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CONTENT

Willingness to pay for air quality improvement in Ho Chi Minh City, Vietnam

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ABSTRACT

This research aimed to estimate people's willingness to pay (WTP) to improve air quality in Ho Chi Minh City (HCMC), Vietnam. Contingent value method (CVM), the single bounded dichotomous choice question format, was employed. Five levels of bid were used, including 10,000; 20,000; 30,000; 40,000; 50,000 VND. A survey was conducted with 600 people, distributed in 8 districts. The results showed that people had limited perception on specific terms, but they understood the phenomenon and the consequence of air pollution. The mean willingness to pay (MWTP) for air quality improvement was 19,147.06 VND/person per month (0.83 USD/person per month) (with protesters) and 28,157.01 VND/person per month (1.22 USD/person per month) (without protesters). The total budget that would be used for air protection in HCMC was 86,927,652,400 VND/month (3,779,463.15 USD/month) (with protesters) and 127,832,825,400 VND/month (5,557,557.62 USD/month) (without protesters). The income, education level and bid level were the factors affecting their WTP.

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1. Introduction

Air pollution is a critical problem at urban area, especially at mega cities. Major sources of pollution are from the transportation and industrial activities. Ho Chi Minh City (HCMC) is the biggest city and the center of development in Vietnam. The economic and social development is obtained at the cost of environmental damage, especially air environment. The city air quality monitoring systems show that air is polluted by dust, noise, CO and NO_2. Transportation and industrial activities are the two main sources of air pollution in HCMC. Transportation contributes most to the pollution. The city has a total of 3,584 roads which are 3,670 km in length and spread over 36 million m^2; 7.1 millions of vehicles within the city; around 1 million of motorbikes and 60,000 cars and trucks moving in and out the city everyday (HDOEP, 2015). Monitoring data shows that 72.36% of monitored sites has an excessive concentration of dust (163.42 – 690.00 µg/m^3), 97.64% of them has excessive noise (> 70 dBA) as compared to the National Standards (HEPA, 2016). Traffic jam is another factor contributing to the consequences. In industrial area, there are 839 industrial sites, 32% of which does not have air treatment systems. Air pollution affects communities' health, especially, children health, and the number of patients related to respiratory diseases is increasing in the past 10 years (HDONRE, 2016).

In order to enhancing the air quality, there are many proposed solutions and all of them need the sustainable finance source. The more diverse financial sources are, the better the long air protection scheme is. In addition, research related to air valuation is limited and the value of environment factor is usually undervalued in making decision process.

Clean air is an environmental good which does not have price. In order to valuate this kind of resource, state preferences methodologies are used. In this group of methods, contingent valuation method (CVM) is the common one. The studies related to air evaluation are often conducted at the air polluted cities and can be divided into different categories. First, there were studies about a certain air pollution event, including WTP to reduce air pollution in Singapore caused by forest fire in Malaysia (Yuan et al., 2017), WTP to reduce smog in China (Chuanwang et al., 2016), WTP to avoid respiratory disease in Turkey (Shihomi & Cem, 2015) and WTP to enhance children health in China (Keran et al., 2015). Second, studies related to people's awareness on air pollution and WTP to reduce air pollution were also conducted (Tifanny et al., 2014; Yutao et al., 2015; Chuanwang et al., 2016). In this group of study, the authors not only used the social-economic characters as explained variables but also awareness variables to figure out the main factors affecting WTP decision. Third, there were studies on certain solutions to improve air quality such as using friendly environment energy instead of the old one (Abdullatif et al., 2016), or using public transport system rather than private vehicles (Nikolaos et al., 2010).

This research aimed to understand people's perception on air environment and their WTP for air quality improvement.

2. Materials and Methods

Clean air does not have the price on the market. In order to measure value of this good, stated preference method is usually used. The CVM belongs to this group. It is used to estimate the value of change in environment. It was developed based on the theory written by Cropper et al., Alberini et al. and Cropper and Freeman (Sarabdeen et al., 2012). As stated in the CVM in measuring the value of culture: The theory can be explained as follows: if initial utility (U_0) is a function of certain levels of income, prices, private goods and public goods, and an increase in the amount of public good supplied increases utility (U_1), then WTP is the difference between U0 and U_1, such that the final level of utility is unchanged.

This study used dichotomous question with 5 levels of price (bids). The survey was conducted by face to face interview at the respondent's house. It took 30-45 min to complete one questionnaire.

2.1. Questionnaire design

The questionnaire comprised 4 sections: introduction; interviewee's information; awareness of interviewee on air pollution; scenario and WTP question.

The designed scenario:

"HCMC is a developed city in Vietnam. Going along with the speedy development of the city is the decrease of its air quality. The main sources of air pollution are transportation and industrial activities. Air pollution is more severe in HCMC. In order to reduce pollution, comprehensive solutions are needed, including doing research, using alternative energy, planting trees, replacing the old-fashion technology, etc.

To implement these solutions, it requires a huge amount of money. Besides the government's budget, it is helpful with the contribution from community. Money generated for air protection fund will be used for reducing air pollution".

Currently, there are environment protection funds (EPF) in HCMC. This air protection fund is an assumed division under EPF management.

WTP question: Are you willing to pay «bid» VND/person per month to improve air quality? There are 5 levels of bid: 10,000; 20,000; 30,000; 40,000; 50,000 VND. These bids were recommended based on 1% of national basic salary of people.

2.2. Sample sites and size

Due to financial constraint, 600 households (HHs) in 8/24 districts of HCMC including Binh Thanh, Phu Nhuan, 1, 3, 5, Binh Chanh, Thu Duc and Hoc Mon (75 HHs/each district) were chosen by stratified random sampling. The distribution of bid levels is shown as Table 1.

Table 1. Distribution of questionnaire in sites and bid categories

Districts Name/WTP bid	10,000 VND	20,000 VND	30,000 VND	40,000 VND	50,000 VND	Total
Binh Thanh	15	15	15	15	15	75
Phu Nhuan	15	15	15	15	15	75
No.1	15	15	15	15	15	75
No.3	15	15	15	15	15	75
No.5	15	15	15	15	15	75
Binh Chanh	15	15	15	15	15	75
Thu Duc	15	15	15	15	15	75
Hoc Mon	15	15	15	15	15	75
Total	120	120	120	120	120	600

2.3. Data analysis

2.3.1. Turnbull estimation

According to Timothy & Kenneth (2002), if the answer is "Yes" to a certain price, the WTP 's respondent is equal to or higher than that price; if the answer is "No", then their WTP is less than that price.

In this study, five bid levels: t1, t2, t3, t4 and t5 were employed. From the number of respondents and the number of respondents answering "Yes" (P1, P2, P3, P4 and P5), WTP was calculated.

Then, we plotted a chart with the bids (tj) and the share of "Yes" answers (Pj) as shown in Figure 1 with tj as the horizontal axis and Pj as the vertical axis. The mean WTP is the area below the bold line in Figure 1.

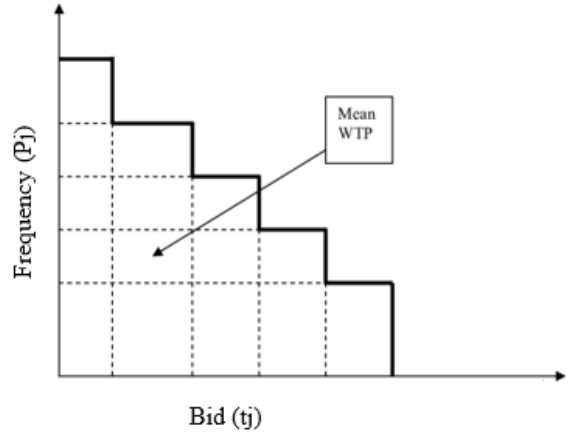

Figure 1. A Kaplan-Meier-Turnbull estimation chart.

2.3.2. Utility random model

The basic model to analyze the ditochomonous question in CVM is random utility model (Timothy & Kenneth, 2002). Basically, random utility model is used to describe an individual utility among the variety choice set. It is assumed that people will choose the alternative that brings more benefits or utility for them (Horowitz et al., 1994). In case of CVM, it will be two choices: agree or not agree to pay (Timothy & Kenneth, 2002). To estimate the random utility model with a linear utility function, a logit model was run with yes/no responses to the WTP question as the dependent variable, and bid and other respon-

Table 2. Variables explanation and expected signs

Variable	Meaning	Expected sign
Income	Monthly income (million VND/month) decides the ability to pay. People who earn more incomes, have more ability to pay	+
Education	Education level is presented by number of years they spend in school (years). The more years they spend in school, the more they want to pay for reducing air pollution	+
Number of year living in Ho Chi Minh City	The habitants who live for a long time in Ho Chi Minh City will have less intention to pay for air pollution as they are familiar with the surrounding. (Unit: Years)	-
Bid	The suggested amount of money: 10,000; 20,000; 30,000; 40,000; 50,000 VND. The higher the bid, the less people want to pay	-

dents' characteristics as the explanatory variables as follows:

$Pr(Yes_j) = a_0 + a_1 bid + \Sigma a_i X_i$

Where:

$Pr(Yes_j)$ is probability of answer "Yes"

a_n is the coefficient associated with the variables

bid is the bid level

X_i is the social-economic variables

2.3.3. Variables explanation and expected signs

Variables explanation and expected signs is shown as Table 2.

2.3.4. Eligible data

There is a question in CVM to distinguish respondents into 2 categories: support or not support for the study (i.e., protesters). This step aims to remove the protesters records which can affect the results.

The question is "Do you agree to contribute to Air Protection Fund?"

⋆ If they do not agree then they are asked for the reasons.

If the reasons for "No" response is any of following the respondents are considered as protesters:

- I don't support this project because environment protection is government duties;
- I don't support this project because the factories should be the ones who pay for the cost;
- I don't believe in this scenario;
- I have to pay many types of fees and taxes;
- I don't believe that the Fund will be used for the air protection;
- I don't know.

If the reason for "No" response is any of following the respondents are considered as supporters:

- I think reducing air pollution is important but I am not able to afford the price.
- I want to pay but the price is too high.

⋆ If they agree then they are asked for the reasons. If the response is "I don't know", then their records are removed from the computation in the case of without protesters.

In addition, this study removed the records of those who are younger than 15 years old to assure that all respondents are in their labor age (HDOS, 2018). Thus, the eligible records were 598/600 in case with protesters and 380/600 in case without protesters.

3. Results and Discussion

3.1. Respondents characteristics

Respondent's characteristics are presented in Table 3.

Table 3. Samples properties

No.	Parameters		Number of respondents (person)	Sharing (%)
1	Gender	Male	333	55.7
		Female	266	44.3
2	Ethic group	"Kinh"	578	96.3
		Others	22	3.7
3	Age	Under 18	12	2
		19 - 60	567	94.5
		Above 61	21	3.5
4	Education	Under High school	309	51.5
		Graduate	250	41.7
		Postgraduates	41	6.8
5	Main labor in households	Yes	329	54.8
		No	271	45.2
6	Career	Student	61	10.2
		Office staffs	110	18.3
		Business	180	30.0
		Homemaker	26	4.3
		Educational field	25	4.2
		Workers	58	9.7
		Others	140	23.3
7	Average income (monthly)	0-5 million VND	188	31.3
		6-10 million VND	286	47.7
		11-20 million VND	101	16.8
		21-30 million VND	15	2.5
		Greater than 31 million VND	10	1.7

Source: data analysis.

Table 4. Definition of air environment

Options	Number of respondents (person)	Sharing (%)
The air surrounding the Earth	426	71.0
The air which is 11-17 km in depth surrounding the Earth	142	23.7
There is no right answer	32	5.3
Total	600	100.0

Source: data analysis.

3.2. People awareness on air environment and air pollution

Air environment definition is not a common term to the community; the right answers occupy only 23.7% the total responses. Similarly, for air quality index (AQI), only 15.5% of respondents have heard about this term and 9.3% and 4% of them can choose the right color presented for best AQI and worst AQI, respectively. The results are shown from Tables 4 to 6.

Regarding to air pollutions consequences, 72.5% of respondents chose the right option, 21.7% chose the insufficient answer and the rest din't know the answer (Table 7).

Respondents were asked to name the air pollutants that they knew. There were 7 air pollutants mentioned, including particle, smoke, NO_x, CO_x, CFC, SO_x and natural substances (Table 8).

They were aware of the consequences of air pollution on human health, construction and vegetation as the results shown in Table 9.

Table 5. The color presented for the best air quality index

	Options	Number of respondents (person)	Sharing (%)
Valid	Don't know	36	6.0
	Green	56	9.3
	Yellow	1	0.2
	Total	93	15.5
Missing	99	507	84.5
	Total	600	100.0

Source: data analysis.

Table 6. The color presented for the worst air quality index

	Options	Number of respondents (person)	Sharing (%)
Valid	Don't know	43	7.2
	Yellow	1	0.2
	Orange	1	0.2
	Red	16	2.7
	Purple	8	1.3
	Brown	24	4.0
	Total	93	15.5
Missing	99	507	84.5
	Total	600	100.0

Source: data analysis.

Table 7. Definition of air pollution

	Options	Number of respondents (person)	Sharing (%)
Valid	Don't know	33	5.5
	The present of harmful substances in the air	130	21.7
	The change in air components	435	72.5
	There is no right options	2	0.3
	Total	600	100.0

Source: data analysis.

Table 8. Air pollutants

		Particle	Smoke	NO_x	CO_x	CFC	SO_x	Natural substances
Valid	Yes	330	335	130	257	59	39	7
Missing	99	270	265	470	343	541	561	593
Total		600	600	600	600	600	600	600

Source: data analysis.

They were asked to list the source of air pollution. Table 10 shows that transportation and industrial sources were mentioned the most.

3.3. People WTP for air quality improvement

3.3.1. Average WTP in case of protest votes

The average willingness to pay (MWTP) for air quality improvement was MWTP = $\Sigma t_j(P_j - P_{j+1})$ = 19,147.06 VND/person per month (0.83 USD/person per month) (with t_j is bid j level and P_j is frequency of bid j) (Table 11).

3.3.2. Average WTP in case without protest votes

The average willingness to pay (MWTP) for air quality improvement in case without protesters was MWTP = $\Sigma t_j(P_j - P_{j+1})$ = 28,157.01

Table 9. Consequences of air pollution

		Respiratory	Skin	Buildings	Vegetation
Valid	Yes	596	398	254	290
Missing	99	4	202	346	310
Total		600	600	600	600

Source: data analysis.

Table 10. Sources of air pollution in Ho Chi Minh City

			Transportation	Industry	Agriculture services	Natural origin
Valid	Yes	588	551	292	311	270
Missing	99	12	49	308	289	330
Total		600	600	600	600	600

Source: data analysis.

Table 11. Turnbull estimation with protest votes

Bid (VND)	Number of respondents (person)	Number of respondents who agree to pay (person)	Sharing %
10,000	119	75	63.0
20,000	120	61	50.8
30,000	120	49	40.8
40,000	120	28	23.3
50,000	119	16	13.4

Source: data analysis.

Table 12. Turnbull estimation without protest votes

Bid (VND)	Number of respondents (person)	Number of respondents who agree to pay (person)	Sharing %
10,000	79	71	89.9
20,000	87	59	67.8
30,000	84	48	57.1
40,000	63	27	42.9
50,000	67	16	23.9

Source: data analysis.

VND/person per month (1.22 USD/person per month) (Table 12).

The reasons of not willing or willing to pay are presented as Tables 13 and 14, respectively. They don't have willingness to pay because they think that government or factories should be the one in charge (14%); they don't believe in the scenario/transparent (12.5%); they have to pay many types of tax (7.5%) and they are not able to afford the bid (26.7%).

Respondents are willing to pay for the reason "it's good for them and their family's health" accounting for 35.8%; 1.2% of respondents agree to pay because the payment will be good for the community in general. Other 0.8% of them are not sure or don't know why they agree to pay.

With the population of 4,540,000 persons in the labor age in Ho Chi Minh City (HDOS, 2018), the total amount of money for air protection that can be collected is 86,927,652,400 VND/month (3,779,463.15 USD/month) (with protester) or 127,832,825,400 VND/month (5,557,948.93 USD/month) (without protester).

3.4. The relations between social-economic variable and WTP

3.4.1. With protes votes

The regression result (with protest votes) and the prediction ability are presented in Tables 15 and 16, respectively. The education, number of years living in Ho Chi Minh City and bid are

Table 13. Reasons of not willing to pay

	Reasons of not willing to pay	Number of respondents (person)	Sharing (%)
Valid	Air protection is government's duty	39	6.5
	Factories should pay for the air pollution	45	7.5
	Don't believe in the scenario	18	3.0
	Already have to pay many types of fee/tax	45	7.5
	Are not able to afford the bid	160	26.7
	Don't believe in the transparent	57	9.5
	I don't know	6	1.0
	Others	3	0.5
	Total	373	62.2
Missing	99	227	37.8
	Total	600	100.0

Source: data analysis.

Table 14. Reasons of willing to pay

	Reasons of willing to pay	Number of respondents (person)	Sharing (%)
Valid	I don't know	5	0.8
	Good for individual and family's health	215	35.8
	Other: good for community	7	1.2
	Total	227	37.8
Missing	99	373	62.2
	Total	600	100.0

Source: data analysis.

Table 15. Regression model with protest votes

	B	S.E.	Wald	df	Sig.	Exp(B)
Education	0.072	0.025	8.080	1	0.004	1.074
Income	0.009	0.010	0.843	1	0.359	1.009
Number of year living in Ho Chi Minh City	-0.012	0.006	4.504	1	0.034	0.988
Bid	-0.558	0.069	66.008	1	0.000	0.572
Constant	0.387	0.397	0.955	1	0.329	1.473

Variable(s): education, income, number of year living in Ho Chi Minh City, bid. R^2 = .193.

the variables affecting people WTP. In particular, people who spend more years in school are more willing to pay for air protection; the less years they live in HCM city, the more they intend to pay; the lower the bid is, the more they want to pay. R^2 is 0.193 meaning that there are more other variables that need to be considered in further research. However, this model can predict the probability of payment up to 68.2%.

3.4.2. Without protest votes

The regression result (without protest votes) and the prediction ability are presented in Tables 17 and 18, respectively. The variables affecting people WTP are education, income and bid. In particular, people who spend more years in school have more willingness to pay for air protection; people who earn more will have the intention to pay; the lower the bid is, the more they want to pay. Table 18 shows that the model can predict decision of payment up to 76.6%.

3.5. Payment collection means and frequency

Table 19 shows that people prefer to pay monthly (51.6%) rather than others. Many of them want to pay directly to air protection fund (51.6%) as presented in Table 20.

Table 16. Predict the decision of payment

	Actual data		Prediction		
			Willing to pay		Accurate prediction (%)
			No	Yes	
Step 1	Willing to pay	No	303	68	81.7
		Yes	122	105	46.3
	Average				68.2
a. The cut value is 0.500					

Source: data analysis.

Table 17. Regression model without protest votes

	B	S.E.	Wald	df	Sig.	Exp(B)
Education	0.101	0.034	8.736	1	0.003	1.106
Income	0.181	0.033	29.912	1	0.000	1.199
Number of year living in Ho Chi Minh City	-0.011	0.008	1.811	1	0.178	0.989
Bid	-0.852	0.105	65.413	1	0.000	0.426
Constant	0.487	0.527	0.854	1	0.355	1.627

Variable(s): education, income, number of year living in Ho Chi Minh City, bid. $R^2 = 0.395$.

Table 18. Predict the decision of payment

	Actual data		Prediction		
			Willing to pay		Accurate prediction (%)
			No	Yes	
Step 1	Willing to pay	No	105	54	66.0
		Yes	35	186	84.2
	Average				76.6
a. The cut value is 0.500					

Source: data analysis.

Table 19. Payment collection means

	Options	Number of respondents (person)	Sharing (%)
Valid	Monthly	114	51.6
	Quarterly	52	23.5
	Annually	55	24.9
	Total	221	100.0

Source: data analysis.

Table 20. Payment collection frequency

	Options	Number of respondents (person)	Sharing (%)
Valid	Attach the bid with electricity bill	86	38.9
	Attach the bid with water bill	15	6.8
	Pay directly to the air protection fund	114	51.6
	Others	6	2.7
	Total	221	100.0

Source: data analysis.

4. Conclusions

People do not know exactly about the specific definitions, but they have awareness on air pollution impacts, air pollutants, etc. The average WTP is 19,147.06 VND/person per month (0.83 USD/person per month) and 28,157.01 VND/person per month (1.22 USD/person per month) in case of with and without protesters. Thus, the city might

have 86,927,652,400 VND/month (3,779,463.15 USD/month) and 127,823,825,400 VND/month (5,557,948.93 USD/month) in case of with and without protesters. People want to pay monthly and directly to Air Protection Fund. The variables which affect people WTP for air protection are education, income and bid. Some concerns arise for further related research, including considering the difference in WTP for different target groups and policy assessment on people who do not agree with the added fee for air environment, especially, people who can not afford to make payments.

Conflict of Interest

All authors have no conflict of interest of this report.

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Combining ability assessment of agronomic characteristics of 8 inbred sweet corn lines

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ABSTRACT

This study was conducted to evaluate 28 sweet corn hybrid combinations to determine the combination ability of 8 lines of sweet corn inbred (K60, R111, N1, N4, N5, N7, N8 and N12) of a S8 generation. The results showed that the yield of hybrid combinations was in a range of 14.2 to 23.7 tons/ha. Especially, the hybrid combination THL9 had a yield of 23.7 tons/ha, reached the soluble solids content of 13.9%. Meanwhile, the yield of the hybrid combination THL14 reached 21.4 tons/ha and its soluble solids content reached 13.6%. This result was higher than Golden Cob which was the control variable in this study, and had a yield of 17.4 tons/ha and a soluble solids content of 12.2%. Evaluating the combining ability of the yield and total soluble solids content of 8 lines of sweet corn showed that the N4 lines had a higher combining ability than the other lines in terms of fresh corn yield and total soluble solids content. R111 line and N4 line could combine good productivity (Ŝij: 2,433*) and total soluble solids content (Ŝij reached 0.963*).

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1. Introduction

Sweet corn (*Zea mays* L. *saccharata*) is a common corn mutant that is characterized by a high sweet and low starch contents that lead to a distinctive flavor, texture and aroma characteristics. The "sweetness" trait is controlled by simple recessive genes or by double or triple binding genes (Nayara et al., 2017). Sweet corn is a high source of tocopherols, vitamin C, carotenoids, and phenolic so it is considered to being an important vegetable for cultivation purposes worldwide (Juvik, 2009). In Vietnam, since many studies on sweet corn are only concerned in the early years of the 21st century, the achievements in research on planting techniques and corn varieties are still limited. Currently, sweet corn varieties on the market are mainly imported ones, seed source is not active at production. So on, high seed prices lead to increased production costs for farmers. Research on the selection of domestically dominant varieties of sweet corn aiming to create potential varieties which are highly adaptable to the natural condition in Vietnam, contributing to proactively source of varieties, reducing seed costs and increasing economic efficiency for farmers (Nguyen & Le, 2018).

Base on a study by Ferh (1987), research on sweet corn breeding, traits related to quality are interested. However, high yield still remains the main goal of a corn breeding program. Accordingly, scientists concern about the methods of yield improvement and quality of sweet corn. So that, developing elite lines is the best strategy to improve those criteria. Evaluation of the individuals in a population selects good strains in

that population. To select good lines in a population, it is necessary to have an assessment of the many varieties in that population. Then use the hybrids to combine the selected lines to create the dominant hybrids. Studies on combining different corn cultivars are an important step in a breeding program as it is crucial to selecting suitable complementary lines to produce the F1 hybrid which achieves high yield and good quality (Solomon et al., 2012). According to this current necessary, the study assessment of the combination ability of 8 sweet corn inbred lines in the S_8 generation in Ho Chi Minh City is conducted to determine the combining ability of the self coordinating lines for high yield and good quality.

2. Material and Methods

2.1. Material

Twenty-eight sweet corn hybrid combinations were bred by the Griffing (1956) (method IV) method from 8 lines of inbred sweet corn in S_8 generation (K60, R111, N1, N4, N5, N7, N8 and N12) which were selected and developed at Nong Lam University Ho Chi Minh City. Golden Cob variety was imported and distributed by East-West Seed Company. This variety was used as a control variable. The sweet corn kernels used were hybrid kernels harvested one month before the sowing date.

2.2. Methods

2.2.1. Experimental arrangement method

The 8 S_8 inbred lines of sweet corn were cultivated in Nong Lam University Ho Chi Minh City in the 2020 Spring-Summer crop and selected individuals to perform the rotation to create 28 single hybrid combinations. The experiment aims to evaluate the growth and yield of 28 F1 sweet corn hybrid combinations which was conducted in the Summer-Autumn crop of 2020. This single-factor experiment was arranged in a randomized complete block design consisting of 29 treatments (28 hybrid combinations and 1 control - Golden Cob); 3 replicates (Table 1). The area of each experimental plot was 5 m x 2.8 m = 14 m^2. Each experimental plot had 4 rows. The planting distance was 70 x 25 cm.

The amount of fertilizer for 1 ha: Lime: 500 kg/ha + 10 tons of cow manure/ha + Inorganic fertilizers (kg/ha): 140 kg N - 80 kg P_2O_5 - 90 kg K_2O. Applied technical procedures and evaluation criteria, monitoring methods were based on the National Technical Regulation (QCVN 01-56: 2011/BNNPTNT, MARD (2011)) on testing for Value of Cultivation and Use of Corn varieties promulgating by the Ministry of Agriculture and Rural Development.

2.2.2. Monitoring criterias

Criteria of growth: Number of leaves, plant height, stalk height, stem diameter, harvesting time.

Criteria of corn morphology, yield and quality: the shape of husk cover, the color of the husk, length of ear, diameter of the ear, weight of ear, number of kernels, the color of kernels, the soluble solids content of kernels (Brix) and yield of the fresh ear.

2.2.3. Data processing method

Data of experiments were collected and calculated on computers according to ANOVA statistical analysis method and LSD ranking test by using SAS software 9.1. Using software of Quantitative Genetics by Ngo & Nguyen (1996) to analyze variance and evaluate inbred combination ability.

2.3. Time and location

The experiment was conducted in Spring-Summer and Summer-Autumn crop in 2020 in Thu Duc, Ho Chi Minh City on sandy loam soils (% sand:silt:clay, respectively 69:11:20); slightly acidic soil (pH H_2O and pH KCl were 6.0 and 5.2, respectively); nutritional content as the organic matter was low (1.3%); low total protein and potassium. Apply additional lime, manure, nitrogen and potassium during the experiment.

3. Results and Discussion

3.1. Agronomic characteristics, productivity and quality of 8 parental lines

The results of monitoring the growth and development of 8 parental lines in Spring-Summer 2020 conditions (Table 2) showed that the difference in time of pollen dispersal and silk emergence of the lines was not large, ranging from 1 - 2

Table 1. Origin of hybrid combinations (THL)

♀ \ ♂	K60	R111	N1	N4	N5	N7	N8	N12
K60		THL1	THL2	THL3	THL4	THL5	THL6	THL7
R111			THL8	THL9	THL10	THL11	THL12	THL13
N1				THL14	THL15	THL16	THL17	THL18
N4					THL19	THL20	THL21	THL22
N5						THL23	THL24	THL25
N7							THL26	THL27
N8								THL28
N12								

Table 2. Morphological characteristics and constituent factors of productivity and quality of 8 parental lines in the Spring-Summer crop 2020

Line	PD (DAS)	NPR (DAS)	H (cm)	Row/Ear	COH	FW (g)	RP (tons/ha)	Brix (%)	COK	Scent
N1	48	49	184.9^{ab}	15.3^{a}	Green	291.2^{ad}	15.8^{ab}	11.5	YO	F
N4	48	50	190.1^{a}	14.9^{a}	Green	311.4^{a}	16.6^{a}	12.0	Y	F
N5	48	50	160.3^{bc}	14.9^{a}	Green	256.7^{cd}	12.5^{bc}	11.8	Y	LF
N7	49	50	187.4a	14.4^{a}	DG	288.9^{ad}	15.5^{ab}	11.3	LY	LF
N8	48	49	150.0^{c}	10.7^{b}	Green	243.5^{d}	11.7^{c}	11.8	LY	LF
N12	48	50	186.4a	15.1^{a}	Green	260.0^{bcd}	13.6^{abc}	11.0	Y	F
K60	47	48	189.5^{a}	14.9^{a}	DG	293.8^{abc}	16.1^{ab}	11.2	YO	F
R111	49	48	173.7^{abc}	17.3^{a}	Green	305.2^{ab}	16.1^{ab}	12.9	YO	F
CV%			5.6	7.6		8.8	12.8	5.3		
F_{value}			7.0^{**}	8.3^{**}		2.9^{*}	2.9^{*}	2.7^{ns}		

In the same column, numbers with the same letter do not had a statistically significant difference; ^{ns}: no significance; *: significant difference at the level of $\alpha = 0.05$; **: very significant difference at the level of $\alpha = 0.01$. PD = Pollen Dispersal, SE = Silk Emergence, H = Height of plant, COH = Color of Husk, FW = Fresh Weight, RP = Real Productivity, COK= Color of Kernel, DAS = Days After Sowing, DG = Dark Green, Y= Yellow, YO = Yellow-Orange, LY = Light Yellow, F = Fragrant, LF = Light Fragrant.

days, the difference between pollen dispersal and silk emergence was so favorable for the process of pollination and fertilization.

Through monitoring some agronomic characteristics of the parental lines, the plant height of the lines ranging from 150 to 190.1 cm. The number of rows/ear of experimental lines varied from 10.7 to 17.3 rows/ear. The line with the highest number of rows/ear was R111 with 17.3 rows/ear and the lowest was that of N8 with 10.7 rows/ear. The color of the husk of the parent lines ranges from green to dark green.

The weight of ear with husk leaves of the sweet corn lines in the experiment ranged from 243.5 to 311.4 g, of which, the N4 line had the largest weight of ear with the largest husk leaves of 311.4 g.

The actual yield of a fresh ear of sweet corn lines in the experiment ranged from 11.7 to 16.6 tons/ha. The N4 line had the highest net yield of 16.6 tons/ha, followed by the R111 and the K60 line at 16.1 tons/ha, the line N1 at 15.8 tons/ha, the line N7 at 15.5 tons/ha. This result was equivalent to the research results of Van et al. (2019) on these pure lines in the S7 self-pollination life. This shows that the 8 lines of sweet corn studied had stable yields.

Corn kernel's color was assessed at the time when the ear was ripe. Yellow to orange color was popular in the market. The lines involved in the experiment had a kernel color ranging from light yellow to yellow-orange. In which, N1, K60, R111 lines were yellow-orange, N4, N5, N12 were yellow.

3.2. Morphological characteristics of 28 corn hybrid combinations in the Summer-Autumn crop 2020

Monitoring the growth and development time of the hybrid combinations in the experiment was

Table 3. Some morphological characteristics of 28 corn hybrid combinations in the Summer-Autumn crop 2020

Hybrid Combination	Harvest date (DAS)	Leaves/plant (Leaves)	Height of plant (cm)	Height of ear set (cm)	Stem diameter (mm)
THL1	72	18.6	203.4^{abc}	98.2^{ab}	2.8
THL2	73	19.0	190.0^{ae}	78.5^{cd}	2.9
THL3	71	18.1	213.0^{ab}	93.3^{ad}	3.2
THL4	73	18.4	198.7^{ad}	92.4^{ad}	2.8
THL5	73	18.6	204.0^{abc}	84.7^{be}	2.9
THL6	72	18.7	198.9^{ad}	87.7^{ad}	2.9
THL7	74	18.2	199.4^{ad}	88.8^{ad}	2.9
THL8	70	18.2	218.6a	100.5^{a}	3.0
THL9	71	19.3	209.1^{abc}	89.3^{ad}	2.8
THL10	72	18.1	206.8^{abc}	92.4^{ad}	2.8
THL11	72	18.1	201.1^{ad}	94.1^{abc}	2.9
THL12	74	18.2	206.0^{abc}	96.8^{abc}	3.0
THL13	72	18.3	188.9^{be}	86.1^{ae}	2.9
THL14	70	19.2	208.9^{abc}	88.0^{ad}	2.8
THL15	72	17.7	196.9^{ae}	96.5^{abc}	2.7
THL16	72	18.1	192.4^{ae}	79.5^{cbd}	3.2
THL17	72	18.1	202.7^{ad}	99.4^{a}	2.8
THL18	73	18.6	201.0^{ad}	90.2^{ad}	2.9
THL19	72	18.3	207.5^{abc}	89.1^{ad}	2.9
THL20	72	18.3	207.3^{abc}	87.2^{ad}	2.8
THL21	74	17.7	209.0^{abc}	84.6^{be}	3.1
THL22	73	18.5	183.2^{cde}	83.8^{be}	2.9
THL23	73	18.2	203.2^{abc}	91.4^{ad}	3.1
THL24	74	17.7	174.2^{de}	73.3^{d}	2.8
THL25	74	17.5	181.8^{cde}	79.6^{cbd}	2.8
THL26	72	18.0	202.5^{ad}	89.9^{ad}	2.9
THL27	72	17.7	203.4^{abc}	95.0^{abc}	2.9
THL28	74	18.6	170.6^{e}	87.3^{ad}	2.8
Golden (Control)	72	16.9	195.9^{ae}	82.4^{ad}	2.9
CV%	-	4.1	7.3	8.2	6.3
Fvalue	-	1.3^{ns}	1.7^{*}	2.4^{**}	13^{ns}

In the same column, numbers with the same letter do not had a statistically significant difference; ns: no significance; *: significant difference at the level of $\alpha = 0.05$; **: very significant difference at the level of $\alpha = 0.01$.

the basis for arranging the structure and planting seasons properly and applying appropriate technical measures for good growth and development. promote seed potential.

Results in Table 3 show that the harvesting time of fresh ear of the hybrid combinations in the experiment ranged from 70 - 74 DAS. The hybrid combinations with the shortest harvest time (70 - 71 DAS) were THL14, THL9, THL8, THL3. The combinations with the longest harvest time (74 DAS) were: THL7, THL12, THL21, THL25, THL24, THL28.

The viability was an important criterion for evaluating the potential of a plant variety. One of the important morphological features of corn was the number of leaves/plant and the height of the plant. The experimental results showed that the total number of leaves/plants of the hybrid combinations was from 17.5 to 19.3 leaves. The hybrid combination THL9 had the highest total number of leaves/plant (19.3 leaves), the lowest was the hybrid combination THL25 (17.5 leaves), the number of leaves of the Golden Cob control variety was 16.9 leaves.

One of the important morphological features of corn was plant height. Through the plant height,

we can preliminarily evaluate the growth and development of hybrid combinations. Plant height and corn stacking height were closely related to the resistance of corn to fall, affecting the yield of the corn variety.

In this experiment, the plant height of the hybrid combinations ranged from 170.6 – 218.6 cm. The plant height of the hybrid combinations showed a statistically significant difference. The THL8 combination had the highest plant height of 218.6 cm, the lowest plant height of THL28 was 170.6 cm.

Plant height and corn stacking height were closely related to the resistance of corn falling, affecting the yield of the corn variety. At the experimental results, the corn set height of the combinations varied according to the plant height, ranging from 73.3 to 100.5 cm. The stem diameter of the hybrid combinations did not have a statistically significant difference, ranging from 2.7 – 3.2 cm. The combination THL3 and THL16 had the largest stem diameter of 3.2 cm, the combination N1/N5 had the smallest stem diameter of 2.7 cm.

3.3. Morphological characteristics of the ear of 28 sweet corn hybrid combination

The length and diameter of an ear were strongly correlated with corn mass. In the experiment, corn length ranged from 18.1 to 21.8 cm, the THL9 combination had the longest corn length of 21.8 cm, the shortest corn length was 18.1 cm of the combination THL25. The corn length of the sweet corn hybrid combinations in this experiment was statistically significant (Table 4).

The ear diameter of sweet corn hybrid combinations ranges from 46.2 to 5.5 mm. The control variety Golden had a length of 20.7 cm and a diameter of 50.2 mm. The difference in ear diameter of the hybrid combinations was not statistically significant.

In the current corn variety selection, the Kernel-row number trait characteristic was an important indicator to evaluate the potential yield of corn variety. The current popular corn varieties usually had 16 rows of kernels/ear. In this study, the hybrid combinations had several rows of kernels ranging from 16.3 to 18.7 rows, with no statistically significant difference.

For the commercial value of fresh ear, the color of kernels was an important criterion to consider and select to meet the goal of selecting corn varieties suitable to the tastes of consumers. The hybrid combinations in this experiment had yellow to orange-yellow kernels, their color was shiny and even, meeting the breeding target.

The color of the husk was an indicator that directly affects the commercial value of fresh corn ear and attracts consumers to choose. Traders often give priority to fresh corn that was bright green from light to dark. For the 28 hybrid combinations in this experiment, all the colors of the husks ranged from light green to dark green, of which only the hybrid combinations THL5, THL8, THL13 had light green husk, meeting market tastes.

3.4. Fresh ear yield of 28 sweet corn hybrid combinations

An important criterion in corn breeding was the high yield of the fresh ear, because this was a synthetic indicator, reflecting the most concentrated, accurate ability to grow and develop as well as the ability to adapt to environmental conditions of each hybrid combination.

The results in Table 5 showed that the weight of corn with husk leaves ranged from 324.8 – 487.5 g. The THL9 combination had the largest amount of corn with the largest husk leaves 487.5 g, the combination THL28 had the lowest weight of corn with the lowest husk leaves at 324.8 g. The ratio of unhusked ear/husked ear (%) was an indicator of interest in breeding. Normally, this ratio was low, the ear with a thick shell was difficult to peel, but it can be preserved for a long time. If this rate was high, the ear was thin, the storage time was short, and the ear quickly grows old. Current commercial varieties of corn had this ratio of around 73-75%. The control variables in the experiment Golden Cob had 75.4% of the ear without husk leaves/corn with husk leaves. While 2 hybrid combinations with high yield, THL9 and THL14 had the rate of unhusked ear/husked ear (%), respectively 77.2 and 73.9%.

The number of effective ears per plant, planting density, and ear weight were factors that directly affect the yield of corn. Actual yield was the result and ultimate goal of the production process or the evaluation and research of hybrid combinations. The actual yield of the hybrid combinations in the experiment ranged from 14.2 to 23.7 tons/ha.

Table 4. Morphological characteristics of corn of 28 sweet corn hybrid combinations

Hybrid Combination	Length of ear (cm)	Diameter of ear (mm)	Kernel-row number	Color of kernel	Husk covering (Point)	Color of husk
THL1	19.1^{bc}	48.8	18.1	Y	VT	Green
THL2	19.3^{abc}	48.0	18.2	Y	VT	Green
THL3	21.2^{ab}	53.1	18.3	YO	VT	Green
THL4	20.6^{abc}	49.3	17.4	Y	T	Dark Green
THL5	19.3^{bc}	48.2	17.6	Y	VT	Light Green
THL6	19.3^{abc}	47.0	16.7	Y	VT	Dark Green
THL7	19.1^{bc}	49.5	16.9	YO	T	Green
THL8	21.3^{ab}	52.8	18.7	YO	VT	Light Green
THL9	21.8^{a}	52.3	16.5	YO	VT	Dark Green
THL10	19.1^{bc}	51.8	16.7	Y	VT	Green
THL11	20.6^{abc}	48.8	16.3	Y	VT	Green
THL12	20.6^{abc}	48.5	18.3	Y	T	Dark Green
THL13	18.3^{c}	51.3	16.5	YO	T	Light Green
THL14	21.8^{a}	53.5	16.8	YO	VT	Dark Green
THL15	19.7^{abc}	51.7	17.7	Y	VT	Green
THL16	19.0^{bc}	46.8	18.0	Y	VT	Dark Green
THL17	19.0^{bc}	49.4	16.5	Y	VT	Green
THL18	18.6^{c}	49.8	17.5	Y	T	Green
THL19	20.3^{abc}	49.8	18.7	Y	VT	Green
THL20	20.6^{abc}	49.9	17.7	Y	VT	Green
THL21	20.4^{abc}	47.5	16.9	YO	T	Green
THL22	18.5^{c}	49.3	17.1	YO	T	Green
THL23	19.8^{abc}	48.6	17.1	Y	VT	Green
THL24	18.4^{c}	46.2	16.4	Y	T	Green
THL25	18.1^{c}	48.9	17.3	Y	T	Green
THL26	19.1b^{c}	49.7	17.8	Y	T	Green
THL27	20.3^{abc}	48.7	16.8	Y	VT	Dark Green
THL28	18.3^{c}	49.2	16.6	Y	T	Green
Golden (Control)	20.7^{abc}	50.2	16.9	Y	VT	Green
CV (%)	5.0	5.2	7.2			
F_{value}	3.64**	1.57^{ns}	1.46^{ns}			

In the same column, numbers with the same letter do not had a statistically significant difference; ^{ns}: no significance; **: very significant difference at the level of $\alpha = 0.01$. Y= Yellow, YO = Yellow-Orange, Husk cover - Point 1: Very Tight, Point 2:Tight.

The hybrid combination THL9 had the highest net yield of 23.7 tons/ha. Next was the hybrid combination THL14 reached 21.4 tons/ha.

The quality of sweet corn varieties was an indicator that consumers always care about. In the evaluation and selection of sweet corn varieties, Brix degree was an important indicator in the evaluation of corn quality. The results in Table 5 shows that the soluble solids content (Brix) of the hybrid combinations was over 11%, ranging from 11.8 – 13.9%. The Brix of the hybrid combinations was not statistically significant. Most of the hybrid combinations participating in the experiment had an aroma and less tip cap when boiled and eaten fresh.

3.5. Determining the combination ability of 8 sweet corn lines

The combining ability of parents to pass on to their offspring the traits of the associated parents in hybrid combinations. The ability to coordinate was divided into two categories: the general combining ability (GCA) and the specific combining ability (SCA).

Table 5. Fresh ear yield of 28 sweet corn hybrid combinations

Hybrid combinations	Weight of husked ear (g)	Weight of unhusked ear (g)	Ratio of unhusked ear/husked ear (%)	Yield (tons/ha)	Brix (%)
THL1	451.3^{abc}	301.7^{ad}	66.8	19.3^{abc}	13.3
THL2	449.8^{abc}	277.5^{be}	61.7	19.1^{abc}	12.2
THL3	453.0^{abc}	338.7^{abc}	74.8	21.4^{ab}	12.8
THL4	380.0^{ae}	285.8^{be}	75.2	18.2^{abc}	13.7
THL5	447.3^{abc}	277.0^{ve}	61.9	19.6^{abc}	12.5
THL6	380.7^{ae}	245.7^{de}	64.5	19.5^{abc}	12.2
THL7	383.7^{ae}	294.2^{ad}	76.7	17.9^{bc}	13.3
THL8	478.7^{ab}	339.7^{abc}	71.0	21.3^{ab}	13.5
THL9	487.5^{a}	376.2^{a}	77.2	23.7^{a}	13.9
THL10	400.8^{ae}	323.7^{ad}	80.7	17.7^{bc}	13.7
THL11	436.2^{ad}	285.3^{be}	65.4	17.8^{bc}	13.0
THL12	425.7^{ae}	302.7^{ad}	71.1	18.1^{abc}	13.5
THL13	413.2^{ae}	298.7^{ad}	72.3	18.6^{abc}	11.8
THL14	487.3^{a}	360.2^{ab}	73.9	21.4^{ab}	13.6
THL15	411.7^{ae}	330.3^{ad}	80.2	18.6^{abc}	12.5
THL16	429.5^{ae}	254.0^{cde}	59.1	19.9^{abc}	13.5
THL17	392.0^{ae}	279.2^{be}	71.2	19.4^{abc}	12.0
THL18	392.7^{ae}	317.2^{ad}	80.8	17.5^{bc}	13.6
THL19	426.2^{ae}	334.8^{abc}	78.6	19.6^{abc}	13.2
THL20	445.2^{abc}	320.0^{ad}	71.9	19.7^{abc}	13.8
THL21	396.8^{ae}	278.3^{be}	70.1	15.9^{bc}	13.2
THL22	431.5^{ae}	260.3^{cde}	60.3	18.2^{abc}	12.2
THL23	375.2^{cde}	302.2^{ad}	80.5	18.8^{abc}	13.4
THL24	335.3^{de}	207.5e	61.9	14.3^{c}	11.8
THL25	349.0^{cde}	276.8^{be}	79.3	15.4^{c}	12.0
THL26	364.3^{cde}	293.2^{ae}	80.5	17.9^{bc}	12.5
THL27	366.3^{cde}	298.3^{ad}	81.4	17.9^{bc}	13.5
THL28	324.8^{e}	260.7^{cde}	80.2	14.2^{c}	12.7
Golden (control)	424.5^{ae}	320.2^{ad}	75.4	17.4^{bc}	12.2
CV (%)	10.1	10.7	-	11.7	9.0
F_{value}	3.2**	3.8**	-	2.7**	1.0^{ns}

In the same column, numbers with the same letter do not had a statistically significant difference; ^{ns}: no significance; **: very significant difference at the level of $\alpha = 0.01$.

In breeding programs, the determination of combining ability helps to select parents to join the breeding program, the specific combining ability assists breeders in identification the potential inbred lines for parental hybrid (Feirreira et al., 2018). Currently, the diallel cross which was developed by Griffing was used quite commonly in sweet corn breeding programs. In the experiment, to evaluate the general and individual coordination ability of 08 sweet corn lines, Griffing 4 model was used through a trait of agronomic properties, yield and quality of sweet corn in Table 6 and Table 7 and Table 8.

3.5.1. The general combining ability of some agronomic traits of 8 sweet corn lines

Analyzing the ability to combine some agronomic traits of 8 sweet corn lines in the experiment (Table 6), the value of plant height, number of leaves/plant, stem diameter, the diameter of the ear showed that the general combining ability of 8 lines was not a statistically significant difference.

Table 6. The general combining ability of some agronomic traits of 8 sweet corn lines

Line \ Combining ability	Plant Height	Leaves/Plant	Stem diameter	Ear length	Ear diameter	Number of row/ear	Yield	Brix
K60	1.954^{ns}	0.262^{ns}	0.026^{ns}	-0.003	-0.558	0.156^{ns}	0.811 ns	-0.121
R111	6.388^{ns}	0.134^{ns}	-0.024	0.497*	1.219 ns	0.122^{ns}	1.050^{ns}	0.318^{ns}
N1	2.476^{ns}	0.143^{ns}	0.009^{ns}	0.152^{ns}	0.817^{ns}	0.511^{ns}	1.173^{ns}	0.018^{ns}
N4	7.605^{ns}	0.234^{ns}	0.045^{ns}	1.113**	1.400^{ns}	0.633*	1.616**	0.357*
N5	-4.418	-0.343	-0.055	-0.320	-0.131	-0.167	-1.283	-0.065
N7	3.065^{ns}	-0.178	0.055^{ns}	0.119	-1.072	-0.167	0.236^{ns}	0.246^{ns}
N8	-5.296	-0.157	-0.010	-0.442	-1.603	-0.5	-1.841	-0.476
N12	-11.235	-0.096	-0.045	-1.115	-0.072	-0.589	-1.761	-0.276

^{ns}: no significance; *: significant difference at the level of $\alpha = 0.05$; **: very significant difference at the level of $\alpha = 0.01$.

In the length of ear, lines R111 and N4 had GCA values of 0.497 and 1.113, respectively, which were statistically significant differences, there was GCA with many other sweet corn lines to create a long corn ear. These two lines can be used as a parent for yield-enhancing hybrid corn seed development programs.

The N4 line also had a statistically significant difference in the combined value of the number of rows per ear, the soluble solids content and the yield. Thus, this line can be used for sweet corn hybrid seed production programs to produce hybrid of corn varieties with both good yield and quality.

GCA is the average performance of a genotype in a series of hybrid combinations. They defined SCA as those cases in which certain hybrid combinations perform better or poorer than would be expected based on the average performance of the parental inbred lines. Parents showing a high average combining ability in crosses are considered to have good GCA while if their potential to combine well is bounded to a particular cross, they are considered to have good SCA (Parviz et al., 2016). The GCA value for low or negative traits, the combined value of that line with other strains was not different from other incarnations, only the lines had GCA value in the properties. The high or positive status will increase the traits of interest to the breeder. This comment was consistent with the results of research in the experiment when the lines R111 and N4 had a high ability to combine in the ear length trait gave high net yield in the hybrid combination R111/N4 (THL9).

However, lines with low GCA values were not excluded from the breeding program, it was only an estimate of the likelihood of association with other lines in the breeding program (Oliboni et al., 2013). This was true with the GCA assessment of the N1 line (low GCA), but when it was the mother in the N1/N4 hybrid (THL 14) hybrid, the hybrid had high yield and good quality.

3.5.2. The specific combining ability in the fresh ear yield and soluble solids content (Brix) traits of 8 lines

The general combining ability (GCA) and the specific combining ability (SCA) estimates for productivity and quality should always be computed together to evaluate the inbred lines. This allows breeders to associate two basic criteria for

Table 7. The specific combining ability in the yield of 8 corn lines

♀ \ ♂	K60	R111	N1	N4	N5	N7	N8	N12
K60		-1.189	-1.482	0.412^{ns}	0.090^{ns}	-0.015	1.922^{ns}	0.262^{ns}
R111			0.512^{ns}	2.433*	-0.712	-2.047	0.283^{ns}	0.720^{ns}
N1				0.010^{ns}	0.125^{ns}	-0.107	1.426^{ns}	-0.484
N4					0.662^{ns}	-0.790	-2.470	-0.257
N5						1.268^{ns}	-1.232	-0.202
N7							0.900^{ns}	0.790^{ns}
N8								-0.830
N12								

^{ns}: no significance; *: significant difference at the level of $\alpha = 0.05$.

Table 8. The specific combining ability of 8 lines in soluble solids content (Brix) trait

♀ \ ♂	K60	R111	N1	N4	N5	N7	N8	N12
K60		0.147^{ns}	-0.687	-0.359	0.236^{ns}	-0.581	-0.192	0.708^{ns}
R111			0.208^{ns}	0.963*	0.458^{ns}	-0.520	0.702^{ns}	-1.231
N1				0.869^{ns}	-0.409	0.247^{ns}	-0.498	0.269^{ns}
N4					-0.014	0.275^{ns}	0.397^{ns}	-0.803
N5						0.230^{ns}	-0.648	-0.581
N7							-0.225	0.575^{ns}
N8								0.463^{ns}
N12								

^{ns}: no significance; *: significant difference at the level of $\alpha = 0.05$.

selecting a population: high mean and the largest genetic variance possible (Cruz et al., 2012).

The value of the specific combining ability in the fresh ear yield traits had a clear difference, the R111 line had a high ability to combine specifically with the N4 line, with the value of 2.433, with a statistically significant difference at the 95% confidence level. Fresh ear yield of R111 strain was 16.1 tons/ha; line N4 was 16.6 tons/ha; the hybrid combination R111/N4 was 23.7 tons/ha; Golden Cob variety was 17.4 tons/ha. The heterosis from the hybrid combination R111/N4 exceeded the parental line average of 44.9% and surpassed the control by 36.2%. This result was in accordance with the conclusion of Oliboni et al. (2013) which indicated that the values of the individual association capacity in the yield traits and the constituent factors for productivity had statistically significant differences. Populations resulting from these lines may produce a crossbred with greater heterosis than the parents.

Assessing the specific combining ability in soluble solids content (Brix), the R111/N4 hybrid combination had the highest specific combination ability value reaching 0.963 and statistically significant differences compared to other hybrid combinations.

4. Conclusions

Under this experimental conditions, all the sweet corn hybrid combinations grew and developed well. The hybrid combinations fell, stem borers, and corn borer were negligible. The N4 line had a high ability to combine both Brix traits and fresh corn yield. The two lines of R111 and N4 had the ability to combine well characteristics of Brix and fresh corn yield.

Experimental results determined that the hybrid combination THL9 (R111/N4) achieved a fresh corn yield of 23.7 tons/ha, a total soluble solids content of 13.9%. The morphology which was shape and color met the needs of the consumer market in Vietnam. It will be contributing to proactively source of varieties, reducing seed costs, and increasing economic efficiency for farmers.

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Seroprevalence of serotype O of foot-and-mouth disease virus in vaccinated pigs and cattle in Ho Chi Minh City

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ABSTRACT

The aim of this study was to assess the seroprevalence against serotype O FMD (foot and mouth disease) virus in vaccinated pigs and cattle in Ho Chi Minh City, as a basis to serve the prevention of FMD epidemics in these animals. A total of 535 pigs and 366 cattle sera were tested by using the Elisa kit for the detection of serotype O FMD antibody (Pirbright, UK). Results of this study showed that most pig farms had the proportions of positive animals for antibodies against FMDV serotype O which met the requirements of Decision no. 07/2016/ Ministry of Agriculture and Rural Development, except Xuan Thoi Thuong, Thai My and Phu My Hung. All sows had high levels of antibodies against serotype O FMD virus. There were no significant differences in the ratios of positive pigs for antibodies against FMDV serotype O among types of pig and age groups. However, there were significant differences in the seroprevalence of vaccinated pigs across herd sizes and days post-vaccination. Meanwhile, the overall seroprevalence of vaccinated cattle against FMDV at individual-level was over 80.00%. No statistical differences were found in the seroprevalence of vaccinated cattle against type O FMDV among regions, types of cattle, herd sizes, age groups and days post-vaccination. In conclusion, pigs raised in farms of Xuan Thoi Thuong, Thai My and Phu My Hung communes should be revaccinated with FMD vaccine to prevent the risk of pigs being infected with FMD virus and reduce the amount of virus produced by an infected animal.

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1. Introduction

Foot-and-mouth disease (FMD) is a trans-boundary animal disease that seriously disrupts regional and international trade in animals and animal products. The disease affects cattle, swine, sheep, goats and other cloven-hoofed ruminants (OIE, 2018). Foot-and-mouth disease virus can

spread rapidly in herds, populations and is difficult to control. Acute FMDV infection is characterized by loss of appetite, fever, and formation of characteristic vesicles on the feet, udders, and in the oral cavity (Orsel et al., 2009). The disease causes serious production losses including weight loss, decrease in milk production, and loss of draught power, the majority of affected animals recovered from FMD disease are often weakened and debilitated (OIE, 2018). FMD outbreaks have been occurring in many continents such as Asia, Europe, Africa, and South America. According to the World Reference Laboratory for Foot and mouth disease in 2020, FMD occurs in 37 countries around the world (WRLFMD, 2020).

In Vietnam, types O, A and Asia 1 were found in infected animals. It was found that 22.3% of sampled ruminants had previously been infected with the FMD virus, of which 10.8% were persistent and asymptomatic carriers (Ferreira et al., 2015). Thereafter, the disease continued to spread throughout the country due to errors in FMD disease control and prevention (Truong, 2017). Risk factors for recurrence of FMD outbreaks were determined including the movement of infected animals, exposure to infected wild animals, long periods between vaccination and infection, proximity to borders, lack of suitable biosecurity (Rweyemamu et al., 2008). According to data reported by MARD (2019), Foot-and-mouth disease outbreaks have been occurring in many provinces: Ha Tinh, Quang Binh, Quang Tri, Quang Ngai causing great damage to the livestock industry in Vietnam. In Vietnam, control of FMD in endemic regions is mainly focused on mass vaccination of all susceptible livestock with a homologous strain vaccine, identification, and testing of animals, establishment of protection and surveillance zones and enforcement of quarantine and biosecurity. FMD vaccine has been recommended in the routine vaccination program for animals according to Decision no. 07/2016/ Ministry of Agriculture and Rural Development (MARD, 2016) about the national program of controlling FMD disease issued in 2016. Besides, the serological survey for antibodies against FMDV was carried out in this program. Therefore, this study aimed to evaluate the seroprevalence of FMD virus in vaccinated pigs and cattle in Cu Chi and Hoc Mon, Ho Chi Minh City.

Descriptive analysis and Chi-square tests were used to compare the difference in proportions of seroprevalence among herd size, breeds, and other variables. If the probability value (P-value) is less than or equal to the set alpha level (0.05) then the result was considered as statistically significant.

2. Materials and Methods

A total of 535 pig and 366 cattle sera were taken from 37 pig farms and 56 households in 11 communes of Cu chi (An Nhon Tay, An Phu, Nhuan Duc, Pham Van Coi, Phuoc Vinh An, Thai My, Tan Thanh Dong, Tan Thanh Tay, Trung Lap Thuong, Trung Lap Ha, Trung An) and two communes of Hoc mon districts (Tan Thoi Nhi, Xuan Thoi Son) Ho Chi Minh City. All animals were originally collected as part of on-going annual disease investigations. These animals in this study were vaccinated against foot and mouth disease. In each pig farm, depending on herd size and the permission of owners, 5 – 100 pigs were collected randomly for blood sampling. Meanwhile, all vaccinated animals per cattle household would be selected for 3-5 mL blood samples to evaluate the seroprevalence of FMD. For each sampled animal, information including regions, herd size, days post-vaccination were also collected to assess their association with FMD seropositivity. All serum samples were tested for the presence of antibodies against FMD antibody detection serotype O (Pirbright, UK).

Descriptive analysis and Chi-square tests were used to compare the difference in proportions of seroprevalence among herd size, breeds, and other variables. If the probability value (P) is less than or equal to the set alpha level (0.05) then the result was considered as statistically significant.

3. Results and Discussion

Table 1 showed that the proportions of positive pigs and cattle for antibodies against FMDV serotype O were over 80.00% which met the demand of the Sub-department of Animal Health Ho Chi Minh scheme (Decision no. 07/2016/TT-BNNPTNT). However, the seroprevalences of vaccinated pigs against FMD virus serotype O in Xuan Thoi Thuong, Thai My and Phu My Hung were 68.18%; 44% and 8.19%; respectively, which were not achieved complete protection from FMD disease. It is likely that poor handling and malpractice in FMD vaccination in farms belonged to Xuan Thoi Thuong, Thai My and Phu My Hung

Table 1. The seroprevalence of antibodies against FMDV type O in vaccinated pigs and cattle by regions

	Communes	Pig		Cattle	
		Number of households	n/N (%)	Number of households	n/N (%)
Cu Chi	Trung Lap Thuong	4	62/71 (87.3)	1	5/5 (100.0)
	Thai My	2	3/10 (30.0)	2	9/10 (90.0)
	Trung An	3	10/10 (100.0)	2	8/10 (80.0)
	Tan Thanh Dong	3	8/10 (80.0)	2	10/10 (100.0)
	Trung Lap Ha	3	10/10 (100.0)	2	10/10 (100.0)
	Pham Van Coi	1	100/100 (100.0)	1	27/27 (100.0)
	An Nhon Tay	5	126/132 (95.5)	3	34/35 (97.1)
	Nhuan Duc	4	70/71 (98.58)	13	43/45 (95.6)
	Tan Thanh Tay	3	15/15 (100.0)	11	44/45 (97.8)
	Phuoc Vinh An	3	7/8 (87.5)	11	42/45 (93.3)
	An Phu	4	15/15 (100.0)	5	58/67 (86.6)
	Phu My Hung	1	5/61 (8.2)	-	-
	Total	36	431/513 (84.0)	53	290/309 (93.9)
Hoc Mon	Tan Thoi Nhi	-	-	1	24/24 (100.0)
	Xuan Thoi Son	-	-	2	31/33 (93.9)
	Xuan Thoi Thuong	1	15/22 (68.2)	-	-
	Total	1	15/22 (68.2)	3	55/57 (96.5)

n: number of positive samples; N: number of serum samples tested.

occurred and this leads to the failure in FMD vaccination in these pigs. In reality, the owners were responsible for vaccination programs in a pig farm and the information about these pigs such as vaccination programs were collected by using questionnaire lists. In addition, Dekker et al. (2016) also indicated that piglets should be vaccinated when maternal antibodies titers are at a very low level to induce a neutralizing antibody titer likely to confer protection in these pigs. To achieve complete protection of pigs at the herd level, the seroprevalence of vaccinated pigs against FMD virus serotype O should be maintained at least at more than 80%; because it is generally considered that vaccination of not less than 80% of the herd is necessary to provide herd immunity (Doel, 1999). In this study, pigs showed low immunogenicity and protective effects compared to those in cattle. In consistent with Orsel & Bouma (2009) and Park et al. (2017), the results indicated that vaccination against FMD seemed to be effective in cattle and sheep, but was less effective in pigs.

Furthermore, in Table 2, no statistical difference was found in the seroprevalence of vaccinated pigs against FMDV serotype O among types of animals and age groups ($P > 0.05$). However, significant differences in the seroprevalence of vaccinated pigs across herd sizes and days post-vaccination ($P < 0.05$) were found. A survey in Minnesota indicated the effect of herd size on the herd protection after vaccination including the efficiency, duration as well as coverage level (Miller et al., 2018).

To manage big herd size, health management and vaccination should be carried out strictly because unvaccinated pig can become a reservoir as well as transmit pathogen to other houses and environments (Lyon et al., 2016). According to Decision 7 of MARD (2016), for post-vaccination surveillance of infectious diseases including FMD disease; Pasteurellosis, Leptospirosis, Tuberculosis in cattle, blood samples should be collected after 21 days to 90 days since the last vaccination. Additionally, a previous study have demonstrated that viruses can persist in the epithelium of pharynx in over 50% of cattle exposed to the virus, even in immunized animals so the longer protection can prevent pig from FMD infection (Kitching et al. 2003). According to Parida (2009), unlike cattle are more susceptible to aerosols and should be vaccinated with single or multiple administration as per requirement in free or endemic areas; pigs can excrete large amount of virus in aerosol, so pigs should been vaccinated to control the spread of virus and then culled of in-contact pig herds.

Table 2. The seroprevalence of antibodies against FMDV type O in vaccinated pigs and cattle based on types of animals

		Number of tested samples	Number of positive samples	Ratio (%)
Types of animals (animals)				
Pigs	Sow	61	61	100.0
	Gilt	64	53	82.9
	Grower	410	332	80.9
Cattle	Dairy	299	280	93.6
	Beef	67	65	97.0
Months of age (months)				
Pigs	6 - 12	64	53	82.9
	12-36	471	393	83.4
Cattle	<12	5	5	100.0
	≥ 12 - < 36	64	62	96.9
	≥ 36 - ≤ 60	297	278	93.6
Herd sizes (animals)				
Pigs	≤ 100	123	94	76.4
	> 100 - ≤ 500	7	7	100.0
	> 500 - ≤ 1000	122	66	54.1
	> 1000 - ≤ 5000	122	122	100.0
	> 5000	161	157	97.5
Cattle	< 20	91	87	95.6
	≥ 20 - ≤ 40	139	134	96.4
	> 40	136	124	91.2
Time of vaccination (days)				
Pigs	≤ 21	15	8	53.3
	> 21 - ≤ 90	258	198	76.7
	> 90	262	240	91.6
Cattle	< 21	91	87	95.6
	≥ 21 - ≤ 40	139	134	96.4
	> 40	136	124	91.2

4. Conclusions

In conclusion, pigs showed low immunogenicity and protective effects compared to those in cattle. However, most farms had positive pigs and cattle for antibodies against FMDV serotype O met the demand of the Sub-department of Animal Health Ho Chi Minh scheme, except Xuan Thoi Thuong, Thai My and Phu My Hung.

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The presence and genetic characteristics of porcine circovirus 3 from pigs in Southern and Central provinces of Vietnam

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ABSTRACT

Porcine circovirus type 3 (PCV3) is an emerging circovirus species that has recently been reported in different countries around the world, suggesting a widespread circulation. This study was carried out in order to investigate the presence and further genetic characteristics of PCV3 from swine herds in Southern and Central provinces of Vietnam. A duplex PCR assay for rapid detection of PCV3 in pigs was established with a pair of specific primers designed between *rep* and *cap* gene segment to amplify full-length ORF2 and another set of primers binding to COX1 gene serving as an internal amplification control (IAC). The resulting duplex PCR was used to examine PCV3 presence in 94 tissue and serum samples. Subsequently, PCV3 was detected in 10 out of 94 cases (10.6%). The infection rate in sows (14.3%) was higher than that in grower pigs (7.7%). Regarding nucleotide sequence comparison, 10 ORF2 genes were selected for nucleotide sequencing and their alignment showed 97.2% - 99.5% homology. According to the phylogenetic analysis and sequence alignment of *cap* gene, all the sequences were clustered into group PCV3a, including 9 strains of sub-group PCV3a1 and only one strain of sub-group PCV3a2. These findings indicated that the PCV3a group is circulating in swine farms in Vietnam. This study provides better insights into epidemiology of this pathogen in the national swine industry.

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1. Introduction

Porcine circovirus 3 (PCV3) is a small non-enveloped virus with single-stranded circular DNA belonging to the genus *Circovirus* in the family *Circoviridae*. Similar to other circoviruses, the capsid protein is considered major structural protein and antigenic characteristics. In 2016, the virus was initially described in sows in the North Carolina (USA) with manifestation of porcine dermatitis and nephropathy syndrome (PDNS) and reproductive failure (Palinski et al. 2017). Despite the novelty, a number of studies associated with the prevalence of PCV3 have been conducted in different pork-producing countries, namely Brazil (Saraiva et al. 2019), China (Guo et al. 2019), Korea (Kim et al. 2018), Thailand (Kedkovid et al. 2018), etc. However, up to date, PCV3-related studies are still limited in Vietnam. Therefore, the objective of this investigation was to establish of duplex PCR assay for detection and amplification of full-length ORF2 of PCV3 in order to determine the presence and genetic characteristics of the virus in pig populations in

Southern and Central areas of Vietnam.

2. Materials and Methods

2.1. Establishment of duplex PCR assay

2.1.1. Primer design and selection

One set of primers designed for PCV3 detection and ORF2-region genome sequencing was generated based on 70 sequences available in the Genbank by Primer3plus software, and validated by NCBI BLAST, OligoAnalyzer 1.0.2, Clustal Omega tool, PCV3-F: 5'-ATGCGAGGGCGTTTACCTG-3', PCV3-R: 5'-TCCCTACAGACCTCCGTGG-3'. Another primer pair designed in COX1 gene (Genbank ID: KY661881.1) was selected to serve as an internal amplification control (IAC), IAC-F : 5'-GCACTGCCTTGAGCCTACTAAT-3', IAC-R: 5'-AACAGGGGTGTTTGGTATTGAG-3'.

2.1.2. Positive and negative DNA controls

DNA template of PCV3 was originally obtained from a field sample followed by sequencing confirmation to be PCV3. The resultant sequence exhibited 99.69% identity to the previously reported PCV3 (Gene ID MN583577.1). The DNA was then quantified to 5×10^6 copies/µL by Biodrop µLITE Spectrophotometer (BiochromTM 80-3006-51, England) and used as positive control for subsequent PCR reactions.

Unrelated DNA used to determine specificity of the primers was DNA viruses or bacteria that are frequently found in swine farm environment and potentially contaminate the samples, including *Salmonella* spp., *Escherichia coli* (*E. coli*) (ATCC 25922), *Streptococcus suis* (*S. suis*), *Staphylococcus aureus* (*S. aureus*) (ACTC 6338), *Clostridium perfringens* (*C. perfringens*); *Pasteurella multocida* (*P. multocida*), *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*), *Haemophilus parasuis* (*H. parasuis*), *Mycoplasma hyopneumoniae* (*M. hyoneumoniae*); Aujeszky's disease virus (ADV), porcine circovirus type 2d (PCV2d). Nuclease free water was used as negative control for all PCR reactions.

2.1.3. Single PCR construction

Initially, to survey annealing temperature of PCV3 primer, the single PCR reactions were conducted in a 20 µL mixture including 10 µL Dream Taq Green PCR Master Mix 2X (Cat#K1081, Thermo Fisher Scientific, USA), 1 µL of each 10 µM PCV3 primer, 6 µL of DNA and nuclease free water was added to reach final volume of 20 µL/reaction. The amplification was performed in a GENE Q Thermal Cycler (Bioer, China) under the following conditions: pre-denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 30 sec, annealing temperature optimized in the range of 50°C to 62°C for 30 sec, extension at 72°C for 1 min and a final extension at 72°C for 5 min. Subsequently, the 10 µL PCR products were visualized by 1.5% agarose gel electrophoresis (Cat#16500100, Invitrogen, Thermo Fisher Scientific, USA) with Midori Green Advance DNA stain (Cat#MG04, NIPPON Genetics Europe, Germany) and ultraviolet light (UV Transilluminators, Korea), using 1Kb DNA Plus ladder (Cat#10787018, Invitrogen, Thermo Fisher Scientific, USA) as the molecular weight markers to indicate the sizes of amplification products. The range of appropriate annealing temperature of PCV3 primers was compared with those of IAC primers to decide the final thermal condition in the mPCR.

2.1.4. Optimization of primer concentration of duplex PCR assay

Entering the upcoming step, to assess mutually compete interfering level of two pairs of primers in duplex PCR assays, the 20 µL duplex PCR reaction encompassed 10 µL Dream Taq Green PCR Master Mix 2X (Cat#K1081, Thermo Fisher Scientific, USA), proper ratio of PCV3 and IAC primer concentration, 4 µL and 1 µL DNA of pig and target pathogen, respectively, nuclease-free water was added to reach the designed volume.

2.1.5. Specificity and sensitivity

In order to prove the specificity, DNA of different viral and bacterial agents was used as unrelated template in the duplex PCR reactions. Regarding the sensitivity, defined as the minimum detectable DNA molecules in a reaction, the PCV3 template was diluted 10-fold serially in nuclease-free water and used in the duplex PCR to determine the detection limit. The applicability of this assay was basically validated by using PCV3-positive samples.

2.2. Application of the duplex PCR to detect PCV3 in field samples

From July 2019 to November 2019, a total of 94 serum and tissue samples (lymph nodes and spleen) were collected from sows (n = 42) and grower farms (n = 52) in 12 provinces of Southern and Central Vietnam. Viral DNA of these samples was extracted using a 'DNeasy Blood and Tissue' Kit (Cat#69504, Qiagen, Germany), and was detected by the duplex PCR assay under optimized conditions as constructed above.

2.3. Genetic characteristics

The full-length ORF2 of PCV3-positive samples was purified and submitted to Nam Khoa Biotek (Vietnam) for sequencing in both directions. The sequences were then assembled by BioEdit version 7.2.5.

To investigate the evolutionary relationship of PCV3 strains detected, full-length cap genes downloaded from GenBank were used together with ORF2 sequences in this study to analyse the similarity by Clustal Omega software and BLAST tool. The genetic distance among strains was also calculated using MEGA X software.

Phylogenetic tree of PCV3 was built based on ORF2 sequences using the neighbor-joining (NJ) method in MEGA X software with bootstrap value of 1000 replicates. The classification of PCV3 in this study was based on four amino acid positions 24, 27, 77 and 150 in cap protein (Fux et al., 2018).

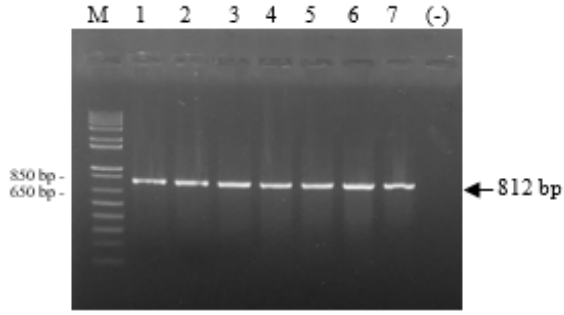

Figure 1. Annealing temperature optimization for single PCR. M: 1Kb plus DNA ladder; (1): 50°C; (2): 52°C; (3): 54°C; (4): 56°C; (5): 58°C; (6): 60°C; (7): 62°C; (-): negative control with nuclease-free water. PCR products were analyzed in 1.5% agarose gel at 90 Volt for 30 min.

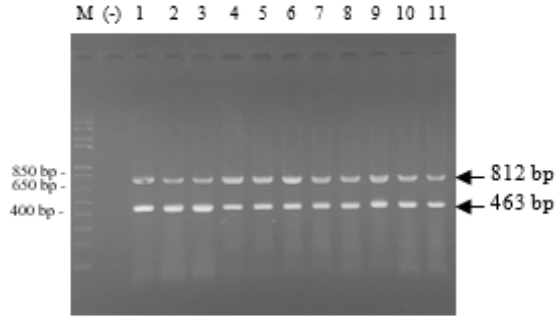

Figure 2. Optimization of primer concentration in duplex PCR assay. M: 1Kb plus DNA ladder; (-): negative control with nuclease-free water; lanes 1-11 are equivalent to ratio of 0.4:0.4, 0.3:0.4, 0.4:0.3, 0.4:0.2, 0.2:0.4, 0.2:0.5, 0.2:0.6, 0.3:0.6, 0.2:0.3, 0.3:0.5 and 0.3:0.7; PCR products were analyzed in 1.5% agarose gel at 90 Volt for 30 min.

3. Results and Discussion

3.1. Establishment of duplex PCR assay

3.1.1. Optimization of duplex PCR assay

Results of the single PCR assay indicated that PCV3 primers worked well in the range of 54°C to 60°C to generate a product of 812 bp as predicted (Figure 1). In addition, optimal annealing temperature for the IAC primers was determined in a separate study to be 60°C. For this reason 60°C was chosen for optimization of the primer ratio in the duplex PCR assay.

To determine the optimal proportion of the IAC/PCV3 primer, different ratios of primers were tested including 0.4:0.4, 0.3:0.4, 0.4:0.3, 0.4:0.2, 0.2:0.4, 0.2:0.5, 0.2:0.6, 0.3:0.6, 0.2:0.3, 0.3:0.5 and 0.3:0.7. Results showed that the ratio of 0.2:0.5 (lane 6) produced the clearest signal and easily distinguishable from each other (Figure 2). It indicates that the concentration of PCV3 primer should be 2.5 times as much as that of IAC primers.

3.1.2. Specificity and sensitivity

As illustrated in Figure 3, the PCV3 primers were specific for the target virus, not binding to DNA from other tested pathogens while the IAC primers were functioning well to confirm the samples to be from swine.

Additionally, Figure 4 showed that the minimum amount of target gene for successful amplification was consistently at 5×10^1. The as-

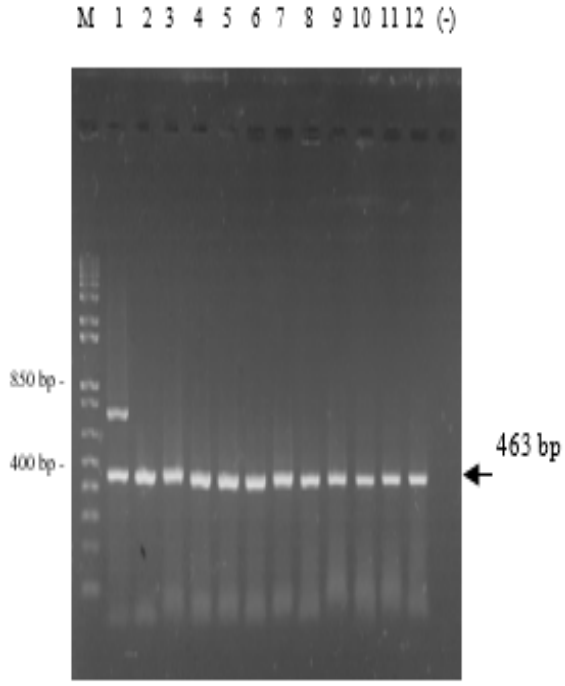

Figure 3. Specificity of duplex PCR assay. M: 1Kb plus DNA ladder; (1): positive control; (2): ADV; (3): PCV2; (4): *M. hyopneumoniae*; (5): *H. parasuis*; (6): *A. pleuropneumoniae*; (7): *P. multocida*; (8): *E. coli*; (9) *Salmonella* spp.; (10): *S. aureus*; (11): *S. suis*; (12): *C. perfringens*; (-): negative control with nuclease-free water. PCR products were analyzed in 1.5% agarose gel at 90 Volt for 30 min.

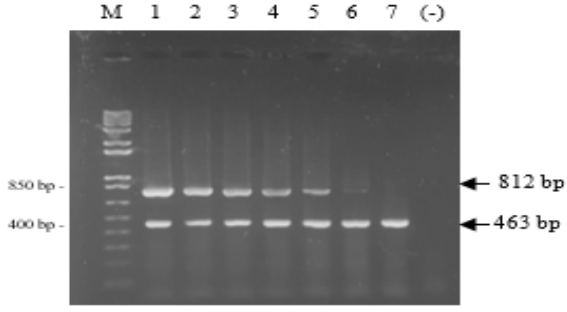

Figure 4. Sensitivity of duplex PCR assay. M: 1Kb plus DNA ladder; lanes 1 – 7 are: 5×10^6; 5×10^5; 5×10^4; 5×10^3; 5×10^2; 5×10^1; 5×10^0; (-): negative control with nuclease-free water.

say was repeated at least three times. The established duplex PCR assay in this study was comparatively sensitive to a SYBR Green-based Realtime quantitative PCR assay (1.73×10^2 copies/reaction) and a conventional PCR assay (1.5×10^2 copies/reaction) reported previously (Wang et al., 2017; Chen et al., 2018). Finally, to assess the applicability of this assay, 5 clinical samples confirmed positive with PCV3 by sequencing method were used. Results showed that the duplex PCR could detect PCV3 in all the five samples as expected (data not shown).

3.2. Application of the duplex PCR in detection of PCV3 in field sample

The established duplex PCR was used to detect PCV3 in 94 field samples collected from pigs. These results revealed 10.6% (10/94) samples positive for PCV3, doubling that reported in Nguyen et al. (2018)'s study conducted in the North of Vietnam which showed 4.44% (6/135) positivity.

3.3. Genetic characteristics

To characterize the genetic information of PCV3 strains detected in this study, the capsid gene of 10 positive samples (BP1_150719, BP2_250919, DN1_300819, DN2_230719, DN3_160719, DL_290819, VT_151119, BD1_160919, BD2_281119, and LD_061119) was sequenced and aligned. Nucleotide sequence analysis of these 10 cap genes demonstrated high sequence similarity, up to of 97.2% to 99.5%. In other words, the genetic distance of these strains was 0.005 to 0.028, which indicated a similar genetic variations compared to the previous study in the North of Vietnam (0.005-0.031) (Nguyen et al., 2018). Furthermore, alignment analysis together with 40 PCV3 ORF2 sequences from the Genbank revealed 97% to 100% sequence identity. Within the 10 strains sequenced in this study, four of them showed high identity to strains detected in Northeast China, Brazil, Italy and Spain at the level of 98.6% - 99.8%. Especially, BP1_150719_Vietnam strain displayed the highest homology to strains reported in Southern China (99.1%). It is in a branch far away from other strains in the phylogenetic tree (Figure 6). Besides, the remaining strains showed a high identity to strains found in Northeast China, Brazil, Italy and Spain.

To classify the subgroup for the 10 strains investigated in this study, amino acid sequences of ORF2 were compared. Based on the subgroup definition recommended by Fux et al. (2018), 9 strains were classified into PCV3a1 subgroup and the other one belonged to PCV3a2 sub-group (Figure 5). These 10 strains differed at 33 nu-

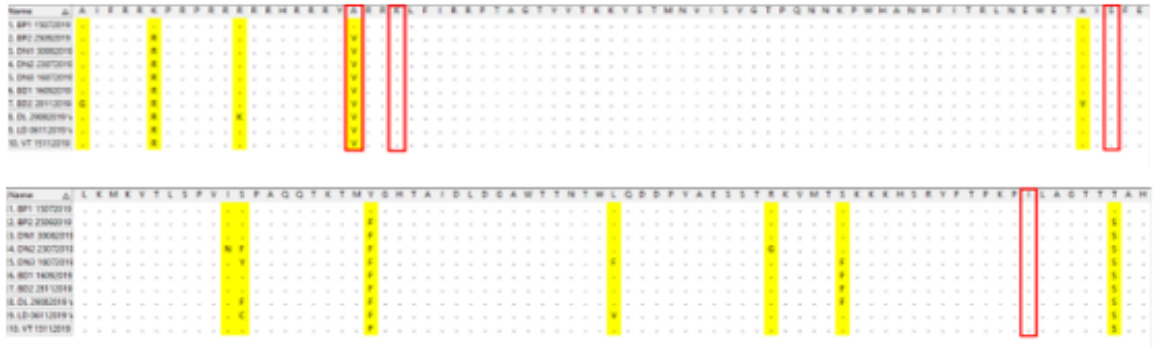

Figure 5. Alignment of the deduced amino acid sequences. Genetic marker at codon positions 24, 27, 77 and 150 were in red frames. Twelve mutations of aa at positions 5, 10, 16, 24, 94, 95, 104, 121, 132, 137 and 156 were shown.

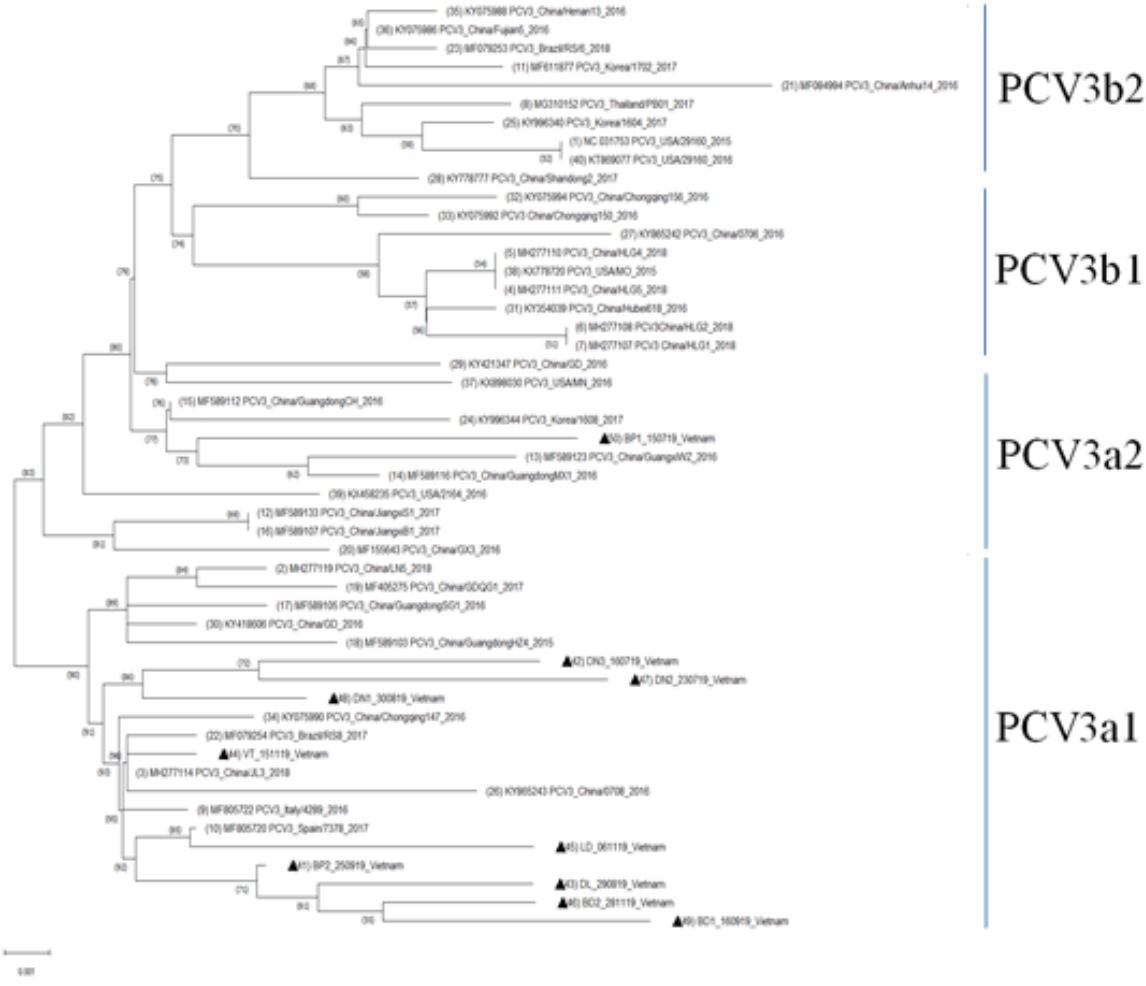

Figure 6. Phylogenetic tree of the full-length ORF2 gene sequences based on 10 different PCV3 strains from this study (black triangles) and 40 reference strains from Genbank. The subgroup of PCV3a1, PCV3a2, PCV3b1, and PCV3b2 was classified based on amino acid motif sequences proposed by Fux et al. (2018).

cleotide positions, leading to 12 non-synonymous mutations of the cap protein (Figure 5). How these nucleotide and amino acid polymorphisms affect the virus life cycle, pathogenesis and its interaction with the host immune responses requires further examinations.

4. Conclusions

The duplex PCR assay was successfully established for the diagnosis of PCV3. In this study, PCV3 was found to be present at a frequency of 10.6% in pigs in the Southern and Central provinces of Vietnam. Furthermore, the circulation rate of PCV3 in sows (14.3%) was twice as high as that in growers (7.7%). Strains of PCV3 found in this study belonged to PCV3a1 and PCV3a2 sub-group.

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Risk factors associated with canine parvovirus disease in dogs: A case-control study

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ABSTRACT

Canine parvovirus type-2 (CPV-2) is one of the most contagious viral agents causing acute enteritis in pups with high rate of morbidity and mortality. The study aimed to investigate the risk factors associated with canine parvovirus (CPV) occurrence in dogs in Ho Chi Minh City, Vietnam. A total number of 132 dogs less than six months of age were recruited in the study and split into two groups: 'CPV diseased dogs' included 44 (33.3%) dogs positive with canine parvovirus confirmed by rapid immuno-migration test, and 'normal dogs' consisted of 88 (66.7%) healthy dogs. Results indicated that the unvaccinated dogs had 11.76 times more risk of CPV infection than vaccinated dogs (OR = 11.76, $P < 0.001$). The risk of CPV in dogs raised with others at the same age was 5.01 times (OR = 5.01, $P = 0.027$) higher than those raised singly. The risk of CPV infection of dogs interacting with neighbour dogs was 3.13 times (OR = 3.13, $P = 0.028$) higher than the dogs having no contact with other dogs living nearby. Briefly, our study highlights potential factors for CPV disease prevention, especially CPV vaccination is the most important driver affecting the occurrence of parvovirus in young dogs.

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1. Introduction

In the 1970s, outbreaks of severe gastroenteritis caused by canine parvovirus type-2 (CPV-2) in dogs were first reported in Europe (1976) and the United States (1978) (Pollock & Coyne, 1993). The emergence of canine parvovirus disease in dogs have been currently spreading worldwide (Decaro & Buonavoglia, 2012). Gastroenteritis and myocarditis are recognized as two common clinical forms of CPV. While myocarditis is rarely found due to maternal effective immunization protecting pups in early period of life (Mohammed et al., 2008), gastroenteritis commonly occurs particularly in less than 6-month aged dogs with clinical signs including bloody foul smelling diarrhoea, vomiting and severe dehydration (Goddard & Leisewitz, 2010). The infection is transmitted by fecal-oral route through direct contact with contaminated feces or exposure to CPV fomites (Lamm & Rezabek, 2008). Canine parvovirus type-2 is highly contagious and stable under a variety of environmental conditions for months to years (Ling et al., 2012). The high mortality due to CPV was mostly found in unvaccinated dogs and up to 91% in cases without therapeutic intervention (Brady et al., 2012).

Previous studies around the world investigated the association between CPV infection and factors related to dogs' general information (i.e.,

age, gender, breed, vaccination status, etc.) (Mohammed et al., 2008; Naveenkumar et al., 2019; Sharma, 2019), dogs' clinical information (i.e., dehydration, rectal temperature, vomiting, diarrhoea, etc.) (Miranda et al., 2015), dog free-roaming access (Hsu et al., 2003), space and time of CPV infection (Brady et al., 2012). In Vietnam, since the first cases of CPV were observed in dogs in 1994 (unpublished data), there have been subsequent reports on parvovirus infection, which mostly focused on the evolution of antigenic CPV (Nakamura et al., 2004), incidence of parvovirus in dogs (Mai et al., 2018), molecular characterization of CPV (Quynh & Dai, 2014; Hoang et al., 2019; Vo et al., 2019). There is still little known about the factors regarding increased occurrence of CPV infection in dogs in Vietnam. Thus, our study aimed to investigate risk factors as potential drivers associated with canine parvovirus in dogs. The results of study will serve to increase awareness of dog owners in CPV prevention and support veterinarians in early treatment of CPV in dogs in Vietnam.

2. Materials and Methods

2.1. Study area and dogs' selection

The study was conducted at a veterinary clinic located in Thu Duc District, Ho Chi Minh city from January 2020 to June 2020. Dogs delivered to the clinic with typically CPV clinical suspicion (i.e., smelly haemorrhagic diarrhea, vomiting, rapid dehydration, lethargy and anorexia) (Oliveira et al., 2018) and confirmed using Rapid Immune-migration (Rapid CPV Ag Test Kit, Zhenrui Biotech Inc., Shenzhen, China) were included in a group named 'CPV diseased dogs'; this group was defined as cases in the study. Besides, another group named 'normal dogs', defined as controls, was healthy dogs carried for other services provided at clinic (i.e., vaccine injection, grooming, spa) with the criteria of normal vital signs (body temperature 38 - 39°C, heart rate 70 - 120 beats/min, respiratory rate 18 - 34 breaths/min) and healthy appearance (moist nose, pink gums, bright eyes without watering, smooth and supple skin, glossy coat, agile signs) (Bukowski & Aiello, 2011). The clinic had a service of weekly calling to dog owners to ask about pet's health condition; healthy dogs were defined in controls were those without any announcement of CPV disease signs after a week from the date dogs brought to the clinic. This criterion helped to define 'normal dogs' were truly healthy and had no infection of parvovirus since the incubation period of CPV in dogs is ranged from 3 - 7 days (Lamm & Rezabek, 2008).

2.2. Detect canine parvovirus by rapid immuno-migration

The test selection was relied on its absolute sensitivity (96.9%) and specificity (96.9%) in a good agreement with hemagglutination inhibition (HI) assay considered as 'gold standard' for detecting CPV in dogs (VETSCAN, 2018.). The principle of the rapid test is to detect specific epitopes of a soluble antigen of all strains of canine parvovirus shed in diseased dog's feces by using Rapid immuno-migration. Samples containing CPV antigen when put into contact with sensitized particles would create a complex which then migrates along a membrane before captured on a sensitized reaction zone where the cumulative concentration could result in a clearly visible purple band. Another purple band located in opposite side of the membrane on the kit is a control band to ensure the test was performed correctly.

2.3. Data collection

The dog owners in both groups of cases and controls were asked to participate in the study and approve providing their responses to structured questionnaire. The questions focused on groups of information could be potential risk factors affecting occurrence of parvovirus in dog including: (1) demographic characteristics of dog owners: age, gender; (2) dog-related information: age, gender, breed of dogs; and (3) dog-caring practice: CPV vaccination status, dog-keeping habits, raised with other dog/s, interaction with neighbour dog/s. Details of participant information sheet (PIS) and information consent form (ICF) have been read carefully and signed by all participants in study.

2.4. Statistical analyses

Risk factor analyses for outcome variable of CPV status (CPV diseased dogs/ normal dogs) were carried out by logistic regression. The explanatory variable investigated were: (1) dog's owner gender (male/female); (2) dog's owner age (years); (3) dog's gender (male/female); (4) dog's

age (months); (5) breed of dog (Vietnamese native/ foreign/ mixed); (6) CPV vaccination status (yes/no); (7) dog-keeping habit (mostly inside the house/ freely outside access/ restrictively outside access); (8) raising with other dog/s (same-aged dog/ different-aged dog/ no); and (9) interaction with neighbour dog/s (yes/no/unknown). Stepwise approach was applied to select a final model of multivariable model. Univariable models were screened and those with $P < 0.20$ were kept as a candidate for final model. Variables of multivariable model with $P < 0.05$ were considered as significant factors (risk/protective) associated with infection of parvovirus in dogs. All statistical analyses were done using R statistical software (The R, 2020).

3. Results and Discussion

3.1. The selection progress of study dogs

Over study period of 6 months, a total number of 44 cases of CPV infection were investigated by the rapid test. In 'normal dogs' group, a total number of 107 dogs participated in the study. However, since all 44 CPV infected dogs were all less than 6 months old, in order to avoid selection bias, we classified 'normal dogs' group and selected only 88 dogs (out of 107) with less than 6-month age as non-cases or controls for epidemiological analyses. The geographical locations of a total number of 132 study dogs (44 cases and 88 controls) are presented in Figure 1.

3.2. Descriptive characteristics of dog's owners and dog-related information

Demographic information of dog's owners and dog-related characteristics are described in Table 1. Out of 44 CPV infected dogs, the proportion of dogs injected CPV vaccine was 25.0% (11/44), whereas 81.8% (72/88) dogs in normal group had vaccination of CPV ($P = 0.005$). More than half of study dogs (50.8%) were foreign breed (i.e., Poodle, Dachshund, Pomeranian, Rockweiler, Cardigan Welsh Corgi, Pekingese, etc.), the remaining dogs were equal (∼25%) in Vietnamese native breed (Indochina dingo, Phu Quoc ridgeback) and mixed breed (Indochina dingo cross-bred with foreign breed); the highest morbidity of CPV infection was in Vietnamese native breed (40.9%), while in foreign and mixed breed the morbidity were 36.4% and 22.7% respectively ($P = 0.005$). Study dogs were mostly kept inside the house, the percentages were 54.6% and 64.8% in both CPV diseased and normal group; however, in case group the percentage of dogs freely outside access was relatively high, accounted for 22.7% (10/44) compared with small percentage 5.7% (5/88) in control group ($P = 0.01$). Dogs raised with the same-aged dogs had morbidity of parvovirus in case group (22.7%) higher than those in normal group (8.0%) ($P = 0.035$). Besides, history of interacting with neighbour dog/s between two groups was significantly different, 50.0% for case group and 33.0% for control group ($P = 0.014$).

3.3. Risk factor analyses

In the multivariable model, three variables of CPV vaccination status, raising with other dogs and interaction with neighbour dogs remained significant from the univariable models (Table 2). Unvaccinated dogs had 11.76 times more risk of getting parvovirus disease than vaccinated ones (OR = 11.76, $P < 0.001$). The risk of CPV infection in dogs raised with other same-aged dogs was 5.01 times (OR = 5.01, $P = 0.027$) higher than those raised singly. Dogs having interaction with neighbour dog/s had 3.13 times (OR = 3.13, P = 0.028) increased risk of getting CPV than dogs without any contact with other nearby dog/s. Variables of Breed and Dog-keeping habit became non-significant in final model since they were confounded by variable CPV Vaccination status (χ^2 Test, $P = 0.001$; Fisher's Test, $P = 0.002$).

3.4. Discussion

To our knowledge, this is the first study investing of factors associated with CPV infection in Vietnamese dogs. We conducted a case-control study with case group was identified using the rapid antigen detection test, and control group was defined based on vital and healthy signs at the time dogs delivered to the clinic and without any announcement of getting disease a week afterward. Control group was therefore appropriate and comparable to the case group in estimates of expected exposures in our study.

In our study, all participants were raising less than 6-month dogs in both groups. The selection bias was considered by exclusion of dogs higher than 6 months of age to get truly representative population from which the cases might have expo-

Table 1. Descriptive characteristics of dog's owners and dog-related information

Characteristics		CPV diseased dogs (n = 44)	Normal dogs (n = 88)	*P*-value
Owner's age* (Median [IQR])		35.0 [29.8-40.0]	33.5 [28.0-39.0]	0.542
Owner's gender** (%)	Male	23 (52.3%)	34 (38.6%)	0.192
	Female	21 (47.7%)	54 (61.4%)	
Dog's age** (%)	$\leq$ 3 months	30 (68.2%)	52 (59.1%)	0.409
	$>$ 3 months	14 (31.8%)	36 (40.9%)	
Dog's gender** (%)	Male	29 (65.9%)	50 (56.8%)	0.415
	Female	15 (34.1%)	38 (43.2%)	
Breed of dogs** (%)	Vietnamese native	18 (40.9%)	14 (15.9%)	0.005
	Foreign	16 (36.4%)	51 (58.0%)	
	Mixed	10 (22.7%)	23 (26.1%)	
CPV vaccination status** (%)	Yes	11 (25.0%)	72 (81.8%)	0.005
	No	33 (75.0%)	16 (18.2%)	
Dog-keeping habit*** (%)	Mostly inside the house	24 (54.6%)	57 (64.8%)	0.010
	Freely outside access	10 (22.7%)	5 (5.7%)	
	Restrictively outside access	10 (22.7%)	26 (29.5%)	
Raising with other dogs** (%)	Yes (same-aged dog/s)	10 (22.7%)	7 (8.0%)	0.035
	Yes (different-aged dog/s)	12 (27.3%)	21 (23.9%)	
	No	22 (50.0%)	60 (68.1%)	
Interaction with neighbour dog/s** (%)	Yes	22 (50.0%)	29 (33.0%)	0.014
	No	15 (34.1%)	57 (64.8%)	
	Unknown	7 (15.9%)	2 (2.2%)	

*Wilcoxon Test, **χ^2 Test, ***Fisher's Test.

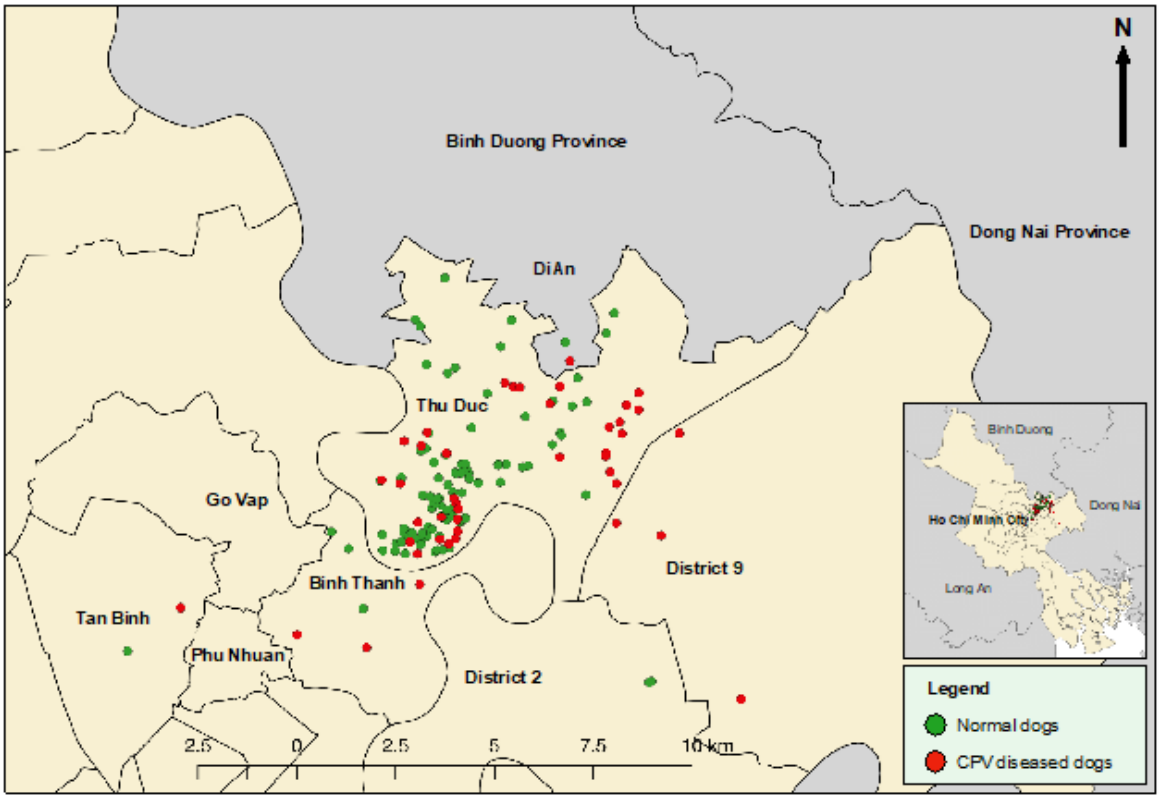

Figure 1. Map of study area in Ho Chi Minh City and participants' geographical locations. The geographical coordinates were collected on Google Map based on the exact addresses provided by participants. Vector layers of quantum geographic information system (QGIS 2.14) were applied to plot the coordinates on the map.

sure (Lamorfe, 2020), but still maintain the statistical power by getting more than one control for every case (the ratio of 2 controls: 1 case in our study) (Lewallen & Courtright, 1998). Besides, participants were raising dogs within duration of up to 6 months, the time was supposed not too long from their memories when they responded to the questionnaire. This helped to reduce recall bias, a common problem in case-control study (Boone et al., 2012).

Our finding indicated the critical importance of vaccination in CPV prevention in dogs. This was consistent with previous studies conducted in Portugal and Nigeria (Mohammed et al., 2008; Miranda et al., 2015) where unvaccinated dogs had a risk of infecting CPV higher than dogs injected CPV vaccine 2.00 and 25.14 times, respectively. In addition, exposure of naive dogs to the feces of CPV-2 infected animal or fomites was also important risk factors of CPV infection (Hsu et al., 2003). Our study found the increased incidence of CPV in dogs who had interactions with other neighbour dogs as well as lived with other same-aged dogs. Therefore, the recommendations for inhibition of dog free outside access and cleaning and disinfection of housing, bedding and other materials in contact with affected animals should be thoroughly performed on a regular basis (Lamm & Rezabek, 2008).

Generally, in Vietnam core vaccination of a multivalent modified-live viral (MLV) vaccine is recommended at 8^{th} - 10^{th} weeks of age, and an additional booster vaccination at 12^{th} - 14^{th} weeks, then a booster vaccination is administered in subsequent years. There were 11 cases of clinical illness in vaccinated dogs in our study with the age median of these dogs was 2.5 months [IQR 1.5-3.0] and all were the first vaccinations. There has been shown that overwhelming viral dose while antibodies yielded from only one shot of vaccination were inadequate to protect dogs (Lamm & Rezabek, 2008). In addition, the susceptibility for CPV infection in pups actually begins 2-3 weeks before the waning of maternal an-

Table 2. Logistic regression models investigating risk factors associated with occurrence of canine parvovirus in dogs

Variables	Univariable Model		Multivariable Model*	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Breed (Baseline=Foreign)				
Vietnamese native	4.10 (1.67 - 10.00)	0.002		
Mixed breed	1.39 (0.54 - 3.52)	0.492		
CPV vaccination status (Baseline=Yes)				
No	13.50 (5.65 - 32.26)	< 0.001	11.76 (4.55 - 30.38)	< 0.001
Dog-keeping habit (Baseline = mostly inside the house)				
Freely outside access	4.75 (1.47 - 15.38)	0.009		
Restrictively outside access	0.91 (0.38 - 2.18)	0.839		
Raising with other dogs (Baseline = No)				
Yes - same aged dog/s	3.90 (1.32 - 11.50)	0.134	5.01 (1.20 - 20.98)	0.027
Yes - different aged dog/s	1.56 (0.68 - 3.69)	0.312	1.85 (0.60 - 5.70)	0.281
Interaction with neighbour dog/s (Baseline = No)				
Yes	2.88 (1.30 - 6.38)	0.009	3.13 (1.13 - 8.68)	0.028
Unknown	13.30 (2.50 - 70.74)	0.241	20.93 (2.91 - 75.72)	0.251

Legend: The tables illustrates only significant variables in univariable models, considered as candidates for multivariable model. *Model intercept = -2.923, SE = 0.526.

tibodies at 8-12 weeks of age (Nandi & Kumar, 2010), the neutralization of early viral vaccine by maternal antibodies could be a reason interpreting for these cases. Besides, the possibility of CPV infection with variant wide-type strains could be eliminated since the demonstration of cross-protection among virus types by multivalent modified-live viral vaccine were reported in previous studies (Truyen, 2006; Glover et al., 2012).

Our study had several limitations: We conducted convenience sampling relied on dogs delievered to the clinic within six months, spatial and temporal data were not fully collected to have a comprehensive description of risks associated with CPV in dogs. Since a lack of information about the time of vaccination in both study dogs and their mothers, the effects of vaccination on CPV disease in dogs, particularly vaccinated dogs, were unable to precisely interpret. However, to a certain extent, we believe that our findings can be extrapolated to other dog populations raised in other areas in Ho Chi Minh city, Vietnam.

4. Conclusions

Our findings highlight that improving the awareness in CPV vaccination and caring practice of dog owners are the crucial factors to reduce the risks of CPV infection in dogs. There is a need for follow-up studies on spatio-temporal epidemiology of CPV to have problem-solving approaches towards canine parvovirus, one of the most currently popular diseases in dogs in Vietnam.

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Conflict of interest declaration

The authors declare no conflict of interest.

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Assessing the status of mechanization and proposing technical solutions for lime farming in the Mekong River Delta

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ABSTRACT

This study was conducted to assess the current status of mechanization in lime growing activities of farmer households in the Mekong River Delta. The current status of mechanization was assessed through different groups of criteria that reflect the scale of mechanization level, resources of farmers applying mechanization, results, and production efficiency. The level of machine and technology application was assessed by building the mechanization index. With a sample of 555 lime farmer households in Long An and Ben Tre, the results showed that the application of machines and equipment in lime growing activities in the study area was quite limited. Households mainly used machines in two stages including land preparation (42% of households applying mechanization) and tree care (93%). The growth rate and mechanization investment of the 2016-2019 period were almost unchanged. The estimated results of mechanization index showed that most of lime growers had a very low mechanization application level with an average value of only 0.09. In fact, 2% of the surveyed households do not invest in mechanization in lime production, 89.8% are in the group with very low mechanization level, 3.1% at low mechanization level, only 5.1% at medium mechanization level and no household had high mechanization application level. From the survey results, potential technical solutions for lime farming in the Mekong River Delta were also proposed, including mechanization for small and medium farms and mechanization considerations for plant care. Finally, in order to improve the quality of lime fruit, the semi-mechanization harvesting systems and appropriate storage facility and suitable packaging are highly recommended.

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1. Introduction

In recent years, lime tree planting activities in the Mekong River Delta have achieved good results and efficiency, bringing income to lime farmers. Especially, limes grown under VietGAP model with good appearance, uniform size, and without pesticide residue are exported. In Long

An, lime export value is always over 30% of the total fruit export turnover of the province. Recently, many households growing limes can earn a profit of 30 - 40 million VND per 1,000 m^2. In the Mekong River Delta, lime trees are grown in Long An and Ben Tre while they are also planted in small areas of some other provinces in the region. Over the years from 2015 to 2019, the lime production area in Long An increased sharply from 6,734.01 ha to 10,825.72 ha. Meanwhile, the planting area of lime in Ben Tre tended to increase from 2015 to 2018 and then has decreased slightly after 2019 (a decrease of 91 ha).

Along with the development of lime growing activities is the promotion of mechanization application in agricultural production according to the Government's orientation. Vietnam's agricultural sector has set a target of a level of 80% - 100% mechanization at stages in agricultural production and of 5 - 6 HP/ha for the average capacity of machinery and equipment nationwide by 2030. In producing areas of agricultural commodities, the level of mechanization is synchronized and automated (Ngoc, 2020). The benefits of applying mechanization and high technology in agricultural production have been studied and confirmed by many authors. According to Bello (2013), agricultural mechanization helps to increase labor productivity, increase production efficiency per unit area, and reduce production costs of manual labor or animals. Singh & Singh (1972) concluded that farms with tractors yielded higher yields of wheat, rice, and sugar cane than farms without tractors. Similarly, Nandal (1986) stated that farms with tractors produced higher yields of wheat and rice, used more production inputs, and managed better uptime. Balishter & Singh (1991) studied farm yields at three different levels of mechanization. The results showed that fully mechanized farms had 10 - 27% higher yields, partially mechanized farms had 2 - 26% higher yields than completely non-mechanized. In addition to the above benefits, agricultural mechanization also created indirect jobs for people involved in the operation, repair, and maintenance of machinery and equipment (Verma, 2006).

However, in the agricultural sector in the Mekong River Delta, the application of high technology in production, processing and preservation remains limited. Sustainable production models, that adapt to climate change, also do not have enough technical and market basis, as well as strong motivation and support to replicate the model. According to the Department of Science, Technology and Environment (Ministry of Agriculture and Rural Development), climate change and sea level rise in the Mekong River Delta are happening faster than the forecast (Thanh, 2019). The effects of sea level rise (SLR) on flooding and drainage in the lowlands of the Mekong River Delta are becoming increasingly apparent. In 2008 and 2009, due to the double impact of SLR and high tide, Kien Giang, Bac Lieu and Can Tho were heavily flooded. Even upland areas such as urban areas, residential areas and roads in some areas have been flooded. According to the Institute of Hydrometeorology, Hydrology and Environment - Imhen (2010), by 2050, the flooded area may have been up to 3,514,403 ha, accounting for about 89% of the whole area of the Mekong River Delta (natural land in the Mekong River Delta is about 3,936,000 ha), about 20% increase compared to the historic flood in 2000. In the context of climate change impacts, the promotion of mechanization brings direct benefits such as increasing productivity and profitability and also brings indirect benefits in contributing to climate change adaptation and mitigation.

According to Sims & Kienzle (2017), farmers, especially smallholders, always had to depend on a temperate climate for farming and food security. Today, due to widespread extreme and erratic weather and the effects of climate change increasing its frequency, small agricultural production systems are becoming more and more insecure. Therefore, Sims & Kienzle (2017) emphasized the role of mechanization to build a climate resilient agriculture that meets three factors including (1) increase productivity to provide food security; (2) adaptation to extreme weather events and long-term weather events affecting agriculture and food security; and (3) mitigate the effects of climate change through GHG (Greenhouse gas) emission reduction. Capaz et al. (2013) reported that the use of machinery in sugar cane harvest would help reduce 39.3% of GHG emissions in Brazil. Ennin et al. (2014) also demonstrated that applying mechanization during soil preparation and fertilization yielded higher yields than farmers' traditional practices in Ghana. Also, mechanization combined with a reduction in the use of piles will significantly reduce the impact of sweet potato production on deforestation and climate change.

In the above context, the objectives of this study were (1) analyzing the current situation and level of mechanization in lime growing activities in the Mekong River Delta, and (2) evaluating the mechanization level and the effectiveness of mechanization in climate change adaptation. The study results provided a general and comprehensive perspective on the development of high-tech agriculture in lime production, from which solutions can be proposed to improve the efficiency of machine application, and promote mechanization in lime production activities in the Mekong River Delta.

2. Materials and Methods

2.1. The concept of machinery and equipment

According to Circular 17/2019/TT-BKHCN of the MOST (2019), machinery and equipment are a complete structure, including details, detail assemblies and parts that are linked together to operate and move according to the intended use.

2.2. The concept of agricultural mechanization

According to Clarke (1997), agricultural mechanization is the process of improving the labor productivity of the farm through the use of agricultural machines and tools, from simple hand tools to high technology machines. Clarke (1997) identified agricultural mechanization as the application of tools, machinery as inputs for agricultural production. There are three resources used including human labor, draft cattle and agricultural machinery and they can be complementary in the production process. Starkey (1998) defined agricultural mechanization as the development of machinery and equipment applied in agricultural production to improve the efficiency of human labor, promptly respond to the seasons. According to Nguyen (2018), agricultural mechanization is the synchronous implementation of infrastructure, machinery, equipment and investment conditions suitable to the scale and production level of each region; above all, they are the large-scale concentrated commodity production areas and the heavy production stages.

Thus, mechanization in agricultural production is the process of using machines, tools and tools to partially or fully replace the force from humans or animals. Moreover, agricultural mechanization contributes to improve the efficient use of input factors such as increasing labor productivity, land productivity, and reducing production costs, resulting in improving income through increasing productivity and increasing production scale. Therefore, agricultural mechanization is also a prerequisite for large-scale production by expanding cultivated area (Bello, 2013).

2.3. Method of evaluating the degree of mechanization application

2.3.1. Evaluation of the status of mechanization application in production

To assess the status of mechanization application in agricultural production in farming households, this study applied the criteria system designed by Nguyen (2018) which was divided into different groups of indicators as follows: the group of indicators reflecting the scale and level of mechanization; group of indicators reflecting the growth of mechanization; the group of indicators reflecting the resources of farming households applying mechanization; and group of indicators reflecting production results and efficiency.

2.3.2. Evaluation of the degree of mechanization application in production

In previous studies, to quantify and classify the degree of mechanization application, the authors often applied a classification based on a mechanization index such as the mechanization level index in planting (Rasouli & Ranjbar, 2008) or the mechanization index reported by Nowacki (1978) and Minli et al. (2017). From the fluctuation of these indicators, it can be seen that the level of mechanization should be established for each study area. According to Nowacki's (1978) approach, the mechanization index is the ratio of machine energy (including fuel energy and machine energy) to the sum of the machine, human, and animal energy. The higher the mechanization index, the more work is done by machine.

To assess the level of mechanization application in lime production in the Mekong River Delta, in addition to statistical criteria such as the number of machines, types of machines, the cost of investment in machinery and equipment, this study estimated the Mechanization Index (MI) for lime growers. The MI index calculation was the basis to evaluate how high the level of application of mechanization of each farmer household was, as

well as help to compare it among different regions accurately and objectively. This study applied the mechanization index calculation formula including the cost factor of Singh (2006):

$$MI_{ij} = \frac{C_{MI_{ij}}}{C_{MI_{ij}} + C_{H_{ij}} + C_{A_{ij}}}$$

In which:

MI_{ij}: mechanization index of product i in household j;

CM_{ij}: the cost of using the machine of product i in household j;

CH_{ij}: labor cost of product i in household j;

CA_{ij}: cost of using the animal power of product i in household j.

2.3.3. Evaluation of the effectiveness of mechanization in climate change adaptation

To evaluate the effectiveness of the application of mechanization and technology in the context of climate change adaptation, the study was based on two assumptions that: (1) the higher level of mechanization applied by households, the higher financial efficiency is achieved; and (2) under the same conditions affected by climate change, households that apply mechanization and technology will achieve higher financial efficiency than households that do not apply mechanization. To test the above assumptions, the study applied the One-way ANOVA test to evaluate the difference among groups of households with different levels of mechanization application. The groups used in the analysis are distinguished as presented in Table 1.

The financial ratios used in this study included revenue, cost, profit, ratio of revenue/cost, rate of profit/cost, rate of profit/revenue. These financial ratios have also been applied by some previous studies to evaluate production efficiency (Nguyen, 2009; Duong & Nguyen, 2014).

2.3.4. Data collection

Secondary data related to the current situation of mechanization and technology application for the Mekong River Delta were collected through reports and statistics from Department of Agriculture and Rural Development and Statistical Offices of the provinces. Primary data in the study were collected through direct interviews with 555 lime growers in Ben Tre and Long An. These are the two provinces with the largest lime production areas in the Mekong River Delta, of which Long An has 9,165 ha, and Ben Tre has 2,300 ha of lime trees (BTSO, 2019; LASO, 2019). In Long An province, Ben Luc and Duc Hue districts are the two typical districts with the largest area of lime cultivation in the region (6,644.75 and 2,629.80 ha, respectively). As for Ben Tre province, Lime is grown prominently in Giong Trom district with 1,966 ha. All collected data were analysed using Microsoft Excel version 16.27 and IBM SPSS software version 25.

3. Results and Discussion

3.1. Assessing the status of mechanization application at lime farming households

3.1.1. Scale and level of mechanization

Most of the stages in the lime planting activity had a very low level of mechanization, the households mainly use machinery in the two stages of land preparation (42% of households applying mechanization) and take care of trees (93%). There were no households using machinery and equipment in the planting and harvesting stages. The results showed that transportation (26%) and the stage of storage and preservation (4%) were used by the households (Figure 1). For lime trees, the application of mechanization is recognized mainly in two stages: soil preparation and tree care. Specifically, to prepare the soil for planting, machines such as cultivators and Kobe excavators are used by farmers. In the tree-care stage, the households have invested in spraying machines, pumps, and weeding to support farming activities, such as discharging water with pumps during the rainy season (water will damage the lime tree without draining).

One feature of the application of mechanization in lime cultivation is that households rent machines or use mechanized services, or invest in the machines themselves. According to the survey results, farmers often rent machinery, or use mechanized services during the land preparation stage, or invest in machinery and equipment themselves during the tree care stage. The cost of hiring a land preparation service varies according to the production area and the type of machine hired. For example, the renting cost of the Kobe excavator is about 2.5 million VND per ha; whereas

Table 1. Classification of different households groups with different levels of mechanization application

Object	Group	Explanation
Households	No mechanization	No mechanization application, MI = 0
	Very low mechanization	$0 < MI \leq 0.25$
	Low mechanization	$0.25 < MI \leq 0.5$
	Medium mechanization	$0.5 < MI \leq 0.75$
	High mechanization	$0.75 < MI$
	Affected by climate change	Households report they are affected by climate change
	Not affected by climate change	Households report they are not affected by climate change

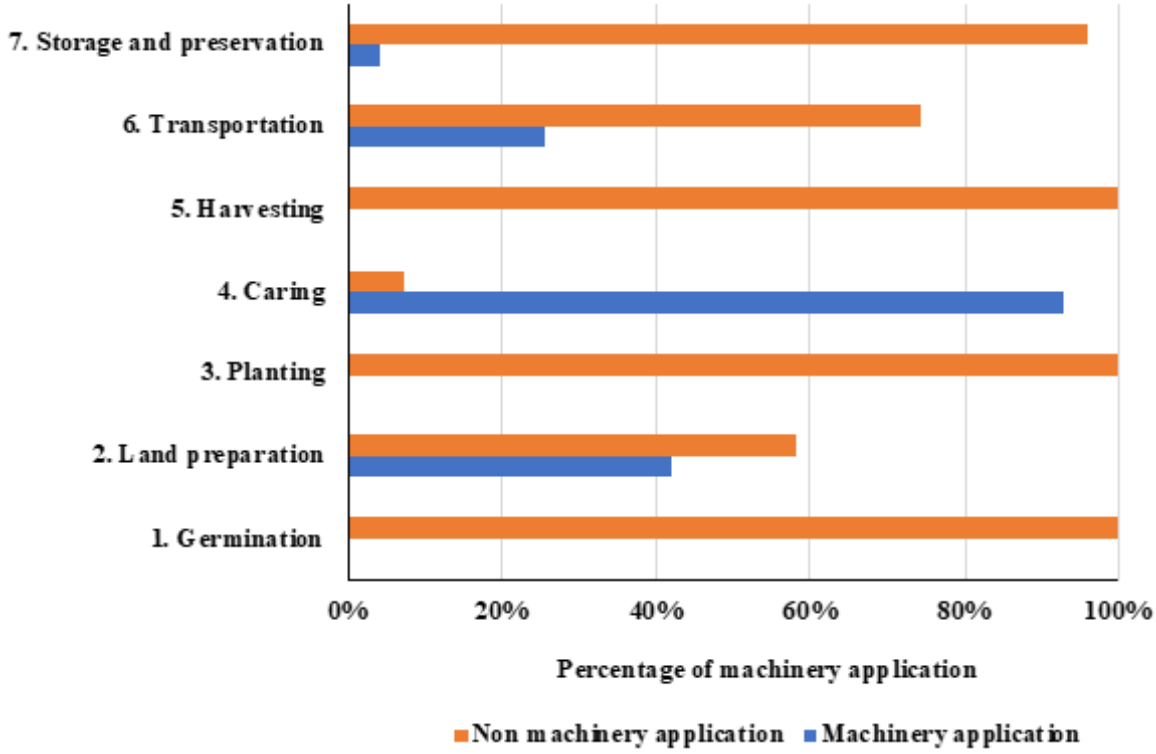

Figure 1. Rate of machinery application in lime growing.

the cost is about 16 million VND per ha for multi-purpose cultivators (50 horsepower, HP). Most of the cultivators are Yanmar brand. The investing cost of machinery during the tree-care stage of lime farmers varies depending on the brands and capacity of machines. On average, the spraying machine is from 4 - 4.5 million, the water pump is from 1 - 8 million depending on the type, the lawn machine is from 6.6 - 8 million.

In Long An, the use of a mist sprayer (airplane, smoke spraying in the wind direction) was tested, but it was ineffective partly due to the design of the machine's operation mechanism. It is due to the characteristics of the lime tree, including large leaf foliage, pests from tree trunks and roots. The stages of planting and harvesting are mainly done by hand. However, it should be noted that the above machines do not completely replace manual labor in the respective stages, only help the lime planting activity shift from full manual labor to the level of mechanization in part and machines. In addition, equipment applied are not completely automated, they are mainly operated by human beings.

The status of mechanization application between the two areas studied also shows noticeable

Table 2. Comparison of mechanization rate between Ben Tre and Long An

		Provinces	
		Ben Tre	Long An
Germinating	Machinery application	0.0%	0.0%
	No machinery	100.0%	100.0%
Land preparation	Machinery application	1.9%	88.9%
	No machinery	98.1%	11.1%
Planting	Machinery application	0.0%	0.0%
	No machinery	100.0%	100.0%
Caring	Machinery application	86.8%	100.0%
	No machinery	13.2%	0.0%
Harvesting	Machinery application	0.0%	0.0%
	No machinery	100.0%	100.0%
Transport	Machinery application	15.1%	37.8%
	No machinery	84.9%	62.2%
Harvesting	Machinery application	0.0%	0.0%
	No machinery	100.0%	100.0%
Storage and preservation	Machinery application	0.0%	8.9%
	No machinery	100.0%	91.1%

differences (Table 2). The general results show that lime growing households in Long An had a higher rate of mechanization than that in Ben Tre (33.7% versus 14.8%). In all production stages, Long An province always has a higher rate of mechanization. The most obvious difference is in the land preparation stage because 88.9% of the households in Long An have used land preparation service, while in Ben Tre only 1.9% of those have used the service, the rest prepare the land with manual labor.

3.1.2. Agricultural mechanization growth

Regarding the agricultural mechanization growth rate over the years, most of the machinery was invested by households before 2016, so the rate of mechanization in the 2016 - 2019 period remained generally unchanged. The reason for the low growth rate in mechanization application can be explained by several reasons. First, the machines being invested by farmers such as pumps, lawn mowers and sprayers were devices of which the structure and design were not too complicated, with a quite long service life (from 5 to 10 years). As such, after the initial investment, farmers could use it for many years. In addition, over 40% of surveyed households said that repairing and maintaining machinery is relatively simple, the repair cost is relatively low. As such, they do not need to invest and/or buy replacement equipment.

Second, about 94% of the households said that they have not received support for the cost of purchasing production support machines and 77% have not undergone training in using machinery and equipment in lime production. According to the survey, most farmers do not have access to new production techniques and technologies, but still produce from traditional experiences. Thus, they do not require equipment in production technology lines or modern farming techniques. About 68% of the farmers said that they do not need to invest in applying additional machinery in the future.

3.1.3. Group of indicators reflecting the resources of households applying agricultural mechanization

A comparison of the resources of lime farmers between the two surveyed areas shows a difference. It is noticeable that the lime planting area and the average value of the machines invested in Long An are significantly higher than those in Ben Tre (Table 3). This figure accurately reflects the real situation. In Long An in 2019, the lime planting area is 10,826 ha, reaching 103% of the plan, as much as 118% compared to 2018; the area of fruiting lime trees is 8,913.4 ha. Lime trees are mainly grown in Ben Luc district (5,393.3 ha), Duc Hue (2,612 ha). In Ben Tre, lime trees are mainly grown in Giong Trom district (2,022 ha). In districts like Cho Lach, Chau Thanh, Ba Tri,

Table 3. Comparison of lime production resources between Ben Tre and Long An

			Provinces	
			Ben Tre	Long An
Education level	Mean		8^{a}	10^{b}
Total household members	Mean		4^{a}	4^{a}
Number of lime growers	Mean		2^{a}	2^{a}
Number of laborers who know how to use machines	Mean		2^{a}	2^{a}
Number of laborers who receive training in machinery	Mean		0^{a}	1^{b}
Number of years growing lime/grapefruit	Mean		18.9^{a}	10.3^{b}
Specialized in lime cultivation	No	Column N%	26.4%	15.6%
	Yes	Column N%	73.6%	84.4%
Lime - Crops	No	Column N%	81.1%	97.8%
	Yes	Column N%	18.9%	2.2%
Lime planting area	Mean		0.4218^{a}	2.1794^{b}
Average machine value	Mean		0.51^{a}	2.09^{b}

Values in the same row and subtable not sharing the same subscript are significantly different at $P < 0$ .05 in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances.[1]
[1]Tests are adjusted for all pairwise comparisons within a row of each innermost subtable using the Bonferroni correction.

Binh Dai, they are only planted with an area of less than 100 ha. The average size of lime orchards of households in Long An is four times larger than that of households in Ben Tre (2.09 ha compared to 0.51 ha). As a result, it can be seen that Long An lime growers spend much more money on production mechanization than in Ben Tre.

3.1.4. Results and production efficiency of household applying mechanization

Regarding the results and efficiency of growing lime (Table 4), Long An province showed better results than Ben Tre. Thanks to the advantages of large scale and high investment costs, the results from lime production of farmers in Long An were significantly higher and different from those in Ben Tre, which were statistically significant in all terms of output, revenue, total cost, and profit (Table 3). Good business results and high profits can be seen as a premise for farmers to continue to reinvest in lime planting, expand the scale and increase investment in machinery and equipment.

For 2019, the estimated total output of lime harvested in Long An province reached 156,126 tons. The price of limes with seeds ranged from 4,000 - 15,000 VND/kg, fell 3,000-10,000 VND/kg over the same period; seedless limes ranged from 10,000 - 20,000 VND/kg, fell 5,000 - 10,000 VND/kg. Farmers had a profit of 70-150 million VND/ha/year. In Ben Luc district of Long An province, the seedless lime tree has become the staple crop of the locality after being piloted by farmers since 2002. Since 2011, the exclusive brand name Ben Luc lime has been built. By 2014, Ben Luc district officially established Thanh Hoa Agricultural Service Cooperative, opening a new direction for Ben Luc lime trees, developing the area scale of seedless lime, building a specialized farming area, orientating the development of production model according to VietGap and GlobalGap standards. The consumption market for limes in the district is quite diverse, such as domestic consumption and export through official channels, through unofficial channels, exported to European markets, Middle East countries and regional countries.

Table 4. Results of lime production of households in Ben Tre and Long An

	Provinces	
	Ben Tre	Long An
Yield of 2019	2.68^{a}	49.90^{b}
Revenue from cultivation	142.58^{a}	535.67^{b}
Total cost of growing lime	30.62^{a}	147.45^{b}
Profits from growing limes	101.67^{a}	380.03^{b}
Revenue/Cost	3.83^{a}	5.60^{a}
Profit/Cost	2.83^{a}	4.60^{a}
Profit/Revenue	0.23^{a}	0.48^{a}

Values in the same row and subtable not sharing the same subscript are significantly different at $P < 0.05$ in the two-sided test of equality for column means.

Table 5. Comparison of mechanization index between Ben Tre and Long An

		Provinces	
		Ben Tre	Long An
Mechanization index of farm households (in groups)	No mechanization	3.8%	0.0%
	Very low mechanization	94.3%	84.4%
	Low mechanization	0.0%	6.7%
	Medium mechanization	1.9%	8.9%
	High mechanization	0.0%	0.0%

In contrast, in Ben Tre, lime is not the most prominent crop among citrus trees. Regarding citrus fruit trees, the most planted trees were grapefruit (8,824 ha), lime (2,300 ha), oranges and tangerines (1,980 ha). In addition, the province also has other prominent fruit trees such as rambutan (5,5330 ha), longan (2,455 ha), durian (2,216 ha), and mangosteen (1,230 ha) (BTSO, 2019). Therefore, the scale, level of investment, intensive farming and production efficiency were lower than those in Long An province.

3.2. Estimating the mechanization index of farming households

The estimated results of mechanization index show that most of the lime growers had very low levels of mechanization application. The mechanization index of the surveyed households ranged from 0 to 0.60, of which the average value was only 0.09. More specifically, 2% of the surveyed households did not invest in mechanization in lime production, 89.8% were in the group with very low mechanization, 3.1% at low mechanization, only 5.1% at medium mechanization level and no household had high level of mechanization application (Figure 2).

The reality of applying mechanization in lime production tends to depend more on the scale of agricultural production than on the resources of the application. Specifically, households, that do not apply mechanization, have 23 years' experience in growing grapefruit. There is only one person in the household participating in growing lime. While households with higher mechanization level have experience in lime production less than 15 years, the number of people involved in lime production per household is 2. The scale of agricultural production in general and lime cultivation in particular increased in the same direction with the application of machinery in production.

Non-mechanization households only have about 0.21 ha of agricultural land, of which 0.125 ha is used for growing limes; while households with very low and low MI indexes have a total agricultural land area of about 1.46-1.48 ha, of which about 1.22-1.46 ha is for lime trees; the medium mechanization households have about 1.92 ha of agricultural land/household, of which about 1.66 ha is for growing limes. Lime production in 2019 also increased corresponding to the levels of the MI index (1.5 tons in non-mechanization households, 16.96 tons in households with very low MI, 19.33 tons in households with low MI, and 166.8 tons in households with medium MI).

The correlation between the scale of agricultural production and the degree of application of machinery in production was again shown through the distribution of the MI index by two provinces (Table 5). In which, households who did not use machines to grow limes were completely distributed in Ben Tre and 94.3% of surveyed households in Ben Tre had very low mechanization. In Long An province, although the majority of lime growers were also very low mechanization (84.4%), there was an additional distribution of households with low MI (6.7%) and medium (8.9%).

3.3. Evaluation of the effectiveness of mechanization in climate change adaptation

For the total number of surveyed households, only 2% of households reflect that they were not affected by climate change at all. Therefore, to evaluate the effectiveness of the application of machines in climate change adaptation, this study examined financial efficiency indicators within groups of households affected by climate change and groups at different levels of mechanization (Table 6). The statistical results indicated that there was non-significant difference in groups with different levels of mechanization af-

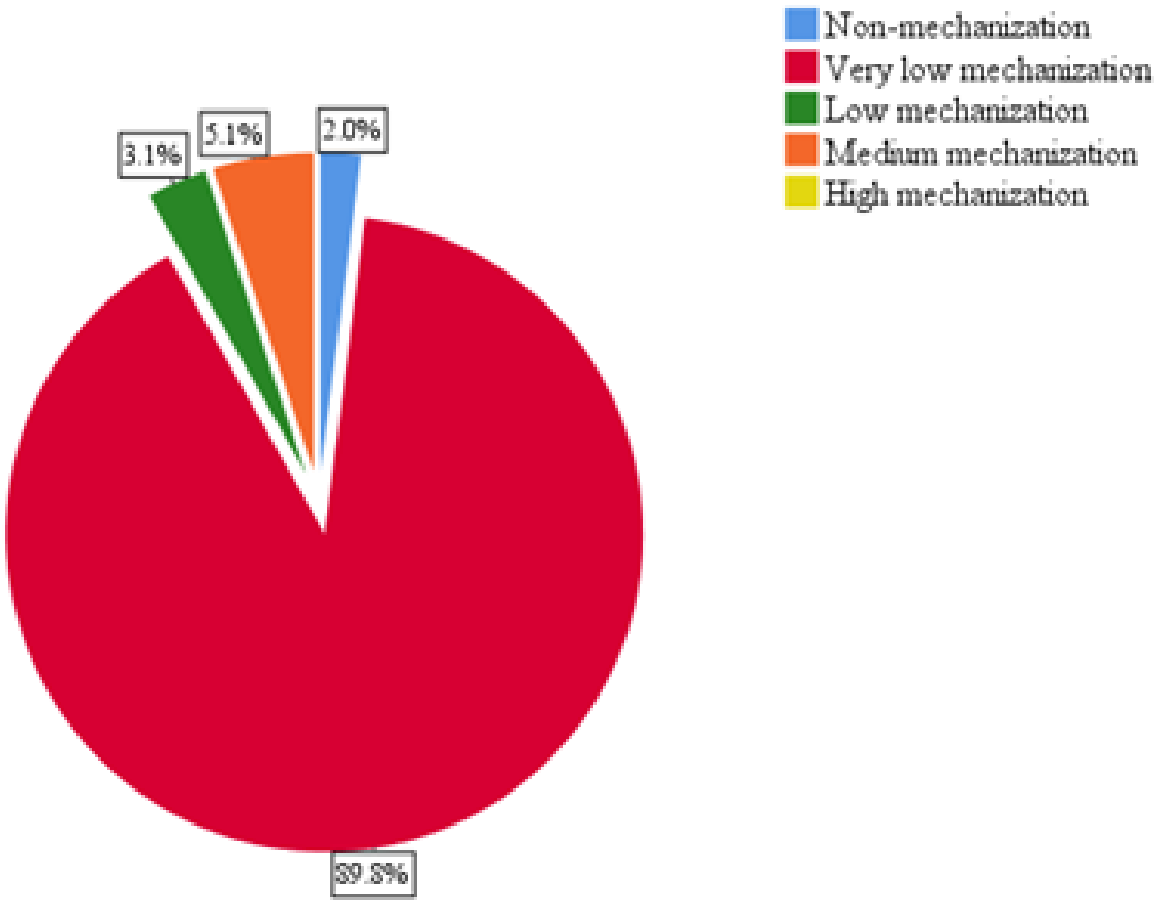

Figure 2. Mechanization index distribution of lime growing households.

Table 6. Results of ANOVA test

	Sum of Squares	df	Mean Square	F	Sig.
Revenue from cultivation	Between Groups	1234717.073	3	411572.358	1.474	0.221
	Within Groups	128728995.119	461	279238.601		
	Total	129963712.192	464			
Total cost of growing lime	Between Groups	58173.027	3	19391.009	1.399	0.243
	Within Groups	6391571.986	461	13864.581		
	Total	6449745.013	464			
Revenue/Cost	Between Groups	283.008	3	94.336	1.141	0.332
	Within Groups	38119.311	461	82.688		
	Total	38402.319	464			
Profit/Cost	Between Groups	283.008	3	94.336	1.141	0.332
	Within Groups	38119.311	461	82.688		
	Total	38402.319	464			
Profit/Revenue	Between Groups	6.248	3	2.083	4.133	0.007
	Within Groups	232.282	461	0.504		
	Total	238.530	464			

fected by climate change in terms of production efficiency, except for the rate of profit/revenue. Thus, it is impossible to confirm that the application of machinery in production really helps

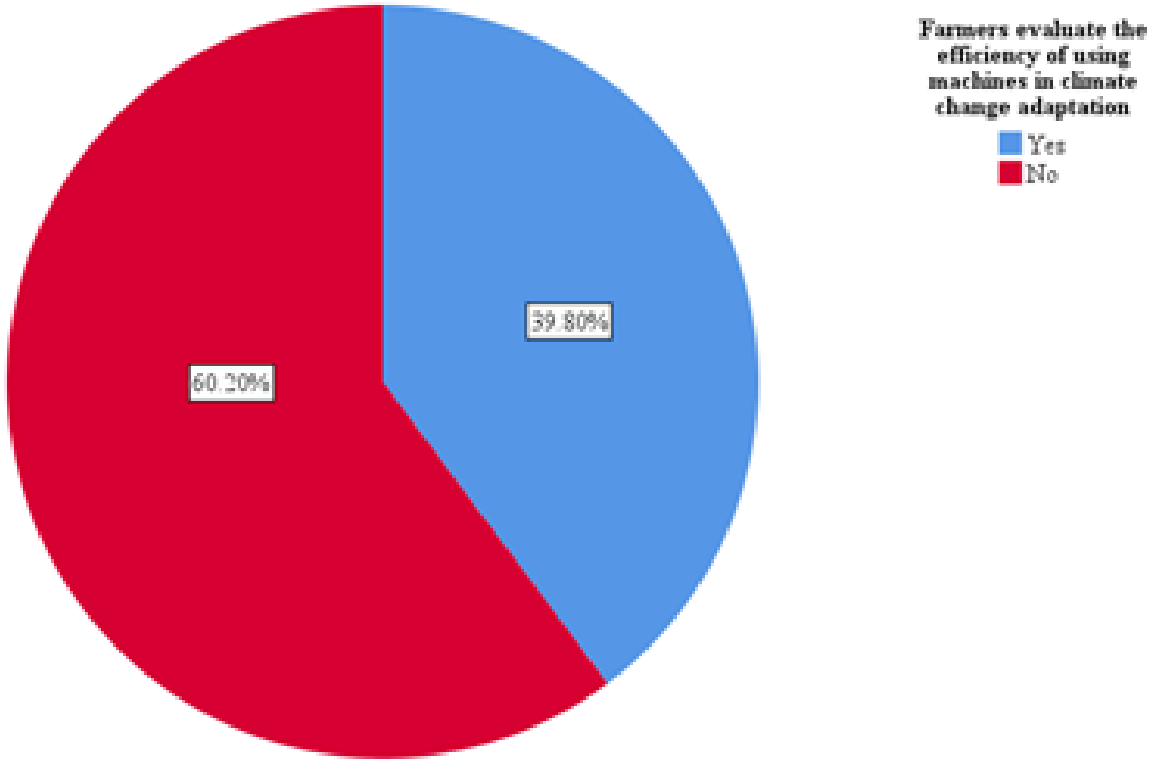

Figure 3. Farmers evaluate the efficiency of using machines in climate change adaptation.

farmers to adapt to climate change.

The negative effects of weather and production conditions that farmers reflected the most are drought, lack of water (68.4%), aluminum contamination (61.2%), and increased disease (43.9%). Although the proportion of households thinks that they are affected by climate change on agricultural production, the majority of farmers self-assess that the use of machinery and equipment is not helpful for climate change adaptation (Figure 3). This assessment is mainly because the machines used at the present are mostly simple ones, supporting and reducing manual labor, the investment in complete machine systems under a certain high-tech model is very little. This result is similar to the reviews by Nguyen et al. (2017).

4. Improvements for the lime production in the Mekong River Delta

The current analyses showed that the mechanization in lime production in the Mekong River Delta is very limited and lacks synchronization. However, the findings also demonstrate that the lime industry is highly potential in large scale production. In this part, the prospects of improvements for the lime production in the Mekong River Delta need to be discussed.

4.1. Mechanization for small and medium farms

The big size of agricultural machines does not always bring benefits. The large machine is more viable when working in large land areas. According to Blackmore et al. (2008), two constraints for applying mechanization of small farms are the unsuitable condition for large machine operation and the too much capital investment for the big machine. The lime farms in the Mekong River Delta area are characterized with small and medium scales (from 0.5 to 2 acres) and thus considerations need to be taken when improving the mechanization. One of the main benefits of mechanization using a big machine is to reduce the labor cost in the labor-shortage areas (Schmitz & Moss, 2015). The Mekong River Delta area has not encountered a lack of labor, and thus the large-scale machines focusing on reducing the labor costs seem not to be very effective. The mechanization in lime production in the Mekong River

Delta is currently far behind the expectation but the feasibility of improvement should focus on the small and smart machine that suitable for small and medium farms.

4.2. Mechanization considerations for plant care

4.2.1. Irrigation and fertilizing using an open hydroponic system (OHS)

The effectiveness of lime production relies much on plant care techniques where the application of novel technology and mechanization become competitive advantages. The advanced techniques in plant care of lime orchards involve in the efficiency of irrigation and nutrient management. The lime growers in the Mekong River Delta may be very experienced in the field but need to be updated with the latest technology to improve productivity. One of the advanced technologies is the open hydroponic system. The OHS (open hydroponic system) has been successfully applied in many crops such as citrus, avocados, peaches, almonds, grapes (Morgan & Kadyampakemi, 2012). The OHS brings many benefits for lime production such as maximize the efficiency of water and nutrient uses, accurately deliver nutrients to improve its availability, modulate roots in the expected zone and reduce nutrient leaching (Morgan & Kadyampakemi, 2012). Though it has not been investigated in lime production yet, the OHS has been reported to be highly suitable for citrus production. Regarding the horticulture practice, the OHS increased the citrus yield by over 20% that has been widely reported (Morgan et al., 2009; Prazeres et al., 2017; Ferrarezi et al., 2020). In particular, five-year observation of using the OHS in the citrus orchards infected by Huanglongbing (HLB) disease showed that the system is suitable for application with higher tree density even under the common disease condition (Ferrarezi et al., 2020). The OHS system has been also documented to be more economical for capital investment. The analysis using net present value (NPV) to evaluate the cost and benefits of OHS demonstrated that within 5 to 7 years of the investment, the OHS will return the positive profits (Roka et al., 2009). The timeframe for the return of investment is considered to be appropriate for the lime production practice in the Mekong River Delta area since many farmers are experienced with more than 10 years involving in growing lime.

The OHS can boost the sustainability of lime production in the era of climate change. The OHS greatly contributes to the sustainability of water use by controlling wastewater treatment using for irrigation and fertilization. Prazeres et al. (2017) proved that the hydroponic system is very promising for wastewater treatment in agriculture of food production. Consequently, the wastewater can be efficiently reused and thus the contamination of surface water and groundwater can be minimized. The main outcome of the system is to minimize the water shortage that may cause shortly. Yet, soil salinity has not affected the lime orchards in the current surveying, the problem would be exacerbated soon since other proximate areas have been experienced with a serious salinity.

4.2.2. Accurate spraying

Another consideration for the application of technology and mechanization for lime production is the pesticide spraying step. The current farming practices in lime production in the Mekong River Delta heavily rely on manual spraying. This practice has been considered as non-uniform, excessive spraying and may cause a reduction in crop yield. The inaccurate also may cause the contamination of soil by the excessive pesticide dripping from the trees (Desale et al., 2019). Farmers' safety issues would also be another consideration when applying the manual spraying (Faiçal et al., 2017) due to the direct contact with those chemical contain. The unmanned aerial vehicle (UAV) has been proposed as an effective option for the lime orchards in preventing the disease. The concept of using the air-assisted sprayer for precision citrus was introduced by Khot et al. (2012). By using the proposed system, the pesticide usage was reduced by 50% whereas the spray deposition was maintained as unchanged. In another study, the cost of applying UAV for accurate spraying of citrus has been analyzed. The effectiveness of the UAV in disease prevention was successfully maintained. The cost of applying UAV was comparable to conventional spraying (Martinez-Guanter et al., 2020). From the aforementioned analyses, it is recommended that UAV would be an appropriate choice for mechanization of lime production focusing on plant care stage.

4.3. Semi-mechanized harvesting

Improving the harvesting practice in lime production is necessary to increase productivity and reduce the labor cost. Many efforts have been made to apply the mechanization in citrus production. Though the mechanical shaking can reduce labor cost and over 90% of fruits can be harvested in a short period (Arenas-Arenas et al., 2017), tree damaging is inevitable that lead to undermining the next crop (Pu et al., 2018). The mechanical vibration for harvesting is also incapable of small and medium lime orchards in the Mekong River Delta. The manual harvesting seems to be suitable for current practice in lime production. However, the improvement focuses on reducing the cost is necessary. The manual labor cost comprises of over 44% of citrus production costs (Costa & Camarotto, 2012). The cost analysis in the current survey in lime production at the Mekong River Delta also suggests that labor cost was the major for the harvesting step. Under this scenario, the semi-mechanized harvesting would be the most appropriate and be recommended. The semi-mechanized harvesting system proposed by Costa & Camarotto (2012) includes four platforms vertically moving along the tree height: from bottom to the highest top to harvest fruits. The fruits were then conveyed and boxed in the same system. The productivity of harvesting using semi-mechanized systems increased by 40%. The increase in productivity of the machine aid system has been also reported in another study of Ferreira et al. (2018). The findings also suggested that the semi-mechanization harvesting systems can also improve the ergonomic of the fruit pickers and reduce the physical stress during harvesting (Costa & Camarotto, 2012; Ferreira et al., 2018). The mobile platform of harvesting thus may be most appropriate for the mechanization of the lime production in the Mekong River Delta.

4.4. Storage

Lime fruit is most commonly marketed in a fresh form that needs an appropriate storage facility and suitable packaging. Unfortunately, most of the lime growers in the Mekong River Delta lack of cold storage facility that leads to stress to be sold before the fruit becomes perishable. The sale price much depends on the season and reaches a peak around February. From December to February the market price can increase by approximately 70% (Ho, 2016). The big lime grower thus can invest a cold storage facility to maintain sustainability in lime production by stabilizing the sale price over a certain period (e.g. two months). Research has proved that lime quality can be extent the shelf-life from 45 to 60 days by cold storage and under certain treatment conditions (MohammadrRezakhani & Pakkish, 2017; Opio et al., 2017; Yousefi et al., 2019). Yet, the cold storage facility may not be applicable for small scale lime production, the medium and big growers are recommended to invest the facility to get the production competitive advantages. The high-quality fruits do not only serve for the freshly-consumed market but also the potential processing industry.

5. Conclusions

The results show that the status of application of machines and equipment in lime growing activities in the survey areas was not really high. Households mainly used machinery in two stages of land preparation (42% households applying mechanization) and tree care (93%). The current situation of mechanization application between the two areas also shows certain differences. The general results showed that lime growing households in Long An had a higher percentage of mechanization than in Ben Tre (33.7% versus 14.8%). In all production stages, Long An province always has a higher rate of mechanization. It is noticeable that the lime growing area and the average value of the machines invested in Long An are significantly higher than those in Ben Tre. The resources of education level and degree of lime specialized cultivation also differ between the two regions. These characteristics lead to better production results and higher efficiency in Long An than in Ben Tre. In addition, the estimated results of mechanization index show that most of the lime growers have very low levels of mechanization application. Regarding the efficiency of the application of machines to cope with climate change, there was statistically insignificant difference in production efficiency. Thus, it is impossible to confirm that the application of machinery in production really helps farmers to adapt to climate change. This result also reflects the subjective assessment of the farmers themselves.

Finally, potential technical solution for lime farming in the Mekong River Delta was also proposed, including mechanization for small and medium farm and mechanization considerations for plant care (irrigation and fertilizing using open hydroponic system and accurate spraying). Furhtermore, the semi-mechanization harvesting systems and appropriate storage facility and suitable packaging are highly recommeded for improving the quality of lime fruit.

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Effects of drying methods on color retention and chlorophyll of celery (*Apium graveolens* L.), spinach (*Spinacia oleracea* L.), Malabar spinach (*Basella alba* L.)

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ABSTRACT

Dried vegetables are considered convenient for storage, transportation and preservation. The different drying techniques could influence the quality of resulting products. This study aimed to evaluate the effects of three distinguish drying methods as hot-air drying, foam-mat drying and microwave drying on the color retention and chlorophyll of green vegetables powder. Fresh spinach (*Spinacia oleracea* L.), celery (*Apium graveolens* L.), Malabar spinach (*Basella alba* L.) were dried by different methods: hot-air at 60°C, foam-mat at 60°C and microwave at 270 W until the samples reached approximately 9% of moisture content (wb). The drying time of the dried samples by microwave, foam-mat and hot-air method were 60, 210 and 240 min, respectively. Foam-mat dried vegetables were found to have the best quality in terms of color and the residual chlorophyll content. The findings suggest that foam-mat drying is promising in dried vegetable processing.

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1. Introduction

Drying is a traditional method of food preservation and has been widely used in the food industry. This process causes a reduction in weight and volume, leading to the minimized packaging, storage, transportation costs, and improving the shelf life of the product (Baysal et al., 2003). Hot-air drying is the process that the convection of air flow is used to dry raw materials (Ratti, 2001). During hot-air drying, water is removed by the heat transferred from the hot air to the product by convection and evaporated water is transported to the air also by convection. Microwave drying is based on the conversion of alternating electromagnetic field energy into heat by the effect of the polar molecules (Vadivambal & Jayas, 2007). Foam-mat drying is used to dry liquid-solid foods (e.g. juice, milk, fruits, beverages and jams), in which the foams undergo air drying temperatures from 50 to 80°C. The foam is produced by mixing the stabilizing agent and or foaming agent (e.g. glycerol monostearate, carboxymethyl cellulose, trichlorophosphate) (Widyastuti & Srianta, 2011; Febrianto et al., 2012; Kandasamy et al., 2012). Color changes are undesirable since the loss of color during drying most likely links to the lower nutritive value of those components building the color (Cui et al., 2004; Konopacka, 2006). Green vegetables are an important source of car-

Table 1. Proximate composition of vegetable samples per 100 g of edible part

Component	Vegetable samples		
	Spinach	Celery	Malabar spinach
Dry matter (g)	7.20	4.70	5.90
Carbohydrates (g)	2.29	2.38	2.79
Protein (g)	2.62	1.34	1.59
Lipid (g)	< 0.30	< 0.30	< 0.30
Fibre (g)	2.11	1.82	2.07
Ash (g)	2.04	0.83	1.29
Calories (kcal)	22.00	16.00	20.00

Samples were analyzed by Eurofins Sac Ky Hai Dang Company Limited.

bohydrates, minerals, and vitamins, particularly fiber. According to Nguyen et al. (2007) and USDA (2019), celery (*Apium graveolens* L.) has a remarkable content of calcium and potassium (325 mg/100 g) while spinach (*Spinacia oleracea* L.) is rich in beta carotene (2147 µg/100 g), fiber (2.4 g/100 g), and Malabar spinach (*Basella alba* L.) contains a high amount of vitamin C (102 mg/100 g). However, because of the limitation of storage time, the use of green vegetables is quite disadvantageous due to the loss in quality. The drying technique is considered as one of the solutions for this problem. This study was aimed to evaluate the effects of three different drying methods on the quality of green vegetable powder.

2. Materials and Methods

2.1. Materials

Spinach, celery and Malabar spinach were bought at the local supermarket (Figure 1). The vegetable samples chosen for the experiment were fresh and carefully packaged. The sample qualities were evaluated by shape, size, color, smell, texture, and especially nutritional value which was shown in Table 1.

2.2. Methods

2.2.1. Sample preparation

The non-edible part of the samples was removed and then the samples were washed with water and drained. Each sample was chopped and ground for 120 seconds by using a multipurpose blender (capacity: 750 W). The moisture and chlorophyll contents were measured. The ground samples were subjected to different drying methods.

2.2.2. Drying methods

In the hot air drying method, 150 g of each sample was spread evenly on the tray (25 x 30 cm) then dried by a hot air dryer at 60°C.

In the foam-mat drying process, a total of 150 g of sample including 1.5% soy protein (SP), 0.5% methyl cellulose (MC) and the vegetable puree was whipped (by using hand mixer HR3705, Philips, Netherlands). For celery and Malabar spinach, the whipping was done in 10 min while the spinach sample was whipped for 15 min to achieve the optimum foam density in the range 0.2 - 0.6 g/cm^3 as described in a study by Hart et al. (1963). The semi-solid foams were spread evenly on the tray (25 x 30 cm) then dried at 60°C.

In terms of microwave drying, 150 g of each ground sample was spread evenly on the tray (25 x 30 cm) before being placed on the microwave (NN-9853, Panasonic, Japan) at the microwave power of 270 W was used in this method.

The moisture was determined every thirty minutes until the moisture content was reached lower than 9% (wet bulk) (Larrauri, 1999). Consequently, dried samples were ground to a fine powder and screened to achieve 0.3 mm diameter particles using a grinder (HR2221/00, Philips, Netherlands). The final powder samples were evaluated for color retention and chlorophyll content.

2.2.3. Moisture content determination

Moisture content was determined using the oven drying method. The samples were dried in the oven at 105°C for 24 h. The dried samples

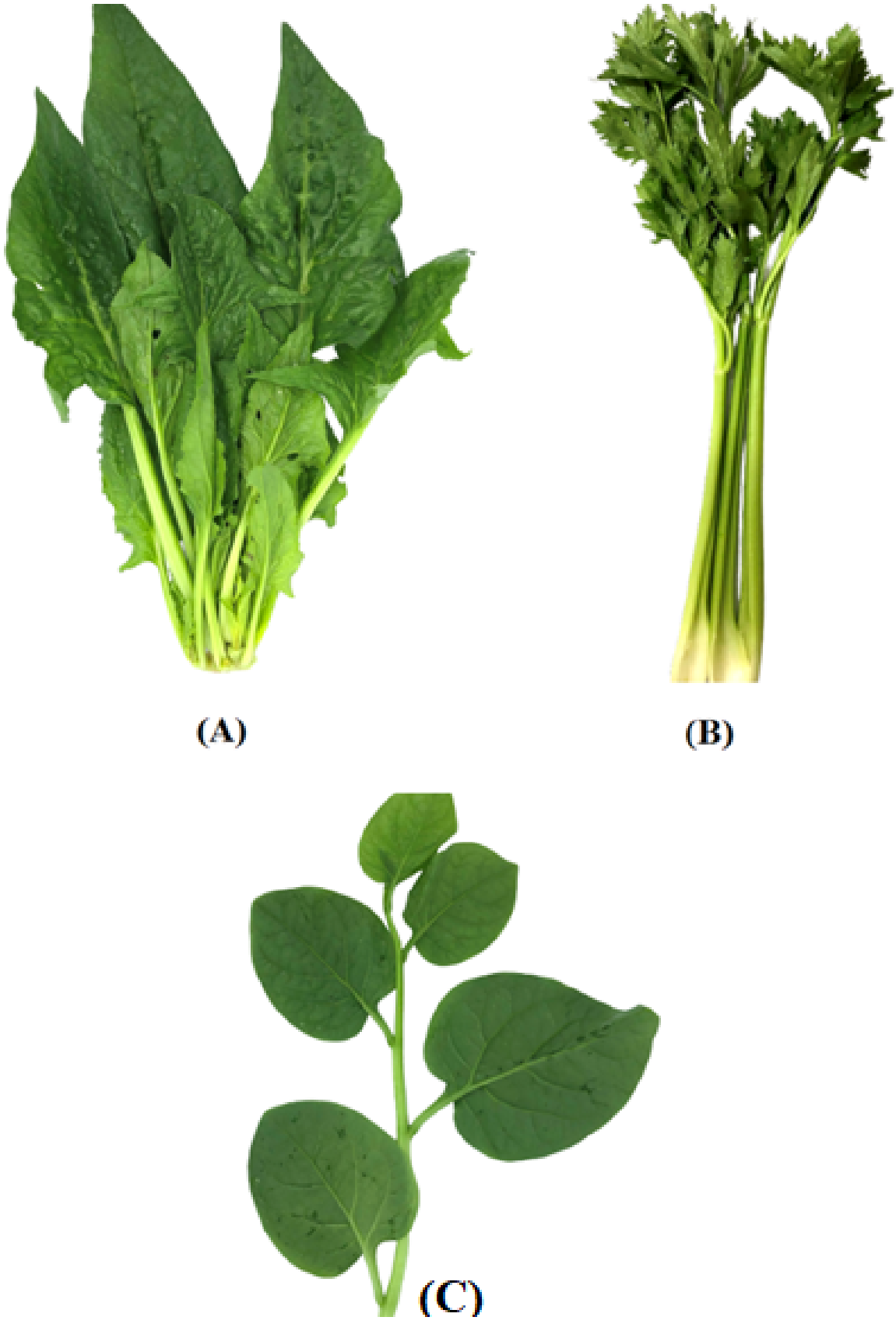

Figure 1. Fresh samples of spinach (A), celery (B), Malabar spinach (C).

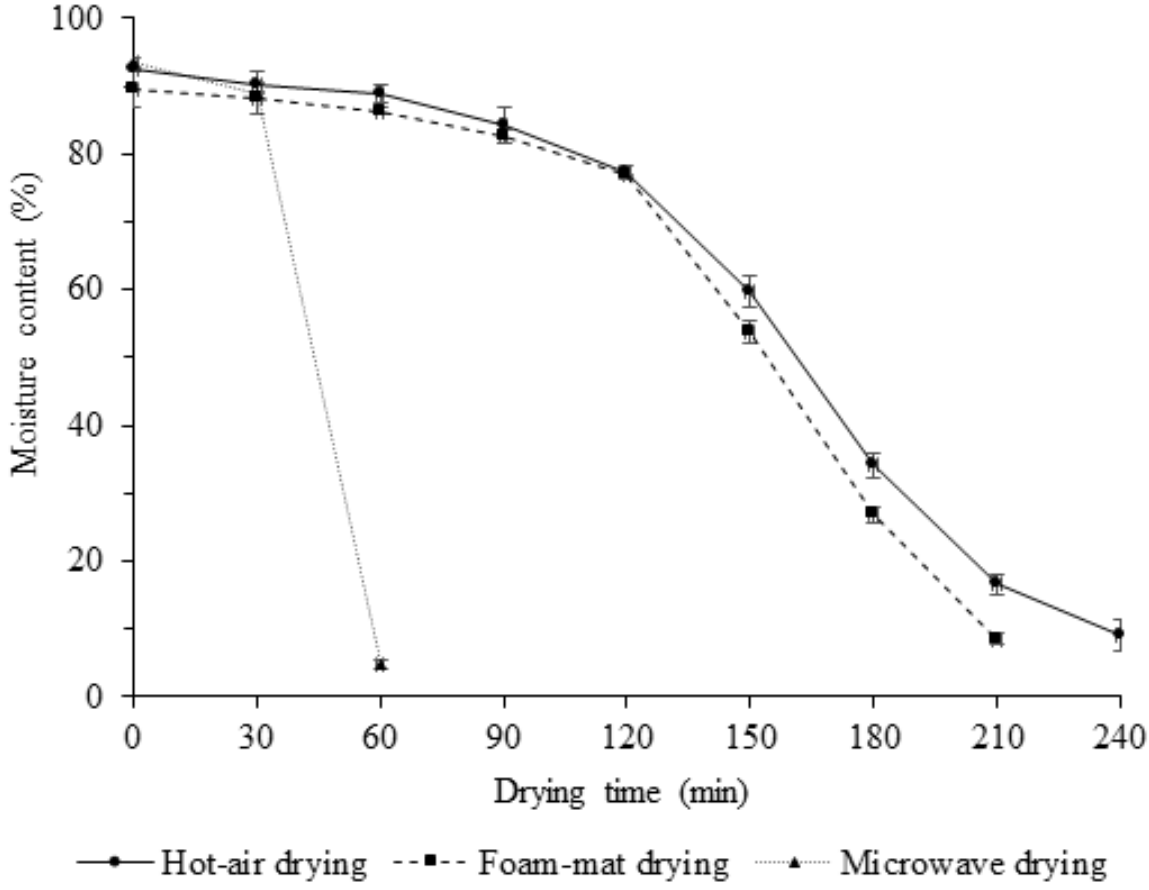

Figure 2. The changes in the moisture content of ground spinach during drying processes.

were cooled in a desiccator for 30 min and the weight was measured (± 0.01 g error). Moisture content was calculated by Formula 1:

$$X = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Where:

X: moisture of samples (%)

W_1: the weight of samples before drying (g)

W_2: the weight of samples after drying (g)

2.2.4. Color analyses

The color was measured by using a colorimeter (CR-400, Konica-Minolta, Japan). The color was expressed in CIELAB color value (L^*, a^*, b^*) whereby L^* stands for the lightness from black (0) to white (100), a^* is from green (-) to red (+) color, b^* is from blue (-) to yellow (+) color. Measurements were done in triplicates. The Browning Index (BI) was calculated using the following expression of (Mohapatra et al., 2010) (Formula 2):

$$BI = \frac{100(X - 0.31)}{0.17} \quad (2)$$

$$\text{With: } X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$$

2.2.5. Chlorophyll content determination

Chlorophyll content was measured by using a colorimetric method described by Sumanta et al. (2014). An appropriate amount of sample (0.5 g for the fresh ground sample and 0.1 g for the powdered sample) was taken and transferred into a 50 mL centrifuge tube then 20 mL of 90% ethanol was added. The sample mixture was centrifuged at 6000 rpm for 10 min. The supernatant was separated and 1 mL of the supernatant was aliquoted and mixed with 4 mL of 90% ethanol. The solution mixture was analyzed for Chlorophyll-a, Chlorophyll-b by using a spec-

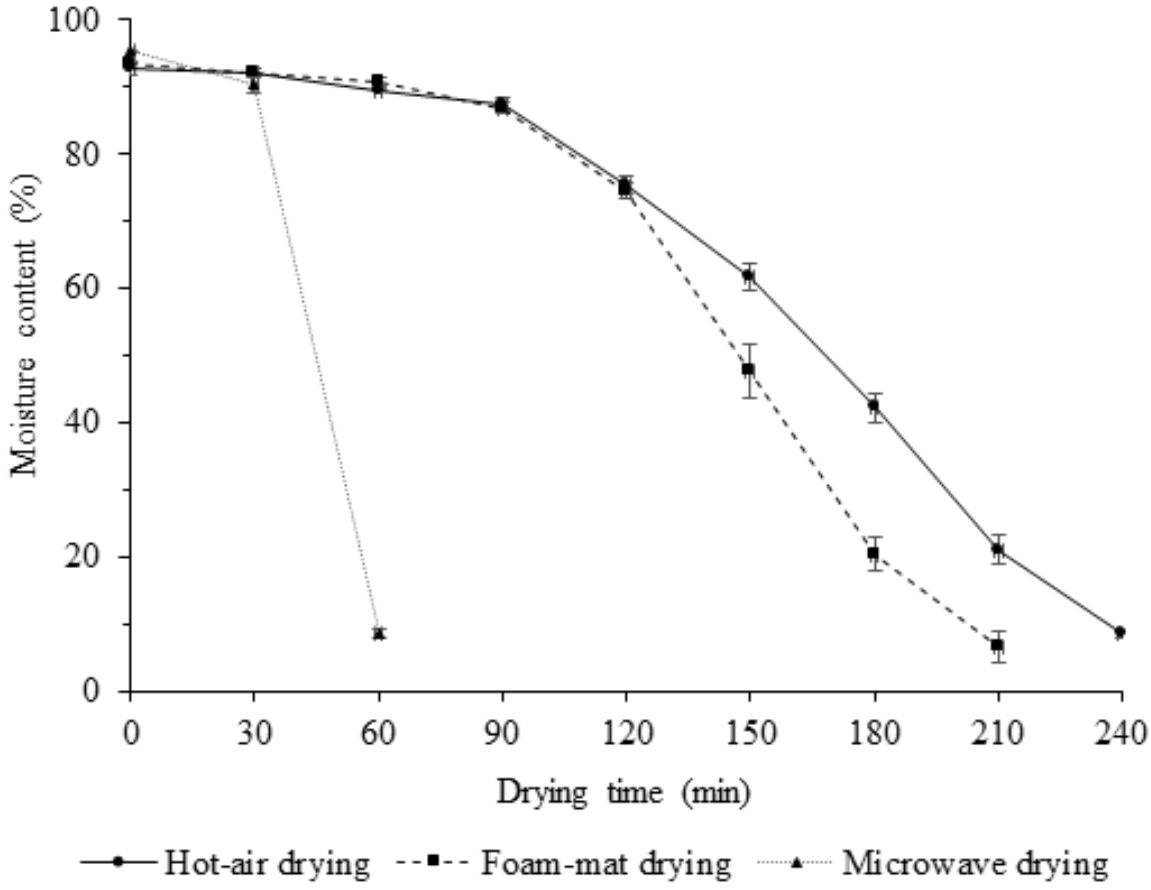

Figure 3. The changes in the moisture content of ground celery during drying processes.

trophotometer (SPECTRO-UV 11, MRC, UK) and was calculated by Formula 3 and 4:

$$\text{Chl-a} = 13.36A_{664} - 5.19A_{649} \quad (3)$$

$$\text{Chl-b} = 27.43A_{649} - 8.12A_{664} \quad (4)$$

Where:

Chl-a: Chlorophyll-a content (µg/mL)

Chl-b: Chlorophyll-b content (µg/mL)

A_{664}: Absorbance at 664 nm

A_{649}: Absorbance at 649 nm

2.2.6. Statistical and data analysis

The data are presented as the mean of three determinations ± standard deviation. The data were analyzed by ANOVA and LSD using JMP statistics software (Version 10.0). Statistical significance for differences was tested at 5% probability level ($P < 0.05$).

3. Results and Discussion

3.1. Changes in moisture content during the drying process

The initial moisture contents of fresh vegetables were measured about 89-95%. The moisture content of the raw material decreased during the drying time. In microwave drying, the shortest drying time (around 60 min) was required to achieve a moisture content of 9% (wb). This trend was observed in all three vegetable samples (Figures 2, 3 and 4). The obtained results in this study were in good agreement with the findings reported by Maskan (2001). The drying time of microwave drying was significantly reduced by up to 89% compared to hot air drying. In a microwave drying system, the microwave can easily penetrate the dry outer layer and be absorbed by the water inside the raw material. The water molecules absorbed microwave energy so they fluctuated at very high frequencies, created great

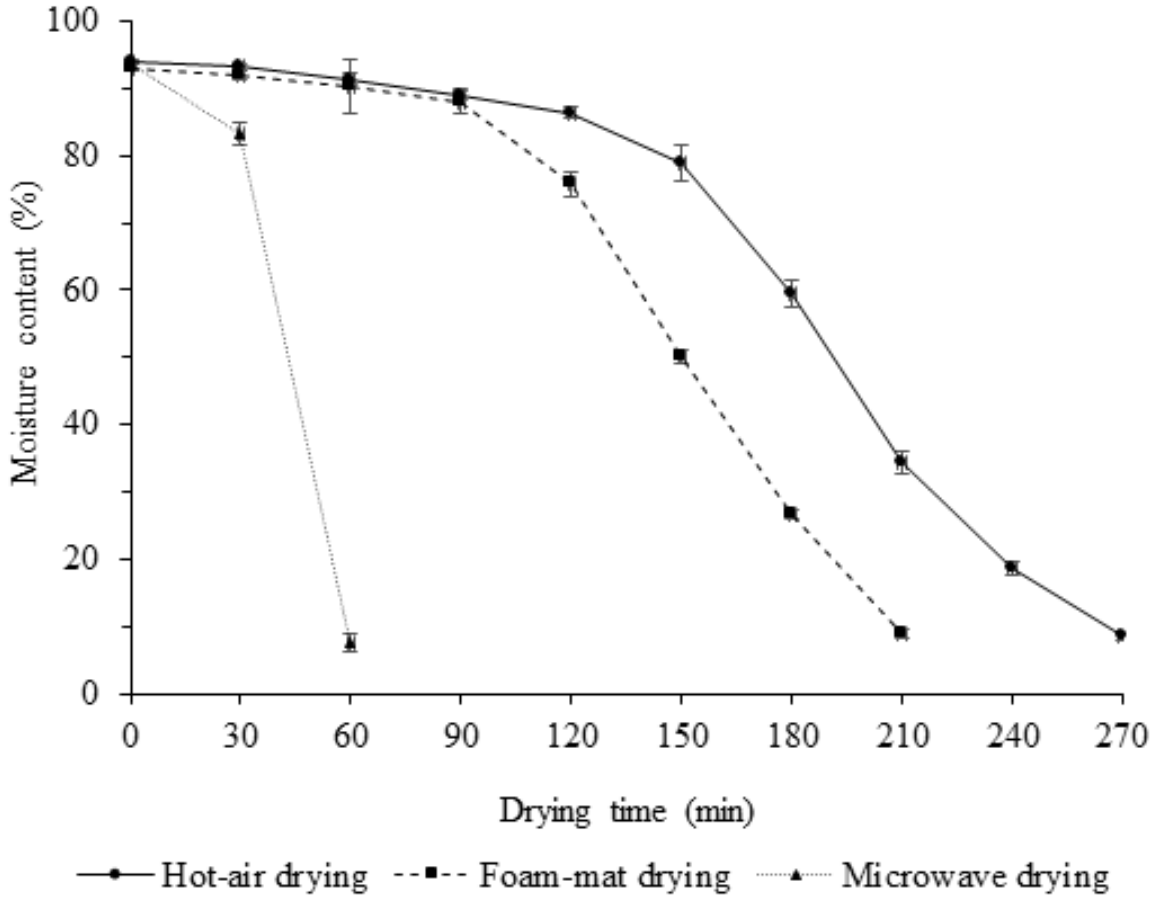

Figure 4. The changes in the moisture content of ground Malabar spinach during drying processes.

friction so the material was heated quickly. The quick energy absorption causes rapid evaporation of water creating an outward flux of rapidly escaping vapor (Lyons et al., 1972). Three vegetable materials in this study seem to obey this hypothesis.

From the drying curves in Figures 2, 3 and 4, it can be observed that the rate of water removal was initially constant for the hot-air method and foam-mat method due to the high moisture content. The moisture contents of hot-air and foam-mat dried samples were dramatically decreased after 90-minute of processing. Compared to conventional hot-air drying, foam-mat drying had at least 30 min shorter drying time (210 vs. 240 min). The moisture reduction rate of foam-mat drying was faster than that of hot air drying could be due to larger the contact surface area. According to (Sangamithra et al., 2015a), the foam generated from whipping has a high surface area that accelerates the dehydration process. Hot-air drying had the longest time of 240 - 270 min, and foam-mat drying was last about 210 min. Longer drying time is unfavorable since it leads to higher energy consumption and possible reduction in product quality (Chua et al., 2002).

3.2. Color analyses

Color is an important attribute of dried leafy vegetables that reflects customer acceptance. Limited changes in color are favorable in the drying process of vegetables. The dried leafy vegetable is expected to have a bright green color. L^* value indicates the brightness of the samples, was measured and the results are presented in Table 2. The L^* values were significantly different between samples obtained from different drying methods ($P < 0.05$).

In comparison, the foam-mat drying method consistently resulted in the brightest color products compared to microwave and hot-air drying

Table 2. Color of vegetable powder using different drying methods

Drying method	Vegetable samples									
	Spinach			Celery			Malabar spinach			
	L*	a*	BI	L*	a*	BI	L*	a*	BI
Hot air drying	$52.6^{b} \pm 0.6$	$-11.2^{b} \pm 0.3$	$22.5a \pm 0.4$	$54.6^{c} \pm 0.5$	$-8.0^{b} \pm 0.2$	$20.5^{c} \pm 0.5$	$54.2^{c} \pm 0.3$	$-9.8^{b} \pm 0.1$	$26.1^{b} \pm 0.6$
Foam-mat drying	$54.6^{a} \pm 0.9$	$-12.7^{c} \pm 0.2$	$22.8^{a} \pm 0.4$	$59.2^{a} \pm 0.1$	$-10.4^{c} \pm 0.2$	$22.3^{b} \pm 0.5$	$58.5^{a} \pm 1.0$	$-11.0^{c} \pm 0.3$	$20.9^{c} \pm 0.7$
Microwave drying	$51.5^{c} \pm 0.2$	$-9.8^{a} \pm 0.3$	$22.4^{a} \pm 0.4$	$57.8^{b} \pm 0.4$	$-6.1^{a} \pm 0.2$	$27.5^{a} \pm 0.7$	$56.3^{b} \pm 0.5$	$-6.0^{a} \pm 0.1$	$27.7^{a} \pm 0.6$

Different letters within the same column denote significant differences at $P < 0.05$, BI: browning index.

methods. The highest value of L* was found in celery product (L* = 59.2) resulted from foam-mat drying. The results in this study are in agreement with previous studies since foam-mat drying generates the appreciable brightness of okra (Falade & Omojola, 2010), yam flour (Falade & Onyeoziri, 2012), and muskmelon (Sangamithra et al., 2015b). The negative value of a* reflects the greenness of the samples which was found lowest ($P < 0.05$) in the foam-mat drying method, regardless of the type of vegetables (Table 2). Within the foam-mat drying method, the lowest value was recorded in spinach (a* = -12.7). This finding implies a high ability to retain the color of leafy vegetables of foam-mat drying method. In contrast, dried vegetables obtained from microwave drying had high a* values. The highest a* value was found in Malabar spinach (a* = -6.0). The microwave drying method, thus, would cause the most serious color changes of dried vegetables. The a* values of products resulted from the conventional hot-air drying were in between compared to that obtained from foam-mat and microwave drying methods. In terms of the green color retention, microwave drying was not superior to the conventional methods. One of the major drawbacks of microwave drying of plant material is the inhomogeneity of heat delivery that leads to overheating of certain local regions of plant tissues (Holtz et al., 2010; Wojdyło et al., 2014). It is possible that the overheating in vegetable tissues in this study occurred which led to the change of sensitive components such as color as observed. The browning during drying is an important factor causing the color change. The browning index (BI) values were calculated (Table 2) to compare the ability of browning during drying. Microwave drying method resulted in high values of BI that seen in celery and Malabar spinach samples. The BI values calculated for microwave drying were significantly higher ($P < 0.05$) than that of foam-mat drying and hot-air drying process. This result conforms to the disadvantage of microwave drying as stated in a study by Lenaerts et al. (2018) since the method can lead to a significant browning.

3.3. Chlorophyll

The favorable green color of vegetables is due to the availability of chlorophyll. The chlorophyll contents of dried vegetables obtained from different drying methods were measured to clarify

Table 3. The remained chlorophyll content of vegetable powders

Drying method	Vegetable samples		
	Spinach	Celery	Malabar spinach
Hot-air drying	$85.82^{a} \pm 0.60$	$81.99^{a} \pm 1.75$	$83.45^{a} \pm 1.08$
Foam-mat drying	$86.65^{a} \pm 0.66$	$81.87^{a} \pm 1.47$	$84.42^{a} \pm 0.38$
Microwave drying	$60.01^{b} \pm 0.42$	$41.16^{b} \pm 1.49$	$48.97^{b} \pm 0.88$

Different letters within the same column denote significant differences at $P < 0.05$

the color changes (the greenness, in particular). The results are presented in Table 3. The remained chlorophyll contents of samples obtained from foam-mat drying were significantly higher ($P < 0.05$) than that obtained from the microwave drying method. Compare to hot-air drying, foam-mat drying retained a higher content of chlorophyll (though statistically insignificant). Generally, the remained chlorophyll contents of the samples dried by hot-air and foam-mat techniques were highest ($> 80\%$) while that of those dried by microwave oven had the lowest content ($< 60\%$). The reduction of greenness color of vegetables during heat processing is involved in conversion of chlorophyll to pheophytin and pyropheophytin (Falade & Omojola, 2010). Chlorophyll was also previously documented to be sensitive to heat (Koca et al., 2007). The result of chlorophyll analysis confirms the previous hypothesis that the microwave drying process, although the drying time is shorter than the others, caused overheating of some vegetable regions that can lead to a significant change in the color building by chlorophyll. Foam-mat drying seems to be the most appropriate drying method to retain the dried leafy vegetables' color.

4. Conclusions

In the present study, the hot-air, foam-mat and microwave drying methods were used to clarify the effects on color and chlorophyll retentions of dried spinach, celery and Malabar spinach. The microwave drying method had the shortest drying time but caused the most unfavorable color change and a significant reduction in chlorophyll content in all vegetables. Conventional hot-air drying had the longest drying time and caused a relative loss in the greenness of vegetables. The foam-mat drying method had a significantly shorter drying time compared to the hot-air drying. The foam-mat drying products were characterised with the highest lightness, greenness and chlorophyll retention. Foam-mat drying resulted in products with the lowest browning index. The foam-mat drying method would be the most suitable for preparing dried vegetables. Nutritional composition retention and other sensory attributes should be compared to further elucidate the potentials of the foam-mat drying method.

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Effect of glycerol concentration on physical and texture properties of edible films prepared from karaya gum

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ABSTRACT

This study formulated edible films based on karaya gum in the presence of glycerol. Physical properties of films were investigated by various methods including texture analysis and differential scanning calorimeter (DSC). The obtained results revealed that glycerol acted as a plasticizer contributing to improve the flexibility, water vapor permeability and heat resistance of karaya films. The best value of tensile strength and puncture force for edible films could be achieved by the combination of karaya gum and glycerol at the concentrations of 4% (w/v) and 10% (w/w), respectively.

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1. Introduction

In recent years, plastic utilization has faced social arguments due to its negative effects on the environment. Plastic bags and other synthesized-polymers-based materials takes many years to decompose. In addition, toxic substances released from plastic wastes combustion can leach into soils, water sources and even the air we breathe everyday, leading to serious public health hazards. Though, a considerable amount of researches have been conducted to figure out the new materials which can replace synthetic packaging, and bio-based polymer films have been considered as the promising approach since the 1980s. In comparison with plastic films made from synthetic-materials, edible films still provide a barrier to moisture, oxygen and solute movement while being eco-friendly (Phan et al., 2005). These films are often produced from natural polymers possessing film-forming ability such as pectin, starch, chitosan, xylan, lignin and cellulose nano fibrils (CNF) which can be extracted easily from various agro-byproducts (Vartiainen et al., 2014). Among biopolymers, polysaccharide gums (Karaya gum, gum Arabic, gum ghatti and larch gum) are potential materials for edible films processing but the studies using this kind of material are quite limited especially when comparing with the others such as agar, pectin and methyl cellulose (Nieto, 2009).

Karaya gum, an exudate gum, is plentifully in the trunk and branches of *Sterculia foetida* plants. This gum contains a lot of complex and branched polysaccharides with a high molecular mass ($16x10^6$ Da) (Lujan-medina et al., 2013;

Mortensen et al., 2016). The main chains of karaya gum are structured by α-D-galacturonic acid and L-ramnose while the branches are connected by the linkages between 1,2-β-D-galactose or 1,3-β-D-glucuronic with galacturonic acid (Verbeken et al., 2003). Some researchers stated that the volume of this gum can swell to 60 times when soaking in aqueous medium (Verbeken et al., 2003; Lujan-medina et al., 2013). Similar with most exudate gums, karaya gum is cheap, biodegradable, eco-friendly and considered safe to consumer's health (Mortensen et al., 2016). Based on these advantages, this biopolymer is widely used as additive in food and pharmaceutical industries (Lujan-medina et al., 2013).

However, the application of karaya gum in food packaging is quite limited due to many challenges. Firstly, the low solubility of karaya gum in water results in a very high viscosity solution which causes many difficulties in preparing films (Sarathchandiran, 2014). Secondly, karaya film has weak tensile strength and high brittleness (Lettre, 2010). To improve film properties, the addition of plasticizers as glycerol can be an appropriate method. Glycerol is a popular hydrophilic plasticizer that helps reduce the intermolecular forces between polysaccharide chains to increase their mobility. Many studies proved that the addition of glycerol could improve the physical properties of edible films like flexibility, water vapor and gas permeability (Farahnaky et al., 2013; Sanyang et al. 2015; Saberi et al. 2016). Nieto (2009) explained the slower disintegration of bio-films when glycerol was locked into the matrix structure, leaving the stable film until product consumption.

Therefore, this study aimed to formulate edible films with improved physical properties including water vapor permeability, mechanical resistance and heat stability. The research provides details of karaya-based films and their characteristics, the effect of the plasticizer in various concentrations and the improvement of properties of karaya-based edible films.

2. Materials and Methods

2.1. Raw materials and chemicals

Gum karaya was collected from local suppliers, glycerol (99% of purity were was purchased from Sigma-Aldrich (Canada) and salts (95% of purity) including NaBr, CH_3COOK and KCl) were purchased from Merck (Germany). Raw karaya gum was grounded and classified using a cutting-grinding head (IKA MF10.1, USA) after removing visible impurities. Particles with a size range of 0.5 – 1 mm were collected and stored in vacuum plastic bags for further experiments.

2.2. Sample preparation

Karaya solution was formed by dissolving raw material into 250 mL distilled water at ambient temperature. Gum solutions at various concentrations (3, 4 and 5%, w/v) were stirred in 2 h using a magnetic stirrer at 800 rpm in 500 mL erlen flasks. Glycerol was then added into gum solution at the concentration of 10%, 15%, 20% and 25% (w/w) based on the dry weight of karaya gum (DW), the mixtures were gently stirred using a glass stick for 15 min at the room temperature. The bubbles formation should be limited during stirring. The solution was spread on the granite plate which was covered by a polyethylene sheet to prevent adhesion. Those plates were dried for 2 h under a vacuum atmosphere (60 mmHg) for 24 h at 55°C using a convection dryer (Memmert, Germany) before being cooled to room temperature. Film samples were stabilized in desiccators for 48 h at the relative humidity of 57% (using sodium bromide saturated solution). Karaya films were kept in vacuum plastic bags before further analysis.

2.3. Film thickness

The film thickness was measured by using a manual slide micrometer Gauge (The Vernier Caliper) to the nearest value of 0.01 mm. Data were collected at 10 random locations for each film sample and an average value was calculated. The thickness value was used for determining the tensile strength and water vapor transmission.

2.4. Water vapor permeability (WVP)

The water vapor transmission of the film was measured according to the modified ASTM E96-80 method (Phan et al., 2005). In the pretreatment step, all samples were equilibrated in desiccators at 25°C and relative humidity of 22% (using potassium acetate saturated solution) during 48 h. Film samples were set up between two Teflon rings of the glass cell which were placed in the desiccators at 25°C for 15 days. The in-

side and outside relative humidity of glass cells are 84% (using potassium chloride saturated solution) and 22%, respectively. WVP was calculated based on the weight changes ($\Delta m/\Delta t$, g/s) of the cell during measurement time using the following equation 1 (Phan et al., 2005):

$$WVP = \frac{\Delta m \times x}{A \times \Delta t \times \Delta p} (gm^{-1}s^{-1}Pa^{-1}) \quad (1)$$

Where x and A are the film thickness (m) and the exposed surface (m^2), respectively while Δp is the partial water vapor pressure difference between 2 sides of samples (Pa).

2.5. Texture analyzer

Tensile strength test and puncture test were conducted by a texture analyzer (TA.XT plus, Stable microsystem, UK) equipped 5 kN load cell. Film samples were cut into rectangular strips of 15.4 mm width x 100 mm length with a thickness of 0.03 mm before stretching. For the puncture test, the film was fixed in a 52.6 mm diameter cell. Both the force and the deformation at the breaking point of samples were measured using a 3 mm diameter aluminum probe. The puncture deformation was calculated using equation 2 (Gontard et al., 1993):

$$\frac{\Delta t}{\Delta l} = \frac{\sqrt{D^2 + l_0^2} - l_0}{l_0} \quad (2)$$

Tensile strength (TS) at breaking, the puncture force (F) and the displacement of the probe (D) were measured using Exponent software (version 6.0, Stable microsystem, UK). The radius of the measurement cell (26.3 mm) was presented as l_0.

2.6. Thermal characteristic analysis

Heat-resistance of karaya film was analyzed using a different scanning calorimeter (DSC) (Q2000, TA Instrument, USA). Samples (5-10 mg) were sealed into aluminum pans and an empty pan was used as a reference. All samples were heated from ambient temperature to 200°C with a rate of 5°C/min.

2.7. Statistical analysis

The experiment was conducted using 2 full-factorial designs with three replications Analysis of variance (ANOVA) and LSD were applied to compare mean value of film's properties with a significance of 0.05. A significant difference was satisfied at 95% probability. SPSS statistics software (version 20, IBM, USA) was used to evaluate the data.

3. Results and Discussion

3.1. Film formation

Preliminary experiments showed that a good appearance of edible films could be obtained if karaya gum concentration in the formulation was limited under 5%. It took about 2 h for raw material as karaya gum completely dissolved in distilled water before casting. Since the air bubbles can be trapped in high viscosity solutions such as karaya gum solution, the pinholes often form during film formation. Therefore, gum solution needs to be kept in a vacuum dryer at 60 mmHg and 55°C for 2 h to eliminate air bubbles before drying 24 h in a convention dryer at the same temperature. The results were in line with Neito's work, in which he stated that the dried films without glycerol were very adhesive to the casting surface while their tensile strength was too weak and brittle to peel off. The addition of 10% glycerol ensured that karaya films could be removed easily from granite plates. Besides, the flexibility of karaya films increased significantly when adding glycerol in a range of 10 - 25% since this additive was a hydrophilic compounds plasticizer (Phan The et al., 2008; Vieira et al., 2011; Jantrawut et al., 2017). At higher glycerol concentrations, the films were hard to form and also required longer drying time.

3.2. Water vapor permeability

Water vapor permeability (WVP) is an important parameter in the evaluation of moisture transmission through barrier of edible films. Various concentrations of the karaya gum and glycerol were investigated to evaluate their effects on WVP of edible films based on karaya gum Two-way ANOVA revealed that both karaya gum and glycerol had significant effects ($P < 0.05$) on the WVP of the films. Generally, a higher concentration of polymer means a denser structure of the film network resulting in a decrease in water vapor transmission. However, WVP of the film with 5% karaya gum was significantly higher than that of films containing 3% and 4% karaya gum at $P =$

Table 1. Physical properties of edible films based on karaya gum and glycerol

Karaya gum (% w/v)	Glycerol (%w/w)	Tensile strength (MPa)	Puncture force (MPa)	Puncture deformation (%)
3	10	46.15 ± 1.80	9.51 ± 2.90	0.72 ± 0.22
	15	37.86 ± 0.26	7.58 ± 0.29	1.07 ± 0.33
	20	16.11 ± 3.25	4.32 ± 1.37	1.98 ± 0.65
4	10	85.89 ± 0.40	10.67 ± 0.47	0.52 ± 0.13
	15	53.59 ± 0.08	10.31 ± 1.27	0.87 ± 0.08
	20	34.61 ± 6.05	4.04 ± 0.20	1.51 ± 0.18
5	10	37.79 ± 0.91	9.66 ± 0.44	2.58 ± 0.30
	15	33.29 ± 1.71	6.67 ± 1.04	5.05 ± 0.09
	20	24.65 ± 1.24	5.68 ± 0.95	5.67 ± 0.10

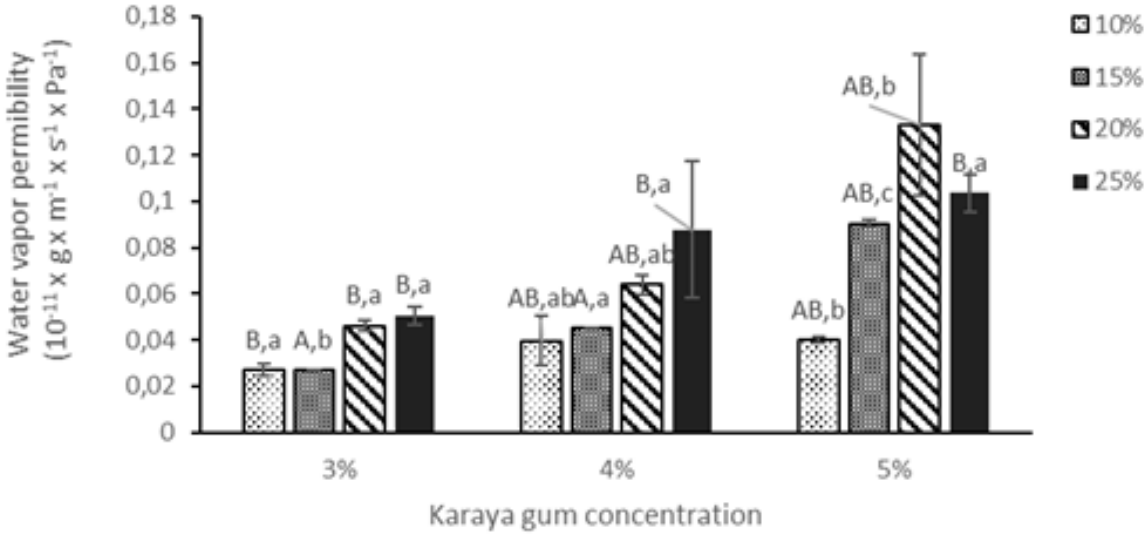

Figure 1. Effect of glycerol concentrations (10, 15, 20 and 25% w/w) on water vapor permeability of karaya films. Different letters are for significantly different groups. The capital letter (A,B) are for comparison of gum content, likewise, lower case (a,b) letters are for comparison of glycerol concentration.

0.05% (Figure 1). The incorporation of polysaccharide chains of gum with water molecules via the intensive hydrogen bonds produces less effective moisture barriers due to its highly hydrophilic property. On the other hand, the addition of hydrophobic ingredients could contribute to the improved water-resistance of gum films while remain their good mechanical properties (Nieto 2009). Figure 1 also revealed the films with above 20% glycerol (w/w) exhibited the high values of WVP among all films tested ($P < 0.05$). There is no significant difference between samples with 10% (w/w), 15% (w/w) glycerol. The results in agreement with several authors who found that glycerol utilization is for increasing the free volume and chain movement through improving molecular mobility (Xiao et al., 2011; Cerqueira et al., 2012; Jouki et al., 2013). As a consequence, the rigidity was reduced, and more water could diffuse through the film structure. On the contrary, some authors reported an opposite trend of WVP for the composite film prepared from plums gum combined with carboxyl methyl cellulose (Shekarabi et al., 2014). Unlike karaya gum, that mixture did not have a good corporation with glycerol resulting in a reduction of the gum cohesiveness.

3.3. Textural properties

Mechanical properties (including tensile strength, puncture force and deformation) of edible films produced from various concentrations of karaya gum and glycerol were investigated

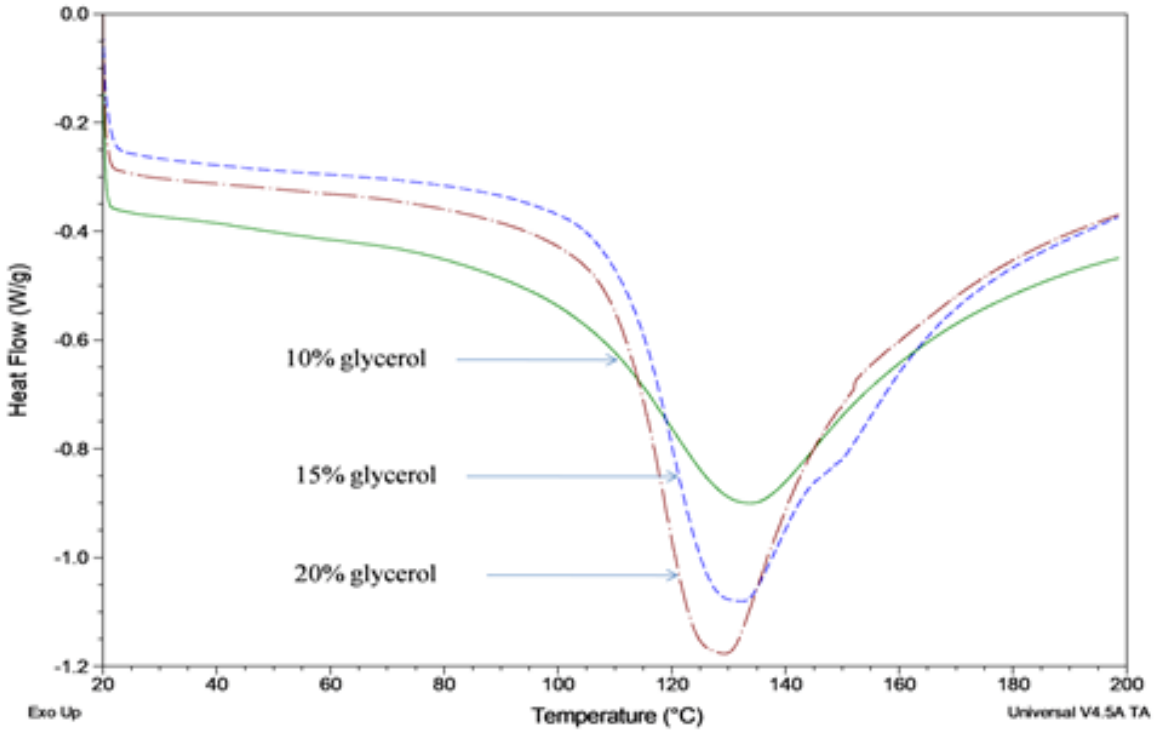

Figure 2. Effect of glycerol concentrations (10, 15 and 20%, w/w) on heat resistance of karaya films (4%, w/v)

and the results are displayed in Table 1. It can be seen that the concentrations of both materials had significant effects on the physical properties of films. According to LSD comparison, 4% karaya gum was the most suitable concentration for producing edible films to obtain the best value of tensile strength and puncture force. A lower concentration of polysaccharide was not enough to stabilize the backbone structure of the films while a higher concentration could increase the brittle.

Although karaya gum has been considered an inappropriate material to form edible films (Nieto, 2009), the result of the current study showed that a combination of karaya gum and glycerol helped form the films with excellent physical properties to possibly apply in packaging technology. Two-way ANOVA and LSD comparison revealed that both tensile strength and puncture force decreased with the increase of glycerol concentration ($P < 0.05$). Similar trends were reported in the previous studies which using glycerol to modify biopolymer films (Bourtoom, 2008; Jouki et al., 2013; Sanyang et al., 2015). As a plasticizer, glycerol was responsible for the decrease of interactions between polysaccharide molecules and the formation of new hydrogen bonds. For examples, glycerol could weaken the macromolecules forces between agar particles (Arham et al., 2016) or increase the free volume between rice starch and chitosan (Sobral et al., 2001). In our study, the addition of glycerol improved the puncture deformation of karaya films. For films containing 4% karaya gum, the deformation could be enhanced to 300% (Table 1) as increasing glycerol concentration from 10 to 20% (w/w). Therefore, it can be confirmed that glycerol contributed to improving the flexibility of karaya films.

3.4. Thermal properties

Thermal analysis was applied to evaluate the heat resistance of edible films based on karaya gum (4% w/v). In all samples, the thermo-curves (Figure 2) showed a major melting peak in a range of 120 - 140°C which should be represented to the release of water in heating. It means that edible films based on karaya gum were thermal-stable below 100°C and hence can be applied as commercial plastic films in food packaging. This

result was in line with the previous study which suggested that the decomposition temperature of raw karaya gum was around 316°C and the water loss occurred around 60°C (Sarathchandiran, 2014). In the current study, it was observed that glycerol played an important role in the water holding capacity of karaya films. For example, the water loss of films adding glycerol only occurred above 100°C instead of 60°C as raw gum. Figure 2 indicated that films with minimum glycerol concentration transform apparently to a viscous rubbery state at a lower temperature than others. Glycerol in karaya films made films more hydrophilic and maintained a high moisture content. The result in this study showed the agreement with Shekarabi's research , in which he reported that the glass transition value of edible films increased sharply with higher glycerol content since new linkages between the polymer chains required more energy to break up and release the aqueous phase (Shekarabi et al., 2014).

4. Conclusions

This work was successful to produce edible films based on karaya gum with a concentration in a range of 3 - 5% (w/v). As expected, the addition of glycerol contributed to improve the water vapor permeability, the flexibility and the textural properties of those films. A combination of 4% karaya gum and 10% glycerol (w/w) was the appropriate ratio to obtain films having the highest values of tensile strength and puncture force while remaining folding ability. Edible films produced from those materials were thermal stable below 100°C and could be applied in food packaging. Further studies can combine karaya gum with other materials such as lignin, chitosan, or pectin to formulate new edible films or improve the physicochemical properties of films.

Conflict of interest declaration

We do not have any conflict of interest.

This study does not involve any human or animal testing.

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