

Imaging Red Light Runners

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Abstract

Traffic lights are routinely ignored by Thai drivers, so we designed a system that allows a single camera attached to a simple PC to monitor a controlled intersection and sends images of vehicles violating traffic lights to a police post further down the road. This allows police to instantly stop motorists, fine them if appropriate but, more importantly, educate them on the importance of following road rules - for the benefit of all drivers. We demonstrated that our system performs with two types of cheap, consumer cameras: it is simple to setup, does not rely upon accurate intersection delineation and can be deployed on intersections that are not well marked. It can view red light runners from the side or rear view thus the only constraint on the camera position is the ability to view the whole intersection and at least one controlling traffic light.

Keywords: visual object tracking, traffic safety

Introduction

Thailand has a very high rate of road accidents: it has been ranked as high as third in the world on a per head of population basis by statistics from the World Health Organization¹. Road traffic accidents have many causes: statistics from the Thai Traffic Police rank the causes of the accident as

1. Highway speeding,
2. Tailgating,
3. Unsafe overtaking and
4. Running a red light².

Although running red lights only causes ~ 10% of accidents in Thailand, this represents ~ 2000 deaths (and many more accidents) annually². However, this problem is international: Retting et al. reports that in the US, 260,000 crashes and 750 fatalities annually were attributed to red light running³. Crashes involving red light runners have greater impact than other crashes causing occupant injuries in 45% of crashes, compared to 30% in other crashes⁴. Internationally, the economic and social cost of road accidents remains high, even in countries with much lower per capita accident rates than Thailand.

For example, in Victoria, Australia, with only 5.4 fatalities per 100,000 head of population, the economic cost is estimated at \$ A4 billion per annum⁵ in a total population of 5.8 million (2014 census), thus the cost in Thailand with 17 fatalities per 100,000¹ the economic cost must be significantly greater. This makes no account of the social cost of deaths and injuries. Despite some campaigns from several sources in Thailand, the accident rate is not decreasing. Thus any system which increases awareness of road hazards will have enormous benefit for the country.

Previous work

Many systems have been developed to monitor traffic violations. Early systems were usually attached to the traffic light control systems, e.g. Abbas and Li used a combination of a PC104 which takes data from the traffic cabinet and cameras storing images with a commercial video capture loop⁶. Other systems, mostly involving cameras linked to traffic cabinets and inductive loop detectors, have been surveyed by Yung and Lai⁷. Washburn and Courage used a commercial video

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recorder with some sensors on the light power cables. Signals from these sensors were encoded on the audio channel of the recorder for later outline

Analysis: the acquired data was used for traffic analysis and was not used for enforcement or education of drivers themselves⁸. Many of these use camera and image processing techniques to assist law enforcement and improve safety as well as improve traffic flow. Klubsuwan et al. used image processing alone to detect traffic light violations without the help of other systems⁹. They correctly detected 80% violations. Several commercial systems are also now available, but they are mainly suited to the well-controlled intersections in western countries¹⁰. One full system developed in Thailand has been reported, but does not appear to have been widely adopted: cost may be a factor as it includes license plate detection¹¹.

1. Extent of the problem

To confirm the extent of the problem of running red lights locally, we made a simple count of the rate of red traffic light violations. On one major highway T-junction in Mahasarakham (a small city in NE Thailand, pop 250,000), we counted the number of vehicles going through traffic lights in light traffic conditions (12 noon on a Sunday): in a one hour period, with 26 light changes (one for each of 3 directions), we noted 79 cars and trucks and 28 motorcycles that did not stop for a traffic light over the three intersections in the T-junction, ie on average one car or truck for every light change and one motorcycle every three changes. This showed that traffic lights are routinely ignored locally and that an automated system that assisted police to detect and educate drivers could make a significant impact on accidents and their cost. We also hypothesized that educating drivers to obey one road rule might encourage them to follow other rules also and reduce accidents from other causes too.

Our system

Our system consists of a single camera attached to a PC which was positioned to monitor a controlled intersection. It monitors the traffic lights themselves and does not require assistance from the traffic controlling hardware and associated road loops. When it detects a red light violation - a vehicle moving through the intersection when the light is red - it captures images of the offending vehicle and transmits a set of images to a police post which can be several hundred meters down the road. This component of the system was designed to follow the very common current Thai police practice of establishing partial road blocks to check vehicles, thus it will require minimal changes to current practices. Our design is also easily adapted to a variety of situations: it can be set up in a few minutes at a new intersection. It just requires a suitable position for a camera mount and a casing for the PC and battery¹. The wireless connection needs a line-of-sight link to the monitor post, say 500m away².

1. Operation

1.1 Setup

Traffic Light Detection initially, the operator needs to place the camera in a stable location so that it can view the intersection and at least one light controlling it. The software detects traffic light candidates which can be anywhere in the field of view and asks the operator to indicate when the correct light has been detected³. Steps in the light detection algorithm are:

1. Identify 'red' areas by comparing high intensities in the red channel of the image with intensities in the other two channels. Typical traffic scenes contain large white areas which show high red intensities, so these are eliminated. Unfortunately, scenes often contain several red patches (red cars seem to be very popular!), so we did not attempt to fully automatically identify lights: previous efforts, e.g. Sooksatra and Kondo¹², found less than 90% correct detections in fairly ideal conditions, as might be expected due to the likelihood of round and red objects appearing in a typical scene⁴. traffic light, false color optical flow map (green indicates highest speed), flow map contour

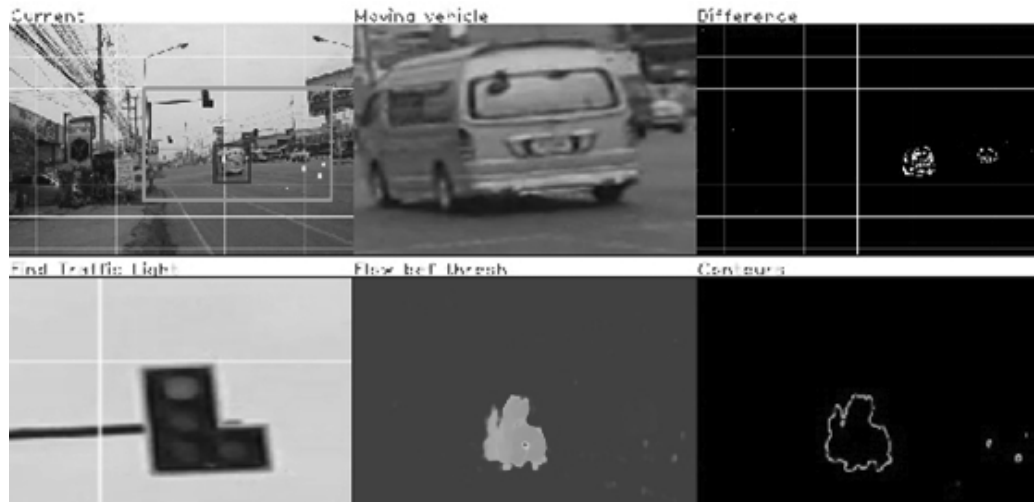


Figure 1 Screen shot after detecting a red light runner using 1280 720 pixel small consumer camera (Canon IXUS 155)
Top row: marked up intersection image, vehicle passing red light, difference image; Bottom row:

2. Select red light candidates by shape and size.
3. Present candidates to the operator, one-by-one
4. Terminate when the operator accepts a candidate or repeat on another image.

Traffic Light Detection The operator then identifies the 'forbidden region' - the area which must not be entered after the light has turned red. Observation showed that many intersections were poorly marked - with markings absent or badly worn - and therefore likely to defeat an automated system. However, an operator can quickly (two mouse clicks) identify the forbidden region - see blue outline in the top left of (Figure 1).

Link to monitor post Then the operator aligns the link between the intersection camera and the monitoring post computer. A reasonable height above ground for the antennae at both ends will ensure trouble-free transmission. Even in quite crowded city areas, transmitters and receivers about 2-3m or more above ground will suffice. Software will verify that significant bandwidth to transmit images of the red light runners is available⁵.

1.2 Automatic Monitoring In monitor mode, the program uses optical flow to detect moving objects in the exclusion zone when the traffic light is red. Candidate algorithms for this are frame differences and optical flow.

1.3 Frame differences This is very fast and meets any real-time constraints, but is susceptible to interference from moving objects (trees are a particular concern, but even traffic lights are affected by the wind. Some examples may be seen in the top panel of (Figure 2) which shows trees and power wires clearly showing up - 'speckle' in the Top row: marked up intersection image, vehicle passing red light, difference image; Bottom row: traffic light, false color optical flow map (green indicates highest speed), flow map contour

background. Even the 'pick up' appearing as a large white area in the difference map is a complex shape with several black 'holes'. So the difference images need considerable intelligent processing to remove noise and non-vehicle movements and we rejected it in favor of optical flow techniques. Although it is not used for primary vehicle movement detection, occasional system artefacts (probably mainly vibrations in the camera base) cause poor frame-to-frame registration, affecting optical flow, which detects anything moving in the scene, and leads to poor discrimination between moving and background objects. So we simply reject frames which are poorly registered: typically more than 30 frames are captured for a red light runner, so this has negligible effect on overall detection.

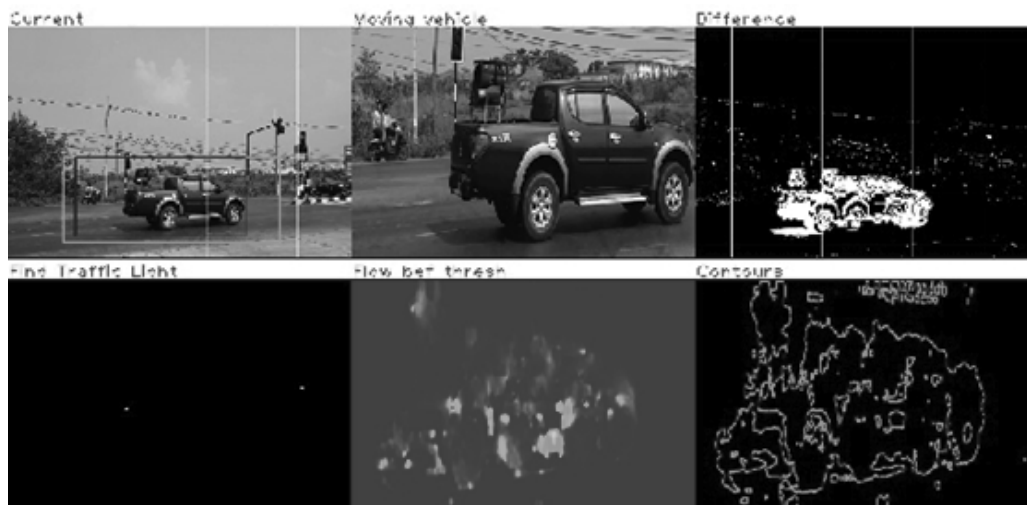


Figure 2 Screen shot after detecting a red light runner using hand held 1900 _ 1080 pixel cell phone camera (Samsung Galaxy Grand Prime)

1.4 Optical flow

Optical flow is unfortunately computationally complex on the high resolution images that are now available. Selection of the forbidden region assists by reducing the area that must be processed. Optical flow images also allow the speed and direction of the moving vehicle to be determined, allowing rejection of vehicles stopped unintentionally in the forbidden region, e.g. blocked by other traffic. We used the Farneback optical flow routine from the Open CV library¹³. In separate trials, we have used the total linear variation algorithm (from Open CV¹⁴) which produces slightly better results but is approximately three time slower. On the bottom middle panel of (Figure 1), a false color map showing which pixels are moving from frame to frame.

Greenish colours encode faster moving pixels. A contour is drawn around the fast moving pixels: small areas are rejected (wind or vibration generated noise) and regions large to represent a vehicle (or motorcycle - the commonest offenders are some intersections) and an image of the offending vehicle extracted from the current scene image for transmission to a monitoring post. For the higher resolution images of (Figure 2), the optical flow algorithm fails to cleanly assign every pixel of the vehicle to a uniform velocity (as seen in the lower middle false colour image of Figure 2), but the moving vehicle is sufficiently correctly identified to allow a complete and clear image of the red light runner (upper middle panel of Figure 2).

Results

Preliminary results for our system have been tested with several video cameras - including one in a cell-phone - have shown that the system reliably detects red light runners. A screen shot from the running program is shown in (Figure 1). Using a Canon IXUS 155 camera (1280_720 pixel resolution), clear images of the red light runner were obtained in typically 300 _ 230 pixel colour images which can easily identify a vehicle. These _ 24 Kbyte images are rapidly transmitted to a monitor post which can capture a short video of the runner.

False detections from low moving and small objects and noise were readily eliminated. Currently the system is being tested using IP links over the cell-phone network, which may prove satisfactory (and considerably cheaper!) in the long run: we note that a red light runner travelling at 60 km/hour needs 30 s to reach a monitoring station 500m down the road and a communications latency of much less than that is readily achieved. A frame rate as low as 10 fps requires a bandwidth of _ 0:25 MB/s to capture 30 frames of a red light runner at 60 km/hour: so a clear picture can still be obtained if many frames are dropped. There is also considerable potential to use higher resolution images but seemingly no need to increase frame rates.

We observed that automatic number plate recognition (as provided in the Smart Vision Technology

system¹¹) would require higher resolution and also restrict position of the camera to be almost directly behind the moving vehicle, but there are only weak constraints of the current system: it can be rapidly set up and only needs a clear view of the intersection and a controlling traffic light. We have tested it successfully from several viewpoints in different intersections.

Conclusions

This work was designed to develop a simple and robust system that was suited to Thai conditions and procedures. It is characterized by a flexible fast setup and thus can be readily redeployed to a new location. Rapid movement may be a key factor in the effectiveness of this system: fixed red light camera locations are widely publicized in several countries, mitigating their effectiveness, but the wide availability of rapidly deployed systems may substantially enhance effectiveness. Tests at several intersections with different camera capabilities have suggested that the system has significant potential to detect traffic light violations in less than ideal conditions with simple set-up procedures and contribute to the serious problems referred to in the introduction that currently exist on all Thai roads. We thus see strong justification for further work to improve its robustness and reliability.

Future work

Robust estimation of the object shape and size from optical flow data needs further study. Further tests in a variety of weakly controlled environments, e.g. partially visible traffic lights, and situations where several competing traffic flows can be observed. Although the system is designed to provide traffic light violations to a human operator and therefore occasional failures are tolerable, confidence and effectiveness of the systems will be improved if its correct behavior is known - and approaches 100%. Statistical analysis of detection accuracy is also required: we have not yet investigated situations where several vehicles run the red light at the same time.

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Causal Relationship Model of Ecological Footprint Integrated with Environmental Education

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Abstract

This research was quantitative research with a survey approach. A questionnaire was used as an instrument for gathering data from a population of 36,009 upper secondary school students under Secondary Service Area Office 28 (Sisaket-Yasothon) in academic year 2014. Cluster Random Sampling technique was implemented to collect 400 upper secondary school students as the sample group. Structural Equation model (SEM) was used for model confirmation. Research results demonstrated that Ecological Footprint (EF), Environmental Education (EE), and Environmental Conservation Inspiration (INS) were able to explicate the variation of cause Environmental Conservation Behavior for Global Warming Alleviation (EnB) with 92.00 percent. EF had the most effect to EnB with 0.69, Subsequences were EE, and INS with 0.48 and 0.40. Additionally, EF and EE were able to explicate the variation of INS with 78.00 percent, and EF had the most effect to INS with 0.36. Subsequence was EE with 0.30. The causal relationship model of EF and EE influencing EnB through INS was confirmed the proposed model and it was fitted with all observed variables in line with criteria of Chi-Square/df value with less or equal to 1.647 and it was less than or equaled to 5.00 ($\chi^2/df \leq 5.00$). RMSEA (Root Mean Square Error Approximation) equaled to 0.045 (RMSEA <0.05) and RMR (Root Mean Square Residual) equaled to 0.047 (RMR <0.05) including index level of model congruent value of Goodness of Fit Index (GFI) equaled to 0.94, and Adjust Goodness of Fit Index (AGFI) equaled to 0.91 which are between 0.90-1.00.

Keywords: model, ecological footprint, environmental education

Introduction

Ecological Footprint (EF) is a measurement of humanities reliance on natural resources. Moreover, EF measures the amount of productive land and water necessary for the production of goods including waste accumulation from the population's activity. EF is a distinctive group of uniqueness, actions, etc., that leave a trace and serve as methods of classification, such as the quantity of productive land suited for average of each person (in the world, a country, etc) for food, water, transport, housing, waste management, and other purposes. The simplest way to

define ecological footprint would be classify it as the impact of human activities measured in terms of the area of biologically productive land and water requisite to produce the goods consumed and to absorb the wastes produced. Basically, it is the quantity of the environment required to produce the goods and services needed to maintain a particular lifestyle.^{8,22}

The EF is rooted in the fact that all renewable resources come from the earth. It accounts for the flows of energy and matter to and from any defined economy and converts these into the corresponding land/water area

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required for nature to support these flows. The Ecological Footprint is defined as “the area of productive land and water ecosystems required producing the resources that the population consumes and assimilate the wastes that the population produces, wherever on Earth the land and water is located.”¹ It compares actual throughput of renewable resources relative to what is annually renewed. Non-renewable resources are not assessed, as by definition their use is not sustainable.

Environmental Education of The Intergovernmental Conference highlighted the Environmental Education functions on preservation and improvement the global environment and required to supply the agenda and directions for environmental education. Environmental education is a learning process that makes human gain more awareness and knowledge on the environment, developing the essential skills and expertise, and cultivating attitudes, and promise for decision making and taking responsibility. Environmental education covers the fundamental component of environmental, knowledge and understanding, awareness for environmental problems, having proper attitude for performing appropriate behavior through repeated practice for environmental conservation with skill and correct decision making with responsibility and participation in environment projects and activities.²⁵

Thiengkamol affirmed that the inspiration of public consciousness or public mind requires no admiration or complement or incentive. Particularly in natural resources and environment conservation, it happens from insight of someone, whilst its occurrence might come from the pleasure in a person as role model or idle, events, situation, environment, media perceiving such movie watching, book and magazine reading, and internet using. In addition, several researches were carried out by her and her colleagues, these have also proved that inspiration of public consciousness or public mind are a critical factor for environmental conservation in diverse environmental management with the integration of the environmental education concept.^{2,3}

The goal of this study was to apply the EF knowledge and EE for the students to conserve the

natural resources and environment via inspiration of public consciousness or public mind. Knowledge and understanding of EF and EE would lead them to be concerned that the ecological capacity has limitations whilst the population growth has increased rapidly because various factors support this growth. A factor such as better medical technology is progress, thus the people have better health. However, there is the problem of a new disease arising from the environmental problems of pollutions. Therefore, understanding the EF would help younger generations to realize the importance of ecological balance and to challenge them to change their environmental behavior of consumption, energy conservation, waste management, recycling, traveling and environmental knowledge transferring. The new generations would be our hope to cure and maintain the ecological system with their public consciousness to meet sustainable development.

Objective

The objective of this research was to develop a causal relationship model of an ecological footprint and environmental education of upper secondary school students under Secondary Service Area Office 28 (Sisaket-Yasothon) in the Northeastern region, Thailand.

Methodology

The research method was conducted following these steps:

1. Population and Sample

Population was 36,009 upper secondary school students under Secondary Service Area Office 28 (Sisaket-Yasothon) of Northeastern region of Thailand in second semester of academic year 2014.

Sample was 400 upper secondary school students that gathered with Cluster Random Sampling technique.

2. Research tool

The content and structural validity of a questionnaire were proved by Item Objective Congruent (IOC) from 5 experts in the fields of ecology, environmental education, social science and social research methodology.

The reliability was tried out by conducting a sample group from 40 upper secondary school students who had the same characteristics with sample group. The reliability was determined by Cronbach's Alpha formula: the ecological footprint composing 42 items, environmental education composing 42 items, environmental conservation inspiration composing 35 items, and environmental conservation behavior for global warming alleviation composing 42 items. The whole questionnaire consisted 161 items. Their reliabilities were 0.816, 0.804, 0.954, 0.957 and 0.938 respectively.

3. Data Collection

The Cluster Random Sampling technique was employed for data collecting of 400 upper secondary school students under Secondary Service Area Office 28 (Sisaket-Yasothon) of Northeastern region of Thailand. The research instrument was a questionnaire, and it was used for data gathering.

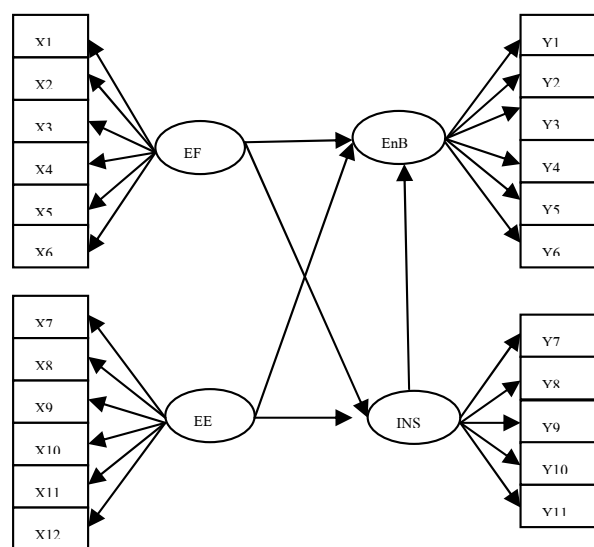
4. Data Analysis

The descriptive statistics were frequency, percentage, mean and standard deviation. Structural Equation Model (SEM) was used for model confirmation with LISREL version 8.30 by considering on Chi-Square value had no statistically significant at level of 0.01 or Chi-Square/df value with less or equal to 5, RMSEA (Root Mean Square Error Approximation) and RMR (Root Mean Square Residual) values with less than 0.05 including index level of model congruent value, GFI (Goodness of Fit Index) and index level of model congruent value, AGFI (Adjust Goodness of Fit Index) between 0.9-1.00.

Conceptual Framework

The exogenous latent variables of Ecological Footprint (EF) and Environmental Education (EE) had direct and indirect effects to Environmental Conservation Inspiration (INS) and Environmental Conservation Behavior for Global Warming Alleviation (EnB). EF was measured by Ecological Footprint for Shelter (X1), Ecological Footprint for Food (X2), Ecological Footprint for Transportation (X3), Ecological Footprint for Cloth (X4), Ecological Footprint for Medicine (X5), and Ecological Footprint for Housing (X6). EE was measured by

Environmental Awareness (X7), Environmental Attitude (X8), Environmental Skill (X9), Environmental Participation (X10), Environmental Responsibility (X11) and Environmental Decision (X12). The endogenous latent variable of EnB was measured by Consumption Behavior (Y1), Energy Conservation Behavior (Y2), Waste Management Behavior (Y3), Recycling Behavior (Y4), Traveling Behavior (Y5), Environmental Knowledge Transferring Behavior (Y6) and INS was measured by Self-Public Mind (Y7), Role Model Impression (Y8), Event Impression (Y9), Environment Impression (Y10), and Media Reception (Y11).



Results

1. Results of Effect among Variables in the Model in Terms of Direct and Indirect Effect

Ecological Footprint (EF), Environmental Education (EE) and Environmental Conservation Inspiration (INS) had effect on Environmental Conservation Behavior for Global Warming Alleviation (EnB) as follows.

- 1) Confirmatory factors of EF had direct effect on INS with statistically significant at level of 0.05 with effect of 0.36. EF had direct effect on EnB with statistically significance at a level of 0.01 with effect of 0.69 and indirect effect on EnB with statistical significant at level of 0.05 with effect of 0.14.
- 2) Confirmatory factors of EE had direct effect on INS with statistical significant at level of 0.05 with effect of 0.33. EE had direct effect on EnB with

statistical significant at level of 0.01 with an effect of 0.48 and indirect effect to EnB with statistical significant at level of 0.05 with effect of 0.13.

3) Confirmatory factors of INS had direct effect on EnB with statistical significant at level of 0.01 with effect of 0.40.

Considering on structural model confirmatory factors of component analysis of EF, EE and INS had effect to EnB with effect of 92.00 %. The structural equation can be written as the following in equation (1).

$$\text{EnB} = 0.40 \cdot \text{INS} + 0.69 \cdot \text{EF} + 0.48 \cdot \text{EE} \dots \dots \dots (1)$$

$$(R^2 = 0.92)$$

Equation (1) factors that had the most effect to EnB was EF with 0.69, subsequences were EE, and INS with effect of 0.48 and 0.40, these were able to explicate the variation of EnB with 92.00 percent.

Considering on confirmatory factors INS of upper secondary school students, it demonstrated that EF had the most effect on INS with 0.36. Subsequence was EE with 0.33, these were able to explicate the variation of INS with 78.00%. The structural equation can be written as follows.

$$\text{INS} = 0.36 \cdot \text{EF} + 0.33 \cdot \text{EE} \dots \dots \dots (2)$$

$$(R^2 = 0.78)$$

Equation (2) factors that had the most effect to INS were EF, and subsequence was EE, these were able to explain the variation of Environmental Conservation Inspiration (INS) with 78.00 percent.

The results of exogenous variables had effect to endogenous variables with direct and indirect effects were demonstrated in (Figure 1) and (Table 1).

Discussion

The results indicated that understanding the concept of Ecological Footprint (EF) was predicted by 6 observed variables of Ecological Footprint for Cloth (X4), Ecological Footprint for Transportation (X3), Ecological Footprint for Shelter (X1), Ecological Footprint for Medicine (X5), Ecological Footprint for Housing (X6) and Ecological

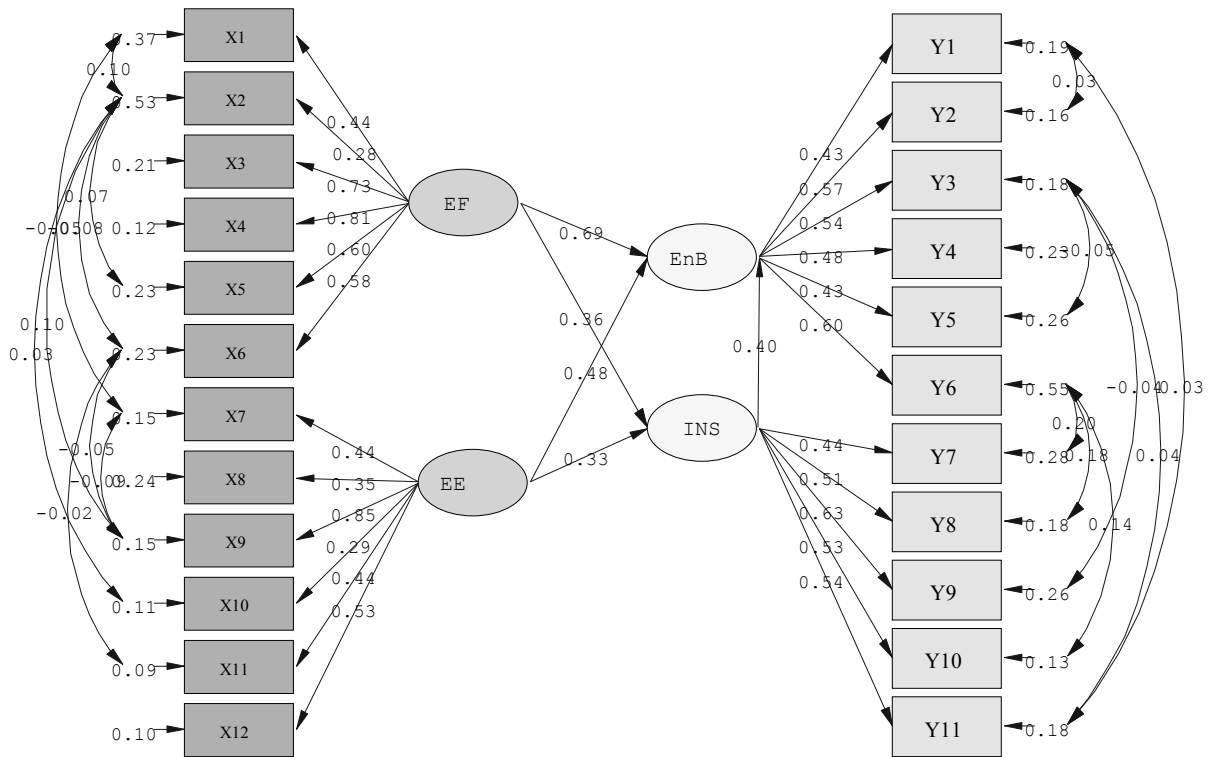
Footprint for Food (X2) with predicting power of 0.83, 0.74, 0.65, 0.64, 0.60, and 0.28 respectively. Moreover, it also had direct effect to Environmental Conservation Behavior for Global Warming Alleviation (EnB) with effect of 0.69, which is a rather high effect, thus it is obviously seen that EF plays an important role to change the environmental conservation behaviors for global warming alleviation that was predicted by Environmental Knowledge Transferring Behavior (Y6), Energy Conservation Behavior (Y2), Waste Management Behavior (Y3), Traveling Behavior (Y5), Recycling Behavior (Y4), and Consumption Behavior (Y1) with prediction power of 0.71, 0.62, 0.62, 0.61, 0.56, and 0.51 respectively. Another essential exogenous variable was Environmental Education (EE) which had direct and indirect effects on EnB with 0.48 and 0.13 and it also was predicted by observed variables of Environmental Skill (X9), Environmental Decision (X12), Environmental Awareness (X7), Environmental Responsibility (X11), Environmental Attitude (X8) and Environmental Participation (X10) with prediction power of 0.89, 0.60, 0.48, 0.47, 0.37 and 0.32 respectively. The findings verified that EF and EE are vital factors that are able to be applied for challenging pro-environmental behaviors of upper secondary school students to act as critical change agents to transfer their environmental knowledge to their friends, family members, and others in society as well including explicit their Energy Conservation Behavior, Waste Management Behavior, Traveling Behavior, Recycling Behavior, and Consumption Behavior as good role model for others too. The results were in the line with Thiengkamol and her colleagues that EE had direct and indirect effect to environmental conservation behaviors for global warming alleviation.

Additionally, Environmental Conservation Inspiration (INS) had direct effect to EnB with effect of 0.40 whereas considering on prediction of correlation of observed variables of Event Impression (Y9), Media Reception (Y11), Environment Impression (Y10), Role Model Impression (Y8), and Self-Public Mind (Y7). These were congruent to different studies of Thiengkamol and her colleagues that the results illustrated that Inspiration of Public Consciousness or public mind influencing pro-environmental

behaviors whether consumption behavior, energy conservation, waste management behavior, recycling behavior, traveling behavior and knowledge transferring and supporting for environmental conservation and so on.

Thus, this research results should be established to inspire upper secondary school students to take action in playing a role as environmental educators to transfer

their environmental knowledge and understanding with public mind to devote for environmental conservation behavior as new generations who take care for ecological balance and maintain environmental quality to meet life quality for achieving sustainable development based on EF and EE concept considerations.



Chi-Square=263.50, df=160, P-value=0.00000, RMSEA=0.045

Figure 1 Model of Direct and Indirect Effect of EF and EE Affecting EnB through INS

Table 1 Direct and Indirect Effect of EF and EE Affecting EnB through INS

Causal variable	Result variables					
	INS			EnB		
	TE	IE	DE	TE	IE	DE
EF	0.36* (0.059)	-	0.36* (0.059)	0.83** (0.075)	0.14* (0.033)	0.69** (0.074)
EE	0.33* (0.059)	-	0.33* (0.16)	0.61** (0.065)	0.13* (0.013)	0.48** (0.069)
INS	-	-	-	0.40** (0.043)	-	0.40** (0.043)

$\chi^2 = 263.50$; df = 160

CN = 234.28

$\chi^2/df = 1.647$

GFI = 0.94; AGFI = 0.91

RMSEA = 0.045

RMR = 0.047

TE: Total Effect, IE: Indirect Effect, DE: Direct Effect

Conclusion

Nevertheless, it might be summarized that latent variables that can be observed from observed variables are influenced through Environmental Conservation Inspiration (INS) composed of Self-Public Mind (Y7), Role Model Impression (Y8), Event Impression (Y9), Environment Impression (Y10), Media Reception (Y11) to Environmental Conservation Behavior for Global Warming Alleviation (EnB) that included behavior of appropriate consumption, energy conservation, waste management, recycling, travelling and knowledge transferring. Thus, the model of EF and EE influencing via INS to cause EnB was verified the proposed model was fitted with all observed variables according to criteria of Chi-Square/df value with less or equal to 5 (). RMSEA (Root Mean Square Error Approximation) equaled to 0.049 (RMSEA < 0.05) and RMR (Root Mean Square Residual) equaled to 0.017 (RMR < 0.05) including index level of model congruent value of Goodness of Fit Index (GFI) equaled to 0.95, and Adjust Goodness of Fit Index (AGFI) equaled to 0.92 which are between 0.90-1.00.

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Acute and Sub-Acute Toxicity Studies of Hawm Nil Brown Rice Kefir Powder

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Abstract

The present study was designed to determine acute and sub-acute toxicities of Hawm Nil brown rice kefir powder (HNKP) in male Wistar rats. In this acute toxicity study, HNKP at doses of 1,000, 2,000 and 4,000 mg/kg were once administered to the rats orally. Symptoms of toxicity and mortality were observed within 24 h and over a further period for 14 days. The results showed that all the doses of HNKP did not produce mortality or symptoms of toxicity. HNKP at a dose of 1,000 mg/kg produced the best body weight gain, food intake and FCR. Moreover, blood biochemistry including TP, Alb, Glob, BS, BUN, CREA, UA, TB, AST, ALT, and ALP, cholesterol, hematological values including WBC, RBC, Hb, Hct, MCH, MCHC, MCV, and Plt, and relative organ weight (ROW) of the rats received all the doses of HNKP did not differ from those in controls. In a sub-acute toxicity study, HNKP at the doses of 500, 1,000 and 2,000 mg/kg were given orally to the rats every 2 days for 14 days. Again, the result showed that all the doses did not produce mortality or symptom of toxicity. HNKP at a dose of 500 mg/kg produced the best body weight gain, food intake and FCR. Furthermore, the rats that received HNKP at this dose had blood biochemistry, cholesterol, hematological values, and ROW close to those in controls. However, the rats received high doses of HNKP (1,000 and 2,000 mg/kg) and long term application altered Alb, Glob, BS, BUN, UA, AST, and ALP levels. These results indicate that long term and high dose application of HNKP can affect renal and hepatic functions ($p < 0.05$). In addition, TG and HDL of the rats that received HNKP were significantly ($p < 0.05$) less than those in controls. Interestingly, the rats that received HNKP had fewer neutrophils while lymphocytes were significantly higher than that in controls ($p < 0.05$). These findings indicate that Hawm Nil brown rice kefir powder had no acute and sub-acute toxicities. However, long term application at high doses (1,000 and 2,000 mg/kg) of HNKP may cause hepatic and renal dysfunctions. Its activity on decreasing neutrophils and increasing lymphocytes resulted in increased globulin leads to improve immunomodulatory activity.

Keywords: Hawm Nil rice, rice kefir, acute toxicity, sub-acute toxicity, kefir powder

Introduction

Kefir is a fermented milk product. It contains lactic acid bacteria, yeasts and acetic acid bacteria that produce jelly-like grain. Kefir grain is white or lightly yellow, gelatinous irregular masses and sized between 0.3-3.5 cm diameter¹⁻². Both bacteria and yeasts are surrounded by a water-soluble branched glucogalactan called kefiran¹. Kefir has been reported to possess antibacterial³⁻⁷, antifungal⁷, antitumor⁸, antioxidant^{5, 9-11}, anti-allergic¹², antineoplastic and pro-digestive¹³⁻¹⁴, antidiabetic¹⁵⁻¹⁶, and

immunomodulatory activities¹⁷⁻¹⁸. Moreover, it is important to anti-inflammatory activity on the liver¹⁹, lung^{12, 16, 20} and colon²¹. Kefir can modulate the intestinal mucosa immune response. It induced the helper T cell type 2 response by increasing the number of immunoglobulin A, interleukins type 4, 6 and 10 cells, and induced simultaneously the production of pro-inflammatory cytokines (IFN γ and TNF α) but without tissue damage¹⁷⁻¹⁸. It can also improve lactose digestion and tolerance²². Fermented milk from kefir has high antioxidant activity and reduces the accu-

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mulation of reactive oxygen species (ROS) including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and nitric oxide ($NO\cdot$)²³. In addition, kefir has high nutritional value as a source of proteins and calcium. It has a long tradition of being regarded as good for health in many countries²⁴. Kefir can be considered as a probiotic resource, as it produces good activities for health.

Recently, the γ -aminobutyric acid (GABA), α -tocopherol, γ -tocopherol and total phenolics compounds (TPC) it is reported, has been found in Hawm Nil rice²⁵⁻²⁶. Moreover, total phenolic content and total anthocyanin content are found in Hawm Nil rice (Black) more than in white and red colored rice²⁷⁻²⁸. In addition, it exerts high levels of antioxidant activity²⁸. Kefir from rice milk has higher antioxidant activity than cow's milk⁵. The antioxidants were higher in Kefir produced from plants as a result of the phenolic compounds presence in the plants²⁹.

A toxicity study of medicines or plant products should be carried out to see whether they are safe for human application. The present study was therefore designed to determine acute and sub-acute toxicity of Hawm Nil brown rice kefir powder in the rats.

Materials and methods

1. Hawm Nil brown rice, fermentation and kefir powder preparation

1.1 Hawm Nil brown rice: Hawm Nil brown rice harvested during the year 2013-2014 from Selaphum, Roi Et province, Thailand was used in this study. The rice was dried, weighed, soaked in distilled water (1:5, W:V) at 25°C for 2 h and thoroughly ground by using a blender and filtrated to obtain rice milk. The rice milk was pasteurized at 70°C for 15 min and then directly cooled at 4°C.

1.2 Hawm Nil brown rice fermentation: A 0.2 g freeze-dried Kefir grain was inoculated into 250 mL flask with 200 mL of Lactobacilli de Man, Rogosa, and Sharpe (MRS) broth and incubated under anaerobic conditions; the flask was put into a 5L anaerobic jar. After that the sample jars were kept at 30°C for 24 h, and then centrifugation (1000×g, 15 min at 4°C) to obtain the cells. The cells were washed and re-suspended in sterile saline solution (0.85% NaCl) and then diluted with sterile 0.85%

NaCl (1:10; V:V). Subculture, kefir starter was inoculated into fresh milk (20:200; V:V) and incubated under aerobic conditions at 30°C for 48 h to obtain activated kefir grain. Then activate kefir grain were cultured and fermented by inoculating into Hawm Nil brown rice milk adding with 2.5% sucrose (100:1,000, V:V) and incubated under anaerobic conditions at 30°C for 24 h to get the final pH of about 4.8-4.9.

1.3 Hawm Nil brown rice kefir powder (HNKP) preparation: the Hawm Nil brown rice milk kefir was freeze-dried and powdered. The kefir powder was kept at -20°C until used.

2. Animals

Forty-eight male Wistar rats weighing 280-300 g were purchased from the National Laboratory Animal Center, Mahidol University, Thailand. The rats were kept in an animal laboratory and acclimated for 7 days in environmental conditions (22-25°C, 50%-55% humidity and under a 12-hour light/dark cycle). The rats were fed with a standard diet (Perfect Companion Group Co., Ltd.) and water *ad libitum*. All experimental protocols were maintained in accordance with the Guidelines of Committee Care and Use of Laboratory Animal Research, National Research Council of Thailand and advice of the Institutional Animal Care and Use Committee, Mahasarakham University, Thailand.

3. Acute toxicity study

Rats were weighed and divided randomly into four groups with 6 rats in each; group 1; rats received phosphate buffered saline (PBS) (control group), group 2, 3 and 4; rats received HNKP 1,000, 2,000 and 4,000 mg/kg respectively³⁰. The doses of HNKP were once administered to the rats orally. Symptom of Toxicity (seizures, vomiting, diarrhea, and nausea) and rat mortality were observed within 24 h and over a further period for 14 days. Body weight and food intake were recorded daily. On day 14, the rats were fasted overnight, weighed and sacrificed by overdoses of chloroform. Blood sample was collected from the rat heart to determine blood biochemistry and hematological values. Visceral organs including liver, lung, heart, kidney and spleen were removed and weighed.

4. Sub-acute toxicity study

The rats were randomly divided into four groups with 6 rats in each; group 1; rats received PBS (control group), group 2, 3 and 4; rats received HNKP 500, 1,000 and 2,000 mg/kg respectively³¹. HNKP was given orally to the rats every 2 days for 14 days. Symptoms of toxicity were observed within 14 days. Body weight and food intake were recorded daily. At the end of experiments, the rats were fasted, weighed and then euthanasia by overdose of chloroform. Blood samples were collected from the rat hearts to determine blood biochemistry values and hematological values. Visceral organs including liver, lung, heart, kidney and spleen were removed and weighed.

5. Relative organ weight and feed conversion ratio

The relative organ weight (ROW) of each animal was calculated using the following equation;

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat (g)}} \times 100 \quad (1)$$

The feed conversion ratio (FCR) of each animal was calculated as follow;

$$\text{FCR} = \frac{\text{Food intake (g)}}{\text{Body weight gain (g)}} \quad (2)$$

6. Determination of blood biochemistry and hematological values

Blood samples were put into heparinized and non-heparinized tubes. Blood was centrifuged at 1500 g for 10 min to separate serum. The serum from the non-heparinized blood was assayed for biochemistry including total protein (TP), blood sugar (BS), blood urea nitrogen (BUN), creatinine (Crea), uric acid (UA), cholesterol (CHO), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), albumin (Alb), globulin (Glob), total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).

Heparinized blood was used for hematological analysis. Hematological analysis included red blood cell (RBC) count, white blood cell (WBC) count, hematocrit

(Hct), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (Plt), neutrophils (Neu), and lymphocytes (Lym).

7. Statistical analysis

The results were presented as mean±SEM and analyzed using one-way ANOVA. The differences among means were detected by using the Duncan's Multiple Range Test and values of $p \leq 0.05$ were considered statistically significant.

Results and Discussion

1. Acute toxicity

1.1 Symptoms of toxicity, body weight gain, food intake and FCR

All the doses of HNKP did not produce any symptom of toxicity and mortality of the rats during 14 days. HNKP at a dose of 1,000 mg/kg produced the best body weight gain, food intake and FCR (Fig.1A–1C). Moreover, the rats received HNKP at a dose of 1,000 mg/kg had hepatic and renal functions (Table 1), lipid profile (CHO, TG, HDL and LDL) (Table 2), hematological values (Table 3), and ROW (data not shown) close to those in controls. However, the rats received all the doses of HNKP had neutrophils less than while lymphocytes were higher than that in controls ($p < 0.05$) (Table 3).

These findings indicate that HNKP has no acute toxicity on rats. Its activity on decreasing neutrophils and increasing lymphocytes resulting in increased globulin leads to improve immunomodulatory activity¹⁷⁻¹⁸.

1.2 Blood biochemistry and ROW

Since, AST, ALT and ALP enzymes are involved in hepatic function and TB, TP, Alb, Glob, BS, BUN, CREA, and UA are involved in renal function³². The results from acute toxicity study revealed that TB, TP, Alb, Glob, BS; BUN, CREA, UA, AST, ALT, and ALP enzymes in the rats received all the doses of HNKP did not differ from those in controls (Table 1), suggesting that HNKP had no effect on hepatic and renal functions.

Hyperlipidemia is well known as one of the major risk factors for atherosclerosis which leads to coronary artery disease (CAD)³³. The total cholesterol

was increased in the rats received HNKP at the doses of 2,000 and 4,000 mg/kg. However, TG, HDL and LDL were not altered in the rats treated with all the doses of HNKP compared to those in controls. These data indicate that HNKP has an effect on total cholesterol but not on TG, HDL and LDL (Table 2). The ROW in the rats treated with HNKP did not differ from that in controls.

1.3 Hematological values

(Table 3), WBC, RBC, Hb, Hct, MCV, MCH, MCHC, and Plt in the rats received HNKP did not differ from those in controls. Interestingly, the rats that received all the doses of HNKP had significantly less

neutrophils while lymphocytes were significantly higher than that in controls ($p < 0.05$), consistent with globulin increased. Since the total WBC count did not change, this result suggests that HNKP acts in opposite way in the differentiation of hematopoietic cells by suppressing neutrophils and stimulating lymphocytes. According to previous reports, kefir induced the helper T-lymphocytes type 2 proliferations by increasing the number of immunoglobulin A (IgA), interleukins type 4, 6 and 10 cells¹⁷⁻¹⁸, in agreement with the increase of lymphocyte and globulin in this study.

Table 1 Blood biochemistry in rats treated with HNKP and PBS from acute toxicity study (mean±SEM).

Blood biochemistry	PBS	HNKP (mg/kg)		
		1,000	2,000	4,000
BS (mg/dl)	182.33±23.55	176.67±38.09	214.83±21.09	235.33±25.04
BUN (mg/dl)	20.17±0.48	19.75±0.37	19.63±0.33	20.62±0.51
CREA (mg/dl)	0.91±0.03	0.88±0.02	0.85±0.02	0.87±0.02
UA (mg/dl)	3.91±0.55	3.72±0.31	3.65±0.31	4.12±0.07
TP (g/dl)	5.65±0.11	5.47±0.17	5.47±0.12	5.63±0.02
Alb (g/dl)	3.47±0.04	3.45±0.08	3.48±0.07	3.47±0.03
Glob (g/dl)	2.20± 0.06	2.25±0.06	2.18±0.05	2.17±0.04
TB (mg/dl)	0.11±0.01	0.09±0.01	0.09±0.00	0.09±0.00
AST (U/L)	147.67±3.95	143.83±4.80	152.83±1.08	143.50±2.79
ALT (U/L)	39.33±1.17	38.17±2.21	35.00±0.68	37.50±0.96
ALP (U/L)	124.33±3.06	118.67±3.01	123.00±2.31	126.00±5.39

TP = total serum protein; Alb = albumin; Glob = globulin; BS= blood sugar; BUN = blood urea nitrogen; CREA = creatinine; UA= uric acid; TB= total bilirubin; AST = serum aspartate aminotransferase; ALT = serum alanine aminotransferase; ALP = alkaline phosphatase.

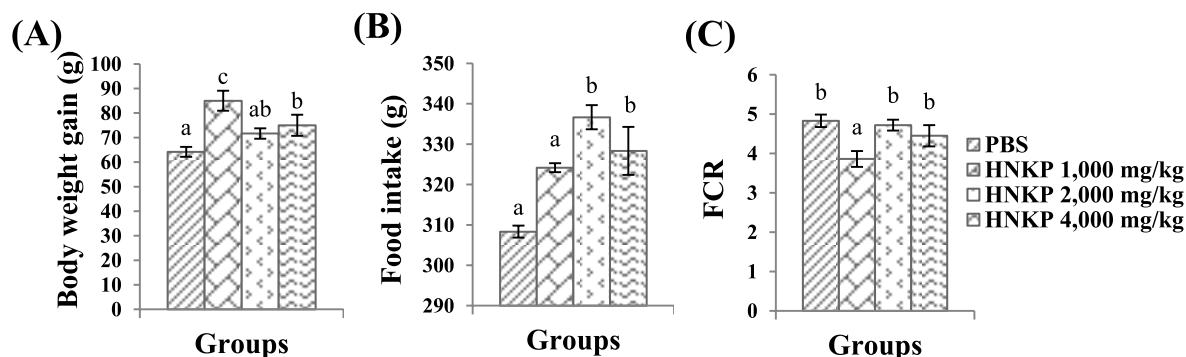


Figure 1 Body weight gain (A), food intake (B) and FCR (C) in rats treated with HNKP and PBS from acute toxicity study at the end experiment (mean±SEM). Mean values with different letters are significantly different, Duncan's test at $p < 0.05$.

Table 2 Cholesterol (CHO), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) in rats treated with HNKP and PBS from acute toxicity study (mean±SEM).

Lipid profiles	PBS	HNKP (mg/kg)		
		1,000	2,000	4,000
CHO (mg/dl)	53.67±1.28 ^a	56.50±1.91 ^{ab}	58.67±0.88 ^b	58.67±1.63 ^b
TG (mg/dl)	130.00±2.62	127.33±4.14	123.50±3.64	123.17±4.73
HDL (mg/dl)	16.48±0.27	16.57±0.55	17.10±0.57	16.62±0.29
LDL (mg/dl)	32.50±2.11	32.00±0.68	32.17±0.98	35.33±0.88

Mean values within each row with different superscripts are significantly different, Duncan's test at $p < 0.05$.

Table 3 Hematological values in rats treated with HNKP and PBS from acute toxicity study (mean±SEM).

Hematological values	PBS	HNKP (mg/kg)		
		1,000	2,000	4,000
WBC (10^3 cell/mm ³)	5.93±0.16	5.55±0.23	5.93±0.22	5.40±0.27
RBC (10^6 cell/mm ³)	8.87±0.13	8.76±0.21	9.00±0.12	8.99±0.05
Hb (g/dl)	17.07±0.40	16.30±0.21	16.35±0.32	16.28±0.28
Hct (%)	53.83±0.87	52.67±1.31	51.83±1.14	51.50±0.22
MCV (fl)	58.33±0.88	59.33±0.21	57.67±1.56	59.67±0.42
MCH (pg)	19.60±0.08	19.53±0.10	19.65±0.26	19.95±0.08
MCHC (g/dl)	32.33±0.34	32.55±0.21	33.07±0.46	33.13±0.18
Plt (10^3 cell/mm ³)	943.17±27.15	904.50±13.18	883.00±23.23	924.83±17.63
Neu (%)	8.50±0.22 ^c	6.17±0.17 ^b	2.50±0.43 ^a	2.33±0.33 ^a
Lym (%)	91.00±1.21 ^a	94.00±0.26 ^b	97.00±1.21 ^c	97.83±0.31 ^c

Mean values within each row with different superscripts are significantly different, Duncan's test at $p < 0.05$. WBC = white blood cells; RBC = red blood cells; Hb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; Plt = platelets; Neu = neutrophils; Lym = lymphocytes.

Table 4 Blood biochemistry in rat treated with HNKP and PBS from sub-acute toxicity (mean±SEM).

Blood biochemistry	PBS	HNKP (mg/kg)		
		500	1,000	2,000
BS (mg/dl)	207.67±11.25 ^c	156.50±19.70 ^b	128.67±9.30 ^b	85.50±7.64 ^a
BUN (mg/dl)	20.45±1.12 ^c	19.27±0.71 ^{bc}	17.53±0.55 ^{ab}	16.47±0.27 ^a
CREA (mg/dl)	0.82±0.03	0.90±0.07	0.78±0.03	0.83±0.03
UA (mg/dl)	2.65±0.34 ^b	2.62±0.28 ^b	1.65±0.25 ^a	1.48±0.19 ^a
TP (g/dl)	5.72±0.13	5.63±0.15	5.43±0.08	5.73±0.18
Alb (g/dl)	3.28±0.06 ^b	2.72±0.05 ^a	2.67±0.03 ^a	2.67±0.05 ^a
Glob (g/dl)	2.48±0.09 ^a	2.73±0.05 ^b	2.73±0.08 ^b	3.02±0.06 ^c
TB (mg/dl)	0.07±0.02 ^{ab}	0.05±0.01 ^a	0.05±0.01 ^a	0.13±0.02 ^b
AST (U/L)	104.00±1.53 ^a	140.00±3.18 ^b	151.67±5.16 ^c	157.33±4.58 ^c
ALT (U/L)	49.17±1.66	47.33±1.41	50.33±1.33	53.83±3.70
ALP (U/L)	132.83±4.66 ^b	119.83±6.82 ^{ab}	116.67±3.37 ^a	112.67±3.25 ^a

Mean values within each row with different superscripts are significantly different, Duncan's test at $p < 0.05$ (N=6). TP = total serum protein; Alb = albumin; Glob = globulin; BS= blood sugar; BUN = blood urea nitrogen; CREA = creatinine; UA= uric acid; TB= total bilirubin; AST = serum aspartate aminotransferase; ALT = serum alanine aminotransferase; ALP = alkaline phosphatase.

Table 5 CHO, TG, HDL and LDL in rats treated with HNKP and PBS from sub-acute toxicity study (mean±SEM).

Lipid profiles	PBS	HNKP (mg/kg)		
		500	1,000	2,000
CHO (mg/dl)	54.50±5.42	46.50±1.43	48.17±3.38	49.33±3.02
TG (mg/dl)	152.00±4.20 ^b	109.00±4.56 ^a	100.67±7.05 ^a	94.33±4.62 ^a
HDL (mg/dl)	22.55±1.04 ^b	18.42±0.42 ^a	16.93±0.84 ^a	17.48±1.44 ^a
LDL (mg/dl)	29.17±1.01	31.83±1.90	33.67±2.93	31.33±2.17

Mean values within each row with different superscripts are significantly different, Duncan's test at $p < 0.05$.

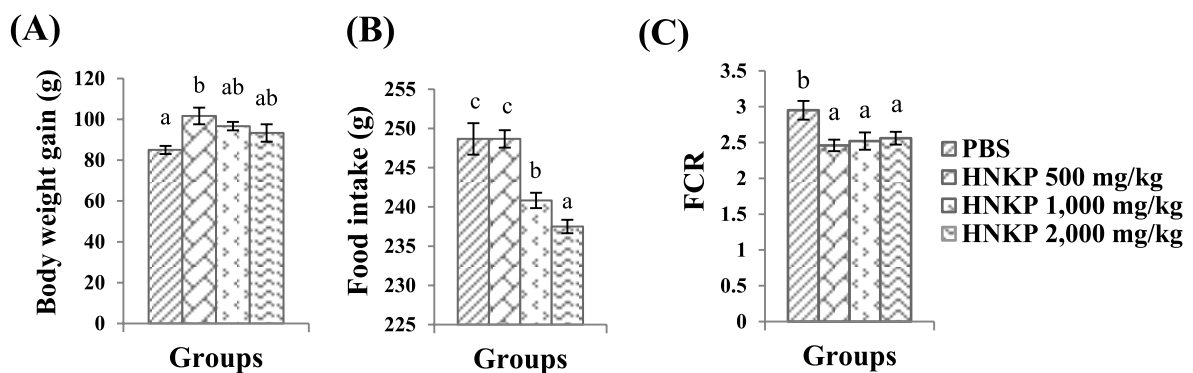


Figure 2 Body weight gain (A), food intake (B) and FCR (C) in rats treated with HNKP and PBS from sub-acute toxicity study at the end experiment (mean±SEM). Mean values with different letters are significantly different, Duncan's test at $p < 0.05$.

Table 6 Hematological values in rats treated with HNKP and PBS from sub-acute toxicity study (mean±SEM).

Hematological values	PBS	HNKP (mg/kg)		
		500	1,000	2,000
WBC (10^3 cell/mm ³)	6.42±0.28	5.77±0.43	6.50±0.26	5.60±0.40
RBC (10^6 cell/mm ³)	8.76±0.13	8.38±0.14	8.63±0.41	8.13±0.18
Hb (g/dl)	17.52±0.23	16.70±0.35	16.88±0.44	16.32±0.47
Hct (%)	53.33±0.76	51.00±0.68	50.67±1.38	50.67±1.36
MCV (fl)	59.33±0.49	59.50±0.43	59.33±0.42	59.33±0.33
MCH (pg)	19.98±0.28	19.90±0.17	19.80±0.27	20.05±0.26
MCHC (g/dl)	33.72±0.35	33.43±0.28	33.37±0.47	33.75±0.36
Plt (10^3 cell/mm ³)	923.17±24.11	887.33±24.78	893.67±42.25	905.67±46.40
Neu (%)	8.83±1.01 ^b	7.33±1.54 ^{ab}	5.83±0.94 ^{ab}	4.83±1.05 ^a
Lym (%)	90.83±1.25 ^a	91.83±1.87 ^{ab}	95.17±0.87 ^{bc}	96.33±0.71 ^c

Mean values within each row with different superscripts are significantly different, Duncan's test at $p < 0.05$. WBC = white blood cells; RBC = red blood cells; Hb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; Plt = platelets; Neu = neutrophils; Lym = lymphocytes.

2. Sub-acute toxicity

2.1 Symptoms of toxicity, body weight gain, food intake and FCR

Again, the results showed that all the doses of HNKP did not produce any symptom of toxicity and mortality of the rats. HNKP at a dose of 500 mg/kg produced the best body weight gain, food intake and FCR (Figure 2A-2C). However, all the doses of HNKP had FCR better than that in controls ($p < 0.05$). Kefir has been widely used in clinical practice to promote growth^{13-14,24}, and may be a source of nutritional compounds. Hawm Nil rice exerts high levels of antioxidant and phytochemical activities than white and red rice²⁵⁻²⁸. Thus, HNKP is suitable for further development as therapeutic agents for growth promotion.

2.2 Blood biochemistry and ROW

The results of this study showed the rats received HNKP at a dose of 500 mg/kg had hepatic and renal functions close to those in controls. Furthermore, the rats received high doses of HNKP (1,000 and 2,000 mg/kg) and long term were altered of Alb, Glob, BS, BUN, UA, AST, and ALP levels. These results indicate that long term and high doses application of HNKP can effect on renal and hepatic functions ($p < 0.05$) (Table 4). The serum AST and ALP activities are widely used as sensitive markers of possible tissue damage, particularly liver toxicity³⁴. Moreover, the triglycerides and HLD of the rats received kefir powder were significant less than that in controls ($p < 0.05$). The decreasing of TG and HLD on rats received HNKP may be cause from hepatic function changing. However, the ROW in the rats treated with HNKP did not differ from that in controls (data not shown).

2.3 Hematological values

In line with the acute toxicity study, WBC, RBC, Hb, Hct, MCV, MCH, MCHC, and Plt in the rats that received HNKP did not differ from those in controls (Table 6). Nevertheless, the rats received all the doses of HNKP had neutrophils significant less than while lymphocytes were significant higher than that in controls ($p < 0.05$). These results confirmed the non-toxicity of the application of Hawm Nil brown rice kefir powder at the doses less than 4,000 mg/kg.

Conclusions

HNKP has no acute and sub-acute toxicities when a dose less than 4,000 mg/kg is administered orally once, or a dose less than 500 mg/kg is administered every 2 days for 14 days. In long term application, the powder at doses higher than 1,000 mg/kg may cause hepatic and renal functions as it produces Alb, BS, BUN, UA, and ALP levels decreasing while Glob and AST levels increase compared to those in controls. Furthermore, its activity on decreasing neutrophils and increasing lymphocytes resulting in increased globulin leads to improve immunomodulatory activity.

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Development of a Bike Trail as a Tourist Attraction in the Area of the Community Forest of Ban Nonhinphueng

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Abstract

A study of ways to develop a of bike trail as a tourist attraction in the area of the community forest of Ban Nonhingphueng, Tambon Dongbang, Amphoe Prachantakam, Prachinburi province was conducted. The study was to investigate tourists' opinions about a bike trail as a tourist attraction in the community. It was found that the tourists having no experience participating in this kind of activity tend to be more satisfied than the experienced ones. With the recommendation provided, ways of development based on a service marketing strategy, such as product varieties, uniqueness, pattern and need conformation, clear signs of tourist attraction and activity trail, increasing garbage dump spot and public relation channel, sale promotion inducing more buying, notifying activity sequence throughout the activity based on the tourists participating in life styles and learning experience with the community in more environmental friendly way, were proposed.

Keywords: development, prototype tourist, service market, creative tourism

Introduction

Based on an evaluation of, Tumbon Dongbang, Amphoe Prachantakam, Prachinburi province conducted by Faculty of Technology and Industrial Management joint with Raks Thai Foundation, a bike trail is proposed as an attraction in the area of the community forest in order to support an ecosystem tourist attraction which beneficially consumes the community's natural resources, develops sustainable careers and provides income for the community. In order to realize whether the trail is appropriate, the prototype activity should be held. Therefore, the researcher joined with Raks Thai Foundation and provided the prototype activity "Ride to the forest, Pick vegetables, and Visit community enterprise" to be aware of the tourists' opinions toward the bike trail in the area of community tourist attraction and to further improve and develop ways of sustainability.

Experimental

1. Tools and population

Questionnaires with a check list form based on Likert's scale and content analysis were mainly used to collect data whereas an interview was used to store data. The population was from the 50 prototype tourists participating in activity provided on 28th February 2015

2. Statistics used in this research

2.1 Significance level at 0.05

2.2 Descriptive statistics such as demographic data in check list form, frequency, percentage, rating scale with data exposing the satisfaction level of prototype activity participation, means, standard deviation, content analysis related to opinions and recommendation showing from highest to lowest.

2.3 Analytical statistics based on t-test (t) is to find the relation between variables of participating in

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riding bike activity for tourism and satisfaction of participating in prototype activity

2.4 Analysis of the average. Evaluating the level of satisfaction.

Average	Interpretation
4.51-5.00	Highest
3.51-4.50	High
2.50-3.50	Moderate
1.51-2.49	Low
1.00-1.50	Least

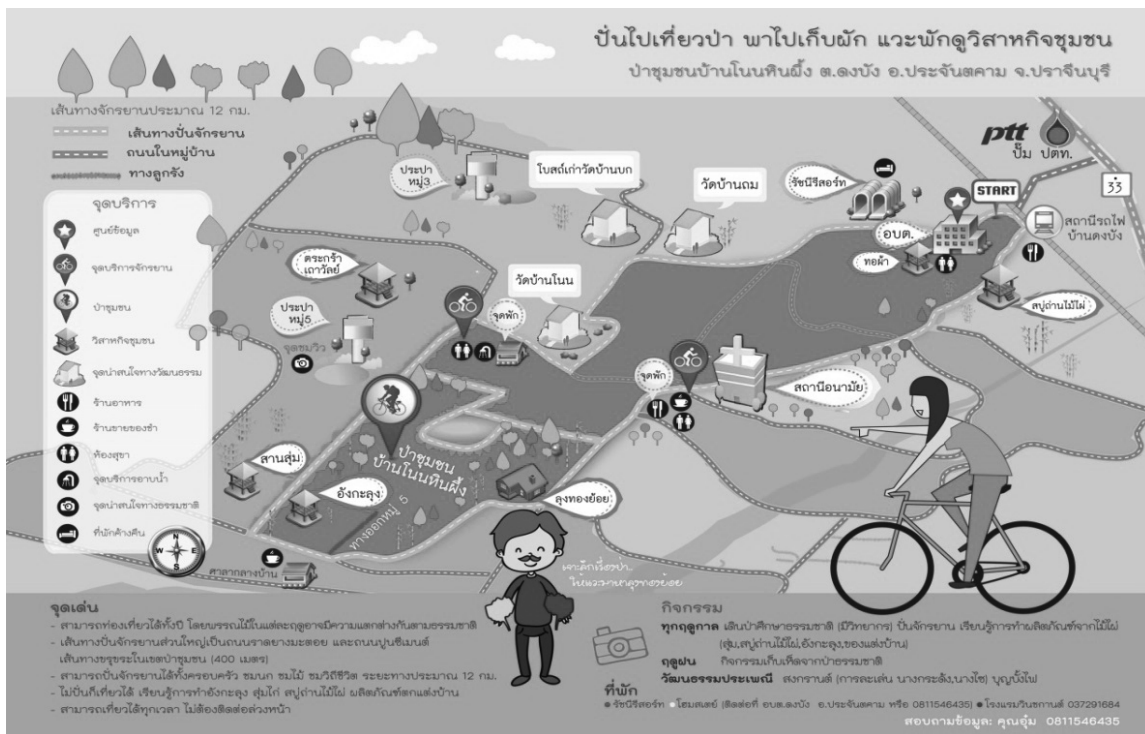


Figure 1 description of bike trail for tourism in the community [1]

Results and Discussions

1. Analysis of general status of the respondents

Table 1 Analysis of general status of the respondents

	Male	More than 45 years	Income/month lower than 15,000 baht	state enterprise officers	Experiencing of similar activity	Experiencing of riding a bike for tourism of mere 5 years
Number	37	31	24	16	35	29
Percentage	74.00	32.00	48.00	32.00	70.00	58.00

According to table 1, the prototype tourists have the following general status as male, age more than 45 years old, income less than 15,000 baht a month, having a career of state enterprise officers, experience of riding a bike for tourism, and experience of riding a bike for tourism of more 5 years.

2. Data analysis of satisfaction of participating in prototype activity and variable relation related to

participating in bike activity for tourism and satisfaction of participating in a prototype activity

According to satisfaction of participating in prototype activity in every aspect, mean is 3.52 indicating high level of satisfaction of participating in prototype activity; data of each aspect can be analyzed and categorized as the (table 2-8) shown.

Table 2 Data analysis showing satisfaction level and relation between product variables

Prototype activities	Data related to satisfaction level			Relation of riding a bike experience and satisfaction			
	Mean	S.D.	Satisfaction	t	Mean Exp.	Mean Inexp.	Sig
Appropriateness of activity	3.76	0.72	High	-1.483	3.53	3.86	0.15
Difference of activity type	3.64	0.75	High	0.573	3.73	3.60	0.57
Various kinds of activity	3.72	0.73	High	0.054	3.80	3.69	0.62
Total aspects of product	3.71	0.61	High	-0.134	3.69	3.71	0.89

According to (Table 2), the prototype tourists have high level of satisfaction to products of prototype activity in every aspect; however, if it is categorized by experience of participating in riding a bike activity for tourism, the satisfaction level is significantly indifferent at 0.05.

Table 3 Data analysis of satisfaction and relation between price variables

Prototype activities	Data related to satisfaction level			Relation of riding a bike experience and satisfaction			
	Mean	S.D.	Satisfaction	t	Mean Exp.	Mean Inexp.	Sig
Appropriateness of activity fee	3.26	1.45	Moderate	0.654	3.47	3.17	0.52
Appropriateness of distributed product	3.48	0.95	Moderate	-1.271	3.13	3.63	0.22
Value acquired by activity participation	4.08	0.72	High	-0.508	4.00	4.11	0.61
Total aspects of price	3.61	0.79	High	-0.428	3.53	3.64	0.67

According to (Table 3), the prototype tourists have high level of satisfaction to the cost of prototype activity; however, the appropriateness of activity fee and price of product distributed has a moderate level of satisfaction. Nevertheless, if it is categorized by the experience of participating in riding a bike activity for tourism, the satisfaction level is significantly indifferent at 0.05.

Table 4 Data analysis of satisfaction and relation between place variables

Prototype activities	Data related to satisfaction level			Relation of riding a bike experience and satisfaction			
	Mean	S.D.	Satisfaction	t	Mean Exp.	Mean Inexp.	Sig
Convenient access to activity	3.86	1.03	High	0.030	3.87	3.86	0.98
Safety of trail and activity participation	3.96	0.73	High	-0.513	3.87	4.00	0.61
Clarity of directional signs and tourist attraction spots	3.48	1.11	Moderate	-1.248	3.13	3.63	0.23
Total aspects of place	3.77	0.77	High	-0.871	3.62	3.83	0.39

According to (Table 4), the prototype tourists have overall high level of satisfaction to place of prototype activity; however, the clear signs and tourist attraction spots have moderate satisfaction. Nevertheless, if it is categorized by the experience of participating in riding a bike activity for tourism, the satisfaction level is significantly indifferent at 0.05.

Table 5 Data analysis of satisfaction and relation between promotion variables

Prototype activities	Data related to satisfaction level			Relation of riding a bike experience and satisfaction			
	Mean	S.D.	Satisfaction	t	Mean Exp.	Mean Inexp.	Sig
Appropriateness of advertising and public relation	3.24	1.33	Moderate	-2.877	2.47	3.57	0.01*
Frequency of activity notification	2.86	1.36	Moderate	-3.203	2.00	3.23	0.00*
Appropriateness of sale promotion such as discount, exchange, give-out, and give in addition	3.14	1.36	Moderate	-1.165	2.80	3.29	0.25
Total aspects of promotion	3.08	1.25	Moderate	-2.576	2.42	3.36	0.01*

According to (Table 5), the prototype tourists have an overall high level of satisfaction to promotion of prototype activity; however, if it is categorized by the experience of participating in riding a bike activity for tourism, the satisfaction level of only one aspect of appropriateness of a sale promotion such as a discount,

exchange, give-out, and give in addition, is significantly indifferent. Nevertheless, there is significant difference between the inexperienced tourists in riding a bike activity for tourism having tendency to have more satisfaction than the experienced tourists in riding a bike activity for tourism at 0.05.

Table 6 Data analysis of satisfaction and relation between personnel variable

Prototype activities	Data related to satisfaction level			Relation of riding a bike experience and satisfaction			
	Mean	S.D.	Satisfaction	t	Mean Exp.	Mean Inexp.	Sig
Appropriateness of manners and courtesy by the service provider.	3.70	1.49	High	-1.450	3.13	3.94	0.16
Full of Knowledge, skill and experience throughout the activity.	3.60	1.38	High	-1.142	3.20	3.77	0.27
Assistance in fixing all kinds of problem throughout the activity session.	3.54	1.47	High	-1.805	2.87	3.83	0.09
Total aspects of personnel	3.61	1.39	High	-1.548	3.07	3.85	0.14

According to (Table 6), the prototype tourists have a overall high level of satisfaction of personnel toward prototype activity; however, if it is categorized by

the experience of participating in riding a bike activity for tourism, the satisfaction level of prototype activity participation is significantly indifferent at 0.05 level.

Table 7 Data analysis of satisfaction and relation between creation and presentation of physical evidence variable

Prototype activities	Data related to satisfaction level			Relation of riding a bike experience and satisfaction			
	Mean	S.D.	Satisfaction	t	Mean Exp.	Mean Inexp.	Sig
Appropriateness of activity duration	3.40	1.41	Moderate	-1.688	2.80	3.66	0.11
Quickness of service provided throughout activity	3.36	1.45	Moderate	-1.596	2.87	3.57	0.12
Appropriateness of tourist attraction and activity pattern	3.50	1.46	High	-1.901	2.80	3.80	0.07
Creation and physical evidence presentation	3.42	1.36	Moderate	-1.769	2.82	3.68	0.09

According to (Table 7), the prototype tourists have a overall moderate level of satisfaction of creation and presentation of physical evidence to the prototype activity; however, the appropriateness of tourist attraction and activity pattern has high level of satisfaction. Nevertheless, if it is categorized by the experience of participating in riding a bike activity for tourism, the satisfaction level of prototype activity is significantly indifferent at 0.05

Table 8 Data analysis of satisfaction and relation between process variable

Prototype activities	Data related to satisfaction level			Relation of riding a bike experience and satisfaction			
	Mean	S.D.	Satisfaction	t	Mean Exp.	Mean Inexp.	Sig
Appropriateness of step of activity participation	3.42	1.30	Moderate	-2.041	2.87	3.66	0.05*
Appropriateness of practical procedure throughout activity	3.32	1.41	Moderate	-1.646	2.73	3.57	0.12
Appropriateness of link between community tourism and nature	3.60	1.40	High	-1.804	3.07	3.83	0.08
Total aspects of process	3.45	1.30	Moderate	-1.755	2.89	3.69	0.10

According to (Table 8), the prototype tourists have overall a moderate level of satisfaction of process toward prototype activity; however, the appropriateness causing the link between community tourist attraction and nature have high level of satisfaction. Nevertheless, if it is categorized by the experience of participating in riding a bike activity for tourism, the satisfaction level of prototype activity of all three aspects is significantly indifferent. However, the significantly different aspect found that the inexperienced tourists riding a bike activity for tourism have more satisfaction level than the experienced ones at 0.05.

Table 9 Data analysis related to opinions and recommendations

Recommendations	Amount
Product	
Distributed clothes should be more colorful or occasional.	6
Various products should be more; samples are not enough.	4
Price	
It is proper.	8
Place	
Directional signs should be added throughout the bike trail.	10
There are not enough garbage bins.	8
Shady construction should be added in every spot and construction maintenance should be regularly done for the tourists' convenience and pleasure.	4
Community forests should be conserved and expanded to the surrounding community.	3
Promotion	
Public relation should be more.	6
Personnel	
They are friendly, helpful, and impressive.	3
Creation and presentation of physical evidence	
There is continuous presentation of public relation of activity through social network and TOT webpage or provincial webpage and activity provision.	4
Process	
Suitable.	3

4. Discussion

The marketing principle of the service market was applied to cover all aspects: product, price, place, promotion, personnel, creation and presentation of physical evidence. The process and creative tourism principle, a tourism pattern, enables the tourists or visitors to develop or use their own potential or others creative skills by participating in with determination and purposefulness of ways and learning experience which the people in the community always do; the tourists only volunteer or take part in.

Except for the study of the provision of prototype trail activity there are other studies with similar results. Panya and his colleges⁴ conducted a research revealing adventurous tourism management by using mountain bikes within the community, and it was found that not only more kinds of activities at the tourist attraction induced more tourists to take community tours but also the community is entitled to participate in their own career promotion and arrange their own community story. These similar results also appeared in the research conducted by the Office of Economic and Social Development Board of North-East⁵ A way of ecosystem tourism promotion by bike at Buddhism Forest of Khoa Eto Waterfall Park, Prachinburi⁶ shows that the environment is not influenced by the pattern of activity provision.

5. Recommendation

The community should develop products with more variety and unique design. However, the activity fee of 300 baht with one free t-shirt would be more appropriate if the pattern and need conformation is met. Moreover, clear directional signs and activity trails, garbage dumping spots, more public relation channels and sale promotion to induce more product buying, and sequence notification of activity process to enhance clear understanding throughout the program should be added.

Conclusion

The community should develop an activity pattern, product, and tourists' needs and community conformation to enhance the tourists' impression and

specify the niche of new tourists who have never experienced this kind of activity since the description shows that the inexperienced tourists tend to have greater satisfaction than others.

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Effect of Calcination Time on Physical and Chemical Properties of CaO- catalyst Derived from Industrial-eggshell Wastes

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Abstract

In this study, eggshell wastes from a hatchery industrial plant were carefully-calcined under air-atmosphere at 800 °C in the range of 1 to 4 hours. Then both physical and chemical properties of calcium oxide derived from calcination of eggshell wastes were systematically characterized by XRF, XRD, SEM, particle size analyzer and gas adsorption experiment. The XRF results and the XRD patterns indicate that all calcination times completely transformed the eggshell wastes to be a crystalline calcium oxide phase with about 98 % w purity of calcium oxide. The SEM characterization results indicate rod-liked porous surface on the obtained calcium oxide and size of surface pore are slightly increased with increasing of calcination times. The Langmuir surface area of the obtained calcium oxides was determined by N₂ adsorption experiment at -195 °C. This experiment showed that the surface area of the obtained calcium oxides is rapidly decreased from 14.9 to 2.0 m²/g with increasing of calcination times from 1 to 4 hour. These results indicate that calcination time is one of the factors determining both physical and chemical properties of calcium oxide. Additionally, this present study also provides a new optimum condition to produce calcium oxide from industrial-eggshell wastes with both physical and chemical properties comparable to commercial calcium oxide.

Keywords: Calcium oxide, Catalyst, Eggshell

Introduction

In Thailand, large hatchery farms generate eggshell wastes (about 6.6 tons per annum) and this waste normally goes to landfill site. This waste management scheme leads to several environmental concerns e.g. landfill leachate, odor and methane emission from landfill. Furthermore, landfill site normally cause a crucial conflict between hatchery farms and their neighbors. So, large amount of eggshell wastes have to be managed properly. Recently, eggshell has been extensively studied as a starting material for preparing low-cost calcium oxide. This is because calcium oxide is normally used as an environmentally-friendly heterogeneous catalyst in a wide range

of processes, e.g. chemical synthesis, petrochemical industry and biodiesel production process. In addition, calcium oxide can be separated from product and can be regenerated/reused in several cycles of production process. Currently, researchers reported that high purity of calcium oxide can be produced from eggshells due to it contain with high content of CaCO₃. Thermal decomposition of eggshell above 700 °C is a facile method to decompose eggshell in to carbon dioxide and calcium oxide. Several synthesis conditions on thermal decomposition of eggshell at different temperatures and under different atmospheric conditions were reported. However, the effect of calcination time on surface properties of

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calcium oxide has not been clearly reported. In this work, the effect of calcination time on physical and chemical properties of CaO-catalyst derived from Industrial-Eggshell wastes was investigated in order to find the optimum condition to produce calcium oxide from industrial-eggshell wastes.

Experimental

1. Raw Material

Chicken eggshell wastes used in this study was obtained from one of the largest hatchery farms in Nakhon Ratchasima province, Thailand. The eggshell wastes were rinsed with water to remove dirt and other impurities and were dried in an oven at 105 °C for 24 hour. The egg-shell membranes were separated manually. Then, the eggshell wastes were crushed in to powder with Panasonic MX-AC400 crushing machine followed by screening through a set of sieves to get a grain size lower than 425 micrometer.

2. Sample Preparation and Characterization

Calcium Oxides (CaO) were prepared by a thermal decomposition method. The eggshell powders were calcined in muffle furnace from room temperature to 800 °C with a heating rate of 50 °C/min. Furnace temperature was maintained at 800 °C for 1 to 4 hour under air atmosphere. Then, the furnace was left to cool down to 120 °C before calcined eggshell wastes were taken to desiccator. All calcined eggshell samples were kept in the vacuum desiccator to avoid the sample reacting with moisture and carbon dioxide in the atmosphere. Crystal structures and elemental composition of the calcined eggshells were determined by X-ray diffractometer (XRD – PW 3040/60 X'PERT PRO Console) with

Cu-K radiation and X-ray fluorescence analyzer (XRF-Bruker S4 Explorer), respectively. Surface morphology of calcined eggshells were investigated by scanning electron microscopy (SEM – LEO 1455VP). Particle size diameter of the samples was analyzed by laser diffraction particle size analyzers (Beckman Coulter LS 230). In addition, Langmuir surface area of the samples was measured by N₂ adsorption/desorption isotherm at -195 °C (Micromeritics-TriStar II 3020).

Results and Discussions

1. Effect of calcination time on chemical properties of CaO derived from industrial- eggshell wastes

XRD patterns and their major peaks of both calcined eggshells from various temperatures and commercial calcium carbonate are presented in (Figure 1) and (Table 1), respectively. The XRD pattern of commercial calcium oxide match with peak data collected from Joint Committee on Powder Diffraction Standards (JCPDS). This indicated that crystal phase of commercial calcium oxide is in both calcite and aragonite form. The XRD patterns and peaks obtained from calcined eggshells are similar to calcium oxide data collected by JCPDS. The results of XRF showed that CaO was the most abundant component in eggshell wastes (68.2 % w). (Table 2) shows the eggshell wastes also contained small amounts of Mg, P₂O₅, Na₂O, SO₃, and K₂O. However, high purity of CaO (about 98.0 % w) can be obtained after eggshell wastes were calcined at 800 °C at various times. Both XRD and XRF results showed that calcined eggshells at 800 °C from 1 to 4 hour, thermally decomposed calcium carbonate in eggshells to carbon dioxide and calcium oxide.

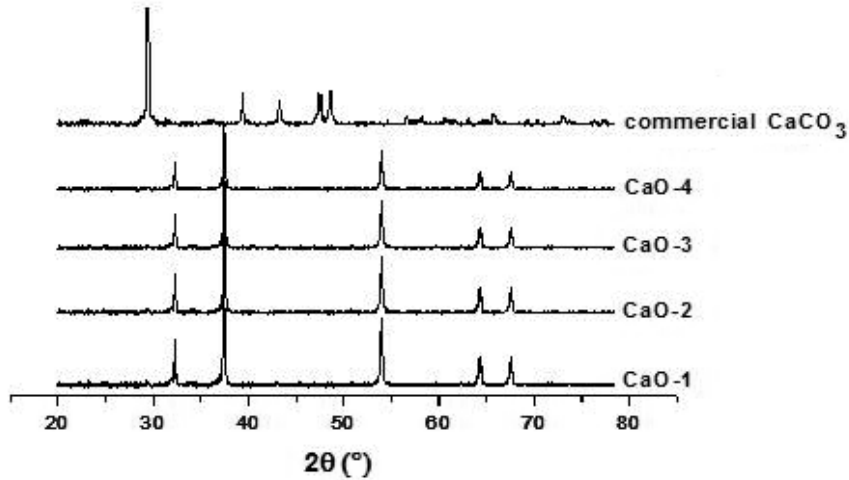


Figure 1 XRD patterns of commercial CaCO₃ and calcined eggshell waste at 800 °C for 1 to 4 hr

Table 1 Major diffraction peaks of commercial CaO and calcined eggshell waste collected by XRD technique.

Sample	Decomposition temperature	Compound	2θ
JCPDS data [1]	-	CaCO ₃	29.4° 39.4° 43.2° 47.4° 48.5°
		CaO	32.2° 37.3° 58.3° 64.1° 67.3°
Egg-shell wastes[2]	800 °C 2h	CaO	32.3° 37.42° 53.92° 64.22° 67.42°
Egg-shell wastes (this study)	As received	CaCO ₃	29.4° 39.5° 43.2° 47.6° 48.6°
	800 °C 1h	CaO	32.2° 37.4° 53.9° 64.2° 67.4°
	800 °C 2h	CaO	32.2° 37.4° 53.9° 64.2° 67.4°
	800 °C 3h	CaO	32.2° 37.4° 53.9° 64.2° 67.4°
	800 °C 4h	CaO	32.2° 37.4° 53.9° 64.2° 67.4°

Table 2 Chemical composition of the samples determined by X-ray fluorescence spectrophotometer.

Element (%w)	CaO-1	CaO-2	CaO-3	CaO-4	CaO [3]	Eggshell
CaO	97.9	98.0	97.9	97.9	97.42	68.2
MgO	0.977	0.984	1.01	1.01	1.63	0.459
P ₂ O ₅	0.543	0.530	0.579	0.532	0.52	0.396
Na ₂ O	0.223	0.162	0.171	0.161	-	0.103
SO ₃	0.111	0.107	0.116	0.120	0.26	0.105
K ₂ O	0.065	0.034	0.045	0.036	0.08	0.0622

2. Effect of calcination time on physical properties of CaO derived from industrial-eggshell wastes

(Figure 2-3) shows SEM photographs of eggshell and calcined eggshell wastes, respectively. It expressed

the rough surface of eggshell with some small pores while calcined eggshell waste obtained from different calcination times showed similar micromorphology of rod-liked or dumbbell shaped. This rod-liked shape occurred during thermal decomposition of carbonate contained in eggshell

and this rod-like shape possibly provides higher surface area than original eggshell waste.

Particle size diameter of the samples is shown in (Figure 4). Initially, particle size diameter of crushed eggshell waste was about 248.4 micron but particle size diameter of the sample gradually reduced to 59.82, 13.43, 13.75, 12.87 micron for CaO-1 to CaO-4, respectively. This indicated that particle size diameter of the sample decreased with increasing of calcination time.

The Langmuir surface areas of CaO derived from different calcination time were shown in (Figure 5). It appeared that surface area of the sample significantly increased about 5.7 times after the eggshell sample was calcined at 800 °C for 1 hour. However, the surface area of the calcined samples significantly decreased from 14.9 m²/g to 2.0 m²/g with increasing calcination time from 1 to 4 hour. Additionally, the surface area of calcined eggshell waste at 800 °C for 1 hour is remarkably higher than surface area of commercial calcium oxide.⁴

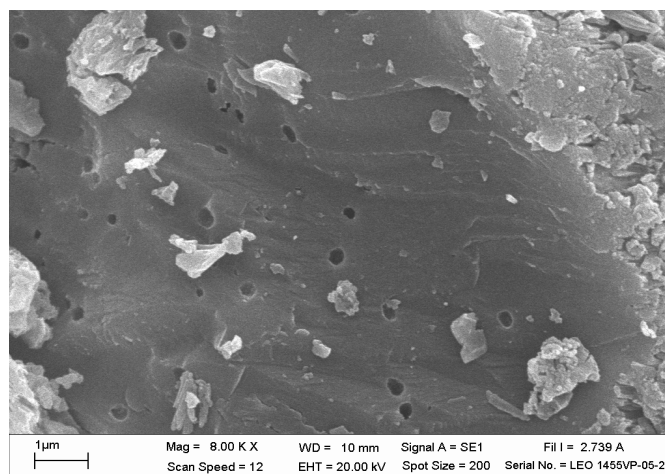


Figure 2 SEM photograph of eggshell waste with 8,000x magnification.

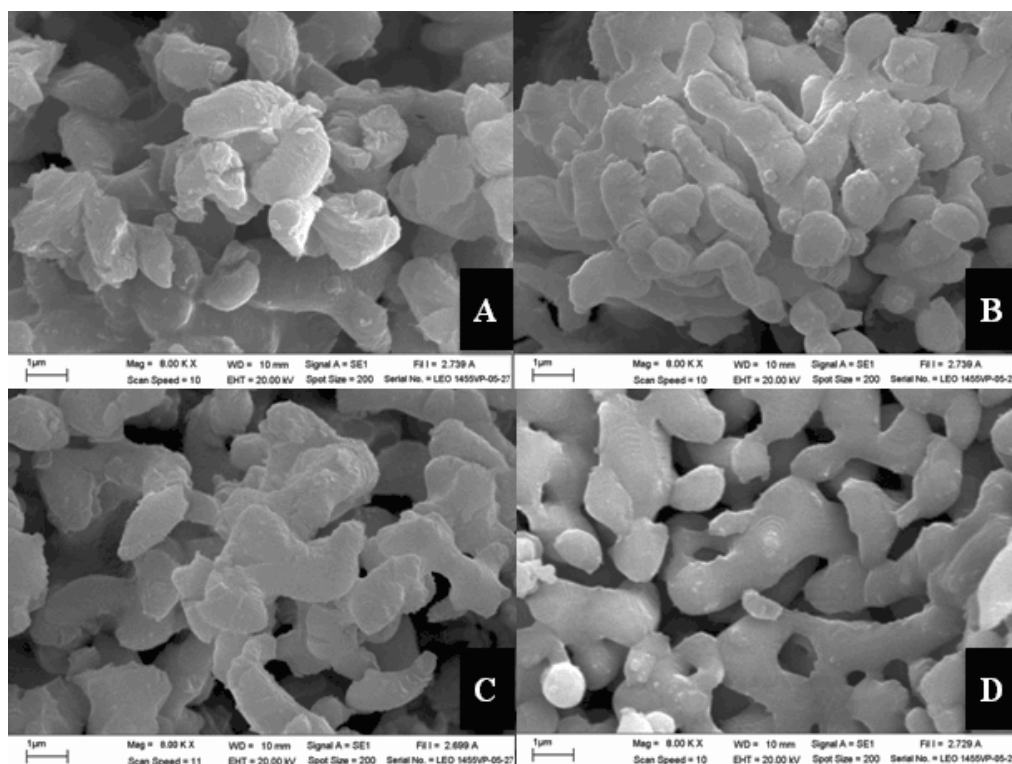


Figure 3 SEM photographs of calcined eggshell wastes at 800 °C for 1 to 4 h with 8,000x magnification, CaO-1 (A), CaO-2 (B), CaO-3 (C) and CaO-4 (D)

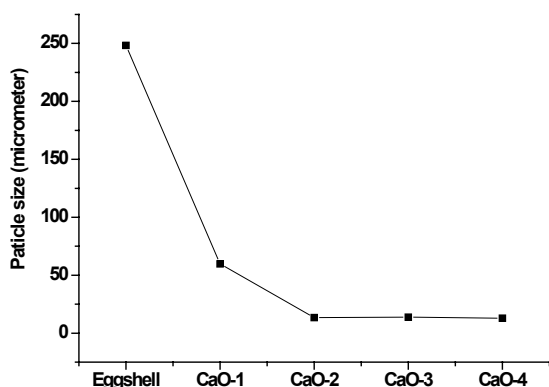


Figure 4 Particle size diameter of samples obtained from various calcination conditions.

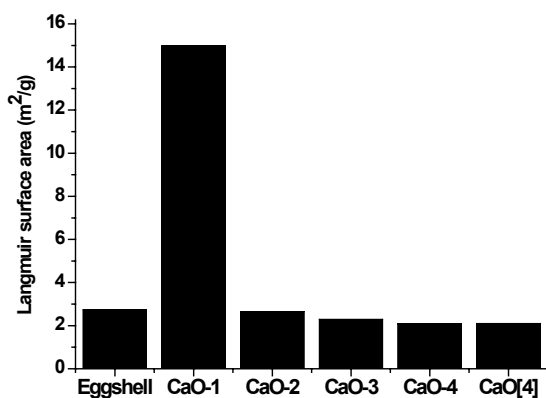


Figure 5 Langmuir surface area of various samples.

Conclusion

In this work, effect of different calcination times on both chemical and physical characteristics of calcium dioxide derived from eggshell wastes was reported. The results indicated that both particle size and surface area decrease with increasing calcination time. In terms of energy conservation, calcination of industrial-eggshell wastes at 800 °C for 1 hour is enough to produce high purity of calcium oxide. In addition, high surface area of calcium oxide can also obtained at this optimum calcination temperature and time. This study also indicated that both physical and chemical properties of calcium oxide obtained from eggshell waste were comparable to commercial calcium oxide.

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