Upscience Vietnam in Binh Duong Province, Vietnam.
[2]Calculated.

perimental unit for live body weight, ADFI, ADG and FCR, whereas one bird was considered the experimental unit for the other parameter. The survival rate between the 2 treatments was compared by the Chi-square test. Treatment effects were considered significant at $P < 0.05$.

7. Results and Discussion

7.1. Growth performance

No differences in the body weight of broilers were found at 1, 14 and 28 days of age between the 2 treatments ($P > 0.05$; Table 6). At d 42, the body weight of birds fed AseaD (2376.1 g/bird) was greater than that of birds fed the control (2355.5 g/bird), but this difference was not statistically significant ($P = 0.598$). There were no differences in ADG and ADFI between the 2 treatments at any phases or for the overall period ($P > 0.05$; Table 7). No differences in FCR were found during 1 to 28 days of age or the overall period ($P > 0.05$). Nevertheless, broilers fed AseaD had a lower FCR than those fed the control from 29 to 42 days of age ($P = 0.016$).

Table 6. Effects of dietary supplementation of AseaD on live body weight of broilers (g/bird)

Age, d	Dietary treatments[1]		SEM	P
	Control	AseaD		
1	42.3	42.2	0.123	0.575
14	441.8	435.0	4.297	0.275
28	1306.2	1301.6	12.93	0.803
42	2355.5	2376.1	27.18	0.598

[1]12 replicate cages/treatment and 9 birds/cage.

Table 7. Effects of dietary supplementation of AseaD on growth performance of broilers

Age, d	Dietary treatments[1]		SEM	P
	Control	AseaD		
D 1-14				
ADFI, g	30.92	30.94	0.356	0.968
ADG, g	28.54	27.87	0.303	0.133
FCR	1.084	1.111	0.011	0.104
D 15-28				
ADFI, g	97.27	97.29	1.234	0.992
ADG, g	61.69	61.70	0.779	0.989
FCR	1.578	1.577	0.014	0.985
D 29-42				
ADFI, g	149.17	144.98	1.969	0.146
ADG, g	75.05	76.71	1.335	0.388
FCR	1.991	1.893	0.026	0.016
D 1-42				
ADFI, g	91.99	90.52	0.994	0.304
ADG, g	54.93	54.89	0.702	0.965
FCR	1.676	1.650	0.012	0.163

[1]12 replicate cages/treatment and 9 birds/cage.

For decades, antibiotics have been used in food animal production for disease prevention and growth promotion. However, in recent years, the use of antibiotics as growth promoters has declined due to an increasing concern about antimicrobial resistance in bacteria. Among several non-therapeutic alternatives, organic acids have been shown to perform antimicrobial activities similar to those of antibiotics (Wang et al., 2009). The combination of MSP and organic acids shows an exponential synergy and presents itself as a potent prophylactic strategy to promote animal health, and thereby reducing the need for antibiotics as the MSP was also found to exhibit an antimicrobial activity (Berri et al., 2016).

In this study, broilers fed AseaD had the same growth performance as those fed the control diet (Tables 6 and 7). This indicates that the MSP enhanced by a blend of organic acids is as efficacious as colistin in maintaining the growth rate of broilers. Interestingly, birds fed AseaD had a better FCR than those fed the control diet from 29 to 42 days of age when colistin was withdrawn from the control diet. These results agree with those of previous studies. The improvement in FCR could be due to a better utilization of nutrients as indicated by numerically lower ADFI and greater ADG in broilers fed AseaD. Previous studies showed that organic acids added to a diet for broilers improved both ME and nutrient digestibility (Garcia et al., 2007; Ao et al., 2009; Ghazala et al., 2011).

7.2. Survival rate

There were no differences in the survival rate between the 2 treatments during 1-14 and 15-28 days of age ($P > 0.05$; Figure 2). From 29 to 42 days of age, the survival rate of broilers fed the control diet (98.1%) was also not statistically different ($P = 0.498$) from that of broilers fed AseaD (100.0%). Over a 6-week study, no differences ($P = 0.701$) were found for the survival rate between the 2 treatments, although the survival rate of birds fed AseaD (97.2%) was numerically greater than that of birds fed the control diet (96.3%).

The AseaD-supplemented diet had the same effects on the survival rate as the control diet. This reflects the effectiveness of the MSP combined with a blend of organic acids which is comparable to 0.02% colistin in maintaining the bird health. It was found that a mixture of fumaric acid, calcium formate, calcium propionate, potassium sorbate, calcium butyrate, calcium lactate was more efficient than enramycin in decreasing intestinal *E. coli* and *Salmonella* spp. of broilers (Hassan et al., 2010). Further, the efficacy of AseaD is due to the combination of the MSP properties, enhanced and completed by the organic acid mixture included in the product. It was reported that the MSP of *Ulva armoricana* green algae exhibited an antimicrobial activity

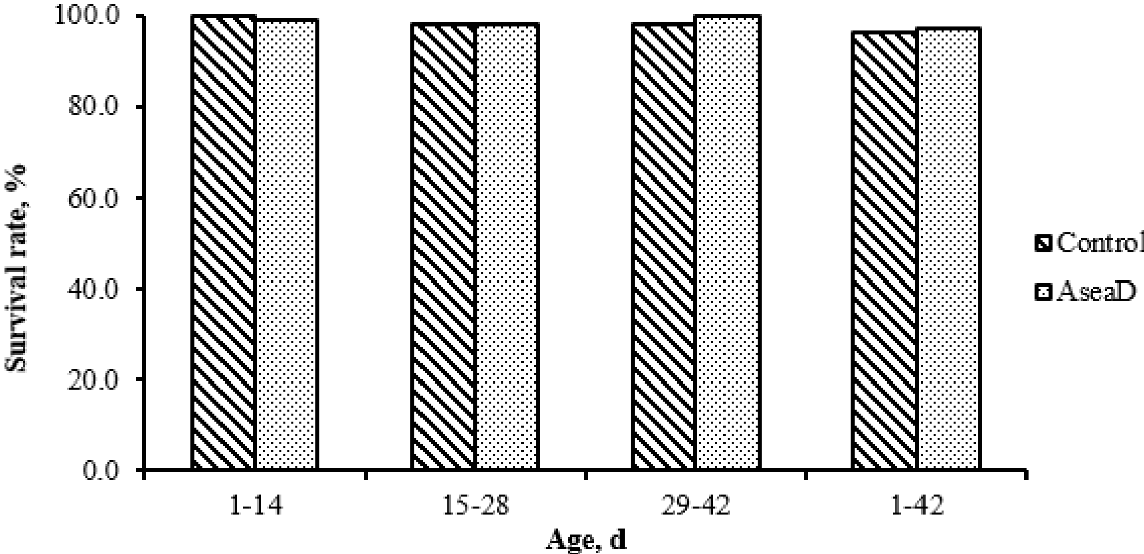


Figure 2. Effects of dietary supplementation of AseaD on the survival rate during the experimental period. There were 108 birds/treatment. No differences were observed for the survival rate during any phases or the overall period between the 2 treatments ($P > 0.05$).

and stimulated cytokine expression by intestinal epithelial cells (Berri et al., 2016). According to Leonard et al. (2011), seaweed extracts (laminarin and fucoidan) added to pig diets improved gut health by reducing the number of colonic *E. coli* and *Enterobacteriaceae*. It has also been proven that certain sulfated polysaccharides from marine algae possess antiviral, antibacterial, antifungal and antioxidant bioactivities (Wang et al., 2011; de Jesus Raposo et al., 2015; Jun et al., 2018). Thus, the suppression of harmful microorganisms by MSP would lead to a better intestinal health, and thereby improving the overall performance and health of birds. At the end of the experiment, both treatments had a high and acceptable bird survival rate (96.3 to 97.2%) like that of previous studies. For example, Rezaei et al. (2018) reported that the survival rate of Ross 308 broilers ranged from 95.5 to 97.3%.

8. Conclusions

Addition of AseaD to a broiler diet resulted in the same growth performance of broilers as a diet supplemented with colistin. The broilers fed the AseaD-supplemented diet had a lower FCR than those fed a diet with colistin from 29 to 42 days of age. These results confirm the potency of AseaD in broiler diets as a potential alternative to colistin used at a concentration of 20 ppm, with significant benefits and interest during the finishing period when colistin is withdrawn from a diet.

Conflicts of interest

The authors declare no conflicts of interest.

References

Adams, C. A. (2004). Nutricines in poultry production: focus on bioactive feed ingredients. *Nutrition Abstracts and Reviews B* 76(4), 1-12.

Ao, T., Cantor, A. H., Pescatore, A. J., Ford, M. J., Pierce, J. L., & Dawson, K. A. (2009). Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. *Poultry Science* 88(1), 111-117.

Berri, M., Slugocki, C., Olivier, M., Helloin, E., Jacques, I., Salmon, H., Demais, H., Goff, M. L., & Collen, P. N. (2016). Marine-sulfated polysaccharides extract of Ulva armoricana green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells. *Journal of Applied Phycology* 28(5), 2999-3008.

Castanon, J. I. (2007). History of the use of antibiotic growth promoters in European poultry feeds. *Poultry Science* 86(11), 2466-2471.

de Jesus Raposo, M. F., de Morais, A. M., & de Morais, R. M. (2015). Marine polysaccharides from algae with po-

tential biomedical applications. *Marine Drugs* 13(5), 2967–3028.

Fascina, V. B., Sartori, J. R., Gonzales, E., Barros De Carvalho, F., Pereira De Souza, I. M. G., Polycarpo, G. V., Stradiotti, A. C., & Pelícia, V. C. (2012). Phytogenic additives and organic acids in broiler chicken diets. *Revista Brasileira de Zootecnia* 41(10), 2189–2197.

Garcia,V., Catalá-Gregori, P., HernáNdez, F., Megiás, M. D., & Madrid, J. (2007). Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. *The Journal of Applied Poultry Research* 16(4), 555-562.

Ghazala, A. A., Atta, A. M., Elkloub, K., Mustafa, M. E. L., & Shata, R. F. H. (2011). Effect of dietary supplementation of organic acids on performance, nutrients digestibility and health of broiler chicks. *International Journal of Poultry Science* 10(3), 176-184.

Hassan, H. M. A., Mohamed, M. A., Youssef, A. W., & Hassan, E. R. (2010). Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. *Asian-Australasian Journal of Animal Sciences* 23(10), 1348-1353.

Jun, J. Y., Jung, M. J., Jeong, I. H., Yamazaki, K., Kawai, Y., & Kim B. M. (2018). Antimicrobial and Antibiofilm Activities of Sulfated Polysaccharides from Marine Algae against Dental Plaque Bacteria. *Marine Drugs* 16(9), 301-313.

Khan, S. H., & Iqbal, J. (2016). Recent advances in the role of organic acids in poultry nutrition. *Journal of Applied Animal Research* 44(1), 359-369.

Leonard, S. G., Sweeney, T., Bahar, B., Lynch, B. P., & O'Doherty, J. V. (2011). Effects of dietary seaweed extract supplementation in sows and post-weaned pigs on performance, intestinal morphology, intestinal microflora and immune status. *The British Journal of Nutrition* 106(5), 688-699.

NRC (National Research Council). (1994). *Nutrient Requirements of Poultry* (9th ed.). Washington DC, USA: National Academy Press.

Panda, A. K., Rama Rao, S. V., Raju, M. V. L. N., & Shyam Sunder, G. (2009). Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. *Asian-Australasian Journal of Animal Sciences* 22(7), 1026-1031.

Rezaei, M., Yngvesson, J., Gunarsson, S., Jonsson, L., & Wallenbeck, A. (2018). Feed efficiency, growth performance, and carcass characteristics of a fast-and a slower-growing broiler hybrid fed low- or high-protein organic diets. *Organic Agriculture* 8(2), 121-128.

Singer, A. C., Shaw, H., Rhodes, V., & Hart, A. (2016). Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Frontiers in Microbiology* 7, 1728.

Tziveleka, L.-A., Pippa, N., Georgantea, P., Ioannou, E., Demetzos, C., & Roussis, V. (2018). Marine sulfated polysaccharides as versatile polyelectrolytes for the development of drug delivery nanoplatforms: Complexation of ulvan with lysozyme. *International Journal of Biological Macromolecules* 118(Pt A), 69-75.

Wang, J. P., Yoo, J. S., Lee, J. H., Zhou, T. X., Jang, H. D., Kim, H. J., & Kim, I. H. (2009). Effects of phenyllactic acid on production performance, egg quality parameters, and blood characteristics in laying hens. *The Journal of Applied Poultry Research* 18(2), 203-209.

Wang, T., Ólafsdóttir, G., Jónsdóttir, R., Kristinsson, H. G., & Johannsson, R. (2011). Functional and nutraceutical ingredients from marine macroalgae. In C. Alasalvar, K. Miyashita, F. Shahidi, & U. Wanasundara (Eds.), *Handbook of seafood quality, safety and health applications*. New York, USA: Blackwell.

Prevalence, antimicrobial resistance profiles and virulence genes of *Vibrio* spp. isolated from shrimp retails in Ho Chi Minh City (Vietnam)

Trinh N. T. Huynh[1*], An T. T. Vo[1], Yen P. T. Nguyen[2] , & Cuong V. Nguyen[2]

[1]Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: January 03, 2019
Revised: April 15, 2019
Accepted: June 11, 2019

Keywords

Vibrio
Shrimp
Prevalence
Antimicrobial resistance
Virulence genes

***Corresponding author**

Huynh Thi Ngoc Trinh
Email: huynhngoctrinht39@gmail.com

ABSTRACT

This study was conducted to determine the diversity of pathogenic *Vibrio* species, the antimicrobial resistance profile and the presence of virulence genes linked to food-borne pathogens of *Vibrio* spp. isolated from shrimp samples in Ho Chi Minh City, Vietnam. A total of 40 raw shrimp batches were collected from retails markets (supermarket and street). All 133 test strains were isolated from 40 shrimp samples. V. parahaemolyticus was the most common species (87.5%), followed by *V. navarrensis* (60%), *V. alginolyticus* (52.5%), *V. cholerae* non-O1 (37.5%), *V. vulnificus* (22.5%), and *V. fluvialis* (10%). *Vibrio* spp. isolates were susceptible to 12 antimicrobial agents. The prevalence of ampicillin resistance was highest (82.7%), followed by cotrimoxazole (18.8%) and 3rd generation cephalosporins (16.5% cefotaxime and 8.3% ceftazidime). Extended-spectrum β lactamase (ESBL) activity was detected in 28.1% *V. parahaemolyticus* isolates. None of *tdh* or *trh* virulence genes were detected. The results of this study indicated the presentation of *Vibrio* species in shrimp samples purchased in Ho Chi Minh City. Therefore, our results could be of great potential for the identification of *Vibrio* infection in shrimp samples taken from different regions to improve food quality and safety.

Cited as: Huynh, T. N. T., Vo, A. T. T., Nguyen, Y. P. T., Nguyen, C. V. (2019). Prevalence, antimicrobial resistance profiles and virulence genes of *Vibrio* spp. isolated from shrimp retails in Ho Chi Minh City (Vietnam). *The Journal of Agriculture and Development* 18(3), 27-34.

1. Introduction

Among several aquatic species, shrimp farming grew quickly in Vietnam, making the country the third greatest shrimp exporter globally (Giang, 2017). The diseases were caused by pathogens such as bacteria, fungi, parasites, and viruses decreased significantly the shrimp production. Bacteria particularly *Vibrio* species, hit global shrimp industry in southern and southeastern Asia (Otta et al., 2001). *Vibrio cholerae* caused cholera, a severe diarrheal disease that could be life-threatening if untreated. It could spread through contaninated water and person to person contact. Non-cholera *Vibrio* spp. (for example, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio vulnificus*) caused Vibriosis. Thermostable direct hemolysin (*tdh*) and tdh-related hemolysin (*trh*) are considered major virulence of *Vibrio parahaemolyticus*. Clinical strain commonly contain either these genes and the presence of these genes is associated with pathogenicity of the strain in human (Baker-Austin et al., 2018). In the late 1990s', *V. parahaemolyticus* was implicated in a large outbreak of enteric disease in central Vietnam, with 523 cases reported (Chowdhury et al., 2004). The antibiotics and other drugs were used for growth promotion and disease prevention and treatment in shrimp culture. The usage of antibiotics in

shrimp culture have increased significantly over the last ten years in Viet Nam (Thuy et al., 2011). The misuse of antibiotics led to the proliferation of antibiotic resistance repoted in *Vibrio* strains (Elmahdi et al., 2016).

The aims of this study were to investigate the prevalence of *Vibrio* spp. in shrimps as well as analyzing the antimicrobial susceptibility profile including the presence of ESBL and their virulence genes.

2. Materials and Methods

2.1. Sample collection

Batches of shrimps (250 - 300 g each) were purchased from 40 different retail sites in 10 districts of Ho Chi Minh City (Vietnam) from March to June 2018. Shrimps which collected in sterile plastic bags (either in live/dead/unfrozen condition) to avoid cross contamination and were transported to the laboratory within 2 h in an ice-containing box. A total of five representative specimens per batch were weighted by using precision scales. three street markets and one supermarket were selected each district. From each retail site, shrimp was purchased in two forms: live or dead (chilled, not frozen). Each batch was collected the information of shrimp species. The heads, legs, and exoskeletons were separated aseptically from the muscle tissue by using a pair of sterile scissors and were subsequently pooled (shell mix). The shell mixes were used to investigate *Vibrio* spp.

2.2. Isolation of *Vibrio* spp. from the samples

Twenty-five gram of shrimp shell mixes from each sample was enriched in 225 mL of alkaline saline peptone water (ASPW) with 2% NaCl (pH 8.6) at 41.5^{0}C for 24 h. After enrichment, a loop of the inoculum was streaked onto Thiosulfate citrate bile and sucrose agar (TCBS) at 37^{0}C for 24 h (Figure 1). The colonies showed typical phenotypic characteristics of *Vibrio* spp. were identified by Maldi-tof (Bruker, Germany).

2.3. Antimicrobial susceptibility testing

The antimicrobial susceptibility of *Vibrio* spp. isolates were determined by using the disk diffusion method. The isolates were classified as resistant, intermediate and sensitive according to the guidelines of the Clinical and Laboratory Standard Institute (M45-A2 2006, CLSI, 2016). Multidrug resistance (MDR) was defined as fully resistant to at least three antimicrobial classes. *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 700603 strain were used for quality control purposes. (Elmahdi et al., 2016).

Briefly, 2 - 3 colonies from MEA were transferred to sterile saline solution (0.9%) by stir loop until the saline solution achieved a turbidity equivalent to a 0.5 McFarland standard. Then, the solution was spread on Mueller Hinton agar by using sterilized swabs. A disk diffusion test was used for susceptibility testing with 12 antimicrobials representative of eight classes of antimicrobials (Oxoid, UK). The full list of antimicrobials investigated is displayed in Tables 1. The plates were inverted and incubated at 37^{0}C for 18 to 24 h. The level of antimicrobial resistance was measured based on comparing the diameter of the inhibition zone on MHA with the standard of CLSI (CLSI, 2016).

The potential production of extended-spectrum β-lactamase (ESBL) was detected by a double disk synergy method using cefotaxime (10 μg), ceftazidime (30 μg) was placed 2 – 3 cm away from a clavulanate acid disk (Oxoid, Hampshire, England). Antimicrobial susceptibility testing results were presented according to the WHO list of antimicrobials ranked by their importance for human health. A clear extension of the edge of the inhibition zone of third generation cephalosporins towards the disk containing clavulanate was interpreted as positive for ESBL production.

ESBL production was confirmed by resistance to a third generation cephalosporin, which was performed by the combination disk diffusion method with cefotaxime and ceftazidime disks alone and in combination with clavulanate (CLSI, 2016).

2.4. Investigation of virulence gene of *Vibrio* spp. by PCR

PCR amplifications was done by the use of *tdh* and *trh* specific primers for detection of pathogenic isolates, as previously described by (Tada et al., 1992). They are genes encoding the thermostable direct hemolysin (*tdh*) and the thermostable direct hemolysin-related hemolysin (*trh*), both described as major virulence of the

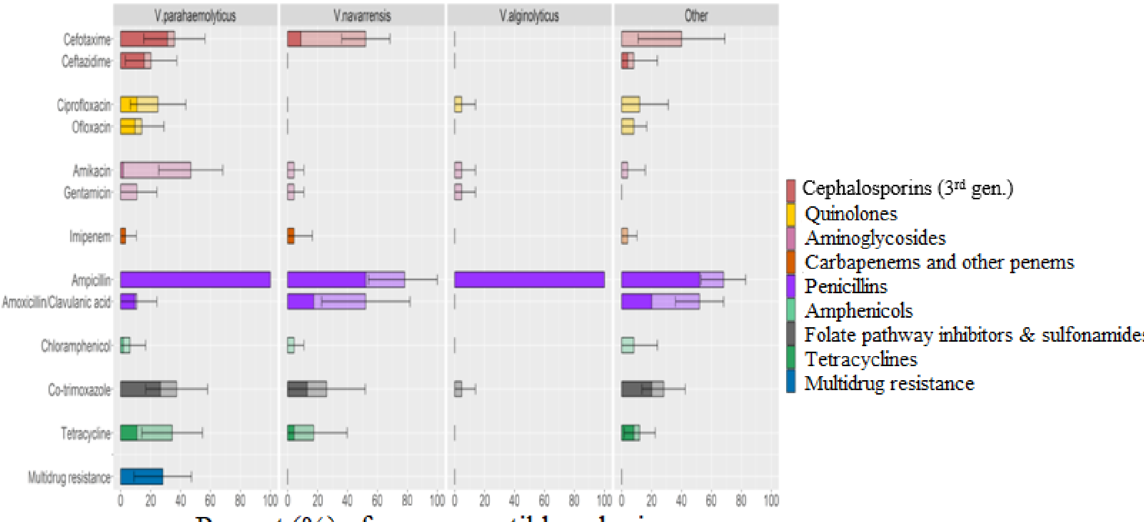


Figure 1. Phenotypic resistance amongst *Vibrio* spp. isolates by the group. The percent of isolates showing intermediate resistance was indicated by pale bars; dark bars indicate the percent of isolates with full resistance.

Table 1. Occurrence of Vibrio spp. in shrimp samples in Ho Chi Minh City, Vietnam

Variable	No. sample	No. of Vibrio species positive samples (%)					
		V.paraamlticus	*V. navarrensis*	*V. alginolyticus*	*V. cholerae non-O1*	*V. vulnificus*	*V. fluvialis*
Type of retail site							
Supermarket	10	9 (90.0%)	7 (70.0%)	4 (40%)	6 (60.0%)	2 (20.0%)	0 (0%)
Street market	30	26 (86.7%)	17 (56.7%)	17 (56.7%)	9 (30.0%)	7 (23.3%)	4 (13.3%)
Shrimp species							
White leg shrimp	30	26 (86.7%)	15 (50.0%)	16 (53.3%)	12 (40.0%)	7 (23.3%)	4 (13.3%)
Giant tiger shrimp	5	4 (80.0%)	5 (100%)	2 (40.0%)	2 (40.0%)	1 (20.0%)	0 (0%)
Other species	5	5 (100%)	4 (80%)	3 (60%)	1 (20%)	1 (20%)	0 (0%)
Condition							
Alive	17	16 (94.1%)	8 (47.1%)	11 (64.7%)	4 (23.5%)	5 (29.4%)	4 (23.5%)
Dead	23	19 (82.6%)	16 (69.6%)	10 (43.5%)	11 (47.8%)	4 (17.4%)	0 (0%)
Total	40	35 (87.5%)	24 (60.0%)	21 (52.5%)	15 (37.5%)	9 (22.5%)	4 (10.0%)

Table 2. Antimicrobial susceptibility of *Vibrio* spp. isolates

Class and antimicrobial	No. isolates and percent resistant (%)						
	V.paraamlticus (n = 64)	*V. navarrensis* (n = 23)	*V. alginolyticus* (n = 21)	*V. cholerae non-O1* (n = 15)	*V. vulnificus* (n = 6)	*V. fluvialis* (n = 4)	Total (n = 133)
Cephalosporins (3rd gen.)	20 (31.3)	2 (8.7)	-	1 (6.7)	-	-	23 (17.3)
Cefotaxime	20 (31.3)	2 (8.7)	-	-	-	-	22 (16.5)
Ceftazidime	10 (15.6)	-	-	1 (6.7)	-	-	11 (8.3)
Quinolones	7 (10.9)	-	-	-	-	-	7 (5.3)
Ciprofloxacin	7 (10.9)	-	-	-	-	-	7 (5.3)
Ofloxacin	6 (9.4)	-	-	-	-	-	6 (4.5)
Aminoglycosides	1 (1.6)	-	-	-	-	-	1 (0.8)
Amikacin	1 (1.6)	-	-	-	-	-	1 (0.8)
Gentamicin	-	-	-	-	-	-	-
Carbapenems							
Imipenem	2 (3.1)	1 (4.3)	-	-	-	-	3 (2.3)
Penicillins							
Ampicillin	64 (100)	12 (52.2)	21 (100)	8 (53.3)	2 (33.3)	3 (75)	110 (82.7)
Amoxicillin-clavulanic acid	6 (9.4)	4 (17.4)	-	5 (33.3)	-	-	15 (11.3)
Amphenicols							
Chloramphenicol	1 (1.6)	-	-	-	0 (0)	-	1 (0.8)
Folate pathway inhibitors							
Trimethoprim/ sulfamethoxazole	17 (26.6)	3 (13)	-	3 (20)	2 (33.3)	-	25 (18.8)
Tetracyclines							
Tetracycline	7 (10.9)	1 (4.3)	-	1 (6.7)	1 (6.7)	-	10 (7.5)
ESBL	18 (28.1)	-	-	-	-	-	18 (13.5)
MDR	18 (28.1)	-	-	-	-	-	18 (13.5)

emergent human pathogen *Vibrio parahaemolyticus*, and other *Vibrio* spp. (Shirai et al., 1990; Terai et al., 1991). The *tdh* and *trh* genes were used to amplified which derived from patients showing typical symptoms of *Vibrio* infection.

DNA extraction process: Strains were subcultured to obtain individual colonies on MEA at 37^0C for 24 h. A single colony was suspended in 500 μL of nuclease-free water. DNA was extracted by heating at 95^0C for 10 min and centrifuged at 14000 rpm. A clear supernatant was used as the DNA template in a PCR.

PCR thermal cycling consisted of a 96^0C hold for 5 min for the initial denaturation, followed by 25 cycles of amplification, with each cycle consisting of denaturation at 94^0C for 1 min and an annealing step at 55^0C for 1,5 min, and an extension at 72^0C for 1,5 min. After the final extension at 72^0C for 7 min, the final hold at 12^0C to preserve all PCR samples.

A negative control containing nuclease-free H_2O. For positive controls, DNA were cloned and

confirmed by sequencing were used, which possessing *tdh* and *trh* genes.

The PCR products were electrophoresed in a 1.0% agarose gel, stained by ethidium-bromide at 160 V for 30 min and photographed. Positive reactions were identified by detecting a 250 bp and 251 bp specific band visualized on agarose gels under ultraviolet light.

2.5. Data analyses

The prevalence of *Vibrio* strains was evaluated in terms of percentage occurrences, in which the positive samples were compared to the total taken samples. The antimicrobial resistance of a group of isolates was calculated as the percentage of isolates among the group that was resistant to a single antimicrobial or a number of antimicrobials. Chi-square tests (χ^2 test) or Fisher's exact tests were used to compare these proportions using online statistical tools (Minitab Software). Statistically significant difference was defined if the value of $P < 0.05$.

3. Results

3.1. Prevalence of *Vibrio* spp. from the samples

The 40 batches included five species of shrimp: white leg shrimp (*Litopenaeus vannamei*) (30), giant tiger shrimp (*Penaeus monodon*) (5), banana shrimp(*Penaeus merguiensis*) (3), greasyback shrimp (*Metapenaeus ensis*) (1), and giant prawn (*Macrobrachium rosenbergii*).

All (100%) samples were positive for *Vibrio* species. Among six *Vibrio* species, the most common species was *V. parahaemolyticus* (87.5%), followed by *V. navarrensis* (60%), *V. alginolyticus* (52.5%), *V. cholerae* non-O1 (37.5%), *V. vulnificus* (22.5%) and *V. fluvialis* (10%). The positive samples for *Vibrio* species results are shown in Table 1.

Statistical analysis was showed there was no significant difference among the sampling sites for the *Vibrio* spp. positive isolates. In addition, *V. navarrensis* prevalence was isolated more in giant tiger shrimp than in white leg shrimp ($P = 0.036$). *V. fluvialis* was also found more in shrimp that bought alive ($P = 0.014$).

3.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing against 12 antibiotic drugs was analyzed in 133 of *Vibrio* spp. isolates and the results are shown in Figure 1 and Table 1. The highest prevalence of resistance was ampicillin (82.7%), followed by trimethoprim sulfamethoxazole (18.8%) and 3rd generation cephalosporins (16.5% cefotaxime and 8.3% ceftazidime). All *V. parahaemolyticus* and *V. alginolyticus* isolates were resistant to ampicillin (100%). In total, the prevalence of resistance against amoxicillin-clavulanic,carbapenems, aminoglycosides, tetracyclines, quinolones and amphenicols was < 11.3% (Table 2).

ESBL was detected in 18/64 (28.1%) *V. parahaemolyticus* strains that were resistant to third- generation cephalosporins (31.3%). producer. The multidrug resistance to more than 3 of antibiotic classes was found in 13.5%. All non-*V. parahaemolyticus* isolates were negative for ESBL (18/133) *Vibrio* spp. isolates including the atypical *V. parahaemolyticus* isolates and a total of 15/18 (83.3%) ESBL positive *V.parahaemolyticus* strains were positive *V. parahaemolyticus* strains were positive for MDR.

3.3. Virulence gene of *Vibrio* spp. in shrimp samples

None of the virulence genes (*tdh* and *tdr*) were presented in 133 *Vibrio* spp. isolates. The representative gel photos for the PCR targeting trh and tdh gene were shown in Figure 2 and Figure 3, respectively.

4. Discussion

4.1. Prevalence of *Vibrio* spp. from the sample

Our study demonstrated that *Vibrio* spp. was dominent in shrimp samples sold in some retails in Ho Chi Minh City, Vietnam (100%). The occurrence of *Vibrio* species in tropical shrimp culture environments such as Vietnam might be expected because the areas with high temperate are an optimal condition for their growth and the infections are directly linked to this pathogen cannot be avoided in shrimp cultures because they are part of the natural microflora of coastal and estuarine environments (Koralage et al., 2012). In a previous study, similar results were ob-

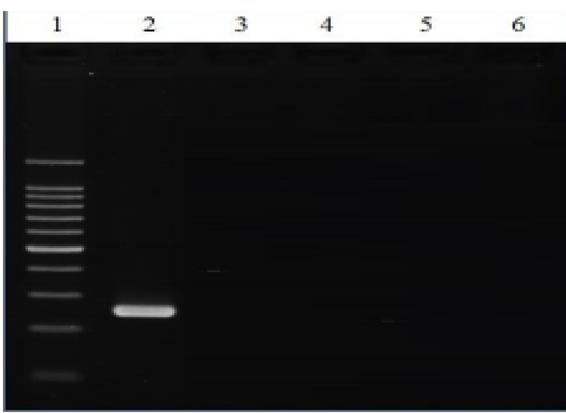


Figure 2. *tdh* gene in *V. parahaemolyticus* with size of 251 bp. Lane 1, 100bp DNA marker; lane 2, positive control; lane 3, negative control; 4-6 *V. parahaemolyticus* isolates.

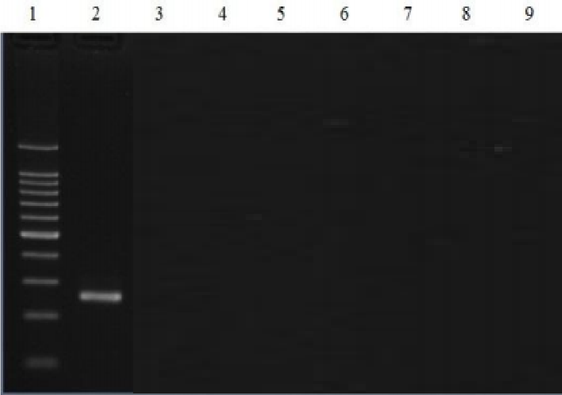


Figure 3. *trh* gene in *V. parahaemolyticus* with size of 250 bp. Lanes 1 and 10, 100bp DNA marker; lane 2, positive control; lane 3, negative control; 4-9 *V. parahaemolyticus* isolates.

tained in northern Vietnam (99.5%) (Tra et al., 2016) and Sri Lanka (98.1%) (Koralage et al., 2012). *V. parahaemolyticus* was the most prevalent species (87.5% samples). The predominance of *V. parahaemolyticus* in our study is similar to the study of retail shrimps in Ha Noi, Vietnam (96.5%) (Tra et al., 2016). However, *V. alginolyticus* was the predominant *Vibrio* species in another study (Sperling et al., 2015). In agreement with our study, *V. vulnificus* has been identified from shrimps in various countries at low prevalence (Gopal et al., 2005; Sperling et al., 2015). *V. parahaemolyticus* plays an important role because it causes diseases and mortality to the shrimp as primary and secondary pathogens. This strain found in previous study was recorded as a primary pathogen to White Spot Disease because population of the bacterial species increases with the onset of this viral disease. In addition, the result from other Antimicrobial susceptibility testing against 12 antibiotic drugs was analyzed in 133 of *Vibrio* spp. isolates and the results are shown in Figure 1 and Table 2. The highest prevalence of resistance was ampicillin (82.7%), followed by trimethoprim sulfamethoxazole (18.8%) and 3rd generation cephalosporins (16.5% cefotaxime and 8.3% ceftazidime). All *V. parahaemolyticus* and *V. alginolyticus* isolates were resistant to ampicillin (100%). In total, the prevalence of resistance against amoxicillin-clavulanic,carbapenems, study confirmed the potential of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* non-O1 as a major foodborne pathogens (Baker-Austin et al., 2017). The high prevalence of *V. parahaemolyticus* in shrimps is considered as potentially hazardous, regarding the probability of pathogenicity among the contaminant strains.

White leg shrimp is one of the most dominant farmed shrimp species in the world because of its fast growth and good toleration rate at high stock densities in different salinity levels (Cornejo-Granados et al., 2017). The white leg shrimps occupy high demand among the most consumed other shrimp in Vietnam markets.

The high prevalence of *V. fluvialis* was found more in shrimp that bought alive that indicated inadequate control in storage temperature which could be the condition in a proliferation of the pathogens. This reflected the scenario of retail outlets. As the cells of this pathogen are very sensitive to freezing and it can grow in the presence or absence of oxygen (Joseph et al., 1982).

4.2. Antimicrobial susceptibility testing

A total of 82.7% of *Vibrio* spp. isolates were resistant to ampicillin including 100% for *V. parahaemolyticus* and *V. alginolyticus*. High antimicrobial resistance to ampicillin and cephalothin were also reported (Lou et al., 2016; Rocha et al., 2016). These results are in agreement with those of other studies that indicated high resistance among *V. parahaemolyticus* isolates from shrimps, especially to ampicillin in northern Vietnam (87.2%) (Tra et al., 2016) and India (100%) (Vaseeharan et al., 2005).

Beta-lactam antibiotics are one of the main

groups used against Gram-negative and Gram-positive bacteria and account for 60% of the antibiotics used worldwide for the treatment of infectious diseases (Livermore & Woodford, 2006). The widespread use of ampicillin and cephalothin in aquaculture resulted in a reduction in the efficacy of treatment (Rocha et al., 2016). The result showed that the high percentage of ampicillin resistance was found in *V. parahaemolyticus* suggests that ampicillin cannot effectively treat infections caused by this organism. Another study reporting that *V. parahaemolyticus* isolated from shrimps in Hong Kong was positive to ESBL (Wong et al., 2012; Liu et al., 2013). The multidrug resistance to more than 3 of antibiotic classes was found in 13.5% (18/133) *Vibrio* spp. isolates including the atypical *V. parahaemolyticus* isolates and a total of 15/18 (83.3%) ESBL-positive *V. parahaemolyticus* strains were positive for MDR. According to results in Brazil, 50% of *V. parahaemolyticus* isolates presented multiple antibiotic resistance (de Melo et al., 2011). We found a high prevalence of MDR among *Vibrio* spp., with a particularly *V. parahaemolyticus* are probably a reservoir of these important resistance genes genes (Wong et al., 2012).

The misuse of antibiotics can increase resistance strains (Elmahdi et al., 2016). *Vibrio* spp. are considered as a genus of human pathogens, so antimicrobial resistance in aquatic environment threaten to human health. A rapid and accurate diagnostic method is necessary to detect the antimicrobial susceptibility of different strains. Management practices should be taken to reduce antimicrobial usage.

4.3. Virulence gene of *Vibrio* spp. in shrimp samples

No two major virulence genes positive *V. parahaemolyticus* strains were detected. Previous research showed the prevalence of *tdh* and *trh* genes in environmental, non- clinical and seafood-related strains was zero or very low (1 to 3%) (Gopal et al., 2005). In a study on 70 shrimp samples from Iran, only 2 (2.8%) *tdh* and 1 (1.4%) *trh* positive strains were identified (Asgarpoor et al., 2018). A total of 385 seafood samples in the Mekong Delta of Vietnam including tdh gene positive *V parahaemolyticus* strains were 22 (5.7%) samples and trh gene positive *V. parahaemolyticus* strains were 5 (1.3%) samples (Tran et al., 2018). However, there was no evidence of these genes among isolates investigated in northern Vietnam (Tra, et al., 2016), or Sri Lanka (Koralage et al., 2012). In addition, the results of other studies indicate that even nontoxigenic *V. parahaemolyticus* (lacking the tdh and trh genes) can induce acute gastroenteritis in humans (Ottaviani et al., 2012). However, there was evidence of *V. parahaemolyticus* was isolated at 8.3% from acute diarrheal patients in the South of Vietnam in 2010, the present of *tdh* gene is 22.2% and the present of of trh gene is 19.4% (The Tai et al., 2011). In the late 1990s', V. parahaemolyticus was implicated in a large outbreak of the enteric disease in central Vietnam, the *tdh* gene was detected in 445 strains (85%) and the *trh* gene was detected in 6 strains (1.2%) (Chowdhury et al., 2004).

5. Conclusion

This study showed the high prevalence of *Vibrio* spp. in retail shrimp products. The AMR that existed among *Vibrio*. *V. parahaemolyticus* strain lacking virulence markers caused infection for the human. The results can be used as reference for more studies in the future. Therefore, more studies should be performed to have a better overview about virulence gene of shrimps. This article can be used as a reference source for public health officials to treat patients after digesting contaminated seafood. In addition, the farmers manage the disease treatment process to avoid exporting problems.

References

Asgarpoor, D., Haghi, F., & Zeighami, H. (2018). Detection and molecular characterization of *Vibrio Parahaemolyticus* in shrimp samples. *The Open Biotechnology Journal* 12, 46-50.

Baker-Austin, C., Oliver, J. D., Alam, M., Ali, A., Waldor, M. K., Qadri, F., & Martinez-Urtaza, J. (2018). *Vibrio* spp. infections. *Nature Reviews Disease Primers* 4(1), 1-19.

Baker-Austin, C., Trinanes, J., Gonzalez Escalona, N., & Martinez-Urtaza, J. (2017). Non-Cholera Vibrios: The microbial barometer of climate change. *Trends in Microbiology* 25, 76-84.

Chowdhury, A., Ishibashi, M., Thiem, V. D., Tuyet, D. T. N., Tung, T. V., Chien, B. T., Seidlein, L. V., Canh, D. G., Clemens, J., Trach, D. D., & Nishibuchi, M. (2004). Emergence and serovar transition of *Vibrio parahaemolyticus* pandemic strains isolated during a diarrhea outbreak in Vietnam between 1997 and 1999. *Microbiology and Immunology* 48, 319-327.

CLSI (Clinical and Laboratory Standards Institude). (2016). *Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria* (3rd ed.). Wayne, USA: Clinical and Laboratory Standards Institute.

Cornejo-Granados, F., Lopez-Zavala, A. A., Gallardo-Becerra, L., Mendoza-Vargas, A., Sánchez, F., Vichido, R., Brieba, L. G., Viana, M. T., Sotelo-Mundo, R. R., & Ochoa-Leyva, A. (2017). Microbiome of pacific whiteleg shrimp reveals differential bacterial community composition between wild, aquacultured outbreak conditions. *Scientific Reports 7*, 1-15.

de Melo, L. M. R., Almeida, D., Hofer, E., Dos Reis, C. M. F., Theophilo, G. N. D., Santos, A. F. das M., & Vieira, R. H. S. D. F. (2011). Antibiotic resistance of *Vibrio parahaemolyticus* isolated from pond-reared *Litopenaeus vannamei* marketed in natal, brazil. *Brazilian Journal of Microbiology* 42(4), 1463-1469.

Diep, T. T., Au, T. V., Nguyen, N. T. N., Nguyen, N. T. K., & Nguyen, L. T. P. (2011). Virulence and antimicrobial resistance characteristics of *Vibrio parahaemolyticus* isolated from environment, food and clinical samples in the south of Vietnam, 2010. *BMC Proceedings* 5(Suppl 1), 94.

Elmahdi, S., DaSilva, L. V., & Parveen, S. (2016). Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: A review. *Food Microbiology* 57, 128-134.

Giang, H. (2017). Action plan to develop antimicrobial resistance phenotype and genotype of pathogenic *Vibrio parahaemolyticus* isolated from seafood. *Food Control* 59, 207–211.

Gopal, S., Otta, S. K., Kumar, S., Karunasagar, I., Nishibuchi, M., & Karunasagar, I. (2005). The occurrence of Vibrio species in tropical shrimp culture environments; implications for food safety. *International Journal of Food Micobiology* 102(2), 151-159.

Joseph, S. W., Colwell, R. R., & Kaper, J. B. (1982). *Vibrio parahaemolyticus* and related halophilic Vibrios. *CRC Critical Reviews in Microbiology* 10(1), 77-124.

Koralage, M. S. G., Alter, T., Pichpol, D., Strauch, E., Zessin, K. H., & Huehn, S. (2012). Prevalence and molecular characteristics of *Vibrio* spp. isolated from preharvest shrimp of the North Western province of Sri Lanka. *Journal of Food Protection* 75(10), 1846-1850.

Liu, M., Wong, M. H. Y., & Chen, S. (2013). Molecular characterisation of a multidrug resistance conjugative plasmid from *Vibrio parahaemolyticus*. *International Journal of Antimicrobial Agents* 42(6), 575-579.

Livermore, D. M., & Woodford, N. (2006). The β-lactamase threat in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. *Trends in Microbiology* 14(9), 413-420.

Lou, Y., Liu, H., Zhang, Z., Pan, Y., & Zhao, Y. (2016). Mismatch between antimicrobial resistance phenotype and genotype of pathogenic *Vibrio parahaemolyticus* isolated from seafood. *Food Control* 59, 207-211.

Otta, & Karunasagar, I. (2001). Bacteriological study of shrimp, *Penaeus monodon* Fabricius, hatcheries in India. *Journal of Applied Ichthyology* 17(2), 59-63.

Ottaviani, D., Leoni, F., Serra, R., Serracca, L., Decastelli, L., Rocchegiani, E., Masini, L., Canonico, C., Talevi, G., & Carraturo, A. (2012). Nontoxigenic *Vibrio parahaemolyticus* strains causing acute gastroenteritis. *Journal of Clinical Microbiology* 50(12), 4141-4143.

Rocha, R. D. S., Sousa, O. V., Vieira, R. H. (2016). Multidrug-resistant *Vibrio* associated with an estuary affected by shrimp farming in North Eastern Brazil. *Marine Pollution Bulletin* 105(1), 337-340.

Shirai, H., Ito, H., Hirayama, T., Nakamoto, Y., Nakabayashi, N., Kumagai, K., Takeda, Y., & Nishibuchi, M. (1990). Molecular epidemiologic evidence for association of thermostable direct hemolysin (TDH) and TDH-related hemolysin of *Vibrio parahaemolyticus* with gastroenteritis. *Infection and Immunity* 58(11), 3568–3573.

Sperling, L., Alter, T., & Huehn, S. (2015). Prevalence and antimicrobial resistance of *Vibrio* spp. in retail and farm shrimps in Ecuador. *Journal of Food Protection* 78(11), 2089-2092.

Tada, J., Ohashi, T., Nishimura, N., Shirasaki, Y., Ozaki, H., N., Shirasaki, Y., Ozaki, H., Fukushima, S., Takano, J., Nishibuchi, M., & Takeda, Y. (1992). Detection of the thermostable direct hemolysin gene (*tdh*) and the thermostable direct hemolysin-related hemolysin gene (*trh*) of *Vibrio parahaemolyticus* by polymerase chain reaction. *Molecular and Cellular Probes* 6(6), 477–487.

Terai, A., Baba, K., Shirai, H., Yoshida, O., Takeda, Y., & Nishibuchi, M. (1991). Evidence for insertion sequence-mediated spread of the thermostable direct hemolysin gene among *Vibrio* species. *Journal of Bacteriology* 173(16), 5036-5046.

Thuy, H. T. T., Nga, L. P., & Loan, T. T. C. (2011). Antibiotic contaminants in coastal wetlands from Vietnamese shrimp farming. *Environmental Science and Pollution Research* 18, 835-841.

Tra, V. T. T., Meng, L., Pichpol, D., Pham, N. H., Baumann, M., Alter, T., & Huehn, S. (2016). Prevalence and antimicrobial resistance of *Vibrio* spp. in retail shrimps in Vietnam. *Berliner und Münchener tierärztliche Wochenschrift* 129(1-2), 48–51.

Tran, L., Nunan, L., Redman, R., Mohney, L., Pantoja, C., Fitzsimmons, K., & Lightner, D. (2013). Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Diseases of Aquatic Organisms* 105(1), 45-55.

Wong, M. H. Y., Liu, M., Wan, H. Y., & Chen, S. (2012). Characterization of Extended-Spectrum-β-Lactamase-Producing *Vibrio parahaemolyticus*. *Antimicrobial Agents and Chemotherapy* 56(7), 4026-4028.

Application of PCR technique in diagnosis of four respiratory pathogenic bacteria in pigs at the slaughterhouse

Han M. Ly*, Trinh T. K. Nguyen, Thiep T. X. Dang, & An T. T. Vo

Department of Animal Husbandry and Veterinary Medicine, Nong Lam Univsersity, Ho Chi Minh, Vietnam

ARTICLE INFO

Research Paper

Received: February 20, 2019
Revised: April 26, 2019
Accepted: May 27, 2019

Keywords

Multiplex polymerase chain reaction (PCR)
Pig lungs
Respiratory bacteria
Slaughterhouse

***Corresponding author**

Ly Mai Han
Email: 14112089@st.hcmuaf.edu.vn

ABSTRACT

The polymerase chain reaction (PCR) for *Actinobacillus pleuropneumonia* (*App*), *Haemophilus parasuis* (*Hps*), *Pasteurella multocida* (*Pm*) and *Bordetella bronchiseptica* (*Bb*) were performed in pure colonies isolated from 114 lung specimens with lesions collected from the Vissan slaughterhouse in Ho Chi Minh City from July 2018 to May 2019. The aim of the experiment was to identify the four respiratory pathogenic bacteria in pigs at slaughterhouse by using PCR technique. The criteria for evaluating the results included the proportion of positive samples with multiplex PCR and percentage of samples co-infected with 2, 3, and 4 bacteria. Among a total of 114 injured lung samples, 21% of the samples was positive to at least one of the four bacteria, 3 samples (2.63%) were positive for *App*, 2 samples (1.75%) were positive for *Hps*, 7 samples (6.14%) were for *Pm*, and 12 lungs (10.53%) were positive for *Bb*. One sample (0.88%) was found co-infected with *Pm* and *Hps*.

Cited as: Ly, H. M., Nguyen, T. T. K., Dang, T. T. X., Vo, A. T. T. (2019). Application of PCR technique in diagnosis of four respiratory pathogenic bacteria in pigs at the slaughterhouse. *The Journal of Agriculture and Development* 18(3), 35-40.

1. Introduction

Respiratory disease in pigs is one of the leading concerns in the livestock industry. The major direct loss effects on the farmer's economy due to respiratory illnesses include increased mortality and morbidity rate, reduced weight gain, long finishing time, and high consumption. expenses for treatment (de Jong et al., 2014). Usually, viral respiratory diseases (PRRS, CSFV, PCV-2, etc.) or some important bacteria such as *Actinobacillus pleuropneumoniae* (*App*), *Bordetella bronchiseptica* (*Bb*) are the primary factors causing diseases. However, the immunodeficiency of infected pigs creates favorable conditions for the arising secondary infections of *Haemophillus parasuis* (*Hps*), and *Pasteurella multocida* (*Pm*) that normally reside in the upper respiratory tract of the animals. The most important respiratory pathogen is *P. multocida* (de Jong et al., 2014). The App causes severe acute pleuropneumonia with very high mortality rates of up to 80%. Infectious rhinitis caused by Bb and Pm is common in commercial pig herds. The *Hps* causes acute infection with characteristic of causing multi-serous inflammation. When these infectious pathogens co-infect, they increase the severity of the disease.

While isolation is time-consuming and requires good laboratory skills, diagnosis by PCR method helps to provide accurate results, high reliability

while saving test time and giving faster results. Thus, the objective of this study was to detect the presence of four respiratory pathogenic bacteria in pigs at the slaughterhouse by using the PCR technique.

2. Materials and Methods

The experiment was conducted from July 2018 to May 2019 at the laboratory of Department of Veterinary Biosciences and the Veterinary Hospital, Faculty of Animal Science and Veterinary Medicine, Nong Lam University. Four bacteria that have significant impact on respiratory diseases in pigs, including *App*, *Hps*, *Pm* and *Bb* were analyzed from 114 swine lung specimens.

2.1. Sample collection

Sample collection was performed at the slaughterhouse of Vissan company in Ho Chi Minh City. Injured lungs with lesions, such as congestion, haemorrhage, and inflammation were separated from the carcass and stored in separate zip bags to avoid contamination and transported to the laboratory for culture.

2.2. Isolation method

Tryptone Soybean Agar (TSA) (Merck Group, Germany) with 5% bovine serum (Gibco, New Zealand) and Nicotinamide adenine dinucleotide (NAD) (Merck Group, Germany) were used to optimize the growth of four bacteria. Before culture, surface of samples and equipment were disinfected by using an alcohol swab to clean the surface of the lung until surface was dry. The scissors and forceps were heated using an alcohol-burner and allowed to cool down before use. To obtain an uncontaminated tissue, lung samples were cut deeply in small tissues. Direct smear of the newly cut tissue was performed into a Petri dish containing the culture medium and a sterile loop to streak the sample was used. Plates were incubated at 37^0C for 24 h in bacteriological incubator (Memmert, Germany). If bacteria growth was seen, the colonies were selected based on colony morphology, catalase reaction (Table 1) and Gram stain (the target bacteria have negative Gram stain). The suitable colonies were transferred into the new TSA medium for pure isolation for the next 24 h.

2.3. Preparation of samples for PCR

Bacterial DNA samples were extracted from whole cells by using thermal shock. Pure colonies were placed into an eppendorf containing 50 μL of Tris EDTA buffer solution (TBR, Vietnam) and went through heat cycles (10 min, 100^0C; 1 min, -20^0C). Cell debris was removed by centrifugation at 12000 rpm in 2.5 min. The supernatant was used directly for PCR process or stored at -20^0C.

The total volume for m-PCR of App, *Pm* and *Hps* was 20 μL. The mixture contained 10 μL of Gotaq G2 Green MasterMix, 2 μL of Nulease-Free water (Promega, USA), 1 μL per each primer x 6 primers (AP-IV (Xiao et al., 2006), KMT1 (Townsend et al., 1998), HPS (Oliveira et al., 2001)) (Table 2) and 3 μL of DNA samples. Bacterial DNA samples were isolated directly from pure colonies by thermal shock. The heat cycle was adapted from Hričínová (2010) research: (1) the initial phase lasted for 5 min at 95^0C, then the denaturation was performed at 94^0C for 30 s. The priming phase lasted for 30 s at 58^0C, followed by the extended phase (72^0C, 45 s) and finally the last 10-min process at 72^0C.

The reaction mixture for s-PCR of Bb was 20 μL including 10 µL of Gotaq G2 Green MasterMix, 1 μL per each primer (*Bb-fla* (Hozbor et al., 1999)) (Table 2), 6 μL Nulease-free water and 2 μL DNA extracted from the sample. The initial phase lasted for 5 min at 95^0C, after which the denaturation took place at 95^0C for 30 s. The priming phase lasted for 30 s at 58^0C, followed by the extended phase (72^0C, 55 s) and finally the last 10-min process at 72^0C (Xue et al., 2009). There were 30 cycles performed for each reaction by the peqSTAR thermal cyclers (peqLAB Biotechnologie GmbH, Germany).

2.4. Electrophoresis

After completing the PCR reaction, 5 μL of each PCR products used for electrophoresis. Seven μL of 100 bp DNA ladder (Promega) was used to identify the approximate size of the PCR products. The steps were performed in 1% agarose (Promega) at U = 150 V, I = 144 mA for 20 min (Xue et al., 2009). *Actinobacillus pleuropneumonia* ATCC 27090 and *Pasteurella multocida* ATCC 12945 were used as positive controls for these two bacteria. Meanwhile, *Haemophilus parasuis* and *Bordetella bronchiseptica* isolated from the field were used as positive control af-

Table 1. Colony morphology of four bacteria on TSA medium after 24-h incubation

Name	Colony morphology	Catalase reaction
Actinobacillus pleuropneumoniae	Circular, raised, smooth, cloudy white, 1-1.5 mm in diameter	Negative
Haemophillus parasuis	Circular, raised, smooth, transparent white, the smallest size in 4 bacteria (< 1 mm)	Positive
Pasteurella multocida	Circular, raised, smooth, opaque white, 3-3.5 mm in diameter	Positive
Bordetella bronchiseptica	Circular, raised, smooth, greyish white, 1-2 mm in diameter	Positive

ter being analyzed by PCR and genotyed at Nam Khoa Biotek Company Limited. The PCR products were observed under Biorad UV2000 (Finetech, Taiwan).

3. Results and Discussion

There were 24 total objective bacteria strains isolated from 114 injured lungs (21.05%) collected at the slaughterhouse of Vissan Limited Company from July 2018 to May 2019. Three isolates of *App* (2.63%), 2 isolates of *Hps* (1.75%), 7 isolates of *Pm* (6.14%) and 12 isolates of Bb (10.53%) were found (Table 3 and Figure 1).

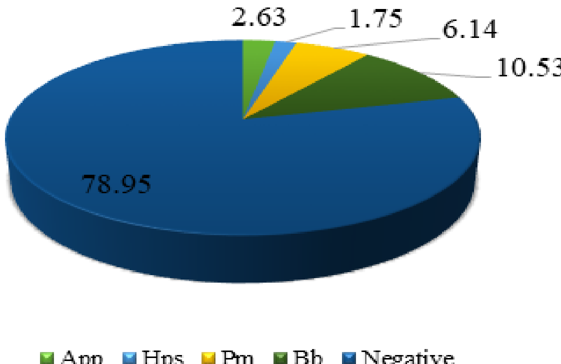


Figure 1. Proportion of positive samples diagnosed with PCR.

3.1. Proportion of positive samples diagnosed with PCR

The results of this study were different from those of other previous ones in different areas. *Bb* caused atrophic rhinitis when co-infecting with *Pm* and resulted in the severity of respiratory in pigs. In this study, Bb had the highest incidence with 10.53% (Figure 1). Zhao et al. (2011) found that 652/3506 lung samples were positive with *Bb* (18.6%). In North India, 8.2% of nasal swabs were positive with Bb by using PCR technique (Kumar et al., 2014). The gel electrophoresis after amplification of Bb is illustrated in Figure 2.

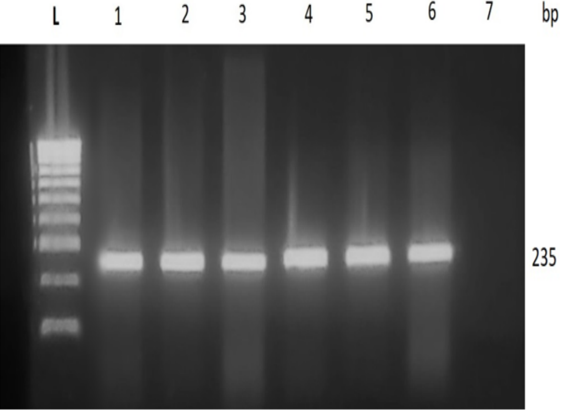


Figure 2. PCR product of Bb - *fla* gene for detection of *Bb* after electrophoresis process L: Ladder (1000 bps); Well: 1 - 5: DNA purified from field samples after cultivation; Well 6: positive control (235bps); Well 7: negative control.

In this study, Pm infection had the second highest proportion of positive samples diagnosed with PCR method (6.14%); however, this figure was lower than those reported by other researchers. In 2017, 296/3212 samples (9.2%) were positive with *Pm* in China (Liu et al., 2017). In other studies, the presence of *Pm* was found in 74.9% of lung samples collected from a slaughterhouse by using m-PCR technique (Hričínová et al., 2010). In Vietnam, Le et al. (2012) found that in Bac Giang, the percentage of Bb was 17.14% in the cases of 245 samples that were confirmed

Table 2. Primer sequences used for PCR

Gene name	Primer name	Sequence (5' ⟶ 3')	Size (bp)	Preference
AP-IV	AP-IVF	ATA CGG TTA ATG GCG GTA ATG G	346	Xiao et al. (2006)
	AP-IVR	ACC TGA GTG CTC ACC AAC G		
KMT1	KMT1 T7	ATC CGC TAT TTA CCC AGT GG	460	Townsend et al. (1998)
	KMT1 SP6	GCT GTA AAC GAA CTC GCC AC		
	AP-IVR	ACC TGA GTG CTC ACC AAC G		
HPS	HPS-F	GTG ATG AGG AAG GGT GGT GT	821	Oliveira et al. (2001)
	HPS-R	GGC TTC GTC ACC CTC TGT		
Bb-*fla*	Bb-*fla*-F	GCT CCC AAG AGA GAA AGG CT	235	Hozbor et al. (1999)
	Bb-*fla*-R	GGT GGC GCC T GC CCT ATC		

positive with porcine reproductive and respiratory syndrome virus (PRRSV). In North of Cao Bang and Bac Giang in 2010, it was found that 5% of the pig herd had Pm*text* (Le et al., 2012). The gel electrophoresis after amplification of Pm is illustrated in Figure 3.

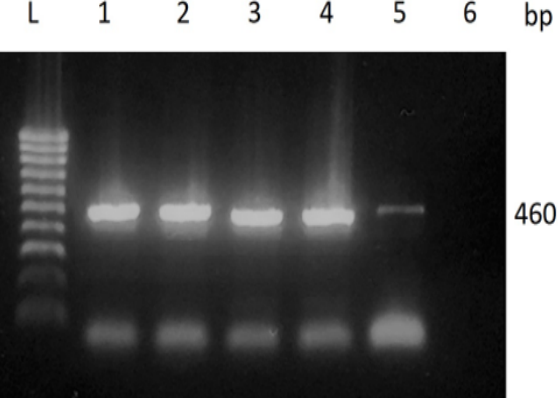


Figure 3. PCR product of KMT1 gene PMT gene after electrophoresis processL: Ladder (1000 bps); Well 1-4: DNA purified from field samples after cultivation; Well 5: positive control (460bps); Well 6: negative control.

App is the causative pathogen of pleuropneumonia in pigs. This bacterium can cause severe lung injuries. The results of this study showed that 2.63% of the samples were positive with this bacterium. This percentage was much lower as compared with those of other studies. In Ben Tre province, the prevalence of App was 24.62% (Thanh et al., 2018) while in Can Tho province, this percentage was 25.9% (Giang et al., 2015) and in Kien Giang, the proportion was 27.69% (Thanh et al., 2017). In some Northen provinces such as Bac Giang, 19.59% of samples positive with PRRSV were also positive with App. According to Hričínová et al. (2010), there was 20.5% of lungs from pigs in slaughterhouse positive to App. The gel electrophoresis after amplification of App is illustrated in Figure 4.

Hps is known as the bacteria causing Glasser's disease and an important agent in the porcine respiratory disease complex. In this study, it was found that only 2/114 lung samples (1.75%) were positive with Hps. Hričínová et al. (2010) found that 1,83% of lung samples from slaughterhouse were positive with Hps. In Thanh Hoa, Hung Yen and Ha Nam, 20/205 samples (9.7%) including nasal swab, tracheal fluid, heart and lungs of

Table 3. Positive samples diagnosed with PCR

	Total sample	Total positive sample	*App*	*Hps*	*Pm*	*Bb*
Number of samples	114	23	3	2	7	12
Percentage	100%	21.05%	2.63%	1.75%	6.14%	10.53%

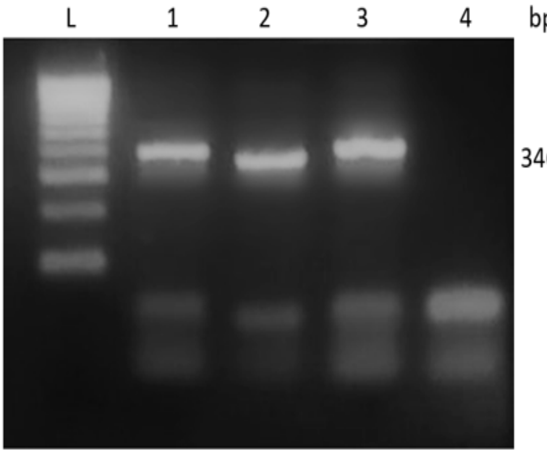


Figure 4. PCR product of the gene AP-IV for detection of App after electrophoresis process. L: Ladder (1000 bps); Well 1-2: DNA purified from field samples after cultivation; Well 3: positive control (346 bps); Well 4: negative control.

Glasser suspected pigs were found positive with Hps (Truong et al., 2018). In China, Zhao et al. (2011) reported that 26.7% samples were found positive with Hps. The gel electrophoresis after amplification of *Hps* is illustrated in Figure 5.

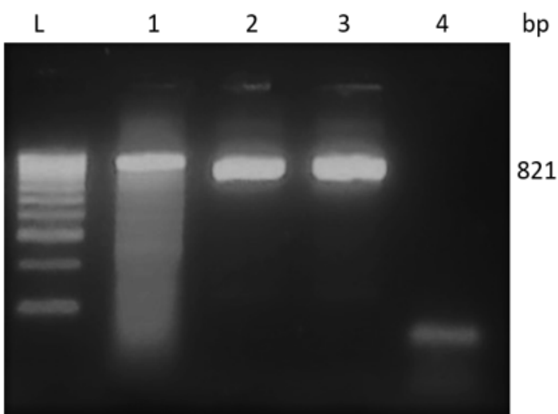


Figure 5. The PCR product of *Hps* gene, *Hps* bacteria after electrophoresis process L: Ladder (1000bps); Well 1, 2: DNA purified from field samples after cultivation; Well 3: positive control (821 bps); Well 4: negative control.

The differences in the percentage of positive samples of the four bacteria in different studies may be associated several factors such as husbandry conditions, weather, and disease pressure in various areas. The method of collecting samples may also affect the results as the bacteria are frequently isolated in the upper respiratory tract of pigs, but they would cause diseases when invading the lower respiratory tract. Another factor that should be considered is pig sources. In previous studies, samples were collected from clinically infected pigs, whereas in this study lungs were taken from pigs in the slaughterhouse with no clinical signs.

3.2. Proportion of samples with co-infection of 2, 3, and 4 bacteria

There was only 1 lung with co-infection of *Hps* and *Pm* (0.88%). Zhao et al. (2011) found the co-infection of *Pm* and *Bb* in all 63 pigs with the atrophy of turbinate bones. So far, the co-infection of those four bacteria has been rarely found in previous studies.

4. Conclusions

The prevalence of the investigated pathogens and their co-infection were not high because pigs at the slaughterhouse were relatively healthy and had no obvious clinical signs. However, it indicates that there is a potential risk for not only naïve herds when they are exposed to the healthy carriers but also the farms which currently have the presence of the pathogens without awareness of the farmers.

Acknowledgements

The authors would like to express their great appreciation to Nong Lam University for supporting this study.

References

de Jong, A., Thomas, V., Simjee, S., Moyaert, H., El Garch, F., Maher, K., Morrissey, I., Butty, P., Klein,

U., Marion, H., Rigaut, D., & Vallé, M. (2014). Antimicrobial susceptibility monitoring of respiratory tract pathogens isolated from diseased cattle and pigs across Europe: The VetPath study. *Veterinary Microbiology* 172(1-2), 202-215.

Hozbor, D., Fouque, F., & Guiso, N. (1999). Detection of *Bordetella bronchiseptica* by the polymerase chain reaction. *Research in Microbiology* 150(5), 333-341.

Hričínová, M., Holoda, E., Mudroňová, D., & Ondrašovičová, S. (2010). Multiplex PCR assay for detection of *Actinobacillus pleuropneumoniae*, *Pasteurella multocida* and *Haemophilus parasuis* in lungs of pigs from a slaughterhouse. *Folia Microbiologica* 55(6), 635-640.

Kumar, S., Singh, B.R., Bhardwaj, M., & Singh, V. (2014). Occurrence of Bordetella infection in pigs in Northern India. *International Journal of Microbiology* 2014, 1-6.

Oliveira, S., Galina, L., & Pijoan, C. (2001). Development of a PCR test to diagnose *Haemophilus parasuis* infections. *Journal of Veterinary Diagnostic Investigation* 13(6), 495-501.

Townsend, K. M., Frost, A. J., Lee, C. W., Papadimitriou, J. M., & Dawkins, H. J. (1998). Development of PCR assays for species- and type-specific identification of *Pasteurella multocida* isolates. *Journal of Clinical Microbiology* 36(4), 1096-1100.

Truong, L. Q., Nguyen, L. T., Nguyen, H. T., & Nguyen, H. T. (2018). Isolation and serotyping of *Haemophilus parasuis* trains from pigs raised in the provinces of Thanh Hoa, Hung Yen, and Ha Nam of Northern Vietnam. *Vietnam Journal of Agricultural Sciences* 16(12), 1068-1078.

Le, D. V., Nguyen, T. Q., Cu, P. H., & Hoang, H. D. (2012). Results of isolation and determination of some biological characteristics of *Pasteurella multocida* in pigs positive with PRRSV at Bac Giang Province. *Veterinary Sciences and Techniques* 19(6), 47-51.

Xiao, G., Cao, S., Duan, L., Wen, X., Ma, X., & Chen, H. (2006). Identification and detection of *Actinobacillus pleuropneumoniae* in infected and subclinically infected pigs by multiplex PCR based on the genes ApxIVA and OmlA. *Agricultural Sciences in China* 5(2), 146-154.

Zhao, Z., Wang, C., Xue, Y., Tang, X., Wu, B., Cheng, X., He, Q., & Chen, H. (2011). The occurrence of *Bordetella bronchiseptica* in pigs with clinical respiratory disease. *The Veterinary Journal* 188, 337-340.

Effects of dietary supplementation of β-mannanase on performance and egg quality in laying hens

Tung M. Che[1*], **Nhan T. M. Nguyen**[1], **& Sarah K. Cervantes-Pahm**[2]

[1]Department of Animal Production, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Elanco Animal Health

ARTICLE INFO

Research Paper

Received: May 31, 2019
Revised: June 18, 2019
Accepted: June 25, 2019

Keywords

β-mannanase
Egg quality
Isa Brown
Laying hens
Performance

***Corresponding author**

Che Minh Tung
Email: tung.cheminh@hcmuaf.edu.vn

ABSTRACT

The objective of the experiment was to evaluate effects of dietary supplementation of β-mannanase (Hemicell®) on productive performance, egg quality, and fecal moisture content in laying hens from 20 to 35 weeks of age. A total of 375 Isa Brown hens (1615.6 ± 76.4 g/bird) were randomly assigned to 5 treatments in a completely randomized design. The 5 dietary treatments included (1) basal diet with a level of 2800 kcal ME and no β-mannanase supplementation (HE, Control), (2) HE + 32 units of β-mannanase/g of feed, (3) HE + 64 units of β-mannanase/g of feed, (4) basal diet with a level of 2700 kcal ME (LE) + 32 units of β-mannanase/g of feed, and (5) LE + 64 units of β-mannanase/g of feed. Each treatment was replicated with 25 cages of 3 hens each. All diets were in meal form and contained no antibiotics. The addition of β-mannanase to HE diets did not affect the egg production of birds as compared with the control ($P > 0.05$). The birds fed LE diets with β-mannanase had the same egg production as those fed the control and β-mannanase-supplemented HE diets ($P > 0.05$). Differences in egg weight, egg quality, survival rate, and fecal moisture content were not significant among the treatments ($P > 0.05$). Briefly, addition of β-mannanase (32 units/g of feed) to LE diets would be beneficial for layers during the early laying period as it resulted in the same performance and egg quality as the HE diet without β-mannanase supplementation.

Cited as: Che, T. M., Nguyen, N. T. M., & Cervantes-Pahm, S. K. (2019). Effects of dietary supplementation of β-mannanase on performance and egg quality in laying hens. *The Journal of Agriculture and Development* 18(3), 41-47.

1. Introduction

Nowadays, prices of feed ingredients are increasing due to the increased use of cereals and plant ingredients such as wheat, soybean meal, sesame meal in animal feeding. However, these ingredients contain high levels of indigestible fiber like β-mannan and non-starch polysaccharides (NSP). According to Reid (1985), β-mannan and its derivatives (β-gaclactomannan or β-glucomannan) are structural components of the cell wall of the legume family. β-mannan present in soybean meal has long been known as an anti-nutritional factor. It was reported that β-mannan reduced egg production, egg weight and daily feed intake in laying hens (Patel & McGinnis, 1985). It has also been found that a diet containing 2 - 4% of β-mannan resulted in decreased growth rate and feed efficiency of broilers (Couch et al., 1967; Ray et al., 1982, Verma & McNab, 1982). Thus, dietary supplementation of β-mannanase would optimize the use of soybean meal because β-mannanase can destroy undigestible β-mannan in diets and would release more energy to be used for egg production (Tucker et al., 2004; Hsiao et al., 2006). The additional energy re-

lease would help producers formulate a diet with a reduced energy level without any adverse effects on egg production and thereby reducing feed cost. Thus, the objective of the experiment was to evaluate effects of dietary supplementation of β-mannanase (Hemicell®) on productive performance, egg quality, and fecal smoiture content in laying hens from 20 to 35 weeks of age.

2. Materials and Methods

2.1. Experimental design, birds, and housing

A total of 375 layers (Isa Brown, 19 weeks old) were randomly assigned to 5 treatments in a completely randomized design. The 5 dietary treatments included (1) basal diet with a high energy level of 2800 kcal ME and no β-mannanase supplementation (HE, Control), (2) HE + 32 units of β-mannanase/g of feed, (3) HE + 64 units of β-mannanase/g of feed, (4) basal diet with a low energy level of 2700 kcal ME (LE) + 32 units of β-mannanase/g of feed, and (5) LE + 64 units of β-mannanase/g of feed. All diets were in meal form and contained no antibiotics. β-mannanase used in this experiment was Hemicell® which contained 160 million units/kg of product and was provided by Elanco Vietnam. The birds were housed in battery cages with raised wire floors in an open-sided house. Each cage measured 0.4 m × 0.45 m × 0.4 m in size. Each treatment had 25 replicate cages with 3 birds each. Birds were brought into the housing facility at 17 weeks of age and allowed to adapt to the new environment for 2 weeks (18, 19) prior to the commencement of the experiment. The initial body weights of birds were 1615.6 ± 76.4 g/bird. The experiment lasted for 16 weeks from 20 to 35 weeks of age.

2.2. Diet, feeding, and lighting program

All diets were formulated to meet or exceed the nutritional requirements of layers during the experimental period (NRC, 1994). Hemicell was added on top of basal diets. The ingredient and analyzed chemical composition of the diets with HE and LE levels is presented in Table 1. Diet and feed ingredients were sampled for determination of approximate composition. Diets were mixed at the Applied Research Farm located on the campus of Nong Lam University and labelled accordingly. Feed and water were provided to allow ad libitum access during the entire experiment.

The lighting regime was 16 h per day and kept constant throughout the experiment. As the natural day length was approximately 12 h per day, the 4-h artificial light was needed. The lights were daily set to be switched on from 4:00 to 6:00 AM and from 6:00 to 8:00 PM.

2.3. Chemical analyses

Feed samples were ground to pass through a 1-mm screen before analysis and analyzed according to the standard methods of AOAC. The feed samples were analyzed in duplicate for DM (930.15), CP (990.03), crude fat (920.39), Ca (968.08), and P (968.08). Amino acids were also analyzed using Phenomenex EZ: faastTM amino acid analysis kit. All analyses were performed by Center of Analytical Services and Experimentation of Ho Chi Minh City, Vietnam. Fecal samples were collected by putting trays under each cage and analyzed for DM according to the AOAC method with modifications. After collection, fecal samples were first dried in an oven at 60°C for 24 h, ground to pass through a 1-mm screen and stored in pillboxes. After that, samples were dried at 105°C until constant weight.

2.4. Assessment of productive performance and egg quality

Egg production, egg weight, and mortality were daily recorded by replicate. Average daily feed intake (ADFI) was weekly determined on a replicate basis. Feed conversion ratio (FCR) was calculated as kg of total feed intake per hen/kg of egg per hen. Production parameters such as egg production, ADFI, and FCR were adjusted for hen mortality. Eggs laid on the last 2 days every 2 weeks were collected for measurement of egg quality. Egg parameters such as egg weight, albumen height, thick albumen weight, thin albumen weight, Haugh units, yolk weight, yolk color, shape index, shell weight, and shell thickness were measured. Albumen height was measured as indicated by Keener et al. (2006). Haugh units were calculated on the input of egg weight and albumen height using the formula of Haugh. Yolk color was determined by using the Roche Color Fan. The egg shape index was calculated by dividing egg length by egg width. Shell thickness was a mean value of measurements at 3 locations on the egg (air cell, equator, and small end), excluding cuticle.

Table 1. Ingredient and chemical composition of the basal diets

Ingredients, %	Basal diets[1]	
	Control (High energy, HE)	Low energy (LE)
Corn	45.36	47.70
Wheat	7.00	7.00
Rice bran, full fat	5.80	5.80
Soybean meal, 44%	26.65	26.19
Soybean oil	3.19	1.31
DL-Methionine	0.154	0.154
Salt	0.34	0.34
Choline chloride 50%	0.30	0.30
Limestone	9.80	9.80
MCP	1.30	1.30
Vit-Min Premix[2]	0.10	0.10
Phytase	0.006	0.006
Analyzed chemical composition		
ME (kcal/kg)[3]	2800	2700
DM, %	90.0	89.6
Crude fat, %	5.92	3.97
Crude protein, %	18.2	18.5
Calcium, %	4.30	4.12
Total phosphorus, %	0.74	0.73
Lysine, %	1.08	1.07

[1]Hemicell was given an energy credit of 100 kcal/kg at 200 or 400 g/MT and added on top of the above diets.
[2]Provided per kg of diet: vitamin A (5000 IU), vitamin D3 (3000 IU), vitamin E (50 IU), Fe (50 ppm), Cu (8 ppm), Zn (60 ppm), Mn (70 ppm).
[3]Calculated.

2.5. Statistical analysis

Data were analyzed as a completely randomized design by ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC). The cage was considered the experimental unit. Treatment differences were compared using the least squares means with a Tukey adjustment. The survival rate was compared by χ^2 analysis. Treatment effects were considered significant at $P < 0.05$.

3. Results

3.1. Productive performance

Over a 16-week study, there were no differences ($P > 0.05$) among the treatments for egg production, egg weight, and egg mass (Table 2). The egg production of laying hens fed different diets ranged from 92.31 - 92.95%. Similarly, laying hens consuming LE diets supplemented with 32 units or 64 units of β-mannanase/g of feed had the same ADFI and FCR as those consuming the control and β-mannanase-supplemented HE diets ($P > 0.05$).

3.2. Egg quality

With regard to egg quality (Table 3), the LE diets supplemented with β-mannanase did not affect ($P > 0.05$) shape index, Haugh units, albumen weight, yolk weight, and yolk color as compared with the HE diets with or without dietary supplementation of β-mannanase. A similar trend was also found among the treatments for shell weight (P = 0.393) and shell thickness ($P = 0.086$).

3.3. Survival rate and fecal moisture content

The control diet (97.3%) had the lowest survival rate as compared with the other treatments (98.7%), but this difference was not significant ($P = 0.845$; Figure 1). Similarly, there were no differences in the fecal moisture content among the treatments ($P = 0.756$; Figure 2). The fecal moisture content of layers in all treatments ranged from 78.0 - 78.7%.

Table 2. Effects of dietary supplementation of β-mannanase on reproductive performance of Isa Brown layers from 20 to 35 weeks of age

Item	Dietary treatments[1]					SEM	P
	A	B	C	D	E		
Egg production, %	92.54	92.31	92.95	92.39	92.59	0.907	0.989
Egg weight, g	54.20	53.51	53.98	54.08	53.77	0.340	0.631
Egg mass, g	50.16	49.42	50.18	49.94	49.78	0.585	0.887
ADFI, g	100.05	98.59	98.22	98.91	100.74	1.049	0.404
FCR, kg/kg[2]	2.000	1.998	1.957	1.982	2.024	0.026	0.464

[1]25 replicate cages/treatment; 3 birds/cage; A: Control diet (no β-mannanase, high energy-HE); B: HE + 32 units of β-mannanase/g of feed (0.02% Hemicell); C: HE + 64 units of β-mannanase/g of feed (0.04% Hemicell); D: Low energy diet (LE) + 32 units of β-mannanase/g of feed (0.02% Hemicell); E: LE + 64 units of β-mannanase/g of feed (0.04% Hemicell).
[2]kg of feed per kg of egg mass.

Table 3. Effects of dietary supplementation of Hemicell on egg quality of Isa Brown layers from 20 to 35 weeks of age[1]

Indicator[2]	Dietary treatments[2]					SEM	P
	A	B	C	D	E		
Shape index	0.790	0.787	0.784	0.789	0.787	0.002	0.462
Haugh units	79.32	78.39	78.70	79.31	78.02	0.120	0.598
Thick albumen weight, %	30.21	30.21	30.15	30.36	29.24	0.370	0.316
Thin albumen weight, %	32.89	32.93	32.97	33.13	33.91	0.340	0.293
Yolk weight, %	24.59	24.72	24.66	24.22	24.62	0.170	0.357
Yolk color	4.40	4.41	4.43	4.46	4.46	0.060	0.200
Shell weight, %	12.31	12.14	12.23	12.29	12.23	0.060	0.393
Shell thickness, mm	0.357	0.354	0.362	0.362	0.358	0.002	0.086

[1]25 replicate cages/treatment; 3 birds/cage.
[2]Mean values of 8 measurements (weeks 21, 23, 25, 27, 29, 31, 33 & 35) for each replicate; A: Control diet (no antibiotic, no β-mannanase, high energy-HE); B: HE + 32 units of β-mannanase/g of feed (0.02% Hemicell); C: HE + 64 units of β-mannanase/g of feed (0.04% Hemicell); D: Low energy diet (LE) + 32 units of β-mannanase/g of feed (0.02% Hemicell); E: LE + 64 units of β-mannanase/g of feed (0.04% Hemicell).

4. Discussion

Over the experimental period, laying hens fed the β-mannanase-supplemented LE diets had the same productive performance as those fed the control or β-mannanase-supplemented HE diets. These findings agree with those of previous studies. Jackson et al. (2004) reported that laying hens fed a LE diet (a reduction by 100 kcal/kg) supplemented with β-mannanase (110 units/g) had the same egg production and egg weight as those fed a diet with a typical energy level without β-mannanase supplementation. Likewise, Maureen (2014) found that diets with 2766 kcal ME/kg and supplemented with β-mannanase (0.04% Hemicell) did not affect the egg production and egg weight of layers as compared with those containing 2866 kcal ME/kg and no β-mannanase. These positive effects may be due to the β-mannanase present in Hemicell which can degrade β-mannan in feed to release more energy for egg production of layers (Nadeem et al., 2005; Bharathidhasan et al., 2008).

The LE diets supplemented with β-mannanase did not also cause any adverse effects on ADFI and FCR as compared with the control. This indicates the efficiency of β-mannanase in improving the nutrient digestion and absorption as the ADFI of hens was not different among the treatments. According to Azarfar (2013), a diet supplemented with β-mannanase increased the crude protein digestibility of broilers. Wu et al. (2005) and Maureen (2014) found that β-mannanase supplementation improved the energy utilization of a corn-soybean meal-based diet for layers. Briefly, addition of β-mannanase to a layer diet at the studied levels would be beneficial as it helps uplift the dietary level of energy by 100 kcal/kg of feed.

In addition, laying hens fed the LE diets with β-mannanase supplementation had the same egg quality as those fed the other diets. This observa-

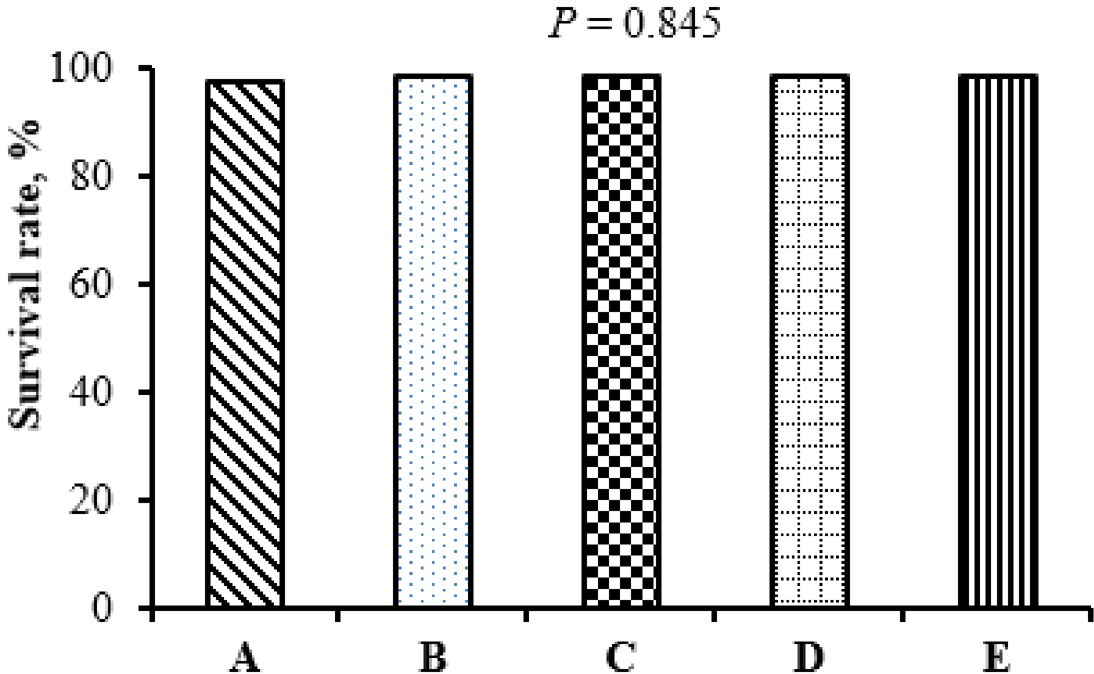


Figure 1. Effects of dietary supplementation of Hemicell on survival rate of Isa Brown layers from 20 to 35 weeks of age. There were 75 birds per treatment. A: Control diet (no antibiotic, no β-mannanase, high energy-HE); B: HE + 32 units of β-mannanase/g of feed (0.02% Hemicell); C: HE + 64 units of β-mannanase/g of feed (0.04% Hemicell); D: Low energy diet (LE) + 32 units of β-mannanase/g of feed (0.02% Hemicell); E: LE + 64 units of β-mannanase/g of feed (0.04% Hemicell).

tion on the enzyme effect in the present experiment is consistent with those of previous studies. For example, Ehsani & Torki (2010) found that Hemicell added to a diet of Lohnman laying hens at a dose of 0.06% did not improve the percentage of eggshell thickness. Torki et al. (2014) also reported that addition of 0.06% Hemicell to diets did not affect the the percentage of eggshell and eggshell thickness of Hy-line layers. Further, it was found that the egg yolk color scores were relatively low across the treatments, ranging from 4.40 - 4.60. This may be explained by the fact that all diets do not contain synthetic pigments, so the formation of egg yolk color is mainly affected by the pigment from corn. According to Cho et al. (2013), when using natural feed ingredients like corn and wheat, the egg yolk color ranges from 4.8 - 5.9 depending on their quality.

β-mannanase has been assumed to reduce the intestinal viscosity through breaking down large molecules of β-mannan into smaller compounds, thereby leading to a reduction of fecal moisture contents. This effect can be seen only when ingredients high in β-mannans, such as guar meal, palm kernel cake, and copra meal are included in a diet (Lee et al, 2013). The results of our experiment showed no differences in the fecal moisture content among the treatments. Rehman et al. (2016) reported that the effectiveness of β-mannanase was relatively low in a diet with ingredients containing low levels of β-mannan such as soybean meal and rice bran. It was shown that the β-mannan amount varied from 30 - 35% in palm kernel cake, 25 - 30% in copra meal, and 3 - 9% in guar meal, whereas there was 1.02 - 1.50% β-mannan in dehulled soybean meal and 1.17 - 2.12% in hulled soybean meal (Dierick, 1989; Hsiao et al., 2006). Furthermore, feeding LE diets with β-mannanase supplementation did not cause any detrimental effects on health of layers as evidenced by a high survival rate of 98.7%. Wu et al. (2005) also showed that the survival rate of Hy-Line W36 layers fed an LE diet (2831 kcal ME/kg) with or without β-mannanase supplementation was not different from that of layers fed an HE diet (2951 kcal ME/kg) in the

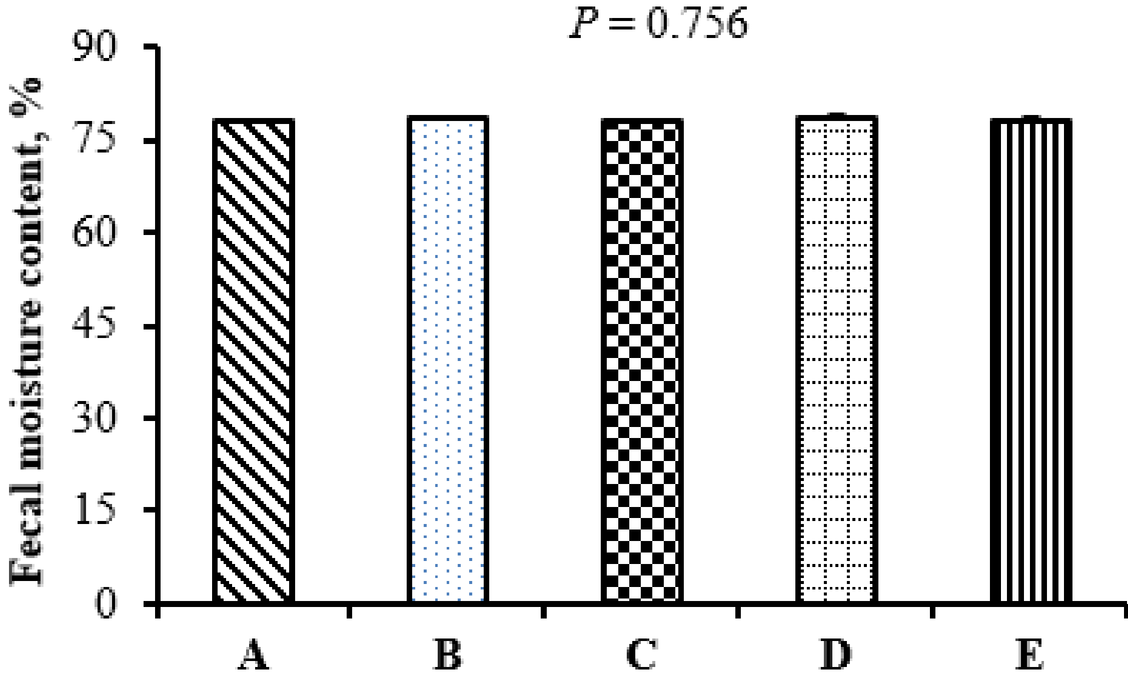


Figure 2. Effects of dietary supplementation of Hemicell on fecal moisture content of Isa Brown layers from 20 to 35 weeks of age. Mean values of 4 measurements (weeks 23, 27, 31, and 35 for each treatment. A: Control diet (no antibiotic, no β-mannanase, high energy-HE); B: HE + 32 units of β-mannanase/g of feed (0.02% Hemicell); C: HE + 64 units of β-mannanase/g of feed (0.04% Hemicell); D: Low energy diet (LE) + 32 units of β-mannanase/g of feed (0.02% Hemicell); E: LE + 64 units of β-mannanase/g of feed (0.04% Hemicell).

absence of β-mannanase. Briefly, addition of β-mannanase to diets with LE or HE levels did not affect the fecal smoiture content and survival rate of laying hens from 20 - 35 weeks of age.

5. Conclusions

Layers fed the low energy diets with β-mannanase supplementation performed equally as those fed the high energy diet without β-mannanase supplementation. No added benefits were obtained when a low enrgy diet was supplemented with 64 units of β-mannanase/g of feed. This indicates that a dietary supplementation of 32 units of β-mannanase/g of feed would be beneficial for layers during the early laying period as it uplifted 100 kcal of ME per kg of feed without affecting the performance and egg quality of layers.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors wish to thank Elanco. This project would not have been possible without Elanco's funding and the support of the Applied Research Farm and Department of Animal Production, Nong Lam University-Ho Chi Minh City.

References

AOAC Int. (Association of Official Analytical Chemists International). (2005). Official Methods of Analysis. 18th ed. AOAC Int., Arlington, VA.

Azarfar, A. (2013). Effect of hemicell enzyme on the performance, growth parameter, some blood factors and ileal digestibility of broiler chickens fed corn/soybean-based diets. *Journal of Cell and Animal Biology* 7(7), 85-91.

Bharathidhasan, A., Chandrasekaran, D., Natarajan, A., Ravi, R., Viswanathan, K., & Ezhilvalavan, S. (2008). Non-Starch polysaccharides and phytate phosphorus content of commonly available poultry feed ingredients. *Tamilnadu Journal of Veterinary and Animal Sciences* 4(6), 219-223.

Cho, J. H., Zhang, Z. F., & Kim. I. H. (2013). Effects of canthaxanthin on egg production, egg quality and egg yolk color in laying hens. *Journal Agricultural Science* 5(1), 269-274.

Couch, J. R., Bakshi, Y. K., Ferguson, T. M., Smith, E. B., & Gregor, C. R. (1967). The effect of processing on the nutritional value of guar meal for broiler chicks. *British Poultry Science* 8(4), 243-250.

Dierick, N. A. (1989). Biotechnology aids to improve feed and feed digestion: Enzyme and fermentation. *Archives of Animal Nutrition* 39(3), 241-261.

Ehsani, M., & Torki, M. (2010). Effects of dietary inclusion of guar meal supplemented by β-mannanase on performance of laying hens, egg quality characteristics and diacritical counts of white blood cells. *American Journal of Animal and Veterinary Sciences* 5(4), 237-243.

Hsiao, H. Y., Anderson, D. M., & Dale, N. M. (2006). Levels of β-Mannan in soybean meal. *Poultry Science* 85(8),1430-1432.

Jackson, M. E, Geronian, K., Knox, A., McNab, J., & McCartney, E. (2004). A dose-response study with the feed enzyme beta-mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. *Poultry Science* 83(12), 1992-1996.

Keener, K. M., McAvoy, K. C., Foegeding, J. B., Curtis, P.A., Anderson, K. E., Osborne, J. A., & Bush, D. J. (2006). Effect of testing temperature on internal egg quality measurements. *Poultry Science* 85(2), 550-555.

Lee, J. Y, Kim, S. Y, Lee, J. H., Lee, J. H., & Ohh. S. J. (2013). Effect of dietary β-mannanase supplementation and palm kernel meal inclusion on laying performance and egg quality in 73 weeks old hens. *Journal Animal Science Technology* 55(2), 115-122.

Maureen, K. C. (2014). *Effects of β-Mannanase on nutrient utilization and peformance of laying chicken* (Unpublished master's thesis). University of Nairobi, Nairobi, Kenya.

Nadeem, M. A., Anjum, M. I., Khan, A. G., & Azim, A. (2005). Effect of dietary supplementation of non-starch polysaccharide degrading enzymes on growth performance of broiler chicks. *Pakistan Veterinary Journal* 25(4), 183-188.

Patel, M. B., & McGinnis, J. (1985). The effect of autoclaving and enzyme supplementation of guar meal on the performance of chicks and laying hens. *Poultry Science* 64(6), 1148-1156.

Ray S., Pubols, M. H., & McGinnis, J. (1982). The effects of purified guar degrading enzyme on chick growth. *Poultry Science* 61(3), 488-494.

Rehman, Z. U., Aziz, T., Bhatti, S. A., Ahmad, G., Kamran, J., Umar, S., Meng, C., & Ding, C. (2016). Effect of β-mannanase on the performance and digestibility of broilers. *Asian Journal Animal and Veterinary Advances* 11(7), 393-398.

Reid, J. S. G. (1985). Cell Wall Storage Carbohydrates in seeds-biochemistry of the seed gums and hemicelluloses. *Advances in Botanical Research* 11, 125-155.

Torki, M., Zangiabadi, H., & Ghasemi, H. A. (2014). Effects of enzyme supplementation on productive performance and egg quality of laying hens fed diets containing graded levels of whole date waste. *Poultry Science* 2(2), 139-151.

Tucker, M. P., Nagle, N. J., Jennings, E. W., Aden, A., Nguyen, Q. A., Kim, K. H., & Noll, S. L. (2004). Conversion of distiller's grain into fuel alcohol and a higher value animal feed by dilute-acid pretreatment. *Applied Biochemistry and Biotechnology* 115(1-3), 1139-1159.

Verma, S. V. S., & McNab, J. M. (1982). Guar meal in diets for broiler chickens. *British Poultry Science* 23(2), 95-105.

Wu, G., Bryant, M. M., Voitle, R. A., & Roland, D. A. Sr. (2005). Effects of β mannanase in corn-soy diets on commercial leghorns in second-cycle hens. *Poultry Science* 84(6), 894-897.

Ecological planning for the conservation and development of pineapple (*Ananas comosus*) in Tan Phuoc district, Tien Giang province

Mai T. Nguyen*, **& Thuy T. P. Doan**

Department of Biology, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: March 11, 2019
Revised: May 19, 2019
Accepted: June 14, 2019

Keywords

Ecological planning
Land assessment
Mekong Delta
Pineapple
Sustainable development

***Corresponding author**

Nguyen Thi Mai
Email: ngtpmai@hcmuaf.edu.vn

ABSTRACT

Land assessment is a specific requirement for the land use. Land assessment results provide the information on land types and natural conditions (land map units) so that we can evaluate the suitability of the land area for agricultural and non-agricultural use. In agriculture, appropriate crops for the land area are usually designed based on the land assessment data. Ecological planning is a process of assessment, evaluation, and decision in order to help authorities design the ideal, appropriate land area and land arrangement for agricultural and non-agricultural purposes. Tan Phuoc district in Tien Giang province is a low and alluvial agricultural area of Dong Thap Muoi. In this area, the income of local people depends majorly on agricultural activities. Pineapple (*Ananas cosmosus*) with its high economic value is widely cultivated in Tan Phuoc and has an important impact on the income of local people. Nevertheless, most of the land area currently used for pineapple production in Tan Phuoc was the land area previously used for cultivation of other crops. Due to this poor land resource planning, the yield of pineapple was low, and the land and environment were polluted. An adequate land assessment for Tan Phuoc is therefore highly needed so that an ecological planning for pineapple would be properly to improve the yield of pineapple, conserve the environment and support the sustainable development in Tan Phuoc.

Cited as: Nguyen, M. T, & Doan, T. T. P. (2019). Ecological planning for the conservation and development of pineapple (*Ananas comosus*) in Tan Phuoc district, Tien Giang province. *The Journal of Agriculture and Development* 18(3), 48-56.

1. Introduction

Tan Phuoc district in Tien Giang province is a low and alluvial agricultural area of Dong Thap Muoi. In this area, the income of the locals depends majorly on agricultural activities. Pineapple (*Ananas cosmosus*) with its high economic value was cultivated widely in Tan Phuoc and has an important impact on the locals' income. Nevertheless, the majority of the land area currently used for pineapple in Tan Phuoc was the land area of other crops. The pineapple farms in Tan Phuoc are formed by spontaneous farming, therefore the efficiency of using land resources is low. Furthermore, in this area the dike systems to protect pineapple farms are incomplete causing flooding causing unwanted affects on the farming productivity and environment. Until now, there has been no assessment of land adaptation and no adaptive zone was determined for pineapple cultivation, the crop that should only farmed in ideal farms with no flooding or flooding in less than 1 day with the submergence level is less than 30 cm. These areas should not be affected by salinity or alluvium; the depth of alluvium layer is above 100 cm, pH $\leq$ 4.0; annual rainfall is 1,000 mm to

1,500 mm (Nguyen, 2014).

An adequate land assessment for Tan Phuoc is therefore highly needed so that a proper ecological planning for pineapple could be designed to improve the yield of pineapple, preserve the environment and support sustainable development in Tan Phuoc.

Ecological planning is a process of assessment, evaluation, and decision in order to help authorities design the ideal, appropriate land area and land arrangement for agricultural and non-agricultural purposes (Huizing, 1992). In order to use land resource efficiently, a proper assessment of land adaptation is highly needed to determine the adaptive area for the optimal growth of crops. A landscape ecological approach to protect the ecosystems and biological resources is an effective approach to ecological planning for sustainable development (Almo, 1998). This approach based on the integration planning between the need for economic development and the sustainable development of the land ecosystem in order to efficient exploit the potential and advantages of land units (FAO, 1976).

We conducted the research to define the adaptive areas and identify potential areas for pineapple cultivation. Our results therefore could be used as a fundamental data for the planning, conservation and development of pineapple to preserve the environment and support sustainable development in Tan Phuoc, Tien Giang.

2. Materials and Methods

2.1. Materials

Our study was conducted in Tan Phuoc, Tien Giang, an area of Dong Thap Muoi, Mekong Delta (Figure 1). This is an agricultural area with low, alum and organic soil formed from the sediments of coastal marshy, hence suitable for the growth of pineapple. Pineapple (*Ananas comosus*) belongs to Bromeliaceae family with the development depend on the depth of the alluvial soil layer (cm), the depth of the alluvial forming layer (cm), the submerged depth (cm) and duration of submergence (day). In Tan Phuoc, pineapple has been farmed since 1983, in Tan Lap 1 and Tan Lap 2 wards. These two areas are notable for the brand "Tan Lap pineapple" with high fruit quality and productivity. The pineapple farms are expanding and there are 16,375.51 ha (DONRE, 2018).

2.2. Methods

2.2.1. Data collection and field surveys

The data include: land map, current land use map, administrative map scale 1: 100,000 was collected and provided by the DONRE, 2018.

Field surveys were conducted based on the data of land units, characteristics and growth conditions of pineapple; factors affecting the pineapple cultivation such as alluvium soil, flooding level etc. (DONRE, 2018).

2.2.2. GIS and mapping

Using GIS - Mapinfo software to create raster maps in Idrisi through data analysis combined with field survey results.

The map of land units, adaptation maps and ecological planning maps by overlapping the component maps (alluvial formation layer, the depth of alluvial layer, submerged depth and duration of submergence) was established based on soil characteristics and ecological factors for pineapple (Carol, 1998).

2.2.3. Data analysis

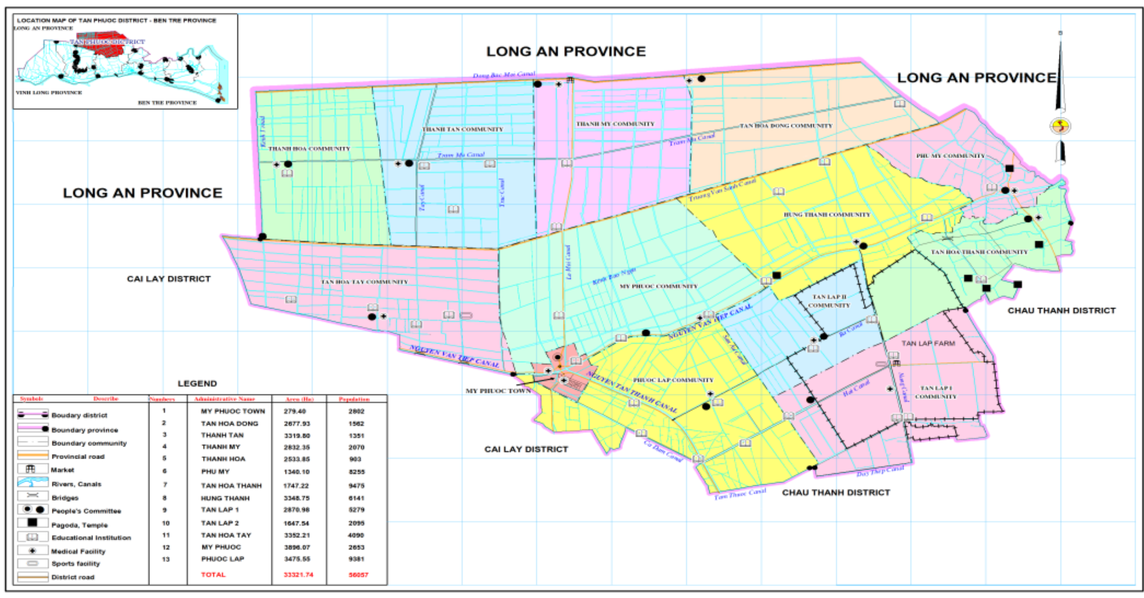
Data was analyzed using Excel và SPSS. All data was analyzed in both natural and economic factors to provide the conclusions and plans that support the developmental potential of the studied area.

2.2.4. Assessment method for the natural, economic and ecological planning for pineapple

The assessment of natural land adaptation according to FAO (1976) using MapInfo 11.0 software included the following steps: (1) Screening and description of land use; (2) Conversion of land characteristics of each land map unit into land quality; (3) Identification of land use requirements for land use patterns and ecological constraints affecting pineapple productivity. (4) Establishment of ecological adaptation chart for pineapple (S1(Highly Suitable), S2 (Moderately Suitable), S3 (Marginally Suitable), N (Not Suitable)). (5) Comparison, adaptation subdivision for pineapple and ecological land.

ADMINISTRATIVE MAP

TAN PHUOC DISTRICT - TIEN GIANG PROVINCE



Scale 1: 100 000

Figure 1. Administrative map of Tan Phuoc, Tien Giang province, Vietnam.

Estimation the adaptation of economic land based on the classification results of natural land adaptations S1, S2, S3 (according to FAO (1976)) and the productivity of the adaptive levels (that was calculated as the average of the optimal productivity based on the results of the field surveys) as follows:

- Productivity of S1: 90% (maximum yield of crops in the studied area).
- Productivity S2: 60% (compared to S1 yield).
- Productivity S3: 30% (compared to S1 yield).

Estimation of economic adaptation (profitability and efficiency of capital using B/C (Benefit/Cost)) and classification of economic factors using the optimal percentage conversion method by FAO (1976), including:

- Highly adaptation S1: $\geq$ 80%.
- Adaptation level S2: $\geq$ 40% to $<$ 80%.
- Adaptation level S3: $\geq$ 20% to $<$ 40%.
- Inappropriate N: $<$ 20%.

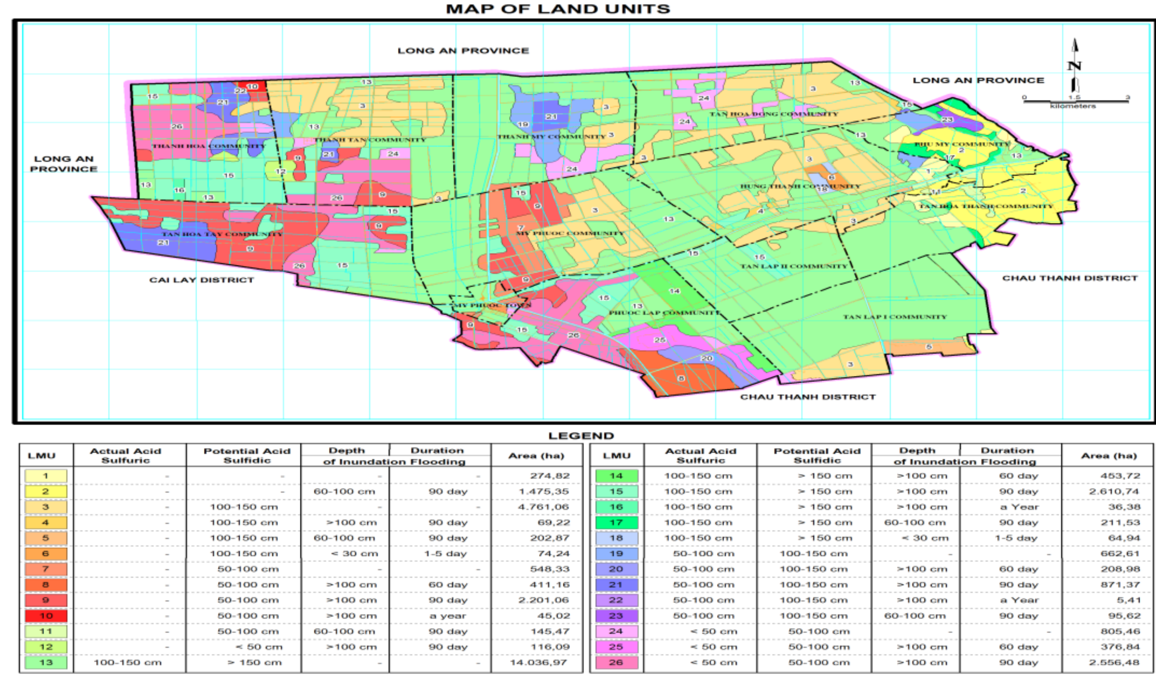
Ecological planning of the specialized area for pineapple: overlapping the ecological map and economical map, exploiting efficiently the natural ecological conditions, developing pineapple at an appropriate level (S1, S2) and less adaptable land (S3), based on the socio-economic development orientation; land use status and land resource characteristics such as soil type; water resources, irrigation system, submerge control, ecological planning for pineapple production area in Tan Phuoc district.

3. Results and Discussion

The overlapping layers of map information of natural ecological factors including soil map, submerged depth, duration of submergence, the depth of alluvial forming layer, the depth of alluvial layer were used to create the land unit map. Areas with the same land features were idenitifed as zone. A zone is an area with mogeneous natural features called land units. Our results showed that there were 26 land units in the studied area (Figure 2).

3.1. Identification of land quality adaptation

Based on the growth characteristics of pineapples, the natural conditions and land quality requirements and the detailed assessment criteria



LMU	Actual Acid Sulfuric	Potential Acid Sulfidic	Depth of Inundation	Duration Flooding	Area (ha)
1	-	-	-	-	274,82
2	-	-	60-100 cm	90 day	1.475,35
3	-	100-150 cm	-	-	4.761,06
4	-	100-150 cm	>100 cm	90 day	69,22
5	-	100-150 cm	60-100 cm	90 day	202,87
6	-	100-150 cm	< 30 cm	1-5 day	74,24
7	-	50-100 cm	-	-	548,33
8	-	50-100 cm	>100 cm	60 day	411,16
9	-	50-100 cm	>100 cm	90 day	2.201,06
10	-	50-100 cm	>100 cm	a year	45,02
11	-	50-100 cm	60-100 cm	90 day	145,47
12	-	< 50 cm	>100 cm	90 day	116,09
13	100-150 cm	> 150 cm	-	-	14.036,97
14	100-150 cm	> 150 cm	>100 cm	60 day	453,72
15	100-150 cm	> 150 cm	>100 cm	90 day	2.610,74
16	100-150 cm	> 150 cm	>100 cm	a Year	36,38
17	100-150 cm	> 150 cm	60-100 cm	90 day	211,53
18	100-150 cm	> 150 cm	< 30 cm	1-5 day	64,94
19	50-100 cm	100-150 cm	-	-	662,61
20	50-100 cm	100-150 cm	>100 cm	60 day	208,98
21	50-100 cm	100-150 cm	>100 cm	90 day	871,37
22	50-100 cm	100-150 cm	>100 cm	a Year	5,41
23	50-100 cm	100-150 cm	60-100 cm	90 day	95,62
24	< 50 cm	50-100 cm	-	-	805,46
25	< 50 cm	50-100 cm	>100 cm	60 day	376,84
26	< 50 cm	50-100 cm	>100 cm	90 day	2.556,48

Figure 2. Land unit map of Tan Phuoc, Tien Giang province, Vietnam.

that affect the land use types were described in Table 1.

The identified adaptation levels for the types of pineapple land use are presented in Table 2 using the data of the land use requirement, the factors affecting the growth of pineapple in combination with the results of the land adaptation and land characterization.

3.2. Identify the adaptation of land based on the natural ecological conditions

The identification of land adaptation was conducted using FAO guidelines (1976). Using Table 2, the evaluation of land quality of land map units for pineapple land showed that the land units number 1, 3, 13 were the land units with highest adaptation; followed by the number 7 and 19 were inadequate adaptation; 6, 18, 24 were less adaptable and finally the land units 2, 4, 5, 8-12, 14-17, 20-23, 25 and 26 were unsuitable for pineapple cultivation (Table 3).

The adaptation of land for pineapple based on the natural ecological conditions:

From the adaptation result of the land use type for each land unit, adaptation zones were identified using following steps: (1) determination of the acceptable levels of land use map units, (2) combinations of land units with similar level of adaptation (Tables 4 and 3).

In that, Zone I: Land units 1, 3, 13 (largest area with 19,072.85 ha) were the land units with highest adaptation. This land units belong to Tan Lap 1, Tan Lap 2, Hung Thanh, My Phuoc, Tan Hoa Đong, Thanh Tan, Thanh My wards.

Zone II: Being 1,210.94 ha included the land units 7 and 19, were medium adaptive with the presence of alluvial layer and the alluvial forming layer in My Phuoc, Thanh My, Thanh Hoa and Thanh Tan wards. Zone II was also the third largest zone after zone I and III.

Zone III: Being 944,64 ha included the land units 6, 18, 24, less adaptive with the presence of the alluvial forming layer and the duration of submergence in part of Tan Hoa Đong, Hung Thanh, Thanh My, Thanh Tan and Thanh Hoa wards. This zone was also the smallest zone.

Zone IV: Being 12,093.31 ha, the second largest zone, included the land units 2, 4, 5, 8-17, 20-23, 25, 26, were unsuitable for pineapple due to the annual floods and partially used for aquaculture. This zone belongs to Tan Hoa Tay, Thanh Hoa,

Table 1. The land use type, land quality requirement and assessment criteria to Pineaple cultivation

The land use type	Land quality	Assesment criteria
Pineapple cultivation	Alluvial risk	The depth of the alluvial layer
		The depth of the alluvial forming layer
	Submerged risk	The submerged depth
		Durarion of submergence

Table 2. The adaptation levels for pineapple

Land type requirement	Affecting factors	Adaptation level			
		S1	S2	S3	N
Alluvial risk	The depth of the alluvial layer (cm)	No alluvium or > 100	50 - 100	< 50	-
	The depth of the alluvial forming layer (cm)	No alluvium or > 100	50 - 100	< 50	-
Submerged risk	Duration of submergence (day)	No submergence	< 1	< 5	> 5
	The depth of submergence (cm)	No submergence	0 - 30	30 - 60	> 60

Table 3. The adaptation of land for pineapple based on the natural ecological conditions

Land unit	The depth of the alluvial layer (cm)	The depth of the alluvial forming layer (cm)	The depth of submergence (cm)	Duration of submergence (day)	Adaptation level
1, 3, 13	S1	S1	S1	S1	S1
2, 4, 5, 14 - 17	S1	S1	N	N	N
6,18	S1	S1	S2	S3	S3
7	S1	S2	S1	S1	S2
8 - 11	S1	S2	N	N	N
12	S1	S3	N	N	N
19	S2	S1	S1	S1	S2
20 - 23	S2	S1	N	N	N
24	S3	S2	S1	S1	S3
25,26	S3	S2	N	N	N

Thanh Tan, Phuoc Lap, Phu My, Tan Hoa Thanh and My Phuoc wards.

3.3. Identify the adaptation of land based on the economical conditions

The assessment of land suitability in terms of economics was evaluated in accordance with the objective of increasing profitability for the adaptive areas. Profit = Total revenue - Cost; Effective use of capital B/C = Profit/Total cost; Total revenue = Productivity * Unit price. The results of field surveys for economic criteria in three regions S1, S2, S3 are summarized in Table 4.

From the results of the land based on the natural ecological conditions (Table 4), the levels of S1, S2, S3 of each land use type for land units and productivity levels are calculated as average optimum productivity according to FAO (1976).

The data from the comparation of the actual conditions in the studied area using the yield at the adaptive levels (after the conversion from the classification of natural ecological adaptation to

Table 4. The adaptation of land for pineapple based on the natural ecological conditions

Zone	Land unit	Adaptation levels	Area (ha)
I	1, 3, 13	S1	19.072,85
II	7, 19	S2	1.210,94
III	6, 18, 24	S3	944.64
IV	2, 4, 5, 8 - 17, 20 - 23, 25, 26	N	12.093,31

Unit: 1.000 VND.

the economic adaptation) was used to establish two economic criteria: profit and B/C, the results were shown in Table 5.

The analysis data on the profit and B/C therefore divided the studied area into 4 economical adaptation levels.

S1: Highly adaptive; S2: medium adaptive; S3: Low adaptive; N: unsuitable. The profitability and capital efficiency were established based on the economic values, at the natural level S1 of the land use type to determine levels of economic adaptability.

The economical adaptation levels by the FAO based on the optimal yield % method according to FAO (1976) for land use were shown in Table 6.

3.4. Identify the economical adaptation of land

The results of economic aggregation (Table 5) and economic decentralization (Table 6) were compared and used to determine the economic suitability of land use patterns for each land unit and zone for pineapple. The results are shown in Table 7 and Table 8.

Accordingly, the economical adaptation levels of land for pineapple were divided into 3 Zones.

Zone I with land units 1, 3, 13. This zone was 19.072,85 ha and in S1 levels for profit and B/C.

Zone II with land units 7, 19. This zone was 1.210,94 ha and in S2 levels for profit and B/C.

Zone III with land units 6, 18 and 24. This zone was 944,64 ha and in S3 levels for profit and B/C.

3.5. Identify the adaptation of land for pineapple based on economy and ecology

The adaptation of land for pineapple based on economy and ecology was established using the results on the adaptation analysis of economy and ecology. The results were shown in Table 9 and the distribution of zones was presented in Figure 3.

• Using the results in table 9, the adaptation of land for pineapple based on the ecological and economical factors were defined in 4 zones (Figure 3):

Zone I: Land units 1, 3, 13 (were in S1 level on the ecology, profit and B/C). This zone was 19,072.85 ha (57,25% of the studied area) including Tan Lap 1, Tan Lap 2, Hung Thanh, Tan Hoa Dong, My Phuoc, Thanh My, Thanh Tan wards. Zone I was highly adaptive for pineapple.

Zone II: Land units 7 and 19 (were in S2 level on the ecology, profit and B/C). This zone was 1,210.94 ha (3,63% of the studied area) distributed in Thanh My, Thanh Tan, Thanh Hoa, My Phuoc, Phu My, Hung Thanh and My Phuoc wards. Zone II was medium adaptive for pineapple.

Zone III: Land units 6, 18 and 24 (were in S3 level on the ecology, profit and B/C). This zone was 944,64 ha (2,83% of the studied area) located in Thanh My, Thanh Tan, Thanh Hoa, Hung Thanh and Tan Lap 1, Phuoc Lap and Tan Hoa Tay wards. Zone III was less adaptive for pineapple.

Zone IV: 12.093,31 ha and accounted for 36,29% the studied area. This zone included land units 2, 4, 5, 8, 9, 10, 11, 12, 14, 15, 16, 17, 20, 21, 22, 23, 25, 26 with are not suitable for pineapple cultivation. Zone 4 distributed mostly in Thanh Tan, Thanh Hoa, Tan Hoa Tay, Tan Hoa Thanh, Phu My wards and partially in My Phuoc, Thanh My, Tan Lap 1 and Tan Lap 2.

• Ecological planning for pineapple cultivation area must:

Align with the agricultural development orientation of the local community.

Align with the land conditions and socio-economical conditions.

Give the priority to the land use types that are medium to high level of adaptation; high yield

Table 5. The adaptation of land for pineapple based on the natural ecological conditions

Land unit	Pineapple cultivation		
	Ecological adaptation level	Profit	B/C
1, 3, 13 (Zone I)	S1	446.179,50	1,80
7, 19 (Zone II)	S2	257.369,40	0,99
6, 18, 24 (Zone III)	S3	104.323,20	0,38
2, 4, 5, 8, 9, 10, 11, 12, 14, 15, 16, 17, 20, 21, 22, 23, 25, 26	N	-	-

Unit: 1.000 VND/ha.

Table 6. The economical adaptation levels for pineapple cultivation in the studied area

Economical criteria	Adaptation level			
	S1	S2	S3	N
Profit	> 339.324,80	169.662,40 - 339.324,80	84.881,20 - 169.662,40	< 84.881,20
B/C	> 1,32	0,66 - 1,32	0,33 - 0,66	< 0,33

Unit: 1.000 VND/ha.

Table 7. The economical adaptation of land for pineapple

Land unit	Pineaple cultivation		Adaptation level
	LN	B/C	
1, 3, 13 (Zone I)	S1	S1	S1
7, 19 (Zone 2)	S2	S2	S2
6, 18, 24 (Zone 3)	S3	S3	S3

Land units: 2, 4, 5, 8, 9, 10, 11, 12, 14, 15, 16, 17, 20, 21, 22, 23, 25, 26 were not suitable for Pineaple cultivation.

Table 8. The economical adaptation of land for pineapple

Zone	Land unit	Adaptation level	Area (ha)
I	1, 3, 13	S1	19.072,85
II	7, 19	S2	1.210,94
III	6, 18, 24	S3	944.64

Table 9. The economical and ecological adaptation of land in the studied area

Land unit	Pineapple cultivation			Adaptation level
	Ecology	Profit	B/C	
1, 3, 13	S1	S1	S1	S1
2,4, 5, 8, 9, 10, 11, 12, 14, 15, 16, 17, 20, 21, 22, 23, 25, 26	N	-	-	N
6, 18, 24	S3	S3	S3	S3
7, 19	S2	S1	S1	S2

and high interest rates; utilize efficiently the land potential, support the protection, maintenance of land for sustainable development.

Our analysis results on ecological and economical adaptation for pineapple land use; the socio-economic development orientation of Tan Phuoc; the current status of land use and land characteristics such as soil type, water characteristics, irrigation system, flood control showed that in order to better utilize the ecological conditions for the development of pineapple farming in Tan Phuoc, the regional planning should be grouped into the areas with highly adaptation level (S1, S2) and the areas with low adaptation level (S3), as follow (Figure 4):

◇ The specialized area for pineapple cultivation: 21,228.42 ha. This is the area with most of land area were bedded. Furthermore, the trans-

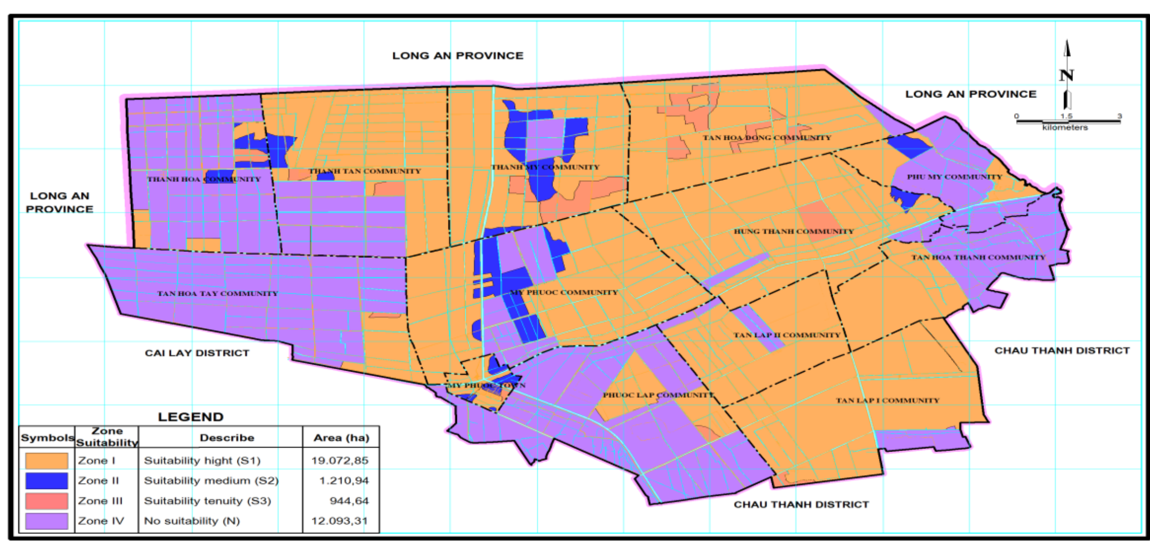


Figure 3. The map of the economical and ecological adaptation for pineapple in Tan Phuoc District, Tien Giang province, Vietnam.

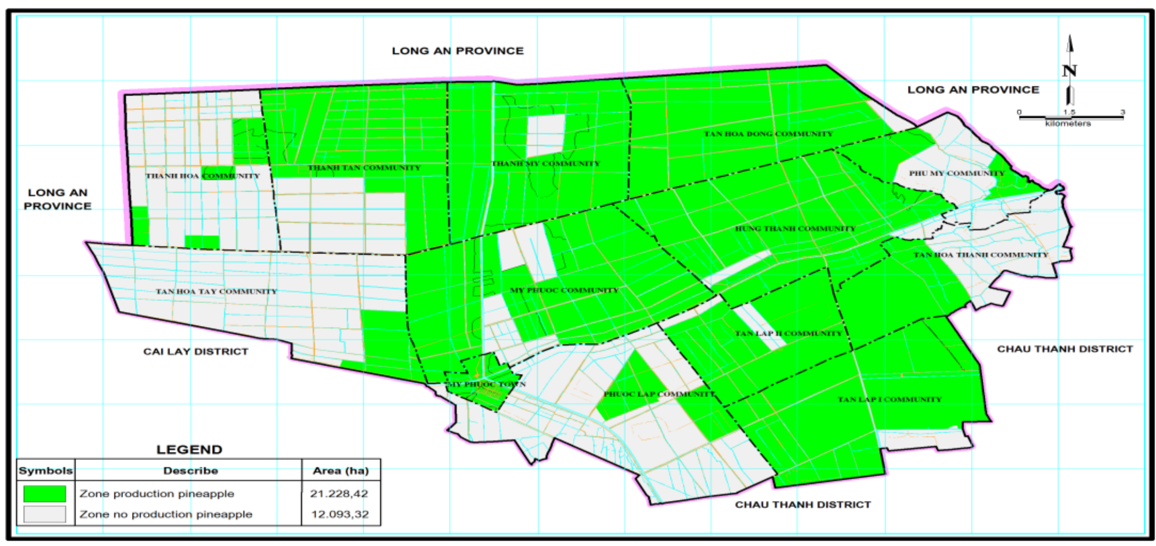


Figure 4. The map of ecological planning for pineapple in Tan Phuoc district, Tien Giang province.

port system, irrigation system and dikes in this area are relatively completed. This is a suitable area for pineapple. This area includes Tan Lap 1, Tan Lap 2, Hung Thanh, My Phuoc, Tan Hoa Đong, Thanh My wards and a part of Thanh Tan ward.

⋄ The unsuitable area for pineapple cultivation: 12,093.32 ha. This is a land area with high level of alum, undeveloped infrastructure, incomplete dike systems. Moreover, this area is frequently flooded and has long flooded periods. This area is not suitable for pineapple cultivation. This area includes Thanh Hoa, Tay Hoa Tay, Phu My, Phuoc Lap and part of Tan Hoa Thanh, Thanh Tan.

4. Conclusions

Application of ecological planning for pineapple cultivation (*Ananas comosus*) helps to uti-

lize efficiently the ecological and economical conditions for the ideal development of pineapple and therefore the optimal pineapple productivity. This study had mapped and identified the pineapple cultivation area of 21,228.42 ha (accounting for 63,7% of the studied area). Our data on the ecological and economical adaptation maps could help to increase pineapple the productivity, protect the environment and support sustainable development in Tan Phuoc district, Tien Giang province.

References

Almo, F. (1998). *Principles and methods in landscape ecology.* London, United Kingdom: Chapman & Hall.

Carol A. J. (1998). *Geographic information systems in ecology.* Oxford, United Kingdom: John Wiley and Sons Ltd.

DONRE (Department of Natural Resources and Environment of Tien Giang province). (2018). Report on environmental monitoring and natural resource management in Ten Giang 2017. Tien Giang, Vietnam: DONRE Office.

FAO (Food and Agriculture Organization of the United Nations). (1976). *A framework for land evaluation.* Rome, Italy: FAO.

Huizing H. (1992). Multiple goal analysis for land use planning. In: *The proceedings of DLD - ITC workshop on GIS and RS nature resource management* (142-153). Enschede, The Netherlands.

Nguyen, H. T. N. (2014). *A guide to the cultivation of pineapple (Queen).* Ha Noi, Vietnam: Agricultural Publishing House.

Extraction and encapsulation of lesser galangal (*Alpinia officinarum*) essential oil using microwave pretreatment and spray drying

Tuan T. A. Nguyen[1], **Dat T. Huynh**[1], **& Natthakan Rungraeng**[2,3*]

[1]Faculty of Food Science and Technology, Nong Lam University, Ho Chi Minh City, Vietnam
[2]School of Agro-Industry, Mae Fah Luang University, Chiang Rai, Thailand
[3]Unit of Innovative Food Packaging and Biomaterials, Mae Fah Luang University, Chiang Rai, Thailand

ARTICLE INFO

Research Paper

Received: May 30, 2019
Revised: June 21, 2019
Accepted: June 28, 2019

Keywords

Alpinia officinarum
Essential oil
Encapsulation
Microwave pretreatment
Spray drying

***Corresponding author**

Natthakan Rungraeng
Email: natthakan.run@mfu.ac.th

ABSTRACT

Galangal (*Alpinia officinarum*) essential oil potentially exerts several health benefits such as antiproliferative, pain relief and fever reduction activities. The essential oil in this study was extracted using the microwave (MW) assisted treatment as an alternative method to conventional extraction. The samples were treated with MW at different power levels (600, 700, and 800 W) for 3 min before subjecting to a hydrodistillation extraction. The effect of sample:water ratio (1:1 and 1:0.5) was also investigated. The MW treatment at 600 W and sample:water ratio of 1:1 resulted in the highest amount of essential oil (0.33%). The MW pretreatment had a positive effect on reducing the extraction time (from 3 h to 2.5 h) observed at all MW power levels. The effect of essential oil loading capacity (10, 20 and 30%) on the encapsulation yield was evaluated. The *in vitro* antioxidative activity of the obtained powder was then measured. The highest encapsulation efficiency (86.5%) was obtained from the 20% loading capacity, suggesting for a suitable amount of essential oil should be loaded for the encapsulation. The *in vitro* antioxidant activity of the encapsulated essential oil powders was determined. The IC_{50} of the powder was 2077 μg/mL using DPPH assay.

Cited as: Nguyen, T. T. A., Huynh, D. T., & Rungraeng, N. (2019). Extraction and encapsulation of lesser galangal (*Alpinia officinarum*) essential oil using microwave pretreatment and spray drying. *The Journal of Agriculture and Development* 18(3), 57-63.

1. Introduction

Lesser galangal (*Alpinia officinarum*) belongs to the *Zingiberaceae* family, which is commonly used as a flavoring ingredient in South East Asian countries. The essential oil of this galangal is documented as rich in phenolic compounds (Tomazelli et al., 2018). Due to the high amount of phenolics, the essential oil from galangal has a number of health benefits such as antibacterial, antifungal, anticancer, antidiabetic, anti-inflammatory, antioxidant (Chudiwal et al., 2010) and antiproliferative (Manosroi et al., 2006).

Hydrodistillation extraction of essential oil is known as the most commonly used method. However, this method is time-consuming, low extraction efficiency and high energy consumption (Khumpirapang et al., 2018). Several innovative techniques of extracting the essential oil can be used to overcome the disadvantages of the conventional method (Danlami et al., 2014; Tongnuanchan & Benjakul, 2014). Microwave pretreatment is a promising method to improve extraction efficiency and reducing the extraction time by losing the cell wall (Kittiphoom & Sutasinee, 2015). By using this technique, the local overheating of the plant tissue can be avoided. Currently, using microwave pretreatment for extracting of essential oil, in particular for lesser galangal essential oil is very limited. Encapsulation of essen-

tial oil using spray drying can be used to improve the application of the essential oil in the food industry. The resulted essential oil powder is suitable for heat-sensitive ingredients in foods and pharmaceuticals (Saénz et al., 2009). The aim of this study was to isolate essential oil from galangal using hydrodistillation with microwave pretreatment. The galangal essential oil was then encapsulated by spray drying to obtain an essential oil powder that is potential for industrial uses.

2. Materials and Methods

2.1. Materials and chemicals

The fresh Lesser galangal (*A.officinarum*) rhizomes were purchased from a local market, Chiang Rai, Thailand. The sample was selected by a firm texture, free from fungi or any damage by the pest.

In this research, microwave (Samsung ME711K) was used to pretreat the samples. A mixture of gum arabic and galangal essential oil was sprayed dried using a JCM Mini Lab SDE-10 spray dryer. In addition, the equipment used for analyzing essential oil and powdered essential oil included UV-Vis Spectrophotometer (Biochrom-Libra S22, UK), Microscope (Motic BA-310, Germany). The analytical grade chemicals DPPH (2, 2 – diphenyl -1-picryhydrazyl), methanol, hexane and ethanol) were purchased from local suppliers. The gum arabic was purchased from Chemipan Corporation Co., Ltd. All other chemicals and solvents used in this study were of analytical grade.

2.2. Methods

2.2.1. Extraction of galangal essential oil

One hundred grams of fresh galangal (*A. officinarum*) was mixed with water at ratio of 1:1 or 1:0.5, which was homogenized using a blender (Philips HR-2115). The mixture was then placed in a 500 mL round bottom flask. The flask was then covered by a plastic film with small holes and heated for 3 min with different power levels (600, 700 and 800 W). The heated samples were subsequently inserted into a Clevenger apparatus for hydrodistillation extraction. The process was conducted for 2.5 h and continued until no more essential oil was obtained for comparison.

The essential oil was collected and transferred into amber colored vials, dehydrated with anhydrous sodium sulfate, and kept at 4^0C in the dark until being analyzed.

The extraction yield of essential oil was calculated according to the equation given:

% Essential oil extraction yield = weight of essential oil extracted (g)/weight of the sample (g) × 100%.

2.2.2. Determined of DPPH free radical scavenging activity

The DPPH assay followed the protocol described by Sahoo et al. (2014) with some modifications. One milliliter of varying concentrations of the sample (10, 20, 50 and 100 μg/mL) in methanol was mixed with 2 mL of 0.1 mM DPPH solution in methanol using a vortex mixer. Then the mixture was put in the dark at ambient temperature for 30 min. The solution was measured at 517 nm using a UV/ visible spectrophotometer (Biochrom-Libra S22, UK). The inhibition percentage of DPPH in the extracts was calculated by using the following formula:

$$\text{Inhibition}(100\%) = \frac{\text{A} - \text{B}}{\text{A}} \times 100\%$$

where A control is the absorbance of the DPPH radical + methanol; B is the absorbance of DPPH radical + essential oil. The IC50 of the essential oil needed to inhibit 50% of the DPPH radicals obtained from the standard curve.

2.2.3. Preparation of the coating material and oil loading

Gum arabic was used as a wall material for the obtained essential oil. The method was conducted according to procedure of Fernandes et al. (2013). The gum arabic solution was prepared at 24% (w/v, in water) in a 250 mL beaker. The solution were pre-mixed for 10 min by a magnetic stirrer. The solution was then allowed to cool to room temperature and kept in a shaking water bath at 25^0C (90 rpm shaking rate) for one night to obtain a complete dissolved solution.

For different oil loading capacities, three ratios of essential oil to the wall material (i.e., 10%, 20% and 30%) were calculated as follow:

$$\text{Oil loading capacity}(\%) = \frac{\text{Core}}{\text{Core} + \text{Shell}} \times 100$$

The mixtures was emulsified in shear homogenizer Ultra-Turrax T-25 (IKA, Werke GmbH & Co. KG, Germany) for 5 min at 10,000 rpm until complete dispersion of the essential oil was obtained (Kausadikar et al., 2015). The prepared emulsions are shown in Table 1.

Table 1. The gum arabic and essential oil compositions in prepared emulsions

Oil loading capacities	10%	20%	30%
Essential oil (core)	6.7 g	15 g	25.8 g
Gum arabic (shell)	60 g	60 g	60 g

2.2.4. Spray drying

The stable emulsions (about 250 mL) were spray-dried using a JCM Mini Lab SDE-10 spray dryer (JCM best technology co., Ltd., Thailand). The dryer was equipped with a two-fluid nozzle atomizer (0.5 mm diameter). The operating conditions of the spray drying were inlet air temperature of 160^0C, outlet air temperature of 80^0C and the feed flow rate was about 500 mL/h. The encapsulated powders were recovered from the collecting chamber. The powders were kept in a desiccator, then vacuum-sealed in high-density polyethylene (HDPE) plastic bags and stored at -4^0C until further analysis.

2.2.5. Determination of encapsulation efficiency

Encapsulation efficiency analysis was conducted to evaluate the ratio of surface oil to the entrapped oil in the capsule. The encapsulation efficiency was calculated using the following equation:

% Encapsulation Efficiency = [total oil content (g)-surface oil content (g)]/[total oil content (g)-surface oil content (g)] × 100

Total oil content in the powder was determined as follow: a 500 mL flat-bottom flask was used to dissolve 5 g of a powder sample in 150 mL of distilled water and distilled for 1 h using Clevenger's apparatus. The essential oil was collected in amber bottles then oil content was obtained from weight measurement.

The method to determinate surface oil was described by Tomazelli et al. (2018). Twenty millilitre of hexane was added to 5 g of a powder and the mixture was stirred at 300 rpm for 10 min. The suspension was filtered through a dried cellulose filter and was washed with 20 mL of hexane three times. The filter was kept in a desiccator under vacuum to vaporize all residual solvent until obtaining constant weight.

2.2.6. Solubility and moisture content

For solubility (Márquez-Gómez et al., 2018), 10 g of a sample was dissolved in 25 mL of solvent (distilled water, vegetable oil and ethanol (95% v/v)), and they were maintained under constant stirring for 5 min at ambient temperature. Then the suspension was observed for the solubility or microcapsule cluster.

The moisture content (MC) of essential oil powder was determined using a thermogravimetric analysis. The sample (2 g) was loaded into the aluminum cups and heated at 105^0C in a hot air oven until a constant weight was obtained.

$$\mathrm{MC_{wb}} = \frac{\mathrm{W_f} - \mathrm{W_i}}{\mathrm{W_i}}$$

where: $\mathrm{W_i}$ is the initial weight of sample; $\mathrm{W_f}$ is the final weight of the sample.

2.2.7. Scanning electron microscopy (SEM) of essential oil powders

Scanning electron microscope (SEM) was operated at 10 kV (LEO/1450 VP) for investigating the microstructure of essential oil powders. Before analysis, samples were sputtered with gold in an anion-sputtering apparatus (Leica EM SCD500, Leica Microsystems, Germany).

2.2.8. Statistical analysis

The results were analyzed by analysis of variance (ANOVA) ($P < 0.05$). When a significant difference was found between the samples, Duncan's Multiple Comparison Test was applied ($P < 0.05$) by using SPSS software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). All tests were carried out in duplicate.

3. Results and Discussion

3.1. Extraction essential oil yield

Microwave pretreatment is assumed to be effective for essential oil extraction from galangal rhi-

Table 2. The essential oil yield (%) and the extraction time[1,2]

	Microwave power and sample: water ratio	Essential oil extraction yield (%)	Extraction time by hydrodistillation (h)
Microwave pretreatment for 3 min	600 W (1:1)	0.33 ± 0.01[a]	2.5
	700 W (1:1)	0.21 ± 0.01[c]	2.5
	800 W (1:1)	0.20 ± 0.01[c]	2.5
	600 W (1:0.5)	0.27 ± 0.01[b]	2.5
	700 W (1:0.5)	0.21 ± 0.01[c]	2.5
	800 W (1:0.5)	0.22 ± 0.02[c]	2.5
Non-microwave pretreatment	Control	0.30 ± 0.01[ab]	3.0

[1]Value are means ± standard deviation.
[2]Value with the same columns followed by different letter are significantly different ($P < 0.05$), based on Duncan multiple using SPSS, version 23.

zome. In this experiment, the pretreatment was applied at different power levels and the results are shown in Table 2.

The MW pretreatment effectively reduced the extraction time by 30 min (from 3.0 h to 2.5 h) while the essential oil yield was insignificant different from that of the untreated sample ($P < 0.05$). The MP may affect the cell wall of the galangal resulting in faster release of essential oil during extraction. Microwave power may provide energy to the cells of the sample so that it can quickly increase in heat and force and loosen the plant matrix cells wall (Proestos & Komaitis, 2008; McMillan et al., 2013). The microscopy imaging of MW pretreated samples was illustrated in Figure 1. The MW pretreatment somehow showed its effects on the cell walls as cracking (red arrows, on the right), suggesting for its role of assistance in essential oil extraction. Thus, the treatment at 600 W resulted in the highest oil yield ($P < 0.05$). The higher power levels (700 W and 800 W) of microwave radiation may lead to losing the volatile compounds during extraction. In MW pretreatment, the lower extraction yield found in high microwave power may be due to high evaporation of volatile compounds (Behera et al., 2004).

The sample:water ratio of 1:1 increased the essential oil yield observed at 600 W treatment (from 0.27% to 0.33%, $P < 0.05$). The water content of plant tissue affects the penetration of microwave (Jack & Cooper, 2005), and cause intensive thermal effects. The sample:water ratio of 1:1 may give adequate moisture for the penetration of MW, resulting in the higher yield as observed.

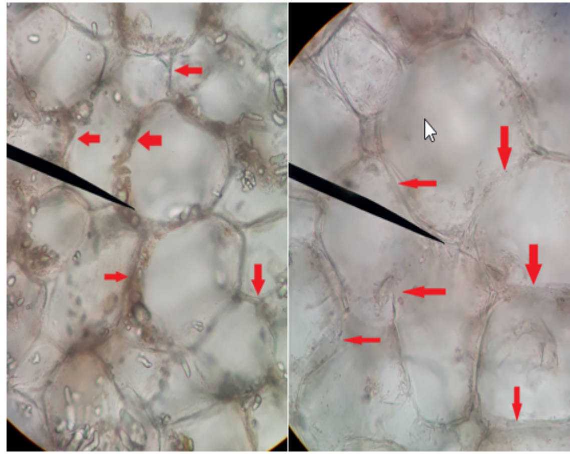


Figure 1. The sample before (left) and after (right) microwave pretreatment, the red arrows show the junctions between galangal rhizome cells.

3.2. Microencapsulation lesser galangal (*A. officinarum*) essential oil by spray drying

The ultimate purpose of the encapsulation of the essential oil is to preserve essential oil quality as well as increase the application in the food industry. In this study, the spray drying method was used to encapsulate the obtained galangal essential oil. The important quality parameter in the encapsulation of oils by spray drying is encapsulation efficiency value (Bae & Lee, 2008). The values of encapsulation efficiency are summarized in Table 3.

For the spray drying encapsulation, the essential oil loading capacity potentially affects the encapsulation efficiency (Ahn et al., 2008; Frascareli et al., 2012). In this study, 20% of essential oil loading capacity gave the highest EE (86.5%).

Table 3. Effect of the oil loading capacity of powder encapsulation of *A. officinarum* essential oil[1,2]

Oil loading capacity (%)	Surface oil content (g/100 g)	Total oil content (g/100 g)	Encapsulation efficiency (%)	Moisture content (%)
10%	3.75 ± 0.35[a]	8.6 ± 0.2[a]	56.6 ± 5.1[b]	5.61 ± 0.83[a]
20%	1.23 ± 0.35[b]	9.5 ± 0.7[a]	86.5± 5.0[a]	4.85 ± 0.64[a]
30%	3.20 ± 0.35[a]	9.2 ± 1.1[a]	64.6± 0.6[b]	6.15 ± 0.78[a]

[1]Value means ± standard deviation.
[2]Value with the same columns followed by a different letter (a to c) are significantly different ($P < 0.05$), based on Duncan multiple.

Surface oil retention is another parameter that can be used to evaluate the process of encapsulation. The presence of surface oil content relates to the physical properties of spray-dried powders because it can lead to rapid lipid oxidation (Fäldt & Bergenståhl, 1995; Jimenez et al., 2004). Surface oil content was found lowest in 20% loading capacity level (1.23%). The surface oil retention was inversely correlated to the encapsulation efficiency (as seen in Table 3). Thus, the higher core material inversely proportionates to the surface oil retention. Interestingly, at 30% oil loading capacity, the surface oil retention was also found to be higher than in the 20% oil loaded sample. This phenomenon can be explained by the wall material:essential oil ratio. At 30% oil loading capacity, the wall material was not adequate to entrap the entire amount of essential oil and consequently resulted in higher surface oil content. Since the high encapsulation efficiency and the low surface oil contents are preferred targets for a promising encapsulation, oil loading capacity at 20% was the most suitable level for essential oil encapsulation by spray drying. There were reported that a ratio of 1:4 (core to wall ratio) provided the most suitable stable to varying volatile cores (Saénz et al., 2009; Fernandes et al., 2013; Kha et al., 2014).

3.3. The moisture content and solubility

The moisture content of the dried powders obtained at difference oil loading capacities was evaluated (Table 3). There was insignificant interaction between the oil loading capacity and the final moisture content ($P > 0.05$). The moisture content is an important parameter for powdered products since oxidative reactions of the oil tend to increase at increasing moisture content. The moisture content of the essential powder under control conditions was less than 7%, which is in range of most dried powders used in the food industry (Kha et al., 2014).

3.4. The antioxidative activity of essential oil powder

In this part, the DPPH assay was used to investigate the antioxidative activity of the essential oil powder and the data is shown in Table 4. It can be seen that the antioxidative activity of the encapsulated powders was significantly lower than that of freshly extracted essential oil ($P < 0.05$). This was observed in all level of oil loading capacities. After spray drying, the IC_{50} of the essential oil extracted from the powders increased 2-fold (Table 4). This means that the antioxidative activity of the extracted essential oil from the encapsulated powder significantly reduced. The negative effect of encapsulation by spray drying on the antioxidative activity of essential oil may be attributed to the thermal degradation of antioxidants during exposure to high temperature (Couto et al., 2012; Fernandes et al., 2014).

Table 4. The IC_{50} of galangal essential oil extracted from encapsulated powders and before encapsulation determined by DPPH assay[1,2]

The per cent oil loading	The IC_{50} value (μg/mL)
10%	2123 ± 138[a]
20%	2077 ± 135[a]
30%	1934 ± 249[a]
Essential oil before encapsulation	1039 ± 66[b]

[1]Mean ± standard deviation.
[2]Value with the same columns followed by a different letter (a to c) are significantly different ($P < 0.05$), based on Duncan multiple.

3.5. SEM microstructure

The scanning electron microscopy (SEM) was used to study the morphologies of the encapsulated essential oil powder. In all samples, the particle size of the powders ranged from 5 to 30 μm (estimated under SEM micrographs, Figure 2).

The shrinkage of the surface was observed in these particles. According to Kha et al. (2014), during drying and cooling, the surface depressions likely result in the shrinkage. The outer surface showed a smooth wall without significant fissures or cracks. The closed structure of the particles can significantly reduce oxygen exposure and thus prevent the oxidation of the core material (essential oil).

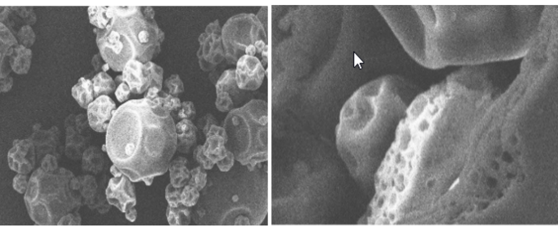


Figure 2. The SEM micrograph of the surface (left) and internal (right) microstructures of the encapsulation powders, the imaging observed at a magnification levels of 1000X and 5000X, respectively.

The cross-sectional SEM images revealed the internal structure of the particles. Homogenous spherical holes were observed indicating an even distribution of the essential oils in gum arabic. The similar results were also documented in the study of Kha et al., (2014). These results again led to a high level of encapsulation effectiveness.

3.6. The solubility of powder in different solvents

The solubility of the powder is an indicator for selecting a suitable medium to dissolve the powder and thus clarify industrial application. The powders obtained by spray drying were completely dissolved in vegetable oil. In distilled water, the gum arabic was mostly dissolved but the essential oil formed a consistent colloid (Figure 3). Such solubility results of the powder were considered to be applicable for most food matrices (Márquez - Gomez et al., 2018).

Figure 3. The solubility of powder in different solvents.

4. Conclusions

The processes of isolating the essential oil from lesser galangal using microwave pretreatment and encapsulation of the essential oil were studied. The highest oil essential extraction (0.33%) was achieved using 600 W microwave power treatment with the sample:water ratio of 1:1. Using the MW pretreatment can reduce the extraction time by 30 min. Excessive microwave radiation power could negatively affect the extracted oil yield. The most suitable encapsulation condition for encapsulating galangal essential oil with gum arabic was 20% loading capacity. At 20% loading capacity, the essential oil powder showed a comparative *in vitro* antioxidative activity with other treatments. The developed product can be further used as an flavoring agent in food and beverage industries.

Acknowledgements

The authors would like to thank The ASEAN International Mobility for Students Programme (AIMS) and Advanced Programme in Food Technology for facilitating collaboration between Mae Fah Luang University and Nong Lam University Ho Chi Minh City.

References

Ahn, J. H., Kim, Y. P., Lee, Y.M., Seo, E. M., Lee, K. W., & Kim, H. S. (2008). Optimization of microencapsulation of seed oil by response surface methodology. *Food Chemistry* 107(1), 98-105.

Bae, E., & Lee, S. (2008). Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. *Journal of Microencapsulation* 25(8), 549-560.

Behera, S., Nagarajan, S., & Rao, L. J. M. (2004). Microwave heating and conventional roasting of cumin seeds (*Cuminum cyminum* L.) and effect on chemical composition of volatiles. *Food Chemistry* 87(1), 25-29.

Chudiwal, A., Jain, D., & Somani, R. (2010). Alpinia galanga Willd.–An overview on phyto-pharmacological properties. *Indian Journal of Natural Products and Resources* 1(2), 143-149.

Couto, R. O., Conceição, E. C., Chaul, L. T., Oliveira, E. M., Martins, F. S., Bara, M. T. F., Rezende, K. R., Alves, S. F., & Paula, J. R. (2012). Spray-dried rosemary extracts: physicochemical and antioxidant properties. *Food Chemistry* 131(1), 99-105.

Danlami, J. M., Arsad, A., Zaini, M. A. A., & Sulaiman, H. (2014). A comparative study of various oil extraction techniques from plants. *Reviews in Chemical Engineering* 30(6), 605-626.

Fäldt, P., & Bergenståhl, B. (1995). Fat encapsulation in spray-dried food powders. *Journal of the American Oil Chemists' Society* 72(2), 171-176.

Fernandes, R. V. d. B., Borges, S. V., & Botrel, D. A. (2013). Influence of spray drying operating conditions on microencapsulated rosemary essential oil properties. *Food Science and Technology* 33, 171-178.

Fernandes, R. V. d. B., Marques, G. R., Borges, S. V., & Botrel, D. A. (2014). Effect of solids content and oil load on the microencapsulation process of rosemary essential oil. *Industrial Crops and Products* 58, 173-181.

Frascareli, E., Silva, V., Tonon, R., & Hubinger, M. (2012). Effect of process conditions on the microencapsulation of coffee oil by spray drying. *Food and Bioproducts Processing* 90(3), 413-424.

Jack, S. A., & Cooper, K. (2005). Microwave endometrial ablation: An overview. *Reviews in Gynaecological Practice* 5(1), 32-38.

Jimenez, M., Garcia, H., & Beristain, C. (2004). Spray-drying microencapsulation and oxidative stability of conjugated linoleic acid. *European Food Research and Technology* 219(6), 588-592.

Kausadikar, S., Gadhave, A. D., & Waghmare, J. (2015). Microencapsulation of lemon oil by spray drying and its application in flavour tea. *Advanced Applied Science Research* 6(4), 69-78.

Kha, T. C., Nguyen, M. H., Roach, P. D., & Stathopoulos, C. E. (2014). Microencapsulation of gac oil by spray drying: optimization of wall material concentration and oil load using response surface methodology. *Drying Technology* 32(4), 385-397.

Khumpirapang, N., Pikulkaew, S., Anuchapreeda, S., & Okonogi, S. (2018). Alpinia galanga oil—A new natural source of fish anaesthetic. *Aquaculture Research* 49(4), 1546-1556.

Kittiphoom, S., & Sutasinee, S. (2015). Effect of microwaves pretreatments on extraction yield and quality of mango seed kernel oil. *International Food Research Journal*, 22(3), 960-964.

Manosroi, J., Dhumtanom, P., & Manosroi, A. (2006). Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Letters* 235(1), 114-120.

Márquez-Gómez, M., Galicia-García, T., Márquez-Meléndez, R., Ruiz-Gutiérrez, M., & Quintero-Ramos, A. (2018). Spray-dried microencapsulation of orange essential oil using modified rice starch as wall material. *Journal of Food Processing and Preservation* 42(2), e13428.

McMillan, J. R., Watson, I. A., Ali, M., & Jaafar, W. (2013). Evaluation and comparison of algal cell disruption methods: microwave, waterbath, blender, ultrasonic and laser treatment. *Applied Energy* 103, 128-134.

Proestos, C., & Komaitis, M. (2008). Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. *LWT-Food Science and Technology* 41(4), 652-659.

Saénz, C., Tapia, S., Chávez, J., & Robert, P. (2009). Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). *Food Chemistry* 114(2), 616-622.

Sahoo, S., Parida, R., Singh, S., Padhy, R. N., & Nayak, S. (2014). Evaluation of yield, quality and antioxidant activity of essential oil of *in vitro* propagated Kaempferia galanga Linn. *Journal of Acute Disease* 3(2), 124-130.

Tomazelli, J. O., Kuhn, F., Padilha, P., Vicente, L., Costa, S., Boligon, A., Scapinello, J., Nesi, C., Dal Magro, J., & Castellví, S. L. (2018). Microencapsulation of essential thyme oil by spray drying and its antimicrobial evaluation against Vibrio alginolyticus and Vibrio parahaemolyticus. *Brazilian Journal of Biology* 78(2), 311-317.

Tongnuanchan, P., & Benjakul, S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of Food Science* 79(7), R1231-R1249.

Effect of different drying methods on the nutritional and physicochemical properties of unpeeled banana flour

Sang Q. Tran[1], Tuyen C. Kha[1], & Khanitta Ruttarattanamongkol[2*]

[1]Faculty of Food Science and Technology, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Faculty of Agriculture, Nature Resources and Environment, Naresuan University, Phitsanulok, Thailand

ARTICLE INFO

Research Paper

Received: April 19, 2019
Revised: June 19, 2019
Accepted: June 28, 2019

Keywords

Banana flour
Drying method
Physicochemical properties

***Corresponding author**

Khanitta Ruttarattanamongkol
Email: khanittar@nu.ac.th

ABSTRACT

This work was conducted to evaluate the effect of drying methods on the nutritional values and physicochemical properties of unpeeled banana flour. Proximate, amylose content, phenolic compound, resistant starch, total dietary fibre, functional properties, pasting properties, and thermal properties of dried banana flour samples were evaluated. Three different drying methods of whole banana with the intact peel were studied including 1) hot-air unpeeled flour (HAU) (dried at 60°C for 2 h in hot-air chamber), 2) microwave-vacuum unpeeled flour (MVU) (36,000 W under vacuum -600 mmHg for 15 min in a pilot microwave-vacuum dryer), and 3) infrared unpeeled flour (IRU) (600 W for 15 min in infrared channel dryer). The HAU and MVU showed the highest yield. Drying methods did not affect the compositions of the flour but significantly affected the total dietary fibre, resistant starch, amylose content and phenolic compound of the flour. Among samples, HAU contained the highest nutritional values with outstanding functional properties, and pasting properties. The unpeeled banana flour can be utilized in various food products such as noodle, bakeries, snack or used as functional ingredients for nutritional purposes.

Cited as: Tran, S. Q., Kha, T. C., & Ruttarattanamongkol, K. (2019). Effect of different drying methods on the nutritional and physicochemical properties of unpeeled banana flour. *The Journal of Agriculture and Development* 18(3), 64-73.

1. Introduction

Banana is used popularly among all classes of people due to its widely available, cheap with great nutritive and medicinal values. In recent times, interest in using banana at the green stage as a food ingredient has been aroused because of its high carbohydrate content, especially resistant starch which is considered as a functional food (Mastro et al., 2007; Mohapatra et al., 2010). The banana peel has been reported to contain a high amount of dietary fibre, minerals, vitamins and polyphenolic compounds (Anjum et al., 2014). Consequently, banana peel is a great potential material that could be considered and developed for further application. However, banana production has been excessive, causing a large amount of bananas to be reduced in price or wasted due to the lack of well-organized and proficient preservation techniques (Maskan, 2000); and banana peel is mostly discarded to be used as fertilizer in landfills, animal feed or waste. Therefore, preservation technique should be investigated to extend the shelf-life and the utilization of banana peel is necessary to maximize the benefit of banana and increase the productivity of banana production.

Hot-air drying is a conventional drying method which has low energy efficiency and a long drying time (Adu & Otten, 1996; Feng & Tang, 1998). For this reason, it may cause substantially undesirable impact on quality of dried products (Lin et al., 1998; Drouzas et al., 1999). Microwave-vacuum and infrared heating are characterized by rapid and uniform heating which could be the alternative for hot-air drying.

In this study, hot-air drying chamber,

microwave-vacuum chamber and infrared channel were investigated as preservation techniques for prolonging the shelf-life of unpeeled banana by lowering its moisture content. Proximate, total dietary fibre, resistant starch, amylose content and phenolic compound were analyzed to figure out the effect of the three different drying methods on nutritional value of banana flour. Functional properties, thermal properties, pasting properties and colour were also evaluated among the three drying methods to understand the physical properties of banana flour.

2. Materials and Methods

2.1. Banana flour processing

The first ripeness stage of banana Pisang Awak Musa obtained from Phitsanulok Province, Thailand was chosen based on the standard ripeness chart (Kader, 1996) for producing banana flour. The preparation of banana flour was made by using the method of Arisa et al. (2013) with a slight modification. Individual banana fruits with intact peel were obtained from banana bunches by cutting and washing. After that, they were sliced into 2 mm thickness using a kitchen slicer and immediately soaked into sodium metabisulfite 0.1% for 5 min. Then, they were separated into 3 parts for being dried using 3 different drying methods including 60°C for 2 h in hot-air chamber (HAU), 36,000 W under vacuum -600 mmHg for 15 min in a pilot microwave-vacuum dryer (MVU), and 600 W for 15 min in infrared channel dryer (IRU). The dried banana slices were ground into powder using a grinder DXFill model DXM 2000 and then screened through a 60 mesh sieve. The banana flour was packed in an aluminium pack and stored at -18^0C.

2.2. Analysis

2.2.1. Production yield

Production yield was determined by dividing the weight of the obtained banana flour (dry basis) by the weight of the fresh banana fruit, and then multiply by 100.

2.2.2. Water activity

Water activity of samples was measured using Lab swift water activity meter.

2.2.3. Proximate

Proximate in terms of moisture, protein, crude fat and ash was analysed by the method described in AOAC (1990).

2.2.4. Amylose content

The amylose content measurement was done according to the method of Almeida et al. (2010) with a slight modification. Briefly, a total of 100 mg sample was homogenized with 1 mL of 95% ethanol and 9 mL of 1 M NaOH. The sample was heated for 10 min in a boiling-water bath to gelatinize the starch. After cooling, it was transferred into a volumetric flask and the volume was made up to 100 mL with water. Then 1 mL of 1 M acetic acid and 2 mL of iodine solution (0.2% I2, 2% KI) was added to a 5 mL aliquot. The solution was made up to 100 mL with water and allowed to stand for 10 min. Spectrophotometric quantification was performed at 620 nm, with a UV-Vis spectrophotometer Shanghai Metash Instrument. The apparent amylose content was calculated using an equation obtained from the standard curve using purified amylose and amylopectin extracted from potato tubers.

2.2.5. Phenolic compound

Phenolic compound was measured using the method of Yang et al. (2014) with a slight modification. Briefly, the sample (5 g) was extracted twice with 50 mL of 80% (v/v) aqueous ethanol for 30 min at ambient temperature and centrifuged at 6000 r/min for 15 min at 20°C. The supernatants were collected, combined, and then evaporated under vacuum -300 mbar at 40°C in Evaporator apparatus (Büchi Rotavapor R-114; Büchi Waterbath B-480, Büchi Vacuum-System B-169) to dry and reconstituted in 100 mL of distilled water. Phenolic compound was measured using Folin-Ciocalteu method.

2.2.6. Resistant starch

Resistant starch was determined by using the method of Megazyme International Ireland. Briefly, the sample was incubated in 4.0 mL of pancreatic α-amylase solution at 37°C with continuous shaking for 16 hours. Ethanol (99% v/v) of 4 mL was added and vigorously stirred on the vortex mixer. The tubes were centrifuged at 3,000

rpm for 10 min (non-capped). The supernatants were carefully decanted and the pellets were re-suspended in 2 mL of 50% ethanol and vigorously stirred on the vortex mixer. Accurately 6 mL of 50% IMS was further added and the solution was centrifuged again at 3000 rpm for 10 min using centrifuge universal 320. The supernatants were decanted. These suspension and centrifugation steps were repeated once more. The pellets were re-suspended in 2 mL of 2 M KOH and stirred for approximate 20 min in an ice/water bath. Accurately 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added. The mixture was incubated in 0.1 mL of AMG at 50°C for 30 min. To measure resistant starch, accurately 0.1 mL aliquots (in duplicate) was mixed with 3.0 mL of GOPOD reagent, the mixture was incubated at 50°C for 20 min. The absorbance of the mixture was measured at 510 nm against the reagent blank.

2.2.7. Total dietary fibre

Total dietary fibre was determined by using the method of Megazyme International Ireland. Briefly, duplicate of samples were mixed with 10ml MES-TRIS solution using magnetic stirring bar. After that, the mixture was incubated in 50 μL thermostable α-amylase solution at 98 - 100°C for 30 min and 100 μL protease solution at 60°C for 30 min successively with continuous agitation. Accurately 5 mL of 0.561 N HCl was dispersed into the mixture and the pH was adjusted with additional 5% NaOH solution. The mixture was incubated in 200 μL amyloglucosidase solution at 60°C for 30 min with continuous agitation. The insoluble fibre in the mixture was filtrated using filter paper Whatman number 4. The soluble fibre in the filtrate was precipitated by incubating in EtOH 95% at 60°C for 60 min. The soluble fibre was filtrated using filter paper Whatman number 4. Each replicate of the insoluble and soluble fibre filter paper was measured for protein and ash. Dietary fibre is the sum of insoluble and soluble fibre.

2.2.8. Functional properties

The water absorption index (WAI), water solubility index (WSI) and swelling power index (SPI) were determined according to a reported method of Tong et al. (2015) with a slight modification. Briefly, about 0.1 g of sample was dispersed in 20 mL deionized water and agitated at 25°C and 100°C for 30 min using shaking water bath JSR model JSSB-30T, respectively. After centrifuging the dispersion at 15,000 g for 30 min using a Hermle Z206A centrifuge, the supernatant was dried in a hot-air oven SNOL at 105°C until a constant weight was obtained. WAI, WSI and SPI were calculated by the following formulas:

$$\text{Water absorption index} = \frac{\text{wet sediment weight}}{\text{dry sample weight}}$$

$$\text{Water solubility index (\%)} = \frac{\text{dried supernatant weight}}{\text{dry sample weight}} \times 100$$

$$\text{Swelling power index} = \frac{\text{wet sediment weight}}{\text{dry sample weight x (1-WSI)}}$$

The oil absorption index (OAI) was determined based on the protocol developed by Kraithong et al. (2018) with a slight modification. The sample of 1 g was mixed with 10 mL of soybean oil bought from Lotus supermarket. The mixture was centrifuged at 4000 rpm for 20 min using a Hermle Z206A centrifuge. After that, the supernatant was decanted while the residue was weighed. The calculation of OAI was as follows:

$$\text{Oil absorption index} \left(\frac{\text{g}}{\text{g}}\right) = \frac{\text{weight of residues(g)-weight of sample(g)}}{\text{weight of dry sample (g)}}$$

2.2.9. Thermal properties

Thermal properties in terms of onset temperature, peak temperature, enset temperature and enthalpy were measured using a Mettler Differential Scanning Calorimetry (DSC). The method was followed a procedure of Nimsung et al. (2007). Approximately 5 mg of the sample (dry basis) was weighed directly in a tared stainless pan at temperature of 20°C and distilled water was added to get the starch:water ratio of 1:2 (5 mg:10 μL). The pan containing the sample was hermetically sealed and allowed to equilibrate for 1 hour at room temperature to complete starch hydration before the analysis. After that, the pan was placed in the DSC and heated from 10 to 130°C at the rate of 10°C/min. An empty pan was used as a reference. The onset (T_o), peak (T_p), completion temperature (T_c), and energy of enthalpy (ΔH) were recorded and computed

using computer software supplied with the instrument.

2.2.10. Pasting properties

The pasting properties in terms of peak viscosity, hot paste viscosity, breakdown, cold paste viscosity, set back, peak time and pasting temperature were determined using a Rapid Visco Analyzer 4500 (Newport Scientific). The method was followed procedure of Nimsung et al. (2007) with a slight modification. The sample of 3 g (dry basis) was weighed into a disposable aluminium RVA canister, and distilled water was added to obtain a total sample weight of 28 g. The sample was held at 50°C for 1 min and heated to 95°C for 4 min and held at 95°C for 2 min, and then cooled from 95°C to 50°C and held at 50°C for 9 min. The RVA parameters including the peak viscosity, hot paste viscosity, breakdown, cold paste viscosity, setback, and pasting temperature were all recorded. All measurements were performed in duplicates.

2.2.11. Statistical analysis

An analysis of variance (ANOVA) was performed. The data were expressed as the mean ± SD and analysed by SPSS (version 19 for windows, SPSS Inc., Chicago, IL, USA) using Duncan's Multiple-Range Test at a significant level of $P < 0.05$.

3. Results and Discussion

3.1. Production yield and water activity

Production yield obtained from 3 drying methods of peeled and unpeeled banana flour is presented in Table 1. There was a small difference in production yield among the drying methods except for MVU and HAU which show higher production yield (25.73% and 25.5%, respectively) than that of IRU. These results were similar to the production yield of green banana flour obtained from 4 types of banana reported by Yani et al. (2013) (*Janten* 35 - 36%, *Kepok Manado* 19 - 20%, *Muli* 16 - 17% and *Raja Nangka* 20 - 21%). Yani et al. (2013) stated that the difference of production yield might be due to the maturity stages of bananas which is related to the starch content and properties. Microwave-vacuum drying and hot-air drying could be considered to apply for producing unpeeled banana flour regarding the economic benefit and production yield.

In general, the water activity of banana flour dried by the 3 drying methods was lower than 0.6 which is the safe level for preserving the product. MVU exhibited the lowest water activity (0.326) and there were not significant differences in the water activity between HAU (0.482) and IRU (0.469). As a result, the most effective drying method for reducing water activity was microwave-vacuum.

Table 1. Production yield (% dry basis) and water activity of banana flour

Samples	Production yield	Water activity
HAU	25.50	0.482 ± 0.060^{a}
MVU	25.73	0.326 ± 0.017^{b}
IRU	22.68	0.469 ± 0.001^{a}

Values are shown as mean ± SD; $^{a-b}$Different letters in the same column are significantly different ($P < 0.05$); HAU: Hot-air unpeeled; MVU: Microwave-vacuum unpeeled; IRU: Infrared unpeeled.

3.2. Proximate analysis

Proximate of banana flour including moisture content, protein, crude fat, ash and carbohydrate is presented in Table 2. The moisture content was highest in IRU (12.14%) and lowest in MVU (5.38%). It might be due to the high processing temperature and shallow depth effect of infrared which caused the cake hardening phenomenon which prevents moisture from diffusion and evaporation; and in microwave vacuum drying, the heat created by ionic polarization or dipole rotation transfer within the food by conduction or convection so that the inner and outer parts of food receive the same energy or the moisture content is removed thoroughly.

Protein ranged from 2.80% in IRU to 3.03% in MVU and there were not significant differences in protein content among different drying methods. The results were close to the range of 2.55 - 3.41% in banana flours reported by Yani et al., (2013), 2.50 - 3.0% reported by Mota et al. (2000) and 1.88 - 4.47% reported by Nimsung et al. (2007). The variance of protein content in banana flour was caused by some factors such as weather, soil nutrient and varieties (Yani et al., 2013) and matuirty stage (Emaga et al., 2007).

The range of 0.23% in IRU and 0.26% in MVU for crude fat were in agreement with Liao & Hung

Table 2. Proximate of banana flour (% dry basis)

Method	Moisture	Protein[ns]	Crude fat[ns]	Ash[ns]	Carbohydrate[ns]
HAU	9.50 ± 0.20[b]	2.84 ± 0.18	0.24 ± 0.02	1.94 ± 0.16	94.98 ± 0.33
MVU	5.38 ± 0.27[c]	3.03 ± 0.33	0.26 ± 0.01	1.68 ± 0.35	95.04 ± 0.07
IRU	12.14 ± 0.13[a]	2.80 ± 0.11	0.23 ± 0.02	1.52 ± 0.16	95.45 ± 0.23

Values are shown as mean ± SD; [a-c]Different letters in the same column are significantly different ($P < 0.05$); [ns]Non-significant; HAU: Hot-air unpeeled; MVU: Microwave-vacuum unpeeled; IRU: Infrared unpeeled.

(2015) and Nimsung et al. (2007) who found that the fat content of banana flour was 0.25% and 1.56 - 4.88%, respectively. The three drying methods did not have an effect on the fat content of banana flour.

The ash was in the range of 1.52% in IRU and 1.94% in HAU. The results also showed that there were not significant differences among the three drying methods ($P > 0.05$). These results were lower than the range of 2.24 - 3.03% and 2.6 - 3.5%, which were reported by Yani et al. (2013) and Mota et al. (2000), respectively. The differences in the ash content might be due to the soil, varieties and planting weather.

Carbohydrate was in the range of 94.98% in HAU and 95.45% in IRU and there were not significant differences among 3 drying methods in the carbohydrate content ($P > 0.05$).

3.3. Total dietary fibre, resistant starch, amylose content and phenolic compound

Total dietary fibre, resistant starch, amylose content and phenolic compound are shown in Table 3. Total dietary fibre ranged from 15.75% in IRU to 18.38% in HAU, these values compared reasonably well with the 6.0 - 15.5% total fibre reported by Mota et al. (2000).

The highest and lowest amounts of resistant starch were found to be in HAU (60.21%) and IRU (34.26%) respectively. The values were well comparable with those of 48.99% reported by Menezes et al. (2011) who followed the method of AOAC 2002.02 and 30.3% reported by Liao & Hung (2015) whose method was based on the approved method 32 - 40 (AACC, 2000). This difference might be caused by the high temperature and temperature fluctuation in infrared drying that reduced the amount of resistant starch.

Amylose content was found to be highest and lowest in HAU (37.95%) and IRU (32.17%) respectively. The difference in amylose content among 3 drying methods might be due to the differences in moisture contents, drying rates, drying temperatures and drying mechanisms. The result shows that there might be a relationship between resistant starch and amylose content since the higher the amylose content, the higher the resistant starch content.

The highest and lowest phenolic compounds were found to be in HAU and IRU respectively. The phenolic content in banana flour was obtained from 20.22 mg GAE/100 g in IRU to 62.66 mg GAE/100 g in HAU. The phenolic content in HAU and MVU was closely and IRU was pretty lower compared to 50.65 mg GAE/100 g reported by Menezes et al. (2011). The difference might be due to the high and inconsistence of the temperature of different drying systems.

The total dietary fibre, resistant starch and amylose content might be sensitive to high temperature and the temperature fluctuation since they were lowest in the flour produced by infrared drying. The impact of hot-air drying on nutritional value of banana flour was the least comparing to microwave-vacuum drying and infrared drying, even though these 2 drying methods were evaluated to be less undesirable on quality of banana flour. It is appeared that optimization of drying condition of microwave-vacuum and infrared drying should be investigated in order to altarnate the traditional drying method.

3.4. Functional, thermal and pasting properties

The functional properties of banana flour including WAI (Water Absorption Index), WSI (Water Solubility Index), SPI (Swelling Power Index) and OAI (Oil Absorption Index) are shown in Table 4. Water Absorption Index (WAI) ranged from 3.15 g/g in MVU to 3.59 g/g in HAU. WAI exhibits the ability of flour to absorb water molecules (Shafi et al., 2016). It is supported by the hydrophilic groups within the starches, which provide the viscosity, smoothness, and softness in the products (Aprianita et al., 2014). The polar side chains in carbohydrates and proteins could

Table 3. Total dietary fibre, resistant starch, amylose content and phenolics of banana flour (dry basis)

Samples	Total dietary fibre (%)	Resistant starch (%)	Amylose (%)	Phenolics (mg GAE/100 g)
HAU	18.38 ±0.03[a]	60.21 ± 0.13[a]	37.95 ± 0.98[a]	62.66 ± 0.04[a]
MVU	17.34 ± 0.16[b]	56.48 ± 0.90[b]	35.07 ± 1.09[b]	57.55 ± 1.82[b]
IRU	15.75 ± 0.12[c]	34.26 ± 0.49[c]	32.17 ± 1.01[c]	20.22 ± 0.34[c]

Values are shown as mean ± SD; [a-c]Different letters in the same column are significantly different ($P < 0.05$); [ns]non-significant; HAU: Hot-air unpeeled; MVU: Microwave-vacuum unpeeled; IRU: Infrared unpeeled.

Table 4. The functional properties of banana flour (dry basis)

Samples	WAI (g/g)	WSI (%)	SPI (g/g)	OAI (g/g)
HAU	3.59 ± 0.04[a]	5.92 ± 0.15[a]	3.82 ± 0.04[a]	2.02 ± 0.01[a]
MVU	3.15 ± 0.02[c]	5.13 ± 0.30[b]	3.32 ± 0.02[c]	1.91 ± 0.04[b]
IRU	3.43 ± 0.01[b]	5.83 ± 0.21[a]	3.65 ± 0.02[b]	2.02 ± 0.03[a]

Values are shown as mean ± SD; [a—c]Different letters in the same column are significantly different ($P < 0.05$); HAU: hot-air unpeeled; MVU: microwave-vacuum unpeeled; IRU: Infrared unpeeled; WAI: Water Absorption Index; WSI: Water Solubility Index; SPI: Swelling Power Index; OAI: Oil Absorption Index.

support the hydrogen bonding of the rice flour (Prasad et al., 2012). The water binding capacity is also encouraged by the negative charges of phosphate groups within amylopectin (Wang et al., 2016). The large particle size could reduce the WAI value (Otegbayo et al., 2013). The amylose-lipid and amylose-protein complexes inhibit the polar and charges group from water binding, which reduce the value of WAI (Falade & Christopher, 2015).

Water Solubility Index (WSI) was obtained in the range of 5.13% in MVU and 5.92% in HAU. It is not significantly different between HAU and IRU. The WSI represents the amount of soluble components which disperse in the aqueous solution during cooking (Shafi et al., 2016). The higher the WSI, the higher adhesive and sticky in the products, however, the lower consistent in the food structure (Wang et al., 2016). Junction zone formation by amylose encourages a rigid structure of starch granules, providing low WSI (Chung et al., 2011). The starch-protein and starch-lipid complexes could reduce the value of WSI because the soluble parts are reduced within the starch molecules (Keawpeng & Meenune, 2012). As a result, the low WSI value is desirable as it indicates the consistent structure of food during cooking (Kraithong et al., 2018).

Swelling Power Index (SPI) was in the range of 3.32 g/g in MVU and 3.82 g/g in HAU. The starch granules absorb water when it is heated to a critical temperature in the presence of excessive water. The starch granules then swell and a part of starch leaches out into the solution. The extend of swelling and leaching are determined by the strength of chemical bonding within the granules. The strong intermolecular bonds and high amylose content create an extensive network which can reduce the extent of swelling. The complete swelling is reached only after amylose has been leached out of the granules, therefore amylose is believed to restrict swelling. Furthermore, swelling index is also affected by the structure of starch granules. The high open structure of waxy starches allows rapid water penetration, swelling, and solubility. The swelling capacity of the starch granules is restricted by the increase of amylose content which can limit the amount of starch exudates leaching into solution. However, there are other factors affecting the swelling and solubility of starch granules (Bhattacharya et al., 1999).

Oil Absorption Index (OAI) was obtained from 1.91 g/g in MVU to 2.02 g/g in HAU and IRU. OAI was the highest and not significant different in HAU and IRU. The OAI value exhibits the ability to retain oil in the starch granules (Kraithong et al., 2018). It is supported by the hydrophobic groups within the starch molecules (Tharise et al., 2014). The flour with high OAI value promotes the mouth-feel, palatability, and flavour retention in the products. However, the rancidity is increased due to high value of OAI (Falade & Christopher, 2015).

The pasting properties including peak viscosity, hot paste viscosity, breakdown, cold paste viscosity, setback, peak time and pasting tem-

perature are presented in Table 5. The pasting profile could be related to the molecular characteristics of the starch components such as lipid and amylose. Furthermore, the morphology of the starch granules could affect the starch property (Nimsung et al., 2007). The variations in pasting properties are accounted by the differences in flour composition (Oko & Ugwu, 2011). The amylose is considered to reduce peak, hot paste, and breakdown viscosities, however, increase the setback, cold paste viscosities, and pasting temperature (Ye et al., 2016). However, the results in this study shows that amylose content had a tendency to increase peak viscosity, hot paste viscosity, breakdown, cold paste and speed up the peak time. The result also demonstrated that resistant starch and total dietary fibre showed the same relationship as amylose content with pasting properties. Protein and lipid also affect the pasting properties. The setback and cold paste viscosities are increased while the peak and breakdown viscosities are reduced by the formations of amylose-protein complex or amylose-lipid complex (Alcazar-Alay & Meireles, 2015). The protein with hydrophilic groups could increases the peak viscosity of rice flour (Hsu et al., 2015). The pasting properties of the flour are determined by the rigidity of starch granules which affects the granule swelling potential.

Peak viscosity values of HAU, MVU and IRU were 404, 313 and 263 RVU respectively and were significantly different ($P < 0.05$). The peak viscosity presents the water binding ability of the starch granule via hydrogen bonds (Otegbayo et al., 2013). The amylopectin content is responsible for high peak viscosity due to its high water holding capacity (Ye et al., 2016). Furthermore, small particle size with large surface area also increases the viscosity (Prasad et al., 2012). Higher peak viscosity is caused by higher breakdown because less heat and shear stress resistance during cooking (Hsu et al., 2015). Hot paste viscosity values of HAU, MVU and IRU were 267, 243 and 222 RVU, respectively. The Hot paste viscosity presents for the minimum viscosity at constant temperature. Breakdown viscosity of HAU, MVU and IRU were 137, 70, and 40, respectively. The high breakdown viscosity is resulted of the composition such as protein, lipid and amylose content. Cold paste viscosity of HAU, MVU and IRU were 408, 385, and 363, respectively. The cold paste viscosity presents for the stability of cooked paste and ability to form gel after cool-

Table 5. Pasting properties of banana flour

Sample	Peak viscosity (RVU)	Hot paste viscosity (RVU)	Breakdown (RVU)	Cold paste viscosity (RVU)	Setback^{ns} (RVU)	Peak Time (min)	Pasting Temp^{ns} (°C)
HAU	404 ± 1^a	267 ± 2^a	137 ± 3^a	408 ± 3^a	141 ± 0	5.00 ± 0.00^c	82.73 ± 0.60
MVU	313 ± 0^b	243 ± 2^b	70 ± 2^b	385 ± 2^b	142 ± 0	5.20 ± 0.09^b	82.83 ± 0.60
IRU	263 ± 1^c	222 ± 2^c	40 ± 1^c	363 ± 0^c	140 ± 2	5.33 ± 0.00^a	83.13 ± 0.04

Values are shown as mean ± SD; $^{a-c}$Different letters in the same column are significantly different ($P < 0.05$); ^{ns}Non-significant; HAU: hot-air unpeeled; MVU: microwave-vacuum unpeeled; IRU: Infrared unpeeled.

Table 6. Thermal properties of banana flour

Samples	Onset (°C)	Peak (°C)	Endset (°C)	Enthapy (J/g)
HAU	74.04	77.83	82.68	4.18
MVU	74.18	77.83	81.49	2.40
IRU	74.74	78.74	83.64	3.14

HAU: hot-air unpeeled; MVU: microwave-vacuum unpeeled; IRU: Infrared unpeeled.

ing. Setback viscosity of HAU, MVU and IRU were 141, 142 and 140, respectively. The setback viscosities of banana flour were not significantly different ($P > 0.05$) among 3 drying methods. The high setback during cooling represents the high retrogradation which is due to the effect of amylose and amylopectin (Nimsung et al., 2007). The retrogradation process occurs faster in the starch which has higher amylose content (Suwonsichon et al., 2011). The increase in setback value is due to the amylose re-association upon cooling which creates a 3-dimensional gel network (Jamal et al., 2016). The peak time was in the range of 5.00 - 5.33 min and the pasting temperature was in the range of 82.73°C - 83.13°C in HAU and IRU respectively.

Table 6 shows the thermal properties of banana flour including onset temperature, peak temperature, endset temperature and enthalpy. The overall gelatinization temperature range of banana flours was 74.04 - 83.64°C, which was comparable to the range (70.70 - 86.18°C) reported by Nimsung et al. (2007) and the range (62.3 - 86.9°C) reported by Mota et al. (2000). The mean onset temperature was 74.32°C, ranging from 74.04°C in HAU to 74.74°C in IRU. The peak temperature ranged from 77.83°C in HAU and MVU to 78.74°C in IRU, with the mean of 78.13°C, while the final temperature showed an average of 82.60°C, ranging from 81.49°C in MVU to 83.64°C in IRU. There was a negative relation between gelatinization and pasting properties in terms of peak viscosity, hot paste viscosity, breakdown and cold paste viscosity. The enset temperature was close to the value of pasting temperature measured by Rapid Visco Analyzer. Gelatinization enthalpy varied from 2.40 J/g in MVU to 4.18 J/g in HAU, with the mean of 3.24 J/g, which was much lower than the values of 10.8 - 13.3 J/g reported by Mota et al. (2000) and 15.16 - 19.62 J/g reported by Nimsung et al. (2007).

The differences in gelatinization temperature were accounted by the differences in amylose content, the distribution, size and form of starch granules as well as the internal arrangement of starch fractions within the granules (Singh et al., 2003). As shown in this study, the amylose content, total dietary fibre and resistant starch were negatively related to gelatinization temperature. The enthalpy represents the melting of amylopectin crystallites. The differences in enthalpy appear to be attributed to the differences in bonding forces between the double helices that form amylopectin crystallites, resulting in different alignment of hydrogen bonds within starch molecule (McPherson & Jane., 1999). The higher values of DSC parameter are encouraged by large amylopectin branches (crystallinity) (Kraithong et al., 2018). According to Alcazar-Alay & Meireles (2015), high energy is required for disrupting the large crystalline regions of high amylopectin rice flour. Besides, amylose-lipid and amylose-protein complex formations also can increase the gelatinization temperature due to their structures (Morales-Martínez et al., 2014). In contrast, the gelatinization of flour with high amorphous regions (high amylose) is accomplished easily because of weak hydrogen bonds (Jamal et al., 2016). The small particle size of rice flour advocates low gelatinization temperature because of large surface areas for binding water molecules (Ye et al., 2016).

4. Conclusion

Productivity was highest in hot-air drying and microwave vacuum drying. Drying method did not have any significant effects on proximate of unpeeled banana flour. Total dietary fibre, resistant starch, amylose content and phenolic compound of the flour produced by microwave-vacuum drying were fairly high, however, these were less than those contained in the flour produced by hot-air drying. Therefore, hot-air drying was still the superior method for preserving those nutritional values. The flour produced by hot-air drying exhibited the highest functional properties such as WAI (3.59 g/g), WSI (5.92%), SPI (3.82 g/g) and OAI (2.02 g/g). Pasting properties of

the flour were also highest for hot-air drying. The gelatinization was highest in infrared drying and the enthalpy was highest in hot-air drying.

References

Adu, B., & Otten, L. (1996). Effect of increasing hygroscopicity on the microwave heating of solid foods. *Journal of Food Engineering* 27, 35-44.

Alcazar-Alay, S. C., & Meireles, M. A. A. (2015). Physicochemical properties, modifications and applications of starches from different botanical sources. *Food Science and Technology* 35(2), 215-236.

Almeida, M. R., Alves, R. S., Nascimbem, L. B. L. R., Stephani, R., Poppi, R. J., & Oliveira, L. F. C. d. (2010). Determination of amylose content in starch using Raman spectroscopy and multivariate calibration analysis. *Analytical and Bioanalytical Chemistry* 397, 2693-2701.

Anjum, S., Sundaram, S., & Rai, G. (2014). Nutraceutical application and value addition of banana (*Musa paradisica* L. Variety, "*Bhusawal Keli*") peel: A review. International *Journal of Pharmacy and Pharmaceutical Sciences* 6(10), 81-85.

Aprianita, A., Vasiljevic, T., Bannikova, A., & Kasapis, S. (2014). Physicochemical properties of flours and starches derived from traditional Indonesian tubers and roots. *Food Science and Technology* 51(2), 3669-3679.

Arisa, N. U., Adelekan, A. O., Alamu, A. E., & Ogunfowora, E. J. (2013). The effect of pretreatment of plantain (*Musa Parasidiaca*) flour on the pasting and sensory characteristics of biscuit. *International Journal of Food and Nutrition Science* 2(1), 10-24.

Bhattacharya, M., Zee, S. Y., & Corke, H. (1999). Physicochemical properties related to quality of rice noodles. *Cereal Chemistry* 76(6), 861-867.

Chung, H. J., Liu, Q., Lee, L., & Wei, D. (2011). Relationship between the structure, physicochemical properties and in vitro digestibility of rice starches with different amylose contents. *Food Hydrocolloid* 25, 968-975.

Drouzas, A. E., Tsami, E., & Aravacos, G. D. (1999). Microwave-vacuum drying of model fruit gels. *Journal of Food Engineering* 39, 117-122.

Emaga, T. H., Andrianaivo, R. H., Wathelet, B., Tchango, J. T., & Paquot, M. (2007). Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. *Food Chemistry* 103, 590-600.

Falade, K. O., & Christopher, A. S. (2015). Physical, functional, pasting and thermal properties of flours and starches of six Nigerian rice cultivars. *Food Hydrocolloid* 44, 478-490.

Feng, H., & Tang, J. (1998). Microwave finish drying of diced apples in a spouted bed. *Journal of Food Science* 63(4), 679-683.

Hsu, R. J. C., Lu, S., Chang, Y. H., & Chiang, W. (2015). Effects of added water and retrogradation on starch digestibility of cooked rice flours with different amylose content. *Journal of Cereal Science* 61, 1-7.

Jamal, S., Qazi, I. M., & Ahmed, I. (2016). Comparative studies on flour proximate compositions and functional properties of selected Pakistani rice varieties. *Proceedings of the Pakistan Academy of Sciences* 53(1), 47-56.

Kader, A. A. (1996). *Banana: Recommendations for maintaining postharvest quality. Perishables Handling #88*. Retrieved March 6, 2019, from http://postharvest.ucdavis.edu/files/259413.pdf.

Keawpeng, I., & Meenune, M. (2012). Physicochemical properties of organic and inorganic Phatthalung Sungyod rice. *Food Research International* 19(3), 857-861.

Kraithong, S., Lee, S., & Rawdkuen, S. (2018). Physicochemical and functional properties of Thai organic rice flour. *Journal of Cereal Science* 79, 259-266.

Liao, H. J., & Hung, C. C. (2015). Chemical composition and in vitro starch digestibility of green banana (cv. Giant Cavendish) flour and its derived autoclaved/debranched powder. *Food Science and Technology* 64, 639-644.

Lin, T. M., Durance, T. D., & Scaman, C. H. (1998). Characterization of vacuum microwave air and freeze dried carrot slices. *Food Research International* 31(2), 111-117.

Maskan, M. (2000). Microwave/air and microwave finish drying of banana. *Journal of Food Engineering* 44, 71-78.

Mastro, N. L., Taipina, M. S., Cohen, V. H., & Rodas, M. A. B. (2007). Avaliação critica da polpa de banana (Musa spp) verde. *Revista Higiene Alimentar* 21, 39-45.

McPherson, A. E., & Jane, J. (1999). Comparison of waxy potato with other root and tuber starches. *Carbohydrate Polymers* 40, 57-70.

Menezes, E. W., Tadini, C. C., Tribess, T. B., Zuleta, A., Binaghi, J., Pak, N., Vera, G., Dan, M. C., Bertolini, A. C., Cordenunsi, B. R., & Lajolo, F. M. (2011). Chemical composition and nutritional value of unripe banana flour (Musa acuminata, var. Nanicão). *Plant Foods for Human Nutrition* 66(3), 231-237.

Mohapatra, D., Mishra, S., & Sutar, N. (2010). Banana and its by product utilisation. *Journal of Scientific and Industrial Research* 69(5), 323-329.

Morales-Martínez, L. E., Bello-Perez, L. A., Sanchez-Rivera, M. M., Ventura-Zapata, E., & Jimenez-Aparicio, A. R. (2014). Morphometric, physicochemical, thermal, and rheological properties of rice (Oryza sativa L.) cultivars indica japonica. *Food and Nutrition Science* 5, 271-279.

Mota, R. V. d., Lajolo, F. M., Ciacco, C., & Cordenunsi, B. R. (2000). Composition and functional properties of banana flour from different varieties. *Starch* 52(2-3), 63-68.

Nimsung, P., Thongngam, M., & Naivikul, O. (2007). Compositions, morphological and thermal properties of green banana flour and starch. *Kasetsart Journal (Natural Science)* 41, 324-330.

Oko, A. O., & Ugwu, S. I. (2011). The proximate and mineral compositions of five major rice varieties in Abakaliki, South-Eastern Nigeria. *International Journal of Plant Physiology and Biochemistry* 3(2), 25-27.

Otegbayo, B., Oguniyan, D., & Akinwumi, O. (2013). Physicochemical and functional characterization of yam starch for potential industrial applications. *Starch* 65, 1-16.

Prasad, K., Singh, Y., & Anil, A. (2012). Effects of grinding methods on the characteristics of Pusa 1121 rice flour. *Journal of Tropical Agriculture and Food Science* 40(2), 193-201.

Shafi, S., Wani, I. A., Gani, A., Sharma, P., Wani, H. M., Masoodi, F. A., & Hamdani, A. M. (2016). Effect of water and ether extraction on functional and antioxidant properties of Indian horse chestnut (Aesculus indica Colebr) flour. *Food Measurement and Characterization* 10, 387-395.

Singh, N., Singh, J., Kuar, L., Sodhi, N. S., & Gill., B. S. (2003). Morphology, thermal and rheological properties of starches from different botanical sources. *Food Chemistry* 81(2), 219-231.

Suwonsichon, T., Chueamchaitrakun, P., Chompreeda, P., Haruthaithanasan, V., & Kasemsamran, S. (2011). Physical properties of butter cake made from mixed Hom-Mali and glutinous rice flours. *Kasetsart Journal (Natural Science)* 45, 295-304.

Tharise, N., Julianti, E., & Nurminah, M. (2014). Evaluation of physico-chemical and functional properties of composite flour from cassava, rice, potato, soybean and xanthan gum as alternative of wheat flour. *International Food Research Journal* 21(4), 1641-1649.

Tong, L. T., Gao, X., Lin, L., Liu, Y., Zhong, K., Liu, L., Zhou, X., Wang, L., & Zhou, S. (2015). Effects of semidry flour milling on the quality attributes of rice flour and rice noodles in China. *Journal of Cereal Science* 62, 45-49.

Wang, L., Guo, J., Wang, R. T., Shen, C., Li, Y., Luo, X., Li, Y., & Chen, Z. (2016). Studies on quality of potato flour blends with rice flour for making extruded noodles. *Cereal Chemistry* 93(6), 593-598.

Yang, L., Zhang, H., Cheng, L., Gu, Z., Hua, D., Qi, X., Qian, H., & Wang, L. (2014). Effect of extrusion on the hydrophilic antioxidant capacity of four whole grains. *Journal of Food and Nutrition Research* 2(2), 80-87.

Yani, A., Arief, R. W., & Mulyanti, N. (2013). Processing of banana flour using a local banana as raw materials in Lampung. *International Journal on Advanced Science Engineering Infomation Technology* 3(4), 26-30.

Ye, L., Wang, C., Wang, S., Zhou, S., & Liu, X. (2016). Thermal and rheological properties of brown flour from Indica rice. *Journal of Cereal Science* 70, 270-274.