e-ISSN 2586-9396

Current Applied Science and Technology

Vol. 18 No. 1
January - April 2018

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

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Each volume comprises three issues (January-April, May-August and September-December).

Current Applied Science and Technology Journal (CAST)

(formerly KMITL Science and Technology Journal)

CONTENTS

Physicochemical Properties of Low Glycemic Index-High Fiber Rice Flour from Storage Rice Grain and Application on Chocolate Chip Cookies as Substitute for Wheat Flour

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Received: 12 July 2017, Revised: 16 March 2018, Accepted: 28 March 2018

Abstract

Chocolate chip cookies are classified as a medium to high glycemic index food (60-75). Thus, the objective of this study was to develop low glycemic index-high fiber chocolate cookies by using low glycemic index-high fiber (LGI-HF) rice flour. The LGI-HF rice flour was produced from two- year storage amylose rice by enzymatically and physical modification. The LGI-HF rice flour had 12.61, 50.45 and 30.66 % of moisture content, degree of crystallinity, resistant starch content and 54.67 of estimated glycemic index, respectively. The LGI-HF rice flour was applied in the cookies formulation at 0, 5, 10, 15 and 20 % substituted for wheat flour. The result showed that 20 % of LGI-HF rice flour substitute of wheat flour was higher on total acceptance score by 30 panelists than the control cookies which contained 14.03, 5.99, 21.94, 1.55, 10.31, and 11.67 % of moisture, protein, fat, ash, fiber, carbohydrate and resistant starch content, respectively. An estimate glycemic index of the developed cookies was lower (60.63) than the control (72.01). Consumers test revealed that 99 % of the consumers accepted and decided to buy the developed chocolate chip cookies with 30 Baht per 100 grams.

Keywords: modified rice flour, glycemic index food, wheat flour

1. Introduction

Food industry is being challenged to re-formulate cookies products for optimal nutritional value, in response to some population sectors with particular nutritional necessities and making them as tasty as or better than the original. One way to achieve a healthy cookies product is to reduce of the calorie-laden ingredients, especially carbohydrate and increase dietary fibre. Carbohydrate-rich foods such as clear wheat flour have high glycemic indexes (GI) and are certainly not good for people with diabetes and cardiovascular disease. Previous studies showed that digested carbohydrate-rich diets resulted in a steady state of hyperglycemia and incidence of type 2-diabetes. While, some carbohydrates break down more slowly, releasing glucose gradually into blood streams and having lower glycemic indexes [1, 2]. The concept of glycemic index (GI) was classify carbohydrate-based foods according to their postprandial glucose responses. The GI is defined as

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the glycemic response in the 2 h immediately subsequent to the consumption of 50 g of carbohydrate, and is expressed as a value relative to that of white bread or glucose [3, 4]. Recently, low glycemic index food ingredients are derived from enzymatically and physical modification of high amylose rice flour in the form of resistant starch type III [5, 6]. Resistant starch (RS) is described as starch that escapes digestion in the small intestine [7]. RS can be found in nature and its contents of RS are varied by botanical source and starch granular structure (resistant starch type I and II). In addition, resistant starch can be produced from retrogradation of gelatinized starch known as resistant starch type III (RS III). RS III has been reported that starch retrogradation could result in a reduction of GI value, due to the increased resistance to digestive enzymes [8]. Thailand is the major rice producing country and the largest rice exporter with the export volume of about 5-6 million tons per year. Recently news form Thai government paddy mortgage project reported that over two-year storage rice from rice milling factory involved in the paddy mortgage project was low quality. Thus, on order to increase the value of this rice by means of utilizing it as raw material for production of low glycemic index-high fiber (LGI-HF) rice flour and its utilization in chocolate chip cookies are interested. Chocolate chip cookies contain complex fat emulsion system including flour, sugar, fat, eggs and baking powder. A proper combination of the ingredients can give a high quality product with desirable flavour and texture. Chocolate chip cookies is a food well-liked by consumers all over the world. However, due to its high glycemic index, over-consumption may contribute to chronic diseases. Previous study [8] revealed that the glycemic indexes of chocolate chip cookies products vary continuously form about 76 to over 80/ 100 g. In order to reduced glycemic index in the product. Thus, the objective of this work was investigate an optimum condition for LGI-HF rice flour production from rice milling factory under Thai government paddy mortgage project. The utilization of LGI-HF rice flour as wheat flour replacement on the physiochemical properties, sensory evaluation, resistant starch content, estimated glycemic index of the developed Chocolate chip cookies were studied.

2. Materials and Methods

2.1 Materials

High amylose rice grain (mixed rice between Hompratum and Chainat varites) which was stored for over 2 years in factort silostorage was supplied by Nitinansupakit rice milling, Phijit province Thailand. All-purpose wheat flour, butter, salt, sugar, baking powder, egg, fructose corn syrup (HFCS-55) brown sugar, dark and white chocolate chip were purchased from supermarket in Lampang province, Thailand.

Food grade pullulanase debranching enzyme (2,000 Units/mL) was obtained from Yojobio, China). Resistant starch assay kit (Megazyme) was obtained from Megasyme International Ireland Ltd., Ireland. Pepsin (EC 3.4.23.1; 2,980 unit/mg), α- amylase (E.C. 3.2.1.1; Type VI-B from hog pancreas; 20.4 unit/mg) and amyloglucosidase (A-3042; 69.65 unit/mg, from *aspergillus niger*), Glucose (GO) assay kit (GAGO-20) were purchased from Sigma Chemical Company, USA.

2.2 Modification of low glycemic index high fiber (LGI-HF) rice flour

The three replications of the over two- year storage rice grain samples were soaked for 4 h and water drain before dried at 50 ºC for 4 h. Afterward the rice grain was ground using a Cyclotec mill (Cyclotec TM 1093, Foss, Sweden) and sieved through a 100 micrometer sieve. The rice flour samples were molecular and physical modification as described in Patsanee *et al*. [9] to produced LGI-HF rice flour as following method. The 50 liter of 15 % gelatinized high amylose rice flour suspension was molecular modified by food grade pullulanase debranching enzyme (5 unit /g rice flour) at 55 °C for 4 h and deactivated enzyme by increased temperature to 95 ºC for 15 mins. Afterward, the debranched rice flour was cooling to 4 ºC followed by one cycle of freeze-thawed process (-10/30 °C). Degree of syneresis, production yield, moisture content and resistant starch content were examined according to Karim *et al*. [10] (2000) and AOAC [11] by Resistant starch assay kit (Megzyme), respectively. Microscopic images of LGI-HF rice samples were examined using SEM with magnifications of 5000x [12]. X-ray diffraction patterns of the four rice flour samples were measured with copper K_2 radiation (λ =0.154 nm) using a diffractometer (JEOL,JDX-3530, Japan), operated at 300 mA and 30 kV, 2θ range from 0 to 50.0 ^{o} with a step size 0.05 ^{o} and a count time of 2s. The data was analyzed with a MDI Jade 6.5 (Japan) program. The crystallinities of the rice flour samples were calculated as the proportion of crystalline area to total area at angles between 12 to 47 ^{o} 2θ [13].

2.3 Preparation of Chocolate chip cookies

Chocolate chip cookies formulation system (100 % component) was set at 37.03, 8.64, 6.12, 18.51, 1.23, 0.6, 12.34 and 15.43 % of wheat flour, chocolate chip, raisin, butter salt, baking soda, vanilla, sugar and egg, respectively. The LGI-HF rice flour was replaced to wheat flour at 0, 5, 10, 15 and 20 %. First, the butter was beaten on level 3 (medium speed) with the paddle attachment in a Kitchen Aid® stand mixer for 5 min and then combined with brown sugar, corn syrup and whole egg and beaten to form moist peaks. Next, sifted wheat flour, LGI-HF rice flour, salt, baking powder and chocolate chip were added and well mixed with beater until all of the ingredients were incorporated into the batter. The mixed batter were wrapped and keep at 10 ^{o}C in refrigerator for 16 h. Afterward, the cookies dough was scooped up a golf ball size with a spoon and dropped onto a cookie sheet and then ten pieces of cookies were measured diameter with digital vernier. Put the cookies in the oven and baked for 10 mins in a 350 °F Hotpoint electric oven. The cookies were allowed to cool for 30 mins and the diameters were measured with digital vernier. The rest of the cookies was packed in low-density polyethylene for future physiochemical analysis and sensory evaluation.

2.4 Physiochemical analysis and sensory evaluations

The spread ratio was calculated using the formula: diameter of chocolate chip cookies divided by height of chocolate chip cookies [14]. Chocolate chip cookies color and hardness value were measured after cooling for 1 h at room temperature. The 3 replications of color value chocolate chip cookies were measured using a Minolta spectrophotometer (Minolta, Co. LTD, Japan) which was measurement of the lightness/brightness or whiteness, (L^*) where black is no reflection and white is perfect diffuse reflection; greenness-redness (a^*), in which negative values indicate green and positive values indicate red, and blueness-yellowness (b^*), where negative values indicate blue and positive values indicate yellow. Furthermore, the hardness value of each chocolate chip cookies sample was measured by using Texture Analyzer (TA-XT Plus, Stable Micro Systems, Surre, UK) within 24 h after baking. Instrument settings were compression mode; sharp cutting blade probe type HDP/BS blade set was used. The parameter used was at pretest speed 2.0 mm/s; post-test speed 5.0 mm/s; test speed 1.0 mm/s; and auto trigger type.

Sensory evaluations were conducted by 30 trained panelists consisting of Rajamangala University of Technology Lana's staff members and students. The chocolate chip cookies samples were evaluated based on likeness score of their appearance, odor, flavors, texture and total acceptance by a hedonic 9-point scale where 9 means most liked and 1 means most disliked [15].

2.5 Nutritive value of chocolate chip cookies analysis

Nutrition compositions of the control and developed chocolate chip cookies were analysed for moisture, protein, fat, ash, crude fiber and carbohydrate content according to AOAC method [11]. Total energies in the control and developed chocolate chip cookies were calculated by multiplying the number of gram of carbohydrate, protein and fat by 4, 4 and 9, respectively. Then sum of the results together to get a total energy as kcal/100 grams cookies [16].

Resistant starch (RS) contents in the control and developed **c**hocolate chip cookies samples were determined using a Megazyme Resistant Starch kit [17]. The samples were incubated in a shaking water bath with pancreatic α-amylase and amyloglucosidase for 16 h at 37 °C to hydrolyzed digestible starch to glucose. The reaction was terminated with 4 ml ethanol and the indigested RS III was recovered by centrifugation (5000 *g*, 10 min). The supernatant was then decanted and washed with 50 % ethanol twice to remove the digested starch. The sediment was solubillized in 2 ml of 2 M KOH in an ice bath, neutralized with 8 ml sodium acetate (1.2 M) and the RS hydrolyzed to glucose with of amyloglucosidase (0.1 ml, 3300 U/ml). The glucose oxidase/peroxidase reaction was used to measure glucose released from the digested and resistant starches. Absorbance was read at 510 nm after a 20 min incubation period at 50 °C. Resistant starch and digested starch were calculated as glucose × 0.9. The total starch was calculated as the sum of resistant starch and digested starch.

In vitro starch hydrolysis and glycemic index (GI) in the control and developed **c**hocolate chip cookies samples were determined according to Goňī, *et al.* [18] with slightly modification. Using the hydrolysis curve (0-180 min), the hydrolysis index (HI) was calculated as the percentage of total glucose released from the samples, to that released from white bread. The glycemic index of the samples was estimated according to the equation: $GI = (39.71+0.549) \times HI$.

2.6 Consumer test

Consumer test was done by 100 consumers from Lampang province, Thailand. Consumer testing consisted of a paper ballot questionnaire probing consumer perception of developed chocolate chip cookie and control cookies products on decided to buy and acceptability of the cookies appearance, odor, flavors, texture and overall preference by a hedonic 9-point scale where 9 means most liked and 1 most disliked data were analyzed according to Lawless and Heymann [15]

2.7 Statistical analysis

Completely Randomized Design (CRD) was used to evaluate the data means of physical properties of chocolate chip cookies samples. Randomized Completely Block Design (RCBD) was used to evaluate the means of sensory scores of the chocolate chip cookies samples. The data obtained for the physical properties and sensory evaluations were subjected to analysis of variance (ANOVA). Duncan's Multiple Range Tests procedure was used to compare differences between treatments [15]. The good quality and the highest total acceptance score of chocolate chip cookies sample were selected for further analysis on nutritional quality and consumer test compared with the control chocolate chip cookies sample. The consumer test data were analyze according to Lawless and Heymann [15].

3. Results and Discussion

3.1 Physicochemical properties of modified LGI-HF rice flour

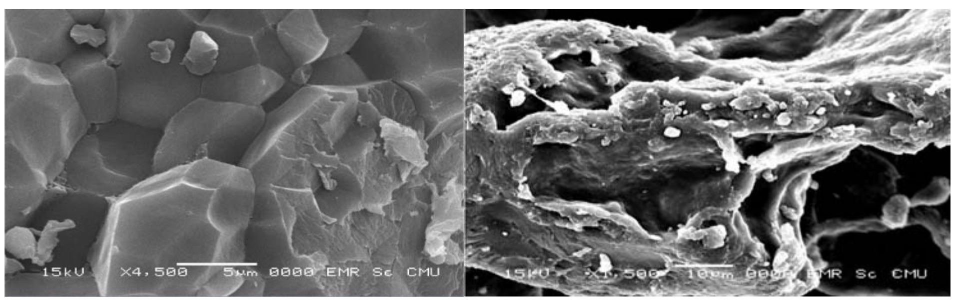
Physicochemical properties of over two-year storage amylose rice grain and modified LGI-HF rice flour samples are shown in Figures 1 to 4. According to the results, an appearance of storage amylose rice grain changed to yellow color after two-year storage at room temperature in rice mill factory, which was classified as low quality rice and cheaper price when compared with new rice grains.

An appearance of modified LGI-HF rice flour is shown in Figure 1. The color of rice flour sample (A) was yellowish, while the modified LGI-HF rice flour (B) was whitening. This results could be due to the process of enzymatically and physically modification of the over two year storage amylose rice grain that improved physical properties and nutritional quality of the rice sample.

(A) (B)

Figure 1. Appearance of samples from two-year storage amylose rice grains and rice flour (A) and modified LGI-HF rice flour (B)

The morphological image of the two-year storage amylose rice flour and modified LGI-HF rice flour presented in Figure 2. Amylose rice flour granules have polygonal shapes with diameters between 3-5 µm. The surface of the granules was smooth with some surface erosion and slightly damaged due to long time storage in warming temperature while the LGI-HF rice flour formed a coarse filamentous network structure which is similar to the finding by Pongjanta *et al.* [7].



(A) (B)

Figure 2. Morphological image of samples from two-year storage amylose rice flour (A) and modified LGI-HF rice flour (B).

X-ray diffraction patterns of amylose rice flour and LGI-HF rice flour samples are shown in Figure 3. The diffraction pattern obtained from amylose rice flour was classified as an A-type pattern as indicated by typical peaks at 15.0, 17.5, 20.0 and 23.2 ^{o} of diffraction angle 2θ. The calculated crystallinity of native rice flour was 35.38 %. The value was in agreement with those reported for amylose rice flour [5, 7]. The amylose rice flour subjected to pullulanase debranching and retrogradation treatments displayed V-type diffraction pattern, with 50.45 % of crystallinity degree. This was attributed to debranching and retrogradation of amylose chain which reorganized the structure of starch into a helical complex to that of V-amylose pattern.

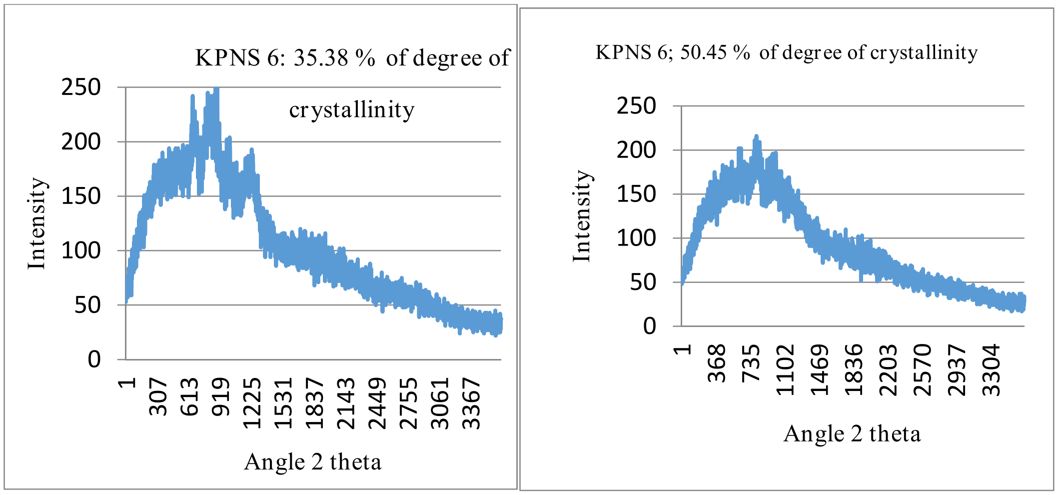


Figure 3. X-ray diffraction patterns of samples from two-year storage amylose rice flour (A) and modified LGI-HF rice flour (B).

Degree of syneresis, production yield, moisture content, degree of crystallinity, resistant starch, digested starch, total starch and estimated glycemic index of LGI-HF rice flour presented in Figure 4. The molecular and physical modified rice flour solution had 67.30 % and 99.78 % of syneresis degree and production yield, respectively. In addition, physicochemical properties of LGI-HF rice flour were 12.61 %, 50.45 %, 30.66 %, 53.14 %, 83.80 % and 54.67 % of moisture content, degree of crystallinity, resistant starch, digested starch, total starch and estimated glycemic index, respectively, which was higher than the previous studied [7, 19-21]. This might be cause by the long time storage of the rice grain sample.

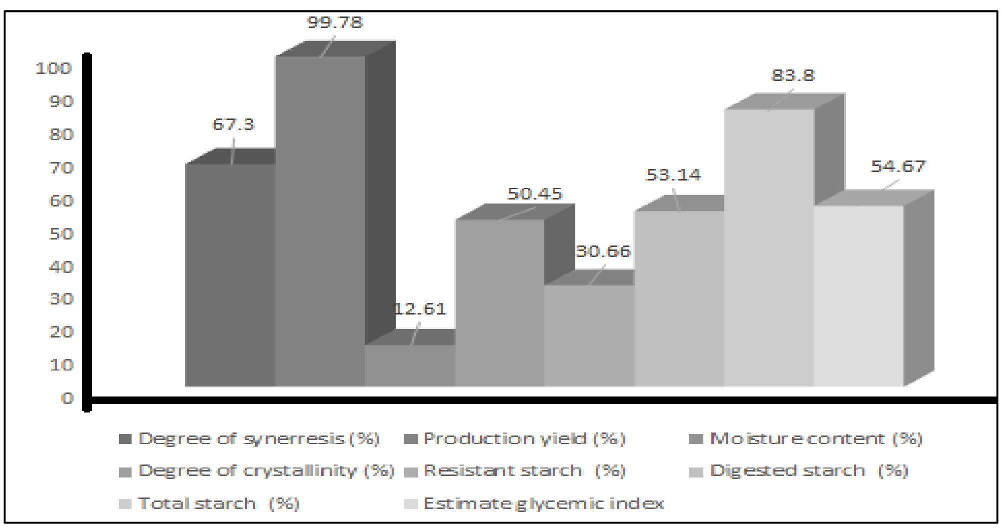


Figure 4. Physicochemical properties of modified LGI-HF rice flour from two-year storage rice grains

3.2 Physical properties of LGI-HF chocolate chip cookies

Table 1 presents the spread ratio, color value and hardness value of chocolate chip cookies with the substituted of LGI-HF rice flour at 0 - 20 % of wheat flour. The spread ratio exhibited non-significant variation between treatments within the range of 1.51-1.51. However, the control chocolate chip cookies gave higher spread ratio than those developed **c**hocolate chip cookies containing LGI-HF rice flour. This might be due to the reduced amount of gluten and the rapid heat input causes less spread of the developed **c**hocolate chip cookies containing LGI-HF rice flour. This result was consistent with the report of Savita *et al*. [14] who indicated that increasing the level of fiber from red and white bran decreased the cookie spread ratio.

The color values for the *L**, *a** and *b** coordinates of the control and LGI-HF chocolate chip cookie were 40.11-43.82, 11.46-13.54 and 26.66-29.83, respectively. The control chocolate chip cookies had the greatest redness (a*), and yellowness (b*) compared to LGI-HF chocolate chip cookies. In addition, the hardness value of LGI-HF chocolate chip cookie was significantly different ($p<0.05$) with the variation of LGI-HF rice flour content. The hardness value increased when the LGI-HF rice flour content increased from 320.68 to 403.27 N. This was because of LGI-HF rice flour producing high absorption dough tend to be hard cookies structure. Similar study reported that more strength was needed to break cookies incorporated with legumes flour with high amounts of resistant starch [22].

Table 1. Physical properties of LGI-HF chocolate chip cookies

LGI-HF rice flour (%)	Physical properties				
	Spread ratio	Color value			Hardness value (N)
		*L**	*a**	*b**	
0 (control)	$1.51±0.15^{ns}$	$41.25±0.29^{ns}$	$13.54±1.88^{bc}$	$29.83±2.04^{ns}$	$320.68±64.5^{c}$
5	1.56±0.11	43.82±1.50	$12.58±1.57^{bc}$	28.02±2.53	$379.62±86.3^{ab}$
10	1.55±0.29	42.78±1.17	$12.97±2.07^{ab}$	28.92±2.86	$348.43±68.2^{b}$
15	1.51±0.13	41.23±0.72	$11.98±3.03^{a}$	27.06±3.67	$357.99±54.6^{b}$
20	1.58±0.18	40.11±7.74	$11.46±1.43^{c}$	26.66±6.63	$403.27±57.8^{a}$
C.V (%)	1.54	6.03	2.37	3.49	27.34

^{ns}Means in a column are not significantly different ($p>0.05$)

^{a,b}Means in a column with a different upper script letter are significantly different ($p<0.05$)

3.3 Sensory evaluation of LGI-HF chocolate chip cookies

The mean sensory evaluation scores for appearance, color, odor, flavor, crust crispy, crumb softness and total acceptance of chocolate chip cookies which wheat flour was replaced with rice flour at 0, 5, 10, 15 and 20 % of LGI-HF rice flour as shown in Table 2. There were not significant ($p>0.05$) scores in the appearance, color, odor, flavor and crumb softness for both experimented cookies. In terms of crust crispy and total acceptance score, the mean scores increased as the level of LGI-HF rice flour increased, with 20 % LGI-HF rice flour having the highest mean score. This indicated that as the percentage of LGI-HF rice flour increased, the crust cookie's crispiness and total acceptance score improved. Thus, the 20 % LGI-HF rice flour replaced of wheat flour and control chocolate chip cookies were selected and subsequently used for nutritional analysis and consumer test.

3.4 Nutritive value of chocolate chip cookies

The chemical composition of control and chocolate chip cookies made from 20 % of LGI-HF are summarized in Table 3. The LGI=HF chocolate chip cookies was higher in moisture content, crude fiber and resistant starch content but lower in carbohydrate and total energy than the control chocolate chip cookies. In addition, the LGI-HF chocolate chip cookies were not significantly different (p>0.05) in fat, ash and protein content from the control chocolate chip cookies which was ranged from 2.14 to 2.16 %, 1.38 to 1.55 % and 5.84 to 5.89 %, respectively.

Table 2. Sensory evaluation result of LGI-HF chocolate chip cookies

LGI-HF (%)	**9 Point Hedonic Scaling Test**						
	Appearance	Color	Odor	Flavor	Crust crispy	Crumb softness	Total acceptance
0 (control)	7.37± 2.14^{ns}	7.27± 1.43^{ns}	7.43± 2.13 ^{ns}	7.33 ±1.84^{ns}	6.23± 2.84^{b}	7.17± 2.15^{ns}	7.01± 2.24^{c}
5	7.17± 2.10	7.17± 2.12	7.20 ±1.28	7.13± 2.44	6.70± 2.42^{ab}	7.4± 2.22	7.34± 2.12^{b}
10	7.33± 1.24	7.40± 3.14	7.3 3± 2.91	7.20± 3.44	6.67± 2.64^{ab}	7.37± 2.24	7.43± 2.62^{ab}
15	7.27 ±2.05	7.10± 1.34	7.43± 4.24	7.13± 4.22	6.67± 3.52^{ab}	7.44± 2.31	7.40± 2.24^{b}
20	7.23± 2.32	6.97± 1.74	7.30± 4.14	7.20± 3.24	6.77± 2.34^{a}	7.50± 2.24	7.57± 2.64 ^{a}
C.V (%)	5.99	2.59	7.33	9.64	5.57	4.36	5.74

^{ns}Means in a column are not significantly different (p>0.05)

^{a,b}Means in a column with a different upper script letter are significantly different (p<0.05)

Table 3. Chemical composition of LGI-HF chocolate chip cookies and control

LGI-HF rice flour (%)	Chemical composition (%)						Total energy (kcal)	Resistant starch content (%)
	Moisture	Fat	Crude fiber	Ash	Protein	CHO		
0 (control)	12.66± 0.52 ^{b}	2.16± 0.51^{ns}	8.54± 1.09 ^{b}	1.38± 0.53^{ns}	5.84± 0.44 ^{ns}	69.42 ± 2.12^{a}	320.48± 4.82^{a}	2.01± 0.22^{b}
20	14.03 ± 0.14^{a}	2.14± 1.06	10.31± 2.09^{a}	1.55± 0.15	5.89 ± 0.092	65.98± 1.24^{b}	179.30± 3.85^{b}	3.63± 0.18^{a}
C.V (%)	2.57	2.31	2.46	3.79	2.30	2.15	4.37	1.37

^{ns}Means in a column are not significantly different (p>0.05)

^{a,b}Means in a column with a different upper script letter are significantly different (p<0.05)

In vitro starch hydrolysis rate and estimated glycemic index of control and LGI-HF chocolate chip cookies were shown in Table 4. An Area Under Curve (AUC) from the starch hydrolysis rated from 0 to 180 min of the LGI-HF chocolate chip cookies samples divided by AUC of white bread revealed that AUC of LGI-HF chocolate chip cookies was significantly (p<0.05) lower than the control chocolate chip cookies. The hydrolysis index of 20 % LGI-HF rice flour in chocolate chip cookies was lower (50.69 %) than the control (58.84 %). Furthermore, an estimated glycemic index (EGI) value for the LGI-HF chocolate chip cookies was significantly ($p\leq0.05$) lower (60.63) than the control chocolate chip cookies (72.01). Jenkins *et al*. [2] classified glycemi index (GI) value that the value at 70 or more is high, a GI of 56 to 69 inclusive is medium, and a GI of 55 % or less is low. Thus, the 20 % LGI-HF rice flour chocolate chip cookie was classified as a mediume GI food.

Table 4. *In vitro* starch hydrolysis rate and estimated glycemic index of the control and LGI-HF chocolate chip cookies.

LGI-HF rice flour (%)	*In vitro* starch hydrolysis rate		
	Area under curve	Hydrolysis index	Estimated glycemic index
0	1,654.87 ±18.05^{a}	58.84±6.43^{a}	72.01±3.53^{a}
20	1,461.85 ±11.37^{b}	50.69±6.44^{b}	60.63±3.54^{b}
C.V (%)	11.03	11.02	4.79

^{a,b}Means in a column with a different upper script letter are significantly different ($p<0.05$)

3.5 Consumer test of the LGI-HF chocolate chip cookies

The questionnaires for the LGI-HF chocolate chip cookies were collected and data were analysed. Most consumers were ladies (71 %) with average age between 20-25 years and had a diplomas and bachelor degrees. Among them were students and private businessman which was had average monthly income of 2,000 to 10,000 baht. Almost 96 % of the consumers had been eating chocolate chip cookies and 55 % of them buying from local supermarket around 2-3 times a week. Most consumers (100 %) have never eaten LGI-HF chocolate chip cookies. The mean scores of 9 point hedonic scale on appearance, color, odor flavor, texture, mouth feel and total acceptance of developed LGI-HF chocolate chip cookies by 100 consumers were medium like within the range of 7.27-7.78 point (Figure 5). In addition, the 100 % of the consumers accepted and decided to buy the LGI-HF chocolate chip cookies products at 30 Baht per 100 grams (Figure 6).

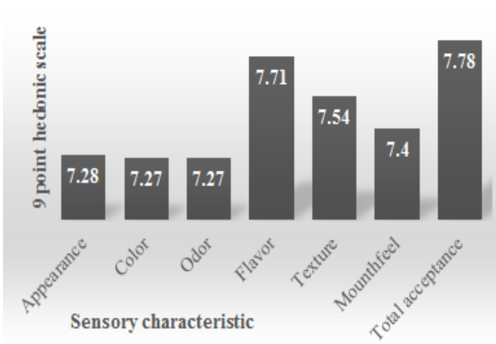


Figure 5. Nine point hedonic liking scale of LGI-HF chocolate chip cookies by 100 consumes.

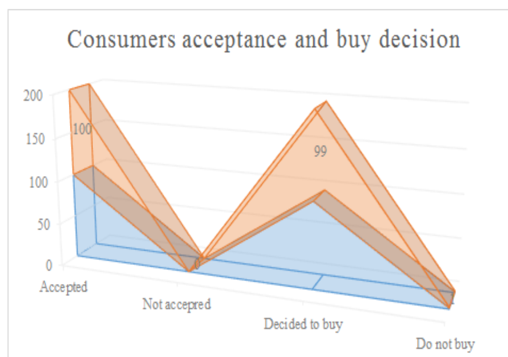


Figure 6. Consumers acceptance and decision to buy the LGI-HF chocolate chip cookies.

4. Conclusions

This study revealed that the enzymatically and physically modified amylose rice grain (two- year storage) enhanced the physical properties and nutrition quality in the form of LGI-HF rice flour. The 20 % of LGI-HF rice flour in substitution of wheat flour significantly enhanced crude fiber content in chocolate chip cookies. The LGI-HF chocolate chip cookies had lower energy content and estimated glycemic index than the control cookies sample. A composite of LGI-HF rice flour and wheat flour increased dietary fiber in the form of resistant starch from 2.01 to 3.63 % per 100 g of the cookies products consumed and reduced estimate glycemic index from 72.01 to 60.63. The

LGI-HF chocolate chip cookies products were accepted by 100 % consumer group and decided to buy the product at 30 Baht per 100 grams.

5. Acknowledgements

This research was supported by the National Research Council of Thailand. The authors acknowledge the valuable laboratory support from the Science and Technology Service Center, Faculty of Science, Chiang Mai U niversity, Thailand and Nitinansupakit Rice Milling, Phichit province, Thailand for supplying amylose rice grains.

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Effects of Transglutaminase and Kappa - carrageenan on the Physical and Sensory Qualities of Fish (*Pangasius hypophthalmus*) Patties

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Received: 12 July 2017, Revised: 15 March 2018, Accepted: 28 March 2018

Abstract

The objective of this research was to study the effect of transglutaminase (TGase) and κ-carrageenan (CG) on the physical and sensorial qualities of a fish patty compared with control (with 0.2 % phosphate). A 3 × 3 full factorial in Complete-Randomized-Design was performed using TGase at 0, 0.3 and 0.6 % with CG at 0, 0.5 and 1 % (w/w), respectively. The results indicated that cooking loss of fish patties containing TGase alone at 0.3 % and 0.6 % was not different from the control. On the contrary, the cooking loss was likely to decrease when 0.3 % TGase was applied with CG at high level compared to other formulations. Regarding the percentage of expressible moisture content (% EMC), the results showed that increasing the level of CG alone has no significant effect on the % EMC ($P > 0.05$), whereas using TGase with CG yielded fish patty with lower % EMC ($P > 0.05$). The lowest % EMC value was found in sample containing TGase/CG at 0.3/1.0 %, (w/w). It had lower Δ cooking loss and higher hardness (4.37 N) than the control (3.14 N) with the overall like score of 6.5 (slightly to moderately like). Providing the health benefit of developed fish patty to consumers, the consumers' purchase intention increased from 60 % to 79 %. This study indicated that TGase and CG could be applied together at the appropriate amount for producing fish patty with selected comparable quality with the control.

Keywords: fish patty, transglutaminase, κ-carrageenan, physical quality, consumer acceptability

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1. Introduction

Increasing on demand for minimally processed, safe and stable products has stimulated the interest in research on alternative technological approaches [1]. Phosphate is a food additive as GRAS (Generally Recognized as Safe) [1]. It enhances the effect of salt properties by improving the water binding capacity, cohesion, and yield of meat products [2].

However, the demand for meat products made without added phosphate is increasing [3]. The presence of excessive amounts of phosphates in the diet may affect the balance of some mineral contents such as calcium, iron and magnesium in the human body as well as can cause the risk of bone diseases [2]. Moreover, higher concentrations of phosphate affect the flavour of the finish product. Transglutaminase (TGase) initiates the formation of covalent bonds between glutamine and lysine residues in proteins [1, 3, 4]. The addition of TGase can improve the thermal stability of meat and fish proteins, imparting desirable properties to reconstructed products during heating [1].

Several researchers reported the positive effect of TGase on the texture of various products such as restructuring pork shoulder, chicken meat, beef and fish [4-6]. In addition, dietary fiber, for instance, carrageenans, pectins and konjac have been used by the meat industry for their gelling properties. The capacity to form a gel and its characteristics depend on various factors such as concentration, temperature, presence of certain ions and pH. Thermo-irreversible gels are used to bind diced meat pieces as cold-set binders and produce restructured meat products [7]. The addition of dietary fibre could improves the nutritional value, textural quality and cooking yield of meat product by increasing water and fat binding capacities [8, 9]. There have been various studies regarding the application of either TGase or dietary fiber in meat products. The TGase was reported to enhance gel strength of meat product after cooking at low-salt levels, while some dietary fiber contributed to water-binding ability improvement and cold-set gel formation. Therefore, combining of TGase with dietary fiber would be the potential advantage of improving the quality of patty product at lower salt level more than using TGase alone [10].

Pangasius hypophthalmus is one of the major fish species in the Mekong River fishery, one of the largest and most important fisheries in the world [11]. However, according to its soft texture, it was still underutilised to produce processed food. The study on the quality of underutilised fish fillet due to its texture by using both enzyme and dietary fiber without phosphate added was limitedly reported. Therefore, objectives of this study were 1) to study the effects of transglutaminase and carrageenan on the quality of underutilised fish fillet and 2) to evaluate the consumer acceptability on the developed fish patty product.

2. Materials and Methods

2.1 Materials

Fish fillet from *Pangasianodon hypophthalmus* was provided by Charoen Pokphand Foods Public Company Limited, Thailand. All ingredients used in the formulation were food grade. Transglutaminase (TGase) with actively of 100 unit/g dry matter was supplied by Ajinomoto Co., (Thailand) Ltd., Bangkok, Thailand and consisted of 99 % maltodextrin and 1 % enzyme on a mass basis.

2.2 Methods

2.2.1 Amino acid profiles of fish (*Pangasianodon hypophthalmus*) fillet

Amino acid profiles of fish fillet were analysed according to AOAC [12].

2.2.2 Determination of pH and TVB-N value of raw material

The pH of fish fillet as raw material was determined according to Choi *et al*. [13] using a pH meter (CyberScan 2500, EUTECH instrument, Singapore). The TVB-N value was measured in duplicate following the method of Hong and Chin [10].

2.2.3 Preparation of Fish patties

To prepare the fish patties, fish fillet was thawed at 4^{o}C for 16 h prior to processing. It was then minced using a grinder. Two hundred grams of each treatment was prepared and 0.25 % of salt was added to the batters. The levels of TGase (0, 0.3, and 0.6 %, w/w) and carrageenan (CG at 0, 0.5, and 1.0 %, w/w) added to the formulations were determined using factorial 3 × 3 in CRD design. The batter was mixed using a hand-blender at low speed (approximately 12,000 rpm) for 2 min. After that, the batters were stuffed into cylindrical aluminium moulds with a diameter of 65 mm and height of 12 mm. Each sample was then heated on the pan to a final internal temperature of 72^{o}C, and thereafter cooled down until reached a core temperature of 25^{o}C. Then patties were subjected to the selected measurements. The fish patty containing 0.2 % sodium tri-polyphospate without TGase and CG was used as the control.

2.2.4 Measurement of fish patty physical property

Cooking loss of fish patties was determined with six measurements for each treatment/batch and calculated by the weight differences between of each patty before and after cooking as the following equations:

$$\text{Cooking loss (\%)} = \frac{[(\text{weight of raw sample (g) - weight of cooked sample (g)})]}{[\text{weight of raw sample (g)}]} \times 100 \qquad (1)$$

$$\Delta\ \text{Cooking loss (\%)} = \frac{[(\text{cooking loss of sample (\%) - cooking of the control (\%)})]}{[\text{cooking of the control (\%)}]} \times 100 \qquad (2)$$

Colour profiles of fish patties were measured in quadruplicates using a Hunter Lab colour meter (ColorFlex E2, Hunter Associates Laboratory, Reston, VA, USA) and reported as the L* value (metric lightness), a* (redness/greenness), and b* (yellowness/blueness).

Textural profile analysis (TPA) of fish patties (core temperature of 72^{o}C) was evaluated in quadruplicates using a texture analyser (LF500, Lloyd instruments Ltd., Fareham, UK). The fish patty slice (30 × 30 × 10 mm^{3}) was compressed to 30 % deformation using a compression probe (diameter of 48 mm) at a cross-head speed of 50 mm/min. The value of hardness, springiness, cohesiveness and gumminess were determined.

2.2.5 Measurement of expressible moisture content

Fish patty samples were determined for expressible moisture content with triplicate measurements following the method of Kaewudom *et al*. [4]. The 5 mm thickness of cylindrical gel samples, weighted accurately (X), were placed between three pieces of Whatman paper No.4 at the bottom and two pieces on the top of the sample. The standard weight (5kg) was placed on the top and held for 2 min. Subsequently, the samples were removed from the papers and weighed (Y). Expressible moisture content was calculated by using the following equation:

$$\text{Expressible moisture content (\%)} = [(X - Y)/X] \times 100 \qquad (3)$$

2.2.6 Evaluation of sensory property

The only selected fish patties were used for sensory evaluation with the criteria of low cooking loss (positive value) as well as hardness values comparable with the control. The respondents were regular consumers who consume fish with no allergic to seafood products. They were served with 3-digit code of samples as counter-balance followed the randomized block design. The sample for each session was not over 4 samples. Respondents (n = 30) provided a liking score for appearance, overall flavour, softness, and overall like using the 9-point hedonic scale. They were provided with white bread and water to rinse their mouths before testing and between samples.

2.2.7 Evaluation of consumer acceptability

Consumer acceptability was conducted in partitioned booths illuminated with cool, fluorescent lights. Consumers (n = 60) who were not allergic to fish or sea food product were recruited. Each consumer was served with the selected fish patty formulation coded with a 3-digit number comparing with the control. They were provided with white bread and water to cleanse their palate before testing and between samples. They were asked to rate acceptability of appearance, texture softness, flavour and overall like using the 9-point hedonic scale on a paper ballot. Consumers were asked to provide the purchase intention before and after providing the benefit of product about using natural food additives. The 5-point Likert scale was used (1= definitely not purchase, 3 = neither purchase nor not purchase and 5 = definitely purchase).

2.2.8 Statistical analysis

All results were subjected to analysis of variance (ANOVA). The Duncan's Multiple Range Test (DMRT) was performed for post-hoc multiple comparison. Statistically significant difference was established at alpha=0.05.

3. Results and Discussion

3.1 Amino acid profile of fish fillet (*Pangasius hypophthalmus*)

Fish fillet from *Pangasius hypophthalmus* was too soft to make processed fish product such as fish ball or fish patty according to its weak protein structure. The fillets could be transformed into high-value products by the restructuring technology [13]. These technologies are used to obtain novel products using additives to improve the mechanical and functional properties.

However, several biochemical and physicochemical considerations regarding muscle proteins must be taken into account to obtain high-quality products. The amino acid profile was conducted to measure focusing on lysine and glutamine amino acid which are the main substrate of transglutaminase (TGase).

The results indicated that *Pangasius hypophthalmus* contained amino acid as shown in Table 1. The highest amount of amino acid found in the fish fillet was glutamic acid and lysine (1369.08 and 903.47 mg/100g), respectively. Moreover, the methionine was found to be at least 175.34 mg/100g. Therefore, TGase could be used to improve the texture of the finish product made from *Pangasius hypophthalmus* fillet, which contains substrates (glutamic acid and lysine) of the enzyme. The TGase catalyses an acyl transfer reaction between protein-bound glutaminyl residues and primary amine [1, 3] and can improve the thermal stability of meat and fish proteins, imparting desirable properties to reconstructed products during heating [1].

Table 1. Amino acid profiles (mg/100g) of fish fillet (*Pangasius hypophthalmus*)

Amino acid	Amount (mg/100g)
Alanine	685.46
Arginine	651.25
Aspartic acid	998.65
Cystine	138.57
Glutamic acid	1,369.08
Glycine	412.06
Histidine	449.22
Hydroxylysine	ND
Hydroxyproline	ND
Isoleucine	407.73
Leucine	791.89
Lysine	903.47
Methionine	175.34
Phenylalanine	380.61
Proline	387.32
Serine	443.16
Threonine	501.73
Tryptophan	203.93
Tyrosine	456.21
Valine	412.39

*ND = Not detected

3.2 pH and total volatile basic nitrogen (TVB-N) of fish fillet

The results showed that the fillet of *Pangasius hypophthalmus* was in the basic condition (pH 8.37) which still meet the quality standard of white fish meat (pH should be above 7.0). Total Volatile Bases Nitrogen (TVB-N) is one of the most widely used methods to estimate the degree of decomposition of fish. The level of TVB-N for white fish is generally considered to be fresh if the TVB-N is less than 20 mg/100 g sample [15]. The results showed that TVB-N of the raw material was of 8.34 mg/100 g which was in good quality and conformed to the standard [15].

3.3 Physicochemical property of the fish patties

The result indicated that pH of the fish patty increased when TGase and CG was added in the different levels as shown in Table 2. As the level of TGase and CG increased, the pH of the fish patties increased. This may be attributed to both enzyme and additive themselves having pH for TGase and CG normally ranged from 7.0 - 10.0 and 6.0 - 8.0, respectively [16].

In respected to the cooking loss, the tendency of decrease in a discrepancy of cooking loss (Δ cooking loss) was observed. Increasing the level of TGase decreased in the Δ cooking loss among all samples comparing with the control. The TGase was reported to induce the acyl transfer between acyl donors to acyl acceptor, in which ε-(γ-glutamyl) lysine linkage could be formed which contributed to the increase in gel strength [4]. Similar results were observed in the study of Moreno *et al.* [17], who reported that the water holding capacity of restructured fish gel from hake increased when 1 % MTGase was added. Trespalacios and Pla [6] also reported that the addition of 0.5 % MTGase, to beef homogenates significantly decreased expressible moisture content as well as the cooking loss. The results indicated that 0.3 % TGase was sufficient to reduce the cooking loss of the samples.

Table 2. The effect of transglutaminase (TGase) and carrageenan (CG) on the selected quality of fish patties

Sample[A] TGase (%)	CG (%)	pH	Δ Cooking loss (%)[B]	L*	a^*	b^*
Control		8.60±0.02c	0.0	69.51±1.18a	-2.82±0.09c	7.90±0.17ab
0.0	0.0	8.27±0.02e	5.9	68.28±0.52a	-2.84±0.14c	6.25±0.35b
0.3	0.0	8.55±0.02d	11.4	69.08±1.77a	-2.72±0.19bc	7.14±0.65ab
0.6	0.0	8.50±0.02d	2.8	69.08±1.54a	-2.81±0.26c	6.77±0.49b
0.0	0.5	8.58±0.02c	22.4	69.67±1.13a	-2.70±0.09abc	7.73±0.49ab
0.3	0.5	8.52±0.01d	17.7	69.05±0.18a	-2.58±0.09abc	6.52±0.52b
0.6	0.5	8.79±0.03b	-7.9	69.79±0.25a	-2.32±0.03a	6.44±0.25b
0.0	1.0	8.50±0.02d	-21.6	68.24±0.70a	-2.45±0.09abc	8.02±0.21ab
0.3	1.0	8.57±0.05c	-5.1	69.06±1.44a	-2.38±0.16ab	8.38±1.54ab
0.6	1.0	9.08±0.04a	5.6	67.25±1.36a	-2.46±0.06abc	9.23±1.50a

Mean ± standard deviation from six measurements for pH and cooking loss and triplicate measurement for colour profiles. Means with the different letters in a column were significant different ($P < 0.05$).

[A]The sample with phosphate served as the control. Sample with 0 % TGase and 0 % CG was the sample without phosphate.

[B](+) Δ cooking loss showed higher cooking loss but (-) Δ cooking loss showed lower cooking loss than the control.

Without TGase, the sample containing only CG at the highest level (1 %) had the lowest Δ cooking loss (-21.6 %). However, when only 0.5 % CG was applied, it had no effect on the reduction of Δ cooking loss of the samples. When the highest level of TGase (0.6 %) and CG (1 %) was incorporated in the formulation, the higher Δ cooking loss was noticeable. This may be attributed to the greater interaction between protein molecules with the denser network in the patty matrix. This led to more free water released from network [18] resulting in higher cooking loss of the sample.

The determination of colour profiles showed that there were no significant differences in lightness of the sample obtained with and without TGase and CG. It was probably due to the colour of CG that was white powder (L* = 90.28), and the inactivation step of enzyme during cooking. The result was similar to the study of Kaewudom *et al.* [4] who reported that there was no difference in whiteness of surimi gel from threadfin bream (*Nemipterus bleekeri*) with increasing levels of MTGase.

In addition, the sample containing TGase and CG at the appropriate levels obtained texture profiles comparable to the control. In the presence of only TGase, the hardness value of sample containing 0.3 % TGase was lower than other concentrations (Table 3).

Table 3. The effect of transglutaminase (TGase) and carrageenan (CG) on the textural quality of fish patties

Sample TGase (%)	Sample CG (%)	Hardness (N)	Cohesiveness	Springiness index	Gumminess
Control		3.14±0.30abc	0.51±0.14ab	0.82±0.02a	1.58±0.39ab
0.0	0.0	3.75±1.25ab	0.46±0.13ab	0.83±0.08a	1.66±0.73ab
0.3	0.0	1.94±0.67c	0.43±0.10ab	0.79±0.03a	0.88±0.49b
0.6	0.0	3.23±0.64abc	0.28±0.09b	0.84±0.03a	0.92±0.36b
0.0	0.5	4.04±0.69ab	0.54±0.11a	0.85±0.06a	2.16±0.55ab
0.3	0.5	3.28±1.39abc	0.42±0.13ab	0.83±0.06a	1.41±0.89ab
0.6	0.5	2.46±0.68bc	0.47±0.15ab	0.83±0.07a	1.17±0.55ab
0.0	1.0	4.56±1.17a	0.48±0.16ab	0.84±0.05a	2.25±1.04ab
0.3	1.0	4.37±1.63a	0.57±0.21a	0.85±0.07a	2.57±1.54a
0.6	1.0	3.11±0.60abc	0.59±0.14a	0.84±0.10a	1.90±0.79ab

Mean ± standard deviation from six measurements for pH and cooking loss and triplicate measurements for color profiles. Means with the different letters in a column were significant different ($P < 0.05$).

Comparing among all samples containing only CG, the level of CG had significant effect on hardness of fish patties. As the level of CG increase, hardness and gumminess value of fish patty significantly increased ($P < 0.05$). This result was in agreement with the study of Cardoso *et al.* [19], who stated that the CG acted as a simple filler of the myofibrillar protein gel, when increasing CG the hardening effect on restructured fish with dietary fibre was considerably observed. Cierach *et al.* [20] also found that CG caused a reduction in cooking loss and increased hardness and gumminess of the low-fat frankfurter. Moreover, Brewer [21] revealed that incorporated into low-fat meat formulations, CG improved the textural characteristics of the product by decreasing toughness and increasing juiciness.

When TGase mixed with CG, the result showed that as the levels of TGase and CG increased, the hardness of fish patties was significantly increased. However, increasing TGase up to 0.6 % did not cause further hardening effect on fish patties more than at 0.3 %. In the presence of 0.6 % TGase and 1.0 % CG, the fish patty had higher Δ cooking loss than the control. This result is consistent with Min and Green [9] who reported that the addition of MTGase increased textural properties such as binding strength, hardness, cohesiveness, chewiness, and springiness, but decreased cooking yield of the patties made from channel catfish (*Ictalurus punctatus*).

3.4 Expressible moisture content

The loss of water may result in shrinking of the gels, changing texture and reducing quality of food. Therefore, water holding capacity is an important criterion in evaluation of the acceptability of food gels [22]. Expressible water content of samples added with CG and TGase at different levels was significantly different as summarized in Figure 1. The expressible moisture content of fish patty decreased as the level of TGase increased. The result showed that the addition of TGase was able to hold water in the patty as observed by the decrease in expressible moisture content.

Additionally, when TGase and CG was incorporated together, it could induce protein cross linking at the appropriate level. The results showed that 0.3 % TGase and 1.0 % CG was sufficient to decrease the expressible moisture content. However, TGase at 0.6% with CG at 1.0 % had no effect on the reduction of expressible moisture content MTGase at the level above 0.4 unit/g did not cause the reduction in expressible moisture content in gel from unwashed mince

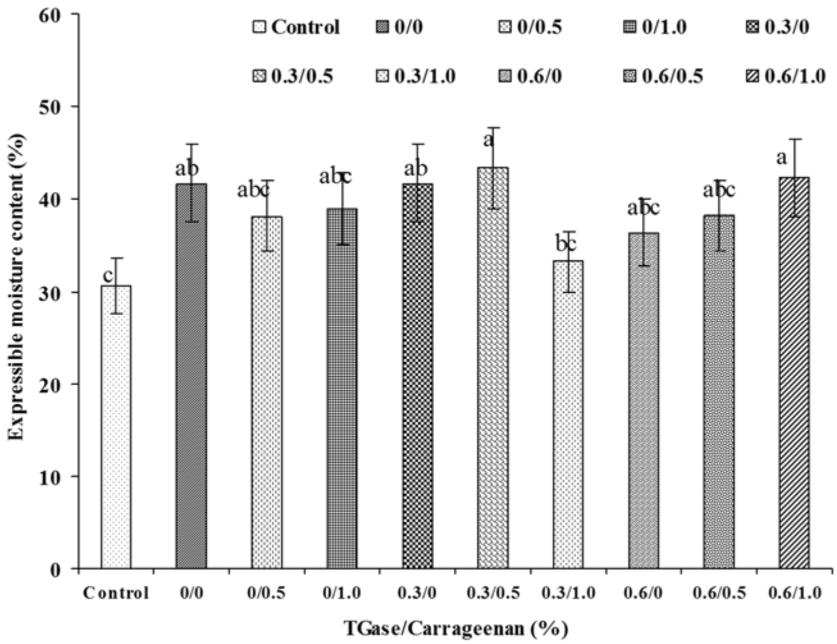

Figure 1. Effect of TGase and CG on the expressible moisture content (EMC) among fish patty formulations compared with the control (sample with1% phosphate)
X/Y = the level of TGase (0, 0.3, and 0.6 %)/CG (0, 0.5 and 1.0 %), for example, 0.3/0.5 = 0.3 % TGase with 0.5 % CG

Indian mackerel fish [15]. However, Zhu *et al.* [23] indicated that the formation of cross-linking was not enhanced significantly in the treatments, and non-covalent interactions could be important roles in producing the microstructure of the gel.

3.5 Sensory quality

The selected fish patty formulations were screened and selected with the criteria of low cooking loss and expressible water comparing with the control. The result showed that TGase and CG significantly affected sensorial quality of fish patty products ($P < 0.05$). The sample without either TGase or CG had lower score of appearance acceptance (5.9 vs 5.6) (Table 4). However, there were no significant effect on the texture and overall like of all formulations compared to the control.

However, the addition of TGase and CG had no effect on the appearance acceptance and a reduced effect on flavour acceptance among all samples. The similar effect was found as the previous study of Dimitrakopoulou *et al.* [2], who reported that transglutaminase significantly affected ($P<0.05$) the overall acceptability of the restructured cooked pork shoulder while it had no effect on the colour, odour and taste of the product.

Additionally, comparing between TGase 0.3 % and 0.6 % at all levels of CG, the result showed that there was no significant difference of all sensory attributes. Thus, the addition of TGase could be incorporated with CG at 0.3 % and 1.0 %, respectively to produce fish patties from *Pangasius hypophthalmus* with desirable texture, cooking loss and acceptability compared with the control. These levels of TGase and CG would be used to further evaluate consumers' acceptability of the developed fish patty.

Table 4. Preliminary sensory evaluation screening fish patty with different levels of TGase and carrageenan (CG) (n = 30)

Sample		Appearance	Texture	Flavour	Overall like
TGase (%)	CG (%)				
Control		6.4 ±1.0ab	6.3±1.1a	6.2±1.4a	6.5±1.1a
0.0	0.5	5.9±1.5ab	6.1±1.3a	5.4±1.8b	6.4±1.2a
0.3	0.0	6.2±1.3ab	6.3±1.2a	5.7±1.6ab	6.8±1.1a
0.3	1.0	6.5±0.9a	6.4±1.1a	6.4±1.2a	6.6±1.1a
0.6	0.0	5.6±1.3b	6.3±1.1a	5.2±1.4b	6.2±1.2a
0.6	1.0	6.4±1.2ab	6.7±1.0a	6.3±1.3a	6.6±1.2a

Mean ± standard deviation with the different letters in the same column was significant different (P < 0.05) based on 9-point hedonic scale.

3.6 Consumer acceptability of the developed fish patty

The addition of TGase and CG could yield the product without negative effect on consumers' acceptability compared with the control (with phosphate) as shown in Table 5. There was no significant difference for all sensory attributes between fish patty containing TGase mixed with CG and the control sample. The acceptance score of all attributes ranged from 6.5 - 6.8 (slightly to moderately like) (Table 5).

Table 5 Consumers' acceptability of the final developed fish patty compared with the control (n = 60)

Sample	Appearance	Texture	Flavour	Overall like
Control	6.7±1.2a	6.8±1.5a	6.5±1.3a	6.8±1.2a
Developed product	6.7±1.4a	6.6±1.6a	6.4±1.2a	6.5±1.4a

*Mean ± standard deviation with the different letters in a row were significant different (P < 0.05) based on 9-point hedonic scale.

**The developed product contained TGase and CG 0.3 % and 1.0 %, respectively without added phosphate.

The purchase intention of consumers was evaluated in this study. The result indicated that before getting information of health benefit of the developed product, consumers' intend for purchasing this product was only 60 %. On the contrary, when consumers are provided with information about health benefit of the product as containing no chemical additives, it would increase willingness for purchasing the developed fish patty up to 79 % which is 19 % over the test before the information was provided (Figure 2). Therefore, TGase and CG would be potential alternative food additives to produce the fish patty with comparable textural quality, acceptability as well as the opportunity to attract consumers' attention with the control.

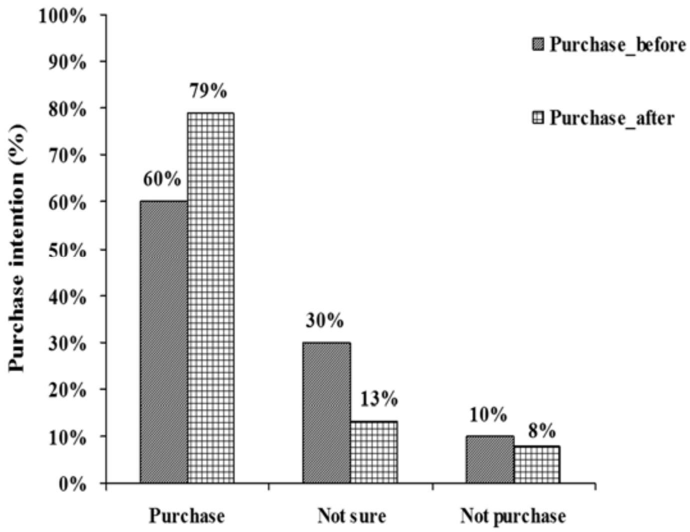

Figure 2. The purchase intention of consumers on fish patties with TGase and CG before ( ) and after ( ) receiving the information on health benefit (n = 60)

4. Conclusions

Based on the results, it could be concluded that TGase and CG affected the physical and sensory qualities of patty from underutilized fish fillet. The results indicated that TGase was able to enhance hardness of the samples at the highest level, while increasing CG contributed to water-binding ability of the samples by lowering cooking loss and expressible moisture content compared with the control. Therefore, combining of TGase and CG would be the potential additive to improve the quality of fish patty without the addition of phosphate. TGase (0.3 %) and CG (1.0 %) was appropriate to produce fish patty with acceptable sensory quality and comparable texture to the control. Consumers' purchase intention was higher when they were provided with the health benefit of additives used in the formulation. This product could be used as the raw material for making alternative healthy product. However, the quality of the product during the storage condition needs further investigation.

5. Acknowledgements

The authors would like to express their appreciation to Faculty of Agricultural Product Innovation and Technology, Srinakarinwirot University for the financial support (Funding No.370/2557). Thanks are expressed to Ajinomoto Co., (Thailand) Ltd. for providing the transglutaminase enzyme and Charoen Pokphand Foods Public Company Limited, Thailand for supporting the fish fillet.

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Quality of Repeated Use of Oil for Frying Chinese Deep Fried Dough (Pa-Tong-Kho)

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Received: 12 July 2017, Revised: 23 March 2018, Accepted: 28 March 2018

Abstract

Thai people frequently consume Chinese deep fried dough and its quality is affected by frying conditions. An experiment was conducted using a 3 × 2 × 3 factorial randomized complete block design. The first factor was the three vegetable oils (palm olein oil (PO), rice bran oil (RO) and soybean oil (SO)). The second factor was the frying conditions (180 °C, 2 min and 160 °C, 4 min) and the third factor was the repeated frying cycle (4, 9, 16). The three factors did not interact. The color, viscosity, polar value (P) and trans fatty acids quantity (TA) of different types of oil were significant ($p \leq 0.05$) difference. The repeated frying oil showed darker color and higher values of the viscosity, P and TA. The color of fried PO and fried RO was not different but fried SO was a lighter yellow. The viscosity of PO was the highest value followed by RO and SO. Polar value of PO was the lowest (10.91 %), followed by RO (11.60 %) and SO (14.40 %). Trans fatty acids quantity of PO was the lowest (0.72 %) followed by SO (0.86 %) and RO (1.42 %). Frying conditions had no significant ($p > 0.05$) effect to color and viscosity while P and TA were significant ($p \leq 0.05$) difference. The higher temperature and shorter time frying resulted of higher P and higher TA of fried oil. However, the fried products were safe to consume because their polar was less than 25 %, and their T was low. PO was better than RO or SO for Chinese dough frying.

Keywords: Chinese dough, Pa-Tong-Kho, repeated frying, polar value, trans fatty acid

1. Introduction

Thai people frequently consume Chinese deep fried dough because the deep-fat frying process generates food with crispy texture and good flavor nevertheless the fried food quality. Quality of food depends on frying conditions including type of oil, temperature, time and repeated frying cycle. The big changes in chemical and physical properties include starch gelatinization, protein denaturation, water vaporization and crust formation [1] will lead to degrade frying oil such as darker color, increased viscosity, decreased smoke point, and increased foaming due to polymerization, oxidation, hydrolysis, and isomerization [2]. The measurements of degradation in frying oils can be determined by many methods such as viscosity measurement, [3], quantity of volatile decomposition products and nonvolatile decomposition products [4], free fatty acid [5], iodine value [6], nonurea-adduct forming ester [3], and total polar materials. Many researchers believe that these measurements are the best indicators for frying oil quality [7]. Paradis and Newar [8] stated that when 25-27 % of total polar materials has accumulated, the frying oil should be discarded.

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Deep fat frying has also been considered as a source for the production of trans fatty acid (TA) [9] that has a relationship to the risk of cardiovascular disease [10]. TA is generally defined as unsaturated fatty acid that contains non-conjugated carbon-carbon double bonds in the trans configuration. TA in foods is derived from the chemical hydrogenation of vegetable and fish oils, the refinement process of edible oils from crude ones and microbial biohydrogenation in the digestive tract of ruminant animals. The main sources of TA for consumers are partially hydrogenated oils. The main objective of this study was to investigate the quality of Chinese dough (Pa-Tong-Kho) repeated frying oil from the different types of oils, frying conditions, and number of repeated frying cycles.

2. Materials and Methods

2.1 Materials for frying

Three refined, bleached and deodorized different vegetable oils, i.e. palm olein (PO), rice bran oil (RO) and soybean oil (SO) were purchased from a local supermarket in Thailand. All purpose wheat flour was purchased from a local supermarket in Thailand. Trans-6-petroselaidic methyl ester ($C_{18:1n6t}$) (Purity ≥ 99.00 %, Supelco), Trans -9-elaidic methyl ester ($C_{18:1n9t}$) (Purity ≥ 99.9, Supelco), Tricosanoic acid methyl ester ($C_{23:0}$) (Purity ≥ 99.8 %, Fluka) were used in this study.

2.2 Frying experiment

The experiment was a 3 × 2 × 3 factorial randomized complete block design with the first factor of three vegetable oils (PO, RO, SO). The second factor was the frying conditions (180 ℃, 2 min and 160 ℃, 4 min) and the third factor was the repeated frying cycle (4, 9, 16). An open pan was filled with 1 liter of oil (using a thermometer for temperature control) and fermented Chinese dough (1.5 kgX. In each frying, the sample was fried repeatedly (16 frying cycles). Oil samples were collected every 4, 9 and 16 cycles. The collected samples of oil and fried Chinese dough were kept at-20 °C until further tests. The CIE color values were measured using a spectrophotometer (Spectraflash 60 0 plus, Datacolor International, USA) and recorded as L* = lightness (0 = black, 100 =white), a*(-a* = greenness, +a* = redness) b* (-b* =blueness, +b* = yellowness), Percent of free fatty acids (FFA) was calculated from acid value [11]. Viscosity (brookfield DV-III Rheometer V3.3 RV and TA were measured using polar value (Testo, Germany) and gas chromatograph, respectively [12]. The data was processed by analysis of variance and Duncan's New Multiple Range Test (DNMRT) for mean comparisons at the 0.05 significance level, using the SPSS statistical software program (SPSS for windows Ver. 12.0, now a part of IBM Corp.; White Plains, NY, USA).

2.3 GC of TA analysis

GC was performed in a CP 3800 Varian Gas Chromatograph fitted with a FID (GC-FID), the column, HP-88, with dimensions of 100 m × 0.250 mm × 0.20 um and Column flow (3.0 ml/min). The initial temperature was 90 °C (hold for 5 min); final temperature was 250 °C (hold for 20 min). The temperature of the detector, FID, was 260 °C. Helium was used as the carrier gas. Split ratio of 100:1 was used and 1 μl of sample was injected in GC for the analysis total trans fatty acid.

1. Percentage of fat $= (M_2 - M_1) \times 100/W$

M_2	=	weight of Round Bottle Flask and Fat (g)
M_1	=	weight of Round Bottle Flask (g)
W	=	weight of sample (g)

2. Area percentage of fatty acid $_x$ = $\frac{100 \times A_x}{A_t - A_{ts}}$

A_x = area counts of fatty acid X
A_t = total area counts for the chromatogram
A_{ts} = area counts of the internal standard

3. Fatty acid, g/100g sample = $F_t \times F_c \times F_a /100$

F_t = total fat (g) in 100 g sample
F_c = correction factor for the conversion of data for fatty acid analyzed as percent of total fatty acid to grams per 100 g edible portion of food (soy bean = 0.93, rice bran = 0.92, palm = 0.956)
F_a = % area fatty acid

4. Total trans fatty acid

Total trans fatty acid $(C_{18}) = C_{18:2n9,\,12t} + C_{18:1n6t} + C_{18:1n9t} + C_{18:1n11t}$

3. Results and Discussion

3.1 Effect of types of frying oils

Due to the three factors were not interactions so the results were from the main factor only. The results of factor of types of oils were shown on Tables 1-3. Color change in frying oils is an indication of the deterioration of oil caused by oxidation led to the formation of nonvolatile decomposition products such as oxidized triacylglycerols and free fatty acids. Increasing viscosity caused by formation of high molecular weight polymers. The more viscous the frying oil, the higher the degree of deterioration. The changes in color or viscosity can be easily observed [13]. The color of fried oil of PO was the darkest followed with RO and SO. The viscosity of fried oil of PO was the highest value followed with RO and SO. Free Fatty Acids (FFA) are the result of the breakdown of oil. The FFA of fried oil of PO was the highest value followed with SO and RO. In most deep fat frying operations, the amount of FFA produced by hydrolysis is too small to affect the quality of the fried food compared oxidation of unsaturated fatty acids because the determination of FFA by titration does not differentiate between acids formed by oxidation and those by hydrolysis, the FFA is not good measure of frying fat deterioration if used alone, however, it can be a good indicator of the extent of fat abuse if used in conjunction with other methods.

P value of fried oil of PO was the lowest value followed with RO and SO. TA of fried oil of PO was the lowest value followed with SO and RO. However the P value of three oils was safe because it was lower than 25 % that is the criteria of Ministry of Public Health of Thailand (Notification of the Ministry of Public Health No. 283). For the color of fried Chinese dough on Table 3 was not significant ($p \leq 0.05$) difference but TA of PO was lowest followed with SO and RO.

All edible oils consist of triglycerides with a variety of fatty acids that differ in chain-length (number of carbon atoms in molecule), degree of saturation, position of double bond within the carbon chain, and geometry of each double bond (*cis* and *trans* isomers) [14]. PO has a composition of low polyunsaturated (9.1 %), 36.6 % monounsaturated and high saturated fatty acid (48.8 %) [15]. RO has a composition of 38 % monounsaturated, 37 % polyunsaturated, and 25 % saturated fatty acids [16] and SO has a composition of high polyunsaturated (58%), 23% monounsaturated and low saturated fatty acid (16 %) [17]. The oxidation rate of oil increased as the content of unsaturated fatty acids of frying oil increased [18-19]. Oleic acid is the most abundant monounsaturated fatty acid in all the common edible oils [14], is more stable to oxidation, compared with polyunsaturated fatty acids, modified oils containing high-oleic acid, low-linoleic and low-linolenic acids produced by various methods including genetic modification [20]. From P value and TA content, these explains why PO with less unsaturated fatty acid than RO or SO with more unsaturated fatty acids and high Oleic acid is a better frying oil than RO or SO. In addition, SO has a highest composition of 7 % linolenic acid while PO has hot found and 2.2 %. of RO. Linolenic acid is critical to the frying performance, the stability of oil, and the flavor of fried food [21-22]. Therefore, low linolenic acid oil by genetic modification was suggested to be a potential alternative to hydrogenated frying oil [23].

Table 1. The color of fried oils from the factor of types of oils (average ± standard deviation)

Oils	L*	a*	b*
PO	92.19±1.06^{b}	-1.89±0.33^{b}	24.24±4.16^{a}
SO	93.41±0.88^{a}	-1.07±0.26^{a}	17.36±4.40^{b}
RO	92.32±1.34^{b}	-1.75±0.64^{b}	24.04±5.13^{a}

Means of each treatment for each attribute (from column) with different letters were significantly different at p ≤0.05.

Table 2. Viscosity, P and TA of fried oils from the factor of types of oils (average ± standard deviation)

Oils	Viscosity (cp)	%FFA	P (%)	TA (%)
PO	67.87±1.62^{a}	0.69±0.05^{a}	10.91±1.77^{c}	0.72±0.60^{b}
SO	52.79±2.40^{c}	0.29±0.03^{b}	14.44±3.16^{a}	0.86±0.71^{b}
RO	64.83±2.14^{b}	0.25±0.02^{b}	11.66±2.58^{b}	1.42±0.66^{a}

Means of each treatment for each attribute (from column) with different letters were significantly different at p ≤0.05.

Table 3.The color of fried Chinese dough from the factor of types of oils (average ± standard deviation)

Oils	L*	a*	b*	TA (%)
PO	58.21±2.22^{a}	7.27±1.65^{a}	22.05±1.78^{a}	0.71±0.41^{b}
SO	58.63±3.30^{a}	7.80±2.02^{a}	22.34±2.99^{a}	0.80±0.19^{ab}
RO	55.09±2.94^{b}	8.26±1.77^{a}	20.93±2.36^{a}	1.01±0.34^{a}

Means of each treatment for each attribute (from column) with different letters were significantly different at p ≤0.05.

3.2 Effect of frying conditions

The results were shown on Tables 4-6. The color, viscosity and acid value of fried oils from factor of high and low temperature were not significant (p≤0.05) difference but P and TA of high temperature were higher than low temperature. The color and TA of fried Chinese dough (Table 6) were not significant (p≤0.05) difference. In line with TA of potatoes fried with commercially canola oil at 160, 180 and 200 °C by the tenth frying operation was increase with increasing temperature [24].

Table 4. The color of fried Chinese dough from the factor of the frying conditions (average ± standard deviation)

Frying conditions	L*ns	a*ns	b*ns
High temperature	92.77±1.35	-1.54±0.48	21.14±5.98
Low temperature	92.50±1.04	-1.60±0.65	22.62±5.04

Means of each treatment for each attribute (from column) with different letters were significantly different at p ≤0.05.

Table 5. Viscosity, Acid value, P and TA of fried oils from the factor of the frying conditions (Average ± standard deviation)

Frying conditions	Viscosity (cp) ns	% FFA ns	P (%)	TA (%)
High temperature	62.04±7.45	0.42±0.40	12.65±3.18 ^{a}	1.34±0.92^{b}
Low temperature	61.62±6.52	0.41±0.47	12.02±2.71 ^{b}	0.66±0.36 ^{a}

Means of each treatment for each attribute (from column) with different letters were significantly different at p ≤0.05.

Table 6. The color and TA of fried Chinese dough from the factor of the frying conditions (Average ± standard deviation)

Frying conditions	L*ns	a*ns	b*ns	TA (%) ns
High temperature	56.41±3.31	8.10±1.70	22.22±2.60	0.48±0.16
Low temperature	58.21±2.91	7.45±1.91	21.33±2.25	0.69±0.39

Means of each treatment for each attribute (from column) with different letters were significantly different at p ≤0.05.

3.3 Effect of repeated frying cycle

The results were shown on Tables 7-9. The more cycles resulted the darker color, the increased viscosity, FFA, P (15.75 %) and TA (1.46 %) of fried oils but the color of fried Chinese dough (Table 9) was not significant (p≤0.05) difference but TA was increased, in accordance with the study of Abdulkarim [13], it was found that Moringa seed oil, canola oil, SO and PO were polar, increasing in duration and number of frying. Increasing of TA in accordace with TA of potatoes fried with commercially canola oil at 160, 180 and 200 °C by the tenth frying operation was 0.99-1.05 g/100 g lipids that was little impact [24].

Table 7. The color of fried oils from the factor of frying cycles. (average ± standard deviation)

Frying cycles	L*	a* ns	b*
4	93.45±0.64^{a}	-1.60±0.72	18.71±4.14 ^{b}
9	93.03±0.65 ^{a}	-1.62±0.53	20.20±4.13^{b}
16	91.43±1.19^{b}	-1.5±0.44	26.74±4.73^{a}

Means of each treatment for each attribute (from column) with different letters were significantly different at p ≤0.05.

Table 8. Viscosity, P and TA of fried oils from the factor of frying cycles. (average ± standard deviation)

Frying cycles	Viscosity (cp)	%FFA	P (%)	TA (%)
0	55.16±5.36^{a}	0.10±0.35 ^{a}	9.96±1.03 ^{d}	0.26±0.29 ^{a}
4	60.23±7.37^{b}	0.39±0.47^{b}	10.88±1.88 ^{c}	0.54±0.39 ^{b}
9	61.23±6.33^{c}	0.41±0.47 ^{b}	12.75±1.94^{b}	0.97±0.27^{c}
16	64.02±6.39^{d}	0.44±0.32 ^{b}	15.75±2.69 ^{a}	1.46±0.25^{d}

Means of each treatment for each attribute (from column) with different letters were significantly different at p ≤0.05.

Table 9. The color and TA of Chinese dough from the factor of frying cycles. (Average ± standard deviation)

Frying cycles	L*ns	a*ns	b*ns	TA (%)
4	58.19±3.61	7.01±1.41	21.58±2.20	0.375±0.12 [a]
9	57.05±3.39	8.27±1.79	22.24±3.11 [a]	-
16	56.7±2.61	8.05±2.07	21.50±2.01 [a]	0.69±0.32 [b]

Means of each treatment for each attribute (from column) with different letters were significantly different at p ≤0.05.

4. Conclusions

Fried Chinese dough was safe from frying with PO, RO, and SO with the conditions of frying 180 °C, 2 min and 160 °C, 4 min and 16 frying cycles because P value of three fried oils was lower than 25 %. PO was better than RO or SO for Chinese dough frying. However, the taste of the products should be tested as well as the shelf life in case of long-term storage.

5. Acknowledgements

Financial support for this research was provided by the National Research Council of Thailand.

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CAST Vol. 18 No. 1 Jan. - Apr. 2018

Effects of Yanang (*Tiliacora triandra*) Gum on Gelation of Waxy Rice Flour

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Received: 12 July 2017, Revised: 20 March 2018, Accepted: 28 March 2018

Abstract

Crude hydrocolloid extract has been prepared from the leaves of Yanang (*Tiliacora triandra*). This research studied effects of Yanang gum on pasting and textural properties of blending of waxy rice flour. Rapid visco analysis (RVA) results showed that the ratio of Yanang extract to water of 1:3 and 1:4 (w/w) significantly decreased the trough, breakdown, and final viscosities of the waxy rice flour whereas the peak viscosities, peak time, and pasting temperatures of the blends were increased. Textural study revealed that the addition of crude Yanang gum enhanced more hardness and springiness of the blending gels than those of waxy rice flour gel, whereas cohesiveness was less affected. These results would be useful as a guideline for developing frozen starch-based food products containing crude Yanang gum.

Keywords: Yanang, hydrocolloid, waxy rice flour, pasting properties, textural properties, RVA

1. Introduction

Retrogradation is a term used for changes that occur in gelatinized starch from disordered state to a more ordered crystalline state and the tendency of starch pastes to thicken and to form stiff gels [1]. Among the cereal, rice is one of the most utilized grains in many forms. Rice flour does not have good handing properties, thus the incorporation of curtain additives as hydrocolloids may be an approach to achieve the desirable properties. Starch/hydrocolloid combinations have been widely used particularly in the food production. The reason for using combinations is because native starches do not generally have ideal properties for the preparation of food products, such as having tendency to syneresis, retrogradation and exhibiting breakdown, either from extended cooking, high shear or acidic conditions, producing weak-bodied, cohesive, rubbery pastes, and undesirable gels [2]. There are extensive studies to overcome these shortcomings by blending of native starches with polysaccharide hydrocolloids (gums), as reviewed by Appelqvist and Debet [3] and BeMiller [4]. Therefore, the mixtures of starch and hydrocolloids have been used to modify and control rheological and textural properties, improve moisture retention, control water mobility, and maintain overall product quality during processing and storage of food products. Yanang (*Tiliacora triandra*) is a climbing herbal plant with deep green leaves and yellowish flowers. It is a species of flowering plant native to mainland Southeast Asia, and is widespread in

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particularly in many cuisines of the northeast of Thailand and Lao PDR especially in bamboo shoot soup. Yanang leaves contain high levels of beta-carotene which has antioxidant activity, fiber, and minerals, such as calcium and iron. The most important properties of the Yanang leaves extract are their viscosity, including thickening and gelling and water binding [5]. Yanang contains hydrocolloid, the major monosaccharide constituent of Yanang gum is xylose, together with substantial amounts of other neutral sugars. The FT-IR spectra of Yanang gum are similar to that of xylan, as reported by Singthong *et al.* [5].

Thai desserts are simply prepared with flour, coconut milk, and sugar. Flour is the main ingredient in these products and minor ingredients commonly added are legumes, vegetables, and fruits as well as roots and tubers. Retrogradation of staple food always occurs when the products are storage at low temperature or at fluctuated temperature condition.

As far as we are aware, there are no studies that compare the effect of crude hydrocolloid from Yanang on the behaviors of rice starch or rice flour. The purpose of this study, therefore, was to observe the effects of extraction ratio of the Yanang (*Tiliacora triandra*) extraction on pasting and textural properties of waxy rice flour. The waxy flour was chosen on the basis starch-based Thai desert. This research would make a practical implications in further applications of Yanang gum in frozen waxy rice based Thai desert, particularly, Bualoy.

2. Materials and Methods

2.1 Materials

Waxy rice flour (WRF) was purchased from Cho Heng Rice Vermicelli Factory Co., Ltd., Nakhon Pathom, Thailand. Yanang leaves were purchased from Ying Charoen Market, Bangkok, Thailand.

2.2 Extraction of Yanang

Yanang leaves were cleaned with water to remove dust and infected leaves were separated. The ratio of Yanang leaves to distil water were calculated to prepare the extracted Yanang. For the gum solutions, calculated weight amount of Yanang or distil water was made. The prepared leaves and water were then blended thoroughly a mixer (Buono®TSK-9355B). The mixture were stained through cheesecloth and stored at refrigerator in a plastic container before use [6].

2.3 Determination of pasting properties

Pasting properties of 6 % (w/w) waxy rice flour without crude Yanang gum with crude Yanang gum and waxy rice flour with crude Yanang gum blends at the ratios of Yanang leaves to water of 1:3 and 1:4 (w/w), respectively as well as Yanang gum (1:3 w/w, ratio) were determined by a rapid visco-analyzer (Model RVA-4C, Newport Scientific Pty. Ltd., Warriewood, Australia). WRF slurries were prepared by dispersing weighed amounts of WRF (dry basis) in distilled water or gum solutions. The slurries weighing 28 g were then poured into aluminum canisters and stirred manually using plastic paddles for 20-30s before insertion into the RVA instrument. The heating and cooling cycles were programmed following the general pasting method (STD 1). The slurry was held at 50 °C for 1 min, heated to 95 °C within 3 min 42s (i.e. a heating rate of 12 °C/min) and then held at 95 °C for 2 min 30s. It was subsequently cooled to 50 °C within 3 min 48s and held at 50 °C for 2 min, while maintaining a rotation speed of 160 rpm. The viscosity was expressed in rapid visco units (RVU). The data were reported as average of triplicate measurement.

2.4 Determination of textural properties

The waxy rice gel with and without polysaccharide were prepared. The exact ingredient quantities of dough sample were 330 g of waxy rice flour and 280 g of boiling water. The ingredients were mixed, knead, and shaped in a small ball with a size of 7 mm diameter. The dough samples were boiled for 1.30 min before adding to prepared syrup. Textural characteristics of the waxy rice gel in the presence or absence of Yanang gum were performed by using Texture analyzer (TA.XT *Plus*, Stable Micro Systems Ltd., Surrey, UK). A set of six pieces of the gel sample after ringing of syrup was placed on a flat metal plate. Instrument settings with a cylindrical 36 mm diameter stainless probe were compression mode, trigger type, pretest speed, 5.0 mm/sec; posttest speed, 10.0 mm/sec; test speed, 2.0 mm/sec; strain, 75 %. From the force-distance curves generated, five texture parameters can be obtained but the highest and lowest values were discarded, thus only three strands were used for data analysis: hardness (g), springiness (ratio), and cohesiveness (ratio).

2.5 Statistical analysis

The experiment was designed in completely randomized design (CRD). Results are expressed as mean ± standard deviations of triplicate analyses for each sample unless otherwise stated. A one-way analysis of variance (ANOVA) and Duncan's test were used to establish the significance of differences among the mean values of RVA data at the 0.05 significance level. Textural properties data were analyzed using anindependent t-test. Statistical analyses were performed using SPSS software.

3. Results and Discussion

3.1 Pasting properties of waxy rice flour and Yanang gum blend

Rapid visco analysis (RVA) was used to study the pasting properties of blends of waxy rice flour and Yanang gum at the various ratios of Yanang leaves to water. Typical RVA pasting profiles of 6 % (w/w) waxy rice flour in the absence of or presence of Yanang gum was shown in Figure 1. Pasting curves for crude hydrocolloid from Yanang at the ratio of Yanang to water of 1:3 (w/w) were almost straight lines with viscosities approximately zero (Figure 1). These results indicated that crude hydrocolloid from Yanang did not develop pasting viscosities under the experimental conditions.

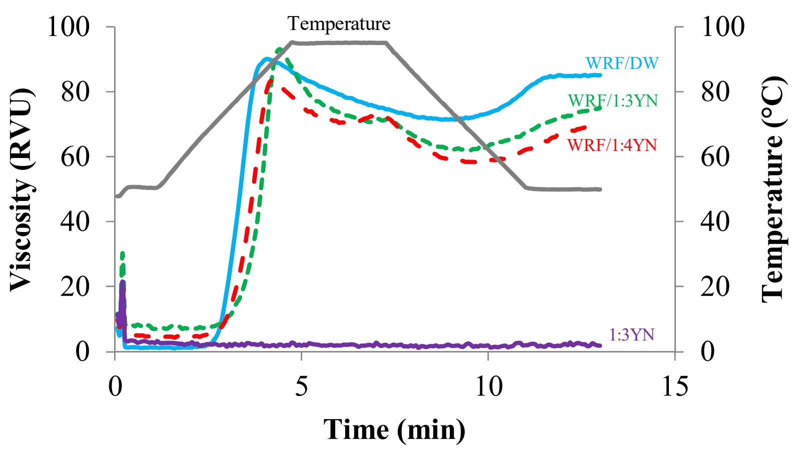

Figure 1. Typical RVA pasting profiles of 6 % (w/w) waxy rice flour in the presence of Yanang gum at the ratios of Yanang to water of 1:3 (WRF/1:3YN) and 1:4 (WRF/1:4YN), w/w respectively, or absence of Yanang gum (WRF/DW) and Yanang gum alone (YN at 1:3, w/w ratio).

The sufficient granules becoming swollen and indicates the water capacity causes a rapid increase in peak viscosity of waxy rice flour in water (WRF/DW) [7]. Addition of crude hydrocolloids from Yanang resulted in a significant decrease in trough, breakdown, and final viscosities of the blends (WRF/1:3YN and WRF/1:4YN) whereas peak viscosities, peak time, and pasting temperatures of the blends were increased ($P \leq 0.05$), except the peak viscosity of waxy rice flour with Yanang gum at the ratio of Yanang leaves to water of 1:4 (w/w), as shown in Table 1. Similar observations were reported by Samutsri and Suphantharika [8] for rice starch with xanthan and guar gum blends. The researchers suggested that because of the interactions between leached starch molecules and hydrocolloid in the continuous phase, resulted in a pronounced increase in the viscosity of the continuous phase and in turn the overall viscosity of the suspension itself, owing to the thickening properties of hydrocolloid added to the thickening produced by swollen starch granules. The peak viscosity of the starch is correlated with the final product quality and it is suggested that high peak viscosity provide the desired product quality [9] and might be used in Thai dessert for good texture. An increase in peak viscosity of waxy rice flour during the hold period of the test at the test minute of 7-8 (Figure 1) therefore hypothesized to be the predominant effect of interactions of protein of waxy rice flour with the other components such as pigment of crude Yanang gum.

Table 1. Pasting properties of 6 % (w/w) waxy rice flour (WRF) in the absence of Yanang gum (WRF/DW) or presence of Yanang gum at the ratios of Yanang to water of 1:3 (WRF/1:3YN) and 1:4 (WRF/1:4YN), w/w respectively.*

Sample	**RVA Viscosity (RVU)**					**Peak time (min)**	**Pasting temperature (°C)**
	Peak viscosity	**Trough**	**Breakdown**	**Final viscosity**	**Setback**		
WRF/DW	119.06 ± 0.9^{a}	92.25 ± 0.8^{a}	26.81 ± 0.5^{c}	109.41 ± 0.9^{a}	17.16 ± 0.1^{a}	4.07 ± 0.0^{c}	70.10 ± 0.5^{c}
WRF/1:3YN	123.00 ± 4.9^{a}	79.64 ± 1.1^{b}	40.36 ± 4.1^{a}	95.72 ± 0.8^{b}	14.08 ± 0.5^{b}	4.40 ± 0.1^{a}	75.33 ± 1.6^{a}
WRF/1:4YN	110.64 ± 2.0^{b}	74.61 ± 0.7^{b}	36.03 ± 2.1^{b}	88.42 ± 0.7^{c}	13.80 ± 0.8^{b}	4.22 ± 0.1^{b}	72.47 ± 0.4^{b}

*Assays were performed in triplicate. Mean ± standard deviation values in the same column for each sample followed by different letters are significantly different ($p \leq 0.05$).

Table 1 shows the pasting properties of waxy rice flour and Yanang gum at the various ratios of Yanang leaves to water. The pasting temperature of waxy rice flour with the crude Yanang gum at the ratio of Yanang leaves to water of 1:3 and 1:4 (w/w) were much higher (75.33 and 73.47 °C) than waxy rice flour without the gum (70.10 °C). Therefore waxy rice flour granules in the presence of crude Yanang gum were swollen at higher degree than waxy rice flour granules without crude Yanang gum, similar resulted was reported by Han *et al.* [10] that the modified waxy rice exhibited reduce pasting temperature, but increased peak viscosities compared with unmodified starch using in traditional Korean waxy rice cake. [11] hypothesized that the spread of highly swollen granules might lower degree of rice starch retrogradation and consequently reducedspongy structure of freeze-thaw gel. Thus, from this study, it was suggested that the spread of highly swollen granules of waxy rice flour granules in the presence of crude Yanang gum might lower degree of rice starch retrogradation of the waxy rice gel.

During cooling, re-association between starchmolecules, especially amylose, will result in theformation of a gel structure and, therefore, viscosity will increase to the final viscosity. This phase is commonly referred to as the setback region and is related to retrogradation and reordering of starch molecules. The setback reveals the gelling ability or retrogradation tendency of the amylose [12]. The highest setback was observed in the control waxy rice flour, suggesting that the highest amylose retrogradation occurred. The setback of waxy rice flour in the presence of crude

Yanang gum (14.08 and 13.80 RVU) were significantly lower than the control (17.16 RVU) ($P \leq 0.05$).

We hypothesized that crude hydrocolloid from Yanang could reduce the retrogradation of the sample and provide the desirable texture due to the highest peak viscosity. The gel of waxy rice and crude Yanang gum at the ratio of Yanang leaves to water of 1:3 (w/w) was chosen for studying of textural properties of gel sample compared to waxy rice gel alone.

3.2 Textural properties of waxy rice flour and Yanang gum blend

The textural properties of waxy rice gel in the absence of crude Yanang gum (WRF/DW) or presence of crude Yanang gum at the ratio of Yanang leaves to water of 1:3 (w/w) (WRF/1:3YN) was shown in Table 2.

Table 2. Textural properties of waxy rice gel in the absence of Yanang gum (WRF/DW) or presence of crude Yanang gum at the ratio of Yanang to water of 1:3 (w/w).*

Sample	Hardness (g)	Springiness (ratio)	Cohesiveness (ratio)^{ns}
WRF/DW	219.58 ± 24.3^{b}	0.1762 ± 0.02^{b}	0.7933 ± 0.09
WRF/YN	398.18 ± 44.2^{a}	0.1919 ± 0.02^{a}	0.7934 ± 0.08

*Assays were performed in triplicate. Mean ± standard deviation values in the same column for each sample followed by different letters are significantly different ($p \leq 0.05$).

It was found that texture analysis hardness and springiness of the waxy rice gels with crude hydrocolloid extracted from Yanang (398.18 ± 44.2 g and 0.1919 ± 0.02) were significantly different as compared with the control gel (219.58 ± 24.3 g and 0.1762 ± 0.02) ($p \leq 0.05$), however, those of cohesiveness was not significantly different ($p > 0.05$). To increase in gel stability, product formation may be developed by employing polysaccharide gums due to enhancement of water holding capacities, viscosity, and cold storage [4]. In fact, the waxy rice gel alone exhibits more brittle gel than that of waxy rice flour with crude Yanang gum, therefore, it is easy to break the gel. When crude Yanang was used in the waxy rice gel, the peak forces of these mixed gels trended to increase [13]. Springiness represents the extent of recovery of from gel surface deformation and is often referred to as elasticity [14]. In this study, the addition of crude Yanang gum in to waxy rice flour seemed to produce a little impact on hardness and springiness of the gels. Increase in the gel hardness and springiness with containing of crude Yanang gum much more obvious than those gels without gum containing. Compared with waxy rice gel without crude Yanang gum, waxy rice gel with crude Yanang gum showed higher gel elasticity. This might be due to the interactions between leached starch molecules and hydrocolloid in the continuous phase [8]. The texture of blend waxy rice gel with crude Yanang gum might be flavored for cold storage condition.

4. Conclusions

The addition of crude Yanang gum was shown to be an effective agent for improving of waxy rice gels. From RVA profile, the waxy rice flour in the presence of crude Yanang at the ratio of Yanang leaves to water ratio of 1:3 (w/w) might provide a low degree of retrogradation and from texture data, good desirable texture was achieved. Data from this study can be used as guidelines in the development of frozen starch-based products that contain crude hydrocolloid from Yanang to improve gel stability. It also provides a basis for preparing crude Yanang in applications of frozen food products. However, further studies are needed to determine the effect of Yanang gum to reduce retrogradation. Thus, studies on the gel textural properties of freeze-thaw waxy rice flour in the presence of gum are required.

5. Acknowledgements

This study was financially supported by the Institute of Research and Development of PhranakhonRajabhat University (IRDP). The authors gratefully acknowledge use of facilities and instrumentations supported by Food Science and Technology program, Faculty of Science and Technology, PhranakhonRajabhat University.

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Understanding Indonesian People: Consumer Acceptance and Emotions Study of Green Tea Products from Thailand

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Received: 12 July 2017, Revised: 11 March 2018, Accepted: 28 March 2018

Abstract

Evaluation of emotions as part of other sensory tests is gaining momentum in the sensory science and consumer. The EsSense Profile® was developed for studying the impact of food on consumer's emotions. Consumer will express their emotions when consuming products. The objective of this research was to measure emotion responses obtaining from Innonesian people by using EsSense Profile®. Fourteen samples of green tea produced from Chinese variety and Assam variety were used in this study. Check-all-that-apply (CATA) method was used to screen the emotion terms. Eight terms were selected from a total of 39 emotion terms as calm, good, good-nature, happy, peaceful, pleasant, tender and warm as key emotion terms that are related to Thai tea products. Consumers were rated their emotions on a 5-point intensity scale before and after drinking tea. It was found that intensity of emotional response of green tea (Assam variety) was sinificantly different ($p < 0.05$) when compare before and after drink whereas green tea (Chinese varitey) was not significantly different ($p \geq 0.05$). Indonesian people accepted green tea produced from Chinese variety (72.1 %) more than Assam variety (52.7 %). Result from logistic regression indicated that color and taste were influential attributes affecting overall acceptance for green tea (Chinese variety) whereas color, aroma, flavor and taste were influential attributes for Assam variety. Indonesian people considered color, flavor and taste as key attributes affecting purchase intent. This result will provide incremental information regarding consumer tests and overall acceptability data and also help connect marketing with product development efforts via consumer emotions and acceptability ratings.

Keywords: Consumer acceptance, Emotions, Green tea

1. Introduction

Tea is one of the world's most consumed beverages, with over 290 billion litres sold in retail and food services. The growth rate of bottled tea consumption is outpacing bottled water, carbonates, beer and coffee. Tea consumption is largely concentrated in Asia, especially in China, India and Pakistan

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Tea leaves (*Camellia sinensis* L.) are the most popular drink. It is a plant rich in polyphenols and flavonoids [1]. Nowadays, tea comsumption is increasing due to their benefits. Polyphenols have beneficial effects such as antioxidant, antibacteria and anticancer [2]. In Thailand, tea products (green tea, oolong tea and black tea) are made from fresh tea leaves of two varieties as Chinese tea (*Camellia sinensis* var. *sinensis*) and Assam tea (*Camellia sinensis* var. *assamica)*. Tea is generally classified into 3 groups base on fermentation process i.e. green tea (non-fermented), oolong tea (semi-fermented) and black tea (fully fermented) [3]. Different tea processing affect to quality and taste.

Sensory evaluation by consumer is important in the development of the product itself for new product development guidance, product improvement and optimization and product maintenance [4]. Sensory perceptions are what consumers associate with food quality and they have great influence in determining consumer acceptance and purchase intent for food products [5]. Consumer acceptability evaluation can provide the most important and reliable information because only consumers can accurately indicate the degree of liking or preference for a product. The expectation of liking and emotions play an essential role in food perception by consumers. Measuring emotions as part of other sensory tests is gaining momentum in the sensory science and consumer. The EsSense Profile®methodology, presented in 2008 [6] and published in 2010 [7], is one of such methods. The EsSense Profile®measures short and relatively intense responses about consumer products. Thus, the objective of this study was to investigate relationship between acceptability and emotions of tea products from Thailand.This result will provide information on consumers' overall acceptability data and help connect marketing with product development efforts via consumer emotions and acceptability ratings.

2. Materials and Methods

2.1 Material

Fourteen samples of green tea that produced from Chinese variety and Assam variety were used in this study. They were collected from tea factory that located in Chiang Rai and Chiang mai. The samples were kept in aluminum foil bag and hermetically sealed.

2.2 Procedure

There were 500 participants (ages 18-50 years) from Bogor Agricultural University (IPB), Indonesia particiapated in this study. Panelists often consuming green tea were qualified.

2.3 Emotional term selection

Check-All-That-Apply (CATA) questionnaire was used to screen emotional term with 100 respondents. List of emotions were used in the EsSense Profile® ballot (Table 1). The 39 emotions were selected from the positive, negative and contextual groups of emotions. These emotions were chosen by consumers based on their appropriateness to tea products. Criteria for term selection was based on $\geq$ 15 % frequency of use on a checklist questionnaire.

Table 1. EsSense Profile® Emotion

Positive		Negative	Unclassified
Active (aktif)	Joyful (sukacita)	Bored (membosankan)	Aggressive (agresif)
Adventurous (petualang)	Loving (mencintai)	Disgusted (menjijikan)	Daring (berani)
Affectionate (diperhatikan)	Merry (ceria)	Worried (menghawatirkan)	Eager (bersemangat)
Calm (kalem)	Nostalgic (nostalgia)		Guilty (bersalah)
Energenic (energik)	Peaceful (damai)		Mild (biasa saja)
Enthusiastic (antusias)	Pleasant (menyenan		Polite (sopan)
Free (bebas)	Pleased (bahagia)		Quite (diam)
Friendly (ramah)	Satisfied (memuaskkan)		Steady (stabil)
Glad (sangat senang)	Secure (aman)		Tame (mudah dikendalikan)
Good (baik)	Tender (lembut)		Understanding (pengertian)
Good-natured (alami)	Warm (hangat)		Wild (liar/tidak terkontrol)
Happy (senang)	Whole (merasa utuh)		
Interested (menarik)			

2.4 Emotion measurement

Selected emotional terms were used to measure and compare for each sample prior and after consumption. Panelists were asked to indicate their emotion (How do you feel before and after consumption). Panelists (n=50) tested samples in individual test booths to prevent them from bias. Two samples of green tea that produced from Assam and Chinese variety were used in this part. A 3 grams of tea leave was infused with 150 ml of hot water, steeped for 5 minutes and served to panelist with 25 ml. A glass of water and cracker were served to each panelist to cleanse the palate between samples. Paneliste were instructed to score using 5-point scale (1 = not at all, 2 = slightly, 3 = moderately, 4 = very much, 5 = extremely). The consumer overall acceptability were evaluated using 9-points hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely).

2.5 Consumer acceptance

Consumer (n=350 between>20-60 years of age) were recruited from Bogor Agricultural University, Indonesia. In this study, a balanced incomplete block design was used. The balanced incomplete block design with t=7, k=4, r=4, b=7, λ=2 was employed due to the number of samples were too much to evaluate at one time. So each consumer was presented with 4 samples. The order of samples was counter-balanced within and across judge. Each consumer received samples with 3-digit coded in a randomized order. A glass of water was also served to each subject to cleanse the palate between samples. Consumers were asked to provide their demographic information.

They provide acceptability ratings for color, odor, taste and overall liking, all on a 9-points hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely) The binomial type questions (yes/no) was used to evaluate overall product acceptance and purchase intent.

2.6 Statistical data analysis

Each of 39 emotional terms were counted frequencies for each. Term selected with 15 % frequency or more were selected to evaluate emotion with/without sample. Principle component analysis was applied to assess the similarity and differences in emotional term of evaluated green tea. Liking score were subjected analysis of variance (ANOVA) using SPSS 16.0 (SPSS Inc., Chicago, U.S.A.). Duncan's multiple range test (DMRT) were performed to locate the differences among samples. Logistic regression are performed to identify sensory attributes influencing acceptance and purchase intent.

3. Results and Discussion

3.1 Screening emotional lexicon

The 39 emotional terms (EsSense Profile®) were classified into 3 groups as positive, negative and unclassified. Positive emotional terms were active, adventurous, affectionate, calm, energenic, enthusiastic, free, friendly, glad, good, good-natured, happy, interested, joyful, loving, merry, nostalgic, peaceful, pleasant, pleased, satisfied, secure, tender, warm and whole. Negative emotional term were bored, disgusted and worried. Unclassified emotional term were aggressive, daring, eager, guilty, mild, polite, quite, steady, tame, understanding and wild. Screening emotional lexicon of Thai green tea was showed in Figure 1. Result indicated that eight emotional term out of 39 (20.52 %) were selected when responding to CATA ballot. These were positive term include clam, good, good-nature, happy, peaceful, pleasant, tender and warm. It may reflect that consuner feel posivitve when they drink tea. These term were selected to measure the emotion before and after drinking tea.

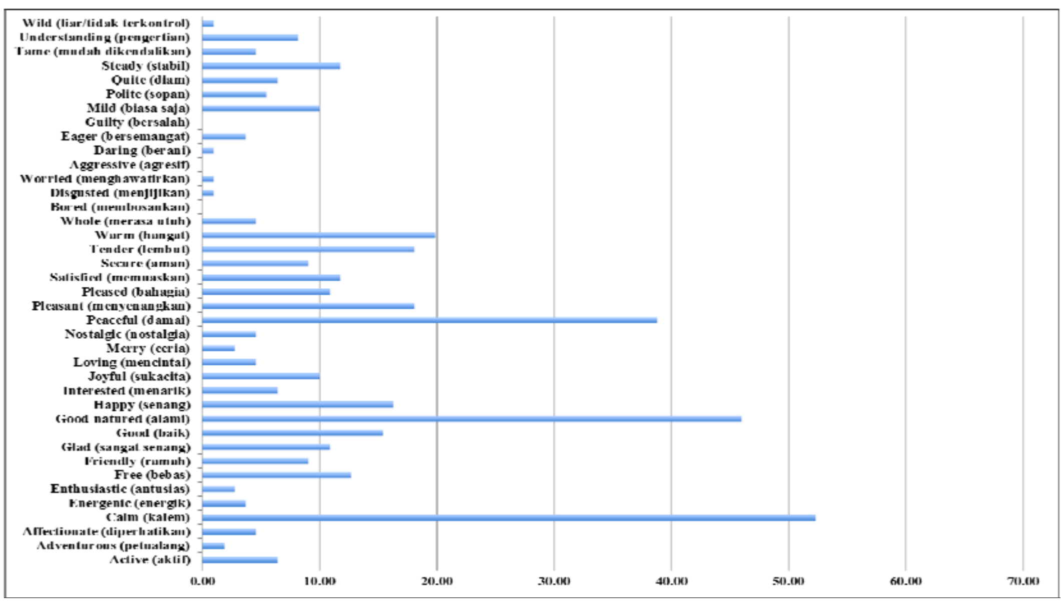

Figure 1. Emotional term screening of Thai green tea by using Check–All–That–Apply (n=100)

3.2 Emotion measurement

Rating scale was used to approach consumer emotions. It was found discimination between before and after consumption (Table 2). Before consumption and after consumption of green tea made from Chinese variety, emotional term was not significantly difference ($p \geq 0.05$) (Table 2) whereas after consumption of green tea made from Assam variety was significantly different ($p < 0.05$) compared with those before consumption.

Table 2. Emotion scores of Thai green tea products (n = 50)

Emotion	Before consumption	After consumption	
	Without sample	Assam variety	Chinese variety
Calm	3.12 ± 1.12^a	2.46 ± 1.01^b	3.16 ± 1.00^a
Good	3.18 ± 0.90^a	2.34 ± 1.14^b	3.20 ± 0.93^a
Good-nature	3.58 ± 1.16^a	3.10 ± 1.23^b	3.56 ± 0.95^a
Happy	3.12 ± 0.87^a	2.14 ± 1.18^b	3.12 ± 1.02^a
Peaceful	3.52 ± 0.86^a	2.60 ± 1.31^b	3.28 ± 1.14^a
Pleasant	3.28 ± 0.86^a	2.32 ± 1.30^b	3.10 ± 1.13^a
Tender	2.78 ± 1.00^a	2.08 ± 1.14^b	2.80 ± 1.16^a
Warm	3.08 ± 1.24^b	3.00 ± 1.20^b	3.48 ± 1.09^a

Means within each row with different letters are significantly different at $p < 0.05$

Principle component analysis (PCA) was used to group emotion terms based on level of correlation that exists among them. PCA biplot as shown in Figure 2, 75.73 % of the variability was emotional term to the first principal component (horizontal axis). The second principal component (vertical axis) accounted for 24.27 % of variability. The PCA result could classify samples into 2 groups according to emotional terms. Before consumption (without sample) and after consumption (Chinese variety) formed one cluster which were similar to each other in clam, good, good-nature, happy, peaceful, pleasant, tender and warm.

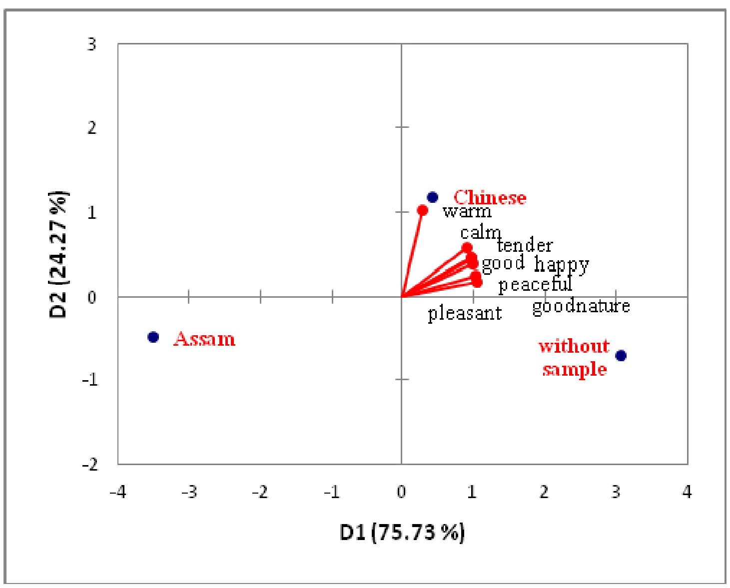

Figure 2. A biplot of the principal component PC1 and PC2, visualizing between before and after consume green tea with various variety and emotional terms.

3.3 Consumer acceptance

Mean sensory score of Thai green tea samples were shown in Tables 3 and 4. Regarding to green tea made from Chinese variety, no differences ($p \geq 0.05$) existed between mean scores of each sample for flavor, taste and overall liking whereas color and aroma were significantly different ($p < 0.05$). Table 5 shows that at least 70 % of consumers accepted Thai green tea made from Chinese Variety. On the other hand, there were differences between Thai green tea made from Assam variety in aroma, flavor, taste and overall liking ($p < 0.05$) whereas color was not significantly difference ($p \geq 0.05$). For purchase intent, Indonesia people decided to buy green tea made from Chinese variety rather than Assam variety due to flavor and taste. Assam variety had strong bitter taste than Chinese variety. Furthermore, it was found that high score of emotion responses may affect buying decision. From previous study [8] mentioned that there is a correlation between overall acceptability and emotional terms.

Table 3. Liking score of Chinese variety (n=175)

Sample	Color	Aroma	Flavor^{ns}	Taste^{ns}	Overall liking^{ns}
Ac	5.92 ± 1.85^{ab}	5.73 ± 1.40^{bc}	5.29 ± 1.65	5.07 ± 1.83	5.37 ± 1.58
Bc	5.42 ± 1.78^{bc}	5.41 ± 1.40^{c}	5.37 ± 1.50	5.23 ± 1.75	5.48 ± 1.44
Cc	5.81 ± 1.56^{ab}	5.96 ± 1.30^{ab}	5.71 ± 1.57	5.42 ± 1.67	5.74 ± 1.53
Dc	5.80 ± 1.58^{ab}	6.27 ± 1.44^{a}	5.43 ± 1.71	5.27 ± 1.84	5.56 ± 1.56
Ec	5.92 ± 1.43^{ab}	5.72 ± 1.56^{bc}	5.52 ± 1.76	5.25 ± 1.79	5.50 ± 1.68
Fc	6.01 ± 1.42^{a}	5.94 ± 1.38^{ab}	5.66 ± 1.62	5.55 ± 1.81	5.75 ± 1.57
Gc	5.28 ± 1.73^{c}	5.62 ± 1.37^{bc}	5.66 ± 1.44	5.70 ± 1.51	5.58 ± 1.38

Means within each column with different letters are significantly different at $p < 0.05$
ns = not significantly different at $p \geq 0.05$

Table 4. Liking score of Assam variety (n=175)

Sample	Color^{ns}	Aroma	Flavor	Taste	Overall liking
Aa	5.94 ± 1.46	6.09 ± 1.30^{a}	4.97 ± 1.80^{b}	4.71 ± 1.81^{b}	5.12 ± 1.62^{bc}
Ba	5.37 ± 1.64	5.46 ± 1.46^{b}	3.59 ± 1.55^{c}	2.98 ± 1.46^{c}	3.64 ± 1.36^{d}
Ca	5.62 ± 1.71	5.04 ± 1.71^{c}	3.25 ± 1.55^{c}	2.73 ± 1.34^{c}	3.22 ± 1.54^{d}
Da	5.81 ± 1.57	6.07 ± 1.16^{a}	5.68 ± 1.56^{a}	5.55 ± 1.70^{a}	5.80 ± 1.44^{a}
Ea	5.94 ± 1.68	5.60 ± 1.80^{b}	4.71 ± 1.99^{b}	4.29 ± 2.05^{b}	4.76 ± 1.89^{c}
Fa	5.94 ± 1.51	6.10 ± 1.39^{a}	5.20 ± 1.58^{b}	4.69 ± 1.80^{b}	5.29 ± 1.49^{b}
Ga	5.85 ± 1.73	6.02 ± 1.48^{a}	4.91 ± 1.60^{b}	4.50 ± 1.64^{b}	4.85 ± 1.49^{bc}

Means within each column with different letters are significantly different at $p < 0.05$

Table 5. Overall acceptance and purchase intent of Thai green tea made from various variety

Variety	Sample	Overall acceptance(%)	Purchase decision(%)
Chinese	Ac	60	40
	Bc	70	42
	Cc	77	51
	Dc	71	46
	Ec	75	52
	Fc	70	46
	Gc	76	54
Assam	Aa	63	36
	Ba	78	59
	Ca	25	11
	Da	60	32
	Ea	52	34
	Fa	63	37
	Ga	24	6

Table 6. Logistic regression statistic of Chinese and Assam variety

Factors	Sensory attributes	P-value		Exp (B)	
		Chinese variety	Assam variety	Chinese variety	Assam variety
Overall acceptance	Color	0.002*	0.011*	0.780	0.818
	Aroma	0.085	0.000*	0.844	0.720
	Flavor	0.474	0.009*	0.920	0.764
	Taste	0.000*	0.000*	0.344	0.357
Purchase intent	Color	0.000*	0.006*	0.742	0.794
	Aroma	0.398	0.085	0.924	0.833
	Flavor	0.001*	0.014*	0.677	0.734
	Taste	0.000*	0.000*	0.413	0.387

The logistic regression analysis (LRA) was used to identify sensory attributes that influenced overall acceptance and purchase intent of Thai tea product (Table 6). Result from logistic regression indicated that color and taste were influential attributes affecting overall acceptance ($p < 0.05$) for green tea (Chinese variety) whereas color, aroma, flavor and taste were influential attributes for Assam variety ($p < 0.05$). Indonesian people considered color, flavor and taste as key attributes affecting purchase intent of Thai tea. Furthermore, the Exp (B) value of the color and taste of Chinese variety was 0.780 and 0.344 respectively. It was implied that a one unit liking score in color and taste increased, the product acceptable were increase 0.780 and 0.344 time. While all attributes of Assam variety should be improved to increase product acceptable. From focus group discussion, consumer said that Assam variety had strong bitter taste than Chinese variety. Furthermore, it was found that lower acceptance score affected lower buying decision. It may imply that Indonesia people accepted green tea made from Chinese variety more than Assam variety.

4. Conclusions

This study was demonstrated that Indonesian people’s expressing emotional terms on Thai tea products were related to tea variety. Different tea variety had different taste that affect acceptability and purchase intent.

5. Acknowledgements

The authors would like to express appreciation to tea company in Thailand for supporting tea samples for this study.

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CAST Vol. 18 No. 1 Jan. - Apr. 2018

Mers Model of Thai and South Korean Population

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Received: 28 February 2017, Revised: 18 November 2017, Accepted: 14 March 2018

Abstract

Coronavirus (MERS-Cov) caused the occurrence of Corona. First infected case was reported in 2012 during a poultry outbreak in Saudi Arabia. After that, there were the reports of the sporadic outbreaks in all regions. In this study, we considered the transmission cycle between two population groups: Thai and South Korea. Each population group was divided into susceptible, exposed, infected, quarantine and recovered groups. The behaviors of the solutions were obtained using a standard dynamical modeling method. The stability conditions for the disease free equilibrium state and disease endemic equilibrium states were determined. The basic reproductive number R_0 is obtained. When $R_0<1$, the disease-free state was locally asymptotically stable. If $R_0>1$, the endemic equilibrium state was locally asymptotically stable. The numerical solutions were shown for supporting the theoretical results. We found that when we decreased α_1 (the rate of susceptible Thai human changes to become an exposed Thai human and μ_1 (the rate at which South Korean population moved out the country), the number of coronavirus case was decreased and outburst of coronavirus epidemic was shorter.

Keywords: Basic reproductive number, mathematical models, MERS, stability, standard dynamical modeling theorem

1. Introduction

The Ministry of Public Health, Thailand had declared Middle East Respiratory Syndrome or MERS as a dangerous communicable disease. MERS was caused by coronavirus [1]. First infected case of MERS-Cov was reported in Saudi Arabia during a poultry outbreak in 2012 [2, 3]. After that, there were the reports of the sporadic outbreak in all regions. In 2016, an epidemic was recognized as the first time occurrence in Thailand. The first case is a traveler, a male from Oman. On 12 June 2015, South Korean confirmed 126 infectious cases with coronavirus and 10 deaths [4]. On 24 January 2016, Thailand confirmed Middle East respiratory syndrome coronavirus (MERS-Cov) disease in a traveler, the second case in the country in the last seven months [5]. Globally cases of this disease had been reported in some countries. On 16 May 2016, World Health Organization (WHO) confirmed infectious cases with coronavirus (MERS-Cov) of 1,723

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laboratory, including at least 628 deaths from 27 countries [1, 6, 7]. Coronavirus (MERS-Cov) had been infected to human and outbreak in many countries. The corona virus transmitted from camel to human and human to human. The pandemic corona virus can be transmitted to human by camel. Human can be infected by direct contact with animals. Human can be also infected through biological fluid contact, hospital setting, family cluster and closed contact cluster. In human, the virus incubated for 2 to 14 days before symptoms appear. Typical symptoms of corona virus included fever, shortness of breath and cough. Pneumonia was also a common presentation [1]. There were many tourists who travel between Thai and South Korea, thus there were the high risk of infections between Thai and South Korean with coronavirus. The numbers of South Korean tourists travel to Thailand from 2012 to 2016 were shown in Figure 1. Figure 2 shows the numbers of Thai tourists traveled to South Korea. We can see that there were many South Korean traveled to Thailand. Thus, in this study, we formulated the mathematical model of MERS considered the moving of South Korean to Thailand.

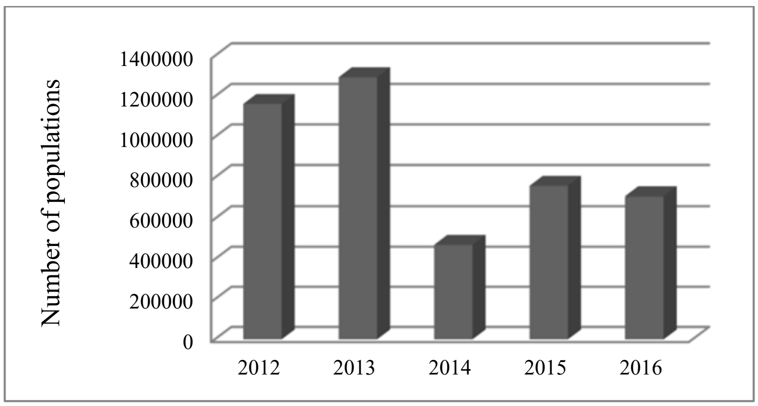
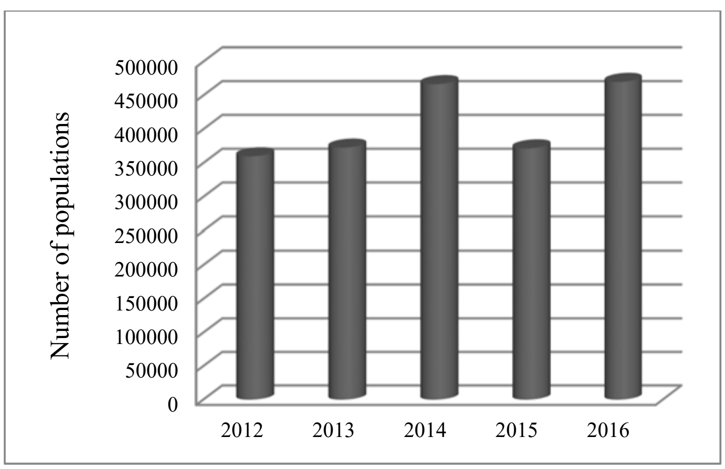


Figure 1. Number of South Korean tourist to Thailand, 2012-2016 [8].



Fiure 2. Number of Thai tourist to South Korea, 2012-2016 [9].

Small *et al*. [10] formulated the mathematical model for describing the transmission of SARS virus and a stochastic small-world network model. Their model studied the behaviors of solutions by using numerical simulations. In 2013, Chowell *et al*. [3] used dynamical transmission model describing the progression of MERS-Cov cases that incorporated community and hospital compartments, and distinguished transmission by zoonotic cases and secondary cases. In 2016, Yong and Owen [11] studied the transmission of MERS-Cov in two areas and formulated SISI model. They used the next generation matrix method to evaluate the basic reproductive number and analyzed the sensitivity of the model. In this paper, we considered the transmission cycle between two population groups: Thai and South Korea. Each population group was divided into susceptible, exposed, infected, quarantine and recovered classes. The standard dynamical modeling method was used for analyzing our model [12]

2. Formulation of the model

The mathematical model of MERS-Cov was formulated by considering the transmission cycle between two population groups (Thai and South Korean human). Each group was divided into susceptible (S_T), exposed (E_T), infected (I_T), quarantine (Q_T) and recovered (R_T) classes. We assumed that there is no Thai human move out the country.

We denoted the variables of our model as follows:

$S_T(t)$ is the number of susceptible Thai human at time t;

$E_T(t)$ is the number of exposed Thai human at time t;

$I_T(t)$ is the number of infected Thai human at time t;

$Q_T(t)$ is the number of quarantine Thai human at time t;

$R_T(t)$ is the number of recovered Thai human at time t;

$S_K(t)$ is the number of susceptible South Korean human at time t;

$E_K(t)$ is the number of exposed South Korean human at time t;

$I_K(t)$ is the number of infected South Korean human at time t;

$Q_K(t)$ is the number of quarantine South Korean human at time t;

$R_K(t)$ is the number of recovered South Korean human at time t;

The diagram for the transmission of this disease can be described as shown in Figure 3:

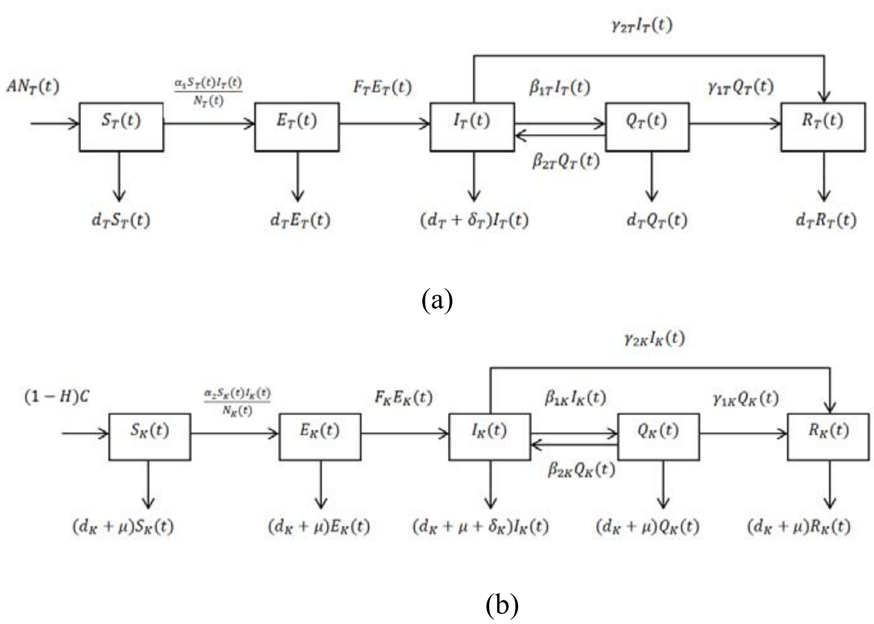


Figure 3. Flow chart of the model (a) for Thai human population (b) for South Korean human population

Rate of change for the number in each class was equivalent to the number entering minus the number leaving. The dynamical equations were as follows:
The number of susceptible Thai human population was increased by new recruitment, but it was reduced through natural death and infection.

$$\frac{dS_T(t)}{dt} = AN_T(t) - \frac{\alpha_1 S_T(t) I_T(t)}{N_T(t)} - d_T S_T(t) \tag{1}$$

The exposed Thai human population was increased by the infection of susceptible humans whereas their reductions were caused by infection and natural death.

$$\frac{dE_T(t)}{dt} = \frac{\alpha_1 S_T(t) I_T(t)}{N_T(t)} - (F_T + d_T) E_T(t) \tag{2}$$

The infected Thai human population was increased by the infection of exposed and quarantine human. Their reductions though quarantine, recovery from the disease, natural death and death due to MERS-Cov. The dynamical equation of infected Thai human population was

$$\frac{dI_T(t)}{dt} = F_T E_T(t) + \beta_{2T} Q_T(t) - (\beta_{1T} + \gamma_{2T} + d_T + \delta_T) I_T(t) \tag{3}$$

The quarantine Thai human population was increased by the infection of infected human but they diminished by infection, recovery from the disease and natural death. The dynamical equation of quarantine Thai human population was

$$\frac{dQ_T(t)}{dt} = \beta_{1T} I_T(t) - (\beta_{2T} + \gamma_{1T} + d_T) Q_T(t) \tag{4}$$

The recovered Thai human population was increased by the recovering of infected human and quarantine, but their reduction through natural death. The dynamical equation of recovered human population was

$$\frac{dR_T(t)}{dt} = \gamma_{1T} Q_T(t) + \gamma_{2T} I_T(t) - d_T R_T(t) \tag{5}$$

The susceptible South Korean human population was increased by new recruitment. Their reductions through natural death, they moved out the country and they were infected. The dynamical equation of susceptible South Korean human population was

$$\frac{dS_K(t)}{dt} = (1-H)C - \frac{\alpha_2 S_K(t) I_K(t)}{N_K(t)} - (d_K + \mu) S_K(t) \tag{6}$$

The exposed South Korean human population was increased by the infection of susceptible humans whereas reduction is caused by natural death, South Korea move out the country and infection. The dynamical equation of exposed South Korean human population was

$$\frac{dE_K(t)}{dt} = \frac{\alpha_2 S_K(t) I_K(t)}{N_K(t)} - (F_K + d_K + \mu) E_K(t) \tag{7}$$

The infected South Korean human population was increased by the infection of exposed and quarantine human and their reduction though quarantine, recovering from the disease, natural death South Korea, move out the country and death due to MERS-Cov. The dynamical equation of infected South Korean human population was shown as

$$\frac{dI_K(t)}{dt} = F_K E_K(t) + \beta_{2K} Q_K(t) - (\gamma_{2K} + \beta_{1K} + d_K + \mu + \delta_K) I_K(t) \tag{8}$$

The quarantine South Korean human population was increased by the infected human who become quarantine but diminished by infection, recovery from the disease, move out the country and natural death. The dynamical equation of quarantine South Korean human was

$$\frac{dQ_K(t)}{dt} = \beta_{1K} I_K(t) - (\gamma_{1K} + \beta_{2K} + d_K + \mu) Q_K(t) \tag{9}$$

The recovered human was increased by the recovery of infected and quarantine human, but their reduction through natural death and South Korean move out the country. The dynamical equation of recovered South Korean human was shown as

$$\frac{dR_K(t)}{dt} = \gamma_{2K} I_K(t) + \gamma_{1K} Q_K(t) - (d_K + \mu) R_K(t) \quad (10)$$

The total number of Thai human populations was the sum of equations (1)-(5)

$$N_T(t) = S_T(t) + E_T(t) + I_T(t) + Q_T(t) + R_T(t) \quad (11)$$

The number of South Korean human population was the sum of equations (6)-(10)

$$N_K(t) = S_K(t) + E_K(t) + I_K(t) + Q_K(t) + R_K(t) \quad (12)$$

Definition of our parameters in our dynamical equations:

Parameters	Definition
A	Birth rate of Thai human population
α_1	Rate at which susceptible Thai human changed to become an exposed Thai human
F_T	Rate at which exposed Thai human changed to become an infected Thai human
β_{1T}	Rate at which infected Thai human changed to become quarantine Thai human
β_{2T}	Rate at which quarantine Thai human changed to become infected Thai human
γ_{1T}	Rate at which quarantine Thai human changed to become recovered Thai human
γ_{2T}	Rate at which infected Thai human changed to become recovered Thai human
d_T	Natural death rate of Thai human
δ_T	Death rate due to MERS-Cov of Thai human
$N_T(t)$	Total Thai human population
H	Percentage of South Korea human who were infectious when they enter into Thailand
C	Recruitment rate of South Korean human
α_2	Rate at which susceptible South Korean human changed to become an exposed Korean human
F_K	Rate at which exposed South Korean human changed to become an infected Korean human
β_{1K}	Rate at which infected South Korean human changed to become quarantine Korean human
β_{2K}	Rate at which quarantine South Korean human changed to become an infected Korean human
γ_{1K}	Rate at which quarantine South Korean human changed to become recovered Korean human
γ_{2K}	Rate at which infected South Korean human changed to become recovered Korean human
d_K	Natural death rate of South Korean human
δ_K	Death rate due to MERS-COV of South Korea
μ	Rate at which South Korea move out the country
$N_K(t)$	Total South Korean human population

We can have the following equations:

$$\frac{dN_T(t)}{dt} = BN_T(t) - \left(S_T(t) + E_T(t) + I_T(t) + Q_T(t) + R_T(t)\right)d_T - \delta_T I_T(t)$$

$$\frac{dN_T(t)}{dt} = BN_T(t) - d_T N_T(t) - \delta_T I_T(t) \tag{13}$$

with the assumption $B = d_T$, then we had

$$\frac{dN_T(t)}{dt} = -\delta_T I_T(t) \tag{14}$$

We normalized Thai population class of our dynamical equations (1)-(5) by letting

$$s_T(t) = \frac{S_T(t)}{N_T(t)}, e_T(t) = \frac{E_T(t)}{N_T(t)}, i_T(t) = \frac{I_T(t)}{N_T(t)}, q_T(t) = \frac{Q_T(t)}{N_T(t)}, r_T(t) = \frac{R_T(t)}{N_T(t)}.$$

Taking the normalized population

$$x_T(t) = \frac{X_T(t)}{N_T(t)},$$

We considered

$$\begin{aligned}
\frac{d}{dt}x_T(t) &= \frac{d}{dt}\frac{X_T(t)}{N_T(t)} \\
&= \frac{1}{N_T(t)}\frac{d}{dt}X_T(t) - x_T(t)\frac{1}{N_T(t)}\frac{d}{dt}N_T(t) \\
&= \frac{1}{N_T(t)}\frac{d}{dt}X_T(t) - x_T(t)\frac{1}{N_T(t)}(-\delta_T I_T(t)) \\
&= \frac{1}{N_T(t)}\frac{d}{dt}X_T(t) - x_T(t)\frac{1}{N_T(t)}(-\delta_T i_T(t) N_T(t)
\end{aligned}$$

$$\frac{d}{dt}x_T(t) = \frac{1}{N_T(t)}\frac{d}{dt}X_T(t) + \delta_T x_T(t) i_T(t) \tag{15}$$

with the above equations, the dynamical equation of normalized Thai populations were as follows:

$$\frac{ds_T(t)}{dt} = A - \alpha_1 s_T(t) i_T(t) - d_T s_T(t) + \delta_T s_T(t) i_T(t) \tag{16}$$

$$\frac{de_T(t)}{dt} = \alpha_1 s_T(t) i_T(t) - (F_T + d_T) e_T(t) + \delta_T e_T(t) i_T(t) \tag{17}$$

$$\frac{di_T(t)}{dt} = F_T e_T(t) + \beta_{2T} q_T(t) - (\beta_{1T} + \gamma_{2T} + d_T + \delta_T) i_T(t) + \delta_T i_T(t) i_T(t) \tag{18}$$

$$\frac{dq_T(t)}{dt} = \beta_{1T} i_T(t) - (\beta_{2T} + \gamma_{1T} + d_T) q_T(t) + \delta_T q_T(t) i_T(t) \tag{19}$$

$$\frac{dr_T(t)}{dt} = \gamma_{1T} q_T(t) + \gamma_{2T} i_T(t) - d_T r_T(t) + \delta_T r_T(t) i_T(t). \tag{20}$$

The normalized Korean populations were found by substituting

$s_K(t) = \frac{S_K(t)}{N_K(t)}, e_K(t) = \frac{E_K(t)}{N_K(t)}, i_K(t) = \frac{I_K(t)}{N_K(t)}, q_K(t) = \frac{Q_K(t)}{N_K(t)}, r_K(t) = \frac{R_K(t)}{N_K(t)}$ to our dynamical equations.

We supposed that the total Korean population has constant size. This means that the rates of change for Korean population of equations (6)-(10) equal to zero or $\frac{dN_K}{dt} = 0$.

Then we obtained the relations: $N_K = \frac{C}{d_K + \mu}$ with the condition

$$s_K(t) + e_K(t) + i_K(t) + q_K(t) + r_K(t) = 1.$$

With the above equation, the dynamical equations of the normalized population were given by

$$\frac{ds_K(t)}{dt} = (1-H)(d_K+\mu) - \alpha_2 s_K(t) i_K(t) - (d_K+\mu) s_K(t) \tag{21}$$

$$\frac{de_K(t)}{dt} = \alpha_2 s_K(t) i_K(t) - (F_K + d_K + \mu) e_K(t) \tag{22}$$

$$\frac{di_K(t)}{dt} = F_K e_K(t) + \beta_{2K} q_K(t) - (\gamma_{2K} + \beta_{1K} + d_K + \mu + \delta_k) i_K(t) \tag{23}$$

$$\frac{dq_K(t)}{dt} = \beta_{1K} i_K(t) - (\gamma_{1K} + \beta_{2K} + d_K + \mu) q_K(t) \tag{24}$$

3. Analytical Solutions

3.1 Equilibrium Points:

To find the equilibrium points, we set the right hand side of equations (16)-(24) equal to zero. We got two equilibrium states, the disease free steady state $E_0 = (1,0,0,0,0,1,0,0,0)$ and the endemic steady state $E_1 = (s_T^*, e_T^*, i_T^*, q_T^*, r_T^*, s_K^*, e_K^*, i_K^*, q_K^*)$

$$s_T^* = \frac{A}{d_T + i_T^*(\alpha_1 - \delta_T)} \tag{25}$$

$$e_T^* = \frac{A\alpha_1 i_T^*}{(d_T + F_T - \delta_T i_T^*)(d_T + i_T^*(\alpha_1 - \delta_T))} \tag{26}$$

$$q_T^* = \frac{\beta_{1T} i_T^*}{d_T + \beta_{2T} + \gamma_{1T} - \delta_T i_T^*} \tag{27}$$

$$r_T^* = \frac{i_T^*\left(\gamma_{2T} + \dfrac{\beta_{1T}\gamma_{1T}}{d_T + \beta_{2T} + \gamma_{1T} - \delta_T i_T^*}\right)}{d_T - \delta_T i_T^*} \tag{28}$$

where i_T^* is the solution of the following equation:

$$F_T e_T^* + \beta_{2T} q_T^* - (\beta_{1T} + \gamma_{2T} + d_T + \delta_T) i_T^* + \delta_T i_T^* = 0 \tag{29}$$

$$s_K^* = -\frac{(-1+H)(d_K+\mu)}{d_K + \alpha_2 i_K^* + \mu} \tag{30}$$

$$e_K^* = -\frac{i_K^* \alpha_2 (-1+H)(d_K+\mu)}{(d_K + F_K + \mu)(d_K + \alpha_2 i_K^* + \mu)}$$

(31)

$$q_K^* = \frac{\beta_{1K} i_K^*}{d_K + \beta_{2K} + \gamma_{1K} + \mu} \tag{32}$$

$$i_K^* = \frac{Z_1}{Z_2} \tag{33}$$

where

$$\begin{aligned} Z_1 = -(&(d_K+\mu)(d_K{}^3 + d_K{}^2(F_K + \beta_{1K} + \beta_{2K} + \gamma_{1K} + \gamma_{2K} + \delta_K + 3\mu) + \mu(\beta_{1K}(\gamma_{1K}+\mu) + (\beta_{2K} + \gamma_{1K} + \mu) \\ &(\gamma_{2K} + \delta_K + \mu)) + F_K(\beta_{1K}(\gamma_{1K}+\mu) + \alpha_2(-1+H)(\beta_{2K} + \gamma_{1K} + \mu) + (\beta_{2K} + \gamma_{1K} + \mu)(\gamma_{2K} + \delta_K + \mu)) \\ &+ d_K(\beta_{2K}(\gamma_{2K} + \delta_K) + \gamma_{1K}(\beta_{1K} + \gamma_{2K} + \delta_K) + 2(\beta_{1K} + \beta_{2K} + \gamma_{1K} + \gamma_{2K} + \delta_K)\mu + 3\mu^2 + F_K((-1+P)\alpha_2 \\ &+ \beta_{1K} + \beta_{2K} + \gamma_{1K} + \gamma_{2K} + \delta_K + 2\mu)))) \end{aligned}$$

$$Z_2 = (\alpha_2(d_K + F_K + \mu)(d_K{}^2 + \beta_{1K}(\gamma_{1K} + \mu) + (\beta_{2K} + \gamma_{1K} + \mu)(\gamma_{2K} + \delta_K + \mu) + d_K(\beta_{1K} + \beta_{2K} + \gamma_{1K} + \gamma_{2K} + \delta_K + 2\mu)))$$

3.2 Local asymptotical stability

The local asymptotical stability of each steady state was determined from the Jacobian matrix. The eigenvalues (λ) were solutions of the characteristic equations. If all eigenvalues had negative real parts, then the steady state were local asymptotical stability [13].

$$\left| J_{E_i} - \lambda I_9 \right| = 0 \quad ,i = 0,1$$

where I_9 was the identity matrix dimension 9×9 .

J_{E_i} was the Jacobian matrix at the equilibrium point E_i for $i = 0,1$.

3.2.1 The disease free steady state E_0, The Jacobian matrix was given by

$$J_{E_0} = \begin{bmatrix} -d_T - (\alpha_1 - \delta_T) i_T^* & 0 & -(\alpha_1 - \delta_T) s_T^* & 0 & 0 & 0 & 0 & 0 & 0 \\ \alpha_1 i_T^* & -d_T - F_T + \delta_T i_T^* & \alpha_1 i_T^* + \delta_T e_T^* & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & F_T & -d_T - \beta_{1T} - \gamma_{2T} - \delta_T + 2\delta_T i_T^* & \beta_{2T} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \beta_{1T} + \delta_T q_T^* & -d_T - \beta_{2T} - \gamma_{1T} + \delta_T i_T^* & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \gamma_{2T} + \delta_T r_T^* & \gamma_{1T} & -d_T + \delta_T i_T^* & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -d_k - \alpha_2 i_K^* - \mu & 0 & -\alpha_2 s_K^* & 0 \\ 0 & 0 & 0 & 0 & 0 & \alpha_2 i_K^* & -d_k - F_K - \mu & \alpha_2 s_K^* & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & F_K & -d_k - \beta_{1K} - \gamma_{2K} - \delta_K - \mu & \beta_{2K} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \beta_{1K} & -d_k - \beta_{2K} - \gamma_{1K} - \mu \end{bmatrix}$$

$$(-d_T - \lambda)^2 (-\beta_{1K}\beta_{2K}(-W - \lambda)(-\beta_{1T}(-B_1\beta_{2T} - \beta 2T\lambda) + (-V - \lambda)(B_1G - F_T\alpha_1 + B_1\lambda + G\lambda + \lambda^2)) + (-Z - \lambda)$$
$$(-F_K\alpha_2(-\beta_{1T}(-B_1\beta_{2T} - \beta_{2T}\lambda) + (-V - \lambda)(B_1G - F_T\alpha_1 + B_1\lambda + G\lambda + \lambda^2)) + (-W - \lambda)(-Y - \lambda)(-\beta_{1T}(-B_1\beta_{2T}$$
$$- \beta_{2T}\lambda) + (-V - \lambda)(B_1G - F_T\alpha_1 + B_1\lambda + G\lambda + \lambda^2))))(-d_K - \lambda - \mu) = 0,$$

where

$B_1 = d_T + F_T$

$G = d_T + \beta_{1T} + \gamma_{2T} + \delta_T$

$V = d_T + \beta_{2T} + \gamma_{1T}$

$W = d_K + F_K + \mu$

$Y = d_K + \beta_{1K} + \gamma_{2K} + \delta_K + \mu$

$Z = d_K + \beta_{2K} + \gamma_{1K} + \mu.$

From evaluating all eigenvalues, the real parts of all eigenvalues had negative signs. We used Routh-Hurwitz criteria when $R_0 < 1$.

$$R_0 = \max\{\frac{D_{T1}}{D_{T2}}, \frac{D_{T3}}{D_{T4}}, \frac{D_{T5}}{D_{T6}}, \frac{D_{T7}}{D_{T8}}, \frac{D_{T9}}{D_{T10}}\},$$

where

$$D_{T1} = F_T\alpha_1 + F_K\alpha_2 + \beta_{1K}\beta_{2K} + \beta_{1T}\beta_{2T}$$

$$D_{T2} = VW + VY + WY + (V + W + Y)Z + G(V + W + Y + Z) + B_1(G + V + W + Y + Z)$$

$$D_{T3} = F_T\alpha_1(V + W + Y + Z) + F_K\alpha_2(V + Z) + \beta_{1K}\beta_{2K}(V + W) + (B_1 + G)(F_K\alpha_2 + \beta_{1K}\beta_{2K}) + B_1\beta_{1T}\beta_{2T} + (W + Y + Z)\beta_{1T}\beta_{2T}$$

$$D_{T4} = GVW + GVY + GWY + VWY + (WY + V(W + Y) + G(V + W + Y))Z + B_1(WY + (W + Y)Z + V(W + Y + Z) + G(V + W + Y + Z))$$

$$D_{T5} = F_T(WY + (W + Y)Z + V(W + Y + Z))\alpha_1 + F_K\alpha_2(GV + GZ + VZ) + \beta_{1K}\beta_{2K}(GV + GW + VW) + (YZ + W(Y + Z))\beta_{1T}\beta_{2T} + B_1(F_K\alpha_2(G + V + Z) + (G + V + W)\beta_{1K}\beta_{2K} + (W + Y + Z)\beta_{1T}\beta_{2T})$$

$$D_{T6} = VWYZ + G(VWY + WYZ + V(W + Y)Z) + B1(VWY + WYZ + V(W + Y)Z + G(WY + (W + Y)Z + V(W + Y + Z))) + (F_K\alpha_2 + \beta_{1K}\beta_{2K})(F_T\alpha_1 + \beta_{1T}\beta_{2T})$$

$$D_{T7} = F_T\alpha_1(VWY + WYZ + V(W + Y)Z) + F_K\alpha_2 GVZ + \beta_{1K}\beta_{2K}GVW + \beta_{1T}\beta_{2T}WYZ\beta_{1T}\beta_{2T} + B_1(F_K\alpha_2(GV + (G + V)Z) + (GV + (G + V)W)\beta_{1K}\beta_{2K} + (WY + (W + Y)Z)\beta_{1T}\beta_{2T})$$

$D_{T8} = GVWYZ + F_T\alpha_1(F_K\alpha_2(V+Z) + (V+W)\beta_{1K}\beta_{2K}) + \beta_{1T}\beta_{2T}(F_K\alpha_2 Z + W\beta_{1K}\beta_{2K}) + B_1(VWYZ + G(VWY + WYZ + V(W+Y)Z) + F_K\alpha_2\beta_{1T}\beta_{2T} + \beta_{1K}\beta_{1T}\beta_{2K}\beta_{2T})$

$D_{T9} = \alpha_1 F_T VWYZ + B_1(F_K\alpha_2 GVZ + \beta_{1K}\beta_{2K}GVW + \beta_{1T}\beta_{2T}WYZ)$

$D_{T10} = \alpha_1 F_T V(F_K\alpha_2 Z + \beta_{1K}\beta_{2K}W) + B_1(GVWYZ + \beta_{1T}\beta_{2T}(F_K\alpha_2 Z + \beta_{1K}\beta_{2K}W)).$

3.2.2 For the endemic steady state E_1, the characteristic equation was given by

$$(-d_T + \delta_T i_T^* - \lambda)(-\beta_{2T}(\beta_{1T} + \delta_T q_T^*)(d_T + (\alpha_1 - \delta_T)i_T^* + \lambda)(d_T + F_T - \delta_T i_T^* + \lambda) + (-d_T - \beta_{2T} - \gamma_{1T} + \delta_T i_T^* - \lambda)$$
$$(-(d_T + (\alpha_1 - \delta_T)i_T^* + \lambda)(d_T + \beta_{1T} + \gamma_{2T} + \delta_T - 2\delta_T i_T^* + \lambda)(d_T + F_T - \delta_T i_T^* + \lambda) + F_T(d_T(\alpha_1 s_T^* + \delta_T e_T^*) + \alpha_1\lambda s_T^*$$
$$+\delta_T e_T^*(\alpha_1 i_T^* - \delta_T i_T^* + \lambda))))(-\beta_{1K}\beta_{2K}(d_K + F_K + \lambda + \mu)(d_K + \alpha_2 i_K^* + \lambda + \mu) + (-d_K - \beta_{2K} - \gamma_{1K} - \lambda - \mu)$$
$$(F_K\alpha_2 s_K^*(d_K + \lambda + \mu) - (d_K + F_K + \lambda + \mu)(d_K + \alpha_2 i_K^* + \lambda + \mu)(d_K + \beta_{1K} + \gamma_{2K} + \delta_K + \lambda + \mu))) = 0,$$

where $s_T^*, e_T^*, i_T^*, q_T^*, r_T^*, s_K^*, e_K^*, i_K^*$ and q_K^* were defined in (25)-(33). From evaluation, all eigenvalues had negative real parts for $R_0 > 1$, where

$$R_0 = \max\{\frac{D_{T1}}{D_{T2}}, \frac{D_{T3}}{D_{T4}}, \frac{D_{T5}}{D_{T6}}, \frac{D_{T7}}{D_{T8}}, \frac{D_{T9}}{D_{T10}}\}.$$

3.3 Numerical Solutions

In this paper, we simulated the numerical solutions to show the behaviors of population. The values of the parameters used in this study were $d_T = d_K = 1/(75\times 365)$ correspond to the life cycle 75 years of Thai human and South Korean human. $F_T = 1/2$ satisfied to the 2 days of infected human. $\beta_{1T} = \beta_{1K} = 1/30$ corresponded to the 30 days of quarantine for Thai human and South Korean human. $\gamma_{1T} = \gamma_{2T} = \gamma_{1K} = 1/30$ corresponded to the average recovered time of 30 days in infectious quarantine Thai human and quarantine South Korean human. $F_K = 1/8$ satisfied to the average incubation 8 days for South Korea and $\gamma_{2K} = 1/14$ satisfies to the average recovered time of 14 days for infectious person. $\alpha_1 = 1/2.5$ satisfied to the average exposed time of 2.5 days for susceptible person. $\alpha_2 = 1/400$ corresponded to the 400 days of exposed for Thai human. $\delta_T = 1/2.7$ corresponded to the average death rate due to MERS-Cov 2.7 days of Thai human. $\delta_K = 1/20$ corresponded to the average death rate due to MERS-Cov 20 days of South Korean human. $\mu = 1/20$ was the rate at which South Korean move out the country.

4. Discussion

We analyzed the model of MERS-Cov disease between Thai and South Korea when there was the traveling of South Korean population to Thailand. We considered the effective contact rate of Thai and South Korean populations. The moving rate of South Korean to Thailand was considered. The results were found by using standard dynamical modeling analysis. The basic reproductive number was defined in the form of R_0 and it was given by.

$$R_0 = \max\{\frac{D_{T1}}{D_{T2}}, \frac{D_{T3}}{D_{T4}}, \frac{D_{T5}}{D_{T6}}, \frac{D_{T7}}{D_{T8}}, \frac{D_{T9}}{D_{T10}}\}$$

Figure 4 shows the numerical solutions of susceptible Thai population, exposed Thai population, infections Thai population, quarantine Thai population, susceptible South Korean population, exposed South Korean population, infections South Korean population and quarantine South Korean population. We can see that the disease free steady state of the eight populations equal to 1,0,0,0,1,0,0 and 0, respectively. Figure 5 shows the numerical solutions of susceptible Thai population, exposed Thai population, infections Thai population, quarantine Thai population, susceptible South Korean population, exposed South Korean population, infections South Korean population and quarantine South Korean population. We can see that the endemic steady state of the eight populations equal to 0.0769, 0.0216, 0.0092, 0.0243, 0.4855, 0.2198, 0.1273 and 0.0241, respectively. From Figure 6 to Figure 7, we simulated the different values of parameters to see the factors effect to the transmission of this disease. From our solutions, we can see that while a susceptible Thai human changed to become an exposed Thai human (α_1) was increased ($\alpha_1 = 1/2, \alpha_2 = 1/4$) and rate at which South Korean population moved out the country (μ_1) was increased($\mu_1 = 1/20, \mu_1 = 1/30$), then the number of MERS-Cov case was decreased and outburst of MERS-Cov epidemic was shorter as shown in Figures 6 and 7.

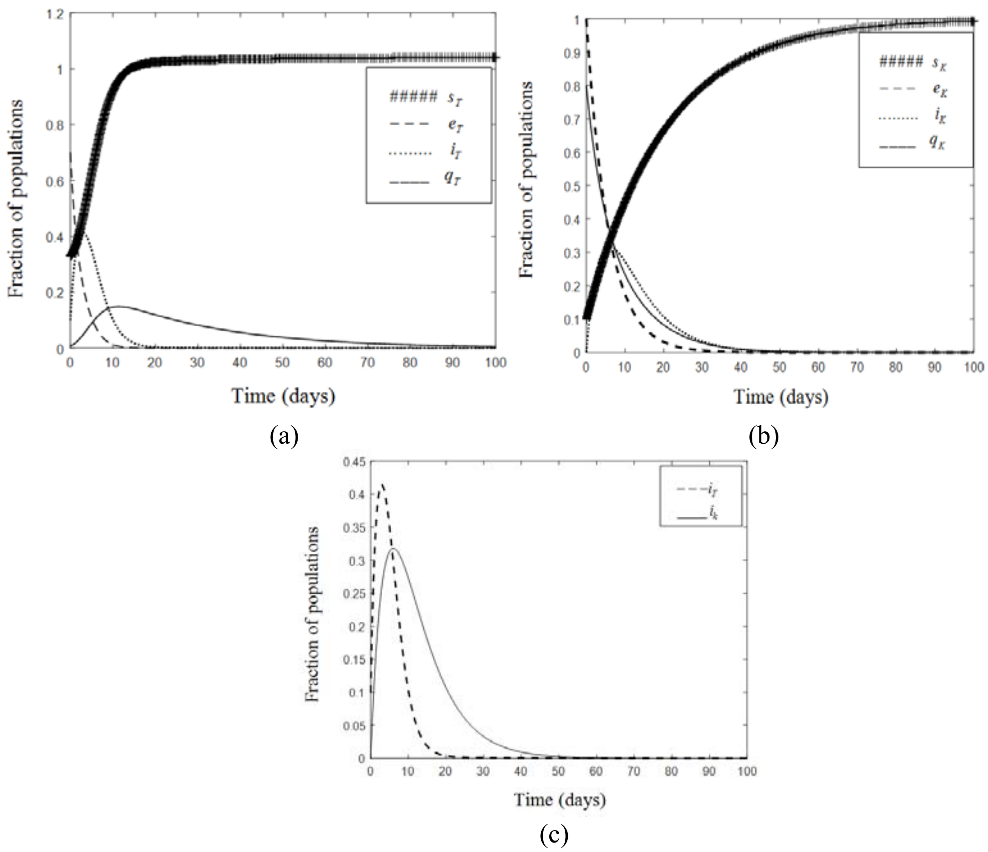


Figure 4. (a) Time series solutions of susceptible Thai population, exposed Thai population, infectious Thai population, quarantine Thai population and recovered Thai population. (b)Time series solutions of susceptible South Korean population, exposed South Korean population, infectious South Korean population, quarantine South Korean population and recovered South Korean population a nd (c) Time series solutions of infectious Thai population and infections South Korean population for $R_0 = 0.055053$.

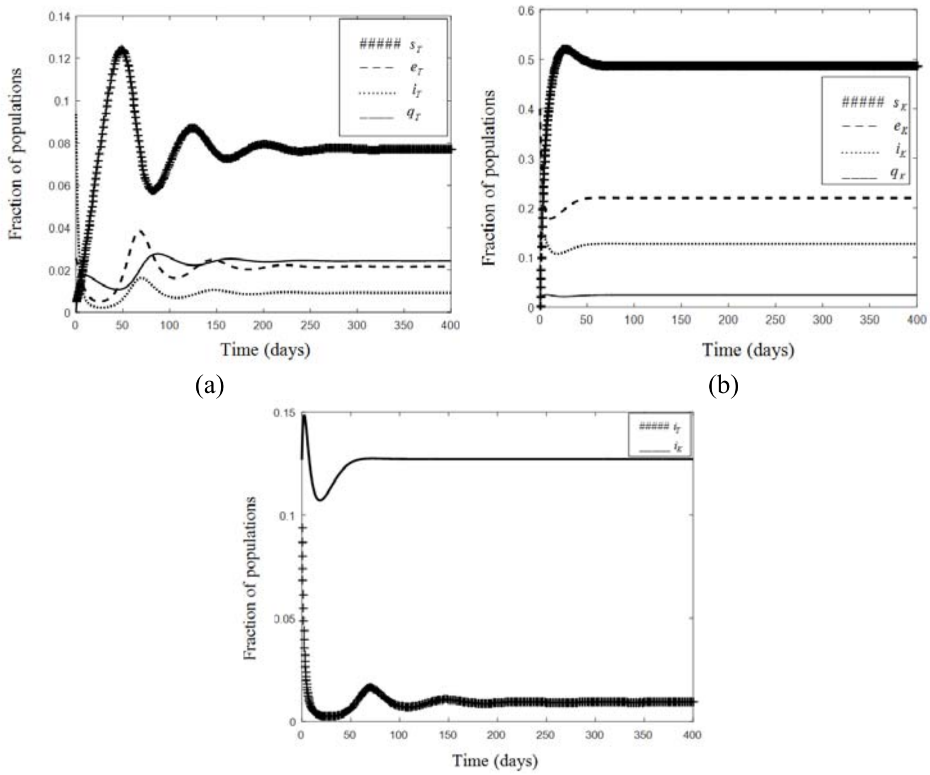


Figure 5. (a) Time series solutions of susceptible Thai population, exposed Thai population, infectious Thai population, quarantine Thai population and recovered Thai population. (b)Time series solutions of susceptible South Korean population, exposed South Korean population, infectious South Korean population, quarantine South Korean population and recovered South Korean population and (c) Time series solutions of infectious Thai population and infections South Korean population for $R_0 = 10.2297$

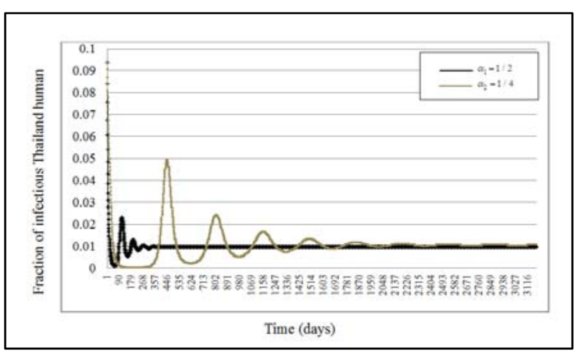


Figure 6. Numerical solution of infected Thai population case when there was the different rate at which susceptible Thai human changes to become an exposed Thai human.

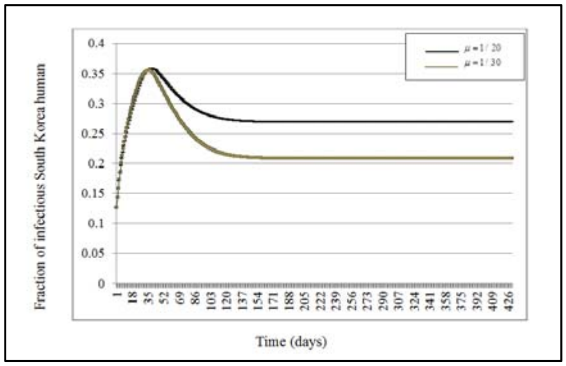


Figure 7. Numerical solution of infected South Korean population case when there was the different rate at which South Korean population moved out the country.

5. Conclusions

We had investigated the effect of repeated introduction of MERS in to Thailand by the entering of South Korea, some of them were infected with MERS. We had two steady state conditions, disease free condition and endemic condition. We can see that the Thai cases can lead to the disease free state in the absence of entering of disease free South Korea. From the numerical simulations, we can see that MERS can become endemic among the Thais when MERS South Korea cases enter in to Thailand.

6. Acknowledgements

This study is supported by Thatphanom College, Nakhon Phanom University, King Mongkut's Institute of Technology Ladkrabang and National Research Council of Thailand. IMT acknowledge the financial support provided by King Mongkut's University of Technology, Thonburi through the KMUTT 55th Anniversary Commemorative Fund.

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1.5” 1.5”

Abstract (12pt)

Maximum 250 words here. (10 pt)

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Clearly explain the nature of the problem, previous work, purpose, and contribution of the paper (10 pt).
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2. Materials and Methods (12 pt)

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3. Results and Discussion (12 pt)

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4. Conclusions (12 pt)

Clearly indicate advantages, limitations and possible applications (10 pt).
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A brief acknowledgement section may be included here (10 pt).
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[1] Barker, R. Kirk, J. and Munday, R.J., 1988. *Narrative analysis*. 3rd ed. Bloomington: Indiana University Press.

[2] Samson, C., 1970. Problems of information studies in history. In: S. Stone, ed. 1970. *Humanities information research*. Sheffield: CRUS, pp. 44-68.

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[4] Ross, A.B., Junyapoon, S., Jones, J.M., Williams, A. and Bartle, K.D., 2005. A study of different soots using pyrolysis–GC–MS and comparison with solvent extractable material. *Journal of Analytical and Applied Pyrolysis*, 74(1-2), 494-501.

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[6] Leonard, Y., Super Sports Limited., 2008. *Tin can manufacture and method of sealing*. Canada. Pat. 12,789, 675.

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[8] NHS Evidence, 2003. *National Library of Guidelines*. [online] Available at: http://www.library.nhs.uk/guidelinesFinder

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